A novel MYOC heterozygous mutation identified in a Chinese Uygur pedigree with primary open-angle glaucoma

Su-ping Cai, Paerheti Muhemaiti, Yan Yin, Hongbo Cheng, A. Di Ya, Maliyamu Keyimu, Xu Cao, Ning Fan, Liqiong Jiang, Naihong Yan, Xiaomin Zhou, Yun Wang, Xuyang Liu

(The first two authors contributed equally to the work)

1Shenzhen Eye Hospital, Jinan University, Shenzhen, P.R.China; 2A Di Ya Eye Hospital, Wulumuqi, Xinjiang, P.R.China; 3Ophthalmic Laboratories, Translational Neuroscience Center, West China Hospital, Sichuan University, P.R.China

Purpose: To characterize the clinical features of a Chinese Uygur pedigree with primary open-angle glaucoma (POAG) and to identify mutations in two candidate genes, trabecular meshwork inducible glucocorticoid response (MYOC/TIGR) and human dioxin-inducible cytochrome P450 (CYP1B1).

Methods: Twenty one members from a Chinese Uygur family of four generations were included in the study. All participants underwent complete ophthalmologic examinations. Five were diagnosed as POAG, four as glaucoma suspects, and the rest were asymptomatic. Molecular genetic analysis was performed on all subjects included in the study. All exons of CYP1B1 and MYOC were amplified by polymerase chain reaction (PCR), sequenced and compared with a reference database. The variations detected were evaluated in available family members as well as 102 normal controls. Possible changes in structure and function of the protein induced by amino acid variance were predicted by bioinformatics analysis.

Results: Elevated intraocular pressure and late-stage glaucomatous cupping of the optic disc were found in five patients of this family. A novel heterozygous missense mutation c.1151 A>G in exon 3 of MYOC was found in all five patients diagnosed as POAG and four glaucoma suspects, but not in the rest of the family members and 102 normal controls. This mutation caused an amino acid substitution of aspartic acid to glycine at position 384 (p. D384G) of the MYOC protein. This substitution may cause structural and functional changes of the protein based on bioinformatics analysis. No mutations were found in CYP1B1.

Conclusions: Our study suggests that the novel mutation D384G of MYOC is likely responsible for the pathogenesis of POAG in this pedigree.

Glaucoma is one of the leading causes of irreversible blindness in the world and is a neurodegenerative disorder characterized by progressive loss of retinal ganglion cells that results in the excavation of the optic disc and gradual constriction of visual field [1]. It is usually associated with elevation of intraocular pressure (IOP) [2]. The most prevalent form of glaucoma is primary open-angle glaucoma (POAG; OMIM 137760) [3]. POAG has two forms: juvenile and adult onset, with the latter most commonly seen. Juvenile open-angle glaucoma (JOAG) may manifest clinically between the ages of 3 and 30 [4,5], while adult POAG usually occurs after the age of 40 [6,7]. Although the exact mechanisms of POAG are not fully understood, it is generally accepted that genetic factors play an important role in its pathogenesis. About 30%–56% of patients with glaucoma or ocular hypertension (OHT) have a positive family history; first-degree relatives of POAG patients are seven to ten times more likely to have POAG [8,9]. Four genes, including trabecular meshwork inducible glucocorticoid response (MYOC), human dioxin-inducible cytochrome P450 (CYP1B1), optineurin (OPTN), and WD repeat domain 36 (WDR36), have been identified as glaucoma-associated genes [10]. Up to date, more than 70 mutations have been detected in MYOC, the first identified POAG causing gene, worldwide [11,12]. About 90% of the mutations were located in exon 3 of MYOC where the olfactomedin-like domain is located [13]. Of particular interest, the MYOC gene has been reported to interact with CYP1B1 through a digenic mechanism, leading to JOAG [14-16]. Both MYOC and CYP1B1 consist of three exons, but in CYP1B1, only exon 2 and 3 encode the protein. In this study, both MYOC (three exons) and CYP1B1 (exon 2 and 3) genes were analyzed.

METHODS

Family recruitment and clinical examination: A four-generation pedigree with POAG (Figure 1) was recruited from a Di Ya Eye Hospital (Wulumuqi, Xinjiang, P. R. China). No consanguineous marriage was noticed in the family. Twenty one members of the family underwent complete ophthalmologic examinations including slit-lamp biomicroscopy, gonioscopy, IOP measurement (Canon TX-F Non-contact tonometer; Canon Inc., Tokyo, Japan), fundus...
examination and visual field test. All individuals in the control group were healthy and with no history of other familial inherited diseases. The study was approved by the Medical Ethics Committee of the Shenzhen Eye Hospital of Jinan University in Guangdong Province, Shenzhen. Informed consent was obtained from all participants according to the principles of Declaration of Helsinki. All subjects were clinically evaluated by glaucoma specialists.

Mutation screening and sequence analysis: Genomic DNA was extracted from 200 μl venous blood using a Qiamp Blood Kit (Qiagen, Hilden, Germany). All the procedures were performed according to the manufacturer’s instructions. DNA integrity was detected by 1% agarose gel electrophoresis. Intronic primers flanking the exons were designed (Table 1) based on gene sequences of MYOC (GenBank AF001620) and CYP1B1 (GenBank U56438), and synthesized by Invitrogen (Carlsbad, CA). Exons of MYOC and CYP1B1 were amplified by PCR using the designed forward and reverse primers. PCR amplification was conducted in a MyCycler thermocycler (Bio-Rad, Hercules, CA). The 30 μl PCR reaction mixtures included 30–40 ng genomic DNA, 1.0 μM of each of the forward and reverse primers, and 15 μl of 2× Taq Master Mix (incubling 1× PCR buffer, 2.5 mM MgCl2, 0.3 mM of each of dNTPs, 1.5 U Pfu DNA polymerase). All reagents used in this procedure were purchased from SinoBio Biotech Co. Ltd, Shanghai, China. The cycling conditions included an initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 58.4 °C for 30 s (the second exon of CYP1B1 at 55 °C), extension at 72 °C for 60 s (the second exon of MYOC for 30 s and the rest for 90 s), and then a final extension at 72 °C for 5 min. The amplified products were purified with a cycle-pure kit (OMEGA; Bio-Tek, Doraville, GA) and sequenced on the ABI 3730XL automated DNA sequencer (Applied Biosystems, Foster City, CA). Sequence data were compared pair-wise with the published MYOC and CYP1B1 sequences. Mutation was named according to the nomenclature recommended by the Human Genomic Variation Society (HGVS).

Bioinformatics analysis: The Clustalw tool was used to align the protein sequences among eight different species. The possible functional impact of an amino acid change was predicted by Polyphen and Sorting Intolerant From Tolerant (SIFT). Garnier-Osguthorpe-Robson (GOR IV) software was used to predict the effect of the mutation on the secondary structure of MYOC [17]. This method infers the secondary structure of a sequence by calculating the probability for each of the four structure classes (helix, sheet, turn, and loop) based on the central residue and its neighbors from the calculated matrices.

RESULTS

Clinical findings of the pedigree: Five out of twenty one members examined in this four-generation family were found to have elevated intraocular pressure and late-stage glaucomatous cupping of the optic disc. Four suspects were found to have elevated intraocular pressure without characteristic glaucomatous optic disc changes (Table 2, Figure 2). The proband (III-8, a thirty-three-year-old female) was diagnosed as JOAG in both eyes at the age of 28, with elevated IOPs (42 mmHg OU), open anterior chamber angle, enlarged cup disc ratio of 0.95/0.8 (OD/OS). No systemic disorders were found. Trabeculectomy was performed for both eyes at the age of 30. Three years later, IOP measured 15–18 mmHg OS. The visual acuity in the right eye worsened...
to no light perception. The proband’s mother (II-2) was diagnosed as POAG at the age of 55. She recalled that she had reduced vision since her early thirties and became totally blind at her early forties. Her IOPs measured 48~50 mmHg OU with the cup/disc ratio of 1.0 OU. Both eyes of the proband’s uncle (II-5) became blind when he was in his early 40s. Since his blood sample was not available, this patient was not included in the study. The proband’s older sister (III-2) was diagnosed as JOAG at the age of 40 with IOPs of 42/50 mmHg (OD/OS). Funduscopy revealed a glaucomatous excavation and atrophy of the optic disc in both eyes with a cup-disc ratio of 0.95/1.0 (OD/OS). She recalled that she had reduced vision in her early thirties. Patient III-12 was diagnosed as JOAG in both eyes at the age of 27. Her IOPs was 35 mmHg OS and 32 mmHg OD. A cup/disc ratio of 0.8/0.8 OU was noticed in the patient (Figure 2). Patient III-13 was diagnosed as JOAG at the age of 25 with the visual acuity of light perception OD, and hand motions OS. His maximal IOPs measured 52 mmHg OD. A cup/disc ratio of 0.8/0.8 OU was noticed in the patient (Figure 2). Patient IV:9, a 9-year-old girl, and IV:13, a 4-year-old boy, were found to have elevated IOP. Possible optic disc damage was found in the 9-year-old girl but was not found in the 4-year-old boy. The visual field results of both of them were unreliable. No ocular abnormalities were found in the rest of the family numbers.

**MYOC and CYP1B1 mutation identification and analysis:**
Sequence analysis of MYOC revealed a heterozygous missense mutation, c.1151 A>G (p. D384G), in exon 3 of all five patients, four glaucoma suspects. No such mutation was detected in other asymptomatic members of the family and 102 normal control subjects included in this study (Figure 3). No other mutations were identified in this gene and in CYP1B1.

**Bioinformatic analysis:** The mutation c.1151 A>G in exon 3 of MYOC would result in replacement of aspartic acid by glycine. Aspartic acid in 384 was highly conserved for MYOC based on analysis of orthologs from eight different species using the online Clustalw tool (Figure 4). The pD384G
Figure 2. Fundography of glaucoma patients and suspects. Fundography of a glaucoma patient III-12 (A, B), two suspects: IV:9, a 9-year-old (C, D), and IV:13, a 4-year-old (E, F).
mutation of MYOC was predicted to be “probably damaging” by Polyphen with a score of 2.189 and “affect protein function” by SIFT with a score of 0.00, which indicated that protein function was affected by amino acid changes (score <0.05 indicating that the amino-acid change may affect the protein function). The results for secondary structure prediction by the GORIV method suggested that the mutant MYOC 384G replace two β sheet “E”s with two coil “C”s at amino acid 384 and 385 (Figure 5).

**DISCUSSION**

MYOC was the first identified gene associated with pathogenesis of POAG [11]. Previous studies showed that MYOC mutations exist in a greater proportion of JOAG and nearly 3% of adult onset POAG patients [18,19]. MYOC mutations have been reported in POAG pedigrees from many different ethnic groups [18-36]. In this study, the MYOC gene was screened for mutations in a four-generation Uygur, an ethnic minority in China, family.

The identified MYOC mutations involved in POAG in different ethnic populations include Pro370Leu, Gly367Arg, Arg158Gln, Gly252Arg, Arg272Gly, Asn 450 Tyr, Gln48His and Asp 384 Asn [11,20-26,35-37]. Most MYOC mutations were located in exon 3, while some in exon 1 [38]. In this study, an A to G transversion at the second base of codon 384 (in exon 3 of MYOC), which resulted in aspartic acid to glycine amino acid substitution, was identified in all affected glaucoma patients (II:2, III:2, III:8, III:12, III:13), and four glaucoma suspects (IV:1, IV:2, IV:9, IV:13), but not in other unaffected members of the family and 102 normal control subjects. The results of GORIV suggested that p.D384G lead to a secondary structure change by replacing two β sheet “E”s with two coil “C”s around the aspartic acid residue 384, which might interfere with the correct folding of the protein. The
MYOC gene mutation exhibited as heterozygous. To date, to the best of our knowledge, this mutation has not been reported in any other ethnic groups. The majority of POAG were juvenile onset in this pedigree since the onset of symptoms occurs before the age of 30 in most patients. Some patients became blind before they came to see glaucoma specialist. This is consistent with previous finding that MYOC mutations exist in a greater proportion of JOAG patients. The D384G mutation of MYOC in this family appears to be the cause of the disease in this family. In this pedigree, all the glaucoma suspects including a 22-year-old female, a 20-year-old male, a 9-year-old girl and 4-year-old boy in the fourth generation were found to harbor this mutation. They were found to have normal optic disc appearance except the 9-year-old girl who may have early stage glaucomatous optic disc. Careful follow up of these individuals will make it possible to identify new patients at the early stage. Early and proper control of their IOP should help to prevent them from having blindness at young age like the JOAG patients with the same mutation in their family. It should be noted that the Xingjiang (Uygur) are very sparsely populated. The inhabitants there usually do not come to see the doctors unless they have to or are required to do so since transportation is very inconvenient.

Recently, another gene, CYP1B1, has been suggested to modify the glaucoma phenotype [10]. It may act as a modifier of MYOC expression or the two genes may interact via a common pathway [39,40]. We also screened for CYP1B1 gene, but no mutation was detected.

In summary, this is the first time that D384G mutation was identified in glaucoma patients. This study added a novel mutation to the existing spectrum of MYOC mutations, suggesting that a mutation in MYOC correlated with glaucoma as observed in this family. These results provide pre-symptomatic molecular diagnosis for the members of the
pedigree and are useful for follow-up of the family. All carriers of the family should undergo ophthalmologic surveillance at regular intervals for the rest of their lives.

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