Letters to the Editor

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Mutations of the *TIGR/MYOC* Gene in Primary Open-Angle Glaucoma in Korea

To the Editor:

Glaucoma affects >3.5 million people in North America. Although treatable in the early stages, often it is not diagnosed and treated in time, which results in irreversible blindness. Primary open-angle glaucoma (POAG), the most common form of glaucoma, represents >50% of glaucoma cases in Western countries (Raymond 1997). POAG is an eye disorder characterized by the progressive excavation of optic disks, typical visual-field defects, and optic-nerve damage. Many families with autosomal dominant POAG have been reported (Brezin et al. 1997). The initial finding of a linkage between the juvenile-onset form of POAG (JOAG) and markers at the GLC1A locus on 1q21–31 (Sheffield et al. 1993) was subsequently confirmed in other families (MIM 137750). Recently, Stone et al. (1997) showed that mutations of the *TIGR/MYOC* (trabecular meshwork–induced glucocorticoid-response protein/myocilin) gene (MIM 601652), which maps at the GLC1A locus, were responsible for JOAG, as well as for middle-age–onset POAG. After publication of that report, other investigators have described various mutations in *TIGR/MYOC* in patients with JOAG/POAG (Adam et al. 1997; Suzuki et al. 1997; Mansergh et al. 1998; Michels-Rautenstrauss et al. 1998). Although a few mutations were found in different exons (Alward et al. 1998), most mutations reported to date are clustered in the third exon. This exon is evolutionarily conserved and bears sequence homology with the olfactomedin gene (Yokoe and Anolt 1993). We investigated whether Korean patients with JOAG/POAG have the mutations of the *TIGR/MYOC* gene. In our report we present two patients whose genomes harbor different *TIGR/MYOC* gene mutations.

After obtaining informed consent, we collected peripheral-blood samples from 45 unrelated patients with POAG who visited the Department of Ophthalmology at the Catholic University Medical Center, Korea. The patients were given diagnoses of POAG on the basis of findings from ocular examinations. Patients with POAG were determined to be affected if intraocular pressure (IOP) was >22 mmHg in both eyes and if the cup/disk ratio was >0.3, with characteristic visual-field loss and gonioscopic grade III or IV. Blood samples were also obtained from 106 patients who had visited the Catholic University Medical Center because of diseases other than POAG and who served as controls. Genomic DNA was extracted from each blood sample by means of DNA-isolation kits for mammalian blood (Boehringer Mannheim). The DNA fragments encoding portions of TIGR/MYOC protein were amplified by means of PCR and were analyzed by cold SSCP. The primers used for PCR are shown in table 1. The nucleotide numbers correspond to those in the work of Nguyen et al. (1998). A PCR reaction was performed in a 30-μl volume containing 50 ng of genomic DNA, 0.2 μM each of forward and reverse primers, 0.19 mM of each deoxyribonucleotide triphosphate (dATP, dCTP, dGTP, and dTTP), 50 mM KCl, 1.5 mM $MgCl_2$, and 10 mM Tris-HCl, pH 8.3. The PCR product was denatured and separated on a 20% polyacrylamid Tris-borate EDTA gel (Novex). The DNA fragments were visualized by staining the gel with ethidium bromide solution. The PCR products exhibiting aberrant SSCP patterns were subcloned and sequenced by means of an ALF express DNA sequencer (Pharmacia) with fluorescent dye–primer chemistry. Multiple clones were sequenced to confirm the presence of both normal and mutant clones.

Of the 45 patients who were screened for mutations, 2 were found to carry variants in the *TIGR/MYOC* gene. We were able to recruit one patient for a family study. This family consists of five members (fig. 1*A*). The proband (individual 3; fig. 1*A*) was given a diagnosis of JOAG at age 15 years. At the time of diagnosis, her IOPs were 29 mmHg (right eye [OD]) and 30 mmHg (left eye [OS]), and she exhibited severe visual-field loss and optic-nerve damage. Because the disease progressed aggressively and medical treatment was not effective, surgery was required to control the progress of glaucoma in both eyes. None of the other family members was given a diagnosis of POAG, although individuals 1, 2, and 4 had slightly elevated IOPs compared with individual 5 (table 2), as well as with those in the control group.

Another patient whom we were unable to recruit for a family study received a diagnosis of POAG at age 59 years and displayed a moderate phenotype. Her IOPs before medication treatment (betaxolol HCl two times a day and dorzolamide HCl three times a day) were 26 mmHg (OD) and 24 mmHg (OS). Severe loss (OU) of visual field was observed, and cup/disk ratios were 0.8 (OD) and 0.6 (OS). Because her sister and one of her daughters were also given diagnoses of POAG, a genetic basis for its etiology could be suggested.

Mutations of the *TIGR/MYOC* gene were detected by SSCP analysis and were confirmed by sequence analysis. SSCP followed by sequence analysis of the proband, individual 3, revealed a C→T transition at nucleotide 201 in exon 1. This alteration resulted in a nonsense mutation at codon 46 (arginine→TAG, amber; fig. 1*Ca*). The complete *TIGR/MYOC* coding region of the proband, individual 3, was sequenced to confirm that there was no alteration other than a C201T transition. This mutation was presumed to result in a truncated protein with 45 amino acids. This proband carried only mutated alleles (fig. 1*B*), which was indicative of homozygosity for the *TIGR/MYOC* gene. Further analysis of her family revealed that her father, mother, and sister were heterozygous for *TIGR/MYOC,* apparently without any detectable symptom. The proband's brother, individual 5, had two normal copies and did not have any symptoms of POAG. The homozygote with this mutation is severely affected, whereas heterozygotes do not display any detectable POAG symptom. Thus, this family shows possible autosomal recessive inheritance of JOAG, whereas other families, which have been described elsewhere, show autosomal dominant inheritance. The heterozygotes may develop POAG later in life, because it is known that late-onset POAG shows age-dependent penetrance. Another possibility is that, because of consanguinity, the proband is homozygous at other loci that may modify the glaucoma phenotype. Importantly, there was an individual with the C201T mutation in the control group (1/106), whose POAG status has not been

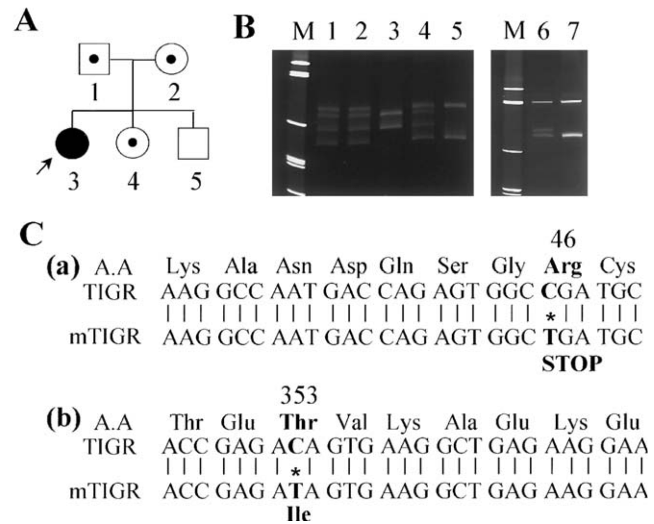

Figure 1 Pedigree of a family, analysis of SSCP, and sequences of regions with mutations in the *TIGR/MYOC* gene. *A,* Pedigree of a family with the C201T mutation. The unblackened symbol denotes a genotypically and phenotypically unaffected individual. The blackened symbol indicates an individual with documented evidence of POAG. A symbol with a dot indicates an obligatory carrier. The arrow indicates the proband. *B,* SSCP analysis of C201T (lanes 1–5) and C1123T (lanes 6 and 7) mutations, described in the text. Lane M, *Hae*III fragments of $\phi \times 174$RF. Lanes 1, 2, 4, and 6, Heterozygotes. Lane 3, Homozygote. Lanes 5 and 7, Normal. *C,* Sequence comparison between the *TIGR/MYOC* gene harboring mutations and the wild type. The altered nucleotides are shown in boldface and are denoted by an asterisk (*). Rows *TIGR,* wild-type sequence. Rows *mTIGR,* sequence from patients with POAG.

Table 1

Primer Sequences for PCR and Conditions for PCR and SSCP

Region (nt[a])	Sequence		Temperature (°C)		Product Size (bp)
	Forward	Reverse	Annealing, for PCR	Running, for SSCP[b]	
88 to 281	5′-TGTGCACGTTGCTGCAGC-3′	5′-ATGGATGACTGACATGGCC-3′	56	10	204
1014 to 1202	5′-ATACTGCCTAGGCCACTGG-3′	5′-CAATGTCCGTGTAGCCACC-3′	62	14	189
1296 to 1493	5′CTGGAACTCGAACAAACCTGG-3′	5′-CATGCTGCTGTACTTATAGCG-3′	60	8	198
−83 to 281	5′-TGGCCACCTCTGTCTTCC -3′	5′-ATGGATGACTGACATGGCC-3′	60		384
177 to int1A	5′- AGGAAGGCCAATGACCAG -3′	5′-TAGGAGAAAGGGCAGGGGAGGC -3′	62		593
int1B to 795	5′-AACATAGTCAATCCTTGGGCC -3′	5′- CGGTGTCTCCCTCTCCACT -3′	56		170
796 to 1316	5′-GATGTGGAGGACTAGTTTGG -3′	5′- CCAGGTTTGTTCGAGTTCCAG -3′	56		521
1296 to 3′UTR	5′-CTGGAACTCGAACAAACCTGG-3′	5′-GCTTGGAGGCTTTTCACATC-3′	60		283

[a] Numbers correspond to those in the work of Nguyen et al. (1998).

[b] Three parts of the MYOC coding region were analyzed by SSCP, and the rest were used for sequencing the *MYOC* gene of the proband, individual 3.

Table 2

Clinical Data on Members of a Family with Familial JOAG and Their Genotypes for the *MYOC* Gene, Determined by SSCP and Sequencing

Pedigree[a]	Age (years)	Vertical Cup/ Disk Ratio		Tension (mmHg)		Gonioscopy	Visual Field[b]	Treatment	Genotype[c]
		OD	OS	OD	OS				
1	42	.5	.5	22	24	IV(D40r)	NA (OU)	None	Heterozygous
2	40	.4	.3	20	20	IV(D40r)	NA (OU)	None	Heterozygous
3	15	.4	.5	29	30	IV(D45r)	Nasal step (OU)	Trabe (OU)	Homozygous
4	13	.4	.5	22	22	IV(D40r)	NA (OU)	None	Heterozygous
5	12	.6	.4	17	17	IV(D40r)	NA (OU)	None	Wild type

[a] As in figure 1*A*.
[b] NA = not affected.
[c] As determined by SSCP and sequencing.

documented. This suggests that the C201T mutation may not be so rare in the Korean population, which supports the notion of autosomal recessive inheritance of JOAG. However, at this point, we cannot exclude the possibility that the proband's parents are genetically related. Whereas further study is required to determine the recessive inheritance for JOAG/POAG, this family provides an opportunity to elucidate a molecular mechanism for *TIGR/MYOC* in POAG pathogenesis.

The mutation identified in a different family was heterozygous and was confirmed to be a C→T transition at nucleotide 1123 (fig. 1*Cb*). None of the 106 patients in the control group was found to contain the same mutation. The change resulted in the conversion Thr353 Ile (fig. 1*B,* lane 6, and *Cb*). This mutation resided in the olfactomedin-homology region in the third exon yet was different from all the mutations in the *TIGR/MYOC* gene that have been reported to date. Threonine at 353 residue is a putative phosphorylation site by protein kinase C, predicted by the PROSITE Pattern DB search program. Thus, the phosphorylation of TIGR/MYOC may play a role in the regulation of IOP in trabecular-meshwork cells.

The prevalence of C201T and C1123T mutations in the *TIGR/MYOC* gene in Korean patients was estimated by screening 45 unrelated patients with JOAG/POAG. The prevalence of each mutation in the *TIGR/MYOC* gene was 1 (2.2%) of 45 in patients with JOAG/POAG; thus the combined prevalence was 4.4%.

With support from another report (Kee and Ahn 1997), the present study indicates that the mutations in the *TIGR/MYOC* gene are responsible for JOAG/POAG in Korean patients. That report also described a phenotypic homozygote with JOAG linked to GLC1A, and it alluded to the autosomal recessive inheritance of JOAG. The analysis of the function of the different mutant forms of *TIGR/MYOC* in the regulation of IOP will enhance our understanding of POAG pathogenesis.

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Electronic-Database Information

The URLs for data in this study are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for POAG [MIM 137750] and *TIGR/MYOC* [MIM 601652])

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