Novel Myocilin Mutation in a Chinese Family With Juvenile-Onset Open-Angle Glaucoma

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Objective: To search for the genetic cause of juvenile-onset open-angle glaucoma (JOAG) in a Chinese family.

Methods: In a 3-generation glaucoma family affected with JOAG or ocular hypertension, we screened myocilin (MYOC) and optineurin (OPTN) for mutations and investigated apolipoprotein E (APOE) polymorphisms in 6 family members, 2 of them patients with JOAG, 2 patients with ocular hypertension, and 2 patients who were asymptomatic. Normal controls included 200 unrelated Chinese subjects. The COS-7 cell line was transfected with expression vectors encoding wild-type or mutated MYOC complementary DNA. Cellular and secreted MYOC proteins were characterized by Western blotting.

Results: One missense MYOC mutation, 734G>A: Cys245Tyr, was identified. It occurred in all 4 family members afflicted with JOAG or ocular hypertension but not in asymptomatic family members. No OPTN variations were observed. APOE polymorphism frequencies were similar to those for controls. The Cys245Tyr MYOC mutation cosegregated with the disorder within the family. It was absent in the 200 control subjects. The Cys245Tyr mutant MYOC protein formed homomultimeric complexes that migrated at molecular weights larger than their wild-type counterparts. These mutant complexes remained sequestered intracellularly in COS-7 cells.

Conclusions: The Cys245Tyr MYOC mutation was the genetic cause of JOAG in this Chinese family. This mutation may alter covalent bonds that formed between MYOC cysteines.

Clinical Relevance: Genetic tests of MYOC mutations may be beneficial to predict new cases of the disease in families with JOAG.

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even normal-tension glaucoma. Among 73 reported MYOC mutations, 63 (86.3%) were located in exon 3, suggesting the olfactomedin-like domain to be important for POAG pathogenesis. The most common MYOC mutation is Gln368Stop, reported in 1.65% of probands with POAG. Our previous screening of MYOC gene in 201 POAG probands and 402 control subjects revealed no Gln368Stop among Chinese but found 3 missense mutations, Arg91Stop, Glu300Lys, and Tyr471Cys, all of which have not been reported in other populations. Here, we report a novel MYOC missense mutation in a Chinese family with JOAG.

METHODS

FAMILY RECRUITMENT

A 3-generation family with JOAG was recruited from the day clinic of the Hong Kong Eye Hospital (Figure 1). The study protocol was approved by the Ethics Committee for Human Research, Chinese University of Hong Kong, Hong Kong. In accordance with the tenets of the Declaration of Helsinki, informed consent was obtained from the study subjects after explanation of the nature and possible consequences of the study. Juvenile-onset open-angle glaucoma was defined as meeting all the following criteria: exclusion of secondary causes (eg, trauma, uveitis, or steroid-induced glaucoma); open anterior chamber angle (grade 3 or 4 gonioscopy); intraocular pressure greater than 22 mm Hg in both eyes; characteristic optic disc damage or visual field impairment were diagnosed with ocular hypertension (OHT). Intraocular pressure and visual field were measured by applanation tonometry and Humphrey perimeter with the Glaucoma Hemifield Test, respectively. In this family, 5 members had JOAG while 2 others were diagnosed with OHT (Figure 1). Blood samples and complete ophthalmic examination were obtained from 6 family members. Clinical information for the other family members was obtained through previous medical records.

MUTATION SCREENING

Genomic DNA was extracted from 200 µL of whole blood using a Qiamp Blood Kit (Qiagen, Hilden, Germany). The coding regions of MYOC, exons 1 through 3, and of OPTN, exons 4 through 16, including their intron-exon boundaries, were screened for sequence alterations using polymerase chain reaction and direct DNA sequencing on an ABI 3730XL sequencer (Applied Biosystems, Foster City, Calif). The cDNA sequences were verified using ABI 3730XL sequencing equipment. COS-7 cells (ATCC), plated at a density of 1.5 × 10^4 per 35 mm, were grown in Dulbecco Modified Eagle Medium high glucose complemented with 10% fetal bovine serum, 100 U/mL of penicillin, 100 µg/mL of streptomycin, and 200 µmol/L of L-glutamine (Invitrogen, Carlsbad, Calif) and incubated at 37°C in a humidified chamber with 5% carbon dioxide. Transient transfections were performed 16 hours later using FuGENE 6 transfection reagent (Roche, La Jolla, Calif). The cDNA sequences were verified using ABI 3730XL sequencing equipment. COS-7 cells (ATCC), plated at a density of 1.5 × 10^4 per 35 mm, were grown in Dulbecco Modified Eagle Medium high glucose complemented with 10% fetal bovine serum, 100 U/mL of penicillin, 100 µg/mL of streptomycin, and 200 µmol/L of L-glutamine (Invitrogen, Carlsbad, Calif) and incubated at 37°C in a humidified chamber with 5% carbon dioxide. Transient transfections were performed 16 hours later using FuGENE 6 transfection reagent (Roche, La Jolla, Calif). The cDNA sequences were verified using ABI 3730XL sequencing equipment. COS-7 cells (ATCC), plated at a density of 1.5 × 10^4 per 35 mm, were grown in Dulbecco Modified Eagle Medium high glucose complemented with 10% fetal bovine serum, 100 U/mL of penicillin, 100 µg/mL of streptomycin, and 200 µmol/L of L-glutamine (Invitrogen, Carlsbad, Calif) and incubated at 37°C in a humidified chamber with 5% carbon dioxide. Transient transfections were performed 16 hours later using FuGENE 6 transfection reagent (Roche, La Jolla, Calif). The cDNA sequences were verified using ABI 3730XL sequencing equipment.

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The proband (III:1) was affected with advanced glaucoma (Table). He was diagnosed with JOAG at 16 years of age and both eyes underwent trabeculectomy. Receiving topical β-blocker (0.5% timolol maleate twice a day OU), his intraocular pressure control was fair. He was 24 years old on his last visit. He had a cup-disc ratio of 0.9, open grade 3 (Shaffer) angles, and typical glaucomatous visual field loss in both eyes. No other obvious anterior segment dysgenesis was noticeable. His mother...
(II:1) was diagnosed with glaucoma at 27 years of age. Since age 30 years, she has been blind in both eyes. Her eyes became phthisical as a result of a repeatedly ruptured descemetocele. The right eye subsequently required enucleation plus artificial eye implantation because of persistent pain and leakage from the descemetocele. We could not obtain any information regarding her optic nerve or intraocular pressure because of her phthisical eyeballs.

The proband’s grandfather (I:1) was deceased at the time of study but was known to have glaucoma since a young age. His grandmother (I:2) never had glaucoma. Last year, the proband’s younger sister (III:2) was diagnosed with OHT but with normal open Shaffer grade 3 angles. Receiving topical -blocker (0.5% timolol maleate twice a day OU), she achieved a median intraocular pressure of 17 mm Hg and below. The proband’s younger brother (III:3) was also diagnosed with OHT with normal open Shaffer grade 3 angles. He chose a conservative treatment and hence was not put on any glaucoma medications at the time. The father (II:2) did not have any eye disorders, and he did not recall any glaucoma cases among his lineage or relatives. In addition, according to old medical records, the mother’s younger sister (II:3) and brother (II:4) were previously diagnosed with JOAG.

One missense mutation, 734G>A: Cys245Tyr (GenBank accession numberAY999052), was identified in exon 3 of the MYOC gene in the mother (II:1) and 3 offspring (II:1, III:2, III:3), all heterozygous. It was not detected in the grandmother (I:2) and father (II:2). In addition, 3 common polymorphisms (-83G>A, Arg76Lys, and IVS2+35A>G) were found in this family. Genotype at MYOC.mt1 was normal with a C at position −1000. None of the 200 unrelated controls carried the mutant MYOC allele of Cys245Tyr. Also in this family, no OPTN sequence alterations were detected, and the APOE polymorphism frequencies were similar to those for controls.

Proteins harboring MYOC variations in their olfactomedin homology domain have been shown to be dated remained sequestered intracellularly. To test whether the Cys245Tyr mutation also inhibited secretion of myocilin polypeptide, we transiently transfected Cys245Tyr MYOC cDNA in cultured COS-7 cells. The wild-type and Lys423Glu mutant myocilin proteins were used as positive and negative secretion controls, respectively. Three myocilin proteins were highly expressed in COS-7 cells (Figure 2). As previously observed for the wild-type and Lys423Glu mutant under native conditions, all 3 proteins formed homodimers as well as homomultimers migrating above 180 kd (Figure 2A). Interestingly, Cys245Tyr MYOC formed complexes that had slower electrophoretic mobility migrating at molecular weights that were higher than those of their wild-type counterparts. For instance, the 2 major mutant complexes, one at about 120 kd and the other at more than 200 kd, were migrating at molecular weights 5% to 10% higher than wild-type complexes. Such slower migration patterns represented the substitution of the 245 cysteine by a tyrosine and the concomitant destruction of a critical disulfide bond. Under reducing conditions, all 3 myocilins were doublets migrating at about 55 and 57 kd and represented glycosylated and unglycosylated forms of the protein (Figure 2B). Regarding secretion, wild-type myocilin was found in the extracellular media (Figure 2C), but both olfactomedin-homology–domain mutant polypeptides were not detected in the COS-7 cell culture media (Figure 2C). Our data therefore demonstrated that the Cys245Tyr mutant MYOC polypeptide remained sequestered intracellularly.
between 2 MYOC monomers while those above were generated by interaction of at least 2 MYOC moieties. Wild-type homo-oligomeric complexes were secreted in the extracellular media of COS-7 cells whereas the Gln368Stop and Lys423Glu mutant/mutant homomultimers and heteromeric wild-type/mutant oligomers remained sequestered intracellularly. The mutated Cys245Tyr protein also formed in nonreducing conditions high molecular weight complexes (Figure 2), most likely generated by multimerization of mutant monomers that remained sequestered intracellularly. These complexes migrated at positions higher than those of their wild-type counterparts. Although cysteine 245 did not impede the homo-oligomerization process, such slower migration patterns may reflect some misfolding resulting from the destruction of the 245 disulfide bridge, thereby changing the structure of the protein complexes. These findings are in agreement with a recent study, which demonstrated that significant migration changes occurred when cysteine 245 is altered to an alanine in addition to cysteine 245Tyr. The OHT patients (III:2, III:3) were only affected due to the homozygosity of the mutation Cys245Tyr in the MYOC gene. It is clear that all 3 offspring (III:2, III:3) obtained the mutant allele from their mother (II:1) because their father (II:2) was free of the mutation. The OHT patients (III:2, III:3) were only neonates, and thus they might progress to JOAG in the future. We excluded the role of the OPTN gene in this family by finding no sequence changes in the OPTN coding regions. The APOE gene has been suggested as a potential modifier for POAG because its variant −491A>T interacted with the MYOC polymorphism, MYOC.mt1. We determined the genotypes for all 4 informative APOE polymorphisms (−491A>T, −27T>C, −219T>G, and ε2/ε3/ε4). The frequencies were similar to controls, indicating no association between APOE and JOAG in this family. Moreover, the genotype of MYOC.mt1 was normal and had no contribution to glaucoma. We concluded that the mutation Cys245Tyr in the MYOC gene was the genetic cause of JOAG in this family. This novel MYOC mutation confirms the key role of the MYOC gene in JOAG and enriches our understanding of the molecular genetic basis of this disease.

Figure 2. Secretion analysis of the myocilin Cys245Tyr mutant. A, Cellular extracts from COS-7 cells transiently expressing the wild-type (WT), Cys245Tyr (C245Y), or Lys423Glu (K423E) myocilin proteins. We analyzed 10 µg of proteins by Western blot under nonreducing conditions. Myocilin was detected with an antimyocilin polyclonal antibody as described in the "Methods" section. B, Detection of cellular myocilin under reducing conditions. Protein samples from COS-7 cells transiently expressing wild-type MYOC, Cys245Tyr MYOC, or Lys423Glu MYOC were treated with 100 mmol/L dithiothreitol (DTT), and 2.5 µg of total proteins were migrated and transferred onto a nitrocellulose membrane. Myocilin was detected using monoclonal antibody as described in the "Methods" section. C, Culture media analyzed using the polyclonal anti-Myc antibody. We analyzed 10 µL of extracellular media from COS-7 cells expressing the wild-type, Cys245Tyr, or Lys423Glu myocilin proteins as in A. MW indicates molecular weight.

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