Mutations in MYOC gene of Indian primary open angle glaucoma patients

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Purpose: Glaucoma is the second leading cause of blindness worldwide after cataract. Defects in the myocilin gene (MYOC) have been shown to be associated with Primary Open Angle Glaucoma (POAG), the most common form of the disease, especially in its juvenile form. Most of the reported mutations in MYOC are in POAG patients of Caucasian origin. A few studies have been reported on Asian patients (such as Chinese, Japanese, and Koreans) but none from the Indian subcontinent. The purpose of this study was to investigate the molecular basis of POAG among Indians, using MYOC as the candidate gene, and broaden our understanding on the pathogenesis caused by MYOC.

Methods: Fifty-six unrelated POAG patients, comprising 39 sporadic cases and 17 patients having familial history for POAG were enrolled in this study. The coding sequence of the gene was amplified by polymerase chain reaction (PCR) using genomic DNA from 30 POAG patients, followed by sequencing of the PCR products. Nucleotide changes were detected by identifying double peaks in the chromatogram due to heterozygosity and pairwise BLAST analysis of the sequence output data against the normal copy of the MYOC cDNA. Alteration of restriction sites due to nucleotide changes was identified. Twenty-six patients (not sequenced) and controls were screened for nucleotide changes by allele specific restriction digestion of the PCR products followed by separation of the digested DNA fragments by polyacrylamide gel electrophoresis.

Results: From a pool of 56 unrelated POAG patients two mutations were identified. A putative novel mutation (144 G->T; Gln48His) of a conserved amino acid was detected in the exon 1 of MYOC from three unrelated patients but none in the 51 control samples examined. The other mutation (1109 C->T; Pro370Leu), located in exon 3 and detected in a family affected with POAG, cosegregated with the disease and was not present in control samples. Two single nucleotide polymorphisms (SNPs) were identified; one in the promoter region (-83 G->A) and the other in the coding sequence (227 G->A; Arg76Lys). These two SNPs were found to be highly heterozygous both in the control (0.480) and the patient (0.477) populations, and were observed to be in linkage disequilibrium.

Conclusions: The presence of a novel non-conservative change in codon 48 of MYOC in 3 POAG patients, but none in the healthy controls, suggests a causal association of the mutation with the disease, either singly or in combination with other genetic loci. The other point mutation (Pro370Leu) detected in the members of an affected POAG family represents a hotspot of mutation in the gene. Two identified SNPs (-83 G->A and 227 G->A) are not associated with the disease phenotype but could be used as highly informative markers in POAG affected families to determine any causal association of MYOC with the disease, and for identification of presymptomatic carriers in the family, where applicable. A comparison of our data with other studies revealed that these two polymorphisms are in complete linkage disequilibrium among Asians, but not among other ethnic groups studied so far.

Glucoma represents a heterogeneous group of neurodegenerative disorders of the eye affecting about 67 million people worldwide [1]. Primary open angle glaucoma (POAG) is the most common form of the syndrome where vision is lost progressively, often silently and can lead to blindness. So far, two different genes, Myocilin (MYOC) and Optineurin [2,3], and four other chromosomal loci have been reported to be linked to POAG [4-7]. Following the discovery of the association between myocilin and POAG in 1997 [2], 49 mutations and a few benign polymorphisms have been detected in different population groups [8-21]. Unfortunately, the pathogenesis of glaucoma continues to elude us. Adult onset POAG is inherited as a non-Mendelian trait, whereas juvenile onset POAG exhibits autosomal dominant inheritance [19]. It has been observed that among the 3 exons of MYOC, the majority of the mutations are clustered in exon 3 (45 mutations) with a few being located in exon 1 (4 mutations) and none detected in exon 2. It has been speculated, based on the above information and additional evidence, that the olfactomedin region of MYOC (encoded by exon 3) is crucial for the normal function of the protein and that the gene might have evolved from two different primordial proteins [22]. It has been well documented that MYOC mutations do not cause glaucoma through haplo-insufficiency but, instead, through dominant negative effects [21]. This observation has been supported by
the creation of MYOC knock out mice that demonstrate normal eye morphology [23]. Although the pathophysiology is unknown, it has been suggested that the mutant MYOC protein obstructs the outflow of the aqueous humour through the trabecular meshwork, resulting in an increased IOP which is frequently associated with glaucoma [24].

There are a few reported studies on defects in MYOC from Asia, including Japan [10,17,25], China [21], and Korea [20]. Recently it has been reported that about 1.5 million people are blind due to glaucoma in India [26]. Though molecular genetic studies on primary congenital glaucoma have been reported from India [27], we are not aware of any report on molecular genetic studies on POAG, despite POAG being the major subtype of glaucoma in this country. Hence, in this study, we investigated the molecular basis of POAG among Indians using MYOC as the candidate gene and attempted to broaden our understanding of pathogenesis caused by mutations in MYOC.

**METHODS**

**Selection of the study subjects:** Indian patients affected with POAG, with or without a family history, were recruited from the glaucoma departments in the Eye Care & Research Center (Kolkata) and the Regional Institute of Ophthalmology (Kolkata). Diagnoses involved clinical ocular and systemic examinations. Ocular examinations included measurement of intraocular pressure by application tonometry (Goldmann). Gonioscopy (Goldmann 3-mirror gonioscope; Shaffer’s grading) identified the angles of the anterior chamber and was used for optic disc evaluation and fundoscopy. The optic disc was further evaluated with +78 D lens; the visual fields were assessed with a Humphrey’s automated perimeter.

For this study, the inclusion criteria were (A) optic disc cupping or visual field changes in patients with an intraocular pressure above 20 mm Hg and (B) optic disc cupping and visual field changes in patients with an intraocular pressure less than 21 mm Hg. Patients were excluded for a history of cardiovascular disease and asthma, the possibility of the data being skewed due to predisposed individuals in the control population would be extremely low in the present study.

**Collection of blood samples and genomic DNA preparation:** With the help of collaborating doctors, 10 ml peripheral blood was collected with EDTA from POAG patients. Similarly, blood samples were also collected from normal individuals with no personal or family history of glaucoma. Written consent was taken from the patients and unrelated normal control individuals for blood collection and relevant eye examinations, as appropriate. The internal review committee on research using human subject cleared the project after proper review as per regulation of Indian Council Medical Research.

Genomic DNA was prepared from fresh whole blood using the conventional phenol-chloroform method followed by ethanol precipitation. Then, the DNA was dissolved in TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) [28] for polymerase chain reaction (PCR) amplification and analysis.

**Polymerase chain reaction (PCR):** PCR was carried out in a total volume of 25.0 µ containing 100 ng genomic DNA, 0.4 µM of each primer, 0.2 mM of each dNTP, 2.5 mM of MgCl₂, and 0.5 unit of Taq polymerase (Invitrogen, Carlsbad, CA) in a thermocycler (GeneAmp-9700, PE Applied Biosystems, Foster City, CA). Each exon was amplified using primers described by Alward et al. [13] under the following conditions; an initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, and extension at 72 °C for 1 min. A final exten-

<table>
<thead>
<tr>
<th>Nucleotide change</th>
<th>Amino acid change</th>
<th>Location</th>
<th>Sequence of primer pairs (5’ to 3’) and PCR condition</th>
<th>Length of PCR product (bp)</th>
<th>Gain (+) or loss (-)</th>
<th>Digestion product (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>144 G-&gt;T</td>
<td>Gln48His</td>
<td>Exon 1</td>
<td>CTTCTGTTGACAGTGCTGCA CTGTCCAAGGTCAATTGGT</td>
<td>313</td>
<td>+</td>
<td>176+137</td>
</tr>
<tr>
<td>1109 C-&gt;T</td>
<td>Pro370Leu</td>
<td>Exon 3</td>
<td>ATACGTGCTAGGCAGCTGGA CAAATGCCTGATAGCACCC</td>
<td>198</td>
<td>-</td>
<td>159+39</td>
</tr>
</tbody>
</table>

A small region of genomic DNA from test samples encompassing the location of suspected mutation was amplified by PCR and digested with restriction site that is lost or gained as a result of mutation as indicated and the digested DNA fragments were separated by electrophoresis in a 6% polyacrylamide gel as described in Materials and Methods.
sion at 72 °C for 3 min completed the reaction. The PCR products (5 µl) were analyzed by electrophoresis in either 6% polyacrylamide gels for DNA fragments less than 800 bp or 1.5% agarose gels for larger DNA fragments and visualized under UV light as detailed in Sambrook et al. [28]. Only those PCR products that demonstrated a single amplification product with no evidence of non-specific amplification was used for DNA sequencing, as described below, using primers nested within the amplified region of DNA [13].

DNA sequencing: The PCR products, free of contaminating bands due to non-specific amplification, were column-purified using Qiagen PCR-purification kits (Qiagen, Hilden, Germany), and bi-directional sequencing was performed using an ABI Prism 377 DNA sequencer with dye-termination chemistry. Nucleotide changes were detected by identifying double peaks in the chromatogram due to heterozygosity of the DNA sample analyzed and confirmed by sequencing from the opposite direction. In addition, the sequences were analyzed using Pairwise BLAST [29] to determine if there were any changes from the normal sequence available in the database.

Restriction enzyme digestion: Mutations identified by DNA sequencing were screened in additional POAG patients and control samples by PCR amplifying the suspected region of genomic DNA (Table 1). The PCR products were digested with appropriate restriction enzymes that distinguished between the mutant and normal alleles (Table 1) under the conditions described by the manufacturer (New England Biolabs, Beverly, MA) in a total reaction volume of 20 µl. Similarly, single nucleotide polymorphisms (see Results section) in the promoter region (pSNP) and coding sequence (cSNP) were screened in both patient and control samples using Ava I and BsmA I restriction enzymes as described by Lam et al. [21].

Detection of a novel putative mutation: A novel change (144 G->T) that would result in a non-conservative substitution (Gln48His) was detected by sequencing exon 1 of MYOC in 3 unrelated sporadic patients who were heterozygous for the change (Table 3 and Table 4). A description of the patients with the mutation is as follows:

First patient: A 70 year old male Hindu patient (GL 41 in Table 3) complaining of dimness of vision, with visual acuity of light perception with projection in the right eye (OD) and 6/24 with glasses in the left eye (OS), had an initial IOP of 24 and 36 mm Hg in right and left eye, respectively. The cup to disc ratio was 0.9 (OD) and 0.5 (OS). Gonioscopy revealed an open angle (grade II-III) in both eyes. The visual field OS showed a diffuse depression with a scotoma in the superotemporal quadrant. The lens showed early cataractous changes. The patient had no other ocular or systemic involvement. He was treated with Betaxolol eye drops and Pilocarpine 2% eye drops (three times daily). On the first follow-up the IOP was 21 mm Hg OD and 26 mm Hg OS. Trabeculectomy was done in OS and the IOP was reduced to 16 mm. On a further follow-up, the IOP varied between 12-16 mm Hg OD and 25 mm Hg OS. The patient had no other ocular or systemic involvement. He was treated with Betaxolol eye drops and Pilocarpine 2% eye drops (three times daily). On the first follow-up the IOP was 21 mm Hg OD and 26 mm Hg OS. Trabeculectomy was done in OS and the IOP was reduced to 16 mm. On a further follow-up, the IOP varied between 12-16 mm Hg OS and visual acuity decreased due to progression of the lental opacity. The patient denied any family history of glaucoma.

Second patient: A 20 year old male Hindu patient (GL 14 in Table 3) was diagnosed with POAG and treated surgically.

### RESULTS & DISCUSSION

Fifty-six unrelated POAG patients, comprising 39 sporadic cases and 17 patients with a family history of POAG were enrolled in this study. Among these 56 patients, 20 had juvenile onset open angle glaucoma (individuals below 30 years of age were selected) and 36 had adult onset open angle glaucoma. The age at diagnosis ranged from 10 years to 67 years, with a mean age (±standard deviation) of 41.98±17.66 years. These patients were not known to have any other eye disorder. The ages of the 51 control subjects enrolled in this study ranged from 18-63 years, with a mean age (±standard deviation) of 36.64±14.65 years. From the patient pool, the coding sequence of MYOC from 30 patients was characterized by DNA sequencing. The remaining 26 patient samples and all 51 controls were examined to detect the presence of nucleotide changes (if any) by PCR and restriction endonuclease digestion. None of the DNA fragments amplified by PCR from patients for either sequencing or for restriction assay had any detectable variation in the size, as compared to healthy controls. This suggested the lack of any deletion/insertion mutation among the examined patient pool. Also, no such mutation in the MYOC coding sequence and splice junctions was detected by DNA sequencing.

### Statistical analysis

Comparison of genotypic distributions of the -83 G->A polymorphism for patients and control groups was performed by a χ² test. The χ² value was calculated from the number of patients and controls for different genotypes, respectively. The p value was calculated by using chi-square to P calculator software.

### Table 2. The distribution of pSNP (-83 G->A) in POAG patients and healthy controls

<table>
<thead>
<tr>
<th>Genotype</th>
<th>POAG</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-G</td>
<td>15 (26.78%)</td>
<td>16 (31.37%)</td>
</tr>
<tr>
<td>G-A</td>
<td>38 (67.85%)</td>
<td>29 (56.86%)</td>
</tr>
<tr>
<td>A-A</td>
<td>3 (5.35%)</td>
<td>6 (11.76%)</td>
</tr>
<tr>
<td>Total</td>
<td>56 (100%)</td>
<td>51 (100%)</td>
</tr>
</tbody>
</table>

χ²=2.0119, Degrees of freedom=2, p=0.3657

### Table 3. Clinical data of patients with missense mutation in MYOC

<table>
<thead>
<tr>
<th>Subject</th>
<th>Gender</th>
<th>Age at diagnosis (yr)</th>
<th>Amino acid change</th>
<th>Diagnosis</th>
<th>Family history</th>
<th>Maximum known IOP</th>
</tr>
</thead>
<tbody>
<tr>
<td>GL 14</td>
<td>Male</td>
<td>25</td>
<td>Gin485His</td>
<td>POAG</td>
<td>No</td>
<td>23 (OD) 14 (OS)</td>
</tr>
<tr>
<td>GL 41</td>
<td>Male</td>
<td>70</td>
<td>Gin485His</td>
<td>POAG</td>
<td>No</td>
<td>24 (OD) 24 (OS)</td>
</tr>
<tr>
<td>GL 32</td>
<td>Female</td>
<td>32</td>
<td>Gin485His</td>
<td>POAG</td>
<td>No</td>
<td>25 (OD) 36 (OS)</td>
</tr>
<tr>
<td>GL 34</td>
<td>Female</td>
<td>18</td>
<td>Pro372Lys</td>
<td>POAG</td>
<td>Yes</td>
<td>18 (OD) 24 (OS)</td>
</tr>
</tbody>
</table>

*Signifies Post operative.
by trabeculectomy in a clinic in another city. The patient came to the Regional Institute of Ophthalmology in Kolkata with complaints of headache. Attempts to get pre-operative clinical records were not successful. It was determined that his visual acuity was 6/6 (OD) and 6/9 (OS) and the intraocular pressures were 20.0 and 14.0 mm Hg. Gonioscopy revealed open angles (grade IV). The cup to disc ratio was 0.9 OU. Perimetry showed glaucomatous field changes OU. No further medical treatment was given. On follow-up, the IOP was almost unchanged and the visual field showed no further progression. He had no other systemic or ocular involvement and there was no history of eye diseases in his family.

Third patient: A 32 year old female Hindu patient (GL 92 in Table 3) came to the clinic with dimness of vision and headache. Her visual acuity was 6/9 (OD) and 6/12 (OS); the acuity OS was 6/6 with correction. The IOP were found to be 28 (OD) and 38 (OS) mm Hg and the cup to disc ratio was 0.5 and 0.7. Gonioscopy revealed open angles (grade III-IV). Glaucomatous field changes were found in both eyes. She was prescribed acetazolamide (250 mg) tablets (3 times daily) and timolol maleate (0.25%) drops twice daily. On the first follow-up, the patient showed a reduction of intra ocular pressure (24 and 32 mm Hg OD and OS, respectively). She has been counseled to undergo surgical intervention and is expected to come to the clinic for further treatment. Following a detailed interview with the patient, it appeared that there is no family history of eye disease.

The three patients having the mutation (144 G->T) belong to three different endogamous caste populations (Brahmin, Mahishya and Gandho Banik) of eastern India and marriage between these castes is not common due to prevailing social restrictions. Hence, from the available information it does appear that the patients are not related. We intend to determine haplotypes in the families of the patient to get a more definitive answer for a common or de novo origin of the putative mutation. It was found that the putative mutation would result in the loss of an Acc I restriction site. A region (313 bp) of exon 1 containing the mutation was PCR amplified from 26 unsequenced patient samples and controls and digested with Acc I to distinguish between the normal and the mutant alleles (Table 1 and Figure 1). An absence of the change in 51 healthy control samples (Table 4) strongly suggested a causal relationship of the putative mutation with POAG. Also, the mu-

<table>
<thead>
<tr>
<th>Sequence change</th>
<th>Codon number</th>
<th>Amino acid change</th>
<th>Location</th>
<th>POAG patients (%) (n=56)</th>
<th>Control subjects (%) (n=51)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>144G-&gt;T</td>
<td>48</td>
<td>Gln-&gt;His</td>
<td>Exon 1</td>
<td>3 (5.35%)</td>
<td>0 (0%)</td>
<td>Novel mutation</td>
</tr>
<tr>
<td>1109C-&gt;T</td>
<td>370</td>
<td>Pro-&gt;Leu</td>
<td>Exon 3</td>
<td>1 (1.78%)</td>
<td>0 (0%)</td>
<td>Hotspot for mutation</td>
</tr>
<tr>
<td>-83G-&gt;A</td>
<td>NA</td>
<td>NA</td>
<td>Promoter</td>
<td>41 (73.2%)*</td>
<td>35 (68.6%)*</td>
<td>pSNP</td>
</tr>
<tr>
<td>227G-&gt;A</td>
<td>76</td>
<td>Arg-&gt;Lys</td>
<td>Exon 1</td>
<td>41 (73.2%)*</td>
<td>35 (68.6%)*</td>
<td>cSNP</td>
</tr>
</tbody>
</table>

*3 and 6 individuals were homozygous for ‘A’ allele in patients and controls, respectively

Abbreviations: NA, not applicable; pSNP, single nucleotide polymorphism in promoter region; cSNP, single nucleotide polymorphism in coding sequence.
tated residue is conserved in all mammalian MYOC characterized so far (i.e., human, rat, mouse, bovine). We suggest that this novel mutation, either singly or in combination with a yet unidentified modifying locus, causes POAG. It is curious that in addition to the novel mutation reported in this study, only four (Gln19His, Arg46Stop, Arg82Cys, Arg91Stop) of a total of 49 reported mutations are located in exon 1, and all of those have been detected only in sporadic cases [8,13,21]. This suggested that the N-terminal region of MYOC, encoded by exon 1, might harbor a functionally less important domain. The importance of such mutations might be better appreciated with a further understanding of the complex pattern of inheritance of POAG.

Detection of a mutation in a hotspot: An 18 year old female Muslim patient (GL 34 in Table 3) with complaints of eye watering and dimness of vision was diagnosed with POAG with a family history (mother, maternal aunt, and her daughter). The IOP were 24 and 32 mm Hg in OD and OS, respectively. The visual acuity was 6/12 (OD) and 6/9 (OS) and the cup to disc ratios were 0.8 and 0.7. Gonioscopy revealed open angles (grade IV). An arcuate scotoma in the superior and inferior halves with nasal steps OD and a scotomatous defect in the superonasal quadrant OS was detected by perimetry. A trabeculectomy operation in both eyes reduced IOP (18 and 24 mm Hg in OD and OS, respectively). She displayed no other ocular or systemic involvement. Timolol maleate (0.25%) eye drops were prescribed. The follow-up was irregular. The patient came back after several years and it was found that she had stopped her medication. A fresh check-up showed that visual acuity had decreased to light perception with projection (OD) and count fingers at 4 m (OS). The IOP varied between 20-22 mm in both eyes. Timolol maleate (0.5%) was resumed in both eyes.

Following a candidate gene approach, the MYOC coding sequence from patient GL 34 was screened for mutations by DNA sequencing. A base change (1109C->T) was detected in exon 3 that would cause a missense mutation (Pro370Leu). This mutation was found in the promoter region (-83G->A) and the other was in the coding sequence of the gene (227 G->A; Arg76Lys). Similar results were also obtained from sequencing of MYOC from 20 control samples, implying that the nucleotide changes were due to single nucleotide polymorphisms (SNP) in MYOC. Thus, among 50 samples sequenced (including patients and controls), 30 were heterozygous (-83G-227G/-83A-227A), 3 were homozygous for polymorphic changes at both locations (-83A-227A/-83A-227A), and 17 were homozygous for normal alleles (-83G-227G/-83G-227G). Simultaneous nucleotide changes (-83A and 227A) in both the locations in all the samples sequenced established that these two SNPs are in total linkage disequilibrium in the eastern Indian population used in this study. It is interesting to note from other studies that these two SNPs are also in linkage disequilibrium in both Japanese and Chinese populations [21,35]. However, in all of the non-Asian populations (Caucasian, African-American, Australian and Canadian) evidence of linkage disequilibrium is not obvious [8,13].

Next, we investigated the allelic variation of the SNP in the promoter region (pSNP) and the coding sequence (cSNP) of MYOC by restriction analysis in both the patient and control groups. We observed that the frequencies of the variant alleles (-83A and 227A) were identical in both the groups. Since these two SNPs were in complete linkage disequilibrium, the allele frequencies (Table 5) and genotype distribution (Table 2) are shown for only one polymorphic marker (i.e. -83 G->A). The heterozygosity of the SNP in patient and control groups was found to be 0.477 and 0.480, respectively. Hence, the two SNPs would serve as a useful informative marker for investigating the cosegregation of MYOC with familial POAG prior to undertaking more labor-intensive mutation screening. Additionally, when evidence of cosegregation of the SNP markers and POAG is evident with statistical significance, one could use the markers as RFLPs.

It is noteworthy that among 49 mutations in MYOC reported so far (in addition to the present study), this mutation has been reported in many independent studies covering different ethnic backgrounds, including French [9], Japanese [10], North Americans [19], and Germans [30]. Based on these findings, and a few other mutations in MYOC (Arg46Stop, Arg82Cys, Gly367Arg, and Thr377Met), which are in a CpG context with recurrent mutation reports in different population groups [8,10,13,16,19,21,30-32], we propose that CpG dinucleotides are active as hotspots for mutations in this gene, presumably due to methylation of the cytosine moieties. This information is relevant in the context of mutation screening in genes where mutations accumulate in CpG context. For example, CpG was not found to be a hot spot in the two genes where the greatest numbers of single base mutations have been delineated; the α-globin gene and the β-globin gene [33,34]. It is likely that such nucleotide changes in MYOC in different population groups have originated by de novo mutation.

Identification of two single nucleotide polymorphisms (SNPs): Mutation screening by DNA sequencing of MYOC revealed single nucleotide changes in 12 of 30 patients screened, in two locations simultaneously. One of these mutations was found in the promoter region (-83G->A) and the other was in the coding sequence of the gene (227 G->A; Arg76Lys). Similar results were also obtained from sequencing of MYOC from 20 control samples, implying that the nucleotide changes were due to single nucleotide polymorphisms (SNP) in MYOC. Thus, among 50 samples sequenced (including patients and controls), 30 were heterozygous (-83G-227G/-83A-227A), 3 were homozygous for polymorphic changes at both locations (-83A-227A/-83A-227A), and 17 were homozygous for normal alleles (-83G-227G/-83G-227G). Simultaneous nucleotide changes (-83A and 227A) in both the locations in all the samples sequenced established that these two SNPs are in total linkage disequilibrium in the eastern Indian population used in this study. It is interesting to note from other studies that these two SNPs are also in linkage disequilibrium in both Japanese and Chinese populations [21,35]. However, in all of the non-Asian populations (Caucasian, African-American, Australian and Canadian) evidence of linkage disequilibrium is not obvious [8,13].
to identify pre-symptomatic individuals in an affected pedigree without any knowledge about the MYOC gene defect.

Glaucoma, in general, represents a complex disease despite some forms of POAG being heritable. A number of polymorphisms in different genes, such as -1000 G>C in MYOC [36], the E allele in APOE [37], and the Arg72Pro polymorphism in p53 have been claimed to have significant association with POAG [38]. This might be dependent on the populations under study [39]. From that perspective we examined the association of the pSNP (-83 G>A) with POAG and did not find any significant association with the disease (p=0.3657, Table 5). It is, however, possible that the identified SNPs might not find any significant association with the disease (p=0.3657, Table 5). It is, however, possible that the identified SNPs might have potential use in the future for monitoring variable penetrance in familial POAG where MYOC might act as a modifier gene, and/or efficacy of potential drugs to treat the disease.

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