Detection of a New TIGR Gene Mutation in a Japanese Family With Primary Open Angle Glaucoma

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Purpose: To describe a new mutation of the trabecular meshwork-inducible glucocorticoid response protein (TIGR) gene in a Japanese patient with familial primary open angle glaucoma (POAG).

Methods: Standard ocular examinations were performed on the 44-year-old patient, his sister, and mother. DNA sequencing was used to identify the mutation. We also developed a DNA diagnostic method for detecting this missense mutation by polymerase chain reaction-induced mutation restriction analysis (PCR-IMRA).

Results: The patient, father, and sister had been diagnosed as having POAG. The patient and his sister had a Thr448Pro mutation (C→A transition at the nucleotide number 1419) in exon 3. This mutation has not been reported before.

Conclusions: Gene analysis is promising for an early diagnosis among the family members of familial POAG patients and will contribute to early therapy before an occurrence of irreversible visual impairment. **Jpn J Ophthalmol 1999;43:85–88** © 1999 Japanese Ophthalmological Society

Key Words: DNA sequencing, missense mutation, PCR-IMRA, primary open angle glaucoma, trabecular meshwork-inducible glucocorticoid response protein.

Introduction

Glaucoma is characterized by a progressive excavation of the optic disc with a loss of retinal nerve fibers leading to visual field defects. Increased intraocular pressure (IOP) is one of the major contributory factors to this condition, although it is no longer considered to be an essential diagnostic criterion. This disease is the second leading cause of blindness in the world.[1]

The candidate gene linked with open angle glaucoma (OAG), especially for juvenile-onset open angle glaucoma (JOAG), is localized on chromosome 1q21-q31 by genetic linkage analysis.[2] In a recent study, the candidate gene was found to be identical to the trabecular meshwork-inducible glucocorticoid response (TIGR) gene.[3] The TIGR gene was cloned from a human ciliary body library,[4] and was also called the "myocilin" gene (MYOC) when cloned from the retina by Kubota et al.[5] The TIGR gene is composed of three exons of 681, 126, and 1323 nucleotides and encodes 504 amino acid residues.[6]

Stone et al[3] found three mutations in exon 3 of the TIGR gene responsible for POAG. In Japan, two other mutations in the TIGR gene were found by Suzuki et al,[7] however, the mutations reported by Stone have not been found in Japanese POAG families. Recently, Adam et al[6] examined the whole coding sequence and showed that all mutations were localized in exon 3, the exon that encodes the olfactomedin-like domain of the TIGR protein. We examined exon 3 of the TIGR gene by DNA sequencing analysis and found a novel missense mutation in a patient with POAG. We studied this muta-

tion in the family members of this patient by DNA sequencing analysis and polymerase chain reaction-induced mutation restriction analysis (PCR-IMRA).

Methods

Subjects

The patient was a 44-year-old man diagnosed as having POAG at age 32. No record of his IOP at the time of diagnosis was available. We know of his condition before 1994 only by a reference letter from a doctor: earlier medical records were not available. Initially he was treated with drugs, but IOP control was poor. Two years after the initial diagnosis, he underwent bilateral trabeculotomy. After the surgery, he obtained good IOP control without medication for 4 years. At age 39, his IOP rose to 50 mm Hg bilaterally. Reintroduction of medications did not control IOP, and at age 40, he was referred to our hospital for surgical treatment. The IOP was 20 mm Hg in the right eye and 28 mm Hg in the left eye. Gonioscopy revealed a widely open angle without sign of congenital glaucoma. Some peripheral anterior synechiae were found at the site of the previous trabeculotomy. The optic disc showed characteristic glaucomatous changes (cup/disc ratio = 0.9). Based on the classification by Aulhorn[8] modified by Greve and Geijssen,[9] the visual field was at stage v in both eyes. Static perimetry (OCTOPUS program G1) showed the end stage visual field loss. The mean sensitivity was 1.8 and the mean defect was 26.5 in the right eye; the mean sensitivity was 0.7 and the mean defect was 27.6 in the left eye. He underwent successful bilateral trabeculectomy in March 1994, and achieved good IOP control around 10 mm Hg without medication. He showed no progression of visual field loss at his last check-up in May 1998.

The patient's 51-year-old sister has suffered from POAG for 9 years. Her IOP at the time of diagnosis was 32 mm Hg in the right eye and 16 mm Hg in the left. The angles were widely open. The optic discs showed characteristic glaucomatous change with a saucer-like appearance (cup/disc ratio = 0.8 in the right eye and 0.3 in the left). Kinetic perimetry of the right eye showed glaucomatous defects with a nasal step and a paracentral isolated scotoma. Perimetry of the left eye revealed no abnormality. She underwent trabeculotomy on the right eye in July 1989 and achieved good IOP control around 12 mm Hg without medication until May 1998. The IOP of her left eye rose to about 20 mm Hg with 2% carteolol in 1990. From 1990 to 1998 her left IOP ranged from 16–45 mm Hg. IOP in her left eye rose to as high as 38–45 mm Hg when she failed to use eyedrops, although it fell to 16–19 mm Hg with medication (0.5% timolol and isopropyl unoprostone).

We learned that the patient's father was also diagnosed as having POAG at age 35, but successfully maintained his visual acuity until his death resulting from renal failure at age 67. The patient's mother, who was under treatment for diabetic retinopathy, showed no signs of glaucoma.

Informed consent was obtained from each person before peripheral blood samples were collected from the patient and his sister and mother. Genomic DNA was purified from the blood samples by use of a Sepa Gene Kit (Sanko Junyaku, Tokyo).

DNA Sequencing Analysis

The coding sequence in exon 3 of the TIGR gene was divided into three segments and these segments were amplified with the primer sets shown in Table 1. The PCR reactions were carried out in a total volume of 25 μL containing 100 ng genomic DNA, 50 pM of each primer, 0.2 mM of each dNTP, 1.5 mM $MgCl_2$, and 0.25 μL Taq polymerase (Ampli Taq Gold; Perkin-Elmer, Foster City, CA, USA).

The amplifications were carried out with an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 minute. A final extension at 72°C for 5 minutes completed the reactions. The products were gel purified, then aliquots (60 ng) were sequenced in an automated fluorescent sequencer (Model 310; Applied Biosystems, Foster City, CA, USA), using dye-primer chemistry.

Table 1. Primers for PCR Amplification

	Sense Primer (5′ → 3′)	Antisense Primer (5′ → 3′)
Exon 3-1	GGATTAAGTGGTGCTTCG	AATACGGGAACTGTCCGTGG
Exon 3-2	ATACTGCCTAGGCCACTGGA	CATGCTGCTGTACTTATAGCGG
Exon 3-3	GAACTCGAACAAACCTGGGA	TGAGCATCTCCTTCTGCC

PCR-IMRA for TIGR Thr448

The genomic DNAs of the family members were subjected to PCR-IMRA with a mismatched sense primer that had one base substitution C→A at nucleotide number 1417 (Figure 2). The sequence was numbered from the transcription initiation site reported by Adam et al.[6] The PCR condition was the same as described above. The amplified 108-bp PCR product was digested with *Sau*3A1 at 37°C for 2 hours. The digests were electrophoresed on a 3% agarose gel.

Results

The patient had a heterozygous A→C mutation at nucleotide number 1419, resulting in an amino acid change from threonine to proline, Thr448Pro (Figure 1). His sister and mother were also examined by DNA sequencing. His sister with POAG had the same gene mutation, whereas his mother did not. We confirmed this missense mutation by PCR-IMRA (Figure 2).

Discussion

The Thr448Pro mutation is a novel TIGR variant. In the previous study, Stone et al[3] described the TIGR gene mutation in the 364th, 368th, and 437th amino acid residues. In nucleotide numbers 1046–1196 and 1331–1483 of exon 3, they showed that the prevalence of these mutations was 4.4% in familial POAG and 2.9% in unselected POAG patients in the United States. Suzuki et al[7] and Stoilova et al[10]

Figure 2. (**A**) Diagram of PCR-IMRA for TIGR Thr448Pro mutation. Sense primer has one substitution, C→A at the nucleotide number 1417 (small arrow). Mutant TIGR gene with the A→C transition at nucleotide number 1419 (*) creates new restriction site for *Sau*3A1, yielding two fragments of 90 and 18 bp on *Sau*3A1 digestion. (**B**) Segregation of Thr448Pro mutation in two-generation family affected with POAG. Black symbols indicate individuals with POAG. White symbol indicates individual who is clinically unaffected. Photograph of an agarose gel is shown below pedigree symbol of family member whose DNA was analyzed on that lane. Data of patient (arrow) and his sister showed two abnormal bands of 90 and 18 bp in addition to normal 108-bp band. Data of his mother and control showed only normal bands. No 18-bp band is visible in this photograph. Base pair sizes of PCR products are indicated on right.

Figure 1. DNA sequencing data for TIGR codon 448. Samples: (**A**) wild and (**B**) mutant types. Upper nucleotide sequences show raw data from which complementary allele of normal or mutant TIGR gene was sequenced. Lower sequences correspond to sense allele. Single base change (A→C) was present at nucleotide number 1419 in mutated TIGR gene. ......, G: - - -, T: -- -- --, A: and --- - ---, C.

reported mutations in the 337th, 367th, and 370th amino acid residues. Recently, Adam et al[6] examined the whole coding sequence and found mutations of the 246th, 477th, 480th, and 499th amino acid residues. All of the mutations reported so far, including the Thr448Pro mutation, are in exon 3, that encodes the olfactomedin-like domain of TIGR protein.

We used DNA sequencing analysis, which is useful to detect randomly distributed unknown mutations and found this novel missense mutation. We

confirmed this missense mutation by PCR-IMRA. Because the TIGR Thr448Pro mutation does not create a new restriction site or abolish an established one, a modified primer with one base substitution was designed to create a new artificial restriction site, which can be directly screened by *Sau*3A1 digestion of the amplified PCR fragment harboring this mutation.

In the families with previously reported TIGR mutation, the mode of inheritance is autosomal dominant with high penetrance. In this family, the patient and his sister with POAG had this mutation, whereas their mother did not. Although we could not examine blood samples of the patient's deceased father, we were able to review his medical records and confirmed that he had glaucoma. With this finding, the mutation in this family is suspected to be inherited in an autosomal dominant manner.

The clinical features of the Thr448Pro mutation in our series were similar to those of eyes with other mutations. At times, the patient's bilateral IOP might be as high as 50 mm Hg. Topical medications were insufficient in controlling his IOP and filtrating surgeries were required. It should be emphasized that the trabeculotomy was effective for 4 years for the patient, and at least 9 years for his sister.

The TIGR gene is highly expressed in trabecular meshwork,[4,6] which is the site of aqueous humor resorption. Although the precise molecular mechanism is still unclear, it is speculated that the amino acid change in codon 448 may have caused structural or functional impairments of the TIGR protein that led to large resistance against the aqueous outflow through the trabecular meshwork and resulted in an elevated IOP.

POAG is one of the most common causes of blindness, but can be successfully treated by controlling the IOP level with existing drugs or surgical approaches in the majority of cases. The main difficulty is in diagnosing this disease. Gene analysis seems promising for detecting the disease among family members of familial POAG patients and could contribute to early therapy before the occurrence of irreversible visual impairment.

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