Objective: To determine the phenotype of an Australian pedigree with the myocilin (MYOC) Gly252Arg mutation, comparing it with other pedigrees carrying the same mutation.

Methods: All recruited subjects underwent a comprehensive clinical examination, including optic disc assessment, applanation tonometry, and visual field measurement. Mutation analysis was performed through direct sequencing. Haplotype analysis was performed using microsatellite markers around the MYOC gene.

Results: Eight Gly252Arg mutation carriers with glaucoma were identified from the same pedigree. Carriers’ mean±SD age at diagnosis was 46.3±11.4 years (range, 31-60 years). Highest recorded intraocular pressure ranged from 27 to 42 mm Hg (mean±SD, 32.4±5.6 mm Hg). Cup-disc ratios in the worst eye ranged from 0.6 to 0.9. Six of the 8 individuals had undergone filtration surgery. A common founding haplotype between MY5 and D1S218 was found for Caucasian individuals tested with this mutation. One subject was compound heterozygotic for the MYOC Gly252Arg mutation and a novel MYOC Gly244Val variant.

Conclusions: Although a common founder for Gly252Arg across Caucasian subjects was found, the phenotype from this Australian MYOC mutation–carrying pedigree is less severe than previously described. The severity of glaucoma caused by the Gly252Arg mutation may be similar to the Thr377Met MYOC mutation, yet is more severe than the most common Gln368Stop mutation.

Clinical Relevance: Since its implication in glaucoma, much work has been performed investigating the clinical features of MYOC-related glaucoma. Given the strong genotype-phenotype correlations with MYOC disease-causing variants, health care professionals armed with such molecular information are able to accurately counsel patients on their likely disease course. Our work suggests that the disease associated with MYOC Gly252Arg is less severe than previously described in other pedigrees with this specific mutation.

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Primary open-angle glaucoma (POAG), the most common form of optic neurodegeneration, is a complex heterogeneous disease. Understanding the genetic pathoetiological mechanisms for POAG will allow predisease risk stratification as well as the development of novel therapeutic modalities.

The myocilin (MYOC) gene was the first gene in which mutations were found to cause glaucoma. Although the precise function of MYOC is unknown, disease-causing mutant forms are relatively detergent insoluble. Mutant MYOC proteins are misfolded and retained in the endoplasmic reticulum of the trabecular meshwork cells, while wild-type MYOC is secreted. Culturing trabecular meshwork cells at a lower than physiological temperature, a condition known to facilitate protein folding, allows secretion of mutant MYOC and reverses the abnormal morphology and resultant cell lysis. MYOC mutations account for most dominant juvenile glaucoma cases and for approximately 2% to 4% of unselected adult-onset POAG. Numerous mutations in MYOC have been identified, with the majority of them being clustered in the conserved olfactomedin domain of exon 3.
Richards and colleagues first identified the MYOC Gly252Arg mutation in a Caucasian patient residing in the United States. As described by Shimizu et al, this patient was diagnosed with glaucoma at the age of 26 years and had a maximum recorded intraocular pressure (IOP) of 62 mm Hg. In keeping with a juvenile-onset glaucoma phenotype, Booth and colleagues described a large Scottish family harboring the MYOC Gly252Arg mutation. The mean ± SD age at POAG diagnosis was approximately 30.8 ± 7.3 years, with a mean ± SD maximum recorded IOP of 39.3 ± 12.5 mm Hg. Five of the 6 mutation-carrying individuals who manifested disease had undergone bilateral trabeculectomy. Willoughby and colleagues have described a 2-generation Chinese pedigree who carried the MYOC Gly252Arg mutation and had juvenile-onset glaucoma. The proband was diagnosed at age 29 years, while her father had been diagnosed at the age of 38 years and had required bilateral trabeculectomy. Interestingly, these subjects were also found to have the Arg545Gln variant in optineurin, the second gene identified to cause POAG. However, this optineurin variant has been found to be distributed equally between Chinese subjects with glaucoma and ethnically matched, normal control subjects and is thus unlikely to be a pathogenetic variant. One person of Japanese ethnicity who was given this diagnosis at age 49 years and had a maximum recorded IOP of 40 mm Hg has been identified to have the MYOC Gly252Arg mutation (T.Y., written communication, March 2006).

The MYOC Gly252Arg amino acid substitution is predicted to have a positive charge change and is Triton assay insoluble. Herein, we describe the phenotype of an Australian pedigree with the MYOC Gly252Arg mutation and show that all known Caucasian subjects with POAG with this mutation have a common founder.

METHODS

This study was approved by the ethics committees of the Royal Victorian Eye & Ear Hospital and the Royal Hobart Hospital. It was conducted in accordance with the revised Declaration of Helsinki; written informed consent was provided by each subject. All previously reported subjects were similarly recruited under appropriate approvals and ethical protections.

Each subject for whom phenotypes were not previously reported underwent a comprehensive clinical examination, which included anterior segment examination, gonioscopy, IOP measurement by Goldmann applanation tonometry, pachymetry, refraction, and a mydriatic optic disc assessment. Simultaneous stereoscopic optic disc photographs were digitalized (Nidek Stereo Fundus Camera 3-Dx/F; Nidek, Gamagori, Japan). All subjects older than 30 years and those younger who had optic disc signs suggestive of glaucomatous damage underwent automated visual field assessment using a computerized perimeter (Humphrey Field Analyzer II; Zeiss-Humphrey, Dublin, Calif.).

The Mann-Whitney U test was used to compare the age at diagnosis and maximum recorded IOP among our subjects with POAG and those presented previously by Shimizu et al and Booth et al. Fisher exact test was used to compare the proportion of subjects who had undergone trabeculectomy in our cohort with that described by Booth et al. Statistical analysis was performed using Intercooled Stata 7.0 for Windows (Stata Corp, College Station, Tex).

Genomic DNA was isolated from peripheral blood samples (Qiagen, Valencia, Calif). The MYOC Gly252Arg mutation was initially detected with the use of single-strand conformation polymorphism analysis. A template of 12.5 ng of DNA was used in an 8.35-μL polymerase chain reaction using primer sequences and conditions previously described. Amplified products were denatured and underwent electrophoresis. Subsequent mutation analysis for other members of the family was performed through direct sequencing. The MYOC exon 3 amplicon containing the MYOC 252 codon was amplified. The polymerase chain reaction products were purified and directly sequenced (Wizard SV Gel PCR Clean-Up System; Promega Corp, Madison, Wis). Sequencing reactions were carried out using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Scoresby, Australia) with 25 cycles of 10 seconds at 95°C and 5 seconds at 50°C, followed by 4 minutes at 60°C, as specified by the manufacturer. Sequencing analysis was performed using a Prism 310 Genetic Analyzer (Applied Biosystems) and was reviewed using Sequencher 4.7 (Gene Codes Corp, Ann Arbor, Mich). To investigate the significance of novel mutations, the coding region of MYOC was fully sequenced in 130 control subjects without glaucoma, who had a mean ± SD age of 81.6 ± 8.6 years.

The haplotype around the MYOC Gly252Arg mutation in affected members of our Australian pedigree was compared with that of subjects with POAG who were known to have the identical mutation. These genotyped individuals comprised 3 pedigrees (North American, Scottish, and Chinese-Canadian families), as well as a single Japanese proband. Genotyping was performed using 9 microsatellite markers (D1S2658, D1S851, MY5, MY3, D1S2813, D1S1619, D1S218, D1S212, and D1S2640), according to previously described methods.

RESULTS

The matriarch and patriarch (born circa 1795) for the 6-generation Australian Caucasian pedigree (GACT02) are known to have 85 descendants. Key individuals are shown in Figure 1. Eight subjects from this pedigree with the MYOC Gly252Arg mutation were found to have glaucoma (Table 1). The mean ± SD age at diagnosis was 46.3 ± 11.4 years (range, 31-60 years). Six of these individuals (75%) had undergone filtration surgery. The highest recorded IOP ranged from 27 to 42 mm Hg (mean ± SD, 32.4 ± 5.6 mm Hg). The mean ± SD central corneal thickness was 520 ± 25 μm. Examples of the optic disc and visual field characteristics for these glaucomatous cases are shown in Figure 2.

An additional 3 subjects with the MYOC Gly252Arg mutation were diagnosed with ocular hypertension, 2 of whom (subjects V:4 and V:6) had commenced taking a prostaglandin receptor agonist at the age of 40 years. Despite being heterozygous for the Gly252Arg mutation, 3 subjects (subjects IV:20, V:1, and V:2; aged 58, 45, and 39 years, respectively) did not manifest ocular hypertension or have reproducible visual field loss (Figure 3).

One affected subject (subject IV:2) was identified as being a compound heterozygote for the MYOC Gly252Arg mutation and a novel MYOC Gly244Val (g.731G>T) variant. Her unaffected brother (subject IV:7) was also found to have this Gly244Val variant. The daughter of subject...
IV:2 has the MYOC Gly244Val change but not the Gly252Arg mutation and was diagnosed with POAG at age 50 years. The other glaucoma-affected daughter of subject IV:2 declined participation in this study. The MYOC Gly244Val variant was not identified in 260 chromosomes from elderly control subjects without glaucoma.

Phenocopy was identified in 2 branches of the pedigree: in subject IV:1 and the son of subject IV:3 (not shown), and in the granddaughter of subject II:5. Subject IV:1 was diagnosed with glaucoma at age 40 years and had undergone trabeculectomy in both eyes. A great grandniece of subject I:2 (not shown) was diagnosed with glaucoma at age 59 years; however, she was found not to have any MYOC coding sequence mutation. Two individuals (subjects V:4 and V:5) were identified as having a synonymous change at codon 285.

A founding haplotype between MY5 and D1S218 was identified across our pedigree and mutation-carrying Caucasian subjects from Scotland and North America (Table 2). This haplotype differed from that of the Chinese-Canadian family. The precise haplotype around MYOC could not be definitively determined from the Japanese subject.

The mean age at onset of glaucoma and ocular hypertension in this Australian pedigree was significantly greater than previously presented (P = .003). This finding remained significant when the ocular hypertension cases were excluded (P = .01). Maximum recorded IOP and the proportion of subjects requiring trabeculectomy did not...

Table 1. Clinical Characteristics of Individuals With the MYOC Gly252Arg Mutation

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Age, y</th>
<th>Most Recent Examination</th>
<th>Diagnosis</th>
<th>Maximum Recorded IOP, mm Hg</th>
<th>Glaucoma Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>V:2</td>
<td>F</td>
<td>Normal</td>
<td>39</td>
<td>...</td>
<td>...</td>
<td>16</td>
<td>None</td>
</tr>
<tr>
<td>V:1</td>
<td>M</td>
<td>Normal</td>
<td>45</td>
<td>...</td>
<td>...</td>
<td>19</td>
<td>None</td>
</tr>
<tr>
<td>IV:20</td>
<td>M</td>
<td>Normal</td>
<td>58</td>
<td>...</td>
<td>...</td>
<td>17</td>
<td>None</td>
</tr>
<tr>
<td>V:6</td>
<td>F</td>
<td>OH</td>
<td>42</td>
<td>40</td>
<td>30</td>
<td>Med</td>
<td></td>
</tr>
<tr>
<td>V:4</td>
<td>F</td>
<td>OH</td>
<td>49</td>
<td>40</td>
<td>26</td>
<td>Med</td>
<td></td>
</tr>
<tr>
<td>V:3</td>
<td>F</td>
<td>OH</td>
<td>51</td>
<td>45</td>
<td>24</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>IV:18</td>
<td>F</td>
<td>HTG</td>
<td>65</td>
<td>31</td>
<td>32</td>
<td>TRAB OU</td>
<td></td>
</tr>
<tr>
<td>IV:13</td>
<td>F</td>
<td>HTG</td>
<td>52</td>
<td>36</td>
<td>30</td>
<td>TRAB OU</td>
<td></td>
</tr>
<tr>
<td>IV:2</td>
<td>F</td>
<td>HTG</td>
<td>76</td>
<td>36</td>
<td>42</td>
<td>TRAB OU</td>
<td></td>
</tr>
<tr>
<td>IV:9</td>
<td>F</td>
<td>HTG</td>
<td>75</td>
<td>42</td>
<td>32</td>
<td>TRAB OU</td>
<td></td>
</tr>
<tr>
<td>IV:16</td>
<td>M</td>
<td>HTG</td>
<td>64</td>
<td>50</td>
<td>40</td>
<td>TRAB OU</td>
<td></td>
</tr>
<tr>
<td>IV:14</td>
<td>M</td>
<td>HTG</td>
<td>71</td>
<td>57</td>
<td>27</td>
<td>TRAB OU</td>
<td></td>
</tr>
<tr>
<td>IV:11</td>
<td>M</td>
<td>HTG</td>
<td>63</td>
<td>58</td>
<td>28</td>
<td>Med</td>
<td></td>
</tr>
<tr>
<td>IV:10</td>
<td>F</td>
<td>HTG</td>
<td>62</td>
<td>60</td>
<td>28</td>
<td>Med</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: HTG, high-tension glaucoma; IOP, intraocular pressure; Med, topical glaucoma medication; MYOC, myocilin; OH, ocular hypertension; TRAB OU, trabeculectomy in both eyes; ellipses, patient did not have disease.
significantly differ between our subjects (P = .11 and P = .55, respectively) and those previously described.9,10

COMMENT

We present the phenotype of an Australian pedigree with the MYOC Gly252Arg mutation. The Gly252Arg mutation alters the charge and is predicted to alter the secondary structure of neighboring residues from a β-strand to an α-helix across a conserved motif.18 Further analysis has revealed that the amino acid alteration renders the protein insoluble on Triton solubility assay.9 The MYOC Gly252Arg mutation has not been identified in any normal control series.3,6,9

A common founder across Caucasian subjects with the MYOC Gly252Arg mutation was identified. Evidence of a common ancestry across other MYOC mutations, such as Gln368Stop and Thr377Met, has been described (Table 2).15,16 Such a finding has important implications for future methods discovering other genes or single nucleotide polymorphisms predisposing an individual to adult-onset POAG. For example, a common founding haplotype has been identified with the complement factor H gene Tyr402His allele, which

Figure 2. Optic disc appearance and Humphrey 24-2 visual field findings of subjects with glaucoma carrying the myocilin Gly252Arg mutation.
Figure 3. Optic disc appearance and Humphrey 24-2 visual field findings of individuals carrying the myocilin Gly252Arg mutation not currently manifesting glaucoma. Note the large optic discs of subject V:1, who has normal intraocular pressures, and that individuals V:4 and V:6 have documented ocular hypertension.

Table 2. Marker Size for Common Myocilin (MYOC) Mutation Haplotypes

<table>
<thead>
<tr>
<th>MYOC Mutation</th>
<th>Ethnicity</th>
<th>Marker Size Base Pairs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gly252Arg</td>
<td>Caucasian</td>
<td>. . . . . . 241 178 228 192 287 . . .</td>
</tr>
<tr>
<td>Gly252Arg</td>
<td>Chinese Canadian</td>
<td>279 184 239 178 226 198 283 123</td>
</tr>
<tr>
<td>Thr377Met</td>
<td>English/Australian</td>
<td>. . . . . . 239 174 232 192 . . . . .</td>
</tr>
<tr>
<td>Thr377Met</td>
<td>Greek</td>
<td>. . . . . . 239 176 230 198 . . . . .</td>
</tr>
<tr>
<td>Gln368Stop</td>
<td>Caucasian</td>
<td>. . . . . . 174 230 192 . . . . .</td>
</tr>
</tbody>
</table>
was first implicated in causing a significant proportion of the genetic liability for age-related macular degeneration through case-control association.\textsuperscript{19,21} Given the substantial evidence for a founding haplotype in many MYOC disease-causing variants, it is likely that other POAG-related genes or risk alleles have also arisen from a common founder and thereby may be identified through case-control whole genome association.\textsuperscript{2}

Despite a common founder for this specific MYOC mutation in Caucasian subjects, the phenotype from this Australian Gly252Arg MYOC mutation–carrying pedigree is less severe than previously described. Although a similar proportion required trabeculectomy, the age at diagnosis for glaucoma in our pedigree is significantly older than that described previously in the literature.\textsuperscript{9,10} Our data suggest that this mutation should be considered in patients with adult-onset glaucoma rather than those solely with juvenile-onset glaucoma and underscores the finding of incomplete penetrance associated with POAG.

From the literature published to date, the Gly252Arg mutation is of comparable glaucoma-causing severity as the MYOC Thr377Met mutation.\textsuperscript{9,10,27} The Gly252Arg mutation results in a more severe case of the disease than the MYOC Gln368Stop mutation but a less severe case of the disease than other mutations such as Pro370Leu or Lys423Glu.\textsuperscript{9,23-25} Using gonioscopy, Booth and colleagues\textsuperscript{10} identified abnormal-angle blood vessels or mesodermal tissue remnants in the series of Gly252Arg MYOC–affected subjects they examined. Interestingly, the drainage angle features described by Booth et al\textsuperscript{10} were not noted in our mutation–carrying patients. Such a difference in angle architecture may be the cause of, or a confounding reason for, the differing age at diagnosis between pedigrees.

The nucleotide change g.731 G>T that results in MYOC Gly244Val is caused by a substitution of the first base of exon 3, which is part of the consensus splice acceptor site. Consequently, this variant may cause exon skipping in mutation transcripts. Nonetheless, the novel Gly244Val variant has a Blosum matrix score of –3, implying that natural selection has a low tolerance for this amino acid substitution.\textsuperscript{26} Codon 244 is relatively well conserved across species (data not shown). Being novel, this variant has not been identified in any control series. It is difficult to decide for certain whether this variant is pathogenic, especially given that an elderly individual carrying it (subject IV:7) was clinically normal, while his niece did manifest the disease at a substantially younger age. Nevertheless, variable penetrance and expressivity is known to occur in MYOC mutations.\textsuperscript{22} The MYOC compound heterozygote subject was relatively young at diagnosis and had the highest maximum recorded IOP compared with other affected members in the pedigree. The MYOC Gly252Arg mutation is a rare allele of large effect, and the Gly244Val variant may be another rare allele with less substantial but nevertheless significant effect. However, phenotypic variability between related individuals and the single case of a much older, unaffected carrier suggests other, probably common alleles of lesser effect, either at the MYOC or at another locus, and/or the action of some unidentified environmental factor.\textsuperscript{27}

As genetic testing for glaucoma is now more readily available, the differentiation between nonimpairing polymorphisms and disease-causing variants becomes more clinically relevant.\textsuperscript{28} Clinical outcome studies are required to correlate specific disease-causing variants with the phenotype, thereby bridging the health care professional or genetic counselor to the laboratory.\textsuperscript{28} Accurate phenotypic descriptions, when compiled with relevant genetic information, should enhance health care professionals’ understanding of the specific natural history of individual patients’ disease.

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