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GENETIC & MOLECULAR BASIS OF PRIMARY OPEN ANGLE GLAUCOMA (POAG) PATHOGENESIS

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ABSTRACT

Herein we compile the different studies regarding the cause of primary open angle glaucoma (POAG). The apoptotic theory of glaucoma suggests that ganglion cells contain protein receptors that, when activated by glutamate, increase intracellular calcium to toxic levels, forming destructive free radicals (ROS) that kill the cells. Neurotrophic nutrient deprivation theory reveals that the deprivation of vital neurotrophic nutrients for the retinal ganglion cells from the lateral geniculate nucleus. We herein also report some of the studies involving structural changes which occur in N-terminal of the myocillin involved extensive insertions and deletions while the C-terminal region involved point mutations, which may also lead to POAG. We also report herein the molecular biological studies, which suggest that mutant protein could be present through heteroaggregates in the aqueous humor (AH) and uveoscleral AH outflow of glaucoma patients that play an important role in the glaucoma pathogenesis. Another study was considered here about the endoplasmic stress that reveals that myocilin-associated POAG can be considered as an ER storage disease, occurring in steps that involves expression of misfolded and non-secreted myocilin, subsequent TM cell death. From the above evidences it might be intriguing to evaluate whether myocillin mediated ER stress induces autophagy in TM cells.

KEYWORDS: POAG, Glaucoma, Myocillin, Mutations, Locus, ER stress.

1. INTRODUCTION

Glaucoma is the chronic, progressive eye disease caused by the damage of the optic nerve, which leads to the visual field loss. It is the second leading cause followed by age related macular degeneration in the western world and cataract in the Indian subcontinent. Trachoma, other corneal opacities, childhood blindness and diabetic retinopathy are among the other known causes of visual impairment in approximately equal magnitude (approx. 4-5%). According to a study carried out by WHO, 161 million people are visually impaired. Among them 37 million are blind (best-corrected visual acuity less than 3/60 in better eyes) and 124 million people having visual impairment less severe than blindness (best-corrected acuity less than 6/18 to 3/60 in the better eye). This data is widely quoted but the uncorrected refractive error as a cause of visual impairment was excluded from the studies. One of the meta-analysis was carried out which include the uncorrected refractive error as a cause of visual impairment, estimates that worldwide about 259 million people are visually impaired with 42 million being blind.[1]

In India, the estimated numbers of blind people, as per the study carried out in 2002, is 6.7 million. According to the study, cataract accounts for 62.4% of the blindness in the people above 50 years of age and glaucoma is the second leading cause of blindness accounting for 9.58% of the cases.[2] This study was deliberately done on people having above 50 years of age because a previous study[3] indicated that more 90 % of the blind people in India belong to this age group. This study also indicated that people over 70-years of age have the greatest risk of blindness.

According to the W.H.O report, 9% of the total blind population in the Indian subcontinent (India, Bangladesh, Nepal and Pakistan) is glaucomatous. According to the latest estimate approximately 12 million people were predicted to be affected with glaucoma by 2010 in India, and with rapidly increasing aging population this figure is expected to rise up to 16 million by 2020.[4] There are multiple reports of region wise population based survey on the prevalence and types of glaucoma in India. Though both Andhra Pradesh and Tamil Nadu are in the southern part of India, the age standardized prevalence of glaucoma in individuals having an age of 50 years or

more is comparatively higher in Andhra Pradesh (6.1%) than in Tamil Nadu (3.2%). A separate study carried out in 4800 subjects in rural Tamil Nadu reports glaucoma to be responsible for 3.79% blindness and 4.29% of bilateral blindness. The same group has reported that the incidence of Primary Open Angle Glaucoma, the most common of the glaucoma subtypes, is more prevalent among urban population than rural populations of Tamil Nadu. Literature reported the incidence of glaucoma to be 3.58% in central India. In the eastern part of India, the prevalence of glaucoma in individuals having age of 50 years or more is 3.4% which is comparable with the single report from Bangladesh (2.4%).[5]

2. Theories supporting primary open angle glaucoma

Some glaucoma patients exhibit elevated levels of the neurotransmitter glutamate within the vitreous. Ganglion cells contain protein receptors that, when activated by glutamate, increase intracellular calcium to toxic levels, forming destructive free radicals (ROS) that kill the cells. This is the *apoptotic theory of glaucoma* - a neurocellular process in which a retinal ganglion cell commits suicide.[6] Another possible cause is *neurotrophic nutrient deprivation theory* in which the deprivation of vital neurotrophic nutrients for the retinal ganglion cells from the lateral geniculate nucleus. The vital nutrient - brain derived neurotrophic factor (BDNF) reaches the retinal ganglion cells from the lateral geniculate nucleus via axoplasmic transport. Elevated intraocular pressure (IOP) and ischemia disrupt axoplasmic transport and deprive the retinal ganglion cells of this vital nutrient.[7] The exact role that IOP played in combination with these other factors and their significance in the initiation and progression of subsequent glaucomatous neuronal damage and cell death over time is still under debate. Glaucoma is now being considered not only as an eye disorder but also as a neurodegenerative disease as it has some commonality with the neurodegenerative disorders like Alzheimer's disease and Parkinson Disease. The drugs which work to prevent Alzheimer's disease can be used to treat glaucoma. This disease does not only affect the retinal nerve fibre layer but also has its extended effects on optic visual sensory pathways of the brain. The recent drugs not target to lower the IOP but to rescue the ischemic condition of the optic nerves by supplying more blood and nutrients to them, which protects the nerve cells from degeneration.

3. Genetic & Molecular basis of primary open angle glaucoma

POAG is transmitted both as a monogenic as well as a complex disease. As mentioned previously, in juvenile and adult onset POAG, genetic linkage analysis in the affected families clearly suggests autosomal dominant inheritance with incomplete penetrance. A recent study suggests that 72% of all the POAG cases have an inherited component in it.[8] Approximately 50% of POAG patients have a positive family history and first-degree relatives of an affected individual have a 3-9 fold increased risk of developing the disease.[9] Pedigrees with familial POAG displaying an autosomal dominant pattern of inheritance with incomplete penetrance and variable expressivity have been described.[10] The varied and complex phenotype suggests POAG has a multi-factorial aetiology and is likely to involve the interaction of one or more genes with environmental factors.[11] It has also been suggested that adult onset POAG is inherited as a non-Mendelian trait, whereas juvenile onset POAG exhibit autosomal dominant inheritance.[12] The genetic relationship of POAG, NTG and JOAG is not yet clear since any given OAG family may show one predominant diagnosis while some family members may have one or both of the other diagnoses.[13] Genotype as well as phenotype studies among glaucoma genes identified so far indicate that in many families, cases of POAG may be identical by descent with the predominant JOAG or NTG cases in those families.[14]

Till date 17 POAG loci have been reported in OMIM database but studies have indicated further heterogeneity in hereditary glaucoma. A genome-wide linkage scan carried out on 182 affected sub-pairs identified six additional regions (19q12, 17q25.1-17q25.3, 14q11.1-14q11.2, 14q21.1-q21.3, 17p13, 2p14) of interest.[15] Additional regions of the genome (10p12.33-p12.1, 2q33.1-q33.3) showed moderate evidence for linkage to OAG in a genome scan of participants in the Barbados Eye Study.[16] Eight Finnish families with POAG were genotyped at glaucoma loci GLC1A-GLC1F and eight other candidate gene regions. Evidence for linkage was not found in any of the tested regions.[17] Report from 2 Chinese families with JOAG indicates a possible linkage with 2p15-16 region, which overlaps with another adult onset POAG locus, GLC1H.[18] An SNP based linkage study identified novel linkage regions on chromosomes 1 and 20, and replicated two previously described loci-GLC1D on chromosome 8 and GLC1I on chromosome 15.[19]

Four of the mapped POAG genes have so far been identified: *Myocilin* (MYOC, MIM601652) at the GLC1A locus,[20] *Optineurin* (OPTN, MIM602432) at the GLC1E locus,[21] *WD-repeat domain 36* (WDR36) at GLC1G[14] and *NTF4* at GLC10.[22] Mutations in MYOC are the most common cause of POAG but still only account for 4% of adult onset cases and 6-36% of juvenile onset cases.[23] Mutations in OPTN are a rare cause of POAG and probably account for less than 1% of the cases.[11] A small number of mutations in OPTN are found in families in which most affected individuals have NTG.[21] Mutations in WDR36 are the first to be found segregating through families in which the more prevalent adult onset glaucoma involving elevation of IOP is predominant.[14]

Genetic linkage studies revealed that PCG mapped to three different loci, *GLC3A* (OMIM # 231300) located at chromosome 2p21,[24] *GLC3B* (OMIM no. 600975, Gene bank accession no. NM_000104) located at chromosome

1p36[25] and *GLC3C* located at chromosome 14q24.3.[26] A study from Pakistan identified a new PCG locus at 14q24.2-24.3.[27] The causal gene at GLC3A locus has been found to be CYP1B1, which encodes cytochrome P450 enzyme.[28] Following the discovery of association between CYP1B1 gene and PCG locus (GLC3A), 42 missense mutations and a few polymorphisms have been identified so far in different populations. It is interesting to note that a substantial portion of the mutations (23/65) also include deletion/insertion implying an inherent instability of the gene. In India, the largest study on PCG has been conducted by L.V. Prasad Eye Institute.[29] Most of the mutations in CYP1B1 were identified in a common haplotype background suggesting a strong founder effect.[30] In addition, a possible digenic mode of PCG causation has also been suggested with CYP1B1 and MYOC mutations.[29]

4. Myocillin structural changes as the cause of POAG

A consensus modeling approach showed that myocilin is structurally characterized of three main regions (i) a N-terminal myosin-like coiled-coil region including a leucine-zipper (between amino-acids 117 and 169); (ii) a flexible linker region (between amino-acids 202 and 243); (iii) a C-terminal OLF domain (between amino-acids 246 and 504).[31] Functional analysis of myocilin showed that the integrity of amino-terminal coiled-coil regions and olfactomedin homology domain are essential for extracellular adhesion and secretion, the N-terminal region being also important for extracellular interactions (ECM and/ or cell surface).[32] Olfactomedin is a secreted polymeric glycoprotein of unknown function, originally discovered at the mucociliary surface of the amphibian olfactory neuro-epithelium and later identified throughout the mammalian brain. The study based on comparison of protein sequences revealed that the evolution of the N-terminal half of the molecule involved extensive insertions and deletions while the C-terminal region evolved mostly through point mutations. It has been reported that myocilin interacts with the regulatory light chain (RLC) of myosin, a component of the myosin motor protein complex, independent of its olfactomedin domain, which implies a role for myocilin in the actomyosin system.[33] One of the studies provided a detailed solution biophysical characterization of MYOC-OLF. MYOC-OLF was found to be stable in the presence of glycosaminoglycans, as well as in a wide pH range in buffers with functional groups reminiscent of such glycosaminoglycans.[34] Circular dichroism (CD) reveals significant P-sheet and p turn secondary structure. Limited proteolysis combined with mass spectrometry revealed that the compact core structural domain of OLF consists of residues 238-461, approximately, which retains the single disulfide bond and is as stable as the full MYOC-OLF construct.

5. Molecular basis of pathogenesis caused by MYOC

The recent studies hypothesized that mutant forms of myocilin are not secreted from the cells and can decrease the secretion of the native protein when two forms are co-expressed.[35] Mutant protein was found to be Triton X100 insoluble, while normal protein was completely soluble. Based on this assay, it was hypothesized that wild-type myocilin can form dimers and possibly multimers and that mutant protein might interfere with wild-type protein through formation of heteromultimers.[36] In order to elucidate the effect of wild-type myocilin on secretion of the mutant protein, a report co-expressed both wild type and four of the mutant myocilin (Gln368Stop, Glu233Lys, Asp380Ala and Pro370Leu) in HEK293 cell line, mimicking the state of heterozygosis in a cell based assay.[37] Their cellular model of heterozygosis showed that co-expression of wild-type and mutant myocilins increases significantly the presence of extracellular mutant molecules and reduces the amount of either extracellular full length or processed wild-type myocilin. These data suggests that the mutant protein could be present through heteroaggregates in the aqueous humor (AH) and extracellular matrix of the TM and uveoscleral AH outflow of glaucoma patients, playing pivotal roles in the pathogenesis of glaucoma.

6. Endoplasmic Reticulum (ER) Stress Response

Mutations in proteins that induce misfolding and proteasomal degradation are common causes of inherited diseases.[38] It has been found that POAG-causing myocilin mutants were misfolded, highly aggregation prone, accumulated in large aggregates in the rough ER of human differentiated primary TM cells[39] and formed typical Russel bodies.[40] Also aggregation of MYOC in the ER activates the unfolded protein response (UPR) in Drosophila disease model.[41]

In TM cells, Pro370Leu mutant myocilin, reported to cause the most severe glaucoma phenotype, was not secreted under normal culture conditions (37°C), and prolonged expression resulted in abnormal cell morphology and cell killing. However, culture of TM cells at 30°C facilitated myocilin folding, promoted secretion of mutant myocilin, normalized cell morphology and, reversed cell lethality.[39] By semi-quantitative PCR analysis,[42] has shown attenuation of ER molecular chaperone Glucose-Regulated Protein (GRP78), which indicates that Pro370Leu mutant MYOC down regulates ER stress response, thereby perturbing the protective mechanism and increasing the vulnerability of HTM (human trabecular meshwork) cells to ER stress. ER stress-induced apoptosis is a pathway to explain the reduction of TM cells in patients with myocilin-caused glaucoma.[40] From these observations, myocilin-associated POAG can be considered as an ER storage disease, consisting in a progression of events that involves chronic expression of misfolded and non-secreted myocilin, subsequent TM

cell death, TM dysfunction and impediment of aqueous humor outflow leading to elevated IOP.[39,40]

In accordance with this observation, several glaucoma-associated MYOC mutations including the Pro370Leu, inhibited calpain II dependent-endoproteolytic processing of full-length myocilin, normally releasing two fragments of ~20 kDa (N-terminal part) and ~35 kDa (C-terminal part), resulting in accumulation of insoluble mutant myocilin aggregates in the ER.[37] This cleavage might regulate extracellular and matricellular protein interactions (e.g., myocilinhevin),[43] contributing to the control of IOP,[44] notably by decreasing myocilin homo-aggregates.[45] Several recent studies have reported that ER stress induces autophagy in mammalian cancer cell lines and mouse embryonic fibroblasts.[46] Autophagy is the cell's major regulated mechanism for degrading long-lived proteins and the only known pathway for degrading organelles.[47] From the above evidences it might be intriguing to evaluate whether myocillin mediated ER stress induces autophagy in TM cells.

CONCLUSION

Mutations in the myocillin gene are the most common cause of primary open angle glaucoma (POAG) mapped as the GLC1A locus and many other mutations have been reported worldwide. Predominantly theories were explaining the cause of POAG like apoptotic theory of glaucoma, Neurotrophic nutrient deprivation theory, etc. Structural studies depict that changes in N-terminal of the myocillin involved extensive insertions and deletions while the C-terminal region involved point mutations. Molecular studies suggest that mutant protein could be present through heteroaggregates in the aqueous humor (AH) and extracellular matrix of the TM and uveoscleral AH outflow of glaucoma patients, playing pivotal roles in the pathogenesis of glaucoma. Cytological studies reveal that myocilin-associated POAG can be considered as an ER storage disease, consisting a progression of events that involves chronic expression of misfolded and non-secreted myocilin, subsequent TM cell death, TM dysfunction and impediment of aqueous humor outflow leading to elevated IOP. From the above evidences it might be intriguing to evaluate whether myocillin mediated ER stress induces autophagy in TM cells.

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