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Identification of *MYOC* gene mutation and polymorphism in a large Malay family with juvenile-onset open angle glaucoma

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Purpose: To screen for mutations in the coding region of the *myocilin* (*MYOC*) gene in a large Malay family with juvenile-onset open angle glaucoma (JOAG).
Methods: A total of 122 family members were thoroughly examined and screened for JOAG. Venipuncture was conducted. Genomic DNA was extracted from peripheral blood leukocytes. The presence of a mutation and a polymorphism was ascertained with PCR amplification followed by the direct sequencing technique.
Results: Thirty-two of the 122 screened family members were identified to have JOAG (11 new cases and 21 known cases). An autosomal dominant inheritance pattern with incomplete penetrance was observed. A C→A substitution at position 1440 in exon 3 that changes asparagine (AAC) to lysine (AAA) was identified in affected family members except two probands (III:5 and IV:6). Six probands were identified as having the Asn480Lys mutation but have not developed the disease yet. An intronic polymorphism IVS2 730 +35 G>A was also identified. There was a significant association between Asn480Lys ($p<0.001$) and IVS2 730+35G>A ($p<0.001$) in the affected and unaffected probands in this family.
Conclusions: The Asn480Lys mutation and the IVS2 730+35 G>A polymorphism increased susceptibility to JOAG in this large Malay pedigree. Identifying the *MYOC* mutations and polymorphisms is important for providing presymptomatic molecular diagnosis.

Glaucoma, characterized by progressive optic neuropathy with specific visual field defects, is one of the main causes of irreversible blindness in the world. Epidemiological studies have determined that a family history of glaucoma increases the risk of developing glaucoma from 13% to 60% [1,2]. Genetic predisposition of glaucoma is further supported by the strong association between glaucoma and endophenotypes of glaucoma in monozygotic twins compared to their spouses [3].

Juvenile-onset of open angle glaucoma (JOAG) is a variant of primary open angle glaucoma (POAG). JOAG is diagnosed earlier before the age of 40 and commonly described as a product of autosomal dominant inheritance [4]. The mode of inheritance in POAG is inconclusive [5]. POAG has been described as oligogenic, polygenic, even multifactorial and is regarded as a complex disease.

The discovery of the *myocilin* (*MYOC;* OMIM 601652) gene as the candidate gene for open angle glaucoma more than a decade ago has provided a new understanding of the genetic basis of glaucoma [6]. *MYOC* consists of three exons separated by two introns and a 5-kb promoter region, which encodes for a 55 to 57 kDa myocilin protein with 504 amino acids and an isoelectric point of approximately 5.21. It is located at the *GLC1A* (OMIM 601652) locus, chromosome 1q23-q25 [7]. *MYOC* mutations are associated with 2% to 4% of POAG [8]. *MYOC* gene mutations are reported to be higher in JOAG (8% to 30%) [9]. The majority of mutations occur in the third exon of the *MYOC* gene, which encodes the olfactomedin-like domain [10].

To date, more than 80 mutations have been identified in *MYOC* [10]. Several *MYOC* mutations have been shown to segregate with glaucoma in a statistically significant manner. These include Tyr437His, Ile477Asn, Gln368Stop, Thr377Met, 396INS397, and Gly364Val. The Gln368Stop mutation was found exclusively among Caucasians, responsible for the disease-causing mutation (DCM), while Arg46Stop was found as the DCM among Asians [10]. There are various reports on *MYOC* mutations in other Asian populations such as Chinese, Filipino, and Indian populations [11-13]. No common DCM has been found. In fact, no mutation was found among a Filipino population [12]. However, there is no report on Malays. Identifying *MYOC* mutations is important for presymptomatic diagnosis in young Malay patients with JOAG. Furthermore, this will enrich the *myocilin* database. The current study reports the first *MYOC* screening in four generations of a large Malay

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family with JOAG. Phenotype and genotype association are also described.

METHODS

Identification of family members: This study was conducted in accordance with the Declaration of Helsinki for research on humans. Ethical approval was obtained from the Ethics Board of Universiti Malaya Medical Centre. The index case was a 34-year-old Malay man who presented to the eye clinic at Universiti Malaya Medical Centre in 2003 for intermittent eye pain and mild headache. Intraocular pressure (IOP) was 28 mmHg on the right eye and 20 mmHg on the left eye. He provided us with an interesting pedigree chart of four generations of affected family members.

The majority of his family members reside on the east coast of Malaysia particularly in the states of Kelantan, Terengganu, and Pahang. A total of 122 out of 152 family members who were still alive were examined. The majority of the family members were screened in hospitals, including Hospital Universiti Sains Malaysia, Kelantan, Hospital Raja Perempuan Zainab II, Kelantan, Hospital Sultanah Nur Zahirah, Terengganu, and Hospital Kuala Lipis, Pahang. A small number of family members were screened at their homes using portable slit lamps and binocular indirect ophthalmoscope, and intraocular pressure was obtained using Tonopen (Medtronic Solan, Jacksonville, FL). If there were any suspicious findings such as vertical cup disc ratio (VCDR) of more than 0.7 or elevated (IOP >21 mmHg), the subject was then reexamined at the nearest tertiary hospital for further confirmation.

Ocular examination in the tertiary hospitals including visual acuity assessment, slit-lamp examination to evaluate the anterior segment, dilated fundus examination to document the structural changes of glaucoma, and gonioscopic examination to evaluate the angle structure was conducted. The central corneal thickness (CCT) was also measured but not for all probands. The Humphrey visual field analysis 24–2 program was also conducted to identify the functional defect of glaucoma. Heidelberg retinal tomography (HRT) II of the optic nerve head was also obtained to further document structural changes in the optic nerve head. Newly diagnosed JOAG is defined as the presence of optic neuropathy with VCDR of >0.7, neuroretinal rim thinning and thinning of the retinal nerve fiber layer thickness on HRT II, glaucomatous visual field defect, grade 3 or 4 of modified Shaeffer classification on gonioscopic examination, and IOP >21 mmHg in at least one eye in any family member aged between 3 and 40 years old.

Clinical record review was also conducted on family members who had been diagnosed with JOAG and received treatment at other tertiary centers. Age at the initial diagnosis, clinical presentation, and subsequent treatment were documented. Ocular examination was also conducted on these family members to obtain their latest clinical findings. The severity of the disease was then categorized according to the mean deviation of the Humphrey visual field analysis of the right eye: mild (<4), moderate (4–7), and severe (>12).

DNA extraction and screening of MYOC: A total of 6 ml of venous blood was obtained during venipuncture. Genomic DNA was extracted from the blood samples using the commercial kit, QIAamp DNA Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. PCR amplification of *MYOC* was done using six pairs of primer for three exons of the *MYOC* gene (Table 1 and Figure 1). The reactions contained 1X PCR buffer, 1.5 mM $MgCl_2$, 0.5 mM dNTPs (Promega, Madison, WI), 0.1 µM of each primer (Sigma-Aldrich, St.Louis, MO), 1 U Taq DNA polymerase (Promega), 50 ng genomic DNA, and double-distilled water in a total volume of 20 µl. The following conditions were used to perform the amplifications: 94 °C for 7 min; followed by 34 cycles of 94 °C for 1 min (denaturation), 1 min for annealing (for temperature, refer Table 1) and 72 °C for 1min (elongation); followed by 72 °C for 7 min (final elongation). The PCR products were evaluated on 2% agarose gel (Promega) and stained with SYBR Green 1 (CambrexBioScience, Walkersville, MD) to confirm the band size.

Amplicons were then purified using a Novagen kit (Merck KGaA, Darmstadt, Germany) according to the manufacturer's instructions. Sequencing was then conducted for the backward and forward primers using ABI Prism. Sequencing analysis was done using Bioedit Sequence Alignment Editor (Ibis Biosciences, Carlsbad, CA).

RESULTS

Clinical findings: The mean age during screening was 26.3±14.4 years, ranging from 6 to 71 years old. Based on the pedigree chart (Figure 1), the inheritance pattern was autosomal dominant in this large family. The first generation consists of the grandmother of our index patient. She died at the age of 60 with a history of bilateral blindness long before her death. She was married twice. She had three children by her first husband and five children from her second marriage. Four out of her eight children were affected by JOAG (II:3, II:7, II:9, II:14). On average, her children had 9.3 children each except the eldest son, who died a bachelor. She had 81 grandchildren. Her granddaughter (III:15) from her first marriage married her affected grandson (II:21) from

Table 1. Sequence and size of primers.

Primer	Sequence	Size	Ta (°C)
MYOC E1A	Fwd: 5′ CCCAGTATATATAAACCTCTCTGG 3′	423 bp	55.9
	Rev: 5′ AAGGTCAATTGGTGGAGGAG 3′		
MYOC E1B	Fwd: 5′ CTTACAGAGAGACAGCAGCAC 3′	453 bp	60
	Rev: 5′ CCTGTAGCAGGTCACTACGA 3′		
MYOC E2	Fwd: 5′ CATCCTCAACATAGTCAATCCT 3′	352 bp	60
	Rev: 5′ AGAGTTCTGTTCCTCTTCTCCT 3′		
MYOC E3A	Fwd: 5′ GATCATTGTCTGTGTTTGGA 3′	570 bp	57.9
	Rev: 5′ AGATTCTCTGGGTTCAGTTTG 3′		
MYOC E3B	Fwd: 5′ ATTGTCCTCTCCAAACTGAAC 3′	554 bp	56.8
	Rev: 5′ AACATCCCATAAATGCTGAC 3′		
MYOC E3C	Fwd: 5′ TGAGGGCGTAGACAATTTCA 3′	609 bp	55.9
	Rev: 5′ TGGATGCTGCTATTTGCTTG 3′		

Ta: Temperature for annealing

her second marriage. They have ten children, but none are affected yet. The majority of the immediate family members of our index patient are affected except III:22 and III:26. The youngest in his family (III:29) is not affected yet but carries the DCM.

We identified a total of 11 newly diagnosed JOAG (9 male; 2 female) probands newly diagnosed with JOAG during our screening (Figure 1 and Table 2). A total of 21 family members had already been diagnosed and treated at various hospitals. The mean age at diagnosis was 25.8±8.0 years (ranging from 16 to 37). Mean IOP before treatment of the right eye was 38.0±13.6 mmHg and 35.8±13.7mmHg of the left eye. This is based on the 32 patients diagnosed with JOAG (11 newly diagnosed and 21 known cases). We were unable to obtain the IOP at the initial diagnosis of ten other known cases. The total prevalence of JOAG in this family was 26.2% of the total family members screened.

Genotyping: We identified C→A at position 1440 in exon 3, which results in a substitution of asparagine (AAC) to lysine (AAA; Figure 2). Asn480Lys was found in all affected individuals except III:5 and IV:6 (Figure 1). Six probands (III:17, III:48, III:58, IV:14, IV:57, IV:58) were not affected by JOAG but carried the DCM, Asn480Lys (Figure 1). However, probands III:5 and IV:6 developed severe disease but without the DCM. The logarithm of the odds (LOD) score was 6.23 calculated using Haploview 4.2. We also found a synonymous polymorphism, G to A substitution, IVS2 730 +35 (Figure 2B,C). IVS2 730 +35G>A was also identified as rs2032555 in the NCBI database. There was a significant association between Lys480Lys and JOAG in this family (Table 3). Similarly, IVS2 730 +35 G>A increased the susceptibility to JOAG significantly (Table 3). The linkage disequilibrium between DCM Asn480Lys and IVS2 730 +35 G>A was 0.619.

Genotype and phenotype association: The analysis was conducted on 32 family members diagnosed with JOAG. Mean age at diagnosis was 29.3±9.2 years old. There was no significant association of the clinical parameters with Asn480Lys and IVS2 730+35G>A (Table 4). However, the JOAG probands with Lys480Lys were diagnosed at a younger age (mid-20s) but with lower mean IOP at diagnosis. The JOAG probands with IVS2 730+35AA were diagnosed in their late 20s and higher mean IOP at diagnosis.

DISCUSSION

Despite the popularity of *MYOC* as a candidate gene for glaucoma especially JOAG, no studies have examined the potential role of *MYOC* in Malay families. *MYOC* mutations are ethnic or population specific, and certain mutations are geographically specific, suggesting the possibility of the founder effect [8,10,14,15]. To the best of our knowledge, this is the first report on *MYOC* screening in a large Malay family with JOAG. Autosomal dominance with variable penetrance was observed in this family. Incomplete penetrance has been reported in most studies that involved families of JOAG [9,11,13]. Penetrance is age dependent and mutation specific [10]. The mean age of the fourth generation of this family is 17.4±7.1 years old. This may explain the incomplete penetrance in this pedigree.

The DCM has been identified mainly at the third exon of *MYOC* [10]. We identified a mutation in the third exon,

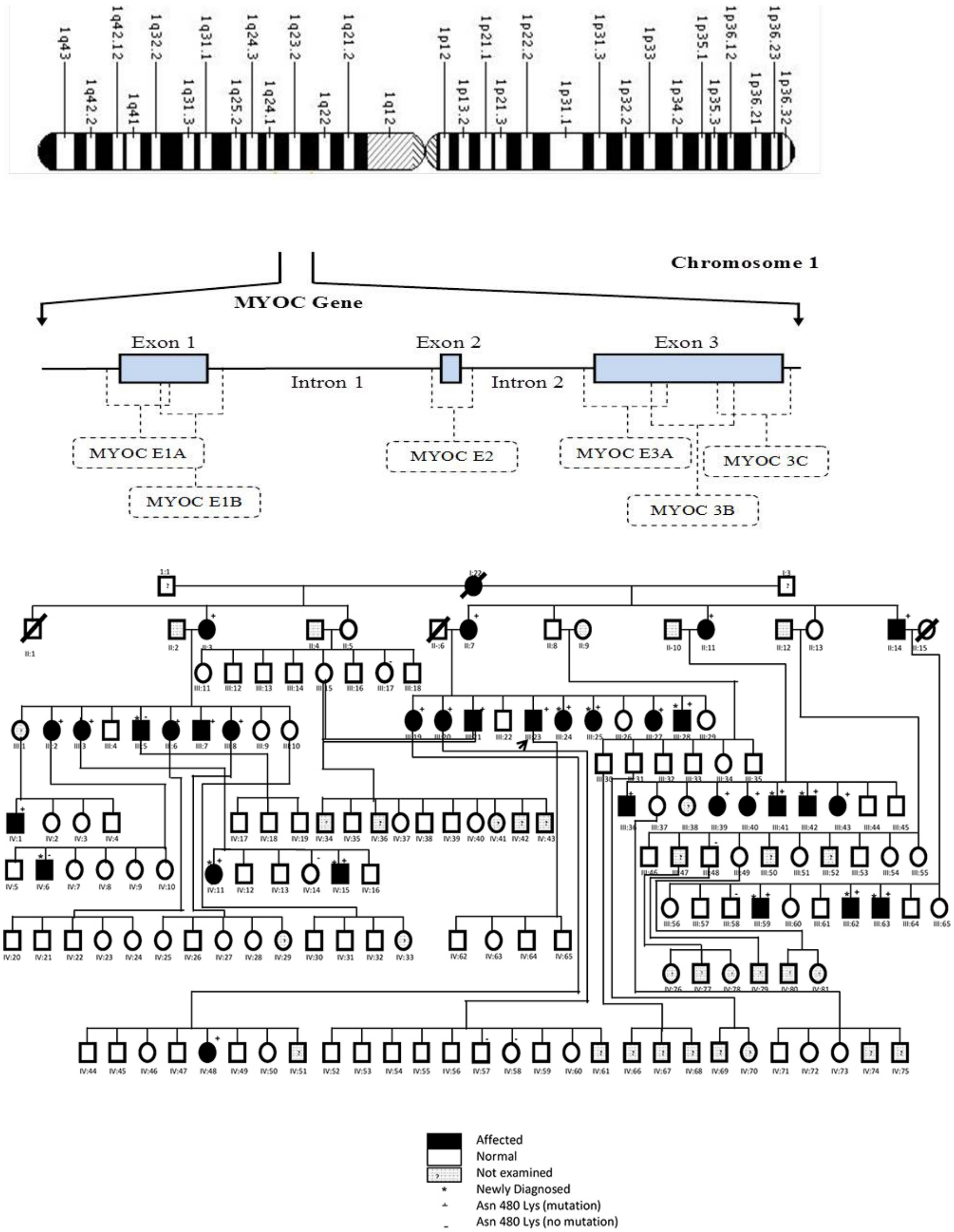

Figure 1. Schematic diagram of the location of the six pairs of primers for *MYOC* screening and the pedigree chart of four generations of a Malay family with juvenile-onset open angle glaucoma.

Asn480Lys, in this family. The Asn480Lys mutation is situated near the casein kinase II site that causes changes in polar amino acid (asparagine) to positively charged amino acid (lysine) and a gain of α-helix in the structural conformation of the MYOC protein [16]. The Asn480Lys mutation has been reported in families with open angle glaucoma in France [17]. The recurrent mutation was then reported in familial open angle glaucoma and case control studies of POAG in Europe, suggesting a possible founder effect [14,15]. Heterozygous C>A of Asn480Lys was reported in patients with POAG in southern India [18]. A variant of this missense mutation at nucleotide 1440 of C>G was reported in Andean families [19]. Heterozygous C>A of Asn480Lys was also seen in the majority of our JOAG probands, but Lys480Lys was observed

Table 2. Clinical characteristic of JOAG probands.

ID	Sex	Visual acuity		Age	IOP		VCDR		CCT		HRT(RNFL thickness)		Visual field		Treatment
		OD	OS		OD	OS	OD	OS	OD	OS	OD	OS	OD	OS	
II:3	F	NPL	NPL	38	35	10	Nil	Nil	Nil	Nil	Nil	Nil	Severe	Severe	No
II:7	F	NPL	6/12	25	Nil	12	0.9	0.9	Nil	510	Nil	Borderline	Severe	Severe	Surgical
II:11	F	6/15	6/15	40	16	20	0.9	0.5	551	527	Abnormal	Abnormal	Severe	Severe	Surgical
II:14	M	NPL	6/7.5	40	58	58	Nil	0.7	557	539	Nil	Abnormal	Severe	Severe	Surgical
III:2	F	NPL	NPL	42	42	42	Nil	Nil	539	480	Nil	Nil	Severe	Severe	No
III:3	F	6/6	6/7.5	40	56	56	0.9	0.9	468	469	Borderline	Normal	Severe	Severe	Medical
III:5*	M	6/7.5	6/7.5	47	24	16	0.8	0.2	515	497	Borderline	Normal	Severe	Severe	Nil
III:6	F	6/6	6/6	40	20	24	0.5	0.4	Nil	Nil	Nil	Nil	Mild	Normal	Medical
III:7	M	NPL	NPL	36	46	45	1	0.9	Nil	Nil	Abnormal	Abnormal	Severe	Severe	Nil
III:8	F	HM	6/24	31	11	17	0.9	0.8	Nil	Nil	Normal	Normal	Severe	Severe	Medical
III:19	F	NPL	6/12	36	60	40	0.9	0.3	533	502	Normal	Nil	Severe	Normal	Surgical
III:20	F	6/6	NPL	34	16	16	0.9	0.9	Nil	Nil	Normal	Normal	Severe	Severe	Surgical
III:21	M	6/7.5	6/7.5	29	32	32	0.7	0.6	466	439	Normal	Normal	Severe	Moderate	Both
III:23	M	6/6	6/6	32	20	23	0.5	0.3	510	512	Normal	Normal	Normal	Mild	Surgical
III:24*	F	6/6	6/6	37	49	33	0.7	0.5	517	509	Normal	Abnormal	Normal	Normal	Medical
III:25*	M	6/6	6/6	34	49	49	0.8	0.9	517	506	Nil	Nil	Normal	Severe	Medical
III:27	F	PL	6/9	17	30	22	0.9	0.9	Nil	Nil	Normal	Normal	Severe	Severe	Surgical
III:28*	M	6/7.5	6/6	23	28	35	0.4	0.5	501	506	Abnormal	Abnormal	Normal	Normal	Medical
III:36	M	6/18	6/6	32	50	50	0.9	0.9	504	499	Normal	Abnormal	Severe	Moderate	Both
III:39	F	6/6	6/6	24	16	14	0.5	0.7	510	513	Nil	Nil	Normal	Normal	Medical
III:40	F	6/9	6/6	25	20	19	0.5	0.3	487	488	Normal	Normal	Normal	Normal	Medical
III:41*	M	6/6	6/6	30	50	54	0.3	0.3	563	560	Normal	Normal	Normal	Normal	Medical
III:42*	M	6/6	6/6	25	29	37	0.6	0.7	557	556	Normal	Normal	Normal	Normal	Medical
III:43	F	6/5	6/5	16	24	22	0.3	0.3	519	529	Nil	Nil	Normal	Normal	Medical
III:59*	M	6/7.5	6/7.5	22	27	25	0.4	0.3	557	565	Abnormal	Borderline	Normal	Normal	Medical
III:62*	M	6/6	6/6	17	36	36	0.8	0.7	537	554	Abnormal	Abnormal	Severe	Severe	Medical
III:63*	M	6/7.5	6/7.5	16	38	40	0.6	0.9	527	532	Abnormal	Abnormal	Moderate	Severe	Medical
IV:1	M	NPL	NPL	16	44	5	0.9	0.9	488	536	Abnormal	Abnormal	Severe	Severe	No
IV:6	M	6/9	6/7.5	33	53	54	0.9	0.9	531	534	Normal	Normal	Severe	Severe	Medical
IV:11*	F	6/6	6/6	31	34	25	0.4	0.4	454	455	Normal	Normal	Normal	Normal	Medical
IV:15*	M	6/7.5	6/7.5	18	26	26	0.6	0.4	465	466	Normal	Normal	Mild	Moderate	Medical
IV:48	F	6/12	6/9	13	33	33	0.4	0.4	565	562	Normal	Normal	Normal	Normal	Both

Newly diagnosed juvenile-onset open angle glaucoma (11 cases); AGE: Age at diagnosis; IOP: Intraocular pressure; VCDR: Vertical cup disc ratio; CCT: Central corneal thickness; HRT: Heidelberg retinal tomography II; RNFL: Retinal nerve fiber layer; Nil: not done or not available; NPL: no perception to light; HM: hand movement.

Figure 2. Electropherogram and nucleotide basic local alignment search tool (BLASTn) showing heterozygous Asn480Lys at position 1440th nucleotide in exon 3 (**A**) and substitution of G to A of IVS2 730 +35 (**B**).

in five of our probands. Lys480Lys was found in our index patient's mother and his family. However, his father refused to participate in the study. We postulated that his father may also have either Lys480Lys or Asn480Lys (heterozygous). The spouse or parent of the majority of the probands may have Asn480Asn resulting in a higher number of heterozygous C>A of Asn480Lys in this pedigree. Unfortunately, the majority of the spouses of the affected probands refused to participate in this study.

Unlike Pro380Leu, Thr377Met, and Gln368Stop that affect the clinical presentation and mode of management of JOAG [8,10], there was no significant association with Asn480Lys with severity of glaucoma in this Malay family. Probands with Lys480Lys may have been detected earlier and treated appropriately compared to Asn480Lys (heterozygous), resulting in less severe glaucoma at the recruitment period of this study. The Asn480Lys mutation has been reported to associate with middle-aged glaucoma and mean IOP at presentation of 39.0±10.6 mmHg [10]. We found that our probands with Lys480Lys were diagnosed at a much earlier age but with lower mean IOP. This is perhaps due to the relatively small number of probands with Lys480Lys (five probands) that may be responsible for the skewness of age, severity, and IOP distribution. However, the lower IOP at the initial diagnosis of these five probands may be due to inaccurate data obtained from the medical records from various hospitals. In addition, there was also some missing data.

Higher baseline IOP was observed in our probands with Asn480Lys (heterozygous). In this study, the IOP was analyzed without adjustment for CCT. This is because CCT

Table 3. Genotype and allele frequency for Asn480 Lys and IVS2 730+35G>A (rs2032555) between affected and non-affected probands.

MYOC variation	JOAG n=32	Not affected n=90	p-value*
Asn480Lsy			
Genotype	N (%)	N (%)	
Asn480Asn	2 (6.3)	84 (93.3)	
Asn480Lys	25 (78.1)	6 (6.7)	<0.001
Lys480Lys	5 (15.6)	0 (0.0)	
Allele frequency			
C	0.45	0.97	<0.001
A	0.55	0.03	
IVS2 730+35G>A			
Genotype	N (%)	N (%)	
GG	5 (15.6)	4 (4.4)	
GA	22(68.8)	23 (25.6)	<0.001
AA	5 (15.6)	63 (70.0)	
	Allele frequency		
G	0.50	0.17	<0.001
A	0.50	0.83	

Table 4. Association between clinical parameters in JOAG probands and genotype frequency of Asn 480 Lys and IVS 2 730+35G>A (rs2032555).

	Genotype							
Clinical parameter		**Asn 480 Lys**		**p-value**		**IVS 2 730+35G>A**		**p-value**
	CC n=2	CA n=25	AA n=5		CC n=5	CA n=22	AA n=5	
Mean age (SD; year) n=32	40.0 (9.9)	29.5 (9.1)	24.4 (8.8)	0.457*	26.0 (6.9)	29.5 (9.1)	28.6 (10.2)	0.742*
	n=2	n=17	n=3		n=3	n=17	n=2	
Mean IOP (SD) (mmHg) n=22	34.5 (16.2)	40.4 (12.8)	27.0 (6.6)	0.284*	42.0 (16.5)	36.6 (12.9)	44.0 (22.6)	0.681*
Mean VCDR	0.56 (0.25)	0.70 (0.24)	0.58 (0.22)	0.280*	0.62 (0.27)	0.69 (0.22)	0.59 (0.28)	0.557*
Visual field defect	n=2	n=17	n=4		n=5	n=17	n=1	
Mean MD (SD) n=23	−23.64 (2.07)	−10.21 (12.11)	−5.28 (6.33)	0.184*	−9.86 (11.53)	−11.20 (12.03)	−2.27 (-)	0.763*
	n=2	n=19	n=4					
Mean CCT (SD; μm) n=25	523 (11)	518 (34)	511 (41)	0.890*	510 (16)	518 (38)	524 (20)	0.834*
Severity								
Early	0	9	3	0.738#	3	8	1	0.839#
Mild	0	2	0		0	2	0	
Moderate	0	1	0		0	1	0	
Severe	2	13	2		2	11	4	

p<0.05 is considered significant based on One way ANOVA* and Fisher exact test#; IOP: intraocular pressure; VCDR: vertical cup disc ratio; MD: mean deviation of Humphrey field analysis; CCT: central corneal thickness.

was not measured in all probands. Furthermore, the predictive accuracy of developing glaucoma is not improved by adjusting the IOP for CCT using the conversion formula [20]. Two probands presented with a severe stage of glaucoma, but they did not have the Asn480Lys mutation. Both probands were detected during the recruitment process in this study. The genotype analysis (even venipuncture) was repeated more than once for these two probands to eliminate any contamination or wrongly labeled EDTA tube.

In this present study, due to financial constraints, only the exons and their flanking regions of *MYOC* were screened. The variations at the promoter, untranslated region, and intronic polymorphisms may have been missed especially in these two probands. There is also the possibility another gene may be responsible. However, six probands had the Asn480Lys mutation but were not clinically diagnosed with JOAG. Their mean age was 23.8±14.3 years (range between 12 and 36 years old). JOAG is age dependent.

We also identified an intronic polymorphism, IVS2 730+35G>A. A similar nucleotide substitution has been reported known as IVS2 +35G>A in case control studies involving Indian and Chinese patients with POAG and controls [21,22]. However, this polymorphism IVS2 730+35G>A had no significant role in either study. There was no similar report in the family with JOAG. There was a significant difference in the genotype and allele frequency of IVS2 730+35G>A between the affected and non-affected probands in our study. To the best of our knowledge, there is no report on the role of this polymorphism as the susceptibility genetic marker for JOAG. There is a possibility that IVS2 730+35G>A may increase the susceptibility of developing JOAG in the Malay population. This is based on the significant difference of allele and genotype frequency of IVS2 730+35G>A between the affected and non-affected probands in this large Malay pedigree. However, IVS2 730+35G>A was not significantly associated with the severity of JOAG.

Based on the linkage disequilibrium, there is a possibility of a combination effect between Asn480Lys and IVS2 730+35G>A as susceptibility markers for JOAG in this family. However, we did not analyze the combination effect of Asn480Lys and IVS2 730+35G>A on the severity of JOAG. Perhaps, the presence of intronic polymorphism IVS2 730+35G>A in the JOAG patient with DCM Asn480Lys may lead to more severe diseases at a younger age. The current study looked into the molecular analysis of *MYOC* in only one large Malay pedigree; perhaps studying multiple families will help in identifying the hotspot mutations of *MYOC* in the Malay population.

The present study provides the first molecular analysis of *MYOC* in a Malay family with JOAG. Identifying the genetic risk factor is important for early detection of JOAG. Early detection and prompt treatment may prevent blindness especially in young patients with JOAG.

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