Occurrence of CYP1B1 Mutations in Juvenile Open-Angle Glaucoma With Advanced Visual Field Loss

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Importance. Juvenile open-angle glaucoma (JOAG) is a severe neurodegenerative eye disorder in which most of the genetic contribution remains unexplained.

Objective. To assess the prevalence of pathogenic CYP1B1 sequence variants in an Australian cohort of patients with JOAG and severe visual field loss.

Design, Setting, and Participants. For this cohort study, we recruited 160 patients with JOAG classified as advanced (n = 118) and nonadvanced (n = 42) through the Australian and New Zealand Registry of Advanced Glaucoma from January 1, 2007, through April 1, 2014. Eighty individuals with no evidence of glaucoma served as a control group. We defined JOAG as diagnosis before age 40 years and advanced JOAG as visual field loss in 2 of the 4 central fixation squares on a reliable visual field test result. We performed direct sequencing of the entire coding region of CYP1B1. Data analysis was performed in October 2014.

Main Outcomes and Measures. Identification and characterization of CYP1B1 sequence variants.

Results. We identified 7 different pathogenic variants among 8 of 118 patients with advanced JOAG (6.8%) but none among the patients with nonadvanced JOAG. Three patients were homozygous or compound heterozygous for CYP1B1 pathogenic variants, which provided a likely basis for their disease. Five patients were heterozygous. The allele frequency among the patients with advanced JOAG (1 in 236 [4.7%]) was higher than among our controls (1 in 160 [0.6%]; P = .02; odds ratio, 7.8 [95% CI, 0.02-1.0]) or among the control population from the Exome Aggregation Consortium database (2946 of 122 960 [2.4%]; P = .02; odds ratio, 2.0 [95% CI, 0.3-0.9]). Individuals with CYP1B1 pathogenic variants, whether heterozygous or homozygous, had worse mean (SD) deviation on visual fields (−24.5 [5.1] [95% CI, −31.8 to −17.2] vs −15.6 [10.0] [95% CI, −17.1 to −13.6] dB; F1,126 = 5.90; P = .02; partial ηp² = 0.05) and were younger at diagnosis (mean [SD] age, 231 [8.4] [95% CI, 17.2-29.1] vs 31.5 [8.0] [95% CI, 30.1-33.0] years; F1,122 = 7.18; P = .008; ηp² = 0.06) than patients without CYP1B1 pathogenic variants.

Conclusions and Relevance. Patients with advanced JOAG based on visual field loss had enrichment of CYP1B1 pathogenic variants and a more severe phenotype compared with unaffected controls and patients with nonadvanced JOAG.
The term glaucoma describes a heterogeneous group of neurodegenerative eye disorders characterized by cupping of the optic nerve and typical visual field defects. Glaucoma is one of the leading causes of irreversible blindness worldwide and affects 3% of the Australian population older than 50 years. Primary open-angle glaucoma (POAG) [phenotype OMIM 137760] is the most common type of glaucoma in which the anterior chamber angle is open and is often, but not always, associated with high intraocular pressure (IOP). We can subclassify POAG into early and late onset; early-onset disease is termed juvenile open-angle glaucoma (JOAG) and is defined arbitrarily by onset before age 30 to 40 years. Primary open-angle glaucoma is a treatable condition, and therapeutic and/or surgical interventions can minimize the loss of visual function.

Family history is one of the strongest risk factors for POAG. First-degree relatives of affected individuals have a risk for developing glaucoma that is 9 times greater than that of the general population. Primary open-angle glaucoma displays a strong heritability but is genetically heterogeneous. The MYOC gene (OMIM 601652) was the first gene identified as causative and accounts for the most cases. The Australian and New Zealand Registry of Advanced Glaucoma (ANZRAG) has previously shown that mutations in MYOC, which are inherited in an autosomal dominant fashion, account for 4% of advanced POAG cases and 17% of advanced JOAG cases. Pathogenic variants in the CYP1B1 gene (OMIM 607771) were first associated with primary congenital glaucoma (PCG; OMIM 231300) and are inherited in an autosomal recessive fashion. Primary congenital glaucoma is a much rarer condition than POAG that results from a developmental defect of the aqueous filtration system and generally manifests in the neonatal or early infantile period. The CYP1B1 gene is a member of the cytochrome P450 superfamily and is involved in the metabolism of endogenous and exogenous substrates. The mechanism by which the gene causes glaucoma is still unknown, but investigators have hypothesized that CYP1B1 pathogenic variants may affect the enzymatic activity or the substrate specificity of the protein, thereby influencing the concentration of metabolites that modulate the expression of targeted genes essential during development.

Pathogenic variants of CYP1B1 have been associated with JOAG among different populations with variable frequencies. However, most studies involving cases of JOAG included small cohorts, and none assessed severe cases as defined by their visual field loss. In this study, we investigated CYP1B1 in a large cohort of patients with severe JOAG to assess the prevalence of mutations in this gene in the Australian population with JOAG and to evaluate whether it was more prevalent in cases with severe visual field loss.

Methods

Recruitment of Participants

We obtained ethics approval for this study from the Southern Adelaide Clinical Human Research Ethics Committee. The study was conducted in accordance with the revised Declara-
30 seconds, and final elongation at 72°C for 1 minute on a thermal cycler (Veriti; Life Technologies).

Residual primers and deoxynucleotides were removed by incubating 10 μL of the PCR products with 5 U of *Escherichia coli* (Exonuclease I; Biolabs) and 1 U of shrimp alkaline phosphatase (USB Corporation). We used cleaned PCR amplicons to perform bidirectional cycle sequencing reactions (BigDye Terminator; LifeTechnologies) with a genetic analyzer (3130XL; Applied Biosystems, LifeTechnologies).

We performed detection of sequence variants using commercially available software (Mutation Surveyor, version 3.10; SoftGenetics LLC). All forward and reverse chromatograms were assembled against the National Center for Biotechnology Information genomic reference sequence NT_022184.16 (GRCh38) containing *CYP1B1*. We used 2 software programs (SIFT [sorting intolerant from tolerant; http://sift.jcvi.org] and PolyPhen-2 [http://genetics.bwh.harvard.edu/pph2]) to predict the potential effect of amino acid substitutions on the protein. We used the HomoloGene system (http://www.ncbi.nlm.nih.gov/homologene) to assess conservation among mammalian species. Allelic frequencies were compared with the population frequencies from the Exome Aggregation Consortium (ExAC) database (http://exac.broadinstitute.org).

Mutation screening of *CYP1B1* for the controls used exome sequencing data. Exome capture was performed using a DNA library (SureSelect system; Agilent). Paired-end libraries underwent sequencing (Illumina HiSeq 2000; Macrogen). All exomes had a mean read depth of at least 60 times, and more than 97% of the genome was covered at 10 times or better. Reads were mapped to the human reference genome (hg19) using the BWA (Burrows-Wheeler Aligner) software package (http://bio-bwa.sourceforge.net), and duplicates were marked and removed using Picard sequencing tools (https://broadinstitute.github.io/picard/picard-metric-definitions.html). Variants were called using the Samtools suite of programs (http://www.htslib.org/) and annotated with the ANNOVAR software tool (http://www.openbioinformatics.org/annovar). Variants were described according to the recommendations of the Human Genome Variation Society (http://www.hgvs.org) and referenced against the ExAC database. All called variants were inspected. They were considered pathogenic if they were predicted to be damaging by SIFT or Polyphen-2 and if they had a minor allele frequency of less than 1%.

### Statistical Analysis
Data analysis was performed in October 2014. We used commercially available software (PASW Statistics, release 18.0.1.2009; SPSS, Inc) for statistical analysis. Data are presented as mean (SD) unless otherwise indicated. We used the Fisher exact and Mann-Whitney tests for the assessment of differences in nonparametric data. We performed multivariate analysis of variance to investigate differences between advanced and nonadvanced cases and between cases positive and negative for *CYP1B1* pathogenic variants. The following 2 groups of correlated dependent variables were identified: IOP, central corneal thickness, and age at diagnosis (group 1) and cup-disc ratio, MD, and trabeculectomy (group 2). When variables were not normally distributed, we applied appropriate transformation. However, age at diagnosis, cup-disc ratio, and MD were not normally distributed and could not be transformed.

### Results
We recruited 160 patients with JOAG who met the entry criteria. The demographic details are presented in Table 2. Advanced JOAG was documented in 118 patients (73.8%) and nonadvanced JOAG, in 42 patients (26.3%). The mean age at recruitment was 56.0 (18.1 [range, 10-86]) years. As expected, we found a statistical difference between advanced and nonadvanced JOAG in the group 2 combined dependent variables ($F_{3,124} = 60.4; P < .001; Pillai trace = 0.59$; partial $η^2 = 0.59$). Using a Bonferroni-adjusted level of .017, the mean

### Table 1. Primers Used for Amplification of the *CYP1B1* Gene

<table>
<thead>
<tr>
<th>Exon</th>
<th>Primer Sequence, Forward/Reverse</th>
<th>Size, Base Pair</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>5′-CACCCAACCGCACTCAGTC-3′/5′-CAGCAGCGCCACCAGCTC-3′</td>
<td>646</td>
</tr>
<tr>
<td>2.2</td>
<td>5′-CCTACCTCGGAGCACTGGAAGG-3′/5′-ACTCAGCATTACCTGTCTCTACTCC-3′</td>
<td>713</td>
</tr>
<tr>
<td>3</td>
<td>5′-AGCCTATTTAAGAAAAAGTGGAA-3′/5′-CTGAATTTTACTCCTCATCTCC-3′</td>
<td>761</td>
</tr>
</tbody>
</table>

### Table 2. Demographic Details of Patients With Primary JOAG

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patient Group*</th>
<th>Advanced JOAG</th>
<th>Nonadvanced JOAG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All (N = 160)</td>
<td>(n = 118)</td>
<td>(n = 42)</td>
</tr>
<tr>
<td>Age at diagnosis, mean (SD) [range], y</td>
<td>31.0 (8.4) [11-40]</td>
<td>31.3 (8.5) [11-40]</td>
<td>30.3 (8.3) [11-40]</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>73 (45.6)</td>
<td>45 (38.1)</td>
<td>28 (66.7)</td>
</tr>
<tr>
<td>Male</td>
<td>87 (54.4)</td>
<td>73 (61.9)</td>
<td>14 (33.3)</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>130 (81.3)</td>
<td>92 (78.0)</td>
<td>38 (90.5)</td>
</tr>
<tr>
<td>Other</td>
<td>30 (18.8)</td>
<td>26 (22.0)</td>
<td>4 (9.5)</td>
</tr>
<tr>
<td>Family history</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>117 (73.1)</td>
<td>82 (69.5)</td>
<td>35 (83.3)</td>
</tr>
<tr>
<td>Negative</td>
<td>42 (26.3)</td>
<td>35 (29.7)</td>
<td>7 (16.7)</td>
</tr>
<tr>
<td>Unknown</td>
<td>1 (0.6)</td>
<td>1 (0.8)</td>
<td>0</td>
</tr>
</tbody>
</table>

Abbreviation: JOAG, juvenile open-angle glaucoma.

*Unless otherwise indicated, data are expressed as number (percentage) of patients. Percentages have been rounded and may not total 100.
CYP1B1 Mutations in Juvenile Open-Angle Glaucoma

Table 3. CYP1B1 Sequence Variants Identified in This Study in Patients With JOAG and Controls

<table>
<thead>
<tr>
<th>Exon Location</th>
<th>Nucleotide Change</th>
<th>Amino Acid Change</th>
<th>Reference SNP No.</th>
<th>SIFT Score</th>
<th>PolyPhen-2 Prediction, HumDiv</th>
<th>Conserveda</th>
<th>Allelic Frequency in ExAC, %</th>
<th>Previously Reported Ethnicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>c.171G&gt;A</td>
<td>p.W57*</td>
<td>rs72549387</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>0.00041</td>
<td>White, Hispanicc</td>
</tr>
<tr>
<td>2</td>
<td>c.2.241T&gt;A</td>
<td>p.Y81N</td>
<td>rs9282671</td>
<td>0.00</td>
<td>Probably damaging</td>
<td>Yes</td>
<td>0.00642</td>
<td>White, Indiand</td>
</tr>
<tr>
<td>2</td>
<td>c.535delG</td>
<td>p.A179Rfs*18</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>0.00004</td>
<td>White, Hispanic, and North Afri[en]</td>
</tr>
<tr>
<td>2</td>
<td>c.685G&gt;A</td>
<td>p.E229K</td>
<td>rs57865060</td>
<td>0.00</td>
<td>Benign</td>
<td>Yes</td>
<td>0.01423</td>
<td>White, Middle Eastern, and Indiani</td>
</tr>
<tr>
<td>2</td>
<td>c.710C&gt;A</td>
<td>p.A237E</td>
<td>0.00</td>
<td>Probably damaging</td>
<td>Yes</td>
<td>NA</td>
<td>Whitee</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>c.868dupC</td>
<td>p.R290Pfs*37</td>
<td>rs67543922</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>0.00005</td>
<td>White, Hispanic, and Middle Easterng</td>
</tr>
<tr>
<td>3</td>
<td>c.1103G&gt;A</td>
<td>p.R368H</td>
<td>rs79204362</td>
<td>0.00</td>
<td>Probably damaging</td>
<td>Yes</td>
<td>0.00616</td>
<td>Asian, white, Hispanic, and Middle Easterni</td>
</tr>
<tr>
<td>3</td>
<td>c.1159G&gt;A</td>
<td>p.E387K</td>
<td>rs55989760</td>
<td>0.00</td>
<td>Probably damaging</td>
<td>Yes</td>
<td>0.00034</td>
<td>White, Hispanicj</td>
</tr>
</tbody>
</table>

Abbreviations: ExAC, Exome Aggregation Consortium database; JOAG, juvenile open-angle glaucoma; NA, not applicable; SIFT, sorting intolerant from tolerant; SNP, single-nucleotide polymorphism.

a A score of less than 0.05 is considered damaging.
b Indicates among mammalian species.

d By source: white, references 24, 28, 31, 32, 34, 41 and 42; Hispanic, reference 43.

e By source: white, references 18, 23, 24, 27, 32, 36; Indian, reference 22.

(11 in 236 [4.7%]) (SD) cup-disc ratio (0.92 [0.08] [95% CI, 0.90-0.94]) vs 0.74 [0.10] [95% CI, 0.71-0.77]; F1,126 = 106.7; P < .001; n1 = 0.46), the MD on visual field test (−20.2 [7.9] [95% CI, −22.0 to −19.2] vs −4.0 [3.6] [95% CI, −6.5 to −2.2] dB; F1,126 = 160.0; P < .001; n1 = 0.56), and the prevalence of trabeculocytosis (74.5% [95% CI, 0.7%-0.8%] vs 33.3% [95% CI, 0.2%-0.5%]; F1,126 = 25.5; P < .001; n1 = 0.17) were all higher in the patients with advanced compared with nonadvanced JOAG. The age at diagnosis, highest IOP, mean central corneal thickness, and family history were not different between the 2 groups. Nineteen patients carried an MYOC pathogenic variant.

We included 80 controls (all white). Forty-four controls (55%) were women, and their mean age at recruitment was 65.7 (18.1) years.

Sequencing of the CYP1B1 gene identified 15 different sequence variants among the patients with JOAG. Six variants were known polymorphisms (eTable in the Supplement). Their allelic frequency did not differ between the patients and controls. The other 9 sequence variants were identified among 10 patients with advanced JOAG (19.4%-24.27,28,31-39) (Table 3 and Table 4). No CYP1B1 variants were identified in the patients with nonadvanced JOAG, nor were any variants identified in individuals carrying an MYOC pathogenic variant.

Two sequence variants were not present in the ExAC database or the literature and were therefore considered novel. The variant p.A64V (c.191G>T) was predicted to be benign by SIFT and PolyPhen-2. Alanine is conserved between mammal, bird, and fish sequences but not amphibian sequences (eFigure in the Supplement). The variant p.M244I (c.732G>T) was predicted to be benign using SIFT and possibly damaging using PolyPhen-2. This position is occupied by methionine or leucine among different species (eFigure in the Supplement). Isoleucine has similar biophysical and chemical properties to methionine and leucine. Based on this evidence, these 2 sequence variants were considered unlikely to be pathogenic.

The other 7 sequence variants were all considered pathogenic based on the findings of mutation prediction software, conservation among species, and previously published literature (Table 3). Therefore, 8 of 160 patients with JOAG (5.0%), including 8 of 118 patients with advancedJOAG (6.8%), had CYP1B1 pathogenic variants. Three patients carried 2 pathogenic sequence variants. Patient AG180 was compound heterozygous for p.A179Rfs*18 and p.E387K, patient AG1751 was homozygous for p.R290Pfs*37, and patient AG1791 was homozygous for p.A237E. Two other sequence variants were present in the heterozygous state in 1 patient each (p.Y81N and p.E229K), and p.R368H was present in 3 individuals.

Apart from the 6 known polymorphisms described above, 6 other variants were identified in controls. They consisted of 4 synonymous (p.L471L, p.G236G, p.V243V, and p.L360L), 1 nonsynonymous (p.A443G), and 1 nonsense (p.W57*) sequence variant. A previous report24,28 described p.A443G as a polymorphism. We identified p.W57* in the heterozygous state in 1 control. As a known pathogenic variant, it has been reported in association with other known mutations in several patients with PCG.24,28,38,39 This finding equates to an allele frequency of CYP1B1 pathogenic variants of 1 in 160 (0.6%) among controls, which is decreased compared with patients with advanced JOAG (11 in 236 [4.7%]) (P = .02; odds ratio, 7.8 [95% CI, 0.02-1.0]).
Table 4. Clinical Details of Patients With JOAG and CYP1B1 Sequence Variants

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Mutation</th>
<th>Sex</th>
<th>Country of Origin</th>
<th>Age at Diagnosis, y</th>
<th>Family History</th>
<th>Dependent Variables, OD; OS</th>
<th>Surgery, Right/Left Eye</th>
</tr>
</thead>
<tbody>
<tr>
<td>AG0180</td>
<td>p.A179Rfs*18/p. E387K</td>
<td>M</td>
<td>Australia</td>
<td>24</td>
<td>Yes</td>
<td>40; 50</td>
<td>20/20; 20/200</td>
</tr>
<tr>
<td>AG0521</td>
<td>p.Y81N</td>
<td>M</td>
<td>Ireland/Germany</td>
<td>25</td>
<td>No</td>
<td>29; 23*</td>
<td>20/125; 20/25</td>
</tr>
<tr>
<td>AG0654</td>
<td>p.R368H</td>
<td>F</td>
<td>Australia</td>
<td>36</td>
<td>Yes</td>
<td>28; 13*</td>
<td>20/20; 20/20</td>
</tr>
<tr>
<td>AG1196</td>
<td>p.R368H</td>
<td>M</td>
<td>Australia</td>
<td>11</td>
<td>Yes</td>
<td>31; 37</td>
<td>20/15; CF</td>
</tr>
<tr>
<td>AG1427</td>
<td>p.E229K</td>
<td>M</td>
<td>Denmark</td>
<td>38</td>
<td>No</td>
<td>NA</td>
<td>20/30; 20/40</td>
</tr>
<tr>
<td>AG1751</td>
<td>p.R290Ps<em>37/p.R290Ps</em>37</td>
<td>F</td>
<td>Italy</td>
<td>16</td>
<td>No</td>
<td>21; 16*</td>
<td>20/40; 20/80</td>
</tr>
<tr>
<td>AG1791</td>
<td>p.A237E/p.A237E</td>
<td>M</td>
<td>Italy</td>
<td>20</td>
<td>No</td>
<td>48; 38</td>
<td>20/60; 20/25</td>
</tr>
<tr>
<td>AG1858</td>
<td>p.R368H</td>
<td>F</td>
<td>India</td>
<td>30</td>
<td>Yes</td>
<td>23; 23*</td>
<td>20/20; 20/40</td>
</tr>
<tr>
<td>KSA008</td>
<td>p.W57*</td>
<td>M</td>
<td>Australia</td>
<td>12</td>
<td>No</td>
<td>12; 12</td>
<td>20/30; 20/30</td>
</tr>
</tbody>
</table>

Abbreviations: BCVA, best-corrected visual acuity; CCT, central corneal thickness; CDR, cup-disc ratio; CF, counting fingers; IOP, intraocular pressure; JOAG, juvenile open-angle glaucoma; LP, light perception; NA, not available. * The IOP at diagnosis not available.

In the ExAC database, 139 CYP1B1 variants were predicted to be pathogenic, occurring 1262 times in 39 657 white individuals (allele frequency of 1.6%). Among all ethnicities, 147 pathogenic variants occurred 2946 times in 61 480 individuals (allele frequency of 2.4%). Among the 8 pathogenic variants identified in our patients and controls, 7 were present in the ExAC database. Our findings showed a higher prevalence of CYP1B1 pathogenic variants in patients with advanced JOAG compared with those in white individuals in the ExAC database ($P < .001$; odds ratio, 3.4 [95% CI, 0.2-0.6]) and when including all ethnicities ($P = .02$; odds ratio, 2.0 [95% CI, 0.3-0.6]).

Clinical variables were compared between the patients with JOAG with and without CYP1B1 pathogenic variants (Table 5). We found a difference in the combined group 1 of dependent variables ($F_{3,120} = 3.15$; $P = .02$; Pillai trace, 0.073; $\eta_p^2 = 0.073$) and in group 2 of dependent variables ($F_{3,126} = 3.51$; $P = .02$; Pillai trace, 0.078; $\eta_p^2 = 0.078$). Using a Bonferroni-adjusted level of .017, patients with CYP1B1 pathogenic variants were younger at diagnosis (mean [SD] age, 23.1 [8.4] [95% CI, 17.2-29.1] vs 31.5 [8.0] [95% CI, 30.1-33.0] years; $F_{3,122} = 7.18$; $P = .008$; $\eta_p^2 = 0.06$) and had worse mean (SD) deviation on visual field tests (−24.5 [5.1] [95% CI, −31.8 to −17.2] vs −15.6 [10.0] [95% CI, −17.1 to −13.6] dB; $F_{3,126} = 5.90$; $P = .02$; partial $\eta^2 = 0.05$) than those without CYP1B1 pathogenic variants. Individuals with the CYP1B1 variants also required trabeculectomy more often (100%) than those without CYP1B1 variants (56.6%). No differences in IOP, family history, cup-disc ratio, or central corneal thickness were identified.

Discussion

Several studies have indicated that CYP1B1 sequence variants may play a role in JOAG and POAG among different populations. However, all studies including cases of JOAG were small, with the largest reporting 61 cases. To our knowledge, the present study includes the largest cohort of patients with JOAG assessed for CYP1B1 sequence variants and is the first to include a cohort of severely affected cases based on visual field loss.

Our findings showed an enrichment of CYP1B1 pathogenic variants among patients with advanced JOAG (6.8%) compared with a control group without glaucoma and compared with the general population. No CYP1B1 mutations were identified in the group with nonadvanced JOAG. The ExAC database reflects the general population that has not been examined for glaucoma, which means that a small percentage might have or might develop JOAG. Our findings are similar to those reported in other white populations (8%-10%) but lower than those in studies from India (12%-20%), Iran (17%-24%), or Saudi Arabia (93%) and higher than those reported in Taiwan (3%) or Japan (0%). The prevalence is expected to be higher in populations with high rates of consanguinity or common founder mutations, such as Iran or Saudi Arabia. However, one has to be cautious when drawing comparisons because most studies included patients with a younger cutoff at diagnosis of JOAG and patients with high-tension glaucoma only. In this study, all individuals diagnosed as having JOAG before age 40 years were included regardless of their IOP or family history.

Three individuals carried 2 CYP1B1 mutations. One individual was compound heterozygous for p.A179Rfs*18 and p.E229K. This combination has been reported to segregate in 2 Spanish siblings, with one diagnosed as having PCG at birth and the other diagnosed as having JOAG at 10 years of age. The other 2 individuals were homozygous. The p.A237E variant has only been reported in the compound heterozygous state in patients with PCG, whereas p.R290Ps*37 has been reported in the compound heterozygous and homozygous states in patients with PCG. Neither variant has been found...
in conjunction with JOAG or POAG. These 3 individuals all received the diagnosis in their third decade of life and do not display the characteristic features of PCG. Genetic modifiers might account for the different phenotypes and ages at onset in this autosomal recessive model.

The ANZRG study previously demonstrated that MYOC pathogenic variants accounted for 17% of advanced JOAG in patients from the Australian population. In comparison, CYP1B1 variants were present in 6.8% of patients with advanced JOAG and in the homozygous/compound heterozygous state in 3 patients (2.5%). However, when considering individuals diagnosed as having advanced JOAG by 25 years of age, 6 of 27 (22%) carried an MYOC pathogenic variant and 3 of 27 (11%) carried 2 CYP1B1 pathogenic variants, all likely to account for their disease. Therefore, our results show that these 2 genes together can explain up to one-third of selected JOAG cases based on severity and younger age at diagnosis.

Five individuals were heterozygous for CYP1B1 mutations. How heterozygous CYP1B1 pathogenic variants are associated with JOAG and whether CYP1B1 acts as a causative gene, or more likely as a contributing modifier gene, remain unclear. Carrier parents of individuals with CYP1B1-enriched PCG have not been shown to be at a higher risk for developing glaucoma, which suggests that variants in the heterozygous state are not sufficient to cause glaucoma. However, Vincent et al described a family with MYOC and CYP1B1 mutations. Among these individuals, those carrying the MYOC variant only had a later age at diagnosis than those carrying MYOC and CYP1B1 variants, suggesting that CYP1B1 may act as a gene modifier for MYOC in patients with JOAG. In our large cohort, no individual carried mutations in CYP1B1 and MYOC, suggesting that this specific combination is an extremely rare cause of disease.

All CYP1B1 pathogenic variants were identified in the group with advanced JOAG, the diagnosis of which was based on central visual field loss. Presumably, a small proportion of patients with JOAG that has not yet progressed to advanced disease will have CYP1B1 mutations; however, a larger study will be required to detect these mutations. Our findings show that individuals with CYP1B1 pathogenic variants had a statistically worse MD on visual field test results and received a diagnosis at a younger age. They all required trabeculectomy to control their glaucoma compared with only 56.6% in the non-CYP1B1 group. A few studies previously reported genotype/phenotype correlations; 1 study found the age at diagnosis to be younger among patients with POAG and CYP1B1 variants, whereas 2 others did not find differences in the age at diagnosis, IOP, disc changes, and visual field defects when comparing both groups. One possible explanation for the severity of glaucoma in CYP1B1 carriers is that most do not have risk factors, such as family history, to prompt a diagnosis in the early stages of disease. Among the 3 patients with 2 CYP1B1 mutations, two had no family history of glaucoma and the relatives of the third received a diagnosis at a much later age. This finding is an important contrast to MYOC-associated glaucoma, in which the rate of positive family history is extremely high and severity of disease typically matches that of other family members.

Conclusions

Our results showed an enrichment of CYP1B1 mutations in patients with advanced JOAG compared with patients with non-advanced JOAG and controls. This finding is in keeping with those of previous smaller studies among white individuals. It reinforces the hypothesis that JOAG is a complex disorder displaying genetic heterogeneity and that heterozygous CYP1B1 variants may act as a modifier to some unknown other genetic factors. We also demonstrated that individuals with CYP1B1 mutations tend to have more severe glaucoma than patients without the CYP1B1 mutations. Because these individuals are at high risk for preventable blindness, early identification through genetic testing for adequate glaucoma intervention is important.
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