REVIEW OF LITERATURE
II. Review of Literature

II.1 Structure of the Eye

The eye can be considered as being optically equivalent to the photographic camera. It has a lens, a variable aperture system (pupil) and a retina corresponding to the film (Guyton, 1996). The globular eye has two segments: a) a large posterior segment and b) a smaller anterior segment. The anterior segment is further divided into the anterior and posterior chambers, and contains the aqueous humor (Williams and Warwick, 1980) (Fig. 1 – page 7). The eye comprises of 3 tunics/layers:

a) **Outer fibrous layer** - this comprises of the sclera, which forms the transparent, non-vascular cornea at the anterior of the eye. The descement’s membrane (of the cornea) disperses over the iridocorneal angle (the angle between the iris and the junction of the cornea and sclera) forming the trabecular tissue/meshwork. The trabecular meshwork (TM) helps to form the Schlemm’s canal, which is the main route of aqueous outflow.

b) **Middle vascular tunic** - this comprises the choroid in the posterior segment and the ciliary body and iris in the anterior segment. The iris divides the aqueous chamber into posterior and anterior chambers (Morrison and Freddo, 1996; Shields, 1998).

c) **Inner neural tunic** - this is composed of the retina (neural retina and non-neural retina). The nerve fibres from retina merge and form the optic nerve that exits through the optic disc. These are supported by the lamina cribrosa (LC) forming the optic nerve head (ONH) (Malwatkar et al., 1999) (Fig. 2 – page 7).

The ciliary body, iris and TM are important with regard to the aqueous humor dynamics. The ciliary body is composed of the anterior pars plicata and posterior pars plana. The pars plicata comprises the ciliary processes that project into the posterior chamber. Each ciliary process is made up of an inner core of capillaries, the surrounding loose stroma and a double layered epithelium (continuous with pars plana) interconnected by specialized intercellular junctions (Kanski et al., 1996; Morrison and Freddo, 1996; Shields, 1998). The ciliary body receives blood via the anterior and posterior ciliary arteries (Morrison and Freddo, 1996).
Fig. 1. Anterior/posterior chambers and the posterior segment (vitreous chamber) of the eye (http://usmlesma.homestead.com/files/1094eye.jpg).

Fig. 2. Horizontal section of the eye with detailed labeling of components (http://www.creativehands.com.sg/hl/eye.jpg).
The TM consists of 3 portions, the uveal meshwork, the corneoscleral meshwork and the juxtacanalicular tissue. The uveal meshwork is the portion adjacent to the aqueous humor in the anterior chamber. The corneoscleral meshwork consists of sheets of trabeculae perforated by elliptical openings (Wordinger and Clark, 1999). The juxtacanalicular tissue is the outermost part of the meshwork, made up of a layer of connective tissue, sandwiched by endothelium. The outer endothelium is the inner wall of Schlemm’s canal (Shields, 1998).

II.2 Intra Ocular Fluid/Aqueous Humor
II.2.1 Aqueous Production

The aqueous humor is secreted by the ciliary processes into the posterior chamber by active transport (Krupin and Civani, 1996). The normal inflow of aqueous is about 2 μl/min (Khaw et al., 2004). The various constituents traverse the capillary wall, stroma and epithelia of the ciliary process to reach the posterior chamber. Transport of substances occurs by one of the 3 mechanisms: a) Diffusion, b) Ultra-filtration and c) Secretion, which is an active process (Shields, 1998). Substances are transported actively across the blood aqueous barrier (non-pigmented epithelium of the ciliary process) to the posterior chamber. This transport depends on the Na+/K+/ATPase pump which secretes Na⁺ into the posterior chamber, creating an osmotic gradient. This leads to movement of other constituents by ultra-filtration and diffusion (Shields, 1998). About 20% of aqueous is produced by ultrafiltration and diffusion (Kanski et al., 1996) (Fig. 3 – page 9).

II.2.2 Outflow of the Aqueous

Initially the 'primary' aqueous is secreted into the posterior chamber. The composition is altered by reabsorption by the iris/ciliary body or by addition of metabolites to form the 'secondary' aqueous, which then enters through the pupil into the anterior chamber. The aqueous exits from here by 2 ways: a) the conventional/direct outflow which takes place through the TM into Schlemm’s canal, through the collector channels into the episcleral and intrascleral veins, b) the unconventional/indirect outflow which takes place via the uveoscleral pathway (Kanski et al., 1996; Lütjen-Drecoll and Rohen, 1996). Approximately 90% of the outflow occurs via the direct/
Fig. 3. Active transport of ions and water across the blood aqueous barrier into the posterior chamber of the eye (http://www.xalatan.com/hcp/image_library/anatomy_and Physiology/G_015_2).
conventional route and 10% through the indirect or unconventional route (Fig. 4 - page 11).

The Schlemm’s canal is a circumferential channel, the inner wall of which is lined with irregular spindle shaped endothelial cells, containing giant vacuoles. These vacuoles form transcellular micro channels through which the aqueous passes into the Schlemm’s canal (Lütjen-Drecoll and Rohen, 1996). From the Schlemm’s canal, collector channels transport the aqueous to the episcleral veins.

The aqueous has been seen to diffuse through virtually every portion of the eye. The uveoscleral tract is the only structure wherein this outflow has been studied in detail other than the conventional pathway (Shields, 1998).

II.2.3 Functions of the Aqueous

The aqueous maintains a proper IOP in the eye (keeping it fully distended). It provides substrates and removes metabolites from avascular parts like the cornea and the lens (Shields, 1998).

II.2.4 Properties of the Aqueous

The aqueous is slightly hypertonic and acidic compared to the plasma. Ascorbate, IgG and a number of growth factors like transferrin, TGF-β, endothelin etc. have been observed in the human aqueous. A high percentage of lower molecular weight proteins are present in the aqueous, while only trace amounts of high molecular weight proteins have been observed (Krupin and Civan, 1996; Shields, 1998). Diurnal variation in the rate of inflow has been observed with the inflow in the ‘morning to noon’ being higher than that from ‘noon to evening’. Aqueous production decreased slightly with increasing age. Diabetes mellitus, inflammation, hypoxia and hypothermia cause a decrease in inflow. A significant increase in aqueous inflow is associated with drinking of water (Kanski et al., 1996; Shields, 1998).
Fig. 4. Movement of the aqueous after secretion into the posterior chamber and the two modes of outflow viz. trabecular and uveoscleral
1.3 Intraocular Pressure (IOP)
1.3.1 IOP - Definition

The intraocular fluid creates a pressure on the eyeball to keep it distended. Factors such as, rate of aqueous secretion, resistance encountered in outflow channels and level of episcleral venous pressure determine the level of IOP. The normal rate of aqueous inflow is about 2 μl/min (Khaw et al., 2004). The normal facility of aqueous outflow is 0.2 μl/min/mmHg and normal episcleral venous pressure is 10 mmHg (Kanski et al., 1996). The normal level of IOP ranges from 11 to 21 mmHg. There is no fixed cut off value of IOP to delimit the normal and the glaucomatous eye. The upper limit of normal IOP is internationally accepted as being 21 mmHg (Shiose, 1990). An increase in IOP with age has been observed with the exception of the Japanese (Shiose, 1990; Kamal and Hitchings, 1998). Women had a higher average IOP than men with a greater tendency for IOP to increase with age.

II.3.2 Factors Influencing IOP

Many factors influence the IOP. These include:

a) Factors exerting long-term influence on IOP:

These factors exert in variable degrees a sustained influence on IOP throughout the lifetime of the individual. These include: i) genetics, ii) age, iii) sex, iv) refractive error (Shiose, 1990; Shields, 1998) and v) race (Shiose, 1991).

b) Factors exerting short-term influence on IOP:

These factors are associated with a rise or fall in IOP, lasting from seconds to months. These include: i) diurnal variation, ii) postural variation, iii) exertional influences, iv) lid and eye movement, v) systemic conditions which include obesity, pulse rate, haemoglobin concentrations, certain hormones, diabetes and systolic BP, and vi) environmental conditions (Shiose, 1990; Shields, 1998).

II.4 Glaucoma Definition and History
II.4.1 Definition

Glaucoma is an optic neuropathy characterized by optic nerve head (ONH) cupping which would lead to loss of peripheral vision followed by total blindness, if not
treated in time. IOP is elevated in most of the cases and is thought to contribute to the nerve damage. The vision loss that occurs is irreversible (Ikezoe et al., 2003).

II.4.2 History

The history of glaucoma may be divided into 3 periods:

i) 400 BC to 1600 AD - During this period glaucoma was considered as a general group of blinding ocular diseases. It was known to cause severe pain and total blindness in the elderly.

ii) 17th century to 19th century (middle)/(1600 – 1854 AD) - The cardinal signs of glaucoma came to light during this period. English Oculist, Bannister in 1626 first mentioned elevation of IOP as a distinct sign of this ocular disease. In the early 1800s firmness of the eye ball was generally accepted as a distinct disease entity which MacKenzie described as glaucoma (Flammer et al., 2002).

iii) 1854 to present - Edward Jaeger was the first to describe the glaucomatous disc and Albrecht Von Graefe, was the first to recognize an elevation of IOP as not just a symptom but as the 'essence' of glaucoma (Tasman and Jaeger, 1996). In 1857, Von Graefe, proposed the concept of a disease process resembling glaucomatous optic neuropathy with normal IOP. Smith in 1885 suggested the involvement of both mechanical and vascular factors (Tanna and Jampel, 2000).

Lately, during the past few decades, the main focus has been on the genetics and pathophysiology. Identification of gene loci/gene(s)/mutations in gene(s) involved in POAG is being carried out. Another main area of focus has been the elucidation of the functional aspects of the proteins said to be involved in POAG, especially the TIGR/Myocilin protein. Further, the non-IOP dependent factors are a major area of interest both pathologically and therapeutically (Borrás et al., 2002; Wiggs et al., 2004).

II.5 Glaucoma - Classification

Glaucoma can be classified into 2 main types:

a) Congenital (developmental) and b) Acquired.

Further sub-classification of the acquired type is based on the mechanism by which aqueous outflow is impaired with regard to the iridocorneal angle recess between
the iris and TM. This results in two sub-types, i) Open Angle Glaucoma (OAG) and ii) Angle Closure Glaucoma (ACG) (Kanski et al., 1996) (Figs. 5 and 6 – page 15).

The above mentioned three types, congenital, open angle and angle closure glaucomas have been classified into 2 further sub-types. These are the a) Primary glaucomas, wherein the events leading to outflow obstruction and IOP elevation have no apparent contribution from other ocular or systemic disorders and b) Secondary glaucomas in which a recognizable ocular or non-ocular disorder alters aqueous outflow which in turn results in elevation of IOP (Kanski et al., 1996; Raymond, 1997; Shields, 1998).

The various types of glaucoma based on these classifications include:

II.5.1 Primary Open Angle Glaucoma (POAG)

Based on the age of onset, there are 2 types of POAG:

1) Juvenile onset POAG (JOAG). This is the less common form, occurs between 3 and 35 years (yrs) of age and manifests with very high levels of IOP (Sarfarazi, 1997).

2) Adult onset POAG (COAG/POAG). The age of onset is after 35 years (middle to late age). It has a slow, insidious course (Morissette et al., 1995; Raymond, 1997; Wirtz et al., 1997). The painless progression (asymptomatic nature) leads to late diagnosis by when the irreversible loss of vision has occurred. Another subtype of POAG is the NTG wherein the patients have normal IOP levels, typical glaucomatous cupping of the ONH with visual field loss and a open angle. This sub-type accounts for 1/3 – 1/5 of all POAG cases (Sarfarazi et al., 1998; Tanna and Jampel, 2000; www.glaucoma.org.au/art_ind.htm).

II.5.2 Secondary OAG

This type of glaucoma is classified into 3 sub-types based on the point at which aqueous outflow is obstructed: a) Pre-trabecular, e.g. neovascular glaucoma; b) Trabecular, e.g. pigmentary glaucoma (Yang et al., 2001), pseudoexfoliation glaucoma (PEG) (Kanski et al., 1996; Ritch, 2001; Foster et al., 2002), red cell glaucoma, ghost cell glaucoma, phacolytic glaucoma, anterior uveitis; c) Post-Trabecular- in this type the aqueous outflow is impaired due to elevated episcleral
Fig. 5. The normal position of the iris and the normal angle in cases of open angle glaucoma (http://www.eyeinstitute.net/../.gonioscopy.html).

Fig. 6. The change in position of the iris and thus a closure of the angle structure resulting in the blockage of the outflow of the aqueous (http://www.eyeinstitute.net/../.gonioscopy.html).
venous pressure (Johnson, 1998) such as in cases of carotid-cavernous fistula and Sturge Weber syndrome (Kanski et al., 1996).

II.5.3 Primary Angle Closure Glaucoma (PACG)

PACG seems to be as common as POAG on a global scale. The most common type is PACG with pupil block wherein the pupil block acts as the precursor for onset of glaucoma. PACG has been seen to be divided into 5 overlapping stages, namely, latent, intermittent, acute, chronic and absolute (Kanski et al., 1996; Johnson, 1998; Shields, 1998). The prevalence is more in Asia (Coleman and Wilson, 2000).

II.5.4 Secondary ACG

In this type, the aqueous outflow is impaired by apposition between the peripheral iris and the trabeculum by either posterior or anterior mechanisms/forces i.e., the iris may be pushed or pulled respectively (Kanski et al., 1996; Shields, 1998).

II.5.5 Primary Congenital Glaucoma (PCG)

In this type, the aqueous outflow is impaired as a result of maldevelopment of the trabeculum (isolated trabeculodysgenesis) including iridotrabecular junction, incomplete development of the meshwork or Schlemm’s canal.

II.5.6 Secondary Congenital Glaucoma

This is the type wherein onset of glaucoma is associated with some other primary ocular conditions such as tumour (like retinoblastoma), intraocular inflammation, trauma etc. (Kanski et al., 1996). Apart from these types, congenital glaucoma also manifests in association with certain syndromes such as Peter’s anomaly, Axenfeld Rieger syndrome etc. (Kanski et al., 1996).

II.6 Glaucoma - Prevalence/Incidence

Thylefors and Negrel (1994) estimated a global glaucoma blindness figure of 5.2 million with about 20 million being affected by glaucoma. An earlier estimate in 1990 has reported a global blindness prevalence of 0.7% (38 million blind) and 145 million
visually impaired (Thylefors et al., 1995). Each year, the number of blind was expected to increase by 2 million and was expected to double by 2020 (Roodhooft, 2002). In India, prevalence of blindness was estimated to be 1% in the year 1990 i.e., 8.9 million blind (Thylefors et al., 1995) (Fig. 7 – page 18). In the year 2002, there were an estimated 6.7 million blind i.e. a decrease of 25% has been reported (Resnikoff et al., 2004). India has a regional burden of blindness (RBB) > 1 and is second on the list of regions with the greatest RBB (Table 1 – page 19) (Thylefors et al., 1995). A regional burden of blindness (RBB) > 1 identifies those regions where the burden of blindness is to be taken into urgent consideration. In India, glaucoma is the second single major cause of blindness, next to cataract.

About one third of the world blindness is observed between the ages of 45 to 59 years (Thylefors et al., 1995). Glaucoma is the leading cause of irreversible blindness in West Africa where ~20% of the people older than 40 years have been estimated to be at risk (Budenz and Singh, 2001). Glaucoma is the second leading cause of blindness globally (Kingman, 2004).

Prevalence of the different types of glaucoma varied with race. PCG has a prevalence of 1:10,000 (western populations); 1:1250 (Slovakian population); 1:2500 (middle east) and 1:3300 in India (Panicker et al., 2002).

II.7 POAG - Primary Open Angle Glaucoma

II.7.1 Definition and clinical features

POAG is described as a multifactorial optic neuropathy that is chronic and progressive with a characteristic acquired loss of nerve fibres. The characteristic changes seen are increased cupping or thinning of neuroretinal rim, disc hemorrhages, asymmetry of cupping in the 2 eyes and loss of retinal nerve fibre. The appearance of ONH is described as the cup/disc ratio. Such nerve fibre losses occur in the presence of an open anterior chamber angle and mostly an elevated IOP, finally resulting in irreversible visual field (VF) loss. IOP can be raised without the accompanying optic disc damage and VF abnormalities and this has been termed as ocular hypertension (OHT) (www.emedicine.com/oph/topic139.htm; Coleman, 1999). POAG is a bilateral
Fig. 7  Graphical representation of the WHO estimation of the distribution of blindness by economic region (1990) (Thylefors et al., 1995) (http://www.who.int/pbd/pbl/Show/BL-Magnitude/Slld008.htm).
Table 1. Regional burden of blindness (RBB) (Thylefors et al., 1995).

<table>
<thead>
<tr>
<th>Region</th>
<th>% of global population (A)</th>
<th>% of global blindness burden (B)</th>
<th>RBB (B/A)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Established Market Economies</td>
<td>15.1</td>
<td>6.3</td>
<td>0.41</td>
</tr>
<tr>
<td>Former Socialist Economies of Europe</td>
<td>6.6</td>
<td>2.9</td>
<td>0.44</td>
</tr>
<tr>
<td>India</td>
<td>16.1</td>
<td>23.5</td>
<td>1.46</td>
</tr>
<tr>
<td>China</td>
<td>21.4</td>
<td>17.6</td>
<td>0.82</td>
</tr>
<tr>
<td>Other Asia and Islands</td>
<td>13</td>
<td>15.3</td>
<td>1.18</td>
</tr>
<tr>
<td>Sub-Saharan Africa</td>
<td>9.7</td>
<td>18.8</td>
<td>1.93</td>
</tr>
<tr>
<td>Latin America and the Caribbean</td>
<td>8.4</td>
<td>6.1</td>
<td>0.72</td>
</tr>
<tr>
<td>Middle eastern crescent</td>
<td>9.6</td>
<td>9.5</td>
<td>0.99</td>
</tr>
</tbody>
</table>
and an insidious painless disease, beginning with the loss of peripheral vision followed by loss of central vision and finally irreversible blindness (Schoevaerdts et al., 2003). It is asymptomatic and hence most of the patients are unaware till considerable vision loss has occurred (Khaw et al., 2004) (Fig. 8 - page 21).

II.7.2 Risk Factors

Several risk factors have been associated with the development of POAG including IOP, age, race, family history of glaucoma, genes, myopia (Wu et al., 1999), history of diabetes, systemic hypertension, vasospasm, migraine and gender (Shields, 1998; Coleman and Wilson, 2000; Leske et al., 2002). Other factors include, genetic mutations, large cup/disc ratio, thin corneas (Khaw et al., 2004), intake of steroids, eye injury (www.2020eyemed.com/glaucoma.php), history of eye pain/redness, headache and multi-coloured haloes, systemic cardiovascular diseases, alcohol, obesity, smoking, stress and anxiety (www.emedicine.com~oph/topic139.html; www.ahaf.org/glaucoma/about/glrisk_body.html).

II.7.3 Incidence/Prevalence of POAG

POAG has been reported to be the most common form of glaucoma (Raymond, 1997; Khaw et al., 2004). This has been reiterated by different researchers in their corresponding study populations (Hyman et al., 2001; Herndon et al., 2002). In fact, adult onset POAG is the most common type of glaucoma (Shields, 1998; Rezaie et al., 2002). POAG is the third commonest cause of blindness in UK (Khaw et al., 2004). In the USA, POAG has an age-adjusted prevalence of 1.55% among the individuals older than 40 years of age. The prevalence of glaucoma was higher in blacks (Quigley and Vitale, 1997). Blacks have a younger age of onset and a more aggressive form of POAG (Budenz and Singh, 2001). Bonomi et al. (1998) reported a prevalence rate of 2% for POAG in Italy, of which 0.6% were NTG cases.

Earlier, an equal prevalence of both OAG and ACG in India had been reported (Quigley, 1996). Jacob et al. (1998) reported a higher prevalence of ACG compared to OAG with the prevalence rate of POAG in their study population being 0.41%. In a later study, a prevalence rate of 1.62% for the ≥30 yrs age group and 2.52% for the ≥40 yrs
The eye is always producing fluid. The eye's drainage areas may become clogged or blocked. Too much fluid stays in the eye. This increases eye pressure.

Fig. 8. Visual field loss as a result of increased IOP (http://www.harthosp.org/eyes/conditions/glaucoma.htm).
and older age group was reported by Dandona et al. (2000) in their study group from Hyderabad, AP, India.

Data on the incidence of OAG are limited and difficult to obtain. Most studies have measured prevalence rates and derived the incidence indirectly. After a 4 year period of study by the Barbados Eye Study (BES) group, an incidence of 1.2% (40-49 yrs), 1.5% (50-59 yrs), 3.2% (60-69 yrs) and 4.2 (> 70 yrs) was reported (Wu et al., 2001). Earlier Wilson (1992) estimated an annual incidence rate of 0.24% in the United States.

II.7.4 Diagnosis

The first step in the diagnosis of POAG is the visualization of the anterior chamber angle. A gonioscope is used for this (Shields, 1998). The angle is represented as open or closed using grades, as per the Scheie, Shaffer or Spaeth grading systems (Kanski et al., 1996; Johnson, 1998). The next diagnostic step is to check the IOP levels using the tonometer. The tonometer is of two types, Applanation and Indentation. Both have the similar principle of measuring the applied force that either flattens or indents the cornea.

Optic disc damage is a key feature of POAG and the fore-runner for visual field loss. This can be diagnosed by an ophthalmoscope – direct or indirect type or by using slit lamp biomicroscope – direct or indirect type. Several instruments are now available to provide alternative perspectives of the ONH and the nerve fibre layer e.g. the Rodenstock scanning laser ophthalmoscope, Heidelberg confocal scanning laser ophthalmoscope, etc. (Coleman and Brigatti, 2001). New methods like retinal thickness mapping, multifocal electroretinogram (mfERG) and pattern electroretinogram (PERG) have been introduced (Schuman and Kim, 2000). PERG is a relatively new technique and is at present the best documented electrophysical technique for detecting early glaucomatous damage and can thus be used in counseling patients with ocular hypertension (Bach et al., 1998; Bach, 2001). A symmetrically enlarged cup/disc ratio of > 0.5 (normal ratio: 0.3 or less) is also a clinical diagnostic criteria. Cup/disc ratio asymmetry between the two eyes of 0.2 or more or highly asymmetric cups
should be regarded with suspicion (Kanski et al., 1996; Distelhorst and Hughes, 2003) (Fig. 9 – page 24).

The last and important feature is the visual field loss. Normally, loss of peripheral vision or tunnel vision is noticed after a loss of > 40% of nerve fibres (Distelhorst and Hughes, 2003). Perimetry is the method to evaluate the VF (Kanski et al., 1996). The traditional method is the "kinetic manual perimetry" which has been replaced by automated perimetry due to improved quality and reproducibility of results. Some of the automated perimeters include, Goldman, Octopus and Humphrey. Several new developments in automated perimetry have contributed to the enhanced diagnosis and management of glaucoma. Four of these include: a) Swedish Interactive Threshold Algorithm and Tendency Oriented Perimetry (SITA TOP), b) Frequency Doubling Technology Perimetry (Johnson 2002), c) Short Wavelength Automated Perimetry (SWAP) (Spry and Johnson, 2000; Johnson, 2002) and d) mfERG and the multifocal Visual Evoked Potential (mfVEP) (Johnson, 2002).

Numerous tests have been developed in an attempt to find additional prognostic indicators of COAG. These are also termed as adjunctive tests. These include Tonography (to measure the facility of aqueous outflow), Provocative tests (PT) which includes Water PT, Dilatation PT and Therapeutic trials (Shields, 1998).

II.8 POAG - Pathophysiology

Development or onset of glaucoma can be visualized to occur in five stages (Shields, 1998) (Table 2 – page 25). The ideology that glaucoma is a condition wherein pressure damages the optic nerve is challenged by cases of NTG and OHT. However, IOP is the only risk factor for which good treatment regimens are available. Patients with nerve damage during diagnosis were more likely to get worse than those without loss. The phenomenon of "delayed functional loss" wherein certain patients had successful therapeutic intervention to decrease IOP but had cupping and VF loss over the years was first observed by Brubaker (1996). Four assumptions were made for the delayed functional loss of vision; a) a process independent of IOP kills ganglion cells, b) unmeasured increased IOP, c) a genetically determined hypersensitivity to IOP, wherein
Fig. 9. Diagramatic representation of a normal and glaucomatous optic cup/disc ratio (Khaw et al., 2004).
Table 2. Five stage onset of glaucomatous visual field loss (Shields, 1998).

<table>
<thead>
<tr>
<th>Stages</th>
<th>Definitions</th>
<th>Pressure- Related</th>
<th>Pressure-Independent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Initiating events</td>
<td>The series of events that may lead to...</td>
<td>Genetic; acquired</td>
<td>Genetic? (less well understood)</td>
</tr>
<tr>
<td>2. Structural alterations</td>
<td>Tissue changes that may lead to...</td>
<td>Alterations in aqueous outflow system</td>
<td>Alterations related to optic nerve head (e.g., vascular, structural, etc.? )</td>
</tr>
<tr>
<td>3. Functional alterations</td>
<td>Physiologic changes that may lead to...</td>
<td>Elevated intraocular pressure</td>
<td>Reduced vascular perfusion, laminar deformity, etc.?</td>
</tr>
<tr>
<td>4. Optic nerve damage</td>
<td>Axonal loss that leads to...</td>
<td>Glaucmatous optic neuropathy</td>
<td></td>
</tr>
<tr>
<td>5. Visual loss</td>
<td>Progressive loss of visual field...</td>
<td>Glaucmatous visual field loss</td>
<td></td>
</tr>
</tbody>
</table>
people loose ganglion cells at pressures within the normal range and d) increased pressure causing indirect changes.

II.8.1 IOP Elevation

The mechanism of increase of IOP is not fully elucidated yet. Increased IOP may result due to the decreased outflow facility through the trabecular meshwork. Theories suggested for this include: obstruction of the TM by foreign bodies, loss of trabecular endothelial cells, loss of giant vacuoles in endothelium of Schlemm’s canal etc. No evidence regarding the actual site of resistance/obstruction is available (Lee, 1995). The exact site and nature of resistance to outflow is uncertain. However, the extracellular matrix (ECM) of the TM is the likely site of resistance to normal aqueous outflow (Acott and Wirtz, 1996). A decrease in the number of giant vacuoles and cell count in Schlemm’s canal have also been observed (Shields, 1998). In POAG, the obstruction is mostly located within the trabecular meshwork. Pores and vacuoles (giant) found in the inner endothelial wall of Schlemm’s canal is decreased or absent in COAG. Collapse of Schlemm’s canal or alteration in the intrascleral channels may cause obstruction to outflow. Abnormal constituents of the aqueous humor may increase resistance to outflow, like increased TGF-β2. This may decrease the cellularity of the TM and enhance build up of ECM material with subsequent resistance to outflow (Tripathi et al., 1994; Lütjen-Drecoll et al., 2001).

II.8.2 Ganglion Cell Death

Two theories have been postulated to account for the damage to the ganglion cell: a) The mechanical hypothesis - changes in the connective tissue structure of the ONH like misalignment of the connective tissue beams in the LC leads to kinking of the bundles of ganglion cell axons and mechanical obstruction of rapid phase axonal transport, b) Vascular hypothesis - ischemia in combination with elevated IOP causes blockage of axonal transports which results due to blood flow interruption of the short posterior arteries. This may be due to accumulation of metabolic tracers in the lamina cribrosa (McKinnon, 1997). Blockage of axonal transport leads to apoptosis of the ganglion cells. Most neurons depend on small peptides such as neurotrophins, neurotrophic factors, cytokines or growth factors for survival e.g. BDNF(Nickells, 1996).
Blocking the flow of BDNF and/or other neurotrophins compromises on the viability of the ganglion cells (McKinnon, 1997).

II.8.2.1 Excitotoxins and Nitric Oxide Pathway

Neurotransmitters under certain conditions exert their toxic effect on the neurons by inducing cell death. These are termed as excitatory neurotransmitters or excitotoxins e.g. glutamate. Glutamate mediated toxicity is a primary response to cellular ischemia (Nickells, 1996). High levels of glutamate stimulates cell surface receptors such as N-methyl D-aspartate (NMDA). In some neurons, activation of nitric oxide synthase occurs leading to generation of nitric oxide (NO). NO forms peroxynitrites which leads to nitrosylation and fragmentation of DNA (Nickells, 1996). Glutamate and the NMDA subtype of glutamate receptors have been shown to be more toxic to larger ganglion cells which have been suggested to be affected first in glaucoma (Glovinsky et al., 1991). However, a reduction in size of large ganglion cells before atrophy had already been indicated (Quigley et al., 1987). RGCs undergo morphologic changes like reduction of cell volume with reduction in size of the axon and dendritic tree (Morgan, 2002).

II.8.3 Other Factors

Involvement of other factors besides IOP was put forward based on many instances that could not be explained by the pressure theory. One sixth of the patients were NTG patients (Sommer et al., 1991). OHT was 10 times more common than glaucomatous damage (Bengtson et al., 1981). Men and women had similar IOP levels but incidence of NTG in females was almost twice as that of males (Flammer and Orgül, 1998). Glaucomatous damage was higher in blacks (Afro-Americans) than whites, though average IOP of both are about the same (Tielsch et al., 1991). In Japan, incidence of damage increased with age but IOP decreased with age (Shiose et al., 1991). Other than IOP, mainly vascular factors have been reported to be associated with glaucoma (Flammer et al., 1992).

II.8.3.1 Ocular Blood Flow (OBF) in Glaucoma Patients

Circulation in a glaucoma patient is on average much reduced in the eye, behind the eye and in the periphery (Flammer, 2001). Reduction in OBF velocities was seen in
both POAG and NTG patients (Hamard, 1994). NTG patients have more pronounced blood flow disturbances (Drance et al., 2001). Blood flow reduction is more pronounced in progressive than non-progressive eyes (Stewart et al., 2000). Two additive but distinct risk factors most probably involved in glaucoma, especially in NTG are systemic hypotension and vasospastic disorders.

II.8.3.2 Systemic Hypotension

The role of systemic hypotension has been emphasized by observations of increased prevalence in patients with progressive damage as well as in NTG patients (Demailly et al., 1984; Kaiser et al., 1993; Bechetoille and Dumont, 1994; Hayreh et al., 1994). Patients with large nocturnal falls in blood pressure (BP) (dippers) were among those whose visual field loss progressed despite normalized IOP (Graham and Drance, 1999). Counter regulatory mechanisms leading to an increased local resistance has been suggested to be additionally involved with the low perfusion pressure. Observations indicate local regulatory mechanisms might be more important than perfusion pressure (Flammer, 1994; Flammer and Orgül, 1998). The perfusion pressure is defined as the difference between arterial and venous pressure. The venous pressure is equal to or slightly higher than the IOP and earlier it was defined as local BP minus IOP in the eye (Flammer, 1994). Perfusion fluctuations rather than a constant decrease in perfusion is a risk factor. This observation is termed as the reperfusion phenomenon (Flammer, 2001). Increased local resistance may be more often due to a functional vascular dysregulation rather than due to structural changes (Flammer and Orgül, 1998; Flammer et al., 1999).

II.8.3.3 Vascular Dysregulation

The pathophysiology of the vascular dysregulation is poorly understood. A dysfunction of the vascular regulatory system at the ONH may lead to hypoperfusion (such as in vasospasm). Vasospasm has been defined as inappropriate constriction or insufficient dilatation in the microcirculation. Many have a genetic predisposition to respond with vasospasm and this has been termed as vasospastic diathesis (Flammer et al., 2001). Conditions wherein dysregulation occurs more globally, involving many organs simultaneously or sequentially are called vasospastic syndrome. Primary
vasospastic syndrome is of importance to ophthalmologists as the spasms mostly involve the eye. Primary vasospastic syndrome is more prevalent in women and in Japanese explaining why NTG is more prevalent in women and Japanese (Flammer et al., 2001). Patients with vasospastic syndrome tend to have lower BP (especially at night) or increased systemic hypotension and may have periods of low perfusion pressure. Glaucoma patients have been observed to be more vasospastic and an association between vasospasm and glaucomatous optic neuropathy (GON) has been observed (Ghergel et al., 2000). The best diagnostic indicator is probably the increased levels of endothelin 1 (Et1) in the circulating blood (Flammer et al., 1992; Flammer et al., 2001).

The vascular endothelium plays a major role in the vasodilation/vasoconstriction or in the development of vasospasm. The vascular endothelium releases vasoactive substances that can be classified as vasodilators (e.g. NO) and vasoconstrictors (e.g. Et - Endothelin) (Flammer and Orgül, 1998). Switch over from the endothelium derived relaxation to that of endothelium derived constriction, may be a major feature of vasospastic disorders (Flammer et al., 1992). NO and Et, both have been shown to be involved in regulation of IOP (Behar-Cohen et al., 1996), local modulation of OBF (Haefliger et al., 1994; Mann et al., 1995) and NO alone in RGC death (Quigley et al., 1988; Quigley et al., 1995).

NO could increase aqueous outflow and thus lower IOP. Et could decrease outflow resulting in an increase in IOP (Haefliger et al., 1999). Increase in IOP in glaucoma might be due to decrease in NO production and excess of endothelin secretion. The role of Et 1 in regulating (increasing/decreasing) the IOP has been in the debate and lately it has been reported that the increase in synthesis and release of Et-1 could contract the TM and thereby reduce, intertrabecular space leading to increased IOP (Zhang et al., 2003). Increase in Et-1 levels in plasma (seen in NTG cases) could cause initial ischemia which could promote detrimental effects in the ONH.

II.8.4 Apoptosis

The functional loss of vision is caused by death of the retinal ganglion cell and
this is at least partially due to apoptosis (Flammer et al., 2002). The exact mechanism inducing apoptosis is not known. Apoptosis leads to loss of ganglion cells and their axons resulting in excavation of the ONH. Observations have revealed that fluctuating blood flow i.e., reperfusion injury might be stronger risk factors for GON than a stable decrease in blood flow (Asrani et al., 2000; Flammer et al., 2002). Reperfusion damage/injury results in a marked increase in free radicals and thereby oxidative stress leading to increased glutamate concentration and finally to excitotoxicity (Flammer et al., 2001; Flammer et al., 2002). Labudova et al. (2000) provided evidence for the mechanism of RGC death occurring due to apoptosis by observing upregulation of p53 in vasospastic NTG patients while the “survivin” (anti-apoptotic gene) was down regulated.

II.8.5 Rheological factors

Decreased erythrocyte deformability, hyper aggregability of erythrocytes and altered RBC membrane integrity have been described in POAG patients. Such changes in constituents of blood could be independent risk factors occurring in some patients. These rheological factors might also be a consequence of the vascular changes (Flammer et al., 2002). Apart from these, increased frequency of psychological disturbances in patients with NTG have been observed (Erb et al., 1999).

II.9 POAG - Genetics

II.9.1 Introduction

Many of the ocular parameters that predispose to development of glaucoma such as IOP, cup/disc ratio etc. have been shown to have a genetic component (Lichter, 1994; Alward et al., 1996; Johnson et al., 1996). A family history of glaucoma represents a major risk factor. Risk indicators of OAG such as cup/disc ratio, vertical optic cup diameter, vertical optic disc diameter have been shown to correlate highly in families (Klein et al., 2004). About 16 – 22% of first degree relatives of POAG patients are at risk to develop the disease compared to about 2% of general population, i.e., a risk of about 8 – 11 times (Alward et al., 1996; Alward, 2000). About one sixth of all glaucoma in the general population is believed to be caused by a genetic component (Wolfs et al., 1998). The more common adult onset POAG has a complex inheritance [AD, autosomal recessive (AR) and multifactorial modes have been suggested], while JOAG has been
suggested to follow an AD mode of inheritance, both exhibiting reduced penetrance (Wiggs et al., 1995; Wiggs et al., 2004).

II.9.2 Genetic Linkage Analysis for Gene Mapping

There are 3 major strategies used to find the location of a disease gene:
a) Clues from karyotypic abnormalities, b) Genetic linkage analysis and c) Candidate gene approach (Blumenthal and Weinreb, 2000).

II.9.3 Linkage Based Studies in POAG Cases

Association of HLA haplotypes to POAG (Blumenthal and Weinreb, 2000), tasters of PTC to traumatic and uveitic glaucoma and association between glaucoma and various blood groups (ABO blood group, Rhesus group, ABH secretion and non-secretion) (Brooks and Gillies, 1988) have been studied. Naturally occurring variations in DNA sequences between individuals provide a virtually unlimited supply of genetic markers such as short tandem repeat markers (STRMs) for linkage analysis (Damji and Allingham, 1997). Johnson et al. (1993) analyzed a family with a 5-generation history of JOAG and suggested an AD mode of inheritance. Linkage to the Rieger’s syndrome locus (4p25) was excluded (Johnson et al., 1993).

II.9.3.1 GLC1A

Sheffield et al. (1993) using linkage analysis (90 STRs) mapped the first locus to chromosome 1q21-q31 in a single large pedigree affected by an AD form of JOAG. A large Caucasian family with AD JOAG was studied and linkage to the reduced interval size of 14 cM between D1S194 and D1S218 was established (Richards et al., 1994). In the following year, linkage to the 1q21-q31 region was demonstrated in 5 (JOAG) pedigrees of Irish, British and German descent. Two families (with juvenile glaucoma) did not show linkage to 1q21-q31, suggesting genetic heterogeneity. These families had IOPs lower than those linked to the 1q21-q31 locus (Wiggs et al., 1995). Two AD JOAG families (one Danish and one Swedish) were investigated in one study. Linkage to 1q21-q31 in the Danish family was ascertained while in the Swedish family no linkage was noted, suggesting AD JOAG to be genetically heterogeneous (Graff et al., 1995).
Morissette et al. (1995) carried out linkage analysis in a huge multi-generational French-Canadian family with both JOAG and adult onset POAG cases. The interval size was reduced to 9 cM. This study first demonstrated linkage of the GLC1A gene to both adult and juvenile onset OAG. Further reports of Johnson et al. (1996), Meyer et al. (1996) and Lichter et al. (1997a) confirmed linkage of AD POAG/JOAG to GLC1A and reduced the interval size to 7 cM (Fig. 10 – page 33). This locus was named as the GLC1A locus by the HUGO genome database. ‘GLC’ is the general symbol for glaucoma genes. 1, 2 and 3 respectively are to represent open-angle, angle closure and congenital types of glaucoma and ‘A’, ‘B’, ‘C’... refer to the first, second or third... gene/gene loci mapped (Raymond, 1997). Genetic heterogeneity reported by Graff et al. (1995) was further confirmed by Richards et al. (1996) and Avramopolous et al. (1996).

II.9.3.2 Other Gene Loci

Linkage to 2cen-q12 region within a 11.2 cM region (GLC1B locus) was observed in some families with moderate to low IOP (Stoilova et al., 1996). Genetic heterogeneity was observed by Allingham et al. (1998a) suggesting the presence of other gene(s)/gene loci. Another locus for adult onset POAG, the GLC1C locus was mapped to 3q21-q24 (within a 11.1 cM region) by linkage analysis (Wirtz et al., 1997). Recently, linkage to the GLC1C locus at 3q21-q24 was established in a large greek pedigree with adult onset AD POAG (Kitsos et al., 2001).

Lichter et al. (1997b) provided indirect evidence for a possible glaucoma locus at 9q34. Meanwhile, Trifan et al. (1998) excluded linkage to the GLC1A, GLC1B and GLC1C loci in a family with adult onset POAG and observed linkage to 8q23 (GLC1D locus) (6.3 cM interval). Sarfarazi et al. (1998) in a genome-wide search established linkage of a large British family with classical adult onset NT-OAG to the 10p15–p14 region, within a interval of 5-11 cM (GLC1E locus). The following year Wirtz et al. (1999), identified a new locus (GLC1F) mapping to 7q35–q36 in a large family with adult onset POAG by linkage analysis. The interval size was limited to 5.3 cM. The GLC1A locus is the only locus which is involved in both JOAG and adult onset POAG and in different populations of the world. Recently, Monemi et al. (2005) have localized
10. Locus intervals mapping to the chromosome 1q21 in POAG patients, based on recombination events observed by various authors (Johnson et al., 1996).
another locus (GLC1G) for adult onset POAG to the 5q22.1 (~2 Mb region). The candidate gene in this locus has also been identified. In a very recent study, early adult onset POAG was linked to the GLC1I locus at 15q11-13 using ordered subset analysis (Allingham et al., 2005).

II.9.4 TIGR/MYOC Gene

The TIGR gene shown to be mutated in POAG patients has been characterized. It consists of 3 exons, separated by 2 intervening sequences or introns. The exon/intron boundaries are in conformity with the GT/AG consensus (Kubota et al., 1997; Nguyen et al., 1998; Tamm and Russel, 2001). A mRNA of 2.37 to 2.5 Kb has been shown by northern blot analysis (Ortego et al., 1997; Fingert et al., 1998; Nguyen et al., 1998; Clark et al., 2001b). The proposed open reading frame of MYOC has two possible start sites (ATG) separated by 42 nucleotides. A signal sequence has been observed following the second ATG site by various authors. Cleavage occurs between amino acids (aa) 32-33 (Ala-Arg) (Kubota et al., 1997; Ortego et al., 1997; Nguyen et al., 1998; Caballero et al., 2000).

Gene sequence analysis revealed presence of 2 major domains in MYOC, a myosin like domain near the N-terminal and an olfactomedin like domain near the C-terminal. The myosin like N-terminal domain has homology of ~25 – 29% aa identity with the heavy chain of myosin of different species, especially the non-muscle myosin of Dictyostelium discoideum (Kubota et al., 1997; Ortego et al., 1997). The C-terminus is homologous to olfactomedin (Adam et al., 1997; Kubota et al., 1997; Tamm and Russel, 2001). Numerous transcription factor binding sites which may be necessary for transcription namely, TATA box, Sac box, AP-1 like sequences, AP-2 sites, E box, NF-kB-related site have been identified (Kirsten et al., 2000; Fingert et al., 2002). Sequences similar to portions of glucocorticoid response elements (GRE) (i.e.) TGTTCT, were seen to be present upstream of MYOC. A number of hormone and cell signaling response elements have been observed within 5 Kb upstream of the TIGR gene. Other gene response elements (both early and immediate) such as serum response element (SRE) and an interferon consensus sequence (ICS) have also been observed at 5 Kb upstream of the MYOC gene (Nguyen et al., 1998).
II.9.5 *TIGR* Mutations

Several genes of the GLC1A locus were considered as candidate genes. Stone *et al.* (1997) first observed mutations in the *TIGR* gene in 5 of the 8 families they analysed using PCR-SSCP methodology. The GLC1A gene was found to be involved in sporadic cases also. Overall missense or nonsense mutations were found in 13 of 330 unrelated glaucoma patients i.e., ~3.9%.

Five novel non-conservative aa substitutions in 8 families were reported by Adam *et al.* (1997). Michels-Rautenstrauss *et al.* (1998) fine localized the *TIGR* gene to lq24.3–q25.2 and observed the Pro370Leu and Gly367Arg substitutions in two families. No mutations were observed in the 100 unselected sporadic patients. In a large study involving 1446 subjects, thirty eight (38) sequence changes (16 probable disease causing) were reported in the *TIGR* gene at a frequency of 4.6% (Alward *et al.*, 1998a).

Two mutations (previously reported) were observed in 2 of 25 JOAG pedigrees i.e., a frequency of 8%. Five of 127 adult onset families had sequence alterations including the Glu352Lys which was suggested to be a rare polymorphism (Wiggs *et al.*, 1998a). Meanwhile, Kennan *et al.* (1998) observed an Asp380Ala change in a GLC1A linked AD JOAG Spanish family. Brezin *et al.* (1998) provided the first documented example of a founder effect for the N480K mutation. A deletion of 4 nucleotides (GACA) at 1177 and insertion of a T (substitution of glutamine and glycine by valine) was seen in 20 individuals showing linkage to GLC1A. Haplotype analysis indicated founder effect (Anguis *et al.*, 1998).

The Lys423Glu mutation was observed in a large French-Canadian family (linked to the GLC1A locus) (Morissette *et al.*, 1998). They also suggested the dominant negative effect of the mutation when present in single dose (Morissette *et al.*, 1998). Mansergh *et al.* (1998) observed a Val426Phe change in a JOAG family and a Gly367Arg in an adult onset POAG family. Three mutations in 5 of 29 families were observed by Allingham *et al.* (1998b). Sequence changes, Pro370Leu, Val426Phe, Glu323Lys and Gly252Arg were seen to co-segregate with both JOAG and POAG cases (Rozsa *et al.*, 1998). Meanwhile, Richards *et al.* (1998) observed linkage to GLC1A
locus in one branch of the family with AD JOAG and the co-segregation of the mutation Ile477Asn. During the same year, Mardin et al. (1999) reported the Gln368Stop mutation in a single patient with normal tension glaucoma.

In a vast study spanning 5 different populations, a total of 1708 patients from Iowa city (707), African Americans from New York city (312), Australia (390), Canada (167) and Japan (107) were analysed by Fingert et al. (1999). Sixty one sequence alterations of which 21 were probable disease-causing variations were recorded. The overall mutation rate was 3.4%. The rates in each of the places individually were Iowa - 4.8%, African Americans from New York city - 2.6%, Japan - 2.8%, Canada - 3% and Australia - 2.8%. Eighteen of the 21 probable disease causing variations were seen to occur in exon III (Fingert et al., 1999).

An AR mode of inheritance in the family with the Arg46Stop mutation was observed. Further, a mutation frequency of 4.4% was reported in Korean patients (Yoon et al., 1999). Damji et al. (1999) reported the Pro370Leu mutation in a Canadian JOAG family with a similar phenotype to that reported earlier (Adam et al., 1997; Suzuki et al., 1997; Stoilova et al., 1998). Taniguichi et al. (1999) characterized the phenotype of two JOAG patients with the Pro370Leu mutation in a Japanese family. Mutations in 9 of 25 JOAG (36%); 2 of 49 (4%) adult onset POAG patients were observed in a study by Shimizu et al. (2000). The functional test of triton X-100 solubility assay was developed for exon III mutations and was carried out for 11 of the 13 variants (Zhou and Vollrath, 1999).

Seven of 25 unrelated Brazilian patients (i.e., 28%) were shown to have a novel mutation Cys433Arg by Vasconcellos et al. (2000). Haplotype analysis suggested presence of founder effect. During the same year Lam et al. (2000) screened the TIGR gene for mutations in 91 Chinese patients and recorded 10 sequence alterations (including the changes in the non-coding region). The Asp208Glu reported earlier in a Japanese patient with OHT was found in 2 POAG patients and a normal subject. Thr353Ileu earlier considered as a mutation (Fingert et al., 1999) was found in 3 controls also. Arg46Stop (in homozygous state) was seen in normal individuals in contrast to the
report of Yoon *et al.* (1999), emphasizing a gain of function effect of the mutation (Lam *et al.*, 2000). Founder effect for the Gln368Stop mutation and presence of other genetic/environmental factors to be associated with glaucoma in pedigrees with the mutation Gln368Stop was suggested in a later study (Craig *et al.*, 2001).

Mukopadhayay *et al.* (2002) observed two mutations in the *TIGR* gene among the 56 unrelated POAG patients screened from Kolkata, India. All mutations were in the heterozygous state. In another study, the *TIGR* gene was screened by PCR followed by denaturing high performance liquid chromatography (DHPLC) in 90 POAG patients from West Africa. Two mutations were observed and a mutation frequency of 4.4% was recorded (Challa *et al.*, 2002). Pang *et al.* (2002) observed 21 sequence changes in 201 POAG patients in China of which only 3 were seen exclusively in POAG patients at a mutation frequency of ~ 1.5%.

Faucher *et al.* (2002) reported 9 of the 20 sequence variants observed, to be disease causing mutations, in a Caucasian population. The frequency of mutations in familial POAG cases was 22.2% and in unrelated POAG cases was 3.8%. Their study showed that *TIGR* mutations may also be associated with other forms of glaucoma. Alward *et al.* (2002) evaluated the prevalence of MYOC mutations in a large consecutive unselected group of 779 patients of which 524 were POAG cases and that included adult onset POAG, JOAG and NTG cases. Seventeen of them (3.2%) had a disease causing variant. 3.3% of adult onset POAG, 6.4% of JOAG and 1.2% of NTG had disease causing variants. Adult onset POAG patients with and without disease causing variants (DCVs) in MYOC were observed to be phenotypically similar.

Colomb *et al.* (2001) reported a promoter polymorphism in MYOC, a novel biallelic polymorphism -1000 C/G, wherein the ‘G’ allele was designated as MYOC.mt1. Prevalence of this allele was found to be similar in patients with adult onset POAG and controls (Alward *et al.*, 2002). Polansky *et al.* (2003) have shown that MYOC.mt1 (+) accelerates worsening of both optic disc and visual field.

Phenotypic analysis of 4 unrelated pedigrees with Thr377Met was carried out by
Mackey et al. (2003). Recently, Bruttini et al. (2003) reported a mutation frequency of 8% in families with POAG/JOAG with AD inheritance. Baird et al. (2003) examined 15 unrelated POAG families who carried the Q368Stop mutation from southeastern Australia. Haplotype analysis indicated a common haplotype/founder. Ikezoe et al. (2003) reported the Gly451Asp in a POAG patient from Japan. In the same year Izumi et al. (2003) screened the MYOC gene for mutations in 80 Japanese NTG cases. Six different nucleotide changes were observed.

Recently, Kanagavalli et al. (2003) screened the TIGR gene for mutations in 107 POAG patients from India and observed 2 patients with heterozygous mutations, Gly367Arg and Thr377Met. The mutation frequency was established to be ~2%. Sripriya et al. (2004) have reported the Gln48His in 2 of 100 patients (mutation frequency of 2%) from India. Meanwhile, only neutral changes were observed in 45 Polish patients (Krawczyński et al., 2004). A recent article has reviewed most of the variants, though some variants which were observed in controls have also been included as disease causing variants. Most of the mutations were seen to lie in the exon III (Gong et al., 2004). Screening of the Myoc gene for mutations using DHPLC in Japanese patients revealed 4 mutations (Phe369Leu – novel; Ile360Asn; Ala363Thr; Thr448Pro), all of which appeared to be specific to the Japanese patients. A mutation rate of 2.9% was recorded (Ishikawa et al., 2004).

Rezaie et al. (2002) found a second gene to be mutated in adult onset POAG namely the OPTN (optineurin) gene mapping to the GLC1E locus. Mutations in OPTN was seen to be responsible for 16.7% of hereditary forms of NTG. The gene has 3 noncoding exons in 5' UTR and 13 exons coding for 577 aa residues. The protein has a molecular weight of 66 kDa and a pI of 5.15 (Sarfarazi et al., 2003). The WDR36 (WD40-repeat36) gene mapping to the newly identified GLC1G locus, has been seen to be mutated in unrelated POAG patients. WDR36 is a novel gene with 23 exons, which encodes for 951 amino acids and a protein with multiple G-beta WD40 repeats (Monemi et al., 2005).
II.9.6 Genetic Heterogeneity/New Loci

Recently, Forsman et al. (2003) ruled out the involvement of the TIGR and OPTN genes in POAG families from Finland suggesting genetic heterogeneity. Linkage to chromosomes 2 and 10 were observed in a number of POAG families by Nemesure et al. (2003). However, further screening of these two regions is essential to draw a conclusion. Wiggs et al. (2000) scanned the genome to identify the genomic location of glaucoma susceptibility genes. Five regions on chromosomes 2, 14, 17p, 17q and 19 were observed to be significant with a MLS > 2.0. Lemmelä et al. (2004) observed linkage to new loci such as 2p14, 2q33-34, 10p, 14q, 17p, 17q and 19q supporting genetic heterogeneity. Wiggs et al. (2004) observed linkage to 9q22 and 20p12 in families that did not have linkage to GLC1A. Evidence for a new AD glaucoma loci mapping to 3p21-22 was reported in a family with POAG. Some members of the family had the Q368Stop mutation in the myocilin gene, exhibiting genetic heterogeneity (Baird et al., 2005).

II.9.7 Other Genes

Lin et al. (2002) suggested the possible role of the codon 72 polymorphism (Arg72Pro) of the p53 gene in POAG. Acharya et al. (2002) found no significant association between the codon 72 polymorphism and POAG patients from India. Genetic heterogeneity between HTG and NTG was suggested as the OPA1 gene was not significantly associated with HTG compared to NTG (Aung et al., 2002). The E 4 allele of the apolipoprotein E gene was more common in NTG and HTG individuals with the frequency being almost twice as that of controls (Vickers et al., 2002). Pressure or stress upregulates many genes in the TM including the Trabecular meshwork inducible stretch response (TISR) gene (Sato et al., 1999). Mutation screening of this gene in Chinese patients could not establish the effect of TISR on glaucoma (Jansson et al., 2003). Recently, an association between the endothelin type A receptor gene polymorphism and NTG risk factors has been suggested (Ishikawa et al., 2005).

II.9.8 Genetics of Other types of Glaucoma

Congenital Glaucoma

An AR inheritance with complete penetrance/multifactorial inheritance has been
said to be associated with PCG (Genčık et al., 1980). Approximately 10% of cases are
familial and show AR inheritance. Penetrance ranges from 40% to 100% (Lakhotia,
2002). This disorder has been linked to at least 2 chromosomal loci; GLC3A mapping to
2p21, (Sarfarazi et al., 1995) and GLC3B mapping to 1p36 region (Akarsu et al., 1996).

Stoilov et al. (1997) identified the gene cytochrome P4501B1 (CYP1B1) in the
2p21 locus to be mutated in PCG patients. The gene was earlier characterized to be a
single copy gene having 3 exons and 2 introns, with a reading frame of 1629 bp (Tang
et al., 1996). Mutations in the CYP1B1 gene of PCG patients from various populations
have been reported (Stoilov et al., 1997; Bejjani et al., 1998; Stoilov et al., 1998;
Plasilova et al., 1999; Mashima et al., 2001; Panicker et al., 2002).

Secondary developmental glaucomas are inherited as AD traits with variable
expressivity (Friedman and Walter, 1999; Wiggs, 2000). Secondary developmental
glaucomas for which genetic information about the gene(s)/loci involved is available
include glaucomas associated with: a) Axenfeld Rieger Syndrome (Friedman and Walter,
1999; Wiggs, 2000; WuDunn, 2002); b) Axenfeld Rieger anomaly (Mears et al., 1998);
c) Aniridia (Alward et al., 1996); d) Peter’s anomaly (Wiggs, 2000); e) Iridogoniodygenesis type I (IRID1) (Mears et al., 1996); f) Familial glaucoma
iridogoniodyplasia (Jordan et al., 1997); g) Iridogoniodygenesis II (Heon et al., 1995;
Walter et al., 1996; Alward et al., 1998b; Kulak et al., 1998); h) Pigment Dispersion
syndrome, two loci for this have been identified, the 1q36 locus and the 18q11-q22 locus
(Blumenthal and Weinreb, 2000); i) Pseudoexfoliation syndrome (PES)/PEG
-Matrilineal i.e., mitochondrial inheritance and dominant inheritance trait linked to
chromosome 2p16 have been suggested (Stefansson et al., 1998; Wiggs et al., 1998b).

PACG: Prevalence of PACG in the first degree relatives is estimated to be 1 to 12%,
(Congdon et al., 1992). Mutations in the MYOC gene have been reported for PACG
patients (Pang et al., 1999; Faucher et al., 2002). Results of a recent study on Chinese
PACG patients did not find evidence to support the role of MYOC mutations in the
pathogenicity of chronic PACG (Aung et al., 2005).
II.10 Myocilin

The myocilin protein was discovered when proteins that could be induced on treatment of cells with dexamethasone were analysed. Earlier reports dating back to 1965 have reported three categories (high responders, moderate responders and non-responders) based on responsiveness to topical administration of a glucocorticoid (dexamethasone). The ocular or systemic administration of glucocorticoids was seen to cause the elevation of IOP (Clark, 1995).

A low level increase in the intensity of the band at the 55 kDa region was observed initially, followed by a major progression in the intensity of the band in accordance to the increase in exposure time of cells to dexamethasone. Induction of a 66 kDa protein was also seen. This appeared to be the variably glycosylated form of the 55 kDa protein (Polansky et al., 1997). Kubota et al. (1997) isolated a human cDNA clone encoding a novel acidic protein of MW 55,000 from retina (photoreceptor cells) which was tentatively named Retina Keio Protein 1 (RKP1) and later named as myocilin. This was the name assigned to the gene and the gene product by HUGO genome database nomenclature committee in 1998. The gene symbol was represented as MYOC (Tamm, 2002). Myocilin was seen to be composed of 504 aa. An isoelectric point (pI) of 5.2 – 5.3 was observed (Ueda et al., 2000). A typical leucine zipper motif, between aa 117 and 166 indicated a functional significance for the motif in homo/hetero dimer/oligomer formation (Tamm and Russell, 2001; Tamm, 2002).

In the TM cell culture supernatant, myocilin formed high molecular mass aggregates, from 110 kDa to 200 kDa which may be dimers or multimers (Nguyen et al., 1998; Fautsch and Johnson 2001; Jacobson et al., 2001). Recently Fautsch and Johnson (2001) using yeast two hybrid system determined the region between aa 117-167 (with the leucine zipper) to be important for dimer/multimer formation. The N-terminal of leucine zipper appeared to be critical for interaction. The olfactomedin region is also important as almost all known mutations are localized to this region. The role of leu at aa 84, 91 and 98 and so also the 2 cys residues at aa position 47 and 61 have been postulated to be involved in the dimer/multimer formation.
Expression of Myocilin

Northern blot analysis detected the MYOC mRNA in TM, ciliary body, sclera, choroid, cornea, iris and ciliary epithelium (Adam et al., 1997; Ortego et al., 1997; Tamm et al., 1999; Takahashi et al., 2000). The TM, sclera and iris had higher expression levels (Adam et al., 1997; Ortego et al., 1997; Tamm et al., 1999). MYOC mRNA was detected in the human ONH using RT-PCR (Ricard et al., 2001). MYOC mRNA was detected in relatively equal amounts in most of the cells of the TM including uveal, corneoscleral and juxtacanalicular regions (Huang et al., 2000; Swiderski et al., 2000; Takahashi et al., 2000; Wang and Johnson, 2000). In situ hybridization have shown expression in most parts of the eye, with highest intensity in the TM comparable to northern blot analysis (Huang et al., 2000; Kim et al., 2000; Swiderski et al., 2000). MYOC mRNA was not detected in the human retina (Adam et al., 1997; Swiderski et al., 2000).

Considerable amounts of myocilin mRNA were detected in human skeletal muscle and heart (Ortego et al., 1997; Fingert et al., 1998; Nguyen et al., 1998). Smaller amounts were observed in mammary gland, thymus, prostate, testis, colon, stomach, small intestine, trachea, bone marrow and thyroid (Adam et al., 1997; Fingert et al., 1998; Tamm and Polansky, 2001).

Localisation of Myocilin

Distribution of myocilin protein has been studied using western blotting and the observations correlated markedly with that of northern blotting. Extracellulary, myocilin was localized between the collagen bundles of the sclera, cornea, keratocytes and in the cells of corneal endothelium and epithelium (Huang et al., 2000; Karali et al., 2000). Myocilin was co-localised with fibronectin, fibrillin, microfibrillar associated glycoprotein and with Type IV collagen and decorin (Tawara et al., 2000; Ueda et al., 2002). Interaction of myocilin and fibronectin was confirmed which could affect the contractility of TM or regulate the formation of the ECM, thereby appearing to be directly involved in regulation of trabecular outflow (Lütjen Drecoll and Rohen, 1996). Myocilin has also been identified as a substantial component of the aqueous
humor in human, monkey and bovine eyes (Rao et al., 2000; Fautsch and Johnson, 2001; Jacobson et al., 2001; Russell et al., 2001).

The presence of myocilin was observed in other regions such as the vitreous, axons of optic nerve ganglion cells, axons of the prelaminar, laminar and post laminar parts of the nerve etc. (Karali et al., 2000; Noda et al., 2000; Clark et al., 2001a; Ricard et al., 2001) indicating that TM might not be the only target of abnormal myocilin/TIGR.

No firm evidence exists that states myocilin to be directly involved in the structural and functional changes associated with the disease. The possibility that the increased expression of myocilin occurs in parallel to the disease causing event cannot be or has not been ruled out. However as long as we do not know the mechanism by which myocilin acts on the resistance to aqueous outflow in the TM, it will remain unclear whether increase of myocilin in TM reflects a cause or symptom of POAG (Tamm, 2002).

Myocilin appears to be a delayed or secondary glucocorticoid response gene (Nguyen et al., 1998; Tamm et al., 1999). Two possibilities have been suggested: a) the glucocorticoid receptor may activate a primary response gene which in turn activates MYOC or b) the receptor may bind directly to MYOC promoter at a yet to be identified secondary glucocorticoid response element.

II.11 Treatment of Glaucoma

The management of glaucoma essentially deals with the prevention or modification of risk factors. Increased IOP is one of the primary risk factors and reduction of IOP is effective in slowing the progression of glaucomatous damage (Ritch et al., 1996; Saxena et al., 2002). POAG remains a syndrome for which all the approved and accepted theories are directed at lowering the IOP (Fetchner and Singh, 2001). The other potentially treatable factor is the vasospastic condition (in NTG cases) (Haeffliger and Flammer, 1997).
II.11.1 Medical Therapy

Antiglaucoma drugs are classified in two types based on the route of administration: a) topical drugs and b) systemic drugs.

a) **Topical agents** include: 
   i) Cholinergic agents or miotics or parasympathomimetic drugs and these may be direct acting (agonists) e.g. Pilocarpine, or Cholinesterase inhibitors, e.g. Ecithiopehtae iodide, Carbachol (Saxena et al., 2002; Costa et al., 2003).
   ii) Adrenergic agonists or sympathomimetics e.g. Epinephrine, Dipinefrin, Apracholidine and Brimonidine.
   iii) β-Adrenergic antagonists (β-blockers) e.g. Timolol, Carteolol, Betaxolol, Levobunolol, Metaprolol etc. (Quigley, 2001).
   iv) Carbonic anhydrase inhibitors (CAIs) e.g. Aminozolamide or 6-amino 2-benzathiozole sulfonamide (Lewis et al., 1986; Kalina et al., 1988). This was the first of the topical CAIs to have an IOP lowering effect. Others include, MK 27 (Higginbotham, 1990), Sezolamide hydrochloride (MK-417) and Dorzolamide hydrochloride (MK 507, L-671152). Dorzolamide was thought to be the most potent topical CAI (Lippa et al., 1992) with brand name of trusopt (Donahue and Wilensky, 1996; Pfeiffer, 1997).
   v) Prostaglandin analogs e.g. Lantanoprost, Unoprostone, PhKA – 85 (Saxena et al., 2002).

b) **Systemic agents** :
   i) CAIs e.g. Acetazolamide, Methazolamide
   ii) Osmotic agents e.g. Mannitol, Glycerol, Urea etc. (Ritch et al., 1996; Saxena et al., 2002).

II.11.2 Surgical Intervention

Laser trabeculoplasty is the first surgical procedure of choice when glaucoma is uncontrolled medically. Discrete burns are applied using an argon or diode laser (Ritch et al., 1996; Shields, 1998; Coleman and Brigatti, 2001). Trabeculectomy is the most common form of filtration surgery wherein a hole is created at the scleral–corneal junction for outflow between the anterior chamber and sub-tenon space. Trabeculectomy
is of two types: a) Partial thickness trabeculectomy and b) Full thickness trabeculectomy (www.emedicine.com/oph/topic139.html; Shields, 1998; Coleman and Brigati, 2001). Trabeculectomy offers the best chance of sustained IOP reduction i.e., progression of the visual field loss though present is much lower, but it has the greatest chance of associated side effects (Fetchner and Singh, 2001). Other surgical interventions include Nd:YAG laser iridotomy and surgical periphery iridectomy (Kanski et al., 1996).

II.11.3 When to Initiate Therapy?

In a typical case of COAG with established visual field loss or ONH damage, treatment to reduce IOP is initiated. No specific level can be defined at which patients with increased IOP but no ONH damage should be treated. However, an IOP of > 30 mmHg has to be monitored carefully (Ritch et al., 1996; Shields, 1998). A target pressure is fixed based on available information of existing risk factors. IOP is lowered to the target level (Realini and Fetchner, 2000; Weinreb, 2001). When a single agent is inadequate to control IOP, combinatorial treatment is tried. When no further escalation of medical treatment is available or inappropriate it is called maximal medical therapy (Fetchner and Singh, 2001). Use of implant devices/artificial drainage shunts as a primary procedure may be used in COAG patients after failure of conventional filtration surgery.

NTG has been a diagnostic and therapeutic dilemma since its first description. Surgical intervention substantially slowed the rate of visual field loss in NTG. Another treatment that has been used for NTG is systemic Ca$^{2+}$ channel blockade (Caprioli, 1998). In older patients, the first step is to start with a topical treatment (Schoevaerdts et al., 2003).

Several compounds that interfere with the degenerative process are being investigated. Neurotrophins (BDNF), glutamate receptor antagonists, Ca$^{2+}$ channel antagonists, alpha agonists, NO inhibitors, antioxidants and inhibitors of apoptosis are being tried (Kwon et al., 2000). Optic nerve regeneration has also been tried (Maclaren et al., 1998). Strategies that use gene therapy to modulate aqueous production and outflow and prevent RGC death are now available (Borrás et al., 2002).