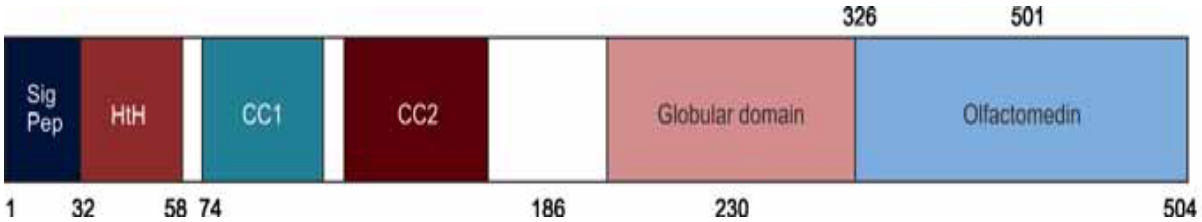
Title- Mutational Analysis of Myocilin in Juvenile Open Angle Glaucoma

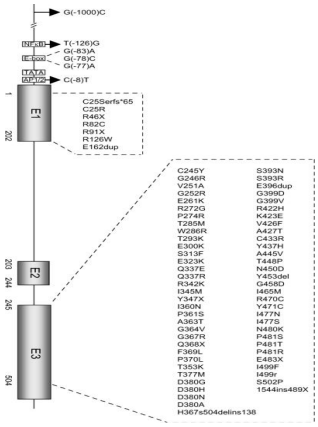
Chitra Sambare, Vidya Chelekar, colin Willoughby.

Background-

JOAG affects individuals between the ages of 5 and 40.Compared to adult-onset POAG, JOAG tends to be more severe and progresses rapidly. High intraocular pressures (IOP > 30 mmHg)need early surgical intervention. It has Autosomal dominant inheritance. Genetic linkage analysis played a crucial role in identifying the GLC1A locus on chromosome 1q23-25 and mutations in the MYOC gene.

- Introduction -MYOC GENE- located within the GLC1A interval. The majority disease-associated MYOC mutations are located within exon 3, where the olfactomedin homology region is localized.





Over 90% are found in exon 3 coding for the olfactomedin homology domain of myocilin. Figure from (Resch and Fautsch. 2009)

AIM

To determine the mutational load associated with myocilin (MYOC; MIM*601652) mutations inpatients with JOAG in Pune region.

Patient Recruitment and DNA extraction (PUNE) - Glaucoma clinics in Pune, India Consent was Obtained from all subjects. Ethical Compliance Followed Declaration of Helsinki Criteria for selection of JOAG: Intraocular pressure >21 mm Hg (in at least one eye) Glaucomatous optic nerve head changes, Open anterior chamber angle,Characteristic visual field defects, No secondary glaucoma conditions, Age was 3-40 years.

MYOC Sequencing (Belfast, UK)

DNA Extraction was done in Pune from peripheral blood leucocytes. PCRAmplification, Sequencing and Analysis using Manual and SeqScape software was dobe in Belfast, UK

Sequence Variant Assessment was done with Myocilin allele-specific glaucoma phenotype database (http://www.myocilin.com/) and PolyPhen-2 and Mutation Taster algorithms.

Structural Effects were evaluated for amino acid substitutions
Conservation Analysis done to assess conservation of affected amino acids across species.

Results - The MYOC mutational load in this cohort was lower 4.5% which suggests further heterogenicity. A 32yr old patient with severe JOAG had two MYOC mutations (compound heterozygote, MYOC Mutations:
Known Pathogenic Mutation: c.1279G>A; p.Ala427Thr
Novel Mutation: c.1129A>G; p.Thr377Ala
The affected patient had three children in whom MYOC was sequenced. The compound heterozygote mutation was inherited by two children.

Conclusion - Cascade screening helps identification of at risk family members and directs targeted surveillance.The MYOC mutational load in this cohort was lower 4.5% which suggests further heterogenicity.

References –

- Wiggs, JL. et al. (1995). Clinical features of five pedigrees genetically linked to the juvenile glaucoma locus on chromosome 1q21-q31. *Ophthalmology* 102(12), 1782-89.
- Johnson, AT. et al. (1993). Clinical features and linkage analysis of a family with autosomal dominant juvenile glaucoma. *Ophthalmology* 100, 524-29.
- Wiggs, JL. et al. (1994). Genetic linkage of autosomal dominant juvenile glaucoma to 1q21-q31 in three affected pedigrees. *Genomics.* 21, 299-303.
- Sheffield, VC. et al. (1993). Genetic linkage of familial open angle glaucoma to chromosome 1q21-q31. *Nat. Genet.* 4, 47-50.
- Richards, J E. et al. (1994). Mapping of a gene for autosomal dominant juvenile-onset open-angle glaucoma to chromosome 1q. *Am. J. Hum. Genet.* 54(1), 62-70.
- Stone, E M . et al. (1997). Identification of a gene that causes primary open angle glaucoma. *Science.* 275, 668-70.
- Hewitt, AW., and Craig, JE. (2006). Complex genetics of complex traits: the case of primary open-angle glaucoma. *Clin Experiment Ophthalmol.* 34(5), 472-84.
- Adam, MF. et al. (1997). Recurrent mutations in a single exon encoding the evolutionarily conserved olfactomedin-homology domain of TIGR in familial open-angle glaucoma. *Hum Mol Genet.* 6, 2091-7.
- Resch, ZT. and Fautsch MP. (2009). Glaucoma-associated myocilin: A better understanding but much more to learn. *Exp. Eye. Res.* 88(4), 704-14.