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PLATFORM 1: Neurodegenerative: Alzheimer

1

Autopsy findings versus biomarker outcomes in a clinical trial of anti-Aβ therapies in dominantly inherited Alzheimer disease

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Background: Clinical trials of anti-Aβ monoclonal antibodies in Alzheimer disease (AD) infer target engagement from Aβ positron emission tomography (PET) and/or fluid biomarkers such as cerebrospinal fluid (CSF) Aβ42/40. However, these biomarkers measure Aβ deposits indirectly and/or incompletely. In contrast, postmortem neuropathologic assessments allow direct investigation of treatment effects on brain Aβ deposits and on many other pathologic features.

Methods: From a clinical trial of anti-Aβ monoclonal antibodies in dominantly inherited AD, we measured immunohistochemistry area fractions (AFs) for Aβ (10D5), tauopathy (PHF1), microgliosis (IBA1) and astrocytosis (GFAP) in 10 brain regions from 10 trial cases–representing gantenerumab (n=4), solanezumab (n=4), and placebo/no-treatment (n=2) arms–and 10 observational study cases. Gantenerumab, solanezumab, and control (placebo/no-treatment/observational) groups were compared based on these AFs, antemortem Aβ PET (11C-PiB) standardized uptake value ratios (SUVRs), and CSF (Aβ42/40, p-tau181, t-tau) biomarkers. Five controls lacked CSF and PET data.

Results: CSF Aβ42/40 showed significant increase in the gantenerumab arm versus controls; CSF t-tau showed a corroborating decrease; CSF p-tau181 showed no significant difference. Aβ PET SUVRs showed significant decreases in the gantenerumab arm versus controls in temporal cortex, caudate, putamen, and thalamus. Strikingly, after continued gantenerumab administration between Aβ PET and autopsy, Aβ AFs were significantly lower in the gantenerumab arm versus control in frontal, temporal, parietal, and occipital cortices, anterior cingulate, hippocampus, caudate, putamen, thalamus, and cerebellar gray matter; only posterior cingulate and cerebellar white matter comparisons were non-significant. In contrast, AFs of tauopathy, microgliosis, and astrocytosis showed no differences across groups.

Conclusions: Our results suggest that gantenerumab treatment, as administered, reduced Aβ deposits–albeit incompletely–in a dose-dependent manner, without significantly altering tauopathy, microgliosis, or astrocytosis. They also suggest that this partial reversal of AD pathology may be optimized with higher doses, more effective anti-Aβ therapeutics, earlier intervention, and/or combined treatments in future clinical trials.

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Cognitive impairment in Primary Age-Related Tauopathy: A community-based autopsy study

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Background: Primary age-related tauopathy (PART) is characterized by neurofibrillary tangles in the medial temporal lobe (MTL), especially hippocampus (HIP), with no or sparse amount of amyloid-beta (Aβ) plaques. However, there is evidence that tau pathology in PART might be present outside of the MTL. Given this restricted pathoanatomical pattern, we aimed to study tau-tangles inside and outside MTL to investigate downstream effects on cognition.

Methods: We included 527 neuropathologically confirmed PART participants (Thal 0-II, Braak I-IV; age-at-death=88 years; women=63%) from community-based cohort studies of aging. All participants underwent annual clinical evaluations including detailed cognitive testing. Tau-tangle density in the MTL, specifically HIP and outside of the MTL, specifically the neocortex of the inferior temporal lobe (IT) was evaluated by AT8-immunohistohemistry. Multivariable linear mixed-effects models examined the association between regional tau-tangles with decline in global cognition and 5 cognitive domains after controlling demographics, LATE-NC, Lewy bodies, and vascular pathologies.

Results: Tau-tangle pathology was very commonly present in the HIP (N=524/527) but also common in the IT (N=449/527). All participants who had tau-tangles in IT also had tangles in HIP. The mean HIP-tau-tangle and IT-tau-tangle density was 2.28(SD=1.29) and 0.69(SD=0.58), respectively. Tangle burden in both regions were separately associated with decline in global cognition (HIP= est=-0.005;SE=0.002,p=0.05 and IT= est=-0.016;SE=0.005,p=0.003) and 2 cognitive domains: episodic and semantic memory. In contrast, when included together in the same model the association of HIP-tangle burden with cognitive decline was attenuated and no longer significant but the association of IT-tangle burden persisted (est=-0.013; SE=0.006, p=0.038). These findings remained robust after controlling Aβ.

Conclusions: These results indicate that tangles outside the MTL are associated with cognition in PART individuals, independent of tangles inside MTL. Future studies on tau-tangle burden outside the MTL are needed to advance our understanding of the extent to which age-related tauopathy changes impact cognitive function.

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Shared Transcriptional Changes in Neurons with Tau Pathology in PART and AD

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Background: Tau pathology is common in age-related neurodegenerative diseases. Tau pathology in primary age-related tauopathy (PART) and in Alzheimer's disease (AD) has a similar biochemical structure and anatomic distribution, which is distinct from tau pathology in other diseases. However, the molecular changes associated with intraneuronal tau pathology in PART and AD, and whether these changes are similar in the two diseases, is largely unexplored.

Methods: Using GeoMx spatial transcriptomics, mRNA was quantified in CA1 pyramidal neurons with tau pathology and adjacent neurons without tau pathology in PART andAD, and compared to control cases without pathology. Transcriptional changes were analyzed for differential gene expression and for coordinated patterns of gene expression associated with both disease state and intraneuronal tau pathology.

Results: Gene expression changes associated with intraneuronal tau pathology were similar in PART and AD. Synaptic gene expression was decreased overall in neurons in AD and PART compared to control cases. However, this decrease was largely driven by neurons lacking tau pathology. Two novel gene expression signatures associated with intraneuronal tau were identified by examining coordinated patterns of gene expression, which were similar in both conditions and shared across excitatory cortical neurons in an independent dataset in AD. Genes in the up-regulated expression pattern were enriched in calcium regulation and synaptic function pathways, specifically in synaptic exocytosis.

Conclusions: PART and AD show similar transcriptional changes associated with intraneuronal tau pathology in hippocampal neurons, raising the possibility of a mechanistic relationship between the tau pathology in the two diseases. Intraneuronal tau pathology was also associated with increased expression of genes associated with synaptic function and calcium regulation compared to tau-negative disease neurons. The findings highlight the power of molecular analysis stratified by pathology in neurodegenerative disease and provide novel insight into common molecular pathways associated with intraneuronal tau in PART and AD.

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Brain Metal Concentrations and Alzheimer's Disease Neuropathological Change in Total Joint Arthroplasty Patients at Autopsy

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Background: The long-term systemic effects of total joint arthroplasty (TJA) are poorly understood relative to the high prevalence of individuals with TJAs. Recent studies suggest that cognitive decline might be slightly faster amongst older patients with TJAs, however the underlying mechanisms and neuropathological changes associated with TJA remain uncertain. One hypothesis is that components of artificial joints shed metal over time, resulting in systemic spread and accumulation in the nervous system, potentially contributing to the mechanisms of neurodegeneration. Herein, we measured metal concentrations in brain tissue and correlated these findings to Alzheimer's Disease Neuropathological Change (ADNC) in an autopsy cohort of patients with and without TJA.

Methods: This study used autopsy tissue and specimens from 88 decedents with TJA and 89 matched non-TJA controls. Ultra-trace metal concentrations (aluminum, chromium, cobalt, manganese, molybdenum, nickel, titanium, and vanadium) were measured from frozen occipital lobe tissues via triple-quadrupole inductively coupled plasma mass spectrometry. Alzheimer’s disease neuropathologic change assessment was performed following National Institute on Aging-Alzheimer’s Association criteria. Analyzes employed ordinal logistic regression models.

Results: The results show that in both TJA patients and controls, elevated concentrations of titanium, manganese, cobalt, and molybdenum were associated with increased odd-ratios of elevated ADNC A and C scores. Cobalt and titanium concentrations were found to be significantly elevated in TJA patients relative to controls (control vs TJA, titanium: 38.57 ng/g and 54.11 ng/g, $p = 0.023$; cobalt: 20.68 and 24.69 ng/g, $p = 0.024$). The increased titanium and cobalt concentrations in TJA patients, also significantly correlated to higher degrees of amyloid pathology, particularly neuritic plaques, relative to controls.

Conclusions: Systemic accumulation of metal debris in TJA patients is well documented. The study observed higher concentrations of cobalt and titanium in the brain, as well as, higher ADNC A and C scores in those with higher metal concentrations.

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Clinical and pathological correlates of ARTAG in the NACC Neuropathology Data set

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Background: The pathoetiology of age-related tau astrogliopathy (ARTAG) is poorly understood. Insights can be gained by analyzing risk factors and comorbid pathologies. Here we addressed the question of which prevalent co-pathologies are observed with increased frequency in brains with ARTAG.

Methods: The study sample was the National Alzheimer's Coordinating Center (NACC) data set. Data from persons with unusual conditions (e.g. frontotemporal dementia) were excluded leaving 504 individual autopsied research participants, clustering from 20 different ADRCs, autopsied since 2020; ARTAG was reported in 222 (44.0%) of included participants.

Results: As has been shown previously, ARTAG was increasingly frequent with older age and in males. The presence and severity of other common subtypes of pathology that were previously linked to dementia were analyzed, stratifying for the presence of ARTAG. ARTAG was relatively more likely to be found in brains with limbic-predominant age-related TDP-43 encephalopathy neuropathologic change (LATE-NC), and in brains with comorbid cerebrovascular pathology (arteriolosclerosis and/or brain infarcts). However, ARTAG was not associated with severe Alzheimer’s disease neuropathologic change (ADNC), or primary age-related tauopathy (PART). In a subset analysis of 167 participants with neurocognitive testing data, there was a trend for ARTAG pathology to be associated with cognitive impairment as assessed with MMSE scores (P=0.07). A limitation of the study was that there were missing data about ARTAG pathologies, with incomplete operationalization of ARTAG according to anatomic region and pathologic.

Conclusions: Overall, ARTAG was not associated with ADNC or PART, whereas prior observations about ARTAG occurring with increased frequency in aging, males, and brains with LATE-NC were replicated. It remains to be determined whether the increased frequency of ARTAG in brains with comorbid cerebrovascular pathology is related to local infarctions or neuroinflammatory signaling, or with some other set of correlated factors including blood-brain barrier dysfunction.

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Environmental-Pathology Interactions in Neurodegenerative Disease Dementia

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Background: Environmental factors, in particular, neighborhood socioeconomic status and air pollution are linked to cognitive dysfunction. Whether the association relates to neuropathologic changes is uncertain.

Methods: In a cross-sectional study with 419 autopsy cases collected from 2011 to 2023, we examined relationships among neighborhood disadvantage measured by categorized Area Deprivation Index (ADI) national rankings, cognitive measures, and 10 dementia-associated proteinopathies and cerebrovascular disease (Thal amyloid phase, Braak stage, CERAD score, Alzheimer's disease neuropathological change (ADNC), Lewy body disease (LBD), limbic-predominant age related TDP-encephalopathy (LATE), large infarcts, amyloid angiopathy, arteriolosclerosis, and cerebrovascular disease in relation to vascular cognitive impairment (VCING)). In a subsequent cross-sectional study with 622 cases from 2000 to 2016, we explored relationships among annual average exposure to fine particulate matter (PM2.5) air pollution estimated at the geocoded residential address for each case for year of death, cognitive measures, and 10 pathologies. All analyses were performed with adjusted regression models.

Results: Higher ADI and PM2.5 concentrations were associated with greater cognitive impairment as measured by Mini-Mental State Examination (MMSE) or Clinical Dementia Rating Sum of Boxes (CDR-SB). While ADI was not associated with an increase in dementia-associated neuropathologic changes, exposure to higher PM2.5 was associated with overall higher neuropathologic burden in major dementia-associated proteinopathies and cerebrovascular disease including higher ADNC level, LBD stage, and VCING level. Finally, the significant association between ADI and cognition was independent of overall neuropathologic changes, while the association between PM2.5 and cognition was largely mediated by increasing ADNC level.

Conclusions: Environmental factors appear to increase risk of dementia through differential pathways. Neighborhood disadvantage appears to reduce cognitive reserve independent of neuropathologic changes, while air pollution appears to affect brain maintenance resulting in exacerbation of ADNC.

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Early Alzheimer's Disease-Like Neuropathology in the Nonhuman Primate Chlorocebus Aethiops: Relationships with Age and Physical Function

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Background: Nonhuman primates are important translational models of human disease due to their conserved genomic sequence, humanoid anatomy, and susceptibility to similar diseases, including cerebral beta amyloidosis. Specifically, Caribbean vervets (Chlorocebus aethiops sabaeus), may serve as an excellent model for AD. We assessed the brains of 18 deeply phenotyped Caribbean vervets for AD-like neuropathology to further evaluate their utility as a model for early AD-like pathology and to investigate the relationships between plaque burden and other AD-risk phenotypes.

Methods: We performed neuropathologic assessments on 18 vervets. CSF was collected and gait speed was measured in 8 of these. At necropsy, we evaluated 23 brain regions for both Aβ and phospho-tau pathology. Plaque burden was assessed and heat maps were generated to show the distribution across the brain. Gait speed was defined as the average of 5 observations of the time it took to walk between two points. The last gait speed prior to death was used in analysis. Statistical analyses were performed to assess the relationships between AD-like neuropathology, age, and gait speed.

Results: Aβ plaques were identified 10 of the 18 animals. A linear regression model demonstrated a direct relationship between age and total plaque counts (p value = 0.012). Average gait speed was significantly slower in animals with beta amyloid plaques compared to those without (p=0.05). Linear regression demonstrated an inverse relationship between total plaque burden and average gait speed (p value = 0.034). Analyses of relationships between plaque load, and fluid biomarkers and cognition are underway.

Conclusions: These results support that Caribbean vervets serve as an excellent model for early AD-like neuropathology. In additional to developing Aβ plaques within the cerebrum in a pattern similar to humans, we have demonstrated a direct correlation with age and plaque burden as well as an inverse relationship between plaque burden and average gait speed.

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Machine learning analysis of Amyloid-β pathologies and their correlations in 131 cases from an Alzheimer's Disease Research Center

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Background: Accurate and scalable quantification of pathologies is crucial for deeper disease phenotyping and furthering research, especially in Alzheimer Disease (AD). This multidisciplinary study addresses the current limitations on neuropathology by leveraging a Machine Learning (ML) pipeline to perform a granular quantification of Amyloid-β (Aβ) deposits and assessing their distribution in the temporal lobe.

Methods: Utilizing available 131 whole-slide-imaging from consecutive autopsied cases at the UC Davis Alzheimer Disease Research Center, our objectives were threefold: 1) Validate an automatic workflow for Aβ deposit quantification in White Matter (WM) and Grey Matter (GM); 2) define the distributions of different Aβ deposit types, and 3) investigate clinicopathological correlates of Aβ deposits with dementia status defined by prior clinical evaluation, and the presence of mixed pathology.

Results: Our methodology highlights the robustness and efficacy of the ML pipeline, demonstrating proficiency akin to experts' evaluations. We provide comprehensive insights into the quantification and distribution of Aβ deposits in the temporal Grey/White Matter, revealing a progressive increase in tandem with the severity of established diagnostic criteria (NIA-AA). We also present correlation of the Aβ load with clinical diagnosis as well as presence/absence of mixed pathology. This study introduces a reproducible workflow, showcasing the practical use of ML approaches in the field of neuropathology, with ML-based workflows offering enhanced diagnostic accuracy in routine clinical settings.

Conclusions: Acknowledging limitations, such as potential biases in the ML model, we propose avenues for future research to refine and expand the methodology. We hope to contribute to the broader landscape of neuropathology advancements, ML applications, and precision medicine, paving the way for deep phenotyping of AD brain cases and establishing a foundation for further advancements in neuropathological research.

PLATFORM 2: Tumors Glial: Part I

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Epigenomic dysfunction and derepressed endogenous retroviral elements promote ATRX-deficient gliomagenesis

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Background: Mutational inactivation of the SWI/SNF chromatin regulator ATRX occurs frequently in diffuse gliomas. Whether and how ATRX deficiency promotes oncogenesis remains unclear, despite its implication across a broad array of physiological processes. We sought to delineate the molecular mechanisms by which global epigenomic dysfunction promotes oncogenic phenotypes in ATRX-deficient glioma models.

Methods: Building on our earlier work implicating chromatin accessibility and transcriptional shifts, we employed integrated epigenomic profiling in Atrx-intact and -deficient murine neuroepithelial progenitor cells (mNPCs), validating findings will functional approaches in human glioma stem cells (GSCs) and induced pluripotent stem cell (iPSC) derivatives of appropriate genotype.

Results: We found that ATRX deficiency dramatically impacted the integrity of large heterochromatin domains genome-wide, with tangible effects on underlying gene expression. This process fundamentally altered chromatin topology, as assessed by Hi-C analysis, and associated superenhancer landscapes, influencing key developmental gene sets like the HOXA cluster. Pharmacological inhibition of HOXA signaling in GSCs selectively reduced proliferation, increased apoptosis, and impaired in vivo growth in the ATRX-deficient context. Finally, our results pointed to derepression of LINE1 endogenous retroviral elements as both key mediators and downstream effectors of ATRX-deficient heterochromatin dysfunction.

Conclusions: Our work implicates novel and targetable molecular mechanisms involving complex epigenomic rewiring and chromatin topology in ATRX-deficient oncogenesis. In doing so, we advance the understanding of a deadly brain tumor along with broader conceptions of epigenetic mechanisms as fundamental drivers in cancer.

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Integrated clinical and molecular characterization of 200 disseminated pediatric low-grade gliomas

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Background: Pediatric low-grade gliomas (PLGG) have excellent outcomes overall but are a major clinical challenge when disseminated. Diffuse leptomeningeal glioneuronal tumor (DLGNT) is a recognised entity both clinically and pathologically, however many disseminated LGG (DLGG) fall outside this diagnosis.

Methods: To better understand the clinical and molecular features of DLGG, as well as risk factors for dissemination, we assembled an international consortium of 30 sites, contributing over 200 patients with clinical annotation, along with genomic and methylation profiling.

Results: DLGG have worse progression-free (PFS) and overall survival (OS) than PLGG overall ($p< 0.0001$). Seventy (35%) presented with a localized mass and secondary dissemination, including some with initial gross-total resection. The most common growth pattern observed (n=77, 40%) is a dominant suprasellar/optic pathway tumor with leptomeningeal drop-metastases involving the brainstem and spinal cord. Only 27 (14%) patients had diffuse tumors (without an identifiable dominant mass), and these had significantly worse OS ($p=0.03$). The most common pathologic diagnosis was pilocytic/pilomyxoid astrocytoma (n=92; 53%). DLGNT comprised a minority of cases (n=23, 14%), with a strong trend ($p=0.056$) towards worse survival compared to other diagnoses. The most frequent molecular alteration was BRAF fusion (78/151 with molecular testing available; 52%), followed by FGFR mutations or fusions (18/151; 12%). BRAF V600E was underrepresented (14/151; 9%). 1p deletion was rare (17 patients) but highly associated with DLGNT. Methylation classification was highly concordant with histologic diagnoses rather than clinical behavior. Importantly, patients that received upfront targeted therapies (BRAF and/or MEK inhibition, n=9) had better PFS compared to those receiving chemotherapy (n=146, $p=0.05$). Furthermore, patients who were treated sequentially with chemo

then targeted therapy had longer PFS on targeted therapy (p=0.011).

Conclusions: This study presents the largest cohort of DLGG to date, expanding our understanding of the clinical, pathologic, and molecular features of this disease, and supports the use of upfront targeted therapy.

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Preanalytic variables impacting clinical evaluation of PI3/AKT/mTOR signaling in diffuse glioma

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Background: Biomarker-driven therapeutic trials and window of opportunity clinical trials are critical to advances in patient outcomes. Yet, evidence-based protocols that minimize preanalytic variables are critical for their successful implementation. In diffuse glioma, phosphatidylinositol 3 (PI3)-kinase/AKT/mTOR (PI3/AKT/mTOR) signaling is an attractive therapeutic target. Yet, the relevant preanalytic variables and optimal tumor sampling methods necessary to measure pathway activity are unknown.

Methods: To investigate potential preanalytical variables we used a murine model for IDH-wildtype glioblastoma (GBM) and human tumor tissue from IDH-wildtype and IDH-mutant diffuse glioma. Cellular phosphoprotein expression was quantified from whole slide images for six phosphoproteins that are common readouts of PI3K/AK/mTOR activity (p-PRAS40, T246; p-mTOR; S2448; p-AKT, S473; p-RPS6, S240/244 and S235/236, and p-4EBP1, T37/46).

Results: First, we determined the impact of delayed time-to-formalin fixation, or cold ischemia time (CIT), on the quantitative assessment of cellular expression of six phosphoproteins that are commonly used as readouts of PI3K/AK/mTOR activity. With CITs ≥2 hours, typical of routine clinical handling, all had reduced or altered expression with p-RPS6 (S240/244) exhibiting relatively greater stability. Patient tumor samples showed a similar pattern with increased sensitivity of p-4EBP1 to delayed fixation relative to p-RPS6 (S240/244). Most clinical trials depend on the exchange of unstained slides for biomarker evaluation. Thus, we evaluated the impact of slide storage conditions on cellular expression of phosphoproteins after 3 or 5 months of storage. Slide storage at -80ºC was required to preserve p-4EBP1 and p-AKT expression and p-RPS6 (240/244) expression was not stable regardless of storage condition. Biomarker heterogeneity is an important factor impacting optimal tumor sampling. In multiple regionally distinct human tumor samples from 8 patients, quantification of p-RPS6 (240/244) expression revealed significant intratumoral heterogeneity.

Conclusions: The clinical assessment of PI3K/AKT/mTOR signaling must overcome both intratumoral heterogeneity and multiple preanalytic factors to accurately assess PI3K/AKT/mTOR signaling in diffuse glioma.

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Activated Signaling Pathways and Therapeutic Responses of Diffuse Leptomeningeal Glioneuronal Tumor with Chromosome 1q Gain

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Background: Diffuse leptomeningeal glioneuronal tumor (DLGNT) with chromosome arm 1q gain (1q+) demonstrates aggressive clinical behavior. Its rarity and the often-minute sample size limit our understanding of the biological basis of its aggressiveness. We sought to determine the activated signaling pathways and responses to current therapeutic options of 1q+ tumors in a multi-institutional cohort of 42 DLGNTs.

Methods: DNA methylation profiles of 13 1q+ tumors and seven tumors without 1q+ were compared to identify differentially methylated regions to suggest activated signaling pathways in 1q+ tumors. Orthogonal validation of the findings was performed using RNA sequencing (seven 1q+; two without), deep proteomics and phosphoproteomics (four distinct 1q+ tumors from a patient), and immunohistochemistry (18 1q+; 12 without). Treatment details and responses were available for 16 patients (nine 1q+; seven without), with progression-free and overall survival data available for all (23 1q+; 19 without).

Results: Gene Set Enrichment Analysis of all differentially methylated regions suggested increased activities of the MAPK, PI3K/AKT/mTOR, WNT, and p53 pathways in 1q+ tumors compared to tumors without. In addition, positive regulators of these pathways were hypomethylated on chromosome 1q as a putative mechanism sustaining their activation. The aberrant activation of these pathways in 1q+ DLGNT was orthogonally supported by RNA sequencing, deep proteomics and phosphoproteomics, and immunohistochemistry data. Among the overexpressed genes/proteins on 1q, CLK2 may contribute to the activated PI3K/AKT/mTOR pathway in 1q+ DLGNT. Our molecular findings suggested that 1q+ DLGNT would resist conventional chemotherapy, single-agent targeted therapy, and radiation. The cohort's treatment response and outcome data supported this notion.

Conclusions: DLGNT with 1q+ were refractory to current treatment options. We identified the activated signaling pathways underlying the aggressiveness and treatment resistance of 1q+ tumors. Our findings suggest therapies targeting CLK2 and the MAPK, PI3K/AKT/mTOR, WNT, and p53 pathways should be further tested for 1q+ DLGNT.

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Comparison of CDKN2A/B homozygous deletion detection by DNA methylation and DNA next generation sequencing

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Background: CDKN2A/B homozygous deletion status is critical for grading of several central nervous system (CNS) tumors, including IDH mutant astrocytoma, pleomorphic xanthoastrocytoma, and meningioma. CDKN2A/B status can be assed using a variety of methods, but the accuracy varies due to technical and analytical variables. Here we sought to compare sensitivity of CDKN2A/B homozygous deletion detection by NGS and DNA methylation array derived copy number analysis.

Methods: We retrospectively analyzed 100 CNS tumors diagnosed at NYU Langone Health between 2020 and 2023. All tumors were profiled by whole genome DNA methylation profiling using the Illumina EPIC array. Copy numbers were analyzed using the 'conumee' R package and visual inspection. Next-generation sequencing (NGS) analysis was performed by NYU Langone Genome PACT, a 510(k) FDA cleared (K202304) matched tumor-normal 607 gene panel. Variant allele frequency of canonical driver mutations was used to molecularly estimate tumor cell content. Discrepant cases were assed using targeted qPCR.

Results: Our cohort of 100 CNS tumors included glioblastoma (N=75), IDH mutant astrocytoma (N=14), oligodendroglioma (N=4), pleomorphic xanthoastrocytoma (N=2), meningioma (N=1), infantile hemispheric glioma (N=1), central neurocytoma (N=1), diffuse midline glioma K27-altered (N=1), and anaplastic pilocytic astrocytoma (N=1). By DNA methylation, CDKN2A/B homozygous deletion was detected in 54 cases, hemizygous deletion was detected in 13 cases, and no deletion was detected in 33 cases. By NGS, CDKN2A/B homozygous deletion was detected in 31 cases, hemizygous deletion was detected in 28, and no deletion was detected in 41 cases. DNA methylation detected a homozygous deletion in 23 cases that were not detected by NGS. All cases of homozygous deletion detected by NGS were also detected by DNA methylation.

Conclusions: DNA methylation derived copy number assessment is more sensitive for CDKN2A/B homozygous deletion than next generation sequencing. This may be due to higher sensitivity of NGS to low tumor cell content for homozygous deletion calls.

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Integrated deep learning model for prediction of DNA methylation and tumor type from histopathology in CNS tumors

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Background: DNA methylation profiles, which capture the methylation status of thousands of individual CpG sites, are data-driven means to enhance diagnostic accuracy, but this technique is not yet routinely available. Deep learning of histopathology images provides an opportunity to obtain potentially useful diagnostic information quickly to assist in the subsequent workup.

Methods: Initial investigations showed that over 60,000 CpG site beta-values could be well-predicted (correlation coefficient 0.4 or higher) from histopathology images. To examine the potential of deep learning in concert with DNA methylation, we developed a model that predicts 10 major categories of CNS tumors from histopathology by integrating three distinct components: 1) prediction directly from histopathology slide images (‘direct model’); 2) predictions of DNA methylation beta-values, which are subsequently used for a classifier (‘indirect model’); and 3) routinely available patient demographics.

Results: Using a model to predict 10 categories of common CNS tumors, trained on an internal dataset of 1,796 patients, we apply this integrated model on three independent external test datasets of 2,156 patients, achieving an overall top-1 accuracy of 95% on samples which are predicted with high confidence, across the three cohorts. For individual cases, the model refined or changed the initial suspected diagnosis, which was then shown to align with the actual methylation classifier result. Additional investigations have expanded a classifier to more than 25 CNS tumor classes, with the inclusion of additional biomarker predictions (e.g., models to predict IDH, H3K27M mutation status and others) as additional features in the overall model.

Conclusions: The methylation status of a subset of CpG sites can be well-predicted from histopathology, and these inferred methylation values could then be used to classify tumors using an integrated model. Such an approach could assist pathologists in initial assessment of DNA tumor diagnosis, by providing suggestions that could then be worked up specifically for individual cases.

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Primary diffuse leptomeningeal gliomatosis in adults: clinical-pathological and molecular findings of 5 cases

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Background: Adult primary diffuse leptomeningeal gliomatosis (PDLG) is a rare, rapidly progressive, and fatal disease marked by distinct leptomeningeal infiltration by a glial tumor in the absence of an identifiable parenchymal mass.

Methods: We describe the clinical, pathological, and molecular features of 5 adult PDLG patients (4 female, 1 male), mean age 60 years (range, 52-68).

Results: All patients had pathologic intracranial leptomeningeal enhancement at time of presentation, 3 with associated FLAIR abnormality in the adjacent brain parenchyma. Of 4 patients with available spine imaging, 3 showed spinal leptomeningeal enhancement upon presentation, and the fourth patient developed it within less than 6 months. In all patients, leptomeningeal biopsy (1 frontal, 1 temporal, 1 thoracic spine, and 2 cauda) showed infiltration by an astrocytic glioma with mitotic activity, but no definite microvascular proliferation or necrosis. GFAP and S100 were focally or diffusely positive in four cases, but negative in one case. All tumors were IDH-wildtype, 4 harboring TERT promoter mutation (1 in conjunction with NF1 mutation) and 1 with PTEN and two TP53 mutations. Chromosomal microarray showed chromosome 7 whole or partial gain in all cases, 9p loss in 4 cases (3 with homozygous CDKN2A/B deletion), and chromosome 10 loss in 3 cases plus additional full and partial chromosome gains and losses, consistent with a high-grade astrocytoma (1 case) or glioblastoma, IDH-wildtype (4 cases). Methylation EPIC array was performed in 1 case and resulted in a no match. Four patients died from the disease: three while under hospice care within a month of biopsy, and one survived 1.4 years after chemoradiation therapy. Additionally, one patient remains alive with two months of post-biopsy follow-up.

Conclusions: Adult PDLG is an aggressive disease with short survival. The clinical, pathological, and molecular features closely resemble those of glioblastoma, IDH-wildtype.

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Rapid Determination of MGMT Methylation Status in Adult-Type Diffuse Gliomas by Droplet Digital PCR Using Intraoperative Smear Preparation

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Background: MGMT methylation is an important predictor of response to chemotherapy in adult-type diffuse gliomas. Currently, MGMT methylation assessment requires formalin-fixed, paraffin-embedded (FFPE) tissue. We developed a digital droplet PCR (ddPCR) MGMT assay performed on bisulfite converted DNA utilizing primers targeting CpG positions with known clinical utility, which we validated against DNA methylation array (98.7% concordance). Smear preparations are routine for intraoperative neurosurgical consultation, thus we sought to validate ddPCR using smear preparations.

Methods: Thirty-six smears were collected during intraoperative consultation. For the first 28 samples, smears were DNA extracted and bisulfite converted, and MGMT promoter methylation was quantified by ddPCR. These results were compared to DNA methylation array. For the final 8 samples, slides were H&E-stained and coverslipped for cellularity assessment prior to DNA extraction, and directly compared with corresponding FFPE specimens.

Results: Of the 28 smears, 25 (88%) were concordant with methylation of corresponding FFPE samples. Two smear preparations were falsely negative (8%). However, this was likely due to sampling as these smears did not show neoplastic cells cytologically. One smear (4%) was positive by ddPCR but negative by conventional methylation of FFPE tissue, although the ddPCR result was only marginally above threshold (4.022% vs 4%). For the eight samples with confirmed adequate cellularity, ddPCR of the smears and FFPE sections all correlated. Furthermore, the percent MGMT promoter methylation matched when normalized to tumor cellularity.

Conclusions: We describe a novel and expedited approach to MGMT methylation assessment using intraoperative smears and ddPCR in adult-type diffuse gliomas. This approach will reduce turn-around time (approximately 2 days) over conventional MGMT analysis with FFPE and could provide clinically pertinent information for treatment planning and clinical trial enrollment options prior to discharge. Furthermore, this approach may also be utilized in those cases wherein limited biopsy tissue is available, which would conserve FFPE tissue for other analyses.

PLATFORM 3: Neurodegenerative: FTLD/Lewy Body/Parkinson, Trauma, Vascular

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ANXA11 is a significant aggregating disease protein in FTLD-TDP Type C and related TDP-43 proteinopathies

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Background: TAR DNA-binding protein 43 (TDP-43) is a RNA binding protein that co-localizes to ribonucleoprotein granules tethered to lysosomes via ANXA11. TDP-43 proteinopathy occurs in 88% of amyotrophic lateral sclerosis (ALS) patients, 55% of Alzheimer's disease patients as limbic-predominant age-related TDP-43 encephalopathy neuropathologic change (LATE-NC), and 42% of frontotemporal degeneration patients as frontotemporal lobar degeneration (FTLD-TDP). FTLD-TDP pathology shows distinct histological subtypes. Type A, B and C are the most common subtypes and Type C may be the most frequent in sporadic FTLD-TDP. ANXA11 inclusions occur in ALS-associated ANXA11 variants, but ANXA11 pathology has not been described in non-variant ALS, FTLD-TDP or LATE-NC cases.

Methods: Neuropathologically diagnosed neurodegenerative disease cases (n= 818) were screened for ANXA11 variants. ANXA11 and TDP-43 immunohistochemistry and double immunofluorescence was performed on putative ANXA11 variant cases and a cohort of sporadic and genetic ALS, FTLD-TDP, LATE-NC and other neurodegenerative diseases (n=332). Immunoblot analysis was performed on sarkosyl-insoluble fractions from post-mortem brains.

Results: TDP-43 and ANXA11 inclusions appear as thick dystrophic neurites in cortex and cytoplasmic inclusions in dentate gyrus and striatum, with similar severity in all FTLD-TDP Type C cases (n=35). ANXA11 inclusions are uncommon in LATE-NC (n=8, 7%), FTLD-TDP Type A and B (n=3, 4%), and ALS cases (n=2, 3%). In a clinical ALS patient with the ANXA11 variant p.G38R and in sporadic ALS and LATE-NC cases, ANXA11 and TDP-43 aggregates co-localize by double immunofluorescence. ANXA11 is the primary pathology in an atypical parkinsonism patient with a novel ANXA11 variant p.P75S in the absence of TDP-43, tau and alpha-synuclein pathology. By immunoblot, FTLD-TDP type C and ANXA11 variant cases show insoluble ANXA11 accumulations including a truncated fragment.

Conclusions: The identification of ANXA11 inclusions in FTLD-TDP Type C cases and related TDP-43 proteinopathies suggests that ANXA11 dysfunction is pathogenic in both sporadic TDP-43 proteinopathies and "annexinopathies" with ANXA11 variants.

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Cellular Dissection of Multiple System Atrophy

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Background: Multiple system atrophy (MSA) is a rapidly progressive neurodegenerative disease that can present clinically with atypical parkinsonism (MSA-P) or cerebellar ataxia (MSA-C). MSA has no established genetic cause and no effective treatment. Pathologically, MSA is characterized by the accumulation of alpha-synuclein in glial cells, predominantly oligodendroglia, and occasionally in subtypes of neurons. It remains unknown how populations of neurons and glia are altered by alpha-synuclein in affected brain regions in MSA patients.

Methods: We used high-throughput single-nucleus RNA-sequencing (snRNA-seq) to generate comprehensive transcriptional profiles of the cerebellum, which contains a high burden of alpha-synuclein pathology in MSA, from a large cohort of cases and age-matched controls.

Results: From the transcriptomes of over 400,000 high-quality nuclei in total, our analysis revealed statistically significant decreases in the relative abundance of specific neuronal populations, including Purkinje neurons in MSA-C. Furthermore, our preliminary results demonstrate extensive transcriptional perturbations in oligodendrocytes, including some shared cellular pathways with excitatory neurons.

Conclusions: Our approach provides a framework for understanding disease mechanisms of MSA in further studies.

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CSF Alpha Synuclein Seed Amplification Assay Performance in Relation to Neuropathological Staging of Lewy Body Diseases and Co-pathologies

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Background: AIMS To examine the predictive value of CSF alpha- synuclein seed amplification assay in an autopsy-confirmed cohort of Lewy body dementia cases

Methods: The US Dementia with Lewy Bodies Consortium (DLBC) is a NIH funded project to longitudinally assess a clinical cohort of Lewy body dementia. DLBC participants were asked to contribute CSF and blood for biomarker testing and collection for the Parkinson’s Disease Biomarker Program (PDBP) biofluid repository (BioSend). DLBC participants who died and consented for autopsy with cerebrospinal fluid (CSF) alpha-synuclein Seed Amplification Assay (SAA) results were assessed for neuropathology of Lewy Body Disease (LBD), Alzheimer's Disease Neuropathologic Change (ADNC), Limbic Associated TDP-43 Encephalopathy (LATE) and vascular pathology.

Results: Currently, the DLBC has enrolled over 160 participants, of which 40 cases have come to autopsy. Of these, 32 are currently available for neuropathologic analysis of staging of LBD, ADNC, LATE and vascular pathology. Three cases did not have Lewy body pathology by H&E and alpha-synuclein immunohistochemistry (LB509) in brainstem, limbic or neocortical regions, and all of three were CSF alpha-synuclein SAA negative. Of the remaining 29 cases, one was SAA negative (amygdala predominant LBD), one was indeterminate (brainstem-predominant LBD) and one additional amygdala predominant LBD case was SAA positive. The remaining 26 limbic and neocortical LBD staged cases were all SAA positive.

Conclusions: CSF alpha-synuclein SAA positivity was highly predictive of limbic or neocortical stage LBD, as confirmed by neuropathologic assessment. In cases with more restricted LBD (e.g., amygdala or brainstem predominant), the SAA findings were inconsistent. Future efforts to increase the sensitivity of SAA may assist in identifying LBD in individuals in this latter circumstance. Importantly, the DLBC autopsy data suggest that other neurodegenerative co-pathologies do not appear to impact the ability of CSF alpha-synuclein SAA to detect limbic or neocortical LBD.

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Neuropathology Indicates Limitations to Clinical Biomarker Based Staging of Lewy Body Disorders

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Background: In recent years, proposals have been advanced to redefine or reclassify Lewy body disorders (LBDs) using clinical criteria including biomarkers. These have either minimized the differences between, or even merged, the long-established entities of Parkinson's disease (PD) and dementia with Lewy bodies (DLB), and without systematically considering comorbid Alzheimer's disease (AD).

Methods: We used a large dataset from the Arizona Study of Aging and Neurodegenerative Disorders to determine whether the groups defined by classical clinicopathological criteria are statistically separable. Clinicopathologically-defined groups included PD (n = 169), PD/AD (n = 83), DLB Consortium High, DLB-H (n = 64), DLB Consortium Intermediate, DLB-I (n = 173), Alzheimer's disease (AD) with amygdala-predominant Lewy bodies, ADLB (n = 294) and AD with no Lewy pathology, ADNLB (n = 486).

Results: Analysis of variance or Chi-square tests showed highly significant ($p < 0.0001$) group differences in age of onset, disease duration, fraction with an apolipoprotein E4 genotype, sex, fraction with severe SN neuronal loss, fraction with peripheral alpha-synucleinopathy, brain alpha-synucleinopathy load, UPSIT smell test score, UPDRS motor score and MMSE score. Groups tended to cluster based on whether their predominant neuropathology was Lewy or AD related.

Conclusions: The ADLB and DLB-I groups are very similar to ADNLB, suggesting the Lewy pathology in these may be secondary to AD. The proposed clinical and biomarker based staging schemes for LBDs do not recognize this neuropathologically defined heterogeneity and fail to note the importance of comorbid AD. Biomarker-based LBD staging is critically needed but must incorporate AD biomarkers in order to approximate the clinicopathological classifications that have effectively guided research and treatment for decades.

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Small Vessel Disease in the Brains of Young Military Service Members

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Background: Small vessel disease, namely arteriolosclerosis, is an important contributor to neurologic morbidity. It is most noted in aged/aging individuals and particularly those with histories of hypertension and/or related cardiovascular risk factors. The Department of Defense/Uniformed Services University Brain Tissue Repository (DoD/USU BTR) is a brain bank dedicated to military brain health, and receives donations from many young Service Members (average age: 48 years) whose health records are accessible, and who have a variety of traumatic brain injury (TBI) exposures.

Methods: We blindly examined H&E-stained sections from frontal lobe (bilateral orbitofrontal and dorsolateral prefrontal), temporal lobe, basal ganglia, brainstem, and cerebellum of 50 consecutive brain donations from individuals aged ≤55 years at the DoD/USU BTR for arteriolosclerosis and perivascular hemosiderin, the severity of which was graded (0-3) on each slide.

Results: Average age was 40.9 years (range: 21-55). 29/50 cases had history of hypertension and/or related cardiovascular risk factors, e.g., diabetes, obstructive sleep apnea, and tobacco/nicotine use. 35/50 had TBI history, whether military-related (e.g., blasts) or civilian (e.g., contact sports). 39/50 had some level of arteriolosclerosis, and 49/50 had some degree of perivascular hemosiderin deposition. Of the 21 cases without hypertension or related conditions, 16 had identifiable arteriolosclerosis, 20 had perivascular hemosiderin deposits, and 15 had TBI history.

Conclusions: We present a high frequency of small vessel disease in brains of young Service Members, many of whom do not have a history of hypertension or related conditions, and many of which have TBI histories. The DoD/USU BTR represents an important opportunity to study the pathophysiology of cerebral microvascular disease over the course of aging, and to assess the contribution of various factors, namely TBI. Disclaimer: The information/content and/or conclusions do not necessarily represent the position/policy of, nor should any endorsement be inferred on the part of, USU, the DoD, or the US Government.

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Differential detection of TDP-43 in FTLD-TDP Type C using antibodies targeting distinct TDP-43 phosphorylated sites

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Background: Frontotemporal dementias are neuropathologically characterized by frontotemporal lobar degeneration (FTLD). Intraneuronal inclusions of TDP-43 are the pathological hallmark of approximately half of the FTLD cases (FTLD-TDP). Based on pathologic phenotypes, FTLD-TDP cases are separated into five subtypes (Types A-E). However, the molecular factors underlying these phenotypes are still poorly understood.

Methods: Proteomic studies have highlighted the significance of TDP-43 phosphorylation in the pathogenesis of TDP-43 proteinopathies. Drawing from this premise, polyclonal antibodies (Abs) targeting TDP-43 peptide sequences that contain phospho-serines (pS) 305, 369, or 375 were developed. The objective was to ascertain if these Abs can differentially recognize TDP-43 in the frontal cortex of individuals affected by various types of FTLD-TDP (Types A-C). Immunohistochemical examinations were carried out on fixed tissue; western blot analyses, and immunoelectron microscopy were carried out on tissue-extracted sarkosyl-insoluble fractions.

Results: Initial investigations focused on FTLD-TDP Type C. Immunohistochemical studies revealed that although all Abs pS305, pS369, pS375, and pS409/410 labeled the neuropil threads, pS305 and pS369 immunolabeled a significantly higher number of them as compared to the other two Abs. Biochemical analyses revealed similar TDP-43 electrophoretic profiles using the pS305, pS369, and pS409/410 Abs, but not with the pS375 Ab. Immunoelectron microscopy studies showed the presence of TDP-43 filaments, which were recognized by the four Abs.

Conclusions: Our studies support developing and validating new Abs for TDP-43 phosphorylated sites to characterize TDP-43 in different TDP-43 proteinopathies. The current findings indicate that in FTLD-TDP Type C, TDP-43 is abnormally phosphorylated at S305, S369, S375, and S409/410. Furthermore, the observation that pS305 and pS369 Abs label a higher number of neuropil threads in Type C than pS375 and pS409/410 Abs raises questions about how these Abs label TDP-43 inclusions in the FTLD subtypes. These findings underscore the importance of extending these investigations to additional phosphorylated epitopes.

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An analysis of cervical dorsal root ganglia hemorrhage in infant decedents

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Background: Cervical dorsal root ganglia (DRG) hemorrhage is reported as a specific autopsy finding in infants with hyperextension/hyperflexion injuries and thought to occur in some cases of abusive head trauma (AHT). However, the relative sensitivity/specificity of cervical DRG hemorrhage has not been examined in larger independent cohorts and evidence of spinal nerve/axonal injury has not been reported.

Methods: A retrospective review of infant deaths examined at the Oklahoma OCME from 2015-2021 was performed, including detailed review of circumstances of death, cause/manner of death, and autopsy findings. A prospective analysis was also performed on 54 infant deaths, with complete neuropathology review, including cervical DRG. Axonal injury was assessed using neurofilament and beta-amyloid precursor protein (bAPP) immunohistochemistry. Iron stains were performed to assess hemorrhage organization.

Results: Cervical DRGs were examined in 20 cases of the retrospective cohort. Cervical DRG hemorrhage was present in 14/16 (87.5%) cases suspicious for AHT, and 0/4 (0%) cases without suspicion for AHT. Within prospective cohort, cervical DRG hemorrhage was present in 5/6 (83.3%) cases with suspected AHT, and only 2/48 (4.2%) cases without AHT suspicion. Axonal bAPP accumulation was observed in spinal nerve roots only in the 5 cases of suspected AHT with cervical DRG hemorrhage, but not in any other case and not in other brain regions in any case. Finally, there was discordance of hemorrhage organization between subdural and DRG hemorrhages in a subset of cases.

Conclusions: Cervical DRG hemorrhages were relatively, though not absolutely, sensitive and specific for AHT, especially when combined with the other elements of the AHT triad. There was abnormal spinal nerve bAPP accumulation in cases with suspected AHT, suggesting functional injury of cervical nerve roots that could be either mechanical and/or ischemic in nature. Organization of subdural and DRG was occasionally discrepant, suggesting a difference in the time frame of when injuries occur.

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Diverse genomic alterations in single neurons after chronic brain trauma

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Background: Chronic traumatic encephalopathy (CTE) is a neurodegenerative disease associated with repetitive head impact (RHI) although little is known about its molecular pathogenesis. Previous studies of single neurons showed that private somatic mutations increase both during normal aging and in neurodegenerative disorders, and show diverse mutational patterns.

Methods: We applied two orthogonal single-nucleus whole-genome sequencing (snWGS) methods to neurons isolated from the prefrontal cortex of 15 individuals with CTE, and 4 individuals with RHI exposure but no CTE diagnosis, and compared mutational rates and spectra with neurons from neurotypical controls and Alzheimer’s disease (AD).

Results: We found a modest but significant elevation of somatic double-stranded single-nucleotide variants (SNVs) that resembles a pattern previously reported in AD. In addition, we found a strikingly large burden of small insertions and deletions (indels) and used duplex sequencing to show that these indels are mainly single-stranded, and again found a similar phenomenon in neurons from AD brain.

Conclusions: Our results suggest that neurons in CTE brain are exposed to stereotyped mutational processes, and that these processes are shared between AD and CTE suggesting potentially shared pathogenic mechanisms. Furthermore, the absence of similar changes in RHI neurons without CTE suggests that the development of CTE entails a mechanism beyond that caused by RHI alone.

PLATFORM 4: Tumors Glial: Part II

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Spatial heterogeneity of glioblastoma based on methylation profiling and impact on imaging

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Background: Recent advances in genome-wide DNA methylation profiling complement central nervous system tumor classification. Glioblastoma, IDH-wildtype (GBM) is characterized by molecular heterogeneity; however, intra-tumoral heterogeneity of methylation profiling is not well established. This study aimed to 1) investigate the intra-tumor spatial heterogeneity of DNA methylation subclasses, 2) investigate the spatial heterogeneity of tumor composition, and 3) examine how these affect radiologic imaging features.

Methods: We conducted methylation profiling on the EPIC array on 112 regions from 30 GBM patients (2-4 regions per patient). We applied two different methylation-based deconvolution methods, which infer the abundance of cell types in the microenvironment and malignant cell states. We derived 6084 radiomic features from multi-parametric MRI in each patient, including histograms, morphologic, and textural descriptors.

Results: Eight patients (27%) showed heterogeneity in GBM subclass on the DKFZ/Heidelberg CNS tumor classifier v12.6. The first deconvolution method showed that the dominant cell subpopulations in each region of the patient sample correspond to the DKFZ/Heidelberg-predicted classes, notably with the MES class displaying a significant myeloid cell presence. The second deconvolution method confirmed the association between the MES class and immune cell infiltration. In addition, it demonstrated an association between the two other classes and the dominant transcriptional subpopulation, with RTK I class associated with a dominant stem-like subpopulation and RTK II associated with a differentiated dominant subpopulation. Intriguingly, stem-like to differentiated cell ratio was preserved across patients, while the estimation of immune cell infiltration varied substantially across regions. We found that tumors enriched in stem-like cells exhibited distinct patterns of imaging features compared to tumors with a low abundance of stem-like cells.

Conclusions: Our findings highlight the importance of considering the heterogeneity in methylation profiling and microenvironment for developing targeted therapies and the potential of specific radiomic features for non-invasively predicting cell states and patient outcomes.

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HOXD12 expression is linked with tumor cell stemness and a clinically aggressive subset of oligodendroglioma, IDH-mutant and 1p19q-codeleted

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Background: Oligodendroglioma, IDH-mutant and 1p/19q-codeleted has the most prolonged expected survival among adult-type diffuse gliomas. However, older age is a significant risk factor. In this study, we sought to discover and characterize molecular factors that are associated with, but independently prognostic of age in oligodendroglioma.

Methods: We analyzed clinical data, DNA methylation array data, bulk and single-nucleus RNA-seq data, and single-nucleus ATAC-seq data from multiple independent oligodendroglioma cohorts, including TCGA, Chinese Glioma Genome Atlas, and cohorts published by Jonsson et al., Capper et al., Wang et al., and Blanco-Carmona et al. Patient age distributions were tested for normality. Unbiased differential gene expression, differential DNA methylation, and patient survival were analyzed in the context of patient age. Univariate and multivariate survival analyses were performed. Single-nucleus RNA and ATAC sequencing were used to analyze gene activity between and within neoplastic and non-neoplastic cells.

Results: Age at diagnosis was not Gaussian distributed in many of the datasets we interrogated. Differential gene expression analysis and subsequent DNA methylation analyses identified elevated HOXD12 gene expression and DNA gene body hypermethylation as age and survival-associated markers in multiple cohorts. Furthermore, in multivariate survival analyses, HOXD12 hypermethylation was independently prognostic of HOXD12 overexpression, patient age, radiographic features, and histologic features such as CNS WHO grade. Single nucleus transcriptomic and chromatin analyses revealed HOXD12 activity was disproportionally higher in neoplastic cells compared to microenvironmental cells, and nuclei with detected HOXD12 activity had enriched stemness scores.

Conclusions: We observed non-Gaussian age distributions in multiple oligodendroglioma cohorts, suggesting oligodendroglioma may be composed of age-associated subclasses. HOXD12 overexpression and DNA hypermethylation were associated with older patients; however, each factor was an independent risk indicator. Lastly, HOXD12 activity was highest in neoplastic cells, and its expression was associated with increased stemness, implicating a developmental biology pathway underlying its aggressive clinical phenotype.

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2D and 3D multiplexed subcellular profiling of nuclear atypia and rupture in human glioblastoma

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Background: Nuclear atypia, including altered nuclear morphology and formation of aberrant structures such as micronuclei (MN), is ubiquitous in cancer cells. Atypical primary and micronuclear envelopes can rupture leading to DNA damage, chromosomal rearrangements, and innate immune signaling. However, the causes and consequences of nuclear atypia and rupture are unknown in most cancers, including glioblastoma.

Methods: The DNA-binding protein BAF rapidly binds cytoplasmic DNA, enabling robust detection of nuclear envelope (NE) rupture. We use BAF immunohistochemistry to characterize NE rupture across major human cancer subtypes (N=145 tumors). We perform live-cell imaging of BAF-GFP glioblastoma patient-derived cell lines (PDCL) (N=4) to analyze the spatiotemporal dynamics of NE rupture. Using highly-multiplexed 2D cyclic-immunofluorescence (CyCIF), we analyze the causes, consequences, and spatial organization of NE rupture in glioblastoma tissue microarrays (N=145) and autopsies (N=4). We develop 3D-super-resolution multiplexed immunofluorescence methods to analyze nuclear morphology and correlate with NE rupture, DNA damage, and immune signaling in GBM tissues (N=5).

Results: BAF immunohistochemistry demonstrates NE rupture in all cancer subtypes, with particularly high rates in glioblastoma (up to >50% of cells). Live-cell imaging of PDCL demonstrates frequent rupture and repair of the NE. 2D and 3D multiplexed imaging of GBM tissues shows that primary and MN rupture are associated with irregular nuclear morphology, low lamin A/C and B1 expression, DNA damage, and activated cGAS-STING and interferon signaling. Multiplexed imaging also reveals lower lamin levels and increased NE rupture in neural-progenitor-cell-like (NPC-like) cells relative to other lineages. Spatial analysis shows that NPC-like cells are predominantly distributed to tumor infiltrating edges.

Conclusions: Nuclear atypia and rupture are core features of human cancer. In glioblastoma, NE rupture is associated with irregular nuclear morphology, reduced lamin expression, NPC-like differentiation, DNA damage, and activation of cGAS-STING/interferon signaling. NE instability and rupture likely contribute to tumor progression and may represent an intrinsic vulnerability in glioblastoma.

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H3K27M mutation loss in a recurrent diffuse midline glioma

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Background: We present the case of a 32-year-old male with a history of a thalamic diffuse midline glioma, H3K27-altered (confirmed with molecular analysis). Subsequent recurrence in the right frontal lobe was notable for the absence of H3K27M mutation (confirmed with molecular analysis).

Methods: Immunohistochemical staining and subsequent molecular analysis were performed on the initial thalamic resection specimen and the subsequent frontal lobe resection specimen.

Results: The initial thalamic specimen was found to have a histone H3 mutation (H3K27M) and a FGFR1 mutation (N577K). Status post radiation and chemotherapy, the patient was found to have a T2/FLAIR hyperintense enhancing focus in the right frontal lobe with interval increase in enhancement. Following resection, the recurrent tumor exhibited morphology similar to the initial resection. However, the immunohistochemical profile of the recurrent neoplasm was unexpected; there was variably retained H3K27 trimethylation and negative H3K27M staining. Molecular analysis failed to detect an H3K27M mutation, but the FGFR1 mutation (N577K) was present. The patient went on to develop additional frontal lobe lesions. Despite extensive disease, the patient survived for two years after initial diagnosis.

Conclusions: Notably, the CNS WHO 2021 states that “[s]omatic heterozygous H3 p.K28 (K27) mutations are invariably maintained during the disease”. The current case demonstrates that the H3K27M somatic mutation is not invariably maintained in recurrence and raises the possibility that a sub-clonal population of tumor cells exist in diffuse midline gliomas that lack the canonical molecular profile of a diffuse midline glioma.

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Histopathological and Molecular Heterogeneity of Tumors classifying as Dysembryoplastic Neuroepithelial Tumour (DNT) by DNA Methylation

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Background: Dysembryoplastic neuroepithelial tumor (DNT) is a glioneuronal neoplasm in the cerebral cortex that occurs in children or young adults and is characterized by activating mutations of FGFR1. The differential diagnosis of DNT is wide, including neoplastic to non-neoplastic. DNA methylation can be used for diagnosis. We aimed to analyze the heterogeneity of lesions classifying as DNT by DNA methylation.

Methods: We analyzed clinical, pathological, and molecular data of 46 tumors that classified as DNT methylation class using the Heidelberg DNA methylation classifier. Results of mutational, copy number and gene fusion analyses were retrieved from clinical next-generation sequencing reports including Oncomine (DNA&RNA), NYU Genome PACT (DNA) and NYU FusionSEQer (RNA). DNA methylation calibrated score was correlated with sample quality, clinical, pathological, and molecular results.

Results: Tumors classifying as DNTs with methylation scores >0.9 (23 cases) carried histological diagnoses such as diffuse astrocytoma, oligodendroglioma, and papillary glioneuronal tumor and showed a flat profile in copy number (18 cases, 78%). DNTs with scores < 0.9 (23 cases) had a variety of histological diagnoses ranging from supratentorial ependymoma to focal cortical dysplasia. Other than FGFR1-TACC1, we detected GPRC5B-PRKCA, TNS3-ETV1, and GNAI1-BRAF fusions. Furthermore, low score tumors had a higher number of CN variations (4 cases with >3 gene alterations), most frequently gain of PDGFRA. Overall, 16/46 (35%) of the histological diagnoses were correctly reclassified as DNTs by DNA methylation.

Conclusions: DNA methylation can accurately classify tumors that are histological mimickers of DNT. However, our data suggest that brain tumor lesions classifying as DNT by DNA methylation with low score represent a diverse group. Furthermore, some FCD can classify as DNT, albeit with low calibrated score and require close correlation.

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Hypoxia and macrophage-secreted TGF-β1 support glioma stem cell enrichment in the peri-necrotic zone of glioblastoma.

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Background: After necrosis develops in glioblastoma, IDH-wildtype (GBM), the tumor microenvironment undergoes significant remodeling. The peri-necrotic zone (PNZ) is characterized by severe hypoxia, influx of tumor-associated macrophages (TAMs), and enrichment of glioma stem cells (GSCs). In prior bioinformatic investigations, Hippo transcriptional co-activators YAP/TAZ have been implicated as master regulators of stemness in the PNZ. We propose that GSCs are enriched through the activation of YAP/TAZ, both directly by hypoxic regulation and by TAM secretion of TGF-β1.

Methods: ELISA of primary macrophage conditioned media and in-silico analyses of GBM and macrophage single-cell RNAseq (scRNAseq) data using NichenetR were performed to determine regulatory cytokines specific to hypoxia. Primary GBM neurospheres were exposed to normoxia, hypoxia, and cytokine candidates, and whole cell, cytoplasmic, and nuclear lysates were analyzed for YAP/TAZ, Hippo readouts (AXL, CYR61), and targets that may regulate stemness. Extreme limiting dilution assays (ELDA) assessed GSC stemness.

Results: Under hypoxia, YAP/TAZ translocated to the nucleus while Hippo readouts AXL and CYR61 were upregulated. Hypoxia likewise upregulated Inhibitor of Differentiation 2 (ID2), a YAP/TAZ target that potentially regulates GSC stemness. Conditioned media from hypoxic primary macrophages also caused upregulation of Hippo readouts, and cytokine analyses showed secretion of IL-8, CXCL5, EGF, and TGF-β1. Computational analyses using NichenetR predicted that hypoxic macrophage-secreted TGF-β1 had the highest regulatory potential among ligands that interact with hypoxic tumor cells and was strongly associated with Hippo target upregulation. Dose-response analysis of TGF-β1 on GBM showed stepwise upregulation of YAP/TAZ translocation and CYR61 under normoxia; GBM treated with TGF-β1 in hypoxia show an additive effect. Using ELDA, we found that TGF-β1 alone enhances GSC stemness and also synergizes with hypoxia.

Conclusions: Hypoxia and TGF-β1 activate Hippo transcriptional activity and enhance maintenance of GSCs, likely contributing to GBM progression and therapy resistance.

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PDE10A haploinsufficiency activates PI3K/AKT signaling independent of PTEN to induce an aggressive glioma phenotype

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Background: Glioblastoma is characterized by frequent somatic copy number alterations (SCNAs) harboring oncogenes and tumor suppressors. In this study, we employed a multi-faceted approach, integrating computational analyses and experimental validations, to uncover a novel clinically relevant tumor suppressor in glioblastoma.

Methods: Logrank survival analysis for SCNAs was performed in the TCGA glioblastoma dataset. Findings were validated in two additional datasets (NYU, REMBRANDT). Combining data from a CRISPR/Cas9 functional screen (neural stem cells/glioma stem cells) with transcriptional data from normal brain (GTEx) and glioblastoma (TCGA, CGGA, IvyGAP), we nominated a potential haploinsufficient tumor suppressor gene in the SCNA region of interest. Utilizing the RCAS/tv-a mouse model of glioblastoma, we tested the nominated gene for its effects in vivo (survival, grade, proliferation) and in vitro (neurosphere formation, proliferation, treatment response). We then combined single nucleus sequencing of mouse gliomas with in vitro assays (Western blot, cell adhesion/migration, pharmacologic pathway inhibition) to determine mechanism of action.

Results: We discovered cytoband 6q27 as an independent poor prognostic marker across multiple glioblastoma datasets. Combined CRISPR-Cas9 data, human spatial transcriptomic data, and human and mouse RNA sequencing data nominated phosphodiesterase 10A (PDE10A) as a potential haploinsufficient tumor suppressor in the 6q27 region. Mouse glioblastoma modeling confirmed that Pde10a suppression induced an aggressive glioma phenotype in vivo and resistance to temozolomide and radiation therapy in vitro. Cell culture analysis showed that decreased Pde10a expression led to increased Pi3k/Akt signaling in a Pten-independent manner. Single nucleus RNA sequencing from mouse gliomas, in combination with cell culture validation further showed that Pde10a suppression was associated with a proneural to a mesenchymal transition that exhibited increased cell adhesion and decreased cell migration.

Conclusions: Our results emphasize the clinical implications of PDE10A loss, identifying a subset (approximately 32%) of glioblastoma patients with worse outcomes, heightened resistance to standard-of-care therapy, and potential sensitivity to PI3K inhibition.

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Prognostic impact of CDKN2A deletion in oligodendroglioma

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Background: Oligodendrogliomas represent approximately 5-10% of gliomas. While molecular advances have defined this entity as 1p/19q-codeleted and IDH-mutant, further studies aimed at molecular markers for grading have had conflicting results. Additionally, studies prior to 2016 often included gliomas mimicking oligodendrogliomas, confounding interpretation of their results.

Methods: We reviewed a series of 184 oligodendroglioma specimens taken at initial resection or recurrence All specimens evaluated were grade 2 by clinical grading criteria at time of diagnosis. Whole exome sequencing (WES) was performed on all specimens, allowing confirmation of 1p/19q-codeletion by inferred copy number variation, as well as IDH-mutant status. Copy number for CDKN2A was inferred from WES data and, in those with potential homozygous loss, CDKN2A status was confirmed by fluorescence in situ hybridization (FISH).

Results: Two of the 107 grade 2 oligodendrogliomas that were evaluated at initial diagnosis had homozygous CDKN2A loss (2%). The median overall survival from time of diagnosis for patients who tumors demonstrated homozygous CDKN2A loss was 46 months while those with intact or hemizygous CDKN2A status had a median overall survival of 252 months. Five of the 77 oligodendrogliomas evaluated at tumor recurrence had homozygous loss of CDKN2A (6%). In this population, the median overall survival from time of initial diagnosis for those whose tumors demonstrated homozygous CDKN2A loss at recurrence was 80 months while those with intact or hemizygous CDKN2A status had a median overall survival of 305 months. Further studies are ongoing, including expanding the cohort by an additional 50 specimens to increase the power, as well as additional analysis of WES data to identify mutations in genes of interest, and any relationship to overall survival. Covariate analysis is also ongoing.

Conclusions: Our current univariate analysis provide support for using CDKN2A status as a grading criteria for oligodendroglioma.

PLATFORM 5: Neurodegenerative: Other

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Multi-OMIC analysis of Huntington disease reveals a neuroprotective astrocyte state
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Background: Huntington's disease (HD) is an incurable neurodegenerative disorder caused by an expansion of CAG repeats in exon 1 of the HTT gene. The disease features neuronal loss and astrogliosis most severely involving the striatum, with spiny projection neurons (SPNs) in the caudate and putamen being severely depleted. Nonetheless, neurodegeneration in HD extends to other brain regions including the cerebral cortex, where specific populations of excitatory neurons are selectively lost. To date, the underlying mechanisms of HD's selective regional vulnerability remains to be fully understood. In this work, the roles of astrocytes in mediating selective regional vulnerability to neurodegeneration will be explored.

Methods: We used a combination of bulk RNAseq, lipidomics, and single nucleus RNAseq on 76 samples from 11 control brains and 20 HD brains. We also performed validation studies using multiplex immunohistochemistry, and in vitro cultures including murine and patient derived neurons.

Results: Using a multi-omic approach, we identified a gene signature driven by the HTT gene CAG repeat length, enriched in astrocytic genes, which implicated fatty acid metabolism. Lipidomic analysis highlighted correlations between lipid species and HD grade, and integration of bulk RNAseq and lipidomics implicated pathways related to protein folding and cell death. Single nucleus RNAseq analysis of over 40,000 astrocytes identified two astrocyte types: protoplasmic and fibrous-like CD44-positive astrocytes. In HD, metallothionein-enriched protoplasmic astrocytes in the caudate were depleted. The abundance of the metallothionein-rich astrocyte cluster was correlated with the abundance of vulnerable SPNs. Conversely, metallothioneins were elevated in HD cingulate astrocytes. Genome wide association studies showed a correlation between elevated metallothionein levels and delayed age of onset in HD. Functional studies demonstrated neuroprotective effects of metallothionein-overexpressing astrocytes in co-culture models with patient derived HD medium spiny neurons.

Conclusions: Our findings unveil a regionally-enriched astrocytic phenotype in less vulnerable brain regions that could be harnessed for neuroprotection in HD.

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Chronic Traumatic Encephalopathy in a Cohort of Military Veterans in the UNITE Brain Bank

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Background: Chronic traumatic encephalopathy (CTE) is a neurodegenerative disease associated with exposure to repetitive head impacts occurring in contact sport and military-related activities. CTE has also been described after blast exposure.

Methods: Here, we report the demographic, clinical, and neuropathological characteristics of a case series of military veterans whose brains were donated to the UNITE Brain Bank. Informants for donors participated in a battery of online questionnaires and phone interviews to retrospectively assess clinical information and athletic and military histories. Neuropathological diagnoses of CTE and other neurodegenerative diseases were based on well-established criteria. Clinical and neuropathological evaluations were conducted blinded to one another.

Results: Among 307 brain donors, 302 (98.4%) of whom were male, mean (SD, interquartile range) age at death was 71.45 (17.43, 65-84) years. Most donors (262 [85.3%]) played contact sports, including 234 (76.2%) who played American football. 84 (27.4%) donors saw combat, 80 (26.1%) suffered military-related traumatic brain injuries (TBI) and 45 (14.7%) were exposed to blasts. 179 (58.3%) donors were diagnosed with CTE. Alzheimer's disease was diagnosed in 84 (27.4%), Lewy body dementia in 68 (22.1%), frontotemporal lobar degeneration in 21 (6.8%), and motor neuron disease in 1 (0.3%). Among 179 with CTE, 175 (98%) were also contact sport athletes. Among the 4 (2.3%) donors with CTE who did not play contact sports, 4 (100%) saw combat, 4 (100%) suffered military-related TBI and 3 (75%) were exposed to blasts. Informant-reported post-traumatic stress disorder symptoms were present in 120 (39.1%) donors, with a similar frequency (42.5%) among those with CTE. Dementia was present in 203 (66.1%) and was more common (74.3%) among those with CTE.

Conclusions: Military brain donors with a history of contact sport play had a high rate of CTE. Contact sports and military service are independent risk factors for CTE, future studies will explore their potential cumulative effect.

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Deciphering the role of the NBIA-mutated protein WDR45 in autophagy and neuroprotection

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Background: Perturbations in autophagy have been implicated in various neurodegenerative disorders including Parkinson's and Alzheimer's disease. WDR45, an autophagy gene is mutated in a group of patients with the familial neurodegenerative disease – Neurodegeneration with Brain Iron Accumulation (NBIA). This disease is characterized by neuronal loss and iron accumulation in basal ganglia and other parts of brain. WDR45 belongs to the WIPI family of proteins with four mammalian members. The precise biological role of WDR45 and how its disruption causes neurodegeneration is unclear.

Methods: To shed light on the function of WDR45, we have generated a deletion mutant for the Drosophila melanogaster WDR45 homolog – CG11975.

Results: These mutants show markedly diminished life span and compromised flight ability. There is extensive accumulation of Ref2 and ubiquitin-positive aggregates in the brains of these animals. These findings suggest that the fly WDR45 mutants may serve as a good model to understand the molecular mechanisms of NBIA. Using a proteomics approach, we have identified putative autophagy cargos that may be degraded with the help of WDR45.

Conclusions: By examining the functional interaction between WDR45 and its putative cargos, we hope to provide novel insights into the physiological roles of autophagy in the brain and the significance of these processes for neurodegenerative diseases, particularly NBIA.

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The New Brunswick Neurological Syndrome of Unknown Cause (NSUC) -- evidence of a fake.

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Background: On March 17, 2021 a press conference in Canada aired featuring a Moncton neurologist and New Brunswick's Chief Medical Health Officer, regarding a cluster of 48 patients in New Brunswick, who had symptoms reminiscent of CJD, and were claimed to have a new neurological disease. Their onset of disease was between 2015 and 2021. 46 of those patients had at that time been reported by aforementioned neurologist. Further news publications suggested that environmental factors were causing this disease: initially a zoonosis of Chronic Wasting disease, and subsequently as beta-methyl-amino-L-alanine from algae bloom. This news has significantly disturbed the (inter)national medical community and the population of New Brunswick.

Methods: Between 2019 and 2021 10 patients died that belonged to the cluster. 8 patients underwent autopsy. 18 brain tissue blocks were examined using H&E, Luxol-PAS, B-Amyloid, Tau, P62, TDP43, Synuclein, and PrP. Frozen tissue of 7 cases processed for PrP Western blot.

Results: The neuropathological exams of 8 showed one case of metastatic carcinoma, one with FTLD-TDP43, two with neocortical Lewy body pathology, 2 cases of Alzheimer's disease, one case of mainly vascular dementia, and one case without significant pathology (consistent with patient's medical history). In these 8 patients no evidence for a prion disease was found by IHC or Western blotting, nor novel pathology.

Conclusions: Statistical evaluation revealed that the varied diagnoses present, predicted similar variability in the entire cluster of 48 patients at a p=0.0001 level (using classical probability theorem methodology). On June 3, 2021 the Oversight Committee was established in New Brunswick reviewing the clinical and epidemiological data of the 48 patients. They ultimately concluded in 2022 that NSUC did not exist. Unfortunately, the aforementioned neurologist still professes the existence of this 'new disease', now with as cause herbicide toxicity, and is still supported by news outlets and reported internationally.

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Efficacy of Routine Scout Sections in the Diagnosis of Spongiform Encephalopathies

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Background: Prionopathies are rare CNS diseases involving aggregation of misfolded prion protein (PrP). They include sporadic/iatrogenic/familial Creutzfeldt-Jakob disease (CJD), variant CJD, other genetic forms like Gerstmann-Straussler-Scheinker disease (GSS), and more. Due to anticipated risk of infectivity, advanced precautions are undertaken in centres handling suspected cases, with some taking limited sampling (scout sections) to render a preliminary diagnosis before referring the brain to a subspecialized centre with dedicated equipment and resources. In Canada, this is the CJD Surveillance System (CJDSS), which receives all such cases nationwide.

Methods: Even sampling scout sections (frontal cortex, temporal cortex, and cerebellar vermis) demands intensive resource commitment. Therefore, it is of interest to compare the accuracy of evaluating scout sections against the diagnosis rendered by the CJDSS. In the London Health Sciences Centre database, there were 111 completed referrals: 88 were positive for a prionopathy (86 sporadic/iatrogenic/familial CJD, 1 GSS, and 1 sporadic panencephalic CJD) and 23 had no evidence of a prionopathy (all of which were correctly determined as such).

Results: Of the 88 confirmed cases, 6 were suspicious for CJD with notable uncertainty due to equivocal PrP immunohistochemistry (IHC), concomitant neurodegenerative diseases, and/or lack of histological findings with clinical suspicion of disease involvement contralateral to the side sampled. The scout sections couldn't delineate specific rarer prionopathies (GSS, sporadic panencephalic CJD), but could distinguish them from typical cases. In some CJD cases, vacuolation was relied upon in place of unreliable PrP immunopositivity. Frontal and temporal cortices were often co-involved, with occasional differences in PrP IHC intensity. Several cases demonstrated isolated involvement of the vermis by IHC-positive plaques and unremarkable neocortices, supporting the necessity of cerebellar inclusion in routine sampling.

Conclusions: Overall, scout samples demonstrate considerable efficacy in the diagnosis and triaging of suspected prionopathy cases, though with some limitations incurred in artifactual immunostaining, multiple disease processes, and rarer prionopathies.

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Differing cognitive profiles in limbic-predominant age-related TDP-43 encephalopathy, frontotemporal lobar dementia, and Alzheimer disease

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Background: Limbic-predominant age-related TDP-43 encephalopathy neuropathologic change (LATE-NC) has recently been recognized as a neuropathologic entity distinct from frontotemporal lobar dementia (FTLD). It is characterized by transactive response DNA-binding protein of 43-kDa with (TDP-43)-immunoreactive inclusions that originate in the amygdala and then progress to the hippocampusi and the middle frontal gyrus. LATE-NC may mimic Alzheimer disease clinically and often co-occurs with Alzheimer disease neuropathologic change (ADNC). We aim to focus on the cognitive effects of isolated and concomitant LATE-NC in comparison to and ADNC and FTLD-TDP.

Methods: Cognitive/neuropsychological, neuropathologic, and demographic variables were analyzed in 28 control, 31 isolated LATE-NC, 62 isolated FTLD-TDP, and 244 isolated ADNC, and 172 concurrent LATE-NC/ADNC subjects from the National Alzheimer's Coordinating Center, as well as a cohort of 2,210 with varying mixed neuropathologies using multivariate logistic regression analysis to disentangle the relative contribution of each disease process and uncover the clinicopathologic characteristics of each neuropathologic entity.

Results: Cases with LATE-NC and ADNC were significantly older than controls, while FTLD-TDP cases were significantly younger. The presence of hippocampal sclerosis was significantly associated with LATE-NC but not ADNC or FTLD-TDP. Both LATE-NC and ADNC exhibited deleterious effects on overall cognition proportional to their neuropathological stages; concurrent LATE-NC/ADNC exhibited the worse overall cognitive effect than either process in isolation. ADNC and FTLD-TDP were associated with impairment in all cognitive domains, while LATE-NC had significant deleterious effects on logical memory and some measures of language and executive function. Multivariate logistic regression analysis determined an independent risk of cognitive impairment for LATE-NC (OR 1.71;$p=0.0051$), ADNC levels (OR 5.72; $p< 0.0001$), and FTLD-TDP (OR 14.30; $p< 0.0001$).

Conclusions: These data add to the existing knowledge on the clinical consequences of LATE-NC, ADNC, and FTLD-TDP pathology and the growing literature on the effects of multiple concurrent neurodegenerative pathologies.

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Brain Digital Slide Archive (BDSA): An Open Source Whole Slide Image Sharing Platform for AD/ADRD Research and Diagnostics

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Background: Neuropathologic evaluation of brain tissue is crucial for diagnosis, yet sharing such data is difficult due to inconsistencies in sample processing and digital pathology analysis across different sites. However, slide digitization and machine learning offer ways to standardize and improve diagnostic accuracy. Building upon the Digital Slide Archive – a versatile platform focused on cancer image analyses – we aim to provide a platform geared to the neurodegenerative neuropathology community, creating a federated open-source Brain Digital Slide Archive (BDSA) platform.

Methods: Our objective is to support brain whole slide images (WSIs) for AD/ADRD research compliant with FAIR (findable, accessible, interoperable, reusable) data principles. FAIR metadata for ADRC participants will be searchable through the NACC Data Front Door. We are developing workflows for sharing WSIs, annotations, and metadata, and enabling training and deployment of image analysis algorithms on multi-institutional datasets. We aim to address variability in practices through harmonized data-driven diagnostic thresholds. WSI data are sourced from an international group comprising 9 AD/ADRD-focused centers. Our goal is to integrate multiple cohorts, datasets, and model training results within a unified online portal.

Results: We convey challenges and opportunities related to harmonizing WSI data across multiple sites which is a “work in progress”. We are crafting software processes specific for AD/ADRD WSIs, performing regular software testing, and incorporating administrative best practices (e.g. universal data use agreements). This will enable the development of secure, high-quality research tools while maintaining control of WSIs at individual institutions. Ultimately, the BDSA will feature standardized and shared scientific, diagnostic, and didactic applications across sites.

Conclusions: We are creating a standardized, accessible, and searchable digital repository of neuropathological data. By providing infrastructure, standards, and reference workflows, we will promote access to, and utilization of, histologic and clinical data to advance our understanding of neurodegeneration.

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Pathologically Based Criteria to Distinguish Essential Tremor from Controls: Analyses of the Human Cerebellum

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Background: Essential tremor (ET) is among the most prevalent neurological diseases. Diagnosis is based entirely on neurological evaluation. Historically, there were few postmortem brain studies, hindering attempts to develop pathologically based criteria to distinguish ET from control brains. However, an intensive effort to bank ET brains over recent years has resulted in postmortem studies involving >200 brains, which have identified numerous degenerative changes in the ET cerebellar cortex. While ET and controls have been compared with respect to individual metrics of pathology, we now sought to derive a combination of metrics to distinguish ET from controls and determine how well this combination performs.

Methods: Analyses included 100 ET brains from the Essential Tremor Centralized Brain Repository and 50 control brains. A standard tissue block from the cerebellar cortex was used to quantify 11 metrics of pathological change that reflected changes in the Purkinje cell and related neuronal populations. Three supervised classification algorithms were investigated, including logistic regression with ridge penalty, random forest and gradient boosted decision tree. Data were divided into training and validation samples

Results: All three algorithms performed similarly to correctly predict a diagnosis of ET. The strongest pathologic predictors were those based on Purkinje cell (PC) loss, climbing fiber abnormalities, PC axonal torpedoes and PC heterotopias. Using logistic regression with a ridge penalty algorithm, the simplest method, sensitivity ranged from 87.5% to 96.64%, with six of eight values >95%, and the specificity ranged from 92.87% to 98.11%, with five of eight values >95%. We developed a web-based application that uses these metric values, and based on specified cut-offs, determines the likely diagnosis.

Conclusions: These analyses illustrate the ability to correctly predict a diagnosis of ET and set the stage for use of pathologically based criteria to distinguish clinically diagnosed ET cases from controls at the time of postmortem.

PLATFORM 6: Tumors Nonglial

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Whole genome cytogenetic analysis and DNA panel sequencing identifies recurrent genetic alterations in epigenetic pineoblastoma subtypes

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Background: Analyses of methylation profiles led to the identification of distinct epigenetic pineoblastoma (PBL) subtypes. The aim of this study was to systematically analyze the genetic alterations of these pineoblastoma subtypes.

Methods: Cytogenetic alterations of tumor samples of 76 patients with a diagnosis of PBL confirmed by reference neuropathology and methylation-based classification were analyzed by high-resolution genome-wide molecular inversion probe (MIP) analysis. 72 cases were screened for mutations by next-generation DNA panel sequencing.

Results: Referring to the proposed epigenetic PBL subtypes (Liu et al., 2021), 48 PBL-miRNA1 (1A=40; 1B=8), 17 PBL-miRNA2, 4 PBL-MYC/FOXR2, and 7 PBL-RB1 samples were identified in our cohort. PBL-miRNA subtype tumors carried characteristic alterations in microRNA-processing genes (DICER1 mutations (n=18), homozygous deletions of DROSHA (n=17) and DROSHA mutations (n=12). Most frequent cytogenetic aberrations in PBL-miRNA were whole chromosome (chr.) 7 gain (n=30) and chr. 14 loss (n=21). DICER1 mutations were significantly associated with chr. 14 loss ($p < 0.001$). 21 cases showed gains of chr. 14, and 8 further cases focal gains of the OTX2 oncogene (chr. 14q). 15 cases of the miRNA subtypes displayed polyploid karyotypes.

Conclusions: The epigenetically defined PBL-miRNA subtypes are characterized by distinct cytogenetic and mutational events. Frequent copy number gain of OTX2 may indicate a potential oncogenic role in the pathogenesis of PBL.

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Molecular testing rarely changes the grade of meningiomas: The two-year experience at a single tertiary care center

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Background: According to the fifth edition of the WHO classification of central nervous system tumors, meningiomas are designated as grade 3 in the presence of a TERT promoter mutation or CDKN2A/B homozygous deletion regardless of histologic features. Our institution changed its practice to include molecular profiling of all meningiomas following publication of these criteria. We reviewed meningiomas at our institution that underwent molecular testing to determine the frequency with which histologically grade 1 or 2 meningiomas became molecularly re-classified as grade 3. We also reviewed cases that were molecularly upgraded to determine whether there is justification for being selective for which meningiomas undergo molecular profiling.

Methods: From January 2022 to February 2024, our institution molecularly characterized 225 consecutive meningiomas from 223 patients. TERT promoter mutational analysis was done by Sanger sequencing and CDKN2A/B status was determined by FISH or chromosomal microarray.

Results: By histology alone, 137 cases (60.9%) were grade 1, 81 (36.0%) were grade 2, 6 (2.7%) were grade 3, and one was not graded. Of the histologic grade 1 meningiomas, only one case (0.73%) showed grade 3 molecular features, with a TERT promoter mutation, 1p loss, and an intragenic DMD deletion but intact CDKN2A/B. Of the histologic grade 2 meningiomas, two cases (2.5%) showed grade 3 molecular features. One case exhibited a TERT promoter mutation, CDKN2A/B homozygous deletion, and 1p loss. The other case had a TERT promoter mutation only. The histologic grade 1 meningioma that was molecularly upgraded showed worrisome radiographic features, including an irregular interface between the tumor and adjacent brain and evidence of rapid growth with vasogenic edema and midline shift, despite absence of brain invasion on reviewed slides.

Conclusions: In our series, molecular testing was most indicated in histologic grade 2 meningiomas and helpful in a histologic grade 1 meningioma with radiographic features suggestive of aggressive behavior.

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Clinical and Molecular Landscape of TERT Promoter Mutant Meningiomas

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Background: TERT promoter (TERTp) mutation in meningiomas has been associated with shorter survival and worse prognosis. The 2021 WHO CNS Classification designated TERTp mutation as a molecular biomarker of WHO Grade 3 Meningioma. However, it is unclear whether TERTp-mutant meningiomas represent a clinically and molecularly homogenous group and how they interact with other molecular biomarkers.

Methods: We analyzed clinical and molecular data of 497 meningiomas from eight institutions that underwent next-generation TERTp sequencing. Copy numbers (CN) were derived from DNA methylation data using the Conumee package. Clinical outcomes were assessed from medical records.

Results: Thirty meningiomas harbored TERTp mutation, all WHO 3, and 468 were TERTp wildtype, of which 21 were WHO 3. Of the TERTp mutants, 50% were in women and 50% in men, with a median age of 66 (range 47-82) years. 76.7% (22/30) of TERTp mutant meningiomas recurred, with a median time to recurrence of 9.5 (range 0.5-175) months. The average mitotic count of TERTp mutants was 9.5 mitoses per 10 high-powered fields. CDKN2A/B locus was lost in 45.8% of TERTp mutants, with homozygous loss in 37.5%, and hemizygous loss in 8.3%. Grade 3 meningiomas with or without TERTp mutations did not differ in terms of recurrence ($p=0.75$) or survival ($p=0.99$). TERTp-mutant meningiomas with CDKN2A/B homozygous deletions demonstrated a significantly shorter post-operative time to progression compared with TERTp-mutant tumors with intact CDKN2A/B (median recurrence time 7 vs. 29 months, $p< 0.02$). Nearly all TERTp-mutated meningiomas (95.8%) showed concurrent CN aberrations, with frequent loss of chromosomes 1p (95.8%) and 22q (91.3%).

Conclusions: TERTp mutation rarely occurs in the absence of other molecular markers of aggressive behavior in meningiomas. The effect of TERTp mutation in WHO grade 3 meningiomas is influenced by these additional alterations, with concurrent CDKN2A/B homozygous loss associated with significantly earlier progression.

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Identification and functional phenotyping of lymphoid aggregates in brain metastases

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Background: Lymphoid aggregates (LA), such as tertiary lymphoid structures (TLS), have been shown to predict overall survival and response to immune checkpoint therapy in non-CNS cancers. Data on tumors of the CNS, however, are sparse.

Methods: Here, we applied CD20 bright-field immunohistochemistry as a screening tool to identify LA in a retrospective cohort of 461 FFPE-brain metastasis samples, derived from the archives of the University Cancer Center (UCT) Frankfurt, Germany, between 2015 and 2022. Stereotaxic biopsies as well as spinal/vertebral metastases were excluded from the study. LA-positive tumors were further analyzed by multispectral whole slide TSA-immunofluorescence (IF) using antibodies for von Willebrand factor, CD3, CD20, CD163, Lamp3, pan-cytokeratins, and DAPI as counterstain. TSL-positive tumors were further characterized by whole exome sequencing, bulk RNA sequencing, spatial transcriptomics and customized highplex-sequential IF panels that included > 40 antibodies for the analysis of tumor immunology and microenvironment.

Results: TLS were observed in 38% of brain metastases and were most common in lung to brain metastasis (46%). Our preliminary data revealed a survival benefit in patients with TLS+ brain metastasis. By RNAseq, spatial transcriptomics and highplex-IF, different types of TLS were identified, based on their cellular and molecular compositions. Interestingly, most of the TLS identified lacked the structural organization reminiscent of a germinal center, suggesting an abrogated immune response.

Conclusions: Further analysis of the TLS subtypes is being conducted to investigate their biological significance and clinical relevance in patients' response to therapy.

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FOXR2-overexpressing CNS tumors exhibit substantial diversity across histological, molecular, and clinical dimensions

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Background: FOXR2 activation is regarded as the pathognomonic molecular signature of CNS neuroblastoma. However, a comprehensive understanding of the landscape for CNS tumors exhibiting FOXR2 activation is lacking.

Methods: Histopathologic, molecular, and clinical data analysis of 36 FOXR2-overexpressing CNS tumors (expression > 1.5 times the interquartile range above the third quartile of all CNS tumors) identified through screening of institutional dataset.

Results: FOXR2-overexpressing CNS tumors showed a broad anatomic distribution throughout the neuraxis. Of the 36 tumors, 17 (47.2%) were gliomas and 17 (47.2%) were embryonal tumors. The gliomas included 16 high-grade gliomas (eight histone H3 K27M-mutant diffuse midline gliomas, seven radiation-associated tumors, and one with MN1::PATZ1 fusion) and one pilocytic astrocytoma. The embryonal tumors included ten CNS neuroblastomas, six pineoblastomas, and one medulloblastoma. Two were difficult-to-classify high-grade neuroepithelial tumors. The gliomas and embryonal tumors demonstrated comparable FOXR2 expression. FOXR2-overexpressing gliomas, but not embryonal tumors, displayed diverse concomitant genetic alterations. The most common mechanisms of FOXR2 activation involved structural alterations causing promoter donation from other genes, frequently incorporating non-canonical non-coding exons, followed by activation of exon -3 promoter or LINE-1 retrotransposon-driven expression. Tumor location, histology, and concomitant alterations correlated with patient outcomes. While FOXR2-overexpressing gliomas were aggressive (5-year overall survival 13.3%), FOXR2-overexpressing embryonal tumors showed a diverse prognosis. FOXR2-activated CNS neuroblastoma showed a 5-year overall survival of 100%. Pineoblastomas with FOXR2 activation in the PB-FOXR2 DNA methylation group showed a 3-year survival of 0% despite multimodal therapy. The one medulloblastoma patient in the cohort is alive three years post-diagnosis.

Conclusions: FOXR2-overexpressing CNS tumors manifest significant histological, molecular, and clinical diversity and can occur as post-radiation tumors. Glioma histology and concomitant genetic alterations correlate with treatment resistance and inferior prognosis while FOXR2-overexpressing embryonal tumors show more heterogeneous outcomes. Integrating histologic and molecular diagnostic approaches is imperative for accurate prognostication and optimal therapeutic decision-making.

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Incidence of CDKN2A/B loss and correlation of MTAP immunohistochemistry in histological grade 1 and grade 2 meningiomas.

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Background: While meningioma grading was historically based on histology, the 2021 WHO classification of central nervous system (CNS) tumors added two grade 3 defining molecular alterations, CDKN2A/B homozygous loss and TERT promoter mutation. Both are associated with increased recurrence rates and poor overall survival. Additionally, CDKN2A/B hemizygous loss shows similar rates of recurrence and survival outcomes to homozygous loss. Assessing for CDKN2A/B alterations often requires costly testing, including chromosomal microarray or fluorescent in situ hybridization (FISH). MTAP, a protein encoded by a gene adjacent to CDKN2A/B on chromosome 9p, has been suggested as a surrogate immunohistochemical marker for CDKN2A/B alterations.

Methods: 152 meningiomas were analyzed, including 22 histologically grade 1 meningiomas with atypical features (“grade 1+”) and 130 histologically grade 2 meningiomas. All tumors were evaluated for CDKN2A/B with FISH. 15 cases were evaluated for MTAP immunohistochemistry.

Results: Homozygous loss of CDKN2A/B was detected in 3.1% of grade 2 meningiomas (4/130), and 0% of grade 1+ tumors (0/22). Localized hemizygous loss of CDKN2A/B was seen in 10.0% of grade 2 tumors (13/130) and 9.1% of grade 1+ tumors (2/22). Hemizygous loss in the setting of monosomy 9 was present in an additional 9.2% of grade 2 tumors (12/130) and 18.2% of grade 1+ tumors (4/22). While rates of homozygous deletion were low, 22.3% of grade 2 tumors and 27.3% of grade 1+ tumors showed some degree of alteration in CDKN2A/B. Immunohistochemical loss of MTAP had a 100% specificity (11/11) but low sensitivity at 25% (1/4).

Conclusions: Molecular testing of grade 1+ and grade 2 meningiomas showed a low incidence of CDKN2A/B homozygous loss with a significant number of tumors showing hemizygous alterations. Results from our single-institution study suggest MTAP immunohistochemistry has low sensitivity for detecting CDKN2A/B alterations and may not be a reliable surrogate.

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Molecular and immunophenotypical analysis of SF1-expressing PitNETs reveals clinically distinct subtypes

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Background: Steroidogenic Factor 1 (SF1 or NR5A1) is a transcription factor (TF) that regulates gonadal and adrenal functions, and contributes to differentiation of gonadotroph cells in the anterior pituitary. According to the CNS WHO 5th edition, SF1 expression defines gonadotrophic lineage for PitNETs. However, SF1 can be expressed aberrantly in other lineages without concomitant expression of FSH or LH. Here, we aimed to characterize landscape of SF1-positive PitNETs.

Methods: We analyzed a cohort of 207 PitNETs operated at NYU Langone Health or Bellevue Hospital. Demographic, clinical, and pathological data was retrieved from clinical records. Tumors were analyzed by DNA methylation using Illumina EPIC array, Next Generation Sequencing (NGS) using NYU Genome PACT, and NYU Fusion SEQER. All tumors were classified using Heidelberg. DNA methylation classifier and copy number profiles assed using conumee package and visual inspection. Unsupervised hierarchical clustering was performed using the 1000 most variable methylation probes. A Chi-squared test was performed for statistical analysis.

Results: Our cohort included 116 PitNETs that expressed SF1 on IHC; 107 (92.2%) expressed SF1 only, eight (6.9%) co-expressed SF1 and Pit1, and one case (0.9%) co-expressed SF1, Pit1, and T-Pit. Clinically, SF1 expression was associated with invasion of adjacent structures ($p=0.03$) and nonfunctioning status ($p< 0.001$) compared to non-SF1 tumors. By DNA methylation, 97% of SF1-only cases classified as PITAD_FSH_LH, whereas only 22% of the multiple TFs cases classified as PITAD_FSH_LH. Unsupervised hierarchical clustering of PitNETs separated the PITAD_FSH_LH tumors into one cluster distinct from the other lineages. This cluster showed a relatively hypomethylated profile and simple CNV patterns ($p< 0.001$). NGS did not show recurrent mutations in the SF1 cluster.

Conclusions: SF1 expressing PitNETs form a clinically and molecularly distinct tumor type characterized by hormonal silencing, tumor invasion, simple CNV profile, and hypomethylation. The gonadotrophic designation may not be clinically informative since they are all nonfunctioning.

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Rethinking Diagnostic Specificity: NKX2.2/CD99 Co-expression in Embryonal Tumor with Multilayered Rosettes

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Background: NKX2.2 and CD99 co-expression by immunohistochemistry (IHC) is considered highly specific for Ewing sarcoma (ES), but to our knowledge, its specificity in the central nervous system (CNS) is not well-established. Recently, we encountered a case of a dural metastasis lacking characteristic rosettes and exhibiting NKX2.2/CD99 co-expression with only patchy LIN28A staining. Molecular studies, including methylation profiling, ultimately confirmed the diagnosis as Embryonal Tumor with Multilayered Rosettes (ETMR). This prompted us to investigate the prevalence of this unexpected marker combination in ETMR.

Methods: We analyzed NKX2.2, CD99, and LIN28A expression in 10 ETMR cases from 8 patients. Two independent pathologists evaluated staining intensity and percentage of positive cells.

Results: All (10/10, 100%) ETMR cases showed at least focal co-expression of NKX2.2 and CD99. All (10/10, 100%) cases also exhibited at least focal LIN28A expression.

Conclusions: NKX2.2/CD99 co-expression was consistently observed in our ETMR cohort, challenging its high specificity for ES. Particularly in the clinical context of dural metastasis, this pattern of immunoreactivity raises the potential for misdiagnosis as ES. Our findings underscore the importance of integrating all available clinical, pathological, and molecular data for accurate diagnosis. Further research with larger and more diverse CNS embryonal tumor cohorts is warranted to validate these observations and refine the diagnostic roles of NKX2.2, CD99, and LIN28A in CNS tumors.

PLATFORM 7: Developmental/Pediatric

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Building a gene expression atlas of developing brainstem motor neurons to study rare congenital neurologic disorders

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Background: Ocular motor neurons (OMNs) in the brainstem mediate eye movement and are differentially affected in some rare congenital neurologic diseases. In cases such as Duane Syndrome, specific OMN subpopulations show disrupted or aberrant innervation while other subpopulations remain unaffected, but mechanisms underlying differential susceptibilities have not yet been identified. Here we generate a transcriptomic atlas to analyze unique gene expression patterns of each developing MN type as a toolbox to help study these disorders.

Methods: We combined multiple mouse genetic reporter lines with intersectional temporal (embryonic days E9.5 to E18.5) and spatial transcriptomics (single cell/nuclei RNA-seq, and Slide-Seq) to isolate and compare eight distinct mouse MN populations: the three oculomotor nuclei (CN3, CN4, CN6) and the other primary MN types (CN5, CN7, CN9/10, CN12 and spinal MNs). Sample integration posed a significant “batch effect” challenge since different types of samples were acquired via multiple methods over many ages. We compared multiple benchmarked high quality integration pipelines to balance batch correction vs. bioconservation. Once cells were identified and labeled we used scDREAMER-SUP, a semi-supervised deep learning algorithm, using cell label annotations as a means to achieve further bioconservation and batch correction. We built a visualization tool to investigate integration quality. Cell clusters were mapped onto spatial transcriptomic slide-seq samples from E11.5 and E14.5 and compared with developmental mouse atlases to confirm cell identities.

Results: Candidate marker genes of each cell population were further validated via database analysis and RNA in situ hybridization. We successfully integrated a developmental time course of mouse MN gene expression from disparate sample types. The resulting atlas can be transposed to label and identify spatial structures within slide-seq datasets to identify cells in time and space.

Conclusions: Overall, this atlas uncovers distinct developmental gene expression patterns and provides new tools to study their differential vulnerability in motor neuron related disorders.

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Disrupted rhombic lip developmental in medulloblastoma oncogenesis: Taking another look at cerebellar dysplasia & heterotopia

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Background: Group 3 and 4 Medulloblastoma (MB) oncogenesis is poorly understood. Recent studies elucidated human rhombic lip cellular populations susceptible to abnormal proliferation and molecular alterations leading to MB formation. We aimed to describe the prevalence of abnormal cellular populations in the rhombic lip across a developmental spectrum via histologic evaluation of the cerebellar vermis in a postmortem cohort.

Methods: Autopsy brains submitted to the pediatric neuropathology (NP) service over a 16 month time period were assessed for the presence of intact cerebellar vermi. When evaluable, parasagittal or horizontal sections to include the nodulus were submitted and histologically reviewed for the presence of cerebellar dysplasia/heterotopia (CbllD/H). We recorded presence of CbllD/H, age, sex, NP and autopsy diagnoses including other developmental anomalies, and available genetic testing results.

Results: Twenty-eight cases had intact cerebellar vermi (age range: 25.3 gestational weeks to 27 years); 5 were intrauterine demises (age range 34.5 to 39.6 gestational weeks), and 9 liveborn infants were less than 40 weeks corrected age at time of death (age range 25.3 to 38.4 gestational weeks). Twelve cases (43%) (age range 25.3 gestational weeks to 6 months) demonstrated vermian CbllD/H and all classification subtypes of CblllD/H were represented; one had additional NP developmental abnormalities. Antemortem genetic testing was performed in 13 cases. Five of these cases had CbllD/H, 3 of which had genetic abnormalities including trisomy 18 (1) and point mutations (2).

Conclusions: As prior studies have described, we confirmed the high incidence of CbllD/H within the vermis in our autopsy cohort. Given recent studies elucidating Group 3 and 4 MB oncogenesis in relation to CbllD/H, additional studies including immunohistochemical characterization of CBllD/H is warranted.

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Autopsy-Based Insights into Pediatric Nontraumatic Intracranial Hemorrhage: A Ten-Year Retrospective Review

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Background: Nontraumatic intracranial hemorrhage (NICH) represents a critical yet understudied pathology in pediatric populations, particularly in the context of hospital autopsies. Existing research predominantly concentrates on radiologic and clinical data, leaving gross and microscopic neuropathologic correlates less explored. This study aims to offer insights into NICH, thereby enhancing our grasp of its broader implications on pediatric health outcomes.

Methods: An IRB-approved 10-year retrospective analysis of autopsies conducted at Children's Hospital Los Angeles from 2014 to 2023 was performed. Inclusion criteria included intracranial hemorrhage, no history of head trauma, and a complete, unrestricted autopsy. Associations were evaluated utilizing the Fisher's Exact Test.

Results: Analysis of 348 autopsies identified 130 cases of NICH (37%). The distribution across different age groups included: 5 fetuses, 46 neonates, 41 infants, 17 children, 8 early adolescents (ages 11-14), 4 middle adolescents (ages 15-17), and 9 late adolescents (ages 18-21). There were 76 males and 54 females. Hemorrhage subtypes included subarachnoid in 61 (47%), parenchymal in 53 (41%), subdural in 39 (30%), interventricular in 38 (29%), germinal matrix in 16 (12%), and epidural in two cases (1.5%) both of which were associated with recent external ventricular drainage. Notably, 17 cases (13%) demonstrated microscopic hemorrhage without gross findings. Associated conditions spanned oncologic, neonatal, developmental, genetic, infectious, and hematologic categories. A vascular malformation was observed in only one case.

Conclusions: Our study identifies NICH in 37% of pediatric autopsies over a decade. While vascular malformations are reportedly a common cause of intracranial hemorrhage, our cohort included only one. Developmental, infectious, and congenital associations were more frequent. Analysis accounting for hemorrhage subtype distribution revealed a proportionally higher incidence of subdural hemorrhage in early adolescents, but further studies are warranted. Collectively, the findings reinforce the importance of autopsy studies in elucidating the complex mechanisms underlying NICH.

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Biomechanical Contributions of Tensegrity in the Formation of Polymicrogyria

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Background: Polymicrogyria is a malformation of cortical development involving complex cortical ribbon undulations with loss of normal laminar architecture. This anomaly is etiologically diverse, with congenital cytomegalovirus encephalitis (cCMVE), genetic (Walker-Warburg syndrome, Zellweger syndrome, COL4A1-mutation, etc.), and vascular/ischemic phenomena at a variety of gestational stages all capable of producing morphologically stereotyped lesions, leading some to favour disruption of structural/biomechanical forces as a unifying mechanism. Tensegrity provides a means of explaining both the complex pattern of normal gyrification and its disruption in polymicrogyria by evoking the interplay between tensile and compressive forces at the cellular and macromolecular level.

Methods: A collection of cCMVE-associated polymicrogyria cases was examined, with gestational ages ranging from 16-39 weeks. These cases were compared to age-matched controls without findings of polymicrogyria. To investigate the possible contributions of tensile forces imparted by neuronal and glial processes, cases were evaluated for relative depletion of projection neuron populations, neuronal and glial process disruption, pial-glial membrane injury, and leptomeningeal heterotopias.

Results: Clinical details and pathological findings indicate that the responsible insults occurred before neuronal migration was complete, lasted several weeks, and led to a variety of structural perturbations to the developing brain. In the affected cases, several findings were almost invariable: subventricular zone necrosis, subventricular germinal cell infection, radial glial disruption, and injuries to the pial-glial border. Subcortical neuronal and leptomeningeal heterotopias were also common.

Conclusions: These cases add to the evidence that polymicrogyria is not solely post-migrational and that a variety of injuries (and timings) can replicate its relatively stereotyped morphology. Furthermore, the associated disruption of projection neurons, radial glia, and pial-glial interface – elements capable of exerting tensile and compressive forces that might influence normal gyrification – provides a biomechanical basis for polymicrogyria formation under the framework of tensegrity.

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Frequent Cerebral Intraparenchymal Calcification in Cases of Sudden Unexplained Death in Pediatrics

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Background: Sudden unexplained death in childhood (SUDC) is a diagnosis of exclusion after complete and thorough death investigation. As a leading cause of death in children, SUDC accounts for more deaths than cardiac disease and cancer. A proposed cause of SUDC is believed to be an underlying disease state accompanied by an environmental stressor. The “underlying disease state”, however, is currently unknown.

Methods: Postmortem examination of the brain was performed on 32 cases of SUDC (Male:Female = 1.67:1; age at death: 0 day-3.5 years [median= 3.5 months]) and 37 cases of age-matched controls (Male:Female = 1.18:1; age at death: 0 day-4.5 years [median= 3.2 months]). Those with evidence of co-sleeping or asphyxiation were designated as the control group.

Results: The brains examined were predominantly grossly unremarkable, with the exception of three cases (9.4%; 3/32) including one Down syndrome case with microcephaly. We identified histopathologic findings in 56.25% (18/32) of the SUDC and 10.81% (4/37) of the control cases in our cohort. The histologic findings seen in SUDC cases included: microcalcification (21.9%; 7/32), acute hypoxic/ischemic changes (12.5%; 4/32, periventricular leukomalacia (9.4%; 3/32), granule cell dispersion (6.25%; 2/32), reactive gliosis (3.13%; 1/32), and hippocampal sclerosis (3.13%; 1/32). In comparison, only 2.7% (1/37) of control cases showed microcalcifications in the brain. The microcalcifications were noted in the subcortical white matter in a perivascular distribution and often associated with microbleeds. They may represent neuronal ferrugination.

Conclusions: Subcortical microcalcification is a frequent finding in SUDC. The etiology and clinical significance are unclear. However, calcification represents findings of chronic or remote injury, which may result from recurrent hypoxic/ischemic insults that predisposes young children to sudden death. This is supported by the relative dearth of findings in our control group.

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Congenital Moebius Syndrome Shows Primary Abducens Motor Nuclei Lesion, Secondary Facial Neuron Loss, and Abnormal Tracts

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Background: Moebius syndrome (MBS) is a rare congenital condition characterized by facial and horizontal gaze palsies, likely due to the absence or underdevelopment of the abducens (CN6) and facial (CN7) cranial nerve nuclei. Proposed etiologies include vascular injury and genetic factors. We utilized histology, MRI/DTI imaging, and whole genome sequencing to investigate MBS.

Methods: We evaluated postmortem brainstems from two adults with MBS against controls. MRI was used for structural evaluation and tract tracing. Histological analysis involved staining for cell morphology, nerve tracts, and a panel of markers. Genomic analysis was conducted on blood and brainstem tissue from the first individual (I1), and on blood from the second individual (I2) and their parents, to identify germline and somatic mutations.

Results: CN6 and CN7 were notably absent in I1 and I2, with bilateral calcified and iron-deposit lesions noted near where CN6 should exist. In I1, CN6 motor neurons were entirely absent, and CN7 motor neurons were largely depleted with gliosis in the surrounding region. Bilateral abnormal dorsal-ventral tracts were identified near the expected paths of CN6, but turned rostrally rather than exiting the brainstem, and were negative for acetylcholinesterase. No definitive candidate genes emerged from genomic analyses.

Conclusions: Our findings suggest initial damage to the dorsal medulla/pons embryonically damages CN6—with secondary CN7 motor neuron degeneration. Pathways involved in abducens/facial control may contribute to the abnormal tracts. MBS may be linked to hypoperfusion resulting from critical developmental vulnerabilities around 6 weeks of gestation, when there's substantial brainstem and vascular remodeling. Our study offers insights into the pathophysiology of MBS-associated cranial nerve anomalies and in the absence of a genetic etiology, underscores a potential vascular cause, possibly linked to early gestation vascular perfusion changes.

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Keratan Sulfate: Chemical Template for Neuroblast Migratory Pathways and Axonal Fascicles

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Background: Keratan sulfate (KS) is a proteoglycan secreted in the fetal brain by astrocytes and radial glia into extracellular parenchyma as granulo-filamentous deposits. KS surrounds neurons except dendritic spines, repelling glutamatergic and facilitating GABAergic axons. The same genes are expressed in both neuroblast migration and axonal growth. This study examines the role of KS in morphogenesis of the normally developing human fetal forebrain.

Methods: 28 normal human fetal brains from 9 to 42 weeks gestational age (wk GA) were studied at autopsy. KS was examined by immunocytochemical reactivity in formalin-fixed paraffin-embedded sections, plus various other cellular markers including NeuN, MAP2, synaptophysin, vimentin, nestin, S100β protein.

Results: Radial and tangential neuroblast migratory pathways from subventricular zone to cortical plate were marked by KS deposits as early as 9wk GA, shortly after neuroblast migration was initiated. During later gestation this reactivity gradually diminished and disappeared by term. Long axonal fascicles of the internal capsule and short fascicles of intrinsic bundles of globus pallidus and corpus striatum also appeared as early as 9-12wk, as fascicular sleeves before axons even entered such fascicles. Intense KS occurs in astrocytic cytoplasm and extracellular parenchyma at 9wk in globus pallidus, 15wk thalamus, 18wk corpus striatum, 22wk cortical plate, hippocampus postnatally. Corpus callosum, and anterior commissure do not exhibit KS at any age. Optic chiasm exhibits reactivity peripherally but not around internal sub-fascicles.

Conclusions: KS forms axonal fascicles before axons enter and is transitory along neuroblast migratory pathways to create a chemical template (not structural scaffold) for axonal trajectories and cellular migration during morphogenesis.

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Multimodal high-resolution whole-brain characterization of holoprosencephaly (HPE) from 13-22 gestational weeks

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Background: Holoprosencephaly (HPE) is a commonly occurring patterning disorder of the forebrain, with a wide phenotypic spectrum of dysmorphism of the face and brain. While the onset of the induction defect is well known to be an early embryonic event (classically affecting the sonic hedgehog pathway), the most detailed neuropathology reports are from third-trimester fetuses, infants or children. We hypothesized that comprehensive analyses from the second trimester would assist in understanding the cellular pathogenesis of the eventual final phenotype.

Methods: We employed a novel, comprehensive multimodal approach, including prenatal ultrasonography, postmortem MRI, whole-brain embedding, with blockface imaging of serial sections, followed by three-dimensional (3D) reconstruction of the digitized whole-mount slides, to characterize 3 HPE brains from 13-22 gestational weeks (GW), comparing them to identically processed age-matched controls.

Results: Aside from the classical failure of cleavage (midline fusion) of the rostral forebrain, our HPE cases showed: 1) established rostral subarachnoid glioneuronal (marginal) heterotopia; 2) thinning of the cerebral mantle posterolaterally, but with an “evenly cellular” cortical plate, conspicuously lacking “glomeruloid” structures as reported previously in more mature cases; 3) “reactive”-appearing astrocytes on GFAP immunostaining of the cortical plate; 4) clustering of migrating cells in posterolateral subcortical cell layers suggesting “incipient” laminar heterotopia, visible even at 13GW; and 5) lateral and horizontal displacement of the hippocampus on 3D segmentation, despite a relatively normal laminar arrangement of cells.

Conclusions: Our approach utilizing multiscale, multimodal 3D analytics provides a complementary perspective on the timing and evolution of the neuropathology of HPE in the second trimester.

PLATFORM 8: Infectious, Demyelinating and Inflammatory, Peripheral Nerve/Muscle, Ophthalmic Pathology

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Multifocal Necrotizing Leukoencephalopathy with a Pontine Predilection Following BCMA CAR-T in a Critically Ill Patient

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Background: The emergence of CAR-T therapy has introduced new neurological complications, notably the clinical syndrome Immune effector Cell-Associated Neurotoxicity Syndrome (ICANS). However, the neuropathology of CAR-T neurotoxicity is not well characterized. Despite increasing clinical classification, the mechanisms and associated neuropathological findings of CAR-T neurotoxicity remain underexplored. Multifocal necrotizing leukoencephalopathy with pontine predilection (MNL-PP) is a neuropathological finding associated with profound immunosuppression and is characterized by microscopic foci of necrosis confined to the white matter of the basis pontis. MNL-PP has previously been associated with congenital and acquired immunodeficiency, chemotherapy, and CD19-directed CAR-T therapy, among other causes. In this case study, we describe the neuropathological findings of a patient with multiple myeloma who received BCMA-directed CAR-T.

Methods: We identified a case of a 57-year-old male patient who received BCMA-directed CAR-T, 6 years after his original diagnosis. Following infusion, he developed cytokine release syndrome (CRS) and immune effector cell-associated hemophagocytic lymphohistiocytosis-like syndrome (IEC-HS), from which he initially recovered. The patient presented one week later in septic shock with bacteremia, viremia, and CAR-T induced aplastic anemia with recurrent/refractory IEC-HS. Despite autologous stem cell boost, the patient continued to decline and ultimately passed.

Results: On autopsy, the patient displayed multi-organ ischemic necrosis involving most visceral organs and findings consistent with aspiration pneumonia. Neuropathological evaluation demonstrated diffuse and marked CD163-positive cells, abundant astrogliosis, and mildly expanded perivascular spaces. The basis pontis showed multiple small foci of white matter spongiosis and necrosis with associated demyelination, patchy axonal loss, and b-APP axonal spheroids.

Conclusions: We describe the postmortem neuropathological findings of a complex and critically ill patient with multiple myeloma who received BCMA-directed CAR-T and was found to have evidence of MNL-PP on postmortem evaluation. Although not directly attributable to CAR-T treatment, we believe this report contributes to the ongoing investigation into the neuropathological findings seen following CAR-T.

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Neuropathologic findings in a community-based autopsy cohort of older, virally-suppressed, people with HIV

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Background: Combination antiretroviral therapy (cART) has reduced the incidence of HIV-related mortality. Consequently, there is a growing population of older, virally-suppressed, people with HIV (PWH). cART has also decreased the prevalence of HIV-associated dementia. However, many PWH experience milder forms of neurocognitive impairment. It is unclear what pathology, if any, is associated with this phenotype and whether this aging population of PWH demonstrates distinct patterns of neurodegenerative pathology compared to uninfected individuals.

Methods: We performed a community-based case-control study where we examined brain tissue from autopsies performed at the Johns Hopkins Hospital between 2015-2023 for 13 virally-suppressed PWH and from 13 HIV-negative subjects. We characterized and performed semi-quantitative analysis of the neurodegenerative pathology, including beta-amyloid pathology, tau pathology, alpha-synuclein pathology, and TDP-43 pathology, in each group.

Results: PWH and uninfected controls had similar demographics, including age (median: 59 vs. 57 years), male sex (54%), and race (100% black). Medical co-morbidities were also similar with a trend towards a higher prevalence of HCV seropositivity in PWH (46% vs. 15%; $p = 0.09$). Common histologic brain findings included atherosclerosis (46% vs. 54%) and infarcts (54% vs. 38%). With respect to neurodegenerative pathology, while no statistically significant differences were found between our relatively small cohorts, PWH demonstrated trends towards increased prevalence of beta-amyloid pathology (69% vs. 54%), caudal entorhinal cortex neurofibrillary tangles (100% vs. 77%), aging-related tau astrogliopathy (23% vs. 0%), and alpha-synuclein pathology (31% vs. 8%); as well as increased severity of diffuse plaque pathology (median density score, 2 vs. 1).

Conclusions: Compared to previous studies that have relied on research study subjects, our community-based cohort of older, virally-suppressed PWH demonstrates trends towards more prevalent and more severe neurodegenerative pathology in PWH. These findings highlight the need larger community-based cohort studies as we aim to characterize and understand the mechanisms of HIV-associated neuropathology in virally-suppressed individuals.

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Comparison of neuropathologies among rabies patients treated with standard, Milwaukee, and/or experimental AAV-RAB strategies

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Background: Symptomatic rabies, treated with supportive medical care, has an essentially uniformly fatal outcome. The Milwaukee Protocol (MP; 2005) is based on the concept that, since rabies is not neuronolytic, induction of coma and prevention of excitotoxicity/seizures could allow host immune clearance of the virus and neurologic recovery. MP has had, however, no meaningful efficacy in scores of reports. Antivirals and, more recently, adenoviral vector delivery of anti-rabies virus antibody (AAV-RAB) have been applied in a few patients without improving outcome.

Methods: We compared the autopsy neuropathology of 3 symptomatic men, all receiving standard (supportive) care and additional treatment as follows: Case 1 (24yo) had a dog bite on the lip; post-exposure prophylaxis (PEP) at 0 and 7d; symptoms at 17d; Favipiravir at 20d; Rabishield intrathecal at 20 and 21d; and death at 21d. Case 2 (17yo) had an unknown exposure and no PEP; MP initiated after 4d of symptoms; death at 26d. Case 3 (57yo) suffered dog bite on leg; no PEP; symptoms at 6mo; MP begun after 6d of symptoms; Favipiravir at 9d; AAV-RAB at 11d; death at 30d. Standard histology was done for Cases 2 and 3, while Case 1 underwent multimodal analysis and 3D histology (PMID 26181725).

Results: In Case 1, inflammation was sparse and correlated with viral nucleocapsid immunopositivity, mapped incrementally from brainstem to diencephalon to cerebral cortex. Case 2 had moderate forebrain inflammation in areas of viral immunostaining. Case 3 had marked lymphoplasmacytic inflammation and neuronophagia in virtually all gray matter sites (cultivable virus recovered postmortem).

Conclusions: Gray matter inflammation was similar between antiviral- or MP-treated cases. By contrast, the MP+antiviral+experimental AAV-RAB case had much greater inflammation. While these differences could be due to rabiesvirus genotypes, host factors including age, and/or

survival intervals, a pro-inflammatory effect of the experimental AAV-RAB protocol urges further analysis of such cases.

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Histological and Immunohistochemical investigation of inflammatory cells in a geographic atrophy case

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Background: Geographic atrophy (GA) is a chronic progressive degeneration of the macula, affecting millions of patients with age-related macular degeneration (AMD), severely impairing central vision. Histologically, GA is characterized by atrophy of outer retinal layer (ORL), retinal pigment epithelium (RPE) and choriocapillaris. Inflammatory damage is proposed to cause GA. However, the histological features and immunohistochemical characteristics of inflammation in human GA eyes are not well understood due to scarcity of human GA eye tissues. Here we characterized a GA eye and evaluated the expression of different inflammatory cell types and compared to normal eye.

Methods: We identified a case of a GA in the left eye collected from an 88-year-old autopsy female. Histologic examination showed focal atrophy of the ORL, RPE and choriocapillaris involving the entire macula, compatible with GA. Additional inflammatory immunohistochemical staining was conducted for CD45RB, CD20, CD3, CD138, CD68, CD163, PU.1, C3D, CD21. Results were compared with stains performed on the control right eye (with no GA).

Results: H&E sections of the left eye showed atrophy of the ORL, RPE and choriocapillaris, compatible with histopathologic diagnosis of GA. Macrophages markers CD163, CD68 and PU.1 highlighted considerable macrophages at the leading edges of the atrophy. By contrast, CD138 plasma marker cell, and CD45RB, CD3, and CD20 markers for lymphocytes were negative at the atrophy. The contralateral healthy right eye did not show positive inflammatory cell markers in the retina. Complement markers (CD21, C3D) were positive in many blood vessels walls in both eyes.

Conclusions: In our histologically-confirmed GA eye, immunohistochemistry supported involvement of macrophages at the edge of the RPE degeneration. By contrast, other inflammatory cell types did not show consistent increase. Activated complement markers labeled blood vessels throughout the choriocapillaris in both GA and healthy eyes. Our findings suggest that macrophage-mediated inflammation may be involved in RPE degeneration during GA pathogenesis.

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Deep immunoprofiling of inclusion body myositis

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Background: Sporadic inclusion body myositis (IBM), the most common idiopathic inflammatory myopathy (IIM) in patients over the age of 50, presents with asymmetric muscle weakness, predominantly involving long finger flexors and quadriceps muscle. In general, CD8+ T cell invasion of muscle tissues is a prominent histological feature of IBM, and highly differentiated cytotoxic T cells are considered to be an important regulator of IBM pathogenesis. However, the drivers of such differentiation remain unclear.

Methods: We performed a deep profiling of peripheral blood mononuclear cells in IBM patients compared to healthy controls by examining the gene expression at single cell level along with complete B cell repertoire (BCR) and T cell repertoire (TCR) analyses. We also performed spatial transcriptomics analysis of immune cell infiltrates of skeletal muscle from the IBM subjects.

Results: We included four patients with IBM, three men, and one woman, with a mean age of 71.5 ± 7.6 years and disease duration of 8 ± 4.4 years in this preliminary analysis. Two healthy controls were recruited (both men, age 47.5 ± 7.6 years). We noted differential gene expression in the IBM B cell compartment that were related to immunoglobulin production, leucocyte migration, and T-cell differentiation. As previously reported, there was a strong gene expression signature of highly differentiated cytotoxic T cells in IBM. Pseudo-time analysis reflected a distinct developmental trajectory of these T cells compared to healthy controls, which associated with a group of transcription factors (TF) including those found in the T-box family.

Conclusions: This preliminary analysis reinforces the autoimmune nature of IBM, further implicating a potential role for both B cells and uniquely developed cytotoxic T cells in the pathophysiology of IBM.

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Comparative study on lupus myositis, dermatomyositis, immune mediated necrotizing myopathy, and antisynthetase syndrome associated myositis

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Background: In a recent study, we showed that lupus myositis (LM) could present in the forms of panfascicular necrotizing myopathy (35%), perifascicular necrotizing myopathy (20%), diffuse necrotizing myopathy (20%), polymyositis (15%), and necrotizing vasculitis (10%). Those various morphologies posed a significant diagnostic challenge on muscle biopsy as they might be histologically indistinguishable from immune mediated necrotizing myopathy (IMNM), dermatomyositis (DM), or antisynthetase syndrome associated myositis (ASyS), respectively. Better immunohistochemical markers are needed to differentiate the above entities.

Methods: Myxovirus resistant protein (MxA), MHC class I (MHC1), MHC class II (MHC2) immunohistochemistry (IHC) as well as detailed histological analyses were performed on the muscle biopsies from clino-serologically proven cases of LM (n= 20), DM (n=14), IMNM (n=22) and ASyS (n=15).

Results: MxA sarcoplasmic reactivity was present in 94.4% DM, 80% LM, 20% ASyS, and 0% IMNM cases. MHC1 sarcoplasmic or sarcolemmal reactivity was present in 90.1% DM, 78.5% LM, 93.3% ASyS, and 35.0% of IMNM cases. MHC1 expression was usually diffuse in LM, compared to perifascicular in DM, ASyS, and patchy/mosaic in IMNM. MHC2 sarcoplasmic reactivity was present in 14.3% DM, 84.2% LM, 100% ASyS and 9.0% for IMNM. MHC2 reactivity was strongest in ASyS and variable in LM. Histologically, mixed T and B lymphocytic inflammation was present in 70% of LM cases, indistinguishable from DM ($p = 1.0$), but significantly more than IMNM ($p=0.0004$).

Conclusions: As a rule, a MxA+/MHC1+/MHC2- IHC profile supports DM; a MxA+/MHC1+/MHC2+ IHC profile supports LM, a MxA-/MHC1+/MHC2+ IHC profile supports ASyS; and a MxA-/MHC1-/MHC2- profile supports IMNM. However, exceptions do exist, and the pathologic findings still need to correlate with clinic-serological findings for a definitive diagnosis.

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Complex presentation of leukoencephalopathy with brain calcifications and cysts (Labrune Syndrome): A rare case report

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Background: Leukoencephalopathy with brain calcifications and cysts (LCC) also known as Labrune syndrome, is an extremely rare leukodystrophy with fewer than 100 cases reported worldwide since it was first described in 1996. It is characterized by progressive deterioration and brain calcifications and cysts, related to mutations in the SNORD118 gene with an autosomal recessive inheritance.

Methods: We present a case of a 62-year-old man with right-sided weakness, facial droop, dysarthria, urinary incontinence, and unintentional weight loss. Imaging studies revealed multiple calcified supratentorial lesions, linear calcifications, a cystic lesion and extensive vasogenic edema, an unusual constellation of findings not typical of metastatic disease, with only some similarity to CNS infection such as neurocysticercosis.

Results: Brain biopsy was performed, and histology showed classic LCC features of brain parenchyma with extensive microangiopathy, ranging from hyalinization, fibrin deposition and occlusion of the lumen. Other features included loss of myelin, perivascular hemosiderin-laden macrophages and reactive astrocytosis, including Rosenthal fibers, and scattered concentric parenchymal concretions. Next generation sequencing identified two germline heterozygous pathogenic variants in the SNORD118 gene associated with autosomal recessive leukoencephalopathy with brain calcifications and cysts.

Conclusions: Labrune syndrome poses diagnostic challenges due to its rarity, diverse clinical and radiological presentation. This case underscores the importance of integrating clinical presentation with imaging, histopathology, and genetic findings for accurate diagnosis. The presence of classic pathological findings, in conjunction with pathogenic SNORD118 variants, is diagnostic of LCC. Management remains supportive, with antiepileptic drugs and steroids as mainstays for symptom control.

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Targeted Next-Generation Sequencing Reveals Frequent Copy Number Alterations in Intraocular Leiomyomas

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Background: Leiomyomas are benign smooth muscle tumors commonly involving the uterus, soft tissue, and gastrointestinal tract, but in rare cases can also arise intraocularly. Notably, some intraocular leiomyomas demonstrate atypia, mitotic activity, and/or necrosis, worrisome for "intraocular leiomyosarcoma," despite the lack of established criteria for malignancy. We performed targeted next-generation sequencing of a series of intraocular leiomyomas, including one with worrisome histologic findings, to investigate for potential recurrent alterations, differences between tumors in anterior versus posterior segment involvement, and possible shared alterations with leiomyomas of other sites.

Methods: We identified eight patients diagnosed with intraocular leiomyoma and performed UCSF500 targeted next-generation sequencing on six cases with sufficient material.

Results: The cohort included 4 female and 4 male patients ranging from age 16 to 70 years. Two tumors limited to the ciliary body did not yield sufficient DNA. The largest tumor in the cohort (30 mm) seen in the youngest patient fills the globe and shows suprachoroidal involvement without a distinct ciliary body attachment and shows mitotic activity (1/10 high-power fields), cytologic atypia and a small focus of necrosis. Sequencing did not reveal any pathogenic single nucleotide variants, deep deletions, amplifications or structural rearrangements involving the 529 cancer genes targeted in this assay. However, copy number analysis demonstrated multiple, recurrent whole chromosome losses (including losses of 1, 2, 3, 10, 15q, 22q).

Conclusions: Loss of numerous whole chromosomes suggestive of near haploidization of the genome is a rare cytogenetic pattern seen in Hurthle cell carcinoma of the thyroid, inflammatory rhabdomyoblastic tumor, and a subtype of mesothelioma, but not reported in leiomyomas of other sites. Intraocular leiomyomas do not harbor MED12 mutations or HGMA2 translocations seen in a subset of uterine and soft tissue leiomyomas. Cytogenetic features of the large tumor with atypia and mitotic activity did not differ from the rest of the cohort.

POSTERS

Posters: Demyelinating/inflammatory

1

Diverse HLA-DR-Immunopositive Cell Morphology in Multiple Sclerosis Lesions and Implications for Pathogenesis

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Background: Multiple sclerosis (MS) features white matter demyelinating plaques categorized as active, chronic active, or chronic inactive based on the degree of demyelination, and inflammatory response, with participation of microglia/macrophages being pivotal. This study investigates the morphological traits of HLA-DR-immunopositive cells within these plaques to advance our comprehension of MS plaque evolution.

Methods: We conducted a comprehensive analysis of 90 plaques from six MS cases, employing various staining techniques (HE-LFB, HLA-DR, GFAP, APP, and NFP). Among these, 77 were classified into three main categories: 30 active, 33 chronic active, and 14 chronic inactive. Additionally, we examined five white matter lesions with vacuolation, two with axonal degeneration, and six challenging lesions. Six control cases were included to assess HLA-DR-immunopositive cell expression across different age groups. These cells were classified based on shape and processes into two categories: round cells without processes (macrophages) and cells with varying shapes and lengths of processes (ramified microglia).

Results: A mixture of macrophages and ramified microglia is present in all lesions, with our primary focus on characterizing the predominant cell type. Among the 30 active plaques, macrophages predominated in 22, while ramified microglia prevailed in 8 plaques. In the center of the 47 chronic plaques, scattered ramified microglia were present in 46 plaques, with one plaque featuring macrophages. Within the 33 chronic active lesions, 31 exhibited ramified microglia in the periphery, while two of them had predominantly macrophages. The intermediate zone within these plaques had a comparable proportion of HLA-DR-immunopositive cells but showed intermediate cellularity between the central and peripheral regions.

Conclusions: The predominance of macrophages in acute lesions is aligned with the phagocytic function in active demyelination. The prevalence of ramified microglia in the periphery of chronic active lesions suggests its potential protective effect in impeding demyelination.

2

Histopathological features of antemortem Marchiafava-Bignami disease with clinical and radiologic correlations

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Background: Marchiafava-Bignami disease (MBD) is thought to be an extremely rare disorder characterized by corpus callosum demyelination and necrosis in the setting of chronic alcoholism, although this damage may also occur with severe malnutrition. To our knowledge there is only one antemortem case report describing the histopathological features.

Methods: Here we describe the clinical, serial radiologic, and histopathologic features of two cases of corpus callosum demyelination identified on biopsy, the first related to chronic alcoholism and the second in the setting of severe malnutrition.

Results: Brain MRI performed on a 53-year-old male with a past medical history of chronic alcoholism, heart failure, and recent development of a right facial droop revealed an expansile T2 signal abnormality with corresponding restricted diffusion and contrast enhancement involving a well-defined region of the body and isthmus of the corpus callosum. A biopsy demonstrated frequent macrophages with myelin debris in their cytoplasm and axonal loss in the area with the densest collection of macrophages. However there was still a degree of axon preservation compared to the degree of myelin loss. The second case is a 46-year-old female with end stage renal disease, severe malnutrition, and pneumonia with pleural effusion. Her hospital course was complicated by encephalopathy and quadriplegia. Brain MRI showed diffuse hyperintense signal, restricted diffusion and patchy contrast enhancement involving the entire corpus callosum and multiple bilateral foci of restricted diffusion in the centrum semiovale and periatrial white matter. Microscopy showed infiltration of sheets of macrophages with foamy cytoplasm and confluent areas of partial demyelination. Prominent myelin debris was observed in the cytoplasm of many of the macrophages with relative preservation of axons.

Conclusions: These two cases with corpus callosum lesions showing demyelination described in the setting of chronic alcoholism and severe malnutrition underscore the diverse etiologic and clinicopathologic features of MBD.

3

Granulomatosis with Polyangiitis with Frontal Cerebral Involvement

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Background: Introduction: Granulomatosis with polyangiitis (GPA; formerly Wegener's Granulomatosis) is a necrotizing, granulomatous vasculitis which affects small- to medium-size vessels, and typically presents in older men with respiratory and/or renal involvement. Cerebral involvement, resulting in neurologic manifestations such as meningitis- or stroke-like symptoms, and psychiatric disturbances such as depression, occur in < 10%.

Methods: Case history: We encountered an unanticipated diagnosis of GPA at autopsy in a 37-year-old man with a history of cocaine use and unspecified remote sinus surgery. He worked as an electrician and was found on the sidewalk outside a job site in a multi-story building. He was not known to have psychiatric illness nor suicidal ideation.

Results: Results: Along with extensive blunt force injuries of the torso, extremities and head, external examination showed a depressed nasal bridge ("saddle nose deformity"). Internally, purulence of the frontoethmoid sinuses and erosion of the cribriform plate were associated with marked dural nodularity and thickening, and underlying encephalomalacia of both frontal lobes. Histopathology revealed typical features of GPA (multifocal vascular fibrinoid necrosis, acute and chronic inflammation, including granulomas with giant cells), extending rostrally from the frontoethmoid sinuses, through the dura and leptomeninges, and into the orbitofrontal cerebral cortex and white matter. Trichrome, elastic-van Gieson, reticulin, and PAS stains confirmed the vasculocentric inflammation. Fragments of foreign material were also identified, likely secondary to ongoing intranasal cocaine but also because of direct communication with the oropharynx. There was no evidence of renal involvement; however, pulmonary vasculature had focal perivascular chronic inflammation and fibrosis involving small vessels with associated elastin fragmentation. The cause of death was multiple blunt impact injuries, and manner undetermined (given uncertainty about the scene circumstances).

Conclusions: Conclusion: GPA can present unexpectedly, evade detection without full autopsy examination, and, remarkably, may cause extensive frontal lobe damage.

4

Rheumatoid Meningitis: 4 cases with emphasis on possible links to Alzheimer disease

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Background: Rheumatoid meningitis (RM) is rare and presents with sufficiently wide-ranging, but non-specific, symptoms (seizures, headaches and cranial nerve palsies) and magnetic resonance imaging (MRI) features of meningeal thickening that is indistinguishable from subacute infectious meningitis. In addition, cognitive alterations due to the systemic inflammation, possibly without structural correlates within the CNS, also are known to occur. Serological testing for rheumatoid factor (RF) and anti-cyclic citrullinated peptide (anti-CCP) are helpful clues for diagnosis, but are not invariably present. Thus, meningeal biopsy may be mandatory for diagnosis. Histological findings for RM commonly include leptomeningitis and small vessel vasculitis, with rheumatoid nodules and pachymeningitis being far less common. We reviewed our experience with RM biopsies, placing them into the context of cases in the literature. Unlike older prior studies, we took into account new studies that show possible links between RM and development of Alzheimer disease (AD), further assessing our cases for amyloid and tau.

Methods: Database searches for the years 2013-2023 to generate cases, coupled with chart review.

Results: 4 RM cases identified: 2 male: 2 female, ages 62-79 years, (median 74 years, mean, 72 years). All patients had leptomeningeal enhancement by MRI and 2 had negative RF serology prior to meningeal biopsy. All 4 leptomeningeal/superficial cortical biopsies revealed diffuse chronic inflammation with patchy distribution of plasma cells, giant cells, vasculitis, and necrotizing rheumatoid nodules. Stains (GMS, Gram, Fite) for microorganisms and IgG4 were all negative. 2 of 4 cases had AD neuropathic changes by amyloid and tau immunostaining, 1 of which developed clinical AD 5 years post biopsy; the second patient was lost to follow-up. The remaining 2 cases had few AD changes on biopsy and remain free of clinically-diagnosed AD at 62 and 54 months post-surgery.

Conclusions: RM is a rare diagnosis with a histologic overlap with AD in a subset of cases.

5

Diffuse lymphocytic encephalomyelitis with stiff-person-like syndrome in multiple myeloma after Teclistamab treatment

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Background: Subacute encephalomyelitis is a complex neurological condition with diverse etiologies, including autoimmune disorders and paraneoplastic syndromes. Although neurological complications such as progressive multifocal leukoencephalopathy has been documented in multiple myeloma (MM), motor neuron disease mimicking stiff-person syndrome has been rarely reported in MM.

Methods: We herein report a 45-year-old man with a 13-year history of IgD lambda MM who underwent allogeneic stem cell transplantation, followed by treatment with Teclistamab, a novel BCMA-targeted bispecific T cell engager. Two years after the initiation of Teclistamab, patient developed gait instability, spasticity, and upper motor neuron-pattern weakness, complicated by chronic lymphocytic meningitis. Anti-GAD65 antibody was mildly elevated in the serum but negative in CSF. Despite a clinical suspicion of stiff-person syndrome, he did not respond to immunotherapies including steroids, rituximab, cyclophosphamide, and methotrexate. The symptoms progressed even after discontinuation of Teclistamab, and he passed away eight months after the onset of these symptoms.

Results: Autopsy revealed diffuse lymphocytic encephalomyelitis with CD8>CD4 T-cell predominance across the brain and spinal cord. The T cells infiltrate all grey matter structures with significant perineuronal aggregates associated with moderate macrophage influx and microglial activation resulting in diffuse neuronal loss and gliosis. Subcortical and periventricular white matter also showed T cell infiltrates with less intensity associated with axonal injury, without definitive evidence of demyelination process. Wallerian degeneration was evident in spinal tracts, highlighting extensive axonal injury. No histology or immunohistochemistry evidence for infectious etiologies were detected. The T-cell receptor gamma gene rearrangement study revealed no neoplastic clonality in the T-cells.

Conclusions: His stiff-person-like symptoms, with low serum GAD65 and negative CSF results, suggest that immunosuppression from MM treatment may mask typical autoimmune markers, complicating diagnosis of stiff-person syndrome. Moreover, Teclistamab-associated encephalitis cannot be ruled out. Further investigation into the complex complications associated with MM treatment is crucial for understanding of its impact.

6

Calcium pyrophosphate deposition (CPPD) of the Spine: An Underrecognized Debilitating Disease

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Background: Calcium pyrophosphate deposition (CPPD) disease is an inflammatory arthritis common in elderly patients. Spinal CPPD however is uncommon and presentation can vary widely as osteoarthritis, chronic inflammatory arthritis, acute pseudogout, or other rheumatologic diseases. Radiographic mimics (e.g. cystic masses, spinal infections, etc.) also pose diagnostic challenges. Accurate pathologic diagnosis is key to improve disease identification and management.

Methods: Between 2014 and 2024, we identified 10 cases of spinal CPPD. Clinical notes, imaging, operative notes, and pathology were reviewed.

Results: We identified 10 patients (age range: 63 to 85 y; 5 M, 5 F), with lumbar (9) and thoracic (1) spinal CPPD, of which 90% presented chronically and 10% acutely, all with pain, 4 with prior instrumentation, and 1 with prior trauma. CPPD was not clinically suspected in any case. Based on imaging, central canal stenosis was suspected in 8 cases followed by neuroforaminal narrowing (5/10), septic arthropathy (4/10), synovial cyst (3/10), degeneration (2/10), and epidural mass/abscess (2/10). Intraoperatively spinal cyst was described most often. Pathology showed purple granular material with anisotropic positively-birefringent rhomboid crystals embedded within fibrocartilage (10/10) with degenerative changes seen in half of cases (5/10). Two patients were treated for CPPDand 2 patients were given antibiotics. Two patients had poor outcomes, with one patient showing CPPD in a wrist joint aspirate 4 years after initial spine pathology revealed crystals.

Conclusions: Spinal CPPD is underdiagnosed and undertreated.Crystal arthropathy was not suspected in any patient that we reviewed, and CPPD was treated in only 2 cases. Variable clinical presentations can be challenging, underscoring the importance of wider recognition of this rare disease among neuropathologists. Improved identification of spinal CPPD, especially in elderly patients with back pain, and/or prior trauma/instrumentation, will promote appropriate therapy and management.

7

Incidental finding of macrophagic myofasciitis in a muscle biopsy with Duchenne muscular dystrophy

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Background: We present the case of an 18-month-old boy who presented with gross motor delay, hypotonia, generalized weakness, myotonic facies, and areflexia. Creatine kinase level was 35,110 U/L. Genetic test revealed an in-frame deletion of exon 5-13 in the Duchenne muscular dystrophy (DMD) gene. Deletions in the DMD gene give rise to both Duchenne and Becker muscular dystrophies. The clinical severity depends on the disruption or maintenance of reading frames. However, there are exceptions to this rule. Deletions in the 5' region of the DMD gene are frequently associated with unpredictable phenotypes. Muscle biopsy of the quadriceps was performed to evaluate the expression of dystrophin protein. The muscle showed moderate fiber size variation with abundant degenerating and regenerating myofibers. Immunohistochemistry demonstrated loss of expression of dystrophin epitopes (rod domain and amino terminus) and attenuated expression of dystrophin carboxy terminus and alpha-sarcoglycan, which supported the diagnosis of Duchenne muscular dystrophy. The in-frame deletion of exon 5-13 might result in the severe phenotype; this information should be considered when counselling cases with such mutations.

Methods: Microscopy

Results: Another unusual finding in this muscle biopsy was extensive lymphoid aggregates and multifocal macrophage-rich inflammation. Extensive inflammation is not a feature of DMD, although mild inflammation is not uncommon. GMS and AFB stains were negative for microorganisms. The morphologic and immunohistochemical findings ruled out lymphoproliferative or histiocytic disorders. Morin stain demonstrated blue-green, granular cytoplasmic fluorescence in the infiltrating macrophages. EM confirmed the existence of the dense spicule-like inclusions within the macrophages. The diagnosis of macrophagic myofasciitis (MMF) was made. MMF is an inflammatory condition associated with intramuscular injection of aluminum adjuvant-containing vaccines. Histologically, it's characterized by aggregates of macrophages with abundant basophilic, PAS-positive, diastase-resistant granules without myofiber damage. The definitive diagnosis of MMF requires demonstration of aluminum within these macrophages.

Conclusions: This highlights a rare case of MMF.

Posters: Developmental/Pediatric

8

A case of meningioangiomatosis with molecular characterization

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Background: A rare hamartomatous proliferation, meningioangiomatosis (MA) often presents with seizure and headache in children and young adults. MA can occur either in isolation, in association with Neurofibromatosis type 2, or in conjunction with meningioma, and may be considered neoplastic in the latter instance.

Methods: Case presentation: A 21-year-old female with no significant medical history developed intermittent numbness involving the tongue and hands. Her symptoms progressed to facial and hand twitching, and later two generalized tonic-clonic seizures. Magnetic resonance imaging showed a 2.0 cm T2/FLAIR hyperintensity involving the juxtacortical left lateral frontal lobe. She underwent left frontotemporal awake craniotomy for resection of suspected low-grade glial neoplasm.

Results: Pathology: Frozen section demonstrated cohesive spindle cell process. Permanent sections showed a meningothelial process extending along perivascular spaces. The meningothelial cells had ovoid nuclei, open chromatin, and eosinophilic cytoplasm with ill-defined borders. These cells showed patchy reactivity for progesterone receptor (PR), but were negative for EMA and glial/neuronal markers, and had low Ki67 (< 1%). No evidence of a glial or glioneuronal neoplasm or cortical dysplasia was found. No distinct meningioma was identified, and the surgeon reported that the lesion was deep from the cortical surface. Single nucleotide polymorphism (SNP) microarray found multiple chromosomal abnormalities including loss of a portion of 1p, gain of 1q, gain of a portion of 9q, and loss of a portion of 22q (including NF2).

Conclusions: SNP array demonstrates a clonal process with a possible molecular driver, as 22q loss of heterozygosity is a common driver of meningiomas. Although clonal proliferations are often considered neoplastic, somatic mutations have also been shown to underlie some vascular malformations. Thus defining MA as hamartoma versus neoplasm can be challenging. In the absence of a definite meningioma component, this lesion with NF2 loss of heterozygosity was favored to represent a non-neoplastic process.

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Comprehensive analysis of MYB/MYBL1-altered pediatric-type diffuse low-grade glioma

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Background: Pediatric-type diffuse low-grade gliomas (pLGG) harboring recurrent alterations involving MYB or MYBL1 are closely related tumors. Detailed treatment and outcome data of large cohorts are still limited. This study aimed to comprehensively evaluate pLGG with these alterations to define optimal therapeutic strategies.

Methods: We retrospectively reviewed details of pLGG with MYB or MYBL1 alterations from patients treated or referred for pathologic review at St. Jude Children's Research Hospital. Tumor specimens were centrally reviewed, and clinical data were collated.

Results: Thirty-three patients (18 male; median age 5 years, range 1.8-40 years) were identified. Two tumors had MYBL1 alterations; 31 had MYB alterations, MYB::QKI fusion being the most common (n=10, 30%). No other genetic alterations were identified in any of the tumors. Most tumors were in the cerebral hemispheres (n=22, 67%). Two patients (6%) had metastasis at diagnosis. Twenty-three (70%) tumors were diagnosed as angiocentric glioma based on their dominant growth patterns. Five tumors (15%) were diagnosed as diffuse astrocytoma, and 5 (15%) as isomorphic diffuse glioma. The median follow-up was 6.1 years. The 5-year event-free survival (EFS) rate was 81.3±8.3%; the 5-year overall survival (OS) rate was 96.4±4.1%. Patients receiving a near-total or gross-total resection had a 5-year EFS of 100%; those receiving a biopsy or subtotal resection had a 5-year EFS rate of 56.6±15.2% ($p < 0.01$). No difference in EFS was observed based on location, histology, or molecular alterations. No histologic evidence of transformation was noted in any subsequent or recurrent samples. However, the tumors that progressed or metastasized may have distinct methylation profiles with evidence of activation of the MAPK and PI3K/AKT/mTOR pathways.

Conclusions: pLGG with MYB or MYBL1 alterations have good outcomes. Our findings suggest that surgical resectability is a crucial determinant of EFS. Further characterization is required to identify optimal treatment strategies for metastatic or progressive tumors.

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Focal Cortical Dysplasia in Proximity to Arteriovenous Malformation: Report of Four Cases and Review of the Literature

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Background: Central nervous system arteriovenous malformations (AVMs) and focal cortical dysplasia (FCD) are congenital lesions originating during fetal development. We present four cases in which FCD was identified adjacent to a surgically resected AVM.

Methods: Clinical and histopathologic features were summarized. A literature search was conducted.

Results: Resection of a clinically diagnosed AVM was performed on four patients aged 25-39 years (two men and two women). Two patients had a history of seizures, with onset ranging from three years before resection to onset at the time of presentation. One patient lacked a seizure history. Resection was undertaken and FCD was identified in intact cortex in proximity to the resected AVM. In each case persistent, radial columns or laminae of neurons (lamination), neurons showing evidence of granular cell and pyramidal cell maturation but lacking horizontal migration in the cortex, consistent with designation as International League Against Epilepsy FCD type Ia were observed in proximity to arteriovenous malformations. One case, a patient with seizures and a family history of seizures, exhibited dysmorphic neurons and balloon cells, consistent with designation as FCD type IIb. Given the presence of FCD in proximity to an AVM, each of these cases can be classified as FCD IIIC. The presence of a cortical AVM might be predicted to lead to dysplastic cortical development. Literature characterizing the frequency of FCD in proximity to AVM is limited. Two large series suggest that the phenomenon is common and perhaps overlooked or not appreciated due on extent of surgical resection.

Conclusions: Both AVMs and FCD can provide a potential anatomic substrate for seizures, making it challenging to designate which abnormality was seizurogenic. In the case of FCD Type IIb with a family history of seizures, FCD was more likely to have been clinically relevant to her seizures.

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Changes in FGF and WNT signaling in Arx mutant mice lead to defects in cortical functional domain development

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Background: The mammalian cerebral neocortex is comprised of a 6 layer sheet with conserved functional domains distributed anterior to posterior and medial to lateral. Coordinated development of these cortical areas, along with the accompanying neural circuits, are essential for normal cognition, emotional responses, motor activity, and social behaviors of the brain. How cortical arealization is established from the naïve neural precursor cells in the early neural tube to the mature brain remains incompletely understood.

Methods: We have established that the aristaless-related homeobox (Arx) gene participates in patterning by suppressing ventral identity during cortical development. To further delineate the role of Arx in maintaining proper levels of morphogen expression, cortical arealization, and cortical circuit formation, we interrogated conditional mutant mice and the WNT and FGF signaling pathways.

Results: Our data show that the loss of Arx leads to disruption in FGF and WNT signals, which in turn alters positional identity within the neural precursors in the telencephalon as well as that of the mature neurons in the neocortex (i.e., shifts cortical area boundaries). These changes also results in defects in cortical circuit formation.

Conclusions: Together our data provide insight into how mutations in ARX cause abnormal brain development that leads to functional defects in human.

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Clinical and neuropathologic findings in a child with NAXE gene variant

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Background: The NAD(P)HX epimerase (NAXE) gene, formerly known as apolipoprotein A-I-binding protein (APOAIBP), encodes an enzyme in the NAD(P)H repair system which prevents accumulation of toxic metabolites. Pathogenic variants in this gene have been reported in progressive encephalopathy and/or neurometabolic diseases. A recent study has found that variants in NAXE are also associated with Leigh syndrome. Leigh syndrome, or subacute necrotizing encephalomyelopathy, is a progressive neurodegenerative disorder primarily presenting in infancy or early childhood. It is neuropathologically characterized by necrotic lesions with vacuolization, gliosis and vascular proliferation, with relative preservation of neurons.

Methods: A 3-year-old female with severe neurodevelopmental delay, autonomic instability, presumed syndrome of inappropriate antidiuretic hormone with profound hyponatremia, and recurrent urinary tract infections due to vesicoureteral reflux, was subsequently found to have a homozygous c.206A>G (p.Asp69Gly) variant in the NAXE gene. She was reportedly normal until 9 months of age when she was diagnosed with a brainstem infarct and became ventilator dependent. A full autopsy was performed after she died from sepsis.

Results: Postmortem neuropathologic examination revealed bilateral cystic and gliotic encephalomalacia with spongy change, diffusely involving the neocortex and brainstem. There was prominent capillary proliferation, with relatively preserved neurons adjacent to the hypervascularity. Patchy, perivascular lymphohistiocytic infiltrate and microglial activation were also present. The pons showed foreign body giant cell reaction surrounding cholesterol clefts and abundant Rosenthal fibers.

Conclusions: Leigh syndrome is caused by mutations in either the mitochondrial or nuclear genome and encompasses a wide clinical and genetic spectrum. This is the first report to link a NAXE variant to Leigh syndrome-like histopathologic findings on an autopsy study. Since clinical and neuropathologic manifestations of this variant are unknown, it is unclear whether these changes can be attributed solely to the pathogenic role of the NAXE variant or sequelae of infarct, possibly reflecting combined effects of both.

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Neuropathologic Features of Pallister-Killian Syndrome: Case Report and Literature Review

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Background: Pallister-Killian syndrome (PKS) is a rare and severe disorder defined by mosaic tetrasomy of 12p. The disease is characterized by dysmorphic features, central nervous system malformations, and cardiac anomalies. The prognosis for PKS is poor.

Methods: A consented autopsy and neuropathologic examination was performed. A literature review for pathologic manifestations of PKS was performed.

Results: Literature review revealed numerous neuroimaging reports, limited autopsies (less than 10), and no dedicated neuropathology reports of patient with PKS. The infant male was born at 27 weeks gestation. PKS was verified by antemortem genetic testing which showed mosaic tetrasomy of 12p. Medical conditions included bronchopulmonary dysplasia, pulmonary hypertension, cardiac defects, and bacterial pneumonia. Neuroimaging revealed progressive cortical and white matter loss, and subdural and intraventricular hemorrhage. He died at 10 months of age. Autopsy revealed dysmorphic features including frontal bossing, high arched cleft-palate, depressed nose, low set ears, and webbed neck. Body measurements demonstrated increased head circumference, short body length, and shortened upper extremities. Neuropathologic examination revealed a low brain weight for age (757 grams), bilateral thin subdural hematomas, polymicrogyria, and globally diminished white matter. The brainstem and spinal cord were grossly normal. Sections showed abnormal cortical development including neuronal marginal heterotopia embedded in interhemispheric leptomeningeal bands, and focal cortical laminar disorganization. Amphophilic globules and dystrophic mineralization were present throughout the cerebrum and spinal cord, highlighted by Von-Kossa stain. Other findings included globoid cardiomegaly, right ventricular hypertrophy, atrial and ventricular septal defects, pulmonary artery dilation, bronchopulmonary dysplasia, absent left adrenal, and atrophic right testicle.

Conclusions: PKS can present with a spectrum of central nervous system findings including structural abnormalities, malformations of cortical development, and white matter disease. In this case, leptomeningeal heterotopia, polymicrogyria, and cortical laminar disorganization suggest disrupted neuronal migration and cortical development. Further autopsy contributions are essential for elucidating PKS's definitive gross and microscopic manifestations.

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Cortical, white matter, and dentate gyrus atrophy associated with infantile myasthenic/hypotonia syndrome and heterozygous mutations in ALG14

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Background: A 4-month-old girl born at term with congenital myasthenic/hypotonia syndrome, infantile spasms and heterozygous mutations (c.220g>A pAsp74Asn; c.203A>G p.Tyr68Cys) in the asparagine-linked glycosylation 14 (ALG14) gene presented for worsening seizure activity, aspiration, emesis and nocturnal hypoxemia. Unfortunately, epilepsy interventions were unsuccessful and the patient was transitioned to comfort care.

Methods: The family requested a full autopsy and donated the patient's right hemisphere and muscle to ongoing congenital disorders of glycosylation (CDG) research at the NIH to contribute better understanding of this rare CDG.

Results: At autopsy, acute bilateral pneumonia was present. The frontoparietal lobes and corpus callosum showed marked atrophy associated with ventriculomegaly. Microscopically, acute and subacute white matter infarcts were present. Moderate loss of granular neurons were present in the dentate gyrus and there was marked cerebellar dentate nucleus injury out of proportion to the concurrent mild global acute hypoxic-ischemic encephalopathy. NIH results are not yet available.

Conclusions: CDGs are multisystem disorders with varying CNS functionality and neurologic symptoms. The ALG14 gene encodes a subunit of UDP-N-acetylglucosamine transferase, an enzyme which adds the second N-acetylglucosamine residue during synthesis of the lipid linked oligosaccharide in the endoplasmic reticulum. Autopsies of ALG14-CDG patients showed an early severe neurodegeneration with myopathic and myasthenic features. Two of these patients showed frontoparietal atrophy and clinical epilepsy. Importantly, one of these patients shared the same heterozygous mutation as this patient. Other patients in the series showed either delayed white matter myelination or subsequent white matter volume loss and ventriculomegaly. While hippocampal dentate gyrus injury was not described in this series, it is well known to be associated with hypoglycemia, which may be present in CDGs. Abnormal metabolic derangement may also be the underlying cause of the cerebellar dentate nucleus injury. Together, these findings strengthen and extend our knowledge of expected abnormalities in rare CDGs with ALG14 mutations.

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Congenital smooth muscle hamartoma overlying a fibrous stalk: A potential cutaneous marker of occult spinal dysraphism

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Background: Spinal dysraphism results from incomplete midline closure during neurulation. Occult spinal dysraphisms (OSDs) often manifest with overlying cutaneous stigmata. Fibrous stalks, a type of OSD, are frequently associated with" cigarette burn" lesions, sacral dimples, lipomas, and cutaneous vascular lesions, including infantile hemangiomas and capillary vascular malformations. We present a case of a fibrous stalk associated with an overlying congenital smooth muscle hamartoma (CSMH).

Methods: A male infant prenatally diagnosed with T11 hemivertebra and rib abnormalities was born at full term with dextroscoliosis, a closed-base sacral dimple, and an erythematous lesion over the lower thoracic spine. Postnatal imaging revealed spinal dysraphism with a subcutaneous fibrous sinus tract and tethered low-lying conus medullaris. At 16-months of age, spinal cord detethering was performed. Intraoperatively, a tract extended from the thoracic cutaneous lesion through the soft tissue and dura and attached to the spinal cord. The intra- and extradural tract, including the skin, was sent en bloc for pathologic examination.

Results: Histologic sections revealed a disorganized proliferation of smooth muscle within the reticular dermis and superficial subcutis, consistent with CSMH. A cord-like stalk of benign fibrous tissue with nerve twigs, meningothelial cells, and focal cartilage extended from the CSMH into the soft tissue. There was no evidence of an epithelium-lined sinus tract, neuroglial tissue, or vascular malformation.

Conclusions: CSMH is an uncommon benign cutaneous lesion composed of a proliferation of smooth muscle thought to arise from arrector pili muscle. Clinically they can be confused with other congenital cutaneous lesions including vascular malformations. CSMHs have not been previously associated with OSDs. We present the first reported case of a CSMH overlying a fibrous stalk. This case illustrates a previously unrecognized cutaneous lesion that could potentially herald an underlying OSD.

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Cortical dyslamination and dysmorphic neurons in POLG-related mitochondrial disorder

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Background: Biallelic variants in the nuclear POLG gene, which encodes the catalytic subunit of mitochondrial DNA polymerase γ, are the most common cause of inherited mitochondrial disorders and result in a wide phenotypic spectrum of diseases including progressive external ophthalmoplegia and sensory ataxic neuropathy, with age of onset ranging from infants to adults.

Methods: We report brain and muscle biopsy findings from a patient with POLG-related disorder with the following POLG variants: maternal allele: c.2243G>C (p.W748S); paternal allele: c.1399G>A (p.A467T). A 29-year-old pregnant female with recent history of mild COVID-19 presented with refractory status epilepticus. Magnetic resonance imaging (MRI) of the brain showed waxing and waning, diffusion restricting T2/FLAIR hyperintense lesions in the periventricular white matter, frontal, parietal, and occipital lobes, and posterior cingulate gyrus as well as mild diffuse pachymeningeal enhancement. Extensive laboratory workup was negative for infectious or autoimmune etiologies. Three separate biopsies/resections of the MRI lesions in the parietal and frontal lobes and a muscle biopsy of the thigh were performed.

Results: The brain showed severe patchy astrogliosis and microglial activation with rare microglial nodules and sparse perivascular lymphocytic infiltrate. Additionally, disorganized cortical lamination with scattered dysmorphic neurons resembling focal cortical dysplasia (FCD) International League Against Epilepsy (ILAE) type 2A was seen. On electron microscopy studies of the parietal lobe, there were rare filiform mitochondria. The muscle demonstrated critical illness myopathy with no COX negative fibers. Ultrastructural studies revealed large areas of myofibrillar abnormality consisting of loss of the A-band, I-band and M-line with some mitochondria showing filiform changes.

Conclusions: Cortical dysplasia can be seen in mitochondrial diseases but has not been reported previously in POLG-related disorder. Although coincidental FCD cannot be excluded, the patient had no history of seizures prior to the current presentation. Our findings expand the neuropathologic spectrum of POLG-related mitochondrial disorder.

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Hyaline Protoplasmic Astrocytopathy in Two Surgical Cases of Developmental and Epileptic Encephalopathy

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Background: Hyaline protoplasmic astrocytopathy (HPA) is a rare condition characterized by astrocytic cytoplasmic inclusions, distinct from Rosenthal fibers, in the cerebral cortex of unknown pathogenesis and clinicopathological significance. We performed immunohistochemical and electron microscopic studies and proteomic analysis of HPA inclusions using FFPE surgical specimens from two patients with developmental and epileptic encephalopathy.

Methods: Case 1 is a 2-year-8-month-old boy presenting with tonic seizures and epileptic spasms since 3 months old, and was diagnosed with West syndrome followed by Lennox-Gastaut syndrome. Brain MRI revealed polymicrogyria in the right frontal lobe. ACTH therapy and total corpus callosotomy (TCC) were ineffective. He became seizure-free after multistage right hemispheric disconnective surgeries. Case 2 is a 10-year-1-month-old boy with Lennox-Gastaut syndrome. He developed epileptic spasms at 7 months old, which resolved temporally with ACTH and other therapies until 5 years old. MRI revealed abnormal gyral formation in the right frontal lobe. The patient became seizure-free after TCC and subsequent right frontal lobe disconnection.

Results: Histopathological examination of the frontal lobe biopsy specimen revealed polymicrogyria with HPA in both cases. Inclusions were brightly acidophilic, PTAH-positive, and non-amyloid, and variably immunoreactive for S-100, glutamate transporter-1, aldehyde dehydrogenase 1 family member 1, cytoglobin, and HSP70; no immunoreactivities were observed for GFAP, filamin A, ubiquitin, p62, or alpha B-crystallin. GFAP expression in most cortical astrocytes was faint or absent. Electron microscopy revealed inclusions consisting of densely packed electron-dense granules of approximately 30-40 nm diameter without unit membranes or filamentous structures, intermingled with occasional mitochondria and Golgi apparatus. Proteomic analysis of macrodissected cerebral cortex by quantitative mass spectrometry detected significantly upregulated astrocytic proteins, including glutamine synthetase and tenascin C, in HPA compared to control cases.

Conclusions: These results suggest that HPA inclusions are derived from organelles and that HPA represents an altered astrocytic glutamate metabolism secondary to intrauterine encephaloclastic mechanisms and neuronal hyperexcitation.

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SEQUENTIAL EMERGENCE AND CONTRACTION OF EPITHELIAL SUBTYPES IN THE PRENATAL HUMAN CHOROID PLEXUS REVEALED BY A STEM CELL MODEL

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Background: As the blood-cerebrospinal fluid interface, the choroid plexus (ChP) mediates body-brain communication throughout life and has broad potential for CNS regenerative medicine. Despite this, relatively little is known about the human ChP or its epithelial cells (CPECs), including their lineages.

Methods: Based on an earlier proof-of-concept method, we devised a simple, efficient, and scalable protocol for CPEC derivation from human pluripotent stem cells. Time-series analysis of single-cell RNA-sequencing data were then performed and validated in independent derivations and perinatal tissues.

Results: In the absence of mesenchymal elements, derived CPECs (dCPECs) acquired canonical properties and displayed dynamic multiciliated phenotypes that impacted Aβ uptake and paralleled those seen in human tissues. Single dCPEC transcriptomes correlated well with human organoid and fetal CPECs, while pseudotemporal and cell cycle analyses revealed dCPEC, neuron, and neural progenitor lineages arising from neuroepithelial cells. In addition, transcriptome analyses defined metabolic (type 1) and ciliogenic dCPECs (type 2) at early stages, followed by type 1 diversification into anabolic-secretory (type 1a) and catabolic-absorptive subtypes (type 1b) as type 2 cells contracted. The sequential emergence and contraction of these subtypes was then confirmed in independent derivations and mapped to distinct prenatal stages using human tissues.

Conclusions: These findings establish an improved protocol for deriving human CPECs, define their prenatal lineage dynamics, and lead to new models of ChP/CSF functions during human brain development.

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Neuropathologic findings in a 32-year-old man with Phelan-McDermid syndrome

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Background: Phelan-McDermid syndrome (PMS), also known as 22q13.3 deletion syndrome, is a rare genetic disorder typically involving the loss of the SHANK3 gene affecting neurological development and leading to facial abnormalities and intellectual disabilities, including features of autism spectrum disorder. Neurofibromatosis type II (NF2) is caused by mutations in the nearby NF2 gene (22q12.2), with well-documented neuropathologic features. In contrast, the tissue neuropathology of individuals with PMS has not been reported.

Methods: We contribute the complete neuropathology of an autopsy case of a 32-year-old man with a diagnosis of Phelan-McDermid syndrome confirmed on antemortem detection of chromosome 22q13 deletion (further details unavailable).

Results: The brain was micrencephalic (weight, 900g; posterior fossa contents, 90g), with slight cerebral and cerebellar atrophy, and meningiomas in the parietal dura (0.3cm) and the glomus of the choroid plexus (0.7cm). The hippocampi were asymmetric, the left having a globular configuration, with subicular widening and expansion of the temporal horn of the lateral ventricle. Microscopic examination further revealed a 0.1cm glial hamartoma in the amygdala, a focus of meningothelial hyperplasia in the basal leptomeninges, and a Schwann cell tumorlet (0.1cm) in the trigeminal nerve. A clear concurrent diagnosis of NF2 by conventional Manchester (NIH) criteria could not be made, however.

Conclusions: Given the proximity of the causative mutations for PMS and NF2 on chromosome 22, we suggest that this decedent may have had a heretofore unrecognized “overlap” syndrome. Investigators have hypothesized that patients with both PMS and NF2 may have a ring chromosome 22 (forming after the loss of genetic material from both the short and long arms), leading to instability of the chromosome during mitosis. Whether other patients with PMS (with or without ring chromosome) also have neuropathologic features of NF2 will require additional autopsy evaluation.

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Autopsy findings in a patient with a severe neurodevelopmental disorder due to compound heterozygous mutations in PNPT1

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Background: Heterozygous mutations in PNPT1 gene (which encodes polynucleotide phosphorylase, a mitochondrial 3'-to-5' exoribonuclease involved in mtRNA processing) cause autosomal dominant spinocerebellar ataxia 25, while patients with bi-allelic PNPT1 mutations exhibit wide clinical heterogeneity, ranging from non-syndromic hearing loss to multi-system Leigh disease-like syndrome. The underlying pathologic changes have not been reported and disease pathogenesis is poorly understood, with mitochondrial dysfunction and systemic inflammation (due to activation of interferon type I response by accumulated double-stranded mtRNA) proposed as possible disease mechanisms.

Methods: Here, we report the autopsy findings from an 11-year-old boy with compound heterozygous PNPT1 mutations, clinically manifesting as cerebral palsy, intellectual disability, dystonia, epilepsy, vision and hearing impairments, scoliosis, dysphagia, constipation, and cyclic vomiting.

Results: At autopsy, the CNS showed severe microcephaly due to cerebral leukoencephalopathy, with widespread white matter attenuation and severe thinning of corpus callosum; pontocerebellar degeneration, with axon loss in the middle cerebellar peduncles and base of pons, patchy loss of Purkinje and dentate nucleus neurons, cerebellar white matter degeneration, and severe atrophy of the cerebellar vermis; and spinocerebellar atrophy, with degeneration of dorsal spinal columns and afferent cerebellar tracts. Cranial and peripheral nerves showed moderate-severe axonal polyradiculoneuropathy with focal demyelinating features, with greater involvement of sensory than motor cranial nerves and dorsal than ventral spinal roots. Skeletal muscles showed ultrastructural mitochondrial abnormalities and chronic neurogenic changes accompanied by ongoing denervation. The gastrointestinal system demonstrated widespread loss of interstitial cells of Cajal and patchy thinning of the colon wall due to smooth muscle degeneration and fibrosis. Superimposed acute ischemic changes were seen in multiple organ systems. Aside from focal bronchopneumonia, no significant inflammation was seen.

Conclusions: Overall, these autopsy findings are consistent with a systemic mitochondrial disorder and do not provide evidence for a concurrent neuroinflammatory disease process, providing important insight into the pathogenesis of this rare disease.

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Artificial Intelligence-Based Statistical Modeling Delineates Cortical Architectural Distortion in Surgically Resected Epileptogenic Foci

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Background: In epilepsy patients lacking radiographic evidence of focal cortical dysplasia (FCD) or other epileptogenic lesions, surgical resections target regions of abnormal electroencephalographic activity. Identifying cortical architectural disorganization in these specimens by conventional histology remains challenging. We explored artificial intelligence (AI)-based cell mapping and morphometry combined with spatial statistical models to evaluate neuronal architectural distortion within and adjacent to regions of FCD.

Methods: Sections from FCD resections (n=29) and matched regions from neurologically healthy autopsy controls (n=19) were stained with NeuN. Whole slide images were generated, and regions of interest (ROI) were annotated in QuPath for surgical samples as FCD (n=33), FCD-adjacent (n=14), Apparently-normal (n=36), and Autopsy True-normal (n=19). The QuPath cell detection algorithm was used to identify NeuN-positive cortical neurons and compared to manual annotations. Extracted spatial and morphometric data for each cell was analyzed in R 4.2.1 using SpatStat. A multinomial, LASSO-regularized regression classifier was trained and tested on an 80/20% training/test split. Kruskal-Wallace tests with non-parametric post-hoc comparisons were used for cell morphologic features, and permutations tests were used to compare summary functions of neuronal spatial distribution.

Results: The cell detection algorithm had an overall sensitivity of 90% and a false positive rate of 12%, with no significant difference in accuracy across categories. The multinomial classifier yielded an accuracy of 70.5 and 82.5% for the ROI classification and overall specimen diagnosis. Parameters related to neuronal clustering, inhomogeneity of neuronal distribution, and nuclear size and regularity factored prominently into the classifier. Subsequent statistical analyses demonstrated significant differences in the intensity of neuronal point patterns ($p=0.01$), the degree of spatial inhomogeneity ($p< 0.001$), and degree and distribution of neuronal clustering ($p< 0.001$) across the annotated categories.

Conclusions: AI-based quantification of cellular morphology and spatial distribution combined with spatial statistical modeling may augment the detection of abnormal neuronal architecture in surgically-resected epilepsy foci.

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A Rare Case Involving Fetal Immunodysregulation Polyendocrinopathy Enteropathy X-linked (IPEX) Syndrome

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Background: This case involved a male fetus that was the product of a 26-week gestation to a 25-year-old primigravid woman. The pregnancy was complicated by multiple anomalies seen on prenatal ultrasound including fetal hydrops. The mother initially presented with severe back pain consistent with contractions and underwent spontaneous vaginal delivery of the fetus. A fetal hydrops genetic panel was pursued, which showed a hemizygous mutation for a likely pathogenic variant in FOXP3 (c.17del, p.Pro6Leufs*56) and heterozygous mutation for a variant of uncertain significance in RPS24 (c.734C>T, p.Pro245Leu).

Methods: At neuroautopsy, the fresh brain weighed 79.2 grams (normal range for 26-weeks gestational age is 105.0 +/- 19.0 grams). External examination of the brain showed a diffuse dusky red-brown discoloration noted throughout the leptomeninges. Coronal sections of the cerebral and cerebellar hemispheres showed moderate-to-severe maceration.

Results: Neuropathologic examination of the tissue sections demonstrated multifocal areas of extensive fresh hemorrhage, transcortical neuronal loss, laminar necrosis with gliosis, small vessel proliferation, and sheets of macrophages. Interestingly, there was a prominent lymphocytic population of infiltrates associated with the areas of organizing cortical necrosis.

Conclusions: This fetus shows a rare presentation of immunodysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome. IPEX syndrome is a rare disorder resulting in aggressive autoimmunity and early death and is due to a mutation in FOXP3 which results in dysfunctional T-regulatory cells. Most patients die in infancy or childhood, though some patients have lived longer with chronic immunosuppression or bone marrow transplants. Patients usually present with infantile diabetes, severe enteropathy, eczema, anemia, and hypothyroidism. Histologic findings are striking, and lymphocytic inflammatory infiltrates of multiple organs. IPEX is an X-linked disease, resulting in female carriers and males with disease. In some families the affected males present earlier and much more severely. In these families the most common presentation is familial intrauterine demise of male fetuses in midtrimester.

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Characterization of a Population of Neural Progenitor Cells in the Developing Hippocampus

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Background: Hippocampal circuitry is critically involved in navigation and episodic memory. The development of the circuit is a finely regulated process which includes the approximation and recession of the lateral ventricle ependyma. As the ependyma retreats, it forms a "seam" below Ammon's horn, which we refer to as the subammonic seam. In this process a population of candidate neural progenitor cells is left hundreds of microns from the ventricle, effectively 'migrating' due to the seam closure. Our study aims to characterize this population of cells.

Methods: Autopsy cases for spontaneous fetal demise or infant death between 20 and 144 weeks post conception were identified. Approximation of the ependyma, seam closure, and position of candidate progenitor cells were assessed. We are evaluating morphologic and immunostaining findings to visualize seam closure and candidate progenitor cell persistence, movement, morphological change, and protein expression.

Results: The subammonic seam begins to close in the third trimester. Candidate neural progenitor cells could be morphologically identified along the closing seam out to 56 weeks post-conception. The subammonic seam can be definitely identified for months after birth until it begins to recede in prominence then vanishes into the white matter of the mature parahippocampal gyrus.

Conclusions: The candidate neural progenitor cells persist along the seam for up to 16 weeks postnatally. This cell population could be implicated in postnatal neurogenesis after the recession of the germinal matrix within this region. Preliminary results suggest some possible trajectories. Further understanding of this cell population may provide a more complete picture of the formation of hippocampal circuitry and potentially of the pathogenesis of diseases such as epilepsy.

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Hyaline Protoplasmic Astrocytopathy of the Neocortex: A Case Study and Literature Overview

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Background: Hyaline Protoplasmic Astrocytopathy (HPA) is a rare disorder of uncertain etiology characterized by the presence of eosinophilic hyaline inclusions within the cytoplasm of protoplasmic astrocytes.

Methods: We examine histology and electron microscopy in brain resection tissue from a patient with chronic epilepsy and HPA, integrating case-specific findings with existing literature.

Results: A 10-week-old girl developed recurrent episodes of head drop associated with arm and leg extension. MRI showed diffuse microgyria and partial agenesis of the corpus callosum. Over subsequent years, she exhibited severe developmental delay, was non-verbal, and G-tube dependent. Extensive genetic and metabolic testing were unrevealing. EEG showed excess background theta slowing, brief bursts of generalized polymorphic delta activity, and abundant independent multifocal epileptiform discharges. Multiple antiepileptics were trialed, and at age 14 she underwent right midfrontal gyrus resection with bilateral centromedian/pulvinar thalamic nuclei responsive neurostimulation placement, all without improvement. Histologic sections showed cerebral cortex and subcortical white matter with numerous glial cells with optically clear nuclei containing abundant glassy, eosinophilic cytoplasmic inclusions present throughout the cortex. The inclusion material was negative for GFAP and weakly immunoreactive for S100. NeuN immunostaining showed focal neuronal depletion and tangential disarray with absence of distinct layering. Electron microscopy of the inclusions demonstrated relatively uniform, non membrane-bound, electron-dense granular material with areas of clearing containing lighter material possibly representing degenerating organelle elements. A diagnosis of Hyaline Protoplasmic Astrocytopathy of Neocortex with Focal Cortical Dysplasia (ILAE type Ib) was rendered.

Conclusions: HPA is a non-specific constellation of findings associated with chronic seizure disorders in various settings, including Aicardi syndrome, tuberous sclerosis, and focal cortical dysplasia. The inclusions stain positive for Filamin A, a regulator of neuronal migration during development, and the glutaminate transporter GLT-1. As a very rare entity, further descriptions of patients with this entity will contribute to a better understanding of the pathophysiology.

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Post-Treatment Neuropathologic Findings in Aicardi-Goutières Syndrome

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Background: Aicardi-Goutières syndrome (AGS) is a rare inherited neurodegenerative disorder associated with interferon overproduction. The syndrome is characterized by inflammatory encephalopathy and cerebral calcifications. Janus Kinase (JAK) inhibitors, such as baricitinib and ruxolitinib, have been explored in the treatment of AGS, however, little is known about the neuropathologic impacts of JAK inhibitor therapy in AGS.

Methods: A consented, unlimited autopsy with formal neuropathologic examination of the brain and spinal cord was performed.

Results: The decedent was a 4-year-old boy with AGS diagnosed in the immediate postnatal period. Genetic testing at two months of age revealed a compound heterozygous mutation in RNASEH2C. He was subsequently initiated on baricitinib. At 4 years of age, he developed septic shock and acute respiratory distress syndrome as a result of aspiration pneumonia. Following compassionate withdrawal of care, he underwent a complete consented autopsy. Externally, body weight was below the 1st percentile for age. Dysmorphic features included microcephaly, equinovalgus feet, and microphthalmia. Internal examination revealed multifocal pneumonia and acute tubular injury. Neuropathologic examination revealed a 219.6-gram brain exhibiting markedly diminished white matter and extensive calcific deposits involving the cerebral cortex, white matter, deep gray nuclei, cerebellum, and brainstem. Microscopic examination revealed astrocytic gliosis and numerous, variably sized calcifications. There were perivascular, intraparenchymal, and leptomeningeal chronic inflammatory infiltrates, composed of CD4 positive T-lymphocytes, rare CD8 positive T-lymphocytes, and rare CD20 positive B-lymphocytes. CD68-positive macrophages were predominately perivascular. Luxol fast blue showed demyelination of the corpus callosum and subcortical white matter. Microinfarction and Bergmann gliosis were identified in the cerebellum. The spinal cord showed diminished bulk of the anterior horn. Calcifications did not involve the spinal column.

Conclusions: Neuropathologic findings in this case of AGS, post-JAK inhibitor therapy, underscore persistent neuroinflammation and degeneration. Further studies are essential to clarify the implications of these results and guide future therapeutic strategies.

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Toxic/Metabolic Leukoencephalopathy in Children

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Background: Toxic-metabolic leukoencephalopathy, characterized by significant white matter damage as the primary finding, is uncommon in children without prior significant neurologic history. We encountered two cases recently with history of a viral prodrome, new-onset seizures quickly progressing to status epilepticus and death, with autopsy findings of non-infectious leukoencephalopathy. On delving into the clinical history, the findings were most consistent with drug toxicity. We decided to review the autopsy cases at our institution over the past 10 years for a diagnosis of leukoencephalopathy/ leukodystrophy and correlated these findings with the clinical history.

Methods: Cases with predominant white matter injury/ myelin loss were identified from the autopsy database maintained by our neuropathologist, and cases with only perinatal hypoxic-ischemic injury were excluded. Complete autopsy reports for cases selected were reviewed.

Results: Seven cases with primary leukoencephalopathy/ leukodystrophy were identified out of 315 cases, with age at autopsy ranging from 6 days to 16 years. 2 had known metabolic disorders, and 2 had findings consistent with drug toxicity. Genetic mutations for metabolic disorders were identified postmortem for 2 cases and the findings on 1 case were suggestive of peroxisomal biogenesis disorder. All the cases had varying severity of associated hypoxic-ischemic injury, and some of them also had abnormal neuronal migration.

Conclusions: Presence of significant white matter damage at autopsy should raise the suspicion for toxic-metabolic disorders. In-depth review of clinical history can offer a clue to the diagnosis of drug- associated toxic leukoencephalopathy. Findings of toxic-metabolic leukoencephalopathy/leukodystrophy at autopsy are still very rare.

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Spectrum of Meningoencephaloceles Presentation: From Massive to Atretic - Case Reports and Literature Review

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Background: Meningoencephaloceles are neural tube defects that affect approximately 0.8-4 per 10,000 live births. They represent congenital malformations with protrusion of central nervous system structures through a cranial defect with associated arachnoid, cerebrospinal fluid, and dura. We report a case of a male infant with a prenatally diagnosed large occipital meningoencephalocele and a female infant with an occult presentation.

Methods: Clinical features were summarized from the patient's medical record. A literature search was undertaken using relevant key words.

Results: Case A: A large meningoencephalocele was identified in utero in a male fetus with elevated maternal alpha-fetoprotein levels. Following Caesarean section delivery, imaging revealed an occipital encephalocele, 10.5 x 9.4 x 4.5 cm, associated with a midline defect in the posterior calvarium. Resection was undertaken and macroscopic examination identified nodules of malformed cerebrum enclosed by cystically dilated meninges associated with skin. Microscopic evaluation identified neuroparenchyma with dystropic lamination and calcifications. No evidence of cerebellar parenchyma or other hindbrain elements was identified. Case B: A 7-year-old girl presented with a midline posterior parietal scalp mass at birth. During early childhood, the lesion would increase in size while crying and was noted to be pulsatile. Imaging revealed normal intra-axial structures with persistent falcine and emissary veins extending to what was interpreted as an extracranial venous malformation in the parasagittal parietal scalp. Radiologic diagnosis was sinus pericranii and treatment was deferred. Tenderness and increased size, to 1.7 x 2.2 x 1.7 cm, lead to resection. Histologic evaluation revealed a minute nodule of dysplastic neuroparenchyma surrounded by arachnoid, dura, and skin.

Conclusions: The prognosis for occipital and parietal encephaloceles is related to the size and location of the encephalocele, the presence or absence of cerebellum or other hindbrain contents within the herniated tissue, the occurrence of hydrocephalus, and the presence or absence of concurrent cerebral malformations.

Posters: Infectious

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A case of racemose neurocysticercosis with molecular diagnosis

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Background: A 40-year-old, otherwise healthy, Spanish-speaking male immigrant from Mexico presented to the Emergency Department with one and a half months of progressive speech difficulty, headaches, and confusion. Neurologic examination was notable for expressive aphasia with intact facial and tongue movement. Computed tomography with angiography demonstrated a massive loculated cystic mass in the left frontotemporoparietal lobes measuring 13.6 x 6.6 cm and causing midline shift and brainstem compression. Magnetic resonance imaging showed that the lesion was non-enhancing except along septa and had a similar intensity to cerebrospinal fluid. The patient subsequently underwent decompressive hemicraniectomy due to acute worsening of his neurologic state.

Methods: Macroscopic evaluation showed a fragmented, tan-pink lesion with both cystic and solid components. H&E-stained sections demonstrated a collapsed, redundant cyst wall with three layers: a brightly eosinophilic cuticle layer reminiscent of broad, blunt villous structures; a more cellular "pseudoepithelial" layer composed of non-human cells; and a loose, reticular layer. Small amounts of reactive brain tissue, foreign body giant cells, and rare calcification were present. No definitive evidence of a parasite, such as hooklets, scolex, or spinal canal, was identified.

Results: Laboratory workup was positive for cysticercosis IgG and echinococcus IgG antibodies. The two IgG's are however known to cross-react, warranting molecular testing. PCR testing on the surgical specimen was positive for Taenia solium, confirming a diagnosis of neurocysticercosis. He was treated with albendazole and praziquantel and had returned to baseline function without recurrence at follow-up of one year.

Conclusions: Neurocysticercosis is the commonest invasive parasitic infestation of the central nervous system but is an infrequent surgical specimen in the United States. Racemose neurocysticercosis is a relatively rare form of neurocysticercosis. Racemose neurocysticercosis forms abundant grape-like clusters of cysts but often lacks a scolex. In this case, molecular testing was used to confirm the infectious organism.

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Intracranial Cerebral Mucormycosis Infection in the Context of Immunosuppression: Comparison of Ischemic vs. Hemorrhagic Complications

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Background: Fungal infection of the central nervous system (CNS) is rare and most commonly affects immunocompromised patients. We report two cases of CNS mucormycosis, presenting variably with infarct or hemorrhage.

Methods: Clinical features were summarized from the patients' medical records. Key histopathologic features were reviewed. A literature search was undertaken using relevant key words.

Results: Patient A: A 75-year-old man with a history of chronic lymphocytic leukemia, undergoing targeted therapy with Imbruvica (ibrutinib), presented with headache, decline in cognitive ability, generalized weakness, and blurry peripheral vision in the right eye. CT and MRI scans identified right maxillary sinus air-fluid levels and left parietoccipital region abnormalities raising the differential diagnosis of a subacute infarct and a high-grade neoplasm and resection was undertaken. Case B: A 23-year-old man with a history of diabetes mellitus type 1 presented with diabetic ketoacidosis. Chest CT scans identified multiple areas of consolidation in his lungs. Initial CT scan of his brain was negative with opacification of the right sphenoid sinus. After being found unresponsive repeat CT scan identified a right frontal lobe parenchymal hematoma which was evacuated. In both cases histologic evaluation revealed angioinvasion by broad, pauciseptate hyphae most consistent with mucormycosis with associated infarct in Case A and hemorrhage in the context of a ruptured, infected artery in Case B. Infection was not in the clinical differential and culture was not performed for either case. While ischemic injury is most commonly identified in the setting of mycotic vasculitis, occasional cases manifesting with hemorrhage have been reported.

Conclusions: This report compares and contrasts two cases of central nervous system mucormycosis that resulted in different cerebrovascular complications, in order to compare precipitating factors and suggest why some patients may be predisposed to aneurysm or intracranial hemorrhage, resulting in progressive disease and poor prognosis.

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HTLV-1 Associated Myelopathy (HAM): Case Report and Literature Review

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Background: HTLV-1 infection is endemic in many areas of the world but there is a low prevalence in the US (about 1%). Here we present an 85-year-old Haitian female with a 15-year history of paraplegia secondary to HTLV-1-associated myelopathy (HAM), also known as tropical spastic paraparesis (TSP).

Methods: The patient presented to Beth Israel Deaconess Medical Center with altered mental status and respiratory failure. Medical imaging showed chronic intracerebral microvascular ischemic changes. Her hospital course was further complicated by sepsis. The patient quickly decompensated and died and a complete autopsy was performed.

Results: There was a macroscopic thinning of the solar and lumbar plexi. There was extensive loss of axons with an associated gliosis in the lateral and anterior columns of the spinal cord and patchy perivascular lymphocytic infiltrates microscopically. The damage extended from the cervical cord to the lumbar cord. There was preservation of the anterior horn neurons. Neurofilament protein immunohistochemistry demonstrated a symmetric loss of axons in the lateral columns of the spinal cord. Cortical sections demonstrated a mild thickening of the leptomeninges and a subpial gliosis.

Conclusions: HAM/TSP is a rare condition within the United States marked by meningomyelitis with axonal degeneration and associated gliosis predominately affecting the lateral columns of the thoracic cord. There can be variable damage to the anterior and posterior columns. In order to establish the diagnosis of HAM, other infectious, autoimmune, and neurodegenerative etiologies of spinal cord degeneration need to be excluded. Many of the infectious agents (i.e., polioviruses) causing transverse myelitis are neuronotrophic and damage the anterior horns. The autoimmune causes (i.e., multiple sclerosis and vasculitis) have variable patterns of distribution, but in general show axonal preservation. The neurodegenerative causes (i.e., amyotrophic lateral sclerosis with or without frontotemporal lobar degeneration) have a similar distribution but have an intracranial component in addition.

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Polymicrobial brain abscesses: a clinicopathological study of 30 cases

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Background: Brain abscesses (BAs) are focal parenchymal infections that are caused by a range of pathogens; despite advances in the diagnosis and treatment during the past decades, they remain a life-threatening condition. Polymicrobial BAs (PBAs) involve two or more infectious microorganisms within their foci; they are complex coinfections with diagnostic and therapeutic challenges.

Methods: We studied 30 patients (ages: 26 ~ 78 years, with a mean of 58 years) with PBAs that were histopathologically examined in correlation with radiological and clinical features.

Results: These patients included 6 females and 24 males; 9 diabetic and 3 other immunocompromised individuals. Common clinical presentations included headache (12/30), confusion (11/30), fever (5/30), hemiparesis (5/30), decreased level of consciousness (5/30), and seizure (4/30). PBAs tended to occur more often in the left (20/30) than the right (10/30) cerebral hemisphere; the most common location was the frontal lobe (17/30), followed by the parietal lobe (6/30); PBAs were unifocal (29/30) except in one case with radiologically multifocal disease within the frontal lobe. While PBA imaging was mostly characterized by a ring-enhancing lesion/mass with central restricted diffusion, some cases showed complex imaging features such as increased heterogeneity, meningeal involvement (10/30) or associated ventriculitis (6/30). PBA histopathological features varied from encephalitis/cerebritis with or without necrosis (9/30, early stages) to encapsulated foci (21/30, late stages); some of which demonstrated complex changes with one pathogen predominating features in certain areas and another pathogen in other areas. The causative pathogens were exclusively bacteria in most cases (25/30); coinfections of bacteria with Toxoplasma (2/30), fungi (2/30) and virus (1/30) were also identified. Streptococcus species (16/30) were the most common pathogen.

Conclusions: Our findings suggest that compared to monomicrobial/overall BAs, PBAs share some similar features such as left-sided predominance, but they are usually unifocal and complex with increased heterogeneity on imaging and histopathology.

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Chronic Meningoencephalitis Caused by Coxsackievirus B3 in a Patient Treated With Ocrevus® (Ocrelizumab) for Multiple Sclerosis: A Case Study

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Background: Coxsackievirus B3 (CVB3) is a common human pathogen that predominantly causes a self-resolving mild flu-like illness. CVB3 neurotropism makes it the leading cause of aseptic meningoencephalitis in young children and a rare complication in patients on immunosuppressive medications, such as rituximab. Less is known about the potential infectious side effects of ocrelizumab, an anti-CD20 monoclonal antibody recently FDA-approved for the treatment of multiple sclerosis (MS).

Methods: We present a case of chronic meningoencephalitis caused by CVB3 in a 50-year-old woman with a 9-year history of MS undergoing ocrelizumab treatment for approximately 3 years. Despite stability of her MS condition, the patient presented with a subacute decline in mental status and cognition. Upon admission, imaging showed predominant bilateral frontal periventricular white matter lesions, and a lumbar puncture revealed elevated protein, normal glucose, 1-2 nucleated cells, no oligoclonal bands, and a negative infectious workup (including HSV, JC virus, CMV, toxoplasma, and bacterial cultures), suggesting a demyelinating etiology. A brain biopsy of these lesions revealed a generalized inflammatory process with lymphocytic infiltration of the meninges without clear evidence of demyelination. After minimal improvement over 3.5 weeks of intravenous steroids, the patient expired and underwent a full autopsy.

Results: Postmortem neuropathologic examination of the brain showed scattered microglial nodules with neuronophagia, multifocal neuronal depletion, and diffuse parenchymal and perivascular lymphocytic inflammatory infiltrates involving the cerebral cortex and midbrain. Further evaluation of CSF using metagenomic sequencing identified Coxsackievirus B3, and RNAScope probes for Coxsackievirus B3 highlighted viral genomic RNA in neuronal bodies and at the center of microglial nodules.

Conclusions: This case study highlights the importance of considering atypical infectious etiologies in patients with neuroinflammatory conditions, particularly in the setting of immunosuppression, and adds CVB3 meningoencephalitis as a rare and unanticipated morbidity associated with ocrelizumab.

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CMV-HSV1 VENTRICULO-ENCEPHALITIS WITH HISTOLOGICALLY OCCULT HSV1

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Background: A 24-year-old man had subacute onset of generalized weakness, confusion, bilateral arm paresthesia, and slurred speech.

Methods: Case report

Results: Head CT demonstrated a 4.4 cm in diameter hyperdense lesion with surrounding hypodensity in the left frontoparietal lobe, suggesting a hemorrhagic lesion with vasogenic edema. The patient was found to have generalized lymphadenopathy and pancytopenia with WBC 0.2 x103 cells/µL, Hgb 7.9 g/dL, and platelets 14 x 103/mL. Tested for HIV-1, he had a plasma viral load of 3 million copies/mL (log10 6.49 cp/mL) and a CD4 count of 159 cells/mm3. He was started on antiretroviral therapy. Worsening clinical status prompted a craniotomy with hematoma evacuation, and histology yielded a diagnosis of EBER-positive DLBCL. The patient received chemotherapy and whole-brain irradiation. The clinical course deteriorating the following six months by disseminated histoplasmosis, CMV viremia, hydrocephalus (due to leptomeningeal involvement), and neutropenia. The patient progressively worsened with continuous altered mental status and emergence of ganciclovir-resistant CMV viremia; he was made DNR in comfort care and expired. Brain autopsy revealed: no active lymphoma, leptomeningeal foci of hemorrhage, necrotizing ventriculo-encephalitis with prominent “owl eye” CMV inclusions, and sub-acute gray matter infarction. This latter with extensive selective neuronal necrosis in the absence of viral inclusions or cellular response. Positivity for CMV Immunostaining was seen in the necrotic regions associated with inclusions. Immunostaining for HSV1, but not CMV, was extensively positive in areas with selective neuronal necrosis, such as the hippocampus, characterizing a rare HSV1-related pseudoischemic change, as described in the literature.

Conclusions: We present a HIV/AIDS young patient with CNS lesions attributable to three Herpesviridae viruses: EBV, CMV and occult HSV1. The areas showing HSV1 immunostaining displayed selective neuronal necrosis without Cowdry type A inclusions. Pseudoischemia is an unusual manifestation of HSV that may not be widely appreciated.

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A Case Of Whipple Disease In The Brain

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Background: Whipple disease is a rare systemic infectious disorder caused by bacterium Tropheryma whipplei. Whipple disease involving central nervous system is a very rare condition, and research on this specific aspect is limited.

Methods: We describe a case of a 69-year-old male who presented with an episode of confusion, weight loss, worsening memory, and right sided weakness over the last month. The patient was started on antibiotics to treat potential urinary tract infection. His further workup included brain MRI which revealed a 2.4 cm enhancing mass centered in the left lentiform nucleus, with extension to the left caudate nucleus.

Results: The patient underwent left insula lesion stereotactic biopsy, and the obtained specimen consisted of multiple small fragments of firm white granular tissue. Microscopy showed granulomatous inflammation with numerous large reactive astrocytes and lymphocytic infiltration with perivascular cuffing. The biopsy tissue was sent for Broad-Range PCR and Next Generation Sequencing, where Tropheryma whipplei was detected. The patient was started on intravenous ceftriaxone. Several days later, the patient underwent duodenal and gastric biopsy, which revealed duodenal mucosa with preserved villous architecture and focal gastric metaplasia, suggestive of peptic injury. Gastric tissue, similarly, exhibited mild reactive changes. PCR analysis of the tissue from both location did not identify any organisms.

Conclusions: The diagnostic criteria for Whipple disease require identification of the organism in the biopsy of the intestine. Alternatively, the diagnosis can also be based on foamy macrophages containing rod-shaped bacilli in affected tissues, particularly in the lamina propria of the small intestine; or molecular methods of detection. Tropheryma whipplei can rapidly become undetectable in response to antibiotic therapy. Therefore, it's important for healthcare professionals to be aware of the limitations of diagnostic tests and to interpret results in the context of the patient's clinical history and presentation.

Posters: Neurodegenerative: Alzheimer

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The Down Syndrome Biobank Consortium: A perspective

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Background: Individuals with Down syndrome (DS) have a partial or complete trisomy of chromosome 21, resulting in an increased risk for early-onset Alzheimer's disease (AD)-type dementia. Of the genetic mutations that cause AD, people with DS represent the largest cohort. Currently, there is a knowledge gap regarding the underlying neurobiological mechanisms of DS-related AD (DS-AD), partly due to limited access to well-characterized brain tissue and biomaterials for research.

Methods: Here, we present an international consortium of brain banks focused on collecting and disseminating brain tissue obtained from persons with DS throughout their lifespan, named the Down Syndrome Biobank Consortium (DSBC).

Results: The DSBC consists of 11 biobanking sites located in Europe, India, and the USA, which combined have more than 100 DS cases and 150 controls. The overall aim is to be an international repository for the collection and distribution of postmortem DS brain, serum, plasma, and CSF to investigate the neurobiological mechanisms and generate novel biomarkers for AD in DS. The DSBC has developed harmonized protocols including minimum recommended brain areas to sample for, neuropathologic diagnosis, staging criteria and storage protocols. A secondary main objective is to promote the use of DSBC samples for research in the DS space. DSBC members are active DS researchers and promote the use of DSBC samples by external researchers. DSBC members review all tissue requests, including site specific local legal requirements. The DSBC also performs educational activities such as clinicopathological conferences.

Conclusions: The DSBC provides a valuable framework to collect brain tissue and biofluids from clinically well-characterized DS and control cases needed to enhance sample availability for DS researchers. We hope to increase collaborative investigations and to raise awareness in the importance of examining postmortem tissue from people with DS and DS-AD as well as increase the quality and quantity of scientific publications in this space.

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Illicit opioid abuse, traumatic brain injuries, and neocortical Alzheimer's disease neuropathology

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Background: Several research studies have demonstrated opioid abuse (ex: heroin) is associated with increased hyperphosphorylation and aggregation of the neurodegenerative protein tau. However, research on younger illicit opiate users is inconsistent with studies focused on older prescription opioid users, where heavy prescription opioid use does not associate with greater Alzheimer's disease (AD) neuropathology. Few studies of opioid use and AD pathology assess traumatic brain injuries (TBI) as a potential confounder, yet it is estimated that in patients with TBI 10-44% have a premorbid history of illicit drug use. Recognizing the complex relationship between opioid use, TBI, and AD pathology, herein we analyzed tau and beta-amyloid in the brains of illicit drug users and matched-controls.

Methods: 2,844 cases were co-enrolled in the Rochester Epidemiology Project and Mayo Clinic Tissue Registry with a date of death between 1/1/05 and 6/18/16. Drug abuse and TBI code set macros were run on diagnostic billing code and death certificate datasets for all cases. 59 cases with an illicit drug abuse diagnosis as well as 59 age- and sex-matched controls without a drug abuse history were selected. Tau and beta-amyloid immunohistochemistry was performed on tissue sections from the frontal neocortex and hippocampus.

Results: Drug abuse cases included 41 men and 18 women and had an average age at death of 47.3 years (range 18.3 – 88.1). In comparing tau pathology and beta-amyloid pathology in the frontal cortex, hippocampus, or both regions to the presence or absence of drug abuse, opioid abuse, TBI, and sex, no statistically significance differences were observed.

Conclusions: Taken together, these preliminary results suggest drug abuse, including specifically opioid abuse, does not appear to be related to AD pathology in two distinct regions of the brain. In this cohort, age at death played a much larger role in the pathogenesis of tau and beta-amyloid pathologies.

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Resistance and resilience to neuropathology in Cognitive SuperAgers

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Background: SuperAgers are persons over 80 with at least normal-for-age global cognition. The Northwestern University SuperAging program was initiated in 2001 and follows SuperAgers with serial neuropsychological testing and brain donation. The goal of this study was to examine neuropathologic features of SuperAgers with a focus on those who showed relative cognitive stability versus decline.

Methods: Thirty-eight SuperAgers underwent annual study visits as part of a longitudinal research program. Study visits included neurocognitive testing, questionnaires, and interviews with study partners; cognitive and functional status were determined in consensus meetings. Average age at death was 91.63 years (range 82-101 years). Average length of follow-up was 12.6 years (range, 3 to 25 years). Autopsy consisted of standard sampling throughout the neuraxis, routine histopathology, thioflavin S, and immunohistochemistry for 4G8, AT8, phospho-TDP 43 (Ser409-410), α-synuclein, and p62. Assessment was per 2012 NIA-AA recommendations.

Results: Of 38 SuperAging participants, 20 showed cognitive stability (52.6%), 18 showed cognitive decline (47.4%). Stable SuperAgers (26.3%) were more often rated ‘B1’ score compared to those who declined (0%; (OR=0.737, $p< 0.05$). Of those who declined, two-thirds had intermediate or greater ADNC; 45% of stable SuperAgers met criteria for intermediate ADNC. Those who declined tended to be rated as “A3” (50%), CERAD “frequent” by Thioflavin S (38.9%), showed Tau positive neuritic plaques (23.5%), and ARTAG (66.7%); though higher than decliners, these features did not differ significantly from stable.

Conclusions: Initial clinicopathological analysis of SuperAgers indicates resistance to proteinopathy and cognitive resilience despite proteinopathy. “High” ADNC was entirely absent. Half of stable SuperAgers had “intermediate” ADNC. Although there was a trend toward stable SuperAgers demonstrating less proteinopathy, neuropathology was not predictive of antemortem cognitive trajectory. More studies are needed to explore the biological basis for resistance and resilience in this population, as well as the biological drivers of cognitive decline with age.

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Neuropathology of Down syndrome: ADNC, ARTAG, and PART

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Background: Down syndrome (DS) is frequently associated with Alzheimer disease neuropathologic change (ADNC). However, studies assessing the full spectrum of neurodegenerative pathologies according to modern consensus and staging criteria are limited.

Methods: We examined 35 brains from 2-70 year-old subjects with DS autopsied between 1986 and 2023 and performed comprehensive neurodegenerative evaluation using contemporary neuropathologic criteria. All cases were stained with thioflavin-S, p-tau (AT8) and amyloid-beta (6E10). Alpha-synuclein and TDP-43 pathologies were assessed only in subjects older than 30.

Results: All subjects over age 33 (n=18) had ADNC, which included 12 (67%) with a high level, 2 (11%) with an intermediate level, and 3 (17%) with a low level, while one case could not be assigned an ADNC level due to incomplete Braak staging. As expected, there was a correlation between age and Braak stage, Thal phase, and CERAD score. The vast majority of cases in this subgroup (15/18, 83%) also had cerebral amyloid angiopathy and a small subset (3/18, 17%) had comorbid aging-related tau astrogliopathy (ARTAG). One case of a 70 year-old subject showed “amygdala predominant” Lewy-related pathology. None of the cases demonstrated limbic predominant age-related TDP-43 encephalopathy. Among the subjects under age 32 (n=17), 6 had no evidence of proteinopathy. The remaining 11 cases did not meet 2012 NIA/AA criteria for ADNC: 4 (24%) harbored only diffuse plaques (DPs), 2 (12%) showed only rare hippocampal pretangles/neurites, 4 (24%) had both DPs and pretangles/neurites, and one case of a 29 year-old demonstrated primary age-related tauopathy (PART), Braak stage IV, with concomitant ARTAG. The youngest subject with DPs was an 11-year-old female, while the youngest subject harboring pretangles/neurites was a 14 -year-old female.

Conclusions: Individuals with DS can develop DP and pretangle/neurite pathology as early as their teenage years, and consistently demonstrate well-developed ADNC in their mid 30s, with comorbid pathologies being relatively uncommon.

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Alzheimer disease and cadaveric human growth hormone therapy: a comparative neuropathologic study

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Background: The causal relationship of cadaveric human growth hormone (cHGH) in the development of Creutzfeldt-Jakob disease is known. Whether Alzheimer disease (AD) pathologic changes may occur related to cHGH treatment is unknown.

Methods: We compared the results of neuropathology analyses and RT-QuIC assays obtained studying the brain of two individuals who died at age 57 and 56 having been affected by early onset dementia.

Results: Patient 1 was a 55-year-old woman, whose past medical history included cHGH therapy during childhood for growth hormone deficiency. She had a two-year history of cognitive decline. She presented with bilateral apraxia of the upper and lower extremities and was evaluated for dementia and parkinsonism. Multiple cerebrospinal fluid (CSF) analyses revealed elevated total and phosphorylated tau, presence of 14-3-3 protein, negative RT-QuIC for prion protein (PrP) and decreased ß amyloid (Aß). The clinical diagnosis was corticobasal syndrome. Patient 2 was a woman, who had a history of head trauma with a laceration of the head around the age of nine and developed cognitive decline at age 54. The clinical diagnosis was AD. She died at age 56. The brain weights were similar being 1011 (Patient 1) and 1009 (Patient 2) grams. Neuropathologic studies revealed AD pathology (A3, B3, C3) and Aß angiopathy of leptomeningeal and intraparenchymal vessels. Immunohistochemistry for PrP was negative. The neuropathologic differences between these cases were that Aß angiopathy was more severe in Patient 1 and that tau inclusions in glial cells within the white matter were present only in Patient 2. Tests of multiple brain regions from the two brains by two different RT-QuIC assays that are broadly reactive with human PrP prion strains were negative. Tau RT-QuIC showed strong tau seeding activity in both brains.

Conclusions: A causal relationship between cHGH treatment and AD is not evident. Cryogenic electron microscopy studies are in progress.

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Building a spatial transcriptomic atlas of Alzheimer's Disease

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Background: Investigation of Alzheimer's disease (AD) biology is limited by its spatiotemporal heterogeneity. Numerous pathological features including neuritic plaques (NP), cerebral amyloid angiopathy (CAA), and neurofibrillary tangles (NFT) each demonstrate a spectrum of maturity across different brain regions, cortical layers, or individual cells within a single brain. Experiments performed on homogenized tissue (bulk or single nuclei) cannot identify which molecular changes associate with each type and stage of pathology. Therefore, we aim to develop a spatial transcriptomic (ST) atlas of AD.

Methods: We evaluated prefrontal cortex samples from AD and control donors using 10x Genomics' Xenium platform, which provides ST at single cell resolution with a customized panel of probes targeting 366 genes. pTau IHC (AT8) was performed on the same section used for ST, and A-beta IHC (6E10) was performed on a serial section and aligned. Computational annotation of IHC features facilitated comparison of genes differentially expressed in neurons and glia associated with each feature. Single nucleus multiomics (snRNA-seq + snATAC-seq) was performed on the same samples and integrated with published literature to support analysis of ST.

Results: ST identified about 100,000 cells per sample, including all cell types in expected proportions. Cells can be confidently classified and scored for behavioral programs, such as "Disease associated microglia". Multiomic analysis generated data for over 2000 nuclei per sample with an average of 2,500 genes per nucleus, which cleanly aligned with published datasets. Scaling up the number of samples is ongoing, including multiple brain regions, Braak stages, and ApoE genotypes. Analyses performed on existing data include comparison of neurons with and without NFTs; glial cells associated with NFTs, CAA, and NPs; and spatial distribution of transcripts from specific genes.

Conclusions: We have developed an effective workflow for integrated ST and multiomics in AD, and are scaling up to develop a comprehensive spatial atlas.

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Amyloid Clearance, Alzheimer's Disease Neuropathologic Change, and Comorbid Pathology in Four Patients Treated with Aducanumab

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Background: Aducanumab is a monoclonal antibody targeting amyloid-β as disease-modifying strategy for Alzheimer's disease (AD). Studies have shown high-dose aducanumab effectively removes amyloid-β as measured by amyloid-PET imaging; however, limited clinicopathologic correlations are available to characterize these novel therapies. We present autopsy findings of four treated patients, the challenges of interpreting the pathology with differing extent of treatments and confounding pathologies, and the clinical correlates.

Methods: Case 1: Male with a five-year history of AD. APOE genotype 4/4. Received aducanumab for 32 months and died 18 months after discontinuation. Case 2: Male with a seven-year history of AD. APOE genotype 3/4. Received placebo therapy before transitioning to aducanumab for 46 months and died three months after discontinuation. Case 3: Female with a nine-year history of AD. APOE genotype 3/4. Received aducanumab for 22 months and died 41 months after discontinuation. Case 4: Male with a seven-year history of AD. APOE genotype 3/4. Received aducanumab for 36 months and died 14 months after discontinuation. Patients 1 and 4 showed evidence of amyloid related imaging abnormalities (ARIA) on brain MRI during trial therapy.

Results: Neuropathologic examination of all four patients demonstrated advanced AD neuropathologic change (A3, B3, C3), moderate to severe cerebral amyloid angiopathy (CAA) without evidence of hemorrhage, and histopathologic features of amyloid clearance described in previous reports. Three of four patients showed clinically relevant comorbid pathology: Case 1, limbic (transitional) Lewy body disease; Case 2, diffuse neocortical Lewy body disease; and Case 3, limbic-predominant age-related TDP-43 encephalopathy (LATE), stage 2.

Conclusions: Four patients treated with aducanumab had histopathologic features of amyloid clearance but declined cognitively during and after treatment and showed advanced AD neuropathologic change. Time on therapy, interval to autopsy, and comorbid pathology confound the evaluation of therapeutic impact on the pathological and clinical diagnoses.

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CAPRIN1 expression and colocalization in human cortical neurons of Alzheimer's disease

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Background: The amyloid cascade of production of amyloid β (Aβ) peptides from amyloid-β precursor protein (APP) is the central event in the pathogenesis of Alzheimer's disease (AD), although the mechanism remains unclear. Recently, cytoplasmic activation/proliferation-associate protein 1 (CAPRIN1) has been reported in neurodegeneration. Tracking CAPRIN1 through cellular organelles in human neurons from AD patient-derived induced pluripotent stem cells (iPSCs), we have demonstrated CAPRIN1 mediates APP degradation through the endosome-lysosome system and thereby regulates Aβ production in AD neurons. Here we report CAPRIN1 expression and its colocalization with APP in the neurons of AD brains.

Methods: AD autopsy brains and age-matched normal brains were obtained from Pathology Laboratory of Indiana University Health. Immunohistochemistry using CAPRIN1 and APP antibodies was performed on cerebral cortex, hippocampus, thalamus, basal ganglia, cerebellum, and brain stem. The expression levels of CAPRIN1 and APP in neurons were quantified. In addition, double immunofluorescence was performed and analyzed by confocal microscopes for colocalization of the proteins in neurons.

Results: Immunohistochemistry revealed that CAPRIN1 is predominantly expressed in the cytoplasm of neurons in normal and AD brains. CAPRIN1 is barely expressed in astrocytes, oligodendrocytes, and endothelial cells of blood vessels. Double immunofluorescence confocal microscopes revealed colocalization of CAPRIN1 and APP in the cytoplasm of the neurons of normal and AD brains. Further qualifications of CAPRIN1 expression will determine the difference of CAPRIN1 expression in neurons in different regions of normal and AD brains.

Conclusions: In this preliminary study, we demonstrated that CAPRIN1 is predominantly expressed and colocalized in the cytoplasm of neurons in AD brains, suggesting the role of CAPRIN1 in APP degradation in the neurons. Further large numbers of normal and AD brain will be analyzed to define the role of CAPRIN1 in AD pathogenesis.

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Investigating lysosomal autophagy pathway dysfunction in tau and TDP-43 synergy

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Background: Although Alzheimer's disease (AD) is pathologically defined by the presence of both amyloid plaques and neurofibrillary tangles, TDP-43 pathology often occurs co-morbidly with AD and correlates with more rapid cognitive decline and faster rates of hippocampal atrophy. We have previously shown that there is a synergistic relationship between tau and TDP-43 in a novel C. elegans model of combined tau and TDP-43 proteotoxicity, however the mechanisms that drive this interaction are unknown.

Methods: To further explore this relationship, we evaluated transcriptomic changes at time-points preceding frank neuronal loss in our C. elegans model of tau and TDP-43 co-expression (tau+TDP-43 Tg). We then followed up our findings with functional assessments in C. elegans as well multiplexed proteomics in human tissue using NanoString GeoMx Digital Spatial technology to relate findings in the model system back to the human disease.

Results: In the C. elegans transcriptomic analyses we found significant differential expression of genes enriched in the lysosomal-autophagy pathway. One of the most significantly differentially expressed genes with a human homologue, cpr-8 (cathepsin-B), is a lysosomal enzyme involved in protein degradation. Subsequent function studies revealed that loss of cpr-8 function phenocopied the tau+TDP-43 phenotype in the tau strain. In our human NanoString experiment we compared neuron and astrocyte protein expression levels in the amygdala from donors with high AD pathology and co-morbid TDP-43 pathology to donors with high AD pathology only. Focusing on a panel of autophagy-related proteins we found significant differences in protein expression in AD+TDP-43 compared to AD alone.

Conclusions: Overall, these results highlight the relevance of lysosomal autophagy pathway dysfunction in the synergistic proteotoxicity of tau and TDP-43. Future work is necessary to further explore the specific mechanisms and timing of this dysfunction.

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Cerebrovascular Lesions in Patients with Neurodegenerative Disease: A Single-Center Autopsy Experience

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Background: Cerebrovascular and neurodegenerative diseases are different in their primary mechanisms and manifestations, but their interplay can have repercussions on clinical diagnoses, prognosis, and therapy. There is growing interest in characterizing cerebrovascular lesions in neurodegenerative disease, in particular in patients with neurodegenerative diseases other than Alzheimer disease (AD).

Methods: We retrospectively reviewed all brain autopsies performed at UCLA between 2014 and 2022 that showed neurodegenerative features and characterized cerebrovascular lesions including atherosclerosis, arteriolosclerosis, cerebral amyloid angiopathy (CAA), infarcts, and hemorrhages. Cases with Braak stage V/VI were classified as pure AD (pure-AD), or mixed AD (mix-AD) based on the absence/presence of other neurodegenerative diagnoses. Cases with Braak stage IV or lower were classified as minimal AD (min-AD) or other neuropathological diagnosis (non-AD) based on the absence/presence of other neurodegenerative diagnoses.

Results: A total of 396 autopsy cases with neurodegenerative features were identified: 163 (41%) were pure-AD (age range: 29-114, mean: 78 years), 70 (18%) min-AD (age range: 59-111, mean: 70 years), 36 (9%) mix-AD (age range: 53-103, mean: 78 years), and 127 (32%) non-AD (age range: 58-98, mean: 71 years). CAA was observed more frequently in pure-AD and mix-AD (87% and 75%, respectively) than in min-AD and non-AD (40% and 25%, respectively). Severe arteriolosclerosis was observed more frequently in pure-AD (14%), min-AD (19%) and mix-AD cases (11%) compared to non-AD cases (3%). Macro-infarcts were observed more frequently among min-AD cases (26%) compared to other groups (pure-AD 11%, mix-AD 8%, non-AD 12%). Mild-to-moderate arteriolosclerosis, atherosclerosis, microinfarcts, vascular calcifications, aneurysms, and hemorrhages were observed in similar proportions between groups.

Conclusions: Cerebrovascular pathology is frequent in patients with neurodegenerative diseases, with CAA more common in those with advanced AD neuropathologic changes and macro-infarcts more common in those with minimal AD. Detailed characterization of cerebrovascular lesions is important in understanding the contribution of comorbid pathology in neurodegenerative diseases.

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Microglial MS4A gene SNPs are associated with cognitively resilient individuals in the Mayo Clinic Study of Aging

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Background: The relationship between cognition and Alzheimer's disease neuropathologic change (ADNC) is complex with a disconnect between cognition and ADNC in a subset of cognitively resilient individuals. Cognitive resilience is influenced by a multitude of mechanisms including genetics, sex, lifestyle, and education levels; however, the association between cognition, neuroinflammation , particularly microglia and microglial-specific genes, is understudied.

Methods: In Mayo Clinic Study of Aging (MCSA), we evaluated microglial-specific genes of interest, including MS4A gene cluster single-nucleotide polymorphisms (SNPs) in two analyses. 1) For those in the MCSA brain bank, we utilized antemortem global cognition z-scores, computed from memory, language, executive function, and visuospatial domain z-scores, to define cognitively intact (CI) participants as those performing in the upper quartile of their respective age groups. From the CI group, cognitively resilient (CR) individuals were identified as those with intermediate to high ADNC based upon neuropathologic evaluation. 2) In those with concomitant global cognition and amyloid PET data, we fit several parsimonious models to evaluate global cognition as a function of age, sex, amyloid PET, and SNPs of interest.

Results: CI individuals (n=118) comprised 23% of the MCSA brain bank (total n=505), with global z-scores mean -0.211. After neuropathologic evaluation, 35/118 (30%) showed no ADNC, 44/118 (37%) low ADNC, 30/118 (25%) intermediate ADNC, and 9/118 (8%) high ADNC, with CR individuals defined as intermediate to high ADNC (39/118, 33%). Of those CR individuals, 11 of 39 (28%) harbored an MS4A6A SNP and showed a protective effect on cognition over time in the amyloid-PET sample (p=0.043).

Conclusions: In MCSA, CR individuals accounted for 7% of total cases with autopsy, which is in keeping with other published proportions. Moreover, microglial specific MS4A6A SNP, which plays a role in modulating microglial activation, was seen in approximately a third of resilient individuals, underscoring the complex interplay between microglia, genetics, and resilience.

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Data-Driven Clinicopathologic Characterization of Parkinsonism and Nigral Neuron Loss in Alzheimer's Disease

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Background: Parkinsonism in Alzheimer's disease (AD) is often attributed to comorbid nigral pathology due to Lewy-related pathology (LRP) or other etiologies. Several studies reported that AD patients with parkinsonism had neuronal loss in substantia nigra (SN) without LRP, but its significance is underestimated due to limited case numbers and methodological limitations. In this study, we applied data-driven approaches to automatically count nigral cells and to collect clinical information to determine clinicopathologic associations of parkinsonism and nigral neuron loss in AD without LRP.

Methods: We included pathologically confirmed cases of AD without LRP or other major neurodegenerative diseases in the Mayo Clinic brain bank. Neurons in the SN were detected on hematoxylin and eosin stained sections using a fine-tuned YOLOv8 model. Clinical symptoms were extracted from medical records using dedicated Python code integrated with fine-tuned ChatGPT 3.5.

Results: We identified 645 AD cases without comorbid neurodegenerative pathologies. Those with bradykinesia and rigidity showed a significant decrease in nigral neuron counts compared to those without these symptoms (median [25th and 75th percentiles]: 358 [277, 458] vs. 456 [328, 556], $P = 0.02$, and 372 [300, 474] vs. 430 [364, 513], $P = 0.01$, respectively). Bradykinesia correlated with the number of pigmented neurons ($P = 0.02$), whereas rigidity correlated with non-pigmented neurons ($P < 0.01$). Cognitive impairment and other parkinsonian features, such as rest tremor and postural instability, did not show significant associations. Cases with TDP-43 pathology in the basal forebrain showed a decreased number of nigral neurons compared to those without it (342 [242, 429] vs. 391 [308, 490], $P < 0.01$).

Conclusions: Our findings suggest that parkinsonism in AD without LRP is associated with neuronal loss in the SN, which might be associated with TDP-43 pathology.

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Complement membrane attack complex in polarized astrocytes propagate T-cell infiltration within the amygdala of pure Alzheimer's disease

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Background: Recent studies demonstrate increased autoimmune co-morbidity and T-cell patrolling in cerebrospinal fluid of patients with neurodegenerative disorders.

Methods: To query whether inflammation in Alzheimer's disease (AD) could be related to autoimmunity we tested substantial-evidence criteria for autoimmunity i.e., presence of adaptive immune cells (T-cells) within the end-organ, its correlation with innate immune activation and autoimmune co-morbidity. We studied AD (N=41) without Lewy pathology, TDP-43 or vascular incidents in the studied regions (pure AD) with thioflavin-S (neurofibrillary tangles and amyloid plaques), phospho-TDP-43, alpha-synuclein (NACP), T-cells (CD3, CD4, CD8), complement membrane attack complex (MAC), inflammasome (NLRP3) and a marker of protoplasmic astrocytosis (AQP4). T-cells were quantified manually on scanned images using a counting tool and validated using a digital algorithm. Clinical records were abstracted retrospectively. Findings were compared to normal controls (N=22).

Results: Controls showed mild T-cell infiltration (median 70 CD3/cm2, range 0 – 225 CD3/cm2, 25% 4.7 CD3/cm2, 75% 111 CD3/cm2). Pure AD showed significantly increased T-cell densities ($p< 0.0001$, median 452 CD3/cm2, range 24 – 2836 CD3/cm2, 25% 238 CD3/cm2, 75% 939 CD3/cm2) which was confirmed using a digital algorithm ($p= 0.0052$). The major T-cell subtype was CD8. Coarse neuritic amyloid plaques within the amygdala but not diffuse or cored within the basal ganglia stained strong-positive for MAC with morphologic features of protoplasmic astrocytes. Immunofluorescence tripe-labeling showed co-localization of MAC, AQP4 and amyloid-beta in coarse but not in diffuse plaques. MAC-positive plaques correlated significantly with T-cell infiltration ($r=0.67$, $p=0.0000017$). The inflammasome stained ramified glia (microglia and fibrillary astrocytes), some amoeboid macrophages but not protoplasmic astrocytes and showed no correlation with T-cell infiltration. Autoimmune co-morbidity was not significantly increased in pure AD.

Conclusions: Astrocytic MAC-polarization in neuritic plaques positively correlates with T-cell infiltration, though autoimmune co-morbidity is not increased. These findings suggest both adaptive and innate immune activation could be intrinsic to Alzheimer's pathoetiology.

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Comorbidities in Early-Onset Sporadic versus Presenilin-1 Mutation-Associated Alzheimer's Disease Dementia

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Background: Autopsy studies have demonstrated that comorbid neurodegenerative and cerebrovascular disease occur in the majority of subjects with Alzheimer disease dementia (ADD), and are likely to alter the rate of cognitive decline, increasing response variability in clinical trials. Generally, comorbidities have been most studied in late-onset sporadic ADD, so we sought to compare ADD comorbidities between subjects with early-onset sporadic ADD (EOSADD; subjects dying under age 60) versus ADD associated with different types of PSEN1 mutations, the most common cause of early-onset autosomal dominant ADD.

Methods: We ascertained the prevalences of ADD comorbidities in PSEN1 cases derived from the United States (US) as well as from Colombia. Data for EOSADD and US PSEN1 subjects (with multiple different mutation types) was obtained from the National Alzheimer Coordinating Center (NACC).

Results: Colombian cases all had the E280A mutation. Of ADD comorbidities, LBD was most common, being present in more than half of all cases in all 3 groups. For TDP-43 co-pathology, the Colombian PSEN1 group was the most affected, at about 27%, vs 16% and 11% for the US PSEN1 and sporadic US cases, respectively. Significant large-vessel atherosclerosis was present in a much larger percentage of Colombian PSEN1 cases, at almost 20% as compared to 0% and 3% of the US PSEN1 and EOSADD cases, respectively. Small-vessel disease, or arteriolosclerosis, was much more common than large vessel disease, being present in all groups between 18% and 37%. White matter rarefaction (WMR) was remarkably common, at almost 60%, in the US PSEN1 group, as compared to about 18% in the EOSADD cases, a significant difference. White matter rarefaction was not assessed in the Colombian PSEN1 cases.

Conclusions: The results presented here indicate that some comorbidities are common even in early-onset ADD subjects and should be considered when planning clinical trials.

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Patient-derived iPSC-neurons recapitulate Alzheimer disease pathologic alterations of β-amyloid and tau in matched patient brain tissue.

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Background: Alzheimer disease (AD) is characterized by the pathologic accumulation of beta-amyloid (Aβ) and phosphorylated tau (pTau) species. The prevailing hypothesis of AD pathogenesis places alterations in Aβ processing upstream of pathogenic tau phosphorylation and subsequent accumulation as neurofibrillary tangles (NFTs). Surprisingly, recent evidence suggests that human induced pluripotent stem cell-derived neurons (iPSC-neurons) from AD patients recapitulate some pathologic features of AD.

Methods: iPSCs derived from fibroblasts of 13 patients with autopsy-confirmed AD (9 sporadic, 4 familial), 4 with Down syndrome, and 3 controls with minimal AD neuropathology were differentiated into iPSC-neurons. Aβ40 and Aβ42 were measured in the cell media by ELISA. AT8 pTau and total tau (tTau) were measured in the cell lysate by Western blot. Semiquantitative measures of NFTs and amyloid plaques, as well as digital-histologic measures of Aβ and pTau immunoreactivity, were examined in frontal cortex (FC), parietal cortex (PC), and hippocampus (HC) of the same patients.

Results: Expectedly, patients with familial or sporadic AD had greater pTau and AB burden in FC by immunohistochemistry. Surprisingly however, these patients also had significantly elevated levels of pTau/tTau and Aβ42/40 in corresponding iPSC-neuron cultures. The iPSC-neuron pTau/tTau measures correlated with densities of tau NFTs in FC (spearman rho=0.38), PC (rho=0.42), and HC (rho=0.54), such that those with moderate/frequent NFTs demonstrated greater pTau/tTau than those with no/rare/sparse NFTs (FC: t=-2.5, p=0.03; HP: t=-3.8, p=0.002). Similarly, iPSC-neuron media Aβ42/40 measures correlated with density of diffuse amyloid plaques in the FC (rho=0.35) and PC (rho=0.39), such that those with moderate/frequent diffuse plaques demonstrated greater AB42/40 than those with no/sparse diffuse plaques (FC: t=-2.7, p=0.02; PC: t=-2.7, p=0.02).

Conclusions: These results indicate that iPSC-neurons derived from patients with Alzheimer disease recapitulate similar tau modifications and Aβ cleavage patterns to the diseased brain, implying patient specific genetic/epigenetic factors that could influence “set-points” for these disease-associated phenotypes.

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The neuropathological landscape of small vessel disease and Lewy pathology in Hispanic and non-Hispanic White decedents with Alzheimer disease

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Background: Cerebrovascular and α-synuclein pathologies are frequently observed alongside Alzheimer disease (AD). The heterogeneity of AD necessitates comprehensive approaches to postmortem studies, including the representation of historically underrepresented ethnic groups.

Methods: In this study, we evaluated small vessel disease pathologies and α-synuclein deposits among Hispanic decedents (HD, n = 92) and non-Hispanic White decedents (NHWD, n = 184) from three Alzheimer's Disease Research Centers: Columbia University, University of California San Diego, and University of California Davis. The study included cases with a pathological diagnosis of Intermediate/High AD based on the National Institute on Aging – Alzheimer's Association (NIA-AA) and/or NIA-Reagan criteria. A 2:1 random comparison sample of NHWD was frequency-balanced and matched with HD by age and sex. An expert blinded to demographics and center origin evaluated arteriolosclerosis, cerebral amyloid angiopathy (CAA), and Lewy bodies/Lewy neurites (LBs/LNs) with a semi-quantitative approach using established criteria.

Results: HD showed more severe Vonsattel grading of CAA in the cerebellum ($p = 0.04$), higher CAA density in the posterior hippocampus and cerebellum (both $p = 0.01$), and increased LBs/LNs density in the frontal ($p = 0.01$) and temporal cortices ($p = 0.03$), as determined by Wilcoxon’s test. Ordinal logistic regression adjusting for age, sex, and center confirmed these findings except for LBs/LNs in the temporal cortex.

Conclusions: Results indicate HD with AD exhibit greater CAA and α-synuclein burdens in select neuroanatomic regions when compared to age- and sex-matched NHWD with AD. These findings enhance the understanding of concurrent CAA and LBs/LNs topography and severity in pathologically confirmed AD, particularly in persons of Hispanic descent, providing insights into precision medicine approaches for AD.

Posters: Neurodegenerative: FTLD/Lewy Body/Parkinson

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Postmortem Neuropathologic Diagnosis of Unknown C9orf72-associated Frontotemporal Lobar Degeneration with Limited TDP43 Pathology

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Background: C9orf72-associated frontotemporal lobar degeneration (FTLD) frequently shows abundant TDP-43 pathology. However, rare cases without significant TDP-43 pathology have been reported.

Methods: We report autopsy findings of a 71-year-old female with history of seizure, anxiety, and early-onset dementia starting at around age 50. Her symptoms included initial loss of executive function that progressed to aphasia, immobility, and behavioral issues. The brain was evaluated with routine histology, immunohistochemistry, and targeted next-generation sequencing.

Results: The fresh brain weighed 1224 grams with mild global atrophy. Microscopic examination revealed moderate neuronal loss and gliosis in the superficial cortical layers and severe neuronal loss and gliosis in the hippocampus. By immunohistochemistry, p62 highlighted frequent neuronal cytoplasmic inclusions (NCI) in all the brain areas examined including the neocortex, cingulate gyrus, basal ganglia, amygdala, hippocampus, brainstem, and cerebellum. Phosphorylated-TDP-43 revealed scattered NCIs in the amygdala and hippocampus but only rare NCIs in the neocortex and no NCIs in the cerebellum and lower motor neurons. These p62-positive NCIs were negative for FUS. There was a low level of Alzheimer's disease neuropathologic change (NIA/AA A1B2C0) and no evidence of an a-synucleinopathy. The overall neuropathological findings were suggestive of C9orf72-FTLD. Per the family's request, an Invatae hereditary ALS, FTD and AD sequencing panel (with C9orf72) was performed on the postmortem brain tissue. Sequencing revealed a pathogenic variant in C9orf72 with characteristic hexanucleotide repeats, confirming the diagnosis of C9orf72-FTLD. Of note, the patient's father also had early-onset dementia with an autopsy performed approximately 30 years ago, which reportedly showed no evidence of ADNC.

Conclusions: TDP-43 pathology may be very limited in C9orf72-FTLD, suggesting that TDP-43 deposition may be a downstream event of the primary pathology in C9orf72-FTLD. In these cases, the presence of p62-positive, TDP43-negative NCIs in the cerebellum, hippocampus, and neocortex provides an important clue to the correct diagnosis.

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Tackling selective neuronal vulnerability: Involved subnuclei in amygdala-predominant Lewy body disease in early-onset Alzheimer's disease

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Background: Lewy body disease (LBD) often co-occurs with Alzheimer's (AD), resulting in more cognitive decline than AD or LBD alone. LBD's hallmarks, asyn-positive Lewy bodies and neurites, propagate from the enteric system or olfactory bulb to the amygdala that gatekeeps spread to other structures. Initially, LBD appears in the central or cortical nuclei, reflecting brainstem or olfactory origins. A third pattern, amygdala-predominant LBD (AP-LBD), is prevalent in sporadic and familial early-onset AD (EOAD), does not conform with the common staging systems for LBD, and has received little attention. We found a link between asyn seeding-competent fibrils in cerebrospinal fluid (CSF) and LBD's transition from the amygdala, supporting its gatekeeper role. The factors enabling asyn propagation in the amygdala remain unknown. Our goal is to map asyn and tau markers in EOAD cases with permissive vs. non-permissive AP-LBD in amygdala subnuclei, identifying spread pathways and vulnerable cell populations.

Methods: We identified all postmortem EOAD cases with AP-LBD from the UCSF Neurodegenerative Disease Brain Bank (n=47), 14 with banked CSF for asyn seeding amplification testing. We are using multiplex immunofluorescence to label: phosphorylated asyn; phosphorylated, mis-conformed, or truncated tau; and all neurons. Markers will be quantified from digital pathology scans; we will analyze the degree of colocalization by region for the central, basolateral, and cortical nuclei.

Results: We will report the pattern of LBD across subnuclei. Of eight CSF samples, 37.5% were positive for asyn seeding; six more samples are pending. Asyn and tau markers by subnucleus will be compared between CSF-positive and -negative cases.

Conclusions: Mapping AP-LBD across amygdala subnuclei may yield insights into selectively vulnerable neurons. This research has the potential to enhance our understanding of AP-LBD and its underlying mechanisms, ultimately contributing to the development of more targeted diagnostic and therapeutic strategies.

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Coexistence of Frontotemporal Lobar Degeneration and Motor Neuron Disease associated with TAF15 proteinopathy

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Background: Motor neuron disease (MND) and behavioral variant frontotemporal dementia (FTD) are known to occur in the same patient; the onset of MND has been previously observed to either precede or follow the onset of FTD. TAF15 proteinopathy associated with MND has not been previously reported. The current study reveals that MND associated with TAF15 proteinopathy may occur early or late in the course of illness.

Methods: Patient 1, a female, began behaving impulsively with judgment and executive skills severely impaired at age 21. She was diagnosed with schizophrenia. At age 27, a brain MRI showed severe bilateral frontal lobe atrophy with knife-like gyri as well as anterior temporal atrophy and she was diagnosed as having FTD; signs of MND were not observed. She died at age 30. Patient 2, a 44-year-old male, had slowing of speech, decreased concentration and memory. Neurologically, spastic dysarthria and muscle denervation in the legs, face, and tongue were observed. Nerve conduction and EMG testing were suggestive of MND. At age 47, he had dysarthria, slurring of speech and dysphagia. Intellectual decline and personality changes consistent with a behavioral FTD were observed. He died at age 51.

Results: Absence of intermediate filament-positive and basophilic inclusions but presence of FUS immunopositive inclusions led to the diagnosis of frontotemporal lobar degeneration (FTLD) and specifically FTLD-FUS. TAF15 and transportin 1 immunopositive inclusions were seen in neurons of the prefrontal cortex and in motor neurons. In Patient 1, degeneration of the lateral cortico-spinal tract was evident. Cryogenic electron microscopy used to determine the amyloid filaments structures extracted from the prefrontal cortex, motor cortex, temporal cortex and brain stem revealed amyloid filaments of the FUS homologue TATA-binding protein-associated factor 15 (TAF15).

Conclusions: These results establishing the association of TAF15 proteinopathy with MND provide a basis for designing diagnostic and therapeutic tools targeting TAF15 proteinopathy.

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A human to mouse stem cell transplantation model of frontotemporal dementia to study the effects of tau filaments in vivo

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Background: Frontotemporal dementia (FTD) is an early-onset dementia without effective treatments. FTD can be caused by an accumulation of hyperphosphorylated forms of the microtubule associated protein tau (MAPT) in various brain regions (FTD-tau), but the exact mechanisms leading to cell death are incompletely understood.

Methods: Here, we developed a model of FTD using human induced pluripotent stem cell (iPSC)-derived neurons from either healthy controls or from patients harboring the FTD-tau-associated MAPT-N279K mutation.

Results: iPSC-derived FTD neurons recapitulate disease hallmarks including tau pathology with increased numbers of phospho-tau positive neurons and elevated 4R tau levels, increased oxidative stress and metabolic alterations, and impaired neurite outgrowth. Addition of tau filaments purified from the brain of FTD patients with the MAPT-N279K mutation to cultured FTD neurons induced upregulation of genes associated with apoptosis, oxidative phosphorylation, proteasome and unfolded protein response. Additionally, genes related to neuroinflammation were upregulated including secreted phosphoprotein 1 (SPP1), a multifunctional cytokine which is also upregulated in the brains and cerebrospinal fluid of FTD patients. We have developed a novel xenotransplantation paradigm where purified filaments from FTD patients or healthy brain preparations were co-injected with either Ctrl iPSC-derived or FTD patient iPSC-derived neural progenitor cells into the forebrain of immune-deficient mice. Transplanted cells were allowed to differentiate in vivo for six months, followed by histological characterization of graft size, extent of graft integration, and interactions at the graft-host interface including measures of reactive astro- and microgliosis. Findings from these analyses will be presented at the conference.

Conclusions: The results of this study will help to clarify the effects of tau aggregates on cell physiology in vivo using a human stem cell transplantation model of FTD.

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Multiple system atrophy with abundant nigral neuronal cytoplasmic inclusions mimicking Parkinson disease

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Background: Although some pathological confirmed multiple system atrophy (MSA) cases who had been clinically diagnosed with Parkinson disease (PD) were reported, the neuropathological differences between typical MSA and PD mimicking MSA were unclear.

Methods: Case report with clinico-pathological discussion.

Results: A Japanese man developed resting tremor on his right leg at age 54. Neurologic examination 4 year after symptom onset revealed cogwheel rigidity on his right limbs and mild bradykinesia. Because of the good response to carbidopa-levodopa treatment and normal brain MRI, he was diagnosed with PD. Truncal and bilateral limb ataxia and severe orthostatic hypotension were observed 13 years after onset. The progressive atrophy of pontine basis and putamen were detectable on brain MRI in his final disease stage. Postmortem examination 14 years after onset revealed sever neuron loss with gliosis and abundant glial cytoplasmic inclusions in the atrophied putamen. Moderate to sever neuron loss was also observed in the substantia nigra. Most of the exiting neurons in the substantia nigra had sickly eosinophilic rounded inclusions in their cytoplasm dense. These neuronal inclusions were positive by Gallyas-Braak silver staining and anti α-synuclein immunohistochemistry and visibly different from Lewy body. Electron microscopy revealed that these neuronal inclusions were composed of randomly orientated granule-coated filaments. Lewy bodies were not observed.

Conclusions: Pure MSA may have slowly progressive levodopa-responsive Parkinsonism, mimicking PD. Abundant nigral NCIs might relate to the slowly progressive degeneration of dopaminergic neuron.

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The spatial landscape of glial pathology and T-cell response in Parkinson's disease substantia nigra

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Background: As there is no known cure for Parkinson's disease, a debilitating neurodegenerative disease characterized by severe loss of dopaminergic neurons, new research avenues are pivotal to filling knowledge gaps of the pathogenesis and progression of the disease. New findings, including identification of specific peripheral T-cell receptor sequences, have sparked interest in analyzing the role of the adaptive immune response in Parkinson's. In this work we focus our attention on the properties of T-cells in the brain regions where neurons degenerate, which are heretofore not well characterized.

Methods: Through analysis of post-mortem brain tissue from PD and control donors in the cingulate cortex and substantia nigra, we provide advanced computational analyses of single nucleus RNA sequencing, spatial transcriptomics and T-cell receptor sequencing.

Results: Taken together, our analyses provide evidence for a CD8+ resident memory and clonally-expanded T-cell phenotype, as well as and altered spatial relationships between T-cells and astrocytes, myeloid cells, and endothelial cells in the PD SN. We further provide descriptions of regional heterogeneity in astrocytic responses to neurodegeneration, and nominate potential molecular and cellular candidates implicated in disease progression and allow a deeper understanding of the pathophysiology of neurodegeneration in PD.

Conclusions: Overall, our work outlines disease phenotypes of T-cells and glia in the Parkinson's substantia nigra. Our findings nominate potential molecular and cellular candidates that allow a deeper understanding of the pathophysiology of neurodegeneration in PD. Together, this work represents a major transcriptional resource for the fields of neurodegeneration and PD.

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Phosphorylated and Non-phosphorylated TDP-43 Inclusions in Skeletal Muscle

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Background: Transactive response DNA-binding protein 43 (TDP-43) proteinopathies, such as Amyotrophic lateral sclerosis (ALS) and ALS with Frontal Temporal Dementia (FTD), exist on a progressive neurodegenerative disease spectrum with clinical heterogeneity and variable etiopathogenesis. In the central nervous system (CNS) of individuals with sporadic disease, TDP-43, mislocalizes to the cytoplasm and aggregates in several configurations within neurons and glia. Recent studies also show TDP-43 aggregates in skeletal muscle of ALS patients. We aim to further explore the morphology and potential implications of TDP-43 inclusions in muscle.

Methods: Between 1993-2021, we identified 15 ALS or ALS-FTD cases, reviewed clinical information, and skeletal muscle pathology from multiple sites with non-phosphorylated (nTDP-43) and phosphorylated TDP-43 (pTDP-43) immunohistochemistry (IHC).

Results: We evaluated muscle pathology from 15 patients (avg 61y; range 24-86y; 8 F, 7 M) with clinical ALS (11), including 2 young patients with suspected early disease, or ALS-FTD (4), and pathologic disease within CNS (7) or muscle tissue (8). Many muscles showed neurogenic atrophy with 20% also showing denervation myopathy. Although TDP-43 morphology showed an overall low inclusion density, a variety of morphologies were observed. All cases showed nTDP-43 inclusions, and most were diffuse (93.3%), followed by dense aggregates (60%) with a minority of juxtanuclear cup-like (20%) and short linear (13.3%) forms. Phosphorylated aggregates were identified (80%) and most were dense (60%) with equal amounts of diffuse and cup-like (40%), and less commonly short linear (26.6%). Aggregates were variably present within subsarcolemmal, juxtanuclear locations, as well as throughout myofibers. ALS-FTD patients (3/4) showed predominantly negative pTDP-43 staining.

Conclusions: TDP-43 muscle aggregates may offer an assessment method for TDP-43 proteinopathy; however, low inclusion density may suggest a need to evaluate greater than one biopsy site. In our sample, dense aggregates were most common. One interesting distinguishing feature between groups may include pTDP-43 negative ALS-FTD cases compared to ALS.

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Clinicopathologic features of frontotemporal lobar degeneration with TDP-43 presenting with progressive supranuclear palsy syndrome

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Background: Frontotemporal lobar degeneration with TDP-43 pathology (FTLD-TDP) can present with frontotemporal dementia, semantic dementia or logopenic progressive aphasia. A less uncommon presentation is corticobasal syndrome, but there are no reports of pathologically-confirmed FTLD-TDP presenting with the clinical syndrome of progressive supranuclear palsy (PSPS). In this study, we examined clinical and pathologic characteristics of patients with PSPS-TDP.

Methods: We reviewed 270 patients with autopsy-confirmed FTLD-TDP from 2000 to 2022. Five patients with FTLD-TDP had a PSPS clinical presentations. For comparison, we selected 10 consecutive patients of PSP presenting with PSPS.

Results: The average age at death in PSPS-TDP was 66 years, and the disease duration was 8 years. The most common clinical symptoms of PSPS-TDP were parkinsonism (80%) and memory loss (80%), followed early falls (60%) and frontal behavioural features (60%). Compared to PSPS-TDP, all patients with PSPS-PSP met the Movement Disorder Society's criteria for probable PSP, but only one PSPS TDP patent met criteria for clinically probable PSP. Two of the five PSPS TDP cases had moderate to marked neuronal loss in the substantia nigra and one had moderate to marked neuronal loss in the putamen and globus pallidus. Three PSPS-TDP patients were classified as FTLD-Type B and two as FTLD-Type A. None were FTLD-Type C.

Conclusions: Although uncommon, FTLD-TDP can sometimes clinically mimic PSP, and should be considered in the differential diagnosis of patients with atypical parkinsonism. Our findings emphasize the need for better clinical and biomarkers for FTLD.

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Mapping Cell Type and Transcriptomic changes associated with TDP-43 Pathology in LATE-NC and FTLD-TDP

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Background: LATE-NC (limbic predominant age-related TDP-43 encephalopathy neuropathologic change) and FTLD-TDP (frontotemporal lobar degeneration with TDP-43 deposition) are two neurodegenerative pathologies which are united by the mislocalization and aggregation of TDP-43, a highly expressed RNA-binding protein. However, the distribution of TDP-43 pathology and the clinical presentations are very distinct; FTLD-TDP is characterized by widespread TDP-43 aggregates and severe language, personality and behavioral disturbances, while in LATE-NC, TDP-43 pathology is limited to mesial temporal structures and is associated with an indolent amnestic cognitive decline. The cellular and molecular features underlying these differences are not understood.

Methods: To explore this question, I used the Xenium ISS spatial transcriptomics platform to profile hippocampal tissue of patients with FTLD-TDP (Type A) and LATE-NC (n=2). This in situ multiplexed microscopy platform allows for analysis of up to 400 transcripts at subcellular resolution and post-analysis immunofluorescence. I used the pre-designed human brain panel, which includes 266 targets that identify major cell types within the nervous system. This panel was augmented with an additional 100 custom transcripts, designed specifically to assess differential expression of TDP-43 targets as well as specific cell types within the hippocampus. By performing post-analysis immunofluorescence for TDP-43, Phospho-Tau, and β-amyloid, transcript levels were correlated with the presence or absence of TDP-43 pathology on a single cell level.

Results: I defined transcriptomic changes selectively in cell types affected by TDP-43 pathology in both FTLD-TDP and LATE-NC.

Conclusions: This work provides insight into factors that discriminate LATE-NC from other TDP-43 proteinopathies.

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Association between cognitive decline progression and α-synuclein oligomers in patients with dementia with Lewy bodies

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Background: Our previous study demonstrated that patients with Parkinson's disease with cognitive impairment showed more α-synuclein oligomers in the hippocampus compared to those without cognitive impairment. We sought to understand the association between cognitive trajectory and α-synuclein oligomers in prospectively assessed patients with dementia with Lewy bodies (DLB).

Methods: Thirteen cases were identified from our brain bank who met the following criteria: (1) Clinical diagnosis of DLB with pathological confirmation of Lewy body disease, (2) Longitudinal neuropsychological follow-up at Mayo Clinic Jacksonville, (3) Underwent Mini-Mental State Examination (MMSE) three or more times, (4) Initial MMSE score ≥ 24, and (5) Final MMSE within three years of death. The annual rate of MMSE decline was calculated for each case. Hippocampal sections from four cases each of the rapid decline group (annual MMSE decline > 4) and the slow decline group (annual MMSE decline < 2) were subjected to α-synuclein proximity ligation assay staining to detect α-synuclein oligomers and phosphorylated-α-synuclein immunohistochemistry. Each pathological burden was assessed semi-quantitatively.

Results: The rapid and slow trajectory groups did not differ in age at death (74±8 vs. 77±4; P=0.51), disease duration (7±2 vs. 10±4; P=0.44), Braak neurofibrillary tangle stage (median 4.5 vs. 2; P=0.06), or Thal amyloid phase (median 4.5 vs. 1.5; P=0.06). The rapid decline group had a higher α-synuclein oligomer burden in the CA1 subfield of the hippocampus compared to those with slow cognitive decline (P = 0.03). There was also a trend for a higher α-synuclein oligomer burden in CA2 (P = 0.11) and CA4 (P= 0.07) in the rapid decline group. No difference in Lewy-related pathology burden was observed in any of these regions between the two groups.

Conclusions: Given the toxicity of α-synuclein oligomers, their accumulation in the hippocampus may accelerate cognitive decline.

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Olfactory bulb gene alterations associated with olfactory dysfunction in Parkinson's disease.

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Background: The olfactory bulb is involved early in the pathophysiology of Parkinson's disease (PD), which is consistent with the early onset of olfactory dysfunction. Identifying the molecular mechanisms through which PD affects the olfactory bulb could lead to a better understanding of the pathophysiology of olfactory dysfunction in PD.

Methods: We specifically aimed to assess gene expression changes, affected pathways and co-expression network by whole transcriptomic profiling of the olfactory bulb in subjects with clinicopathologically defined PD. Bulk RNA sequencing was performed on frozen human olfactory bulbs of 20 PD and 20 controls without dementia or any other neurodegenerative disorder, from the Arizona Study of Aging and Neurodegenerative Disorders.

Results: Differential expression analysis revealed 2164 significantly differentially expressed genes in PD. Significantly downregulated pathways included oxidative phosphorylation, olfactory transduction, metabolic pathways, and neurotransmitters synapses while upregulated pathways were involved in the immune and inflammatory responses as well as cellular death. An overrepresentation of microglial and astrocyte-related genes was observed amongst upregulated genes, and excitatory neuron-related genes were overrepresented amongst downregulated genes. Co-expression network analysis revealed significant modules highly correlated with PD and olfactory dysfunction that were found to be involved in the MAPK signaling pathway, cytokine-cytokine receptor interaction, and cholinergic synapse. LAIR1 (leukocyte associated immunoglobulin like receptor 1) and PPARA (peroxisome proliferator activated receptor alpha) were identified as hub genes with a high discriminative power between PD and controls. Olfactory identification test score positively correlated with expression of genes coding for G-coupled protein, glutamatergic, GABAergic, and cholinergic receptor proteins and negatively correlated with genes for proteins expressed in glial olfactory ensheathing cells.

Conclusions: This study reveals gene alterations associated with neuroinflammation, neurotransmitter dysfunction, and disruptions of factors involved in the initiation of olfactory transduction signaling that may be involved in PD-related olfactory dysfunction.

Posters: Neurodegenerative: Other

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Autopsy case of familial ALS with UBQLN2 (P494L) mutation

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Background: Ubiquilin-2 (UBQLN2) mutations are now considered linked to the pathogenesis of amyotrophic lateral sclerosis (ALS). However, autopsy reports of ALS mutations are rare. Here, we report a Japanese autopsy case of ALS with the UBQLN2 (P494L) mutation.

Methods: The autopsy subject was a 51-year-old Japanese male. He had been diagnosed with ALS at age 44, had undergone gastrostomy and tracheostomy with ventilation, had difficulty walking at age 45, been bedridden since age 47, and communicated only with the orbicularis oculi muscle. The cause of death was disseminated intravascular coagulation. No notable psychiatric or behavioral disorders were observed. Later, the patient's daughter was diagnosed with ALS at age 48.

Results: Brain weight was 1360 g. Atrophy of the frontal lobe, precentral gyrus, hippocampus, and amygdala was observed. Histologically, there was severe neuronal loss in the motor cortex, lower motor neuron, and Bunina bodies in the remaining neurons. Neuronal loss was also seen in the temporal pole, subiculum, amygdala, substantia nigra, globus pallidus, and subthalamic nucleus. The corticospinal tract was severely degenerated. Phosphorylated TDP-43 (pTDP-43) immunostaining showed round inclusions and skein-like inclusions in the lower motor neurons and many pTDP43-positive neuronal cytoplasmic inclusions (NCIs), short dystrophic neurites, and glial cytoplasmic inclusions in the motor cortex, hippocampus, and subiculum. UBQLN2 immunostaining revealed UBQLN2-positive NCIs in the hippocampal dentate gyrus and round inclusions in the nuclei and dendrites of CA1 neurons. UBQLN2 (P494L) mutation was detected in the patient's frozen brain and in his daughter's DNA. Frozen-brain immunoblot analysis revealed a TDP-43 type-B band pattern. No amyloid beta, tau, or alpha-synuclein was observed.

Conclusions: ALS with UBQLN2 (P494L) mutation shows wide degeneration of the upper and lower motor neurons, hippocampus, amygdala, and pallido-nigro-luysian system, with pTDP-43 and UBLN2-positive inclusions. UBQLN2 is closely associated with the pathogenesis of ALS.

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Human Neuropathology Ontology (HNO): Bridging Knowledge Gaps for Advanced Machine Learning in Neuropathology

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Background: Biomedical ontologies in histopathology are a promising approach to standardize and facilitate the sharing, analysis, and integration of data towards the digitization of knowledge in neuropathology. For example, the Gene Ontology (GO), Systematized Nomenclature of Medicine Clinical Terms (SNOMED CT), and Human Phenotype Ontology (HPO) are essential for data annotation, integration, and analysis in clinical and research settings. However, existing ontologies are not tailored to human neuropathology. There is a critical need to develop a structured neuropathology specific ontology to better enable neuropathologists in applying advanced machine learning algorithms to morphological datasets.

Methods: To develop the ontology, we reviewed authoritative textbooks in neuropathology, which we treated as gold standard sources of data. From these sources, we extracted relevant terms, which were then categorized by individuals with expertise in neuropathology to form the foundation of the ontology structure. This included concepts (or classes), relationships, properties (or attributes), instances, axioms, annotations, hierarchy, logical consistency, standardized terminology and versioning. These terms were then linked to annotated patches from digital whole slide images of histopathological specimens from a spectrum of disease contexts.

Results: Our preliminary neuropathology-specific ontology, which we call the Human Neuropathology Ontology (HNO), categorizes key terms across anatomy, diagnoses, approaches, and morphological terms, establishing a detailed framework for subsequent analysis. The ontology incorporates diverse categories such as neuroanatomy, disease-specific diagnoses, clinical and pathological examination approaches, and technical terms including routine stains and immunohistochemistry, facilitating a holistic approach to neuropathological assessment.

Conclusions: This ontology marks a significant advance, providing a structured framework that paves the way for enhanced data sharing, analysis, and the application of machine learning algorithms in digital histopathology research and diagnostics. This framework provides a foundation, holding promise for expanded integration of neuroinformatics and neurohistology image data which will allow for the training and validation of multidimensional machine learning algorithms.

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Repetitive head impacts induce neuronal loss and neuroinflammation in young athletes

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Background: Repetitive head impacts (RHI) sustained from contact sports are the largest risk factor for chronic traumatic encephalopathy (CTE). Currently, CTE can only be diagnosed after death through identification of hyperphosphorylated tau (p-tau) aggregates in neurons around blood vessels at the depth of the cortical sulcus. To date, the multicellular cascade of events that trigger initial p-tau deposition are still unclear and symptoms endorsed by young individuals with early disease are not fully explained by the extent of p-tau deposition, severely hampering development of therapeutic interventions.

Methods: We used grey matter from the dorsolateral frontal cortex sulcus from 8 non RHI-exposed controls, 9 RHI-exposed individuals without CTE, and 11 RHI-exposed individuals with diagnosed with mild CTE. All cases were under the age of 51. We then performed single nucleus RNA sequencing for cell type specific analysis. Phenotypes of interest were validated using antibody staining and in situ hybridization.

Results: We identified inflammatory microglia, dysregulated endothelial cell angiogensis, reactive astrocytes, and altered synaptic gene expression were already present in athletes with exposure to RHI but no CTE pathology. Similar types of degenerative changes were observed in athletes with early stage CTE, just at a more intense severity. Microglia exhibited high expression of SPP1 and Hif1a, suggesting a hypoxia response element. We also identified an astrocytic phenotype similar to the previous published disease associated astrocytes. Finally, we observed ~50% loss of cortical layer 2/3 neurons specific to the sulcus. This loss was independent of CTE pathology and scaled with years of contact sports play.

Conclusions: These results provide robust evidence that RHI induces lasting cellular alterations that underlie p-tau deposition and possibly the clinical symptoms endorsed in young athletes with contact sport exposure. Furthermore, these data provide new starting points for the development of diagnostics and therapeutics targeting cellular responses occurring before the onset of CTE.

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Tagging ARTAG In The Aging Indian Population

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Background: Tau inclusions in neuronal and glial cells are typical of several neurodegenerative tauopathies. Neuronal tau accumulation also occurs in normal aging. On the other hand, glial (astrocytic) accumulation of tau in older age, termed aging-related tau astrogliopathy (ARTAG), is less frequently recognized, and recent guidelines to harmonize evaluation and nomenclature, have been formulated. Thorn-shaped astrocytes (TSA) are typically subpial, perivascular, subependymal, and in the white matter, whilst granular/fuzzy astrocytes (GFA), are mostly in the grey matter.

Methods: In this study we applied the guidelines to examine the prevalence, distribution, morphology, and severity of ATRAG in aging Indian brains (> 65years, n=16). Additionally, aging non-degenerative neurological disorders (>65 years, n=7) were also examined. Samples were sourced from the human brain tissue repository, department of neuropathology, NIMHANS, India. Immunohistochemistry for pTau (AT8 clone) was performed on representative brain regions.

Results: ARTAG was noted in 68.75% of aging brains (n=11/16), with prevalence of 68%, 83%, and 57% in seventh, eighth and ninth decades respectively. Amongst the ARTAG-positive cases, TSA were found in 10 cases (90.9%), across all the decades, whilst GFA were seen in 3 (27.3%), only in the seventh decade and above. The non-degenerative neurological group (n=7) showed ARTAG in two cases (28.6%) both in the eighth decade. One displayed TSA in the subependymal and perivascular regions, and the other showed few white matter TSA. Summary of the detailed morphological examinations showed that TSA were often perivascular, subpial and subependymal, the latter especially prominent along the lateral ventricle adjacent to amygdala. GFAs were seen in the temporal regions, parahippocampal gyrus and basal ganglia.

Conclusions: Herein we have identified and documented in detail, the recently proposed ARTAG, in the Indian aging population, focusing on presence, type and localization, highlighting the feasibility and reproducibility of findings that may aid insights into functional relationship between astrocytes and neurons.

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HIF-1α Alterations Associated with Vascular Dementia Lesion Burden in VCID Progression

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Background: Vascular contributions to cognitive impairment and dementia (VCID) encompasses all types of cerebrovascular cardiovascular disease-related cognitive decline. Multiple lesions (e.g. arteriolosclerosis, microinfarcts) are present in the brains of individuals with VCID. VCID is associated with myelin disruption and oligodendrocyte dysfunction. Oligodendrocytes are particularly vulnerable to hypoxia, implicating them in VCID pathophysiology. Cerebral hypoperfusion/ischemia induce a hypoxic microenvironment, activating pathways with cytoprotective roles including the hypoxia-inducible factor-1 alpha (HIF-1α) pathway, which has been extensively studied in other diseases. However, its activation in VCID-related oligodendrocytes remains poorly understood. We aim to quantify and delineate HIF-1α alterations within oligodendrocytes in the development of dementia that are associated with the presence of co-existing vascular lesions. Assessments were made in clinically and neuropathologically characterized postmortem brain samples.

Methods: Multiplexed immunofluorescence staining for HIF-1α and the oligodendrocyte-specific marker ASPA was performed on the basal ganglia of human brain autopsy samples from the Honolulu Asia Aging Study and Nun Study (n=64) with matched Alzheimer's Disease Neuropathologic Change (ADNC): including background of Not, Low, Intermediate, and High ADNC and increasing numbers of co-existing chronic microinfarcts (0-9) or macroinfarcts (0-3), in addition to increasing memory impairment. HIF1-α—ASPA co-localization was quantified using object count with a positive cell detection threshold used for HIF1-α detection and quantification. Analyses included t-tests, Spearman's correlation, and ANOVA ($P < 0.05$).

Results: Considerable increases in HIF-1α staining was observed in micro and macroinfarct cases with co-existing Not/Low ADNC compared to controls (Not/Low ADNC and 0 micro or macro infarcts). A threefold increase in positivity from 3.2% in controls to 9.6% in VCID cases was observed.

Conclusions: The HIF-1α pathway plays a significant role in VCID pathogenesis suggesting its potential as a viable therapeutic target for drug intervention. Manipulation of HIF-1α pathways may improve oligodendrocyte response in VCID by preserving myelin function.

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Early-Stage Moderate Alcohol Consumption Dysregulates Brain Metabolic Hormones: Potential Links to Neurodegeneration, including AD

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Background: Alzheimer's disease (AD), the most prevalent cause of dementia, is mainly sporadic in occurrence but driven by aging and other cofactors. Studies suggest that excessive alcohol consumption may increase AD risk. Our study examined the degree to which short-term moderate ethanol exposure leads to broad molecular pathological changes of neurodegeneration, including those identified in AD.

Methods: Long Evans male and female rats were fed for 2 weeks with isocaloric liquid diets containing 24% or 0% caloric ethanol (n=8/group). Neurobehavioral testing was used to evaluate deficits in memory and cognition. The frontal lobes were used to measure immunoreactivity to AD biomarkers, white matter myelin/oligodendrocyte glycoproteins, insulin-related endocrine metabolic molecules, and mTOR pathway signaling by duplex or multiplex ELISA.

Results: Neurobehavioral testing demonstrated ethanol-impaired performance on the Open Field and Novel Object Recognition tasks. Molecular and biochemical studies revealed mixed pathologies in cortical (increased phospho-tau), white matter (reduced myelin-oligodendrocyte glycoproteins-MAG1, MBP, and PLP), and endocrine-metabolic pathways (reduced ghrelin, glucagon, leptin) in the ethanol-exposure group. Those complex responses were associated with significantly inhibited mTOR pathway signaling in the brain.

Conclusions: Short-term effects of chronic ethanol feeding included cortical, white matter, neuroendocrine, and metabolic signaling abnormalities, similar to the findings in human neurodegenerative diseases, including AD. The findings suggest that chronic alcohol consumption rapidly establishes a platform for impairments in energy metabolism that occur in both the early stages of AD and alcohol-related neurodegeneration.

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Extracellular Vesicle Detection of AD White Matter Pathology: Opportunities for Liquid Biopsy Monitoring of Disease

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Background: AD targets white matter such that atrophy and degeneration begin in the early stages of disease and contribute to cognitive decline. White matter pathology is likely mediated by degeneration of oligodendrocytes and myelin with attendant oxidative stress and neuroinflammation, but the lack of biomarkers has compromised understanding of the mechanisms and potential treatments. However, growing evidence suggests that CNS pathology can be accessed via extracellular vesicles (ECV).

Methods: This study compared AD-associated alterations in myelin glycoprotein expression in cerebral white matter versus O4+ oligodendrocyte-derived ECVs in human ApoE3.3 AD and control cases. Glial-neuronal marker expression was measured by PCR array analysis and ELISA. ECVs were isolated using an ultracentrifugation protocol and confirmed by NanoSight tracking and pan-tetraspanin ELISA.

Results: Quantitative RT-PCR analyses of white matter tissue demonstrated significant ($p <= 0.05$) or trendwise ($0.05 < p < 0.10$) reductions in nestin, PDGFRA, PLP, MOG, MAG1, MBP, RTN4, and CSPG4 mRNA in AD white matter. In addition, AD samples had reduced expression of Olig2, NKX2-2, and NKX6-2 and increased SOX transcription factor expression relative to controls. Regarding ECVs, all samples exhibited pan-tetraspanin immunoreactivity but the levels were significantly higher in AD ($P=0.0003$). Concerning myelin/oligodendrocyte glycoproteins, the mean ECV levels of PLP ($p < 0.0001$), MOG ($p=0.02$), MBP ($p=0.0076$), and GAL-c ($p=0.0002$) were significantly elevated in AD, whereas MAG1 ($p=0.004$), PDGFRA ($p=0.06$), and Nestin ($p < 0.0001$) were reduced.

Conclusions: Significant AD-associated white matter molecular pathology is detectable in both tissue and oligodendrocyte-derived ECVs with concordant reductions in PDGFRA, MAG1, and Nestin. The broad downward shifts in myelin-oligodendrocyte glycoprotein mRNA levels, together with reductions in key transcription factors indicate that white matter degeneration is mediated in part at the level of transcription. These results provide new evidence that white matter degeneration in AD can be detected and monitored using non-invasive liquid biopsy approaches such as CSF- or serum-based ECV analyses.

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Biochemical Evidence for a Pathological Variant of Progressive Supranuclear Palsy
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Background: Tau proteins aggregate into distinct lesions in tauopathies. These signature lesions result from deposition of tau proteins that are distinctly modified for each disease strain. Identifying the unique tau protein abnormalities that lead to specific neurodegenerative diseases is beneficial in their diagnosis and treatment. This is especially true for Progressive Supranuclear Palsy a primary tauopathy with clinical symptoms mirroring Parkinsonian syndromes and frontotemporal dementias.

Methods: Biochemical analysis of tau aggregates from patients with Progressive Supranuclear Palsy (n=60) demonstrates that the tau protein in this disease is specifically modified and may form the basis of this tauopathy strain. Here data collected using the sarkosyl-insoluble tau fractions from tissue samples of pathologically diagnosed patients with Progressive Supranuclear Palsy without comorbidities allows for the direct observation of the modifications. Most interesting is the variability of the tau proteins that actually accumulate for this disease opening up the possibility that tau strains may exist within any given tauopathy even as the clinical manifestations appear similar. This indicates that early and specific identification and detection of these strains is critical for the development of therapies for these diseases.

Results: Extraction and examination of sarkosyl-insoluble tau proteins from deceased patients pathologically diagnosed with Progressive Supranuclear Palsy indicates that multiple tau strains exist within this primary tauopathy. Western blotting of these extracts from patient tissues demonstrates a unique banding pattern of insoluble tau in a subset of the cases that exhibit extreme hyperphosphorylation as indicated by additional high molecular weight tau banding. These patients also had an earlier age of onset and age of death indicating a more aggressive disease strain. Additional data is presented comparing the clinical presentations and pathological distribution of tau in these Progressive Supranuclear Palsy patients compared to those with typical onset and insoluble tau signature.

Conclusions: Multiple pathological tau strains exist in Progressive Supranuclear Palsy.

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Lessons Learned from 25 Years of Progressive Supranuclear Palsy Brain Banking of at Mayo Clinic Florida

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Background: The brain bank for progressive supranuclear palsy (PSP Brain Bank) at Mayo Clinic in Florida was created in 1997 with assistance from the Society for Progressive Supranuclear Palsy (SPSP), later incorporated as CurePSP. Since its inception until February 2024, the PSP Brain Bank has received 1,856 brains from persons with a clinical diagnosis of PSP (n=1565) or PSP in the differential diagnosis (n= 291).

Methods: In almost all cases, both fixed and frozen hemibrains were received for diagnosis and research. All cases received a systematic and standardized neuropathologic evaluation that included thioflavin S fluorescent microscopy to assess Alzheimer type pathology and immunohistochemistry for phospho-tau (CP13) in sections of basal ganglia, thalamus, brainstem, cerebellum, as well as limbic and neocortices.

Results: Of those with clinical diagnosis of PSP, a pathologic diagnosis was confirmed in 1302 cases (83% diagnostic accuracy). Of those with PSP in the differential diagnosis, 168 had PSP (58% diagnostic accuracy). The most common misdiagnoses were Lewy body disease (LBD) (n=119), corticobasal degeneration (CBD) (n=116) and multiple system artophy (MSA) (n-45).

Conclusions: Samples from the PSP brain bank have contributed to molecular characterization of PSP. The PSP Brain Bank was one of the major sources of pathologically-confirmed PSP in several multicenter studies, including genome wide associations studies, as well as whole genome and whole exome studies of PSP. The resource has also been valuable in clinicopathologic studies addressing diagnostic accuracy, comorbid pathologies and neuroimaging correlates of pathology.

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Neuropathology in the LifeAfter90 Study: 2024 update on an Ethnically Diverse Cohort Study of Oldest-Old

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Background: Examining the neuropathology of the oldest-old has significantly advanced understanding of the multiple etiologies in very late life. Most studies have included exclusively White decedents with limited ethnoracial diversity. Our goal was to characterize neuropathology in a cohort of ethnically and racially diverse oldest-old decedents.

Methods: The LifeAfter90 study is an ongoing cohort study of Kaiser Permanente Northern California members, aged 90+ with targeted recruitment of individuals across different ethnoracial groups with no prior diagnosis of dementia in their medical record. Interviews and cognitive assessments occur approximately every six months. Brain donation was available to all consenting participants. Neuropathology was assessed using National Alzheimer's Coordinating Center Neuropathology forms and NIA-AA guidelines for diagnoses.

Results: As of January 2024, 340 participants(39%) have enrolled in autopsy(22% Asian, 18% African American, 17% Latino, 9% Multiracial/Other, and 34% White). Of the 340 participants, 91 had died and neuropathological evaluations were completed. The median age of death was 95 years(range 90-105), 51(56%) were female, 12 Asian, 10 Black, 16 Latino, 44 White, and 8 Multiracial/Other. At final clinical exam, 25 participants had dementia(28%), 24 had cognitive impairment but were not demented(42%), and 41 were cognitively normal(45%). Alzheimer disease (AD) and vascular pathologies were the most frequent findings. Nineteen percent of participants did not have AD and High likelihood of AD was found in four participants. For vascular pathologies, 54% has moderate/severe white matter rarefaction and 76% had moderate/severe arteriolosclerosis. Thirty-three percent had Lewy bodies; two cases had hippocampal sclerosis. One case had pathologic evidence of progressive supranuclear palsy and was demented at last diagnosis.

Conclusions: This diverse cohort of oldest-old individuals reveals numerous brain pathologies are present with advanced age, with AD and vascular pathologies being the most common. This confirms previous findings that with increases in cognitive impairment there is increasing pathology severity especially for AD pathologies.

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TAF15 pathology in motor neuron disease

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Background: The FET protein family comprises three predominantly nuclear DNA/RNA binding proteins named fused in sarcoma protein (FUS), Ewing's sarcoma protein (EWS), and TATA-binding protein associated factor 15 (TAF15). The mislocalization of FUS into cytoplasmic inclusions along with nuclear retention of EWS and TAF15 is characteristic of amyotrophic lateral sclerosis (ALS) patients harboring pathogenic FUS mutations (ALS-FUS). On the other hand, cytoplasmic mislocalization of all three FET proteins is characteristic of sporadic tau- and TDP-43-negative motor neuron disease (MND) and/or frontotemporal lobar degeneration (FTLD) cases known as FTLD-FUS, which is made up of three distinct disease subtypes: atypical FTLD with ubiquitin-positive inclusions (aFTLD-U), neuronal intermediate filament inclusion disease (NIFID), and basophilic inclusion body disease (BIBD). The recent, unexpected identification of abundant TAF15 amyloid fibrils in four aFTLD-U patients, including two patients who presented with clinical ALS, has emphasized the importance of TAF15 in neurodegeneration.

Methods: Given these discoveries, we sought to better understand the role of TAF15 in patients with tau- and TDP-43-negative motor neuron disease. From a series of 41 FTLD/ALS-FUS patients, we identified 15 with MND, including 1 ALS-FUS (R521G), 1 sporadic ALS, 1 sporadic primary lateral sclerosis (sPLS), 5 BIBD, and 7 NIFID patients. TAF15 and FUS pathology was semiquantitatively evaluated in the motor cortex, hypoglossal nucleus, and anterior horns of the spinal cord.

Results: In nearly all cases, TAF15 pathology was similar or slightly greater than FUS pathology. We observed relatively greater FUS pathology in the spinal cord of one BIBD case and no TAF15 pathology in the ALS-FUS case. We observed frequent TAF15 pathology compared to sparse FUS pathology in the motor cortex of sPLS, which suggested the name PLS-TAF15 instead of PLS-FUS.

Conclusions: These findings suggest that TAF15 may play a similarly important role in the pathogenesis of sporadic MND as has been described in aFTLD-U.

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Case report: Novel Variant in CSF1R Associated with Adult-Onset Leukoencephalopathy with Axonal Spheroids and Pigmented Glia (ALSP)

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Background: Adult-Onset Leukoencephalopathy with Axonal Spheroids and Pigmented Glia (ALSP) is a progressive disorder of microglia that affects the white matter of the brain and frequently manifests in the 4th and 5th decades of life. Former entities now recognized as ALSP include pigmentary orthochromatic leukodystrophy (POLD) and hereditary diffuse leukoencephalopathy with axonal spheroids (HLDS). The disease is caused by a mutation in the CSF1R gene (colony-stimulating factor receptor type 1), which is involved in the regulation of microglia. Typical initial symptoms are behavior and mood abnormalities later followed by neurologic deterioration. Neuroimaging can show bifrontal patchy or confluent white matter changes. Hallmark microscopic findings include vacuolization of white matter with pigmented microglia and axonal spheroids.

Methods: We present a case of a 54-year-old woman with sub-acute onset of progressive confusion and speech difficulties followed by gait and motor abnormalities. Past medical history included recent COVID-19 infection near time of onset, systemic lupus erythematosus treated with Plaquenil, and marginal B cell lymphoma (in remission since 2012). Brain MRI showed diffuse leukoencephalopathy characterized by extensive confluent and symmetric T2/FLAIR hyperintensity throughout the bilateral cerebral white matter, bilateral posterior limb of the internal capsule, and bilateral cerebellar white matter. Stereotactic brain biopsy of the frontal lobe was performed and sections were evaluated by H&E; immunohistochemical staining with GFAP, neurofilament, and CD163; and special staining with PAS and luxol fast blue/PAS counterstain. Invitae Leukodystrophy and Genetic Leukoencephalopathy panel was also performed.

Results: Microscopic evaluation showed white matter with vacuolar/spongiform leukoencephalopathy, reactive gliosis, macrophage infiltration, and axonal dystrophy. Genetic testing revealed a variant of uncertain significance (VUS) in CSF1R. Taken together, the radiologic, microscopic, and genetic findings lead to a diagnosis of ALSP.

Conclusions: Overall, this case illustrates a novel variant in the CSF1R gene resulting in a case of ASLP with hallmark neuroimaging findings and supportive microscopic features.

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Convolutional Neural Network-Derived Neurofibrillary Tangle Classifier Performance on Tau p217-stained Whole Slide Images

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Background: Neurofibrillary tangles (NFTs), the intraneuronal accumulation of hyperphosphorylated tau in Alzheimer Disease (AD), exist along a spectrum of maturation (pre-NFTs, intraneuronal iNFTs, extraneuronal eNFTs) with morphologic evolution that parallels a series of post-translational modifications. While AD staging does not require maturation stage differentiation, the advent of biomarkers capable of diagnosing and/or monitoring disease progression has motivated the investigation of individual maturation stages, necessitating a method of sorting NFTs. Here we report the performance of a convolutional neural network (CNN) trained to distinguish between maturation stages.

Methods: A deep-learning CNN (HALO AI v3.5 DenseNetV2) was trained on whole-slide images of autopsy collected hippocampal and entorhinal cortex sections stained with tau p217 IHC and compared against gold-standard pathologist annotated sections for assessment of precision, recall, and F1 score for pre-NFTs, iNFTs, and eNFTs. A total of 38 images were divided into training (n=32 (9M/23F), mean age 77.2 ± 9.5, Braak stages III-IV to VI and 2 non-AD controls) and testing sets (n=6 (3M/3F), mean age 75.3 ± 7.5, Braak stages ranging from V-VI to VI). 3,120/1,164/879 and 2,476/619/648 annotations were created for pre-NFTs/iNFTs/eNFTs in the teaching and testing sets, respectively.

Results: The CNN demonstrated strongest performance identifying pre-NFTs (Precision: 0.79, Recall: 0.6, F1 Score: 0.68), followed by eNFTs (Precision: 0.61, Recall: 0.59, F1 Score: 0.57), and iNFTs (Precision: 0.44, Recall: 0.14, F1 Score: 0.19). Identification of eNFTs was improved (Precision: 0.64, Recall: 0.56, F1 Score: 0.58) by combining annotations of preNFTs and iNFTs into a single class (nucleated NFTs) and retraining the CNN.

Conclusions: We describe a novel CNN trained to distinguish NFT maturation stages with precision that may be integrated into a workflow designed to collect enriched populations of NFTs for downstream molecular characterization, especially pre-NFTs. Performance may be improved with increased annotations, number of annotators, sample size, and anatomic regions sampled.

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Clinical heterogeneity of Creutzfeldt-Jakob Disease: An Autopsy Study of 28 Patients

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Background: Creutzfeldt-Jakob disease (CJD) is a fatal neurodegenerative disorder characterized by rapid progressive dementia and motor dysfunctions due to accumulation of misfolded prion proteins in the brain. Clinical heterogeneity sometimes leads to misdiagnosis as other neurodegenerative diseases.

Methods: This study examined 28 autopsy-confirmed cases of CJD from the Mayo Clinic brain bank. Review of medical records, histopathological examination with immunohistochemistry for prion proteins, and genetic and biochemical analyses were conducted to compare the characteristics between patients with antemortem diagnosis of CJD and non-CJD.

Results: Within our cohort, only 12 patients (43%) were correctly diagnosed with CJD before death, while the remaining 16 (57%) were clinically diagnosed with other diseases, such as progressive supranuclear palsy (PSP; n = 5), dementia with Lewy bodies (DLB; n = 3), and Alzheimer's disease (AD; n = 3). No significant difference was observed in the age of onset between patients diagnosed with CJD and those with non-CJD diagnoses (median: 73.0 vs. 67.0 years); however, the former group had significantly shorter disease duration than the latter group (median: 1.0 vs. 3.0 years). Symptomatically, misdiagnosed patients exhibited features characteristic of their clinical diagnoses, such as oculomotor dysfunction and postural instability in PSP cases. Genetic and biochemical analysis revealed that eight patients who had either octapeptide repeats (7-repeat and 4-repeat) or variably protease-sensitive prionopathy had significantly longer disease duration than those without these features (median: 4.0 vs. 1.0 years). Seven of the eight patients were clinically diagnosed with non-CJD diseases. Neuropathologically, no significant difference was observed in Braak neurofibrillary tangle stage and Thal amyloid phase, yet Kuru-like plaques were more frequent in the non-CJD group than those with a CJD diagnosis (65% vs. 9%).

Conclusions: This study reaffirms the heterogeneity of CJD, highlighting the need for refined diagnostic approaches. It suggests that diverse clinicopathological, genetic, and biochemical features may necessitate further subclassification.

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Gross and microscopic manifestations of Wernicke encephalopathy in a 67-year-old female

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Background: Wernicke encephalopathy (WE) is a neurodegenerative disorder caused by thiamine deficiency; it is usually associated with alcoholism, but can also be caused by starvation, chronic emesis, malabsorption, and inflammatory disorders. Many cases of WE are diagnosed at autopsy, since many patients do not exhibit the "classic" triad of encephalopathy, ophthalmoplegia, and ataxia at presentation.

Methods: We performed a retrospective review of the electronic medical record. Gross examination of the brain was performed at autopsy in 2023, and key brain structures were microscopically examined using H&E and targeted immunohistochemical stains. These findings were then correlated with clinical history and pre-mortem brain imaging from the 2016 and 2023.

Results: The patient was a 67-year-old female with a history of alcoholism and remote mechanical falls who presented with several weeks of nausea, emesis, and confusion. She became persistently encephalopathic, requiring intubation. WE was clinically suspected due to the patient's history of excessive alcohol intake, but she did not respond to high-dose thiamine supplementation and died approximately 1 month after admission. Autopsy examination of the brain revealed evidence of remote left frontal lobe contusion and bilateral subdural neomembranes over the cerebral convexities, consistent with remote history of traumatic subdural hemorrhage and contusion. Grossly, there were subtle lesions in the bilateral mammillary bodies, in the periaqueductal midbrain, and in the floor of the 4th ventricle. These regions demonstrated subacute necrosis with a minor degree of microscopic acute hemorrhage. Extensive chronic gliosis was observed in the anterior thalamic nuclei.

Conclusions: This combination of findings is most consistent with acute on chronic changes of thiamine deficiency; taken together with the patient’s clinical neurological manifestations, a diagnosis of Wernicke-Korsakoff syndrome was made. This case highlights the importance of suspecting WE even when a patient fails to exhibit the "classic" clinical presentation and does not respond to high-dose thiamine supplementation.

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Neuropathologic evaluation of a rare case of frontotemporal lobar degeneration with combined GRN and SQSTM1 mutations

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Background: Frontotemporal lobar degeneration (FTLD) is one of the most common causes of early onset dementia and is clinically, pathologically, and genetically heterogeneous. Pathologically, approximately half of FTLD is associated with TAR DNA-binding protein 43 (FTLD-TDP) and most remaining cases are associated with tau (FTLD-tau). Although most patients with FTLD have no known genetic cause, approximately 10–25% carry a genetic mutation. While FTLD-tau is only associated with mutations in MAPT, there are a number of genes with rare variants that cause FTLD-TDP, including GRN and, more rarely, SQSTM1. The heterogeneity of FTLD has contributed to the challenge studying this complex disease; however, examination of individual FTLD patients and families have proven invaluable in advancing our understanding of the pathophysiology FTLD.

Methods: Here we report the clinicopathologic features of a single patient clinically diagnosed with early onset frontotemporal dementia (FTD) who had a mutation in GRN (c.991C>T (p.Gln331*)) and SQSTM1 (c.1175C>T (p.Pro392Leu)), two genes independently associated with autosomal dominant FTLD-TDP. Neuropathologic examination included extensive evaluation for pTDP-43 pathology, other protein aggregates commonly associated with neurodegenerative disease (Abeta, tau, alpha-synuclein, ubiquitin), and TMEM106B.

Results: The patient first presented at age 30 with complaints that she could no longer stay on task or complete household chores. Her symptoms progressed relatively quickly, and she died at age 35. Postmortem neuropathologic examination revealed severe neurodegenerative changes, including marked cortical thinning, neuron loss, gliosis, and parenchymal vacuolation of the frontal and temporal lobes. Unexpectedly, no TDP-43 pathology was identified. Only minimal ubiquitin pathology was identified. Interestingly, the post prominent finding was TMEM106B immunoreactivity, which has recently been described in a number of neurodegenerative diseases, most notably FTLD-TDP with GRN mutations.

Conclusions: This case provides a unique opportunity to further interrogate the role of GRN and SQSTM1 in TDP-43 and TMEM106B proteinopathy and the clinical-genetic-pathologic correlations of FTLD.

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Neuropathological correlates of dementia in cases with Braak neurofibrillary stage IV

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Background: The density and neuroanatomical localization of neurofibrillary tangles (NFT) contribute to cognitive impairment. Autopsied subjects with NFTs restricted to subcortical regions may have had no cognitive impairment, mild cognitive impairment, or dementia, whereas subjects with widespread NFTs in neocortex most often have dementia. In this study we aimed to understand why some subjects with NFTs restricted to limbic areas, specifically Braak stage IV, develop dementia, while others do not. We hypothesized that comorbid non-AD pathologies could be contributing to dementia in these subjects.

Methods: Subjects with neuropathological Braak NFT stage IV were selected from the Arizona Study of Aging Neurodegenerative Disorders. We used multiple logistic regression models to predict the presence of dementia or no dementia. Independent variables included Lewy body pathology (LB), cerebral amyloid angiopathy (CAA), cerebral white matter rarefaction (CWMR), argyrophilic grains (ARG), TDP-43, number of microinfarcts and neuritic plaque densities. All models were adjusted for sex and age.

Results: From a total of 394 subjects, 240 were demented and 154 were not demented. Age was not significantly different between groups, while a higher percentage of males had dementia. Demented subjects had higher densities of LB, CAA, neuritic plaques and number of cases with TDP-43. The total number of different pathologies significantly predicted dementia and plaque, LB and CWMR were all significant independent predictors of dementia. When subdividing cases based on their plaque densities, we found that LB, CWMR and ARG were independent predictors of dementia in the low neuritic plaque density group (LPD; zero to sparse), while LB and plaque density predicted dementia in the higher plaque density group (HPD; moderate to frequent).

Conclusions: These results demonstrate that the presence of co-pathologies contribute to dementia observed during life.

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FAM76B regulates NF-κB-mediated inflammatory pathway by influencing the translocation of hnRNPA2B1

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Background: FAM76B has been reported to be a nuclear speckle-localized protein with unknown function

Methods: In this study, we produced FAM76B overexpression and knockout macrophage cell lines, as well as FAM76B knockout mice and studied the roles of FAM76B in regulating inflammation and neuroinflammation. We also evaluated changes in microglial FAM76B expression by immunohistochemistry in human brains with different disorders.

Results: FAM76B was first demonstrated to inhibit the NF-κB-mediated inflammatory pathway by affecting the translocation of hnRNPA2B1 in vitro. We further showed that FAM76B suppressed inflammation in vivo using a traumatic brain injury (TBI) mouse model. Lastly, FAM76B was shown to interact with hnRNPA2B1 in human tissues taken from patients with acute, organizing, and chronic TBI, and with different neurodegenerative diseases. The results suggested that FAM76B mediated neuroinflammation via influencing the translocation of hnRNPA2B1 in vivo during TBI repair and neurodegenerative diseases.

Conclusions: In summary, we for the first time demonstrated the role of FAM76B in regulating inflammation and further showed that FAM76B could regulate the NF-κB-mediated inflammatory pathway by affecting hnRNPA2B1 translocation, which provides new information for studying the mechanism of inflammation regulation.

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Stochastic expression of innate immune response genes in murine models of neurodegeneration and aging

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Background: Several murine models of the human neurodegenerative disease Aicardi-Gutiérrez Syndrome (AGS) have been created by introducing mutations in RNA editing genes. Mice carrying a homozygous knock-in mutation in ADAR1 demonstrate a deficit in RNA editing that leads to MDA-5 mediated activation of the innate immune response (IIR). Despite months of elevated interferon stimulated gene (ISG) expression, there is essentially no inflammatory infiltrate and limited neuropathology of deep gray matter mineralization developing between 8 and 12 months of age. Previously we used in situ hybridization (ISH) to describe the regional and cellular distribution of IIR activation. Rather than a uniform global increase in IIR gene expression, consistent upregulation was observed consistently in specific regions, along with a robust stochastic distribution of loci throughout the brain. ISH also demonstrated that different cell lineages demonstrated specific IIR gene expression.

Methods: To further characterize the nature of this aberrant IIR and its relationship to neurodegeneration, we employed Digital Spatial Profiling (DSP) of the murine whole transcriptome atlas (WTA). DSP allowed us to assess regional and temporal differences in gene expression of young adult and aged wild type (WT) and ADAR1 mutant mice.

Results: DSP directed WTA of brain regions that showed elevated expression of ISG-15 or CXCL-10 by in situ hybridization demonstrated elevation of those and other innate immune response genes previously shown in RNA extracted from whole brain homogenates. The WTA of ADAR1 mutant mice permitted an unbiased assessment of all altered gene expression and definition of a set of genes differentially associated with neurodegeneration. A similar gene set was differentially expressed in young adult compared to aged WT mice, with aged WT mice showing areas of expression similar to lesional areas in young mutant mice.

Conclusions: These results suggest a novel pathway associated with age related neurodegeneration, heretofore not visible with traditional degenerative markers.

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TDP-43-regulated cryptic exon expression in chronic traumatic encephalopathy

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Background: We have recently shown that a history of repetitive head impacts is associated with TDP-43 pathology and that TDP-43 inclusions are frequently present in the frontal cortex of chronic traumatic encephalopathy (CTE) cases. Studies in human stem cell-derived motor neurons have demonstrated that depletion of TDP-43 leads to perturbations in RNA processing. For instance, TDP-43 loss-of-function results in reduced expression of stathmin 2 (STMN2), a neural-enriched transcript that encodes for a microtubule-binding protein, and increased expression of an aberrant truncated form of STMN2. This disease-associated STMN2 cryptic exon transcript is detected in several TDP-43 proteinopathies, including ALS and FTLD. We hypothesized that TDP-43 pathology in CTE is associated with TDP-43 loss-of-function phenotypes (i.e. cryptic exon expression) that may contribute to neurodegeneration following brain injury.

Methods: A total of 133 brain donors with CTE (n=97) or controls (n=36) were examined for the presence and distribution of TDP-43 inclusions. Quantitative RT-PCR for cryptic exons in STMN2, UNC13A (Unc-13 homolog A), and others was validated in human stem cell-derived cortical neurons and then conducted in post-mortem dorsolateral prefrontal cortex in CTE and controls. Analysis of RNA-sequencing results to examine selected cryptic exons, such as STMN2 cryptic exon, in CTE was also performed.

Results: Out of 97 with CTE, 42 (43%) had TDP-43 inclusions within the hippocampus, frontal cortex, or both (CTE-TDP). Mis-spliced cryptic exons were detected for STMN2 and UNC13A within the prefrontal cortex of CTE-TDP cases as compared to CTE without TDP-43 and to controls.

Conclusions: TDP-43 loss-of-function molecular phenotypes are a feature of a subset of CTE cases. This suggests that candidate biomarkers based on cryptic exons that are being developed for TDP-43 proteinopathies, such as ALS, may also be used to assess TDP-43 pathology in CTE.

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An Analysis of Glial cytoplasmic Inclusions in Patients With Multiple System Atrophy

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Background: Multiple system atrophy (MSA) is a rare neurodegenerative disorder characterized by progressive degeneration of multiple brain regions, including the basal ganglia, midbrain and cerebellum, as well as the autonomic nervous system (1,2). MSA varies widely among patients, with motor symptoms such as bradykinesia, rigidity, and postural instability. Autonomic dysfunction manifests as orthostatic hypotension, urinary incontinence, and erectile dysfunction. Cerebellar involvement leads to gait ataxia, dysarthria, and limb coordination deficits. Neuropathologically, MSA is characterized by the presence of glial cytoplasmic inclusions (GCIs) primarily composed of α-synuclein aggregates, found in oligodendrocytes throughout the central nervous system. This study aimed to elucidate the protein composition of GCIs in MSA patients using LCM followed by proteomic analysis, using methods we have previously published (3-5).

Methods: GCIs were identified by α-synuclein histology (Clone Syn303), followed by microdissection. Dissected regions included the cerebral peduncle of the midbrain and the white matter of the cerebellum from each case. Subsequent proteomic analysis was conducted to identify proteins associated with GCI pathology in MSA when compared to control cases.

Results: From neuropathology analyses of aggregated α-synuclein, six MSA cases were identified. The MSA cases were compared to age and sex matched control cases (n = 5) without neurological disorders. From proteomics analyses, 2393 proteins were detected. This demonstrates a protocol for obtaining cells affected in MSA by LCM for proteomics analyses. There were a number of altered proteins in MSA compared to control cases.

Conclusions: GCIs represent a neuropathological hallmark of MSA, GCIs contribute to neuroinflammation, oxidative stress, and neuronal loss, leading to progressive neurodegeneration. Yet the precise molecular mechanisms underlying their formation and pathogenicity remain elusive. We are hoping this study will shed light on the molecular landscape of GCIs in MSA, providing valuable insights into the pathogenesis of this devastating disorder and identifying potential therapeutic targets for intervention.

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A Novel Strategy to Screen for Genetic Determinants of Selective Neuronal Vulnerability In Vivo

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Background: Specific types of neurons display increased vulnerability to damage and death in various neurodegenerative diseases, yet the reasons behind this selective susceptibility remain unclear. CRISPR-based screening platforms enable high-throughput discovery of neuronal susceptibility genes in cell culture, but they fall short in capturing specific neuronal subtypes. The mouse nervous system, mirroring human diversity in neurons and regional neuroanatomy, offers a more representative model to study selective neuronal vulnerability. However, applying high-content CRISPR screening in the mouse brain in vivo faces challenges related to the delivery and recovery of sgRNAs in brain tissue in a cell type-specific manner.

Methods: We developed a CRISPR-based genetic screening strategy utilizing adeno-associated virus (AAV) and a Cre recombinase-dependent workflow, allowing for the cell type-selective identification of neuron-essential genes directly within a mouse brain. Alongside a neuron-specific Cre, we delivered an AAV sgRNA library targeting over 2000 genes into neonatal mice with CRISPR machinery and harvested the brains after 4-12 weeks to isolate the remaining AAV genomes, followed by their sequencing to evaluate sgRNA dropout to indirectly assess neuronal death.

Results: The screen identified several neuron-essential genes within distinct biological categories, including aminoacyl tRNA synthetases, vacuolar ATPases, and cholesterol synthesis. The hits were highly reproducible across individual mice and showed strong concordance with a previous screen in human stem cell-derived glutamatergic neurons, while additionally revealing new hits. We validated a new top hit, Hspa5, as an essential neuronal chaperone in primary cultures and in vivo.

Conclusions: We devised an efficient and scalable workflow that enables identification of essential genes in the mouse brain, capitalizing on AAV's widespread distribution, strong neuronal tropism, and simplified retrieval of viral genomes, as well as a Cre-dependent switch for cell type-specific evaluation. This innovative platform offers a unique avenue to identify susceptibility genes in distinct neuronal populations using existing neurodegenerative disease mouse models.

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Military Veterans with Early-Onset Dementia, Blast Exposure and Interface Astroglial Scarring

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Background: Clinical and neuropathologic findings in 5 PAVAHCS patients who died from 2008 to 2023 with early-onset dementia (EOD), likely or definite wartime blast exposure (BE), and CNS interface GFAP+ astroglial scarring are reported.

Methods: Cases 1 and 2 served in combat during Gulf War 1. Both died in 2008. Case 1 (50 yo female) had a possible family history of dementia; she was diagnosed with schizophrenia, dementia, and catatonia 4 years (y) prior to death (PTD); she had frontotemporal lobar degeneration (FTLD-A, brain weight (BW): 1075 g). Case 2 (46 yo M) had acute psychosis and mutism; he was diagnosed as “atypical Pick disease” 8 years PTD. He had a severe FTLD (?type, non-tau, non-TDP43; BW: 840 g). Cases 3-5 had multiple documented military training- and/or combat-related BE in Iraq/Afghanistan. Case 3, a 34 yo M with sleep disorder, chronic pain and depression was diagnosed with mild cognitive impairment 5 y PTD (suicide, 2018). He had incidental focal cortical dysplasia but his brain was otherwise grossly unremarkable (BW: 1835 g). Cases 4 (59 yo M, died 2020) and 5 (58 yo M, died 2023) had EOD with behavioral disturbances diagnosed 14 y PTD and 7 y PTD, respectively. They had severe ADNC (Case 4: A2B3C3, BW: 940 g; Case 5: A3B3C3, BW: 900 g). Both also had CTE neuropathologic changes; the others did not.

Results: All cases showed dense interface astroglial scarring in subpial (neocortical [predominantly gyral crown], basal forebrain, mamillary body, brainstem, cerebellum and spinal cord (3/3), and in all brain subependymal areas.

Conclusions: Genetic, family history and BE data are incomplete but BE likely contributed to early-onset cognitive impairment, behavioral/psychiatric manifestations and diverse, severe neuropathologic processes in these cases. These findings complement those of polypathology (Iacono et al, 2020), and expand the range of BE-related neuropathology in military veterans with EOD.

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Genetic Creutzfeldt-Jakob disease associated with PRNP V180I mutation

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Background: Genetic Creutzfeldt-Jakob diseases (gCJD) have diverse clinicopathological phenotypes, depending on the genetic variant. More than 600 cases of gCJD having PRNP V180I mutation have been identified in Japan. The average age of onset is almost 80 years old. Although the disease is associated with genetic mutations, the family history of gCJD (V180I) is not always clear. The clinical course is more gradual than that of sporadic CJD.

Methods: Since the number of pathologically confirmed V180I cases is limited, we provide the neuropathologic results of 18 autopsied cases at a single institution.

Results: There were 5 males and 13 females with a mean age at death of 84 (69-103) years. Codon 129 polymorphism was found in 13 cases (72%) with MM and 5 cases (28%) with MV, and MV was more common than in the Japanese population. Gross pathology showed moderate cerebral atrophy, thinning of the cerebral cortex, as well as decreased volume and brownish color changes of the cerebral white matter. Histologically, the cerebral cortex showed severe spongiform degeneration and/or status spongiosis. The cerebellar cortex showed mild gliosis in the molecular layer. Immunostaining with anti-prion antibodies revealed fine granular, synaptic-type deposits, in the cerebral cortex. In some cases, dot-like and coarse deposits were observed in the cerebellar cortex. In addition, anti-prion antibodies occasionally showed positive findings along the dendrites of Purkinje cells. In the retina, bilaminar anti-prion antibody-positive findings were consistently observed mainly in the outer and inner reticular layers. Alzheimer's disease pathology, senile plaques and neurofibrillary tangles, were observed in most of the cases, and the degree of these changes was moderate or higher. Biochemical analysis by Western blotting showed a type 2 pattern but lacked a deglycosylated band.

Conclusions: The V180I mutation may have a very characteristic clinical course as hereditary CJD and appears superimposed on age-related pathological changes.

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Neuropathology of Patient-Customized Antisense Oligonucleotide Therapy in the Setting of CLN7 Batten Disease

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Background: The neuronal ceroid lipofuscinoses (NCLs) are a group of thirteen genetically defined lysosomal storage disorders that are collectively known as Batten disease. While rare, NCL is the most common cause of neurodegeneration in children and can manifest clinically with rapid psychomotor regression, seizures, and vision loss. NCL is characterized by accumulation in neurons and other cell types of autofluorescent storage material resembling lipofuscin, leading to severe cerebral atrophy. NCL also frequently affects other tissues such as the skin, muscle, rectum, and eye. Our current understanding of the pathogenesis and pathologic findings of NCL is limited. This project focuses on a patient case for which a novel “n of 1” CLN7 genetic therapy, an antisense oligonucleotide (ASO) called milasen, uniquely targeting a patient’s disease-driving mutation was developed and administered.

Methods: We examined H&E stains of cerebral, ocular, and other tissues pertaining to this patient case. We performed RNAscope localization to analyze the biodistribution of the investigational drug across tissues.

Results: We observed widespread intracytoplasmic inclusions throughout the brain. Our studies of the eye revealed extensive atrophy of the outer retina with a thickened nerve fiber layer. Cone and rod outer segments of the retina were essentially completely absent. We also observed migration of the retinal pigment epithelium into the inner layers of the retina. RNAscope localization of milasen revealed strong localization in the cytoplasm and nuclei of neurons throughout the prefrontal cortex of the brain. We also identified leptomeningeal macrophage infiltration as an ASO-therapy-associated histologic feature, and RNAscope visualization supported histologic and ultrastructural evidence of specific ASO engulfment in leptomeningeal macrophages. Lastly, we observed linkage between milasen drug levels and splice site rescue in in distinct central nervous system sites.

Conclusions: Taken together, these results allow us to begin to characterize the microscopic changes associated with this rare neurodegenerative disease in the pediatric setting.

Posters: Ophthalmic Pathology

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Aggressive Periorbital Epithelioid Hemangioma with GATA6::FOXO1 Fusion and Low-level KRAS Mutation

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Background: Epithelioid hemangioma (EH), a rare benign vascular tumor, is even rarer in the periorbital area. Both clinical and radiographic findings are non-specific, making diagnosis challenging. Although FOS/FOSB fusions are identified in approximately one-third of EH cases, presence of GATA6::FOXO1 fusion is exceptionally rare, documented in only several cases, none of which demonstrated aggressive behavior. Herein, we report a case of an aggressive periorbital EH with GATA6::FOXO1 fusion and KRAS mutation. Case: A 15-year-old male presented with a gradually enlarging lateral canthal nodule. MRI revealed a 1.4cm periorbital lesion with soft tissue extension and periosteal reaction. Excision showed a vascular neoplasm with epithelioid and spindled endothelial cells, focal hobnail morphology, scattered eosinophils, and focally increased mitotic activity. Immunohistochemistry analysis showed vascular marker expression, focal FOSB positivity, and slightly elevated Ki67, with negative STAT6, HHV8, and CAMTA1 expression.

Methods: Whole transcriptome RNA sequencing (RNA-seq), OncoKids, and vascular anomaly panels were performed on formalin-fixed paraffin-embedded tissue.

Results: RNA-seq revealed a GATA6::FOXO1 fusion, without FOS/FOSB fusion, alongside a low-level (1.8% variant allele frequency) KRAS mutation (c.35G>C, p.Gly12Ala), confirming the diagnosis of EH. Despite a trial of steroids and propranolol, symptoms persisted. MRI suggested residual tumor, prompting re-excision with negative margins. However, back pain developed, and persistent bleeding ensued from a lesion protruding through the right inferior conjunctiva. MRI orbits showed surgical bed enhancement, suspicious for recurrent/residual tumor. Spine MRI showed enhancing lesions, concerning for metastatic EH. Workup for spinal lesions is ongoing.

Conclusions: Unusual location, genetic aberrations, and aggressiveness of this case broaden our understanding of EH's clinicopathologic and molecular characteristics. Presence of GATA6::FOXO1 fusion and/or a KRAS mutation may play a role in the tumor's aggressivity. Further studies are needed to understand their clinicopathological significance and to ascertain whether EH harboring GATA6::FOXO1 fusion and/or KRAS mutation represents a more aggressive subtype or potentially a low-grade malignancy.

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Ocular surface fibroma as a differential diagnosis in conjunctival lesions

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Background: Ocular surface fibromas are lesions arising from fibroblasts in the tarsal plate, conjunctival substantia propria, and Tenon's capsule. This lesion was first described in 1983, and there have only been 13 cases reported in the literature. Due to their infrequency, the clinical and histopathologic diagnostic and prognostic characteristics of ocular surface fibromas are not well understood.

Methods: We report a case of a 17-year-old patient presenting with 2-year history of a painless, growing, well-circumscribed pink bulbar conjunctival lesion on the right eye associated with a foreign body sensation upon blinking. Clinically, the differential diagnosis included pyogenic granuloma, squamous papilloma, ocular surface squamous neoplasia, atypical conjunctival nevus or lymphoid infiltration. The lesion was surgically excised and submitted for histopathologic evaluation. Additional immunohistochemistry staining was conducted for CD34, CD117 (c-kit), Sox10, CD68, CD163, Ki67, Desmin, Actin Smooth Muscle, and ERG.

Results: Microscopic sections showed a benign polypoid fibrous conjunctival lesion with benign spindle cells in an abundant collagen-rich substantia propria. CD117(c-kit) staining highlighted many mast cells infiltrating the lesion. Sox10 staining highlighted scattered benign melanocytes at the base of conjunctival epithelium. CD34 staining highlighted fibroblasts and endothelium in lesion. CD68 and CD 163 staining highlighted rare macrophages. Ki67 was positive for scattered basal epithelial cells. Desmin staining was negative, and Actin smooth muscle staining highlighted vascular endothelial cells. ERG transcription factor staining highlighted endothelial cells.

Conclusions: Histopathological and Immunohistochemistry findings support the diagnosis of an ocular surface fibroma, and there was no evidence of squamous neoplasia, melanocytic proliferation, lymphoproliferative process, or pyogenic granuloma. CD34 staining positivity confirms the likely fibroblastic origin of the lesion. Ocular surface fibromas should be considered in the differential diagnosis of bulbar conjunctival lesions. Notably, this case represents the youngest documented occurrence of ocular surface fibroma.

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Unraveling The Mystery Behind a Dry Eye

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Background: Primary lacrimal gland tumors are rare. Mass lesions of the lacrimal gland are more likely to have infectious, autoimmune, or idiopathic etiologies.

Methods: A 55-year-old man presented with 4-month history of right eye dry sensation, and numbness involving his eyebrow and forehead with extension to the scalp. MRI orbits showed diffuse enlargement (3 x 2.6 x 1.4 cm) of the right lacrimal gland with partial encasement of the superior and lateral rectus muscles. The radiologic differential included dacryoadenitis, autoimmune disease, idiopathic inflammation, granulomatous disease, amyloidosis, and neoplasm.

Results: H & E-stained sections showed large epithelial cells with abundant eosinophilic cytoplasm, pleomorphic nuclei, and prominent eosinophilic nucleoli. Tumor cells formed occasional glands and a rare focus of comedo necrosis was noted. Tumor was intimately associated with the lacrimal gland. Immunohistochemical stains showed the tumor stained with epithelial markers, cytokeratin AE1/AE3, and cytokeratin 7. Apocrine differentiation was noted with androgen receptor (AR) and GCDFP15. HER2Neu was also positive. p63, p40, calponin, S100, SOX10 and cytokeratin AE1/AE3 were negative. Mucicarmine was also negative.

Conclusions: Since the tumor showed androgen differentiation by immunohistochemistry, the differential included primary ductal adenocarcinoma of the lacrimal gland, metastatic salivary duct carcinoma and metastatic prostatic adenocarcinoma. There was no history of a salivary gland neoplasm and PET scan did not reveal tumor at other sites. Therefore, the findings were consistent with primary ductal adenocarcinoma of the lacrimal gland. Less than 50 cases of primary ductal adenocarcinoma of the lacrimal gland have been reported in the literature. Here we add another case to the growing literature which may help to inform diagnostic, prognostic and therapeutic decision making in the future.

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Rhinosporidiosis of the bulbar conjunctiva presenting as pyogenic granuloma

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Background: Rhinosporidiosis is a rare infection caused by Rhinosporidium seeberi, an endosporulating microorganism that affects mucous membranes, causing a slow growing chronic granulomatous disease. The organism is in the class Mesomycetozoea, which includes microorganisms with features of both animals and fungi. Contact of damaged epithelium with contaminated water or inhalation of spore contaminated field dust are the proposed mechanisms of infection. Rhinosporidiosis is considered an emerging infectious disease, with the majority of reported cases from India, followed by endemic parts of South America, with additional cases in tropical and subtropical areas of North America and Europe.

Methods: We present a case of Rhinosporidiosis in a 62 year old man with a bulbar mass lesion clinically consistent with pyogenic granuloma. This painless exophytic globoid red mass protrudes from the left temporal bulbar conjunctiva, with numerous feeder vessels. The patient's history does not identify any exposure events or risk factors for an infectious process. The lesion is completely excised.

Results: Histologically, the conjunctival tissue contains necrotizing granulomas with large encysted microorganisms. The cysts are highlighted by GMS, PAS, and mucicarmine special stains. These large sporangia contain endospores, and are morphologically consistent with Rhinosporidia. The sporangia are in various stages of degeneration, and involve the epithelium and substantia propria.

Conclusions: Rhinosporidiosis should be considered in patients with painless conjunctival lesions clinically resembling pyogenic granuloma. Complete surgical excision and examination of sinonasal mucous membranes are recommended to assess for additional lesions.

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Ciliary Body Melanocytoma with Novel Genetic Profile

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Background: A 56-year-old woman without significant past medical history presented with chronic, painless, and progressive vision loss of the left eye over several years. Medications included daily vitamin-D3.

Methods: Dilated fundus examination demonstrated a large, hyperpigmented ciliochoroidal mass in the superonasal quadrant, posterior to the lens. No subretinal fluid or orange pigments were observed. Ultrasound revealed a 11.14 x 12.8 x 10.62 mm mass with mid-to-high internal reflectivity, and cystic spaces – ciliochoroidal melanoma was the clinical impression. Size and location directed enucleation with concurrent fine needle aspiration (FNA) for molecular testing.

Results: The enucleated eye measured 22.5mm antero-posteriorly, with oval-shaped, hyperpigmented ciliary-body mass abutting the lens. Preservation of choriocapillaris was observed with an intact Bruch's membrane; additionally, no intra/extrascleral extension of the lesion was identified. Peroxide bleaching revealed cytologic details of heavily pigmented cells with bland round-oval nuclei, sheets of polyhedral cellular structures, normal nuclear-to-cytoplasmic ratio, and no mitotic figures. Histology was compatible with ciliary body melanocytoma. The tumor's immunohistochemistry further supported benign uveal melanocytic proliferation with positive nuclear staining for BRCA1-associated Protein 1 (BAP1) and no expression of preferentially expressed antigen in melanoma (PRAME).

Conclusions: Genome expression profile (GEP) of the FNA showed a class IA melanocytic lesion. A 7-gene sequencing panel for known uveal melanoma genes showed no mutations in GNAQ, GNA11, CYSLTR2, PLCB4, SF3B1, EIF1AX, and BAP1. An absence of genetic alterations was further confirmed for GNAQ, GNA11, CYSLTR2, SF3B1, and BAP1 by next generation sequencing (NGS) via Stanford Actionable Panel for Solid Tumors on the enucleation. The NGS also identified 3 mutation events of unknown significance in genes ABL1, NTRK2, and TACC3, previously reported in cutaneous melanomas but not uveal melanocytic neoplasms. Interestingly, ABL1 can activate the MEK/ERK pathway, similar to GNAQ/GNA11. Our case suggests that additional genetic mutations, such as ABL1, may drive pathogenesis of uveal melanocytic neoplasms.

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Orbital Intramuscular Vascular Anomaly

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Background: Intramuscular vascular anomalies [IVA] ("intramuscular angiomas or hemangiomas") are proliferations of benign appearing vascular channels intercalated through mature skeletal muscle. They commonly occur in the lower extremities and trunk, but they are occasionally found within muscles of the head and neck region. Their occurrence within orbital skeletal muscles has been described very rarely.

Methods: Microscopy, immunohistochemistry, NGS

Results: Here we describe a case of a 62-year-old male with orbital pain, excessive lacrimation, erythema, and visual loss in his right eye. Serial brain MRI scans showed a mass of markedly increasing size, most recently seen to measure 1.6cm in size with replacement of the right superior rectus muscle. The radiologic differential diagnosis included idiopathic myositis or autoimmune condition such as IgG4 disease. Intraoperatively, surgeons observed a medusa-like sprawl of vessels over the lateral aspect of the levator muscle and an abnormal beige color of the enlarged superior rectus muscle. Histologic examination showed skeletal muscle transversed by a mixture of small-to-medium sized arteries, arterioles, and derivative capillary channels. Blood vessels showed variable wall thickness and were frequently cuffed by dense lymphoid aggregates. These anomalous structures were surrounded by myxoid stroma and thick fibrous connective tissue. Scattered atrophic muscle fibers were aligned in parallel to the abnormal vessels. On immunohistochemical examination, SMA highlighted thick blood vessel walls and CD31 labeled endothelial cells while D2-40 was negative. Perivascular lymphoid aggregates were highlighted by the T cell marker CD3. IgG4 and IgG stains did not support IgG4 disease.

Conclusions: Overall, the clinical, radiographic, and histologic findings of this unusual lesion were most consistent with a benign vascular anomaly primarily involving the superior rectus muscle.

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Eyes or lies? Navigating ocular pathology controversies in infant head trauma.
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Background: The medical community has long identified specific ocular pathologies, such as retinal hemorrhages and macular folds, as indicators of inflicted pediatric head injury. These findings, carrying significant medicolegal weight, were traditionally linked to the "vitreoretinal traction" (VRT) theory, positing that shearing forces from shaking or impact trauma are causative.

Methods: This study undertakes a critical review of literature spanning four decades, coupled with case analyses, to challenge the prevailing opinion that these ocular pathologies are unequivocal evidence of child abuse. The methods involve scrutinizing the foundational research underpinning the VRT theory and examining alternative explanations for the pathologies observed.

Results: The review suggests that the association of ocular pathology with deliberate head trauma is not exclusive. The foundational studies supporting the specificity of these pathologies in child abuse cases have been criticized for circular logic and bias. Our findings indicate that alternative etiologies may explain the presence of intra-ocular hemorrhages without the necessity of intense force.

Conclusions: Given the complexity and sensitivity of diagnosing child abuse based on ocular pathology, a comprehensive and cautious approach is advocated. The pathophysiological basis attributed to VRT in the context of retinal abnormalities is contested, necessitating further research and a more nuanced understanding of ocular injuries in pediatric head trauma.

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Illuminating Blurry Vision: Visualization of Corneal Protein Deposition with Immunofluorescence in Two Illustrative Cases

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Background: Monoclonal gammopathy of unknown significance (MGUS) is an asymptomatic premalignant disease with a progression rate of 0.5-1% per year to multiple myeloma. MGUS is often discovered by routine laboratory tests. Although the disease does not require treatment, it can rarely present with significant ocular symptoms in the context of crystalline keratopathy, necessitating multiple medical and surgical treatments. Lattice corneal dystrophy type I, a rare inherited disorder caused by mutations of TGFBI, manifests with amyloid deposition within the corneal stroma, and penetrating keratoplasty may be needed for vision symptoms. Here, we pictorially highlight protein deposition using immunofluorescence performed on formalin-fixed paraffin-embedded (FFPE) blocks in a case of a 66-year-old man with MGUS and blurry vision (Case 1) and an 80-year-old woman with unexplained blurry vision (Case 2), both having underwent penetrating keratoplasty.

Methods: Patient medical records were reviewed. Hematoxylin and eosin, special staining, immunohistochemistry, and immunofluorescent techniques were performed on FFPE. Literature review was performed.

Results: Case 1: Eosinophilic accumulations of the cornea were highlighted with PAS-D (Figure 1, A) and IgG-kappa by immunohistochemistry (B). IgG lambda immunohistochemistry and Congo red were negative. Immunofluorescence (IF) technique demonstrated IgG-kappa (2+) staining in the stroma with rare globules in the epithelium (C). Lambda (1+) was largely limited to the stroma (D) as was IgG (1-2+) and IgM (1+). Case 2: Amorphous, eosinophilic deposits within the corneal stroma (Figure 2, A) were congophilic (B) with apple-green birefringence on polarized light (C). Thioflavin T highlighted the amyloid through immunofluorescence (D). Mass spectrometry detected a peptide profile consistent with ATGFBI-type amyloid deposition.

Conclusions: Though immunofluorescence is usually performed on frozen samples, these cases demonstrate the utility of such techniques on limited FFPE corneal tissue that may be helpful for demonstration of abnormal protein deposition in select, rare cases.

Posters: Peripheral Nerve/Muscle

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Unmasking of neuromuscular paraneoplastic syndrome by pembrolizumab leading to rapid respiratory failure and death

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Background: Paraneoplastic syndromes are rare autoimmune conditions caused by an underlying tumor, most commonly lung cancer. Paraneoplastic neuromuscular syndromes can affect the brain, spinal cord, dorsal root ganglia, peripheral nerves, muscles, or a combination of the above. Treatment involves immunosuppression and managing the underlying neoplasm.

Methods: We present the case of a 69-year-old man with a history of orthopnea, dysphagia, and peripheral neuropathy which began around the same time as discovery of a large polypoid mass in the hepatic flexure of the colon. The following year, he was diagnosed with metastatic colon cancer and started on chemotherapy. Due to disease progression, therapy was eventually changed to pembrolizumab, an immune checkpoint inhibitor. Just over 2 weeks after this, he presented to the emergency department with dyspnea and dysphagia, and these symptoms progressed until his death approximately 1 week later. A full autopsy was performed.

Results: Nodules of Nageotte with neuronal loss and degeneration and lymphocytic infiltration were seen in the dorsal root ganglia, indicating sensory neuronopathy. The phrenic nerves showed multifocal inflammatory demyelination and decreased axons in a fascicle. The diaphragm showed denervation changes. The sural nerves showed moderate and patchy axonal loss with evidence of regeneration and foci of inflammatory demyelination. The psoas muscles showed denervation atrophy, evidence of previous reinnervation, and type 2 fiber atrophy. Additionally, the diaphragm and psoas muscles showed mild myopathic features.

Conclusions: The proximity of the decedent’s malignancy to the onset of his breathing and swallowing problems raises the possibility of paraneoplastic syndrome, which was further supported by the autopsy findings. Pembrolizumab activates the immune system and is known to worsen or unmask paraneoplastic syndromes. The acute worsening of the decedent's known neuromuscular difficulties following initiation of pembrolizumab therapy underscores the importance of being aware of this potentially rapidly fatal complication.

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Late Onset Multiple Acyl-CoA Dehydrogenase Deficiency with Two Variants in ETFDH Gene

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Background: Multiple acyl-CoA dehydrogenase deficiency (MADD) is a rare metabolic disorder divided into three types depending on presentation. Type III is the late onset form and the most common presentation with symptoms including exercise intolerance, muscle pain, or weakness. Workup includes laboratory/ biochemical testing, neuro/muscular imaging, muscle biopsy, and molecular testing. Diagnosis is ultimately established by identification of pathogenic (or likely pathogenic) variants in ETFA, ETFB, or ETFDH genes.

Methods: Our patient is a 28-year-old man with six months of progressive muscle weakness in the proximal lower extremities. He had dysphagia and dysarthria with elevated LFTs and CPK with concern for rhabdomyolysis. Given new dysphagia and muscle weakness, EMG, laboratory studies, imaging, and muscle biopsy were performed. EMG was abnormal, suggesting progressive motor neuron disease versus genetic/metabolic neuron disease.

Results: Laboratory findings showed elevated esterified carnitine level with low total and free levels and organic acids in the urine. Muscle biopsy showed abundant myofiber necrosis associated with lipid globules in myofibers, and myofibers showing advanced stages of myophagocytosis. Genetic testing revealed two heterozygous variants in the ETFDH gene – one likely pathogenic, not paternally inherited [c.1084G>A (p.Gly362Arg)], and one of uncertain significance, paternally inherited [c.1213A>G (p.Lys405Glu)]. These genetic findings coupled with the patient's other findings are consistent with a diagnosis of late-onset MADD.

Conclusions: We report a rare case of late onset MADD. Muscle biopsy showed characteristic findings of lipid storage myopathy. However, these are not consistently found in all cases of MADD. Electron microscopy may yield further diagnostic information from biopsies in these cases. Genetic testing identified variants in the ETFDH genes, allowing for diagnosis of MADD. One of the two variants identified has not been previously reported, and further studies may provide insight into a potential pathogenic role in this condition.

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Anti-SRP antibody-positive immune-mediated necrotizing myopathy diagnosed by reevaluation 40 years after symptom onset

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Background: Immune-mediated necrotizing myopathy (IMNM) is one of the most common forms of idiopathic inflammatory myopathies. However, cases with juvenile-onset can be challenging to distinguish from muscular dystrophy, especially when they become chronic.

Methods: We report a 45-year-old Japanese woman who presented with generalized muscle weakness. Her motor development was normal until the age of 4 years, but by age 5, she exhibited Gowers' sign. Pediatric evaluation at that time revealed proximal muscle weakness, winged scapulae, and gait disturbance, with creatine kinase levels elevated to 6069 IU/L. Biopsy from the left biceps brachii led to the diagnosis of muscular dystrophy. Her condition progressed, necessitating the use of a wheelchair during her elementary school years. At age 41, she was transferred to our department for reevaluation, which revealed symmetric proximal muscle weakness, muscle atrophy, marked spinal deformity, and bilateral pes cavus. Marked myogenic changes were detected in needle EMG study. Muscle MRI showed marked atrophy and fatty infiltration in the proximal muscles.

Results: Re-biopsy from the left tibialis anterior revealed marked myofiber atrophy without evident necrotic and regenerating fibers and inflammatory cell infiltration. Immunohistochemical staining for muscular-dystrophy-related proteins, HLA-ABC, and p62 was normal. However, reevaluation of the first muscle biopsy revealed widespread expression of HLA-ABC and granular sarcoplasmic p62 staining, leading to the diagnosis of IMNM. Despite a negative ELISA screen, anti-signal recognition particle (SRP) antibodies were detected by RNA immunoprecipitation assay.

Conclusions: This case represents a rare instance of juvenile-onset IMNM that went untreated for an extended period. The absence of diagnostic features in the biopsy conducted 40 years after the onset highlights the challenges in diagnosing such cases. Reassessment of initial biopsy specimen and antibody testing can be crucial for accurate diagnosis in similar situations.

Posters: Trauma

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Chronic Subdural Hematoma and the institutionalized Civil War Veteran
H Ames, ; University of Maryland

Background: Chronic subdermal hematoma was a pathologic enigma in the late 19th century, as Virchow himself declared this condition to be of inflammatory, rather than traumatic, origin. Without imaging technology to link injuries to hemorrhage, the granulation tissue - rich with inflammatory cells - was equated to a purulent infection. In 1897, Dr. Isaac Blackburn of St. Elizabeth's Hospital, a government psychiatric institution, published 197 cases of "internal pachymeningitis" in the hospital's annual report. The project is a review of his report with modern knowledge and historical context.

Methods: A large proportion of the patients who died at St. Elizabeth's were current or former soldiers. The autopsy patients in this study were linked to soldiers in the National Park Service's Civil War database. Where possible, these soldiers were linked to pension files, discharge files, and medical records for the appropriate time period.

Results: Eighty-seven of the autopsied soldiers could be linked to a documented service record. Eleven (8%) of these patients had reported gunshot wounds in the service. Additionally, many of these autopsy reports also describe skull fractures, remote contusions, and even one case of a trephination scar from the field. In many of these cases, epilepsy, then considered a psychological condition, was the primary symptom.

Conclusions: These cases show the medical and social impact of deficient and erroneous information regarding symptoms and etiology of chronic subdural hematoma. These soldiers suffered decades of illness for traumatic brain injuries that would be fully treatable following modern standards. Given the technical limitations of 19th century medicine and despite interpretive errors, Dr. Blackburn's thorough observations are interpretable in the modern era in a way that allows us to reflect on the events of history in a new light.

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Reactive Tauopathy Following Severe Traumatic Brain Injury

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Background: The hypothesis that traumatic brain injury (TBI) causes neurodegenerative disease dates back more than 100 years. The support for this concept is based largely on hypothesis-generating studies; large scale epidemiology has shown mixed results, with no evidence that TBI is causal for any specific neurodegenerative disease. Whether TBI initiates a progressive, Alzheimer's disease-like proteinopathy remains an open question.

Methods: A 62-year-old man was found unresponsive and pronounced dead. He had required long term care following an assault requiring neurosurgery and leading to a coma for three months, 22 years earlier. He had been bed-bound since, and could speak only in short sentences. Medical records described residual hemiparesis/quadriplegia (right worse than left), dysarthria, dysphagia, and neurocognitive/communication deficits that were stationary.

Results: The gross brain showed neomembranes and showed multiple foci of encephalomalacia. Of note was a slit-like cavitation with hemosiderin staining consistent with remote shearing injury in the pons. H&E demonstrated neurofibrillary tangles (NFTs), often globose, involving brainstem nuclei adjacent to the lesion. AT8 immunohistochemistry demonstrated predominantly astrocytic tau encircling the lesion, along with NFTs. Immunohistochemistry for 3R and 4R tau showed a mixed 3R/4R tauopathy involving neurons and 4R tauopathy involving astrocytic tau. No cortical tau patterns suggestive of "CTE" were noted. Braak stage was II. There were no amyloid plaques.

Conclusions: The findings demonstrate reactive, predominantly astrocytic tauopathy in the brainstem adjacent to a remote traumatic lesion. This suggests a traumatic etiology to the tau aggregates. The clinical course, however, suggests morbidity dictated by mechanical trauma, with no meaningful role of proteinopathy. This may explain in part the absence of correlation between proteinopathies in "repetitive head impact" scenarios and long-term mental health or neurological problems. The co-localization of trauma and astrocytic tau further suggests that astrocytic tau may be more relevant to trauma than neuronal tau. More research is needed.

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Blunt truths: Innovations in identifying neck injuries from infant head trauma.
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Background: Evidence from radiologic and pathological studies, enriched by case practice, has indicated that blunt head trauma in infants is often associated with concurrent injuries to the neck structures. Initial findings pointed to shearing forces affecting cervical nerves, especially at the dorsal root ganglia, but further research has highlighted additional injuries to other neck structures.

Methods: This study introduces an enhanced ex situ cervical spine evaluation method. Evolving from previous techniques, this method allows for a detailed macroscopic and microscopic examination of the cervical spine, both in its natural state and following spinal cord removal. The method's efficacy in detecting injuries to cervical soft tissues, chondro-osseous structures, and neuroanatomy was assessed.

Results: The application of the advanced ex situ method revealed a broader spectrum of neck injuries than previously detected by conventional in situ examinations. These included, but were not limited to, hemorrhaging or swelling in the nuchal ligament, interspinal ligaments, and the junction between the anterior arch of C1 and the odontoid process.

Conclusions: The enhanced ex situ cervical spine evaluation method offers a significant improvement in identifying neck injuries in cases of infant head trauma. By providing a more comprehensive analysis of the internal neck anatomy, this method improves the accuracy and reliability of diagnosing associated injuries. Its adoption may lead to better-informed conclusions in the intricate field of infant head and neck trauma research.

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Coexistence of CTE and Alzheimer Folds of Tau in the Brain of a Former Soccer Player with Alzheimer Disease: a Neuropathologic and Cryo-EM Study

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Background: Chronic traumatic encephalopathy (CTE) is a neurodegenerative disease associated with repetitive head impacts, often sport-related. CTE can also result from exposure to blast waves. Results of studies that combined neuropathology and cryo-electron microscopy (cryo-EM) of brain tissue of an individual who had played soccer and developed dementia later in life have not been reported. A male, who played soccer for several years in childhood and as a professional between 18 and 21 years of age, began experiencing word-finding difficulties followed by cognitive decline at age 69. At age 71, neuropsychological, neurological, and neuroimaging studies led to the diagnosis of dementia, consistent with Alzheimer disease (AD). He died at age 76.

Methods: The brain postmortem weighed 1,145 grams and showed moderate atrophy. The tissue was studied using histology, immunohistochemistry, and Cryo-EM.

Results: The diagnosis was AD with neuropathologic changes scored as A3, B3, C3, according to the NIA-AA guidelines; however, tau immunohistochemistry, using antibodies AT8, anti-RD3 and anti-RD4, revealed a severe glial pathology in the neuropil and frequently around blood vessels located along the subependymal tissue adjacent to the ventricles. Glial pathology also occurred beneath the pia mater over the surface of brainstem and spinal cord. Frozen specimens of frontal and temporal cortices, amygdala, and hippocampus were used for Cryo-EM, which revealed the presence of tau filaments with the Alzheimer and CTE folds; these were most abundant in amygdala and hippocampus. The latter were areas where neurofibrillary tangles coexisted with tau immunopositive astrocytes and astrocytic processes in perivascular location. Glial tau was best seen by anti-RD4. Of interest was the presence of numerous ghost tangles, that were best recognized by anti-RD3 in the amygdala and the hippocampus.

Conclusions: This study presents the first Cryo-EM results of the coexistence of AD and CTE tau filaments in a former soccer player’s brain. (KN, CQ: Equal contribution)

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University of Washington Experience with Chronic Traumatic Encephalopathy (CTE) Pathology in Community-associated Traumatic Brain Injury

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Background: The characteristics of chronic traumatic encephalopathy (CTE) pathology have primarily been investigated in cohorts of brain donors focusing on specific exposures such as contact sports and military history or in cohorts examining aging and neurodegenerative disease.

Methods: At the University of Washington (UW), we have established strong relationships with the local medical examiners to obtain brain donation and better evaluate community-associated traumatic brain injury (TBI) with a range of exposures. In this Pacific Northwest Brain Donor Network, we evaluated 112 cases with a history of TBI and found 28 cases have a diagnosis of CTE.

Results: These patients range in age from 20 to 77 years old (mean 53) and all 28 are male. The main trauma exposures include contact sports (14/28), assault (12/28), motor-vehicle accidents (8/28) and military service (8/28) with 3 patients with known blast exposure(s); a little over half of cases had multiple exposures (16/28). We examined tau deposition in 17-32 cortical regions with sulci per case and observed 1 to 16 CTE lesions per case (mean 3.94) and 20 cases had multiple lesions (71.4%). We observed the highest number of lesions in the inferior parietal lobule (17.1% of all lesions), superior frontal gyrus (16.2%) and superior/middle temporal gyri (15.3%). 16 patients had low stage (60.8%) and 11 had high stage CTE (39.2%). When we examined the incidence of astrocytic tau in canonical CTE lesions, we found that 16 patients (57.1%) had co-occurrence of tau in both neurons and astrocytes (N+A), while 12 had only neuronal tau (42.9%). Interestingly, the presence of N+A versus neuronal only was independent of age, vascular disease, Thal, C-score or LATE stage.

Conclusions: In summary, CTE pathology in this community-based cohort is higher than expected at 25% of cases and these cases often show neuronal only lesions without clear association with other pathologies or age.

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Histologic and Ultrastructural Evidence of Astrocyte Injury Following Blast Exposure

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Background: Astrocytes functions include those of water/ion homeostasis (particularly via Aquaporin-4 [AQP4]), and blood-brain barrier integrity. Many of these occur at the level of astrocyte endfeet (AEs), which envelope blood vessels (BVs). We have demonstrated astrocyte dysmorphology, characterized by truncated, beaded processes, at cortical grey-white interfaces in blast-exposed human brains.

Methods: We performed EM analysis of AEs enveloping BVs at frontal grey-white interfaces in brains from five blast-exposed Service Members with beaded astrocyte dysmorphology based on histology (GFAP), paired with controls. BVs enveloped by AEs were identified, and BV and AE surface areas and diameters were compared. To further characterize astrocyte dysmorphology at cortical grey-white interfaces in blast-exposed cases versus controls, we performed immunohistochemistry for astrocyte proteins GFAP, AQP4, and connexin-43.

Results: EM analysis revealed increased AE:BV surface area ratios ($t(4)=2.796$, $p=0.049$) in blast cases. There was reduced occurrence of full ensheathment of BVs by AEs in blast cases ($t(4)=3.556$, $p=0.024$), and a general increase in BV thickness in blast cases. Morphologically, AEs showed frequent fragmentation and irregularities in thickness/contour. There was altered astrocyte immunoreactivity at the grey-white interface of blast cases: controls showed two dominant populations, labeling as either GFAP+ or AQP4+ only, whereas blast samples showed a third dominant population, particularly dysmorphic astrocytes, co-labeling with GFAP and AQP4. The GFAP+ beaded processes in dysmorphic astrocytes showed additional AQP4 staining, and co-labeling with phosphorylated connexin-43, indicating an inflammatory phenotype.

Conclusions: We show histomorphologic and ultrastructural alteration in astrocytes at cortical interfaces in blast-exposed brains. These findings indicate direct damage to astrocytes by blast, and potentially compromised function. Disclaimer: The information/content and/or conclusions do not necessarily represent the position or policy of, nor should any endorsement be inferred on the part of, USU, the DoD, the U.S. Government, or the Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc.

Posters: Tumors: Glial

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Astrocytoma, IDH-mutant, CNS WHO grade 3 with novel non-canonical IDH2 mutation

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Background: IDH-mutant astrocytoma is an infiltrating IDH1- or IDH2-mutant glioma with frequent ATRX and/or TP53 mutations, MGMT promoter hypermethylation, and absence of 1p/19q codeletion. Approximately 83-91% of IDH-mutant gliomas harbor the canonical IDH1 p.R132H mutation, followed by IDH1 p.R132C, p.R132S, and p.R132L. IDH2 mutations are uncommon, p.R172K being the most frequent, with rare cases harboring p.R172W, p.R172M, and p.R172S.

Methods: We discuss the case of a 32-year-old male with headaches, nausea, and vomiting found to have a 4.2-cm, T2/FLAIR hyperintense, solid and cystic left posterior temporal mass with regional enhancement on MRI. Craniotomy with resection was performed with histologic evaluation, including immunohistochemistry, next-generation sequencing (NGS), MGMT assessment by methylation-specific PCR and pyrosequencing, 1p/19q FISH, and tumor methylation profiling at the National Institutes of Health.

Results: Histologic sections showed an infiltrating astrocytoma without definitive microvascular proliferation or necrosis. Up to 8 mitoses per 10 HPF were identified. The Ki67 proliferation index was elevated at 10-15%. By immunohistochemistry, the tumor was positive for GFAP and Olig2, negative for IDH1 p.R132H mutant protein expression, and demonstrated retained nuclear ATRX expression. 1p/19q was intact by FISH analysis. NGS revealed an uncharacterized IDH2 p.R172_H173delinsSN variant. Initial MGMT testing was negative for promoter hypermethylation. Tumor methylation profiling matched with high confidence to the class “IDH glioma, subclass astrocytoma” and confirmed lack of MGMT promoter hypermethylation. He received adjuvant radiation and temozolomide. Surveillance brain MRI fifteen months post resection demonstrated no evidence of recurrence or progression.

Conclusions: We present a case of IDH-mutant astrocytoma with an atypical molecular presentation, including a previously uncharacterized IDH2 mutation, retained ATRX expression, and lack of MGMT promoter hypermethylation. The prognostic significance of the latter two findings is unclear, with conflicting data in the literature. Though it has not been biochemically or functionally validated, tumor methylation profiling is supportive of this novel IDH2 variant as tumorigenic.

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Mechanisms of glioma induced neuronal hyperexcitability

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Background: Cognitive impairment is a common and debilitating symptom in patients with diffuse glioma. Recent evidence from the emerging field of glioma neuroscience suggests that infiltrating tumor cells remodel the underlying neural circuitry, leading to hyperactive circuits and cognitive impairment while simultaneously promoting tumor progression. However, the precise mechanisms by which tumor cells modulate the underlying neural circuitry remain unclear.

Methods: We studied the intrinsic biophysical properties of neurons and spontaneous activity patterns using whole-cell patch clamp recordings in two model systems: 1) a patient-derived glioblastoma (GBM) xenograft mouse model and 2) primary mouse neuron-patient tumor cell co-culture. In the xenograft mouse model, we prepared acute slices from 4–5-month-old mice and recorded from both peritumoral neurons and neurons from the contralateral, non-injected hemisphere as a control. For our in vitro co-culture model, E17 mouse brains were dissociated to prepare cortical neuronal cultures, primary GBM cells were added to the mouse cortical neuronal culture after 6 days (DIV 6), and we performed patch-clamp recordings on neurons from co-cultured and primary cell only culture at DIV 11-14.

Results: Our preliminary data suggest that neurons adjacent to tumor xenografts tend to have increased excitability at the level of both single cells and neural circuits. Specifically, tumor-adjacent neurons demonstrate a higher firing rate for a given current injection and increased spontaneous synaptic activity. The impact of specific gene knockdown on these phenotypes is currently under investigation.

Conclusions: Our preliminary data suggests that peritumoral neurons show increased intrinsic excitability and enhanced synaptic activity. Insights into the neural mechanisms underlying cognitive impairment in patients with diffuse glioma may identify therapeutic targets to improve cognition and slow tumor progression.

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Digital Spatial Profiling of H3 G34-mutant Gliomas Reveals Interneuron Gene Signatures and Potential Markers of Invasiveness

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Background: Diffuse hemispheric gliomas with H3 G34 mutations occur in the young (median age of diagnosis 15.8 years), and are postulated to arise from interneuron precursors. To date, gene expression profiling of G34-mutant gliomas has been conducted through bulk RNA-seq and scRNA-seq methods, but given their infiltrative growth pattern, the incorporation of spatially resolved data may provide further insights.

Methods: We conducted a spatially-resolved whole transcriptome analysis of three G34R-mutant glioma samples using Nanostring's digital spatial profiler, GeoMx. Targeted sampling was performed from regions of solid tumor, infiltrative edge, and adjacent histologically uninvolved cortex ("normal"). An 18,000 gene target cohort was first analyzed unfiltered, then subsequently filtered into relevant gene subsets including markers of interneuron lineage and established markers of glioma invasiveness. We tested for the existence of statistically significant differential expression of genes among the three tumor density categories (normal, infiltrative, solid).

Results: Established interneuron-associated genes were expressed within regions of tumor involvement, as anticipated. ANOVA and Post Hoc analysis (Tukey HSD) revealed 36 differentially expressed neurodevelopmental and/or glioma-associated genes, such as PDGFRA, after pairwise comparison (normal/solid; normal/infiltrative). Univariate linear regression analyses showed significant effect sizes by tumor density on the expression of the 36 targets respectively. Focused analysis of invasive edge samples showed significant ($p < 0.05$) increased expression of specific genes known to be associated with cell proliferation, cell migration, and/or poor prognosis (FABP7, SLC1A3, GNB1, and MT1X).

Conclusions: Spatially-resolved expression data reliably indicates both the existence of interneuron gene signatures among G34R-mutant glioma samples and differential expression of multiple genes associated with the tumor's invasive edge. Continued research will focus on further validating the data set through the analysis of contributed variance by random effect factors, gene set enrichment analysis, and incorporation of tumor exome sequencing data to determine effects attributable to underlying mutations.

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EGFR transcript variants: potential diagnostic biomarker for glioblastoma, IDH-wildtype?

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Background: EGFR transcript variants (EGFRtv) were primarily described in morphologically defined glioblastoma, IDH-wildtype (GBM-IDHwt), often with EGFR amplification. We investigate the distribution of EGFRtv among central nervous system (CNS) tumors and the potential diagnostic utility of EGFRtv as a molecular biomarker for GBM-IDHwt.

Methods: CNS tumors clinically tested by a 187-gene DNA mutation and RNA fusion/tv targeted neuro-oncology next-generation sequencing panel (2018-2022) with EGFRtv were selected. A subset had chromosomal microarray (n=101) and/or methylation array (n= 10) data.

Results: Four-hundred-thirty-one cases were identified. Median age was 60 years (range, 9-92; < 18, n=2), with 1F:1M, and predominant non-midline location (393/412, 91%; 19 unavailable). All tumors were histologically reported as high-grade gliomas: GBM-IDHwt (n=426, 99%), astrocytoma, IDH-mutant, CNS WHO grade 4 (n=2), diffuse pediatric-type high-grade glioma, IDHwt and H3wt, NOS (n=1), diffuse midline glioma, H3 K27M-altered (n=1), and high-grade glioma, consistent with EP300::BCOR fusion glioma by methylation (n=1). EGFRtv comprised vIII (exon 2-7 deletion; 83%), vIVa (exon 25-27 deletion; 23%), vII (exon 14-15 deletion; 10%), and vIVb (exon 25-26 deletion; 9%). EGFRtv co-occurrence was observed in 20% (n= 88, mostly vIII+vIVa, n=54), concomitant EGFR mutations in 22% (n=95), and additional fusions in 11% (n=46; EGFR fusions, n=40) of cases. Concurrent molecular diagnostic biomarkers in GBM-IDHwt included TERTp mutation (82%), EGFRamp (99/101, 98%), +7/-10 (66/101, 65%). By methylation profiling using NCI/Bethesda v2 classifier, all 8 GBM-IDHwt matched to superfamily glioblastoma (7 matched and one suggested to GBM_RTK_II class) and one astrocytoma, IDH-mutant matched to class A_IDH_HGG. Three (0.7%) cases (all GBM-IDHwt) failed (n=1) or had insufficient specimen (n=2) for mutation testing.

Conclusions: Our findings indicate that EGFRtv exclusively occur in high-grade gliomas, confirming that EGFRtv is a hallmark of GBM-IDHwt. Although EGFRtv rarely occurs in other CNS tumor types, EGFRtv testing may be diagnostically useful in cases with suspected GBM-IDHwt, especially when available tissue is limited.

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Study of Neurotransmission Signaling Pathways in Glioblastoma

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Background: Glioblastomas (GBM) are the most common malignant primary brain tumors and are incurable. Identifying cancer cell survival- and proliferation-associated mechanisms may help uncover new therapeutic targets. Several lines of evidence suggest that gamma-aminobutyric acid (GABA) and glutamate, along with other molecules pertaining in neurotransmitter signaling, are involved in gliomagenesis. Their communication networks within the tumor microenvironment (TME) remain nonetheless poorly defined. We hypothesize that the modulation of GABA, glutamate, and calcium signaling pathways may control tumoral aggressivity. Here, we aimed at identifying vulnerable biomarker genes for GBM, and assessing their validity in in vitro and clinical studies.

Methods: scRNAseq data of nine resected new-diagnostic, and five recurrent IDH-wildtype GBM was used to characterize cell populations, and gene expression of the TME using a novel algorithmic cell type identification approach. Vulnerabilities were identified through gene mutability analysis following GABA, glutamate, and calcium gene extraction. A GABA-treated GBM cell line (U87) underwent RNA sequencing followed by differential and enrichment analysis. Fifty resected GBM samples were utilized to associate extracted new genes, as well as previous markers C5AR1, VGAT, GAD1, and GABAB, to histologic characteristics comprising necrosis, angiogenesis, and infiltration. Immunohistochemical alignment between our targets and clinical markers, including MIB1, was performed.

Results: scRNAseq data unveiled significant cellular heterogeneity within the environment, with distinct population variations as the tumor progressed. Neurotransmission expression was highly dependent on cell and tumor type. However, a few dozen genes consistently displayed notable regulatory patterns. Some transcriptional responses varied enough to represent possible vulnerabilities. In vitro RNAseq data suggested that GABA regulates the transcription of cancer-associated pathways, such as survival and metabolism. Comparison of RNAseq and scRNAseq data demonstrated significant modulation of identified genes in the TME, correlating with increased GABA levels in MIB1-rich zones in histopathological analyses.

Conclusions: This study introduces GABA-, glutamate-, and calcium-associated biomarkers as GBM therapeutic vulnerabilities.

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Histologic and Molecular Features of Methylation Class Diffuse Leptomeningeal Glioneuronal Tumor

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Background: Genome-wide DNA methylation profiling can help identify many types of CNS tumors, including diffuse leptomeningeal glioneuronal tumor (DLGNT). Due to the infrequency of this entity, DLGNT remains poorly understood.

Methods: To help resolve uncertainty regarding DLGNT, we examined epidemiologic, histologic, and molecular features of 31 (20 newly profiled, 11 publicly available) tumors matching to DLGNT by the DKFZ classifier (v12, score >=0.85).

Results: Of these 31 tumors, 25 matched to DLGNT subtype 1 and six to subtype 2. Subject median age was 14 (range 2-44), with 15 females and 16 males. Spinal cord was the most frequently involved site (n=21), but intracranial lesions were observed (n=5), and one tumor was found in the lateral ventricle. Leptomeningeal involvement was noted in only three of 11 tumors with available imaging descriptions. In the subset of cases available for histologic review (n=18), oligodendrocyte-like morphology was present in over half (n=11), and piloid features (n=10), eosinophilic granular bodies (n=9), and myxoid stroma (n=8) were also common. Microvascular proliferation was uncommon (n=5), and necrosis was rare (n=2). Chromosome 1p loss was observed in 28 (90%), chromosome 1q gain was observed in 11 (35%), and 1p/19q co-deletion was observed in 18 tumors (58%). In the subset of tumors with fusion testing (n=15), KIAA1549::BRAF fusion was present in 12 (80%), and two harbored alternative MAPK pathway activating alterations (one QKI::RAF1 fusion, one BRAF p.V600E mutation). Four additional tumors harbored BRAF mutations, and one additional tumor harbored a break-point in BRAF without a known fusion partner. In nine subjects with clinical follow-up, median overall survival was 6.4 years (range 2 months to 19 years) with one death after 3.1 years.

Conclusions: Presentation of DLGNT without noted leptomeningeal involvement appears common. Further characterization of subject outcomes will help clarify the clinical behavior of DLGNT.

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Patterns of N6-Methyladenosine RNA Modifications in the Progression Disease in Glioblastoma

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Background: In the realm of glioblastoma (GBM) treatment challenges, the presence of tumor heterogeneity is the result of variability in molecular modification. N6-methyladenosine (m6A) is the most abundant regulator in RNA modifications. Our investigation is thorough into the m6A methylation sites in gene expression patterns of Progression Disease (PD) versus pseudo-progression disease (psPD) in GBM.

Methods: We retrospectively evaluated 92 GBM patients (PD=59; psPD=33) who were confirmed pathologically as either recurrent glioblastoma or reactive changes in Discovery (n=36) and did not pursue second resection confirmation in Validation (n=56) cohorts. Molecular analysis involving m6A RNA modifications was performed by RNase MazF by Arraystar m6A Single Nucleotide Array technique. Calculating fold change and p-values for differential methylation transcript (DMT) and expression gene (DEG) was conducted for each m6A transcript, followed by enrichment analysis, protein network interactions, and constructing gene co-regulation interactions in clinical patterns applying Weighted Gene Correlation Network analysis.

Results: No differences were revealed in clinical, and survival-related clinical biomarkers such as EGFR, ki-67, MGMT, and p53 between the groups among cohorts. Quantile Normalization with the ComBat method was employed to mitigate the effects of batch and normalize the data enhancing results precision. We observed 87 transcripts differential methylated, and 14 of those were related differentially gene expressed. The GO and KEGG enrichment analysis identified histone monoubiquitylation as the most significant biological process associated with m6A modification. Notably, the magenta module network highlighted key co-expressed genes, including SMAD3 and BCL9L, which regulate transcription, vesicle organization, and Wnt signaling in GBM among the PD group. In addition, the number of m6A site-specific was not associated with DEG by Pearson Correlation.

Conclusions: Our study brings to light the process of m6A RNA methylation unveiling intricate links to biological pathways and gene expression in GBM among PD patients, enhancing the management for prognostic assessment and therapeutic approaches.

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Pediatric Cerebellar IDH1 R132H-Mutant Astrocytoma

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Background: Although gliomas as a group represent the most common pediatric brain tumors, IDH-mutant astrocytomas are uncommon, particularly in children under age 10 years.

Methods: Herein we present an unusual glioma harboring IDH1 R132H mutation arising within the cerebellar vermis of a 6 year old male.

Results: He presented with several weeks of difficulty making out fine print at school, but no balance issues or nausea / vomiting. Ophthalmologic examination detected bilateral papilledema, prompting neuroimaging studies. Magnetic resonance imaging (MRI) of the head documented a large T2-hyperintense mass with mild enhancement centered in the cerebellar vermis. There was additionally ventriculomegaly and multiple areas of nodularity within the lateral and third ventricles. MRI of the spine detected leptomeningeal enhancement at cervicothoracic levels. Surgical resection yielded tissue for pathologic examination which demonstrated a moderately cellular astrocytic neoplasm with variably solid to infiltrative growth pattern. Areas with alternating compact and loose / microcystic architecture were present, mimicking pilocytic astrocytoma. There was abundant microcalcifications and focal hyalinization, and Rosenthal fibers and eosinophilic granular bodies were absent. Mitotic figures were rare. Microvascular proliferation and necrosis were not seen. Lesional cells were diffusely positive for GFAP, SOX10, and OLIG2, with blush synaptophysin positivity. Immunostains for ATRX, p53, H3 K27M, and BRAF V600E yielded wildtype results, whereas IDH1 R132H-specific immunostain was diffusely positive. Whole exome sequencing confirmed IDH1 R132H mutation together with in frame insertion mutation of PDGFRA. No pathologic gene fusions or copy number alterations were detected. The lesion could not be assigned to a defined methylation class, though was potentially suggestive of low grade glial / glioneuronal tumor. MGMT was unmethylated. Cerebrospinal fluid taken from ventricular drain prior to resection yielded tumor fragments similar to the cerebellar glioma.

Conclusions: To our knowledge, pediatric cerebellar IDH1 R132H-mutant low grade astrocytoma has not previously been described.

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IDH-Mutant Astrocytoma in Persons Age 55 year and older: Survival differences vs younger age group

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Background: Mutations in the isocitrate dehydrogenase (IDH1/2) genes are common genetic alterations predictive of a better outcome compared to IDH-wildtype diffuse astrocytomas. Although the peak incidence is in young and middle aged adults , IDH1/2 mutations can occasionally present in persons age 55 years and older. Few studies have reported the clinical, histologic, and prognostic differences in tumors in those age >55 years. We now extend our original studies on this topic (JNEN 2017;76(2):151-154).

Methods: Search of databases for IDH-mutant astrocytoma, 2014-2024 with follow up. Survival, date of death, or recurrence at last follow-up were noted. Cohorts were stratified into adults < 55 years versus >/=55 years.

Results: Of the 78 identified patients, 51 were < 55 and 27 >/=55 years. The latter cohort comprised 10 WHO grade 2, 6 WHO grade 3, and 11 WHO grade 4 tumors. When equal grades were compared by Kaplan-Meyer survival analysis, those >/=55 years showed a worse prognosis, although differences were relatively minor. Chart review indicates similar treatment regimens for both cohorts with standard external beam radiotherapy and temozolomide (Stupp protocol). Thus, treatment differences were not apparent. For those patients in either cohort with at least 5 years follow-up, 5-year survival was 38% in those >/=55 years and 66% in those < 55 years.

Conclusions: Despite CNS WHO 2021 noting “adverse prognosis in patients >/=55 years”, in our cohort, survival of 5 years or more was seen in over 1/3rd of patients >/=55 years. Based on our chart review, it appeared that a high percentage of the older patients at our institution were able to receive optimal therapy comparable to younger aged patients. However, factors associated with somewhat adverse prognosis in patients >/=55 years require further study.

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EGFR/CEP7 High Polysomy is Separate and Distinct from EGFR Amplification in Glioblastoma as Determined by Fluorescence In-Situ Hybridization

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Background: No standardized guidelines exist for determining EGFR amplification or gain of chr 7. Clearly defined criteria for establishing the presence of EGFR amplification can help standardize the diagnosis of GBM and may be critically important as additional therapies are developed targeting EGFR.

Methods: We identified 1,143 cases analyzed by FISH. An EGFR/CEP7 copy number of ≥ 5 with ratio < 2 was utilized as our cut-off criteria for high polysomy. Patient information, tumor characteristics, molecular test results, and clinical outcome data was collected and analyzed.

Results: The highly polysomic cases comprised 1.9% (22/1,143) of the total FISH results. Four cases had insufficient clinical data and the remaining cases were diagnosed as GBM, IDH-wildtype, WHO Grade 4 (n=15), IDH-mutant astrocytoma (n=2), and a high-grade glial neoplasm, NOS (n=1). Clearly EGFR amplified and unamplified cases were identified and used as control groups. The median age between the three groups of amplified, highly polysomic, and non-amplified were 62, 50.5, and 62 years, respectively. NGS was available on 3 highly polysomic cases. All 3 of the highly polysomic cases had a TP53 mutation and only 1 of the 3 cases carried a TERT promoter mutation. The median survival was 42.86, 66.07, and 41.14 weeks for the amplified, highly polysomic, and non-amplified groups, respectively.

Conclusions: Highly polysomic EGFR and CEP7 cases are rare and only comprise ~1.9% of cases submitted to a major reference lab. In our small sample group they appear to be dissimilar to amplified tumors that have been defined by an EGFR/CEP7 ratio of ≥ 2. These findings support distinguishing high polysomy cases from outright EGFR amplified cases. We recommend calling these cases as non-amplified or indeterminate with a comment describing that if the diagnosis or treatment hinge on the EGFR status additional testing should be undertaken.

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An Institutional Experience of Methylation Profiling of Unusual Historical Cases: Solving Mysteries Up to 46 Years After Initial Diagnosis

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Background: We highlight instances where DNA methylation profiling proved pivotal to resolve challenging cases.

Methods: Methylation profiling was performed at the NIH.

Results: A 33 year-old experienced headaches and word-finding difficulty secondary to a left frontal enhancing solid/cystic mass. Histology revealed nested cells with clear cytoplasm, foci of sclerosis, rare rosettes, mitoses, and necrosis. The original diagnosis was malignant astroblastoma. The tumor recurred multiple times where diagnosis alternated between malignant astroblastoma and anaplastic ependymoma, sometimes with both entities in the diagnostic line. Sixteen years later, the patient requested diagnostic clarification. Methylation profiling matched the tumor to the astroblastoma class with suggestion of an MN1 fusion. A 62 year-old experienced leg weakness with subsequent fall. Imaging showed a circumscribed enhancing and hemorrhagic right parietal lesion. A diagnosis of ependymoma, grade 2 was rendered with recurrence seven years later. Histology showed a glial tumor with ependymal features. Sequencing revealed an IDH-wildtype tumor with TERT promoter and FGFR1 mutations. Numerous copy number alterations, including loss of chromosome 13, were identified. Methylation profiling categorized this tumor as high-grade glioma with pleomorphic and pseudopapillary features, a new ependymoma-like entity not in existence at the time of original diagnosis. A 48 year-old with reported history of optic chiasm pilocytic astrocytoma subtotally resected at age 2 status/post radiation without interval follow-up presented with new vision abnormalities. A sellar/suprasellar mass, concerning for recurrence, was identified. Histology showed monotonous round cells with neuronal phenotype, some with perinuclear clearing, abundant eosinophilic granular bodies, focally increased mitotic activity, and KIAA1549::BRAF fusion with TERT promoter mutation. A diagnosis of anaplastic pilocytic astrocytoma was entertained. Methylation profiling revealed a match to diffuse leptomeningeal glioneuronal tumor. Co-deletion of 1p/19q was subsequently identified.

Conclusions: Methylation profiling solved a diagnostic dilemma, re-categorized a tumor, and changed a diagnosis underscoring its importance as an adjunct modality for characterization of unusual cases.

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Granular cell astrocytoma, IDH-wildtype, CNS WHO grade 4 involving the optic nerve and mimicking a histiocytic neoplasm: A Case Report

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Background: Granular cell astrocytoma/glioblastoma (GCA) is an astrocytic neoplasm with histopathologic features resembling histiocytes but with molecular findings similar to glioblastoma, IDH-wildtype, CNS WHO grade 4.

Methods: Electronic medical records were used to obtain clinical history and radiologic findings. Histopathologic evaluation and next generation sequencing (NGS) were performed at UCSF.

Results: A 46-year-old man presented with worsening left eye vision and headache. Brain MRI demonstrated an expansile optic pathway mass including the prechiasmatic optic nerves, optic chiasm, and optic tracts ganglia, characterized by FLAIR MRI signal abnormalities, contrast enhancement and elevated perfusion. A biopsy demonstrated small fragments of CNS tissue involved by a solid-looking moderately cellular neoplasm with loosely cohesive cells. Atypical cells had enlarged nuclei, prominent eosinophilic nucleoli and abundant amphophilic vacuolated cytoplasm. Most tumor cells were CD68 and S100 immunoreactive with BCL-1 expression in a subset of tumor cells. GFAP, OLIG2, PAS, neurofilament, CD1a, OCT2, BRAF V600E, ALK, HMB45, SOX10, desmin, SMA, and CAM5.2 stains were negative, suggestive of a histiocytic neoplasm. NGS demonstrated a nonsense mutation in PTEN, a hotspot mutation in the TERT promoter, focal 12q amplifications of the CDK4 and MDM2 genes, and combined whole chromosome 7 gain and 10q loss. An integrated final diagnosis of granular cell astrocytoma (GCA)/glioblastoma, IDH-wildtype, CNS WHO grade 4 was rendered.

Conclusions: GCA can have histiocytoid/epithelioid morphologic features with solid growth, CD68, S100, and EMA immunoreactivity, yet lack of GFAP and OLIG2 expression, leading to potential misdiagnosis. Molecular findings of GCA are identical to glioblastoma, IDH-wildtype, CNS WHO grade 4, and the molecular profile of this case confirmed the correct diagnosis.

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Chordoid glioma with strong keratin expression and lacking mucinous stroma: a diagnostic challenge

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Background: Chordoid glioma is a well-circumscribed glial neoplasm with cords of GFAP-expressing epithelioid cells with intervening mucinous stroma and with recurrent PRKCA p.D463H missense mutation. Less commonly, tumors show sheet-like organization without significant mucinous stroma.

Methods: A highly diagnostically challenging case underwent next generation sequencing (NGS) and DNA-methylation profiling (DNAMP).

Results: A 33-year-old man presented with a multilobulated, enhancing suprasellar mass. Histopathology showed a solid epithelioid neoplasm arranged in sheets and focal papillary architecture. Tumor cells were relatively uniform, with ovoid nuclei, small nucleoli, fine chromatin, and moderate amphophilic cytoplasm. Rare foamy cells were seen. No mitoses, necrosis or microvascular proliferation was identified. Tumor cells showed focal weak GFAP, patchy weak cytoplasmic EMA and D2-40 and patchy strong CK7 immunoreactivity. Rare tumor cells showed TTF1, p63 and S100 positivity. Mucicarmine, synaptophysin, OLIG2, BRAF V600E, CK20, CAM5.2, transthyretin and SSTR2A stains were negative. NGS demonstrated PRKCA p.D463H missense mutation and a hotspot mutation in MAP2K1. DNAMP indicated a match to chordoid glioma.

Conclusions: Chordoid gliomas may rarely present as a solid/papillary growth tumor composed of keratin-positive epithelioid cells with no intervening mucinous stroma and only weak-to-absent GFAP expression. This histopathology is challenging to distinguish from meningioma, choroid plexus neoplasms, craniopharyngioma and metastatic carcinoma. The tumors originating from the ventricular zone/lamina terminalis such as chordoid glioma can have TTF1 immunoreactivity, and the combination with GFAP immunoreactivity can support a chordoid glioma diagnosis even in the absence of other characteristic features.

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Natural history of an adult thalamic diffuse midline glioma without clinical intervention
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Background: Diffuse midline glioma (DMG), H3 K27-altered are aggressive glial tumors occurring both in children and adults. DMG occurring in the adolescent and adult population tends to occur in the spinal cord and thalamus, rather than the brainstem, and has been shown to have overall longer survival than in pediatric patients. We present a case of DMG with H3 K27M mutation with 7-year survival without treatment.

Methods: A 35-year-old woman initially presented in 2016 with headache. MRI brain performed at that time revealed a T2 hyperintense lesion involving the medial left thalamus measuring approximately 8 mm in diameter. No follow up was pursued until September 2023. MRI brain performed at this time demonstrated a 4.2 x 3.1 x 2.5 cm T2/FLAIR signal non-enhancing lesion encompassing the left thalamus, extending to the left basal ganglia and pons. The differential diagnosis included low-grade glioma versus demyelinating process. The patient presented for stereotactic biopsy.

Results: Biopsy of the thalamic mass revealed an infiltrating astrocytic glioma with low mitotic index and without necrosis or microvascular proliferation. By immunohistochemistry, the tumor cells were positive for GFAP and OLIG2; IDH1 p.R132H immunohistochemistry was negative for mutant protein, and ATRX showed retained nuclear staining. H3 K27M immunohistochemistry was positive. Mutational analysis by a 225-gene next-generation sequencing panel confirmed the p.K28M alteration in H3-3A; additional alterations in NF1 and TP53 were identified. No alterations in the MAPK pathway, including BRAF or FGFR, were identified.

Conclusions: To our knowledge, this is the first case of diffuse midline glioma, H3 K27M-altered, with unusually prolonged survival in the absence of co-occurring MAPK alterations and without clinical intervention.

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Novel QKI-TOX fusion in a pediatric high-grade glioma

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Background: Pediatric high-grade gliomas (pHGG) are a heterogeneous group of tumors that are relatively under-investigated. Herein we report a case of a pHGG harboring a novel QKI-TOX fusion. The QKI gene, known for its role in RNA splicing and involvement in myelination and angiogenesis, and the TOX gene, crucial for immune system regulation and lymphocyte development, have been independently studied for their roles in cancer and neurobiology, but their fusion is not yet reported in gliomas, although QKI gene fusion with MYB is characteristic of angiocentric glioma.

Methods: We report a case of a 12-year-old girl who presented with seizures and was found to have a large heterogeneous intra-axial left parietal mass with patchy enhancement on imaging. She underwent craniotomy for tumor resection. Histologic evaluation and ancillary studies were performed for tumor characterization.

Results: Routine histology showed a diffusely infiltrative moderate- to markedly-hypercellular glioma with variable appearance, including fibrillary and piloid phenotypes, microcysts with myxoid material and frequent psammomatoid calcifications. Few areas had perivascular pseudo-rosetted arrangement. Focally increased pleomorphism, mitoses (up to 5/10 hpf) and incipient necrosis were identified. The tumor demonstrated reactivity for glial markers but was negative for neuronal and ependymal differentiation and had variable Ki-67 (< 1% to 17.4%). Mutant IDH1(R132H), H3K27M, and BRAF(V600E) were negative and ATRX was retained. Targeted DNA sequencing showed no reportable genomic alterations, although chromosomal losses of 13 and 22 were present. RNA exome fusion panel identified QKI-TOX fusion, and no match was found on methylome profiling.

Conclusions: This report identifies a novel QKI-TOX fusion in pHGG setting, underscoring the significance of using an integrated approach for diagnosis, as well as highlights an unmet need in our understanding of pHGG. Further investigation is warranted to determine this fusion's role in tumorigenesis and its potential diagnostic or therapeutic role.

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Multidimensional characterization of supratentorial adult-type diffuse gliomas that migrate to the brainstem

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Background: We previously showed that cerebral diffuse gliomas usually spread to the brainstem at the final stages of disease, and that this, not tonsillar herniation, most likely accounts for brainstem-type symptoms commonly observed at the end of life (PMID: 31711239). It remains unknown whether glioma cells that spread to the brainstem are different from glioma that remains near the original tumor site ("stationary").

Methods: We analyzed original premortem tumors, postmortem stationary tumors, and postmortem tumors that infiltrated the brainstem from 20 patients (5 IDHmut astrocytomas, 15 IDHwt GBM). Each site was analyzed for expression of key markers by immunohistochemistry, genomic DNA methylation profiling (including deconvolution analysis of immune populations), and whole exome sequencing.

Results: Compared to stationary tumors, glioma subclones that migrated to the brainstem had higher Ki67 proliferation indices (P=0.003), expressed fewer mesenchymal markers (P< 0.001), lost OLIG2 expression (P< 0.001), and were normoxic as indicated by HIF1a (P=0.03). Brainstem tumors also had fewer admixed macrophages and lymphocytes (P< 0.05), except for patients who received immunotherapy during their disease courses. In those cases, lymphocytes and macrophages were elevated in the brainstems (but not in stationary tumors) relative to the brainstems of patients without immunotherapy (P< 0.01). Microvascular proliferation was not found in any glioma-infiltrated brainstems. Whereas IDHmut astrocytomas retained their methylation profiles, 40% of the RTK-II subset of IDHwt GBM shifted methylation subclass, most commonly to RTK-I. There was no consistent pattern of mutations in brainstem subclones vs. premortem or stationary tumors, and tumor mutation burden was similar in all three settings (P=0.17).

Conclusions: Together, these data suggest that, while brainstem subclones mostly retain the molecular patterns of their supratentorial origins, protein expression shifts in consistent patterns, perhaps as a consequence of the glioma cells having moved to a new microenvironment. This study sheds new light on the characteristics of end-stage glioma spread.

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The G-quadruplex stabilizer CX-5461 effectively combines with ionizing radiation to selectively target ATRX-deficient malignant glioma

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Background: Mutational inactivation of α-thalassaemia/mental retardation X-linked (ATRX) represents a defining molecular feature in large subsets of both adult and pediatric malignant glioma, where standard-of-care management has remained largely stagnant for the past 30 years. ATRX deficiency gives rise to abnormal G-quadruplex (G4) DNA secondary structures at GC-rich regions of the genome, altering chromatin accessibility of and enhancing DNA damage. Building on earlier work, we sought to assess the extent to which pharmacological G4 stabilization would selectively enhance DNA damage and cell death in preclinical models of ATRX-deficient glioma.

Methods: Deploying the G4 stabilizer CX-5461 in patient-derived glioma stem cells (GSCs), we evaluated efficacy as both a single agent and in combination with ionizing radiation (IR), a central element of current treatment standards. Flank and intracranial xenograft paradigms were employed. We also applied genomic/epigenomic profiling and functional assessments in vitro to further elucidate the CX-5461 mechanism of action.

Results: We found that CX-5461 promoted dose-sensitive lethality in ATRX-deficient GSCs relative to ATRX-intact controls. Mechanistic studies revealed that CX-5461 disrupted histone variant H3.3 deposition, enhanced replication stress and DNA damage, activated p53-independent apoptosis, and induced G2/M arrest selectively in ATRX-deficient GSCs. These data were corroborated in ATRX-deficient and -intact GSC flank xenograft mouse models, treated with either vehicle, CX-5461 alone, IR alone, or CX-5461 and IR. Excitingly, we demonstrated that combinational treatment led to profound tumor growth delay exclusively in ATRX-deficient flank tumors. Multiplexed immunofluorescence of CX-5461-treated tumors, either alone or in combination with IR, revealed enhanced G4 induction, replication stress, and DNA damage, recapitulating in vitro findings. Intracranial xenograft models also demonstrated significant pharmacodynamic effects of G4 stabilization, despite reduced blood-brain-barrier penetration.

Conclusions: In its totality, our work substantively demonstrates efficacy and defines mechanisms of action for a novel therapeutic strategy targeting ATRX-deficient malignant glioma, laying the groundwork for clinical translation.

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Transformation of Infant-type hemispheric glioma harboring ALK fusion to gliosarcoma after treatment with Lorlatinib

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Background: Infant-type hemispheric glioma is a recently characterized unique glioma group that occurs in the cerebral hemispheres harboring tyrosine kinase gene fusions resulting in constitutive activation of oncogenic pathways. Despite high-grade aggressive histologic features and large size at presentation, treatment with receptor tyrosine kinase inhibitors (RTKI) has shown favorable prognosis compared to high-grade gliomas in older children. Due to the paucity of cases and lack of prospective outcome data, this entity has no assigned WHO grade.

Methods: Our patient is a 2-year-old girl diagnosed with Infant-type hemispheric glioma. She presented with vomiting and macrocephaly. Brain MRI revealed a 10 cm mass in the left frontal lobe. Following gross total resection, pathology showed a highly cellular high-grade glial neoplasm with variable infiltration, well-demarcated edges, necrosis, brisk mitoses and microvascular proliferation. Immunostaining for GFAP was variably positive, while ALK showed diffuse strong expression. Genetic analysis revealed a SPECC1L::ALK fusion, supporting diagnosis of infant-type hemispheric glioma.

Results: Initial therapy was focused proton beam radiation therapy. Subsequently, the patient began ALK-inhibitor therapy with Lorlatinib. Five months into the treatment, MRI revealed disease progression with a 5.5 cm heterogeneous enhancing mass in the left occipital lobe. Surgical resection confirmed recurrence of infant-type hemispheric glioma with widespread gliosarcomatous mesenchymal metaplasia. Tumor showed expression of ALK and patchy GFAP expression, while the mesenchymal component diffusely expressed myogenin, MyoD1 and desmin. The Ki-67 proliferative index was >50. Diagnosis was gliosarcoma demonstrating rhabdomyosarcomatous features.

Conclusions: We report a unique case of infant-type hemispheric glioma harboring ALK fusion with recurrence exhibiting gliosarcoma following treatment with Lorlatinib. Recurrence of gliosarcoma with well-differentiated rhabdomyosarcomatous features is exceedingly unusual. The association to prior RTKI treatment and whether this treatment can be associated with transformation of these tumors remains to be explored.

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A recurrent neuroepithelial tumor with MAP2K1 mutation and CDKN2A/B homozygous deletion

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Background: Concomitant presence of MAP2K1 mutation and CDKN2A/B homozygous deletion is not commonly reported in central nervous system tumors.

Methods: We present a unique case of a 24-year-old female with a history of a left temporal neuroepithelial tumor exhibiting an unusual genetic profile featuring an activating MAP2K1 mutation and a homozygous deletion of CDKN2A/B.

Results: The initial pathology in 2021 characterized the tumor as low-grade, with low proliferative activity and without necrosis or vascular proliferation. The tumor showed diffuse CD34 expression, variable NeuN expression, foci of tumor cells with xanthomatous change, and negative reticulin staining. She didn’t receive any chemotherapy or radiation therapy at that time. A recurrence in 2024 revealed a tumor with two regions with distinct histology: one part of the specimen exhibited high cellularity, frequent mitoses, pseudopalisading necrosis, and microvascular proliferation, with Ki67 labeling index up to 30-40%. This part of the tumor was CD34-negative. The second histomorphology showed fewer mitoses and a low Ki67 index with positive staining for synaptophysin, NeuN, and CD34 (patchy).

Conclusions: MAPK pathway alterations are common in pediatric-type glioneuronal or glial neoplasms. They are often low-grade with rare malignant transformation. This unusual neoplasm underscores the unpredictable progression of gliomas with concurrent MAP2K mutations and CDKN2A/B deletion.

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Pigmented Ganglioglioma

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Background: Like cutaneous tumors, neural tumors may show pigmentation due to their shared neuroectodermal origin. Pigmentation in these tumors may be due to melanin or melanin-like pigments or due to metabolic or blood degradation byproducts. The most commonly encountered pigmented lesions in the neurosurgical setting are primary central nervous system melanocytoma, melanoma, and melanotic schwannoma. Very rarely, gross pigmentation can be seen also in gangliogliomas.

Methods: Microscopy, immunohistochemistry, NGS, DNA methylation profiling

Results: Here we describe a 37-year-old male patient who presented with headache and vision changes. Brain MRI showed a superficially located, 6.5-cm, left parietal-occipital mass with a large cystic component. Intraoperatively, the lesion showed dark red-brown discoloration through intact leptomeninges. Slide from the resection specimen showed a neoplasm composed of cells with variably glial or neuronal features. Cells with ganglion cell morphology demonstrated enlarged nuclei, prominent nucleoli, indistinct to eosinophilic cytoplasm, and occasional binucleated forms. Immunohistochemical stains showed labeling of ganglion cells with glial and neuronal markers. Interestingly, a large subset of these cells contained brown cytoplasmic pigment. Glial-appearing tumor cells were intermixed with the neuronal component and showed round-to-ovoid nuclei and predominantly clear cytoplasm; these labeled with SOX10 and GFAP. HMB-45 and MelanA were negative in tumor cells. No mitotic activity was seen, and the Ki67 proliferation rate was low overall. Next generation sequencing was negative for mutations, while DNA methylation profiling identified superfamily “low-grade glial/glioneuronal/neuroepithelial tumors” and subclass “supratentorial pilocytic astrocytoma”.

Conclusions: In summary, we present a comprehensive view of in situ, histologic, and molecular analysis of a rare case of ganglioglioma with prominent pigmentation.

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Quantification of subcellular YAP and TEAD1 expression in gliomas using digital immunohistochemistry

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Background: Glioblastoma (GBM) is an aggressive brain tumor lacking curative treatment. Recent studies have implicated Hippo pathway effectors YAP (Yes associated protein) and TEAD1 in GBM proliferation, invasion, and therapeutic resistance; supporting an ongoing early-phase clinical trial with the YAP-TEAD inhibitor Verteporfin in EGFR-mutant GBM patients. The oncogenic activity of YAP-TEAD is restricted to the nucleus, as cytoplasmic phosphorylation of YAP leads to its degradation. Assessing nuclear YAP-TEAD expression within and across GBM types is crucial to predict and monitor therapeutic response; however, most YAP quantification studies have relied on mRNA or total protein (nuclear and cytoplasmic) expression. Here, we developed robust digital quantification for nuclear-only YAP and TEAD1 immunohistochemical expression.

Methods: We analyzed 51 gliomas with diverse drivers, including 41 IDH-wildtype GBM (EGFR/NF1/PDGFRA-altered), 4 IDH-mutant high-grade, 2 H3K27M-altered diffuse midline, and 4 low-grade gliomas. Nuclear TEAD1 and YAP expression were quantified as percent-positive of total in highest tumor density area, via digital immunohistochemistry (QuPath). Expression was correlated to genomic driver and MGMT methylation status ($p < 0.05$ Student T-test considered significant).

Results: In GBM, nuclear YAP and TEAD1 were expressed in most cells within a tumor, and across tumor subtypes (mean 63-73% for YAP, 59-66% for TEAD1), independent of MGMT status ($p=0.5$). Abundancy of nuclear YAP expression was significantly higher in EGFR-altered vs. EGFR-wildtype GBM ($p=0.04$ one-tailed), in IDH-wildtype vs. IDH-mutant high-grade gliomas ($p=0.0002$), and in GBM vs. non-GBM ($p=0.0002$). PDGFRA-altered GBM ($n=7$) tended to exhibit lower YAP1 expression, although not statistically significant. TEAD1 expression was significantly higher in high-grade vs. low-grade gliomas ($p=0.01$).

Conclusions: GBM tumors exhibit frequent YAP and TEAD1 nuclear expression across subtypes, with highest abundance seen in EGFR-mutant cases, suggesting widespread vulnerability to YAP-TEAD inhibitor therapy. Such digital quantification of oncogene expression with subcellular resolution can be leveraged to predict and monitor response in targeted glioma therapies.

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Diagnostic challenges in early evolving glioma

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Background: Early evolving glioma poses numerous diagnostic challenges. On MRI, early-stage glioma often demonstrates only ill-defined FLAIR or T2 hyper-intensities, and there is considerable overlap between the radiologic features seen in early-stage glioma and other non-neoplastic conditions, so tissue diagnosis becomes imperative. On histologic exam these specimens often have low tumor cellularity which may make the distinction between glioma, reactive gliosis, and other non-neoplastic conditions difficult.

Methods: We review a series of early-stage diffusely infiltrating glioma to investigate the usefulness of various diagnostic modalities. We performed histomorphologic and immunohistochemical analysis on 8 cases, 2 were IDH1R132H mutant and 6 were IDH1R132H wildtype. The immunohistochemistry panel included GFAP, IDH1R132H, p53, ATRX, p16, BRAFV600E, H3K27M, H3K27me3, Ki-67, Olig2, mismatch repair proteins, and SOX11 among others. Next generation sequencing (NGS) was performed on all suspected gliomas with a panel of 324 genes (Foundation one).

Results: Imaging showed diverse features including non-enhancing lesions and T2/FLAIR hyperintensities. Histologic examination revealed mildly hypercellular brain parenchyma with scattered atypical glial cells in all cases. The immunohistochemistry panel identified neoplastic glia in all cases, IDH1R132H in 2 cases, Ki-67 in 4 cases, p53 in 2 cases, and Olig 2 in all 8 cases. NGS identified tumor-specific alterations in 6/8 cases, in the other 2 cases it failed due to low tumor cellularity. TERT promoter mutations were found in 3/8 cases.

Conclusions: Immunohistochemical and molecular analysis are vital in early glioma detection. IDH1R132H, p53, ATRX, Ki-67, Olig2, and SOX11 markers highlight neoplastic cells. Additional stains like BRAFV600E, H3K27M, and H3K27Me3 aid if altered. NGS identifies early genetic events in gliomagenesis and clonal evolution, including PTEN, TERT, IDH1/2, TP53, and 1p-19q co-deletion. NGS identified early molecular changes in 6/8 suspected cases. Cerebrospinal fluid cell-free DNA sequencing could complement cases unresolved by immunohistochemistry and NGS.

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FGFR1 fusions in genomically and epigenetically bona fide glioblastoma, IDH-wildtype

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Background: Initially reported in a small subset of histologically-defined glioblastoma, FGFR1 fusions are not considered typical of glioblastoma, IDH-wildtype (GBM-IDHwt), per 2021 CNS WHO classification. We present three cases with clinical, radiographic, histopathologic, genomic, and epigenetic findings typical for GBM-IDHwt, all harboring FGFR1 fusions.

Methods: We searched clinically tested cases by the Mayo Clinic neuro-oncology next-generation panels (2017-2022) that were GBM-IDHwt (provided histological features and IDH/H3-wt status) and had reportable FGFR1 fusions. Clinical, radiological, additional genetic testing, and follow-up data were collected from electronic medical records. Methylation profiling (EPIC array v1) was analyzed using the NCI/Bethesda classifier v2.

Results: Among 1655 cases, three (0.2%) harbored an FGFR1 fusion: FGFR1::TACC1 (n=2) and FGFR1::HOOK3 (n=1). FGFR1 exon 18 was involved in all cases, fused with exons 7 and 9 of TACC1 and exon 9 of HOOK3. Ages at diagnosis were 49, 63 and 69 years (2F:1M). All cases were supratentorial, non-midline, enhancing tumors, histologically characterized as mitotically active diffuse astrocytic gliomas with necrosis and microvascular proliferation. Tumors exhibited frequent TERT promoter (n=2) and PTEN (n=2) mutations. Clinically relevant copy number changes (methylation array, n=3; chromosomal microarray, n=1) included whole chromosome 7 gain (n=3; one case with EGFR amplification), whole chromosome 10 loss (n=2, copy neutral loss of heterozygosity n=1), and chromosome 9p loss, including CDKN2A/B (n=3; possible homozygous deletion in two cases). All tumors matched and clustered with glioblastoma methylation class (calibrated score 0.99 for each): two with subclass GBM_RTK_II (scores 0.89 and 0.97) and one with GBM_RTK_I (score 0.99). MGMT promoter was methylated in two of three cases, as predicted by methylation array. All patients underwent gross total resection with adjuvant chemoradiation using temozolomide and died, with survival ranging from 12- 17 months.

Conclusions: We confirmed that FGFR1 fusions do occur in typical GBM-IDHwt and do not appear to impact clinical outcome.

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Unraveling astrocytoma not elsewhere classified (NEC) with molecular analysis and methylation profiling

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Background: A small number of adult-type diffuse glioma does not fit within a definite WHO CNS 2021 category even after a complete immunohistochemical and molecular workup. This applies to histologically low-grade IDH-wildtype diffuse glial neoplasm (LGG IDH-WT) which lack molecular criteria for glioblastoma IDH-WT, such as TERT promoter mutation, EGFR amplification, and chromosomes 7+/10-. Those cases are referred as astrocytoma not elsewhere classified (NEC). In this scenario, methylation profiling and correlation with patients' survival could help for a more accurate categorization.

Methods: Forty-five primary adult diffuse glioma (IRB#275/2013) were retrieved and analyzed according to WHO CNS 2021 criteria. Methylation profiling was performed using the Infinium Methylation EPIC v1.0 Array. Raw methylation data of the DKFZ series of CNS tumors were downloaded from GEO (GSE109381) and used to build and validate the methylation-based classifier using a random forest algorithm (epi-classifier). This prediction model was then used to analyze our cohort.

Results: A total of 10 cases of LGG IDH-WT were identified; among those, 3 did not meet criteria for GBM and were classified as astrocytoma NEC. According to the epi-classifier, one NEC glioma was labeled as low-grade glioneuronal tumor (LGG-DNT) and two as normal controls (CONTR-Hemi). The tumor-purity of these cases was >70%. Regarding patients' survival status, the LGG-DNT is alive after 61 months from surgery, one CONTR-Hemi is alive after 91 months but relapsed after 84 months as GBM, and the other CONTR-Hemi died 48 months from surgery. Six out of the 7 molecular-defined GMB were classified as GBM of various sub-classes and one as control (CONTR-Hypthal).

Conclusions: Methylation profiling can support the re-classification of LGG IDH-wildtype if molecular tests are unavailable. Nevertheless, more studies are needed for a precise clinical categorization of astrocytoma NEC; correlation with patients' survival is crucial to understand the clinical behavior of those rare and heterogeneous tumors.

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Adult-Type Diffuse Glioma with Extra-Cranial Metastases: A Series of 3 Cases

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Background: Extra-cranial metastases from central nervous system tumors are rare, and metastases from adult-type diffuse gliomas are exceptional. We report 3 cases of extracranial metastases from adult-type diffuse gliomas.

Methods: Case 1 was a 60-year-old male with a contrast-enhancing, centrally necrotic intra-axial lesion in the right temporal lobe and a lung nodule discovered simultaneously. Case 2 was a 71-year-old male with a history of glioblastoma IDH-wildtype, WHO grade 4 which presented 1 year after the initial diagnosis with local recurrence and multiple lung nodules. Case 3 was a 46-year-old male with an oligodendroglioma, IDH-mutant and 1p/19q codeleted, WHO grade 3 which presented 3 years after the initial diagnosis with multiple vertebral and costal lesions.

Results: At histopathological examination, the intra-axial lesion of case 1 consisted of spindle cells haphazardly arranged in disorganized fascicles, with plurifocal epithelioid and pleomorphic aspects, and focal necrosis; no microvascular proliferation was noted. The lesion was heterogeneously positive for OLIG2, and focally for GFAP. The pulmonary nodule showed a population of OLIG2-positive, GFAP-negative epithelioid dischoesive cells. Molecular analyses demonstrated TERT promoter mutation and absence of IDH1 and IDH2 mutations. Those findings were coherent with gliosarcoma, IDH-wildtype WHO grade 4 with pulmonary metastasis. A lung nodule of case 2 showed a GFAP-positive, OLIG2-negative glial neoplasm, consistent with a metastatic glioblastoma IDH-mutant, WHO grade 4. A costal biopsy of case 3 showed an oligodendrocitic neoplasm with IDH1 mutation and 1p/19q codeletion, consistent with a metastasis of oligodendroglioma, IDH-mutant and 1p/19q codeleted, WHO grade 3.

Conclusions: Metastatic gliosarcoma is very rare, and metastases usually present secondarily; concurrent metastasis at the time of diagnosis has seldom been described. Metastatic glioblastoma and oligodendroglioma are exceptional. Nevertheless, in a patient with a history of adult-type diffuse glioma, a localization should be considered for the differential diagnoses of extra-cranial neoplasms.

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Post-mortem visualization of micro and macroscopic glioma heterogeneity using whole slide and magnetic resonance imaging

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Background: Gliomas are notoriously heterogenous tumors that can be difficult to properly characterize due to unclear tumor boundaries and spread from initial site of disease. To better understand tumor heterogeneity, our group uses a unique post-mortem processing protocol for brain cancer patients enrolled in our neuro-oncology brain bank.

Methods: Since 2012, 129 patients have been enrolled in this study. Patients enrolled underwent routine clinical imaging prior to death for brain cancer treatment. At time of death, the final MR-imaging session was used to create a digital brain mask for designing and 3d-printing custom brain cages meant to prevent tissue distortion during the formalin fixation process. The brain model was also used to create a brain slicing jig for use at autopsy that aligns knife slots with alternating slices from the patient's final axial MRI acquisition. All 3D-print files are manually created using the open-source software Blender. With help from the neuropathology team, large 2-inch by 3-inch tissue samples were taken from brain slices of interest. These tissue samples were processed, paraffin-embedded, hematoxylin and eosin stained, and digitized at 40x magnification using a sliding stage Huron microscope. In 3 select cases, digital histology images were oriented back into alignment and stitched together for a whole brain slice. Finally, custom MATLAB software was developed in-house to align all patient's digital histology back into MR-space giving a view of the whole brain slice histology and MRI examination.

Results: To date, 221 tissue samples have been processed using this method. By allowing whole brain slice sectioning at autopsy, we can view the entire tumor burden at several levels of the brain as well as align this back into the clinical axial MR-space for future research purposes.

Conclusions: Our unique process allows visualization of tumor heterogeneity compared to the patients clinical MRI scan.

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Diagnosis of Brain Tumors Using Wide-Field Imaging Mueller Polarimetry

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Background: Brain cancer is a global health problem with a substantial mortality rate, especially in high-grade gliomas. Neuro-oncological surgery, the primary treatment, faces challenges due to the invasive nature of gliomas. The desired radical brain tumor resection is unattainable due to the lack of contrast between healthy fiber tracts and neoplastic tissue as well as the need to prevent neurological damage, highlighting the importance for precise tumor margin delineation to enhance prognosis. Current intraoperative techniques exhibit limitations, underscoring the demand for innovative approaches. Mueller polarimetry, a method analyzing light polarization to extract structural information, is a promising method for brain tissue characterization and demonstrated effectiveness across various organs.

Methods: This study marks a crucial stride for developing Mueller polarimetry in diagnostic neuropathology, focusing on the characterization of brain tumors polarimetric properties. We employed our wide-field Imaging Mueller Polarimetry system to measure and analyze the polarimetric properties of 45 fresh brain tumor samples encompassing various tumor types with an emphasis on gliomas. A novel neuropathology protocol was introduced to integrate histological data, serving as a reliable ground truth for tissue identification, and polarimetric data. A custom image processing pipeline aligned histological and polarimetric images, enabling the overlay of histological annotation masks on polarimetric parameter maps. The polarimetric parameters of depolarization, linear retardance and azimuth of the optical axis, proven as representative for brain tissue characterization, were quantified to assess differences in regions showing variability in tumor cell content.

Results: Results revealed drops in depolarization and linear retardance in the white matter of neoplastic tissue. We also observed a pronounced randomization of the azimuth of the optical axis, a proxy for brain fiber orientation, in tumor regions, offering potential advancements in brain tumor segmentation.

Conclusions: This study lays the foundation for developing machine learning-based tumor segmentation algorithms using polarimetric data, ultimately enhancing intraoperative diagnosis

during neurosurgery.

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Molecular Confirmation of Glioblastoma with Multiple Extracranial Metastases: A Case Report and Literature Review

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Background: Glioblastoma, IDH wildtype (GBM) is the most common malignant primary brain tumor. Metastases from GBM are exceedingly rare, with a reported incidence of approximately 0.5%. Multiple extracranial metastases are rarer still.

Methods: In this report, we present the case of a 45-year-old man with multiple extracranial metastases seven months following resection and adjuvant therapy of GBM.

Results: A 45-year-old man presented acutely with a right-sided facial droop and was found to have a heterogeneous ring-enhancing mass within the anterior left temporal lobe. He then underwent a craniotomy for resection of the mass which was diagnosed as glioblastoma, IDH wildtype. This was followed by adjuvant radiation and chemotherapy. Seven months later, he began to experience throbbing pain in his hips and shoulders as well as nausea and diarrhea. He brought himself to the emergency room, where imaging demonstrated multiple lesions in the liver, lungs, and bone. The liver and bone lesions were biopsied and received for histopathologic review. The patient's initial brain tumor resection showed typical features of GBM, though some areas demonstrated spindle cell morphology and other mesenchymal features. The tumor in the liver and bone biopsies consisted almost entirely of poorly differentiated spindle cells with only focal GFAP positivity. Ancillary molecular testing showed identical alterations in TP53, TERT, CHEK2, and CDK4 in all three lesions. Additionally, the brain and bone tumors showed similar clustering on DNA methylation array. Together, these results indicate that the extracranial tumors were metastases from the patient's primary GBM.

Conclusions: Metastatic GBM is a rare occurrence despite the aggressive nature of the tumor, and multiple extracranial metastases, as in this case, is even rarer. This case highlights the importance of ancillary molecular testing and opens up the possibility of identifying a molecular basis for why certain GBMs metastasize, a mechanism which has heretofore been poorly understood.

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Epigenetic characterization of glioblastoma, IDH-wildtype/TERT promoter-wildtype harboring MAPK/ALT pathway alterations

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Background: Approximately 10-15% of glioblastoma, IDH-wildtype (GBM, IDHwt) lacks TERT promoter mutation (i.e., TERTpwt) and has alternative lengthening of telomere (ALT) and/or MAPK pathway gene alterations. We investigated if GBM-IDHwt/TERTpwt harboring MAPK/ALT pathway alterations could epigenetically represent tumor types with overlapping morphology, like high-grade astrocytoma with piloid features (HGAP).

Methods: We collected 75 reportedly primary morphologically defined GBM, IDHwt/TERTpwt with evidence of MAPK and/or ATRX/DAXX clinically relevant alterations that had been tested by neurooncology NGS panels at the Mayo Clinic, Laboratory Genetics and Genomics (2017-2023). Epigenetic characterization was performed using EPIC v1 array and the NCI/Bethesda classifier v2.

Results: Median age was 55 years (range, 19-92; >56 [51%], < 41 [33%]), with 3F:2M. Most tumors were reportedly non-midline (63/71; 89%). MAPK pathway alterations (n=64) involved NF1 (58%), BRAF (19%), FGFR1 (11%), PTPN11 (8%), CBL (3%) and NTRK1 (2%); 23 (31%) cases had ATRX and 4 (5%) had DAXX alterations. Among “matched” (n=49; 65%) and “suggested” (n=25; 33%) methylation profiling results, 51% epigenetically aligned with GBM (21 GBM_MES_TYP, 2 GBM_MES_ATYP, 8 GBM_RTK_I, 5 GBM_RTK_II, 2 GBM). Other classes included PXA (n=9, all “matched”), HGAP (n=8, 3 “matched”), pedHGG (n=6, 3 “matched”), HGG_E (n=2, 1 “matched”), Intermediate_grade_IDH_widtype_gliomas (n=2; 1 “matched”), HPAP (n=1, “matched”), 5 CONTR, and one “suggested” case each of BCOR_altered_tumors, DMG_H3K27_altered and LCH. Most tumors with mutations in NF1 (23/37; 62%) and ATRX (13/23; 57%) and all 4 tumors with DAXX mutations epigenetically aligned with GBM. Conversely, 8 (of 9) BRAF-mutant tumors matched to methylation class PXA and all 6 FGFR1-mutant tumors epigenetically aligned with a non-GBM class (3 with HGAP).

Conclusions: GBM, IDHwt/TERTpwt harboring MAPK/ALT pathway alterations are epigenetically heterogeneous. Half of cases epigenetically align with GBM, mainly with GBM_MES_TYP class and FGFR1-mutant cases primarily align with a non-GBM methylation class. Methylation array profiling may be diagnostically useful particularly in selected mutational contexts.

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Exploring the Endothelial Glycocalyx in Human Brain Tumor Vasculature

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Background: The endothelial glycocalyx, lining the luminal surface of vascular endothelial cells, plays a crucial role in vascular homeostasis and is implicated in cancer progression, including tumor cell adhesion, formation, and growth. While tumor blood vessels generally exhibit increased permeability, some resemble normal cerebral vessels, maintaining a drug barrier function termed blood-tumor barriers. Neuropathologists have contributed significantly to understanding brain tumor blood vessel microstructure, yet little is known about the endothelial glycocalyx in human brain tumors. This study aims to visualize and discern structural disparities in the endothelial glycocalyx across distinct brain tumor types.

Methods: Surgical specimens from our hospital's tumor resection patients were utilized, with tumor vessels scrutinized via electron microscopy employing lanthanum nitrate.

Results: Human brain tumor vasculature was found to host a distinct endothelial glycocalyx.

Conclusions: Our investigation successfully visualized the endothelial glycocalyx utilizing lanthanum nitrate. Future elucidation of its role may offer insights enhancing drug treatment efficacy for brain tumors.

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Genetic, transcriptional, and epigenomic investigation of an IDH-mutant high-grade astrocytoma with epigenetic glioblastoma features

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Background: The hypermethylated DNA profile associated with IDH mutation distinguishes IDH-mutant glioma from IDH-wildtype glioblastoma. DNA methylation profiling of an IDH-mutant astrocytoma with extreme histologic anaplasia classified the tumor as an IDH-wildtype glioblastoma with primitive neuronal component. However, infiltrative areas with less pleomorphism classified as astrocytoma, IDH-mutant, high-grade. Molecular investigation of this unique glioma reveals insights into epigenetic classification and differences between IDH-mutant and IDH-wildtype astrocytomas.

Methods: (1) Sequencing and spatial transcriptomics of primary tissue to investigate the two distinct areas. (2) Deep single-cell RNA sequencing and immunohistochemistry analysis of patient-derived glioblastoma organoids (GBOs). (3) Comparison of DNA methylation patterns within the two areas in both primary tissue and paired GBOs.

Results: Deconvolution and comparison of the two areas of the glioma give deeper understanding of the biology, which is correlated with spatial transcriptomic results. The primitive anaplastic areas have a greater number of copy number changes, including gain of chromosomes 1 and 14, and loss of chromosomes 3, 10, and 13. Genes involved in maintaining the progenitor stage are enriched in the pleomorphic area while transcripts directing differentiation and synaptogenesis are seen in the infiltrative component. Although immunohistochemistry findings were uniform throughout the surgical specimen, additional staining and deep single-cell RNA sequencing of GBOs identified two distinct cell populations, consistent with the two distinct subpopulations in the clinical specimen, including loss of chromosome 10 in the pleomorphic cells.

Conclusions: Transcriptional and copy number differences between the two tumor areas have implications related to evolutionary trajectories and epigenetic characteristics of high-grade gliomas.

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Posterior fossa ependymomas with ZFTA fusions are molecularly distinct from PFA, PFB and ZFTA-fused supratentorial ependymomas

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Background: Ependymomas are a heterogeneous group of neoplasms that can develop from infancy through adulthood and occur throughout the neuraxis. The molecular drivers of these tumors are correlated with both patient age and tumor location. Fusions involving ZFTA drive a majority of supratentorial ependymomas (ST-EPN), which are seen across a wide age range. Posterior fossa ependymomas (PF-EPN), in contrast, are typically driven by EZHIP overexpression leading to loss of H3 K27 trimethylation (PF-EPN-A) or genome-wide copy number alterations (PF-EPN-B). PF-EPN in infants and young children are almost exclusively PF-EPN-A, whereas the PF-EPN-B subtype predominates in late adolescence and adulthood. A few PF-EPN harboring ZFTA fusions have been reported in the literature. How these rare tumors relate to other more common ependymoma subtypes is not known.

Methods: Two infantile posterior fossa ependymomas with ZFTA::MAML2 fusions were analyzed by whole transcriptome sequencing (RNA-seq; TruSeq RNA Exome kit, NextSeq 2000, Illumina) and whole genome methylation array testing (Infinium MethylationEPIC v1.0 or v2.0 BeadChip, NextSeq 550, Illumina). Methylation data were analyzed using the DKFZv12.5 or v12.8 and Bethesda v2 (NCI) classifiers. RNA-seq data underwent gene expression analysis and principal component analysis (PCA).

Results: One tumor matched to methylation class ST-EPN-ZFTA with a high calibrated score by DKFZv12.5 but not NCI, while the other matched to ST-EPN-ZFTA with a moderate score by both DKFZv12.8 and NCI. Neither matched to PF-EPN-A or PF-EPN-B. Despite the epigenetic similarities between ZFTA-fused PF-EPN and ZFTA-fused ST-EPN, gene expression and PCA analysis showed these tumors are distinct from both ST-EPN and PF-EPN subtypes. Although EZHIP expression was not observed in the ZFTA-fused PF-EPNs, these tumors expressed PAX3, JUN, ATF3, HIF1A and VEGFA, which are all relatively specific to PF-EPN-A.

Conclusions: These results suggest that PF-EPN with ZFTA fusions may represent a rare but molecularly distinct ependymoma subtype in the posterior fossa.

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Supratentorial Ependymoma, ZFTA Fusion-Positive, with Extensive Mesenchymal Differentiation

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Background: Supratentorial ependymomas are glial neoplasms thought to arise from the radial glia. Many of these tumors harbor a ZFTA fusion, often ZFTA::RELA. Ependymomas can rarely undergo mesenchymal (sarcomatous) differentiation. In this report, we describe a case of supratentorial ependymoma, ZFTA fusion-positive, with extensive mesenchymal differentiation ("ependymosarcoma").

Methods: The patient was a 20-month-old infant who presented with a several months' history of left-sided weakness. The patient had initially been meeting developmental milestones and began walking at 16 months of age. At 18 months of age, she began developing left lower extremity weakness and was noted to 'drag' her left leg. At 20 months of age, she began having left arm weakness and loss of tone, at which point she was referred to the clinical neurology service for evaluation. An MRI demonstrated a large, heterogeneously enhancing right hemispheric mass involving the basal ganglia and thalamus and extending into the brainstem.

Results: The patient subsequently underwent resection of the mass. By microscopic examination, the tumor was predominantly composed of fascicles of spindle cells with brisk mitotic activity. A few interspersed areas showed lobulated nests of tumor cells with round to ovoid nuclei. Focal perivascular anucleate zones ('pseudorosettes') were also noted. By immunohistochemistry, the tumor cells within the nested areas showed GFAP expression, limited Olig2 expression, and focal perinuclear dot-like EMA expression, together supporting ependymal differentiation. Chromosomal microarray demonstrated 11q loss adjacent to RELA. RNA fusion testing demonstrated a ZFTA::RELA fusion. Genome-wide DNA methylation profiling demonstrated a match to "Supratentorial ependymoma, ZFTA fusion-positive" with a high confidence score (0.9999).

Conclusions: Given these histologic, immunohistochemical, and molecular features, the tumor was determined to be a supratentorial ependymoma, ZFTA fusion-positive, with extensive mesenchymal differentiation, CNS WHO Grade 3. Given the sparse body of literature on this entity, the biological behavior of the tumor and overall patient prognosis is uncertain.

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Molecularly-defined supratentorial ependymosarcoma, ZFTA fusion-positive

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Background: Case report: Ependymomas harbor distinct genetic drivers within the supratentorial, posterior fossa, or spinal cord compartments across the neuraxis. Among supratentorial ependymomas, ZFTA fusion is the most common molecular alteration in children. Here, we describe a supratentorial ependymoma arising in a 7-year-old male who presented with progressive headache. MR imaging demonstrated a 5.3 cm avidly enhancing multiloculated cystic - solid mass within the right parietal lobe. Intraoperatively, the cystic component was decompressed and the solid component was firm and well-demarcated from adjacent brain parenchyma. Postoperative imaging was consistent with a gross total resection.

Methods: Histologic sections revealed a solid, cellular tumor composed of epithelioid to spindled cells arranged in large nests, lobules, and fascicles within a variably collagenous stroma. Only focal arrangement of tumor cells in pseudorosettes along hyalinized blood vessels was noted. Mitotic figures were easily identified, along with foci of necrosis. The tumor cells demonstrated heterogeneous immunoreactive for GFAP while negative for OLIG2. EMA demonstrated focal dot-like positivity, CyclinD1 was diffusely positive, p16 expression was lost, and reticulin deposition was extensive.

Results: Ancillary molecular studies demonstrated the presence of RELA::ZFTA fusion along with CDKN2A homozygous deletion. DNA methylation profiling demonstrated a high-confidence match to "supratentorial ependymoma, ZFTA fusion-positive, subtype ZFTA::RELA fused, subclass A". The integrated diagnosis was “supratentorial ependymosarcoma, ZFTA-fusion positive, WHO grade 3”. The patient is currently undergoing treatment with proton radiation therapy.

Conclusions: Discussion: We report a supratentorial ependymosarcoma, ZFTA fusion-positive. Molecularly characterized examples in the existing literature are sparse. While CDKN2A homozygous deletion has been shown to be an unfavorable genetic event in ZFTA fusion-positive supratentorial ependymoma, the prognostic significance of sarcomatous change in molecularly-defined supratentorial ependymoma is not clear and optimal treatment paradigms for this scenario, including possible targeted therapy approaches, are not well-defined. The underlying initiator of sarcomatous transformation in supratentorial ependymoma remains unknown.

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Aggressive Epithelioid Glioblastoma with BRAF Mutation, Homozygous CDKN2A Deletion, Monosomy of Chromosome 10 and Polysomy of chromosome 7

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Background: Epithelioid glioblastoma (EG) is characterized by well demarcated, loosely cohesive aggregates of large epithelioid to rhabdoid cells with abundant cytoplasm, large vesicular nuclei and prominent macronucleoli. It has been known that EG with BRAF mutation and homozygous CDKN2A deletion is a prognostically favorable molecular subclass.

Methods: We present a case of an aggressive EG with BRAF mutation, homozygous CDKN2A deletion, monosomy of chromosome 10, and polysomy of chromosome 7. A 22-year-old female presented with complaints of headache worsening over several weeks. MRI of brain revealed a heterogeneous enhancing lobulated mass in the inferior left frontal lobe measuring 4.9 x 6.0 x 4.7 cm. The tumor showed moderate surrounding vasogenic edema with moderate left to right midline shift. The patient underwent an image-guided left frontotemporal craniotomy and resection of brain tumor with use of the operating microscope.

Results: The histologic sections demonstrated a relatively uniform population of epithelioid cells showing focal loss of cohesion, a distinct cell membrane, abundant eosinophilic cytoplasm, and eccentric or centrally located nuclei with extensive tumor necrosis. The tumor cells were focally positive for GFAP, S100, and Olig2; and negative for IDH1. The methylation-based tumor profiling showed that the methylation profile did not match any methylation class in current CNS classifier (NIH). The next generation sequencing analysis (Caris-NGS) showed BRAF mutation, homozygous CDKN2A deletion, and no mutations of IDH1 and IDH2. FISH analysis (UCLA) showed that there was monosomy of chromosome 10 and polysomy of chromosome 7.

Conclusions: These molecular profiles indicated that this tumor cannot be classified into a prognostically more favorable tumor of molecular subtype (BRAF mutation and homozygous CDKN2A deletion). This case highlights the importance of molecular profiling in EG including NGS, methylation profiling, and FISH to predict an EG biological behavior.

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Leptomeningeal gliomatosis : Unusual presentation of a Rosenthal fiber rich, H3K27M mutated Spinal Glioma

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Background: : Primary leptomeningeal gliomatosis rare, and not a known presentation of H3K27M mutated tumors

Methods: Analysis of clinical records, histopathologic, immunohistochemical and molecular studies, and evaluation of relevant medical literature.

Results: A 31y old male with known H3K27M mutated spinal glioma, diagnosed outside 6 years earlier, was s/p post-chemoradiation. MRI images did not reveal any neoplastic brain lesions during initial admission to our hospital 4 years later, but later started revealing small subependymal metastases, presumed CSF seeding. After an infected groin lesion led to septic shock, the patient passed away. At autopsy, gross brain examination revealed only small nubbings within the lateral ventricles, but the striking finding was a thick infiltrate ensheathing the whole spinal cord, entirely confined to the subarachnoid space. Histological examination revealed a GFAP+ astrocytic tumor with very low Ki67 indices (< 1%), composed of piloid cells accompanied by unusually massive deposits of Rosenthal fibers, nearly obscuring the underlying glioma. The infiltrate was confined exclusively to the leptomeninges of the cord, brainstem, and cerebellum. There was no involvement of the underlying parenchyma, except in the subependymal portion of the basal ganglia, where there was a focal infiltrate corresponding to the foci of CSF seeding. Molecular analysis of autopsy tissue is pending, but the original tumor had a p.K28M hotspot mutation in the H3F3A gene, with a truncating frameshift mutation in the NF1 gene, accompanied by loss of remaining wild type allele

Conclusions: This report highlights the rare presentation of a morphologically distinct neoplasm. The intriguing finding, notwithstanding the diffuse leptomeningeal spread and Rosenthal fiber predominance, was the complete absence of high grade morphology such as mitoses, vascular proliferation, or necrosis. Regardless of its low-grade appearance, the tumor seems to have steadily progressed from its primary subarachnoid spinal location to cause massive leptomeningeal spread, leading to a fatal outcome

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A wide range of fusions and alterations are detected in MYB- or MYBL1-altered gliomas

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Background: Diffuse astrocytoma, MYB- or MYBL1-altered are rare pediatric low-grade gliomas, reported in young adults that are often associated with refractory seizures and generally located in the supratentorial compartment. While GFAP is consistently expressed, interestingly OLIG2 expression (another glial marker) is not a common finding. Here we describe the various associated genetic alterations.

Methods: Fourteen cases matching to methylation class (MC), Diffuse astrocytoma, MYB- or MYBL1-altered, were analyzed for structural variants/fusion partners.

Results: The median age was 16 years (range 1-64), with male: female ratio of 1:1. All but one was supratentorial (one located in pons). On histology, some showed an angiocentric pattern while others a had distinctive histopathology of a low-grade diffuse glioma. OLIG2 was negative in 3/6 cases and focally positive in 3/6. RNA analysis detected MYB/MYBL1 alterations in 13/14 cases. Five cases had a productive fusion: MYB-QKI in 2/5, MYB-EYA4 in 1/5, MYB-PCDHGC3 in 1/5, and MYBL1-KHDRBS3 in 1/5. Of the remaining 9 cases, productive fusions involving MYB or MYBL1 was not detected, although gene truncations/non-productive fusions could be found in 8/9 cases. No fusion or mutation was detected in 1 of these 9 cases.

Conclusions: MYB- or MYBL1-altered tumors are unusual but have distinctive appearances on histopathology. OLIG2 staining is variable, indicating that this may not be a reliable marker. Genetic alterations involving MYB or MYBL1 are heterogenous including the MYB-QKI fusion which is typically found in angiocentric gliomas. Our case series also found additional fusions including MYB-EYA4, MYB-PCDHGC3 and MYBL1-KHDRBS3. Interestingly, the absence of productive fusions in tumors matching to methylation class MYB/MYBL1-altered glioma is relatively common, with a high proportion of cases showing MYB/MYBL1 truncation mutations.

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“Pleomorphic xanthoastrocytoma,” “glioblastoma,” or “high-grade glioma, BRAF-altered, NEC”?

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Background: A 34-year-old female presented with new seizures. MR imaging revealed a 4.5 cm avidly enhancing left frontal lobe mass.

Methods: Histologic sections revealed a densely cellular tumor composed of epithelioid cells arranged predominantly in sheets and nests with brisk mitotic activity and large areas of necrosis. The tumor cells were focally immunoreactive for GFAP while negative for OLIG2. Synaptophysin highlighted entrapped axons, consistent with an infiltrative growth pattern. While BRAF p.V600E immunostain was positive, CD34 was negative.

Results: An exhaustive work up including melanocytic, epithelial, and hematolymphoid markers ruled out other somatic malignancies and favored a high-grade glioma. Next-generation sequencing confirmed the presence of BRAF p.V600E mutation along with a TERT c.124C>T mutation and CDKN2A homozygous deletion in the absence of IDH1/2 mutations. DNA methylation profiling did not result in a consensus match to a single methylation class but entertained the possibilities of pleomorphic xanthoastrocytoma (PXA - Heidelberg; with high classifier scores on v11 [0.93] and v12[0.88]) and glioblastoma, IDH-wildtype (GBM - NCI/Bethesda; with high classifier score of 0.98). Uniform Manifold Approximation and Projection dimensionality reduction analysis placed the tumor in the PXA cluster. Given the ambiguous molecular results, the integrated diagnosis was “high-grade glioma, BRAF-altered, NEC”.

Conclusions: We report a high-grade glioma with genetic and epigenetic features overlapping between GBM and PXA. The histologic appearance in isolation would be most consistent with GBM, but the possibility of a high-grade PXA cannot entirely be excluded given the patient demographics and ambiguous DNA methylation signature. While BRAF-altered GBM and PXA have overlapping genetic alterations (BRAF and TERT activation, CDKN2A inactivation), they typically harbor unique epigenetic signatures. This case emphasizes the need for more robust molecular signatures to differentiate between these two entities to enable accurate prospective classification of high-grade BRAF-altered gliomas, especially in young adults.

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Glioblastoma Presenting with Diffuse Spinal and Cranial Leptomeningeal Disease

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Background: A 45-year-old man with no significant past medical history presented two months prior to his death with back pain, progressing quickly to photophobia, insomnia, confusion/confabulation, nausea/emesis, nystagmus, myoclonus, and eventually blindness. Spine MRI showed diffuse leptomeningeal and nodular nerve root enhancement. Brain MRI showed T2/FLAIR hyperintense, diffusion-restricting lesions along both lateral ventricles. CSF showed pleocytosis, elevated protein, and hypoglycorrhachia. A broad workup, including for autoimmune and infectious etiologies, was negative. A right superior frontal lobe superficial brain biopsy, performed at an outside facility prior to transfer to our facility, was non-diagnostic. Empiric immune-modulating therapies with corticosteroids and plasma exchange provided no substantial/sustained benefit. The patient passed away and his family consented for autopsy.

Methods: Neuropathologic gross and microscopic evaluation of the dura mater, brain, and spinal cord was performed.

Results: Gross examination showed marked thickening and opacification of the leptomeninges and multifocal fusiform enlargement of several spinal nerve roots. The lateral ventricles were dilated with ragged lining, and there was focal blurring of the parieto-occipital lobe gray-white matter junction. Routine histology showed widespread involvement of the leptomeninges by a glial proliferation, with significant atypia that encased/involved spinal nerve roots and lateral ventricles. A focus of diffuse high-grade glioma was discovered in the left parieto-occipital cortex in an area of gray-white matter blurring, with no MRI correlate. Tumor cells were partially positive for GFAP and negative for mutant IDH1 (R132H). FISH showed polysomy 7 and monosomy 10. Methylation profiling matched to glioblastoma, IDH-wildtype, mesenchymal subtype.

Conclusions: This case highlights an unusual clinical presentation of a common primary brain tumor and alludes to its possible consideration in the differential diagnosis of unexplained diffuse leptomeningeal enhancement. Biopsy in such a case should target areas of increased enhancement to maximize diagnostic utility.

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Clinical feasibility of pharmacogenetic analysis in brain cancer patients

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Background: Pharmacogenetic testing can be used to predict rates of drug metabolism allowing practitioners to tailor drug therapies based on unique genetic profiles. However, pharmacogenomics is not currently utilized for management of patients with brain tumors. We performed a feasibility study to determine pharmacogenomic profiles in brain tumor patients.

Methods: We analyzed normal blood-derived DNA from 43 brain tumor patients diagnosed at NYU Langone Health, including glioblastoma (n= 9, 21%), meningioma (n=10, 23%), schwannoma (n=5, 12%), pituitary adenoma (n=4, 9%), astrocytoma (n=4, 9%), ependymoma (n=2, 5%), and low-grade glioma (n=9, 21%). Analysis was performed using the Illumina genome-wide global diversity array, (GDA), and analyzed using a pharmacogenetic workflow integrated with Dragen Array PgX Star Allele Annotation software. Samples were compared based on genetic variants associated with drug metabolism, and classified based on predicted measures of metabolism rates, ranging from slow to rapid. In patients who underwent adjuvant therapy (n=8), we correlated adverse effects and survival outcomes with metabolism status in fifteen target genes.

Results: Using GDA, we classified each patient into one of eight metabolizer categories: ultrapoor, poor, normal, intermediate, likely-intermediate, rapid, or ultrarapid. Patient 1 classified as ultrapoor and poor metabolizer in CYP2C19 and CYP2D6, respectively, with moderate adverse effects and a 14-month survival. Patient 3 classified as poor metabolizer in CYP2B6 and CYP3A5 genes, and intermediate and likely-intermediate in CYP2D6 and CYP2C19 genes, respectively, and showed severe adverse symptoms and a short survival (2 months). Patient 7 classified as intermediate and poor in CYP2C9 and CYP3A5, respectively, had the highest number of normal metabolizer genes, and extended survival (21 months).

Conclusions: Our data suggest that pharmacogenetic-based testing is feasible in routine clinical workflow and provides the potential for individualized therapeutic management. Larger studies are required to establish the effect of metabolizer status and response to adjuvant therapy in various brain tumors types.

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Molecular Profiling in the Diagnosis of a Case of a Metastatic Glioblastoma to the Liver
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Background: Glioblastomas mostly remain confined to the central nervous system until the death of the patient; however, they do have the potential for metastasis. Here, we present the case of a 74-year-old woman with a history of breast carcinoma who presented with left-sided weakness.

Methods: Imaging showed a 5.4 cm right parietal brain mass with associated vasogenic edema. The patient underwent a craniotomy and resection of the mass. One month following the procedure she re-presented with new syncopal episodes. During workup for her new symptoms, she developed a new-onset transaminitis. Subsequent imaging of the liver showed multiple lesions, and she underwent a percutaneous biopsy of one of the liver lesions.

Results: The right parietal resection demonstrated a glioblastoma, IDH-wildtype, WHO grade 4. Immunohistochemistry demonstrated no staining for IDH1 (R132H), positive staining for GFAP and OLIG2, and strong, diffuse staining for SOX2. The liver biopsy demonstrated a round blue cell tumor initially concerning for a metastatic high-grade neuroendocrine carcinoma. Immunohistochemistry demonstrated focal staining for GFAP, no staining for OLIG2, and strong, diffuse staining for SOX2. Next-generation sequencing performed on the two samples revealed identical mutations in several key genes, including EGFR, TERT promoter, TP53, and CDKN2A, consistent with a metastasis from her glioblastoma.

Conclusions: This case represents an example of a rare complication of glioblastomas (approximately 0.5% of glioblastoma cases), as well as demonstrating the diagnostic challenge posed by such cases. It also highlights the value of SOX2 immunostaining in diagnosing glial neoplasms with unusual presentations. The nearly identical molecular signatures of the two processes underscores the importance of molecular testing in complex pathological diagnoses.

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SOX2 as a Potential Marker for Distinguishing Between Gliomas and Reactive Gliosis

S Schwartz, H Varma; Beth Israel Deaconess Medical Center/Harvard Medical School

Background: The challenge of distinguishing glial neoplasms from reactive gliosis underscores the need for a reliable histological marker. While some gliomas can be identified via immunohistochemistry for IDH1 R132H or H3 K27M, no such reliable marker has been identified for a large majority of gliomas.

Methods: SOX2 is a stem cell marker that is expressed in glial cells under certain conditions such as gliomas while not being expressed in normal brain astrocytes. We first confirmed that SOX2 was strongly expressed in fetal brain germinal matrix (positive control) and not expressed in meningiomas (negative control) using a commercially-available antibody. We then evaluated SOX2 expression via immunohistochemistry in a range of neoplastic and reactive processes; both the presence or absence of staining, as well as the distribution and intensity, were assessed.

Results: We confirmed that glioblastomas, as well as other gliomas such as astrocytomas, ependymomas, and other rare gliomas consistently demonstrated strong, diffuse staining for SOX2. In contrast, reactive glial tissue demonstrated weak to modest staining. SOX2 staining proved useful in cases that presented diagnostic challenges, including a granular cell glioblastoma and a metastatic glioblastoma, where routine GFAP and OLIG2 stains were equivocal or negative.

Conclusions: These results showcase the potential of SOX2 as a reliable immunohistochemical marker for gliomas and its utility as a supportive immunohistochemical stain in diagnostically challenging gliomas where routine glial markers such as GFAP and OLIG2 are either negative or only focally positive. Furthermore, the extent and quality of staining can provide some distinction between gliomas and reactive gliosis.

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Low-Grade Neuroepithelial Tumors with FGFR2 fusion: Histologic, Molecular, and Epigenetic Characterization

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Background: FGFR2 fusions have been reported in a subset of low-grade neuroepithelial tumors (LGNETs) with epigenetic similarities, most commonly polymorphous low-grade neuroepithelial tumor of the young (PLNTY) but also ganglioglioma (GG). Here, we report a series of LGNETs with FGFR2 fusions and examine their histologic and molecular features, including methylation classification.

Methods: LGNETs with FGFR2 fusions on molecular testing performed at our institution were identified. Histologic features were reviewed. Nucleic acid was extracted from formalin-fixed, paraffin-embedded tissue. Next generation sequencing (NGS) was performed using a custom targeted panel on the Ion S5 XL (Thermo Fisher Scientific). Whole transcriptome sequencing (RNA-seq) was performed using the RNA Prep with Enrichment (L) Tagmentation kit on the NextSeq 2000 (Illumina). Whole genome methylation array testing was performed using the Infinium MethylationEPIC v2.0 BeadChip on the NextSeq 550 (Illumina). Data was analyzed using the DKFZ v12.8 and Bethesda v2 (NCI) classifiers.

Results: Nine tumors with FGFR2 fusion were identified, seven with CTNNA3 as the 3' partner and one each with INA and SHTN1[KIAA1598]. Patient age range was 4 to 33 years. All tumors were hemispheric and showed varying overlap between morphologic features of PLNTY and GG. Eight tumors were sufficient for methylation profiling. Four matched to GG with high calibrated score on both classifiers, three matched to GG with moderate score on DKFZ v12.8 and high score on Bethesda v2, and one matched to PLNTY with high score on DKFZ v12.8 and moderate score on Bethesda v2.

Conclusions: Identified LGNETs with FGFR2 fusions demonstrated a spectrum of morphologic features and did not consistently methylate to PLNTY using multiple classifiers, even in the setting of suggestive morphology and FGFR2::CTNNA3 fusion. These results highlight the importance of a comprehensive evaluation for such tumors and the limitations of methylation profiling to resolve closely related entities.

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Histiocyte-rich Pleomorphic Xanthoastrocytoma (PXA): Case Report and Diagnostic Challenges

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Background: In recent years, molecular analysis has indicated that Pleomorphic xanthoastrocytomas (PXAs) may have a more heterogeneous histomorphology than previously believed. Herein we describe a case of PXA (CNS WHO Grade 3) that harbors a prominent population of infiltrating macrophages and CD163-positive neoplastic glial cells.

Methods: A 43-year-old male presented with headache and new-onset seizure. Brain imaging revealed a large heterogeneously enhancing mass in the right temporoparietal lobe causing compression of the right lateral ventricle. The tumor underwent gross total resection and subsequent pathological evaluation, including methylation profiling on distinct tumor components.

Results: Histologic sections showed a hypercellular neoplasm with heterogeneous morphological features. A prominent component consisted of scattered large multinucleated cells amidst a population of macrophages, neutrophils and lymphocytes. Large cells showed occasional multinucleation and intracytoplasmic neutrophils, and were negative for glial, histiocytic and neuronal markers, however showed immunoreactivity for a BRAF (p.V600E). A smaller portion of the tumor consisted of monomorphic small epithelioid cells or had a fibrillary appearance, with immunoreactivity for GFAP, BRAF, and CD163. Palisading necrosis and elevated mitotic activity (7 mitoses per 10 hpf) were present. Methylation profiling was performed on separate components of the tumor. While the more convincing glial regions showed a high confidence score match for the methylation class PXA, the region including the large neoplastic cells with prominent inflammation showed a match to “Control_Inflammatory” tissue.

Conclusions: This case demonstrates several unusual features that may add to the histopathologic spectrum of PXA. The presence of a prominent macrophage component surrounding large neoplastic cells, which themselves show apparent emperipolesis, can raise consideration for a histiocytic neoplasm. The presence of CD163 positivity within neoplastic glial cells, along with the difficulty in obtaining molecular results with robust inflammation, can add to the diagnostic challenge. In this case, BRAF immunohistochemistry was helpful in delineating neoplastic cells from background inflammation.

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De novo ultra-hypermutation high-grade glioma: an unusual case presentation
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Background: De novo ultra-hypermutation high-grade gliomas can be seen in the setting of Lynch syndrome or constitutional mismatch repair syndrome, but most often in pediatric patients. Hypermutated IDH-wildtype glioblastomas can also be seen in the setting of recurrent glioblastoma after treatment with temozolomide.

Methods: We describe here a rare case of de novo ultra-hypermutation IDH- and H3-wildtype high-grade glioma in a patient over the age of 55 without prior temozolomide therapy.

Results: Our patient is a 61-year-old woman with a remote history of ovarian cancer, infiltrating ductal breast carcinoma, and colon polyps who presented with complaints of sudden left extremity paresthesia, numbness, and weakness. Brain MRI showed a parenchymal hematoma centered in the right basal ganglia with peripheral enhancement and slightly increased T2 hyperintensity superiorly. Targeted stereotactic biopsy was performed. On histology, brain parenchyma was infiltrated by a highly cellular glial neoplasm characterized by nuclear atypia, enlargement, and pleomorphism with scattered multinucleated giant cells. Mitotic figures were frequent; microvascular proliferation and early necrosis were also present. Immunohistochemistry supported a preliminary diagnosis of diffuse high-grade glioma negative for H3 p.K27M and IDH1 p.R132H mutations. Molecular testing revealed a very high tumor mutational burden at 268.5 mutations/Mb as well as high microsatellite instability (MSI-high) and a total of 260 variants. Among these, pathogenic variants in ATRX, BRCA2, MSH2, MSH6, PALB2, and TP53 were found at a high allele frequency. These results prompted additional mismatch repair immunohistochemistry. MSH2 and MSH6 showed complete and partial loss in neoplastic cells, respectively, with retained staining in nonneoplastic cells. MLH1 and PMS2 were positive.

Conclusions: This pattern was concerning for Lynch syndrome due to a germline MSH2 variant(s). Unfortunately, the patient passed away approximately 2 months after diagnosis and was unable to start treatment or receive genetic testing.

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Metabolic profiling of adult and pediatric gliomas reveals distinct age-associated profiles independent on histology and molecular drivers

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Background: Gliomas are the most common primary brain tumors and a major source of mortality and morbidity in adults and children. Genomic studies have identified multiple molecular subtypes; however metabolic characterization of these tumors has thus far been limited.

Methods: All tumors were collected fresh and frozen in -80C upon obtaining informed consent . We performed metabolic profiling of 114 adult and pediatric primary gliomas using LC/MS and integrated metabolomic data with transcriptomics and DNA methylation classes. Normalized batch corrected data was used to perform unsupervised principal component analysis (PCA) and generate heatmaps by examining variance and calculating Z-scores respectively. Normalization was performed across each comparison. Heatmaps were produced using ComplexHeatmap R package.

Results: We identified that pediatric tumors have higher levels of glucose and reduced lactate compared to adult tumors regardless of underlying genetics or grade, suggesting differences in availability of glucose and/or utilization of glucose for downstream pathways. Differences in glucose utilization in pediatric gliomas may be facilitated through overexpression of SLC2A4, which encodes the insulin-stimulated glucose transporter GLUT4. Transcriptomic comparison of adult and pediatric tumors suggests that adult tumors may have limited access to glucose and experience more hypoxia, which is supported by enrichment of lactate, 2-hydroxyglutarate (2-HG), even in isocitrate dehydrogenase (IDH) wild-type tumors, and 3-hydroxybutyrate, a ketone body that is produced by oxidation of fatty acids and ketogenic amino acids during periods of glucose scarcity. Our data support adult tumors relying more on fatty acid oxidation, as they have an abundance of acyl carnitines compared to pediatric tumors and have significant enrichment of transcripts needed for oxidative phosphorylation.

Conclusions: Our findings suggest striking differences exist in the metabolism of pediatric and adult gliomas, which can provide new insight into metabolic vulnerabilities for therapy.

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Heavily lipidized glioblastoma: Clinicopathological study of three cases

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Background: Lipidization is one of the features that characterize gliomas including glioblastomas (GBs). They perdominantely have been designated as malignant gliomas with heavily lipidized tumor cells or heavily lipidized GB (HLGB). Since its first description, only a very small number of cases have been reported.

Methods: To investigate of HLGB, clinical history, histopathology, and genetics were reviewed for three unusual cases.

Results: Case 1 was a 38-year-old man with a history of epilepsy. A MRI showed a neoplastic lesion in the right temporal lobe. Case 2 was a 42-year-old man with right hemiparesis and motor aphasia. A MRI revealed a mass lesion in the left frontal lobe. Case 3 was a 61-year-old woman with a 10-year history of unruptured aneurysm under follow-up MRI. At 11 years of follow-up, a ring-shaped contrast lesion was found in the right temporal and occipital lobes. All patients underwent gross total resection and received postoperative radiochemotherapy. They are under postoperative follow-up at 2, 5, and 1 year, respectively, and no recurrence has been observed. Histopathologically, all cases showed infiltrative growth of tumor cells that were fat-rich cytoplasm. They were also accompanied by necrosis and microvascular proliferation. On the other hand, in case 2, some tumor cells had changed to a fibrosarcoma appearance, and in case 3, many tumor cells had eosinophilic granules in their cytoplasm. Immunohistology showed that tumor cells showed IDH-1/2, and H3K27M negative findings while GFAP, ATRX and H3K27me3 showed positivity in all cases. Genetic analysis showed TERT mutations in all cases. In case 1, a BRAF mutation was found. Based on these findings, case 1 was considered to be in the category of GB with BRAF mutation, while cases 2 and 3 were considered to be in the category of IDH wild-type GB.

Conclusions: These results suggest that HLGB could be not a single genetic entity.

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Unraveling Pathways: CDKN2A/B Alterations and Alternative Routes in Disease Progression of IDH-Mutant Astrocytomas

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Background: Mutations in IDH1/2 genes are the most prevalent genetic alterations in adult diffuse low-grade gliomas (LGGs). Although slow-growing, these low-grade IDH-mutant gliomas tend to recur and progress to high grade. Homozygous deletion (HD) of CDKN2A/B is an independent factor associated with shorter survival and its presence imparts grade IV clinical behavior, irrespective of low-grade histological features. However, it remains unclear whether, in addition to CDKN2A/B HD, there are alternative pathways leading to disease progression in IDH-mutant astrocytomas.

Methods: To address this question, we examined 53 patients with matched primary and recurrent IDH-mutant astrocytomas (total 132 specimens) over the past ten years at Columbia University Medical Center (CUMC). Disease progression was evaluated by histological grading. A combinatorial approach including immunohistochemistry, CDKN2A/B alteration by FISH, DNA methylation profiling and NGS analyses was performed.

Results: Our preliminary data, derived from 20 patients, suggest that CDKN2A/B alterations (including HD, heterozygous deletion, and partial deletion) were only detected in cases with either histological grade IV in the primary resection or in recurrent cases with disease progression. These alterations accounted for the majority (~71%) of recurrent astrocytomas that developed disease progression. While relatively fewer, a smaller number of cases (21%) showed heterogeneous IDH-mutant expression status in recurrent specimens, suggesting an IDH-mutant independent pathway underlying disease progression. This heterogeneity correlated with lower VAFs on NGS and distinct methylation profiles in the recurrent tumor. However, no evidence of loss of heterozygosity of IDH-mutant was found. Neither CDKN2A/B alteration nor heterogenous IDH-mutant status was observed in patients with low-grade histology showing no disease progression. Further expanded studies on the entire longitudinal cohort are ongoing.

Conclusions: Collectively, out data, though preliminary, unveil two distinct pathways driving disease progression in IDH-mutant astrocytomas. The majority of cases exhibit CDKN2A/B alterations, while in a minority of cases, heterogeneity on IDH-mutant status constitute an alternative biological process.

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Spatial genomic analysis reveals the architecture of glioblastoma

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Background: Single-cell genomic analyses have revealed that glioblastoma contains malignant cells in diverse cellular states, but their tissue distribution in human samples remains uncharted.

Methods: We combine spatial transcriptomics with spatial proteomics and novel computational approaches to define glioma cellular states at high granularity in human samples and uncover their organization.

Results: We found that malignant cell states tend to be spatially clustered, such that tumors are composed of small local environments that are each typically enriched with one major malignant cellular state and that specific pairs of glioblastoma states preferentially reside in proximity across multiple scales. Pairwise interactions that we detect collectively define a global architecture composed of five layers. Necrosis/hypoxia drives this 5-layered organization, as it is both associated with unique states of surrounding cells and with a long-range organization that extends from the necrotic/hypoxic core to the infiltrative edge of the tumor.

Conclusions: In summary, we provide an atlas of spatial transcriptomics for glioblastoma and a conceptual framework for its organization at the resolution of cellular states and highlight the role of hypoxia as a long-range tissue organizer.

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Synchronous presentation of two gliomas: Oligodendroglioma and Astrocytoma

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Background: A 39-year-old male with a history of thyroid carcinoma, status-post thyroidectomy and radiation 10 years ago was evaluated for recent seizures.

Methods: Case report

Results: MRI demonstrated a 3 cm infiltrating cortically based lesion at the right temporal, occipital junction, characterized by T1 signal hypointensity, T2 and FLAIR signal hyperintensity, nonenhancing, with mild mass effect—no restricted diffusion. Moreover, a second 1.3 cm lesion with similar signal characteristics is seen within the left frontal operculum. Considerations were multifocal glial or glial-neuronal tumors. The patient underwent resection at Mount Sinai for the right parietal tumor. Histology shows an infiltrating glial tumor with astrocytic morphology, increased mitotic activity, and no atypical microvascular proliferation or tumor necrosis. Immunohistochemistry (IHC) was positive for IDH1-R132H-mutant protein, GFAP, and OLIG2; there was prominent p53 staining in 60% of cells, and Ki-67 proliferation index was 5.6%, while ATRX was lost (mutant). FISH did not detect 1p/19q co-deletion. Next-generation sequencing (NGS) confirmed the IDH1 R132H mutation gene, ATRX, and TP53 mutation, besides revealing BRCA2 and SMARCA4 mutations. The diagnosis of Astrocytoma IDH-mutant, CNS WHO Grade 3 was unequivocal. After one month, the patient underwent resection for the other lesion at Columbia. The resection of the left frontal operculum lesion revealed an infiltrating glial neoplasm with oligo-astrocytic morphology, with no mitotic activity, necrosis, or microvascular proliferation. The tumor has IDH1-R132H mutation by IHC and was again positive for GFAP but had no significant p53 positivity (wild type), and ATRX was intact. FISH results demonstrated 1p/19q co-deletion and further NGS detected TERT and IDH mutations. The second tumor was unequivocally an Oligodendroglioma IDH-mutant CNS WHO Grade 2.

Conclusions: Multicentric presentation and/or progression of gliomas is common; however, synchronous gliomas of different genetic make-up, morphology, and grade are unusual. To our knowledge, this is the third report characterizing distinct neoplasms arising concurrently in one patient.

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A new entity in glial tumors: CD34-expressing glioma with MEN1 alteration.

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Background: A 43-year-old male with numbness and paresthesia for 5 months, found to have a cavernous malformation per radiology and underwent resection.

Methods: Case report

Results: MRI demonstrated a left frontal lesion abutting the temporal lobe most consistent with a cavernous malformation with what appeared to be a likely developmental venous anomaly. The patient underwent resection. Permanent sections show a clonal expansion of glial cells underlying the vascular malformation. The clonal cells had compact growth pattern, low mitotic activity, and displayed perivascular pseudorosetting. Immunohistochemistry shows KI67 2.4%, GFAP and OLIG2 positive, P53 positive in 3% of cells (wildtype), IDH1-R132H negative for mutant protein, BRAFV600E negative, EMA negative, and ATRX intact. CD34 had strong and diffuse positivity. At this point, the differential included polymorphous low-grade neuroepithelial tumor of the young (PLNTY) and next-generation sequencing (NGS) was performed. However, NGS did not reveal the expected MAPK-pathway alterations. Instead, it unveiled a MEN1 alteration, which is seen in ependymomas. The morphology and EMA staining did not support ependymoma. The case was sent to a second opinion and DNA methylation profiling. DNA Methylation demonstrated a tumor signature falling close to pleomorphic xanthoastrocytoma (PXA) but was not a perfect fit and was far from ependymal tumors. Since PXA is not suggested by histomorphology this tumor could be a new entity.

Conclusions: Recent advancements in DNA methylation profiling of glial tumors are expanding the classification of CNS tumors and has re-classified old entities into new categories based on the methylation signature. The present report is the first in literature of a CD34-expressing glioma with MEN1 alteration. Given the low mitotic activity and the compact growth pattern, most likely, the behavior will be similar to that of a CNS WHO grade 1 glial tumor. However, a grading system is not yet known for this entity and close follow up was recommended.

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Dysembryoplastic Neuroepithelial Tumor: An Institutional Review With Emphasis on Complex Histology and Molecular Features

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Background: We have previously shown that targeted fusion analysis can aid in the classification and treatment of pediatric tumors (PMID 31595628); in this study, we expand our findings to pediatric cases of dysembryoplastic neuroepithelial tumor (DNET). DNETs demonstrate diverse histologic features including “simple” and “complex” forms, the latter showing regions resembling pilocytic astrocytoma or oligodendroglioma. Most carry FGFR1 alterations, although some reports suggest a significant percentage with BRAF V600E mutations. We retrospectively reviewed histological and molecular findings to determine 1) what proportion of our cases were complex, 2) what molecular alterations were present and 3) whether molecular results allow reclassification of cases retrospectively.

Methods: Database search, coupled with review of records for diagnoses of “dysembryoplastic” and “DNET”, 2010-2023, with recurrence defined by neuroimaging. Histological features were correlated with molecular test results, where available.

Results: 28 tumors from 25 patients were identified. M:F=2.13, median age 10 years (range 3-19 years). Temporal lobe was most often involved (n=14; 56%), followed by frontal (n=8; 32%), parietal (n=2), and occipital (n=1). Simple (19) predominated over complex and diffuse patterns (3 each). Of 11 cases with molecular results, 2 had FGFR1 internal tandem duplication, a likely pathogenic alteration in BLM was present in 1, and PDGFRB::LRP1 in 1. 6/11 showed no alterations. One case with complex histology had mutations in FGFR1, NF1, and PIK3R1, suggestive of rosette-forming glioneuronal tumor. 7 patients had recurrent epilepsy following resection or ablation with 5/7 demonstrating tumor recurrence, 1 of which showed complex histology.

Conclusions: In our cohort, complex histology showed no increased recurrence compared to simple histology. One complex case had a mutation pattern suggestive of an alternate diagnosis. While complex histology has not been shown to have clinical significance, review of the genetic features is warranted.

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Are PDFGRA Dinucleotide Alterations Definitional for Myxoid Glioneuronal Tumor? Report of PDFRA Alteration in a Neonatal High-Grade Glioma

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Background: Central Nervous System tumors are increasingly being defined by the molecular alterations that they harbor. This shift to molecular diagnostics promises to increase precision and reduce subjectivity but discordant cases are increasingly being reported. Myxoid glioneuronal tumor (MGNT) is a WHO grade 1 tumor histologically similar to dysembryoplastic neuroepithelial tumor and characterized by dinucleotide mutations in PDGFRA (K385L or K385I). Here, we report a case of a high-grade glioma in a neonate with K385L molecular alteration suggesting that this alteration is not specific to MGNT.

Methods: Histologic and immunohistochemical analysis with a large, targeted gene panel mutation and fusion analysis.

Results: The patient was a male neonate born via caesarean section at 35 weeks gestation due to hydrocephalus. MRI showed a dysmorphic brain with lobulated tissue replacing large portions of the cortices. He experienced progressive hypoxia and intracranial hemorrhage and died on day 37 of life. The brain showed a hemorrhagic tumor involving bilateral hemispheres and the ventricular system. Histologically, the tumor demonstrates cells with atypical nuclei, indistinct cytoplasm, and a myxoid background infiltrating parenchyma and layering over ventricular surfaces. Some regions also show distinct mucin pools and monomorphic oligodendroglioma-like cells. Numerous mitotic figures and foci of microvascular proliferation are present. The cells show diffuse expression for OLIG2 and GFAP with no expression of synaptophysin. INI-1 is retained. MIB-1 shows a proliferative index of ~60%. NGS showed PDGFRA K385L with a variant allele frequency of 83.6%. The pons additionally showed neuronal necrosis.

Conclusions: This report of a PDGFRA mutation in a neonatal high-grade glioma suggests that this alteration is not specific to MGNT. Alternatively, it may indicate that in rare cases, MGNT may undergo malignant transformation. As few cases of MGNT are available in the literature, we recommend caution in classifying a tumor as MGNT in the presence of PDGFRA alteration.

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Myxoid Glioneuronal Tumor in the Fourth Ventricle: An Atypical Location of a Rare Tumor

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Background: Myxoid glioneuronal tumor (MGNT) is a WHO grade 1 tumor characterized by oligodendroglial-like cells demonstrating mixed glial and neuronal phenotype in a myxoid background. Originally, cases were classified as dysembryoplastic neuroepithelial tumor (DNET) of the septum pellucidum, given their archetypal septum pellucidum location. Today, MGNT is recognized as being molecularly distinct from DNT, with dinucleotide mutations in PDGFRA (K385L or K385I). Our group has previously reported well-documented cases arising in midbrain tectum and temporal lobe sites (PMID: 34297434, 35562106). Here we report one arising in 4th ventricle.

Methods: Case was assessed by histology, immunohistochemistry (IHC), and next generation sequencing, with target enrichment of the regions of interest was carried out by a hybridization-based methodology using long biotinylated oligonucleotide probes followed by polymerase chain reaction and sequencing on an Illumina NovaSeq 6000 generating 150bp paired reads. Only exons and canonical splice sites were captured.

Results: The patient is a 13-year-old male who presented following a sports-related head injury. MRI demonstrated a non-enhancing 4th ventricle mass, and he underwent a biopsy. Histologic sections show oligodendroglial-like cells infiltrating around axons and neurons within a myxoid background. No high-grade features were present. Immunohistochemical stains for GFAP and Olig2 showed diffuse expression. Synaptophysin was negative. MIB–1 proliferative index was < 1%. Rosette-forming glioneuronal tumor was favored with a differential including pilocytic astrocytoma and DNET. Sequencing demonstrated a PDGFRA K385L mutation, diagnostic of myxoid glioneuronal tumor. 4 months after initial identification, no significant tumor growth or dissemination was noted.

Conclusions: Although generally associated with favorable outcome, MGNT can be a cause of morbidity through ventricular dissemination and poor response to therapy. Tumors typically occur in the lateral ventricles/septum pellucidum, albeit sometimes intraparenchymal. This is the first case report of a 4th ventricular MGNT, adding yet another site or origin for these tumors.

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METASTATIC MYXOPAPILLARY EPENDYMOMA TO THE CEREBELLUM: CASE REPORT AND REVIEW OF THE LITERATURE.

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Background: Myxopapillary ependymomas are slow-growing gliomas, arising almost exclusively in conus medullaris and filum terminale, are associated with a relatively favorable prognosis, though many people live with persistent disease, because of the difficulties of gross total resection and/or cerebrospinal fluid seeding of the thecal sac or more rostral neuroaxis. Metastatic myxopapillary ependymomas can be encountered at the time of diagnosis and many years after the diagnosis.

Methods: A 57 year old male with a history of a L5 myxopapillary ependymoma resection in 1990, had local recurrence in 2001, which was resected followed by adjuvant radiation, presented 22 years later in 2023 with pain and numbness in lower extremities, progressive gait instability. MRI demonstrated a ventral spinal canal mass at the T12/L1 interspace and L5/S1. MRI of brain and cervical and thoracic spine identified a 1.9 cm left lateral cerebellar mass, a ventral spinal mass at C7, and a cluster of lesions from T7-T9. The cerebellar and the C7 masses showed metastatic myxopapillary ependymoma. Metastatic myxopapillary ependymoma with this long disease free interval and outside of the spinal canal is rare, thus we report this case and review the literature.

Results: We reviewed the literature and identified 30 cases of extraspinal canal metastasis of myxopapillary ependymomas: 21 males (63%) with slight male over female predominance. The longest period since diagnosis to metastasis is 29 years in a 17 yo female patient (metastasis to the 4th ventricle, retroperitoneum, lung, pleura, and para-aortic lymph node), reported in 1970 by Rubinstein. Other sites of metastasis included cerebellopontine angle, liver, mediastinum, pleura, chest wall, retroperitoneum, cervix, internal auditory canal. Most common site of metastasis is cerebellum (7/30 = 23%), including the case presented.

Conclusions: Myxopapillary ependymoma is an indolent tumor, can recur or have distant metastasis many years since the initial treatment. Thus long term follow up is recommended.

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A report of recurrence Diffuse Glioneuronal Tumor with Oligodendroglioma-like features and Nuclear Clusters with unique genetic abnormalities

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Background: Diffuse glioneuronal tumor with oligodendroglioma-like features and nuclear clusters (DGONC) is a rare brain tumor included as a provisional diagnosis in the 2021 5th World Health Organization (WHO) Classification of Tumors of the Central Nervous System (CNS). Although only a few cases of DGONC have been reported following the initial description of the tumor, they occur in the pediatric age group, have a distinct DNA methylation pattern, and share a recurrent chromosomal finding of monosomy 14. The natural history and optimal post-surgical adjuvant therapy are unknown.

Methods: We report a 7-year-old boy presented with a new onset of tonic-clonic seizures. Other than exhibiting some aggressive behavior, his history was otherwise negative, and his physical exam was non-focal. Magnetic resonance imaging (MRI) of the brain revealed a mass lesion in the left para midline inferior frontal/suprasellar area extending along the planum sphenoidal, medial left middle cranial fossa, and left optic apparatus. The patient underwent a craniotomy and a gross total resection of the tumor, from which he recovered uneventfully.

Results: As with many cases of DGNOC, imaging characteristics, and initial traditional pathologic evaluation were somewhat confusing with high-grade features. DNA microarray analysis revealed an unusual near-tetraploidy, albeit with only two copies of chromosome 14. DNA methylation analysis was performed and showed a consensus match to DGONC. Post-operatively, he was observed with surveillance scans and received no further adjuvant therapy. A follow-up MRI scan almost a year later showed recurrence both in the original tumor bed and in the ventricular system. The disease in the original tumor bed was again resected with pathology again consistent with DGONC. Adjuvant radiotherapy is now planned.

Conclusions: This case adds to the evolving understanding of the spectrum of genetic abnormalities seen in DGONC.

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FGFR alterations in pediatric, young adult, and adult brain tumors: a comparison of histologic, molecular, and clinical features

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Background: Fibroblast growth factor receptor (FGFR) alterations are found in a variety of CNS tumors including both low- and high-grade gliomas.

Methods: The frequency of FGFR alterations, their histologic and clinical associations, and patient outcomes were investigated.

Results: In pediatric/young adult (P-YA) subjects (< 40years), FGFR-altered tumors (N=57) comprised 11% of all sequenced low-grade glial/glioneuronal/neuronal tumors. Diagnoses included various glial/glioneuronal tumors and extraventricular neurocytoma. FGFR alterations were also present in high-grade tumors in P-YAs including diffuse midline glioma and medulloblastoma. FGFR1 alterations were most frequently observed (N=43), followed by FGFR2 (N=9) and FGFR3 (N=5). The most common alterations were fusions (N=21) and single nucleotide variants (N=28). Co-occurring alterations were present in 48% of in P-YA cases; NF1, PIK3CA, and PTPN11 were most frequent. In adult subjects (≥40years), FGFR alterations were observed in only 3% (N=40) of all sequenced gliomas. 91% were high-grade gliomas; glioblastoma was the most frequent diagnosis in adults. 65% of FGFR-altered adult high-grade gliomas harbored FGFR3::TACC3 fusions, and essentially all adult tumors had molecular alterations in addition to FGFR. Across all subjects, FGFR-altered low-grade tumors presented at a mean age of 14.6 years (range 1-36 years) and high-grade tumors presented at a mean age of 52.9 years (range 8-81 years). 98% of patients with low-grade tumors were alive with a median overall survival (OS) of 2.3 years and progression-free survival (PFS) of 2.0 years. 26% of low-grade tumors had radiographic evidence of progression; many of these tumors were hypothalamic region pilocytic/pilomyxoid astrocytomas. 75% of all subjects with high-grade tumors were deceased with a mean PFS and OS of 0.6 and 1.1 years, respectively. Targeted therapy was utilized in 12 patients with progressive disease.

Conclusions: FGFR alterations are present in a range of primary CNS tumors and not specific to tumor type and behavior, highlighting the importance of histologic diagnosis and grading.

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Concurrent presentation of brain arteriovenous malformation and BRAF-mutated low grade glioma

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Background: Diffuse low-grade glioma, MAPK pathway-altered (including BRAF p.V600E-mutant), is a low grade glioma generally occurs in childhood and is less common primary central nervous system tumor in adults. Interestingly, similar BRAF mutations are frequently observed in brain arteriovenous malformations (AVMs), a distinct vascular condition. The simultaneous occurrence of low-grade glioma and brain AVM is exceedingly rare, with limited reports available. This ambiguity extends to whether these conditions coexist or develop sequentially.

Methods: Patient clinical information was extracted from the electronic medical record, and histologic staining was conducted following standard protocols.

Results: We report a unique case of a 24-year-old male presented with a seizure, and was subsequently found to have a 7 mm enhancing lesion in the right occipital lobe on MRI. He has past medical history of attention deficit hyperactivity disorder and depression. The lesion, initially thought to be vascular in nature, raised differential diagnoses including AVM, angioma, or aneurysm, with a neoplasm or infection deemed less likely. Subsequent angiography did not reveal typical vascular anomalies but follow-up image showed stable enhancement and increased edema around the lesion, coinciding with additional seizure episodes. Excision was performed, revealing an AVM intertwined with infiltrating glial proliferation with pleomorphism, characteristic of diffuse low-grade glioma. The glioma is negative for IDH and H3K27M, with a slight increase in Ki-67, and next-generation sequencing identified BRAF p.V600E mutation. These findings confirmed the diagnosis of diffuse low-grade glioma, BRAF pV600E-mutant, with concurrent of an AVM.

Conclusions: This case contributes to the sparse literature on the co-occurrence of low-grade glioma and AVM, highlighting the need for further research to understand their pathophysiological relationship and molecular underpinnings.

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Do pilocytic astrocytomas with anaplasia still exist? – Report of a case

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Background: Pilocytic astrocytoma (PA) is a WHO grade 1 neoplasm that is generally associated with a favorable prognosis. While the WHO Classification of CNS Tumors (2021) recognizes PA with histological features of anaplasia as a variant, it also states that the majority (81%) of such histologically defined tumors harbor the methylation profile of high-grade astrocytoma with piloid features (HGAP).

Methods: We present a well-documented case of recurrent supratentorial PA with anaplastic transformation where the workup included NGS and methylation profiling.

Results: A 28-year-old male presented with headaches, nausea, and vomiting. MRI showed a heterogeneously enhancing mass measuring 4.2 x 2.5 x 2.6 cm within the occipital horn of the left lateral ventricle. A gross total resection was achieved. The tumor was histologically low grade and showed several features supporting PA, including piloid astrocytic cytologic features on the intraoperative smear prep, biphasic architecture with solid and microcystic areas, low cellularity, rare Rosenthal fibers, prominent hyalinized vessels, and noninfiltrative growth. No adjuvant therapy was administered. Nineteen months later, an MRI showed a rapidly enlarging, enhancing mass measuring 2.8 x 2.5 cm adjacent to the site of the previous resection. Histologically, the recurrent tumor contained both low-grade areas consistent with PA and similar to the previous specimen as well as anaplastic areas characterized by high cellularity, pronounced nuclear atypia, and a high mitotic index ranging from 8/10 HPF to 14/10 HPF. Both areas were strongly immunopositive for the BRAF V600E mutation. NGS confirmed BRAF V600E and also showed a TERT promoter mutation. Methylation profiling from the anaplastic portion (NCI/NIH) suggested the possibility of glioblastoma, mesenchymal subtype; however, the confidence score was below the established cutoff from all classifiers.

Conclusions: Even in the era of NGS and methylation profiling, cases of anaplastic PAs that are distinct from HGAPs still exist, particularly in adult patients with supratentorial PAs.

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Glioneuronal tumor with FGFR1::TACC1 fusion: Time to reconsider the extra-ventricular neurocytoma (EVN) terminology?

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Background: Extra-ventricular neurocytoma (EVN) is a rare neuroepithelial tumor that can arise in multiple locations within the central nervous system outside of the ventricles. While it can histologically resemble a central neurocytoma, these tumors have been reported to present with a wide morphological spectrum including oligodendroglia-like cells to ganglion or ganglioid cells, as well as GFAP or Olig2 positive components suggestive of glial differentiation.

Methods: Here we describe a case of a 46-year-old male to female transgender women with a multi-year history of an occipital brain tumor that had been followed serially with slow interval growth for 11 years prior to biopsy. Initially the patient was asymptomatic but began developing headache and visual symptoms of left hemianopsia as the tumor grew prompting them to eventually biopsy the lesion.

Results: The biopsy was characterized with alternating cellularity with areas of clustered tumor cells and areas of paucicellularity, with a variable spectrum of cell morphology such as pleomorphic round to oval tumor cells, scattered multinucleated cells, and a subset with peri-nuclear haloes resembling oligodendroglioma. Immunohistochemistry showed these tumor cells were positive for Synaptophysin and Olig2 with scattered positivity for GFAP in reactive astrocytes. Subsequent molecular testing showed a FGFR1::TACC1 fusion, a recurrent molecular feature of the diagnosis of EVN. Consistent with this diagnosis, methylation sequencing analysis showed a strong match score (0.8386) in the DKFZv12 classifier for EVN.

Conclusions: This case highlights the histologic characteristics of tumors harboring FGFR1::TACC1 fusions, including the presence of nuclear clusters, multinucleated cells, and glial elements, and suggests perhaps it is time to reconsider the term extraventricular neurocytoma in favor of a molecularly defined entity such as glioneuronal tumor with FGFR1::TACC1 fusion.

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The Molecular Characteristics of Granular Mitosis in Glioblastomas

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Background: Glioblastoma is the most common malignant neoplasm of the central nervous system (CNS) and has a poor prognosis with an average survival of 6 months. A high proliferative index, such as an increased mitotic rate, is a key feature of these high-grade tumors. “Granular mitosis” (GM) is a unique type of atypical mitotic figure observed exclusively in glioblastoma, but not other tumors. The molecular etiology of GM formation in glioblastoma is largely unknown.

Methods: The Cancer Genome Atlas (TCGA) Glioblastoma Multiforme dataset (N=619) was used. GM was annotated on the WSI to training a machine learning model. Differential gene expression, methylation, mitochondrial (mt) DNA, and copy number variation (CNV) analyses were conducted on the patient's corresponding genomic data. Downstream in silico overrepresentation analysis was performed to identify pathways, and multi-Omics Factor Analysis (MOFA) is conducted to detect patterns by integrating these datasets to gain an in-depth understanding of the factors associated with granular mitosis.

Results: There were 82 glioblastoma slides reviewed, and 53 glioblastoma cases were included for analysis (35 GM positive, 18 GM negative). The transcriptomic analysis results show that 80 genes are differentially expressed between glioblastoma patients who exhibit granular mitosis and patients who do not (FDR < 0.05; fold change >2). Functional enrichment through Gene Ontology (GO) analysis has identified associations with nucleosome assembly and organization ($p = 3.19e-10$), as well as chromatin structure ($p = 1.18e-11$).

Conclusions: Our findings demonstrate a molecular difference between glioblastoma with and without granular mitosis, including pathways in nucleosome assembly, organization and chromatin structure.

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Early truncating mutations in ASXL1 drive reduced H3K27me3 across various central nervous system tumors

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Background: ASXL1 encodes a chromatin remodeling protein. While ASXL1 inactivation leading to epigenetic dysregulation is a well characterized molecular driver of myeloid malignancies, ASXL1 inactivation in central nervous system (CNS) tumors remains poorly characterized.

Methods: We hypothesize that CNS tumors harboring ASXL1 mutations will demonstrate aberrant H3K27me3. Among 895 CNS tumors clinically sequenced at JHH, we identified 7 cases harboring somatic ASXL1 alterations, and 4 additional cases were retrospectively identified at MSKCC. We performed and interpreted H3K27me3 immunohistochemistry in all 11 cases. Assessment of H3K27me3 status in comparator ASXL1-wildtype tumors is also in process.

Results: Tumor types included IDH-wildtype glioblastoma (n=3), IDH-mutant astrocytoma (1), oligodendroglioma (3), H3K27-mutant diffuse midline glioma (1), PFA ependymoma (2), and medulloblastoma (1). Of these, nine harbored truncating ASXL1 mutations localizing to either exon 11 (p.R404*, p.Q428fs) or the 5’ region of exon 12 (p.G646fs x2, p.R693* x2, p.T822fs, p.S846fs, p.Q1074*). These cases demonstrated reduced H3K27me3 by immunohistochemistry. The single diffuse midline glioma case exhibited an oncogenic H3F3A p.K27M mutation. The two glioblastoma cases harboring mutations localizing to the 3’ region of exon 12 (p.R1148H, p.G1150fs) demonstrated retained H3K27me3 by immunohistochemistry.

Conclusions: Early truncation of ASXL1 is an uncommon mechanism for reduced H3K27me3 across various CNS tumors. Reduced H3K27me3 is observed in a subset of clinically aggressive oligodendrogliomas, and ASXL1 inactivation may be the underlying mechanism in some cases. We additionally identify ASXL1 mutations in two entities defined by H3K27me3 loss – diffuse midline glioma and PFA ependymoma. While ASXL1 has been reported in a small subset of these tumors, how ASXL1 mutations may synergize with either H3 p.K27M mutations in diffuse midline glioma or EZHIP overexpression in PFA ependymoma to modulate H3K27me3 remains to be explored. Cases harboring ASXL1 mutation leading to reduced H3K27me3 may present diagnostic challenges, with differential considerations including other H3K27me3-altered entities.

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Combined targeting MEOX2 and SRGN in glioblastoma stem cells disrupts malignant progression.

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Background: Glioblastoma (GBM) is the most lethal primary brain tumor with the intra-tumoral hierarchy of glioblastoma stem cells (GSCs). The heterogeneity of GSCs within GBM inevitably leads to treatment resistance and tumor recurrence. Here, we found that classical (CL) and mesenchymal (MES) GSCs are enriched in the reactive immune region and high CL-MES signature informs poor prognosis in GBM. Through RNA-sequencing datasets, we identified specific GSCs targets, including MEOX2 for the CL GSCs and SRGN for the MES GSCs. MEOX2-NOTCH and SRGN-NFκB axes play important roles in promoting proliferation and maintaining stemness and subtype signatures of CL and MES GSCs, respectively.

Methods: 1 Culture of glioblastoma stem cells and other cell models 2 Single-cell library preparation and sequencing 3 Plasmids and lentiviral transduction 4 Proliferation and neurosphere formation assays 5 RNA isolation and quantitative RT-PCR 6 Western blotting 7 Immunofluorescence 8 Immunoprecipitation

Results: CL and MES GSCs are enriched in reactive immune region regions and high CL-MES signature informs poor prognosis in GBM. MEOX2-NOTCH and SRGN-NFκB axis maintain stemness and subtype signatures of CL and MES GSCs, respectively. MEOX2 and SRGN mediate the resistance of CL and MES GSCs to macrophage phagocytosis. Targeting MEOX2 and SRGN via FDA-approved drugs can overcome GSCs heterogeneity.

Conclusions: Our investigation discovered that the representative targets MEOX2 and SRGN in CL and MES GSCs. We provided a deep understanding of the distinct regulatory mechanisms of CL and MES GSCs. Our data demonstrated that using genetic and pharmacologic techniques, combined therapy with FDA-approved agents against GSCs achieved the most effective tumor control.

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IFI35 regulates non-canonical NF-κB signaling to maintain cancer stem cells.

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Background: Glioblastoma (GBM) is the most aggressive malignant primary brain tumor characterized by a highly heterogeneous and immunosuppressive tumor microenvironment (TME). The symbiotic interactions between glioblastoma stem cells (GSCs) and tumor-associated macrophages (TAM) in the TME are critical for tumor progression. Here, we identified that IFI35 (Interferon-Induced Protein 35), a transcriptional regulatory factor, plays both cell-intrinsic and cell-extrinsic roles in maintaining GSCs and immunosuppressive TME.

Methods: Glioblastoma tissues of GSCs were obtained from excess surgical resection samples. In vivo tumorigenesis and animal models: Intracranial xenograft models were established by implanting BALB/c-Nude mice, RNA-seq and data analysis. Statistical analysis: All numerical data are presented as the mean ±SD from at least three independent experiments.

Results: Combined multiomics analyses reveal preferential expression of IFI35 in GSCs and GBM. IFI35 supports GSC proliferation and self-renewal. IFI35 promotes in vivo tumor growth and has therapeutic potential in GSCs.

Conclusions: The results identified IFI35 as both a transcriptional and translational regulatory factor in maintaining GSCs and recruitment of TAMs in GBM. Upregulated IFI35 levels in GSCs play both cell-intrinsic and cell-extrinsic roles in maintaining the immunosuppressive TME. Targeting IFI35 and its downstream signaling effectors may provide effective therapeutic strategies for GBM treatment.

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Primary intradural/extramedullary lumbar Ewing sarcoma: a case report

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Background: Ewing sarcoma (ES) is a malignant neuroectodermal tumor that usually affects children and adolescents in the second decade of life. It is considered a primary bone malignancy. Ewing sarcoma can occur along the spinal column as an osseous or extraosseous tumor. Primary intradural extramedullary Ewing sarcoma (IEES) is an exceedingly rare entity. We report a case of intradural extramedullary Ewing sarcoma (IEES) of the lumbar spine.

Methods: A 57-year-old male presented with progressive lower back pain with urinary urgency followed by complete loss of bladder function. Magnetic resonance imaging (MRI) of the spine showed multilevel lumbar spondylosis and two separate, large, intradural, extramedullary lesions at L2-3 and L5-S2 vertebral levels with significant displacement of the cauda equina. Consult with radiology colleagues noted intradural/extramedullary lesions without any bone involvement, with differential diagnosis including myxopapillary ependymoma, nerve sheath tumor, paraganglioma, and drop metastases. He underwent laminectomy with tumor resection.

Results: Sections showed monotonous small round blue cells with small inconspicuous nucleoli and scant cytoplasm with sheet-like growth and vague possible perivascular rosettes. No necrosis was appreciated, and the Ki-67 proliferation index was estimated at 15-30%. A panel of immunohistochemical markers showed the tumor cells were positive for CD99 (diffuse, membranous), focally positive for S100 and negative for CAM5.2, Pan-CK, EMA, CK7, CK20, SOX10, MART-1, tyrosinase, HMB45, Synaptophysin, INSM1, GFAP, Olig2, Inhibin, TLE3, Desmin, SMA, CD45. Fluorescence in situ hybridization (FISH) analysis revealed EWSR1 gene rearrangement. Subsequent sarcoma fusion next generation sequencing (NGS) confirmed the fusion partner as EWSR1-FLI1confirming a diagnosis of Ewing sarcoma

Conclusions: In conclusion, we report a rare case of primary intradural extramedullary ES which is an extremely rare malignancy in this location. This case reminds pathologists to consider rare possibilities and do the appropriate workup for monotonous small round blue cell tumors even in unlikely locations

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PLEKHH2::ALK Fusion-Positive Primary Intracranial Mesenchymal Neoplasm: A Case Report

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Background: We present the case of an 18-year-old female with no significant medical history who presented with progressive right-sided blurry vision and morning headaches. MRI revealed a 5.2 cm heterogeneous mass within the left frontal lobe.

Methods: Histologic examination revealed a haphazard proliferation of monomorphic spindled-to-stellate cells embedded in a myxoid background, with a pronounced hyalinized vascular network. The tumor was predominantly moderately cellular with rare hypercellular foci showing fascicular growth and a collagenized background. Mitoses were increased in hypercellular areas (5 mitoses per 10 HPF). Focally, the tumor was seen invading the adjacent brain parenchyma. Sparse chronic inflammation was noted in the background. Tumor necrosis was not observed.

Results: Immunohistochemically, the tumor cells were positive for CD34, S100, ALK, PR (focal), and EMA (focal), and were negative for SSTR2, GFAP, OLIG2, SOX10, STAT6, CAM5.2, SMA-D, Desmin, MUC4, and BCOR. INI1 and H3K27me3 were intact/retained. Ki67(MIB1) showed approximately 1-4% proliferative activity. Chromosomal microarray identified multiple copy number abnormalities, including homozygous loss of CDKN2A/B and chromothripsis of chromosome 2 showing breaks in PLEKHH2 and in/near ALK genes. Fusion panel testing detected a PLEKHH2::ALK fusion transcript.

Conclusions: Overall, the tumor exhibited findings like those described in an emerging family of mesenchymal tumors with similarities to NTRK-rearranged tumors. This specific ALK fusion is rare and to our knowledge has not been reported in an intracranial tumor to date. This case underscores the importance of molecular characterization in mesenchymal neoplasms as it provides valuable insights for diagnosis and potential targeted therapies. Further studies are warranted to elucidate the clinical significance and optimal management of such tumors.

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Unraveling Complexity: A Case of Intra-Cranial Malignant Peripheral Nerve Sheath Tumor

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Background: We present a rare case of an intra-axial malignant peripheral nerve sheath tumor (MPNST) in a 60-year-old male who presented with seizures preceded by a week of unsteady gait, dizziness, and weakness. The patient had a history of lung and brain masses two decades prior, addressed surgically and with chemoradiation therapy. Pathologic records from those lesions were not available. MRI revealed a heterogeneous enhancing solid mass in the left paramedian frontal lobe measuring 3.6 x 3.1 x 2.9 cm.

Methods: Histological examination demonstrated a spindle cell proliferation exhibiting extensive infiltration of the background brain. The tumor displayed a myxoid background with distinct islands of collagen. There was no tumor necrosis or microvascular proliferation; rare foci showed up to 7 mitoses per 10 HPF.

Results: Immunohistochemically, tumor cells were focally positive for SSTR2, CD34, CD99, Desmin, MyoD1, and myogenin, and negative for S100, GFAP, Olig2, IDH1(R132H), p53, SOX10, STAT6, EMA, ERG, Cam 5.2, SMA, and Cyclin-D. ATRX and INI1 were intact/retained. NeuN, synaptophysin, and neurofilament highlighted neurons. Ki67 was approximated at 15%. H3K27me3 immunohistochemistry revealed loss of nuclear staining in tumor cells. Next generation sequencing detected mutations in NF1, CHEK2, and ARID1A. Chromosomal microarray detected multiple copy number abnormalities including chromothripsis of chromosomes 4pq and 5q, an amplification of chromosome 4q containing the PDGFRA gene, whole arm losses of chromosomes 10p and 10q, and regional losses of chromosomes 9p, 13q, and 17q respectively containing CDKN2A/B, RB1, and NF1 genes. DNA methylation-based tumor classification classifier indicates a match for malignant peripheral nerve sheath tumor (confidence score of 0.90).

Conclusions: This case highlights the diagnostic challenges associated with intra-axial MPNST and the importance of comprehensive molecular characterization, including DNA methylation profiling, in the workup of such rare and enigmatic cases.

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Prevalence of seizure development in patients with meningioma and its predictors

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Background: Meningioma is the most common primary central nervous system tumours. The clinical presentation of meningioma are variables according to the site of the meningioma. Seizure is reported in around 30% of patients with meningioma. The aim of this study is to assess the prevalence of seizure as a presentation pf meningioma in patients from Saudi Arabia

Methods: We retrospectively reviewed patients with meningioma from King Abdulla Medical City from 2017 to 2023. Data collected included patients age, gender, location of meningioma, presentation, pathological grade and the presence of oedema.

Results: A total of 88 patients with meningioma were included with a mean age of 51.2 ± 12.4 years old. the most reported diagnoses were WHO grade 1. Meningothelial meningioma (31.8%), WHO grade 1. Transitional meningioma (30.7%), WHO grade 2. Atypical meningioma (18.2%), and WHO grade 1. Angiomatous meningioma (4.5%). The least reported types were WHO grade 1. A total of 27 (30.7%) cases with meningioma had seizures while most of them (69.3%; 61) had no seizures. None of the patient's bio-clinical factors showed a significant relation with having seizures. Seizures were insignificantly higher among older patients, male patients and patients who had edema ($P>0.05$ for all)

Conclusions: the prevalence of seizure in association with meningioma is 30.7%. No significant association of seizure development to the patient's bio-clinical factors.

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Meningothelial hamartoma in an 8-month-old male and a 9-year-old male: Clinical summary and key diagnostic points

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Background: Meningothelial hamartoma is a rare entity reported as a deep scalp lesion, with most occurrences in neonates and young children. Histologic descriptions involve round to spindle-shaped epithelioid meningothelial cells with an infiltrative pattern associated with connective tissue elements, emphasizing vascular channel associations. Case studies have expressed concerns about angiosarcoma in these lesions' workup due to the epithelioid cells forming structures resembling anastomosing vascular channels, referred to as "pseudovascular spaces." Here, we examine two cases of posterior scalp lesions in an 8-month-old male (Case 1) and a 9-year-old male (Case 2).

Methods: The resection specimens were serially sectioned and then entirely submitted. Our lab performed the H&E staining per protocol and then the following immunohistochemical stains on representative blocks: Case 1: EMA, Glut-1, D2-40. Case 2: EMA, CD34, PR, D2-40, vimentin, AE1/3.

Results: H&E staining demonstrated that both lesions were subcutaneous and notable for infiltrating patterns of round-to-ovoid meningothelial cells lining small capillary-like pseudovascular spaces embedded in a collagenous stroma. Case 1 demonstrated variably solid architecture with concentrated pseudovascular spaces in some areas, giving it a sieve-like pattern. Associations with vascular channels were conspicuous. Case 2 was a more spread-out, infiltrative distribution of meningothelial cells with scattered pseudovascular spaces discernible from anastomosing vasculature on H&E sections. Immunohistochemical stains read as follows: Case 1: EMA+, Glut-1(-), D2-40(-). Glut-1 and D2-40 stained vasculature. Case 2: EMA+, CD34+, PR+, D2-40+, vimentin+, AE1/3(-).

Conclusions: Our findings were adequate for diagnosing meningothelial hamartoma in both cases. Excluding vascular lesions was of higher priority for Case 1 because of the denser and more sieve-like areas of pseudovascular spaces and the more intimate vascular association. In addition to highlighting some distinguishing features, this report supports the need for consideration for diagnosing meningothelial hamartoma in the differential diagnosis of deep scalp lesions.

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Primary CNS Histiocytic Sarcoma: Case Report with Literature Review

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Background: Primary Central Nervous System (CNS) Histiocytic Sarcoma (HS) is a rare and challenging diagnosis with peculiar histopathological features. To date, there have been many reported cases of HS, however, primary involvement of the CNS is exceedingly uncommon, with less than 40 reported cases worldwide and it piques one's interest as it masquerades several other conditions both radiologically and histopathologically.

Methods: A 42-years old female patient presented with loss of consciousness and ataxia. Examination was only significant for an ataxic gait. Magnetic Resonance Imaging (MRI) Brain with contrast revealed a left cerebellar space-occupying lesion with features of high-grade pathology. Whole-body computed tomography (CT) scan was not significant. A sub-occipital craniotomy with gross-total excision of the lesion was performed. There was significant improvement in her ataxic gait and MRI showed complete resection of the lesion.

Results: Histopathological features of the lesion were of malignant histolytic-rich lesion and further analysis with immunohistochemsitry and molecular studies which confirmed the diagnosis of Primary CNS Histocytic Sarcoma.

Conclusions: Primary CNS histolytic sarcoma is a rare and aggressive malignancy with poor prognosis. The aim of this report is to add pivotal data to the literature given that the condition is frequently overlooked and can be confused with other common brain lesions. Hence, we emphasize the importance of a multidisciplinary approach to brain lesions. To date, there is no standard treatment option for HS and data in the literature on the condition is scarce. More studies are needed to determine the background of disease and the best available management options.

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Diffuse Spinal Cord Necrosis and Peripheral Neuropathy Due To Neuroleukemiosis in the Setting of Menin Inhibitor Treatment

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Background: Neuroleukemiosis is a rare leukemic complication wherein blasts infiltrate peripheral nerves leading to variable clinical symptoms that may overlap with treatment-related effects, complicating therapeutic decisions.

Methods: Report of a case: A 15 year old male with B symptoms was diagnosed with KMT2A-translocated t(9;11) acute myeloid leukemia (AML), and underwent induction and consolidation. Facial palsy development led to imaging showing a right frontal lobe mass; biopsy confirmed CNS involvement of AML. In addition to marrow, soft tissue, and cutaneous involvement by AML, masses in right sciatic nerve distribution were noted. He received radiation and intrathecal chemotherapy with methotrexate +/- cytarabine several times per week and developed progressive weakness and limb pain requiring opiates. Nerve conduction studies showed marked sensory nerves abnormalities and absent motor nerves responses in all extremities. He began Menin inhibitor SNDX-5613 with decreased size of myeloid sarcomas and absent blasts on bone marrow. Spinal MRI showed aberrant signal in dorsal columns from C1 to L1 without cord expansion/compression or enhancement, with a differential of subacute combined degeneration vs drug effect from SNDX-5613. The menin inhibitor was discontinued with rapid recurrence of peripheral blasts and expansion of myeloid sarcoma; the patient died of septic bacteremia.

Results: Autopsy demonstrated widespread involvement of marrow, soft tissue, lymph nodes, visceral organs and brain by AML. The spinal cord was diffusely necrotic and compressed by subdural and leptomeningeal blasts. Brachial plexus and sciatic nerve branches showed neuroleukemiosis and axonal loss without demyelination, with acute and organizing injury and marked fibrosis.

Conclusions: While clinical symptoms worsened during SNDX-5613 treatment, postmortem neuropathology favored combined effects of neoplastic spinal cord compression and neoplastic vascular and peripheral nerve involvement as the etiology of neurologic symptoms. Differentiating neuroleukemiosis from treatment effect is diagnostically challenging but an important consideration when withdrawing life-prolonging therapy.

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Evaluating MCM2 immunohistochemistry across meningioma histologic subtypes

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Background: Minichromosome Maintenance Complex Component 2 (MCM2) is a key factor involved in genome replication, whose expression has been associated with an aggressive molecular subtype of meningioma. Previously, we have shown that nuclear MCM2 is an independent immunohistochemical marker for aggressive meningiomas across CNS WHO grades. Here, we investigate the distribution of MCM2 indices across meningioma histologic subtypes.

Methods: Meningioma samples from various histologic subtypes reviewed and gathered. Immunohistochemistry for MCM2 was performed using a Leica BOND Rxm autostainer. Stained slides were digitized with a Hamamatsu 360 scanner. Automatic immunohistochemical quantification was performed using QuPath software. The highest regions of MCM2 percentage per slide were used for quantification.

Results: Forty meningioma cases were identified for inclusion, with histologic subtypes as follows: Clear Cell (n=10), Chordoid (n=3), Angiomatous (n=7), Secretory (n=2), Transitional (n=5), Meningothelial (n=7), Microcystic (n=3), and Atypical (n=3). The range of nuclear MCM2 percentages were (0.6-52.0%) across all samples. Clear Cell and Atypical meningioma subtypes had the highest MCM2 expression (2.3-52.0% and 9.6-44.6%, respectively). Mean MCM2 values for the histologic subtypes were: Clear Cell (16.9%), Atypical (24.5%), Angiomatous (6.3%), Secretory (3.6%), Transitional (10.3%), Meningothelial (4.6%), and Microcystic (13.0%). While there was a trend towards differences in MCM2 expression across subtypes, there was no statistical difference with one-way Analysis of Variance (ANOVA) and pairwise testing. Of note, a previously determined threshold of 40% positivity was only present in three tumors (two Clear Cell meningiomas and one Atypical meningioma).

Conclusions: Our preliminary findings indicate that Clear Cell and Atypical histologic subtypes have higher MCM2 indices than traditional CNS WHO grade 1 subtypes, consistent with their reported aggressive clinical behavior. Including more cases in the future will be useful in determining overall utility of MCM2 immunohistochemistry across meningioma histologic subtypes.

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A Rapidly Progressive Case of Primary CNS T-Cell Lymphoma

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Background: Primary CNS lymphoma (PCNSL) accounts for 2-4% of all primary brain tumors. CNS T-cell lymphoma is a rare subtype of PCNSL that can present diagnostic and treatment challenges.

Methods: We describe clinical and histologic findings in a case of rapidly progressive primary CNS T-cell lymphoma.

Results: A 64-year-old man with a history of juvenile psoriatic arthropathy and chronic eosinophilic dermatitis presented with multiple cranial neuropathies, perineuritis, and scleritis, concerning for CNS involvement by vasculitis. He was treated with corticosteroids and rituximab with some improvement of visual symptoms. Imaging showed extensive leptomeningeal and parenchymal enhancement involving the cerebellar folia, cervical spinal cord, cingulate gyrus, and cranial nerves. CSF cytology demonstrated large, atypical lymphoid cells. CSF flow cytometry showed no evidence of a monoclonal B-cell population, and molecular studies were negative for MYD88 mutation. IGH clonality testing was suspicious for a clonal B-cell population, while TCR clonality testing detected multiple TCR-gamma rearrangements. Infectious causes were excluded after an extensive workup. An autoimmune encephalitis panel was negative. Right frontal brain biopsy showed foci of perivascular atypical lymphoid cells that were CD2+, CD3+, and granzyme B+, suggestive of T-cell lymphoma. The patient's visual symptoms worsened with development of right-sided optic neuritis and new subacute cerebellar ischemic infarcts. Imaging showed bilateral T2/FLAIR hyperintense cerebellar lesions. Cerebellar biopsy revealed a dense infiltrate of large, atypical lymphoid cells that were CD2+, CD3+, CD7+, CD8+, CD56+, granzyme B+, and perforin+, extensively involving the cerebellar parenchyma and leptomeninges, consistent with a diagnosis of peripheral T-cell lymphoma (PTCL-NOS) with a cytotoxic phenotype. The patient expired after diagnosis (one month after admission) before methotrexate treatment could be initiated.

Conclusions: This case highlights the diagnostic challenges of identifying PCNSL, and the need for developing tools towards earlier and more accurate diagnosis, as well as awareness of rarer types of PCNSL such as T-cell lymphomas.

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Large B-cell Lymphoma with IRF4 Rearrangement Presenting as a Primary Central Nervous System Lymphoma in the Brain

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Background: Large B-cell Lymphoma (LBCL) with IRF4 rearrangement (LBCL-IRF4r) constitutes < 0.1 % of LBCL, typically presents in the head and neck, specifically the Waldeyer ring, with a diffuse and/or follicular growth pattern, and is more common in pediatric/young adult population and rare in adults. To our knowledge, it has not been reported in the central nervous system in the English literature.

Methods: Department of Pathology files were searched for cases of LBCL-IRF4r between 2016-2024.

Results: One case was identified. A 27-year-old man presented to the Emergency Department with headache, double vision, and dizziness, and a right ventricular/basal ganglia mass with surrounding edema was identified. Biopsy showed diffuse sheets of large lymphoid cells with mildly-irregular nuclei, open chromatin pattern, and occasional nucleoli/chromocenters. Immunohistochemically, MUM1, CD20, BCL6, BCL2, and CD10 were positive. Ki-67 proliferation index was about 90%. FISH identified a DUSP22::IRF4 rearrangement, and no rearrangement of BCL2, BCL6, or MYC. Flow cytometry of the cerebrospinal fluid was negative. Bone marrow biopsy was negative for lymphoma by morphology and flow cytometry. PET/CT showed no evidence of systemic disease, indicating a primary central nervous system (CNS) LBCL-IRF4r. The patient received four cycles of MATRIX chemotherapy with significant response, and BCNU-Thiotepa-based autologous stem cell transplant. At two-year post-transplant follow-up, his MRI showed no evidence of disease. He continues to be in complete remission.

Conclusions: Included as a provisional entity in the 2016 revision of the World Health Organization Classification of Lymphoid Neoplasms, LBCL-IRF4r is rare. Histologically, it may resemble high-grade follicular lymphoma or diffuse LBCL. Strong expression of IRF4/MUM1 and BCL6 is characteristic, a clue for further investigation. IRF4 rearrangement can be seen in other hematolymphoid neoplasms, such as plasma cell neoplasms, but their histology is different. Familiarity with such updates and unusual entities is important for pathologists in the work-up of hematolymphoid neoplasm of the CNS.

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Metastatic extra-axial, right frontal convexity olfactory neuroblastoma, an uncommon entity

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Background: Olfactory neuroblastoma (ONB) is a locally aggressive malignant neuroectodermal neoplasm originating from sensory olfactory cells of the upper nasal cavity, representing 2-6% of intranasal tumors. Whereas local recurrence and contiguous extension of ONB are not unusual, metastatic spread to the meninges, both distant from and not connected to the primary tumor, is an infrequent occurrence. The reported gene mutation profiles of ONB show significant variation and the molecular profile of intracranial metastatic ONB is not well described.

Methods: We report the clinical presentation, histologic analysis and molecular findings of a metastatic right frontal convexity, extra-axial ONB in a patient with prior resected and treated nasal cavity ONB.

Results: A 45-year-old female presented with anosmia, proptosis, and double vision due to a sinonasal mass involving bilateral ethmoid sinuses extending into the anterior cranial base, diagnosed as an ONB. The patient showed poor response to neoadjuvant chemotherapy, then underwent surgical excision and focused radiation therapy with no local recurrence observed on radiological follow-up. One year later, MRI revealed a 4 mm enhancing mass along the right frontal cerebral convexity, clinically suspected as a meningioma. Resection of this mass revealed "small round, blue cells" with a high nuclear to cytoplasmic ratio and positive staining for chromogranin, synaptophysin and CD56, resembling the primary ONB. Immunohistochemistry for EMA, SOX-10 and S-100 was negative. The final diagnosis was metastatic distant meningeal olfactory neuroblastoma. Molecular profiling revealed a TP53 gene point mutation.

Conclusions: ONBs are locally aggressive with a tendency for local recurrence, but distant metastasis to leptomeninges is rare. Although TP53 mutations in ONB are associated with a poorer prognosis, the molecular features of distant metastases are not well-explored. The identification of a TP53 mutation in this metastatic ONB suggests that such mutations may indicate an unfavorable prognosis, potentially involving distant intracranial spread.

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Hemorrhagic presentation: A characteristic of Primary Intracranial Sarcomas, DICER-1 mutant ?

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Background: Primary intracranial sarcomas (PIS) DICER-1 mutant tumors are rare, originating from multipotent primitive mesenchymal cells within leptomeninges or dura. This report highlights its tendencies to present with serious hemorrhagic manifestations and masquerade as a primary vascular event.

Methods: Analysis of clinical records, histopathologic, immunohistochemical and molecular studies, and evaluation of relevant medical literature.

Results: A young 26-year-old male with a history of anxiety, alcohol and cannabis use disorder presented for severe headaches. Imaging revealed right frontoparietal intracranial hemorrhage requiring decompressive craniectomy. The initial diagnosis was a ruptured arteriovenous malformation. A digital subtraction angiogram demonstrated evidence of venous congestion and arteriopathy, but no definite nidus was identified. Areas of enhancement along the margin of hemorrhage were concerning for neoplasia, but a vascular etiology seemed more likely, since metastatic workup was negative. One month later, the patient re-presented with acute nausea, lethargy, and aphasia. Imaging revealed another large right frontoparietal hematoma. Despite embolization, the patient experienced persistent neurologic deficits, and during evacuation, an abnormal mass was encountered. Histologic examination, besides prominent hemorrhagic areas, revealed a high-grade spindle-celled neoplasm with sarcomatous features. There were areas with pleomorphism, including multinucleated giant cells, but the characteristic eosinophilic globules were identified only focally in a subsequent resection. Marked tumor vascularity was noted with lakes of blood, and necrosis, including those of vessel walls. Immunohistochemically, glial and meningeal markers were negative. Methylation profile classifiers confirmed a high-grade primary intracranial sarcoma, DICER1-mutant. An additional alteration included a pathogenic variant on PDGFRa.

Conclusions: This report highlights a relatively new tumor entity that can present with serious hemorrhagic manifestations, posing challenges and delays in diagnosis. Although anecdotal, at least six more instances of PIS with intra-tumoral or subdural hemorrhage were identified in the recent literature, and eight more in cases prior to the recognition of an associated DICER-1 mutation in primary intracranial sarcomas.

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Two cases of B-Cell Lymphoma Masquerading as an Inflammatory Pseudotumor of the CNS with Concurrent EBV and HSV-1 Positivity

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Background: Inflammatory pseudotumor is group of rare lesions of unknown etiology. Primary CNS involvement was rarely described presenting commonly as supratentorial nodule with meningeal attachment. Histologically, it shows mixed inflammatory mononuclear cells and high association with Ebstein-Barr virus. We are reporting two cases of CNS B-cell lymphoma presenting as inflammatory pseudotumor showing polyclonal inflammatory infiltrate by H&E, immunohistochemistry and flow cytometry in 12- and 56-year old patients. B cell lymphoma was diagnosed by clonal immunoglobulin heavy chain (IgH) gene rearrangement. One case was positive for EBV and HSV1.

Methods: H&E, immunohistochemical staining and flow cytometry for representative tumor sections were done according to our laboratory protocol in Westchester Medical center (Valhalla, NY). Cases were sent for B-cell gene rearrangement by PCR to Neogenomics (Aliso Viejo, CA) and Genoptix (Carisbad, CA).

Results: H&E sections showed involvement of brain parenchyma by a mixed infiltrate of lymphocytes, plasma cells and macrophages in one case. The other case showed cerebellar/periventricular mixed lymphoid infiltrate. Immunohistochemistry showed that the cells are polyclonal with CD3 positive T cells and CD20 positive B cells. B cells were also positive for CD79a and PAX-5, BCL-6 and negative for BCL-2. Flow cytometry showed mixed population of cells with no evidence of lymphoma. Gene rearrangement studies showed clonal IgH gene rearrangement by PCR with a diagnosis of B-cell lymphoma. One case showed EBV and HSV-1 positivity by PCR.

Conclusions: We are describing two cases of B-cell lymphoma diagnosed primarily as inflammatory pseudotumor based on H&E, immunohistochemistry and flow cytometry. However, The diagnosis of B-cell lymphoma was based on clonal IgH gene rearrangement detected by PCR. To our knowledge, this is the first reported cases of primary CNS lymphoma mimicking inflammatory pseudotumor. This also raises a question whether lymphoma arose from the mixed chronic inflammatory process or lymphoma triggered a reactive inflammatory response.

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Expression of CD163 in Meningioma and its correlation with WHO grading: A single institution case cohort series

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Background: Meningioma is the most common primary brain tumor and is classified into low grade/WHO grade 1, atypical/WHO grade 2 and anaplastic/WHO grade 3 types. Studies have shown CD163, transmembrane protein of monocytes/macrophages, to be aberrantly expressed in some cancers. It was also shown to inhibit apoptosis in rectal cancer suggesting relation with cancer aggressiveness. However, CD163 expression in meningiomas especially in higher grades and its expression in normal Meningothelial cells is not well documented. We studied CD163 expression in all meningioma grades. We also correlated CD163 expression with three grades of meningioma and examined the expression of CD163 in normal meningothelial cells.

Methods: We performed immunohistochemistry on 60 human meningioma specimens of different WHO grades to assess the expression of CD163 in meningioma. We scored the expression of CD163 in the positive cases then, we did correlation analysis between grades of meningioma and CD163 expression. We are also performing CD163 immunohistochemistry on 15 dural autopsy specimens to examine its expression in normal meningothelial cells. Correlation was done by using correlation coefficient and statistical analysis was done by using graphpad prism software.

Results: On immunohistochemistry, CD163 was found to be positive in ~84% of meningioma cases with different degrees. There was a positive correlation between the three grades of meningioma and the positivity of CD163 and also with degree of expression in positive cases. The only low-grade meningioma showing extremely high CD163 expression degree (~100%) was the lymphoplasmacytic-rich meningioma.

Conclusions: Our study shows expression of CD163 in meningioma. To our knowledge, this is the first study to perform correlation analysis including three grades of meningioma and CD163 expression. We also measured percentage of CD163 positivity in different meningioma types of low grade/WHO grade 1 meningioma. Results from this study is highlighting CD163 as a potential valuable marker in meningioma.

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Primary Intracranial Sarcoma, DICER1-Mutant in a Child with Neurofibromatosis Type 1: A Case Report

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Background: Among the mesenchymal, non-meningothelial primary intracranial tumors described by the 5th edition of the WHO Classification of Tumors of the Central Nervous System, primary intracranial sarcoma, DICER1-mutant, represents a newly recognized, rare entity. These tumors are most characteristically defined by pathogenic alterations in the DICER1 gene which can be either somatic or germline, the latter as part of DICER1 syndrome. Additionally, these tumors generally have MAPK signaling pathway activating alterations, including mutations in NF1, and a rare association with neurofibromatosis type 1 has also been observed.

Methods: We present a case of a 16-year-old boy with neurofibromatosis type 1 and previous history of pilocytic astrocytomas who presented with a rapidly enlarging left frontal intracranial mass which was diagnostic on pathology of primary intracranial sarcoma, DICER1-mutant.

Results: The tumor had characteristic histology of primary intracranial sarcoma, DICER-1 mutant, with molecular work-up demonstrating mutations in DICER1, NF1, and TP53, hallmark alterations providing key molecular diagnostic confirmation. Finally, DNA methylation profiling demonstrated a consensus methylation class suggestive of “primary intracranial sarcoma, DICER1-mutant”, further providing diagnostic support.

Conclusions: This case is particularly notable in that it contributes additional evidence of an association of this tumor with neurofibromatosis type 1, an only occasionally described finding, as well as providing another report of this unusual neoplasm including comprehensive molecular profiling.

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Erdheim-Chester disease presenting in the foramen magnum with mass effect of a 39-year-old male

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Background: Erdheim-Chester disease (ECD) is a rare clonal histiocytosis neoplastic condition. This condition is characterized by a non-specific infiltration of foamy macrophages in multiple organs, such as the bone or skeletal system (>95%), retroperitoneum, kidneys, and heart, resulting in inappropriate inflammation, fibrosis, and multi-end organ dysfunction. central nervous system (CNS) is present in a minority of cases. This condition is extremely rare with only a few hundred cases reported in medical literature.

Methods: We present a case of ECD in a 39-year-old male with past medical history for type-2 diabetes mellitus, neuropathy, and low testosterone on replacement therapy who experienced worsening migraines and was found to have a homogenously enhancing craniocervical mass on brain magnetic resonance imaging (MRI; greatest dimension 3 cm). Mass effect was present with displacement of the inferior brainstem and cervical cord to the right. Clinical differential included primary CNS tumors like meningioma, and surgical resection for definitive diagnosis and management was pursued. Intraoperatively, it was very fibrous and difficult to resect. It encased the vertebral artery and so only a limited resection could be performed.

Results: Grossly the mass appeared orange-yellow to pale-tan with a soft texture. Microscopically numerous histiocytes with ranging degrees of foamy cytoplasm in a background of increased fibrosis was identified. Histiocytic markers such as CD163 and PU.1 were diffusely positive in these cells, while CD1a and Langerin were both negative. No microorganisms were identified on special stains either. The patient was found to be BRAF mutant positive, supporting a diagnosis of ECD. Abdominal imaging and a biopsy of a renal mass was also consistent with ECD. The patient was appropriately started on Vemurafenib.

Conclusions: This case highlights the importance of considering ECD when histiocytosis is identified.

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Germ cell tumors in the central nervous system: a 16-year-single-institution experience
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Background: Germ cell tumors (GCTs) in the central nervous system (CNS) are rare neoplasms, which can be either primary or metastatic; this is a retrospective study of their clinical and pathological characteristics.

Methods: The institution`s neuropathology archives were searched for primary and metastatic germ cell neoplasms diagnosed between 2007 and 2023. Relevant clinical and pathological data was collected from the electronic medical record.

Results: Twenty-three cases from eighteen patients were identified (17 male, 1 female). The mean age at diagnosis was 28 years (range, 13-59 years). Eleven tumors were primary and twelve were metastatic (11 testicular, one undetermined). Five cases presented with imaging findings of intracranial hemorrhage; the rest were mass lesions. Histologically, all primary GCTs showed predominantly a germinoma morphology and all metastatic GCTs were non-seminomatous. The latter occurred mostly in non-midline locations. The average clinical follow-up was 46 months (range, 0.5 – 168 months); seven patients had no evidence of disease, seven were dead of disease, two were alive with disease and for two follow-up data was unavailable.

Conclusions: In our cohort, GCTs in the CNS affect predominantly young and middle-aged males and have variable outcomes. A non-germinoma morphology and non-midline location strongly suggest metastatic disease.

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An unexpected intraventricular neoplasm

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Background: Neoplasms involving the ventricles represent a heterogenous group of neoplasms. Tumors such as ependymoma, subependymoma, central neurocytoma, subependymal giant cell astrocytoma, chordoid glioma of the third ventricle, rosette-forming glioneuronal tumors, and other glial/glioneuronal tumors are thought to arise from components of the ventricular wall and septum pellucidum. Ventricular components, such as the choroid plexus, can also give rise to choroid plexus papillomas and carcinomas. In addition, mesenchymal and leptomeningeal components may give rise to sarcomas and meningiomas. Here we report an unusual and unexpected intraventricular neoplasm.

Methods: A 59-year-old woman with no significant past medical history presented with sudden onset diplopia, unilateral hearing loss, and ataxia. MRI revealed two T1 isointense, T2 slightly hypointense, avidly enhancing masses (right: 1.2 x 1.4 x 1.4 cm; left: 2.0 x 2.1 x 1.5 cm) in the region of the bilateral foramina of Luschka extending into the cerebellomedullary cisterns. The radiologic differential included choroid plexus neoplasms such as papilloma or carcinoma, ependymoma, and metastases. No mass lesions were identified in other areas of the body.

Results: Microscopy revealed choroid plexus and cerebellar tissue involved by single and small clusters of lymphoid cells having oval, indented or irregular nuclei, vesicular chromatin, small nucleoli and moderate amount of cytoplasm. Tumor showed positivity for CD45, CD20, BCL-6, BLC-2, MUM1 and CD19. Tumor was negative for pan-cytokeratin.

Conclusions: The immunohistochemical staining pattern supported a diagnosis of primary CNS diffuse large B cell lymphoma. This case underscores the complexity of diagnosing primary CNS lymphomas, especially when they present in atypical locations like the choroid plexus of the fourth ventricle. It highlights the indispensable role of immunohistochemical profiling in distinguishing primary CNS lymphoma from other intraventricular neoplasms. Awareness of such presentations is crucial for neuropathologists and neurosurgeons, as it broadens the differential diagnosis for fourth ventricle masses and informs appropriate therapeutic strategies.

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Central Neurocytoma: 20-year retrospective with clinical follow up

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Background: Central neurocytoma (CN) is a rare, intraventricular, midline nervous system tumor with a unique DNA methylation profile, but no recurring somatic mutational alterations, making it distinct from extraventricular neurocytomas (EVN) that are most commonly characterized by FGFR1::TACC1 fusion. EVNs can show considerable “disconnect” between histological features and DNA methylation, often requiring the latter for confident diagnosis, whereas CNs manifest sufficiently stereotypic histological features that further assessment by methylation and next generation sequencing is usually not performed and indeed, unnecessary for confident diagnosis. CNs manifest monotonous sheets of uniform round cells with speckled chromatin and scant NeuN/synaptophysin immunoreactivity, without full ganglionic differentiation. Although WHO grade 2 is shared between EVN and CN, it is unclear if prognosis is equal. Indeed, clinical implications, if any, for “atypical central neurocytoma”, defined by WHO 2021 as showing: “atypia, mitotic figures, vascular proliferation, necrosis, and/or elevated proliferative index (Ki-67/MIB-1)” have not been clarified.

Methods: Institutional database text word searches, 2007-2023, coupled with chart review.

Results: 20 cases identified: 12 male: 8 female, ages 22-78. Mean age at the time of diagnosis was 36.25 years, median 34.5 years. 5/20 cases had MIB-1 labeling > 3%, with 2 cases having a MIB-1 of 4-6%, and the remaining 3 cases having a MIB-1 of 8% as well as scattered mitotic figures. 2/5 cases were lost to follow up, 1 case is 14 years poster surgery and the remaining 2 cases were recently performed. 15/20 had sufficient clinical information to provide meaningful follow up; these 15 were all alive and well, with a post-surgical range of 3 weeks to 20 years. Longest survivals were 19 and 20 years.

Conclusions: We conclude that although the terminology of “atypical central neurocytoma” is being utilized, we could not detect influence of MIB-1 labeling index or mitotic rate on patient outcome in our small series.

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Papillary Tumor of the Pineal Region Lacking Papillary Features in a 29-Year-Old Man: An Unusual Manifestation of a Rare Neoplasm

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Background: Primary pineal region neoplasms are uncommon. Most occur in children and young adults. Herein, we describe a case of a rare neoplasm, a papillary tumor of the pineal region (PTPR).

Methods: A literature search using key words including “primary pineal neoplasm” and “papillary tumor of the pineal region”.

Results: A 29-year-old man who presented with intermittent headaches and dizziness. Magnetic resonance imaging of his head identified (1) a pineal region mass, 1.8 X 1.3 X 1.4 cm, T2/FLAIR hyperintense, with contrast enhancement, with mild mass effect on tectum and minimal narrowing of the cerebral aqueduct which remained patent, (2) mild to moderate ventriculomegaly without significant edema, and (3) a retrocerebellar cyst with imaging features consistent with an arachnoid cyst with mild hypoplasia of the vermis and lateral displacement of the hemispheres. Resection revealed a neoplasm with ependymoma-like histologic features, lacking papillary architecture, with expression of cytokeratin and lack of expression of glial fibrillary acidic protein. Loss of chromosomes 1, 3, 9, 10, and 14 was noted and methylation analysis was diagnostic of PTPR, subclass A, World Health Organization grade 2. A literature review found that PTPRs are rare, neuroepithelial lesions, most likely arising from specialized ependymal cells located in the subcommissural organ. First described in 2003, they have been included as an entity in the World Health Organization Tumors of the Central Nervous System since the 2007 edition and constitute fewer than 1% of all central nervous system neoplasms with fewer than 200 described in the literature to date

Conclusions: PTPR is a rare neoplasm and should be in the differential diagnosis of a pineal region lesion. The lack of papillary architectural features in this case with immunohistochemistry and molecular evaluation findings diagnostic of PTPR could represent limitations of sampling or reflect one morphologic phenotype of the neoplasm.

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An Autopsy Case of Tumor-to-Tumor Metastasis Involving Bilateral Dysplastic Cerebellar Gangliocytomas in Cowden Syndrome

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Background: Tumor-to-tumor metastasis (TTM) is defined by the presence of two histologically distinct tumors at one location, not otherwise explained by contiguous growth, collision, or embolization. Although a rare phenomenon, TTM may occur more frequently in patients with genetic tumor predisposition syndromes. Cowden syndrome is an autosomal dominant disorder caused mainly by germline PTEN mutations and is characterized by hamartomas and a high risk of malignancy, including carcinomas of the breast and thyroid gland.

Methods: We present the autopsy findings of a 49-year-old woman with Cowden syndrome. Antemortem genetic testing confirmed a germline pathogenic variant of PTEN (missense mutation c.359C>A, p.A120E). The patient had a history of trichilemmomas, papillary thyroid carcinoma (status post total thyroidectomy), multiple meningiomas, bilateral dysplastic cerebellar gangliocytomas, and bilateral invasive ductal carcinoma (status post mastectomy). At the time that she presented with generalized weakness, the patient had biopsy-proven metastatic breast carcinoma involving the lungs and multiple enlarging intracranial masses, which were complicated by worsening hydrocephalus and cerebellar tonsillar herniation prior to death.

Results: Postmortem examination demonstrated metastatic carcinoma involving the bilateral lungs, liver, bilateral adrenal glands, and bone. Neuropathologic examination revealed cerebellar tonsillar herniation and acute hypoxic-ischemic changes of the brainstem. Histologic examination confirmed the presence of bilateral dysplastic cerebellar gangliocytomas and multiple meningiomas, all of which demonstrated TTM by metastatic breast carcinoma. Additionally, there was metastatic breast carcinoma involving the calvarium, dura, and cerebrum.

Conclusions: To our knowledge, this is the second reported autopsy case of metastatic carcinoma to dysplastic cerebellar gangliocytoma in Cowden syndrome. The preferential colonization of lesional over normal cerebellar tissue sheds light on the altered microenvironment. Further, it favors neoplasm over hamartoma with regards to the histogenesis of dysplastic cerebellar gangliocytoma.

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Chondroid Chordoma with RICTOR Amplification: A Case Report

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Background: Chordomas are slow growing, locally invasive, malignant bone neoplasms that arise in the axial skeleton from remnants of the embryonic notochord. Chondroid chordoma is a characteristic histological type of conventional chordoma that contains an extracellular matrix mimicking hyaline cartilage. The mutational landscape of chordomas is heterogeneous; and the molecular mechanisms of the notochordal tumoral transformation are not well understood.

Methods: We present the case of a 61-year-old female with a chondroid chordoma arising from the C2 vertebral body. One year after surgical resection and adjuvant radiation, the patient developed weakness of both hands; and imaging showed tumor recurrence. Given its treatment resistance and local progression, the tumor from the initial resection was interrogated with next-generation sequencing using the Illumina TruSight Oncology 500 assay.

Results: Histopathology showed chondrocyte-like tumor cells embedded within a hyaline articular-like cartilaginous matrix. Many areas were necrotic; however, no mitotic figures and no areas of dedifferentiation were observed. Immunohistochemical staining of the tumor showed diffuse positivity with cytokeratin AE1/AE3, variable positivity with S-100, uniform nuclear positivity with brachyury, and retained SMARCB1 (INI1) expression, consistent with a conventional chondroid chordoma. Next-generation sequencing of the tumor demonstrated microsatellite stability, low tumor mutational burden (3.13 mut/Mb), and homologous recombination proficiency (HRD score: 14). Potentially pathogenic alterations included amplifications of RICTOR, FGF10 and MDM4. Variants of unknown significance included a CCNE1 promoter mutation (c.-993G>A) and missense substitutions in POLD1 (c.16C>T, p.R6W), CIC (c.2918C>A, p.T973K), FLT4 (c.2228C>T, p.A743V) and SPTA1 (c.3398G>A, p.R1133Q).

Conclusions: Amplifications of RICTOR, FGF10 and MDM4 are known oncogenic alterations and associated with poor prognosis in different cancer types. To our knowledge, this is the first report of a chordoma with RICTOR amplification, which was co-amplified with FGF10 on chromosome 5p13. RICTOR amplification may represent a clinically significant alteration in chordoma and predict response to targeted therapy with RICTOR/mTORC2 inhibitors.

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A Rare Case of a Low-Grade, Multinodular and Vacuolated Neuronal Tumor

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Background: This case involves a 54-year-old female who presented with intermittent headaches, nausea, and left upper extremity numbness. Brain magnetic resonance imaging (MRI) revealed a non-enhancing, intra-axial, lobulated lesion (2.6 x 2.5 x 1.3 cm) in the anterior right parietal lobe near the frontoparietal junction involving the cortex and subcortical white matter.

Methods: Tumor sections demonstrated a neoplasm of low cellularity with nodular-like organization composed of neuronal tumor cells within a background of vacuolated neuropil. Mitotic activity was inconspicuous. No high-grade features of necrosis or microvascular proliferation were identified.

Results: Immunohistochemical stains were ordered with synaptophysin showing positivity in a subset of tumor cells while simultaneously highlighting the background neuropil with diminished staining noted in the pale nodular areas. Tumor cells were negative for GFAP, IDH1-R132, NeuN, and CD34. The MIB1 proliferation index was estimated at less than 2%. Based on the characteristic histopathologic features, a diagnosis of multinodular and vacuolating neuronal tumors (MVNTs) was established. Sections of the tumor were sent out to the National Institutes of Health (NIH) for DNA methylation profiling, which demonstrated mildly hypercellular brain tissue with focal edematous changes.

Conclusions: MVNTs are rare, low-grade tumors that were most recently described in a 2013 study by Huse et al. Shortly after this study, they were acknowledged as separate, independent entities and given a Central Nervous System World Health Organization (WHO) Grade 1 designation. A fundamental aspect unique to this case that warrants further discussion is the NIH methylation results subclassifying the tumor as normal brain tissue. In combination with the radiological findings of a non-enhancing lesion and vacuolar changes observed on microscopy, MVNTs could pose a diagnostic challenge making them hard to distinguish from other entities such as focal cortical dysplasia or Creutzfeldt-Jacob Disease. This further highlights the importance of the morphological diagnostic criteria in accurately recognizing these rare entities.

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Embryonal tumor with multilayered rosettes demonstrating neuronal differentiation after chemotherapy and radiation

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Background: Embryonal tumor with multilayered rosettes (ETMR) is a rare embryonal neoplasm most commonly occurring in the cerebral hemispheres in patients less than 4 years of age. ETMRs characteristically have C19MC alterations or DICER1 mutations. They are rapidly growing tumors with an aggressive clinical course. Treatment includes gross total resection, chemotherapy, and/or radiotherapy which may prolong overall survival. Post-treatment maturation and neuronal differentiation have been reported in few cases which may contribute to favorable outcomes.

Methods: Our patient is a 4-year-old girl diagnosed with ETMR at the age of three. She presented with headache, vomiting, lethargy, and incontinence. MRI revealed a 7.9 cm mixed cystic/ solid enhancing mass in the right frontal lobe. Following gross total resection, pathology showed a densely cellular embryonal neoplasm with scattered multilayered true rosettes and ependymoblastic rosettes in addition to large areas of necrosis, and brisk mitotic activity. Molecular testing revealed amplification of C19MC in 49% of cells, supporting diagnosis of ETMR.

Results: She was treated per ACNS0334 reg A NOS with intrathecal topotecan, as well as autologous stem cell transplants, and proton beam radiation. One month after completing radiation, surveillance MRI suggested recurrence. She underwent another resection, and pathology now showed residual neoplasm with nodular architecture and mature neuron-like neoplastic cells in a neuropil-rich stroma. No embryonal cytologic features, rosette formation, or mitotic activity were identified. Repeat molecular testing showed three to eight copies of C19MC in 30.5% of cells.

Conclusions: This case contributes to the few reports to date of post-treatment maturation/differentiation in ETMR. Similar to previously reported cases, C19MC alterations were found in a fewer percentage of cells which may correlate with the neuronal differentiation. Treatment regimens vary across reported cases, so the association with particular treatments remains to be explored.

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Two patients with WHO Grade 3 Solitary Fibrous Tumors who reside in neighboring municipalities.

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Background: Solitary fibrous tumors (SFTs) within the central nervous system (CNS) are uncommon neoplasms with rare instances of WHO Grade 2 and Grade 3 SFTs (approximately 0.4% of all CNS neoplasms). Here we present two patients: a 35-year-old male with ongoing headaches for one month and a 43-year-old male with two days of nausea, headache, and intermittent left eye blurry vision. Of note, both patients resided in neighboring municipalities and presented a month apart.

Methods: Medical imaging for the 35-year-old male showed a 9.2 cm heterogeneously enhancing mass arising from the right lateral ventricle, with minimal surrounding vasogenic edema and multiple foci of susceptibility signal abnormality suggesting blood products. Imaging for the 43-year-old male showed an avidly enhancing multilobulated extra-axial supratentorial and infratentorial 7.8 cm mass centered in the region of the straight sinus and vein of Galen with a suspected dural tail arising from the left tentorial leaflet.

Results: The findings from the occipital resection for the first patient and parietal resection for the second patient were diagnostic of a solitary fibrous tumor, WHO grade 3. Immunohistochemistry for both resections demonstrated positive staining for STAT-6, CD34, and CD99.

Conclusions: High grade CNS SFTs are rarely encountered. The presence of a NAB2-STAT6 fusion can help confirm the diagnosis as CD34 immunohistochemistry can be lost in these high-grade tumors. High-grade SFTs are associated with TP53 and TERT promotor mutations. One tumor did not show either of these alterations while the second tumor demonstrated loss of heterozygosity on chromosome 17 which can be seen in the setting of TP53 mutations. While there are no known associated environmental risk factors or syndromic associations in our current understanding of SFTs, the close physical proximity and time of presentation of these tumors raise the possibility of shared etiologies or risk factors.

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Modulation of macrophage immune checkpoint regulators involved in phagocytosis of group 3 medulloblastoma cells.

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Background: Immune cells constitute a significant component of the medulloblastoma microenvironment. Investigating how these immune cells are activated and interact with each other is essential for understanding the initiation and progression of medulloblastoma. Most immune cells in brain tumors are tumor-associated macrophages and microglia (TAM), which can make up to 30% of the tumor mass. CD47, overexpressed in many types of cancer, is a ligand for SIRPa, a protein expressed on macrophages. It imparts a “don’t eat me” signal to the macrophages, preventing them from phagocytosing a cancer cell. In this presentation, we investigate mechanisms of human adult peripheral blood (APBM) and umbilical cord blood-derived macrophages (UCDM) activation with respect to their ability to phagocytose medulloblastoma cells in the presence and absence of immune checkpoint inhibitors and present additional immunomodulating mechanisms based on macrophage RNASeq data.

Methods: The medulloblastoma lines D341, D425, and D458 were generously provided by Siddhartha Mitra (Children’s Hospital Colorado, University of Colorado, School of Medicine) and confirmed to be group 3 via molecular subtype markers. We performed RNAseq at the HCI High Throughput Genomics Facility, University of Utah.

Results: Addition of anti-CD47 antibodies increases phagocytosis of medulloblastoma cells by BMDM. Interestingly, UCDMs have higher basal and anti-CD47 antibody-induced phagocytosis levels, which prompted us to investigate potential mechanisms by RNASeq. While different isoforms of Fc gamma receptors are expressed in adult macrophages at a higher level than in umbilical cord-derived macrophages, several innate immunity receptors are expressed at a higher level in UCDMs, suggesting possible involvement of innate immunity-related mechanisms. A delicate balance of self-inhibitory and pro-activation signals was uncovered, especially in SIRPa-SIRPb and B7H-B7-CD28 axes

Conclusions: In addition to anti-CD47 immune checkpoint inhibitors, other immunomodulators should be considered for further upregulation of phagocytosis of medulloblastoma and more efficient downstream activation of T cells.

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Histologic and molecular characterization of a case of primary intracranial sarcoma, DICER1-mutant

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Background: Primary intracranial sarcoma, DICER1-mutant, included as a new diagnostic entity in the 2021 WHO classification of central nervous system tumors, is a rare but aggressive neoplasm generally identified in the supratentorial forebrain. The prognostic implications of these uncommon tumors, their relationship to DICER1 syndrome, and optimal treatment strategy remain unclear.

Methods: Review of the electronic medical record and case analysis was performed, including histologic characterization by hematoxylin and eosin-stained sections and a targeted panel of immunohistochemical stains. Molecular characterization was pursued including targeted next generation sequencing and RNA sequencing panels and DNA methylation profiling.

Results: A 19-year-old female was found unresponsive after reporting a severe headache. CT demonstrated an intra-axial, mass-like hemorrhage in the left temporal lobe which was subsequently resected. The tumor was relatively demarcated from the adjacent brain parenchyma and demonstrated varying cellularity. The tumor cells had hyperchromatic nuclei with a spindled to round appearance. Numerous mitoses, interspersed islands of mature hyaline cartilage, and scattered eosinophilic globules associated with cells with marked nuclear atypia were noted. The tumor cells were positive for desmin, myogenin, and SMSA (focal) and negative for other lineage markers, suggestive of a mesenchymal neoplasm with myogenic differentiation. Immunohistochemistry for multiple fusion proteins (STAT6, HEY1, SS18-SSX) was negative. Next generation sequencing revealed DICER1 (P1805fs and E1705K) and KRAS (Q61H) variants; the composite methylation profile prompted a final diagnosis of Primary Intracranial Sarcoma, DICER1-mutant.

Conclusions: A wide differential was considered for this unusual case, including embryonal rhabdomyosarcoma, mesenchymal chondrosarcoma, Ewing sarcoma, and gliosarcoma. This tumor also shares histologic similarity with pleuropulmonary blastoma, which is associated with DICER1 syndrome. Robust immunohistochemical testing and additional clarification from multiple radiologists was useful in narrowing the differential diagnosis while awaiting molecular information, a key diagnostic tool. This patient’s initial presentation of acute headache with intratumoral hemorrhage is consistent with previously reported cases.

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A Rare Case of an Adult with a Somatic DICER1-altered Primary Intracranial Sarcoma: Application of the CNS and Sarcoma DNA Methylation Classifiers

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Background: Sporadic tumors with somatic DICER1-alterations, in the absence of germline mutations, classically present similarly to tumors associated with DICER1-syndrome. The most common CNS manifestation of this syndrome is metastatic pleuropulmonary blastoma mainly seen in the pediatric population, but primary intracranial sarcomas with DICER1-mutation can also been observed.

Methods: A 42-year-old female presented with lethargy and altered mental status. Imaging of the brain showed a right frontal region brain mass with associated intracranial hemorrhage. Resection of the mass was performed, and histology showed sheets of poorly differentiated tumor cells that were positive for Fli-1 and vimentin, and negative for immunostains ruling out lymphoma, melanoma, carcinoma, glioma, or other common sarcomas. Sarcoma targeted gene fusion panel did not identify fusions. Next generation sequencing studies revealed mutations in TP53, ATRX, KDM5C and RAD51C. Significantly, two DICER1 variants were also identified in the tumor tissue. Germline testing was negative, therefore confirming somatic biallelic DICER1-alterations present. DNA methylation profiling for CNS tumors was not able to classify this tumor but copy number variation analysis showed multiple gains and losses. Furthermore, DNA methylation profiling using the sarcoma classifier showed a class match within the “sarcoma-RMS” group, at a high calibrated score of 0.935. The molecular hallmark of this class is the presence of DICER1-mutations.

Results: Overall, primary intracranial sarcomas are rare, especially those with associated DICER1-alterations. Somatic and/or germline mutations in association with DICER1-syndrome can be identified, with a predominance of patients in the pediatric age group. Investigation of clinical prognosis is limited due to the rarity of these cases. Utilizing DNA methylation can further assist in classifying more of these cases for advancement in treatment and clinical outcomes.

Conclusions: We present a challenging and rare case of an adult with a primary intracranial sarcoma showing somatic biallelic mutations in DICER1, that was further confirmed using DNA methylation classifier.

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Methylation in CNS Tumors: A Systematic Review of Discordant Histopathology/Methylation Profile Cases & Unclassifiable Cases with Case Example

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Background: DNA methylation profiling has seen increasing relevance in central nervous tumor diagnostics as a tool in neuropathologists' armamentarium. However, limitations of available methylation-based techniques and unsupervised tumor clustering algorithms have yet to be thoroughly characterized, including in cases not associated with existing methylation classes using the most recent classifications.

Methods: Systematic review of the literature and pediatric illustrative case. Original studies from Medline database including adult/pediatric patient cases with CNS tumors (primary or metastatic) undergoing DNA methylation profiling without restriction in classifier algorithms used, published in English or French, from 2018 to February 2024, were retrieved with assistance from an information specialist. Manuscripts reporting on outcomes of interest were included: (1)rates of cases with discordance between histopathological diagnosis and methylation class, and/or of (2)cases with methylation profiling attempted but no methylation class attributed e.g., due to technique limitations, or categorized as unclassifiable ("no class match").

Results: 3728 articles (title/abstract) were retrieved from Medline. DNA methylation classes were discordant with pre-existing histopathology in up to 25% of CNS tumor cases in original cohort studies. We present a case of a 5-year-old male with a voluminous left-sided intra-axial frontotemporal mass, who underwent multiple resections/immunotherapy/proton therapy. Histopathology showed a myxoid mesenchymal tumor, not elsewhere classified. Molecular analyses demonstrated FGFR1 (30%;Tier 2) duplication, RET variant (NM_020975.6(RET):c.2371T>A (p.Tyr791Asn);Tier 3) and gain of multiple chromosomes, with lack of identifiable fusion (including no FET::CREB) or amplification of CDK4/MDM2/HMGA2. Methylation profile using DKFZ-CNS v12.5 was unclassified, but, using DKFZ-sarcoma v12.6, the tumor was clustered to well-/dedifferentiated liposarcoma (confidence score 0.8832), without key supporting molecular features.

Conclusions: DNA methylation profiling must be considered with available histopathological/molecular data for integrated diagnosis. Iterative improvement of methylation-based algorithms and better characterization of novel entities not yet included in the 5th WHO CNS classification will help drive precision medicine approaches in patients with rare CNS tumors.

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Ependymoma-misleading ‘CNS tumor with BCOR-altered’

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Background: ‘CNS tumor with BCOR internal tandem duplication (ITD)’ is an emerging malignant embryonal tumor with ITD in exon 15 of the BCOR gene.

Methods: A comprehensive investigation was conducted through clinicopathological review, whole genome sequencing (WGS), RNA sequencing analyses, and Infinium MethylationEPic850K macroarray utilizing validated methodologies and analysis algorithms.

Results: A 44-year-old female presented with a two-week history of headaches. MRI revealed a 6.0.x 5.4 cm heterogeneously enhancing round mass in the right temporal lobe. Histopathology of the initial tumor was composed of perivascular pseudorosettes with uniform oval nuclei. Ten years later, the tumor recurred, displaying numerous true rosettes or canals. The mitotic rates and the Ki-67 indices of the initial and recurrent tumors were 12 and 13 per 10 HPF, and were 13% and 11.2%, respectively. No microvascular proliferation or necrosis was observed. Ultrastructurally, tumor cells exhibited euchromatic nuclei and cytoplasmic processes with a jigsaw puzzle-like interlocking pattern of the cytoplasm and long intermediate junctions. WGS and RNA sequencing revealed a 'BCOR::CREBBP Fusion,' and the methylation profile matched the CNS_BCOR_ITD by DKFZ v12.5 methylation class (score: 0.89485). Both the initial and recurrent tumors were treated with radiation therapy following gross total removal. Four years after the second operation, the patient's health deteriorated and he died.

Conclusions: This case presents several unique features, including an embryonal tumor occurring in adulthood, distinctive histopathology characterized by the formation of true ependymal rosettes, a latent relapse after 10 years, and the presence of BCOR::CREBBP fusion without BCOR ITD. It is noteworthy that BCOR ITD and BCOR fusion are mutually exclusive, and this particular case did not exhibit BCOR ITD. Therefore, it is recommended to reclassify the tumor name from 'CNS tumor with BCOR ITD' to 'CNS tumor with BCOR/BCORL1-altered' to accurately reflect its molecular characteristics.

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Clinicopathological spectrum of desmoplastic infantile astrocytoma/ganglioglioma (DIA/DIG)

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Background: Desmoplastic infantile astrocytoma/ganglioglioma (DIA/DIG) is a low-grade glioneuronal tumor, predominantly found in the cerebral hemispheres of infants. This tumor is primarily associated with MAPK pathway activation, frequently activated by BRAF mutation or receptor tyrosine kinase (RTK) gene fusions.

Methods: A retrospective clinicopathological analysis was conducted at Seoul National University Hospital, from 2018 to the present, coinciding with the establishment of NGS studies employing a brain tumor-targeted gene panel. In one case, the Infinium Methylation EPIC850k microarray was used to enhance the diagnostic precision.

Results: Among the three cases reviewed, two manifested huge solid and cystic masses in the cerebral hemisphere, while the third case developed in the medulla oblongata and cervical spine. The tumor background exhibited dense staining with Masson's Trichrome and Reticulin stains. Patient 1 (8 mo./M) exhibited a BRAF V600delinsDL, distinct from the more common V600E mutation observed in patient 2 (10 yr./F). While the tumor of patient 1 displayed a high Ki-67 index (25.4% at the hotspot) and high mitoses (12/10 HPF.), the tumors of patients 2 and 3 (9 mo./M) did not exhibit these characteristics. Patient 2 is undergoing an adjuvant BRAF inhibitor treatment in a clinical trial due to a residual mass in the medulla oblongata and upper cervical spine with perilesional edema. Patient 3, initially diagnosed with infant-type hemispheric glioma, CNS WHO grade 1, with VIM::MET fusion, underwent reclassification through a methylation study, which revealed clustering with DIG (score: 0.99743, DKFZ v12.5). All three patients remain alive and well, with annual MRI follow-up examinations.

Conclusions: These cases provide distinct features, including a DIA/DIG manifestation in older children, methylation study validation of the diagnosis, an unconventional BRAF mutation (V600delinsDL), and a MET gene fusion. The findings contribute crucial insight into the wide spectrum of molecular pathology of DIA/DIG, and expands the age demographic for potential encounters by neuropathologists.

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Mixed pituitary adenoma-gangliocytomas: immunohistochemistry contributions

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Background: Mixed pituitary adenoma/PitNET-gangliocytomas (PA/PitNET-GC) have been reported in small series over the past 20 years, some with limited immunohistochemistry (IHC) and many predating use of pituitary transcription factors. The largest, 2022 study identified 34 examples (of 12,673 pituitary cases): GC with 9 sparsely granulated growth hormone (SG-GH), 20 mixed GH-prolactin (PRL), 2 sparsely granulated PRL, 2 corticotroph, 1 null cell tumors. We interrogated our 20-year experience focusing on patterns of GC component and IHC contributions, especially anti-neurofilament protein (NFP) and CAM5.2.

Methods: Database text-word search, 2003-2023, review of histological features, and IHC for anterior pituitary hormones, transcription factors, NFP and other neuronal markers, and CAM5.2.

Results: Search generated 19 cases, 7M :12F, ages 20-71 years, 17 macroadenomas, 1 giant adenoma, 1 microadenoma. GC was associated with 4 corticotroph, 2 densely granulated lactotroph, 5 mixed lactotroph-somatotroph (2/4 with PRL>>GH), 8 SG-GH (all with minor lactotroph component). In some cases, PRL IHC+ was preferentially seen in ganglionic cells, suggesting the major contribution to metaplasia from lactotrophs. Patterns were discrete nodular foci of GC (8/19), extensive GC differentiation (7/19) that preferentially, but not exclusively, occurred in corticotroph tumors (3/4, in 2 of which GC greatly overshadowed the PA/PitNET component), and axonal-rich neuropil and metaplastic ganglion cells intimately admixed within the PA/PitNET (4/19). NFP often identified significantly greater axonal content, even in adenoma areas, than was apparent on H&E and helped identify small cohesive regions of neuropil that were inapparent on H&E. CAM5.2 highlighted neuronal morphology, including triangular shape and ganglionic processes, sometimes to greater extent than NFP. Co-existent PIT1-SF1 IHC+ was found in several somatotroph-containing cases.

Conclusions: We conclude that the metaplastic nature of PA/PitNET-GC is best disclosed by combination use of NFP and CAM5.2+, given the latter is sometimes more extensive and discloses differing morphological features of the neuronal and axonal component than NFP.

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Re-assessment of Plurihormonal Pituitary Adenomas

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Background: Plurihormonal pituitary adenomas/neuroendocrine tumors (PA/PitNETs) express multiple pituitary hormones and/or transcription factors (PH/TFs), as determined by immunohistochemistry (IHC). 3 types exist based on Endocrine WHO 2022 classification. Tumors formerly designated Plurihormonal PIT1-adenomas today stratify into mature and immature types with the two sharing an IHC profile (PIT1, GH, PRL, TSH), with cytological atypia and less IHC+ in the latter. Immature variants are said to have an "aggressive' behavior, but for individual patients it is unclear what this translates into clinically. A third type exists with unusual combinations of PH/TFs. We reviewed our experience with plurihormonal adenomas since 2018, recategorizing them by Endocrine WHO 2022 and providing endocrinological, neuroimaging, and followup insights.

Methods: Database search, with retrospective review of medical charts, 2018 to 2023.

Results: 22 cases were identified: M 9:F 13, mean age at surgery 51 +/- 16 years. Most common symptoms at initial presentation were headaches and/or vision changes (6/22), acromegaly (5/22), seizures (2/22) and hypogonadal symptoms (2/22). All tumors were macroadenomas, mean size 25 +/- 17 mm; 12/22 (55%) had cavernous sinus invasion. More than 70% of tumors clinically secreted at least one hormone and 27% tumors secreted at least two different hormones. 5 patients required more than one surgical intervention within 1 year of initial resection. Reclassification yielded 7 immature and 3 mature PIT1-lineage, with the remainder true plurihormonal, most often with SF1/PIT1 or TBX19/PIT1 expression with additional mixed-lineage PH (eg., FSH, PRL). More than 50% of cases (12/22) had residual tumor after initial surgery. MIB-1 labeling was < 3% in most cases, (2 negligibly higher at 3-4%). 3/5 cases requiring multiple surgeries were immature PIT1-lineage PA/PitNETs.

Conclusions: “Aggressive” behavior in immature PIT1 plurihormonal tumors translates into requirement for repeated surgical resections over short intervals in a significant percentage of cases. Some unusual true plurihormonal tumors can also be aggressive.

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Plasmacytoma: Yet Another Rare Sellar Region Mass

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Background: We have a longstanding interest in unusual sellar region masses, including B cell lymphomas (JNEN; 2019;78(8):673-684); others have also rarely identified sellar lymphomas (2/316 pituitary region cases; PMID: 31209729). Plasma cell neoplasms are however, seldom reported in this location, with fewer than 80 reported examples.

Methods: We report a 73-year-old male who presented with headaches, left cranial nerve 3 palsy, and weight loss, and was found to have a 3.4 x 3.8 x 3.6 cm destructive mass in skull base and sellar region. The mass replaced the marrow and eroded the clivus with extension into both cavernous sinuses, sphenoid sinus and submucosal nasopharynx. Neuroimaging diagnoses included metastatic disease, plasmacytoma, chordoma, versus meningioma. Preoperative evaluation revealed hyperprolactinemia (18.7 ng/mL, normal: 3-13) and hypogonadism (total testosterone 51 ng/dL, normal: 300-720).

Results: Transsphenoidal biopsy proved plasma cell neoplasm/plasmacytoma with lambda light chain restriction and co-expression of CD56, CD117, and focal cyclin D1. Further workup revealed an abnormal kappa:lambda free light chain ratio and a paraprotein lambda IgG on serum protein electrophoresis. Positron emission tomographic scan demonstrated a moderately FDG-avid skull base mass (SUVmax 6.2) with multiple FDG avid lytic lesions in the manubrium, left iliac crest, and spine. Bone marrow biopsy confirmed the diagnosis of multiple myeloma with 60-70% involvement by clonal plasma cells. Patient will undergo radiation therapy to the sellar region and systemic treatment with Daratumumab, Lenalidomide and Dexamethasone.

Conclusions: Amongst the cases reported with sellar plasmacytomas, only 37% of these cases have newly diagnosed multiple myeloma at the time of initial evaluation (PMID: 28251542). Although rare, neuropathologists should include both lymphoma and plasmacytoma in their differential considerations for sellar region masses.

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Primary intracranial sarcoma, DICER1-mutant, report of two cases and review of literature

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Background: Primary intracranial sarcoma, DICER1-mutant is a rare neoplasm of the central nervous system (CNS). According to the 5th edition WHO Classification of CNS Tumors, the diagnosis requires a primary intracranial sarcoma AND pathogenic DICER1 mutation, AND (for unresolved lesions) a DNA methylation profile aligned with this entity.

Methods: We present two cases of Primary intracranial sarcoma, DICER1-mutant. One case occurred sporadically in a 70-year-old woman; the other case occurred in a 34-year-old man with Xeroderma Pigmentosa.

Results: Case 1: A 70-year-old woman presented with headache and a left homonymous hemianopia. Magnetic resonance imaging (MRI) showed a 4.4 cm extra-axial right parietal lobe mass. The resection specimen showed a spindle cell neoplasm with fascicular architecture, abundant mitoses, and extension into Virchow-Robin spaces with brain parenchymal invasion. Molecular studies identified mutations in DICER1 and KRAS. DNA methylation profiling matched to the methylation class Malignant peripheral nerve sheath tumor. Case 2: A 34-year-old man with Xeroderma Pigmentosa presented with progressive headache and a 4.8 cm enhancing, extra-axial right frontal lobe mass on MRI. The resection specimen showed a well-circumscribed neoplasm composed of spindled tumor cells with abundant mitoses and necrosis. Next generation sequencing identified mutations in DICER1, ATRX, PDGFRA and XPC. DNA methylation profiling matched to the methylation class sarcoma (RMS-like).

Conclusions: Primary intracranial sarcoma, DICER1-mutant, is a rare malignancy that may occur in patients with DICER1-related tumor predisposition syndrome or neurofibromatosis type 1, or they may occur sporadically. Diagnosis requires integration of histopathology and multiple molecular modalities. We herein describe two additional cases, including one arising in Xeroderma Pigmentosa, which is previously unreported for this entity.

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Medulloblastoma in mixed germ cell tumor: characterization by methylation analysis

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Background: Germ cell tumors (GCT) of the CNS account for 3-4% of primary intracranial neoplasms in children. Rarely, somatic malignancy can exist in mixed GCT, and their molecular features and clinical implications are not well described. We encountered a 2-year-old patient who presented with vomiting and ataxia and was found to have a large posterior fossa mass with intracranial and CSF metastasis. Serum AFP was elevated; CSF tumor markers were normal. The patient underwent gross total resection of the primary tumor.

Methods: The clinical chart, imaging studies, and pathology slides were reviewed. Targeted exome sequencing, chromosomal microarray analysis, and methylation profiling were obtained as part of the clinical workup.

Results: The pathologic findings were consistent with a malignant mixed GCT composed of teratoma with embryonal tumor, embryonal carcinoma, and endodermal sinus tumor. Targeted exome sequencing and chromosomal microarray performed on the area of embryonal tumor showed group 3 medulloblastoma, MYC amplified. Methylation array analysis indicated a match to medulloblastoma, group 3. Based on these findings, the patient was treated for high-risk medulloblastoma with an infant embryonal tumor chemotherapy protocol, including tandem autologous stem cell transplantation and intensification with intrathecal chemotherapy. There was rapid tumor recurrence, and a second resection was performed followed by photon CSI. The patient died with widespread leptomeningeal disease. Autopsy confirmed disseminated medulloblastoma.

Conclusions: Molecular methods are infrequently used for the subclassification of CNS germ cell tumors. In this case, the molecular characterization of the somatic malignancy led to the patient being treated with a modified protocol that targeted the medulloblastoma. This case report suggests that molecular characterization of somatic malignancy in mixed germ cell tumors may influence the clinical management.

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Prominent Cerebral Corpora Amylacea Deposits Mimicking a Low-grade Glioma

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Background: Corpora amylacea [CA] (“wasteosomes”) are spherical polyglucosan corpuscles that appear commonly in the periventricular, subpial, and perivascular regions of the human brain during aging. It is believed that they entrap waste products from neurons, astrocytes, oligodendrocytes, and blood. They are also seen in brain tissue affected by infections and neurodegenerative diseases. Here we describe the finding of abnormal brain tissue rich in CA that clinically and radiologically simulated a low-grade neoplasm.

Methods: Microscopy, immunohistochemistry, NGS

Results: A 55-year-old male presented with headaches, confusion and word finding difficulties. Brain MRI showed two abnormal areas: a mass-like lesion in the right middle temporal gyrus resulting in thickened gray matter with extension into the white matter, and smaller lesions in the left occipital and left parietal lobes with similar radiological features. The radiologic differential diagnosis included low-grade glioma versus cortical dysplasia or an inflammatory process. The resection specimen included gray matter showing large numbers of subpial CA and associated gliosis. Rare neurons with dysmorphic features including nucleomegaly, Nissl substance peripheral dispersion, and maloriented neurites were found. The white matter also showed frequent CA forming large aggregates around blood vessels. Oligodendroglial cell clustering was seen around neurons and around CA. No neoplasm was identified. No abnormalities were identified by the next generation sequencing or chromosomal microarray analysis.

Conclusions: In summary, we present a rare case of massive brain CA accumulation mimicking a neoplasm.

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PitNETs Demonstrate Differential SSTR2 Staining by Lineage

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Background: PitNETs are among the most commonly encountered tumors in routine neuropathology. While most PitNETs demonstrate indolent behavior, invasion of surrounding structures may complicate radiographic identification and surgical management. Radiopharmaceuticals such as Ga68-DOTATATE been developed targeting SSTR2 receptors have shown utility in the management of neuroendocrine tumors and meningiomas via PET imaging. We show here that PitNETs and autopsy controls demonstrate differential labeling for SSTR2 stratified by hormonal lineage.

Methods: TMAs were generated from archival PitNET/adenomas and autopsy control pituitary materials sampled at WCM between 2003 and 2018 and stained for SSTR2 according to established protocols. Tissue cores were semi-quantitatively scored by a neuropathologist on a scale of 0+ (no staining) to 5+ (strong diffuse staining). Staining scores for each case were then averaged, and correlated with hormonal status, transcription factor lineage, and age.

Results: A total of 177 PitNETs (107 gonadotrophs, 32 prolactinomas, 23 corticotrophs, 12 mammosomatotrophs, 3 somatotrophs, 1 thyrotroph, 1 null-cell) and 20 autopsy controls had sufficient clinical information and diagnostic material for analysis. Compared to autopsy controls (mean SSTR2 staining of 2.7 +/- 0.4), pitNETs stratified into high-staining in mammosomatotrophs (4.0 +/- 0.8) and somatotrophs (3.0 +/- 1.0), intermediate staining in corticotrophs (2.75 +/- 1.4) and prolactinomas (2.65 +/- 1.3), and low-labelling in gonadotrophs (2.1 +/- 0.9). Thyrotrophs and null-cell adenomas were underrepresented in this dataset, but single cases demonstrated high (4.7) and low (2.0) staining intensity respectively. In autopsy controls, weak age-related trends towards increases in women and decreases in men were observed, which were not statistically significant.

Conclusions: SSTR2 expression in PitNETs and pituitary autopsy controls broadly stratified into high-staining (mammosomatotroph, somatotroph, and thyrotroph), intermediate-staining (autopsy control, corticotroph, and prolactinoma), and low-staining (gonadotroph and null-cell). These findings suggest differential SSTR2 expression is appreciable in pitNET of different lineage, with implications for SSTR2-targeted PET in the management of pitNETs.

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Congenital Myofibroma in the Central Nervous System

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Background: Prenatal CNS tumors present difficult questions for management, including whether to pursue tissue diagnosis following elective termination. We present a rapid autopsy case performed for tissue procurement on a fetus found to have a left posterior fossa mass on routine prenatal screening, found to represent an myofibroma.

Methods: A 31-week fetus conceived via in vitro fertilization to a 38-year-old female was found to have a 5.3 cm posterior fossa lesion on prenatal screening. Following elective termination with KCl, consent was obtained for rapid autopsy and tissue procurement. Routine histology was performed, and the tumor was characterized with TSO500 sequencing.

Results: The tissue was noted to be densely adherent to the left aspect of the petrous temporal bone, occipital bone, and tentorium cerebelli, displacing the brainstem and cerebellum and complicating removal. The tumor was noted to be firm, fleshy, and demonstrated marked internal hemorrhage and necrosis. Histopathologic examination demonstrated a tumor with mesenchymal morphology, including angulated vasculature reminiscent of solitary fibrous tumor. Widespread hemorrhage and necrosis were identified, and frequent mitotic figures were seen. The tumor cells were immunoreactive for CD34, nonreactive for STAT6, and showed preserved nuclear staining for H3K27me3 and INI-1. Next-generation sequencing revealed a complex insertion-deletion within exon 11 of PDGFRB, rearrangement of exon 14 of CD36, and KTMT2D R3127H mutation. The morphologic, immunohistochemical, and molecular features supported a diagnosis of myofibroma.

Conclusions: Infantile myofibromas often demonstrate morphologic features overlapping with solitary fibrous tumors, are molecularly distinct. While myofibromas are most common in infants, fetal cases are uncommon and location within the neuraxis is rare. Activating PDGFRB alterations are common in sporadic and congenital myofibromas. Sporadic myofibromas are typically benign, but infantile cases are associated with increased mortality. Genetic counselling should be recommended to families with history of prenatal tumors to guide screening for future pregnancies.

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Mixed Gangliocytoma-Pituitary Adenomas Match Pituitary Adenoma Family by DNA-Methylation Analysis with no Matching for Neuronal Tumors

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Background: Mixed gangliocytoma-adenomas (MGAs) are rare pituitary tumors composed of gangliocytic and adenomatous elements. Most MGAs are associated with endocrinopathies, acromegaly and infrequently Cushing disease and hyperprolactinemia. Recent studies have shown shared features between adenomatous and ganglionic cells, with co-expression of neuronal-associated proteins and pituitary cell lineage transcription factors supporting the etiological hypothesis of a common origin or transdifferentiation of neuroendocrine cells into neuronal elements [PMID: 28079576; 28079577]. In this study, we investigated if MGAs are constitutively characterized by a mixed cell or a single cell population by tumor DNA methylation analysis questioning in which methylation class/family these tumors would fit.

Methods: Twenty cases with 13 female and 7 male patients were collected. Tumors were classified according to the 2022 WHO Classification by immunohistochemistry for pituitary hormones and cell lineage transcription factors. Tumors were analyzed by DNA methylation profiling using the Heidelberg and the NCI/NIH classifiers. Nine cases have been previously reported [PMID: 23611590; 28079576].

Results: The series includes 15 sparsely granulated somatotroph MGAs (SMGA); 4 corticotroph MGAs (CMGA); and 1 lactotroph MGA (LMGA). Thus far, 12/20 cases have been analyzed by methylation assays of which 11/12 had enough materials for analysis. Amongst the 9 SMGAs, 5 tumors matched somatotroph-producing adenoma methylation class (MC), 2 tumors were suggestive of pituitary adenoma MC with a low score, and 2 tumors did not match with any MC. 2/4 CMGAs did not match with any MC. However, UMAP analysis of all cases showed clustering within the pituitary adenoma family MC. None of the tumors matched with neuronal tumor MC.

Conclusions: Our preliminary results reaffirm the current concept that MGAs are neuroendocrine tumors with differentiation into a neuronal phenotype rather than a definite neuronal cytogenesis. Therefore, these tumors should be classified as pituitary neuroendocrine tumors/adenomas with focal neuronal differentiation rather than the current proposed name mixed gangliocytoma-adenoma.

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Mixed Gangliocytoma-Pituitary Adenoma: A case report of a rare entity

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Background: Mixed gangliocytoma-Pituitary adenoma is an extremely rare entity of the sellar region that accounts for < 0.5% of sellar tumors. Approximately 100 cases have been reported in the medical literature. These neoplasms consist of a gangliocytoma cellular component admixed with a hormone secreting pituitary adenoma, with growth hormone being the mostly encountered, followed by prolactin and others.

Methods: We present a case with a mixed gangliocyoma and somatotroph adenoma. The patient is a 46-year-old female who was noted to have a pituitary mass on workup for neck pain. She was also noted to have increasing hand and feet size over several years. Endocrinology workup revealed elevated growth hormone consistent with acromegaly. MRI brain w/wo iv contrast showed a sellar mass measuring approximately 2.5 cm in largest dimension which partially encased carotid siphons bilaterally. The signal intensity was heterogenous with nearly homogenous contrast enhancement.

Results: Histologic evaluation showed a neuroendocrine neoplasm with sheets of chromophobic cells effacing the nested organization of the adenohypophysis. Neoplastic cells exhibited dense eosinophilic cytoplasmic inclusions (ie. fibrous bodies) and within some areas, large ganglion-like cells were intermixed with the adenoma cells. Immunostaining showed that the neoplastic cells were diffusely positive for Synaptophysin, Cytokeratin CAM5.2 (perinuclear dot-like pattern) and hGH (large subset), and negative for prolactin, TSH and GFAP. The Ki67 proliferative index was 1-3%. Ganglion-like cells were immunoreactive for neurofilament and negative for hormonal expression.

Conclusions: Mixed Adenomas are rare entities that has been reported mostly in case reports. Recent studies of these mixed gangliocytomas and somatotroph adenomas have suggested that the histogenesis of the mature neuronal-like cells are derived from transdifferentiation of neuroendocrine cells within pituitary adenomas. Understanding the molecular drivers of these neoplasms into transdifferentiation may be a targetable therapeutic approach to treat these aggressive subtypes of pituitary neuroendocrine neoplasms and warrant further exploration.

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A multiomics approach for the diagnosis and prognosis of anaplastic meningioma

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Background: While the WHO 2016 classification of meningiomas defined anaplastic meningiomas using histological criteria only, the WHO 2021 classification scheme introduces molecular alterations that define this group: CDKN2A/B homozygous deletion (HD) and TERT promoter (TERTp) mutations. With limited studies examining the prognostic value of these markers, we sought to further characterize the prognostic implications of these and other alterations in a cohort of anaplastic meningiomas using both the 2016 and 2021 classification schemes. Furthermore, we investigated the utility of p16 and MTAP as surrogate immunohistochemical (IHC) markers for detection of CDKN2A/B HD.

Methods: Demographic, clinical and histopathological information were obtained from the electronic medical records of anaplastic meningioma cases resected at a tertiary center between 2007-2020. Tumors underwent molecular testing for TERTp mutations, CDKN2A/B HD, and methylation profiling. IHC was performed for H3 K27me3, BAP1, p16, and MTAP. The p16 and MTAP IHC staining pattern were compared with CDKN2A/B status.

Results: 15 cases were included in the study with 8/15 classified as anaplastic by WHO 2021 criteria and 7 additional tumors by WHO 2016 criteria (6/15 rhabdoid and 1/15 papillary). One rhabdoid tumor exhibited BAP1 loss and demonstrated an aggressive clinical course. Of the WHO 2021 anaplastic meningiomas, five tumors harbored a TERTp mutation and/or CDKN2A/B HD while three tumors had no molecular alterations. Anaplastic meningiomas that harbored TERTp mutations and/or CDKN2A/B HD were associated with significant reductions in survival compared to cases with elevated mitotic index (≥20/10 HPF) alone. Meningiomas with CDKN2A/B HD showed consistent loss of p16 and MTAP immunoreactivity.

Conclusions: Anaplastic meningiomas with TERTp mutations and/or CDKN2A/B HD demonstrate significantly reduced survival compared non-mutated anaplastic meningiomas, supporting their utility for prognostication. Furthermore, p16 and MTAP IHC are promising surrogate markers for prediction of CDKN2A/B status.

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Cribriform Neuroepithelial Tumor: Case report and Literature Review

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Background: Cribriform neuroepithelial tumors (CriNET) were first introduced as a provisional entity in the 2021 WHO classification of tumors of the central nervous system. CriNET mainly occur in children and are very rare intraventricular embryonal tumors, characterized by the presence of primitive non-rhabdoid cells with distinctive cribriform architecture, genetic alterations affecting the SMARCB1 gene, and a favorable prognosis. Only thirteen cases have been reported to date. Diagnosis can be challenging, as these tumors show significant morphologic, immunohistochemical, molecular, and epigenetic overlap with more common tumors, such as atypical teratoid rhabdoid tumor and choroid plexus carcinoma.

Methods: Here, we report a case of a 4-year-old female presenting with symptoms of elevated intracranial pressure, found to have a 4th ventricular mass. The patient underwent an excisional biopsy of the lesion. The original institution diagnosed the tumor as a choroid plexus carcinoma by morphology and the case was sent to Children’s Hospital of Pittsburgh for review.

Results: The histopathological examination of the lesion showed a well demarcated hypercellular neoplasm composed of primitive appearing cells, displaying a solid to papillary arrangement with scattered cribriform strands and ribbons. Mitotic figures and necrosis were present. Immunohistochemical analysis of the tumor was positive for S100, EMA, pancytokeratin, and Cam 5.2, with focal expression of GFAP and Olig2. INI BAF47 stain showed complete nuclear loss in the tumor cells. Proliferative activity was elevated. The case was sent to the National Institute of Health in Bethesda, Maryland, for DNA-methylation profiling. Array studies demonstrated chromosome 22 loss in the area of SMARCB1, and consensus DNA-methylation classification demonstrated a match to cribriform neuroepithelial tumor, SMARCB1-altered.

Conclusions: A final diagnosis of cribriform neuroepithelial tumor, SMARCB1-altered was rendered. The patient in this case unfortunately passed away from complications relating to treatment. We report this unusual case with a review of the relevant literature and potential diagnostic pitfalls.

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A paraspinal soft tissue tumor with neuroendocrine-like morphology and β-catenin mutation: A case report

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Background: The patient is a 78-year-old female with no significant past medical history presenting with persistent radiculopathy and paraparesis. Spine MRI demonstrates enhancing contiguous soft tissue masses along the right posterolateral spine with an intradural component extending from C4 to T1 (approximately 9.7 x 3.6 x 3.1 cm).

Methods: The clinical workup was performed as detailed below.

Results: Microscopic evaluation: H&E sections show a morphologically-distinctive tumor with pseudopapillary to pseudoglandular architecture, in which neoplastic cells exhibit monotonous, ovoid nuclei with speckled chromatin, and are frequently arranged around hyalinized vessels and extracellular hyaline eosinophilic globules. Mitotic activity is overall low (1 mitosis in 10 high power fields) with a variable Ki67 index (up to 10-15% in discrete areas); no necrosis is identified. The tumor is immunohistochemically notable for nuclear expression of beta-catenin (suggestive of an underlying CTNNB1 gene alteration), and diffuse expression of S100 and CD34. It shows a limited focus of reactivity for synaptophysin, while essentially negative for other markers of neuroendocrine (chromogranin, INSM-1), epithelial (OSCAR, CK AE1/AE3), meningothelial (EMA, PR, SSTR2), glial (GFAP, OLIG2), and myogenic (SMA, Desmin) differentiation. Molecular studies: Mutation analysis for β-catenin (CTNNB1) reveals the following clinically relevant mutation: c.97T>G (Exon 3), Amino Acid Change: p.S33A (Ser33Ala) (VAF: 44.4%). DNA methylation-based tumor classification and dimensionality reduction with UMAP were non-contributory.

Conclusions: Here we presented a paraspinal soft tissue tumor in an older adult patient with unique histopathological features. Despite showing the morphology of a well-differentiated neuroendocrine neoplasm, this tumor was negative for markers of neuroendocrine and epithelial differentiation. These features have recently been identified in a newly described entity: Pseudoendocrine sarcoma. The diagnosis is supported by CTNNB1 hotspot mutations on next generation sequencing that can be reflected by nuclear β-catenin expression in most cases. Of note, CTNNB1 mutations has not been consistently reported in other round cell sarcoma tumors.

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Intratumoral non-necrotizing granulomatous inflammation involving a meningioma

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Background: Inflammatory lesions may arise in extra-axial locations of the central nervous system (CNS) and are among the differential diagnoses for meningioma. Co-existing inflammatory lesions and neoplasms at the same site are unusual and may present challenges in diagnosis.

Methods: We report an unusual case of non-necrotizing granulomatous inflammation within a meningioma in a patient without a prior history of systemic granulomatous disease.

Results: A 72-year-old woman with a past medical history of hypertension, migraine headaches, and hepatitis C infection presented with complaints of intermittent headaches lasting for 2 weeks. Imaging studies revealed an extra-axial, 2.6 cm, left cerebellopontine angle/cerebellar hemispheric mass thought to represent a meningioma. Subsequent follow-up revealed a gradual increase in the size of this avidly enhancing mass to 3.9 cm, and she underwent a left retrosigmoid craniotomy for resection. Histopathologic examination revealed a spindle cell lesion arranged in parallel and interlacing bundles within a collagen-rich matrix with scattered psammomatous calcifications. Additionally, numerous non-necrotizing granulomata with multinucleated giant cells, histiocytes, lymphocytes, and plasma cells were interspersed. Immunohistochemical staining demonstrated a focally epithelial membrane antigen (EMA)-positive fibrous meningioma and CD68-positive, EMA-negative multinucleated giant cells and histiocytes. Stains for acid-fast and fungal organisms were negative, and no cholesterol clefts or polarizable foreign material were present.

Conclusions: The significance of intratumoral non-necrotizing granulomatous inflammation in meningioma remains unclear in this patient. It is uncertain whether the granulomata represent a metaplastic change (xanthomatous/xanthogranulomatous meningioma), are the result of an occult underlying systemic granulomatous process such as sarcoidosis with incidental involvement of the meningioma, or are secondary to degenerative changes or necrosis within the meningioma.

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Central nervous system involvement by ALK-positive histiocytosis with PPFIBP1-ALK fusion: a case report and review of the literature

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Background: ALK-positive histiocytosis is a recently recognized entity demonstrating the ALK fusion with different partners. It can present as a single organ or as a multisystemic disease with central nervous system (CNS) involvement. Here we report a case of ALK-positive histiocytosis with PPFIBP1-ALK fusion presenting as a brain mass.

Methods: A 36-year-old male patient was found to have a 1.4 x 1.3 x 1.3 cm well-circumscribed solitary mass in the right temporoparietal gray-white junction associated with vasogenic edema on MRI during the workup for dizziness. The resected mass demonstrated a proliferation of atypical spindle cells with mild to moderate cytological atypia. Histiocytes, small lymphocytes and plasma cells were present in the background. PPFIBP-ALK fusion was detected by DNA sequencing. Further workup demonstrated that the atypical spindle cells were positive for ALK-1, CD163 and CD68, but negative for glial cell markers. The findings were consistent with ALK-positive histiocytosis. Recent followup MRI showed no recurrence.

Results: This is the first case of CNS only involvement by ALK-positive histocytosis with PPFIBP-ALK fusion. ALK-positive histiocytosis has been reported as a localized lesion in CNS or as part of the systemic involvement. Histologically it can mimic as a glial neoplasm (e.g. gliosarcoma).

Conclusions: It is important to recognize ALK-positive histocytosis involving CNS, especially when it is presented as a localized disease.

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Atypical teratoid rhabdoid tumor in adults: Report of 3 cases

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Background: Atypical teratoid rhabdoid tumor (ATRT) is a rare embryonal CNS malignancy typically diagnosed in infants under 6 months old and characterized by genetic alteration of SMARCB1 (INI1) or, on rare occasions, SMARCA4 (BRG1). In adulthood, this malignancy is extremely rare and may occur anywhere throughout the neuroaxis, with cerebral hemispheres and suprasellar region as the two most common locations. Given the rarity of this tumor in adults, we sought to better characterize adult ATRT by reporting cases of such found within our pathology archives.

Methods: Medical records were retrospectively reviewed and formalin-fixed paraffin-embedded sections were retrieved to document histopathologic characteristics. Next generation sequencing (NGS) using brain tumor-targeted gene panel and methylation class studies are included.

Results: We identified a total of three tumors, one in a man and two in women, with ages ranging from 39 to 52 years old. These tumors occurred in pineal, cerebellum, and suprasellar regions, and all demonstrated predominantly nested sheet-like architecture with rhabdoid cells identified at least focally. Immunohistochemically, all demonstrated INI-1 loss, a finding supported by results of NGS. By methylation profiling, these tumors all together clustered best with ATRT-MYC subtype.

Conclusions: This report contributes to the very limited number of cases of adult ATRT in the literature and expands on the differential diagnoses considering location, histology, and molecular characterization.

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Intracranial inflammatory myofibroblastic tumor with DCTN1-ALK fusion, a rare entity

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Background: Inflammatory myofibroblastic tumor (IMT) is a neoplasm recently classified as a distinct disease entity with intermediate biological potential, due to its tendency for local recurrence, risk of metastasis, and the common gene rearrangement involving anaplastic lymphoma kinase (ALK) gene. IMTs are mainly extracranial. Only rare cases have been reported involving the central nervous system (CNS). However, such intracranial cases are particularly difficult to treat, and diagnostic challenges remain due to their rarity as well as both radiologic, and histopathologic mimicry to other intracranial diseases.

Methods: A 33-year-old previously healthy male underwent craniotomy with a near total resection for a 4.4 cm lobulated enhancing extra-axial lesion centered within the left cavernous sinus. The surgical specimen was collected for histological, immunohistochemical, and molecular characterization.

Results: Histological examination revealed a spindle cell neoplasm arranged in fascicles and loose aggregates with low mitotic activity, admixed with prominent lymphohistiocytic inflammatory infiltrate in a vascular stroma. Immunohistochemistry showed spindled cells were positive for muscle actin (HHF-35), CD99, and ALK, while negative for meningothelial, epithelial, melanocytic, and hemotolymphoid markers. RNA sequencing detected a DCTN1-ALK fusion, and the ALK-rearranged IMT diagnosis rendered in conjunction with the histomorphology. Post craniotomy, the patient completed radiotherapy and remains progression free for 6 months.

Conclusions: Herein, we present to our knowledge the second case of ALK-rearranged intracranial IMT with DCTN1 as its partner gene. The spindle cell morphology and enriched inflammatory infiltrate of the lesion emphasize the diagnostic challenges for this rare entity. Given its aggressive course and recurrence rate, IMT should be considered in the differential diagnosis, and warrants evaluation for ALK expression status.

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Add CAPNON (Calcifying Pseudoneoplasms of the Neuraxis) to your differential diagnosis of ventricular tumors.

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Background: Choroid plexus papilloma, meningioma, and gliomas are in the differential diagnoses of intraventricular tumors, and calcifying pseudoneoplasms of the neuraxis (CAPNON) should also be added. CAPNON are rare, benign tumors that manifest anywhere along the neuraxis, but more predominantly in the posterior fossa. Diagnosing them can pose significant challenges, particularly during intraoperative consultations, where extensive calcifications alongside small cellular components can complicate the diagnosis. CAPNON exhibits diverse histological patterns, including calcifications, epithelioid cells, myxoid extensions, and focal osseous metaplasia. Imaging studies often fail to provide conclusive evidence, as CAPNON can mimic other conditions.

Methods: This case is to report a patient diagnosed with a CAPNON in the fourth ventricle. Both frozen/intraoperative consultation and permanent histologic sections were performed.

Results: We present a case of a 42-year-old patient who presented with worsening headaches and frequent paresthesia in the right upper extremities, accompanied by an increasing mass size in the right and fourth ventricle. A small biopsy at frozen section showed a firm, knobby, transparent tissue that crumbled apart on touch. Frozen section showed calcifications and scant cellular component. Permanent sections reveal small cell epithelioid component giving rise to cytoplasmic radial projections that form granular calcifications. Small cell component accompanied by stellate reticulum, along with spindle cells and bone formation. Lesional cells were immunopositive with EMA and negative with GFAP, SSTR2A, and SOX-10. These findings aided in the exclusion of meningioma, subependymoma, gliomas, and choroid plexus papilloma as differential diagnoses.

Conclusions: This case emphasized the importance of histological examination of entire lesion for definitive diagnosis, with immunohistochemistry stains aiding in excluding alternative diagnoses. Given CAPNON’s multiple histologic components, this tumor may exhibit similarities to gliomas and meningioma, and it should be considered in the differential diagnosis of ventricular lesions.

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CNS Involvement by Non-Nodal Mantle Cell Lymphoma Masquerading as Autoimmune Encephalitis vs Paraneoplastic Syndrome

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Background: Mantle cell lymphoma (MCL) is a rare malignancy of mature B-cells. CNS involvement is a serious complication present in 2-4% of cases. We report an unusual case in a 67-year-old female who initially presented with neurologic symptoms mimicking autoimmune encephalitis and paraneoplastic syndrome.

Methods: Chart review and autopsy with brain examination were performed. FISH and sequencing studies were conducted.

Results: Rather than a typical MCL presentation (e.g., B symptoms, lymphadenopathy, splenomegaly, lymphocytosis), the patient developed progressive oculomotor abnormalities, dysphagia, intermittent hypothermia, and episodes of reduced consciousness. Brain MRI revealed T2 hyperintensities in the hypothalamus and midbrain, yet CSF cytology and flow cytometry were unrevealing. A differential diagnosis of autoimmune encephalopathy vs. paraneoplastic syndrome was entertained due to mildly elevated anti-TG and anti-TPO antibodies and concurrently discovered anal squamous cell carcinoma. Her clinical condition deteriorated despite treatment with high-dose steroids, intravenous immunoglobulin, plasma exchange, and chemoradiation for the squamous carcinoma. At autopsy, gross examination revealed discoloration of the pons. Microscopic examination revealed CD20+/CD5-/Cyclin D1+/SOX11- atypical lymphoid infiltrates involving the hypothalamus and dorsal brainstem with associated gliosis. FISH identified a t(11;14) CCND1::IGH fusion, diagnostic of MCL. Sequencing revealed a pathogenic TP53 mutation. The absence of nodal involvement and negative SOX11/CD5 immunoreactivity are suggestive of the non-nodal MCL subtype; this subtype is generally indolent, yet TP53 mutation correlates to aggressive disease.

Conclusions: To our knowledge, this is the first report documenting CNS involvement in the non-nodal subtype of MCL. This case highlights the diagnostic challenge when neurologic symptoms are the initial presenting feature of MCL, especially given that ~16% of cases with CNS involvement have negative CSF workup. Future studies may examine how disease subtype and TP53 mutation status correlate with CNS involvement in MCL.

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Rosai-Dorfman Disease Involving the Central Nervous System

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Background: Rosai-Dorfman Disease (RDD) is a non-Langerhans cell histiocytosis with nodal or extra-nodal painless, slow-growing lesions, characterized by large, S100-positive histiocytes with round nuclei, prominent nucleoli, and emperipolesis. The most common extra-nodal site is skin with central nervous system (CNS) involved in < 5% of cases. We present CNS lesions and discuss pathologic pitfalls.

Methods: Department files were searched for RDD in CNS. Slides/stains and clinical data were reviewed.

Results: Four patients (M:F=3:1; mean age=41.5, range=31-52 years) between 2009-2024, presented with vision/hearing loss, headache, seizures, and dura-based extra-axial, enhancing mass lesions in the frontotemporal skull-base (n=2), temporal (n=1) and parietal lobe (n=1). Skull-base lesions were widespread, involving cavernous sinus, optic nerve, sphenoid wing, and sellar region; convexity lesions compressed the brain, mimicking meningioma. Superficial brain invasion was present in the temporal lobe lesion. In addition to characteristic diagnostic light-microscopic features, all cases showed a prominent plasma cell (PC) population; three had further work-up, showing increased numbers of IgG4-positive PC (IgG4/IgG=20-30%). Three cases tested were negative for BRAF V600E mutations. One case in a 52-year-old man recurred three years later. Molecular testing in two cases showed an NRAS mutation in the recurrent/persistent skull base lesion; another had no other mutations. No Langerhans cell histiocytosis (LCH) or lymphoma was identified.

Conclusions: While RDD is typically self-limiting, requiring intervention for end-organ compromise, identification of a MAPK/ERK pathway mutation in about 50% of cases suggests a neoplastic proliferation, and provides therapeutic options for recurrent/persistent disease. Emperipolesis may be inconspicuous in extra-nodal sites, along with the presence of foamy histiocytes and fibrosis, making diagnosis challenging. Some cases may be familial. Some may coexist with LCH or lymphoma. Prominent IgG4-positive PC population mimics IgG4-related disease; however, other features of IgG4-related disease are not present. Clinico-radiologic presentation mimics meningioma, creating a diagnostic challenge. Patients should be investigated for systemic involvement.

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DNA Methylation Profiling in a Case of Papillary Tumor of the Pineal Region

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Background: Pineal tumors comprise less than 1% of intracranial neoplasms in adult patients, with papillary tumors of the pineal region accounting for a small subset of all pineal tumors. Here, we present the case of a 52-year-old woman who presented with sudden-onset severe headache and vomiting.

Methods: Imaging demonstrated a 1.8 cm heterogeneously enhancing hemorrhagic mass centered in the pineal region with resultant obstruction of the cerebral aqueduct and secondary hydrocephalus. The patient underwent an endoscopic third ventriculostomy and biopsy of the mass.

Results: Neuropathological examination of the mass demonstrated a papillary tumor without identifiable mitotic figures or necrosis. The Ki-67 proliferative index was approximately 1%. Immunohistochemistry demonstrated the neoplastic cells to be positive for SOX2 and CK18 and negative for CRX. These findings were consistent with the diagnosis of papillary tumor of the pineal region, WHO grade 2. DNA methylation profiling further supported the diagnosis of papillary tumor of the pineal region, subtype A, with high confidence.

Conclusions: This case represents an example of a rare tumor entity with classic microscopic findings. It also highlights the importance of DNA methylation profiling in prognostication. In the context of this patient, methylation profiling helped guide clinical intervention through interval follow-up with subtype A having a length of progression-free survival roughly three times that of subtype B (125 months versus 43 months). This information provided by DNA methylation profiling can be crucial in guiding the selection of clinical intervention and would not be obtainable through other methodologies.

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Anaplastic Transformation of Pituitary Neuroendocrine Tumors (PitNETs): the UCSF Experience

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Background: Pituitary neuroendocrine tumors (PitNET)/adenomas that metastasize comprise 0.2% of adenohypophyseal tumors, are aggressive, and are challenging to treat. Non-metastatic tumors may also be aggressive and some histologic features may suggest aggressive behavior.

Methods: We review our experience with metastatic PitNETs, “pituitary neuroendocrine carcinomas” (PitNECs), and PitNETs with sarcomatous transformation as identified through an archival search at UCSF. A tissue microarray was constructed for additional immunohistochemistry (IHC), including transcription factors. Tumors were subtyped using the Endocrine WHO 2022 and the 2018 IARC grading scheme for neuroendocrine neoplasms (NEN).

Results: We identified 17 specimens from 11 patients (9 male, 2 female) with median age 54 years (range 31-73) at diagnosis. Histologic subtypes included acidophil stem cell, null cell, thyrotroph, corticotroph, gonadotroph, and immature PIT-1 lineage tumors. In some patients, anaplastic transformation from PitNET to PitNEC (N=4) and/or sarcoma (N=4) was seen, and one case qualified as grade 3 NET using IARC criteria. Median Ki-67 labeling index was 25% (range 7-54%). Tumors with losses of p16 or RB1 expression were seen in 2 patients each. Tumors from 3 patients had strong diffuse p53 positivity. Molecular analysis in 3 tumors variably included TERT-promoter alterations, CDKN2A homozygous deletion, aneuploidy, and mutations of PTEN, TP53, PDGFRB and/or PIK3CA. Seven (64%) died of disease and six had CSF/systemic dissemination. Worrisome features including aggressive histologic subtype, high mitotic count, and/or high Ki-67 were seen in all primary tumors. Further evidence of transformation includes loss of neuroendocrine and/or hormone markers by IHC, carcinomatous and/or sarcomatous histology, RB1 loss, p16 loss, TP53 mutation/diffuse p53 staining, TERT promoter alterations, and homozygous CDKN2A deletion.

Conclusions: We conclude that metastatic PitNET is not the only high-grade pituitary NEN. Anaplastic transformation to PitNEC or sarcoma may also occur. If further confirmed, these features could represent evidence of biological aggressiveness and be applied towards a future grading scheme.

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PAS+/PASD- intracytoplasmic glycogen is not specific for clear cell meningioma

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Background: Intracytoplasmic glycogen is highlighted by periodic acid-Schiff (PAS) and is removed by diastase (PASD) digestion. The presence of intracytoplasmic glycogen has traditionally been used to confirm a suspected diagnosis clear cell meningioma, a histologic subtype associated with younger age and poorer patient outcomes. However, its presence in other meningioma subtypes has not been extensively studied.

Methods: We performed PAS with and without diastase on 243 non-clear cell meningiomas using tissue microarrays (156 CNS WHO grade 1 meningiomas, 74 CNS WHO grade 2 meningiomas, and 13 CNS WHO grade 3 meningiomas).

Results: A total of 85 meningiomas (35%) contained PAS+/PASD- intracytoplasmic glycogen (49 CNS WHO grade 1 [58%], 33 CNS WHO grade 2 [38%], and 3 CNS WHO grade 3 [4%]). PAS+ staining was diffuse in 53% and patchy in 47% of glycogen-containing meningiomas. Histologic subtypes with PAS+ material included transitional, angiomatous, fibrous, lymphoplasmacyte-rich, secretory, psammomatous, meningothelial, and microcystic. Meningiomas with focal chordoid or rhabdoid features were also noted to have PAS-positivity. PAS also highlighted pseudopsammoma bodies, eosinophils, macrophages, and basement membranes. These pose potential pitfalls in PAS stain interpretation.

Conclusions: PAS staining in meningiomas is not specific to the clear cell variant and can be seen in a variety of other histologic subtypes.

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An uncommon isolated cavernous sinus ALK-positive histiocytosis in an adolescent

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Background: Anaplastic lymphoma kinase (ALK)-positive histiocytosis is a distinct and rare systemic disease characterized by the proliferation of histiocytes that exhibit ALK positivity, frequently due to genetic mutation like the KIF5B/ALK gene fusion. This entity was first delineated in 2008. The involvement of the central nervous system (CNS) by this disease has been documented with increasing frequency, and the posterior fossa is the most common site in the single CNS system involvement.

Methods: Patient clinical information was extracted from the electronic medical record, and histologic staining was conducted following standard protocols.

Results: The patient, a 14-year-old male with a history of stable vocal and motor tics and attention deficit hyperactivity disorder, exhibited new-onset symptoms including right eye deviation and facial weakness. Initial diagnostic imaging through brain MRI identified a 2.2 x 1.1 cm enhancing lesion within the right cavernous sinus, suggesting a differential diagnosis that ranged from nerve sheath tumors/ schwannoma to inflammatory processes. Upon subsequent evaluation two months later, the lesion had enlarged to 2.7 x 1.3 cm, yet no significant lymphadenopathy or masses were detected elsewhere in the body. This led to an expanded differential diagnosis that included considerations for lymphoma and histiocytosis. A biopsy was performed, revealing predominantly of CD68+ and CD163+ histiocytes, with a notable expression of nuclear and cytoplasmic ALK1. The diagnosis of ALK-positive histiocytosis was conclusively established following the identification of the KIF5B/ALK gene fusion through next-generation sequencing.

Conclusions: This case underscores the critical importance of a collaborative diagnostic approach, involving thorough clinical evaluation by physicians, radiological assessment, and precise pathological analysis, to navigate the complexities of rare diseases. Furthermore, it expands the clinicopathological landscape by adding a rare site of involvement to the existing case pool, thereby broadening the understanding of this uncommon disorder and its varied clinical presentations.

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A Rare Case of a Diffuse Large B-Cell Lymphoma Arising in the Pineal Gland

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Background: A 14-year-old male with no significant past medical history presented with slurred speech, weakness in the right hand, and gait imbalance. Magnetic resonance imaging (MRI) of the brain revealed a 2.5 cm solid/cystic pineal mass and additional 2.4 cm mass along the right lateral margin of the fourth ventricle.

Methods: Routine hematoxylin and eosin and immunohistochemical stains were performed on formalin-fixed, paraffin-embedded sections of the tumor.

Results: Sections showed an angiocentric proliferation of undifferentiated atypical cells characterized by medium-to-large cells with irregular nuclear contours, multiple small nucleoli, and scant amount of eosinophilic to amphophilic cytoplasm. There were multiple foci of necrotic and crushed cells. The differential diagnosis included germ cell tumor, pineoblastoma, central nervous system (CNS) embryonal tumor, high-grade glioma, and metastasis. Immunohistochemical stains revealed the tumor was CD20+, CD10+, MUM-1 partial+, BCL6+, BCL2-, c-MYC+, BCOR partial+, CD3-, CD30-, TdT-, ALK-, EBV ISH-, with a Ki-67 of 80%, consistent with a diagnosis of primary CNS diffuse large B-cell lymphoma, germinal center phenotype. Epithelial, glioneuronal, and germ cell markers were negative. Fluorescence in situ hybridization (FISH) lymphoma panel analysis showed no evidence of BCL6 gene rearrangement or deletion, MYC/IGH translocation, MYC gene rearrangement (performed per reflex protocol) or IGH/BCL2 translocation in any of the cells examined.

Conclusions: Germinal center immunophenotype is present in < 10% of primary CNS diffuse large B-cell lymphoma cases; CD10 is more frequently positive in systemic diffuse large B-cell lymphomas. Bone marrow biopsy and imaging did not detect any other potential primary source. The unusual location posed additional complexity on frozen and permanent sections, as the pineal gland can harbor several tumors with overlapping histology including germ cell tumors, pineoblastoma, metastatic tumors. This case emphasizes the need to include hematolymphoid malignancies in the CNS, particularly diffuse large B-cell lymphoma, in neuropathology practice.

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A Case For Renaming: Malignant Intracerebral Nerve Sheath Tumor Confirmed by Methylation Array Profiling

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Background: Malignant peripheral nerve sheath tumors (MPNSTs) are rare, aggressive mesenchymal neoplasms that occur sporadically or in association with neurofibromatosis type 1 (NF-1). These tumors can rarely arise from the central nervous system (CNS) parenchyma. Here, we present a case of malignant intracerebral nerve sheath tumor (MINST), for which methylation classification was critical in confirming the diagnosis.

Methods: A 73-year-old male presented with expressive aphasia and headache. Magnetic resonance imaging identified a 3.8 x 2.4 x 3.0 cm enhancing mass in the posterior left temporal lobe. Left temporal craniotomy with complete surgical resection of the mass was performed. Next-generation sequencing (NGS) and genome-wide DNA methylation array profiling using the DKFZ classifier were conducted. A PubMed.gov literature search was performed for cases of primary intracranial malignant nerve sheath tumors using appropriate key words.

Results: The resected tumor was encapsulated and consisted of hyper- and hypocellular regions of spindle cells negative for neuronal, glial and meningioma markers, with a high proliferation index, and loss of nuclear H3K27me3. NGS showed wild-type NF1, loss of CDKN2A/B and PTEN, and a TERT promoter activating variant. Methylation-based profiling revealed a high-fidelity match to MPNST.

Conclusions: Among intracranial malignant nerve sheath tumor cases published to date, 23% were not associated with cranial nerves, and 60% of these were intracerebral (MINST). The one- and five-year survival rates for MINST were 71.5% and 41.4%, respectively. Here we present the second reported case of MINST to be identified by methylation profiling, which highlights the critical role of methylation-based classification in accurately diagnosing rare intracranial nerve sheath tumors. To standardize classification, we propose use of the term “malignant nerve sheath tumor” to broadly describe neoplasms of this type, with subcategorization into MPNST or intracranial malignant nerve sheath tumors, including MINST, depending on location.

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Recurrent Langerhans Histiocytosis of the Skull: Case Report and Review of the Literature

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Background: Langerhans cell histiocytosis (LCH) is a hematologic disease process where Langerhans cells proliferate as localized or disseminated disease, most commonly occurring in children under 15 years of age with an incidence rate of 4.5 per million children. Herein, we present a case of a child with a history of disseminated LCH with recurrence in the skull.

Methods: The patient's electronic medical records were reviewed and a literature search was undertaken using appropriate key words.

Results: A five-year-old boy initially presented at age two with left orbital swelling. Imaging of his skull revealed (1) a lesion in the right orbit with mass effect on the left globe, and (2) a left superior occipital lytic skull lesion. An excisional biopsy of the skull lesion revealed LCH with a BRAF V600E mutation. Right iliac crest bone marrow biopsy revealed involvement. He was treated with chemotherapy and subsequent imaging showed a stable, subtle T2 hyperintensity within the left orbit but no clear evidence of persistent disease. He subsequently developed headaches along with a new, palpable bump on his head. CT and MRI imaging of his head revealed a left parietal skull lesion, lytic, and avidly contrast-enhancing. The lesion was resected and histologic and immunohistochemical evaluation revealed recurrent LCH.

Conclusions: LCH can present in the skull as a localized lesion or as part of a disseminated disease process. The recurrence rate after treatment varies from 5.7% recurrence in unifocal bone disease, 12.5% reactivation in single system bone disease, and 23.8% recurrence in multifocal bone disease. When disseminated, with involvement of bone marrow, liver, or spleen, treatment is more challenging and survival is reduced. This case illustrates the challenges of effective treatment of LCH, particularly when disseminated, and the need for vigilant follow-up.

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Breast neuroendocrine tumor presenting as a suprasellar pituitary mass: An unusual presentation of a rare entity posing a diagnostic challenge.

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Background: Primary neuroendocrine tumors of the breast (NETB) is a rare subtype of breast tumors. Only two cases of metastatic NETB to the CNS have been reported. Metastasis to the pituitary can be diagnostically challenging due to morphologic similarities to pituitary adenomas. We report a case of NETB to the pituitary with an initial presentation as a suprasellar mass with clinical and radiologic features mimicking pituitary adenoma. The Identification of the primary tumor's location was challenging due to lack of suspicion of a breast primary, the negative usual breast immunohistochemistry markers and the relatively small size of the breast mass.

Methods: A 63-year-old Caucasian female presented with panhypopituitarism and bitemporal hemianopia. MRI showed a 9 mm suprasellar mass involving pituitary gland and the stalk with extension to the hypothalamus. A transsphenoidal resection of the pituitary lesion was performed.

Results: Histology revealed nests and sheets of monomorphic tumor cells with neuroendocrine features and scattered mitoses (2/ 10 high power fields). Immunohistochemistry is positive for synaptophysin and cytokeratin Cam5.2 and negative for pituitary hormones and transcription factors. The tumor also expresses MOC31 and TTF1. Breast markers (GATA3, mammoglobin, ER and PR) were negative. Molecular studies revealed MYCL amplification. PET scan was subsequently done and revealed prominent cervical and axillary lymph nodes. A screening breast ultrasound revealed a 1.5 cm mass in the left breast and enlarged axillary lymph nodes. A left partial mastectomy was performed, confirming NETB with morphology and immunophenotype identical to the pituitary tumor. The patient received postsurgical adjuvant chemotherapy and radiotherapy. Follow-up imaging revealed an interval increase in the size of the intracranial tumor.

Conclusions: NETBs with metastases to the pituitary is diagnostically challenging with the risk of misdiagnosis as pituitary adenoma. Close attention to morphologic details, high index of suspicion and extended immunohistochemistry panel may help in identification of these lesions.

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Atypical meningioma with brain invasion, increased mitotic activity and angiomatous features: a case report with review of the literature

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Background: Meningioma is the most common primary brain tumor in adults with at least 15 subtypes. However, the features of more aggressive growth can arise in any of these subtypes and the criteria for atypical or anaplastic meningioma should be applied regardless of the underlying subtypes. Here we report an angiomatous meningioma with brain invasion and increased mitotic activity, corresponding to WHO grade 2.

Methods: A 69-year-old female with progressive headache was found to have a large bifrontal dural based, extraaxial mass growing into the falx and anterior superior sagittal sinus. The tumor was resected and morphologically compatible with angiomatous meningioma. However, focal brain invasion (supported by GFAP immunostain) and increased mitotic figures (up to 4-5 mitoses/10HPF) were present, consistent with a diagnosis of atypical meningioma, WHO grade 2.

Results: Angiomatous meningioma is one of the subtypes that is usually WHO grade 1 tumor. We believe this is the first report of this subtype of meningioma with brain invasion and increased mitotic activity. The occurrence of atypical features, such as brain invasion or increased mitotic activity is extremely rare in meningiomas with a classic WHO grade 1 histologic subtype.

Conclusions: Features for atypical meningioma, like brain invasion or increased mitotic figures can be seen in meningiomas with a classic WHO grade 1 histological subtype. Extensive sampling and careful examination of the entire specimen is important to avoid undergrading of the meningioma.

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Metachronous Diffuse Large B-cell Lymphoma of the Ovary and the Brain in an HIV-positive Woman.

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Background: DLBCL is an aggressive lymphoma and the ovary is a rare primary site. DLBCL may also secondarily involve the CNS (SCNSL) usually involving the dura and choroid plexus.

Methods: We present a case of a 30-year-old HIV positive woman who presented with a left ovarian diffuse large B cell lymphoma - germinal center B cell subtype, without bone marrow and lymph node involvement. She underwent 6 cycles of R-EPOCH and was in remission. Five years later, she presented with altered mental status and stroke-like symptoms and was found to have an enhancing mass in the posterior corpus callosum, which was diagnosed as a diffuse large B cell lymphoma- activated B-cell subtype.

Results: The ovarian tumor showed germinal center differentiation [CD20(+), BCL-6(+), MUM1(-)] and no rearrangement of BCL6, BCL2 or MYC, while the CNS tumor featured an activated B-cell phenotype [CD20(+), MUM1(+)] and was positive for BCL-6 and IGH rearrangements, suggesting two metachronous diffuse large B cell lymphomas.

Conclusions: Involvement of the CNS and other extranodal sites by DLBCL can be seen in ~30-40% of cases. While synchronous and metachronous hematopoietic malignancies have been reported, to our knowledge this is the first example of two metachronous diffuse large B cell lymphomas in the ovary and CNS.

Posters: Tumors: Vascular/Stroke

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ApoE expression in neurons as a marker of early ischemic change
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Background: Apolipoprotein E (ApoE) is a lipoprotein that is involved in lipid metabolism and membrane homeostasis in the CNS. It is synthesized predominantly by astrocytes and can also be detected in neurons in case of injury. The goal of this study is to identify whether cytoplasmic ApoE expression in neurons can serve as a marker of early ischemia in cases that are negative for acute eosinophilic change on hematoxylin and eosin (H&E) staining.

Methods: Anti-ApoE immunohistochemical staining was performed on hippocampal and cerebellar sections from 42 randomly selected recent hospital autopsy cases. Stained slides were manually reviewed to identify the correlation of neuronal ApoE expression with evidence of hypoxic/ ischemic neuronal injury on H&E stained sections.

Results: All cases with acute neuronal eosinophilia in the hippocampus or cerebellum by H&E staining showed strong and diffuse neuronal cytoplasmic ApoE positivity. In addition, many but not all cases that did not show neuronal cytoplasmic eosinophilia in these sites were found to express some degree of neuronal cytoplasmic ApoE. Additional analyses showed a strong correlation between ApoE expression in the hippocampal CA1 sector and Purkinje neurons.

Conclusions: ApoE is expressed consistently in neurons with morphologic changes of ischemia and in a subset of cases in which ischemic injury is suspected but not conventionally histologically apparent. We hypothesize that ApoE immunohistochemistry may serve as a sensitive technique for detection of early ischemic change in the brain.

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Survey of Neuroanatomic Sampling and Staining Procedures for Cerebral Vascular Disease across Alzheimer's Disease Research Centers

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Background: Cerebral vascular disease (CVD) is common in post-mortem examination of autopsy brains, highly heterogeneous, and associated with cognitive and dementia outcomes. Despite its high prevalence in autopsied brains, CVD lacks standardized methodology for pathologic evaluation and a coding system in which to grade it. In this study, we surveyed 37 Alzheimer's Disease Research Centers (ADRCs) on the various methods used to quantify CVD. The objective was to identify similarities and differences with approaches with an ultimate goal of developing harmonized, cost-effective, and reproducible methodologies of evaluations.

Methods: A survey was sent in 2023 to 37 ADRC neuropathology cores (NPC) across the USA to assess current practices in evaluating CVD within their ADRC participants that had come to autopsy. The results were compiled in RedCap.

Results: 35/37 (95%) ADRCs responded to the survey. Thirty-one centers (89%) used the NPC v11 to document CVD, and 27 (77%) linked these findings to pre and/or post-mortem MRI findings. The vascular lesions documented included cerebral microhemorrhages (43%), macrohemorrhages (34%), microinfarcts (51%), lacunar infarcts (69%), and large infarcts (40%). Amyloid beta was the most common IHC stain, used in 97% of the centers to evaluate cerebral amyloid angiopathy (CAA). For white matter pathology, 34% used luxol fast blue, while 27% used hematoxylin and eosin staining. For large vessel evaluation, all respondents incorporated gross examination of the circle of Willis, while only 17% submitted large vessels for microscopic examination. For assessment of arteriolosclerosis, some centers generated a global measurement, while others reported the most severe region. The majority of centers used a semi-quantitative approach to grade arteriolosclerosis (91%) and CAA (89%).

Conclusions: Our survey had a high response rate and showed that the ADRCs have a broad agreement in terms of CVD documentation and evaluation of arteriolosclerosis and CAA, and a fair agreement for the identification of specific CVD lesions.

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A Mysterious Cause of Death: Rapidly Progressing Idiopathic Neutrophilic Vasculitis with Fibrinoid Necrosis of the Central Nervous System.

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Background: Neutrophilic vasculitis with fibrinoid necrosis encompasses a group of disorders caused by various etiologies including autoimmune, medication-induced, infectious, and paraneoplastic. These disorders typically present with skin and/or systemic manifestations. Isolated central nervous system (CNS) manifestations without systemic involvement are very rare.

Methods: We present a unique case of a 75-year-old male who developed isolated, rapidly progressing idiopathic neutrophilic vasculitis with fibrinoid necrosis. He was found unresponsive at home but regained consciousness when paramedics arrived. Later, he rapidly became unresponsive again and was subsequently intubated and sedated. His past medical history includes diabetes, hypertension, mitral valve replacement, HFpEF, and malignant fibrous histiocytoma/pleomorphic undifferentiated sarcoma of the scalp diagnosed and treated five years prior with brachytherapy and without any evidence of recurrence. Initial imaging revealed findings consistent with edema from an early subacute infarct and a pontine infarct with hemorrhagic conversion. Follow-up imaging was suggestive of rhombencephalitis. Blood cultures were negative, and the patient did not receive antibiotics during the hospital course. Despite aggressive efforts, he progressed essentially to near brain death within 36 hours and expired five days from the initial presentation.

Results: Gross examination of the brain revealed severe global edema (1630 grams), uncal and tonsillar herniation, and a dorsal pontine hematoma. On histopathologic examination, severe neutrophilic vasculitis with fibrinoid necrosis was found involving the cerebrum, brainstem, and cerebellum, along with acute parenchymal and leptomeningeal, most prominent in the brainstem and cerebellum. No evidence of bacterial, fungal, viral, or parasitic infection was identified by H&E, AFB, PAS, GMS, and Gram special stains, and immunohistochemical stains for HSV and toxoplasma. There was no evidence of neoplasia. No similar vascular lesions were identified in any other organ system evaluated at autopsy.

Conclusions: To our knowledge, no previous reports have described similar histopathologic findings associated with such unique radiographic findings and rapid clinical decline.

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Automating Microinfarct Screening in Hematoxylin and Eosin-stained Human Brain Tissues: A Machine Learning Approach

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Background: Microinfarcts, characteristic lesions of vascular dementia (VaD), are heterogenous and vary in appearance, which pose a considerable challenge for VaD grading as there is great interrater variability in microinfarct assessment. We propose a novel application of machine learning (ML) in the automated screening of microinfarcts, addressing a gap in the post-mortem analysis of VaD in whole slide images (WSIs) from human brain.

Methods: Our study adapts a patch-based pipeline with convolutional neural networks (CNNs) to automate microinfarct screening in WSIs. We compare performance and computational cost of different fields-of-view (FOV) for the patch-based method. Additionally, we propose a postprocessing step and leverage multiple FOVs to mitigate false positives. This study is validated against 66 annotated WSIs (N = 40 infarcted, N = 26 un-infarcted) from Frontal, Parietal, and Occipital white matter regions across 22 cases. Annotations are from a single trained expert, who delineated microinfarct regions and graded white matter rarefaction.

Results: We report screening performance, i.e, the ability to distinguish infarct-positive from infarct-negative WSIs, and detection performance, i.e, the ability to localize the microinfarct regions within a WSI. Despite the inherent challenges of the inexact boundaries of microinfarcts, our models demonstrate notable efficacy in screening WSIs for infarcts, with ResNet-18 achieving 100% accuracy at WSI level. However, the sensitivity in detecting infarct regions is below 50%, based on Intersection over Union thresholds above 20% overlap (a performance metric used to evaluate the accuracy of object detection models). Leveraging multiple FOV improves both detection and screening performances, reducing false positives.

Conclusions: This work presents a proof-of-concept pipeline driven by machine learning to automate microinfarct screening & detection in WSIs. This workflow provides new avenues in the automated analysis of neuropathological images of microinfarcts, bringing potential advancement in ML-based dementia and neuropathology research.

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Cerebral Hemorrhage in Setting of Cerebral Arteriovenous Malformation with Concurrent Metastatic Renal Cell Carcinoma: A Novel Co-occurrence

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Background: Renal cell carcinoma (RCC) constitutes approximately 8% of brain metastases. RCC's propensity for metastasis is linked to its robust vascularity and angiogenic profile and has a highly vascular appearance on imaging. Distinction of vascular malformations from hypervascular neoplasms by imaging is complex due to shared vascular features. We report a case of RCC metastatic to the vicinity of a brain arteriovenous malformation (AVM).

Methods: Electronic medical records and imaging studies were reviewed. A literature search was conducted.

Results: A 69-year-old woman presented with sudden-onset headaches. Brain imaging revealed a right parietal intracranial hemorrhage. Cerebral angiogram identified a right parietal arteriovenous malformation, fed by middle cerebral artery branches. Histological examination revealed an abnormal collection of blood vessels within the subarachnoid space, gliotic neuroparenchyma, and neoplasm. Arterial-type vessels displayed size variation with thickened media and internal elastic lamina, while vein-type vessels exhibited fibrosis and obliteration within admixed neuroparenchyma, consistent with an arteriovenous malformation. Approximately 20% of the specimen was composed of neoplastic cells with clear cytoplasm and a nested within a rich vascular network. Neoplastic cells showed expression of cytokeratin CK8/18, CD10, PAX-8, and RCC, consistent with metastatic renal cell carcinoma. Whether the hemorrhage arose from a ruptured AVM or hemorrhage within the neoplasm was unclear. Whole body CT scan identified left kidney masses and left nephrectomy revealed a clear cell renal cell carcinoma and an angiomyolipoma. Several reports of metastasis of lung carcinomas to a brain AVM have been reported. To our knowledge, this is the first report of metastasis of RCC to an arteriovenous malformation in the brain.

Conclusions: The differential diagnosis of a highly vascular lesion on imaging includes vascular malformation and metastatic neoplasm. Metastatic RCC often appears as a highly vascular lesion on imaging mimicking a vascular malformation. Metastasis to a vascular malformation is rare.

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Venous Malformations of the Brain: Not Always Benign

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Background: Cerebral vascular malformations include venous malformations (VMs; developmental venous anomalies or venous angiomas), cavernous hemangiomas, arteriovenous malformations, and capillary telangiectasias. VMs are the most common with autopsy incidence of 3%. They are considered “benign”, with little effect on cerebral circulation. Our goal was to review autopsy VMs and their relationship to symptomatology and death.

Methods: We screened our database from 7/2016 through 12/2023, for brain VMs, analyzing the clinical manifestations and relationship to death.

Results: We identified 22 cases (ages 7-74 years, median 37; 17 male). VMs occupied the following: frontal lobe (n=6 [1 bilateral, involving basal ganglia]); parietal lobe or occipital lobe (n=2 each); parieto-occipital (n=1); multifocal bilateral hemispheres including thalamus (n=1, with perinatal disruptive lesions); brainstem (n=3); cerebellum (n=6); brainstem and cerebellum (n=1). Fourteen had capillary components (venocapillary malformations, VCMs), of which 6 had associated focal cortical dysplasia (FCDIIIc). In 8 subjects with known seizure disorder, VMs or VCMs were the likely substrate in at least 5, 3 of whom also had hippocampal sclerosis. Three VCMs had associated hypotrophy and mineralization; 2 had additional features of cerebral dysgenesis elsewhere. Local hemorrhages were noted in 4, all infratentorial: 1 dorsal midbrain (old); 3 cerebellum (2 acute; 1 acute-on-old with the underlying dural venous anomaly demonstrated in situ). Cause of death was attributed to complications of seizure disorder (3 supratentorial) or to VM rupture and hemorrhage (3 infratentorial). Sixteen VMs were “incidental” (unrelated to death).

Conclusions: An appreciable subset of VMs is associated with death due to seizure disorder in supratentorial locations, or hemorrhage in infratentorial sites. Moreover, capillary components of VMs, especially in the cerebral cortex, should prompt evaluation for FCD (type IIIc). Finally, broad lobar involvement, including deep gray structures, may occur, raising the prospect of a radial telencephalic developmental defect involving embryonic vascular and neuronal organization.

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Retinal Vasculopathy with Cerebral Leukoencephalopathy (RVCL) presented as brain mass lesion.

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Background: Retinal vasculopathy with cerebral leukoencephalopathy and systemic manifestations (RVCL-S) is a small-vessel disease that affects highly vascularized tissues including the retina, brain, liver, and kidneys, defined by autosomal dominant hereditary pattern and TREX1 mutation. Herein, we report a case with initial manifestation as an acute brain mass effect.

Methods: N/A

Results: 42-year-old gentlemen who presented to emergency department complaining severe headache with generalized throbbing, nausea, vomiting and photophobia. Past medical history include pancreatitis, ADHD, hypertension, GERD. Brain CT and subsequent MRI showed multiple enhancing supratentorial lesion with edema, the largest in the left basal ganglia (2.3 cm) demonstrating rim-enhancing mass lesion with central necrosis, concerning for primary or metastatic tumor. However, biopsy showed negative for neoplasm. Instead, we observed macrophage-predominant chronic inflammation, necrosis, reactive astrocytosis, myelin loss with axonal damage, raising differential diagnosis for infection, primary demyelinating disorder and vasculopathy/vasculitis. Tissue microbiological multiplex PCR and CSF analysis failed to detect significant contributing microorganism. CSF analysis failed to detect oligoclonal band. CT angiogram failed to detect features of vasculitis and stenosis. A further inquiry revealed possible family history of ‘stroke’, therefore, small vessel disease is suspected. Genetic test revealed TREX1 mutation, RVCL was considered very probable. Ophthalmic examination showed right fundus sclerotic vessels, peripheral avascular with neovascularization at inferior region but essentially normal left fundus. At the time of this writing, no evidence of renal or liver disease has been identified.

Conclusions: RVCL-S is a rare autosomal dominant hereditary vasculopathy involving multiple organs. The clinical course varies but typically starts from retinopathy, kidney and liver diseases; cerebral deficit usually comes at a later stage (Pelzer et al. J intern Med 2019; 285:317-332). In this unique case, cerebral deficit was the first manifestation that brought to medical attention, mimicking tumor mass on imaging and mimicking inflammatory demyelinating disorder in pathological finding.

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You Don't Have to Look Fahr: Three Cases of Fahr's Disease/Syndrome in a Medical Examiner Office

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Background: Fahr’s disease, also known as familial idiopathic basal ganglia calcifications, is a rare neurologic disorder with a wide range of clinical presentations. The disease has primary causes, whereas Fahr’s syndrome is due to secondary metabolic or endocrine disorders, tumors, autoimmune disease, and infections. The clinical presentation can vary from completely asymptomatic to marked extrapyramidal or neuropsychiatric symptoms, beginning as early as the fifth decade. Here we report the postmortem diagnosis of Fahr’s disease/syndrome in a large medical examiner office, highlight the varied clinical presentations of each, and explore forensic considerations.

Methods: Three decedents (ages 44, 58, and 78; 2 males) were identified following a database search from 1/2020 through 1/2024.

Results: The first was a 78-year-old man with a history of memory and cognitive decline, without features of movement disorder, neuropsychiatric symptoms, or family history (i.e., sporadic Fahr’s disease). Another was a 44-year-old woman with a history of anxiety, hypoparathyroidism, abnormal calcium deposits, and suspected secondary (metabolic) Fahr’s syndrome. Finally, we identified a 58-year-old man with a more classic clinical presentation including history of schizoaffective disorder, hypothyroidism, and antemortem imaging suggestive of Fahr’s syndrome. The neuropathologic examination in each was notable for vessel calcification in the basal ganglia, white matter, and cerebellum, in excess of that expected for age.

Conclusions: Our three cases of Fahr’s disease/syndrome each illustrate differing underlying etiologies and varied clinical presentations. When this diagnosis is rendered, we recommend seeking records of antemortem imaging, and ancillary studies such as molecular genetics or metabolic studies to further characterize the pathologic process. With regard to the influence of Fahr’s disease/syndrome on death certification, psychiatric manifestations may result in completed suicide and therefore constitute a contributing factor. Finally, given the potential familial occurrence, family members may benefit from early in vivo diagnosis and symptomatic treatment.

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Neuropathologic Correlates of Cerebral Amyloid Angiopathy in Older Adults

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Background: Associations between cerebral amyloid angiopathy (CAA), apolipoprotein E (ApoE) ε4, and Alzheimer's disease pathology (AD) are well documented, although the causes of CAA in older adults are unclear. Here, we examine the odds of CAA in persons with variable neuropathologic indices.

Methods: This study evaluated CAA in older participants who were followed in one of two longitudinal studies of aging and dementia. Postmortem neuropathology evidence and severity of CAA as well as other cerebrovascular pathologies including cerebral atherosclerosis, arteriolosclerosis, and infarcts; neurodegenerative diseases including AD, limbic-predominant age-related TDP-43 encephalopathy (LATE), and Lewy body disease; and age-related brain lesions including β-amyloid plaques, PHF-tau tangles, TDP-43-positive inclusions, Lewy bodies, and hippocampal sclerosis was assessed. Multiple regression models were used to analyze the association of cerebrovascular and neurodegenerative pathologies and lesions with CAA controlling for demographic factors.

Results: In total, 1968 older adults were studied. Participants died at mean age of 89.7 years (SD=6.6 years). Individuals were characterized as having absent-to-mild CAA (1207 persons, 62.5%) or moderate-to-severe CAA (724 persons, 37.5%). The odds of moderate-to-severe CAA was higher in persons who were older (odds ratio (OR), 1.03 [95% CI, 1.02–1.05]), ApoE ε4 carriers (OR, 3.62 [95% CI, 2.90–4.52]), or had AD (OR, 4.14 [95% CI, 3.28–5.23]). No associations were found with cerebrovascular pathologies with the exception of cortical infarcts. Similarly, no associations were identified with Lewy bodies or Lewy body disease, but higher odds of CAA was also unexpectedly found in persons with LATE, controlling for age, education, and AD (OR, 1.12 [95% CI, 1.03–1.21]). No interactions were identified between CAA and demographic factors or presence of AD.

Conclusions: This study confirmed higher odds of moderate-to-severe CAA in persons who are older or have AD, and unexpectedly identified higher odds of CAA in persons with LATE. The association of cortical infarcts with CAA requires further investigation.

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Fibromuscular Dysplasia Involving the Cervicocephalic Arterial Tree Highlighting the Neuropathological Findings

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Background: Three types of fibromuscular dysplasia (FMD) have been described based on the dominant arterial wall layer that has been affected, include - intimal (10% of cases), media (75-80% of cases), and adventitia (1% of cases). Histologically, intimal FMD corresponds to circumferential deposition of collagen within the intimal layer with the internal elastic lamina often being fragmented or duplicated. Medial FMD is by far the most common subtype and involves altering areas of a thin medial layer and thickened collagenous fibromuscular ridges. This results in multiple stenotic areas with aneurysmal outpouching giving the vessel the classic “string-of-beads” appearance as seen on magnetic resonance angiography.

Methods: Provide detailed gross and microscopic illustrations of the neuropathological findings associated with intracranial involvement of FMD within the Circle of Willis.

Results: This case involved a 27-year-old female with a complicated hospital course due to FMD causing multifocal angiopathic associated luminal stenosis, dissections, bulbous arterial dilatation, and associated thrombi within the Circle of Willis. Downstream affects observed as a direct result from the FMD affected vessels showed numerous hemorrhagic infarcts within the cerebrum, cerebellum, and brainstem. At neuroautopsy, the Circle of Willis was dissected out of the interpeduncular cistern of the subarachnoid space located on the basilar surface of the brain for extensive microscopic examination. Microscopic examination of the vasculature demonstrated alternating areas of increased fibroblastic-like transformation of the smooth muscle cells within the tunica intima and media leading to arterial wall tearing, luminal stenosis, occlusion, and recanalization.

Conclusions: In conclusion, the goal of this report is to demonstrate the histopathological findings associated with FMD within the Circle of Willis while highlighting the diagnostic value of special stains when assessing the morphological features and accurate subtyping for FMD.

Posters: Other

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Crystal-storing Histiocytosis in the Central Nervous System
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Background: Crystal-storing histiocytosis is characterized by intracytoplasmic monoclonal immunoglobulin light chain crystals in histiocytes, most commonly of kappa type. There are limited reports of central nervous system (CNS) involvement in the setting of lymphoplasmacytic disorders and inflammatory conditions such as gout, Crohn's disease, and primary CNS angiitis.

Methods: We report a 27-year-old female with common variable immunodeficiency and systemic sarcoidosis presenting with headache, dizziness, and left-sided weakness. Brain MRI showed a 1.1 cm confluent T2-hyperintense right frontal operculum lesion with central contrast enhancement. The clinicoradiological differential diagnosis included demyelinating disease, neurosarcoidosis, and a lymphoproliferative or glial neoplasm. A stereotactic biopsy was performed.

Results: Histologic sections showed sheets of CD68+ histiocytes with abundant brightly eosinophilic PAS-negative granules in a background of extensive CD138+ plasma cells and predominantly perivascular CD3+ T-lymphocytes. Negative staining for PAX-5, CD20, CD79a, and OCT2 argued against the presence of a B-cell population. Kappa and lambda in situ hybridization highlighted plasma cells with a kappa/lambda ratio of approximately 3:1. The LFB-PAS stain revealed focal areas of demyelination. GFAP highlighted reactive astrocytes while IDH1 p.R132H was negative and ATRX was retained. Immunofluorescent stains for IgM and kappa revealed granular staining in histiocytes, while lambda was negative. The workup for systemic hematologic malignancies including serum protein electrophoresis, bone marrow aspiration biopsy, and positron emission tomography scan were negative. NeoGenomics B-cell/T-cell receptor beta and gamma gene rearrangement, next-generation sequencing (NGS), and UCSF500 NGS showed no evidence of a clonal process.

Conclusions: Crystal-storing histiocytosis in the CNS should prompt further immunohistochemical, immunofluorescent, and molecular studies to rule out an underlying inflammatory or hematologic disorder.

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Impact of Postmortem Interval on Brain Weight in Adult Autopsies

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Background: Postmortem interval (PMI) can affect tissue integrity. Understanding its impact on brain weight is crucial, especially in neurodegenerative studies where lower weight often indicates atrophy.

Methods: We retrospectively analyzed 742 adult autopsies performed between January 1, 2013 to December 31, 2023 at a county and private hospital. Brain weight, age, sex, race/ethnicity, and PMI were analyzed. We compared groups (t-tests, chi-square) and evaluated associations (linear, ordinal logistic, multiple linear regressions) adjusting for covariates (Stata/SE 18.0).

Results: Demographics differed between institutions, but overall brain weights were similar (private hospital: 1315.02 ± 148.71 grams; county hospital: 1313.07 ± 165.19 grams, $p = 0.892$). PMI ranged from a few hours to two months, with the county hospital having a significantly longer average PMI (≥ 28 days: 42.3%, $p = < 0.0001$) compared to the private hospital (PMI > 3 - 7 days: 57.1%, $p = < 0.0001$). While brains with longer PMIs (>3 - 7 and >14 - 21 days) were slightly heavier than those with PMI ≤1 day, the differences were insignificant (adjusted β: 27.07, 95% CI: -32.55 to 86.69, $p = 0.373$; and β: 2.95, 95% CI: -65.76 to 71.66, $p = 0.933$ respectively). PMI durations exceeding one day showed no significant association with brain weight changes (all $p > 0.05$). Importantly, after adjusting for confounders (sex, race/ethnicity, institution), there was not sufficient evidence to show a significant association between PMI duration and brain weight changes.

Conclusions: Despite anticipated tissue deterioration, our study found no significant association between PMI and brain weight, even after adjusting for potential confounders. These findings suggest that, within study limitations, PMI may not significantly influence brain weight measurements in hospital autopsies, potentially supporting the use of postmortem brain weight data for further research.

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DNA methylation and copy number analysis of breast carcinoma metastases to brain identifies discrete molecular clusters

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Background: Stage 4 breast cancer metastasizes to the brain 10-15% of the time, and is associated with resistance to systemic therapies and poor outcome. We aimed to analyze the epigenetic landscape of breast cancer brain metastases.

Methods: We analyzed 22 metastatic brain tumor samples from 21 patients. Clinicopathologic data, hormone receptor (HR) status, and HER2 status were retrieved from chart review and previous pathology reports. DNA methylation was profiled using Illumina EPIC array, and analyzed by unsupervised clustering using top 1000 most variable probes, tSNE. Copy number (CN) profiles were visualized using conumee package followed by visual inspection. CN profiles were scored based on number of chromosomes altered and presence/absence of chromotripsis.

Results: There were 21 females, 1 male. The average age at diagnosis of metastatic disease was 59.3 years. 11 patients were white, 4 black, 3 asian, and 4 lacked ethnicity information. There were 13 ductal adenocarcinomas, 1 lobular adenocarcinoma, and 8 of unknown type. Out of 19 cases with complete IHC, 11 were HR+, 1 was Her2+, three were triple negative, and 4 cases co-expressed HR and Her2. DNA Methylation classified metastases into 3 clusters, which were independent on clinical or IHC variables. However, only methylation Cluster 1 included tumors with simple karyotypes and 83% of cases showed no evidence of chromotripsis, while Cluster 3 was largely composed of cases with highly complex karyotypes and 70% of cases showed chromotripsis. Tumors in Cluster 2 had complex karyotypes, and 50% showed evidence of chromotripsis.

Conclusions: Our data suggest that DNA methylation analysis can subclassify breast cancer brain metastasis into distinct subgroups that are independent of current biomarkers. Molecular classification can be used for future therapeutic stratification and management.

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Comparative Analysis of Digital Pathology Software for Phospho-Tau Quantification

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Background: Digital pathology enables precise and unbiased quantification to be performed. Non-neoplastic brain tissue presents challenges for digital image analysis software (DIAS) that are typically optimized for cancer samples. We compared leading DIAS to assess their accuracy in quantifying phosphorylated-Tau pathologies (tangles, neuritic plaques [NPs]) in postmortem brains (n=37) with known CERAD NP scores and Braak tangle stages in hippocampus and middle frontal gyrus (MFG).

Methods: Slides were immunohistochemically stained with AT8, digitized using the Aperio Scan Scope XT at 20x, and analyzed with QuPath and HALO IndicaLabs software. Optical density (OD) and AT8 tissue percent-positivity were measured, stratifying by brain region and Braak stage: 0-2 (G1), 3-4 (G2), and 5-6 (G3). Analyses included t-tests, Spearman's correlation, and ANOVA ($P< 0.05$).

Results: No differences were observed between QuPath and HALO AT8 percent-positivity across Braak stages in either region ($P>0.05$). Hippocampal OD differences were observed between QuPath and HALO only in G1 ($P=0.002$) and G2 ($P=0.0002$). MFG OD measures differed between QuPath and HALO across Braak stages (all $P< 0.05$). Intergroup comparisons showed differences in hippocampal AT8 percent-positivity across Braak stages for QuPath and HALO (all, $P< 0.001$). Hippocampal OD differed across Braak stages when using QuPath ($P=2.2\times10\text{-}4$), whereas no OD difference was observed with HALO ($P=0.09$). Similarly, differences were observed in MFG AT8 percent-positivity across Braak stages using QuPath and HALO, in addition to QuPath's OD measures (all $P< 0.001$). However, no differences were identified across Braak Stages with HALO MFG OD measures ($P=0.18$). Highly positive correlations were found between AT8 percent-positivity and semi-quantitative CERAD NP scores with QuPath and HALO for both regions (all, $P< 0.001$).

Conclusions: QuPath and HALO exhibited strong correlations with CERAD NP scores, supporting their utility in accurately assessing Tau pathologies. Interestingly, QuPath was more

effective than HALO at differentiating between Braak stages by OD measures in both regions.

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Protecting Pathologists from Negligence while Working with Artificial Intelligence

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Background: As Artificial Intelligence (AI) making advancements in supporting histopathological analyses, there is a growing concern regarding the consequences of pathologists' negligence while working with AI. This negligence may involve accepting erroneous AI recommendations or overlooking correct ones. Therefore, it is crucial to investigate whether pathologists appropriately utilize AI and find effective approaches to reduce negligence incidents to avoid adverse outcomes.

Methods: We conducted a user study involving 22 pathology participants, which included eleven neuropathologists/neuropathology fellows, six pathologists, and five senior resident trainees. Participants were required to label mitoses in 48 high-power-field images selected from H&E meningothelial meningiomas. They detected mitoses manually, first, and with AI assistance after a wash-out period. A combinatorics (the mathematics of counting and arranging) table was created to track and categorize participants' negligence incidents. Additionally, we compared their decisions with majority-voting results, which were synthesized from a randomly selected group of three participants. Precision, recall, and F1 scores were computed for participants' and majority-voting decisions.

Results: On average, participants made 15.32 negligence incidents (SD=1.21, CI95= [13.00, 17.82]) while working with AI. The average precision, recall, and F1 scores of the 22 participants were 0.811, 0.824, and 0.817, respectively. Majority-voting decisions from three participants reduced negligence incidents to 5.55 (SD=0.20, CI95= [5.15, 5.94]). The average precision, recall, and F1 scores of majority voting results were 0.901, 0.843, and 0.871.

Conclusions: AI can be a powerful tool to assist histopathological examination. However, ensuring the proper use of AI is critical for achieving safe and reliable outcomes. The supervision of AI-assisted diagnoses emerges as a vital quality control measure. Majority voting, which combines the expertise of pathologists with diverse backgrounds, holds the potential to achieve precise diagnoses. Therefore, incorporating second or even third opinions should be considered to reduce "AI negligence" incidents and enhance overall performance.

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Histopathological features of Dyke-Davidoff-Masson syndrome: three surgically resected cases

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Background: Dyke-Davidoff-Masson syndrome (DDMS) is a neurological condition consists of diffuse unilateral cerebral atrophy-hypoplasia accompanied by unique bony changes. It is thought to occur as a result of intrauterine or peri/postnatal ischemia due to intrauterine stroke, vascular malformation, complicated delivery, and infections such as Rasmussen encephalitis. DDMS has a strong association with epilepsy. Despite the widely acknowledged association of seizure foci with the atrophic hemisphere, histological correlation has not been well established, especially within the context of updated classification of epileptogenic abnormalities suggested by International League Against Epilepsy (ILAE).

Methods: We describe histopathological findings of three cases of mesial temporal lobe epilepsy with radiologic features of DDMS which underwent temporal lobectomy based on the result of stereotactic electroencephalogram (sEEG).

Results: We encountered three cases of DDMS clinically presented with mesial temporal lobe epilepsy; 60-year-old female with left cerebral hemiatrophy, 59-year-old female with right cerebral hemiatrophy, 33 year-old female with right cerebral hemiatrophy all of whom underwent temporal lobectomy for their treatment-resistant epilepsy at our institution. Histological examination of these three cases shared some common features such as: moderate Chaslin gliosis and scattered disorganized neurons in the resected temporal lobe and amygdala, hippocampal neuronal loss, and abundant corpora amylacea in subpial region of the temporal lobe, amygdala, and hippocampus.

Conclusions: Taking into consideration our current understanding of the pathogenesis of DDMS, the histological findings in the presented cases are most likely secondary to perinatal hypoxic-ischemic injury, which is a precursor to DDMS development. The increased number of corpora amylacea, suggests the association of the injury and dysfunction of the glymphatic system, which may contribute to the difficult-to-treat seizures in these patients. This case series increases our understanding of the histopathologic features of DDMS related epilepsy.

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Precision diagnostics in CNS tumors: Clinical impact of the JAX OncoMethyl

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Background: In accordance with WHO guidelines, the diagnosis and management of central nervous system (CNS) tumors are undergoing a paradigm shift to incorporate molecular data. To address this, our group validated the JAX OncoMethyl assay, incorporating the classifier developed by DKFZ with the Illumina EPIC methylation array to classify tumors, determine MGMT promoter methylation status, and identify copy number alterations (CNAs).

Methods: We assessed 145 clinical samples using the JAX OncoMethyl assay. In addition, subsets of samples were also analyzed for MGMT promoter methylation using high-resolution melt (HRM) analysis (N=40), and/or JAX SOMASEQ, a next-generation sequencing panel (N=42). The pathologist's diagnosis and that obtained from the classifier were reviewed by two neuro-oncologists to determine the clinical impact of the result. Concordance between assays was assessed.

Results: JAX OncoMethyl and MGMT HRM analysis were concordant for 29 samples (72.5%). CNA analysis between JAX OncoMethyl and JAX SOMASEQ demonstrated consistent results. Of the 145 JAX OncoMethyl samples, 22 (15.2%) did not pass the clinical reporting thresholds and were reported as "no match". Notably, 52 of the remaining 123 samples (42.3%) yielded diagnoses that would impact clinical approach or alter treatment regimen relative to pathology results alone. In cases where brain-derived specimens did not reveal CNS classification, the assay's "no match" result provided invaluable diagnostic clarity, directing clinicians towards potential alternative diagnoses.

Conclusions: The JAX OncoMethyl provides CNS tumor classifications, MGMT promoter methylation status, and CNA information to aid in the diagnosis of CNS tumors. Discrepancies between OncoMethyl and HRM approaches to MGMT are likely due to different numbers of MGMT markers assessed. The CNA calls for OncoMethyl and SOMASEQ assays are consistent and yield crucial diagnostic information. Most importantly, the JAX OncoMethyl assay significantly influenced therapeutic decisions and clinical approach. In summary, the JAX OncoMethyl and other molecular tests will improve clinical management in CNS tumors.

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Neuropathology Outreach in Ethiopia

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Background: Ethiopia has a population of 123 million people and an average doctor-to-patient ratio of 1:10,000. In the last two decades, the neurosurgeon density has grown more than 20-fold, become self-sustaining and is now present in 12 urban centers. Yet, there are currently no pathology fellowships offered within the country and there is no dedicated neuropathologist. Here we present a two-week short course format of neuropathology outreach performed at the St. Paul Hospital Millennium Medical College (SPHMMC) in Addis Ababa.

Methods: In February of 2024, two board-certified neuropathologists traveled to Ethiopia to give two-weeks of daily neuropathology teaching sessions. This outreach consisted of a total of 20 approximately 1.5-hour didactic lectures and interactive sessions covering the WHO 2021 brain tumor classification system, neuroautopsy, neuroinfectious disease, neurodegenerative disease, neuromuscular disease, neurodevelopment, and unknown case conferences. A survey of 14 questions including 12 multiple choice questions and two short answer questions was used to assess each component and the overall satisfaction of participants.

Results: Four brains were examined at two neuroautopsy conferences and five consultations on current cases were performed upon request. Survey results showed statistically significant increased appreciation of the neuroautopsy and unknown case conferences. Comments were positive and focused on the dedicated time, access to experienced diagnosticians and importance of molecular updates. Suggestions for improvement included adding more morphology and immunohistochemistry in lectures as well as increasing the number of slide sessions. Participants also desired forensic neuropathology sessions and identified the need for continuing in-person outreach in neuropathology.

Conclusions: This experience provides a useful and well-appreciated approach to delivering international neuropathology outreach. Inclusion of the WHO 2021 brain tumor classification system and new/emerging tumor updates as well as a broad neuropathology curriculum were well-received. Ongoing opportunities include periodic short-term in-person outreach and consultation on challenging cases.

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An unusual cause of Wernicke's Encephalopathy – Case Report and Review of Literature

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Background: Wernicke's encephalopathy is classically associated with chronic alcohol use. Rare non-ethanol related causes have been described including hyperemesis gravidarum, gastric malignancy, AIDS, gastric bypass, or prolonged periods of emesis.

Methods: We herein report a case of a young male with findings of Wernicke's encephalopathy at autopsy.

Results: A 25-year-old male presented one month prior to death with progressive fatigue, lethargy, lower extremity weakness, and episodes of confusion and altered mental status. In the month prior to he had experienced protracted nausea, recurrent vomiting, and somnolence. He was admitted with hypotension, tachycardia, leukocytosis, anion gap metabolic acidosis, and hypokalemia. Investigations for viral encephalitis, acute intermittent porphyria, and autoimmune/rheumatologic illnesses were all negative. His admission was complicated by his elevated BMI (50kg/m2) limiting neuroimaging, pulseless electrical activity arrests, ventilator acquired pneumonia, bilateral lower extremity deep venous thromboses, pulmonary emboli, new right caudate hemorrhagic infarct, new onset atrial fibrillation, and acute respiratory failure. Following thrombectomy for saddle pulmonary embolism, the patient experience progressive ventilatory support, shock, and cardiac arrest without resuscitation. At autopsy no significant gross pathology was identified on examination of visceral organs. Gross examination of the brain revealed only a 1.0 cm hemorrhage of the right caudate nucleus. Microscopically, subacute lesions consisting of endothelial hypertrophy, neuropil destruction, variable/incomplete neuronal loss, and abundant macrophages were identified in the mammillary bodies, medial thalamus (periventricular), and periaqueductal gray matter. These lesions were notable for a rim of subependymal sparing. These findings were favored to be manifestations of Wernicke's Encephalopathy.

Conclusions: Wernicke's Encephalopathy is a neurologic manifestations of Thiamine/Vitamin B1 deficiency, the other being Beriberi. Though classically associated with alcohol use disorder, any cause resulting in impaired gastrointestinal absorption may result in similar changes. The cause of death in this individual was ruled sequelae of acute gastroenteritis, noting the unusual presentation for Wernicke's Encephalopathy.

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Pellagra in a Forensic Setting: Not Simply a Historical Disease

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Background: Pellagra is a condition caused by the deficiency of niacin (vitamin B3), which serves as a crucial precursor for coenzymes involved with cellular metabolism, synthesis of DNA, and energy metabolism. In the early 20th century, pellagra emerged as a public health crisis in the Southern United States, where economic challenges led to stagnant and inexpensive diets comprised of niacin-deficient foods such as corn. The classic manifestations include cutaneous (photosensitive dermatitis), gastrointestinal (diarrhea), and neurological/psychiatric symptoms (dementia), and if left untreated, may result in death ("the four Ds"). Fortification of foods with niacin consequently reduced the incidence of pellagra in the United States, although it continues to be endemic in regions that lack access to good nutrition or have maize and jowar as dietary staples. The aim of this study is to review the diagnosis of pellagra in a medical examiner setting and assess decedent characteristics.

Methods: We performed a retrospective database search from 1/2020 through 1/2024 for cases we diagnosed with pellagra, and analyzed the clinical manifestations, co-morbidities, and relationship to death in a large metropolitan medical examiner office.

Results: We identified 5 cases (ages 36-82, mean 65.4 years; 2 male). Comorbidities included dementia (40%), marked malnutrition (40%), and alcohol use disorder (20%). The most common neuropathologic finding included histologic evidence of ballooned neurons in the brainstem. Classic cutaneous and gastrointestinal manifestations were not established ante- or postmortem. Most cases (60%) had complications of malnutrition or dementia as a cause of death and most manners were natural.

Conclusions: Despite advances in nutritional supplementation, pellagra is not simply a disease of historical significance and should be a differential for at-risk populations. Medical examiners and neuropathologists working in a medical examiner setting should consider the diagnosis of pellagra especially in vulnerable persons with severe malnutrition, alcohol use, or uncharacterized dementia.

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Autopsy Findings of L1 Syndrome: A Rare Syndrome Causing Hydrocephalus and Agenesis of the Corpus Callosum

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Background: L1 syndrome is a syndrome that results from mutations in the L1CAM gene located at Xq28. It has a wide clinical spectrum and may manifest as: 1) X-linked hydrocephalus with stenosis of the aqueduct of Sylvius (HSAS) 2) Mental retardation (intellectual disability), Aphasia (delayed speech), spastic paraplegia (shuffling gait), and adducted thumb syndrome including X-linked complicated hereditary spastic paraplegia type 1 and 3) X-linked complicated corpus callosum (CC) agenesis.

Methods: We present autopsy findings of a 20-month-old male with a clinical history of bronchopulmonary dysplasia, congenital hydrocephalus, laryngomalacia, tracheomalacia, and severe obstructive sleep apnea. Molecular testing detected L1CAM mutation and alpha thalassemia. The patient expired succumbing to viral infection and concomitant acute bacterial pneumonia superimposed on chronic lung disease and upper airway obstruction and respiratory failure.

Results: Autopsy findings included findings consistent with bronchiolitis with superimposed acute bacterial pneumonia, multiorgan acute hemorrhages, trilobar left lung, and no dysmorphic features (including thumb abnormalities). Autopsy brain findings included a low normal brain weight, posterior parietal and occipital polymicrogyria, absence of corpus callosum, and marked hydrocephalus. Coronal sections showed marked dilatation of the lateral ventricles with dysmorphic subcortical grey and white matter. Postmortem viral and bacterial cultures of lung and liver tissue were negative. Brain findings included marked agenesis of the CC, polymicrogyria, and marked ventricular dilatation. Microscopic examination of representative sections showed marked attenuation of the deep white matter tracts and agenesis of the CC. Brainstem examination revealed abnormal configuration of the pons. No stenosis of the aqueduct of Sylvius was seen.

Conclusions: L1 syndrome is very rare with a wide spectrum of manifestations and can have severe neurological abnormalities. Genetic testing for L1CAM can help confirm the diagnosis. Further studies are needed to better understand the pathophysiology of this entity.

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Helianthus annuus: A Forensic Neuropathologic Description of Histologic Findings in a Case of 'Sunflower Syndrome'

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Background: We report on the histopathologic findings of the neuropathologic autopsy of the rare 'sunflower syndrome'. The history involves a 22 female with a known clinical history of sunflower syndrome who tragically passed in an accident.

Methods: On neuropathologic autopsy, the brain of the decedent showed edema (brain weight 1600 grams) but otherwise no gross anatomic architectural pathology. Histologic examination of the primary visual cortex showed evidence of neuronal delamination with foci of large dysmorphic neurons in cortical layers 3 and 4 consistent with a focal cortical dysplasia (FCD) type IIA pattern.

Results: This morphologic finding was further supported by Neu, confirming, and further highlighting dyslamination, and Neurofilament stain showing abnormal cytoplasmic accumulation of NF in a subset of neurons and dysmorphic neurons.

Conclusions: FCD is a known cause of seizure, though traditionally found in other regions of the CNS. As the epilepsy of sunflower syndrome is triggered by sunlight and the finding of FCD is associated with seizure, we conclude its presence in the visual cortex adequately explains this case of seizure syndrome. There is essentially no neuropathologic description characterizing Sunflower Syndrome in the literature. More studies are required to interrogate if FCD is a pathognomonic finding for sunflower syndrome or if there is a range of cytoarchitectonic and physiologic disturbances to explain this rare form of epilepsy.

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Image Analysis Workflow for Analysis of Cortical and White Matter Cellularity and Cellular Composition Using QuPath

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Background: The ever-expanding advancements in digital pathology facilitate the analysis of scanned slide images of tissue, offering a myriad of opportunities for precise, quantitative, and comprehensive investigation. We explore the application of digital pathology techniques, utilizing the tools QuPath and StarDist, to quantify CNS cell counts, cell density, and cell types in the cortex and subcortical white matter of autopsy brain tissue.

Methods: We employed high-resolution scanning of the H&E-stained slides from 29 samples, encompassing 29 frontal border zone regions and, capturing cytoarchitectonics. QuPath, a digital pathology analytic software, was utilized for image processing, annotation, and segmentation. The areas corresponding to the cortex (GM) and subcortical white matter (WM) were delineated using pixel classification and a degree of manual adjustment.

Results: Subsequently, StarDist, a deep-learning-based tool for object detection, was employed for cell segmentation (>= 75 Area µm^2) within the delineated areas. The combination enabled precise identification and separation of individual cells; rapidly overcoming challenges posed by tissue heterogeneity. Quantitative analysis was performed to evaluate the cellularity within the GM (overall density: range 6.8-15.4) and neuron density, (i.e., range: 0.2-1.9, ratio range: 2-24%). WM analysis (i.e., cell density range: 9.99-19.18) was performed utilizing cell counts and cellular area. Cell counts and cell density metrics were computed, providing valuable insights into the cellular composition and distribution patterns (i.e., Neurons to glial cells range: 1-12%, overall ratio GM v WM range: 49-94%).

Conclusions: The findings of this project highlight the utility of digital pathology technologies in elucidating the complex cytoarchitectonics of brain tissue. Using technologies like whole slide scanning, QuPath, and StarDist researchers can quickly and effectively quantify cell populations, assess cellular densities, and characterize cell types in areas of interest. Integration and use of such technologies for qualitative and quantitative analyses hold promise for expediting and advancing scientific research and clinical applications.

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How long are cellular structures maintained in fluid preserved brains?

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Background: Fluid preservation is widely used in brain banking to store fixed tissue specimens for future research studies. However, the effects of long-term immersion on neural circuitry and biomolecules are not well characterized. There is a critical need to synthesize existing data on fluid preservation of brain tissue and build up additional data to fill in gaps in the literature.

Methods: We searched PubMed and other databases to identify studies measuring the effects of long-term fluid preservation on nervous system tissue. We categorized 91 studies based on the fluid preservative used: formaldehyde solutions, buffer solutions, alcohol solutions, storage after tissue clearing, and cryoprotectant solutions. To corroborate our literature findings, we also present histological data on brain tissues stored in phosphate buffer and 0.1% sodium azide.

Results: When present, the most frequent alteration reported was decreased antigenicity, most commonly during storage in solutions containing formaldehyde, which may be due to prolonged crosslinking and other chemical alterations in biomolecules over time. Other studies reported different types of artifacts, such as local areas of tissue rarefaction, “myelin-like” whorls, nuclei degradation, and a general decrease in structural preservation quality. These artifacts were each reported only in up to a few studies each and may be associated with the preservative used and other storage conditions. Our empirical results suggest that alterations over long periods of storage can affect tissue staining properties.

Conclusions: Further research is needed to optimize fluid preservation protocols and identify the visualization methods that are most compatible with long-term fluid preserved tissue.

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Brain Bank for Aging Research for the furute of neuroscience

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Background: In 1999, Brain Bank (BB) for Aging Research (BBAR) was established in Tokyo Metropolitan Institute of Gerontology and Geriatrics (TMIGG), the first institute dedicated for aging population in Japan. In 2012, BBAR recruited the members of the Japanese Society of Neuropathology (JSNP), with the consensus of longitudinal clinical studies, brain donation system, open resource, and quality control of neuropathological findings, and established the Japanese Brain Bank Network for Neuroscience Research (JBBNNR). In 2020, BB for Neurodevelopmental, Neurological and Psychiatric Disorders (BBNNPD, Osaka University) was established for the registry of cases from forensic autopsy.

Methods: The BBAR project comprised longitudinal clinical studies of clinical findings, neuroimages and biomarkers, in addition to the coordination to brain donation and final recovery of postmortem brains, spinal cords, peripheral nerves, skeletal muscles and general organs. JBBNNR is based on full autopsy providing neuropathological diagnosis by JSNP certified neuropathologists. Neuropathological examination follows the Japanese Prion Surveillance System, which includes routine histology, immunohistochemistry, Western blotting, and genomic analysis as well as EM. BBNNP starts registry of suicide victims and accidental death cases of developmental disorders. In 2020, JSNP stared board certification test for young neuropathologists, whose main aim is to inherit the Japanese brain bank network system to the next generation.

Results: BBAR recovered 1,100 resources and recieved Moore Award in 2008. JBBNNR now consists of BBAR, BBNNPD and BBs of NCNP, Mihara Memorial Hospital and Fukushimura Hospital. BBNNPD has to overcome cultural and religious barrier. JSNP now welcomed 13 board certified young neuropathologists. Biobank Japan (BBJ) is one of the major biobanks which started in 2003, including 199,998 patients with 340,298 DNA and serum, whose 86 participants were registered to BBAR. In 2023, we started comprehensive genomic studies of these patients

Conclusions: BBAR will continue to make every effort to keep brain bank system for the future of neuroscience.

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Brain Death: Some Additional Neuropathological Findings

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Background: In 1982 some of the members of our group described the pathological findings in a man who had been clinically brain dead for 68 days. What we found was ischemic necrosis of the entire brain and necrosis of the cervical spinal cord except at the lowermost levels, where there was relative sparing surrounding the anterior median fissure.

Methods: We have examined the brains and spinal cords in many cases of brain death, to explain these spinal cord findings and to find a simple and effective way of confirming the clinical diagnosis of brain death in cases of much shorter duration.

Results: We found sparing of the ventromedial spinal cord at lower levels (T4 or lower) in all cases of brain death of more than 2-3 days' duration. This area is the portion of the spinal venous system that drains into the anterior median spinal vein (Gillilan, 1970). Below this level were circumferential, radially oriented, perivenous hemorrhages, signifying impaired leptomeningeal venous drainage. Additionally, we observed that the proximal portion of the optic nerve (adjoining the brain stem) is infarcted, whereas the distal portion (attached to the globe) is spared. Also, the pituitary gland, in our experience, always shows acute focal ischemic necrosis.

Conclusions: Brain death represents total brain infarction. In a prolonged brain dead state the infarcted brain will undergo revascularization from the extracranial scalp vessels. Spinal cord necrosis is due to cerebellar tonsillar herniation, leading to venous infarction since the spinal leptomeningeal veins normally drain upward into the cranial cavity (Di Chiro and Doppman, 1970). Finally, we determined that the simplest way to confirm the clinical diagnosis of brain death is to demonstrate focal ischemic necrosis within the pituitary gland.

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Manual and deep learning analysis of 3 choroid plexus pathologies show increases with age, but not Alzheimer's disease

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Background: The choroid plexus (ChP) plays an important role in brain homeostasis and its dysfunction may have a role in brain aging and diseases such as Alzheimer's disease (AD). However, many aging and disease-related changes within the ChP are understudied. In this study, we used both manual and deep-learning (DL) AI approaches to characterize three age and disease-related ChP pathologies: large lipid droplets, fibrosis, and a ChP-unique amyloid referred to as Biondi bodies.

Methods: We quantified the prevalence and spatial distribution of these pathologies using H&E and Thioflavin S-stained sections of ChP autopsy tissue from over 100 individuals across lifespan. We developed a rigorous manual counting methodology to measure the prevalence of each pathology and data from this analysis was used to train DL models based on ResNet, RetinaNet, and UNet architectures.

Results: Manual quantification found that all three pathologies significantly increase with age and can appear quite early in life, but no association was found with AD. Additionally, manual spatial analysis of three individuals revealed that these pathologies can spatially cluster within the ChP. DL models trained from our manual analysis performed well compared to human annotators and recapitulated the findings from the manual study. The models were then used to expand our spatial analysis to all individuals in our study. The models revealed significant clustering of all pathologies in almost all individuals studied and showed an inverse relationship between fibrosis and the other two pathologies.

Conclusions: Our results show DL models can be a valuable tool for automating data collection and reveal that many ChP pathologies can appear relatively early in life and that different pathologies impact each other.

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Target-enriched enzymatic methylation sequencing for classification and copy number profiling of clinical brain tumor specimens

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Background: DNA-methylation profiling using Illumina Infinium BeadChips performs well on both fresh-frozen (FF) and formalin-fixed, paraffin-embedded (FFPE) tissues and has substantially enhanced tumor diagnosis and classification. However, reliance on BeadChip arrays necessitates specific instrumentation which may not be available in many molecular pathology laboratories, limiting wider adoption of methylation profiling. To address this issue, we evaluated Target-enriched enzymatic methyl-seq (TEEM-seq) using the Twist Human Methylome capture panel as a sequencing-based alternative capable of being performed using equipment available in most molecular pathology laboratories.

Methods: In this pilot study, we used DNA from FF and FFPE tissues collected from 20 known diagnostic brain tumors samples and 1 control with matched DNA methylation array data with technical replicates (n=50). Methylation detection from DNA libraries constructed for EM-seq was targeted to 3.98M CpG sites using the Twist Human Methylome Panel. We developed and validated a bioinformatics pipeline, including a custom classification model, to process and classify brain tumors using TEEM-seq data. The clinical utility for tumor classification and copy number profiling on clinical samples were assessed.

Results: The coverage depth ranged from 66 to 346x with at least 45x mean per-base coverage. Our pilot study showed a high correlation in methylation detection between replicate samples (pairwise correlation coefficients > 0.98) and a highly accurate classification of tumor samples into their correct molecular classes. The approach effectively identified copy number abnormalities, aligning with array-based methods.

Conclusions: TEEM-seq using the Twist Human Methylome Panel and our bioinformatic pipeline proved effective for DNA methylation profiling from FFPE and FF samples, showing compatibility for clinical diagnostics. This method could lower the barriers to adopting DNA methylation profiling in clinical labs, offering a viable alternative to current array-based methods.

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Learned resizing with efficient training (LRET) improves the performance of large-scale digital histopathology image classification models

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Background: Histologic examination plays a crucial role in oncology research and diagnostics. The adoption of digital scanning of whole slide images (WSI) has created an opportunity to leverage deep learning-based classification methods to enhance diagnosis and risk stratification. Due to the massive size, each WSI must be partitioned into smaller patches prior to training or applying deep learning models. The input dimensions of Deep Convolutional Neural Network (DCNN) models are small compared to the typical pathologist field of view, degrading performance by excluding important architectural features.

Methods: We introduce a novel approach that addresses the main limitations of traditional histopathology classification model training. Our method, termed Learned Resizing with Efficient Training (LRET), couples efficient training techniques with image resizing to facilitate seamless integration of larger histology image patches into state-of-the-art classification models while preserving important structural information. We used the LRET method coupled with two distinct resizing techniques to train three diverse histology image datasets, using multiple diverse DCNN architectures. The model performances were compared using the metrics of accuracy, precision, recall, and F1-score.

Results: We demonstrate that the LRET method is compatible to diverse input patch sizes and DCNN architectures. Our findings demonstrate a substantial enhancement in classification performance and training efficiency. Across the spectrum of experiments, LRET consistently outperforms existing methods, yielding improvement of 15-28% in accuracy for a large scale, multiclass brain tumor classification task consisting of 74 distinct brain tumor types. LRET not only elevates classification accuracy but also substantially reduces training times, unlocking the potential for faster model development and iteration.

Conclusions: We have developed LRET methods to facilitate efficient training of a large DCNN model with improvements of up to 28% in accuracy in the largest WSI dataset. The method makes future development of large comprehensive brain tumor classification models from histology images feasible.

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Established in 1902: a brief history of the Institute of Neurology (Edinger Institute) in Frankfurt, Germany

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Background: The Institute of Neurology was established by the Jewish neurologist Ludwig Edinger on the premises of the Dr. Senckenberg Institute of Pathology in Frankfurt. Edinger was considered to be the leader in comparative neuroscience of its time by the Spanish Nobel prize winner Ramon y Cajal. Edinger was among the founders of the “Königliche Universität zu Frankfurt“ (later renamed as Goethe University) in 1914 and was appointed as the first Professor of Neurology in Germany. In 1919, the Ludwig Edinger-Foundation was established.

Methods: During the “Third Reich”, Jewish scientists, including Edinger’s daughter Tilly and Edinger´s successor, Kurt Goldstein, were forced to leave the Institute. The next director, Ernst Scharrer, together with his wife Berta, although gentile, left Germany in 1937 and emigrated to the USA. As a consequence, scientific activities in the Institute of Neurology ceased almost completely.

Results: After WWII, the new director Wilhelm Krücke was among the founders of the German Society of Neuropathology in 1950. In 1962, the Max-Planck Institute for Brain Research, successor of the Kaiser-Wilhelm Institute for Brain Research was established. As a consequence of the merger of these institutions, specimens of victims of the Nazi-regime were transferred to Frankfurt. The provenience of these specimens was only detected by the meticulous work of historians. In 1990, all specimens were buried in a ceremony hosted by the Max-Planck-Society.

Conclusions: 2002 marked the 100th anniversary of the Edinger Institute. Several members of the Edinger family returned to Germany for the first time after WWII on this occasion. In 2024, 122 years after its establishment, the Institute of Neurology continues to serve as the department of neuropathology, hosting three professors and six research groups, consisting of neuropathologists, scientists and research staff to provide a unique, lively environment for translational neuropathology, coming close to how it was initially intended by Ludwig Edinger.

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First report of neuropathology of Mitchell syndrome (ACOX1 N237S)

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Background: Pathogenic variants in ACOX1 represent a rare subtype of peroxisomal disorder. Most ACOX1 pathogenic variants show autosomal recessive inheritance and result in loss of enzymatic activity of acyl-CoA oxidase leading to accumulation of very long chain fatty acids (VLCFAs) and leukodystrophy. In contrast, ACOX1 N237S, the variant implicated in Mitchell syndrome, is thought to result in an autosomal dominant gain-of-function, also leading to leukodystrophy. While this variant has been reported in 25 patients worldwide, the neuropathologic features remain unpublished.

Methods: Here we report a case of a patient with a history of progressive myeloneuropathy secondary to de novo heterozygous ACOX1 N237S variant. He initially presented at age 11 with neurotrophic ocular keratopathy, clumsiness, sensory ataxia, and hyporeflexia. Imaging showed a longitudinally extensive lesion preferentially involving the dorsal columns with eventual progression to involve the anterolateral cord. At age 19, he developed neuromuscular respiratory failure and encephalopathy. Brain MRI showed new, diffuse, bilateral white matter FLAIR hyperintensities in the cerebrum consistent with disease progression. The patient passed away soon after elective extubation to comfort care, and underwent complete autopsy.

Results: Histologic assessment demonstrated extensive white matter pathology affecting large areas of the spinal cord and portions of the cerebral cortex. Within the spinal cord, there was severe injury to the dorsal columns with near total axon loss. There was significant neuronal loss and atrophy within the posterior horn of the spinal cord and in the dorsal root ganglia. The anterior and lateral corticospinal tracts showed active tract degeneration as evidenced by prominent macrophage infiltrate with phagocytosis of myelin contents. Within the cerebral cortex there was demyelination and axonal damage in the subcortical white matter of the occipital lobe and cingulate gyrus with sparing of the U-fibers.

Conclusions: Histologically, Mitchell syndrome demonstrates similar neuropathology to other ACOX1 variants, despite the distinct mechanism.

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Subgemmal Neurogenous Plaque: Pitfalls in Normal Neural Anatomy

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Background: The subgemmal neurogenous plaque (SNP) is a normal structure found in the foliate, fungiform, and circumvallate papillae of the human tongue. It is a subepithelial neural plexus associated with a taste bud, which is normally composed of nonmyelinated nerve fibers communicating with myelinated fibers in the papillae connective tissue core. SNPs encountered on biopsy can be misinterpreted as a neural lesion, leading to diagnostic difficulties if this normal neural structure is not recognized.

Methods: We present three cases received as external consults in the last 4 years of SNPs thought to represent neural lesions.

Results: All patients were adults with no syndromic diagnosis and, of the two patients with available clinical information, both presented with throat pain and were noted to have tongue “lesions” that were biopsied. In two cases the outside pathology was previously signed out as neurofibroma and in one case even resulted in genetic testing for Neurofibromatosis type 1, which was negative. The third case was sent in consultation with a question of neuroma. In all three cases, the biopsy showed a taste bud with a prominent subgemmal neurogenous plaque. The subepithelial nerve plexus shows a classic biphasic pattern: a superficial layer displayed a fibrillar neurofibroma-like pattern, while the deep zone is composed of organized neuronal bundles intermingled with ganglion cells. However, the superficial biopsies in this series generally lacked the deeper organized bundles, simulating a neural lesion on histology. S100 and SOX10, performed on one case each, highlighted the fibrillar neurofibroma-like pattern of the nerve plexus.

Conclusions: SNPs are a normal structure associated with taste buds which, when hyperplastic, can rarely be sampled as a “lesion”. Recognition by pathologists as a normal anatomical structure is important to avoid misdiagnosis as a neural neoplasm.

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Bing-Neel Syndrome Confirmed at Autopsy: A Case Report

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Background: Bing-Neel Syndrome (BNS) represents a rare neurological complication of lymphoplasmacytic lymphoma (LPL), marked by infiltration of malignant lymphoplasmacytic cells into the central nervous system. This syndrome is an uncommon manifestation of Waldenström's macroglobulinemia (WM), a B-cell lymphoma that is consistent with LPL, involves the bone marrow, and produces a monoclonal IgM paraprotein. The incidence of BNS is less than 1% in those with WM. Less than 50 case reports contribute to current clinical understanding.

Methods: We present a novel case of a 77-year-old man with history of WM, who succumbed to BNS. The patient had a prolonged hospitalization for septic shock and encephalopathy secondary to COVID-19-related acute respiratory distress syndrome and ventilator-associated pneumonia. Although the patient improved with treatment for sepsis, his encephalopathy persisted, and he remained comatose. MRI demonstrated pachymeningeal enhancement in a pattern concerning for BNS. Premortem molecular testing of serum for the MYD88 L265P mutation was positive, a finding present in up to 90% of cases. Unfortunately, the patient expired before correlate MYD88 studies of the cerebrospinal fluid could be obtained. The patient’s next of kin requested and consented to a brain only autopsy to confirm the diagnosis and increase knowledge of BNS.

Results: Autopsy revealed diffuse atypical lymphoplasmacytic infiltration of the pachymeninges and focal involvement within the basal ganglia. Immunohistochemistry and in-situ hybridization studies confirmed WM infiltration, establishing the postmortem diagnosis of BNS.

Conclusions: This case underscores the elusive nature of BNS and the importance of considering it in patients with WM who present with neurological symptoms. Autopsy serves a significant and critical role in confirming the diagnosis when premortem work-up is incomplete. This case contributes to the limited pool of knowledge on BNS.

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Utilization of Multimodal Molecular Testing to Classify Metastases of Unknown Primaries – A Case of a Patient with no Known Medical History

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Background: Metastases are the most commonly diagnosed brain tumors in adults. In up to 15% of brain metastases, the source of primary malignancy remains unknown despite a thorough workup.

Methods: Here, we present a case of a 25-year-old male who presented with intracranial hemorrhage and a mass in the left occipital lobe. At the time, his past medical history was unknown to all care providers. He underwent emergent craniotomy for resection of the tumor. Multimodal advanced molecular diagnostic testing, including next-generation sequencing, chromosomal microarray analysis, and DNA methylation profiling using Northwestern Memorial Hospital's Cancer of Unknown Primary (CUP) classifier were performed to classify the neoplasm and identify the primary source for his metastatic tumor.

Results: Histopathologic sections showed brain parenchyma with acute hemorrhage intermixed with small fragments of a primitive neoplasm. The immuno-profile of the tumor was suggestive of germ cell differentiation. The tumor harbored multiple molecular and cytogenetic alterations including amplification of CCND2 and 12p, gain of chromosomes X, 1q, 7 and 8 and loss of chromosomes Y, 1p, 16 and 22. DNA methylation profiling did not match (i.e. score >0.9) with any tumor entity on the CUP classifier, however the tumor clustered closest to non-seminomatous testicular germ cell tumors on the t-distributed stochastic neighbor embedding (t-SNE) plot.

Conclusions: This case highlights the value of utilizing multimodality advanced molecular testing when facing a diagnostically challenging case. While none of the molecular tests performed was independently diagnostically conclusive, in concert, they pointed to a definitive diagnosis. Upon diagnosis, imaging revealed a surgically absent left testicle and multiple lung metastases. Patient confirmed diagnosis of a testicular cancer few years prior to his current presentation. He is undergoing systemic treatment for metastatic non-seminomatous testicular germ cell tumor.

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The Molecular Characterization Initiative: A clinical, pathologic and genomic resource for the international pediatric brain tumor community

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Background: The Molecular Characterization Initiative (MCI) launched in 2022 under the NCI Childhood Cancer Data Initiative (CCDI) in partnership with the Children's Oncology Group (COG). MCI represents a national strategy facilitating appropriate clinical and molecular characterization of childhood cancers while building an accessible data ecosystem. Since activation, 2400+ pediatric patients with CNS tumors have enrolled from 184 COG sites across the U.S. and globally. MCI provides free comprehensive genomic profiling for newly diagnosed CNS tumors, with results returned to physicians within 21 days. De-identified clinical and genomic data are deposited in the CCDI repository and may be accessed via the database of Genotypes and Phenotypes through a controlled data access request. Digital whole slide images corresponding to the extracted tissues are accessed through NCI Imaging Data Commons.

Methods: Patient enrollment occurs through the COG cancer registry Project:EveryChild, facilitating the collection of genomic and longitudinal clinical data. Centralized processing is performed in the CLIA-certified Biopathology Center (BPC) at Nationwide Children's Hospital. Molecular assays, including tumor/normal exome sequencing, methylation array, and targeted RNA fusion analysis, are performed at Nationwide Children's Hospital's Institute for Genomic Medicine.

Results: De-identified demographic, staging, therapeutic, and genomic data are included in the CCDI repository. At least 67 unique CNS tumor types are represented in the dataset (~2200 patients). Initial genomic analysis of germline samples revealed pathogenic mutations in 13.6% of participants, while somatic analyses revealed pathogenic variants in 46.9% of tumors and medically-informative CNV-LOH events in 75.8%. The most frequent diagnoses comprise 54% of total cases (medulloblastoma, 23%; pilocytic astrocytoma, 19.8%; posterior fossa ependymoma, 6.5%; and diffuse midline glioma, H3 K27-altered, 5%).

Conclusions: MCI encompasses a national comprehensive profiling strategy for pediatric CNS tumors and unprecedented resource of genomic and clinical data to improve diagnosis and support pediatric cancer research, especially for rare tumors for which large-scale collaborative studies are needed.

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Neurosarcoidosis with Non-Pulmonary Symptoms: Hydrocephalus and Vasculitis-Related Stroke as an Uncommon Presentation of a Common Disease

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Background: Sarcoidosis is a multisystem, granulomatous disease of unknown etiology. While pulmonary symptoms are present and predominate at the time of presentation in most patients with sarcoidosis, symptoms related to disease in other organ systems can be the primary presentation in some patients. We report a patient who presented with both hydrocephalus-related and stroke-like manifestations.

Methods: Clinical features were summarized from the patient's medical record. Literature searches were undertaken using relevant keywords.

Results: A 27-year-old man with an unremarkable past medical history presented with intermittent headaches with dizziness, ataxia, and blurred vision. Imaging of his head identified (1) communicating hydrocephalus, (2) subacute, bilateral cerebellar hemisphere infarcts, (3) chronic infarcts involving occipital lobes and basal ganglia, and (4) leukoencephalopathy. A thoracic CT scan identified enlarged mediastinal and axillary lymph nodes. Serum calcium was elevated. Serum and cerebrospinal fluid (CSF) angiotensin-converting enzyme (ACE) levels were normal. CSF evaluation was negative for meningitis/encephalitis and paraneoplastic encephalopathy by a screening panel. A frontal lobe brain biopsy identified multifocal, non-caseating, granulomatous inflammatory infiltrates with subarachnoid and parenchymal blood vessel wall damage consistent with vasculitis. Special stains for acid-fast bacilli and fungi were negative. Combined findings were consistent with sarcoidosis. A ventriculoperitoneal shunt was placed and immunosuppressive therapy was initiated.

Conclusions: Central nervous system manifestations as the initial presentation of sarcoidosis are uncommon, with neurosarcoid most commonly seen with concomitant systemic features. Cranial nerve symptoms are the most common CNS manifestation. Hydrocephalus, parenchymal vasculitis, and stroke are rare presenting manifestations. The diagnosis of neurosarcoidosis is made in the context of a thorough workup including CT scans of the chest, abdomen, and pelvis. Brain biopsy may be necessary given the low sensitivity of serum and CSF ACE levels and other tests.