Myocilin mt1 promoter polymorphism in Turkish patients with primary open angle glaucoma

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Purpose: To evaluate the association of the myocilin gene promoter variant -1000C>G (MYOC.mt1) with primary open angle glaucoma (POAG) and its possible role on the phenotype and the severity of glaucoma in Turkish patients.

Methods: Eighty-eight POAG patients and 123 healthy subjects were included in the study. All subjects were genotyped by PCR-RFLP. Allele and genotype frequencies between healthy subjects and glaucoma patients were compared by the χ2 test. The age at diagnosis, the age at inclusion, the maximum IOP at diagnosis and the number of antiglaucomatous medications were compared between MYOC.mt1 carriers and non-carriers using the Student’s t-test; C/D ratio, mean deviation (MD), and pattern standard deviation values were compared with the Mann-Whitney U-test. Statistical significance was defined as p<0.05.

Results: MYOC.mt1 genotype and allele frequencies did not differ in POAG and healthy subjects (p=0.204 and p=0.083, respectively). In the control group, 17.1% of the subjects were MYOC.mt1 carriers, while 27.3% of the POAG patients were MYOC.mt1 carriers (p=0.107). The odds ratio for CG was 1.859 (95% CI: 0.9-3.7; p=0.084) and for GG 1.594 (95% CI: 0.31-8.13; p=0.575). The phenotype variables were quite similar in MYOC.mt1 carriers and non-carriers. Gender by itself or with the MYOC.mt1 did not have any effect on IOP, C/D ratio, or MD values (univariate analysis of variance, p>0.05). No significant difference was found in the distribution of genotypes between different stages of glaucoma groups (p=0.93).

Conclusions: Our results suggest that in our Turkish glaucoma patients, MYOC.mt1 is not a risk factor for the development of POAG and is not associated with the phenotype and severity of glaucoma.

Glaucoma is one of the leading causes of vision loss and blindness in the world. It is suggested that over 67 million people are affected by glaucoma worldwide [1]. The most common form of the glaucoma is adult-onset primary open-angle glaucoma (POAG), with a prevalence of about 1-3% over age 50 in white populations [1-4]. It is characterized with optic nerve head cupping, neuroretinal rim loss, and visual field defects, usually accompanied with high intraocular pressure (IOP). It is known that elevated IOP is the major risk factor for POAG and lowering IOP is the primary goal of the treatment [5]. The etiology of POAG is not clearly understood, but current data suggest that POAG is a complex disease in which several genetic and environmental factors are involved in its development and pathology. Genetic studies performed to understand the molecular basis of glaucoma have identified several candidate loci for POAG, but causal mutations have only been identified in a small proportion [6-13]. The first gene associated with POAG was MYOC (TIGR/MYOC), at the GLCIA locus on chromosome 1, encoding the protein myocilin [11]. Two other genes, optineurin [14] in the GLCIE on 10p15-14 and WDR36 [15] on 5q22.1 (GLCIG), have been reported to cause adult-onset POAG. In a recent study [16], a linkage was identified to an 11 cM region on chromosome 15q11-13, designated GLCII, in a subset of families with an early-onset form of POAG. The genetic heterogeneity of glaucoma might affect the phenotypic features of glaucoma like age at onset, intraocular pressure, resistance to antiglaucomatous medications, and severity.

MYOC was identified by cloning mRNA from cultured trabecular meshwork cells after stimulation with corticosteroids [17]. Myocillin is expressed in many ocular tissues, including trabecular meshwork and optic nerve head of POAG patients [18-20]. The deposition of high amounts of myocilin in trabecular ECM could affect aqueous outflow either by physical barrier and/or through cell-mediated process leading to elevation of IOP. The clinical features in POAG patients linked to TIGR/MYOC are more severe and relatives of probands have increased risk of developing glaucoma [21]. MYOC mutations account for 2% to 5% of POAG cases worldwide [11,22-26]. In the juvenile-onset form of POAG, the frequency of these mutations is much higher [11,27,28]. Recently a new autosomal dominant glaucoma locus at 3p21-22 which shows an association with the Q368STOP mutation of myocilin was identified in a large six generation Tasmanian family with POAG exhibiting genetic heterogeneity [13].

The investigation of possible effects of MYOC gene variants on the clinical status of glaucoma has become a major topic of discussion. MYOC gene promoter variant -1000C>G, also designated as MYOC.mt1, concerns a much larger proportion (15%) of the population when compared with MYOC mutations. Polymorphisms of the MYOC gene promoter re-
tion, or medical staff with no ocular problems. They had no family history of glaucoma and their IOP measurements were lower than 21 mm Hg on 2 different visits. All glaucoma patients and controls were unrelated.

**DNA analysis:** The study was approved by the Ethics Committee of Hacettepe University Faculty of Medicine and adhered to the tenets of the Declaration of Helsinki. After written informed consent was obtained from all participating individuals, peripheral venous blood was collected for molecular analysis. The genotyping was done in a masked fashion related to diagnosis and clinical features. Genomic DNA was isolated from 400 µl peripheral blood using the phenol-chloroform extraction method. For determination of the promoter variant at position -1000C>G of MYOC, the PCR-restriction fragment length polymorphism (PCR-RFLP) method was used. PCR was performed using the primer sequences 5'-CCA TTA CGA ATA GAG CCA TAA-3' and 5'-TGG TGA AAT CTG GGG AAC TC-3' (forward and reverse, respectively). After PCR, products were checked in 1% agarose gel prior to restriction digestion. For RFLP analysis, 10 µl PCR product was digested with 3 Units of AlwN1 at 37 °C overnight. Digested DNA fragments were separated on 2% agarose gel electrophoresis. Genotyping was carried out on the gel image. CC was diagnosed as non-carrier of the MYOC.mt1 variant, CG was heterozygous for the MYOC.mt1 variant, and GG was homozygous for the MYOC.mt1 variant. CG and GG were also grouped as MYOC.mt1 carriers. Allele and genotype frequencies of the MYOC.mt1 variant were determined in patients and controls for statistical evaluation.

**Statistical analysis:** SPSS 11.5 for Windows was used in statistical analysis. Allele and genotype frequencies between the healthy subjects and glaucoma patients were compared by the χ² test. Logistic regression analysis was done and odds ratios (ORs) and 95% confidence intervals (CI) were calculated for genotypes and alleles. The distribution of genotypes in different stages of glaucoma was further analyzed using the χ² test. The age at diagnosis, the age at inclusion, the maximum IOP at diagnosis and the number of antiglaucomatous medications were compared between MYOC.mt1 carriers and non-carriers using the Student’s t-test and C/D ratio, MD values and pattern standard deviation (PSD) values with the Mann-Whitney U-test. For all analyses, we used only the patients’ worse eye. These parameters were also compared between women with and without MYOC.mt1. The mean age of the

### Table 1. Comparison of the MYOC.mt1 Promoter Variant Genotypes and Alleles in POAG Patients and Control Group

<table>
<thead>
<tr>
<th>MYOC.mt1</th>
<th>Healthy subjects</th>
<th>Glaucoma subjects</th>
<th>Odds ratio (95% confidence intervals; p value)</th>
<th>p value (χ² test)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>102 (82.9 %)</td>
<td>64 (72.7 %)</td>
<td>referent</td>
<td>0.204</td>
</tr>
<tr>
<td>CG</td>
<td>18 (14.6 %)</td>
<td>21 (23.9 %)</td>
<td>1.659 (0.9 - 3.7; p = 0.084)</td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>3 (2.4 %)</td>
<td>3 (3.4 %)</td>
<td>1.594 (0.31 - 8.13; p = 0.575)</td>
<td></td>
</tr>
<tr>
<td>C allele</td>
<td>222 (90.2 %)</td>
<td>149 (84.8 %)</td>
<td>referent</td>
<td>0.083</td>
</tr>
<tr>
<td>G allele</td>
<td>24 (9.8 %)</td>
<td>27 (15.3 %)</td>
<td>1.67 (0.53 - 5.01; p = 0.385)</td>
<td></td>
</tr>
</tbody>
</table>

The comparison of the MYOC.mt1 promoter variant genotypes and alleles in POAG patients and the control group. Genotype frequencies, although quite different, were not statistically significant (p=0.107).
patients in different stages of glaucoma was compared using one way ANOVA and Tukey HSD tests. The correlation between age and glaucoma severity was evaluated with the $\chi^2$ test. Univariate analysis of variance was done to evaluate the effects of gender and MYOC.mt1 on maximum IOP at diagnosis, C/D values, MD, and PSD values and the interaction between these two factors. Statistical significance was defined as $p<0.05$.

RESULTS

There were 56 females and 32 males with a mean age of 62.66±10.32 (SD) years (range between 39-84 years) in the POAG group and 67 females and 56 males with a mean age of 61.67±10.12 (SD) years (range between 41-84 years) in the control group. There were no statistically significant differences for age and gender between the groups ($p=0.487$ and $p=0.183$, respectively). The patients had been followed up for a minimum of 2 years (between 2 and 29 years; with a median of 8 years). The highest IOP ranged between 21 and 37 mm Hg. In the control group, 102 (82.9%) were non-carriers of MYOC.mt1, while 18 (14.6%) were heterozygous and 3 (2.4%) were homozygous for the MYOC.mt1 variant. On the other hand, in the POAG group, 64 (72.7%) were non-carriers, while 21 (23.9%) were heterozygous and 3 (3.4%) were homozygous for the MYOC.mt1 variant. There was no significant difference between the healthy subjects and POAG cases for MYOC.mt1 promoter variant ($p=0.204$). The distribution of genotypes in control and glaucoma groups, odds ratios and confidence intervals (95%) are given in detail in Table 1.

In both POAG and control groups, the genotype frequencies did not differ between females and males ($p=0.676$ and $p=902$, respectively). When the logistic regression analysis was done taking sex and age into consideration, the odds ratio for females was 1.39 (95% CI: 0.78-2.47; $p=0.260$) and the odds ratio for age was 1.00 (95% CI: 0.98-1.03; $p=0.660$). In the control group, 17.1% of the subjects were MYOC.mt1 carriers, while 27.3% of the POAG patients were MYOC.mt1 carriers. Although the frequencies were quite different and POAG patients had a higher frequency of the MYOC.mt1 variant, it did not reach to a statistically significant level ($p=0.107$). The frequency of allele G was 9.8% in the control group and 15.3% in the glaucoma group and the difference was not significant ($p=0.083$).

The influence of MYOC.mt1 on clinical features of the glaucoma group was examined. The age at diagnosis, the age at inclusion, the maximum IOP at diagnosis, C/D ratio at inclusion, MD, and PSD values, the percentage of trabeculectomy, and family history were quite similar in MYOC.mt1 carriers and non-carriers (Table 2). The influence of sex on MYOC.mt1 on the maximum IOP value at diagnosis was tested with univariate analysis of variance. However, no significant effect of MYOC.mt1 or sex on IOP could be found and there was no interaction between these two factors ($p=0.321$). Sex by itself or with the MYOC.mt1 did not have any effect on C/D ratio, MD, and PSD values. The parameters did not differ between MYOC.mt1(+) and MYOC.mt1(-) females ($p>0.05$). MYOC.mt1 did not show any association with a family history of glaucoma. In patients with positive family

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**Table 2. Comparison of the phenotype variables between POAG patients with and without the MYOC.mt1 variant**

<table>
<thead>
<tr>
<th>MYOC.mt1</th>
<th>Carriers (24 patients)</th>
<th>Non-carriers (64 patients)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>at diagnosis (years)</td>
<td>51.58 ± 9.47</td>
<td>53.94 ± 9.73</td>
<td>0.313</td>
</tr>
<tr>
<td>at inclusion (years)</td>
<td>61.96 ± 10.88</td>
<td>62.92 ± 10.17</td>
<td>0.699</td>
</tr>
<tr>
<td>Mean IOP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>at diagnosis (mm Hg)</td>
<td>27.29 ± 3.9</td>
<td>26.14 ± 3.4</td>
<td>0.182</td>
</tr>
<tr>
<td>at diagnosis in females</td>
<td>27 ± 3.18</td>
<td>25.84 ± 3.13</td>
<td>0.213</td>
</tr>
<tr>
<td>at diagnosis in males</td>
<td>28 ± 5.5</td>
<td>26.62 ± 3.82</td>
<td>0.455</td>
</tr>
<tr>
<td>Mean C/D ratio (median)</td>
<td>0.58 ± 0.21 (0.60)</td>
<td>0.54 ± 0.21 (0.50)</td>
<td>0.524</td>
</tr>
<tr>
<td>Mean mean-deviation (median)</td>
<td>-7.08 ± 6.8 (-4.73)</td>
<td>-8.98 ± 7.15 (-5.4)</td>
<td>0.307</td>
</tr>
<tr>
<td>Mean pattern standard deviation (median)</td>
<td>4.90 ± 4.16 (2.62)</td>
<td>5.16 ± 4.03 (2.45)</td>
<td>0.793</td>
</tr>
<tr>
<td>Mean number of medications</td>
<td>1.9 ± 0.8</td>
<td>1.7 ± 0.8</td>
<td>0.375</td>
</tr>
<tr>
<td>Family history (number and percentage)</td>
<td>5 (20.8%)</td>
<td>14 (22.2%)</td>
<td>0.889</td>
</tr>
<tr>
<td>Trabeculectomy (number and percentage)</td>
<td>2 (8.3%)</td>
<td>8 (12.5%)</td>
<td>0.721</td>
</tr>
</tbody>
</table>

Comparison of the phenotype variables between POAG patients with and without the MYOC.mt1 variant. The influence of the MYOC.mt1 polymorphism on different clinical parameters did not differ between MYOC.mt1 carriers and non-carriers.
history of glaucoma, 73.7% were non-carriers and 26.3% were heterozygous carriers, while in patients with no family history, 72.1% were non-carriers, 23.5% were heterozygous carriers and 4.4% were homozygous MYOC mt1 carriers.

When the POAG patients were classified by the stage, 39 patients had early glaucoma, 26 had moderate glaucoma, and 23 had severe glaucoma. The mean age of patients with severe glaucoma (67.30±8.63 years) was higher than patients with early glaucoma (60.72±10.02 years; p=0.038) and patients with moderate glaucoma (61.46±11.18; p=0.111). Glaucoma severity did not differ according to sex (p=0.869). Genotype frequencies in different stages of glaucoma is given in Table 3. No significant difference was found between the groups (p=0.93).

### DISCUSSION

Besides mutation detection studies, research for determination and comparison of the phenotypic differences between patients carrying different polymorphisms has become an important approach in understanding the molecular pathology of diseases. Patients with particular polymorphisms in the coding or promoter sequences of some genes might be more susceptible to disease development, have a more severe phenotype or might show resistance to medical treatment when compared to subjects who do not have that polymorphism. When Colomb et al. [29], reported the association of a single nucleotide polymorphism (SNP) in the TIGR/MYOC gene promoter (MYOC.mt1) with the severity of POAG, researchers were prompted to screen the DNA of patients for this promoter variant. A commercial genetic test for this variant soon became available as an indicator of disease severity and resistance to therapy. Polansky et al. [31] showed an association between MYOC.mt1 promoter and worsening of glaucoma with time. However, Alward et al. [32] and Fan et al. [33] showed no relation between the MYOC.mt1 promoter polymorphism with the development and severity of POAG and the debate is heating up. In this respect, we thought it noteworthy to screen our cohort of Turkish POAG patients and a control group recruited from a single center for MYOC.mt1 polymorphism.

As MYOC.mt1 genotype and allele frequencies were statistically not important in Turkish POAG patient population and controls, we think that the MYOC.mt1 variant is not a risk factor for the development of glaucoma. The distribution of the MYOC.mt1 genotype frequencies determined in our study is also similar with the results of other populations studied. Colomb et al. [29] reported the frequency of CG genotype as 16% in control group and 15.5% in POAG and the frequency of GG as 0% in controls and 2.1% in POAG. Alward et al. [32] found the MYOC.mt1 polymorphism as 23.9% in healthy subjects and 15.5% in POAG. In the study of Polansky et al. [31], the percentage of mt1(+) among POAG patients was 14%. In the study of Fan et al. [33], the frequency of the polymorphism was found to be higher both in POAG (39.15%) and healthy subjects (36.20%) in a Chinese population. However, their glaucoma group included both juvenile-onset and adult-onset glaucoma, as well as high-tension and normal-tension glaucoma.

In our study, POAG patients who harbored the rare allele (G) of the MYOC.mt1 promoter polymorphism were no different from those with the more common variant in any measure of glaucoma phenotype. Our results were consistent with the findings of Alward et al. [32] and Fan et al. [33], who also could not find any association between MYOC.mt1 promoter polymorphism and POAG phenotype variables. However, in the study of Colomb et al. [29], MYOC.mt1 was found to be associated with increased IOP at inclusion (p=0.0004) and greater visual field score (p=0.02), Patients with this polymorphism had a higher IOP at inclusion than those without the polymorphism and they claimed that the presence of this polymorphism might lead to an unresponsiveness to glaucoma treatment. We did not use the IOP at inclusion and the difference between IOP at diagnosis and IOP at inclusion as parameters showing glaucoma severity and response to treatment, since the patients were not treated with the same medications or with the same protocol. Polansky et al. [31], also found no discernible effects of mt1(+) variant on IOP levels, either at baseline or subsequently and the baseline optic disc and visual field scores did not differ between patients with and without mt1(+) variant. There was no remarkable resistance to medical treatments. Therefore, it is impossible to conclude that MYOC.mt1 carriers do not respond favorably to medical therapy without elucidating the effect of type and number of medications.

The differences between the study of Colomb et al. [29] and others may be the result of demographic and clinical differences in the patient samples included. Colomb et al. [29], evaluated a sample whose glaucoma is diagnosed at a younger age (average 45 years) than the samples studied by other groups. In the study of Colomb et al. [29], the effects of MYOC.mt1 on IOP and visual field were predominant in females. Therefore, we performed further analysis to evaluate the effect of both MYOC.mt1 and gender on IOP, C/D, MD, and PSD scores and the interaction between them. We found that sex by itself or with the MYOC.mt1 did not have any effect on these parameters. The clinical features were similar in women with and without MYOC.mt1. Alward et al. [32] also could not show any significant differences in glaucoma severity measures between women with and without the polymorphism.

In the study of Polansky et al. [31], patients with POAG were followed up for approximately 15 years and Cox proportional hazards regression model was used for time-to-event analyses to show if mt1(+) variant was associated with more...
rapid progression of the glaucoma. They showed that the mt1(+) variant accelerated worsening of both optic disc and visual field measures of glaucoma progression. We could not perform time-to-event analysis in our patients, since the number of mt1 carriers followed more than 5 years was low. We studied only a single point in time. It would be valuable to follow up these patients to see the effect of the mt1(+) variant on the clinical course.

We must note that although the mt1 variant seemed to have no significant effect on the phenotype of POAG by itself in our study, it might interact with other SNPs in the promoters and/or genes. APOE (-491T), which is highly interacted with MYOC (-1000G), is found to be associated with increased IOP and resistance to IOP lowering agents in patients with POAG [30]. Although we must consider that there were only 5 patients carrying both of the above SNPs, still their results are intriguing. The lack of promoter activity assay at present is a huge handicap and certainly it would be valuable to know the role of the mt1 variant on the MYOC gene expression profile. The effect of other candidate genes and other sequence variations suggested by recent reports to have role in POAG is a huge handicap and certainly it would be valuable to know the role of the mt1 variant on the MYOC gene expression profile.

This is the first genetic report on myocillin polymorphisms in Turkish POAG patients. Our results suggest that MYOC mt1 is not a risk factor for the development of POAG and is not associated with the phenotype and severity of glaucoma in our patients. As most of the studies found no significant difference in the frequency of MYOC mt1 polymorphism between POAG patients and healthy subjects, MYOC mt1 screening appears to have no value in predicting people at risk for developing POAG. However, diagnosed POAG patients with MYOC mt1(+) should be followed-up carefully for glaucoma progression. More comparative studies must be made in different populations with larger numbers of glaucoma patients and healthy subjects and most importantly, the functional analysis of the mt1 variant must be undertaken with haste to see the end of this debate.

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REFERENCES


