INTRODUCTION
I. Introduction

Most of the information about our ambience comes from vision and hence, the eye is considered the 'window of the brain'. The eye is responsible for approximately 38% of the neuronal input to the brain. It has a delicate, complex anatomy and disruption of any of the ocular tissues can lead to ocular discomfort, compromised visual function or loss of vision (Clark and Yorio, 2003). The eye can be divided into the anterior and posterior segments. They have the aqueous and the vitreous humor respectively. The ciliary body in the aqueous chamber is responsible for the secretion of the intraocular fluid (aqueous humor/aqueous) and the trabecular meshwork in the anterior part of the aqueous chamber is responsible for the outflow. The formation and outflow of the aqueous humor regulates the intraocular pressure (IOP). The upper limit of normal IOP level has been reported to be 21 mmHg. Various factors like age, race etc. have been reported to influence the IOP (Shiose, 1990; Bourne et al., 2001).

Glaucoma has been defined as progressive optic neuropathy involving characteristic structural changes to the optic nerve and irreversible visual field loss (Gupta and Weinreb, 1997). Though elevated IOP was earlier considered as the major essential diagnostic criteria for glaucoma, presently it is considered as a risk factor for glaucoma because only about 10% of the patients with an elevated IOP have glaucomatous damage (Gupta and Weinreb, 1997; Coleman and Brigatti, 2001). The history of glaucoma dates back to the period of Hippocrates i.e., 400 BC.

. Glaucoma can be classified into two types - primary and secondary glaucomas based on the absence or presence of other ocular or systemic diseases respectively. Furthermore, each of the two types of glaucoma can be sub-divided as open angle glaucoma and angle closure glaucoma based on the configuration of the iridocorneal angle (Coleman and Brigatti, 2001). Another subset of glaucoma is the congenital glaucoma which is identified based on the onset of glaucoma at intrauterine stage or immediately after birth. Primary open angle glaucoma (POAG) is the type wherein there is no other ocular or systemic disease that could have resulted in glaucoma. POAG is associated with an open iridocorneal angle.
Glaucoma is the second leading cause of blindness in the world (Kingman, 2004). POAG is reported to be the most common of all the types of glaucoma, affecting almost 2% of the world's population (Pang et al., 2002). POAG has been said to account for 60 – 70% of primary glaucomas (Raymond, 1997). Quigley, (1996) have placed India at the second position in the list of world regions harbouring the most number of glaucoma patients, with an equal prevalence of POAG and primary angle closure glaucoma (PACG). In a later study, a prevalence rate of POAG (1.62%), similar to that reported in other populations (North American, European and Australian) was observed in Andhra Pradesh (AP) of southern India (Dandona et al., 2000). Recently, Balasubramanian, (2002) had estimated about 1.5 million people to be blind in the year 2000 in India as a result of glaucoma.

POAG can be classified into two types based on the age of onset of the disease: a) middle to late onset POAG or chronic open angle glaucoma (COAG) or (POAG); b) juvenile onset POAG (JOAG) (Morissette et al., 1995; Raymond, 1997). Another subset of POAG is the normal tension glaucoma (NTG) (Kamal and Hitchings, 1998; Tanna and Jampel, 2000; www.glaucoma.org.au/art_ind.htm). POAG is characterized by the presence of an open chamber angle (iridocorneal angle), optic disc damage and visual field loss (Kanski et al., 1996; Distelhorst and Hughes, 2003). IOP may or may not be elevated in POAG patients. The elevation of IOP may be due to the decreased facility of the aqueous outflow through the trabecular meshwork (Distelhorst and Hughes, 2003).

The mechanism of the optic disc damage or the corresponding loss in retinal ganglion cells (RGCs) has been said to occur in two steps; a) the primary destructive events, followed by, b) secondary degeneration or RGC death (Kwon et al., 2000). Two hypotheses have been proposed which bring about the primary destructive events. These are the mechanical hypothesis and the vascular hypothesis. The mechanical hypothesis considers increased IOP as the main event. The vascular hypothesis considers systemic hypotension and vascular disorders (ocular blood flow dysregulation) as the most probable causal and treatable factors (Flammer and Orgil, 1998). The vascular factors may act alone or in concert with increased IOP to cause
ganglion cell death. Both the mechanical and the vascular mechanisms impede the axoplasmic flow within the RGCs depriving it of the essential neurotrophins like, brain derived neurotrophic factor (BDNF). These primary events lead to the secondary degeneration which may occur due to apoptosis. Evidence for apoptosis is now available in human glaucomas (Kwon et al., 2000). An abnormal increase in levels of neurotransmitters like glutamate is another pathway in the events leading to apoptotic ganglion cell death. This phenomenon has been termed as excitotoxicity (Kwon et al., 2000).

Glaucoma was first recognized as being familial as early as 1842 by Benedict (Alward et al., 1996). A family history of POAG is considered as a major risk factor for POAG (Alward et al., 1996; Wolfs et al., 1998; Alward, 2000). JOAG is mostly seen to have an autosomal dominant (AD) mode of inheritance while the adult onset form of POAG has a more complex mode of inheritance (Lichter, 1994). The first locus for POAG was mapped to 1q21-q31 by linkage analysis in a JOAG family with an AD mode of inheritance (Sheffield et al., 1993). Further studies on JOAG families from various populations confirmed linkage of POAG to this locus (Richards et al., 1994; Graff et al., 1995; Morissette et al., 1995; Wiggs et al., 1995). The locus identified was termed as GLC1A by the human genome organization (HUGO) genome database. Morissette et al. (1995) observed the involvement of this locus in adult onset POAG cases also. Further studies confirmed linkage of POAG to the GLC1A locus (Johnson et al., 1996; Meyer et al., 1996; Lichter et al., 1997a). Five different loci for the adult onset POAG were mapped by linkage analysis in succession following this first locus. These loci were GLC1B at 2cen-q13 (Stoilova et al., 1996), GLC1C at 3q21-q24 (Wirtz et al., 1997; Kitsos et al., 2001), GLC1D at 8q23 (Trifan et al., 1998), GLC1E at 10p15-p14 (Sarfarazi et al., 1998) and GLC1F at 7q35-q36 (Wirtz et al., 1999). Recently, the GLC1G locus was mapped to 5q22.1 (Monemi et al., 2005), and the GLC1I locus has been mapped to 15q11-13 (Allingham et al., 2005).

Mutations in the Trabecular Meshwork Inducible Glucocorticoid Response (TIGR) gene of the GLC1A locus were first observed in POAG cases by Stone et al. (1997). A mutation frequency of 3.9% (~3% in unselected POAG patients) was
reported. Further, reports of mutations in the TIGR gene in both JOAG and POAG patients from various parts of the world have been published (Adam et al., 1997; Alward et al., 1998a; Fingert et al., 1999; Lam et al., 2000; Shimizu et al., 2000; Alward et al., 2002). The role of the TIGR gene in disease causation has also been discussed by many authors. Recently, reports of mutations in the TIGR gene of POAG patients from India have been published (Mukhopadhyay et al., 2002; Kanagavalli et al., 2003; Sripriya et al., 2004). Further, mutations in another gene OPTN or optineurin mapping to the GLC1E locus at 10p15 – p14 have been reported in 16.4 % of families with NTG (Rezaie et al., 2002).

During the period, when the TIGR gene was identified to be mutated in POAG patients by Stone et al. (1997), another set of researchers had been working on the TIGR protein. The TIGR protein was first described when the effects of dexamethasone (a corticosteroid) on cultured human TM cells was analyzed (Polansky et al., 1997). Kubota et al. (1997) in parallel, yet independent studies, isolated and sequenced the cDNA clone from the human retina and designated it as myocilin (due to homology to myosin in the N-terminal region). Ortego et al. (1997) identified a cDNA from the human ciliary body cDNA library. This was identical to the TIGR cDNA described earlier, indicating that both of them coded for the same protein. The HUGO genome database designated the term myocilin to the TIGR protein and the gene was termed as MYOC (Tamm, 2002). Myocilin consisted of 504 amino acids with a molecular weight of 55-57 kDa and a pI of 5.2 (Kubota et al., 1997; Karali et al., 2000; Ueda et al., 2000). The proposed open reading frame consists of two start sites with a signal sequence following the second start site (Ortego et al., 1997; Nguyen et al., 1998). N-glycosylation sites, leucine zipper motifs, potential O-glycosylation sites, phosphorylation sites, hyaluronan binding sites and glycosaminoglycan initiation sites have been observed (Polansky et al., 1997; Nguyen et al., 1998).

The TIGR/MYOC gene consists of 3 exons (Kubota et al., 1997; Nguyen et al., 1998; Tamm and Russell, 2001). Homology to the myosin gene at the N-terminal and to the olfactomedin gene at the C-terminal have been observed. About 90% of
mutations identified lie in the exon III (Fingert et al., 1999). Fifty two of 59 mutations reported have been shown to lie on exon III (Markandaya et al., 2004).

The present study was aimed at screening the ‘Hot Spot’ region of the TIGR/MYOC gene (namely exon III and initial part of exon I [till the 71st amino acid]) for mutations among randomly selected POAG patients from south India, using PCR-SSCP methodology, so as to confirm the importance of this ‘Hot Spot’ region in these patients as well.