CLINICAL SCIENCE

Phenotype of cytochrome P4501B1 gene (*CYP1B1*) mutations in Japanese patients with primary congenital glaucoma

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Aim: To investigate the phenotypes associated with cytochrome P4501B1 gene (*CYP1B1*) mutations in Japanese patients with primary congenital glaucoma (PCG).

Methods: 66 Japanese patients with PCG were screened for sequence mutations in the *CYP1B1* gene using single strand conformation polymorphism analysis followed by automated DNA sequencing. 11 cases had a *CYP1B1* mutation in both alleles (the mutation group) and 21 cases did not have a *CYP1B1* mutation (the "no mutation" group). The clinical features, such as age of onset, sex, intraocular pressure, and Descemet's membrane rupture, of the two groups were compared.

Results: The clinical symptoms and signs did not differ for the two groups. The mean age at onset was 1.7 months in the mutation group and 3.1 months in the no mutation group, and the male:female ratio was 6:5 in the mutation group and 19:2 in the no mutation group. Both of these differences were statistically significant.

Conclusions: In clinically diagnosed cases of PCG, a subgroup shows a *CYP1B1* gene mutation. Age at onset was earlier in PCG patients with *CYP1B1* mutations than in patients without mutations. Women were more prevalent among patients with mutations than those without mutations.

Primary congenital glaucoma (PCG; gene symbol, GLC3) is a group of disorders characterised by an improper development of the eye's aqueous outflow system.[1–4] PCG is detected clinically in neonates and infants by the increased intraocular pressure (IOP). Because the coating of the infantile eye is elastic, it stretches in response to the elevated IOP, resulting in an enlarged globe (buphthalmos).[3 4]

PCG is inherited as an autosomal recessive trait, and the incidence varies geographically; one in 5000 to one in 22 000 newborns in Western countries, one in 2500 in the Middle East, and one in 1250 in the gipsy population of Slovakia, among whom PCG is the major cause of blindness.[1 5–8] In Japan, the incidence of PCG has not been determined, but most cases are sporadic.[9]

Two PCG mutation loci have been identified—the GLC3A locus which maps to chromosome 2p21[10] and the GLC3B locus which maps to chromosome 1p36.[11] Recently, several mutations in the cytochrome P4501B1 gene, *CYP1B1*, have been reported in families with PCG linked to the GLC3A locus.[9 12–18] *CYP1B1* gene mutations were detected in over 85% of families with PCG in Saudi Arabia, Turkey, and Slovakia.[13–15] Recently, we screened the *CYP1B1* gene in 65 unrelated Japanese probands with PCG and identified 11 novel mutations in 13 probands (20%).[9]

We present a more detailed investigation of the relation between the phenotype of Japanese patients with PCG and the genotype of *CYP1B1* mutation.

SUBJECTS AND METHODS

Blood samples were collected from 66 patients (65 families) with PCG at the following hospitals: 28 at Keio University Hospital, 16 at Tokyo University Hospital, 15 at the National Children's Hospital, three at Kyoto University Hospital, and four at Tenri Yorozu Hospital. Patients with elevated IOP associated with other ocular or systemic anomalies were excluded. None of these Japanese probands was the offspring of a consanguineous marriage. All cases except for one pair of twin girls (cases 8 and 9) in the group with mutations appeared to be sporadic with no family history of glaucoma. Informed consent was obtained from the parents of each child as well as their own participation in the study. This investigation was performed according to the guidelines of the Declaration of Helsinki.

Genomic DNA was prepared from leucocytes by proteinase K-phenol-chloroform extraction. We first screened the *CYP1B1* gene (GenBank accession number U56438) by polymerase chain reaction (PCR) amplification followed by single strand conformation polymorphism (SSCP) analysis as described previously.[9]

Clinical features at initial presentation

The clinical features at the onset of PCG were ascertained in 32 cases by examining the clinical records at each hospital. Of the 32 PCG patients, 11 had a *CYP1B1* mutation in both alleles (the "mutation group"), while 21 had no mutation (the "no mutation" group). We compared the mutation group with the no mutation group in terms of sex, age at diagnosis of PCG, and appearance of anterior segment (for example, corneal opacity, Descemet's membrane rupture, and gonioscopic appearance). For analysis, the onset of PCG was defined as the time of the initial diagnosis.

RESULTS

All cases in the mutation and no mutation group had typical symptoms and signs of PCG such as tearing, photophobia, and corneal enlargement. The clinical and genetic information of the 11 patients with *CYP1B1* mutations is summarised in Table 1.

The male:female ratio was 6:5 in the mutation group and 19:2 in the no mutation group (Table 2). This difference was significant (Fisher's exact probability test: $p < 0.05$). In comparison, previous reports have estimated that male cases account for approximately 65% of PCG cases overall.[3 6] Patients with bilateral PCG included 10 (91%) of 11 cases with the

Table 1 Clinical and genotypic information in 11 Japanese patients with primary congenital glaucoma (mutation group)

Case	Sex	Age at onset	Affected eye	Corneal opacity	Ruptures in Descemet's membrane	Mutations in CYP1B1 gene	
1	F	2 weeks	B	R+/L+	R+/L+	4776 ins AT (Frameshift)	G 7927 A (Val 364 Met)
2	M	2 months	B	+/–	–/–	4776 ins AT (Frameshift)	G 7927 A (Val 364 Met)
3	M	1 month	B	+/+	–/–	C 4645 A (Cys 280 stop)	G 8168 A (Arg 444 Gln)
4	F	at birth	B	+/+	–/–	3964del C (Frameshift)	G 8168 A (Arg 444 Gln)
5	M	at birth	B	+/+	+/+	G 7927 A (Val 364 Met)	G 8168 A (Arg 444 Gln)
6	M	at birth	B	+/+	–/+	A 4380 T (Asp 192 Val)*	
7	F	at birth	B	+/+	+/–	4776 ins AT (Frameshift)	G 7927 A (Val 364 Met)
8	F	1 week	B	+/+	–/–	G4793T,C4794T (Ala 330 Phe)	G 7927 A (Val 364 Met)
9	F	1 week	B	+/+	–/+	G4793T,C4794T (Ala 330 Phe)	G 7927 A (Val 364 Met)
10	M	2 months	B	+/+	–/–	A 4380 T (Asp 192 Val)	G 7927 A (Val 364 Met)
11	M	11 months	L	–/+	–/+	C3130T (Unknown)†	G 4763 T (Val 320 Leu)

B = bilateral, R = right, L = left.
*Homozygous; †Probable mutation in the noncoding region of exon 1.

Table 2 Clinical signs in primary congenital glaucoma patients with or without *CYP1B1* mutations

	Male: female*	Bilateral: unilateral	Mean age at onset**
Mutation group	6:5	10:1	1.7 months (0–11)
No mutation group	19:2	17:4	3.1 months (0–8)

*Fisher's exact probability test; $p<0.05$.
**Mann-Whitney test; $p<0.05$.

CYP1B1 mutation, and 17 (81%) of 21 cases without the *CYP1B1* mutation (Table 2). This finding without mutation is in agreement with previous reports that the disease is bilateral in approximately 75% of cases.[3]

The PCG was always diagnosed at birth or within the first year of life. A significant difference was evident in mean age at onset (3.1 months in the no mutation group and 1.7 months in the mutation group, Table 2; Mann-Whitney test, $p<0.05$). More specifically, 10 cases in the mutation group (all except for one case with a C3130T mutation, case 11) were diagnosed as PCG within 2 months of birth.

Most cases had corneal opacity and ruptures in Descemet's membrane (Table 3). No differences in the clinical findings were found between the two groups. Gonioscopy showed that most cases had a high insertion of the iris (Table 3), but we could not strictly classify anterior angle appearance because of corneal opacities and the retrospective, multicentre design of the study. One case had an atypical, unilateral uveal ectropion on the pupillary margin (case 4).

We present the clinical features of two atypical cases (4 and 11), and a unique pair of identical twins (8 and 9) with the *CYP1B1* mutation.

Case 4

A newborn Japanese girl was referred to Kyoto University Hospital for evaluation of corneal opacities of both eyes. No contributory family history or consanguinity was present. The IOPs, measured during sleep were 25 mm Hg for right eye and 30 mm Hg for left eye. The horizontal corneal diameter was 10.5 mm for right eye and 11.0 mm for left eye. Diffuse corneal opacities were present in both eyes, but no rupture in Descemet's membrane was present. Corneal opacities prevented a clear examination of the anterior chamber angles by gonioscopy. But the pupils were round and both irises were slightly atrophic in colour. The crystalline lenses and the vitreous cavity were normal. The optic discs showed slight glaucomatous cupping, with cup to disc ratios of 0.3 for right eye and 0.5 for left eye.

Genetic analysis showed that she was a compound heterozygote with both 3964delC and Arg444Gln mutations.

The patient underwent trabeculotomies once in the right eye and twice in the left eye, and the IOPs were controlled at 9–18 mm Hg (right eye) and 12 to 22 mm Hg (left eye) for 5 years.

When she was 3 years old, a uveal ectropion was observed at the pupillary margin of the left eye. Corrected visual acuity on a recent examination was 0.5 for right eye and 0.02 for left eye.

Case 11

An 11 month old Japanese boy, who was a compound heterozygote with both the C3130T and Val320Leu mutations, was referred to the National Children's Hospital for evaluation of a corneal enlargement and opacity in left eye. No contributory family history or consanguinity was present. The IOPs were 13 mm Hg for right eye and 50 mm Hg for left eye. The patient was diagnosed with PCG and subsequently examined under anaesthesia. The horizontal corneal diameter was 11.5 mm in the right eye and 12.5 mm in the left eye. Ruptures in Descemet's membrane were observed in the left eye. Gonioscopy revealed that the anterior chamber angles were widely open with anterior iris insertion, most markedly in the left eye. The crystalline lenses and the vitreous were normal. The left optic disc had glaucomatous cupping with the cup to disc ratio of 0.8; the right disc appeared normal.

Trabeculotomy was performed on the left eye, and the IOP was controlled between 8 and 18 mm Hg for 6 years. The corrected visual acuity on a recent examination was limited to 0.05 because of amblyopia probably caused by corneal opacity.

Cases 8 and 9

These identical twin girls, who were compound heterozygotes with both Ala330Phe and Val364Met, were referred to the

Table 3 Anterior segment appearance in primary congenital glaucoma with or without *CYP1B1* mutations

	Corneal opacity	Ruptures in Descemet's membrane	High insertion of iris
Mutation group	20/21 eyes (95.2%)	8/21 eyes (38.1%)	17/21 eyes (80.9%)
No mutation group	35/38 eyes (92.1%)	16/38 eyes (42.1%)	32/38 eyes (84.2%)

Tokyo University Hospital as newborns for evaluation of corneal opacities in both eyes. Both cases were affected bilaterally with PCG, and the increased IOP was detected within 1 week of age. The IOPs were elevated in both eyes, and clinical features such as corneal opacities and gonioscopic appearance were very similar in the twins. Goniotomies were performed on both eyes of the twin. But the IOPs could not be controlled on the left eye of case 9, and finally Scheie's sclerostomy was performed after two goniotomies. The final corrected visual acuity was 0.8 for right eye and 0.7 for left eye in case 8, and 0.1 for right eye and no perception of light for left eye in case 9.

DISCUSSION

The rarity of PCG and its differing frequency among ethnic groups impedes analysis of the phenotype and genotype of *CYP1B1* mutations. We are not aware of a previous report discussing the phenotypes of patients with and without *CYP1B1* mutations. In our Japanese patients with PCG, 11 cases had a *CYP1B1* mutation in both alleles and 23 cases did not have a mutation in the *CYP1B1* gene.

CYP1B1 gene mutations have been identified in over 85% of PCG affected families in Saudi Arabia, Turkey, and Slovakia.[13–15] However, a 27% prevalence of *CYP1B1* mutation has been reported in cases with sporadic occurrence.[16] This finding is similar to that of our earlier study, in which a 20% mutation rate was found in the Japanese PCG patients.[9]

In our present investigation, comparisons between the *CYP1B1* mutation group and the no mutation group revealed significant differences in sex preponderance and age at glaucoma onset. Interestingly, 10 cases in the mutation group, all except case 11, had bilateral involvement, and the first signs of disease appeared within 2 months of birth. In addition, compared to the no mutation group, the proportion of female patients was significantly higher in the mutation group. These features such as sex preponderance and bilateral incidence were very similar to PCG patients with the *CYP1B1* mutations in Saudi Arabia, Turkey, and India.[13 14 17] It is not clear why the sex ratios are so different between the two groups, as opposed to the fact that males account for approximately 65% of PCG cases overall.[3 6] We suggest that there may be another subtype of PCG with a higher preponderance in males, and these patients may have mutations not linked to the *CYP1B1* but to an unknown genetic locus.

Maldevelopment of the anterior segment may involve the trabecular meshwork alone or the trabecular meshwork in combination with the iris and/or cornea. Although PCG is a specific term referring to eyes that have an isolated maldevelopment of the trabecular meshwork without other ocular developmental anomalies or diseases that can raise the IOP, the terminology for glaucomas affecting infants has been inconsistent and sometimes confusing.[3 4] In our case 4, a unique uveal ectropion was observed in one of the affected eyes. Thus, a new classification of glaucomas affecting infants might be based on the presence or absence of a *CYP1B1* gene mutation.

A male patient (case 11), who was a compound heterozygote with both C3130T and Val320Leu alterations, was diagnosed relatively late in life as having unilateral glaucoma. In this case, the initial signs of glaucoma were identified when the patient was 11 months old, a relatively late detection time, and the anterior chamber developmental anomalies were found in both eyes (although pressure was elevated in only one eye). Thus, this C3130T mutation may partially inhibit normal cytochrome P4501B1 expression, leading to incomplete anterior chamber development and be manifested as a relatively mild case of PCG.

We had also a pair of identical twin girls with the *CYP1B1* gene mutation (cases 8 and 9). There was a concordance in the phenotypic features due to the same genetic factor, but the final visual acuity was different for the two cases. Although we should consider both the surgical efficacy and the influence of amblyopia, some environmental factors as well as genetic factors might modulate the severity of the disease.

In conclusion, among Japanese patients clinically diagnosed with PCG, we found a subtype with *CYP1B1* gene mutations. Clarification of how the *CYP1B1* gene is related to anterior chamber development may lead to better understanding of the mechanism of PCG occurrence and fundamental changes in the treatment of PCG.

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REFERENCES

1 **François J**. Congenital glaucoma and its inheritance. *Ophthalmologica* 1980;**181**:61–73.
2 **Hoskins HD**, Shaffer RN, Hetherington J. Anatomical classification of the developmental glaucomas. *Arch Ophthalmol* 1984;**102**:1331–6.
3 **Dickens CJ**, Hoskins HD. Congenital glaucoma. In: Ritch R, Shields MB, Krupin T, eds. *The glaucomas*. 2nd ed. St Louis, MO: Mosby, 1996:727–49.
4 **Stamper RL**, Lieberman MF, Drake MV. Developmental and childhood glaucoma. In: *Becker-Shaffer's diagnosis and therapy of the glaucomas*. 7th ed. St Louis, MO: Mosby, 1999:361–411.
5 **Travers JP**. The presentation of congenital glaucoma. *J Pediatr Ophthalmol Strabismus* 1979;**16**:241–2.
6 **Gencik A**, Gencikova A, Ferak V. Population genetical aspects of primary congenital glaucoma. I: Incidence, prevalence, gene frequency, and age of onset. *Hum Genet* 1982;**61**:193–7.
7 **Jaffer MS**. Care of the infantile glaucoma patient. In: Reineck, RD ed. *Ophthalmology annual 15*. New York: Raven Press, 1988.
8 **Wagner RS**. Glaucoma in children. *Pediatr Clin North Am* 1993;**40**:855–67.
9 **Mashima Y**, Suzuki Y, Sergeev Y, *et al*. Novel cytochrome P4501B1 (CYP1B1) gene mutations in Japanese patients with primary congenital glaucoma. *Invest Ophthalmol Vis Sci* 2001;**42**:2211–6.
10 **Sarfarazi M**, Akarsu AN, Hossain A, *et al*. Assignment of a locus (GLC3A) for primary congenital glaucoma (buphthalmos) to 2p21 and evidence for genetic heterogeneity. *Genomics* 1995;**30**:171–7.
11 **Akarsu AN**, Turacli ME, Aktan SG, *et al*. A second locus (GLC3B) for primary congenital glaucoma (buphthalmos) maps to the 1p36 region. *Hum Mol Genet* 1996;**5**:1199–203.
12 **Stoilov I**, Akarsu AN, Sarfarazi M. Identification of three different truncating mutations in cytochrome P4501B1 (CYP1B1) as the principal cause of primary congenital glaucoma (buphthalmos) in families linked to the GLC3A locus on chromosome 2p21. *Hum Mol Genet* 1997;**6**:641–7.
13 **Bejjani BA**, Lewis RA, Tomey KF, *et al*. Mutations in CYP1B1, the gene for cytochrome P4501B1, are the predominant cause of primary congenital glaucoma in Saudi Arabia. *Am J Hum Genet* 1998;**62**:325–33.
14 **Stoilov I**, Akarsu AN, Alozie I, *et al*. Sequence analysis and homology modeling suggest that primary congenital glaucoma on 2p21 results from mutations disrupting either the hinge region or the conserved core structures of cytochrome P4501B1. *Am J Hum Genet* 1998;**62**:573–84.
15 **Plasilova M**, Stoilov I, Sarfarazi M, *et al*. Identification of a single ancestral CYP1B1 mutation in Slovak gypsies (Roms) affected with primary congenital glaucoma. *J Med Genet* 1999;**36**:290–4.
16 **Sarfarazi M**, Stoilov I. Molecular genetics of primary congenital glaucoma. *Eye* 2000;**14**:422–8.
17 **Panicker SG**, Reddy AB, Mandal AK, *et al*. Identification of novel mutations causing familial primary congenital glaucoma in Indian pedigrees. *Invest Ophthalmol Vis Sci* 2002;**43**:1358–66.
18 **Stoilov IR**, Costa VP, Vasconcellos JP, *et al*. Molecular genetics of primary congenital glaucoma in Brazil. *Invest Ophthalmol Vis Sci* 2002;**43**:1820–7.