Summary

Glaucoma affects approximately 70 million people and is the second leading cause of irreversible blindness worldwide. Among the various subtypes, Primary open-angle glaucoma (POAG) is the most common form of this disease. POAG is a multifactorial complex disorder where both environmental and genetic factors precipitate the disease. It has been suggested that 72% of POAG cases have some familial component, but on rare occasion it follows a Mendelian pattern of inheritance. It is characterized by progressive loss of retinal ganglion cells and atrophy of the optic nerve head, often associated with elevated intraocular pressure (IOP) caused by the reduced outflow of aqueous humor through the trabecular meshwork (TM), a meshwork of tissue lining the outflow pathway at the iridocorneal angle of the anterior chamber of the eye.

To date, 33 loci have been reported to be linked with POAG, but only four underlying genes have been identified: viz. Myocilin (MYOC), Optineurin (OPTN), WDR36 and NTF4. In addition, recent studies show involvement of CYP1B1 in POAG, in spite of its being a candidate gene for primary congenital glaucoma (PCG). MYOC has been reported to be responsible for 2-4% of the adult onset cases of the disease and estimated to be responsible for 33% of juvenile open angle glaucoma (JOAG), whereas involvement of OPTN, WDR36, and NTF4 with POAG appears to be more restricted based on the investigation reported so far. A second major risk factor for glaucoma is aging. It has been observed that the progression and incidence of glaucoma increases with age even at baseline IOP. This suggests that the vulnerability of the optic nerve gradually increases with aging, which ultimately results in the death of the retinal ganglion cells and degeneration of the optic nerve. Such pathophysiology has also been observed in aged rodents. To date, no mechanism has been elucidated which explains the relationship between age and neuronal vulnerability. However, there is increasing evidence which suggests that oxidative stress and mitochondrial dysfunction may play a key role in predisposing to neuronal cell death in age-related neurodegenerative diseases such as glaucoma. My research plan entails molecular genetics studies to unravel the disease causing variants and functional implication of the suspected variants to understand the disease pathogenesis.

The objectives of my study for this dissertation are:
1. Analysis of the causal involvement of MYOC in Eastern Indian POAG patients with a familial history of glaucoma or high intraocular pressure.
2. Functional evaluation of variants identified in Myocilin and characterisation of the ERE elements present in the MYOC promoter for better understanding of its function and to delineate its possible role in glaucoma pathogenesis.

3. Screening of mitochondrial genome of POAG patients and controls for nucleotide variants with potential for disease pathogenesis.

The study on the eastern Indian POAG cohort (n=450) from this group led to identification of 17 changes including 10 novel variations, 3 reported mutations and 4 reported polymorphisms in MYOC. The mutations include 5 nonsynonymous changes, 2 deletions and 1 nonsense mutation identified in 12 patients (2.6%), which is consistent with the world average (2-4%). Most of the mutations (5/8) are located in exon 3 which codes for the olfactomedin domain, reported to be important for the functionality of the protein. Among all the mutations reported so far from India Gln48His is most common and has not been detected yet in any other country. In the patient cohort I screened, Gln48His was found in 4 POAG patients. This study, according to our knowledge, is the largest study to date carried out in a single cohort in the Indian population.

Two myocilin mutants Gln48His and Pro370Leu (highly penetrant mutation across the world) were selected for downstream functional studies. Triton-X solubility assay showed that the Gln48His form significant aggregates and remain insoluble in Triton-X similar to Pro370Leu (positive control). These aggregates were inferred to give rise to Endoplasmic Reticulum (ER) stress as evident from the level of p-eIF2α. With the attempt to identify the potential cell death mechanism underlying MYOC mutant mediated pathogenesis we identified that myocilin mutants trigger autophagy in trabecular meshwork cells, as observed by the conversion of LC3-I to LC3-II, the hallmark of autophagy and Beclin 1 upregulation. However, further studies are required for substantiating the observation.

Over expression of wild type myocilin is also believed to be involved in glaucoma pathogenesis. In a previous study from the lab using deletion constructs of MYOC promoter region with reporter gene, it was observed that estrogen response elements (EREs) upstream to MYOC gene are functionally active (Ref: PhD Thesis of Dr. Suddhail Mookherjee, IICB, Kolkata). As a follow up of the study, it was further shown that estrogen receptor (ER) competitor 4-OH tamoxifen could inhibit the effect of 17β-estradiol stimulation of reporter gene by MYOC promoter construct. Also, an increase in myocilin level was found in TM cells on 17β estradiol treatment providing
support to the observation made by reporter gene assay. The direct evidence for physical interaction of 17\(\beta\)-estradiol-ER\(\alpha\) complex with the EREs on MYOC promoter region was obtained by chromatin immune precipitation (ChIP) assay.

To investigate the possible involvement of the mitochondrial genomic variants in POAG pathogenesis; an unexplored territory especially in Indian population, we evaluated the entire mitochondrial genome in 101 POAG patients and 71 controls. The preponderance of transversion changes was higher in the patients than controls. Analysis of the coding region showed that the non-synonymous changes in Complex I of mtDNA are more frequent in patients compared to control. ND5 represents the potential gene where 48\% of the changes clustered in patients compared to 31\% in controls. This study for the first time identifies ND5 as the potential gene involved in POAG. In addition, the frequency of changes in 12SrRNA was significantly higher in patients.

In addition to the studies presented in the dissertation, I have been involved secondarily, along with other co-workers, in various studies to investigate the etiology of POAG. These include analysis of the role of WDR36 (Mookherjee et al, Mol Vis, 2011), CYP1B1 (Bhattacharjee et al, Mol vis, 2008) and IL-1 (Mookherjee et al, BMC Med Genet, 2010) in POAG pathogenesis among Indian patients. In addition, I participated in a study elucidating the possible common pathway where MYOC and CYP1B1 might interact in a “digenic” inheritance in POAG (Mookherjee et al, Under Revision)

The published papers and communicated manuscripts from the studies I have been involved are listed below:


2. Suddhasil Mookherjee, Moulinath Acharya, **Deblina Banerjee**, Ashima Bhattacharjee, Kunal Ray. Molecular basis for involvement of CYP1B1 in Myocilin mediated glaucoma pathogenesis (*Under Revision*).


Summary


7. Indian Genome Variation Consortium. Genetic landscape of the people of India: a canvas for disease gene exploration. *Journal of Genetics*, 87,1,:3-20, 2008. [Contributed in the study as a member of Indian Genome Variation Consortium and as mentioned in the paper].

8. EGLN1 involvement in high-altitude adaptation revealed through genetic analysis of extreme constitution types defined in Ayurveda. *Proc Natl Acad Sci*; 2010, 107(44):18961-6. [Contributed in the study as a member of Indian Genome Variation Consortium and as mentioned in the paper].