



# Absence of *myocilin* and *optineurin* mutations in a large Philippine family with juvenile onset primary open angle glaucoma

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**Purpose:** To analyze the role of the two primary open angle glaucoma (POAG) genes, *myocilin* (*MYOC*) and *optineurin* (*OPTN*), in a large Philippine family segregating autosomal dominant juvenile onset open angle glaucoma (JOAG).

**Methods:** The coding sequences of the *MYOC* and *OPTN* genes were screened in 27 family members by polymerase chain reaction and direct sequencing. The specific *MYOC* promoter polymorphism (*MYOC.mtl*) was identified by restriction endonuclease assay. All of the ABI MD-10 microsatellite markers on chromosomes 1, 2, 3, 7, 8, and 10, which harbor the six known POAG loci, were analyzed for linkage with POAG.

**Results:** No mutation was identified in this large kindred. Instead, three polymorphisms (-80G->A, -1000G->C, R76K) in *MYOC* and four polymorphisms (T34T, M98K, R545Q, IVS7+24G->A) in *OPTN* were found. All markers flanking the six known POAG loci gave LOD scores not more than 1.1. Non-parametric linkage analysis for all these markers resulted in p values more than 0.05.

**Conclusions:** Both mutation testing and linkage analysis provide strong evidence against *MYOC* and *OPTN* being the causative gene in this large family. It indicates that unidentified genes will underlie the occurrence of glaucoma in this family.

Glaucoma consists of a heterogeneous group of progressive neurodegenerative disorders of which together account for one of the leading causes of blindness in virtually every country [1]. Progressive loss of vision can lead to blindness. Primary open angle glaucoma (POAG) is the most common form of glaucoma. There are both sporadic and familial POAG, the latter shows strong evidence of complex inheritance, with variable severity and phenotypic expression. POAG can be arbitrarily subdivided into two different categories depending on the age of disease onset; juvenile onset open angle glaucoma (JOAG) and adult onset open angle glaucoma [2]. In affected families of both groups of POAG, genetic linkage analysis clearly suggests autosomal dominant inheritance with incomplete penetrance [3]. It has also been suggested that adult onset POAG is inherited as a non-Mendelian trait, whereas JOAG exhibits autosomal dominant inheritance [4,5].

To date, six chromosomal loci; 1q23 (*GLCIA*), 2cen-q13 (*GLC1B*), 3q21-q24 (*GLC1C*), 7q35-q36 (*GLC1F*), 8q23 (*GLCID*), and 10p15-p14 (*GLC1E*) have been identified for POAG [5-12]. There are two known POAG genes, *MYOC* in *GLCIA* and *OPTN* in *GLC1E*. When mutated, *MYOC* causes

severe open angle glaucoma, mostly in its juvenile onset form and less commonly the adult onset form. More than 70 *MYOC* mutations and a few benign polymorphisms have been detected in different population groups [3,13-15]. Most mutations cause an early onset, severe glaucoma, whereas others account for a less typical late onset or even normal tension glaucoma (NTG) [15-17]. Although the exact mechanism is unknown, the *MYOC* gene has been implicated in causing obstruction of aqueous outflow through the trabecular meshwork, resulting in increased intraocular pressure (IOP) [15]. Defects in the *OPTN* gene are responsible for moderate to mild forms of late onset glaucoma. It has been suggested that *OPTN* is specific for NTG [12]. So far, 5 mutations and a few polymorphisms have been reported [12,18]. Ethnic specific *OPTN* mutation patterns may exist. *OPTN* mutations were found in 12% of sporadic Caucasian POAG patients [12]. While we identified *OPTN* mutations in 1.6% of sporadic Chinese POAG patients [18], no disease causing mutations in *OPTN* was detected among 148 NTG and 165 POAG patients in the Japanese population [19]. The wild type *OPTN* protein, operating through the TNF- $\alpha$  pathway, is speculated to play a neuroprotective role in the eye and optic nerve. But when defective, it produces visual loss and optic neuropathy as typically seen in NTG and high tension glaucoma [3,18,20].

Two studies have investigated the role of *MYOC* and *OPTN* simultaneously, one on Finnish families [21] and the other on Barbados glaucoma families of African descent [22].

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Except for a few polymorphisms in *MYOC* and *OPTN*, no mutation was identified in these families. In this study, we evaluated the role of the *MYOC* and *OPTN* genes in a large Philippine family.

**METHODS**

*Study subjects:* One five generation kindred segregating JOAG was recruited from the Philippines (Figure 1). We determined the clinical characteristics of 95 family members. Among them 27 subjects were given a complete ocular examination, and peripheral venous blood was collected and stored at -20 °C for less than 3 months prior to DNA extraction. The clinical status of the other individuals was traced in medical records. JOAG was observed in 22 out of 95 individuals, and 11 of them were male.

Affected individuals were typically diagnosed before the age of 35 years. JOAG was diagnosed as meeting all of the following criteria; (1) exclusion of secondary causes (e.g., trauma, uveitis, or steroid induced glaucoma), (2) anterior chamber angle open (grade III or IV gonioscopy), (3) elevated IOP greater than or equal to 22 mm Hg, (4) characteristic optic disc changes (e.g., vertical cup to disc ratio greater than or equal to 0.7, disk hemorrhage, or thin or notched neuroretinal rim), and (5) characteristic visual field changes with refer-

ence to Anderson’s criteria for minimal abnormality in glaucoma [23]. Visual acuity was determined using a Snellen eye chart. IOP and visual field were measured by applanation tonometry and Humphrey perimetry with the Glaucoma Hemifield Test, respectively. These criteria also covered glaucoma diagnosis in hospital records or other ophthalmologists’ records.

The study was approved by the Ethics Committee for Human Research, the Chinese University of Hong Kong, and followed the tenets of the Declaration of Helsinki. All participants were given an informed consent after explanation of the nature and possible consequences of the study.

*Mutation screening:* Genomic DNA was extracted from 200 µl of blood using a commercial kit (Qiamp Blood Kit; Qiagen, Hilden, Germany). The coding exons and adjacent sequences of *MYOC* and *OPTN* were screened for sequence alterations by polymerase chain reaction, as previously reported [14,18], followed by direct sequencing using an ABI 377XL automated DNA sequencer (Applied Biosystems, Foster City, CA). Sequence data were aligned using sequence Navigator analysis software, version 1.0.1 (Applied Biosystems) and compared to the published *MYOC* (AB006686) and *OPTN* (AF420371) sequences. For the *MYOC*.mtl polymorphism, subjects were screened by PCR,

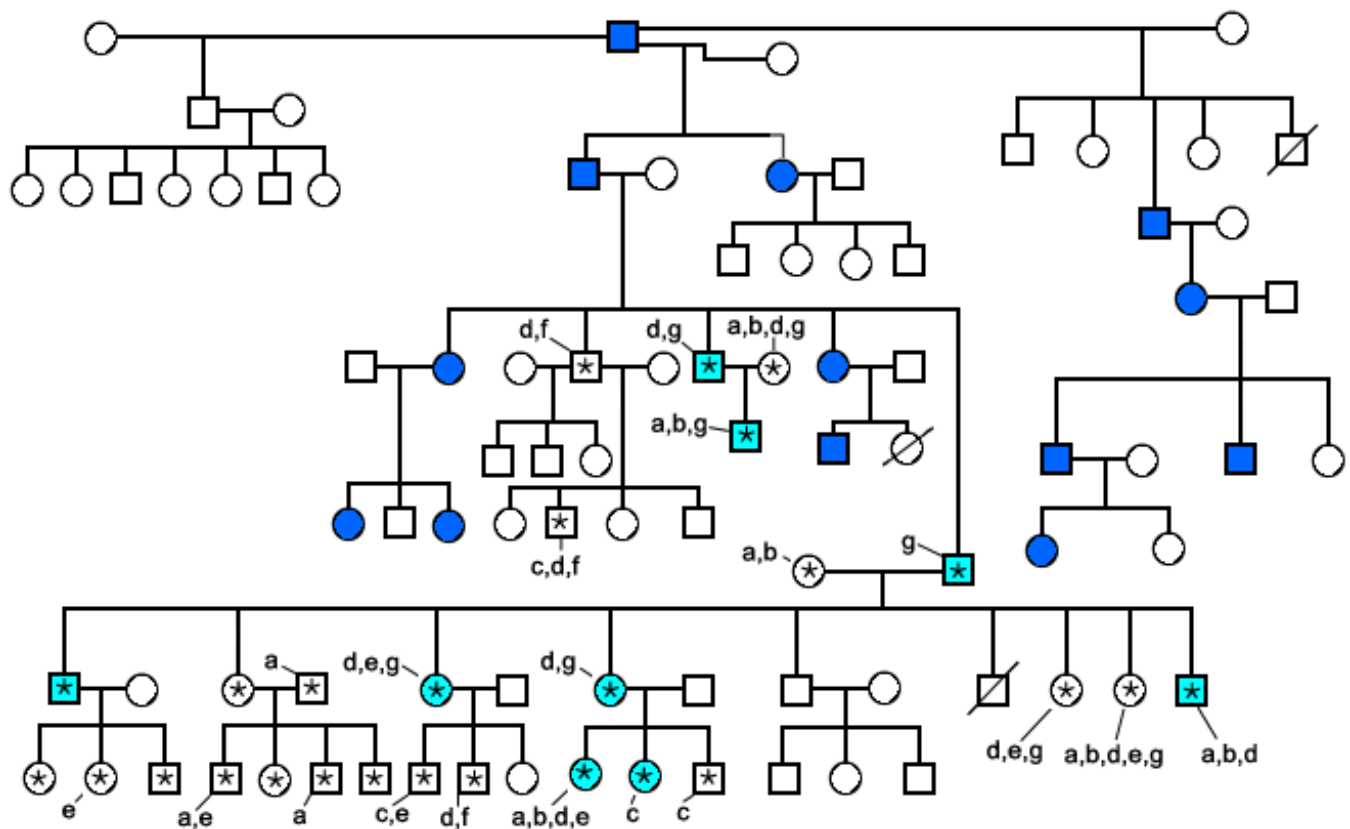


Figure 1. Pedigree of the Philippine JOAG family. The asterisk inside certain cells indicates that a DNA sample was available for analysis. The *MYOC* and *OPTN* genes were sequenced and, within the image, the “a” indicates the R76K polymorphism, the “b” indicates the -83G->A polymorphism, the “c” indicates the -1000C->G polymorphism, the “d” indicates the T34T polymorphism, the “e” indicates the M98K polymorphism, the “f” indicates the R545Q polymorphism, and the “g” indicates the IVS7+24G->A polymorphism. Square cells refer to males and circle cells refer to females. Cells filled with dark blue are JOAG by history and the cells filled with light blue are JOAG by analysis.

followed by restriction endonuclease assay. The PCR products were digested with the restriction enzyme *AlwNI* at 37 °C for 4 h prior to polyacrylamide gel electrophoresis.

Statistical analyses were carried out using SAS statistical software version 8.2 (SAS Institute, Cary, NC). Significance of the difference in distribution of each sequence alteration between affected and unaffected subjects was determined by  $\chi^2$  tests or using Fisher's exact tests.

**Linkage analysis:** Microsatellite markers of ABI PRISM Linkage Mapping Set MD-10 (Applied Biosystems) on chromosomes 1, 2, 3, 7, 8, and 10 were genotyped on 27 participants. The Genescan and Genotyper software packages (Applied Biosystem) were used to call genotypes. The GenoPedigree and GenBase software packages (Applied Biosystem) were used to draw pedigree and to export data for linkage analysis. Prior to performance of linkage analysis, we eliminated all Mendelian inconsistencies in the pedigree data using the PedCheck program [24]. The JOAG gene frequency was set as 0.0001. An autosomal dominant mode of inheritance was used with one liability class with penetrance values 0, 1, 1, respectively. Two point analyses were performed with the MLINK and ILINK programs from the FASTLINK version 4.1P software package [25]. Nonparametric linkage analyses were carried out with Simwalk2 version 2.83 [26].

## RESULTS

Segregation analysis showed autosomal dominant inheritance in this family. We screened a total of 27 subjects (9 with JOAG) for sequence alterations in the coding regions of *MYOC* and *OPTN*. No disease causing mutations were identified in *MYOC* in this family. Instead, three neutral polymorphisms, -1000C->G, -83G->A, and R76K, were found (Figure 1). The most common polymorphism was R76K, detected in 33% (9/27) of subjects. No significant difference was found in all polymorphisms between affected and unaffected individuals ( $p>0.05$ ; Table 1).

For *OPTN* also, no mutation was identified. However, 4 known polymorphisms, T34T, M98K, R545Q, and IVS7+24G->A were found (Figure 1). The most common polymorphism was T34T identified in 40% (11/27) of study subjects. There was also no significant difference in all polymorphisms between affected and unaffected individuals ( $p>0.05$ ; Table 1).

**TABLE 1. *MYOC* AND *OPTN* VARIANTS IDENTIFIED IN THE PHILIPPINE FAMILY**

Gene	Location	Sequence change	Codon change	Allele frequency (%)		Genotype frequency	
				JOAG (n=18)	Normal (n=36)	JOAG (n=9)	Normal (n=18)
<i>MYOC</i>	Exon 1	c.227G>A	R76K	3(17)	7(19.4)	0/3/6	1/5/12
	Promoter	-80G>A	-	3(17)	4(11.1)	0/3/6	1/2/15
	Promoter	-1000C>G	-	1(5.6)	3(8.3)	0/1/9	0/3/15
<i>OPTN</i>	Exon 4	c.412G>A	T34T	5(27.8)	6(16.7)	0/5/4	0/6/12
	Exon 5	c.603T>A	M98K	2(11.1)	5(13.9)	0/2/7	0/5/13
	Exon 16	c.1944G>A	R545Q	0(0)	3(8.3)	0/0/9	0/3/15
	Intron 7	IVS7+24G>A	-	5(27.8)	4(11.1)	0/5/4	1/2/15

The  $\chi^2$  test or Fisher's exact test was used to compare the frequencies of various alleles and genotypes between JOAG and normal subjects. No polymorphism was statistically significant ( $p>0.05$ ).

Linkage analysis of markers flanking all six POAG loci showed no linkage with this family. For each of these six chromosomes, the highest two point LOD scores were detected in D1S484 ( $Z_{\max} = 0.89$  at  $\theta=0.0$ ), D2S2211 ( $Z_{\max} = 1.07$  at  $\theta=0.05$ ), D3S1278 ( $Z_{\max} = 0.74$  at  $\theta=0.0$ ), D8S258 ( $Z_{\max} = 0.53$  at  $\theta=0.20$ ), D10S548 ( $Z_{\max} = 0.68$  at  $\theta=0.0$ ), and D7S517 ( $Z_{\max} = 1.06$  at  $\theta=0.0$ ), respectively (Figure 2). Non-parametric linkage analysis resulted in  $p$  values more than 0.05 for all markers on these chromosomes.

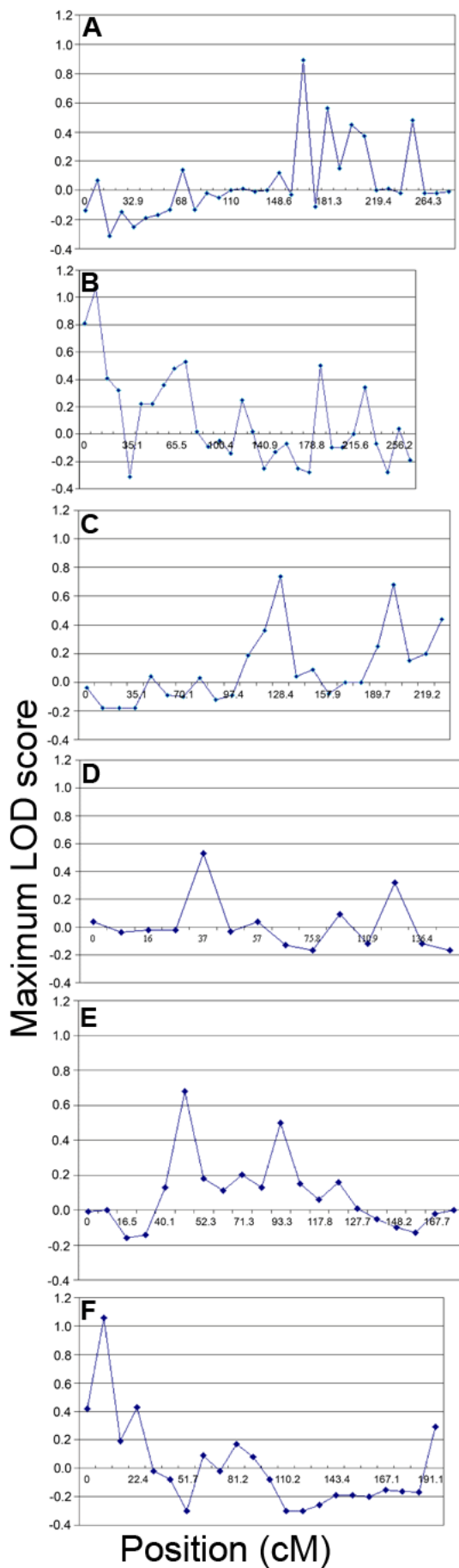
## DISCUSSION

In this study on a large five generation family, which segregates JOAG in an autosomal dominant fashion, no disease causing mutations were identified in *MYOC* and *OPTN*. Only three known *MYOC* polymorphisms (-1000C->G, -83G->A, and R76K) and four known *OPTN* polymorphisms (T34T, M98K, R545Q, and IVS7+24G->A) were found. These sequence alterations were not classified as causative mutations because of similar frequencies in JOAG patients and the unaffected family members.

In the *MYOC* gene, the most common polymorphism R76K was not associated with glaucoma in this family. R76K has no critical effect. It is located outside the olfactomedin-like region and the leucine zipper region (codons 117-166) which has been implicated as another potential site for *MYOC* dimerization and oligomerization [15]. However, R76K and -83G->A are in linkage disequilibrium in Chinese, Japanese, and Indian populations [3,14,15]. In contrast, evidence for linkage disequilibrium is not obvious in Caucasians and African-Americans [13,16]. In the present study of Philippine subjects, the linkage disequilibrium of these two polymorphisms is obvious, consistent with other populations in Asia.

Polymorphism -1000C->G in the *MYOC* gene, also designated as *MYOC.mt1*, did not segregate with the disease phenotype in the present study. In one report, it was associated with increased IOP and greater visual field damage, especially in women [27]. Time to event analysis showed that *MYOC.mt1* accelerated deterioration of both optic disc and visual field [28]. However, the association of this specific polymorphism with the severity of POAG is still controversial. One reported study showed no significant difference of the distribution of *MYOC.mt1* in any measure of disease severity from 393 POAG patients [29]. Our recent study on the *MYOC* promoter in unrelated individuals also indicated that *MYOC.mt1* is not associated with the risk and severity of POAG [30].

For the *OPTN* gene, the most common polymorphism was T34T (c.412G->A). This does not alter the amino acid sequence and has been reported as a synonymous codon change and neutral polymorphism [12,18]. We also found no segregation of M98K and R545Q with JOAG in this family, although they had been reported as mutation or risk associated genetic factor for glaucoma [12,31]. Since M98K is located within a putative bZIP domain and is conserved in macaques, it may represent a risk associated factor or a dominant susceptibility allele. M98K also did not segregate with POAG in a study on Caucasian families [32], but it could be a common polymorphism in Chinese and Japanese [18,20]. R545Q is not part of



a known protein domain, it is situated near the only zinc finger motif within optineurin. This motif is normally seen in transcription factors. The R545Q variant appears to be a neutral polymorphism that is much more prevalent among Asians than Caucasians [20].

In the present study, *MYOC* IVS7+24G->A did not show a significant association with JOAG. In our previous report, the mutation was associated with an increased cup to disc ratio and was potentially related to disruption of optic nerve [18]. However, before it can be concluded as a glaucoma causing mutation, it still has to be affirmatively linked with glaucoma through segregation in families, sequence analysis of relevant cDNA regions, assessment of the level of the mature transcript, or expression studies.

To date, the genes that underlie two of the six named POAG loci have been identified [6-11]; *MYOC* is the gene for *GLC1A* and *OPTN* is the gene for *GLC1E*, whereas genes underlying the other reported loci have not yet been identified. In families with mixed onset POAG containing both JOAG and adult onset open angle glaucoma, the *MYOC* mutation prevalence is 31% [33]. *MYOC* mutations have also been identified in 2-4% of unrelated individuals with POAG in various populations [3,6,14,15]. Meanwhile, *OPTN* mutations had been found in 16.7% of the hereditary form of NTG [12] and in about 1-2% of sporadic POAG patients [3,18,20]. All these findings show that *MYOC* and *OPTN* mutations only account for a minority of POAG.

Apart from the lack of *MYOC* and *OPTN* mutations, our linkage analysis also showed no evidence for linkage to any of six known POAG loci. All markers flanking these POAG loci gave LOD scores of not more than 1.1, and p values more than 0.05 were obtained by non-parametric linkage analysis for all these markers. Furthermore, no *MYOC* and *OPTN* mutations had been found in studies on Finnish and Barbados POAG families [21,22]. Linkage analysis was also carried out in the Barbados study, in which glaucoma did not show linkage with markers near the *MYOC* and *OPTN* regions. Although both our study and the above studies did not exclude a role for *MYOC* and *OPTN* in POAG, they did not support a major role for any of them as a cause of POAG. Further studies with familial and sporadic glaucoma patients are needed to detect susceptibility genes for POAG in the future. A genome wide linkage analysis in this family to discover the disease loci for JOAG is in progress.

#### ACKNOWLEDGEMENTS

We are grateful to the family that participated in this study. The work described in this paper was partially supported by a block grant from the Chinese University of Hong Kong and by the Mrs. Annie Wong Eye Foundation, Hong Kong.

Figure 2. Two point linkage analysis. All ABI-MD markers on these chromosomes were genotyped. The X-axis indicates the genetic positions for ABI-MD markers, the Y-axis indicates maximum LOD scores. A: Chromosome 1. B: Chromosome 2. C: Chromosome 3. D: Chromosome 8. E: Chromosome 10. F: Chromosome 7.

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