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Gln48His is the prevalent myocilin mutation in primary open angle and primary congenital glaucoma phenotypes in India

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Purpose: Myocilin gene defects have been originally implicated in primary open angle glaucoma (POAG). Based on multiple reports for the occurrence of Gln48His mutation (c.144G>T; HGMD accession number CM023962) among Indian POAG patients, we wanted to estimate the prevalence of this mutation in primary open angle and primary congenital glaucoma (PCG) in India and assess its role in the causation of the disease.

Methods: Two hundred cases each of POAG and PCG were screened for the Gln48His mutation by RFLP (*AccI*) analysis of the PCR amplicons followed by confirmation of the c.144G>T change by direct sequencing.

Results: The Gln48His mutation was detected in 9 different glaucoma patients (four POAG and five PCG). While all four POAG cases were heterozygous, among PCG cases, four were heterozygous and one exhibited homozygous genotype for the mutation. One each of POAG and PCG patients was detected to be heterozygous for *CYP1B1* mutation (c.1656C>T, Pro437Leu) and (c.1449G>A, Arg368His), respectively. None of the 300 ethnically matched normal controls contained either the *MYOC* or *CYP1B1* mutation(s).

Conclusions: The myocilin mutation, Gln48His, represents an allelic condition involving a spectrum of glaucoma phenotypes in Indian populations, and could be a potential risk factor towards disease predisposition among patients of Indian origin. The study also highlights the role of *MYOC* as a candidate in different glaucoma subtypes that needs to be investigated further.

Glaucoma, the second leading cause of blindness worldwide, represents a group of disorders with varied clinical symptoms [1]. The underlying molecular mechanism is still unknown although 7 chromosomal loci (*GLCIA* to *GLC1G*) have been mapped for primary open angle glaucoma (POAG) and 3 (*GLC3A* to *GLC3C*) for primary congenital glaucoma (PCG), of which only *GLCIA* (Myocilin), *GLC1E* (Optineurin) and *GLC3A* (*CYP1B1*) have been characterized [1-3]. The myocilin gene (*MYOC*) exhibits a wide spectrum of mutations and accounts for 2-5% cases of POAG [1]. Some pathogenic mutations (e.g., Gln368Stop) are widely prevalent while others are recurrent (Gly252Arg, Gly367Arg, and Pro370Leu) in varying frequencies in different populations [1-3]. The limited studies done on Indian POAG patients suggest that the Gln48His mutation in *MYOC* recurs in different ethnic groups but is restricted to the people of Indian origin according to the published literature [4,5]. In this context, we attempted to investigate the prevalence of the myocilin mutation (Gln48His) among Indian glaucoma patients comprising of POAG and PCG cases.

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METHODS

The study protocols adhered to the tenets of the Declaration of Helsinki and were approved by the Institutional Review Board. Two hundred cases each of POAG and PCG were recruited from the southern (37.5%), eastern (35.5%), western (13.5%), and northern (23.5%) parts of India.

Cases were enrolled as POAG on the basis of an elevated intraocular pressure of >21 mm Hg and/or glaucomatous disc changes in the presence of typical field defects, along with an open angle on gonioscopy and no other secondary causes. Cases of ocular hypertension were excluded from this category. On the other hand, PCG cases were included on the basis of an increased corneal diameter (>12.0 mm) along with raised intraocular pressure (>21 mmHg) and/or presence of Haab's striae, or optic disc changes (where examination was possible). The ages of onset ranged from 0-1 years and symptoms of epiphora, photophobia, and rupture in the Descemet's membrane were the corroborating factors. Three hundred ethnically matched normal individuals without any signs or symptoms of glaucoma and other systemic diseases served as controls. Their visual acuity ranged from 20/20 to 20/40 and IOP was <21 mm Hg. Clinical examination on stereo biomicroscopy did not reveal any changes in the optic disc suggestive of glaucoma.

Collection of blood samples and genomic DNA preparation, polymerase chain reaction (PCR), and the Gln48His

mutation screening by digesting the PCR amplicons with *AccI* restriction enzyme were done as described earlier [4]. The loss of the *AccI* site suggested presence of the mutation (c.144G>T) which was confirmed by direct sequencing. The patients containing the mutant *MYOC* allele was screened for the *CYP1B1* mutation by direct sequencing as described earlier [6].

RESULTS & DISCUSSION

Among 200 POAG cases we identified 4 individuals carrying the *MYOC* Gln48His mutation (Table 1), including 3 mutants reported earlier [4]. One of the patients was also heterozygous for a *CYP1B1* mutation (c.1656C>T; Pro437Leu) suggesting a digenic inheritance, as shown in a JOAG family [2], which could not be investigated further because one of the proband's parents and his siblings were deceased. In addition, other studies from India have reported two other POAG cases harboring the same mutation [5]. These observations clearly establish that Gln48His is a common mutation among Indian patients which, however, has not yet been reported from any other population.

We also screened 200 PCG cases for the *MYOC* Gln48His mutation and identified 5 cases harboring the mutant allele (Table 1), which also included one homozygote (Table 2). Among Indian PCG cases about 40% are *CYP1B1* mutants [6]. Interestingly, 4 of the 5 PCG cases harboring *MYOC* mutation lacked any *CYP1B1* defect. The presence of Gln48His in the homozygous state in one PCG case devoid of any *CYP1B1* mutation, and absence of the Gln48His mutation in 300 ethnically matched normal controls strongly argue for the role of the mutant *MYOC* protein causing PCG. However, no study has yet described the functional mechanism for the involvement of *MYOC* in PCG; although an earlier study showed that heterozygous *MYOC* and *CYP1B1* mutations cause JOAG through a digenic mechanism. It was also hypothesized that *CYP1B1* may be a modifier of *MYOC* expression and these two genes might act through a common biochemical pathway [2]. It is worthwhile to mention here that there are examples of single gene defects (e.g., RDS/peripherin) manifesting clinically distinguishable eye diseases [7].

The PCG proband homozygous for the *MYOC* mutation (Gln48His) was born out of a consanguineous marriage, as evident by homozygous genotypes of markers in the patient (data not shown). However, we did not have the opportunity

TABLE 1. DISTRIBUTION OF MYOCILIN MUTATION GLN48HIS AMONG THE INDIAN GLAUCOMA PATIENTS

Phenotype	Number of individuals	Number of Gln48His mutations
POAG	200	4 (2.0%)
PCG	200	5 (2.5%)
Normal	300	0

In addition to the data presented below, Sriprya et al. [5] reported a Gln48His mutation in two Indian POAG patients out of 100 screened for defects in *MYOC*.

to investigate the segregation of the *MYOC* mutant alleles in this family because the parents and siblings were deceased. Clinically this patient had a relatively severe phenotype (Corneal diameter 14 mm, total cupping, and an IOP of 74 and 50 mm Hg in the right and left eyes, respectively) compared to other PCG patients with the heterozygous *MYOC* mutation [8]. The outcome in terms of vision and IOP control (on treatment) was also poor. Interestingly, it has been shown in a large French-Canadian family that homozygotes for a *MYOC* missense mutation (Lys423Glu) are asymptomatic while heterozygotes are affected with POAG suggesting a dominant negative effect in single dosage of the defective *MYOC* rather than haploinsufficiency in this family [9]. Thus our observation in homozygous *MYOC* mutant (Gln48His) is remarkably different, which suggests that accumulation of much larger dataset and functional studies might shed more light to decipher the biology of pathogenesis of glaucoma.

It is possible that for the other 3 PCG cases lacking the *CYP1B1* mutation, some other yet unidentified locus together with the *MYOC* Gln48His mutation might be involved in the causation of the disease. In one PCG patient having one mutant allele each for *CYP1B1* (c.1449G>A; Arg368His) and *MYOC* (c.144G>T; Gln48His), the disease might be caused by digenic inheritance, as proposed for JOAG [2]. The father and the mother of this patient were heterozygous for the mutant *MYOC* and *CYP1B1* alleles, respectively, and did not manifest any glaucomatous symptoms [8]. Although it has been hypothesized that *CYP1B1* could be a modifier of *MYOC* expression and that these two genes might act through a common biochemical pathway [2], there are no functional evidences so far to support this point. The genotypes of all nine patients with Gln48His mutation are described in Table 2.

Although we cannot ascribe causality of all glaucoma phenotypes to the Gln48His mutation alone, it is likely to be a potential risk factor towards disease predisposition. Hence we recommend the screening for this *MYOC* mutation in all glaucoma patients of Indian origin. The study presented here sug-

TABLE 2. GENOTYPE AND PHENOTYPE OF GLAUCOMA PATIENTS WITH THE MYOC GLN48HIS MUTATION

Patient Number	Phenotype	Age at symptom onset	Genotypes	
			MYOC (c.144)	CYP1B1 (c.1449/c.1656)
1	POAG	37 years	(G, 'T')	(G,G)/(C, 'T')
2	JOAG	-	(G, 'T')	(G,G)/(C,C)
3	POAG	-	(G, 'T')	(G,G)/(C,C)
4	JOAG	-	(G, 'T')	(G,G)/(C,C)
5	PCG	at birth	('T', 'T')	(G,G)/(C,C)
6	PCG	at birth	(G, 'T')	(G,G)/(C,C)
7	PCG	at birth	(G, 'T')	(G,G)/(C,C)
8	PCG	4 months	(G, 'T')	(G,G)/(C,C)
9	PCG	at birth	(G, 'T')	(G, 'A')/(C,C)

Among all the nine patients harboring the myocilin (NM_000261) mutation, two were also heterozygous for *CYP1B1* (NM_000104) mutations. Three of the POAG patients (patient 2, 3, and 4) have been described before [4]. The mutant alleles are enclosed by apostrophes. *MYOC* mutation: c.144G>T, Gln48His; and *CYP1B1* mutations: c.1449G>A, Arg368His and c.1656C>T; Pro437Leu.

gests that the *MYOC* mutation could be associated to different subtypes of glaucoma that need to be further investigated to better appreciate the role of *MYOC* in glaucoma pathogenesis.

ACKNOWLEDGEMENTS

We thank the patients and their families and the normal volunteers for participating in this study. KK, SK, KRD, MA, and AM thank the Council of Scientific Research, Government of India, for pre-doctoral research fellowships. This study was supported by grants from the Department of Biotechnology, Indian Council of Medical Research, and the Council of Scientific and Industrial Research, Government of India. We thank Prof. D. Balasubramanian for his critique of the manuscript.

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