Letters to the Editor

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Prevalence of Mutations in TIGR/Myocilin in Patients with Adult and Juvenile Primary Open-Angle Glaucoma

To the Editor:

Primary open-angle glaucoma (POAG) is an important cause of irreversible blindness worldwide (Quigley 1996). The disease results in a characteristic degeneration of the optic nerve that is usually associated with an elevation of intraocular pressure. Pressure within the eye is dependent on the rate of production of a fluid (aqueous humor) by the ciliary body and on the rate of removal of the fluid by the trabecular meshwork.

Relatives of POAG patients have an increased risk of developing glaucoma, which suggests that genetic factors are an important component of POAG susceptibility (Leske 1983). Adult-onset POAG is inherited as a non-Mendelian trait, whereas forms of juvenile-onset POAG exhibit autosomal-dominant inheritance (Wiggs et al. 1995). One locus for juvenile glaucoma was initially mapped to 1q23 (Sheffield et al. 1993; Richards et al. 1994; Wiggs et al. 1994) and was subsequently refined to a 3-cM interval (Belmouden et al. 1997). In recent studies, evaluation of candidate genes mapped to this region has led to the identification of mutations in the TIGR/Myocilin gene (Stone et al. 1997). This gene was originally cloned, from cultured trabecular meshwork cells, as a steroid-response protein, named "trabecular meshwork-induced glucocorticoid response protein" (TIGR; Nguyen et al. 1993). The gene was isolated subsequently from a retinal cDNA library and was shown to be localized to the cilium connecting the inner and outer segments of photoreceptor cells (named "myocilin"; Kubota et al. 1997). Mutations have been detected in the TIGR/myocilin gene in juvenile- and adult-onset glaucoma pedigrees, and in populations of sporadic adult- and juvenile-onset patients (Adam et al. 1997; Stone et al. 1997; Suzuki et al. 1997; Alward et al. 1998; Morissette et al. 1998). Because the prevalence of mutations in TIGR/myocilin has not yet been investigated in a large number of pedigrees affected by juvenile- and adult-onset glaucoma, we have performed SSCP and sequence analysis in 152 affected families and in 104 individuals with macular degeneration but with normal intraocular pressures and optic nerves.

The pedigrees used for this study are all of North American origin and were ascertained and sampled at the New England Medical Center and the Duke University Medical Center. The diagnostic criteria for POAG included intraocular pressure >22 mm/Hg and glaucomatous optic-nerve damage with consistent visual-field loss. Gonioscopic evaluation showed open angles (at least grade III) without any associated abnormalities. Individuals were identified as affected by adult-onset POAG if onset of the disease occurred after age 35 years, and as affected by juvenile-onset POAG if onset occurred before age 35 years. All pedigrees included in this study had at least two affected individuals. The control population underwent a complete ocular examination and did not show evidence of elevation of intraocular pressure or of optic-nerve disease.

For mutation detection, a BAC clone (244L10) containing the TIGR/myocilin gene was identified by the screen of an arrayed BAC library (Research Genetics, Inc.). The BAC DNA was used as a source for the genomic sequence, and three exons separated by two introns were identified. Oligonucleotide primers (sequences available on request from Janey L. Wiggs), developed from the intron sequences flanking the intron/exon boundaries and from the cDNA sequence, were used to selectively amplify overlapping fragments of the coding sequence and the exon/intron splice sites. Sixty-eight families (25 juvenile-onset and 43 adult-onset) were screened for mutations in the entire coding sequence of the gene, and an additional 84 adult-onset families were screened for mutations in the third exon. Abnormal SSCP patterns were observed in 17 of the 152 families screened. To identify sequence variants, direct DNA sequencing was performed bidirectionally, by use of the Amersham Life Science dideoxy sequencing kit. All DNA sequence alterations that resulted in a change of amino acid were found in the third exon of the gene. Deletions, insertions, or splice-site mutations were not found. In the population of individuals without glaucoma, nine individuals had a T→C transition at position 1041, which resulted in a wobble mutation

(TYR347TYR). No other base-pair changes were found in the control population.

Two different missense mutations were found in 2 of the 25 pedigrees affected by juvenile-onset POAG (table 1). One of these (TYR437HIS) has previously been identified in two pedigrees from North America (Stone et al. 1997; Alward et al. 1998). A second mutation (PRO370LEU) was identified in a three-generation POAG pedigree of English ancestry that settled in Maine >100 years ago. This mutation has also been identified in one Japanese family (Suzuki et al. 1997) and in two unrelated French families (Adam et al. 1997). The recurrence of this mutation in pedigrees of varied ethnicity suggests that the loss of the proline at this position may severely affect the function of the protein. One juvenile pedigree had a C→T transition at position 366, which resulted in a wobble mutation (122GLY122).

In our analysis, only 8% of juvenile-onset pedigrees had identifiable mutations in the TIGR/myocilin gene. This prevalence is lower than that reported in a study by Adam et al. (1997) that showed mutations in five of eight pedigrees, demonstrating linkage to the GLC1A locus. In our study, the majority of pedigrees are too small for accurate detection of linkage to any particular locus. The small number of pedigrees, in our study, with mutations in TIGR/myocilin suggests that additional genes are likely to be responsible for this disease. Genetic heterogeneity of juvenile-onset POAG has previously been suggested (Graff et al. 1995; Wiggs et al. 1995; Avramopoulous et al. 1996; Richards et al. 1996; WuDunn et al. 1996).

Sequence alterations that resulted in a change of amino acid were identified in 5 of 127 families affected by adult-onset POAG. Three of these families had the GLN368STOP mutation, previously reported in pedigrees of North American origin (Stone et al. 1997; Alward et al. 1998). The three pedigrees we studied do not have common ancestry. We did not detect the GLN368STOP mutation in any individuals affected by juvenile-onset glaucoma.

Two adult-onset pedigrees have sequence alterations that do not segregate with the phenotype. Neither of these sequence changes were seen in 104 control patients. Three members of pedigree 27 had a C→T transition, which resulted in a change of the threonine at amino acid 377 to a methionine. Of the four affected individuals in this family, only three were found to have this alteration. No abnormalities in this gene were found in the remaining affected individual (fig. 1), and it remains a possibility that individual 27-3 is a phenocopy. Pedigree 5039 was found to have a DNA sequence-pair change, which caused the glutamate at position 352 to be replaced by a lysine. Of the four affected individuals in this family, one has the mutation, whereas three do not (fig. 1). Of the four unaffected individuals, one has the mutation but, at age 48 years, does not have any evidence of the disease. This sequence change could represent an extremely rare polymorphism, not present in our study or control populations. In support of this hypothesis, this family is of African American origin, whereas 90% of the study and control populations are Caucasian. The TYR437TYR wobble variant was found in eight adult-onset pedigrees.

Previous reports have suggested that 3%–5% of patients with adult-onset glaucoma have mutations in the TIGR/myocilin gene (Stone et al. 1997; Suzuki et al. 1997; Alward et al. 1998). Our results confirm that mutations in the TIGR/myocilin gene are an uncommon cause of adult-onset POAG. These results are consistent with the current heterogeneity of adult-onset POAG. In recent studies, five loci for adult-onset POAG have been discovered: GLC1B (Stoilova et al. 1996), GLC1C (Wirtz et al. 1997), GLC1D (Trifan et al. 1998), GLC1E (Sarfarazi et al. 1998), and GLC1F (Wirtz et al. 1998). The identification of these and other genes responsible for various forms of glaucoma may lead to valuable

Table 1

Mutations Identified in Juvenile- and Adult-Onset POAG

POAG Type and Pedigree	Mutation	Proband Age at Diagnosis (years)	Proband Intraocular Pressure at Diagnosis (mmHg)
Juvenile-Onset POAG:			
4	Pro370Leu (1109 C/T)	6	38
18	Tyr437His (1309 T/C)	16	35
Adult-Onset POAG:			
27	Thr377Met (1131 C/T)	42	24
125	Gln368STOP (1102 C/T)	49	24
5052	Gln368STOP (1102 C/T)	78	28
5055	Gln368STOP (1102 C/T)	53	31

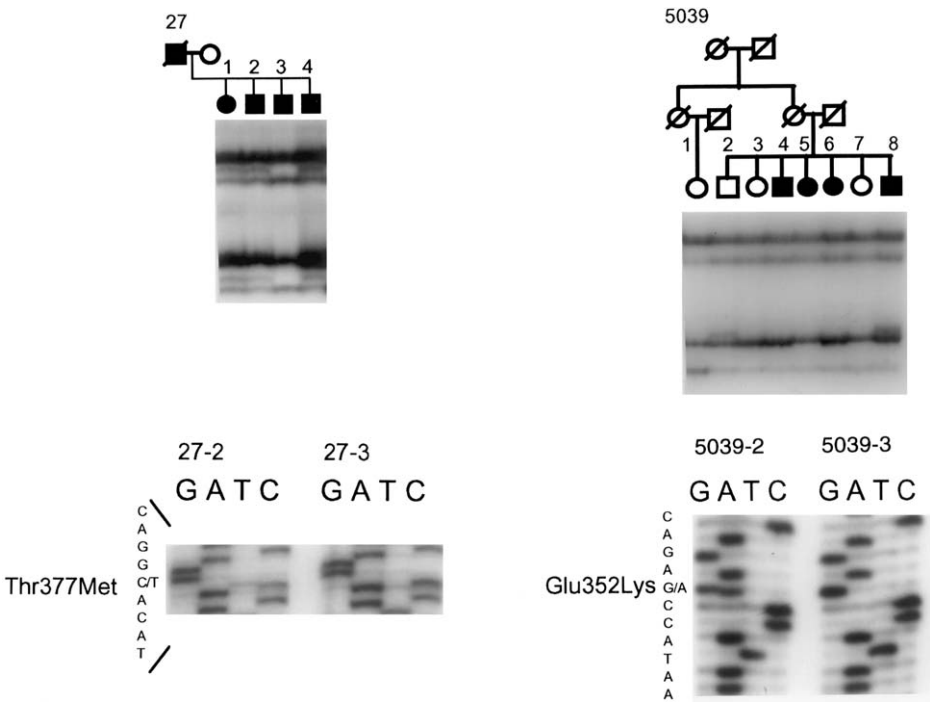

Figure 1 SSCP and DNA sequence analysis of POAG pedigrees 27 and 5039. Affected individuals are shown as blackened circles or squares. SSCP variants are seen in 27-1, 27-2, 27-4, 5039-2, and 5039-8. The DNA sequence of the sense strand shows aT→C transition in 27-2 that results in a change in amino acid 377 (threonine→methionine). The same sequence change is found in 27-1 and 27-4 (data not shown). This sequence change is not found in 27-3. The DNA sequence of the sense strand shows a G→A transition in 5039-2 that is not found in 5039-3, which results in a change in amino acid 352 (glutamate→lysine). This sequence change is also found in 5039-8 but is not found in 5039-1, 5039-4, 5039-5, 5039-6, or 5039-7 (data not shown). All DNA sequence changes were confirmed by sequencing the antisense strand.

insights into the pathophysiology of these important blinding disorders.

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