TIGR/MYOC Gene Sequence Alterations in Individuals with and without Primary Open-Angle Glaucoma

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PURPOSE. To discover sequence alterations in the TIGR/MYOC gene associated with primary open-angle glaucoma (POAG) in Chinese subjects.

METHODS. Two hundred one unrelated Chinese patients with POAG and 291 unrelated individuals without glaucoma, aged 50 years or more, were screened for sequence alterations in the TIGR/MYOC gene by polymerase chain reaction, conformation sensitive gel electrophoresis, and DNA sequencing. Up to 111 more control subjects were screened for some of the alterations.

RESULTS. Fourteen sequence variants that lead to amino acid changes were identified. Seven were novel: Pro16Leu, Ala17Ser, Leu35Pro, Leu215Pro, Gly300Lys, Gln214Lys, and Tyr471Cys. Of these, Gly300Lys and Tyr471Cys were found only in POAG. Arg46Stop was found in 4 patients with POAG (2.0%) and 9 of 402 control subjects (2.2%); one control subject was homozygous. IOP showed a trend (P = 0.11) toward a decrease of 1.5 mm Hg among the control subjects, with Arg46Stop compared with matched control subjects without Arg46Stop. Gly12Arg occurred four times as frequently in control subjects as in patients, but the difference was not statistically significant.

CONCLUSIONS. Gly12Arg might be negatively associated with POAG, suggesting a protective effect. Three patients with POAG had a sequence change not found in control subjects, for a frequency of possible disease-causing TIGR/MYOC mutations of 1.5% (95% confidence interval [CI] = 0.3%–4.3%). Arg46Stop occurred with similar frequency in patients with POAG and control subjects, suggesting that the reduced amount of TIGR/MYOC predicted to result from this truncation does not dramatically increase or decrease risk of glaucoma.

Glaucoma, a degeneration of the optic nerve fibers, is one of the major contributors to visual field loss and blindness. Primary open-angle glaucoma (POAG) is the major type of glaucoma, affecting almost 2% of the world’s population.2 Onset of the disease can occur at a young age or later in life, but the prevalence increases with age. The causes of POAG are not known, although risk factors include high intraocular pressure (IOP), positive family history, age, and the presence of diabetes and/or hypertension.3–6

The first identified gene with mutations that cause POAG was TIGR/MYOC.7 Approximately 2% to 4% of POAG cases are probably due to TIGR/MYOC mutations.8–11 Most tissues of the eye express TIGR/MYOC protein, including trabecular meshwork, sclera, ciliary body, and retina and nonocular tissues such as the lungs, heart, and pancreas.12–14 At the cellular level TIGR/MYOC is present in the extracellular matrix of both glaucomatous and normal trabecular meshwork, colocalizing with fibronectin, laminin, and type IV collagen.15,16 The function of TIGR/MYOC is unknown. In fact, it is not necessary for apparently normal eye development or function, and its absence does not cause glaucoma.11,17 The mechanism whereby mutations in TIGR/MYOC lead to POAG is also unknown, but the finding that mutant TIGR/MYOC proteins form aggregates that are not secreted suggests that mutant TIGR/MYOC accumulates in trabecular meshwork cells and disturbs normal cell function, resulting in impaired outflow of aqueous humor.18,19 Administration of glucocorticoids induces expression of TIGR/MYOC and secretion by trabecular meshwork cells, and topical glucocorticoid treatment increases IOP in some individuals.20 TIGR/MYOC protein with a normal sequence can aggregate and block outflow when injected into the anterior chamber.21 Thus, one hypothesis is that high levels of normal, secreted TIGR/MYOC protein may be a cause of aqueous outflow obstruction,20 which subsequently increases IOP and causes POAG.

Many sequence alterations have been found in TIGR/MYOC, some occur exclusively in patients with POAG and segregate with disease in POAG-affected families, suggesting they are disease-causing mutations.7–10,15,25–32 These are mainly missense alterations in exon 3, although many exon 3 sequence alterations have also been found that are not associated with disease. However, the majority of patients with sporadic POAG do not have mutations in TIGR/MYOC.8–11 Discovery of the range of mutation types and locations may shed light on the role of TIGR/MYOC in POAG and normal eye function. Because some mutations may be ethnicity specific, it is important to conduct case–control mutation studies in various populations. Only one large study on TIGR/MYOC mutations in the Chinese has been published, in which we examined 91 patients with POAG, 132 control subjects, and family members of some patients.11 To uncover further detail of the function of TIGR/MYOC, we examined an additional 110 patients with POAG, 270 control subjects, and some family members and analyzed them in combination with the earlier subjects.

MATERIALS AND METHODS

Study Subjects

Two hundred one unrelated patients with POAG were recruited from the Eye Clinic of the Prince of Wales Hospital, Hong Kong. These included 91 patients as previously reported.11 POAG was diagnosed on the fulfillment of all the following criteria: exclusion of secondary causes (e.g., trauma, uveitis, or steroid-induced or neovascular glaucoma), open anterior chamber angle, IOP more than 21 mm Hg,
characteristic optic disc changes (e.g., vertical cup-to-disc ratio more than 0.3, thin or notched neuroretinal rim or disc hemorrhage), and characteristic visual field changes with reference to Anderson’s criteria for minimal abnormality in glaucoma.52

Some relatives of the index patients were also examined and geno-
typed, but were not included in statistical analyses. Four hundred two
unrelated control subjects were recruited from people at least 50 years
of age who attended the clinic to accompany patients or to obtain
treatment for conditions other than glaucoma, including cataract, re-
fractive errors, and itchy eyes. All study subjects were given a complete
characteristic visual

TIGR/MYOC Sequence Analysis

Genomic DNA was extracted from 200 μL of blood using a kit (Qiamp
Blood Kit; Qiagen, Hilden, Germany). The three coding exons and adja-
cent sequences of TIGR/MYOC were screened for sequence alter-
tations by polymerase chain reaction in seven amplicons, as previously
reported,11 followed by conformation-sensitive gel electrophore-
sis.36 For some sequence alterations, 97 or
sequenced on an automated DNA sequencer (model 377; Applied
Biosystems, Foster City, CA). 36 For some sequence alterations, 97 or

Statistical Analysis

The computer and several software programs (SPSS; SPSS Inc., Chi-
cago, IL; Epi6, Centers for Disease Control, Atlanta, GA; and Excel;
Microsoft, Redmond, WA) were used for statistical calculations. Signi-
icance of the difference in distribution of each sequence change
between patients and control subjects was determined by χ² tests
when all expected values were 5 or more and by the Fisher exact
two-sided test otherwise. Signi-

RESULTS

Twenty sequence alterations were identified in our study sub-
jects (Table 1): 2 were nonsense sequence changes, 11 were mis-

Table 1. Number of Study Subjects with Sequence Alterations in TIGR/MYOC, Probabilities for Association with POAG, and Previously Unreported Alterations

<table>
<thead>
<tr>
<th>Sequence Alteration</th>
<th>Location</th>
<th>POAG Patients (n = 201)</th>
<th>Control Subjects (n = ≥291)</th>
<th>P</th>
<th>Novel</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–83 (G→A)</td>
<td>Promoter</td>
<td>28 (14.0)</td>
<td>70/388 (18.0)</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td>Gly12Arg (34G→C)</td>
<td>Exon 1</td>
<td>1 (0.5)</td>
<td>8/388 (2.1)</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td>Pro16Leu (47C→T)</td>
<td>Exon 1</td>
<td>0 (0.0)</td>
<td>1/388 (0.3)</td>
<td>1.0</td>
<td>Yes</td>
</tr>
<tr>
<td>Ala17Ser (49G→T)</td>
<td>Exon 1</td>
<td>0 (0.0)</td>
<td>1/291 (0.3)</td>
<td>1.0</td>
<td>Yes</td>
</tr>
<tr>
<td>Arg46Stop (136C→T)</td>
<td>Exon 1</td>
<td>4 (2.0)</td>
<td>9/402 (2.2)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Arg76Lys (227G→A)</td>
<td>Exon 1</td>
<td>29 (14.4)</td>
<td>70/388 (18.0)</td>
<td>0.27</td>
<td></td>
</tr>
<tr>
<td>Arg91Stop (271C→T)</td>
<td>Exon 1</td>
<td>1 (0.5)</td>
<td>0/388 (0.0)</td>
<td>1.0</td>
<td>Yes</td>
</tr>
<tr>
<td>Leu95Pro (284C→T)</td>
<td>Exon 1</td>
<td>0 (0.0)</td>
<td>1/291 (0.3)</td>
<td>1.0</td>
<td>Yes</td>
</tr>
<tr>
<td>Thr123Thr (369C→T)</td>
<td>Exon 1</td>
<td>2 (1.0)</td>
<td>5/291 (1.7)</td>
<td>0.71</td>
<td></td>
</tr>
<tr>
<td>Asp208Glu (624C→G)</td>
<td>Exon 2</td>
<td>2 (1.0)</td>
<td>3/388 (0.8)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Leu215Pro (644T→C)</td>
<td>Exon 2</td>
<td>0 (0.0)</td>
<td>1/291 (0.3)</td>
<td>1.0</td>
<td>Yes</td>
</tr>
<tr>
<td>(730+35A→G)</td>
<td>Intron 2</td>
<td>65 (31.3)</td>
<td>117/388 (30.2)</td>
<td>0.77</td>
<td></td>
</tr>
<tr>
<td>Ala260Ala (780A→G)</td>
<td>Exon 3</td>
<td>2 (1.0)</td>
<td>1/291 (0.3)</td>
<td>0.57</td>
<td></td>
</tr>
<tr>
<td>Ile280Ile (864C→T)</td>
<td>Exon 3</td>
<td>0 (0.0)</td>
<td>4/388 (1.4)</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>Glu500Lys (898G→A; 900G→A)</td>
<td>Exon 3</td>
<td>1 (0.5)</td>
<td>0/291 (0.0)</td>
<td>0.41</td>
<td>Yes</td>
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<tr>
<td>Gln309Gln (927G→A)</td>
<td>Exon 3</td>
<td>0 (0.0)</td>
<td>1/291 (0.3)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Thr553Ile (1058C→T)</td>
<td>Exon 3</td>
<td>4 (2.0)</td>
<td>6/388 (1.6)</td>
<td>0.74</td>
<td></td>
</tr>
<tr>
<td>Glu414Lys (1240G→A)</td>
<td>Exon 3</td>
<td>0 (0.0)</td>
<td>1/291 (0.3)</td>
<td>1.0</td>
<td>Yes</td>
</tr>
<tr>
<td>Tyr471Cys (1412A→G)</td>
<td>Exon 3</td>
<td>1 (0.5)</td>
<td>0/291 (0.0)</td>
<td>0.41</td>
<td>Yes</td>
</tr>
<tr>
<td>1515-73 (G→C)</td>
<td>3′ UTR</td>
<td>2 (1.0)</td>
<td>10/291 (3.4)</td>
<td>0.13</td>
<td></td>
</tr>
</tbody>
</table>

Data are number of subjects, with the percentage of the total group in parentheses.
* Previously reported only in our study on a subset of these subjects.11

- Table 1. Number of Study Subjects with Sequence Alterations in TIGR/MYOC, Probabilities for Association with POAG, and Previously Unreported Alterations

- RESULTS

Twenty sequence alterations were identified in our study sub-
jects (Table 1): 2 were nonsense sequence changes, 11 were mis-

Statistical Analysis

The computer and several software programs (SPSS; SPSS Inc., Chi-
cago, IL; Epi6, Centers for Disease Control, Atlanta, GA; and Excel;
Microsoft, Redmond, WA) were used for statistical calculations. Signi-
icance of the difference in distribution of each sequence change
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two-sided test otherwise. Signi-

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between patients and control subjects was determined by χ² tests
when all expected values were 5 or more and by the Fisher exact
two-sided test otherwise. Signi-

and 3 men. The average IOP was 11.8 ± 2.7 mm Hg in the control subjects with Arg46Stop and 13.3 ± 2.2 in those without Arg46Stop. Comparison of IOPs of these groups by two-tailed unpaired t-test resulted in P = 0.11 or P = 0.17 by paired t-test (each triplet without Arg46Stop was averaged for pairing). The average age of patients with Arg46Stop was 46.5 ± 30 years and 46.8 ± 24 years without. They consisted of two men and two women with Arg46Stop and six men and six women without. The average IOP (measured before treatment) was 25.9 ± 4.1 with Arg46Stop and 27.6 ± 4.8 without. Comparison of IOPs of these groups by two-tailed unpaired t-test resulted in P = 0.52 or P = 0.58 by paired t-test. When the patients and control subjects were combined, the average age was 66 ± 21 years with Arg46Stop and 66 ± 19 years without. The average IOP was 16.2 ± 7.4 with Arg46Stop and 17.7 ± 7.4 without. Comparison of IOPs by two-tailed unpaired t-test resulted in P = 0.52 or P = 0.15 by paired t-test.

**DISCUSSION**

Three patients with POAG in this study each had a unique sequence change—Arg91Stop, Glu300Lys, and Tyr471Cys—not found in control subjects, for a frequency of possible disease-causing TIGR/MYOC mutations of 1.5%. Two of these sequence changes are novel and one, Arg91Stop, has been reported. The 95% CI for this proportion, 0.3% to 4.3%, is consistent with the range of 2% to 4% reported in various ethnic groups. Mutations are disease-causing sequence alterations. However, these three sequence changes have not been reported in other populations and have not been linked with glaucoma by statistics, segregation in families, or biochemical studies. Therefore, they cannot yet be conclusively termed mutations. Because the nearby truncation Arg46Stop may not affect disease risk, Arg91Stop may also not affect the risk for POAG.

Aasp208Glu and Thr353Ile, which have been reported as possible glaucoma-causing mutations, were found in control subjects in this study. Therefore, these are not disease-causing mutations, although it remains possible that they affect the risk for POAG. It is possible that other such assignments of perhaps harmless sequence changes as disease-causing mutations have occurred, suggesting that reported frequencies of disease-causing TIGR/MYOC mutations may be somewhat overstated. This study has identified seven predicted protein sequence alterations not reported elsewhere, including our earlier study of a subset of these subjects. Five of these alterations were found in control subjects, lending some support to the above assertion that many rare sequence alterations in TIGR/MYOC may not be disease-causing mutations.

**TABLE 2. Clinical Features of Patients with POAG Who Had TIGR/MYOC Sequence Alterations**

<table>
<thead>
<tr>
<th>Codon Change</th>
<th>Subject</th>
<th>Sex</th>
<th>Age at Diagnosis (y)</th>
<th>Visual Acuity</th>
<th>Highest Known IOP (mm Hg)</th>
<th>Cup-to-Disc Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arg91Stop*</td>
<td>P184</td>
<td>M</td>
<td>50</td>
<td>20/20</td>
<td>20/50</td>
<td>0.3</td>
</tr>
<tr>
<td>Glu300Lys*</td>
<td>P387</td>
<td>M</td>
<td>75</td>
<td>20/30</td>
<td>20/40</td>
<td>0.6</td>
</tr>
<tr>
<td>Tyr471Cys*</td>
<td>P394</td>
<td>M</td>
<td>66</td>
<td>20/200</td>
<td>20/100</td>
<td>0.6</td>
</tr>
<tr>
<td>Arg46Stop</td>
<td>P161</td>
<td>M</td>
<td>4</td>
<td>LP</td>
<td>20/50</td>
<td>1.0</td>
</tr>
<tr>
<td>Arg46Stop</td>
<td>P475</td>
<td>F</td>
<td>48</td>
<td>20/50</td>
<td>20/50</td>
<td>0.3</td>
</tr>
<tr>
<td>Arg46Stop</td>
<td>P512</td>
<td>M</td>
<td>73</td>
<td>20/40</td>
<td>20/40</td>
<td>0.8</td>
</tr>
<tr>
<td>Arg46Stop</td>
<td>P515</td>
<td>F</td>
<td>61</td>
<td>7/200</td>
<td>7/200</td>
<td>0.3</td>
</tr>
</tbody>
</table>

LP, light perception.

* Sequence alterations not found in control subjects.

**TABLE 3. Average IOP of Control Subjects with TIGR/MYOC Sequence Alteration Arg46Stop**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>Age (y)</th>
<th>IOP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57</td>
<td>F</td>
<td>78</td>
<td>10.5</td>
</tr>
<tr>
<td>C155</td>
<td>F</td>
<td>66</td>
<td>17.5</td>
</tr>
<tr>
<td>C177</td>
<td>F</td>
<td>77</td>
<td>11.0</td>
</tr>
<tr>
<td>C182</td>
<td>F</td>
<td>79</td>
<td>11.5</td>
</tr>
<tr>
<td>C203</td>
<td>F</td>
<td>61</td>
<td>15.0</td>
</tr>
<tr>
<td>C221</td>
<td>M</td>
<td>70</td>
<td>11.5</td>
</tr>
<tr>
<td>C238</td>
<td>F</td>
<td>80</td>
<td>9.5</td>
</tr>
<tr>
<td>C311</td>
<td>F</td>
<td>89</td>
<td>10.0</td>
</tr>
<tr>
<td>C362</td>
<td>F</td>
<td>72</td>
<td>10.0</td>
</tr>
</tbody>
</table>

* This subject is homozygous for Arg46Stop.

**FIGURE 1.** IOPs of age- and sex-matched control subjects versus genotype at codon 46. IOPs of patients were measured before treatment. All IOPs in patients are higher than 20 mm Hg (○), whereas those of all control subjects are less than 20 mm Hg (●).
Arg76Lys, the most common protein sequence polymorphism, was not associated with POAG. It usually occurred with the promoter polymorphism 1-83 (G→A), as in a Japanese study38 (although a few subjects had either polymorphism without the other), suggesting that this haplotype has entered the population recently.

Our earlier study of a subset of these subjects found a trend toward an association of the Gly12Arg sequence alteration with the absence of POAG.14 The additional subjects reported herein allowed us to reexamine this trend. When unrelated control subjects were compared with patients with POAG (Table 1), there was still a trend toward an association of Gly12Arg with the absence of POAG (Fisher exact two-tailed test, \( P = 0.18 \)). Although the difference in frequency of Gly12Arg between POAG and control subjects was large (odds ratio = 0.24), the low frequency of this sequence change resulted in a power to achieve \( P < 0.05 \) at these frequencies of only 16%, even with our increased sample sizes. Gly12Arg has been reported only in the Chinese and Japanese. Combining our results with those from two studies of Japanese patients with POAG and control subjects,9,38–40 which found Gly12Arg in 1 of 226 patients with POAG and 2 of 149 control subjects, showed a frequency of 0.5% in POAG and 1.9% in control subjects (\( P = 0.053 \); odds ratio = 0.25). These results suggest Gly12Arg may protect against POAG. Because it is located near the hydrophobic signal sequence, the change to a positively charged amino acid may hinder secretion. Because injection of a nonglycosylated bacterial TIGR/MYOC recombinant protein into the anterior chamber of a perfused anterior segment culture increases IOP,21 and because glucocorticoids can both stimulate TIGR/MYOC expression and increase IOP,20 an increase in the level of secreted TIGR/MYOC may increase IOP and cause POAG. Conversely, a decrease in TIGR/MYOC secretion may reduce risk for POAG. However, predictions of the signal sequence and cleavage site locations and probabilities did not differ between TIGR/MYOC sequences with Gly and with Arg at position 12. For both Gly- and Arg-containing sequences, the probability of cleavage between amino acids 52 and 33 was 61%,39,40 Another possible mechanism for an effect of N-terminal TIGR/MYOC sequence changes on disease risk is suggested by the finding that the region between amino acids 15 and 138 is needed to localize TIGR/MYOC to microtubules inside cells.41 Sequence changes in this region may alter subcellular localization and function of TIGR/MYOC or of trabecular meshwork cells.

Another missense change that increases the positive charge has been reported in the signal sequence. Gln19His was found in 1 of 1703 patients with POAG and 1 of 238 control subjects in a multietnic study.9 The control was homozygous. The frequency is too low to determine statistical significance, but these results are consistent with those of Gly12Arg. Although not found in our original study,14 this expanded study revealed a total of two other sequence changes in or near the signal sequence Pro16Leu and Ala17Ser. Both of these previously unreported alterations were found only in control subjects, consistent with Gly12Arg. These three signal sequence changes, when combined, show a trend toward a frequency difference between patients and control subjects (1/201 = 0.5% vs. 10/388 = 2.6%, odds ratio = 0.19, Fisher exact two-tailed \( P = 0.11 \)), also suggesting a protective effect. Transfected cell culture studies may show whether these sequence alterations affect secretion of TIGR/MYOC or localization.

If TIGR/MYOC protein containing Arg46Stop were translated and processed, it would be only 13 amino acids long after cleavage of the signal sequence.20 However, mRNA carrying nonsense mutations often undergoes nonsense-mediated decay. Thus, Arg46Stop may essentially reduce expression of TIGR/MYOC by half in heterozygotes and eliminate expression in homozygotes. To test the hypothesis that blocking TIGR/
Acknowledgments

The authors thank Fion Lau for contacting subjects and collecting blood samples.

References