Different Optineurin Mutation Pattern in Primary Open-Angle Glaucoma

Yuk Fai Leung,[1,2] *Bao Jian Fan,*[1,2] *Dennis S. C. Lam,*[1] *Wing Shan Lee,*[1] *Pancy O. S. Tam,*[1] *John K. H. Chua,*[1] *Clement C. Y. Tham,*[1] *Jimmy S. M. Lai,*[1] *Dorothy S. P. Fan,*[1] *and Chi Pui Pang*[1]

Purpose. The optineurin gene (*OPTN*) is the second gene besides *MYOC* in which mutations have been identified to be associated with primary open-angle glaucoma (POAG). In this study, sequence alterations in the *OPTN* gene associated with POAG in Chinese subjects were investigated.

Methods. All the coding exons of *OPTN* were screened, including the intron–exon boundaries, for sequence alterations in a Chinese sample of 119 sporadic patients with POAG and 126 unrelated control subjects by polymerase chain reaction–conformation-sensitive gel electrophoresis and DNA sequencing.

Results. Sixteen sequence changes were identified: 3 had been reported (T34T, M98K, and R545Q) and 13 were novel (T49T, E103D, V148V, P199P, T202T, H486R, IVS6-5T→C, IVS6-10G→A, IVS7+24G→A, IVS8+20G→A, IVS13+21C→G, IVS15+10G→A, and IVS15-48C→A). Among them, only E103D, H486R, V148V, and IVS13+21C→G were found exclusively in patients with POAG, whereas P199P, T202T, and IVS8+20G→A were present only in control subjects. The genotype of IVS7+24G→A showed a significant association with POAG ($P = 0.02$, Fisher two-tailed exact test) and with and increased cup-to-disc ratio in these patients ($P = 0.005$, Mann-Whitney test).

Conclusions. The findings in the current study enrich the evidence on the *OPTN* gene as a causative gene for POAG and suggest a different mutation pattern of *OPTN* in Chinese than in whites. The wide spectrum of putative mutations detected in this study suggests that both structural and functional disruptions in OPTN may contribute to the pathogenesis of glaucoma. (*Invest Ophthalmol Vis Sci.* 2003;44:3880–3884) DOI:10.1167/iovs.02-0693

Glaucoma is a group of eye disorders characterized by progressive degeneration of the optic nerve. This degenerative change results in peripheral and central visual loss[1] and is the second leading cause of blindness worldwide. It has been estimated that the disease affects 66.8 million people, and 6.7 million of these have bilateral blindness.[2] A cross-sectional population study showed that glaucoma is the major cause of blindness among adult Chinese, and primary open-angle glaucoma (POAG) is the predominant form of the disease.[3]

Although the molecular basis of POAG is not completely known, it is probably a genetically heterogeneous disorder attributed to the interaction of multiple genes and environment influences.[4,5] Risk factors like family history, age, smoking, and myopia are also implicated in the pathogenesis of POAG.[6,7] Six chromosomal loci, *GLC1A*, *GLC1B*, *GLC1C*, *GLC1D*, *GLC1E*, and *GLC1F*, were found to link with POAG.[8–10] The first gene identified with mutations that cause POAG was *MYOC*.[11,12] In whites, approximately 2% to 4% of POAG cases are due to *MYOC* mutations,[13–15] although it can be as high as 36% in families with juvenile hereditary POAG.[16] In Japanese, the frequency of *MYOC* mutations in adult-onset POAG range similarly—approximately 3% to 4%.[17] An intriguing finding in our previous screening of the *MYOC* gene in Chinese was that the mutation pattern was very different from that in other populations.[18,19]

Recently, the candidate gene for *GLC1E* on the short arm of chromosome 10, region p14-p15 has been identified as optineurin (*OPTN*). Mutations in *OPTN* have been found in approximately 17% of families with hereditary and adult-onset POAG, most of whom had intraocular pressure (IOP) less than 22 mm Hg and a small proportion with moderate IOP between 22 and 26 mm Hg.[20] The *OPTN* gene consists of 3 noncoding exons in the 5′ untranslated region and 13 exons that code for a 577-amino-acid protein. It is also known as FIP2 (14.7-kDa interacting protein)[21] and NEMO-related protein (NRP).[22] It was found to interact with Huntingtin protein,[23] transcription factor IIIA,[24] and RAB8.[25] Expression of the human OPTN transcript had been found in ocular tissues, retina, trabecular meshwork, and nonpigmented ciliary epithelium,[20] as well as nonocular tissues, such as heart, brain, placenta, liver, and skeletal muscle.[20,21] OPTN is also present in aqueous humor of a wide variety of species including humans, cattle, pigs, goats, and sheep, suggesting it to be a secreted protein.[20]

In this study, we sought to identify sequence alterations of *OPTN* in the Chinese population and their association with POAG and corresponding clinical features.

Subjects and Methods

Case and Control Study Subjects

Unrelated patients with POAG were recruited from the Eye Clinic of the Prince of Wales Hospital, Hong Kong. Diagnosis was based on meeting all the following criteria: exclusion of secondary causes (e.g., trauma, uveitis, or steroid-induced glaucoma); anterior chamber angle open (grade III or IV gonioscopy); characteristic optic disc changes (e.g., vertical cup-to-disc ratio of >0.5, disc hemorrhage, or thin or notched neuroretinal rim); and characteristic visual field changes with reference to Anderson's criteria for minimal abnormality in glaucoma.[26] Visual acuity was determined using the Snellen eye chart. IOP

From the [1]Department of Ophthalmology and Visual Sciences, the Chinese University of Hong Kong, Hong Kong, China.

[2]Contributed equally to the work and therefore should be considered equivalent senior authors.

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Corresponding author: Chi Pui Pang, Department of Ophthalmology and Visual Sciences, The Chinese University of Hong Kong, Hong Kong Eye Hospital, 147K Argyle Street, Kowloon, Hong Kong; cppang@cuhk.edu.hk.

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and visual field were measured by applanation tonometry and a perimeter (Humphrey Field Analyzer; Carl Zeiss Meditec, Dublin, CA) with the Glaucoma Hemifield Test, respectively. The patients with POAG in this study included those with low-tension glaucoma (LTG) or high-tension glaucoma (HTG), but patients with congenital glaucoma or with a family history of glaucoma were excluded. Unrelated control subjects were recruited from people who attended the clinic for conditions other than glaucoma, including senile cataract, floaters, refractive errors, and itchy eyes. Glaucoma was ruled out in the control subjects by the same diagnostic criteria and the same ophthalmic examination as were used in the patients.

The patient group was composed of 119 sporadic patients with POAG: 66 males and 53 females, with age at diagnosis ranging from 11 to 86 years (mean ± SD: 55.7 ± 16.7). The IOP at diagnosis ranged from 13 to 58 mm Hg (mean ± SD: 27.3 ± 8.3). Among these 119 patients with POAG, 47 (39.5%) were LTG with IOP less than 22 mm Hg. There were 126 unrelated control subjects, 53 men and 73 women, with age ranging from 50 to 84 years (mean ± SD: 66.4 ± 10.9), and IOP less than 22 mm Hg. All study subjects were given a complete ocular examination. Venous blood was obtained and stored at −20°C for less than 2 months before DNA extraction. The study protocol was approved by the Ethics Committee for Human Research of the Chinese University of Hong Kong and adhered to the tenets of the Declaration of Helsinki. Informed consent was obtained from all study subjects after explanation of the nature and possible consequences of the study.

PCR and High-Throughput Conformation-Sensitive Gel Electrophoresis

Genomic DNA was extracted from 200 μL of whole blood by a commercial procedure (Qiamp Blood Kit; Qiagen, Hilden, Germany). All coding sequences of *OPTN*, including intron–exon boundaries, were screened for sequence alterations using PCR followed by high-throughput conformation-sensitive gel electrophoresis (HTCSGE).[27] PCR was performed on a thermal cycler (model 9700; Applied Biosystems, Foster City, CA), with an initial denaturation step of 12 minutes at 94°C, followed by 35 cycles of 94°C for 40 seconds, a touchdown annealing temperature of 62°C minus 0.2°C per cycle for 1 minute, and 72°C for 40 seconds, and a final extension step of 72°C for 7 minutes. Each 25-μL reaction contained 1.5 mM $MgCl_2$, 1 U *Taq* polymerase (Ampli*Taq* Gold; Applied Biosystems, Inc.), 200 μM dNTPs, 1 μL genomic DNA, and 400 pM primers. We also screened all DNA samples in this study for *MYOC* sequence alterations, as described in our previous reports.[18,19]

Direct DNA Sequencing

The samples with altered electrophoretic mobility in HTCSGE were sequenced using a cost-saving sequencing protocol[28] on an automated DNA sequencer (model ABI 377XL; Applied Biosystems, Foster City, CA). Sequence data were aligned with sequence-analysis software, (Sequence Navigator, ver. 1.0.1; Applied Biosystems, Inc.) and compared with the published *OPTN* gene sequence (GenBank accession number AF420371; http://www.ncbi.nlm.nih.gov/Genbank; provided in the public domain by the National Center for Biotechnology Information, Bethesda, MD). A portion of samples not showing altered HTCSGE pattern were also selected at random for direct sequencing to affirm the reliability of the HTCSGE screening procedure.

Statistical Analysis

Statistical analyses were performed on computer (SAS ver. 8.2; SAS Institute, Cary, NC). The χ^2 test or the Fisher exact test was used to compare the frequencies of various alleles or genotypes between patients and control subjects. $P < 0.05$ was considered significant. The confidence intervals for odds ratios (OR) were calculated by the logistic regression method. The Mann-Whitney test was used to test the association between each phenotype variable and the genotype. Multivariate analyses were performed with a logistic regression model to confirm the association between all the phenotype variables and the genotype. All the phenotypes were set as numerical variables, whereas all the genotypes were set as categorical variables (homozygote, 2; heterozygote, 1; wild type, 0). Multiple logistic regression models were built and optimized using a stepwise approach. A final optimal model was determined by balancing the significance of overall model and phenotype variables.

Results

OPTN Variants Detected in the Study Subjects

Sixteen sequence changes were identified in patients and control subjects (Table 1). Four were missense changes, five were synonymous codon changes, and seven were changes in the noncoding sequences. Apart from T34T, M98K, and R545Q, the other 13 sequence changes were novel. E103D, H486R, V148V, and IVS13+21C→G were found exclusively in patients, all with adult-onset POAG. However, P199P, T202T, and IVS8+20G→A were found only in control subjects. M98K was identified from in 34 patients with POAG and 31 control subject, and R545Q in 8 patients and 11 control subjects. Several homozygotes were identified: IVS6-5T→C and IVS15-48C→A each in one patient with POAG, and IVS7+24G→A in one control subject who was a 50-year-old woman. There were three homozygous patients and one homozygous control subject who had M98K (Table 1).

Distribution of *OPTN* Variants in POAG Patients and Control Subjects

Allele frequencies of the 16 variants between patients and controls were not significantly different ($P > 0.05$), whereas IVS7+24G→A showed an inclination toward an association with POAG ($P = 0.08$; Table 2). However, IVS7+24G→A was found to be associated significantly with POAG ($P = 0.02$; OR 4.12; 95% CI 1.46–12.68) when comparing the genotypes between two study groups, whereas the genotypes of other variants were not associated with POAG significantly ($P > 0.05$; Table 1).

Genotype–Phenotype Correlations

While the genotype of IVS7+24G→A showed a significant association with POAG, univariate analysis showed it was also significantly related to an increased cup-to-disc ratio ($P = 0.005$; Table 2). There was no significant association between IVS7+24G→A and age at inclusion, age at diagnosis, IOP at inclusion, IOP at diagnosis, or visual acuity. Because some of these phenotype variables may be linked, the relationships between all the phenotypic features and the genotype of IVS7+24G→A were investigated by stepwise logistic regression analysis. Still, only the cup-to-disc ratio remained independently associated with IVS7+24G→A ($P = 0.02$). The model was deemed satisfactory, in that the deviance and Pearson goodness-of-fit statistics were 0.97 and 0.90 respectively, and the likelihood ratio, score, and Wald global model testing statistics were 0.02, 0.04, and 0.07 respectively.

Discussion

Apart from T34T, M98K, and R545Q, which were previously identified in the study by Rezaie et al.,[20] 13 of the 16 sequence changes found in this study of Chinese subjects were novel.

TABLE 1. OPTN Variants Observed in 119 Patients with POAG and 126 Control Subjects

Location	Sequence Change	Codon Change	Frequency in Study Subjects (%)		Allele Frequency (%)		Genotype*	
			POAG n = 119†	Control n = 126†	POAG n = 238‡	Control n = 252‡	POAG n = 119†	Control n = 126†
Exon 5	603T→A	M98K	34 (28.6)	31 (24.6)	37 (15.5)	32 (12.7)	3/31/85	1/30/95
Exon 5	619G→C	E103D	1 (0.8)	0 (0)	1 (0.4)	0 (0.0)	0/1/118	0/0/126
Exon 14	1767A→G	H486R	1 (0.8)	0 (0)	1 (0.4)	0 (0.0)	0/1/118	0/0/126
Exon 16	1944G→A	R545Q	8 (6.7)	11 (8.7)	8 (3.4)	11 (4.4)	0/8/111	0/11/115
Subtotal			44 (37.0)	42 (33.2)	47 (19.7)	43 (17.1)		
Exon 4	412G→A	T34T	19 (16.0)	12 (9.5)	19 (8.0)	12 (4.8)	0/19/100	0/12/114
Exon 4	457C→T	T49T	1 (0.8)	5 (4.0)	1 (0.4)	5 (2.0)	0/1/118	0/5/121
Exon 6	754G→A	V148V	1 (0.8)	0 (0)	1 (0.4)	0 (0.0)	0/1/118	0/0/126
Exon 7	907T→C	P199P	0 (0)	1 (0.8)	0 (0.0)	1 (0.4)	0/0/119	0/1/125
Exon 7	916G→A	T202T	0 (0)	1 (0.8)	0 (0.0)	1 (0.4)	0/0/119	0/1/125
Subtotal			21 (17.6)	19 (15.1)	21 (8.8)	19 (7.5)		
Intron 6	IVS6−5T→C	—	2 (1.7)	4 (0.8)	3 (1.3)	4 (1.6)	1/1/117	0/4/122
Intron 6	IVS6−10G→A	—	2 (1.7)	4 (0.8)	2 (0.8)	4 (1.6)	0/2/117	0/4/122
Intron 7	IVS7+24G→A	—	13 (10.9)	5 (4.0)	13 (5.5)§	6 (2.4)	0/13/106‖	1/4/121
Intron 8	IVS8+20G→A	—	0 (0)	1 (0.8)	0 (0.0)	1 (0.4)	0/0/119	0/1/125
Intron 13	IVS13+21C→G	—	1 (0.8)	0 (0)	1 (0.4)	0 (0.0)	0/1/118	0/0/126
Intron 15	IVS15+10G→A	—	4 (3.4)	4 (3.2)	4 (1.7)	4 (1.6)	0/4/115	0/4/122
Intron 15	IVS15−48C→A	—	6 (5.0)	4 (3.2)	7 (2.9)	4 (1.6)	1/5/113	0/4/122
Subtotal			28 (23.5)	22 (17.5)	30 (12.6)	23 (9.1)		
Total			93 (78.2)	83 (65.9)	98 (41.2)	85 (33.7)		

* The numbers indicate homozygote/heterozygote/wildtype.
† Total number of subjects.
‡ Total number of alleles.
§ $P = 0.08$ with χ^2 test.
‖ $P = 0.02$ with the Fisher exact test (two-tailed).

E103D, V148V, H486R, and IVS13+21C→G were detected exclusively in patients with POAG. The four patients with any of these mutations were LTG, with IOP lower than 22 mm Hg in both eyes. IVS7+24G→A showed a significantly higher frequency in patients with POAG than in control subjects (Table 1), suggesting it as a glaucoma-associated change. Although V148V is a synonymous codon change, whereas IVS13+21C→G and IVS7+24G→A are noncoding sequence alterations, they may affect mRNA stability and protein function, although the mRNA levels are still to be assessed.[29] The two IVS variations may cause splice site alterations thereby changing the *OPTN* coding sequences. Among these five sequence changes, E103D and H486R are more likely to be disease causing, accounting for 1.6% (2/119) of sporadic patients with POAG in this study. If all five were confirmed causative mutations, they would have affected 14.3% (17/119) of our patients. Meanwhile, all these sequence changes are still to be affirmatively linked with glaucoma by statistics, segregation in families, sequence analysis of relevant cDNA regions, assessment of the level of the mature transcript, or expression studies before they can be concluded as glaucoma-causing mutations.

TABLE 2. Genotype–Phenotype Relationships in Patients with POAG

Phenotype Variable	IVS7+24G→A	
	Heterozygous A/G (n = 13)	Wild-type G/G (n = 106)
Age at diagnosis (ys)	53.6 ± 16.4	56.0 ± 16.8
IOP at diagnosis (mm Hg)	27.6 ± 9.5	27.2 ± 8.1
Cup-to-disc ratio (×10)	7.8 ± 0.8*	6.7 ± 1.4*
Visual acuity (×10)	3.8 ± 2.0	4.1 ± 2.8

* $P = 0.005$ with Mann-Whitney test; $P = 0.02$ with logistic regression analysis.

M98K and R545Q were found to have intriguingly similar frequencies among glaucoma patients and control subjects, although they were reported as glaucoma-causing mutations by Rezaie et al.[20] In our case, M98K was found in 28.6% patients (34/119) and 24.6% control subjects (31/126), or 12.7% (32/252) of normal chromosomes. Both frequencies were higher than in the white population,[20] especially in the control subjects, which was approximately 10 times higher. R545Q was a probable glaucoma-causing mutation detected only in patients with POAG,[20] but it was found in 8.7% (11/26) of our control subjects, or 4.4% (11/252) of normal chromosomes. It is possible M98K and R545Q are common polymorphisms specific in the Chinese population. However, because the range of age in our control group was from 50 to 84 years, it remains possible that a subset may eventually undergo development of glaucoma and consequently affect the results of our statistic analysis. Although large-scale screening is needed to affirm the allelic frequencies of *OPTN* variants, pathogenic and functional effects of these sequence alterations have to be affirmed by family linkage analysis and expression studies.

It is noteworthy that in our study subjects we did not find E50K (c.458G→A) and c.691_692insAG, which were reported as disease causing.[20] The identification of a large number of novel sequence variants and the difference in allelic frequencies in our Chinese subjects when compared with the reported study of whites,[20] suggest a different *OPTN* mutation pattern in Chinese.[30]

The phenotype variables in this study including age at diagnosis, IOP at diagnosis, cup-to-disc ratio, and visual acuity were not normally distributed, as verified by the Kolmogorov-Smirnov test. As a result we used a nonparametric test (i.e., the Mann-Whitney test) rather than a parametric test (e.g., Stu-

dent's *t*-test) to test the association between each phenotype variable and the genotype. Because some of phenotype variables may be linked, a confounding bias might be introduced if the phenotype variables were analyzed individually by the univariate analysis method. To address this problem, we adopted a multivariate analysis method (i.e., logistic regression analysis) that can effectively control the confounding bias, to investigate the association between all the phenotype variables and the genotype of IVS7+24G→A. These two analysis methods consistently revealed that IVS7+24G→A was associated with an increased cup-to-disc ratio, which is one of the major parameters to assess optic nerve damage in patients with POAG.

Although there have been intensive investigations on the genetic basis of POAG, its pathogenesis is still elusive. *MYOC* was the first identified disease-causing gene for POAG.[11] In our study on the relationship between the *MYOC* gene and POAG in a Chinese Han population, we found that the T353I mutation was significantly related to an increased risk of POAG.[19] Mutations of *MYOC* have been detected in many populations,[10,11,15,18,19] although they accounted for only 2% to 4% of patients with POAG.[15] The *OPTN* gene is an important causative gene for POAG.[20] Our report enriches the evidence on the relationship between the *OPTN* gene and POAG because it provides new opportunities for understanding the disease's pathogenesis. It was responsible for only approximately 12% of sporadic patients with POAG who mainly had LTG, according to Rezaie et al.[20] and for between 1.6% to 14% of our patients, 60% of them having HTG. However, because we used HTCSGE to screen for the sequence alterations, it is possible that additional sequence alterations could still be missed. In addition, Copin et al.[31] found that *APOE* gene interacted with *MYOC* gene and suggested *APOE* gene was a potential modifier for POAG. Besides, Torrado et al.[32] reported that the optimedin and myocilin may interact with each other and may be another candidate gene for POAG. None of the study subjects in this present study carry a MYOC mutation that causes glaucoma. We shall in future attempt to conduct *MYOC* and *OPTN* mutation analysis in all our available patients with POAG and control subjects for detail statistical analysis of the genotype-phenotype associations after fine division of genotype and phenotype subgroups. The function of OPTN and its role in POAG pathogenesis are not known at this moment, although it is probable that it is a protein that is involved in a number of signal transduction pathways.[22,24] A study on the interaction between genetic factors and environmental factors would shed new light on the pathogenesis of POAG.

We have shown E103D, H486R, V148V, IVS7+24G→A, and IVS13+21C→G are possibly linked with glaucoma. E103D and H486R may alter protein structure, function, or stability, whereas V148V, IVS7+24G→A, and IVS13+21C→G may alter mRNA stability. IVS7+24G→A is associated with an increased cup-to-disc ratio and is potentially related to disruption of optic nerve. These different mechanisms may ultimately affect the normal signal transduction function of OPTN and potentiate optic nerve apoptosis. At the same time, the deregulation of the signal transduction pathway can also be mediated through post-translational modifications. One likely candidate is phosphorylation, which decreases OPTN half-life.[22] It would be interesting to investigate whether the change in OPTN half-life associates with glaucoma pathogenesis and how this pathway is regulated. It may finally turn out that the upstream regulators of OPTN also play important roles in the pathogenesis of glaucoma.

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