DISCUSSION
VI. Discussion

Glaucoma is the second leading cause of blindness (Kingman, 2004) with POAG being the most common form of all the glaucomas (Khaw et al., 2004). Though glaucoma is a very old disease, there still is a severe lack of awareness about the disease especially in developing countries like India. Many concepts/features related to the genetics and pathophysiology of the disease remain at the hypothetical level. The TIGR gene has been found to be mutated in POAG patients of different populations from all over the world. The function of the protein (myocilin) is still unclear (Tamm, 2002; Gould et al., 2004). Since a positive family history is a major risk factor for POAG, the need to unravel the genetics of glaucoma arises. Identification of mutations in the genes and the associated phenotypes in glaucoma is essential. This will help us to develop mutation detection as a clinical diagnostic tool and help the ophthalmologist to start therapy as soon as necessary. Knowing all the possible mutations in the gene(s) involved in glaucoma is essential to evolve diagnostic and therapeutic strategies.

A WHO estimate in 1990 reported 1% of the population to be blind in India i.e., 8.9 million blind (Thylefors et al., 1995). Another recent estimate has reported only about 6.7 million blind i.e., a decrease of ~25% (Resnikoff et al., 2004). This indicates that awareness is spreading and blindness is being prevented. However, some other reports have stated an increase in the prevalence of blindness. A report from the state of AP, south India, showed that the prevalence of blindness has increased from 1.5% in late 1980s to 1.84% in 2000 (40% increase) (Dandona et al., 2001). The prevalence of
POAG in AP is comparable to or more than that observed from other parts of the world especially North America, Europe and Australia (Dandona et al., 2000).

When this work was planned and started in 1999, there was no report available on genetic studies with regard to the TIGR/MYOC gene and POAG, from India. During the course of this work, other independent groups started work on similar fields in different population groups of India. Reports of genetic analysis have started to come since the late 2002.

Glaucoma has a high prevalence after middle age (Thylefors et al., 1995). This fact is not known by many patients and their family members. This, supplemented with the lack of early symptoms, may be the main cause of the severe damage observed in many patients involved in this study. Most of the patients of this study had a severe glaucomatous cupping or visual field loss by the time they were diagnosed. The findings of the present study may be reflective of a severe disease phenotype in patients of this region or may be due to delayed or poor access to eye care. Also, absence of routine eye check up by the patients may be a causative factor. The individuals have sought medical care after a subjective loss of vision occurred.

In an earlier report (Dandona et al., 2000) on Indian patients, it was noted that a vast majority of the persons were diagnosed to have glaucomatous damage at their first visit to the clinic. In certain clinics in KK district, the only facility to diagnose glaucoma was the IOP measurement. Though this was the scenario in the rural area, in Chennai (urban region) only a few doctors were not really aware of the importance of
the risk factors such as systemic hypotension etc. Now the awareness about glaucoma is fast catching up with the advertisements in the visual media about glaucoma and its ill effects.

VI.1 Differential Sex Ratio

Analysis of the sex ratio of the samples revealed a preponderance of male patients over female patients. The increase in male patients was nominal in KK district (55 males : 45 females) and in Chennai the difference was vast (71 males : 30 females). This corroborates the earlier reports wherein males have been reported to be affected more, in terms of number, than females. Leske et al. (1994) have reported a higher prevalence in men (8.3%) than women (5.7%) in the residents of Barbados (West Indies). The prognostic significance of sex was however less clear than age, race etc., (Shields, 1998).

VI.2 Age Distribution

Reports available so far have revealed that the prevalence of POAG increased with increasing age (Wensor et al., 1998; Dandona et al., 2000; www.glaucoma.net/gany/about/index.html). This feature was also observed in our patient population wherein most of the patients were in the 50 - 70 yrs age group. This age group was based on the age at diagnosis of the disease. The number of patients in the age group of 40 - 50 yrs was also higher, though not at the level of the higher age groups (50 – 70 yrs).
VI.3 Sequence Alterations

The PCR-SSCP analysis resulted in identification of 10 variants or sequence alterations from 9 patients. Whereas, sequencing results indicated that a total of 11 sequence variants/changes were present. Thus, 9 patients harbored 11 sequence variants. This was because, 2 patients had 2 sequence alterations each. This phenomenon of double alterations/changes within a gene seems to be not a frequent finding in POAG patients, though two mutations in one POAG patient has been reported recently (Faucher et al., 2002). They reported the presence of Arg126Trp in a POAG patient who already had the Lys423Glu in the family/pedigree. The Arg126Trp was reported in controls also. The Arg426Trp was suggested to be a late onset disease causing mutation and/or a genetic modifier accelerating the pathogenesis in combination with TIGR/MYOC or other glaucoma gene mutations. This individual had an aggressive form of JOAG (Faucher et al., 2002).

The 11 sequence alterations were of 7 different types. Of the 7 types of variants two were polymorphisms (only single base change in DNA and no change in amino acid). The remaining 5 were probable mutations, one of which resulted in a conservative amino acid change but had a variation in the predicted secondary structure of the mutant protein. Of the 5 (including the conservative amino acid change), 4 were seen to lie in exon III (80% of mutations were in exon III). This is in conformity with earlier reports (Fingert et al., 1999).

VI.3.1 Gln48His (144 G>T) Alteration

The alteration/change 144 G>T (G to T transversion) was observed in two
unrelated patients (Eg 10 and Eg 17) from Chennai population. This resulted in a non-conservative amino acid substitution of glutamine to histidine. In both the patients, this variation was in heterozygous state. None of the control subjects had this change. These features i.e., alteration of the amino acid sequence and absence of it in the controls, indicate that this variant might play a disease causative role, reiterating the importance of the TIGR gene in POAG patients of India.

This mutation has been recently reported in two different studies on Indian patients. One of the studies has observed the mutation in a north Indian patient and in one patient native of Chennai, southern India. The ages at onset were 17 years and 65 years respectively in the two individuals. The IOPs were 33/26 mmHg in one patient and 21/23 mmHg in the other. Cup/disc ratio varied from 0.4 to 0.6 (Sripriya et al., 2004). Another study has reported this mutation in 3 of 56 patients from Kolkata, eastern India. The ages at onset were 20, 32 and 70 yrs. The IOP values ranged from 14 – 38 mmHg and cup/disc ratios ranged from 0.5 to 0.9. The damage was extensive in one eye, while the other was relatively normal i.e., unilateral damage (Mukhopadhyay et al., 2002). Yet another study that constituted patients from Madurai, farther south to Chennai did not report the presence of this mutation (Kanagavalli et al., 2003). In the present study also, none of the patients from farther south of Chennai i.e., KK district had this mutation.

This mutation has a varied age of disease onset, ranging from 17-70 years (including the patients of the present study), with an average age of onset of 44.4 years. This average age of onset is less, compared to the age of onset for the most common
mutation, Gln368Stop, reported from all over the world, especially in Caucasian populations (average age of 59 years) (Alward et al., 1998) indicating a comparatively severe phenotype. The damage was mostly unilateral.

This mutation (144 G>T) has so far been reported only in India and has not been reported from any other population in the world. Therefore, at present it could be said that this mutation is specific for the Indian sub-continent. The phenotypes observed for this mutation in the present study correlated well with the other reports from India (Mukhopadhyay et al., 2002; Sripriya et al., 2004).

Possible Role of the Gln48His Variant in Disease Causation

This mutation occurs at the 48th aa. A PATTINPORT search revealed a 66% homology to GAG attachment site at aa 44-47, close to this mutation. The 44th-46th aa also form a putative PKC site. Predictions using bioinformatics tools also revealed many changes in the secondary structures of the mutant protein (See Table 6 in page 131). The resulting changes in the predicted secondary structure overlapped with the important region of aa 44 – 46. This indicates a plausible causal effect of the mutation at aa 48 in the disease manifestation/progression.

The cysteine at 47th aa has been proposed to be involved in the oligomerization of TIGR (Fautsch and Johnson, 2001). The Gln48His mutation results in a change from glutamine, a relatively simple structured aa, to histidine, an aa with a bulky side chain in the form of the 5-C ring structure. This bulky side chain could play a role in hindering the oligomerization via the cysteine at 47, resulting in aberrant myocilin
protein. However, until further evidence on the formation of multimers is available, the impact of the Gln48His on the adjacent aa cysteine will remain unknown.

Another possible effect of change in N-terminal MYOC/TIGR sequence on disease risk is suggested by the finding that the region between aa 15 - 138 is necessary to localize TIGR/MYOC to microtubules in cells (Mertts et al., 1999). Sequence changes in this region may thus alter the sub-cellular localization and thereby the function of TM cells.

It could not be predicted whether the presence of this Gln48His mutation in certain groups of Indian patients and their absence in patients from the southern most region of the peninsula (KK district) is due to demographic factors or due to a founder effect. So far (including the present work), there are no reports of the variant being present in familial cases. A larger cohort of patients especially including familial cases will have to be screened from both the places before a final conclusion can be arrived at. Also the possible role of the influence of other genes/environmental factors have to be ascertained.

VI.3.2 Thr325Thr Alteration

A G>A transition at nucleotide 975 (Thr325Thr) was one of the two polymorphisms (silent mutations) in the present study. This was observed in one patient, Ngl 12, who also had the Ser331Thr variation. Thus, along with Ser331Thr, the G>A transition at nucleotide 975 might play a causative role in the disease.
VI.3.3 Ser331Thr Variation

The mutation Ser331Thr was observed in one patient, Ngl 12. The same individual harboured the polymorphism (SNP), G>A transition at nucleotide 975 (Thr325Thr).

Possible Role of Ser331Thr as a Mutation

The Ser331Thr observed in this study is a conservative change of serine to threonine, both of which are polar amino acids. However its absence in the controls and the effect of this change on the predicted secondary structure of the protein suggests that it might have a causal effect on the onset of glaucoma. Though there is no direct evidence of this being a causal factor of glaucoma, still the benign nature of this variant cannot be assumed. Hence based on the available reports on the basis of defining a mutant and a polymorphism, also on the fact that this change has not been reported elsewhere so far, it can be suggested that this is a mutation or a probable disease causing variation. Further, in an earlier study, though the non- conservative changes were presumably more injurious to the functioning of the myocilin protein than conservative changes, the conservative changes have also been classified as probable disease causing mutations if they were absent in the controls (Fingert et al., 1999).

VI.3.4 Tyr347Tyr Alteration

The synonymous Tyr347Tyr as a result of the 1041 T>C transition is the other polymorphism (silent mutation) that was observed in the present study. This was seen in 4 patients, N 45, N 39, Ney 3 and Ngl 9 - all from KK district.
These patients had a varied range of disease features. Most of the patients had a considerable damage at least in one eye and a varied age at onset/diagnosis ranging from 19 to 70 yrs. This polymorphism was also in the heterozygous state in all of the patients. This polymorphism has never been reported in any study on Indian patients, though the same has been reported from other populations abroad.

The translationally silent polymorphism in codon 347 has a relatively high incidence in subjects of KK district (= 4%). These observations indicate this to be a frequent polymorphism found in POAG patients of this part of India. This was not seen in the Chennai patients or in the controls from both the places. It is possible that it might play a role as a modifier of the disease phenotype with relation to other gene(s) that might be involved in the causation of glaucoma.

VI.3.5 Thr353Ileu Alteration

This was seen in the patient Bsr 16; the change was a heterozygous, C>T transition in the nucleotide 1058. This change has been reported earlier in a Japanese patient (Fingert et al., 1999). Later some reports have shown the frequency of this change to be equal or more in controls compared to POAG patients (Lam et al., 2000; Pang et al., 2002). The presence of the change in the codon 353 in POAG patients and its absence in the controls in the present study suggests a disease causal role of this variant.

The patient seems to have a typical case of AD-JOAG with an early age of onset of glaucoma (diagnosed at 14 yrs) and a severe course of the disease. The patient
has a family history of the disease with the mother and her brother being affected at the ages of 45 and 19 yrs, respectively. Both had to undergo surgery to control the progression of the disease.

Earlier reports have characterized this mutation to confer a less severe disease characteristic with a later age of onset compared to the patient in this study. Yoon et al. (1999) reported a patient with this mutation who was diagnosed at the age of 59 years and had an IOP level of 26 mmHg in the right eye (OD) and 24 mmHg in the left eye (OS) prior to medication. Severe loss of visual field and a cup/disc ratio of 0.8 and 0.6 in the right and left eyes respectively were observed. Other authors reported this change in both controls and patients (Lam et al., 2000; Pang et al., 2002). These patients had a varied age at diagnosis ranging from 16 to 69 yrs. The IOPs ranged from 25 to 37 mmHg. The cup/disc ratio was seen to vary from 0.3 to 1.0. However, in the present study none of the controls had this change.

An interesting aspect of this variation is that this change has always been reported in the Asian populations such as Japanese, Chinese, Korean (Fingert et al., 1999; Yoon et al., 1999; Pang et al., 2002) and now perhaps for the first time in an Indian patient. Thus the geographic/demographic criteria may play a role in the disease causation. The role of this mutation as a causative factor in glaucoma is still in doubt. In the present study, this variation was found only among the patients, hence, it may be considered as a probable DCV/mutation.
However, the criteria of defining a DCV and a non-DCV are not fool proof and would fail to recognize a low penetrance glaucoma susceptibility allele that is common in both POAG patients and controls. They may also inappropriately assign pathogenicity to a rare polymorphism (non-DCV) that just happens to alter the amino acid sequence of the myocilin protein product (Fingert et al., 1999).

**Possible Effect of the Sequence Change**

The mutation results in a change from threonine to isoleucine, which results in change in polarity. Threonine is a polar aa, while isoleucine is a hydrophobic aa. This change is at the exon III which is the most conserved part of the TIGR/MYOC, with homology to the olfactomedin protein. The threonine at 353 position is a putative phosphorylation site by PKC. The phosphorylation of the TIGR may play a role in the regulation of the IOP. To know the effect of these phosphorylation sites on the function of the TIGR, the function of the normal TIGR protein has to be elucidated. The Chou-Fasman prediction revealed a gain of turn ‘T’ at a nearby aa 351.

**VI.3.6 Asn480Lys Variation**

An interesting result of the present study is that two patients had two different sequence alterations each. One of the two patients’ (Bsr 16) had two mutations within exon III and the other, Ngl 12, had a silent/same sense variation and a mutation in exon III. The Asn480Lys was observed in the patient Bsr 16, who also had the Thr353Ile mutation. Both the alterations were in the heterozygous state. In the present work, none of the controls exhibited these two variations.
The Asn480Lys mutation has been reported in individual patients and mostly in patients with a family history of the disease. Founder effect for this mutation has been demonstrated in families (Adam et al., 1997). Of the three families studied the median age of onset has been reported to vary between 30 and 35 yrs. Another study has reported a varied phenotype ranging from mild to severely affected cases with the age at diagnosis being 30, 34 and 40 yrs respectively in three of the patients studied (Hulsman et al., 2002). Brezin et al. (1998) found this mutation to be present in 6 families with juvenile and middle age onset POAG. The patients were of the age ranging from 10 to 65 yrs and the IOP levels ranged from 22 to 50 mmHg. The mutation has been said to be associated with the disease of intermediate severity.

**Possible Disease Causing Effect of Asn480Lys**

This mutation results in a C>A transversion which results in a change from asparagine (polar aa) to lysine (positively charged aa). Thus a change in charge is the result of this mutation. Further the CK2 site at aa 475 – 478 was seen to have gained an α helix due to the mutation at aa 480. These changes may possibly play an inhibiting role in the normal functioning of the protein. The exact role of the normal protein is unknown and also the role of these motifs is not clear.

The very early age of onset and rapid progression into a severe phenotype in the individual (Bsr 16) of the present study compared to the previous reports of individuals with either one of these two variants, indicates that these two mutations could have complemented each other to lead to the severe/aggressive form of the disease. Such co-occurrence of two sequence changes are perhaps not very common. This type of severe
phenotype correlating with the presence of two variants in a single individual had been reported recently (Faucher et al., 2002). The Thr353Ile considered as a polymorphism in other studies could thus have a role in aggravating the phenotype of the mutation Asn480Lys. The primary disease causing variant and the supporting variant could not be elucidated based on this information. However, the severe phenotype in this patient suggests the combinatorial role of these variants in disease causation/progression.

VI.3.7 Pro370Leu Alteration

This change was observed in the patient Rose 6, who had a severe disease phenotype with a very early age of onset of the disease (diagnosed at 16 years), rapid progression of the disease and a positive family history of the disease. The severe nature of the disease associated with this variant correlates well with earlier reports. Samples from three of the proband’s uncles and her brother were analysed and none had any band shift on SSCP. This suggests that the mutation Pro370Leu found in the patient Rose 6 may not be associated with a familial kind of the disease at least in this case. However this conclusion may not be fool proof. The samples have been stored for future analysis by DHPLC and/or sequencing.

Phenotype

The early age of onset and the rapid progression of the disease in the patient in the present study correlates to earlier reports wherein the individuals with this mutation had a very early age of disease onset, range of 6-14 yrs (median age – 10 yrs); 7-27 yrs (median age at diagnosis – 11 yrs) in two families with a severe course of the disease
poorly controlled by topical drugs (Adam et al., 1997). Rozsa et al. (1998) reported a JOAG family with the Pro370Leu variation and having an age at diagnosis ranging from 5 – 27 yrs (mean = 12 yrs) with a high average IOP of 45 mmHg (ranging from 25 – 66 mmHg). Three individuals within one family with ages at diagnosis of 17 (proband), 3 and 8 yrs (offsprings) had the Pro370Leu mutation, with rapid disease progression and needed surgery to control the progression (Damji et al., 1999). Two patients with ages at onset of 13 and 26 yrs were reported by Taniguichi et al. (1999). These two patients also exhibited high IOP and poor response to medical treatment.

Further, an Indian patient with an age at diagnosis of 18 yrs has been reported (Mukhopadhyay et al., 2002). The patient had pressures of 24 and 32 mmHg, OD and OS respectively and cup/disc ratios of 0.8 (OD), 0.7 (OS) respectively. Vision tests revealed presence of an arcuate scotoma in the superior and inferior halves with nasal steps OD and a scotomatous defect in the superonasal quadrant OS (Mukhopadhyay et al., 2002).

The aggressive nature of the disease in patients with this mutation suggests that this is a strong mutant allele of TIGR/MYOC. Also, this mutation has been reported in Indian, English, French, North American, Japanese and German populations. The occurrence of this mutation in pedigrees of varying ethnicity suggests that the loss of proline at this position may severely affect the formation/function of the protein (Wiggs et al., 1998a).
Effect of the Pro370Leu Change on the Protein

The change is a C>T transition at nucleotide 1109, resulting in a change from proline (a polar aa) to a hydrophobic leucine molecule. This occurs in the CpG dinucleotide. The importance of CpGs in the disease causation has been suggested earlier (Mukhopadhyay et al., 2002). Proline has a side chain that inhibits α helix formation and fits poorly in the α helix conformation while the leucine is one of the good α helix formers (Brandon and Tooze, 1999).

Apart from the secondary structures of the mutant protein that were seen to be altered using bioinformatics tools, a casein kinase II (CK2) phosphorylation motif was seen to be present across aa residues 377 – 380 (Thr-Asp-Ile-Asp). Accessibility to this phosphorylation site may be altered since there is loss of turn near the CK2 site (Damji et al., 1999). Data suggests that the phosphorylation of Thr (aa 377) at the CK2 site is important for normal TIGR/MYOC function. However, a substantial difference in age at onset for Pro370Leu and Thr377Met (Pro370Leu resulted in an earlier onset of the disease) suggests that the Pro370Leu conformation change may bring about more than just a failure of CK2 modification (Rozsa et al., 1998).

All of the mutations presented here are predicted to affect the myocilin protein’s charge or the secondary structure. Since the function of the TIGR/MYOC gene is not yet fully elucidated, the effects of single amino acid changes cannot be precisely predicted and can only be hypothesized (Michels-Rautenstrauss et al., 1998). However, the fact that some of the patients with very severe phenotype i.e., a near total blindness/total blindness at a very young age and harbouring mutations in the TIGR/MYOC gene substantiates the fact that severe phenotypes may positively correlate with the MYOC
mutations. So far, the other parallel studies from the Indian subcontinent have dealt with smaller number of patients, this study included the largest number of patients (201) screened in an Indian study.

The mutations Pro370Leu, Thr353Ile etc., are predicted to have an impact in the conserved phosphorylation motif. The TIGR gene has been reported to be phosphorylated but it is not yet determined if the predicted phosphorylation motifs near the mutations are the residues that are actually modified in vivo (Shimizu et al., 2000).

VI.4. Myocilin and POAG

The mutant forms of TIGR may cause POAG by different mechanisms. The mechanisms hypothesized are a) haploinsufficiency, b) dominant negative effect (by competing or binding normal myocilin) and c) gain of function (interacting abnormally with other proteins, may acquire a longer half-life) (Allingham et al., 1998b; Alward et al., 1998). Evidence against haploinsufficiency and supporting the later two mechanisms were reported by Morissette et al. (1998), Wiggs and Vollrath, (2001) and Kim et al. (2001).

Observations that missense mutations have a earlier age of onset of glaucoma and significantly higher IOPs than nonsense mutations such as Gln368Stop suggests a dominant negative mechanism for at least some of the mutations rather than haploinsufficiency (Alward et al., 1998). However haploinsufficiency was suggested by observation of a severe phenotype in a homozygous Arg46Stop individual by Yoon et al. (1999). This has been contradicted by reports of Lam et al. (2000), Kim et al. (2001), and Wiggs and Vollrath, (2001). Haploinsufficiency was ruled out, as the loss
of TIGR gene product alone is not enough to result in glaucoma. This was evidenced based on the finding of the Arg46Stop mutation in controls raising doubts as to whether TIGR is essential for normal functioning of the eye or whether other proteins can serve the function of TIGR (Lam et al., 2000).

Overall, a reduced myocilin in the aqueous of MYOC-glaucoma patients has been observed. No mutant myocilin in humor was detected especially in patients with the Gln368Stop mutation (Jacobson et al., 2001). Similar results were observed when mutant myocilin lacking the olfactomedin domain was expressed (Caballero and Borras, 2001). It has been suggested that the mutant forms are not secreted (Caballero et al., 2000; Jacobson et al., 2001) thereby contradicting the earlier hypothesis that MYOC may plug out flow pathways (Polansky et al., 1997; Nguyen et al., 1998). The mutants may alter secretion of normal MYOC or other proteins whose function may be to maintain integrity of TM and extracellular matrix.

Generally misfolded proteins in the ER are degraded. If this mechanism fails, mutant/misfolded proteins aggregate, congest secretory pathways and finally elicit ER dysfunction and finally death of the cell by apoptosis (Hampton, 2000). Such a situation might happen in cells of patients with mutant myocilin especially in those where myocilin expression in TM is high (Caballero and Borras, 2001).

Normal TIGR/myocilin can form dimers/multimers (Nguyen et al., 1998) suggesting that mutants may interfere with normal protein through formation of hetero-multimers and thereby decreasing the secretion of the normal protein (by retaining it inside the cells). The disruption by mutant protein of the processing of normal TIGR/
Myocilin through binding and sequestration of other proteins and/or cell death due to the accumulation of insoluble protein complexes has also been hypothesised (Zhou and Vollrath, 1999).

VI.5. Mutation Frequency

The total mutation frequency in our study group was ~2.5% (5/201). The mutation frequency of patients originating from our study area is 2% (4/200) (one of the patients with a mutation was from Kolkata, eastern India). The mutation rate amongst POAG patients of Chennai and KK district were the same at 2% each. Thus the mutations and the mutation frequency coincide with that reported from other parts of India and the world. This study is a preliminary work to establish the importance of the region of the TIGR/MYOC that is most often mutated in other populations. Thus, this study has dealt with screening of the exon III and initial part of exon I (till the 71st aa) only. However, it is essential to screen the whole gene in further studies before the actual mutation frequency can be suggested.

The overall mutation frequency among the primary open angle glaucoma patients reported from other parts of the world range from 1.4 – 4.6% (Alward et al., 1998a; Fingert et al., 1999; Pang et al., 2002; Aldread et al., 2004). The mutation rate in the present work is comparable to the other research studies that came up during the same period from other Indian populations (Kanagavalli et al., 2003; Sripriya et al., 2004). Meanwhile one study on an Indian population has reported a higher mutation frequency of ~7.1% (Mukhopadhyay et al., 2002). Moreover, of the five mutations found in the present study, 2 were present in JOAG cases and the remaining three in adult onset POAG cases. Thus, 2 of 27 JOAG i.e. 7.41% had a mutation. This is
comparable to the earlier reports of Wiggs et al. (1998a) and Alward et al. (2002), who have reported a mutation frequency in JOAG cases as 8% and 6.4% respectively. As reported earlier (Fingert et al., 1999) that most of the mutations are present in the exon III, out of 5 variants (mutations) observed in this study, 4 have been localized to exon III.

VI.6. Future Work

*TIGR/MYOC* has been reported to be mutated in families with JOAG and also in sporadic POAG patients (Alward et al., 2002; Hulsman et al., 2002). However, there are many sequence alterations that are not associated with the disease and also majority of the sporadic POAG patients do not have mutations in the *TIGR/MYOC* gene. Hence, genetic analysis of the POAG patients (sporadic and familial) with regard to the *TIGR/MYOC* gene and other gene loci like *OPTN* and *WDR36* is essential.

It would be useful to study the impact of MYOC sequence and structural changes associated with POAG in various ethnic groups of India for advancing the molecular genetics based diagnosis and evaluation of POAG (Kanagavalli et al., 2003). This has been emphasized by the observation of the specific mutation for Indian populations alone that has not been identified in any other part of the world, namely the Gln48His. This is a specific mutation for the Indian subcontinent and DNA based diagnosis of such specific mutation is feasible and cost effective provided the rate of this mutation is high in Indian patients. Further, discovery of the range of mutation types and locations may shed light on the role of *TIGR/MYOC* in POAG and normal eye function. It is also essential to identify other gene loci/ gene(s) likely to be involved in the causation of POAG.