Sex Bias in Primary Congenital Glaucoma Patients with and without *CYP1B1* Mutations

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Purpose: To investigate variations in sex ratio among Iranian primary congenital glaucoma (PCG) patients with and without mutations in the *CYP1B1* gene and to evaluate possible clinical variations associated with sex in these two groups.
Methods: Phenotypical data on 104 unrelated Iranian PCG patients who had previously been screened for *CYP1B1* mutations were analyzed. Emphasis was placed on analysis of sex ratios among patients with and without *CYP1B1* mutations. In addition to sex, familial and sporadic incidence and clinical features including age at onset, bilateral/unilateral involvement, corneal diameter, intraocular pressure, and cup-disc ratios were compared between these two groups. Information on phenotypical parameters was available for most but not all patients.
Results: Among the 93 PCG patients whose sex was recorded, 57 were male (61.3%) and 36 were female (38.7%) (P=0.03). Patients with *CYP1B1* mutations included 37 male (66.1%) and 29 female (43.9%) subjects (P=0.30), while patients without the mutation included 20 (74.1%) male and 7 (25.9%) female individuals (P=0.013). Our data did not provide conclusive evidence on difference in severity of the disease between those with and without *CYP1B1* mutations, nor between the two sexes.
Conclusion: Consistent with data on PCG patients from other populations, the overall incidence of PCG in Iran seems to be higher among male subjects. The difference in incidence between the two sexes was not significant among patients whose disease was due to mutations in *CYP1B1*. The overall higher incidence of PCG among male subjects seems to be attributable to a higher incidence in male patients not harboring *CYP1B1* mutations, suggesting that other genes or factors may be involved in manifestation of PCG phenotypes in a sex dependent manner.

Key words: Primary Congenital Glaucoma; Cytochrome P-4501B1; Sex Ratio; Phenotype

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INTRODUCTION

Primary congenital glaucoma (PCG; Online Mendelian Inheritance in Man [OMIM] no. 231300) is a severe form of glaucoma characterized by an anatomical defect in the trabecular meshwork (trabeculodysgenesis) in neonates and infants generally before the age of 3 years.[1] The clinical features of PCG include increased intraocular pressure (IOP), globe en-

largement (buphthalmos), corneal enlargement, Descemet membrane ruptures, corneal edema and opacification, and optic nerve damage. Details of the pathogenic pathways are not completely understood. PCG occurs in both sporadic and familial patterns. Sporadic occurrence may reflect incomplete penetrance of potentially disease causing mutations.[1,2] In familial cases, the inheritance is usually autosomal recessive. The incidence of PCG is geographically and ethnically variable, ranging from 1:10,000 in Western countries to much higher frequencies in several inbred populations.[1] Its incidence in the Middle East is estimated at 1:2500.[2]

Although three loci have been found to be linked to PCG, only the gene associated with one of them, *CYP1B1* (OMIM 601771), has been identified.[3] This gene is a member of the cytochrome P450 superfamily of genes and encodes cytochrome P4501B1. Presumably, mutations in *CYP1B1* result in aberrant metabolism of an endogenous substrate involved in the pathogenesis of PCG. The proportion of patients with PCG attributable to *CYP1B1* mutations is variable among different populations, ranging from as low as about 20% in Japan[4] to approximately 50% in Brazil,[5] France,[6] and India,[7] and as high as 100% in Slovakia Roma[8] and Saudi Arabia.[2] In a recent study, *CYP1B1* mutations were identified in approximately 70% of Iranian patients.[9]

Steroid hormones may somehow be relevant to the expression of the *CYP1B1* gene or to the function of the coded protein. Transcription of the gene is induced by the aryl hydrocarbon receptor.[10-13] Furthermore, estradiol can act as a substrate for the CYP1B1 protein and mutations in the gene affect hydroxylation of this substrate.[14] It seems reasonable to attribute the higher incidence of PCG among male subjects to these observations.[15] Male patients account for approximately 65% of PCG cases.[16] Although some authors have reported correlations between phenotypes of PCG and various mutations in *CYP1B1*, few have addressed sex differences.[9,17] To the best of our knowledge, sex ratio comparisons between patients with and without *CYP1B1* mutations have been presented in only one report on Japanese patients.[18] Herein, we compare phenotypic features among a relatively large number of Iranian PCG patients who were previously shown to harbor or not to harbor *CYP1B1* mutations, with an emphasis on sex ratios.

METHODS

Data of 104 unrelated Iranian PCG patients previously genotyped by sequencing of the coding region of *CYP1B1* were analyzed.[9] The research was performed in accordance with the Helsinki Declaration. Diagnosis was made by experienced glaucoma specialists familiar with PCG based on elevated IOP ($\geq$21 mmHg) before treatment, corneal edema, Descemet membrane rupture, megalocornea (corneal diameter >12 mm), and optic nerve head changes suggestive of glaucomatous damage. Clinical features were ascertained during examination at the time of patient recruitment or from hospital records. IOP measurements were obtained using Goldmann applanation tonometry or the Tono-Pen in cases with limited cooperation or central corneal scars. The condition was considered familial if the parents were consanguineous or if relatives affected with PCG were reported. The remaining cases were considered sporadic. Information on phenotypical parameters was available for most but not all patients. Statistical comparisons were made using the Chi-square test or Chi-square test contingency tables with significance level set at $P<0.05$.[19]

RESULTS

Table 1 summarizes the distribution of patients based on sex, familial/sporadic involvement and the presence or absence of *CYP1B1* mutations. Twelve comparisons were made, and 3 showed statistically significant differences in distribution. Overall, the incidence of PCG was higher in male subjects (P=0.03). Similarly, PCG incidence was higher in male patients among cases without *CYP1B1* mutations (P=0.013) and in male subjects among familial cases without *CYP1B1* mutations (P=0.05). Notably, there was no significant difference between PCG incidence in males and females harboring *CYP1B1* mutations (P=0.30).

Clinical features of the patients based on gender and presence of *CYP1B1* mutations are presented in Table 2. There was a notable difference in mean age at presentation between male (5.58 months) and female (0.32 months) subjects harboring *CYP1B1* mutations. The incidence of unilateral PCG was higher in male subjects both in cases with and without mutations. Average corneal diameter in female patients with and without *CYP1B1* mutations was 13.6 mm versus 12.9 mm, respectively. Average IOP was higher in both sexes among subjects with *CYP1B1* mutations as compared to those without these mutations.

Table 1 Distribution of patients based on sex, familial/ sporadic patterns and the presence of *CYP1B1* mutations

	Numbers	P value
Male/Female		
Overall	57/36	0.03*
Familial	30/20	0.80*
Sporadic	27/16	0.10*
Overall with mutations	37/29	0.30
Familial with mutations	20/17	0.65
Sporadic with mutations	17/12	0.35
Overall without mutations	20/7	0.013
Familial without mutations	10/3	0.05
Sporadic without mutations	10/4	0.1
Familial/Sporadic		
Overall	50/43	0.80*
With mutations	37/29	0.3
Without mutations	13/14	~1

P values were obtained with Chi-square test (*) or using Chi-square contingency tables.

Table 2 Clinical features based on gender and presence of *CYP1B1* mutations

CYP1B1 mutations	Mean Age at onset (months)	Uni-lateral (%)	Mean corneal diameter (mm)	Mean maximum IOP (mmHg)	Mean C/D
Male with	5.58	16	13.3	27.4	0.54
Female with	0.32	9	12.9	25.2	0.54
Male without	2.73	33	13.2	22.1	0.65
Female without	3.17	0	13.6	23.1	0.44

IOP, intraocular pressure; C/D, cup-disc ratio.

DISCUSSION

The higher male to female ratio observed in our series is consistent with comparisons made on patients from other populations.[15,18,20] We found that male predominance was statistically significant in patients without *CYP1B1* mutations, but not in those with *CYP1B1* mutations. We found only one publication in the literature comparing sex ratios among patients with and without *CYP1B1* mutations, and the authors of the study reported the same trend.[18] The observations of that study were made on 32 patients, whereas our data was obtained from 93 patients. Furthermore, it is important that the same conclusions on sex ratios among patients with and without *CYP1B1* mutations are being made on two distinct populations i.e. a Japanese and an Iranian population. Despite the fact that steroids are relevant to *CYP1B1* gene expression and CYP1B1 protein function, it is interesting that sex related differences in incidence were not observed among patients harboring mutations. *CYP1B1* mutations are known to have variable expressivity and some apparently unaffected family members of PCG probands harboring *CYP1B1* mutations have been observed to have the same genotype as their affected siblings. Consistent with the absence of a sex-dependent effect on PCG incidence in subjects with *CYP1B1* mutations, we have observed equal numbers of males and females among phenotypically unaffected individuals who are actually "affected" according to *CYP1B1* genotypes (unpublished data). The higher male to female ratio among patients not harboring *CYP1B1* mutations suggests that one or more genes other than *CYP1B1* may be involved in the etiology of PCG in a sex dependent manner and that these genes may account for the higher incidence of PCG among male subjects observed in various populations.

Clinical features among male and female patients harboring and those not harboring *CYP1B1* mutations revealed some differences, particularly in terms of age at presentation between male and female patients harboring *CYP1B1* mutations. Nevertheless, we feel that our data do not provide conclusive evidence on differences in severity of disease between subjects with and without *CYP1B1* mutations, and also between male and female patients. Yet more detailed clinical evaluations on a larger number of patients must be made to ascertain such possible differences.

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