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A sequence change (Arg158Gln) in the leucine zipper-like motif region of the MYOC/TIGR protein

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Abstract The myocilin/trabecular meshwork-inducible glucocorticoid response (*MYOC/TIGR*) gene was identified as a gene that caused open angle glaucoma (OAG). Single-strand conformation polymorphism analysis and subsequent sequence analysis were performed for the *MYOC/TIGR* gene in 120 unrelated Japanese OAG patients with increased intraocular pressure (IOP), 116 unrelated OAG patients without increased IOP, and 106 unrelated control subjects without glaucoma. An Arg158Gln sequence change in the leucine zipper-like motif (LZM) region in the myosin-homology domain was found in 2 OAG patients with or without increased IOP, and in a 56-year-old control subject without glaucoma. This is the first report of missense sequence change in the LZM region of the MYOC/TIGR protein in subjects showing various phenotypes, including a control subject. These findings suggest that Arg158Gln in the LZM region is probably a rare nondisease-causing polymorphism, despite its important role in this region, because it was found in a control subject, although Arg158Gln was previously reported as a probable disease-causing mutation.

Key words The *MYOC/TIGR* gene · *GLC1A* · Open angle glaucoma · Japanese · Arg158Gln · Leucine zipper-like motif

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Introduction

Glaucoma is one of the most common eye diseases and can potentially result in bilateral blindness. It was estimated that nearly 66.8 million people worldwide would be affected by glaucoma by the year 2000 (Quigley 1996). The disease is characterized by an excavated optic nerve head and the progressive loss of visual field.

The myocilin/trabecular meshwork-inducible glucocorticoid response (*MYOC/TIGR*) gene was identified as a gene that caused open angle glaucoma (OAG) (Stone et al. 1997). It consists of three exons and encodes 504 amino acid residues (Adam et al. 1997). The amino acid sequence is highly homologous to the myosin in the N-terminal region and to the olfactomedin in the C-terminal region (Kubota et al. 1997). The myosin-homology domain contains a leucine zipper-like motif (LZM) (Kubota et al. 1997), and this kind of motif found in the tropomyosin-like zipper protein is thought to be involved in interactions with myosin (Bikle et al. 1993). These findings suggest that LZM in the MYOC/TIGR protein may play an important role in maintaining the normal function of the MYOC/TIGR protein. Kubota et al. (2000) reported that missense sequence change (Arg158Gln) in this motif region was probably responsible for OAG, although the olfactomedin-homology domain of the MYOC/TIGR protein appears to be the focus of pathogenic mutations in patients with OAG (Adam et al. 1997). In this study, we found Arg158Gln in subjects showing various phenotypes, including a control subject without glaucoma, we report the genotype and phenotypes of these patients.

Subjects and methods

Subjects

Informed consent was obtained and peripheral blood was collected from 120 unrelated Japanese OAG patients with increased intraocular pressure (IOP), 116 unrelated

Table 1. Characteristics of subject population

	OAG patients with increased IOP (n = 120)	OAG patients without increased IOP (n = 116)	Control subjects (n = 106)
Age at diagnosis (years; mean ± SD)	53.1 ± 15.9	59.0 ± 13.1	64.8 ± 11.1
Range	13–87	22–84	41–84
Sex			
Male	77 (64.2)	44 (37.9)	33 (31.1)
Female	43 (35.8)	72 (62.1)	73 (68.9)
Family history of glaucoma	27 (22.5)	24 (20.7)	0 (0)
Maximum known intraocular pressure (mmHg; mean ± SD)	29.3 ± 9.9	18.6 ± 1.9	15.0 ± 2.8
Range	22–74	14–21	8–20

Data values are numbers (percentages) unless otherwise indicated
OAG, Open angle glaucoma; IOP, intraocular pressure

Japanese OAG patients without increased IOP, and 106 unrelated Japanese control subjects without glaucoma. The study protocol was approved by the Ethics Committee of Yamanashi Medical University. The age at diagnosis in the OAG patients with increased IOP ranged from 13 to 87 years (mean ± SD, 53.1 ± 15.9 years), and the age in the OAG patients without increased IOP ranged from 22 to 84 years (mean ± SD, 59.0 ± 13.1 years). In the OAG patients with increased IOP, 27 patients had a family history of glaucoma (22.5%), and in the OAG patients without increased IOP, 24 patients had a family history of glaucoma (20.7%). The mean maximum known IOP was 29.3 ± 9.9mmHg (range, 22 to 74mmHg) in the OAG patients with increased IOP, and the mean maximum known IOP was 18.6 ± 1.9mmHg (range 14 to 21mmHg) in the OAG patients without increased IOP. The age in control subjects ranged from 41 to 84 years (mean ± SD, 64.8 ± 11.1 years); their mean maximum known IOP was 15.0 ± 2.8mmHg (range 8 to 20mmHg) (Table 1).

Mutation analysis

Genomic DNA was purified with a DNA Extractor WB Kit (Wako, Osaka, Japan), and screened for the *MYOC/TIGR* gene mutations, using single-strand conformation polymorphism (SSCP) analysis. The three exons of the *MYOC/TIGR* gene were amplified by dividing the two longer exons into overlapping polymerase chain reaction (PCR) products. The 13 PCR amplicons were obtained using the primer pairs reported by Alward et al. (1998). The PCR reactions were carried out in a total volume of 20μl, containing 100ng genomic DNA, 4pmol of each primer, 0.2mM of each dNTP, 1.5mM of $MgCl_2$, and 0.5U of Taq polymerase (TaKaRa Taq; Takara, Tokyo, Japan) in a thermocycler (model AB-1820; ATTO, Tokyo, Japan). Amplification was carried out with an initial denaturation at 94°C for 5min, followed by 25 cycles of denaturation at 94°C for 30s, annealing at 55°C for 30s, and extension at 72°C for 1min. A final extension at 72°C for 7min completed the reactions. After amplification, 4μl of stop solution (90% formamide, 5mM ethylenediamine tetraacetic acid (EDTA), 0.05% bromophenol blue, 0.05% xylene cyanol) was added to 4μl of each sample. Amplification products were loaded on polyacrylamide gels (GeneGel Excel 12.5/24 Kit; stacking gel, T = 6%; C = 3%; separating gel, T = 12.5%; C = 2%; Amersham Pharmacia Biotech, Amersham Place, UK), after denaturation for 5min at 95°C, and electrophoresed at 20W for 1h and 40min at the most appropriate temperature for amplification products (5°C, 10°C, or 15°C), using an electrophoresis unit (GenePhor; Amersham Pharmacia Biotech). After the electrophoresis, the gels were stained with silver nitrate in an automated gel stainer (Hoefer; Amersham Pharmacia Biotech). Abnormal PCR products identified by SSCP analysis were sequenced using fluorescent dideoxynucleotides in an automated sequencer (model 310; ABI Prism Biosystem, Foster City, CA, USA). All sequencing was bidirectional.

Results

Of the 236 OAG patients, 2 had a heterozygous G-to-A change at nucleotide 473 in the first exon of the *MYOC/TIGR* gene, resulting in an amino acid change from arginine to glutamine: Arg158Gln (Fig. 1). This sequence change was also found in 1 of the 106 control subjects without glaucoma. These 3 subjects simultaneously had heterozygous G-to-A changes at 83bp upstream of the promoter site and at nucleotide 227, resulting in an amino acid change from arginine to lysine: Arg76Lys, which was found in 14 of the 236 patients with OAG and in 8 of the 106 control subjects.

Case 1

A 43-year-old man with no family history of glaucoma had had blurred vision in his right eye at the age of 38, when the IOP was high in both eyes. He was referred to us because of insufficient reduction in IOP despite his having used topical antiglaucoma medications. At the initial examination, his best corrected visual acuity was 1.2 in each eye. His IOP was 23mmHg in the right eye and 28mmHg in the left eye with 0.5% timolol maleate (Timoptol; Banyu, Tokyo, Japan) and 0.1% dipivefrine hydrochloride (Pivalephrine; Santen, Osaka, Japan) twice daily. Gonioscopy revealed a widely

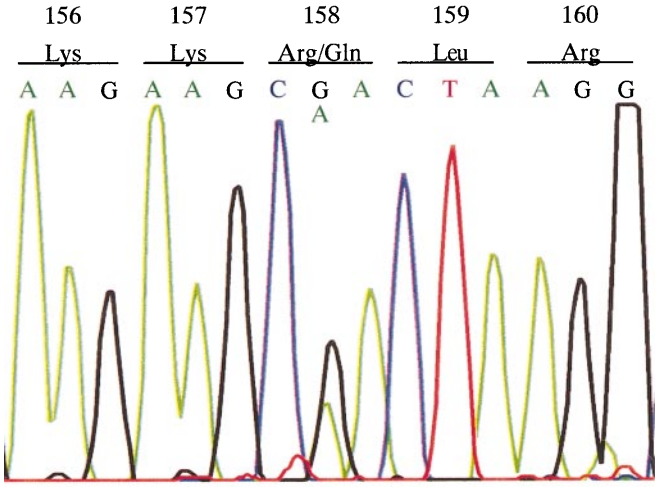

Fig. 1. Electrophorograms resulting from automated sequencing, showing Arg158Gln. A heterozygous G-to-A change at nucleotide 473 in the first exon of the *MYOC/TIGR* gene, resulting in an amino acid change from arginine to glutamine

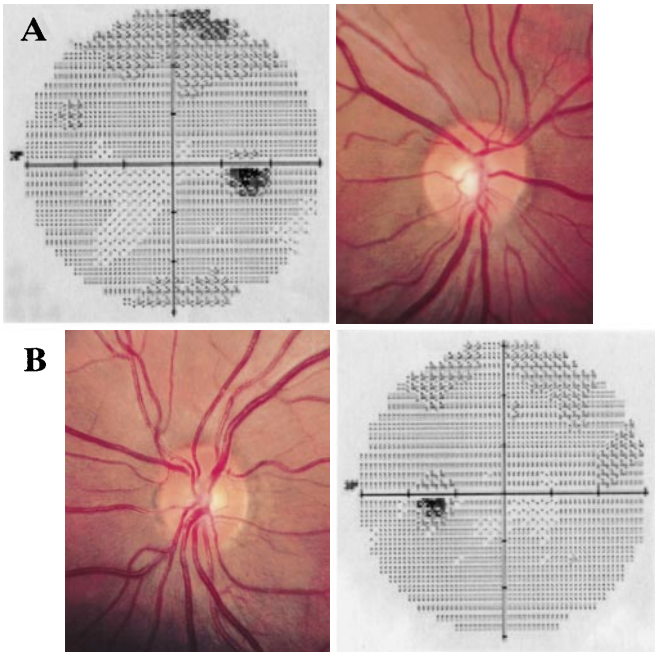

Fig. 2A,B. Visual field and optic disc of patient (case 1) with Arg158Gln in the MYOC/TIGR protein. **A** Right eye; **B** left eye. Although glaucomatous disc cupping and visual field defect were not detected, his maximum known intraocular pressure (IOP) was remarkably high (36 mmHg)

open angle in both eyes. Although ophthalmoscopic examination revealed no glaucomatous changes in the optic disc, and automated static perimetry (Humphrey Visual Field Analyzer 30-2 [HFA 30-2]; Humphrey Instruments, San Leandro, CA, USA) demonstrated no visual field defects in either eye (Fig. 2A,B), he was diagnosed with OAG because his maximum known IOP was remarkably high (36 mmHg).

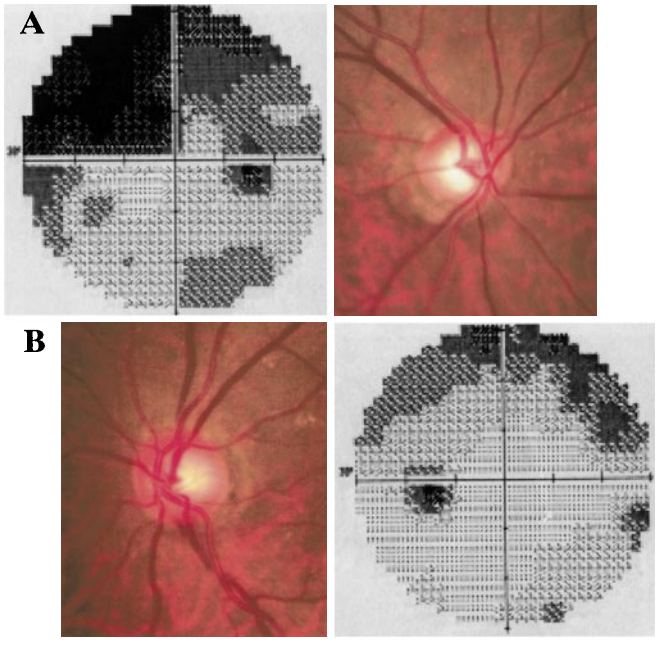

Fig. 3A,B. Visual field and optic disc of patient (case 2) with Arg158Gln in the MYOC/TIGR protein. **A** Right eye; **B** left eye. Glaucomatous disc cupping and visual field defect were observed. Her maximum known IOP was 16 mmHg

Case 2

A 76-year-old woman was seen in 1998, when she complained of visual loss. She had no family history of glaucoma. At the initial examination at the age of 74, her best corrected visual acuity in the right eye had been hand motion, because of cataract, and 1.0 in the left. After cataract surgery, her best corrected visual acuity in the right eye improved to 1.0. Ophthalmoscopic examination revealed moderately excavated optic discs in both eyes, with a splinter hemorrhage at the inferotemporal edge of the left optic disc (Fig. 3A,B). HFA30-2 showed an altitudinal visual field defect in the right eye and an arcuate defect in the left eye (Fig. 3A,B). Gonioscopy revealed a widely open angle in both eyes. The IOP never exceeded 16 mmHg, without medication, and the visual field defects were stable during the following 2 years. An X-ray computerized tomography study revealed no signs of intracranial disease that would cause visual field defects. She was diagnosed with OAG without increased IOP.

Case 3

A 56-year-old man with mild cataract with no family history of glaucoma served as a normal control subject. His best corrected visual acuity was 1.0 in each eye. No signs of glaucoma were found in the optic disc in either eye, with an IOP of 13 mmHg in his right eye and an IOP of 15 mmHg in his left eye.

The family members

The family members of these three individuals (cases 1, 2, and 3) were requested to undergo ophthalmoscopic and genetic studies. Only one of the five children of case 2, a 49-year-old man, was studied. His IOP was 14 mmHg in the right eye and 13 mmHg in the left eye, with no glaucomatous changes in the optic disc in either eye. He did not have the Arg158Gln sequence change in the MYOC/TIGR protein.

Discussion

Twelve amino acid sequence changes in the MYOC/TIGR protein have been reported in Japanese patients with OAG (Suzuki et al. 1997; Fingert et al. 1999; Yokoyama et al. 1999; Kawase et al. 2000; Kubota et al. 2000). Of these 12 sequence changes, 5 were found only in Japanese. Arg158Gln may also be a specific sequence change in Japanese, because it has not been found, despite the screening of more than 2000 patients with OAG and more than 1000 control subjects, in other ethnic groups (Adam et al. 1997; Stoilova et al. 1997, 1998; Allingham et al. 1998; Angius et al. 1998; Brezin et al. 1998; Kennan et al. 1998; Mansergh et al. 1998; Michels-Rautenstrauss et al. 1998; Morissette et al. 1998; Richards et al. 1998; Wiggs et al. 1998; Damji et al. 1999; Fingert et al. 1999; Yoon et al. 1999; Lam et al. 2000).

Arg158Gln was reported as a probable disease-causing mutation because it has previously been found only in a 12-year-old OAG patient without increased IOP (Kubota et al. 2000). The MYOC/TIGR protein contains an LZM, which consists of two subgroups (amino acid residues 85–99 and 117–166) in which leucine residues appear three and eight times, respectively, at every seventh position (Adam et al. 1997). Arg158Gln occurs in the latter subgroup of LZM and may be responsible for OAG, by preventing the normal function of the LZM by altering the charge in this motif region. The LZM is considered to play an important role in the interaction between proteins, in polymerization as well as dimerization (Alber 1992). Previous studies have reported that kidney renin-binding protein with mutations in the LZM neither bound to renin nor formed a homodimer (Inoue et al. 1991), and that mutations in the LZM of human immunodeficiency virus type 1 gp41 dominantly inhibited infectious virus production (Chen et al. 1998). Because MYOC/TIGR proteins are thought to form homodimers (Nguyen et al. 1998), it is possible that Arg158Gln may interfere with their dimerization.

However, in this study, Arg158Gln was found not only in two OAG patients but also in a 56-year-old control subject without glaucoma, which means that Arg158Gln is probably a rare non-disease-causing polymorphism that just happens to alter the amino acid structure of the MYOC/TIGR protein, as it was found in a control subject. Mouse and rat have Gln in this position, and this observation supports the nondisease-causing polymorphic nature of this amino acid change in codon 158.

Although these findings suggest that Arg158Gln in the LZM region of the MYOC/TIGR protein is not responsible for OAG, despite its important role in this region, other missense sequence changes without Arg158Gln in this motif region, especially a sequence change in the leucine residue composing zipper-like motif, may be responsible for OAG. Further investigation will be needed to elucidate the effect of amino acid sequence changes in the LZM region of the MYOC/TIGR protein.

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