**Gln368STOP Myocilin Mutation in Families with Late-Onset Primary Open-Angle Glaucoma**

R. Rand Allingham,¹ Janey L. Wiggs,² Monica A. De La Paz,¹ Doug Vollrath,³ Deidre A. Talleyt,¹ Bob Broomer,¹ Katherine H. Jones,¹ Elizabeth A. Del Bono,² Jeremy Kern,² Kara Patterson,² Jonathan L. Haines,⁴ and Margaret A. Pericak-Vance¹

**PURPOSE.** To examine families ascertained for late-onset primary open-angle glaucoma (POAG) to determine mutations in the gene coding for myocilin.

**METHODS.** The diagnosis of late-onset POAG was defined as age at diagnosis more than 35 years, intraocular pressure (IOP) 22 mm Hg or more in both eyes or 19 mm Hg or more while the patient was taking two glaucoma medications, glaucomatous optic neuropathy in both eyes, and visual field loss consistent with optic nerve damage in at least one eye of the proband. Two of three criteria were required in other family members. DNA from all families was screened for polymorphisms in myocilin using single-strand conformation polymorphism analysis. All polymorphisms were sequenced for mutations.

**RESULTS.** Eighty-three affected people in 29 families with late-onset POAG were screened for mutations. Three mutations, two novel missense (Thr377Met and Glu352Lys) and one nonsense (Gln368STOP), were identified. The missense mutations did not segregate with the disease phenotype in these families. The nonsense mutation was found in 3 of 29 unrelated families with POAG. All affected family members and 8 of 12 in whom glaucoma was suspected had the Gln368STOP mutation. All people with this mutation had elevated IOP, and 78% had POAG by age 70.

**CONCLUSIONS.** Three mutations were identified in the gene coding for myocilin in families with late-onset POAG. Of these, the Gln368STOP mutation was highly associated with the development of glaucoma. All people with this mutation had glaucoma or elevated IOP by age 70. In the United States, the Gln368STOP mutation in myocilin is strongly associated with the development of late-onset POAG. However, factors in addition to the presence of this mutation seem to play a role in the development of ocular hypertension and glaucoma in these families. (Invest Ophthalmol Vis Sci. 1998;39:2288-2295)

---

Glaucoma is a group of disorders characterized by progressive excavation of the optic nerve head with associated loss of the visual field. Although unnecessary for diagnosis, elevated intraocular pressure (IOP) is often associated with glaucoma. Glaucoma is a highly prevalent disorder and is estimated to be the third most common cause of blindness worldwide.¹ Primary open-angle glaucoma (POAG) is the most common form. It is estimated that POAG is responsible for blindness in as many as 116,000 people in the United States and in 3,000,000 worldwide.²,³

Late-onset POAG is a bilateral disease that typically occurs after the fourth decade of life. The clinical course usually consists of mild to moderately elevated IOP, although a significant number of patients may not have a measured IOP higher than normal.¹ Optic nerve damage and visual field loss, which may culminate in blindness, occurs over many years, usually decades after onset of the disease. The mode of inheritance for late-onset POAG is unknown. However, it is thought that POAG is a complex trait that results from interaction of multiple genes in conjunction with environmental influences.

Juvenile-onset POAG is an uncommon autosomal dominant form of glaucoma. The age of onset is most often before the fourth decade of life and the phenotype is clinically more severe than late-onset POAG. A locus for juvenile-onset POAG was mapped to chromosome 1q23 to 25 and confirmed by others.⁵,⁶ More recently, a candidate gene was localized to chromosome 1q23-25, which codes for the protein described as trabecular meshwork-induced glucocorticoid response protein (TIGR)⁹⁹ and myocilin.¹⁰ The Human Genome Organization Genome Database Nomenclature Committee has recently adopted the term myocilin. Mutations in myocilin have been identified in families with juvenile-onset POAG. One mutation in myocilin (Gln368STOP) has been identified in people with late-onset POAG.¹¹ In this article we report on the presence of mutations in myocilin in families ascertained for late-onset POAG.
METHODS

Family Collection and Ascertainment

Families were ascertained through the Departments of Ophthalmology at the Duke University Medical Center in Durham, North Carolina, and the Tufts New England Medical Center in Boston, Massachusetts. Some family members were ascertained by referring ophthalmologists. Predominantly, families were identified from the southeastern United States and New England. Informed consent was obtained from all participating family members. This study was approved by the Duke University and New England Medical Center Investigational Review Boards. All procedures used in the execution of this study adhered to the tenets of the Declaration of Helsinki.

All family members were personally examined or had their medical records reviewed (JLW or RRA). Examiners were masked to family members’ mutation status. Ascertainment for proband, affected (late-onset POAG), suspected glaucoma, and unaffected was defined as follows. All those affected had age at diagnosis of more than 35. Proband had IOP measured by applanation tonometry in both eyes of 22 mm Hg or more or 19 mm Hg or more while taking two glaucoma medications, glaucomatous optic neuropathy in both eyes, and visual field loss consistent with optic nerve damage in at least one eye. To be considered glaucomatous optic neuropathy, documented progression of optic nerve cupping or two of the following were required: focal thinning of the neuroretinal rim, vertical cup-to-disc ratio more than 0.7 in both eyes, optic cup asymmetry more than 0.2, or presence of an optic disc hemorrhage. To be considered affected (other than proband) required two of the three ocular criteria just described. Those with suspected glaucoma had to have IOP of 22 mm Hg or more or optic nerves that seemed glaucomatous to the examiner. Visual field evaluations were performed on all family members who had elevated IOP or optic nerves that raised suspicion of glaucoma. Unaffected people had IOP in the normal range (<22 mm Hg) and normal appearing optic nerves. People with a history of incisional surgery (e.g., cataract extraction) before a history of incisional surgery (e.g., cataract extraction) before documentation of glaucoma status, a history of penetrating or blunt ocular trauma, or any secondary form of glaucoma were not included in the initial screen for mutations in myocilin. Families with any member identified with pseudoxfoliation or pigment dispersion syndrome were excluded from analysis. All families had at least two siblings affected by POAG. Age at diagnosis, race, sex, visual acuity, applanation tonometry, slit lamp examination, gonioscopy, fundus examination, visual fields, refraction, and available ophthalmic medical and surgical history were obtained on all family members.

All available members of families identified with the myocilin Gln368STOP mutation were examined and sampled. Affected status was classified according to the described criteria with the exception that people with previous ocular surgery were included in the final analysis. DNA was obtained from people with age-related macular degeneration without personal or family history of glaucoma to be used as control subjects.

Single-Strand Conformation Polymorphism and Sequencing Analyses

DNA from all family members and control subjects was screened for myocilin mutations using single-strand conforma-
FIGURE 1. Pedigrees of families with late-onset primary open-angle glaucoma in which the Gln368STOP myocilin mutation was identified. Age at last examination is shown.

jects. No other mutations were identified in the control population. The missense mutations occurred in small families and did not segregate with the disease phenotype. These mutations will be discussed in more detail elsewhere (Wiggs et al., unpublished results).

The Gln368STOP mutation consisted of a CAG to TAG bp change at codon 368 in all 3 families. Pedigrees of these families are depicted in Figure 1. In family 5052 there were three members with the Gln368STOP mutation, all of whom were affected with POAG. The fourth family member had...
Mutations in Myocilin in Families with POAG

Mutations in the myocilin gene have been identified that are associated with the development of POAG, usually of juvenile onset.11 We have identified three mutations in myocilin in families screened for late-onset POAG. Two missense mutations (Thr377Met and Glu352Lys) were found in one family each. Of note, neither mutation segregated with the POAG phenotype in these families. These mutations may represent rare polymorphisms, although they were not found in the control population nor have they been described elsewhere. It is possible that factors in addition to these mutations may be required to produce the POAG phenotype in these families. The third mutation was a nonsense mutation (Gln368STOP) and was found in three families. The missense mutations found in these families will be described in more detail elsewhere (Wiggs et al., unpublished). The primary focus of this article is on families with the Gln368STOP mutation.

In all families in which the Gln368STOP mutation was identified, all affected people with POAG had the mutation. All family members older than 70 years with the Gln368STOP mutation had elevated IOP, and 78% of these had glaucoma. The association between mutation status and classification as a glaucoma suspect was less clear. Four of 12 of those with suspected glaucoma and 3 of 11 with ocular hypertension did not have the Gln368STOP mutation, although those with suspected glaucoma who had the Gln368STOP mutation had a higher average IOP than those without the mutation. In addition, there was significant overlap in the age at diagnosis of affected people, people with suspected glaucoma, and the ages of unaffected people with the Gln368STOP mutation in these families. Therefore, it seems that there are important genetic and/or environmental factors in these families that play an important role in the pathogenesis of ocular hypertension and glaucoma.

These data suggest that the Gln368STOP mutation in myocilin, a novel protein, is associated with the development of glaucoma in families with late-onset POAG. Little is known regarding the function of myocilin. The deduced amino acid sequence of myocilin shares significant homology with nonmuscle myosin at the N terminus and neural olfactomedin at the C terminus.10,13 Evidence to date suggests that myocilin may be a membrane-associated cytoskeletal protein, an extracellular protein, or both.9,10 The gene for myocilin has three exons.10,15 To date, all mutations associated with the open-angle glaucoma phenotype are located in the third exon or that region coding for the olfactomedinlike domain.11,14,15 All these are missense mutations, which produce amino acid substitutions in the protein, except for one nonsense mutation, Gln368STOP. This mutation codes for premature truncation of 135 amino acids of the C terminus of the myocilin protein, or approximately half of the olfactomedinlike domain.

Excluding the Thr377Met and Glu352Lys mutations described that do not segregate with the disease phenotype, the mean age at glaucoma diagnosis with missense mutations is under age 35 (Table 3). In contrast, the age at diagnosis for the Gln368STOP mutation occurs at least 3 decades after these missense mutations.

The reason for this striking difference in the age at diagnosis of POAG between missense mutations and the Gln368STOP mutation is unclear. Although gene expression studies are needed to answer many of these questions, it is reasonable to speculate that this observation is attributable to differences in expression or function between proteins with amino acid substitutions and those that are prematurely truncated. Missense and nonsense mutations alter the tertiary struc-
tured of proteins, which in turn can alter their function, half-life, and interaction with other proteins.\textsuperscript{16} Altered forms of myocilin may compete with or bind normal myocilin produced by the normal homologue or interact with other cellular proteins. Truncated proteins, because of greater alterations in structure, are more likely to be recognized as abnormal and are often rapidly eliminated by the cell.\textsuperscript{17-19} Therefore, the protein coded for by the \textit{Gln368STOP} mutation may be eliminated more rapidly, producing a relative deficiency of myocilin. Abnormal myocilin produced by missense mutations, particularly if these have a produced by missense mutations, particularly if these have a

\begin{table}[h]
\centering
\caption{Genotype and Clinical Data of Study Participants in Families with \textit{Gln368STOP} Myocilin Mutation}
\begin{tabular}{|c|c|c|c|c|c|c|c|}
\hline
Family/Individual Number & \textit{Gln368STOP} & Age at Diagnosis & Sex & Visual Acuity OD & OS & IOP Highest (mm Hg) & Vertical Cup-to-Disc Ratio & Surgery \\
Number & Mutation & & & & & & & \\
\hline
\textbf{Affected with POAG} & & & & & & & & \\
5055/0001 & + & 53 & M & 20/30 & 20/40 & 31/32 & 0.7/0.7 & LTP \\
5055/0108 & + & 61 & F & 20/20 & 20/20 & 29/32 & 1.0/1.0 & CE; TRAB \\
5055/0056 & + & 65 & F & 20/30 & 20/30 & 28/24 & 0.9/0.9 & LTP; RS \\
5052/0101 & + & 75 & F & 20/30 & 20/30 & 30/24 & 1.0/0.9 & LTP; TRAB; CE \\
5052/0102 & + & 73 & M & 20/20 & 20/20 & 30/24 & 0.7/0.7 & CE \\
5052/0105 & + & 41 & M & 20/20 & 20/20 & 34/28 & 1.0/1.0 & LTP; TRAB; CE; \\
125/0001 & + & 56 & M & 20/20 & 20/20 & 25/25 & 0.7/0.7 & \\
125/0100 & + & 56 & M & 20/20 & 20/20 & 25/25 & 0.7/0.7 & \\
\hline
\textbf{Possible POAG} & & & & & & & & \\
5055/0100 & + & 61 & F & 20/25 & 20/25 & 24/24 & 0.3/0.5 & \\
5055/8138 & + & 23 & M & 20/15 & 20/15 & 25/20 & 0.1/0.1 & \\
5055/0076 & + & 59 & F & 20/20 & 20/20 & 30/32 & 0.7/0.5 & \\
5055/0023 & + & 41 & F & 20/20 & 20/20 & 25/25 & 0.2/0.2 & \\
5055/0101 & + & 72 & F & 20/300 & 20/300 & 30/34 & 0.5/0.5 & \\
5055/0078 & + & 59 & F & 20/20 & 20/20 & 27/27 & 0.5/0.4 & \\
125/9002 & + & 39 & F & 20/15 & 20/15 & 24/27 & Tilted & \\
125/9000 & + & 42 & M & 20/20 & 20/20 & 24/25 & 0.3/0.3 & \\
5055/9036 & + & 72 & M & 20/30 & 20/30 & 18/19 & 0.6/0.6 & CE \\
5055/9042 & + & 64 & F & 20/25 & 20/25 & 22/19 & 0.3/0.25 & \\
125/9005 & + & 41 & M & 20/25 & 20/25 & 26/19 & 0.1/0.1 & \\
5052/0100 & + & 73 & M & 20/30 & 20/30 & 23/23 & 0.1/0.1 & \\
\hline
\textbf{Unaffected by POAG} & & & & & & & & \\
5055/8136 & + & 35 & M & 20/20 & 20/15 & 15/16 & 0.4/0.3 & \\
5055/9002 & + & 58 & F & 20/20 & 20/20 & 18/18 & 0.5/0.5 & \\
5055/9007 & + & 59 & F & 20/20 & 20/20 & 19/19 & 0.3/0.3 & \\
5055/9005 & + & 43 & F & 20/20 & 20/20 & 18/17 & 0.2/0.3 & \\
5055/9016 & + & 55 & M & 20/20 & 20/20 & 13/14 & 0.2/0.2 & \\
5055/9019 & + & 50 & M & 20/20 & 20/20 & 18/18 & 0.25/0.25 & \\
5055/9070 & + & 67 & F & 20/20 & 20/25 & 17/18 & 0.2/0.2 & \\
5055/9054 & + & 49 & F & 20/15 & 20/20 & 14/14 & 0.4/0.3 & \\
5055/8134 & + & 58 & F & 20/20 & 20/20 & 17/18 & 0.1/0.1 & \\
125/9006 & + & 36 & F & 20/25 & 20/40 & 17/17 & 0.3/0.3 & \\
5055/9058 & + & 63 & F & 20/20 & 20/25 & 18/18 & 0.3/0.3 & \\
5055/9004 & + & 49 & F & 20/25 & 20/20 & 16/16 & 0.2/0.2 & \\
5055/8139 & + & 31 & F & 20/20 & 20/20 & 16/16 & 0.2/0.2 & \\
5055/8131 & + & 28 & F & 20/15 & 20/15 & 16/17 & 0.2/0.2 & \\
5055/8114 & + & 47 & M & 20/20 & 20/20 & 16/11 & 0.3/0.3 & \\
5055/8116 & + & 45 & F & 20/15 & 20/15 & 16/17 & 0.2/0.2 & \\
5055/8141 & + & 40 & M & 20/20 & 20/20 & 13/13 & 0.2/0.1 & \\
5055/8027 & + & 32 & M & 20/20 & 20/30 & 18/16 & 0.2/0.1 & \\
5055/9044 & + & 62 & F & 20/25 & 20/100 & 17/21 & 0.2/0.2 & \\
5055/8088 & + & 42 & F & 20/20 & 20/20 & 15/16 & 0.3/0.3 & \\
5055/8056 & + & 42 & F & 20/15 & 20/15 & 12/12 & 0.3/0.3 & \\
5055/8059 & + & 39 & F & 20/15 & 20/15 & 15/15 & 0.3/0.3 & \\
5055/0002 & + & 55 & F & 20/25 & 20/50 & 18/19 & 0.1/0.1 & \\
5055/0074 & + & 61 & F & 20/25 & 20/25 & 16/16 & 0.25/0.25 & \\
5055/9020 & + & 34 & M & 20/30 & 20/30 & 15/15 & 0.2/0.2 & \\
5055/115 & + & 91 & M & 20/30 & 20/100 & 12/14 & 0.3/0.3 & CE \\
\hline
\end{tabular}
\end{table}

POAG, primary open-angle glaucoma; IOP, intraocular pressure; LTP, laser trabeculoplasty; CE, cataract extraction; TRAB, trabeculectomy; RS, retinal surgery; RL, retinal laser; LP, light perception; NLP, no light perception; CF, counts fingers.
Regardless, these data suggest that chronic reduction of or interference with normal myocilin function produced by these mutations is associated with increased risk of IOP elevation and glaucoma. It is well known that the administration of exogenous glucocorticoids is also associated with increased risk of elevated IOP and glaucoma, particularly in people with POAG and their close relatives.20–23 Of interest, glucocorticoids increase production of myocilin in human trabecular cells.9 Although the time course in steroid-induced IOP elevation is measured in weeks to months versus decades in people with mutations in myocilin, it is interesting that abnormal myocilin and excess levels of myocilin are both associated with increased IOP and glaucoma.

In family members with the Gln368STOP mutation, the age of patients at diagnosis of POAG is approximately 10 years more than that of those with suspected glaucoma. Additionally, all with suspected disease who had this mutation and those affected by glaucoma had elevated IOP. It appears that elevated IOP precedes optic nerve damage by many years in people with this mutation in myocilin. It is impossible to determine the precise risk of development of elevated IOP or glaucoma from this small number of families; however, it is important to note that fully 60% of those family members with the Gln368STOP mutation who are aged more than 35 years had elevated IOP or glaucoma. Of additional interest, the three people without this mutation have elevated IOP. It is clear that prospective longitudinal studies are needed to define further the risks and appropriate methods for observing people with these mutations.
Figure 4. Visual fields of eyes in Figure 2 affected with primary open-angle glaucoma. Generalized depression and peripheral constriction are present in both eyes. Superior visual field loss is present in the right eye.

Table 2. Age at Diagnosis, Mutation Status, Sex, and Mean IOP of Individuals with the Gln368STOP Mutation of Myocilin

<table>
<thead>
<tr>
<th>Mutation Status</th>
<th>Number</th>
<th>Age* (M/F)</th>
<th>Mean IOP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>POAG†</td>
<td>8</td>
<td>61.8</td>
<td>4/4</td>
</tr>
<tr>
<td>Suspected Glaucoma</td>
<td>12</td>
<td>53.8</td>
<td>5/7</td>
</tr>
<tr>
<td>Gln368STOP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ mutation</td>
<td>8</td>
<td>49.5</td>
<td>2/6</td>
</tr>
<tr>
<td>− mutation</td>
<td>4</td>
<td>62.5</td>
<td>3/1</td>
</tr>
<tr>
<td>Unaffected by POAG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gln368STOP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ mutation</td>
<td>28</td>
<td>47.1</td>
<td>8/20</td>
</tr>
<tr>
<td>− mutation</td>
<td>10</td>
<td>49</td>
<td>3/7</td>
</tr>
<tr>
<td>− mutation</td>
<td>18</td>
<td>46</td>
<td>5/13</td>
</tr>
</tbody>
</table>

POAG, primary open-angle glaucoma; IOP, intraocular pressure.
* Values are means at time of diagnosis for those with POAG and those with suspected POAG and at time of last examination for those who were unaffected.
† All Gln368STOP mutation +.

We identified the Gln368STOP mutation in more than 10% (3/29) of probands and none of 104 control subjects. This association was highly significant (P = 0.0002). Stone et al.11 found the Gln368STOP mutation in 2.6% (6/227) of probands. Because their sample included many families with juvenile-onset glaucoma this figure may underestimate the percentage.

Table 3. Mean Age of Diagnosis for TIGR and MYOC Mutations

<table>
<thead>
<tr>
<th>Mutation (Type)</th>
<th>Mean Age at Diagnosis (y)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gln368STOP</td>
<td>62-64</td>
<td>Current study, 11</td>
</tr>
<tr>
<td>Pro370Leu</td>
<td>10-11</td>
<td>15, 14</td>
</tr>
<tr>
<td>Ile477Ser</td>
<td>33</td>
<td>15</td>
</tr>
<tr>
<td>Asn480Lys</td>
<td>30-35</td>
<td>15</td>
</tr>
<tr>
<td>Ile499Phe</td>
<td>31</td>
<td>15</td>
</tr>
<tr>
<td>Gly246Arg</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>Tyr437His</td>
<td>22</td>
<td>11</td>
</tr>
<tr>
<td>Gly364Val</td>
<td>33</td>
<td>11</td>
</tr>
<tr>
<td>Lys423Glu</td>
<td>30</td>
<td>15, 24</td>
</tr>
</tbody>
</table>
that would have been found in families with late-onset POAG. Therefore, the actual prevalence of the Gln368STOP mutation in families with late-onset POAG may be greater than previously thought. If true, this would have significant implications regarding population screening for mutations and the approach to medical intervention.

In summary, we have identified three mutations in the gene coding for myocilin in families with late-onset POAG. Of these, the Gln368STOP mutation is highly associated with the development of the disease phenotype. Glaucoma developed or was suspected by age 70 in all people with this mutation. However, factors in addition to the presence of this mutation appear to play a role in the development of ocular hypertension and glaucoma in these families.

Acknowledgments

The authors thank the families who enthusiastically participated in this project; David L. Epstein, W. Daniel Stamer, and Brian S. McKay for their critical review of the manuscript; and the Center for Human Genetics of the Duke University Medical Center for permitting the use of resources and staff.

References


Mutations in Myocilin in Families with POAG