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RESEARCH REPORT

Clinical and genetic characterization of a large primary open angle glaucoma pedigree

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ABSTRACT

Purpose: To both characterize the clinical features of large primary open angle glaucoma (POAG) pedigree from a village in southern India and to investigate the genetic basis of their disease.
Materials and methods: Eighty-four members of a large pedigree received complete eye examinations including slit lamp examination, tonometry, gonioscopy, and ophthalmoscopy. Some were further studied with perimetry. Those diagnosed with POAG were tested for disease-causing mutations in the myocilin and optineurin genes with Sanger sequencing.
Results: Fourteen of 84 family members were diagnosed with POAG, while eight were clinically judged to be POAG-suspects. The family structure and the pattern of glaucoma in the pedigree are complex. Features of glaucoma in this pedigree include relatively early age at diagnosis (mean 50 ± 14 years) and maximum intraocular pressures ranging from 14 to 36 mm Hg with a mean of 23 mm Hg ± 6.5 mm Hg. Patients had an average central corneal thickness (mean 529 ± 37.8 microns) and moderate cup-to-disc ratios (0.74 ± 0.14). No mutations were detected in myocilin, optineurin, or TANK binding kinase 1 (*TBK1*).
Conclusions: We report a five-generation pedigree with a complex pattern of POAG inheritance that includes 22 POAG patients and glaucoma suspects. Although the familial clustering of POAG in this pedigree is consistent with dominant inheritance of a glaucoma-causing gene, mutations were not detected in genes previously associated with autosomal dominant glaucoma, suggesting the involvement of a novel disease-causing gene in this pedigree.

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Introduction

Glaucoma afflicts millions in India and is a common cause of blindness. In 2002, an estimated 6.7 million Indians were blind due to glaucoma.[1] The prevalence of glaucoma in India has been estimated to be 1.6–4.0%[2–4] and the majority of glaucoma in India is classified as primary open angle glaucoma (POAG).[3] Glaucoma and POAG are clearly important public health problems in India.

Primary open angle glaucoma (POAG) has a strong genetic component to its pathogenesis and is highly heterogeneous.[5] Some forms of POAG are caused by the combined action of many genetic risk factors. Genome-wide association studies have identified many such genetic risk factors for POAG, including *CAV1/CAV2*,[6] *CDKN2B-AS1*,[7–10] *TMCO1*,[11] *ATOH7*,[7] *SIX1/SIX6*,[7,10] *GAS7*,[11] chromosome 8q22 locus,[9] *ABCA1*,[12,13] *AFAP1*,[12] *GMDS*,[12] *PMM2*,[13] *FNDC3B*,[14,15] *TFGBR3*,[16] *TXNRD2*,[17] *ATXN2*,[17] and *FOXC1I*.[17] While these variants are commonly detected in healthy individuals, they are observed at statistically higher frequencies in glaucoma patients. Other forms of glaucoma are caused primarily by mutations in a single gene, such as myocilin (*MYOC*),[18] optineurin (*OPTN*),[19] or TANK binding kinase 1 (*TBK1*).[20] Each of these genes was discovered with studies of large pedigrees that have autosomal dominant inheritance of POAG. In the current study, we report clinical characterization of another large POAG pedigree with Egyptian heritage from southern India (Tamil Nadu). Genetic analyses were also conducted to determine if the gene that causes disease in this family could be identified.

Materials and methods

POAG subjects and controls

Informed consent was obtained from all family members after explanation of the nature and possible consequences of the study. The study protocol was approved by the Institutional Review Board at the University of Iowa, and the Aravind Medical Research Foundation/Aravind Eye Hospitals. This study followed the tenets of the Declaration of Helsinki.

After screening 240 people during a field trip to Kayalpatanam, a total of 84 members of a single family

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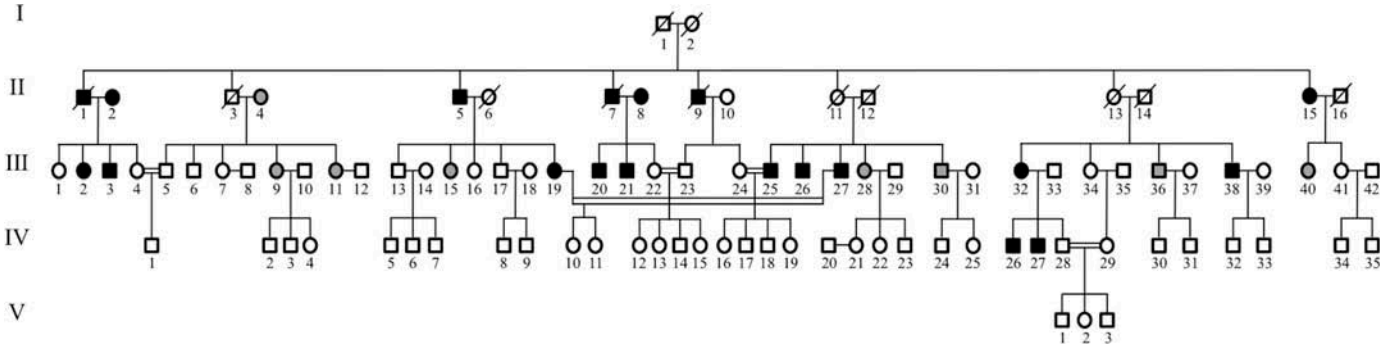

Figure 1. POAG pedigree from southern India. Family members diagnosed with POAG are indicated with symbols shaded black, while those that are glaucoma-suspect are indicated with symbols shaded grey. Family members and spouses who had normal eye examinations, didn't meet the criteria for POAG, or had suspect status are indicated with a symbol shaded white.

were enrolled (Figure 1). All the family members underwent comprehensive ocular examination which included applanation tonometry, pachymetry, slit-lamp examination, gonioscopy, and optic disc examination with 90 D lens. Eleven family members diagnosed with glaucoma or who were judged to be glaucoma suspects were further evaluated at the Aravind Eye Care System and Institute of Ophthalmology at Tirunelveli, Tamil Nadu, where additional testing was conducted including standard automated perimetry with a Humphrey Visual Field Analyzer (Zeiss-Humphrey Systems, Dublin, CA) using SITA 24-2 and 10-2 algorithms. Family members with open angles on gonioscopy (Shaffer grade III or IV) and glaucomatous optic disc cupping and corresponding visual field defects were diagnosed with POAG as previously described.[21] Elevated IOP was not required for a diagnosis of POAG. Exclusion criteria included potential causes of secondary glaucoma: pigment dispersion syndrome, exfoliation syndrome, inflammation, ocular trauma, ocular surgery, or developmental abnormalities. Patients were judged to be glaucoma suspects if they did not meet threshold criteria for POAG but had ocular hypertension (IOP > 21 mm Hg), or had suspicious appearing optic discs or suspicious visual fields.

Sample preparation and mutation screening

A volume of 5–10 ml of peripheral venous whole blood samples were collected in EDTA by venipuncture tube from each participant. We extracted DNA using the salt precipitation method as previously described.[22] The isolated DNA was quantified and further preceded for mutational screening known candidate genes (*MYOC, OPTN* by bi-directional Sanger sequencing and *TBK1* by qPCR) as previously described.[21,23,24]

Statistical analysis

Age, maximum IOP, CCT (average between eyes), and cup-to-disc ratio were compared using two-tailed unpaired T-test. The threshold for significance was set at $p = 0.05$.

Results

Clinical characteristics of glaucoma in the pedigree

A total of 84 members of a large family from southern India, all with Egyptian heritage (Figure 1), had complete ophthalmic examinations. Fourteen of the 84 family members were diagnosed with POAG, while eight family members were judged to be glaucoma suspects (Figure 1). Clinical features of these family members are described in Table 1. The POAG in this family has a relatively early age of onset with a mean of 50 ± 14 years and a range of 23–68 years. The maximum recorded IOP in family members with POAG ranged from 14 to 36 mm Hg with a mean of 22.5 ± 6.5 mm Hg. Six (43%) of the 14 family members with POAG had a maximum recorded IOP < 21 mm Hg. Central corneal thickness (CCT) in family members with POAG had a mean value of 529 ± 37.8 microns and cup-to-disc ratios at first examination (mean of OD and OS) ranged from 0.6 to 0.9 with a mean of 0.74 ± 0.14. Moderate to severe visual field loss was detected with SITA 24-2 and 10-2 testing algorithms with a Humphrey Visual Analyzer.

Eight of 14 family members (Figure 1) were judged to be glaucoma suspects because their examinations were suggestive of glaucoma, but did not meet the threshold for a diagnosis of disease. The clinical features of the glaucoma suspects are described in Table 2. The maximum IOP of the glaucoma suspects ranged from 13 to 22 mm Hg and the mean maximum IOP of 18.0 ± 2.7 mm Hg is nominally lower than the mean maximum IOP of POAG patients, 22.5 ± 6.5 mm Hg ($p = 0.079$). The glaucoma suspects in the family had a mean CCT of 554 ± 37.9 microns which is nominally thicker than CCT of the POAG patients, 529 ±37.9 microns ($p = 0.16$). The cup-to-disc ratio of the glaucoma suspects at first examination (mean of OD and OS) was 0.59 ± 0.088 which is significantly smaller than the mean cup-to-disc ratio of the family members with POAG, 0.74 ± 0.14 ($p = 0.00064$).

Known candidate gene screening

Patients of this pedigree with POAG had maximum recorded IOPs that ranged from 14 to 36 mm Hg and 6 (43%) of 14 patients have never had a measured IOP > 21. Consequently,

Table 1. Clinical features of the family members diagnosed with POAG.

	Age at diagnosis	Max IOP	CCT	Cup-to-disc ratio at first exam		Humphrey Visual Field Analyzer Data (SITA 24-2/10-2[a])					
Pedigree symbol	(years)	(mm Hg)	(microns)	OD	OS	MD OD (dB)	PSD OD (dB)	MD OS (dB)	PSD OS (dB)	Glaucoma surgeries	Co-morbidity
III-2	23	24	522	0.7	0.4	−2.89	8.07	−0.26	1.10	None	None
III-3	34	26	527	0.7	0.8	−8.36	8.36	−10.37	10.01	None	None
II-2	65	15	506.5	0.8	0.8	−6.05	2.42	−9.05	4.93	None	None
III-20	45	21	565.5	0.7	0.6	−7.32	4.07	−9.64	5.71	None	None
III-21	40	14	519	0.6	0.6	NA	NA	NA	NA	None	None
II-5	56	17	NA	0.9	NA	NA	NA	NA	NA	Trabeculectomy	CRVO
III-19	51	24	552	0.5	0.6	−8.8	6.61	−10.09	8.37	None	None
III-32	NA	26	519	0.9	0.9	NA	NA	NA	NA	None	None
IV-26	NA	36	528.5	0.9	0.9	NA	NA	NA	NA	Trabeculectomy	CRVO
IV-27	NA	18	617	0.8	0.8	NA	NA	NA	NA	None	None
III-38	68	22	535	0.6	0.6	−3.63	1.63	−4.42	2.24	None	None
III-26	66	18	486	0.8	0.8	−14.24[a]	8.12[a]	−12.56[a]	9.56[a]	None	CRVO
III-25	48	20	470	0.9	NA	−26.62[a]	9.97[a]	NA	NA	Trabeculectomy	CRVO
II-15	53	34	NA	NA	0.9	NA	NA	−23.57	8.25	Trabeculectomy	CRVO
Mean	50	22.5	529	0.74		−6.18	5.19	−9.63	5.80	NA	NA
Std Dev	14	6.5	37.8	0.14		2.46	2.90	7.19	3.31		

[a]Visual field loss was detected in these individuals with 10-2 testing algorithms with a Humphrey Visual Field Analyser.
IOP, intraocular pressure; CCT, central corneal thickness; OD, right eye; OS, left eye; SITA, Swedish Interactive Thresholding Algorithm; MD, mean deviation; dB, decibels.

Table 2. Clinical features of family members who were glaucoma suspects.

	Age at diagnosis	Max IOP	CCT	Cup-to-disc ratio at first exam		Humphrey Visual Field Analyzer Data (SITA 24-2)			
Pedigree symbol	(years)	(mm Hg)	(microns)	OD	OS	MD OD (dB)	PSD OD (dB)	MD OS (dB)	PSD (dB)
III-15	51	19	552	0.6	0.65	NA	NA	NA	NA
II-4	58	20	552	0.6	0.6	−6.71	4.997	−12.76	10.02
III-9	40	16	601	0.6	0.6	NA	NA	NA	NA
III-11	36	19	601	0.65	0.65	NA	NA	NA	NA
III-36	45	22	545	0.6	0.6	−3.7	1.19	−3.28	1.43
III-30	52	13	527	0.6	0.3	0.91	1.41	0.7	1.47
III-28	49	18	486	0.6	0.7	−3.17	2.85	−3.86	1.34
III-40	43	17	568	0.6	0.5	NA	NA	NA	NA
Mean	47	18.0	554	0.59		−3.17	2.61	−4.80	3.57
Std Dev	7.1	2.7	37.9	0.088		3.13	1.75	5.68	4.30

IOP, intraocular pressure; CCT, central corneal thickness; OD, right eye; OS, left eye; SITA, Swedish Interactive Thresholding Algorithm; MD, mean deviation; dB, decibels.

members of this pedigree were tested for disease-causing mutations in genes previously associated with POAG with high IOP (myocilin) and in genes previously associated with POAG with low IOP (optineurin and *TBK1*).

All 14 family members with POAG were tested for myocilin mutations with Sanger sequencing. One non-synonymous variant (Arg76Lys) was detected in the myocilin gene, which has been previously reported as a benign, non-disease-causing variant.[21] POAG patients were also tested for the glaucoma-causing optineurin mutation (Glu50Lys),[19] however, this variant was not detected. Finally, the family members with POAG were tested for the previously reported copy number variations (duplications and triplications) of the *TBK1* gene that are associated with normal tension glaucoma.[20] No *TBK1* copy number variations were detected.

Discussion

This family has POAG with variable clinical features and age at diagnosis and maximum IOP (Table 1). Although the average age at diagnosis was 50 years and most family members were diagnosed with POAG in their fifth or sixth decade, others were diagnosed at an earlier age (i.e. at 23 and 34 years old). Similarly, members of this pedigree with POAG had a mean maximum IOP of 22.5 mm Hg, but six (43%) of these family members had never had an IOP recorded above 21 mm Hg. While members of this glaucoma pedigree together have average ages at diagnosis and average maximum IOPs typical for a diagnosis of POAG, some members have an onset before age 40 years which is suggestive of juvenile-onset open angle glaucoma. Likewise most families have maximum IOPs greater than 21 mm Hg, but a sizeable fraction, 43%, have maximum IOPs less than 21 mm Hg consistent with diagnosis of normal tension glaucoma. This variability in age at diagnosis and maximum IOP is not uncommon among large glaucoma pedigrees. However, it is possible, although less likely, that disparate phenotypes may suggest that one or more family members is a phenocopy (i.e. has a different cause of glaucoma than the rest of the family).

Pedigrees with many (i.e. more than ten) family members diagnosed with POAG are rare, but several have been reported.[19,20,25–32] The POAG in each of these large pedigrees has a relatively early age at diagnosis and is inherited in an autosomal dominant pattern. Genetic studies of these pedigrees have had a profound influence on the investigation of glaucoma pathogenesis. Studies of one group of these large POAG pedigrees ultimately led to the discovery that mutations in myocilin gene cause 4% of

POAG cases.[18,21] Subsequent studies of myocilin biology in human patients, organ culture systems, and transgenic mice suggest that some cases of glaucoma are caused by accumulation of abnormal myocilin protein within trabecular meshwork cells.[33–35] Genetic studies of other large POAG families similarly led to the discovery of optineurin[19] and *TBK1*[20] as glaucoma-causing genes and have implicated defects in auotphagy as another important mechanism in glaucoma pathogenesis. Consequently, there is great interest in characterizing the clinical features of POAG pedigrees such as ours (Figure 1) and searching for the genes that cause their glaucoma. This current report shows that our large POAG pedigree does not harbor mutations in the three genes currently known to cause POAG and suggests that further studies of this pedigree have the potential to identify a new glaucoma-causing gene.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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