TIGR Gene in Primary Open-angle Glaucoma and Steroid-induced Glaucoma

Changwon Kee, M.D. and Byung-Heon Ahn, M.D.

Department of Ophthalmology, Samsung Medical Center, College of Medicine, Sung Kyun Kwan University, Seoul, Korea

To identify TIGR gene mutation in two Korean pedigrees of primary open-angle glaucoma (POAG), and in 25 steroid-induced glaucoma patients, TIGR gene assay was performed. Genomic DNA was extracted from peripheral blood and the TIGR gene was amplified by polymerase chain reaction (PCR). The PCR amplification products were evaluated by single-stranded conformation polymorphism and direct DNA sequencing. In POAG families, not only those patients clinically diagnosed as glaucoma, but also most of their siblings, in whom glaucoma had not yet been detected, had a proline-to-serine (TCC-to-CCC) mutation in codon 334. However, no steroid-induced glaucoma patients showed TIGR gene mutation. TIGR gene mutation, in familial POAG, was found in a different codon from that previously reported. The TIGR gene does not, however, seem to be related to steroid-induced glaucoma.

Key words: TIGR gene, primary open-angle glaucoma, steroid-induced glaucoma, mutation

INTRODUCTION

Clinically, patients with juvenile-onset open-angle glaucoma (JOAG) are identical to patients with primary open-angle glaucoma (POAG) in that both groups exhibit elevated intraocular pressure and optic nerve cupping in the presence of a gonioscopically normal trabecular meshwork, and the mode of inheritance is autosomal dominant.1-4

A genetic locus associated with JOAG (GLC1A) was identified on chromosome 1q21-q31 through genetic linkage analysis.5 Continuous linkage analysis isolated the interval containing the GLC1A gene to a 3-centimorgan region of chromosome 1q.6 Further evaluation of marker haplotypes showed that the GLC1A gene lies between markers D1S1619 and D1S3664.7 There are several genes mapping to the GLC1A region of chromosome 1, among which the TIGR (Trabecular Meshwork Inducible Glucocorticoid Response) gene is thought to be a candidate for glaucoma.8 This gene, which regulates the expression of glucocorticoid receptor protein, is expressed in the ciliary body9 and trabecular meshwork.8 The TIGR gene product has been postulated to cause increased intraocular pressure by obstruction of the aqueous humor outflow channel.10

TIGR gene mutation has recently been reported in POAG,7 and we therefore attempted to detect this mutation in Korean familial POAG patients.

In addition, since the TIGR gene is associated with glucocorticoid receptor protein, we also investigated the association between its mutation and steroid-induced glaucoma.

Reprint requests to Changwon Kee, M.D., Department of Ophthalmology, Samsung Medical Center, 50 Ilwon-Dong, Kangnam-Ku, Seoul 130-230, Korea.
SUBJECTS AND METHODS

Two pedigrees of POAG and 25 steroid-induced glaucoma patients were enrolled in this study. Tonometry, gonioscopy, optic disc examination, and perimetry were performed in all patients.

The criteria for diagnosis of POAG are enlargement of cup-to-disc ratio, glaucomatous visual field defect, and intraocular pressure of above 21 mmHg.

All steroid-induced glaucoma patients had a history of using steroid eye drops for more than six months and met the above criteria for diagnosis of POAG.

Three ml of peripheral blood was withdrawn and genomic DNA was extracted by a genomic DNA isolation kit (Wizard genomic DNA purification kit, Promega Co.). Amplification of the TIGR gene was performed by polymerase chain reaction (PCR). Briefly, a 15 ng sample of each patients DNA was used for the template in a 50 μl PCR mixture containing the following: 5 μl of 10 × buffer (100mM tris-HCl, pH 8.3, 500mM KCl, 15mM MgCl₂); deoxynucleotides dCTP, dATP, dGTP, and dTTP (300 M of each); 1 pmol of each primer; and 0.25 units of Taq polymerase. Samples were denatured for 5 minutes at 94°C and incubated in a DNA thermocycler for 35 cycles under the following conditions: 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds. The amplification products were evaluated by single stranded conformation polymorphism (SSCP) analysis. Briefly, 5ml of stop solution (95% formamide, 10mM NaOH, 0.05% bromophenol blue, 0.05% xylene cyanol) was added to each PCR sample. Amplification products were denatured for 3 minutes at 94°C and electrophoresed on 6% polyacrylamide-5% glycerol gels at 20 W for 3 hours. After electrophoresis, the gels were dried and autoradiography was performed.

PCR products were bidirectionally sequenced by a Sequenase version sequencing kit 2.0 (Amersham Life Science).

RESULTS

The first POAG pedigree involved three generations and the second pedigree, two generations (Fig. 1).

In the first pedigree, glaucoma had already been diagnosed in two second generation females, and they had undergone treatment. The father of these two patients had already died, so testing for the TIGR gene was not possible; the daughters stated, however, that he did suffer from glaucoma. Their mother was not suffering from glaucoma and no

Fig. 1. Two POAG pedigrees. Squares = males; circles = females; solid symbols = clinically and genetically affected individuals; open symbols = unaffected individuals; dots within the open symbols = genetically affected individuals; and a diagonal line through the symbol = individual is deceased.

Fig. 2. A photograph of SSCP gel. Mutant TIGR genes showed four bands, whereas, normal TIGR genes showed only two bands.
It has been reported that TIGR gene mutation was isolated in 2.9% of non-familial POAG patients, suggesting that this gene plays a partial role in all open-angle glaucoma cases. Since, however, Stone et al. evaluated only a portion of this gene, associated mutation may have occurred in more than this portion of POAG patients. Besides, since it has been postulated that glaucoma is a multifactorial disease, genes other than TIGR may also play a causative role.

Diagnosis made on the basis of DNA study shows that the prevalence of most genetic disease is usually low; unfortunately, however no treatment is available for these conditions and so the patient can be offered only genetic counselling. In contrast, glaucoma patients are frequently encountered, and in most cases, can be managed with existing drugs or surgery. Since the early detection of a patient at risk for glaucoma can provide an opportunity for management before the development of glaucomatous damage, detection of TIGR gene mutation is therefore significant.

The TIGR gene of glaucoma patients contains four identified loci of mutation, namely tyrosine-to-tyrosine (TAT-TAC) mutation in codon 340, glycine-to-valine (GGC-GTC) mutation in codon 357, glycine-to-stop (CAG-TAG) mutation in codon 361, and tyrosine-to-histidine (TAC-CAC) mutation in codon 430. In our study, however, the above codons were normal, and instead, proline-to-serine (TCC-CCC) mutation in codon 334 was detected in all genetically affected POAG patients. This may be a specific racial difference.

In steroid-induced glaucoma patients, no TIGR gene mutation was detected. This suggests that the TIGR gene is not related to steroid-induced glaucoma, but we evaluated only a portion of the TIGR gene, and the other portion, or other genes, might be associated with steroid-induced glaucoma.

In conclusion, we found that TIGR gene mutation in familial POAG was located in a different codon from that previously reported; however, the TIGR gene does not seem to be related to steroid-induced glaucoma. If early detection of TIGR gene mutation is followed by close observation and the provision of glaucoma therapy, significant visual loss can be prevented.
REFERENCES


