Clinical Relevance of GWAS and Pedigree-based studies

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No conflicts to disclose

POTENTIAL BENEFITS OF GENETIC STUDIES

- **Diagnostic utility**
  - Early detection
  - Confirm clinical evidence of disease
- **Prognostic utility**
  - Predict disease course
  - Predict response to therapy
- **Family counseling**
  - Risk for offspring

- **Gene-directed therapies**
  - Medications that target a gene defect
  - Gene therapy (viral delivery of normal genes)
  - Genome editing (in vivo gene repair)
- **Regenerative therapies**
  - Stem cell therapy (replacement of tissue)

TWO LARGE CLASSES OF GLAUCOMA GENES

Some cases are "caused" primarily by a SINGLE gene

- Three are known: *MYOC*, *OPTN*, *TBK1*
- 5% of POAG is cause by one of these 3 genes
- Mutations are essentially seen only in patients
- Familial glaucoma (autosomal dominant inheritance)

If glaucoma genes were coins and it took a dollar to get glaucoma…

MYOC, *OPTN*, and *TBK1* would each be dollar coins

TWO LARGE CLASSES OF GLAUCOMA GENES

Other cases of glaucoma are the result of the combined action of MANY genetic and environmental risk factors

- Risk factors are commonly detected in POAG patients (often in 25% or more)
- Risk factors are observed in normal subjects too (less often than in patients)
- > 35 Risk factor genes have been discovered
- Act in concert – NO SINGLE RISK FACTOR CAN CAUSE DISEASE ON ITS OWN

If glaucoma risk factors were coins and it took a dollar to get glaucoma…

- Each risk factor would be a penny
- Many are required for glaucoma

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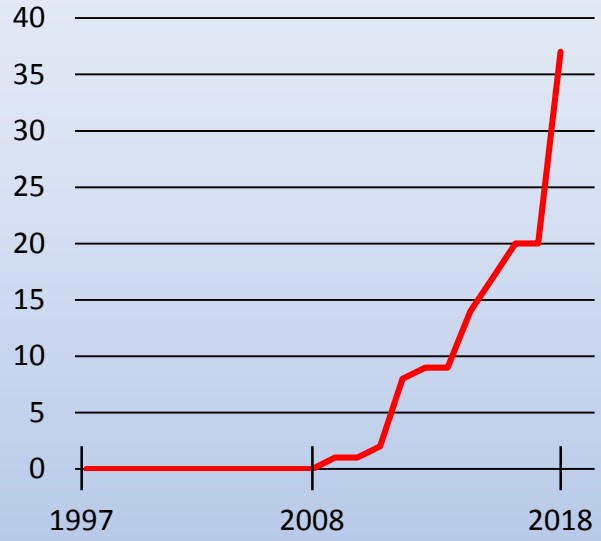
RISK FACTOR GENES

Together they cause glaucoma, but each contribution small risk

ABCA1
AFAP1
ANKRD55-MAP3K1
ANKH
ARHGEF12
ATOH7
ATXN2
CADM2
CAV1/CAV2
CDKN2B-AS1
CHR 8q22 locus
CRYGS
DGKG
ELOVL5
EXOC2
FDNC3B
FMNL2
FOXC1
GAS7
GMDS
HMGA2
IKZF2
LHPP
LMO7
LMX1B
LOXL1
MEIS2
MYOF
PDE7B
PMM2
SIX6
SRBD1
TFGBR3
TLR4
TMCO1
TMTC2
TXNRD2



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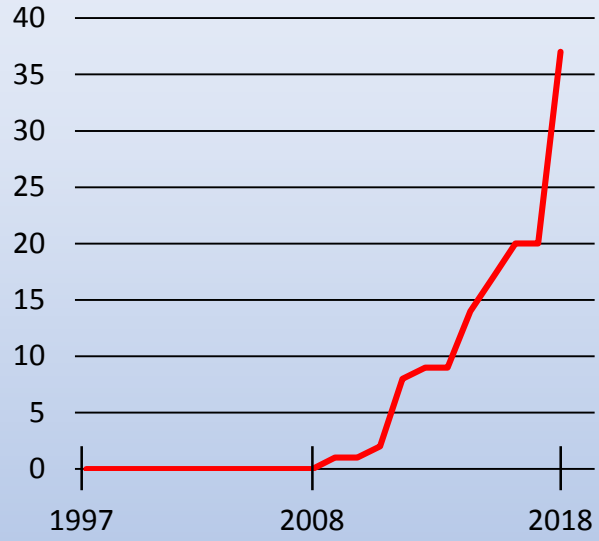
GLAUCOMA RISK FACTOR Example 1 (*TMCO1*)

ABCA1
AFAP1
ANKRD55-MAP3K1
ANKH
ARHGEF12
ATOH7
ATXN2
CADM2
CAV1/CAV2
CDKN2B-AS1
CHR 8q22 locus
CRYGS
DGKG
ELOVL5
EXOC2
FDNC3B
FMNL2
FOXC1
GAS7
GMDS
HMGA2
IKZF2
LHPP
LMO7
LMX1B
LOXL1
MEIS2
MYOF
PDE7B
PMM2
SIX6
SRBD1
TFGBR3
TLR4
TMCO1
TMTC2
TXNRD2



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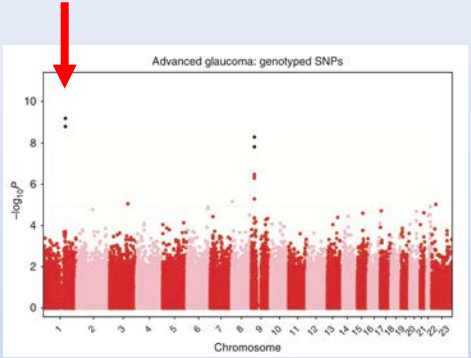
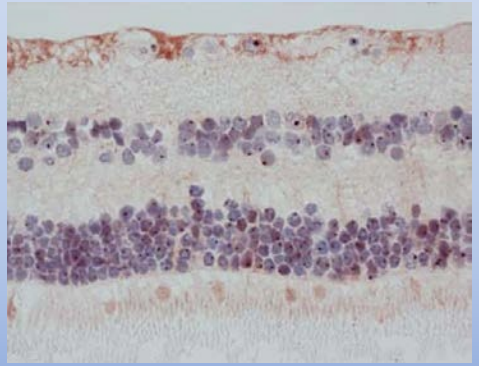
TMCO1 and POAG risk

TMCO1 GENE

- Identified as a glaucoma risk factor in 2011
- GWAS hit with odds ratio of 1.51
  - Burdon KP, Macgregor S, et al. Nat Genet, 2011; 43: 574-578.
- Encodes a transmembrane protein
- Retina / RGC / optic nerve expression
  - Burdon KP, Macgregor S, et al. Nat Genet, 2011; 43: 574-578.
  - Van Koolwijk LME, Ramdas WD, et al., PloS Genet, 2012; 8: e1002611.
  - Sharma S, Burdon KP, et al., IOVS, 2012; 53: 4917-4925.





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TMCO1 in the OHTS
Confers significant risk for POAG

OCULAR HYPERTENSION TREATMENT STUDY

- Prospective, multicenter, controlled, treatment trial
- 1637 participants with ocular hypertension
  - Treatment arm - 20% reduction in IOP
  - Observation arm – Placebo
- Visual fields every 6 months / annual disc photos
- Comparison of incident glaucoma between groups

OHTS
Ocular Hypertension Treatment Study

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Gordon MO, Kass MA, et al, The Ocular Hypertension Treatment Study: design and baseline description of the participants, Arch Ophthalmol, 1999, 117:573-583.

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TMCO1 in the OHTS

Confers significant risk for POAG

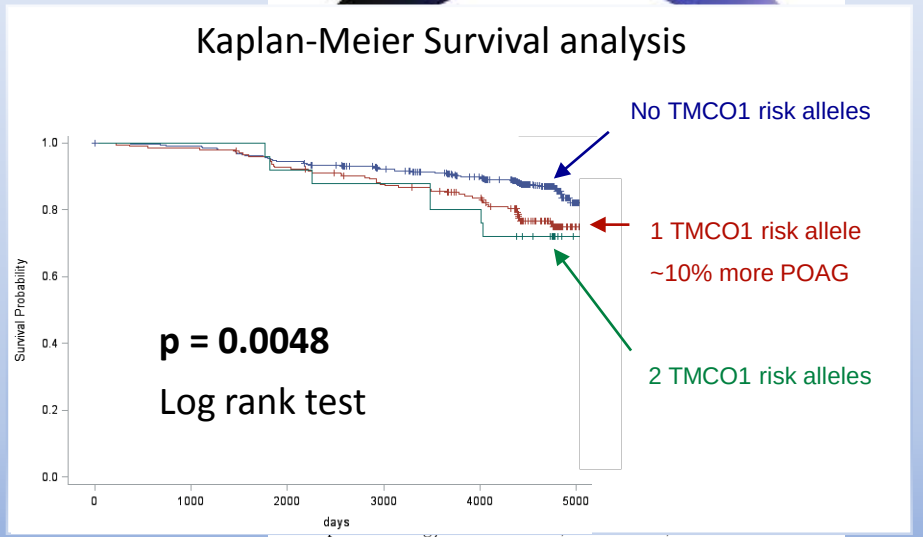
OCULAR HYPERTENSION TREATMENT STUDY

Genetics Ancillary Study

- DNA from 1055 of 1636 participants
- Genetic study of POAG risk factors in OHTS

TMCO1 risk alleles vs incident POAG

- 14 year follow up (OHTS-I and OHTS-II)
- *TMCO1* risk alleles are powerful



KAPLAN MEIER ANALYSIS:

10% HIGHER PROBABILITY FOR POAG AT 14 YEARS

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TMCO1 in the OHTS
Confers significant risk for POAG

OCULAR HYPERTENSION TREATMENT STUDY

Genetics Ancillary Study

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TMC01 risk alleles vs incident POAG

- 14 year follow up (OHTS-I and OHTS-II)
- *TMCO1* risk alleles are powerful

COX PROPORTIONAL HAZARDS:

RISK ON PAR WITH POTENT CLINICAL FACTORS

Cox proportional hazards ratio analysis

752 non-Hispanic whites	Hazards ratio	95% confidence interval	p value
Age (decade)	1.39	1.14 – 1.71	0.0012
Gender (male)	1.55	1.07 - 2.24	0.021
IOP (per mm Hg)	1.08	1.01 – 1.15	0.029
CCT (per 40 micron)	1.58	1.29 – 1.94	0.000014
VCDR (ratio per 0.1)	1.28	1.15 – 1.42	0.0000029
TMCO1 (per allele)	1.66	1.24 – 2.22	0.00061

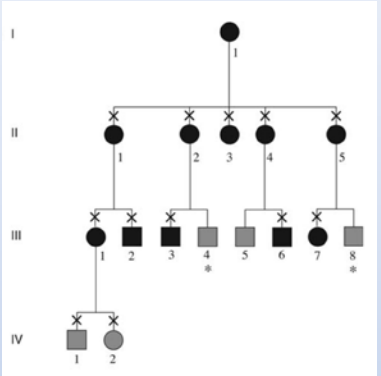
Calculating Glaucoma Risk

- One risk factor (*TMCO1*) has a strong influence on glaucoma
- More precise, personalized risk assessments will be possible
  - Combined analyses of more genetic factors
  - Combined analyses with clinical factors
- Now a work in progress

Mendelian (single gene) POAG

discovered with pedigree studies

- Myocilin (*MYOC*) 1997
  - 16 to 63% JOAG cases
  - 3-4% of POAG cases
  - 1% of NTG cases
- Optineurin (*OPTN*) 2002
  - 1% of NTG cases
- TANK binding Kinase 1 (*TBK1*) 2011
  - 1% of NTG cases



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Rezaie T, Child A, Hitchings R et al. ***Science***. 2002; 295(5557):1077–9.

Fingert JH, Robin AL, Ben R Roos et al. ***Hum Mol Genet.*** 2011; 20(12):2482–94.

Mendelian (single gene) POAG

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  - 1% of NTG cases
- TANK binding Kinase 1 (*TBK1*) 2011
  - 1% of NTG cases

Key features

- One gene causes glaucoma
- High penetrance
  - Most carriers develop POAG
- Virtually absent from controls
- Autosomal dominant

GLAUCOMA-CAUSING GENE
Example 1

20 years old male – routine exam

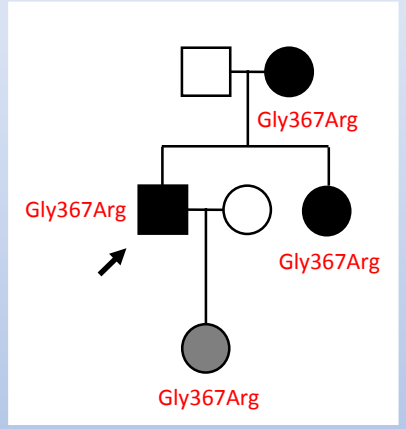
- HIGH IOP: 36 mm Hg OD / 38 mm Hg OS
- LARGE CUPS (OS > OD)
- VF LOSS (OS > OD)

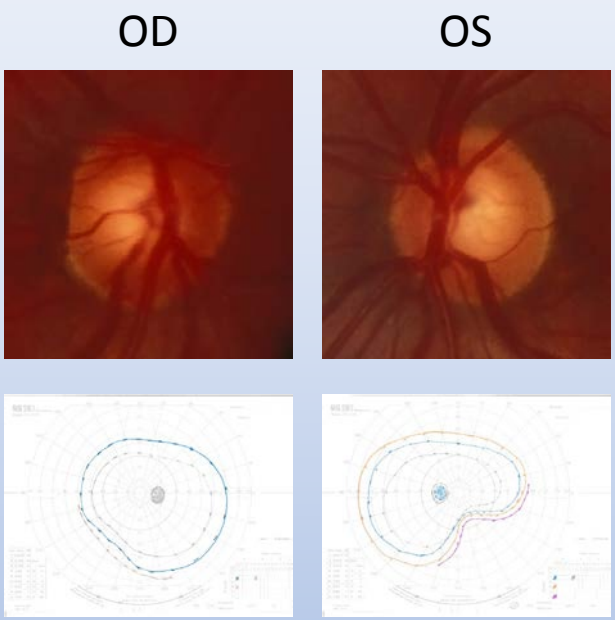
Diagnosis of JOAG

- Positive family history

Genetic Testing

- Positive (GLY367ARG MYOC mutation)





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MYOC GLN367ARG and JOAG

Previously detected in JOAG

- Europe, USA, Australia, India, and Japan

Clinical features

- Mean age at diagnosis: 18-37 years
- Mean maximum IOP: 35-51 mm Hg
- Surgery usually required for IOP control

- Mansergh FC, Kenna PF, Ayuso C, et al. **Human Mut**; 1998, 11: 244-251.
- Michels-Rautenstrauss KG, Mardin CY, Budde WM, et al. **Human Genet**; 1998, 102:103-106.
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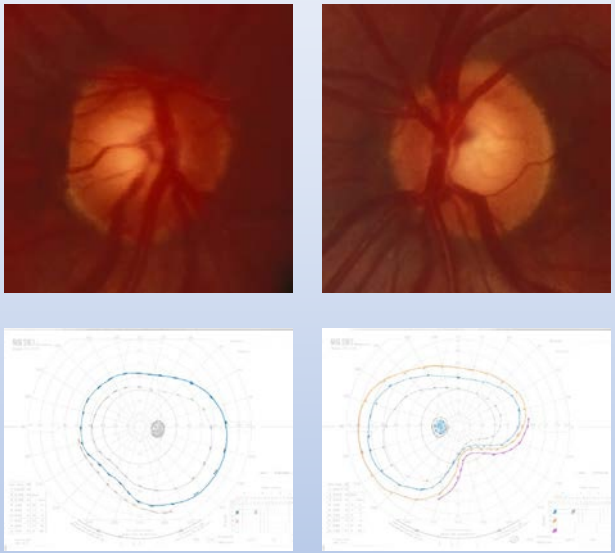
- Iliev ME, Bodmer S, Gallati S, et al. **Eye**; 2008, 22: 880-888.
- Souzeau E, Burdon KP, Dubowsky A, et al. **Ophthalmology**; 2013, 120: 1135-1143.

MYOC TESTING
OFTEN USEFUL FOR JOAG PATIENTS

- High rate of positive tests (8-63% cases)
- Key features
  - Early onset
  - High IOP
  - Strong family history
- Positive test may help direct management
  - Careful surveillance, more rapid move to surgery
  - Testing may be offered to relatives at risk



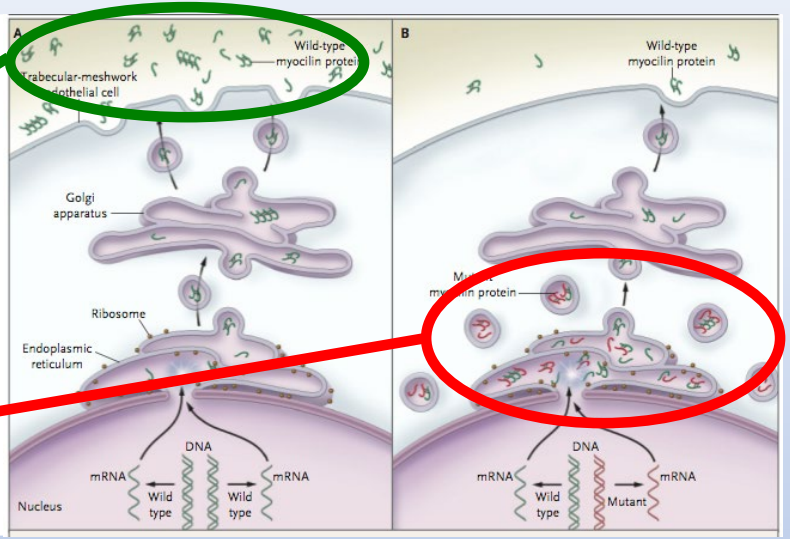
High rate of positive *MYOC* tests (up to 63%) in a rare form of OAG (<1% of OAG)

MYOC testing in POAG
with more typical adult-onset disease

- Mutations are less common in adult-onset glaucoma
  - 3 to 4% positive test rate
  - 1 to 2% of POAG cases due to a single *MYOC* mutation Gln368Ter
- High risk patients may benefit from testing
  - Relatives of known MYOC+ patients (up to 50% risk of positive test)
  - Patients with strong history of familial glaucoma
- Unselected testing probably not yet warranted (except for research)

Mechanism of disease:
How *MYOC* mutations cause glaucoma

- **Normal MYOC protein properly folded and is secreted**
- **Mutant MYOC misfolds and stays within cells**



Kwon YH, Fingert JH, et al. N Engl J Med, 2009, 360:1113-1124.

Abnormal intracellular accumulation of mutant MYOC → TM cell dysfunction or death → Reduced aqueous outflow → Elevated IOP and glaucoma

Mechanism of disease:
How *MYOC* mutations cause glaucoma

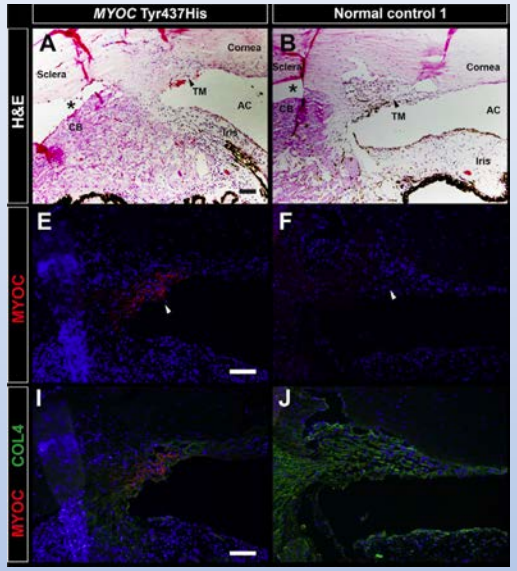
Donor eye from a patient with myocilin glaucoma

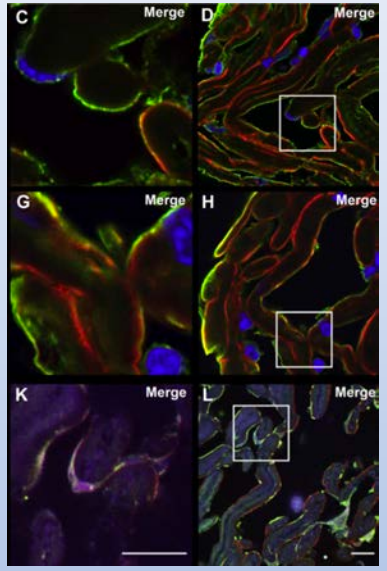
- Tyr437His mutation

More MYOC is retained in TM

Colocalizes with intracellular proteins (WGA, ConA, BiP)

Confirms disease model of mutant MYOC retained in TM cells



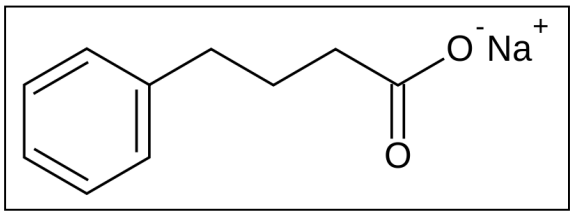


Van der Heide CJ, Alward WLM, et al. Ophthalmol Glaucoma, 2018; 1: 132-138.

MYOC-directed therapies
drugs and genome editing

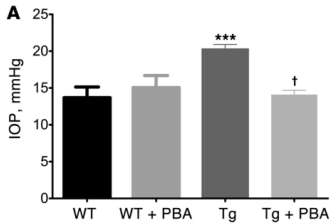
Drug (phenylbutyrate) cures *MYOC* glaucoma in Tg-mice

- Chemical chaperone promotes proper folding and secretion of mutant MYOC
- Lowers IOP / prevents nerve damage
- Potential utility for human therapy

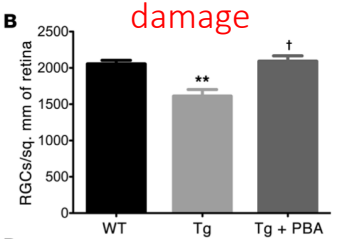


Phenylbutyrate

PBA lowers IOP



PBA prevents nerve damage



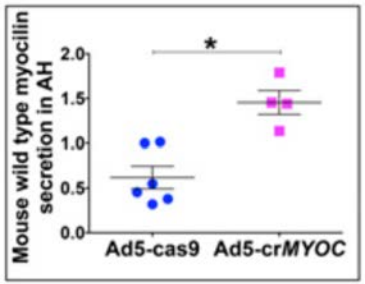
Zode G, Kuehn MH, J Clin Invest, 2011; 121: 3542-3553.

MYOC-directed therapies
drugs and genome editing

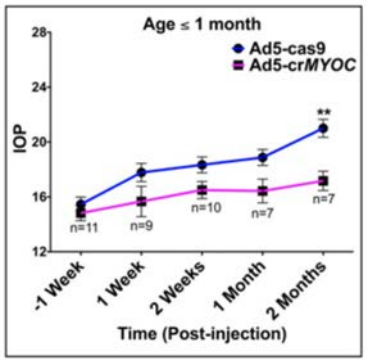
CRISPR/Cas9 editing cures *MYOC* glaucoma in mice

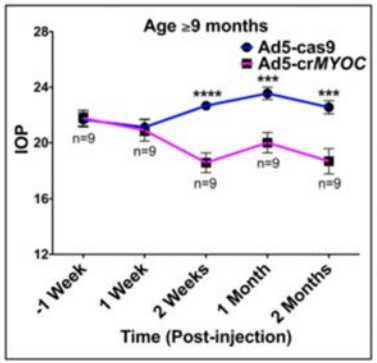
- Blocks expression of mutant protein
- Lowers IOP / prevents nerve damage
- Potential utility for human therapy

Genome editing increases MYOC secretion



Genome editing lowers IOP





Jain A, Zode G, et al. Proc Nat Acad Sci, 2017; 114: 11199-11204

GLAUCOMA-CAUSING GENE
Example 2

33 year old male – routine exam

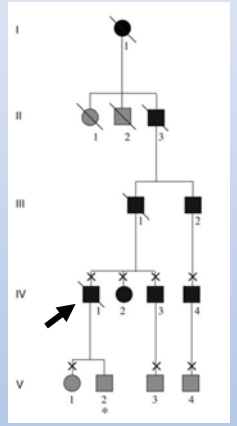
- NORMAL IOP: 16 mm Hg OD / 16 mm Hg OS
- LARGE CUPS OU
- VF LOSS Severe OU

Diagnosis of JOAG / NTG

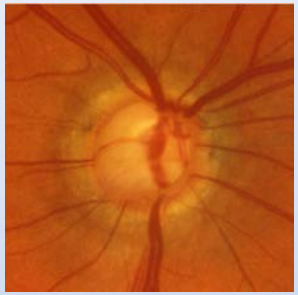
- Strong positive family history

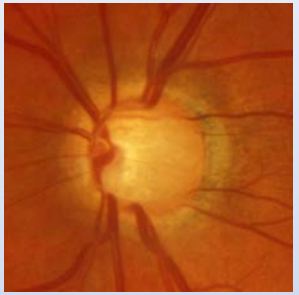
Genetic Testing

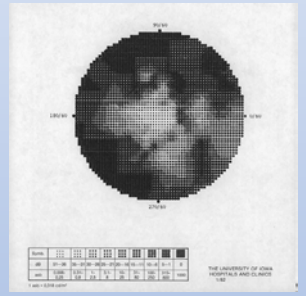
- *TBK1* gene TRIPLICATION mutation

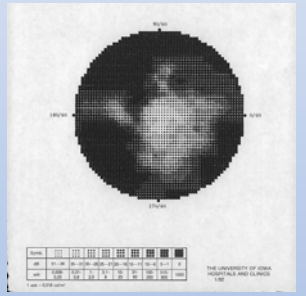


OD OS









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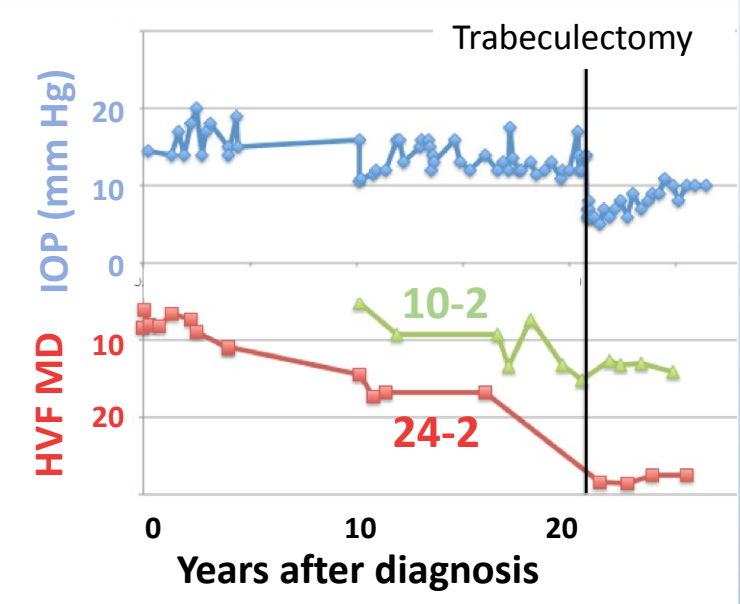
TBK1 gene triplication and NTG

Detected in NTG

- Caucasian ancestry (USA and Australia)
- *TBK1* duplications in patients of African, Asian, and Caucasian ancestry (US), Asian ancestry

Clinical features

- Mean age at diagnosis: 29 years (Triplication)
  36 years (Duplication)
- Mean maximum IOP: 18 mm Hg (Triplication)
  18 mm Hg (Duplication)
- Some patients progress at low IOP



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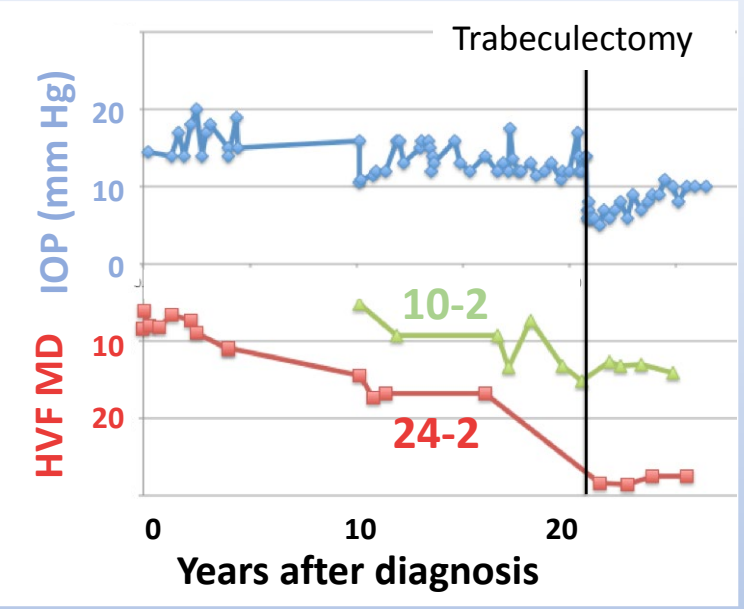
TBK1 TESTING MAY BE USEFUL FOR ***SELECT*** NTG PATIENTS

- Rare + tests in unselected NTG
  - 0.4 – 1.2%
- Higher utility in select patients
  - Early onset
  - Presentation with severe disease
  - Strong family history of NTG
- *TBK1* positive patients may benefit from lower IOP target



GLAUCOMA-CAUSING GENE
Example 3

GLAUCOMA-CAUSING GENE
Example 3

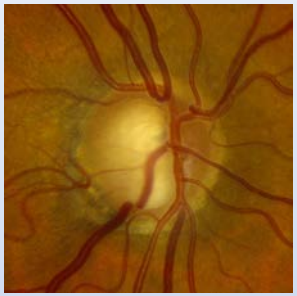
45 year old female – exam due to FHx

- Mother, sister, several others
- IOP 15 mm Hg OD and 16 mm Hg OS
- Large cup to disc ratios
- Glaucomatous VFs OU

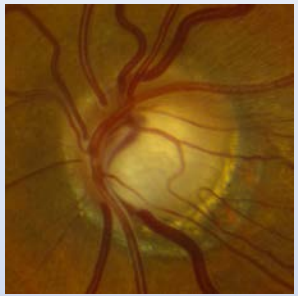
Genetic Testing for NTG:

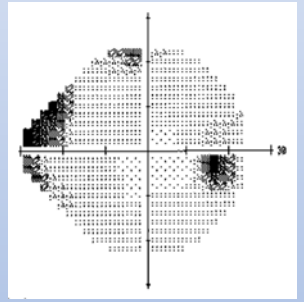
- *OPTN* testing: positive (GLU50LYS mutation)

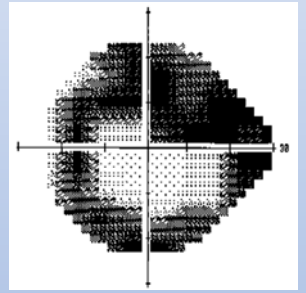
OD



OS







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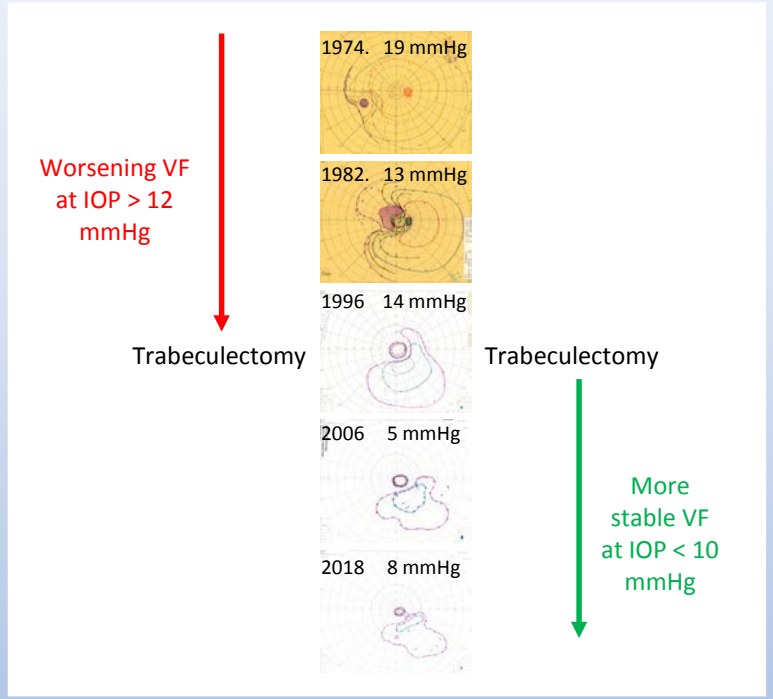
GLU50LYS *OPTN* mutation and NTG

Glu50Lys has been detected in NTG

- Caucasian (USA, Europe) and Asians (Japan)

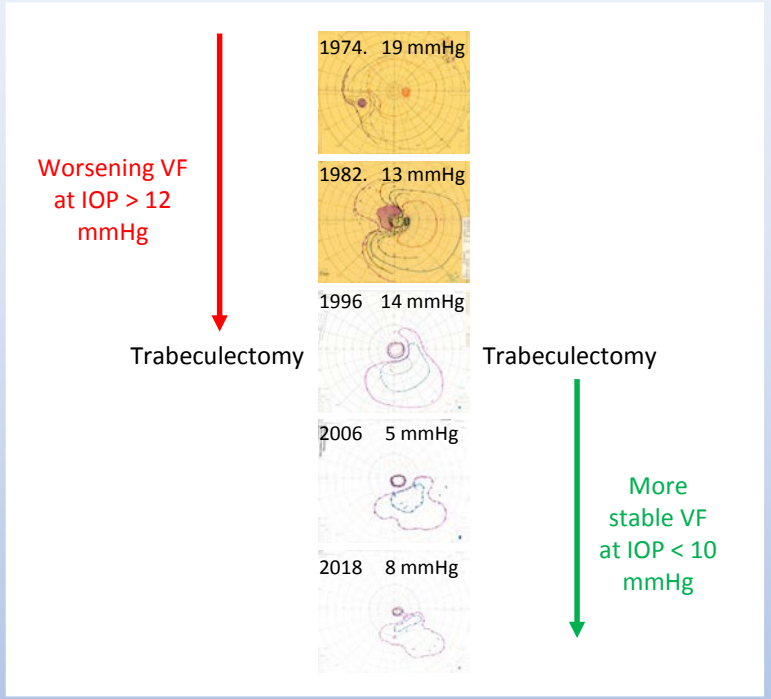
Clinical Features

- Mean age at diagnosis: 41 years
- Mean maximum IOP: 17 mm Hg
- More frequent need for trabeculectomy (3X)
- Some may benefit from low IOP (i.e <10 mm Hg)



OPTN TESTING MAY BE USEFUL FOR ***SELECT*** NTG PATIENTS

- **Rare + tests in unselected NTG**
  - 1.0 – 3.0%
- **Features that may improve utility**
  - Early onset / severe presentation
  - Strong family history of NTG
  - Perhaps more common in Caucasian populations
- ***OPTN*** **positive patients may benefit from lower IOP target**



Genes are important in glaucoma and are becoming more important in the care of your glaucoma patients

Acknowledgements

Fingert Lab

- Ben Roos
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- Ting Aung
- CC Khor
- Eyes From Africa Consortium

Funding Support

National Eye Institute

The Glaucoma Foundation

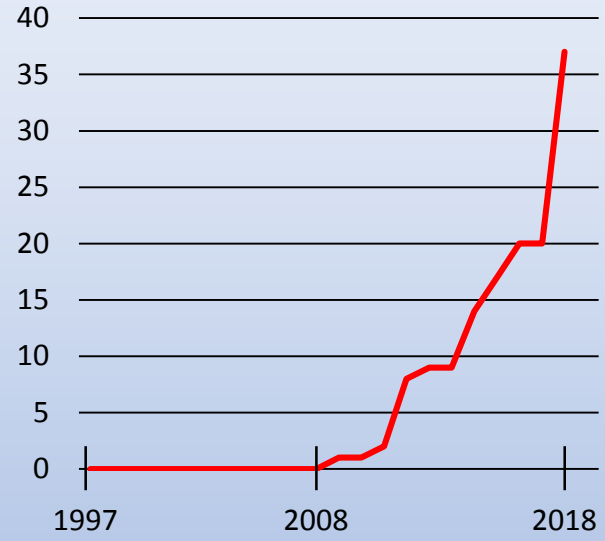
Research to Prevent Blindness

GLAUCOMA RISK FACTOR Example 2

ABCA1
AFAP1
ANKRD55-MAP3K1
ANKH
ARHGEF12
ATOH7
ATXN2
CADM2
CAV1/CAV2
CDKN2B-AS1
CHR 8q22 locus
CRYGS
FDNC3B
FMNL2
FOXC1
GAS7
GMDS
HMGA2
LMX1B
LOXL1
MEIS2
MYOF
PDE7B
PMM2
SIX6
SRBD1
TFGBR3
TLR4
TMCO1
TMTC2
TXNRD2

Newly identified factor

APBB2



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A new risk factors

POAG in patients of African ancestry

We
stu

- M
- Ti
- Chris Girkin (UAB)
- Ey

GV

- 23
- 2121 African control subjects

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AMERICAN ACADEMY OF OPHTHALMOLOGY

Genetic Architecture of Primary Open-Angle Glaucoma in Individuals of African Descent

The African Descent and Glaucoma Evaluation Study III

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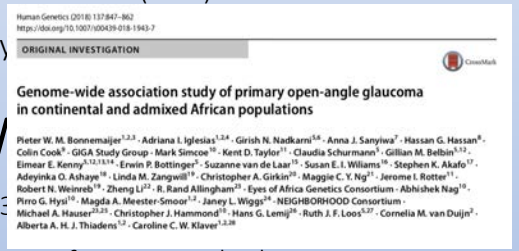

Human Genetics (2018) 137:847–862
https://doi.org/10.1007/s00439-018-1943-7

ORIGINAL INVESTIGATION

Genome-wide association study of primary open-angle glaucoma in continental and admixed African populations

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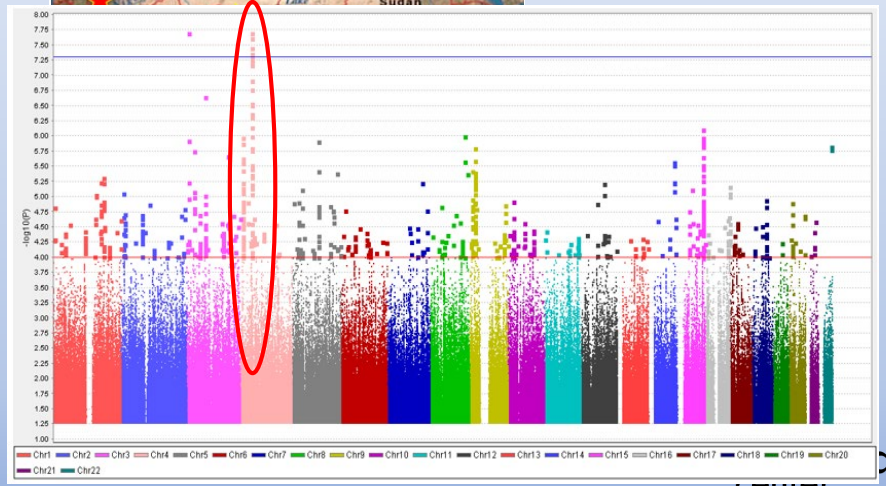
APBB2

1st African risk factor



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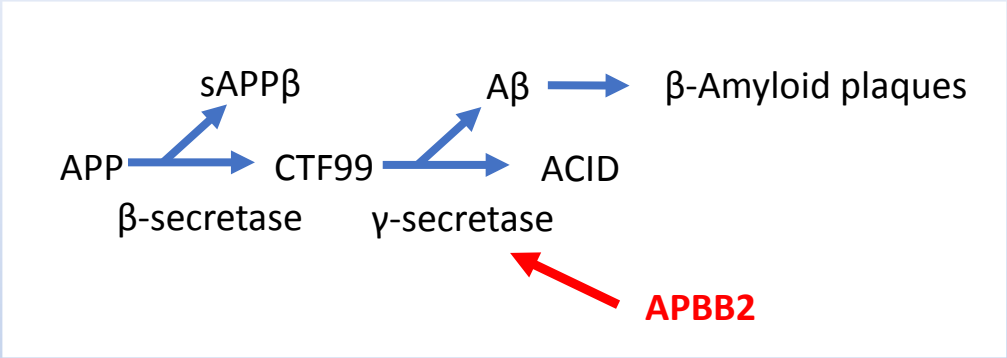
What does *APBB2* do?

amyloid beta precursor protein binding family B member 2 (*APBB2*)

- Amyloid precursor protein (APP)
  - Required for retinal development
- Improper processing of APP
  - Formation of β-amyloid plaques, as seen in Alzheimer's disease
- APBB2 stimulates γ-secretase digestion
  - Promotes β-amyloid formation



Hypothesis: New risk factor promotes glaucoma via:

- Increasing APBB2 production
- Increasing toxic β-amyloid formation in retinal ganglion cells

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Sections of human donor eyes

Labeled with an antibody against APBB2

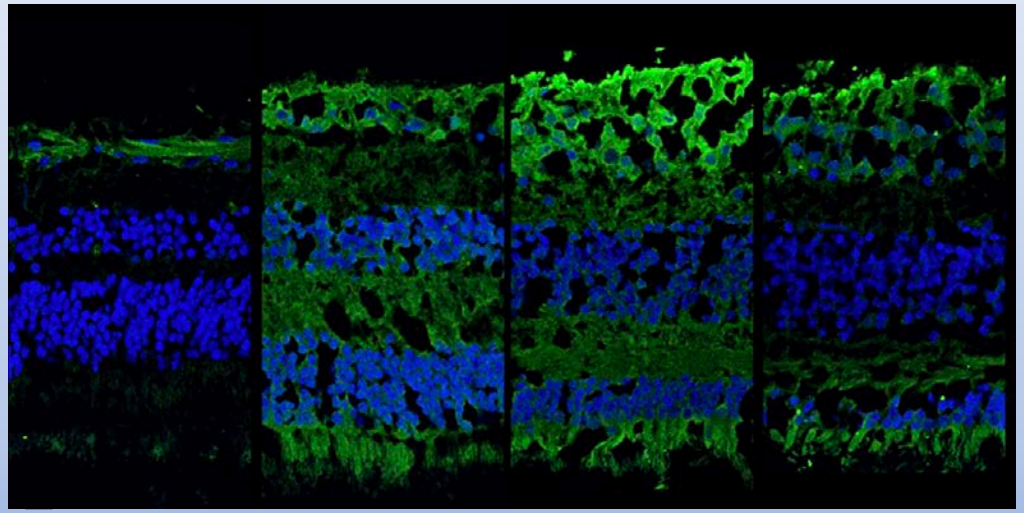
No APBB2 risk alleles | 1 APBB2 risk allele

APBB2 risk alleles result in more APBB2 protein in human retina

Sections of human donor eyes

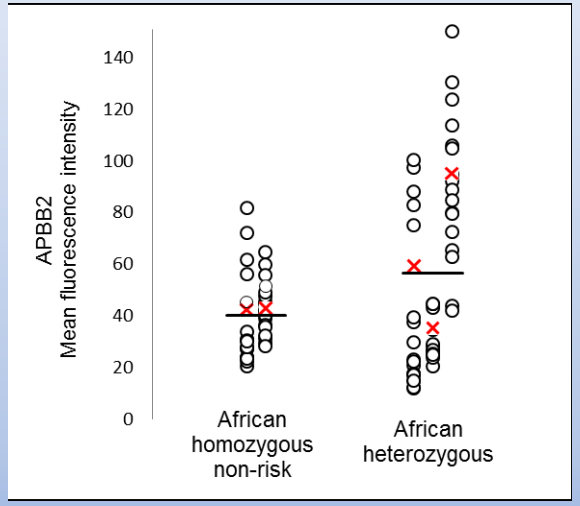
Labeled with an antibody against β-amyloid

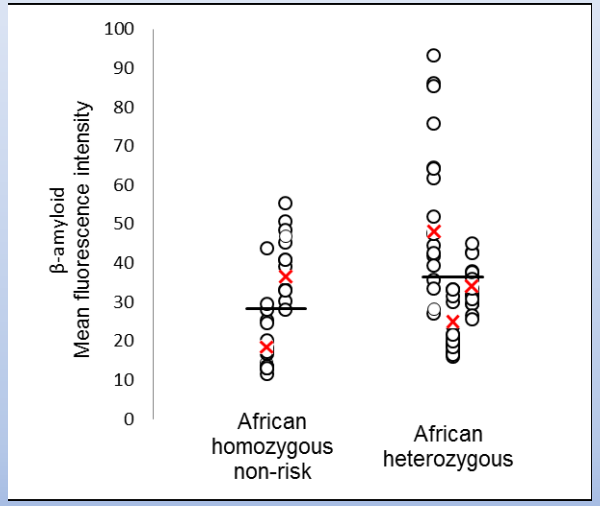
No APBB2 risk alleles | 1 APBB2 risk allele



APBB2 risk alleles result in more β-amyloid in retinal ganglion cells

APBB2 risk factors lead to more APBB2 and β-amyloid in the retina





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Genetic factor discovery suggests new disease mechanism

- APBB2 risk allele
  - Associated with increased retinal expression of APBB2 protein
  - Associated with increased retinal expression of toxic β-amyloid
- Suggests links with Alzheimer's disease
- Suggests pathophysiology (β-amyloid biology)
- Suggests new avenues for glaucoma therapy