Introduction
1.1. VISION AND BLINDNESS

Eyes are nature's greatest creation. Rays of light illuminate and unravel the mysteries of nature for eyes to see and mind to appreciate. Sight is essential for creatures. For all of us the eye provides the most important link to the world by enabling us to visualize shapes and colors. Some animals and plants only sense changes in light without seeing specific images. The eye and brain process visual information to link our inner being to the world and beyond. Hence to preserve this immense spectacle of nature, man has engaged himself to fight the curse of blindness for ages.

1.2. CAUSES OF VISUAL IMPAIRMENT

1.2.1. GLOBAL SCENARIO

According to the global data on visual impairment, the largest proportion of blindness is related to aging. Globally cataract is the leading cause of blindness accounting for almost half of the cases. Glaucoma 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) (Resnikoff et al. 2004) (Figure 1.1, Panel A). This data is widely quoted but the uncorrected refractive error as a cause of visual impairment was
excluded from the study. A recent meta-analysis carried out by Dandona et al, which include the uncorrected refractive error as a cause visual impairment, estimates that worldwide about 259 million people are visually impaired with 42 million being blind (Dandona et al. 2006).

1.2.2. INDIAN SCENARIO
In India, the estimated numbers of blind people, as per the study carried out in 2002, is 6.7 million (Balasubramanian 2002). According to the study carried out by Murthy et al in 2005, 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 (Murthy et al. 2005). This study was deliberately done on people having above 50 years of age because a previous study carried out by Mohan et al in 1989 indicated that more 90% of the blind people in India belong to this age group (Mohan 1989). This study also indicated that people over 70-years of age have the greatest risk of blindness (Figure 1.1 panel B).

The present study focuses on the second largest blinding disorder “glaucoma”, for better understanding and management of the disease pathogenesis.

1.3. GLAUCOMA
1.3.1. PREVALENCE OF GLAUCOMA
As stated in the previous section, Glaucoma is the second leading cause of blindness globally. According to the report by Quigley et al in 1996, glaucoma affects about 67 million people worldwide (Quigley 1996). In a much recent study, Quigley and Broman in 2006 estimated that there will be 79.6 million with primary glaucoma by 2020 and bilateral blindness will be present in 11.2 million people (Quigley et al. 2006).

The study carried out by Balasubramanian et al in 2002 estimated that about 1.52 million people in India are blind due to glaucoma (Balasubramanian 2002). According to the W.H.O report, 9% of the total blind population in the Indian subcontinent (India, Bangladesh, Nepal and Pakistan) is glaucomatous (Resnikoff et al. 2004). According to the latest estimate by Quigley et al in 2006, 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 (Quigley et al. 2006). 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%) (Dandona et al. 2000; Ramakrishnan et al. 2003). 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 (Vijaya et al. 2006). 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 (Ramprasad et al. 2008). Palimkar et al reported the incidence of glaucoma to be 3.58% in central India (Palimkar et al. 2008). In the eastern part of India, the prevalence of glaucoma in individuals having age of 50 years or more is 3.4% (Raychaudhuri et al. 2005) which is comparable with the single report from Bangladesh (2.4%) (Rahman et al. 2004).

1.3.2. Historical Perspective of Glaucoma

Glaucoma - the term was first coined by Hippocrates in approximately 400 BC. In the Hippocratic Aphorisms the term glaucoma was used to describe blindness coming on in advancing years associated with a glazed appearance of the pupil. The word glaucoma came from ancient Greek, meaning “clouded or blue-green hue”, most likely describing a person with a swollen cornea or who were rapidly developing a cataract, both of which may be caused by long-term elevated pressure inside the eye. The Hippocratic writings did not make a clear distinction between cataract and glaucoma. Both Classical and Alexandrian Greeks did not recognize the specific disease that we now call glaucoma. In etymological lexicons, one finds entries such as: glaucoma - 1643, from Gk. Glaucoma "cataract, opacity of the lens" (cataracts and glaucoma were not distinguished until 1705 AD).

The definition of glaucoma has changed drastically over time. The first recognition of a disease associated with a rise in intraocular pressure and thus corresponding to what is now known as glaucoma occurs in the Arabian writings, “Book of Hippocratic treatment”, of At-Tabari (10th century). In European writings, it is Dr. Richard Bannister (1622), an English ophthalmologist and author of the first book of ophthalmology in English, who makes the first original and clear recognition of a disease with a tetrad (four) of features: eye tension, long duration of the disease, the absence of perception of light and the presence of a fixed pupil.

It was not until the beginning of the 19th century that the first excellent description of glaucoma with raised ocular tension was given by the French physician Antoine-Pierre Demours (1818). Thereafter, the central concept of a rise in the
Intraocular pressure (IOP) became fully established. In London, Dr. G.J. Guthrie (1823) recognized hardness of the eye as a characteristic feature of glaucoma. Finally, the essential feature of raised eye tension was fully established by Dr. William McKenzie, a Scottish clinician (1835) who, in the second edition of his classical and widely read textbook, ascribed the raised tension in both chronic and acute glaucoma. The final clinical observation in this era was the unifying concept of Dr. Donders (1862) where he described an incapacitating increased eye tension occurring without any inflammatory symptoms as Simple Glaucoma. The concept of glaucoma has been further refined, particularly over the last 100 years. Dr. Drance (1973) provided for the first time the definition of glaucoma as a disease of the optic nerve (Drance 1973) caused by numerous factors, called risk factors (courtesy: Dr. Nick Mantzioros, Ophthalmologist, Melbourne from http://www.glaucoma.org.au).

Currently, glaucoma refers to a heterogeneous group of optic neuropathies, with a complex genetic basis. It is a multifactorial optic disc neuropathy in which there is a characteristic acquired loss of retinal ganglion cells and atrophy of the optic nerve. These neuropathies gradually reduce vision (Figure 1.2) without warning and often without symptoms. At least half of the affected individuals are unaware of having it for lack of symptoms; making untreated glaucoma a leading cause of irreversible blindness.

1.3.3. Present Concepts and Diagnosis

Glaucoma is currently defined as a disturbance of the structural or functional integrity of the optic nerve that causes characteristic atrophic changes in the optic nerve, which may also lead to specific visual field defects over time (Figure 1.2 & Figure 1.3). The front part of the eye is filled with a clear fluid (called aqueous humor) made
by the ciliary body. Normal intraocular pressure (IOP) levels range from 12-15 mm Hg (millimeters of mercury). Positive pressure, generally above 6 mm Hg, is needed to keep the retina attached to the back of the eye and to nourish the cornea and lens which have no blood vessels. This fluid flows out the eye’s drainage system (trabecular meshwork of drainage canals around the outer edge of the iris). Production, flow, and drainage of this fluid, is an active, continuous process. In most types of glaucoma, the eye’s drainage system becomes clogged so the fluid cannot drain out. As this fluid builds up, it causes increased pressure inside the eye.

Axons of retinal ganglion cells travel through the optic nerve carrying images
from the eye to the brain. Damage to these axons causes ganglion cell death with resultant optic nerve atrophy and patchy vision loss. Elevated IOP (in unaffected eyes, the average range is 11 to 21 mm Hg) plays a role in axonal damage, either by direct nerve compression or diminution of blood flow. However, the relationship between pressure and nerve damage is variable. Of people with IOP > 21 mm Hg (i.e. ocular hypertension), only about 1 to 2% per year (about 10% over 5 yr) develop glaucoma. Additionally, about one third of patients with glaucoma do not have IOPs > 21 mm Hg (known as low-tension glaucoma or normal-tension glaucoma). In general, the outflow pathway of the fluid from the eye is blocked or obstructed which cause a rise in the IOP resulting in degeneration of the optic nerve heads, a condition known as 'glaucomatous cupping' (Figure 1.4). The generic term glaucoma should only be used in reference to the entire group of glaucomatous disorders as a whole, because multiple subsets of glaucomatous disease exist.

Figure 1.4: The glaucomatous condition
Panel A: showing the direction of rising pressure in a glaucomatous eye;
Panel B: showing the route of aqueous humour outflow through trabecular meshwork (indicated by the blue arrow) along with the local anatomy;
Panel C: Comparison of the optic disc under normal and glaucomatous conditions (courtesy: website of National Eye Institute, USA).
Glaucoma patients have characteristic loss of visual fields, which enlarge as the disease progresses. Thus, glaucomatous optic neuropathy may not be a chronic degeneration of the whole of optic nerve and ganglion cell stomata but rather a series of acute losses of individual, or groups of, ganglion cells. The neuro-protecting agents should render some beneficial effects against the progression of ganglion cell

![Nerve Fibre thickness Map](image1)

![Deviation Map](image2)

**Figure 1.5:** Evaluation of glaucomatous thinning by scanning laser polarimetry (SLP). Panel A: output data of SLP analysis for glaucomatous and normal eyes to generate nerve fiber thickness map and deviation map. Thinning of nerve fiber was plotted on deviation map for proper quantification of the extent of thinning. Arrows in the nerve fiber-thinning map of the glaucomatous person indicate the area of thinning. The letters T, S, N & I in the map correspond to the four quadrants—temporal, superior, nasal and inferior— in which the eye is divided to define its anatomical position. Panel B: Evaluation of visual field damage by automated perimetry of a typical glaucoma patient; In the left, the color scheme used in graded scale to estimate the field damage is shown (red, damage; and blue, normal).
Introduction
dearth in glaucoma because of its characteristic slow and variable rate of
degeneration (Osborne et al. 1999).

The definition of glaucoma has evolved over time. Previously one of the main
criteria for the diagnosis of glaucoma was elevated intraocular pressure. But later it
was observed that some people show definitive glaucomatous degeneration in retinal
nerve fiber layer (RNFL) while having normal intra-ocular pressure (IOP). Thus, it is
believed nowadays that the primary and sufficient condition to diagnose a patient
with glaucoma is not only the IOP and cupping of the optic disc but also the
characteristic visual field damage and detection of retinal nerve fibre layer loss.

It is estimated that there are approximately one million nerve fibres in a
normal eye. Normal individuals lose ganglion cells with age at an estimated rate as
high as 5000 axons per year, which may translate to a considerable axon loss during
a 70 years life span. In glaucomatous eye these cells are lost at an accelerated rate.
However, the inbuilt redundancy in the visual system makes it difficult to ascertain
retinal nerve fibre damage with traditional diagnostic methods, until a significant
proportion of ganglion cells (>50%) have been lost (Quigley et al. 1982).

Recent advances in optical imaging technologies have resulted in techniques
that provide measurements of optic disc and retinal nerve-fibre layer (RNFL) loss
with micron scale sensitivity e.g. Visual field analysis, Scanning laser polarimetry,
Heidelberg retinal tomography. Visual field analysis is an indirect method of RNFL
analysis. In this method no definitive loss become apparent before 50-70% of the
retinal nerve fiber is lost. But by Scanning laser polarimetry and Heidelberg retinal
tomography, it is possible to recognize the RNFL loss almost 5 years ahead of
detection by Visual field analysis. Scanning Laser Polarimetry (SLP) can detect early
minimal loss of optic nerve axons among many high-risk patients who are apparently
normal by standard procedures. In Figure 1.5, SLP results of a normal and an
affected individual have been shown.

1.3.4. Subtypes of Glaucoma

Glaucoma can be classified according to etiology (primary vs. secondary), anatomy
of the anterior chamber (open angle vs. closed angle) and the time of onset (infantile
vs. juvenile vs. adult) (Sarfarazi 1997). In general glaucoma is broadly classified into
three major groups: (i) Primary Open Angle Glaucoma, (ii) Primary Congenital
Glaucoma and (iii) Primary Angle Closure Glaucoma (Figure 1.6). In addition, a
number of ocular conditions that are generally postulated to be the result of an
abnormal differentiation of neural crest cells are also reported to be associated with
glaucoma (Table 1.1). Of special interest is pseudoxfoliating glaucoma which is the major form secondary glaucoma worldwide.

1.3.4.1. PRIMARY OPEN ANGLE GLAUCOMA (POAG)

Primary open angle glaucoma (POAG) is a phenotypically complex disease that causes progressive optic nerve degeneration resulting in irreversible blindness. Synergistic effects of environmental and genetic factors precipitate the disease. It is described distinctly as a multifactorial optic neuropathy that is chronic and progressive with a characteristic acquired loss of optic nerve fibers. Primary Open Angle Glaucoma is the most common of the glaucoma subtypes and will be emphasized in the subsequent sections.

1.3.4.1.1. Signs and Symptoms

Although early and moderate POAG patients are virtually asymptomatic, there are definitive signs: progressive enlargement of the optic cup at the expense of the neuroretinal rim (either diffusely or focally) and repeatable visual field loss. Other glaucomatous signs include elevated intracocular pressure in many cases, nerve fiber layer (NFL) loss, notching of the neuroretinal rim at the inferior or superior poles, advancing peripapillary atrophy and NFL hemorrhages emanating from the optic disc.

POAG may further be subdivided depending upon age of onset (juvenile or adult onset) and intraocular pressure (normal tension and high tension). The patients whose IOP is in the normal range (<21mm Hg) but still show definitive symptoms of glaucoma are classified as normal tension glaucoma (NTG) patients and those having IOP >21mmHg are high tension glaucoma (HTG) patients. Patients having
Introrfaicttorv glaucoma typically before 40 years of age are classified as juvenile open angle glaucoma (JOAG) and those having it after 40 years are classified as adult onset open angle glaucoma cases.

1.3.4.1.2. Pathophysiology
Even today much remains elusive about the pathophysiology of POAG. The exact cause of glaucomatous optic neuropathy is not known, although many risk factors have been identified viz.: elevated IOP, family history, race, age older than 40 years, and myopia.

Figure 1.7: Mechanism of Optic Nerve head cupping in glaucoma
Panel A: Schematic representation of optic disc cupping in glaucomatous condition. Panel B: The axons of RGC exit through lamina cribrosa. It is a porous structure which supports the RGC and is made up of elastin and collagen. Panel C: In glaucomatous condition when IOP is raised, it disrupts the Lamina Cribrosa resulting in blockage of retrograde and anterograde traffic through axons. Panel D: This phenomenon ultimately results in death of RGC and glaucomatous cupping.

Elevated IOP is the most studied of these risk factors because it is the main clinically treatable risk factor for glaucoma. Multiple theories exist concerning how IOP can be one of the factors that initiates glaucomatous damage in a patient. Two of the major theories include the following: (1) onset of vascular dysfunction causing ischemia to the optic nerve, and (2) mechanical dysfunction via cribiform plate compression of the axons.

Optic disc cupping is due to thinning of the nerve fiber layer. The lamina cribrosa (Figure 1.7 Panel B) is made of collagen and elastin and serves as a
supporting tissue for the transition from the intraocular contents to the optic nerve. The axons of the retinal ganglion cells (RGCs) go through the holes in the lamina cribrosa in bundles (Figure 1.7; Panel B). According to the mechanical theory of POAG, elevated IOP affects the structure of the lamina cribrosa (Figure 1.7; Panel C) in such a way so as to change the shape of the pores as a result of the cupping (Figure 1.7; Panel A; and Figure 1.4) associated with the glaucomatous process. This affects the axons of the RGCs, which are coursing through these pores. The blood vessel and RGC axon are essentially pinched off, which can result in a disruption in the transport of neurotrophins (Johnson 2000) along the axon as well as vascular ischemia, both of which may contribute to RGC death (Figure 1.7, Panel D). The vascular theory suggests that with elevated IOP, reduced blood flow to the optic nerve starves the cells of oxygen and nutrients.

Recent studies suggest another mechanism of ganglion cell death. 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 (Reactive oxygen species or ROS) that kill the cells. This is the apoptotic theory of glaucoma - a neurocellular process in which a retinal ganglion cell will commit "suicide." Another possible event is 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 IOP and ischemia disrupt axoplasmic transport and deprive the retinal ganglion cells of this vital nutrient. The exact role that IOP plays 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; the precise mechanism is still a hot topic of discussion.

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 (Guo et al. 1997). 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 (Figure 1.8). The recent drugs are no longer targeted only 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.
1.3.4.1.3. Risk factors for Primary Open Angle Glaucoma

The risk factors identified with primary open-angle glaucoma include the following:

1. Elevated intraocular pressure
2. Family history of glaucoma
3. Race (People of African American descent are at an increased risk)
4. Age above 40 years (The risk of glaucoma increases with age)
5. Nearsightedness (myopia)
6. Genetic factors
1.3.4.1.4. Molecular Genetics

POAG (OMIM # 137760) 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 (Sarfarazi 1997). A recent study by Gong et al suggests that 72% of all the POAG cases have an inherited component in it (Gong et al. 2007). 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 (Tielsch et al. 1994; Wolfs et al. 1998; Allingham et al. 2009). Pedigrees with familial POAG displaying an autosomal dominant pattern of inheritance with incomplete penetrance and variable expressivity have been described (Sarfarazi et al. 1998). The varied and complex phenotype (Tielsch et al. 1994) suggests POAG has a multi-factorial aetiology and is likely to involve the interaction of one or more genes with environmental factors (Challa 2008)

It has also been suggested that adult onset POAG is inherited as a non-Mendelian trait, whereas juvenile onset POAG exhibit autosomal dominant inheritance (Wiggs et al. 1998). 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 (Morissette et al. 1995; Richards et al. 1996; Shimizu et al. 2000; Monemi et al. 2005). Genotype-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 (Morissette et al. 1995; Richards et al. 1996; Shimizu et al. 2000; Monemi et al. 2005).

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 sib-pairs identified six additional regions (19q12, 17q25.1-17q25.3, 14q11.1-14q11.2, 14q21.1-q21.3, 17p13, 2p14) of interest (Wiggs et al. 2000). 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 (Nemesure et al. 1996). Eight Finnish families with POAG were genotyped at glaucoma loci GLC1A–GLC1F (Table 1.1) and eight other candidate gene regions. Evidence for linkage was not found in any of the tested regions (Lemmela et al. 2004). Report from 2 Chinese families with JOAG indicates a possible linkage with 2p15–16 region, which overlaps with another adult onset POAG locus, GLC1H (Lin et al. 2008). A recent SNP based linkage study by Crooks et al,
identified novel linkage regions on chromosomes 1 and 20, and replicated two previously described loci — GLC1D on chromosome 8 and GLC11 on chromosome 15 (Crooks et al. 2011). Together a total of 33 loci have been identified, the details of which is summarized in Table 1.1.

Four of the mapped POAG genes have so far been identified: Myocilin (MYOC, MIM601652) at the GLC1A locus (Stone et al. 1997; Nguyen et al. 1998), Optineurin (OPTN, MIM602432) at the GLC1E locus (Rezaie et al. 2002), WD-repeat domain 36 (WDR36) at GLC1G (Monemi et al. 2005) and NTF4 at GLC10 (Pasutto et al. 2009). Mutations in MYOC are the most common cause of POAG but still only account for 4% (Table 1.2) of adult onset cases (Shimizu et al. 2000; Alward et al. 2002) and 6-36% of juvenile onset cases (Shimizu et al. 2000). Mutations in OPTN are a rare cause of POAG and probably account for <1% of the cases (Challa 2008). A small number of mutations in OPTN are found in families in which most affected individuals have NTG (Rezaie et al. 2002). 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 (Monemi et al. 2005) but the involvement of this gene has been excluded by others (Hewitt et al. 2006; Fingert et al. 2007; Mookherjee et al. 2011) and the locus remains controversial (Allingham et al. 2009). Similarly, sequence variants in NTF4 have been excluded as a cause of POAG (Liu et al. 2010; Rao et al. 2010). Much remains to be understood about the underlying mechanisms by which mutations in these genes cause glaucoma and most cases cannot be accounted for by these three genes.

1.3.4.2. PRIMARY CONGENITAL GLAUCOMA (PCG)

Primary congenital glaucoma (PCG) is an ocular developmental anomaly that occurs due to the obstruction in the drainage of the aqueous humor outflow caused by the abnormal development of the trabecular meshwork (TM) and the anterior chamber angle (Kupfer et al. 1979).

1.3.4.2.1. Pathophysiology

It is a rare genetic disorder that usually manifests itself at birth or within the first year of life, but may emerge up to the age of three (Francois 1980; Gencik 1989; Dickens et al. 1996). Characteristic clinical features of PCG include tearing, photophobia, megalocornea, corneal opacity with characteristic "Haab's striae" and enlargement of the eyeball (buphthalmos meaning 'ox eye') resulting from elevated IOP.
Table 1.1: Genetic loci associated with glaucoma

<table>
<thead>
<tr>
<th>Glaucoma Subtype</th>
<th>Locus</th>
<th>Location</th>
<th>OMIM #</th>
<th>Gene (GenBank Accession#)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary Open Angle Glaucoma</td>
<td>GLC1O</td>
<td>12q14</td>
<td>613100</td>
<td>NTF4</td>
<td>(Pasutto et al. 2009)</td>
</tr>
<tr>
<td>GLC1P</td>
<td>4q35.1-q35.2</td>
<td></td>
<td></td>
<td></td>
<td>(Fingert et al. 2001)</td>
</tr>
<tr>
<td>GLC1Q</td>
<td>19q12</td>
<td></td>
<td></td>
<td></td>
<td>(Porter et al. 2011)</td>
</tr>
<tr>
<td>GLC1R</td>
<td>17q25.1-25.3</td>
<td></td>
<td></td>
<td></td>
<td>(Wiggs et al. 2000)</td>
</tr>
<tr>
<td>GLC1S</td>
<td>14q11.1-11.2</td>
<td></td>
<td></td>
<td></td>
<td>(Wiggs et al. 2000)</td>
</tr>
<tr>
<td>GLC1T</td>
<td>17p13</td>
<td></td>
<td></td>
<td></td>
<td>(Wiggs et al. 2000)</td>
</tr>
<tr>
<td>GLC1V</td>
<td>2q33.1-q33.3</td>
<td></td>
<td></td>
<td></td>
<td>(Nemesure et al. 2003)</td>
</tr>
<tr>
<td>GLC1W</td>
<td>2p14</td>
<td></td>
<td></td>
<td></td>
<td>(Wiggs et al. 2000)</td>
</tr>
<tr>
<td>GLC1X</td>
<td>1p32</td>
<td></td>
<td></td>
<td></td>
<td>(Charlesworth et al. 2005)</td>
</tr>
<tr>
<td>GLC1Y</td>
<td>10q22</td>
<td></td>
<td></td>
<td></td>
<td>(Charlesworth et al. 2005)</td>
</tr>
<tr>
<td>GLC1Z</td>
<td>2p15-16</td>
<td></td>
<td></td>
<td></td>
<td>(Lin et al. 2008)</td>
</tr>
<tr>
<td>GLC1AA</td>
<td>1q23.2</td>
<td></td>
<td></td>
<td></td>
<td>(Crooks et al. 2011)</td>
</tr>
<tr>
<td>GLC1AB</td>
<td>4q25</td>
<td></td>
<td></td>
<td></td>
<td>(Crooks et al. 2011)</td>
</tr>
<tr>
<td>GLC1AC</td>
<td>14q11.2</td>
<td></td>
<td></td>
<td></td>
<td>(Crooks et al. 2011)</td>
</tr>
<tr>
<td>GLC1AD</td>
<td>6q15</td>
<td></td>
<td></td>
<td></td>
<td>(Crooks et al. 2011)</td>
</tr>
<tr>
<td>GLC1AE</td>
<td>3q13.32</td>
<td></td>
<td></td>
<td></td>
<td>(Crooks et al. 2011)</td>
</tr>
</tbody>
</table>
### Table 1.1: Genetic loci associated with glaucoma (continued)

<table>
<thead>
<tr>
<th>Glaucoma Subtype</th>
<th>Locus</th>
<th>Location</th>
<th>OMIM#</th>
<th>Gene (GenBank Accession #)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary</td>
<td>GLC3A</td>
<td>2p21</td>
<td>231300</td>
<td>CYP1B1 (NM_000104)</td>
<td>(Sarfarazi et al. 1995)</td>
</tr>
<tr>
<td>Congenital glaucoma</td>
<td>GLC3B</td>
<td>1p36</td>
<td>600975</td>
<td>-</td>
<td>(Akarsu et al. 1996)</td>
</tr>
<tr>
<td></td>
<td>GLC3C</td>
<td>14q24.3</td>
<td>-</td>
<td>-</td>
<td>(Stoilov et al. 2002)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14q24.2-24.3</td>
<td>-</td>
<td>-</td>
<td>(Firasat et al. 2008)</td>
</tr>
<tr>
<td>Other anomalies associated with glaucoma</td>
<td>Locus</td>
<td>Location</td>
<td>OMIM#</td>
<td>Gene (GenBank Accession #)</td>
<td>References</td>
</tr>
<tr>
<td>Reiger syndrome</td>
<td>RIEG1</td>
<td>4q25</td>
<td>180500</td>
<td>PITX2 (NM_000325)</td>
<td>(Heon et al. 1995)</td>
</tr>
<tr>
<td></td>
<td>RIEG2</td>
<td>13q14</td>
<td>601499</td>
<td>-</td>
<td>(Vincent et al. 2001)</td>
</tr>
<tr>
<td>Iridogoniodygenesis</td>
<td>IGDA</td>
<td>6p25</td>
<td>601631</td>
<td>FKL7 (NM_144769)</td>
<td>(Phillips et al. 1996)</td>
</tr>
<tr>
<td></td>
<td>IRID2</td>
<td>4q25-q26</td>
<td>137600</td>
<td>PITX2 (NM_000325)</td>
<td>(Lehmann et al. 2000)</td>
</tr>
<tr>
<td>Pigment dispersion syndrome</td>
<td>PDS</td>
<td>7q35-q36</td>
<td>274600</td>
<td>-</td>
<td>(Andersen et al. 1997)</td>
</tr>
<tr>
<td>Nail-patella syndrome</td>
<td>NPS</td>
<td>9q34.1</td>
<td>161200</td>
<td>LMX1B (NM_002316)</td>
<td>(Dreyer et al. 1998)</td>
</tr>
</tbody>
</table>
Table 1.2: Mutations in MYOC gene

<table>
<thead>
<tr>
<th>Region of the gene</th>
<th>Domain</th>
<th>Missense and Nonsense changes</th>
<th>Population</th>
<th>Del/Ins changes</th>
<th>Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 1</td>
<td>Myosin-like (including leucine zipper domain)</td>
<td>9</td>
<td>Indian, Japanese, Chinese, Korean, American, Australian, Caucasian</td>
<td>2</td>
<td>African American, Caucasian</td>
</tr>
<tr>
<td>Exon 2</td>
<td>Linker region</td>
<td>None</td>
<td></td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Exon 3</td>
<td>Olfactomedin like</td>
<td>80</td>
<td>Indian, Japanese, Chinese, Korean, Spanish, French, Scottish, Irish, German Canadian Afro-American (NY), North American, Australian</td>
<td>3</td>
<td>American-Australian, Italian, African-American</td>
</tr>
</tbody>
</table>

The mutation details have been adapted from www.mycilin.com (Last Updated: 28th Sept, 2011)
1.3.4.2.2. Prevalence and Mode of Transmission
The incidence of PCG varies geographically, for example 1:10,000 in western countries (Francois 1980), 1:2500 in Middle East (Jaffar 1988), 1:1250 in Romany population of Slovakia (Gencik et al. 1982), and 1:3300 in the Indian state of Andhra Pradesh (Dandona et al. 2001). The high rate of consanguinity among Slovakian Gypsies accounts for the increased prevalence of PCG. Only about 10% PCG is hereditary usually with autosomal recessive mode of transmission (Turacli et al. 1992).

Apparently vertical transmission in some families is also observed which may be explained by pseudo-dominance (Stoilov et al. 1997). Also, incomplete penetrance (Bejjani et al. 2000), sex bias (Gencik et al. 1980) and polygenic character (Gencik et al. 1982) have been documented for PCG.

1.3.4.2.3. Molecular Genetics
Genetic linkage studies revealed that PCG mapped to three different loci, GLC3A (OMIM # 231300) located at chromosome 2p21 (Sarfarazi 1997), GLC3B (OMIM no. 600975, Genbank accession no. NM_000104) located at chromosome 1p36 (Akarsu et al. 1996) and GLC3C located at chromosome 14q24.3 (Stoilov et al. 2002) (Table 1.1). Recently a study from Pakistan identified a new PCG locus at 14q24.2-24.3 (Firasat et al. 2008). The causal gene at GLC3A locus has been found to be CYP1B1, which encodes cytochrome P450 enzyme (Stoilov et al. 1997). A large number of genes have been identified in the chromosomal region (1p36) that contains GLC3B locus but none of these has been reported to segregate with PCG (Sarfarazi 1997). 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 (Table 1.3). 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 (Panicker et al. 2002; Panicker et al. 2004; Reddy et al. 2004; Kaur et al. 2005; Chakrabarti et al. 2006; Chakrabarti et al. 2009). Most of the mutations in CYP1B1 were identified in a common haplotype background suggesting a strong founder effect (Chakrabarti et al. 2006). Apart from CYP1B1, minor role of mutation in FOXC1 has also been identified in PCG pathogenesis (Chakrabarti et al. 2009). In addition, a possible digenic mode of PCG causation has also been suggested with CYP1B1 and MYOC mutations (Kaur et al. 2005).
Table 1.3: Mutations in CYP1B1 gene in association with PCG

<table>
<thead>
<tr>
<th>Location</th>
<th>Domain</th>
<th>Missense/Nonsense changes</th>
<th>Family History</th>
<th>Population</th>
<th>De/Vn</th>
<th>Family History</th>
<th>Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 1</td>
<td>Untranslated region</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exon 2</td>
<td>Hinge region, Helix I</td>
<td>37</td>
<td>All positive</td>
<td>Indian, Turkish, Japanese, Hispanic, Brazilian, Canadian, Arabian</td>
<td>20</td>
<td>All positive</td>
<td>Indian, Turkish, Brazilian, Japanese, Indonesian, European, Hispanic</td>
</tr>
<tr>
<td>Exon 3</td>
<td>Helix J, Helix K, Meander, Helix L, Heme binding region</td>
<td>27</td>
<td>All positive</td>
<td></td>
<td>14</td>
<td>All positive</td>
<td>American, Turkish, British, Brazilian, Hispanic, Indian</td>
</tr>
</tbody>
</table>

Courtesy: HGMD database (http://www.hgmd.cf.ac.uk)
1.3.4.3. PRIMARY ANGLE CLOSURE GLAUCOMA (PACG)

Primary angle closure glaucoma occurs when the peripheral iris physically opposes the trabecular meshwork or corneal endothelium and impedes aqueous outflow.

1.3.4.3.1. Pathophysiology

Several mechanisms might lead PACG. The most common etiology of angle closure is pupillary block, whereby the flow of aqueous humor from the posterior to anterior chamber is inhibited, causing iris bombé. Another mechanism which may induce angle closure involves an abnormal configuration of the iris, the so-called "plateau iris syndrome."

1.3.4.3.2. Prevalence

Primary angle closure glaucoma is visually destructive with high rates of blindness (Aung et al. 2002). The disease is responsible for most bilateral glaucoma blindness in Mongolia (Foster et al. 1996), Singapore (Foster et al. 2000) and China (Foster et al. 2001). A recent study from China reported its prevalence to be 0.5% (95% CI 0.3 - 0.7) (Liang et al. 2011). Among the three landmark studies conducted in India, the Vellore eye survey reported a prevalence of 4.32% for PACG (Dandona et al. 2000). The Andhra Pradesh eye disease survey reported a prevalence of 0.71% for PACG and occludable angles accounted for 1.41% of the study population. The Aravind comprehensive eye survey reported a prevalence of 0.5% for PACG (95% CI 0.3 - 0.7) (Ramakrishnan et al. 2003). Despite near similar study populations, a meaningful comparison is difficult because of the differences in patient selection, methodology and definitions used.

1.3.4.3.3. Molecular Genetics

Very little information is available regarding genetic factors related to PACG. Aung et al has identified a locus on chromosome 10 (LOD score > 3 at θ = 0.0) for a large PACG family from Singapore (Aung et al. 2003). In 1987, a report described a seemingly 'new' autosomal recessive syndrome, a progressive pigmentary retinal degeneration with nanophthalmos, cystic macular degeneration and angle closure glaucoma (MacKay et al. 1987). Nanophthalmos, an autosomal dominant disease, has a significant association with PACG (Othman et al. 1998). Two chromosomal loci have been identified which are linked to nanophthalmos (Othman et al. 1998; Morle et al. 2000). Recently, major research efforts have been undertaken to unravel the underlying genetic mechanism of PACG, particularly in the south-east Asian
countries including India. In 2006 Sripriya et al reported 4q31 to be a possible locus linked with PACG in one of the India PACG family with a LOD score >1 (Sripriya 2006).

1.3.5. Genes Involved in POAG

1.3.5.1. Myocilin (MYOC)

1.3.5.1.1. Gene Mapping of MYOC

In 1993, Shefield et al, identified the first locus associated with POAG, denominated as GLC1A, in a five generation family having JOAG in the long arm of chromosome 1 (Sheffield et al. 1993). In 1997 Stone et al identified the gene to be Myocilin (OMIM # 601652), a TM-inducible glucocorticoid response gene (TIGR), located in the GLC1A interval on chromosome 1q23-q24, from families affected with autosomal dominant JOAG and POAG (Stone et al. 1997). The gene spans about 17 kb region in the genomic DNA and contains three exons (Figure 1.9, Panel A).

![Figure 1.9: Panel A: Structure of the Myocilin gene, number of mutations (if reported), and the characterized/putative protein domains are shown. The number of identified mutations in each exon is shown in parenthesis. The 3'-untranslated regions (UTR) is indicated by white area of the boxes while the protein coding regions are shaded. Panel B: Representation of the primary structure of myocilin locating different domains.](image)

1.3.5.1.2. Regulation and expression of MYOC

The induction of the MYOC gene expression was initially observed in cultured TM cells following treatment with glucocorticoids such as dexamethasone (DEX) (Polansky et al. 1997; Nguyen et al. 1998). It is well known that the long-term use of topical ophthalmic steroids results in elevated IOP with glaucoma, known as steroid-induced glaucoma (SIG) (Francois 1980). Interestingly, the profile of MYOC up-regulation by DEX was dose- and time-dependent, very similar to the course of development of Steroid Induced Glaucoma (Nguyen et al. 1998). Nevertheless, the
association between myocilin expression and steroid-induced IOP was not evident (Fingert et al. 2001).

Fingert et al (Fingert et al. 1998) showed by northern blot analysis that MYOC is expressed as a 2.3 kb transcript and the translated product is predicted to contain 504 aa (57 kDa). Western blot analysis shows that its expression is found in many human tissues e.g., heart, stomach, thyroid, bone marrow, thymus, prostate, colon, its highest abundance appeared to be restricted to ocular tissues such as iris, ciliary body, optic nerve, aqueous humor and TM. However, no expression of the gene is found in brain, placenta, liver, kidney, spleen, or leukocytes (Karali et al. 2000; Rao et al. 2000; Swiderski et al. 2000; Russell et al. 2001; Wistow et al. 2002; Wistow et al. 2002; Tomarev et al. 2003). MYOC mRNA was expressed in cultured human lamina cribrosa (LC) cells and optic nerve head (ONH) astrocytes, two different cell types found in the optic nerve head. MYOC transcripts were found in high abundance in cells lining the laminar sheets of the LC as well as in the surrounding sclera, dura mater, arachnoid, pia mater and the perivascular connective tissue surrounding the central retinal artery and vein (Clark et al. 2001).

The study of regulatory mechanisms governing glucocorticoid-mediated MYOC induction in human TM cells, showed that (i) the promoter region between −2548 and −1541 bp, is required for DEX induction of MYOC expression; (ii) MYOC is a delayed secondary glucocorticoid-responsive gene; (iii) MYOC mRNA is intrinsically quite stable (Joe, Vis. Res. 2011). This MYOC mRNA stability can be regulated by over-expression of optineurin, another protein associated with glaucoma (Park et al. 2007).

1.3.5.1.3. Structural characterization of MYOC

MYOC gene is composed of three exons, 604, 126, and 782 bp, respectively (Fingert et al. 1998), and spans a region of 17 kb (Tomarev et al. 1998), along with 5' and 3' untranslated regions. Putative sequences found for the MYOC promoter, in the 5kb region sequenced upstream, contained TATA and CAT boxes, MIR (Mammalian Interspersed Repeat) and Alu repeat sequences, binding sites for multiple hormone e.g. multiple hormone and cell signaling response elements including seven glucocorticoid response element (GREs), three negative glucocorticoid response element (nGREs) (four GREs and two nGRE are proximal, within 2.5-kb promoter region), three ERE (estrogen response element), a PRE (progesterone response element), and a TRE (proximal thyroid response element), early and immediate gene response elements including an SRE (serum response element), three AP-1 sites
and an AP-2 site, and an ICS (interferon consensus sequence) and cell signalling response elements, but lacks SP1-binding sites (Nguyen et al. 1998). The 3'-UTR contains three polyadenylation signal sequences (Kong 2001).

The encoded protein, myocilin, is a glycoprotein that exists in glycosylated and nonglycosylated forms, with molecular weights of 57 kDa and 55 kDa respectively, and detected in both intracellular and extracellular locations (Polansky et al. 1997; Nguyen et al. 1998). It has two major domains: a myosin-like domain at the N-terminal and an olfactomedin-like domain (OLF) at the C-terminal encoded by exons 1 and 3, respectively (Polansky et al. 1997; Nguyen et al. 1998). In silico analysis shows that MYOC has a unique bipartite structure i.e. α-helical structure in the N-terminal region and β-sheet in the C-terminal region. Furthermore, it has been observed in aqueous humor and ocular tissues that human wild type MYOC is proteolytically cleaved between Arg226 and Ile227, resulting in a 35 kDa fragment containing the C-terminal OLF domain and a 20 kDa fragment containing the N-terminal leucine zipper domain (Aroca-Aguilar et al. 2005) by Calpain –II protease in the lumen of endoplasmic reticulum (ER) (Sanchez-Sanchez et al. 2007) (Figure 1.10). Experiments show that

Figure 1.10: Model of the endoproteolytic processing of myocilin by calpain II

According to this model the proteolytic cleavage of myocilin is carried out by a subpopulation of calpain II in the lumen of the ER, producing two myocilin fragments: one containing the leucine zipper-like domain (LZ) and another one containing the olfactomedin-like domain (OLF). It is proposed that two different regions of myocilin are involved in this processing. The olfactomedin-like domain could act as the substrate binding site to calpains, while the putative linker domain (LINK) contains the cleavage site. Full-length myocilin and the olfactomedin-like domain resulting upon processing by calpain are secreted and the processed N-terminal region mainly remains in the ER (adopted from Sanchez-Sanchez et al, JBC, Vol282, pp21810-27824. 2007).
the native conformation of myocilin rather than amino acid sequence motifs is required for this cleavage. The authors postulated that the N-terminal domain is not functionally important and is degraded post cleavage, which explains the higher incidence of POAG pathogenic mutations in exon 3 of myocilin, coding the olfactomedin domain.

Interestingly, a knowledge-based consensus modelling approach (Ortego et al. 1997; Tamm 2002) showed that myocilin is structurally characterized of three main regions (Figure 1.9, Panel B): (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). However, this model was somehow in partial discordance with a deletion experiment study (Gobeil et al. 2006) that revealed a (i) coiled-coil domain located between the amino-acids 78-105; (ii) leucine zipper region between the amino-acids 114-183; (iii) C-terminal domain between the amino-acids 245-504. 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) (Gobeil et al. 2006). To date the 3D structure of the myocilin is unknown. Indeed, cellular studies have demonstrated temperature-sensitive secretion of myocilin mutants, but difficulties in expression and purification have precluded biophysical characterization of wt MYOC and disease-causing mutants in vitro (Burns et al. 2010).

The protein has been reported to have high level of sequence similarity to non-muscle myosin of Dictyostelium discoideum in the N-terminal region and to olfactomedin of the bullfrog in the C-terminal region (Kubota et al. 1998). It has been further hypothesized that mammalian MYOC has evolved from fusion of genes from two different primordial proteins (Mukhopadhyay et al. 2002). 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 (Karavanich et al. 1998; Karavanich et al. 1998). 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, suggesting evolutionary constrains in the C-terminal region for a predictably important functional role. 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
(Wentz-Hunter et al. 2002).

A recent study provides 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. Circular dichroism (CD) reveals significant
β-sheet and β 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 (Orwig et al. 2011).

The apparent molecular mass of the protein present in the aqueous humour
(>250 KDa) appears to be greater than a dimeric protein and the gel filtration studies
indicate the protein may be present as an oligomer. The aggregation of the molecule
is also supported by the hydrophobic nature of the protein (Russell et al. 2001). In
eye, MYOC may be produced in greater amount at times of stress (Johnson 2000). It
has been speculated that myocilin may be involved in a protective role like a
molecular chaperone (Johnson 2000).

1.3.5.1.4. Myocilin Is Phylogenetically Well Conserved
Comparison of full-length myocilin primary sequence of different species showed that
there are very few gaps in the myocilin sequences (Mukhopadhyay et al. 2002;

![Figure 1.11: Primary sequence alignment of myocilin from different mammalian
species. Protein sequence conservation appears in blue.](image)
Menaa et al. (2011). A stretch of 14 amino acids residue present at the N-terminal end of human MYOC was observed to be absent in other seven species (Figure 1.11). This short amino-acid sequence corresponds to the peptide signal (Figure 1.9, Panel B), suggesting human specificity in relation to the myocilin protein cellular trafficking (Menaa et al. 2011).

1.3.5.1.5. The chaperone activity of MYOC
Myocilin is thought to be a stress response protein, but exact molecular functions have not been established. A recent study by Anderssohn et al, revealed that the level of myocilin is increased in cells in response to mechanical stress, heat shock, TNFα, or IL-1α. It was found to display general molecular chaperone activity by protecting Drd I, citrate synthase, and GAPDH from thermal inactivation. Myocilin also suppresses the thermal aggregation of citrate synthase. Thus, one function of myocilin may be to serve as a molecular chaperone (Anderssohn et al. 2011).

1.3.5.1.6. Molecular defects in MYOC in glaucoma patients
Several mutations in MYOC gene are continuously described in families with POAG from different ethnic or geographic origins. The most commonly encountered mutation type is missense (~85%), with nonsense mutation accounting to ~6.8% and 2.3 % are indels (www.myoclin.com). It has been observed that among three exons of MYOC, majority of the mutations (86 mutations, over 90%) are clustered in exon 3, rarely in exon 1 (11 mutations) and none has been detected so far in exon 2. Myocilin mutations are generally associated with juvenile or early-adult form of POAG (Figure 1.12). The frequencies of these mutations are usually higher in family studies than in unrelated ones (Faucher et al. 2002).

In vitro and in vivo studies showed that several OLF domain mutations prevented myocilin secretion in physiological temperature conditions (37°C), but when cells were cultured at 30°C, a process known to facilitate protein folding, some sequestered mutants were released in the extracellular medium (Gobeil et al. 2006).

To date, the largest study has been conducted in 1703 POAG patients from five different populations representing three racial groups, which led to the identification of 61 different MYOC sequence variations, 21 of which were designated as probable disease-causing mutations. The most common mutation observed was Gln368Stop, in 27 of the 1703 (1.6%) glaucoma probands and was also
Figure 1.12: Variants reported in Myocilin. (Data was obtained from http://www.myocilin.com/variants.php, a comprehensive myocilin mutation database). The red letters indicate the glaucoma causing mutation, magenta for mutation with uncertain pathogenicity and the blue letter indicate the neutral polymorphism. Total 192 variants were collated in the database from 129 published literature describing the myocilin variants in glaucoma (Hewitt et al. 2008)
reported to be associated with strong founder effect among Caucasians (Fingert et al. 1999). This mutation has also been identified in two eastern Indian families but unlike the Caucasians, here Gln368Stop mutation did not show any founder effect (Bhattacharjee et al. 2007). This mutation is mainly common among the Caucasians (Bhattacharjee et al. 2006) with a prevalence of 29.3% (www.myocilin.com).

To date, studies done on MYOC suggest that haploinsufficiency is not a critical mechanism for POAG in individuals with mutations in the gene. There has been a lack of discernable phenotype in both Myoc-heterozygous and Myoc null mice (Kim et al. 2001). A genetic study identified an individual who was homozygous for Arg46stop mutation on MYOC but did not develop glaucoma (Lam et al. 2000). Morissette et al. (1998) described an interesting MYOC-mutation (Lys423Glu) in a French-Canadian family, which resulted in a dominant-negative effect when present in single dosage but had no phenotypic effect when present in both copies of the gene (Morissette et al. 1998). The findings represent the first example of autosomal dominant 'metabolic interference,' as suggested by Johnson (Johnson 1980). In this system homozygosity for the normal allele XX, and the mutant allele xx, give a normal phenotype. Only the heterozygous condition Xx produces an abnormal phenotype because the two alleles, when present together, interact to elicit a harmful effect (Johnson 1980). The proposed model for this theory in case of structural proteins is illustrated in Figure 1.13. However autosomal recessive inheritance was reported to be caused by a nonsense mutation in MYOC exon-1 in Koreans (Yoon et al. 1999), but another study from China identified a normal individual who was homozygous for the same mutation (Lam et al. 2000).

Figure 1.13: Mutation affecting a multi-subunit structural protein
X and x are two alleles of a gene. The hypothesis proposed by Johnson et al. in 1980 suggests that the multi-subunit gene product resulting from homozygous alleles of either type (Panels A & C) has normal biological function but the protein comprised of peptides translated from heterozygous alleles has abnormal biological function (Panel B) due to abnormal interaction between the two allele products (Johnson, Am J Hum Genet, Vol 32, pp374-386, 1980).
Among these mutations, Hewitt et al (Hewitt et al. 2007) have studied the founder effects associated with Thr377Met mutation in 24 POAG affected members from 9 families from Greece, India, Finland, USA and Australia. Their result suggests that the Thr377Met mutation arose at least three times worldwide (Figure 1.14) and summarized that the Australian families from Greece have a common founder. However, the British, Finnish and Indian families have a distinct haplotype from the Greek one, suggesting that the Thr377Met mutation has occurred de novo more than once (Figure 1.14). This mutation has also been reported as a predominant mutation in Veli Guda and Croatian patient cohorts (Zgaga et al. 2008).

![Figure 1.14: The worldwide spread of the Thr377Met MYOC mutation. Stars indicate location of known origin, solid arrows indicate known migrations and the dashed arrow indicates an inferred migration (adopted from Hewitt et al, MolVis, Vol 13, pp487-492. 2007).](image)

Interestingly, mutations in both MYOC and CYP1B1 genes were described in a Canadian family segregating both autosomal dominant adult and juvenile onset-POAG. CYP1B1 is the most important gene associated with primary congenital glaucoma. It encodes a member of the cytochrome P450 super family and is co-expressed with MYOC in the iris TM, and ciliary body of the eye (Discussed in Section 1.3.5.4). All affected family members carried the MYOC mutation (Gly399Val) but those who also had the CYP1B1 (Arg368His) mutation had juvenile onset-POAG, whereas those with only the MYOC mutation had the adult-onset form. The mean age at onset of disease among carriers of the MYOC mutation alone was 51 years, whereas those with both MYOC and CYP1B1 mutations had an average
age of only 27 years. Individuals carrying only CYP1B1 mutation were not clinically affected, implying the fact that in this family, CYP1B1 could act as a modifier of MYOC (Vincent et al. 2002).

Among MYOC mutations identified in Indian POAG patients (Mukhopadhyay et al. 2002; Kanagavalli et al. 2003; Markandaya et al. 2004), Gln48His represents an allelic condition involving a spectrum of glaucoma phenotypes and could be a potential risk factor towards disease predisposition among patients of Indian origin (Chakrabarti et al. 2005). In a study carried out in our lab, the mutation has been identified in 3 out of 56 unrelated POAG patients who were found to be heterozygous for the change (Mukhopadhyay et al. 2002). In addition, other studies from India have reported two other POAG cases harboring the same mutation (Sripriya et al. 2004). These observations clearly establish that Gln48His is a common mutation among Indian patients and has not yet been reported in other populations (Kaur et al. 2005). The mutation has also been detected in heterozygous state in a digenic PCG case also having heterozygous mutation in CYP1B1 (Kaur et al. 2005).

1.3.5.1.7. Misfolding and Aggregation of Myocilin in POAG Etiology

Myocilin has long been established as a candidate gene for glaucoma (Stone et al. 1997), but the function of the protein still remains largely unknown. However, recent studies have partially unraveled the potential molecular basis of pathogenesis caused by MYOC. It is hypothesized that mutant forms of myocilin are not secreted from the cells and can diminish the secretion of the native protein when two forms are co-expressed (Jacobson et al. 2001). Mutant protein was found to be Triton X-100 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 (Zhou et al. 1999).

In order to elucidate the effect of wild-type myocilin on secretion of the mutant protein, Aguilar et al (Aroca-Aguilar et al. 2005) co-expressed both wild type and four of the mutant myocilin (Gln368Stop, Glu233Lys, Asp380Ala and Pro370Leu) in HEK-293 cell line, mimicking the state of heterozygosis in a cell based assay. 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. The model is described in Figure 1.15. Recent studies using a mouse model with the human myocilin gene implicate the involvement of a peroxisomal targeting signal-1 receptor (PTS1R), which is absent from the mouse gene, in the disease process (Shepard et al. 2007).

Figure 1.15: Model of the secretion and proteolytic processing of wild-type/mutant myocilin heteroaggregates. Heteroaggregation takes place in the lumen of the ER. For simplicity the diagram shows a heterodimer but the aggregates are composed of multiple myocilin monomers (indicated by dots in the heteroaggregate) linked by disulphide bonds (short black lines in the heterodimer). According to this model heteroaggregation has three major effects: 1) increases the retention of wild-type myocilin in the ER via a dominant negative effect (DNE); 2) increases secretion of mutant myocilin along with a reduction of extracellular wild-type myocilin, and 3) reduces the amount of extracellular wild-type myocilin (olfactomedin fragment) under conditions known to increase the proteolytic cleavage of myocilin by calpain II such as 96 h of culture (Sanchez-Sanchez F, 2007). The N-terminal fragment that arises after the cleavage mainly remains in the ER [Sanchez-Sanchez F, 2007]A possible gain of function (GOF) of mutant myocilin (both full-length and cleaved olfactomedin fragment) that could contribute to glaucoma pathogenesis is also indicated. The leucine zipper (yellow), linker (red), and olfactomedin (blue) domains of myocilin are also indicated in the heterodimer. The ray indicates mutant myocilin. Question marks indicate a hypothetical dominant negative effect and/or gain of function of the mutant protein (Adapted from Aroca et al, MolVis, Vol14, pp2097-2108. 2008)
Endoplasmic Reticulum Stress Response

In cells, under normal condition, ER monitors the folding of secretory proteins through association of ER chaperones with misfolded and even unfolded polypeptide chains (Ellgaard et al. 2003). Proteins unable to assume native structure fail to transit to the golgi compartments and are subjected to ER-associated degradation (ERAD) via retrotranscript to the cytosol followed by ubiquitination and proteasomal degradation. Often proteins carrying mutations which affect the native folding are not efficiently degraded and form ER or cytoplasmic aggregates (Kopito 2000). ER retention has been implicated in the pathogenesis of various diseases (Rutishauser et al. 2002). Mutations in proteins that induce misfolding and proteasomal degradation are common causes of inherited diseases (Yam et al. 2005). 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 (Liu et al. 2004) and formed typical Russel bodies (Yam et al. 2007). Also aggregation of MYOC in the ER activates the unfolded protein response (UPR) in Drosophila disease model (Carbone et al. 2009).

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 (Liu et al. 2004). However, culture of TM cells at 30°C facilitated myocilin folding, promoted secretion of mutant myocilin, normalized cell morphology and, reversed cell lethality (Liu et al. 2004).

By semi-quantitative PCR analysis Wang et al have 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 (Wang et al. 2007). Such a mechanism is similar to the pathogenic role of mutant PS1 (Presenilin-1), which is linked to familial Alzheimer Disease on the signaling pathway of UPR (Katayama et al. 1999; Sato et al. 2001). Amyloid-β, the major constituent of senile plaques in Alzheimer disease has been reported to be implicated in the development of RGC apoptosis in glaucoma, with evidence of caspase-3-mediated abnormal APP (Amyloid Precursor Protein) processing and increased expression of Aβ in RGCs in experimental glaucoma (McKinnon 1997) and decreased vitreous Aβ levels (consistent with retinal Aβ deposition) in patients with glaucoma (Yoneda et al. 2005). Guo et al (2007) has shown that Aβ induces significant RGC apoptosis in vivo and suggests that targeting
the Aβ pathway provides a therapeutic avenue in glaucoma management (Guo et al. 2007).

ER stress-induced apoptosis is a pathway to explain the reduction of TM cells in patients with myocilin-caused glaucoma (Yam et al. 2007). Indeed, the presence of myocilin aggregates induced the UPR proteins BiP and phosphorylated ER-localized eukaryotic initiation factor-2alpha kinase (PERK) with the subsequent activation of caspases 12 and 3 and expression of C/EBP homologous protein (CHOP)/GADD153, leading to apoptosis (Yam et al. 2007).

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 (Liu et al. 2004; Yam et al. 2007).

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 (Aroca-Aguilar et al. 2005). This cleavage might regulate extracellular and matricellular protein interactions (e.g., myocilin-hevin) (Li et al. 2006; Aroca-Aguilar et al. 2011), contributing to the control of IOP (Sanchez-Sanchez et al. 2007), notably by decreasing myocilin homo-aggregates (Aroca-Aguilar et al. 2010).

Several recent studies have reported that ER stress induces autophagy in mammalian cancer cell lines and mouse embryonic fibroblasts (Ding et al. 2007; Kouroku et al. 2007). Autophagy is the cell’s major regulated mechanism for degrading long-lived proteins and the only known pathway for degrading organelles (Levine et al. 2004). During autophagy, an isolation membrane forms, presumably arising from a vesicular compartment known as the preautophagosomal structure, invaginates, and sequesters cytoplasmic constituents including mitochondria, endoplasmic reticulum, and ribosomes. Degradation of the sequestered material generates nucleotides, amino acids, and free fatty acids that are recycled for macromolecular synthesis and ATP generation. Recent studies have reported a role for autophagy under a variety of pathophysiological conditions, including cancer (Kondo et al. 2000; Liang et al. 2011), defence against infections (Kirkegaard et al. 2004), neurodegeneration (Hara et al. 2006; Komatsu et al. 2006) and heart failure (Nakai et al. 2007). The role of autophagy in cell death thus depends on the context.
Introduction

in which it occurs: it either constitutes a stress adaptation aimed at preventing cell death or conversely contributes to cell death. From all the above evidences it might be intriguing to evaluate that whether Myocilin mediated ER stress induces autophagy in TM cells.

Mitochondria Connection

MYOC has been found to be localized to both intra and extra cellular sites in Trabecular Meshwork (TM) cells. Immunofluorescence has shown that the intracellular form of MYOC is distributed in the cytoplasm including perinuclear regions (Tamm 2002). Subcellular fractionation indicated that intracellular MYOC in TM cells is associated not only with ER, Golgi apparatus, but also with mitochondria (Wentz-Hunter et al. 2002). The mitochondrial association was visualized by immune electron microscopy (Ueda et al. 2000). Sakai et al. (2007) has shown that level of MYOC imported into the TM cell mitochondria is dramatically higher than into the mitochondria from corneal fibroblasts and mouse liver, consistent with the notion that MYOC processing and localization may be distinct in TM cells (Sakai et al. 2007). MYOC is predicted to contain mitochondrial transit peptide at its N-terminus (amino acid residues 1 - 47). Both N and C-terminus contains a Lysine and Arginine rich mitochondrial tethering domain (amino residues 33-46 and 460-504) (Sakai et al. 2007). Pro370Leu mutant MYOC also damages mitochondrial function of HTM cells, as indicated by decline of \( \Psi_m \), indicator of mitochondrial energy state. The latter is hypothesized to occur because of disruption of outer mitochondrial membrane (Wang et al. 2007) and facilitates the release of pro apoptotic factors like Bcl-2 family proteins from mitochondria (Sakai et al. 2007).

The chaperone effect

In cell culture studies, the toxicity of mutant myocilins caused by ER-retained aggregates can be reduced chemical chaperones e.g. 4-phenylbutyric acid (4-PBA) (Yam et al. 2007) or osmolytes e.g. trimethylamine N-oxide (TMAO) (Jia et al. 2009). In addition, reduction of ER stress by phenylbutyric acid (PBA), prevented glaucoma phenotype in transgenic mouse model of POAG carrying Y437H MYOC mutation (Zode et al. 2011). It was also observed that the compromised stability of myocilin mutants can be restored with chaperones like sarcosine and trimethylamine N-oxide, thus promoting secretion to the extracellular matrix, to better control intraocular pressure and ultimately delay the onset of myocilin defect induced glaucoma.
1.3.5.1.8. Myocilin-Molecules Interactions in POAG Etiology

Biochemical and immunoelectron microscopic studies data indicated that myocilin may interact with itself and/or with several intracellular and extracellular matrix (ECM) proteins (e.g., flotillin-1, γ-synuclein, hevin-1, optiomedin, GAPDH, fibronectin, fibrillin-1 or type VI collagen) (Menaa et al. 2011), but the biological significance and clinical relevance of such interactions in the POAG etiology remain unclear. Thereby, myocilin forms in vivo (e.g., human aqueous humor, human TM) high molecular weight complexes, ranging from 120 to 180 kDa, due to interaction with itself and other myocilin binding proteins (Fautsch et al. 2001; Kim et al. 2001).

Among the possible molecular interactions that may modulate IOP and thus prevent or contribute to POAG, are: (i) the myocilin-myoeilin, involving the amino-acids 117-166 in the leucine zipper domain, which might be necessary for the myocilin-RLC (myosin regulatory light chain) interaction, suggesting a role for myocilin in the actomyosin system (Fautsch et al. 2001; Wentz-Hunter et al. 2002); (ii) the interaction of myocilin with components of the Wnt signaling pathways (e.g., Wnt receptors of the Frizzled (Fzd) family, Wnt antagonists of the secreted Frizzled-related protein (sFRP) family and Wnt inhibitory factor 1 (WIF-1), which modulates the organization of actin cytoskeleton stimulating the formation of stress fibers, critical for the contractility of the TM and IOP regulation (Kwon et al. 2009). Interestingly, the absence of a glaucoma phenotype resulting from Myocilin null mutation (Myoc-/-) in the eye could also be explained by the compensatory action of Wnt proteins (Kwon et al. 2009).

An unexpected interacting partner, PTS1R, for misfolded myocilin. PTS1R was identified by yeast two-hybrid analysis using TM or heart cell cDNA libraries as binding partners with C-terminal half WT or mutant myocilin (Fig. 1B and C), and this interaction was confirmed by mammalian two-hybrid analysis (Shepard et al. 2007). Co-immunoprecipitation data also suggest that early onset severe myocilin mutants such as Tyr437His interact with PTS1R more effectively than later onset, milder myocilin mutants such as Gln368Stop. Evidence for PTS1R shuttling of mutant myocilin to peroxisomes was seen by direct visualization of fluorescent protein-tagged myocilin and PTS1 in TM cells. The expression of human mutant myocilin in the eyes of mice caused the relevant glaucoma phenotype of elevated IOP, and the degree of IOP elevation was mutation specific. The observation correlated well with the clinical IOP phenotypes of MYOC glaucoma patients. WT myocilin is secreted from TM cells and is found in the aqueous humor. In contrast, mutant myocilin is
misfolded, retained in the ER and not secreted from TM cells. Within the ER, misfolded myocilin is likely recognized by the ERAD pathway and dislocated to the cytoplasm for ubiquination and proteosome mediated degradation. However, exposure of the normally cryptic PTS1 signal in mutants would allow interaction of myocilin with PTS1R (Figure 1.16). Therefore, there is likely to be competition between ubiquitin–proteasome degradation and PTS1R interaction of the mutant myocilin proteins. Several mechanisms may be responsible for the mutation specific interaction with PTS1R and degree of IOP elevation. Myocilin normally exists as homodimers and higher order multimers, which in MYOC glaucoma patients would lead to WT myocilin-mutant myocilin heterodimers. Interaction of WT myocilin with the misfolded mutant myocilin may or may not expose the normal cryptic PTS1 signal

![Figure 1.16: Proposed model for mutant myocilin-induced PTS1R interaction.](image)

**Panel A:** Properly folded WT myocilin containing a cryptic C-terminal PTS1 motif is normally synthesized, oligomerized and transported through the ER-Golgi pathway and secreted from the cell. **Panel B:** Misfolded mutant (Mut) myocilin (e.g. Y437H or G364V) with an exposed PTS1 motif, is synthesized and oligomerized in the ER, retro-translocated from the ER into the cytosol, and either ubiquitylated and degraded by the proteosome or bound by PTS1R and shuttled to the peroxisome. Misfolded mutant myocilin that lacks the PTS1 motif (e.g. Q368X or S502P), associates by heteroligomerization with WT myocilin causing misfolding and exposure of its partner WT myocilin PTS1 motif.

on WT myocilin. The higher degree of interaction between some mutant myocilin eg.Tyr437His and PTS1R may be due to exposure of the cryptic PTS1 sites on both the mutant and WT myocilin molecules, essentially doubling the chance for binding PTS1R. Other MYOC mutations may expose the PTS1 site only on the mutant myocilin molecule. Interaction of WT myocilin with mutant myocilin proteins that lack a PTS1 site (Ser502Pro, Glu368Stop) may expose the cryptic signal on WT myocilin
Introduction

(Figure 1.16). The latter two scenarios may lead to weaker interactions with PTS1R. In addition, specific myocilin mutations may lead to varying degrees of myocilin misfolding, some of which are better recognized by the ubiquitin-proteasome degradation pathway causing decreased opportunity to interact with PTS1R and a milder IOP phenotype.

However, future studies are required for (i) physical and functional characterization of wt MYOC versus mutant MYOC in different micro-environmental conditions; (ii) characterization of dynamic molecular interactions between myocilin-protein complexes; (iii) unravelling of the 2D/3D myocilin structure (wt versus mt MYOC); (iv) continuous determination of myocilin variants pattern, clinically relevant among worldwide patients populations in order to establish groups at risk for POAG and allow early diagnosis; (v) efficient therapy-based control of the IOP – without major post-treatments complications – to avoid potential disease progression.

1.3.5.2. OPTINEURIN (OPTN)

1.3.5.2.1. Current Knowledge on OPTN

In 1998, Sarfarazi and his colleagues (Sarfarazi et al. 1998) reported linkage of a normal-tension glaucoma phenotype in a large British family to a locus (p15-14) on chromosome 10 (GLC1E). The 16 affected patients in this family were diagnosed at ages ranging from 23 to 65 years, with a mean of 44 years. These patients had

---

Figure 1.17: Genes associated with glaucoma.

Structure of the genes associated with glaucoma, number of mutations (if reported), and the characterized/putative protein domains are shown. In each case, the exon number of the gene is indicated above the exon (boxes) and the number of identified mutations therein in parenthesis. The 5'- and 3'- untranslated regions (UTRs) are indicated by white areas of the boxes while the protein coding regions are shaded. The protein domains are identified by black shades within the coding region.
normal or near-normal intraocular pressure, ranging from 15-25 mm of Hg. Of the 16 affected members, 12 never had a recorded IOP higher than 22 mm of Hg. In 2002, Sarfarazi and associates (Rezaie et al. 2002) reported that sequence variations in optineurin gene (OMIM # 602432) were associated with the development of familial NTG in the original GLC1E family as well as eight other families with NTG. A total of 16.7% of the 52 families in their report were found to have putative disease-causing OPTN variations with an additional attributable risk factor of 13.6% in both familial and sporadic cases. These families all included at least one member with NTG.

OPTN spans ~ 37 kb region and contains 3 non-coding exons in the 5'-region and 13 exons that code for a 577-amino acid protein (Figure 1.17). Alternative splicing generates at least 3 different isoforms. The mouse optineurin gene codes for a 584-amino acid protein (67 kDa) that has 78% identity with its human homologue (66 kDa). The same gene has also been reported earlier as FIP-2 (Li et al. 1998) and NRP (Schwamborn et al. 2000).

Li et al. observed expression of FIP-2 in heart, brain, placenta, liver, skeletal muscle, kidney and pancreas (Li et al. 1998). Expression of the gene at lower level was recognized in trabecular meshwork, non-pigmented ciliary epithelium and retina by RT-PCR (Rezaie et al. 2002). Northern blot analysis revealed a major 2.0-kb transcript in human trabecular meshwork and non-pigmented ciliary epithelium and a minor 3.6-kb message that was 3 to 4 times less abundant. Optineurin expression was also detected in aqueous humor samples suggesting that it is a secreted protein. Immunocytochemistry showed that optineurin is localized to the Golgi apparatus (Rezaie et al. 2002). Recent reports suggest that in response to an apoptotic stimulus, optineurin changes subcellular localization and translocates from the golgi to the nucleus. This translocation is dependent on the GTPase activity of Rab8, an interactor of optineurin. Furthermore it has been demonstrated that the expression of OPTN protects cells from H$_2$O$_2$ induced cell death and blocks cytochrome c release from the mitochondria. A mutated form of OPTN, Glu50Lys, loses its ability to translocate to the nucleus and when over-expressed compromises the mitochondrial membrane integrity resulting in cells that are less fit to survive under stress conditions (De Marco N. et al. 2006). Later Glu50Lys mutation was found to be responsible for high ROS generation which ultimately leads to cell death (Chalasani et al. 2007). It has been shown that over-expression of OPTN in TM cells resulted in prolonged turnover rate of MYOC mRNA but had little activity on MYOC promoter (Park et al. 2007). The authors have speculated the interaction through control of mRNA stability. Recently it has been found that expression of optineurin is regulated
by NF-κB. On the other hand, optineurin negatively regulates NF-κB (Sudhakar et al. 2009).

1.3.5.2.2. Molecular Defects in OPTN in Glaucoma Patients
An initial report on 52 POAG families reported the involvement of OPTN mutations in 16.7% along with an additional risk to 13.6% in familial and sporadic cases due to the intragenic SNPs (Rezaie et al. 2002). Following this discovery, a large number of studies were undertaken to identify the defects in OPTN causal to NTG and POAG (Alward et al. 2003; Aung et al. 2003; Weisschuh et al. 2005). One such study screened 1,048 patients for variations in OPTN reportedly associated with glaucoma. Among the previously reported mutations (Rezaie et al. 2002), they detected one individual with familial NTG and British ancestry to have the Glu50Lys mutation. One of the OPTN variants, Arg545Gln, reported by Rezaie et al. (2002) as a mutation has been described in this study as a polymorphism that is not associated with the disease (Alward et al. 2003). Similarly, other reports have made conflicting claims regarding association of specific allelic variant of OPTN for its association with POAG and/or NTG (Table 1.4). Originally a report from India on evaluation of OPTN, carried out in our lab, showed limited role of the gene, if any, in glaucoma pathogenesis (Mukhopadhyay et al. 2005). Another study from south India found an association of Met98Lys variant with NTG (Sripriya 2006). Later, another study from India has reported a novel Thr202Arg mutation in OPTN detected in 1 out of 251 POAG patients from Karnataka (South India) (Kumar et al. 2007). It is worth noting that unlike the mutation spectrum in MYOC causing POAG, there are relatively fewer mutations in OPTN that would lead to POAG or NTG (Sarfarazi et al. 2003). By large the nucleotide variants observed across populations have been SNPs that are infrequently associated with the disease phenotype. It is therefore not surprising that despite discovery of the gene with glaucoma pathogenesis in 2002, the HGMD database (http://www.hgmd.cf.ac.uk) do not yet include any entry other than those from the discovery paper. Although studies have been undertaken to decipher the effects of missense mutations in OPTN at molecular level, current knowledge of genetic studies suggests that the gene might not have any major role in causation of POAG in general.
Table 1.4: Distribution of the Arg545Gln variant in OPTN in patients and controls across globe

<table>
<thead>
<tr>
<th>Populations</th>
<th>Number analyzed</th>
<th>Variants present in</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patients (n)</td>
<td>Controls (n)</td>
<td></td>
</tr>
<tr>
<td>Australia</td>
<td>27</td>
<td>94</td>
<td>0</td>
</tr>
<tr>
<td>UK</td>
<td>315</td>
<td>95</td>
<td>0</td>
</tr>
<tr>
<td>Germany</td>
<td>112</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>USA</td>
<td>46</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>USA</td>
<td>86</td>
<td>80</td>
<td>0</td>
</tr>
<tr>
<td>USA</td>
<td>1048</td>
<td>251</td>
<td>12</td>
</tr>
<tr>
<td>Canada</td>
<td>66</td>
<td>184</td>
<td>1</td>
</tr>
<tr>
<td>China</td>
<td>119</td>
<td>126</td>
<td>8</td>
</tr>
<tr>
<td>Japan</td>
<td>154</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>Japan</td>
<td>411</td>
<td>218</td>
<td>26</td>
</tr>
<tr>
<td>India</td>
<td>200</td>
<td>200</td>
<td>6</td>
</tr>
<tr>
<td>Brazil</td>
<td>99</td>
<td>100</td>
<td>22</td>
</tr>
</tbody>
</table>
1.3.5.3. WD Repeat Domain 36 (WDR36)

1.3.5.3.1. Current Knowledge on WDR36

A linkage study in two large Caucasian families led to the identification of another novel locus, designated as GLC1G, in chromosome 5q22.3 (Monemi et al. 2005). The underlying gene was characterized as WDR36 (OMIM # 609669). The gene spans about 34.7-kb genomic region and contains 23 exons (Figure 1.17), expressed predominantly as two transcripts (5.9 kb and 2.5 kb). The full length protein contains 950 aa (Mr ~105 kD) harboring four conserved domains: (a) nine WD40 repeat domain; (b) Utp21 domain; (c) AMP dependant synthetase and ligase domain, and (d) cytochrome cd1-nitrite reductase-like domain. It is expressed in human ocular and nonocular tissues as well as in embryonic and adult mouse tissues and is also found to be upregulated during human T-cell proliferation (Mao et al. 2004).

This gene was previously reported to be uniquely involved in T-cell activation and highly co-regulated with interleukin 2. A study by Skarie et al. (2008) provides an insight into the functionality of the protein. This study reveals WDR36 is a multifunctional protein. WDR36 is required for ribosomal rRNA processing and maintaining the proper nucleolar morphology. The protein is also important for proper development of brain, eye and gut. It has also been noted that loss of WDR36 function in mouse leads to an activation of the p53 stress–response pathway (Skarie et al. 2008).

1.3.5.3.2. Molecular Defects in WDR36 in Glaucoma Patients

Monemi et al (2005) identified 4 mutations in the WDR36 gene among 17 unrelated POAG subjects, 11 with high-tension and 6 with low-tension glaucoma. The mutations were absent in a minimum of 200 normal control chromosomes and the residues were conserved between WDR36 orthologs in mouse, rat, dog, chimpanzee and human. Specific ocular expressions and observed mutations were consistent with a role for WDR36 in the etiology of both high and low tension glaucoma (Monemi et al. 2005).

When other groups extended the study of WDR36 to different populations, the relationship between WDR36 and glaucoma appeared more complex. Originally described disease-causing variants have been found in control individuals with an equal frequency as patients with POAG (Hauser et al. 2006; Hewitt et al. 2006; Fingert et al. 2007; Pasutto et al. 2009). Hauser et al reported probable disease causing variants in 17% of the patients, but the distribution of WDR36 variants in the
Introduction

pedigrees did not show consistent segregation with the disease. They found WDR36 sequence variants to be more frequent in patients with more severe disease. They concluded that defect in WDR36 is not sufficient for POAG causation rather the gene acts as a modifier locus for POAG (Hauser et al. 2006). Studies by Reiss et al and Weisschuh et al in German POAG and NTG patients concluded that WDR36 play a minor role in POAG pathogenesis (Weisschuh et al. 2005; Pasutto et al. 2008). A case control study in Australian population with one of the predominant mutation of WDR36 viz. D658G in Caucasians revealed the presence of this mutation in both patient and control groups neutralizing its role in POAG pathogenesis (Hewitt et al. 2006). Also, study by Fingert et al did not find any association of WDR36 with POAG (Fingert et al. 2007).

As far as Asian populations are concerned, two studies had been published until recently—one from Japanese population (Miyazawa et al. 2007) and another from Chinese population (Fan et al. 2009). These two studies have identified one disease-causing variant each viz. Ser664Leu and Ile713Val, respectively (Miyazawa et al. 2007; Fan et al. 2009). Also, two SNPs (Ile264Val and c1965-30A>G) were found to be associated with high tension glaucoma in Japanese cohort and a single SNP (c.1965-30A>G) was found to be associated with HTG in Chinese cohort (Miyazawa et al. 2007; Fan et al. 2009). Recently, another study from our lab in Indian cohort did not reveal any disease causing mutation in WDR36 coding exons but a single intronic SNP (c.710+30C>T, rs10038177) was found to be associated with HTG (Mookherjee et al. 2007).

1.3.5.4. Cytochrome P450 family 1 subfamily B polypeptide 1 (CYP1B1)

1.3.5.4.1. Current Knowledge on CYP1B1

CYP1B1 was primarily identified to cause primary congenital glaucoma (PCG) in autosomal recessive mode of inheritance (Sarfarazi et al. 1995; Stoilov et al. 1997). It represents the first example where mutations in a member of the cytochrome P450 superfamily results in a primary developmental defect (Stoilov et al. 1997). Mutations in this gene has also been found in Peters anomaly (Vincent et al. 2001), Axenfield Reiger syndrome (Chavarria-Soley et al. 2006). However, studies show the involvement of the gene in POAG as well.

CYP1B1 (OMIM # 601771) genomic region, located on chromosome 2 (2p21), spans for more than 12 kb and contains three exons (Figure 1.17). Exon 1 represents untranslated region while exons 2 and 3 encodes for a protein containing 543 amino acids (Stoilov et al. 1997). The conserved structural elements include four
helix-bundles (helices D, I and L and the anti-parallel helix E), helices J and K, β-sheets 1 and 2, the heme-binding region and the “meander” region just N-terminal of the heme-binding domain (Stoilov et al. 1998).

It belongs to cytochrome p450 group of proteins and is functionally diverse. It is involved in drug metabolism, fatty acid metabolism and steroid metabolism. CYP1B1 is a membrane bound enzyme involved in the phase I detoxification pathway. CYP1B1 is the main CYP450 enzyme responsible for the 4-hydroxylation of estradiol to the corresponding catechol, a metabolite shown to be carcinogenic in animal models (Liehr et al. 1986; Shimada et al. 1996). Therefore, it is conceivable that an increase in CYP1B1 activity might increase risk of cancer and POAG due to the formation and accumulation of carcinogenic catechol estrogens. The molecule is also hypothesized to be involved in metabolism of signaling molecules important in ocular development (Sarfarazi et al. 2000). CYP1B1 oxidizes all-trans-retinol to all-trans-retinal which is a rate limiting step in retinoic acid biosynthesis (Figure 1.18) (Chen et al. 2000). The latter is involved in transcriptional activation of molecules involved in eye angle development. Thus, mutation in CYP1B1 may affect the production/activation, degradation/ deactivation of a key biological molecule involved in anterior segment development of eye. CYP1B1 null mice have grossly normal

**Figure 1.18: Presumptive role of CYP1B1 in eye angle development.**
CYP1B1 converts all-trans-retinol to all-trans-retinal, which in turn activates Tcfap2a. The latter is responsible for inducing the synthesis of Tyrosine Hydroxylase which is one of the enzymes involved in conversion of tyrosine to L-DOPA which is believed to take part in angle development (Adapted from Gould et al, Int J Dev Biol, Vol 4. 2004).
phenotype but develop focal malformation of the iridocorneal angle (Libby et al. 2003; Jiang et al. 2008). In the affected region, the malformation can include hypoplastic trabecular meshwork, abnormally located basal lamina in the trabecular meshwork and iridocorneal adhesions. Interestingly, the developmental malformation as seen in some PCG cases with CYP1B1 mutations resembles that of CYP1B1 null mouse (Gould et al. 2004). But the exact role of CYP1B1 in ocular development is still lacking.

1.3.5.4.2. Molecular Defects of CYP1B1 in POAG

CYP1B1 has been demonstrated as a modifier locus for POAG that together with MYOC mutation expedite the disease progression from adult onset to a juvenile form in a digenic mode of inheritance. In a Guyanese family, a heterozygous Arg368His mutation in the CYP1B1 gene actually modified the age of onset of primary open angle glaucoma (POAG) caused by a heterozygous Gly399Val mutation in the MYOC gene (Figure 1.19) (Vincent et al. 2002). Screening CYP1B1 in 236 unrelated French Caucasian POAG patients unraveled mutations in 4.6% (n=11) of the patients with no mutation in MYOC. In two families, occurrence of PCG and POAG in members of a single sibship was found, all of whom were compound heterozygous

![Figure 1.19: Digenic form of POAG](image)

A pedigree of a Guyanese family affected with POAG, and carrying CYP1B1 (Arg368His) and MYOC (Gly399Val) mutations is shown. Solid symbols, JOAG; Open symbols, unaffected / unknown status; and Hatched symbols; POAG. Arrow indicates the proband. The number above each symbol identifies the person in different generations indicated on the left side. The numbers below the symbols represent genotypes for MYOC (top row) and CYP1B1 (bottom row) indicating the wild type ("1") and mutant ("2") alleles.
for mutations in the CYP1B1 gene (Melki et al. 2004). Mutations in CYP1B1 were typically associated with juvenile and middle-age onset POAG with a median age at diagnosis of 40 years (range: 13–52 years), significantly earlier than in patients not carrying CYP1B1 mutation. Moreover, although the age at diagnosis in the whole group of patients also was young (median: 48; range: 6–81; interquartile range: 37–58), the age at diagnosis of CYP1B1 carriers was nonetheless younger than that of non-carriers (Melki et al. 2004). Excepting one, all mutations detected in POAG patients were previously associated with PCG.

Interestingly, Kaur et al (2005) have postulated a digenic case of congenital glaucoma based on the patient being double heterozygous - Gln48His in MYOC and Arg368His in CYP1B1 (Kaur et al. 2005). Thus, heterozygous Arg368His mutation in CYP1B1 has been found in both the digenic cases of glaucoma. It was found to give rise to JOAG when present with Gly399Val mutation in MYOC and caused PCG when present along with Gln48His in MYOC, a well documented POAG/JOAG causing mutation in India. Considered collectively, these observations suggested that MYOC and CYP1B1 may interact through a common pathway and MYOC function may be influenced by changes in CYP1B1 (Vincent et al. 2002). Thus mutations of CYP1B1 might be a cause of POAG or at least a risk factor for this disease. Recently we have observed that CYP1B1 mediated estrogen metabolism can alter the expression of MYOC. We found that putative EREs in the MYOC promoter are active and myocilin expression can be induced by 17β estradiol treatment. Any mutation in CYP1B1 that affects its estrogen metabolism activity could upregulate MYOC expression by prolonging the presence of estrogen in the cells. With the help of three mutation of CYP1B1, that have lower estrogen metabolism activity, we observed that these mutants have the ability to upregulate MYOC expression (S. Mookherjee and K. Ray, unpublished data), and overexpression of MYOC can lead to POAG.

In addition, heterozygous mutations in CYP1B1 in POAG, monogenic involvement of the gene in the disease has been reported (Acharya et al. 2006). A novel homozygous CYP1B1 variant (Arg523Thr) of a conserved residue, detected in a familial juvenile onset POAG (JOAG) patient (lacking MYOC or OPTN mutations), co-segregated with the disease locus in an autosomal recessive fashion. Based on these observations it has been suggested that CYP1B1 mutations pose not only as a significant risk factor for early-onset POAG or modifier of glaucoma phenotype, but can also monogenically cause disease in patients who apparently do not carry a mutation in any other gene.
Coding SNPs within the gene have been found to be associated with predisposition towards complex diseases like different types of cancer (Watanabe et al. 2000; Chang et al. 2003; Sasaki et al. 2003). Apart from having direct causal association, SNPs in the gene can pose as susceptible factors for predisposition towards complex diseases like POAG and cancer. CYP1B1 SNP (Asn453Ser) in French POAG patients has been reported to be associated with glaucomatous clinical features like optic disc cupping and visual field alteration (Melki et al. 2005). Another CYP1B1 coding SNP (Leu423Val) has been found to be associated with POAG where Val432 acts as a risk factor towards disease predisposition. Incidentally, Val432 variant was reported to generate higher amount of ROS than its Leu432 counterpart (Bhattacharjee et al. 2008).

1.3.6. INVOLVEMENT OF MITOCHONDRIAL GENOME

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 (Leske 2007). This suggests that the vulnerability of the optic nerve gradually increases with aging, which ultimately result in the death of the retinal ganglion cells and degeneration of the optic nerve (Lee et al. 2011). Such pathophysiology has also been observed in aged rodents (Katano et al. 2001). Till date, no mechanism has been elucidated which explains the relationship between aging and neuronal vulnerability. However, there is increasing evidence that suggests (Olanow 1992; Joseph et al. 2000) mitochondrial dysfunction may play a key role in predisposing to neuronal cell death in age-related neurodegenerative diseases such as glaucoma (Olanow 1992; Joseph et al. 2000). It has been suggested that mitochondria consume more than 90% of the free oxygen molecules, 15% of which is converted to ROS under normal physiological conditions. The mean respiratory activity of mitochondria decreases with age resulting in higher production of ROS and free radicals in mitochondria (Sacca et al. 2007). This is supported by the observation that mitochondrial ATP production decreases and ROS increases in mitochondria in aged rodents (Navarro et al. 2007; Boveris et al. 2008) and human (Cooper et al. 1992; Ojaimi et al. 1999). Several investigations suggest that the mitochondrial abnormalities are important cellular changes which occur in both early and late-onset neurodegenerative diseases (e.g. Amyotrophic lateral sclerosis, Alzheimer’s disease, and Parkinson’s disease) (Reddy et al. 2011). These mitochondrial abnormalities include defects in oxidative phosphorylation, increased accumulation of mitochondrial DNA defects, impaired calcium influx, accumulation of mutant proteins in
mitochondria, and mitochondrial membrane potential dissipation. Altered mitochondrial fission and fusion mechanism might also play a role in disease pathogenesis as it controls the structure, morphology and number of mitochondria in a cell (Bossy-Wetzel et al. 2003; Knott et al. 2008). Therefore, the health and activity of the mitochondria are central in the aging process. Nevertheless, uncertainty prevails over the fact, whether accumulation of mitochondrial mutations leads to a decline in mitochondrial function.

The proposed mechanism of RGC death through apoptosis in a murine model is similar to other optic neuropathies associated with mitochondrial dysfunctions (Calandrella et al. 2007). It has been demonstrated that mitochondrial dysfunction and AIF (Apoptosis Inducing Factor) translocation from mitochondria may play crucial roles, both in RGC death and in axonal degeneration, the primary target of IOP elevation in experimental rat glaucoma models (Munemasa et al. 2010). Studies in mice subjected to ocular hypertension, have shown COX reduction, mitochondrial fission, and cristae depletion (Ju et al. 2007). In addition, an increase in IOP has been correlated with alteration in OPA1 expression and induction of OPA1 release, a protein which plays a crucial role in mitochondrial inner membrane fusion (Ju et al. 2009). A recent study by Khaled et al, reported a spectrum of mitochondrial abnormalities in 27 POAG patients, including a decrease in the mean respiratory activity of mitochondria in patients compared to controls (Abu-Amero et al. 2006). Another study by He et al (2008), reported defects in Complex I contributed to progressive loss of the TM cells in POAG patients by promoting excessive mitochondrial ROS production and by decreasing mitochondrial membrane potential and ATP synthesis. These events result in accelerated aging of the TM cell in POAG patients, thereby driving the cell towards apoptosis (He et al. 2008). In addition, there are reports of involvement of mitochondrial protein-coding genes in Normal Tension Glaucoma (Mabuchi et al. 2007; Wolf et al. 2009). These findings further substantiate a major role of mitochondrial dysfunction in glaucomatous optic nerve degeneration.

1.3.7. Complex Etiology of POAG

POAG is a complex disease in which a number of genetic and environmental factors act together to precipitate the disease. It has been suggested that single-gene mutations must reside in a permissive genetic background, modulated by modifier genes, for a disease phenotype to manifest (Haider et al. 2002). In case of glaucoma, the digenic disease caused by defects in both MYOC and CYP1B1 exemplifies interplay of multiple genes, which would be appreciated better, with the discovery of
new candidate genes and functional studies to unravel cross talk between multiple proteins. The interaction of MYOC with other proteins and the probable effects of gene variants in both MYOC and its interactors on such interaction partly underlie the genetic complexity and heterogeneity associated with POAG. In addition, the study of disease modifiers in mice and the investigation to find whether those modifiers play a similar role in humans might illuminate the path that leads to unravel the complex genetic background for a multifactorial trait in glaucoma. Recent observations suggest that, in addition to RGC, glaucoma patients contain neurodegenerative lesions deep into the brain supporting the speculation of its being a neurodegenerative disorder (Ray et al. 2009).

In addition to the candidate genes, many other genes have been proposed to be associated with POAG (Table 1.5). Most of the studies demonstrate an association in single population group and in some cases conflicting results has been published in multiple studies done on the same population. Therefore, it is difficult to judge whether the variability in the results of the association studies are due to population difference, sample size, study design or clinical heterogeneity between different cohorts of patients. The involvement of such predisposing factors can be better elucidated by studies done on large samples, study design applied to multiple cohorts of patient samples and/or functional studies deciphering the molecular basis of pathogenesis.

An interaction was observed between TNF-α −863A/C and OPTN 603A/T (Met98Lys) polymorphisms (Funayama et al. 2004). TNF-α induces OPTN expression through NFκ-B and their role in POAG has also been described (Sudhakar et al. 2009). Possible interactions have been reported between SNPs of MYOC and OPTN with APOE (Fan et al. 2005). These investigators observed two sets of interaction for HTG patients of OPTN IVS15+10G/A and IVS5+38T/G with MYOC Thr353Ile and APOE −491A/T respectively and three sets of interaction for NTG patients of OPTN Arg545Gln with APOE 2/ 3/ 4, MYOC -83G/A with APOE 2/ 3/ 4 and MYOC IVS2+35A/G with APOE −219T/G. Another interaction was also found between OPTN and OLFM2 (Olfactomedin2) in POAG (Funayama et al. 2004). Incidentally, OLFM2 was predicted to be a probable candidate gene for POAG by in silico analysis from our lab (Mukhopadhyay et al. 2004).

Proteomics and microarray expression studies in different tissues and cells (e.g., TM cells and tissue, retina, optic nerve head, astrocytes, retinal ganglion cells, etc.) have identified a large number of differentially expressed genes.
### Table 1.5: Genes reported to be associated with POAG

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Gene name</th>
<th>OMIM #</th>
<th>Location</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGTR2</td>
<td>Angiotensin II receptor, type 2</td>
<td>300034</td>
<td>Xq22–q23</td>
<td>(Hashizume et al. 2005)</td>
</tr>
<tr>
<td>APOE</td>
<td>Apolipoprotein E</td>
<td>107741</td>
<td>19q13.2</td>
<td>(Copin et al. 2002)</td>
</tr>
<tr>
<td>IL1A</td>
<td>Interleukin 1 alpha</td>
<td>147760</td>
<td>2q14</td>
<td>(Wang et al. 2006)</td>
</tr>
<tr>
<td>EDNRA</td>
<td>Endothelin receptor, type A</td>
<td>131243</td>
<td>4q31.2</td>
<td>(Ishikawa et al. 2005)</td>
</tr>
<tr>
<td>GSTM1</td>
<td>Glutathione S-transferase, mu-1</td>
<td>138350</td>
<td>1p13.3</td>
<td>(Juronen et al. 2000)</td>
</tr>
<tr>
<td>IGF2</td>
<td>Insulin-like growth factor II</td>
<td>147470</td>
<td>11p15.5</td>
<td>(Tsai et al. 2003)</td>
</tr>
<tr>
<td>IL1B</td>
<td>Interleukin 1 beta 5, 10- methyltetrahydrofolate reductase</td>
<td>147720</td>
<td>2q14</td>
<td>(Lin et al. 2003)</td>
</tr>
<tr>
<td>MTHFR</td>
<td>MTHFR</td>
<td>607093</td>
<td>1p36.3</td>
<td>(Junemann et al. 2005)</td>
</tr>
<tr>
<td>NOS3</td>
<td>Nitric oxide synthase 3</td>
<td>163729</td>
<td>7q36</td>
<td>(Tunny et al. 1998)</td>
</tr>
<tr>
<td>NPPA</td>
<td>Natriuretic peptide precursor A</td>
<td>108780</td>
<td>1p36.2</td>
<td>(Tunny et al. 1996)</td>
</tr>
<tr>
<td>OCLM</td>
<td>Oculomedin</td>
<td>604301</td>
<td>1q31.1</td>
<td>(Fujisawa et al. 2003)</td>
</tr>
<tr>
<td>OLFM2</td>
<td>Olfactomedin</td>
<td>605290</td>
<td>3q28–q29</td>
<td>(Aung et al. 2002)</td>
</tr>
<tr>
<td>TAP1</td>
<td>Transporter, ATP-binding cassette, major histocompatibility complex, 1</td>
<td>170260</td>
<td>6p21.3</td>
<td>(Lin et al. 2004)</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumor necrosis factor</td>
<td>191160</td>
<td>6p21.3</td>
<td>(Lin et al. 2003)</td>
</tr>
<tr>
<td>OPTC</td>
<td>Opticin</td>
<td>605127</td>
<td>1q32.1</td>
<td>(Acharya et al. 2007)</td>
</tr>
<tr>
<td>COCH</td>
<td>Cochlin</td>
<td>603196</td>
<td>14q12–q13</td>
<td></td>
</tr>
<tr>
<td>CYP2D6</td>
<td>Cytochrome P450, Subfamily IID, Polypeptide 6</td>
<td>124030</td>
<td>22q13.1</td>
<td>(Yang et al. 2009)</td>
</tr>
<tr>
<td>PON1</td>
<td>Paraoxonase 1</td>
<td>168820</td>
<td>7q21.3</td>
<td>(Inagaki et al. 2006)</td>
</tr>
<tr>
<td>CDH-1</td>
<td>Cadherin 1</td>
<td>192090</td>
<td>16q22.1</td>
<td>(Lin et al. 2006)</td>
</tr>
<tr>
<td>LMX1B</td>
<td>Lim Homeobox Transcription Factor 1</td>
<td>602575</td>
<td>9q34.1</td>
<td>(Park et al. 2009)</td>
</tr>
<tr>
<td>ANP</td>
<td>Atrial natriuretic polypeptide</td>
<td>108780</td>
<td>1p36.2</td>
<td>(Tunny et al. 1996)</td>
</tr>
<tr>
<td>P21</td>
<td>P21</td>
<td>116899</td>
<td>6p21.2</td>
<td>(Tsai et al. 2004)</td>
</tr>
<tr>
<td>HSPA1A</td>
<td>Heat shock 70 kDa protein 1A</td>
<td>140550</td>
<td>6p21.3</td>
<td>(Tosaka et al. 2007)</td>
</tr>
<tr>
<td>TLR4</td>
<td>Toll-like receptor 4</td>
<td>603030</td>
<td>9q32–q33</td>
<td>(Shibuya et al. 2008)</td>
</tr>
<tr>
<td>CYP4F2A1</td>
<td>Cytochrome P450, Family 46, Subfamily A, Polypeptide 1</td>
<td>604087</td>
<td>14q32.1</td>
<td>(Fourgeux et al. 2009)</td>
</tr>
<tr>
<td>PAI-1</td>
<td>plasminogen activator inhibitor-1</td>
<td>173360</td>
<td>7q21.3–q22</td>
<td>(Mossbock et al. 2008)</td>
</tr>
<tr>
<td>ADRB1</td>
<td>beta-adrenergic receptors 1</td>
<td>109630</td>
<td>10q24–q26</td>
<td>(Inagaki et al. 2006)</td>
</tr>
<tr>
<td>TMCO1</td>
<td>Transmembrane and coiled-coil domains protein 1</td>
<td>614123</td>
<td>1q24.1</td>
<td>(Burdon et al. 2011)</td>
</tr>
<tr>
<td>CDKN2B-AS1</td>
<td>CDKN2B Antisense RNA</td>
<td>613149</td>
<td>9p21.3</td>
<td>(Burdon et al. 2011)</td>
</tr>
</tbody>
</table>
Based on the information available on the involvement of different genes and pathways, a recent review from our lab has summarized the unfolding network of events in POAG shown in Figure 1.20.

**Figure 1.20**: Schematic overview of potential involvement of different pathways in POAG pathogenesis. The scheme presented summarizes the unfolding network of events in primary open angle glaucoma (POAG) and has been created based on the information available on the involvement of different genes and pathways in its pathogenesis. The major nodes in the network i.e., mutation in the candidate gene(s) or in the modifier genes, associated SNPs, reduced antioxidants and rise in ROS generation, vascular dysregulation, glutamate excitotoxicity, immune dysregulation, mechanical stress which ultimately leads TM cell dysfunction and RGC loss are highlighted in yellow. Abbreviations used: ROS reactive oxygen species; NOS, nitric oxide synthetase; UPR, unfolded protein response; NO, nitric oxide; ONOO, peroxy-nitrate species; AIF, apoptosis inducing factor; MEF2C, MADS box transcription enhancer factor 2, polypeptide C; RGC, retinal ganglion cells. (Adapted from K.Ray, J Genet, 2009).
1.4. PERSPECTIVE OF THE STUDY UNDERTAKEN FOR THE DISSERTATION

POAG is a multifactorial complex disorder where both environmental and genetic factors precipitate the disease. It is primarily an adult onset disease whose incidence increases with age. It has been suggested that 72% of POAG cases have some familial component, but on rare occasion it follows a Mendelian pattern of inheritance. Early identification of genetic susceptibility provides the opportunity to arrest its onset and/or controls further deterioration of pathogenesis by appropriate medical and surgical intervention. From this perspective identification of susceptible loci and the genetic variant therein are important. On the other hand, functional studies are necessary for understanding the pathology of the disease and development of suitable drugs.

My research plan entails molecular genetics studies to unravel the disease causing variants in Indian POAG patients and functional implication of the suspected variants to understand the disease pathogenesis. During the tenure of this dissertation I studied MYOC, the most penetrant of all candidate genes for the disease, for its causal role in disease pathogenesis. In POAG, the mechanism by which mutant Myocilin cause a dominant glaucoma phenotype is not known. Thus I attempted to investigate the role suspect Myocilin mutants towards disease causation with downstream in vitro studies to functionally validate and relate the observations with glaucoma pathogenesis.

As already discussed in the introduction section, glaucoma pathophysiology may include chronic retinal ganglion cell apoptosis causing progressive damage to axons at the optic nerve head. The subacute retinal ganglion cell apoptosis and axonal injury at the optic nerve head in Leber hereditary optic neuropathy (LHON) are associated with mitochondrial abnormalities. While several studies till date have reported the structural and functional alterations in mitochondria and their metabolites in the pathogenesis of neurodegenerative diseases, the relationship of inherited variants in the mitochondrial genome with POAG has not been implicated in large prospective cohorts. Given that only a minority of risk factors for POAG have been identified, which includes nuclear genetic abnormalities; I explored the possible link between mitochondrial abnormalities and POAG by identifying mtDNA nucleotide changes in cases and controls in lab cohorts.

Thus this dissertation provides a glimpse on the studies I pursued to better understand the genetics of POAG in the context of its occurrence in India.