Chapter 1

Introduction
1. Introduction

1.1 Glaucoma

Glaucoma is a complex eye disease leading to irreversible blindness worldwide. It is a heterogeneous group of optic neuropathies characterized by retinal ganglion cell death, visual field defects and degeneration of the optic nerve head (Shields et al., 2005). According to the WHO statistics on global burden of visual impairment (Resnikoff et al., 2004), glaucoma is the second major cause of blindness (12.3%) after cataract (47.8%). Since the peripheral vision is lost first and the disease is typically pain free, it goes unrecognized until substantial degree of vision loss occurs. Hence, glaucoma is called ‘the sneak thief of sight’. Although raise in intraocular pressure is a major risk factor, there is no set threshold for intraocular pressure that causes glaucoma. If left untreated, it leads to permanent damage of optic nerve head and loss of visual field and permanent blindness.

1.1.1 Aqueous humor outflow pathway

The anterior chamber (the space between the cornea and the iris) of the eye is filled with a liquid called aqueous humor (AH). After being produced by the ciliary body, the aqueous humor enters the anterior chamber and finally enters the venous cavity through a sieve like region called the trabecular meshwork (TM) and Schlemm’s canal. The fine balance between its production and drainage maintains the intraocular pressure of the eye within its physiological range (10-22mm Hg). In certain pathological states, the aqueous humor gets accumulated in the eye, leading to a raise in intraocular pressure (IOP). This
causes damage to the optic nerve and visual impairment. The loss of vision is because of degenerative changes in the retina, particularly retinal ganglion cells—the sensory cues of the eye. The aqueous humor outflow is depicted in Figure 1.1A.

1.1.2 Optic nerve head cupping and glaucomatous neurodegeneration

The optic nerve head (optic disc) consists of axons from approximately one million ganglion cells that have their cell bodies in the retina (Weinreb et al., 2004). The axons converge on the optic disc to form the optic nerve (Figure 1.2A). The fibers exit the eye through lamina cribrosa and synapse in the lateral geniculate nucleus of the brain. The convergence of the axons in the optic disc form a central depression called the optic cup. The neuroretinal rim of the optic nerve head is pink in colour and surrounds the cup.

Glaucomatous progression is characterized by a decrease in the width of the neuroretinal rim with a concomitant enlargement of the cup (Weinreb et al., 2004). As axons within the nerve die (largely through apoptosis) and the plates of the lamina cribrosa sclerae (a specialized area of sclera) collapse due to IOP or ischemia, loss of optic nerve tissue produces a characteristic excavation or “cupping” of the optic nerve head (Figure 1.2A&B). Thinning or notching of the disk rim, or disk hemorrhages might also be seen. When a vertical cup-to-disk ratio of 0.6 or greater is seen, glaucoma should be suspected. The cupping of the optic nerve head is directly related to the loss of one’s peripheral visual field leading to tunnel vision. With peripheral vision loss, a person can see in front of him- or herself but has lost the vision to the side (Figure 1.2C).
Figure 1.1: Diagram showing aqueous humor outflow pathway.
(A) Normal aqueous humor outflow pathway.
   http://www.ahaf.org/glaucoma/about/aqueousflowBorder.jpg
(B) Illustration showing normal angle glaucoma and angle closure glaucoma.
   http://catalog.nucleusinc.com/generateexhibit.php?ID=8908&ExhibitKeywordsRaw=&TL=&A=2
Figure 1.2: Normal and abnormal optic nerve head in glaucoma.
(A) (i) The optic nerve is divided into tenths and the cup is compared to the entire optic nerve (optic disc) to obtain the cup-to-disc ratio. This C/D ratio here is 0.4. (ii) Normal optic nerve (iii) abnormal optic nerve. http://www.medrounds.org/glaucoma-guide/2006/02/section-1-b-meaning-of-cupping.html
(B) Picture showing progressive loss of vision in glaucoma. http://www.eyesoftexas.us/cataracts/fig_02.jpg
(C) Vision of a normal person and an advanced glaucoma patient, who has "tunnel" vision.
1.1.3 Types of glaucoma

Glaucoma can be classified based on different criteria and one of the classifications based on the mechanism is mentioned below (Shields et al., 2005).

a. Primary open-angle glaucoma (POAG)

POAG is classified based on the visibility of the anterior chamber angle structures, TM, scleral spur and ciliary body upon gonioscopy (Shields et al., 2005). POAG is the most common clinically defined subset of glaucoma. As the term open angle suggests the angle between the cornea and iris is unimpeded, yet there is obstruction in the outflow of aqueous humor. Based on the age of onset, POAG is divided into juvenile onset POAG (JOAG) and adult onset POAG. JOAG occurs during the ages of 5-35 and adult onset POAG occurs at an older age. POAG patients have IOP consistently above 22mm Hg, which is hence also called high tension glaucoma (HTG). Approximately, one-third (33%) of POAG patients have IOP within normal range (<22mm Hg), or normal tension glaucoma (NTG) or low-pressure glaucoma (LPG) (Kamal & Hitchings, 1998). But the ultimate theme in both HTG and NTG is retinal ganglion cell death and visual field loss whose mechanism is still unclear.

b. Primary angle closure glaucoma (PACG)

In PACG, the iridocorneal angle (angle between iris and cornea) is closed, and hence a blockage for the aqueous humor to flow through the trabecular meshwork into the venal system. As a result IOP rises and causes optic nerve head cupping and blindness. ACG is more common in people who have
shallower anterior chambers and narrower angles, like patients with hypermetropia (far sightedness). It is more common in people with Asian racial background. ACG can be either chronic or acute. Unlike POAG, patients with ACG may have intermittent symptoms like eye ache with cloudy vision, nausea, headache or vomiting. Patients with ACG can be provided relief by performing a surgery called laser iridotomy, in which a hole is made in the iris to allow normal outflow of AH through the exit channels. The defect is depicted in Figure 1.1B.

c. Developmental glaucoma

This is mainly classified based on the developmental abnormalities in the structures of conventional aqueous outflow pathway such as trabecular meshwork and Schlemm’s canal. Congenital glaucoma is a type of developmental glaucoma and, as the name suggests, occurs at birth, and is due to an improper development of the eye’s drainage channel (trabecular meshwork). Although individual clinical manifestations exist for the various types of glaucoma mentioned, the ultimate pathology in all of these involves the degeneration of retinal ganglion cell axons and soma, which is manifested as progressive visual field defects.

1.1.4 Risk factors for glaucoma

Awareness and early detection is very important in treating glaucoma because the disease can be well managed if diagnosed early. While everyone is at risk for developing glaucoma, some are at a higher risk and need regular eye check-ups to take care. High IOP is the strongest known risk factor for glaucoma (Sommer et al., 1991). Apart from IOP, other risk factors include increasing age
(Mitchell et al., 1996), family history of glaucoma (Tielsch et al., 1994; Wolfs et al., 1998), and high myopia (Xu et al., 2007). Other risk factors, particularly for NTG, include race (Suzuki et al., 2006), primary vascular dysregulation (Flammer et al., 1999), and low blood pressure (Kaiser and Flammer, 1991).

1.1.5 Molecular genetics of glaucoma

Glaucoma is a genetically heterogeneous disease and involves multiple factors. While a smaller proportion follows monogenic inheritance, majority of the cases involve multiple factors. Although autosomal dominant and recessive glaucomas exist, the disease is much more complex in many individuals, thus making it difficult to understand the molecular genetics. The most common forms of glaucoma do not exhibit Mendelian inheritance but the immediate relatives of the proband are at higher risk than general population. Multiple genetic and environmental factors play a major role in its etiology. Molecular geneticists have identified 17 chromosomal loci till date, which have been shown to be linked with various forms of glaucoma (Fan et al., 2006) (Table: 1.1), but only 4 genes are identified. The 3 known POAG genes include Myocilin/ TIGR (Stone et al., 1997), Optineurin (OPTN) (Rezaie et al., 2002) and WDR36 (Monemi et al., 2005). CYP1B1 is the candidate gene for congenital glaucoma (Stoilov et al., 1997).

Myocilin, also called TIGR (Trabecular meshwork induced glucocorticoid response) was the first gene discovered as a POAG gene (Stone et al., 1997), and is located as GLC1A locus at the position 1q23-25. Mutations in MYOC are responsible for approximately 3% of adult onset POAG and a major proportion of JOAG (Libby et al., 2005). The protein’s structure includes an amino terminal signal sequence, a myosin-like domain, a leucine zipper domain and an
olfactomedin domain (Tamm, 2002). Most of the mutations reported till date lie in the olfactomedin domain. MYOC is expressed in both ocular and non-ocular tissues and the wild type protein is said to be secreted (Tamm, 2002). It was reported that the mutant proteins of MYOC form aggregates in the cytoplasm, lose the capacity to get secreted and hence its accumulation in the cell causes cellular stress (Caballero et al., 2000; Joe et al., 2003). Though the importance of MYOC in glaucoma is known, the function of normal MYOC protein in aqueous humor physiology remains an enigma.

**OPTN** was identified as a candidate gene for POAG, particularly NTG. Since the main focus of the project is on optineurin, a detailed introduction will be given about the same later.

The third candidate gene in POAG, **WDR36**, mapped onto chromosome 5q22.1 (**GLC1G**) (Monemi et al., 2005), comprises 23 exons and codes for a 951 amino acid residue protein containing a domain of the dipeptide WD repeated 36 times with several conserved residues. These domains are involved in protein-protein interactions. Mutations in **WDR36** were initially reported in 17% of POAG cases (Hauser et al., 2006) but some studies could not replicate its association to POAG (Fingert et al., 2007).

**CYP1B1** (Cytochrome P4501B1) was the first gene of the CYP450 superfamily whose mutations are accounted for autosomal recessive primary congenital glaucoma (Stoilov et al., 1997; Bejjani et al., 1998; Martin et al., 2000). This protein is expressed in the tissues of the anterior chamber angle of the eye and in several other cell types. It is an autosomal recessive trait with incomplete penetrance. Mutations in this gene have been observed in different populations,
accounting for 20-100% of all primary congenital glaucoma (PCG) patients (Mashima et al., 2001, Reddy et al., 2004). Molecular modeling experiments suggest that mutations in CYP1B1 lead to loss of integrity of the protein and inherent instability; this is because substantial portion of the mutations reported are either deletions or insertions.

Only a small proportion of the genetics of glaucoma is accounted for. Even the cell biology aspects remain poorly understood. Much more research has to be focused on the genetic and linkage studies of the disease in order to have a better understanding. These efforts must be combined with genomic and proteomic approaches to speed up the rate at which glaucoma genes can be identified. Also there appears to be genetic alteration by genetic modifiers, and some mutations may cause the disease only when present in a susceptible genetic context. So this complexity in phenotype-to-genotype associations makes it difficult to clearly understand the specific allele responsible for the disease. Moreover, since multiple factors are involved, using animal models would be useful, whereby the effect of specific alleles in a defined genetic background and controlled environment can be studied. There are a few glaucoma models in mice, eg., C57BL/6J (Danias et al., 2003) and GLAST<sup>−/−</sup> and EAAC1<sup>−/−</sup> mice (Harada et al., 2007). C57BL/6J is an inbred mouse model and is reported to lose almost 50% of its retinal ganglion cells during aging. The OPTN ortholog of this strain has a glutamine at residue 552 and a lysine at residue 98 (respectively orthologous to the POAG-associated variants R545Q and M98K) (Rezaie et al., 2002). It is intriguing that this mouse has an extensive RGC loss despite a normal IOP, and hence represents an experimental mouse model for NTG (Libby et al., 2005).
GLAST (glutamate/aspartate transporter) and EAAC1 (excitatory aminoacid carrier) are the glutamate transporters in Muller cells and retinal ganglion cells, respectively. In mice knocked out for either of these transporters, RGC and optic nerve degeneration are observed in the absence of high IOP. Hence, these mice represent NTG models, which offer a powerful system for determining mechanisms and evaluating new treatments for NTG (Harada et al., 2007)

1.1.6 Understanding the mechanism of glaucoma pathogenesis

Although apoptosis of retinal ganglion cells is known to be the main event occurring in glaucoma, the events leading to the cell death are not known. Elevation of intraocular pressure was earlier considered to be indispensable for glaucoma to occur. But later observations show that there is a subset of glaucoma, where IOP is in normal limits, and yet the disease progression occurs, namely in normal-tension glaucoma (NTG). Thus, understanding the etiology of the optic neuropathy in such cases remains complicated. Several pathophysiological mechanisms have been hypothesized to play a role in causing RGC death in glaucoma. Cellular and molecular biological studies are now focused on understanding the changes in patients in the trabecular meshwork, optic nerve head and retinal ganglion cells. The pathogenesis of glaucoma has been heavily debated since mid-nineteenth century and the changing concepts over the years led to the proposal of three theories (Flammer et al., 2002), which attempt to explain both IOP-dependent and independent mechanisms of RGC death.
In 1858 Muller proposed the **Mechanical theory** where abnormally elevated intraocular pressure relative to normal pressure of an eye causes mechanical deformation of the cribriform plates of the lamina cribrosa, which includes posterior bulging and deformation of the pores (Flammer *et al.*, 2002); the deformation of the lamina cribrosa causes compression of the optic nerve bundle which in turn leads to glaucomatous changes in the optic nerve. Alteration of axoplasmic flow may therefore be associated with the disease progress. Such changes are observed in eye with high IOP and the degree of damage depends on the severity of the IOP elevation (Yamamoto & Kitazawa, 1998).

The second theory is **vascular theory/ ischemic theory**; here elevation of IOP causes compression of optic nerve fibers and the optic arteries at the lamina cribrosa which results in alterations in the blood supply to the optic nerve head, leading to ischemic damage of optic nerve head and progressive death of retinal ganglion cells. The main evidence for this theory comes from (i) blood flow incompetence at ONH in glaucoma patients (Chung *et al.*, 1999), and (ii) presence of retinal blood flow abnormalities in glaucoma patients (Arend *et al.*, 2004).

**Excitotoxicity theory** suggests that like any other neurodegenerative disease, glaucoma too is characterized by excitotoxicity. It occurs when the glutamate receptor gets overstimulated, leading to excessive glutamate intake leading to an abnormal calcium ion influx. This leads to free radical formation, as well as the activation of enzymes causing cellular damage. If neurons are injured or unable to properly control glutamate levels, secondary damage can result, even if the primary cause is not related to glutamate. Injured neurons are more vulnerable to even normal levels of glutamate, become overstimulated and die.
Glutamate is the major excitatory neurotransmitter in the retina and its levels have been shown to be increased in glaucomatous eyes. An interesting observation is that persistent levels of glutamate cause death of RGC, independent of elevated IOP (Casson et al., 2006). Excitotoxic damage is suggested to be one of the mechanisms of RGC damage during glaucoma, and hyperactivity of NMDA receptors has been described in glaucoma. A partial NMDA receptor antagonist is being tested as a potential therapeutic agent in glaucoma (Lipton, 2003).

1.1.7 Factors contributing to glaucomatous neurodegeneration

Apart from rise in IOP, some other factors play a major role in glaucomatous neurodegeneration. These include ischemia, glutamate excitotoxicity, aberrant immunity and alteration in glial cells (Weinreb and Khaw, 2004) (Figure 1.3). The relative significance of each specific factor varies between patients depending on the magnitude of IOP elevation, oxidative stress and individual life style (John, 2005).

a) Ischemia: Elevated IOP induces ischemia, which results in blockage of axoplasmic flow and obstruction in normal flow of growth factors leading to RGC death. Optic nerve blood flow is compromised in glaucomatous patients (Flammer et al., 1994). Ischemia enhances the levels of reactive oxygen species (ROS), glutamate release, and nitric oxide production, which increase the levels of Ca2+ that show detrimental effect on RGC cells (Cioffi, 2005).

b) Excitotoxicity: Glutamate is an essential excitatory amino acid in the central nervous system and in the retina as well. In several neurological diseases,
Figure 1.3: Factors contributing to the pathophysiology of glaucomatous neurodegeneration. Apart from IOP, other contributing factors for glaucoma include ischemia-hypoxia, excitotoxicity, alterations in glial cells and aberrant immunity (Weinreb et al., 2004).
excessive glutamate induces over-excitation of neurons, which ultimately leads to the death of neuronal cells. It has recently been shown that glutamate excitotoxicity plays an important role in the pathogenesis of glaucoma (Vorwerk et al., 1999). The evidence comes from the fact that (i) vitreal glutamate levels are elevated in experimental and clinical glaucoma (Dreyer et al., 1996), (ii) ischaemia plays a role in glaucoma (Lam et al., 1997), (iii) neuroprotection based animal studies and (iv) secondary degeneration of RGCs after optic nerve injury.

c) Autoimmunity: Franz et al. (2004) showed circulating autoantibodies against several ocular antigens in glaucoma patients, and suggested the role of autoimmunity in glaucoma. The auto-antibodies observed include heat shock proteins (HSPs), rhodopsin, γ-enolase, glutathione-S-transferase (GST), tumor necrosis factor-α, and γ-synuclein. These antibodies may play role in the formation of inflammation that can induce apoptosis of RGCs.

d) Glial cell activation: Glial cells are important structural and functional components of the optic nerve head or ONH. The glial cells present in ONH and retina include astrocytes, oligodendrocytes and microglia. These glial cells support neuronal tissue by supplying metabolites and growth factors, and by scavenging toxic metabolites. Therefore, glial activation in glaucomatous eyes may initially represent a cellular attempt to limit the extent of neuronal injury and to promote tissue repair. However, activated glial cells may also have toxic effects on neuronal tissue by creating mechanical injury and/or changing the microenvironment of neurons. The activated glial cells in glaucomatous eyes produce neurotoxic substances such as nitric oxide synthase and TNF-α. In addition, in vitro experiments have provided direct evidence that glial cells are
activated in response to glaucomatous stressors such as elevated pressure and ischemia, and are directly involved in facilitating the apoptosis of retinal ganglion cells due to increased production of apoptosis-promoting substances, including nitric oxide and TNF-\(\alpha\) (Tezel et al., 2000).

1.1.8 Diagnosis and treatment strategies for glaucoma

Early diagnosis is the key to successful management of glaucoma. Since glaucoma occurs without any signs or symptoms, regular eye-check ups are a must particularly for people above the age of 40. The diagnosis of glaucoma no longer relies simply on the presence of pressure within the eye; it requires that there be optic nerve damage or a strong suggestion of damage, which can be clearly seen during a dilated eye examination of the optic nerve. In general, the hallmark sign of this condition is a loss of peripheral vision. Glaucoma evaluation has several components. Apart from measuring the IOP by tonometry, the health of the optic nerve is also examined by ophthalmoscopy and visual field test. In order to determine early damage in the optic nerve, a number of diagnostic instruments have been developed to assess the nerve fiber layers at the back of the eye (the fundus) and to check for optic disk cupping. The cup of the optic disc is the center portion, which enlarges as nerve damage progresses. A cup-to-disc ratio is critical when evaluating glaucoma. The cup-to-disc ratio is the amount of the entire nerve head that has been cupped out or where glaucoma has caused damage. Readings range from 0 meaning no cupping at all to 1.0 where the entire optic nerve is cupped out. Many people have some cupping- which is normal. Gonioscopy helps in examining the intactness of the anterior part of the eye using a specific lens.
The treatment strategies for glaucoma usually include medicated eye drops which lower the IOP by either decreasing aqueous humor production or increasing its outflow. In extreme cases when the medications do not improve the condition, surgeries are performed such as trabecuoplasty, where high energy laser beam is used to shrink one part of the trabecular meshwork so that the other half stretches and allows drainage of aqueous humor, or a conventional surgery like trabeculectomy. In this surgery the trabecular meshwork is excised from the patient completely so that the aqueous humor can easily leave the eye through the hole. In a few cases like secondary glaucoma or for children with glaucoma, drainage implants are inserted for effective drainage of aqueous humor. While the above surgical techniques are applied for open angle glaucoma, angle closure glaucoma needs a different surgery called laser iridotomy.

The primary goal of glaucoma treatment is to preserve vision. However, current knowledge of the factors causing optic nerve damage and visual loss is limited. Proven existing therapies focus mainly on IOP reduction although elevated IOP is not always a diagnostic feature for glaucoma. So, a multidrug approach will be of use which includes agents targeted towards lowering IOP as well as an agent directed at preserving and protecting the optic nerve from glaucoma.

1.1.9 Complete therapy

Glaucoma is a neurodegenerative disease. Current therapeutic agents under investigation include neuroprotectants, which target the disease progress manifested by death of retinal ganglion cells, axonal loss and irreversible loss of
vision. Neuroprotectants, when used in conjunction with IOP-reducing therapy, form a complete therapy for glaucoma management. Till date, no such medications have been approved. Drugs which protect RGC degeneration belong to different groups, which include NMDA antagonists such as memantine and dexanabinol, inhibitors of inducible nitric oxide synthase (NOS-2), neurotrophic factors such as nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), and neurotropin 3 (Rudziniski et al., 2005). One interesting drug which has reached phase 3 (final stage) clinical trial is memantine, which is a N-methyl-D-aspartate receptor blocker. Similarly, trophic factors are used to protect degenerating RGC neurons. Trophic factors such as BDNF or NGF act on the tyrosine kinase (Trk) receptor signaling, but this kind of cell signaling is not specific to only RGCs. They might affect others as well, which might lead to unwanted side effects.

Thus, there is a need to search for compounds which show maximum advantage, with minimum or no side effects. One such possibility is the search for natural anti-oxidants, which can also work through different and multiple mechanisms, such as oxidative stress induced or excitotoxicity induced mechanisms of RGC degeneration in glaucoma.

1.2 Oxidative stress in glaucoma

Oxidative stress is caused by an imbalance between the production of reactive oxygen species (ROS) and a biological system's ability to readily detoxify the reactive intermediates or easily repair the resulting damage (Mozaffarieh et al., 2008). All forms of life maintain a reducing environment within their cells. This reducing environment is preserved by enzymes that maintain the
reduced state through a constant input of metabolic energy. Disturbances in this normal redox state can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA. DNA damage is involved in the pathogenesis of various chronic-degenerative diseases like cancer, atherosclerosis and neurodegenerative diseases (De Flora et al., 1996). Increasing experimental evidence indicates that oxidative stress plays a major role in the pathogenesis of glaucoma (Izzotti et al., 2003).

Since the major risk factor for the disease is rise in IOP, initial therapeutic management was focused on drugs that help in lowering the IOP. However, although IOP lowering treatment can provide neuroprotection and retard the disease progression in many glaucoma patients, it is not always sufficient to fully prevent disease progression. This is just one reason why recent efforts have focused on the development of alternative treatment strategies for neuroprotection. In addition, IOP is not always elevated in the eyes exhibiting glaucomatous degeneration. Clinical (Tezel et al., 1996) and histopathological (Wax et al., 1998) findings in the glaucomatous eyes with either elevated or normal IOP indicate that there is also an IOP-independent component of neuronal damage in glaucoma. It becomes more important in the cases of normal tension glaucoma, where patients suffer from the disease in spite of normal IOP. In such cases, increased glutamate levels (Shen et al., 2004), oxidative stress (Moreno et al., 2004), vascular alterations (Chung et al., 1999) can be considered as other concomitant factors associated with the disease.

The ability of oxidative damage to alter several structures of the eye has been recognized as an etiopathogenetic factor in various ocular diseases like
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cataract, age-related macular degeneration (AMD) (Ohia et al., 2005) and only recently in POAG (Izzotti et al., 2003). Increasing evidence supports that the glaucomatous tissue stress initiated by elevated IOP or tissue hypoxia also involves an oxidative component (Bonne et al., 1998; Tezel, 2006). Multiple pathogenic mechanisms have been proposed for glaucomatous neurodegeneration which include axonal injury, which may result in neurotrophin deprivation, mechanical injury, glutamate excitotoxicity, and vascular ischemia. Most of these mechanisms appear to be associated with a common pathway of oxidative injury. Alvarado et al. have suggested that the progressive loss of trabecular meshwork cells in patients with glaucoma may be attributable to the long term effect of free radical damage (Alvarado et al., 1984). This hypothesis has been supported indirectly by experimental studies. Experimental elevation of IOP induces oxidative stress in the retina of experimental rat models with glaucoma (Ko et al., 2005). The inner retinal layers, particularly retinal ganglion cells show, high carbonyl reactivity indicating that the RGCs are prominently exposed to oxidative stress in the ocular hypertensive eyes (Tezel et al., 2005). Additional supportive evidence for the role of oxidative stress in glaucomatous degeneration comes from studies on glaucoma patients. It is shown that the ROS production is more in the trabecular meshwork of POAG patients than in the age matched controls (Izzotti et al., 2003). Yang et al. have detected autoantibodies against GST in the glaucomatous patients’ sera; GST which belongs to a major group of detoxifying enzymes gets regulated in vivo by the levels of ROS (Yang et al., 2001). Moreover, a recent study reports increased mRNA and protein levels of the iron-regulating proteins transferrin, ceruloplasmin, and ferritin in an experimental monkey model of glaucoma (and in human glaucoma) suggesting
the involvement of iron and copper metabolism and associated antioxidant systems in the pathogenesis of glaucoma (Farkas et al., 2004).

One of the harmful consequences of ROS generation in glaucoma is the oxidative modification of many important retinal proteins like HSP72 and glutamine synthetase (Tezel et al., 2005). Adult GLAST$^{-/-}$ and EAAC1$^{-/-}$ mice show increased lipid hydroperoxides in retina suggesting the involvement of oxidative stress in RGC loss (Harada et al., 2007). EAAC1 and GLAST are glutamate transporters located in different regions of the retina. Glutamate transporters play an important role in the removal of excess glutamate from the extracellular fluid of the retina (Harada et al., 2007). Indeed, these mice models were the first NTG animal models developed to investigate IOP-independent mechanisms of RGC loss. However, whatever the effect is seen, it largely reflects the balance between a variety of intracellular signaling pathways linked to cell survival or death that are activated in response to the oxidative insult (Kamata and Hirata, 1999). The major signaling pathways known to be involved in regulating the cellular response to oxidative stress include MAPKs, PI3-kinase/Akt pathway, heat shock protein expression, p53 signaling, and NF-κB signaling (Martindale and Holbrook, 2002). All the studies mentioned above focus on oxidative stress to be a key component in both IOP-dependent and independent cases of glaucomatous neurodegeneration.
1.3 Optineurin

1.3.1 Identification as a candidate gene for glaucoma

The gene *OPTN* (accession numbers: NM_001008211.1, protein NP_001008212.1) was identified as a candidate gene for glaucoma where mutations in the coding region are responsible for NTG and POAG (Rezaie *et al*., 2002). In fact, *OPTN* remains the only candidate gene reported for NTG till date. In humans the gene is located within the GLC1E locus at chromosome 10p14-p15 (Sarfarazi *et al*., 1998). The initial study was conducted in 54 families and sequence alterations in *OPTN* were identified in 16.7% of the families with hereditary POAG, including a proportion with normal IOP (Rezaie *et al*., 2002).

1.3.2 History of optineurin before identification as a glaucoma candidate gene

Optineurin was initially discovered as FIP-2 because of its interaction with adenoviral 14.7kDa protein (Li *et al*., 1998). Later FIP-2 was shown to interact with diverse cellular proteins like huntingtin (HYPL) (Faber *et al*., 1998), Rab8 (Hattula & Peranen, 2000) and TFIIIA (Moreland *et al*., 2000) and the protein sequence shows 56% similarity to NEMO which is a central molecule for NF-κB activation (Schwamborn *et al*., 2000). Hence optineurin is also called NEMO related protein (NRP).

1.3.3 Structural features, alternative splicing and sub-cellular localization

Optineurin has a total of 16 exons; the first 3 are non-coding at the 5'-UTR and the rest 13 exons code for a 577- amino acid protein. Sequence analysis
suggests that the protein has several putative domains, including one bZIP motif, two leucine zippers, coiled-coil motifs and a c-terminal C2H2-type zinc finger domain (Figure 1.4). Alternative splicing at the 5'-UTR generates three mRNA forms all coding for the same protein (Genbank accessions AF420371 to AF420373). The OPTN homologs from monkey, mouse and rat share substantial degree of similarity to human OPTN. The localization of endogenous protein remains a controversy still as there are opposing reports on its cellular distribution. Optineurin was initially shown to be a Golgi localizing protein (Schwamborn et al., 2000; Rezaie et al., 2002) but later report shows that the protein shows diffused cytoplasmic distribution and is by and large not associated with Golgi (Park et al., 2006).

1.3.4 Expression of optineurin in various ocular and non-ocular tissues

Various teams have studied optineurin expression in human, mouse, monkey and chicken species at the mRNA and protein levels. In humans, optineurin exists as three different transcripts. The levels of optineurin vary among various tissues, e.g., heart, brain, placenta, lung, skeletal muscle, liver, kidney and pancreas, with maximum levels in skeletal muscle (Li et al., 1998). The difference in the sizes of the three message forms is most likely because of alternative spicing at the 5’- end of the gene. Further studies showed that optineurin shows expression in human trabecular meshwork, non-pigmented ciliary epithelium, retina, adrenal cortex, lymphocyte and fibroblast (Rezaie et al., 2002).

In mouse, the highest expression of optineurin mRNA is in liver, heart and testis, although expression is seen in the eye and muscle. Similar to the human
Figure 1.4: Schematic representation of the protein optineurin, showing predicted structural motifs and known binding sites for cellular proteins.

The locations of some of the mutations found in NTG or POAG are also shown. The binding sites (amino acids) for cellular proteins are: Rab 8 (141-209), metabotropic glutamate receptor (mGluR1a, 202-246), transcription factor IIIA, huntingtin (Htt, 411-461), myosin VI (412-520), Rip1 (424-509). In addition adenoviral E3-14.7 kDa protein binds in the region of residues 395-577.
counterpart, optineurin here exists as at least three major transcripts. Within the eye, optineurin shows most intense expression in the pigmented epithelium and also in the retinal ganglion cells of the retina. In the anterior eye, expression is seen in cornea, trabecular meshwork and iris (Rezaie and Sarfarazi, 2005; Kroeber et al., 200; De Marco et al., 2006). In situ hybridization studies revealed optineurin expression in the developing mouse eye at E10.5, but not at the late developmental stage till adult phase (De Marco et al., 2006; Rezaie et al., 2007). Optineurin transcripts were also detected in the forelimb buds of the mouse embryo (Rezaie et al., 2007). The relative abundance of optineurin in the RGCs emphasizes the importance of the protein in the context of glaucoma where apoptosis of RGC is observed.

In monkey, optineurin is present as two different transcripts and the highest level of mRNA expression is in skeletal muscle, similar to its human counterpart. Expression in heart, brain, kidney, lung is comparable to human and mouse (Rezaie et al., 2005). Immunohistochemistry revealed maximum expression in retinal pigmented epithelium and retinal ganglion cells of the retina apart from neighbouring surrounding cell types similar to the mouse counterpart. In the anterior segment optineurin expression is seen in the iris, trabecular meshwork and non-pigmented ciliary epithelium (Rezaie et al., 2005).

In chicken, in contrast to other species, there is a single optineurin transcript and it is expressed in muscle, spleen, kidney, brain, liver and lung (Stroissnigg et al., 2002).

1.3.5 Optineurin mutations
Mutations in OPTN were initially reported in 16.7% of families with hereditary POAG, with most of them having NTG (Rezaie et al., 2002). Also, 13.6% of risk-associated alterations were observed in both familial and sporadic individuals. Out of 16.7% of mutations, the major proportion of patients, i.e. 13.5% of them carried a missense mutation [Glu$^{50} \rightarrow$ Lys (E50K)] where glutamic acid at position 50 was mutated to lysine. Here, 81.5% of the E50K patients had normal intraocular pressure (IOP) suggesting that this mutation was majorly responsible for NTG. The recurrent E50K mutation is located in the putative bZIP motif of optineurin which is conserved in mouse, macaque and bovine genomes. This motif is a transcription factor domain and is involved in DNA binding and protein dimerization, so the E50K mutation located in the bZIP motif may have a dominant negative effect (Rezaie et al., 2002). Later, this mutation was observed in a patient with positive family history of NTG in a larger study comprising of 1048 mixed glaucoma patients (Alward et al., 2003). Moreover, patients with the most common mutation E50K appear to have a more severe form of NTG than those glaucoma patients without this mutation (Aung et al., 2005).

Another mutation which accounts for 2.2% of the disease was a 2-bp AG insertion (c691-692insAG) leading to premature truncation. All the patients carrying this mutation had normal IOP. This mutation truncates the protein by 76% and hence the disease in this case may be attributed to haploinsufficiency or loss of function. R545Q was reported initially as a disease causing mutation (Rezaie et al., 2002), but was later observed in normal controls in the Chinese (Leung et al., 2003) and Japanese populations (Toda et al., 2004; Funayama et al., 2004). Later screening studies have revealed one interesting mutation H486R [His$^{486} \rightarrow$ Arg] which represents the first reported OPTN mutation responsible for
juvenile open angle glaucoma (JOAG), although this mutation is also seen in NTG cases (Willoughby et al., 2004).

In the Indian scenario, an earlier study did not implicate OPTN as a candidate gene in POAG (Mukhopadhyay et al., 2005); however a possible role of SNPs rather than mutations in OPTN is suggested to be implicative in glaucoma pathology (Sripriya et al., 2006). Table 1.2 represents the disease-causing mutations of optineurin reported till date.

1.3.6 Functional studies on optineurin

1.3.6.1 Optineurin - component of TNF-α and NF-κB signaling pathway

TNF-α is a multifunctional proinflammatory cytokine that belongs to the tumor necrosis factor (TNF) superfamily. It signals through two distinct cell surface receptors, TNFR1 and TNFR2. NF-κB is a transcription regulator that is activated by various intra and extra cellular stimuli including cytokines like TNF-α. The TNF-α induced NF-κB activity involves the five mammalian NF-kappaB/Rel proteins: c-Rel, NF-kappaB1 (p50/p105), NF-kappaB2 (p52/p100), RelA/p65, RelB. In the absence of TNF-α stimulation, NF-κB is associated with the inhibitor IkappaB in the cytoplasm. TNF-α induced activation of NF-kB largely relies on phosphorylation dependent ubiquitination and degradation of inhibitor of kappa B (IκB) proteins. The inhibitor of kappa B kinase (IKK) complex, a multiprotein kinase complex is responsible for the TNF alpha induced phosphorylation of IκB. The free NF-κB translocates to the nucleus and induces expression of certain genes. TRADD adaptor molecule interacts with TNFR1 and recruits the additional adaptor proteins like RIP, TRAF2, and FADD, which in turn recruit
additional key components to TNFR1 responsible for initiating downstream events and mediating programmed cell death signaling and NF-κB activation. This pathway is involved in the regulation of a wide spectrum of biological processes including cell proliferation, differentiation, apoptosis, lipid metabolism, and coagulation.

Optineurin was initially identified as FIP-2 (E3-14.7K interacting protein) while screening for interacting partners of E3-14.7K adenoviral protein. Upon overexpression of TNFR1 (TNF-α receptor 1; TR55) intracellular domain which is a potent inducer of apoptosis and E3-14.7K, the latter showed a protective effect on TR55 induced cytolysis. But in the presence of optineurin, the protective effect was reversed. On the basis of this observation it was suggested that optineurin is a component of TNF-α signaling pathway, although no effect of optineurin on TNF-α-induced or TR55-induced apoptosis was reported (Li et al., 1998). Interaction between TNF-α and optineurin is further supported by findings that show an increase in the expression of optineurin in trabecular meshwork cells (Vittitow and Borras, 2002) and HeLa cells (Schwamborn et al., 2000) following TNF-α treatment (Vittitow and Borras, 2002) and a possible interaction between polymorphisms in the OPTN and TNF-α genes that appear to increase the risk for glaucoma (Funayama et al., 2004).

As mentioned earlier, a multi-subunit complex called IKK complex (comprising of IKKα/β/γ) plays the major role in Iκ-B phosphorylation and degradation. IKKγ, also called NEMO (NF-κB Essential Modulator), forms the structural and regulatory subunit of this high molecular kinase complex. Optineurin (called as NRP or NEMO-related protein) shares 53% aminoacid
similarity to NEMO and is structurally homologous to it (Schwamborn et al., 2000). Though there is significant similarity between these two proteins, it appears that NRP cannot substitute for NEMO nor can associate with IKKβ or NEMO (Schwamborn et al., 2000). But a later study showed that optineurin has a ubiquitin binding domain (Wagner et al., 2008) just like NEMO and competitively antagonizes NEMO’s binding to poly ubiquitinated RIP and inhibits TNF-α induced NF-κB activation (Zhu et al., 2007). Moreover, a recent study reports that optineurin mRNA levels get induced by S100A4, metastasis promoting protein, in a NF-κB dependant manner (Boye et al., 2008). This was also supported by earlier evidence which shows that optineurin is a direct NF-κB target; a deletion of putative NF-κB binding site in the promoter region of optineurin completely abolishes the enhancing action and modulatory effect of NF-κB on optineurin (Mrowka et al., 2008). Together, all the investigations discussed above indicate that optineurin is a part of the negative feed back system that is important in regulating NF-κB activity.

1.3.6.2 Regulation of transcription and gene expression by optineurin

Optineurin interacts with transcription factor IIIA (TFIIIA) at its C-terminus and in turn facilitates TFIIIA-dependent 5S gene transcription (Moreland et al., 2000). Moreover, optineurin knock down by siRNA leads to the identification of genome wide- expression changes (Weisschuh et al., 2007). A wide variety of differentially expressed genes belonging to categories, like genes involved in cell morphology, organelle movement and organization of the actin cytoskeleton were obtained on optineurin knockdown. Hence, this study further emphasizes the role of optineurin in gene expression directly or indirectly.
1.3.6.3 Role of optineurin in Golgi organization and vesicular transport

Rab8 is a small GTPase molecule of around 203 amino acids and is involved in vesicular trafficking. It is a unique Rab member which promotes radical changes in cell shape by actin and microtubule reorganization. It controls polarized membrane transport by modulating cellular morphogenesis (Peranen et al., 1996). Studies on FIP-2 (an earlier name of optineurin) shows that it mediates the interaction of huntingtin (huntington’s disease protein) with Rab8 forming a complex that regulates membrane trafficking and cellular morphogenesis (Hattula and Peranen, 2000). In this process, the amino-terminal region of optineurin (position 141-209) is essential for binding to Rab8 and the carboxy terminal region 411-461 binds to huntingtin. Coexpression of FIP-2 and huntingtin relocates huntingtin to Rab8-positive vesicular structures. Optineurin is shown to protect NIH3T3 cells from H$_2$O$_2$ induced cell death and during this process it translocates to the nucleus which is mediated by the GTPase activity of Rab8 (De Marco et al., 2006). While wild type optineurin shows protection, the disease associated mutant, E50K, does not show any protection on apoptotic stimulus and no translocation to the nucleus. Therefore, Rab8 may have a role in mediating response to stress which needs further investigation.

Optineurin also interacts with myosin VI, a Golgi protein involved in the steady-state organization of Golgi complex and post-Golgi membrane traffic. When optineurin is knocked down by siRNA in HeLa cells, Golgi gets fragmented and exocytosis is hampered. Also optineurin targets myosin VI to the Golgi complex and is responsible for colocalization of myosin VI and Rab8 in the perinuclear region around the Golgi and vesicles underneath the plasma membrane, both of which are important regulators of membrane trafficking.
(Sahlender et al., 2005). The authors suggest that optineurin plays two major linker functions: (a) optineurin together with huntingtin links via the minus end directed motor dynein to the microtubule network and by directly interacting with myosin VI, is linked to the actin cytoskeleton, thus playing a co-ordinated microtubule and actin based motor activity around the Golgi complex, (b) optineurin might link myosin VI to the Rab8 involved in sorting molecules to the exocytic pathway at the trans-Golgi network and in membrane fusion at the plasma membrane (Sahlender et al., 2005).

1.3.6.4 Optineurin and metabotropic glutamate receptor signaling

Glutamate is the major excitatory neurotransmitter in the retina (Kuehn et al., 2005). Glutamate is thought to mediate excitatory synaptic transmission at the photoreceptor/bipolar cell synapses and at the bipolar/ganglion cell synapses, acting through activation of glutamate receptors (Nakanishi, 1992). These receptors have been divided according to their primary signal transduction mechanism into ionotropic glutamate receptors and G-protein coupled metabotropic receptors. Glutamate is released by the presynaptic cells and acts through various postsynaptic receptors including N-methyl-D-aspartate (NMDA) receptors. If excessive amount of glutamate is released or glutamate clearance is insufficient, neuronal cell death occurs though a process known as excitotoxicity. Group I metabotropic glutamate receptors (mGLuRs) play an important role in regulating neurotoxicity and neuroprotection. These G-protein coupled receptors (GPCRs) are coupled to inositol 1,4,5-triphosphate formation and the release of Ca\(^{2+}\) stores, play an important role in regulating neuronal function. G-protein coupled receptor kinase-2 (GRK2) is an essential GPCR protein that protects cells from receptor overactivation and is present at low levels in striatal tissue.
Optineurin is shown to interact with mGLuR and inhibits the latter’s signaling to form inositol triphosphate (Anborgh et al., 2005). An optineurin interacting partner, huntingtin also interacts with mGLuR and a mutant form of huntingtin enhances optineurin mediated inhibition of mGLuR signaling. A mutant form of optineurin, H486R, does not interact with mutant huntingtin and in turn fails to attenuate mGLuR mediated inositol triphosphate formation. The study shows a novel mechanism for mGLuR desensitization by optineurin in striatal tissue where GRK2 is at low levels and an additional biochemical link between glutamate receptor signaling and huntington’s disease - a neurodegenerative disease.

1.3.6.5 Optineurin in the context of glaucoma

Various risk factors are associated with glaucoma, and a few unrelated risk factors are shown to induce optineurin gene expression levels (Vittitow and Borras, 2002). One risk factor, namely, elevation of IOP is associated with elevation in optineurin mRNA level (Vittitow and Borras, 2002), although a later report contradicts the increase in optineurin mRNA levels after an increase in IOP (Kamphuis and Schneemann, 2003). The role of TNF-α in the eye and its potential contribution to eye disease has been well studied. In the human glaucomatous optic nerve head, expression of TNF-α and its receptor, TNFR1 are significantly upregulated (Yuan and Neufeld, 2000). Increase in TNF-α levels has been associated with elevated hydrostatic pressure (Tezel and Wax, 2000) and retinal degeneration (Cotinet et al., 1997). Moreover evidence exists for the possible interaction between polymorphisms in the optineurin and TNF-α genes that would increase the risk for glaucoma (Funayama et al., 2004). Patients
treated with glucocorticoids develop steroid-induced glaucoma and
dexamethasone is the most commonly used steroid which is shown to induce
optineurin levels (Vittitow and Borras, 2002). The induction in mRNA levels of
optineurin by TNF-α (and by interferon-γ) has been reported earlier (Li et al.,
1998; Schwamborn et al., 2000).

Incidentally, a recent report shows that optineurin, on overexpression,
dramatically induced the mRNA levels of myocilin (MYOC, first candidate gene
for glaucoma) (Park et al., 2007). This suggests a possible molecular interaction
between these two glaucoma genes, which may have pathological
consequences.

The functional studies discussed above do not explain the possible role of
optineurin and its mutants in the eye, particularly in the retinal ganglion cells. It
has been speculated that optineurin has a neuroprotective function, but it is not
experimentally demonstrated in the eye or cells that are relevant for glaucoma.
Hence, the main aim of my research was to study the role of optineurin in the eye
and to elucidate the functional association between optineurin mutations and
glaucoma phenotype.

1.4 Background and objectives of the current study

One of the genes associated with normal tension glaucoma (NTG), as well
as primary open angle glaucoma (POAG), is OPTN, which codes for the protein
optineurin. Certain missense mutations in the coding region of OPTN are
associated with adult onset as well as juvenile NTG and E50K is the most
common disease-causing mutation of optineurin reported so far. Though there
are a few reports on the functional aspects of optineurin, the role of the protein in
the eye has not been explored.

Hence, we chose to investigate the function of optineurin and its mutants
in the ocular environment. Since the primary defect in glaucoma is the death of
retinal ganglion cells, we analyzed the effect of expression of optineurin and its
mutants on the survival of retinal ganglion cells.

The main objectives of this work have been:

A) To understand the function of wild type optineurin, and

B) To elucidate the mechanism by which optineurin mutations such as E50K,
   H26D, H486R and R545Q cause glaucoma.

Chapter 1 provides information on the disease glaucoma, the genes involved
and on optineurin, the main gene of interest in the current study.

Chapter 2 provides information on the reagents and materials, as well as a
detailed description of methodologies used in the study.

Chapter 3 describes results, where we show that E50K, the severe mutant of
optineurin, induces death of retinal ganglion cells on overexpression and that this
death is cell type specific as it does not cause death of other cell lines tested,
viz., Cos-1, HeLa, IMR-32 (non-ocular cell lines) and D407 (ocular cell line).
Other mutants of optineurin tested (H26D, H486R and R545Q) did not induce
death of RGC. E50K induced cell death shows features of apoptosis and involves
caspases-1 & 9. Antioxidant treatment rescued the retinal ganglion cells from
E50K induced cell death and there was increased ROS production on E50K
overexpression. Also, both wild type and E50K mutant potentiate TNF-α induced cell death in RGC-5 cells. Optineurin does not seem to have a generalized cytoprotective function as speculated because under certain conditions of stress, (e.g., TNF-α here), even wild type optineurin kills retinal ganglion cells.

Chapter 4 describes the identification of novel optineurin interacting proteins using the yeast two-hybrid approach. In addition the interaction of these optineurin interacting proteins with mutants of optineurin was also analyzed. We obtained three NF-κB regulators as optineurin-interacting partners, which suggests that optineurin has a role in the regulation of NF-κB pathway. Apart from the above mentioned proteins, proteins involved in vesicular transport, immune response and transcriptional repression were also identified as interacting partners. Hence, it is seen that optineurin interacts with diverse cellular proteins. E50K and H486R showed altered interaction with some of the optineurin-interacting proteins whereas H26D and R545Q behaved like the wild type. Finally, we observed that two of the interacting partners of optineurin lie in the region of known glaucoma loci.

Chapter 5 describes results of experiments designed to explore the possible mechanisms involved in cell death caused by E50K in RGC-5 cells. Since the mutant shows death only of retinal ganglion cells, we chose to see if there is defect in RGC-5 in responding to oxidative stress. We observed that the cell line does not show a generalized defect in responding to oxidative stress and antioxidant treatment; however there might be a selective defect in the inducibility
of MnSOD. On E50K expression, one of the genes, PGC1α, which normally gets induced upon oxidative stress showed a decrease in mRNA levels. Hence, a defect in the mRNA levels of this gene might be one possible reason for not eliciting protective response by this cell line on E50K overexpression. Also, we observed that E50K shows subtle alteration in interaction with Rab8 (a protein involved in membrane recycling) compared to wild type optineurin. While, optineurin interacts with Rab8, it does not interact with Rab11 suggesting therefore that E50K might affect Rab8 mediated membrane recycling specifically.
### Table 1.1 Candidate genes/loci involved in glaucoma

<table>
<thead>
<tr>
<th>Candidate Loci</th>
<th>Gene</th>
<th>Phenotype</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLC1A (1q24.3-q25.2)</td>
<td>Myocilin (MYOC)</td>
<td>JOAG</td>
<td>Stone et al., 1997</td>
</tr>
<tr>
<td>GLC1B (2cen-q13)</td>
<td>-</td>
<td>POAG</td>
<td>Stoilova et al., 1996</td>
</tr>
<tr>
<td>GLC1C (3q21-q24)</td>
<td>-</td>
<td>POAG</td>
<td>Wirtz et al., 1997</td>
</tr>
<tr>
<td>GLC1D (8q23)</td>
<td>-</td>
<td>POAG</td>
<td>Trifan et al., 1998</td>
</tr>
<tr>
<td>GLC1E (10p15-p14)</td>
<td>Optic neuropathy inducing protein (OPTN)</td>
<td>NTG</td>
<td>Rezaie et al., 2002</td>
</tr>
<tr>
<td>GLC1F (7q35-q36)</td>
<td>-</td>
<td>POAG</td>
<td>Wirtz et al., 1999</td>
</tr>
<tr>
<td>GLC1G (5q22.1)</td>
<td>WD repeat-containing protein 36 (WDR 36)</td>
<td>POAG</td>
<td>Monemi et al., 2005</td>
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<tr>
<td>GLC1H (2p16.3-p15)</td>
<td>-</td>
<td>POAG</td>
<td>Suriyapperuma et al., 2007</td>
</tr>
<tr>
<td>GLC1I (15q11-q13)</td>
<td>-</td>
<td>POAG</td>
<td>Allingham et al., 2005</td>
</tr>
<tr>
<td>GLC1J (9q22)</td>
<td>-</td>
<td>JOAG</td>
<td>Wiggs et al., 2004</td>
</tr>
<tr>
<td>GLC1K (20p12)</td>
<td>-</td>
<td>JOAG</td>
<td>Wiggs et al., 2004</td>
</tr>
<tr>
<td>GLC1L (3p21-22)</td>
<td>-</td>
<td>POAG</td>
<td>Baird et al., 2005</td>
</tr>
<tr>
<td>GLC1M (5q22.1-q32)</td>
<td>-</td>
<td>JOAG</td>
<td>Fan et al., 2007</td>
</tr>
<tr>
<td>GLC1N (15q22-q24)</td>
<td>-</td>
<td>POAG</td>
<td>Wang et al., 2006</td>
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<td>19q12-14</td>
<td>-</td>
<td>POAG</td>
<td>Wiggs et al., 2000</td>
</tr>
<tr>
<td>GLC3A (2p21)</td>
<td>Cytochrome P450 1B1 (CYP1B1)</td>
<td>PCG</td>
<td>Stoilov et al., 1997</td>
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<tr>
<td>GLC3B (1p36.2-p36.1)</td>
<td>-</td>
<td>PCG</td>
<td>Akarsu et al., 1996</td>
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<tr>
<td>GLC3C (14q24.3-q31.1)</td>
<td>-</td>
<td>PCG</td>
<td>Stoilov, 2002</td>
</tr>
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</table>
Table 1.2 Reported optineurin mutations and their percent occurrence

<table>
<thead>
<tr>
<th>Mutation</th>
<th>cDNA change</th>
<th>Percent of mutations</th>
<th>Type of glaucoma</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Disease causing alterations</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>E50K</strong></td>
<td>c.458G&gt;A</td>
<td>13.5%</td>
<td>NTG &amp; POAG</td>
<td>Rezaie et al., 2002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.5%</td>
<td>NTG subset</td>
<td>Alward et al., 2003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.5%</td>
<td>NTG subset</td>
<td>Aung et al., 2003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.5%</td>
<td>NTG subset</td>
<td>Hauser et al., 2006</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3%</td>
<td>NTG subset</td>
<td>Ayala-Lugo et al., 2007</td>
</tr>
<tr>
<td><strong>Premature stop</strong></td>
<td>c.691-692insAG</td>
<td>2.2%</td>
<td>NTG subset</td>
<td>Rezaie et al., 2002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.46%</td>
<td>POAG</td>
<td>Ayala-Lugo et al., 2007</td>
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<tr>
<td><strong>H26D</strong></td>
<td>c.386C&gt;G</td>
<td>1.12%</td>
<td>POAG</td>
<td>Fuse et al., 2004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5%</td>
<td>POAG</td>
<td>Funayama et al., 2004</td>
</tr>
<tr>
<td><strong>H486R</strong></td>
<td>c.1767A&gt;G</td>
<td>1.5%</td>
<td>JOAG</td>
<td>Willoughby et al., 2004</td>
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<tr>
<td><strong>A336G</strong></td>
<td>c.1317C&gt;G</td>
<td>0.9%</td>
<td>NTG</td>
<td>Weisschuh et al., 2005</td>
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<tr>
<td><strong>A377T</strong></td>
<td>c.1439G&gt;A</td>
<td>0.9%</td>
<td>NTG</td>
<td>Weisschuh et al., 2005</td>
</tr>
<tr>
<td><strong>Risk-associated alterations</strong></td>
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<td></td>
<td></td>
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<tr>
<td><strong>M98K</strong></td>
<td>c.603T&gt;A</td>
<td>13.6% in patients and 2.2% in controls</td>
<td>NTG &amp; POAG</td>
<td>Rezaie et al., 2002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16.9% in POAG, 15.4% in NTG and 5% in controls</td>
<td>NTG &amp; POAG</td>
<td>Fuse et al., 2004</td>
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<td></td>
<td></td>
<td>17% in POAG, 22.1% in NTG and 16.5% in controls</td>
<td>NTG &amp; POAG</td>
<td>Funayama et al., 2004</td>
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<tr>
<td></td>
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<td>28.6% in POAG and 24.6% in controls</td>
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<td>9.1% in NTG, 16.3% in POAG and 2.9% in controls</td>
<td>NTG &amp; POAG</td>
<td>Willoughby et al., 2004</td>
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<tr>
<td></td>
<td></td>
<td>14.5% in POAG, 14.2% in NTG and 1.7% in controls</td>
<td>NTG &amp; POAG</td>
<td>Umeda et al., 2004</td>
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### Polymorphisms

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Frequency</th>
<th>Condition</th>
<th>Reference</th>
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<tbody>
<tr>
<td>R545Q c.1944G&gt;A</td>
<td>2.2%</td>
<td>NTG</td>
<td>Rezaie et al., 2002</td>
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<tr>
<td></td>
<td>5.7% in POAG, 6.9% in NTG and 5% in controls</td>
<td>POAG &amp; NTG</td>
<td>Funayama et al., 2004</td>
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<td></td>
<td>1.5% in NTG</td>
<td>NTG</td>
<td>Fuse et al., 2004</td>
</tr>
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<td></td>
<td>1.5% in NTG and 1.6% in controls</td>
<td>NTG</td>
<td>Willoughby et al., 2004</td>
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