GLAUCOMA BIOMARKERS… HIDING IN THE AQUEOUS?

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FINANCIAL DISCLOSURE

- I have no financial interests to disclose.

WHAT IS A BIOMARKER?

- A characteristic that is **objectively measured** and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention
- Any substance, structure, or process that can be **measured** in the body or its products and influence or **predict** the incidence of outcome or disease
- A naturally occurring molecule, gene, or characteristic by which a particular pathological or physiological process, disease, etc. can be identified.

NIH Biomarkers Definitions Working Group, 1998
WHO International Programme on Chemical Safety
Biomarkers in Risk Assessment: Validity and Validation. 2001.

EXAMPLES OF BIOMARKERS

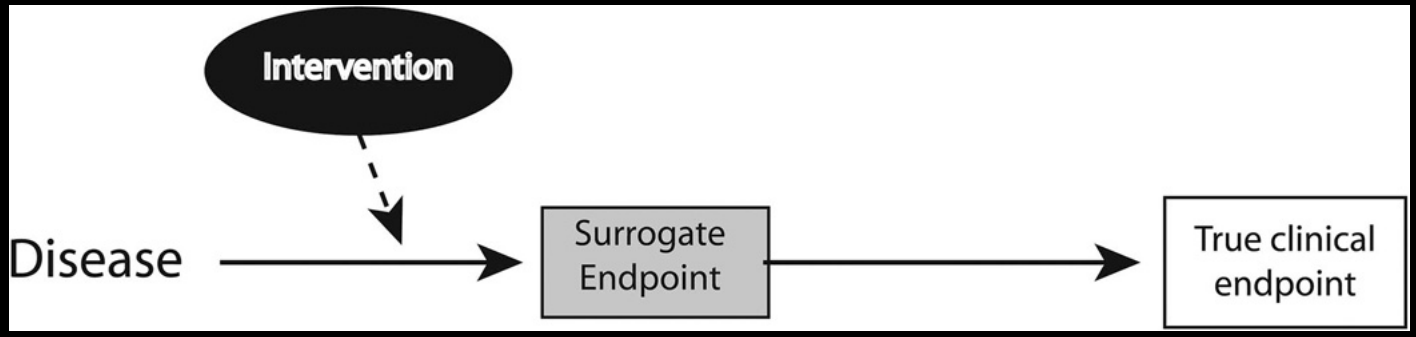
- Pulse, blood pressure
- Blood analytes
- ”Surrogate endpoints” in clinical trials
  - Solid scientific evidence (e.g., epidemiological, therapeutic, and/or pathophysiological) that a biomarker consistently and accurately predicts a clinical outcome, either a benefit or harm

CHARACTERIZATION AND EVALUATION OF BIOMARKERS

- Relevance -ability to appropriately provide ***clinically relevant*** information on questions of interest to the public, healthcare providers, or health policy officials
- Validity -need to characterize a biomarker's effectiveness or utility as a surrogate endpoint
  - Precision, reliability and accuracy of measurement
  - External validation in other populations
  - ? Broader application to other clinical endpoints
    - Ex: Anti-arrhythmics and cardiac disease- surrogate endpoint for mortality

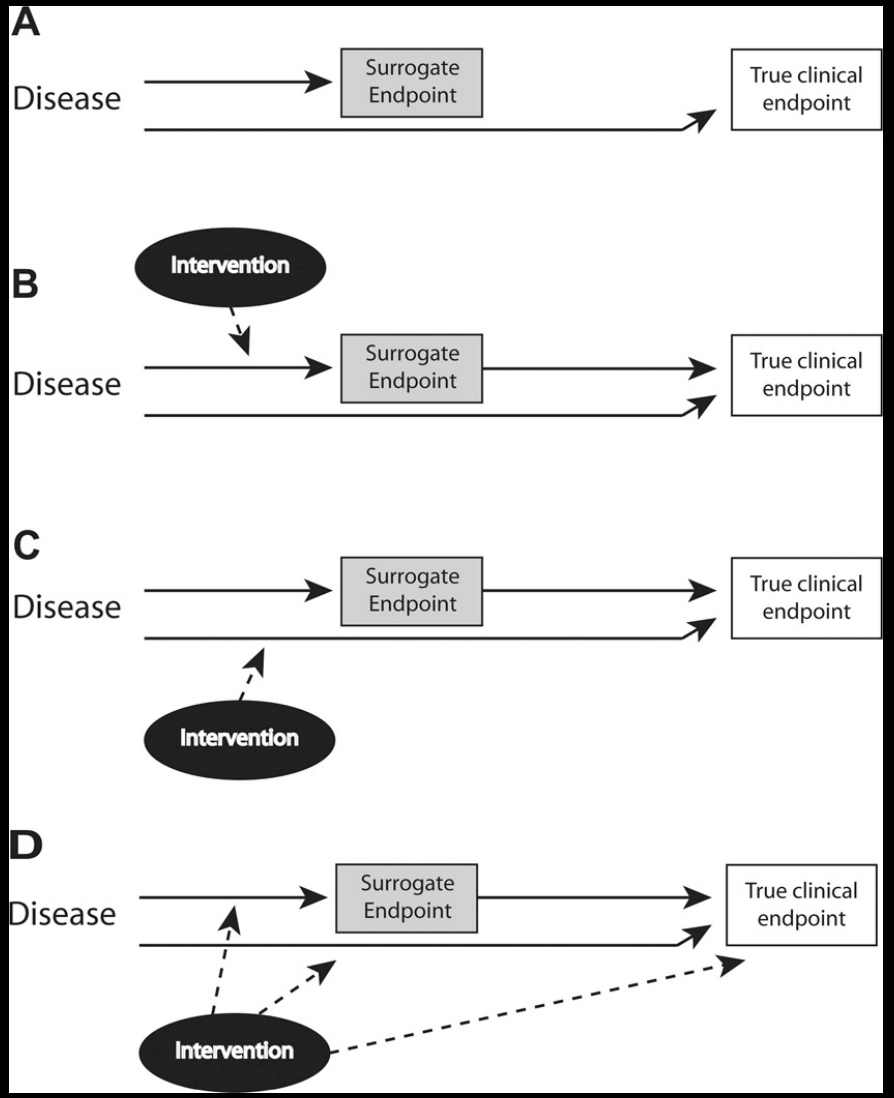
ENDPOINT REPLACEMENT

- Need for complete understanding of:
  - Normal physiology of biological process
  - Pathophysiology of that process in the disease state
  - Effects of an intervention – pharmacological, device…



Madeiros F, BJO 2015

INVALID SURROGACY



Madeiros F, BJO 2015

GLAUCOMA BIOMARKERS

- IOP
- Visual field loss
- RNFL thickness / optic disc topography
- Combination of structure/function

Madeiros F, BJO 2015

GLAUCOMA BIOMARKERS

There is a critical need to identify specific molecular markers that predict or measure glaucomatous neurodegeneration.

GLAUCOMA BIOMARKERS

- Oxidative stress and antioxidants (aqueous and serum)
  - 8-hydroxydeoxyguanosine
  - Malonyldialdehyde
  - Ascorbic acid
  - Nitric oxide synthetase
  - Superoxide dismutase
  - Catalase
  - Glutathione peroxidase
  - Protein carbonyls and advanced glycation end products
- Cytokines (tear)
  - IL
  - TNF
  - IFN
- miRNA's

GLAUCOMA BIOMARKERS

- Proteomic markers
  - Crystallins
  - Heat shock protein 60 (HSP 60) and HSP 90
  - Myotrophin
  - Apolipoprotein B and apolipoprotein E
  - Endothelial leukocyte adhesion molecule-1
  - Myoblast determination protein 1
  - Myogenin
  - Vasodilator-stimulated phosphoprotein,
  - Ankyrin-2
  - Transthyretin
  - Cochlin
  - Prostaglandin H2 D-isomerase and caspase-14

GLAUCOMA BIOMARKERS

- Neurodegenerative factors
  - APO AI, APO CIII, APO E, TTR
  - a2-macroglobulin, Cystatin-C
  - Endothelin 1
  - Erythropoeitin
  - CD44

AUTOIMMUNITY AND GLAUCOMA

- Accumulation of autoantibodies in retinas of patients with glaucoma
- Potential effect on local immune homeostasis
- Activation of microglia may result in increased levels of IL-8 and TNF-alpha
- **Antibody profiles in serum** of glaucoma patients can identify disease (>93% sensitivity and specificity)
- Protective effect of auto-antibodies on RGCs
  - anti-GFAP
  - anti-γ-synuclein

Hohenstein-Blaul N et al. Eye 2017

GDF15 is elevated in mice following retinal ganglion cell death and in glaucoma patients

Ban N, Siegfried CJ, Lin, Shui Y-B, Sein J, Pita-Thomas W, Sene A, Santeford A, Gordon M, Lamb R, Dong Z, Kelly SC, Cavalli V, Yoshino J, Apte RS, JCI Insight, 2017;2(9):e91455.

JCI insight
Published by The American Society for Clinical Investigation

PURPOSE

- To identify specific markers of RGC death that could potentially be used to accurately and reliably predict or measure glaucomatous neurodegeneration.

METHODS

- All animal studies were performed in accordance with the Washington University in St. Louis School of Medicine Animal Care and Use guidelines after approval by the Animal Studies Committee.
- The research protocol for the human study was approved by the Washington University School of Medicine Human Research Protection Office and the IRB in compliance with HIPAA guidelines and the tenets of the Declaration of Helsinki.

METHODS

- Strong association between neuroinflammation and glaucomatous optic neuropathy
- Retinal cytokine/growth factor–focused PCR array consisting of 88 genes (ILs, TNF and TGF superfamilies, growth factors, interferons, chemokines, and others) to identify factors whose expression correlates specifically with RGC death
- To identify factors unique to RGC death, we used 3 different murine models of disease:
  - Optic nerve crush (ONC)- model of axonal injury and RGC-specific death (glaucoma)
  - Light-induced retinal degeneration (RD)-model of photoreceptor-specific cell death
  - Endotoxin-induced uveitis (EIU) -model of ocular inflammation

METHODS

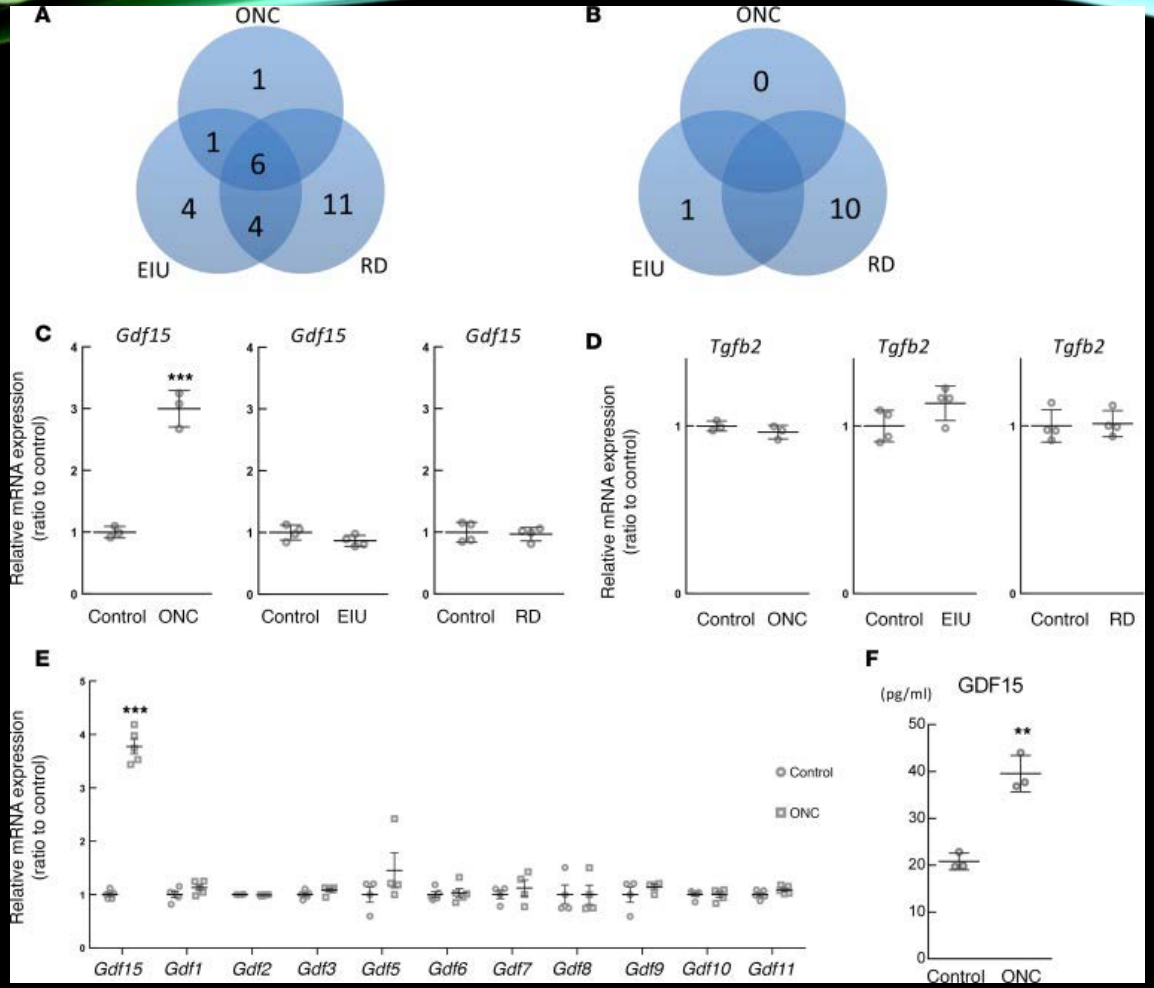
- Animals
  - Six-week-old C57BL/6J mice - ONC and EIU
  - Six-week-old 129S1/SvImJ mice were used -RD experiment
  - Six-week-old and 18-month-old C57BL/6J mice were used for young and old mice comparison
  - Three-month-old and 1-year-old DBA/2J mice were used as a chronic glaucoma model/control
  - Eight-week-old Sprague Dawley rats - ONC experiment

- Patients
  - Undergoing cataract, glaucoma, or combined cataract/glaucoma surgery
  - Demographic information / medications
  - Aqueous specimen collection (50-100µl)
  - Storage at -80º in gas phase of liquid nitrogen tank

METHODS

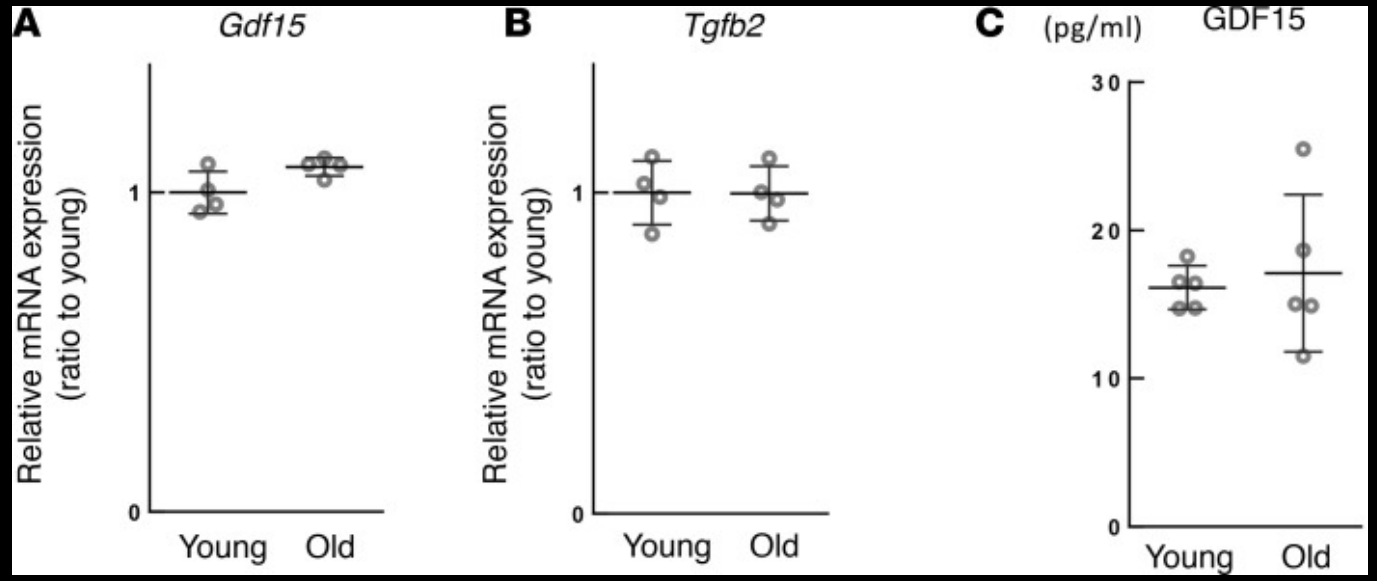
- ELISA
  - Quantikine Mouse/Rat GDF15 ELISA Kit (R&D Systems)
  - Human GDF15 Quantikine ELISA Kit (R&D Systems)
  - Quantikine ELISA Human TGFB2 (R&D Systems)
  - Minimum detection level = 4 pg/ml

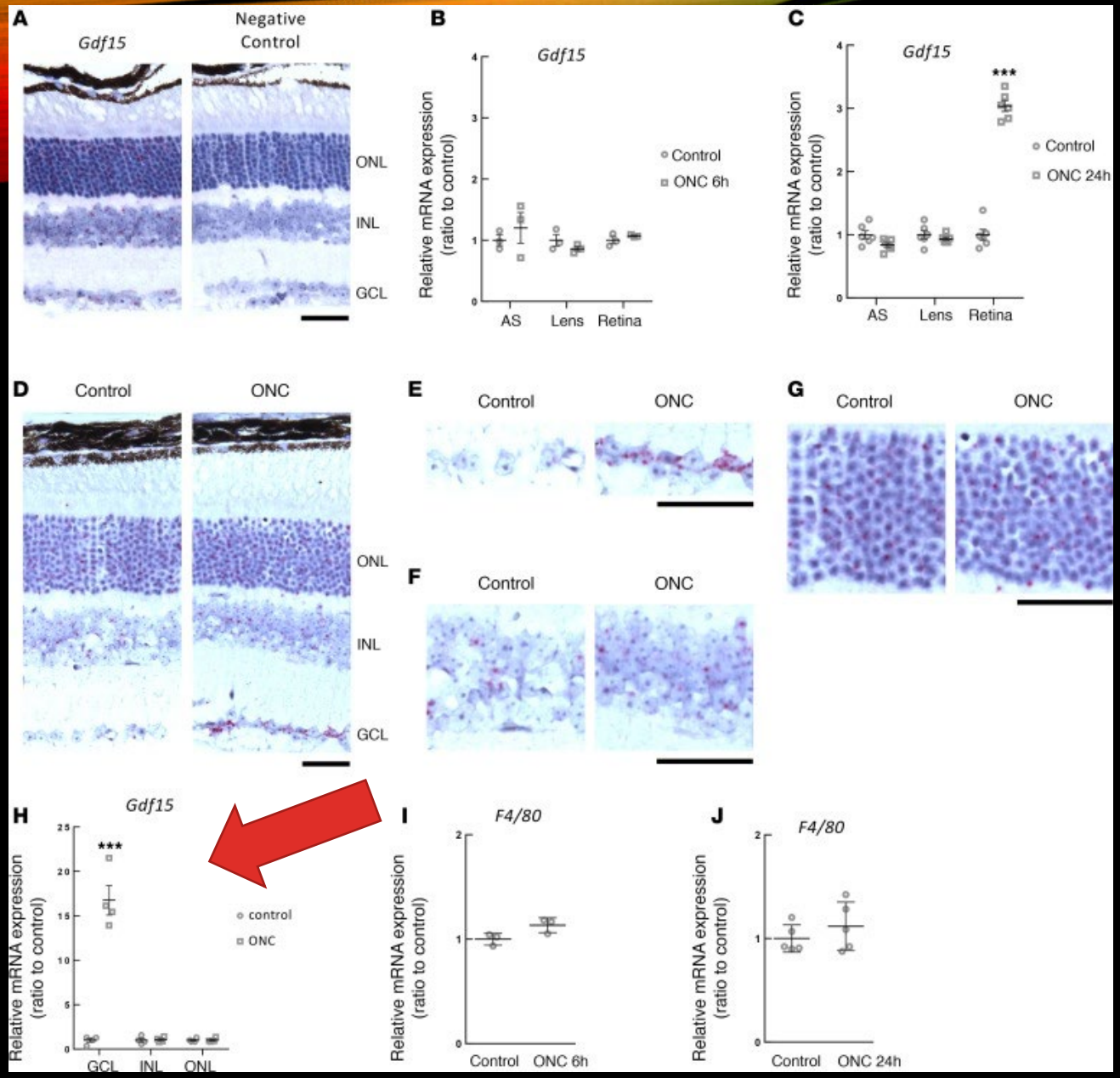
PCR Array and gene expression data



- Only **one** gene significantly altered in retinas (24 hours after ONC but not after RD and EIU)
- Protein confirmed in aqueous humor after ONC
- Not found with TGF-beta 2 or other growth factors

GENE EXPRESSION AND AQUEOUS HUMOR PROTEIN LEVELS- UNAFFECTED BY AGING



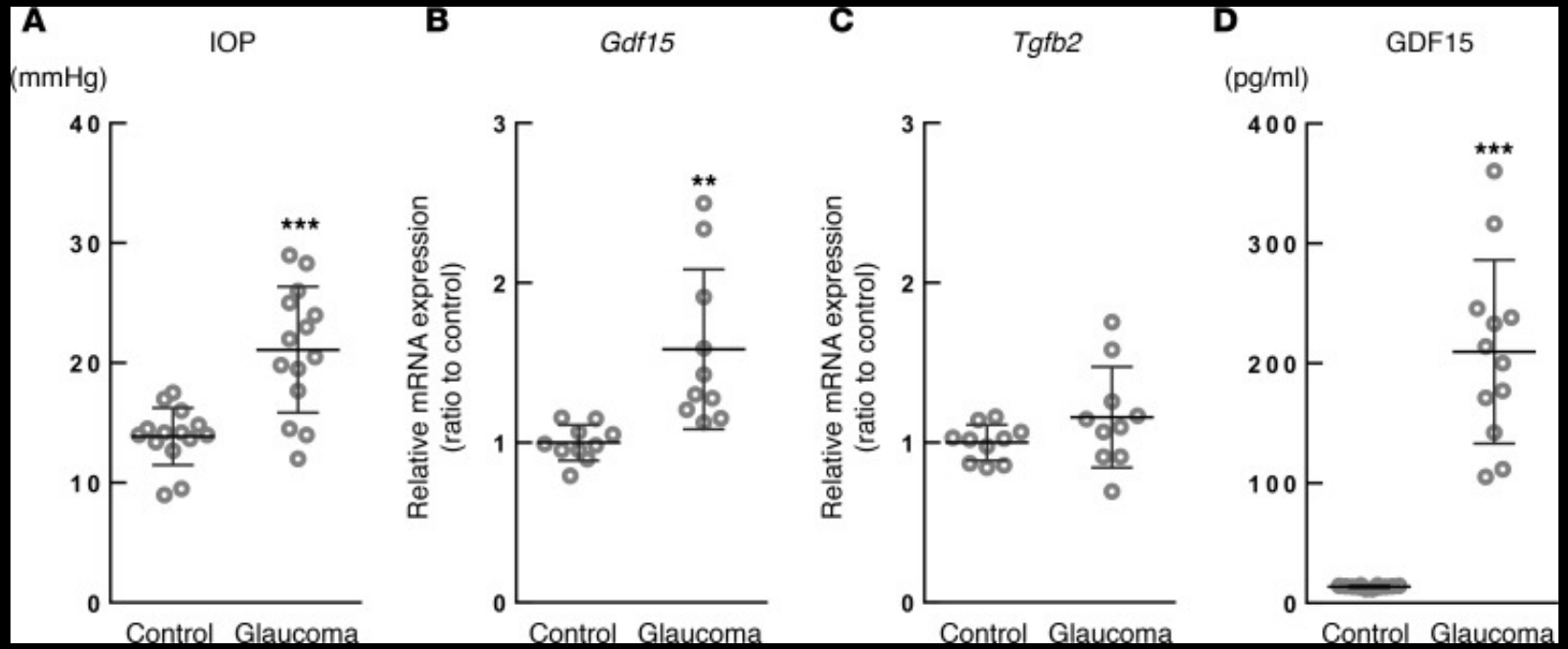


In Situ Hybridization

- Increased *Gdf15* expression in ganglion cell layer after optic nerve crush
- Observed only in the retina 24 hours after ONC
- Analysis of cornea, trabecular meshwork, iris, ciliary body, and ciliary processes), lens, and retina.
- No change in F4/80 expression (macrophage infiltration)

CONFIRMATORY ASSESSMENTS

- Gene expression and aqueous humor levels in rats
- Aqueous humor of murine glaucoma model (DBA/2J)- 1 year old vs. 3 month old controls



THE HUMAN MODEL

- Classification into 3 groups (Hodapp Anderson Parrish criteria) n=80
- No significant differences in age, sex, race, diabetic status

Table 2. Characterization of primary open angle glaucoma (POAG) patients (stage 1 to stage 3) and controls

		All patients	Control	POAG Stage 1	POAG Stage 2	POAG Stage 3
Age[A]	Mean ± SD	66.9 ± 14.9	65.8 ± 11.7	70.4 ± 8.8	70.5 ± 8.5	70.1 ± 12.0
	Range	47-90	47-88	52-88	56-82	52-90
Sex[B]	male	27	9	5	6	7
	female	53	14	18	9	12
Diabetic status[B]	no DM	57	16	17	13	11
	DM	23	7	6	2	8
Race[B]	ED	57	15	16	14	12
	AA	23	8	7	1	7

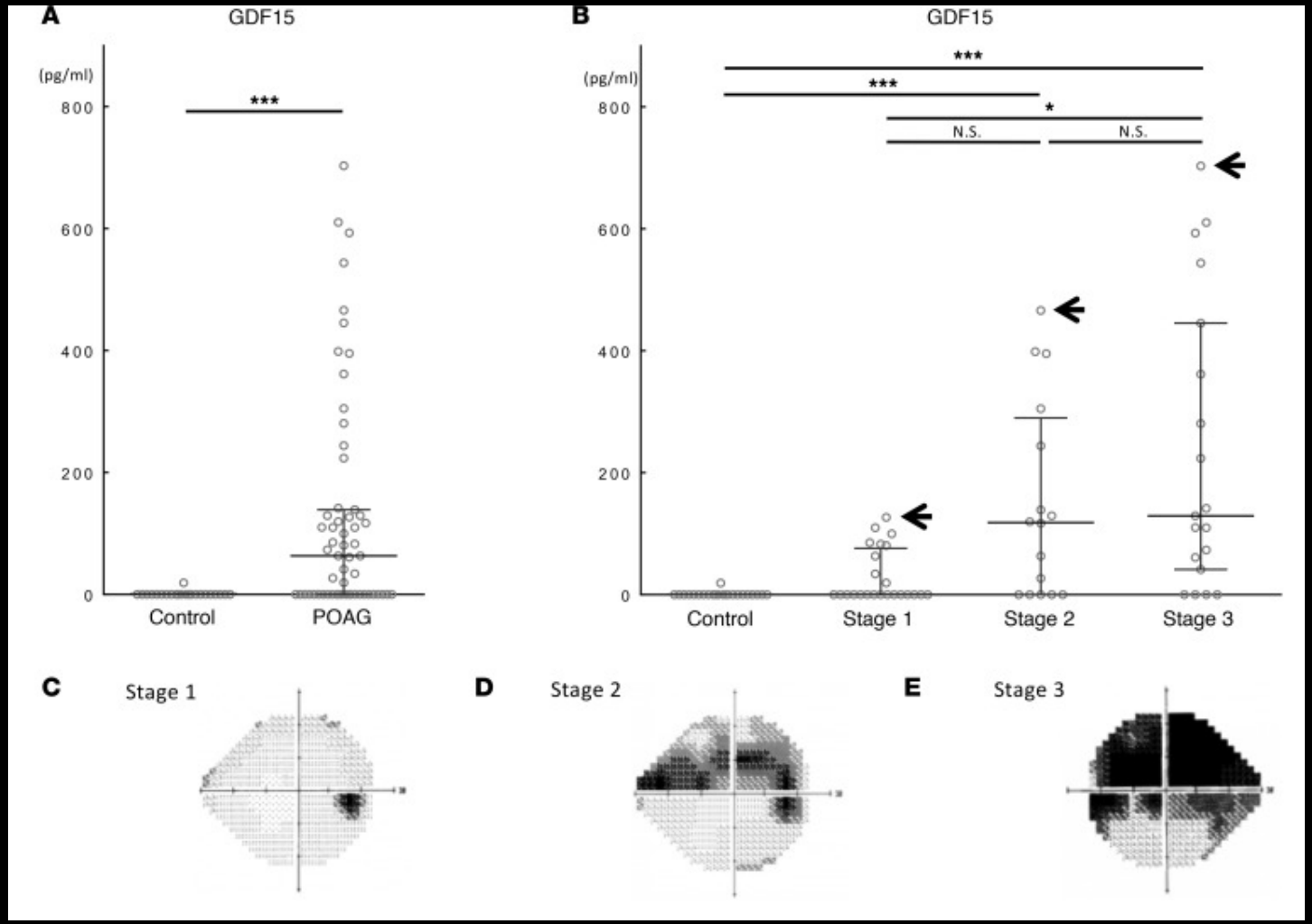
[A]There were no significant differences between different groups (controls, POAG stage 1, stage 2, and stage 3) by one-way ANOVA test. [B]There were no significant differences between different groups by chi-square test. DM, diabetes mellitus; AA, African American; ED, European descent.

AQUEOUS HUMOR LEVELS OF GDF15

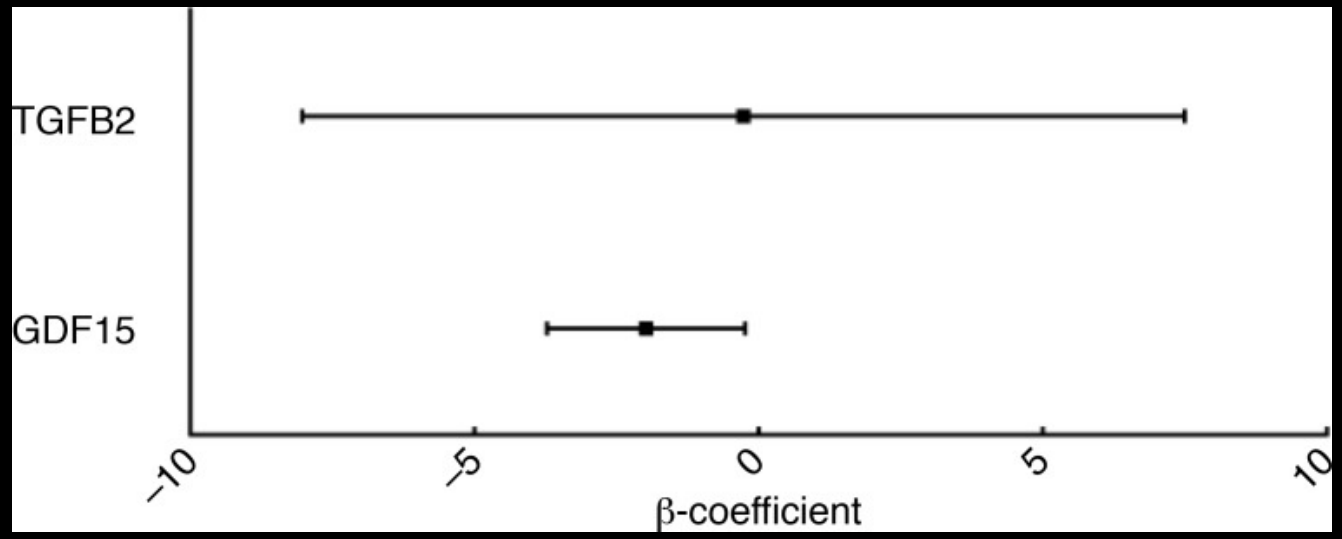
- POAG vs. Controls
***$P < 0.001$ by Mann Whitney *U* test

- $P < 0.001$ by Kruskal-Wallis test. *$P < 0.05$ and ***$P < 0.001$ by Dunn's multiple comparison.

- Control (n=23)
- Stage 1 (n=23)
- Stage 2 (n=15)
- Stage 5 (n=19)



TGF BETA 2 VERSUS GDF15



Multivariate linear regression model:
Log-transformed GDF15 (β = –2.0, 95% CI: –3.7 to –0.2, *t* = –2.3, *P* = 0.027) predict worse mean deviations on VF testing after controlling for age, race, intraocular pressure (IOP), and diabetic status.

Not with TGF beta 2 levels (β = –0.3, 95% CI: –8.0 to 7.5, *t* = –0.1, *P* = 0.945)

WHAT IS GDF15?

- Member of TGF-β superfamily
- Expressed in both the CNS and the peripheral nervous system
- Neurotrophic or neuroprotective effect in CNS and PNS
- Demonstrated to be upregulated in the retina after axonal injury to the optic nerve
- Magnitude and time course of RGC death after axonal injury was not different in mice that are genetically deficient in GDF15 compared with littermate controls, which suggests that GDF15 did not influence the temporal progression of RGC death
- GDF15 provides independent prognostic information on cardiovascular events beyond previously identified cardiovascular risk factors and other markers of chronic and acute coronary disease

Wang X, et al. Cell Tissue Res 2015
Charalambous P, et al. Cell Tissue Res 2015
Wollert KC, et al. Circulation 2007

TGF BETA2 VS. GDF15

- Ciliary epithelium or lens is main source
- Increased levels in human glaucomatous AH, not murine glaucoma model
- Functional changes of trabecular meshwork in humans

STUDY LIMITATIONS

- Animal models do not reflect usual biological process of human glaucoma
  - ONC (acute) vs Glaucoma (chronic)
  - DBA/2J model of pigmentary glaucoma (phagocytosis, inflammation)
- Selection bias of human study
  - Exclusion of Stage 4 and 5
  - Stability vs severity of disease process
- Failure to detect glaucoma at all stages of disease

CHALLENGES

- Complexity of disease
- Patient heterogenicity
- Spectrum of specific molecules
- Need for powerful and validated screening strategies
- General limitations to techniques of analysis
  - Lack of common operating procedures for proper banking of biological tissues
  - Analytical insensitivity
  - Multiplexed complicated assays
  - Regulatory landscape

CONCLUSIONS

- Validation of biomarkers for glaucoma will be beneficial to serve as surrogate endpoints in clinical trials
- Our data suggest that measurement of GDF15 in AH may provide quantitative information about glaucomatous neurodegeneration
- In addition to conventional IOP measurements, VF assessments, and ON evaluation, this information may enhance current treatment algorithms for glaucoma
- Further prospective validation studies (sequential/longitudinal) will be necessary to determine if GDF15 in AH is correlated with glaucoma progression and can be used in clinical practice