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.

PHENOTYPE OF CYTOCHROME P4501B1 GENE (CYP1B1) MUTATIONS IN JAPANESE PATIENTS WITH PRIMARY CONGENITAL GLAUCOMA

Y Ohtake, T Tanino, Y Suzuki, H Miyata, M Taomoto, N Azuma, H Tanihara, M Araie, Y Mashima

Primary congenital glaucoma (PCG; gene symbol, GLC3) is a group of disorders characterised by an improper development of the eye’s aqueous outflow system. 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). 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. In Japan, the incidence of PCG has not been determined, but most cases are sporadic.

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

We present a more detailed investigation of the relation between the phenotype of Japanese patients with PCG and the genotype of CYP1B1 mutation.
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 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. 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 3964delIC 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 cavity 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

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

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age at onset</th>
<th>Affected eye</th>
<th>Corneal opacity</th>
<th>Ruptures in Descemet’s membrane</th>
<th>Mutations in CYP1B1 gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>2 weeks</td>
<td>B</td>
<td>R+/L+</td>
<td>R+/L+</td>
<td>4776 ins AT (Frameshift)</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>2 months</td>
<td>B</td>
<td>+/-</td>
<td>+/-</td>
<td>4776 ins AT (Frameshift)</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>1 month</td>
<td>B</td>
<td>+/-</td>
<td>+/-</td>
<td>4645 A (Cys 280 stop)</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>at birth</td>
<td>B</td>
<td>+/-</td>
<td>+/-</td>
<td>3964delC (Frameshift)</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>at birth</td>
<td>B</td>
<td>+/-</td>
<td>+/-</td>
<td>7927 A (Val 364 Met)</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>at birth</td>
<td>B</td>
<td>+/-</td>
<td>+/-</td>
<td>4380 T (Asp 192 Val)</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>at birth</td>
<td>B</td>
<td>+/-</td>
<td>+/-</td>
<td>4776 ins AT (Frameshift)</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>1 week</td>
<td>B</td>
<td>+/-</td>
<td>+/-</td>
<td>4793T,C4794T (Ala 330 Phe)</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>week</td>
<td>B</td>
<td>+/-</td>
<td>+/-</td>
<td>7927 A (Val 364 Met)</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>2 months</td>
<td>B</td>
<td>+/-</td>
<td>+/-</td>
<td>4380 T (Asp 192 Val)</td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>11 months</td>
<td>L</td>
<td>+/-</td>
<td>+/-</td>
<td>3130T (Unknown)†</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>Male: female*</th>
<th>Bilateral: unilateral</th>
<th>Mean age at onset**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutation group</td>
<td>6:5</td>
<td>10:1</td>
<td>1.7 months [0-11]</td>
</tr>
<tr>
<td>No mutation group</td>
<td>19:2</td>
<td>17:4</td>
<td>3.1 months [0-8]</td>
</tr>
</tbody>
</table>

*Fisher’s exact probability test: p<0.05. **Mann-Whitney test: p<0.05.

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

<table>
<thead>
<tr>
<th></th>
<th>Corneal opacity</th>
<th>Ruptures in Descemet’s membrane</th>
<th>High insertion of iris</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutation group</td>
<td>20/21 eyes (95.2%)</td>
<td>8/21 eyes (38.1%)</td>
<td>17/21 eyes (80.9%)</td>
</tr>
<tr>
<td>No mutation group</td>
<td>35/38 eyes (92.1%)</td>
<td>16/38 eyes (42.1%)</td>
<td>32/38 eyes (84.2%)</td>
</tr>
</tbody>
</table>

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 3964delIC 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...
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 mutation in the CYP1B1 gene.

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

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

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