Molecular Analysis of the Myocilin Gene in Chinese Subjects with Chronic Primary-Angle Closure Glaucoma

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PURPOSE. Mutations in the myocilin (MYOC) gene have been implicated in juvenile as well as late-onset primary open-angle glaucoma (POAG). Overall, MYOC mutations account for 3% to 5% of cases of POAG worldwide, making it the most significant gene identified so far in glaucoma. Although there are some similarities in the phenotype of POAG and in particular chronic primary angle-closure glaucoma (PACG), little is known about the role of MYOC in the causation of PACG. To address this, the MYOC gene was screened in a cohort of 106 patients with chronic PACG.

METHODS. Genomic DNA was extracted from leukocytes of the peripheral blood and exons 1 to 3 of the MYOC gene were PCR amplified and subjected to bidirectional sequencing and analysis.

RESULTS. One hundred six patients with chronic PACG of Chinese ethnicity were studied. Sequencing of the MYOC gene in these patients revealed eight sequence variants. Of these, one was a nonsense change, three were missense changes, two were synonymous codon changes, and two were changes in noncoding sequences. These included the Arg346Stop and Thr353Ile mutations, which have been reported in individuals with POAG. However, all the sequence alterations identified have been found in normal Chinese subjects.

CONCLUSIONS. The results of this study do not support a role for MYOC mutations in the pathogenesis of chronic PACG in the Chinese. (Invest Ophthalmol Vis Sci. 2005;46:1303–1306) DOI:10.1167/iovs.04-1163

Glaucoma, a group of heterogeneous optic neuropathies characterized by progressive visual field loss, is the leading cause of irreversible blindness worldwide.1,2 Categorized according to the anatomy of the anterior chamber angle, there are two main forms of glaucoma: primary open-angle (POAG) and primary angle-closure (PACG) glaucoma. PACG is a major form of glaucoma in Asians,3–4 compared with POAG, which is the predominant disease among whites and Africans.5–6 The disease is responsible for most bilateral glaucoma-caused blindness in Singapore, China, and India, and it is estimated that PACG blinds more people than POAG worldwide.9–12

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Singapore. Written informed consent was obtained from all subjects, and the study had the approval of the Ethics Committees of the two hospitals and was performed according to the tenets of the Declaration of Helsinki. Standardized inclusion criteria for chronic PACG were used:

1. The presence of glaucomatous optic neuropathy, which was defined as disc excavation with loss of neuroretinal rim tissue with a cup-to-disc ratio of 0.7 or greater, when examined with a 78-D biomicroscopic lens.

2. Visual field loss detected with static automated white-on-white threshold perimetry (program 24-2 STFA, model 750; Humphrey Field Analyzer; Carl Zeiss Meditec, Dublin, CA) that is consistent with glaucomatous optic nerve damage. This was defined as Glaucoma Hemifield Test results outside normal limits and/or an abnormal pattern SD with P < 5% occurrence in the normal population.

3. A closed angle on indentation gonioscopy. A closed angle was defined as the presence of at least a 180° angle in which the posterior pigmented TM was not visible on gonioscopy, and with evidence of peripheral anterior synchia in any part of the angle.

Subjects with a history of acute symptomatic angle closure as well as cases of secondary angle closure such as neovascularization of the iris, uveitis, trauma, lens intumescence, or subluxation were excluded.

Genomic DNA was extracted from leukocytes of the peripheral blood and exons 1 to 3 of the myocilin gene were amplified by polymerase chain reaction (PCR) with a thermocycler (DNA Thermal Cycler 9700; Applied Biosystems, Inc. [ABI], Foster City, CA). Primers for sequence reactions were the same as those for the PCR reaction. Variations were identified by automated bidirectional sequencing with dye terminator chemistry (BigDye Terminator, ver. 3.1; ABI). An automated DNA sequencer (Prism 3100; ABI) was used. Primers for sequence reactions were the same as those for the PCR reaction.

**RESULTS**

A total of 106 subjects with chronic PACG were studied. All subjects were of Chinese ethnicity (Table 1). There were 69 (65%) women, and the mean age was 73.5 ± 8.1 years (range, 54–94).

Eight MYOC sequence alterations were identified in our study subjects (Table 2). Of these, one was a nonsense change, three were missense changes, two were synonymous codon changes, and two were changes in noncoding sequences. Only one sequence change, an intronic variant (604 +16G > T), was novel. All other sequence alterations have been reported earlier in Chinese samples. The promoter polymorphism I-83 G → A always occurred with the Arg76Iys polymorphism. This haplotype may be specific to the Asian population, since it has only been observed in Chinese and Japanese populations.

The Arg46Stop change was found in one patient with chronic PACG. The patient concerned was an 82-year-old lady who got with POAG, which suggests autosomal recessive inheritance. However, Pang et al. later identified this change in a 77-year-old homozygous individual who had no POAG and later still in heterozygote individuals with and without POAG.

In 1999, Fingert et al. defined a disease-causing mutation in MYOC as one that alters the amino acid sequence of MYOC, is present in one or more subjects with glaucoma but in <1% of the general population, and is absent in normal control subjects. According to these criteria, the results of this study indicate that MYOC mutations do not play a major role in the causation of chronic PACG, as none of the sequence variations found are thought to be disease causing. This is in contrast to POAG, where MYOC mutations are thought to account for 3% to 5% of all cases. It is likely that POAG and PACG, though both leading to glaucomatous optic neuropathy, have different underlying mechanisms. In PACG, the main predisposing factor appears to be pre trabecular obstruction of the outflow channels. Glaucoma probably develops secondary to the high IOP induced by this obstruction. In contrast, trabecular obstruction is absent in POAG, and other factors are likely to be involved in the pathophysiology of the glaucomatous process, such as increased outflow resistance at the trabecular level or increased susceptibility of the optic nerve to damage from raised IOP.

Several sequence variations in the MYOC gene were identified in this panel of PACG subjects. The Arg46Stop sequence variant, found in one patient with PACG, has already been identified in POAG, normal-tension glaucoma, and normal individuals. Currently, there is some debate as to whether this variant is a disease-causing mutation, a polymorphism, or a modifier, with respect to glaucoma disease phenotype. Yoon et al. first identified this change in a homozygote with POAG, which suggests autosomal recessive inheritance. However, Pang et al. later identified this change in a 77-year-old homozygous individual who had no POAG and later still in heterozygote individuals with and without POAG.

The Arg46Stop variation may thus reduce the expression of MYOC by half in heterozygotes and eliminate expression in homozygotes. However, as this change was prevalent in 1.5% of the general population, and is absent in normal control subjects, the possibility however remains that Arg46Stop, possibly in combination with other genetic or environmental influences, may affect the disease phenotype.

The Thr353Ile change was found in three patients with PACG in this study. This change occurs in a residue located in the olfactomedin homology region of the protein, which is conserved in all mammalian MYOC protein sequences tested (data not shown). However, the fact that this change has been identified in normal individuals suggests it is non-disease-causing, though again the possibility remains that Thr353Ile also affects risk of PACG.
Although the amounts of myocilin mRNA observed in the retina and optic nerve are considerably lower than in the anterior segment of the eye,\textsuperscript{29–42} it is possible that MYOC not only acts on the TM but also on the optic nerve. Studies have shown that myocilin is expressed in astrocytes of the optic nerve head at the lamina cribrosa\textsuperscript{42–45} and is a component of the myelin sheath that surrounds postlaminar optic nerve axons.\textsuperscript{43} The fact that MYOC knock-out mice grow normally with normal IOP and ocular morphology\textsuperscript{46} and that a person missing one copy of the MYOC gene had normal IOP and no glaucoma,\textsuperscript{47} suggests that total absence of myocilin is harmless to optic nerve function. It is important to investigate further the influence of normal and “mutant” myocilin on the optic nerve, since some variants of MYOC may still increase susceptibility to retinal ganglion cell damage at high IOP. Such studies may also resolve and identify common pathogenic processes between PACG and POAG.

Since Tornquist\textsuperscript{48} first suggested that PACG was transmitted by a single, dominant gene in 1953, there has been a paucity of research into the genetic basis of PACG. To date, a genetic locus for PACG has not been published, and there have been no other reports of candidate-gene-association studies related to PACG. This is probably related to the high prevalence of the condition in populations in which glaucoma research has not been a major focus. There may be underreporting of the family history, as in POAG,\textsuperscript{46} leading to the impression that most affected patients are isolated cases. The late onset of the disease and the lack of accurate clinical information on previous generations are further obstacles to determining the genetics of the disorder. It is hoped that further research into the genetic basis of PACG will lead to improved understanding of the molecular basis of this major worldwide cause of blindness.

References


