Case Report
CYP1B1 gene mutations with incomplete penetrance in a Chinese pedigree with primary congenital glaucoma: a case report and review of literatures

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Abstract: To investigate the cytochrome P4501B1 (CYP1B1) mutations in a three-generation Chinese Han family with PCG, the 2 and 3 coding exons of CYP1B1 gene were amplified by PCR, and were directly sequenced using Sanger bidirectional sequencing reactions. The mutation c.517 G>A p.E173K was detected in all the affected individuals (which showed homozygous AA genotype) and not in all the unaffected ones except one individual. The mutation c.517 G>A p.E173K is associated with disease causing in this pedigree. And the possible genetic model is recessive inheritance. One apparently unaffected individual had mutations and haplotypes identical to her affected sibs suggested incomplete penetrance in this pedigree.

Keywords: CYP1B1 gene, primary congenital glaucoma, Chinese pedigree, incomplete penetrance

Introduction

Primary congenital glaucoma (PCG) is the most frequent childhood glaucoma and can lead to blindness during neonatal or early infantile period. Molecular genetic studies conducted during the last several years have confirmed that PCG is an autosomal recessive trait. Currently, four chromosomal loci have been implicated in PCG on GLC3A harboring the cytochrome P4501B1 (CYP1B1) gene [1], GLC3B, GLC3C and a new locus GLC3D harboring the LTBP2 gene. Of which, mutations of the CYP1B1 on GLC3A is identified to be implicated in the majority of PCG cases.

The spectra of CYP1B1 mutations causing PCG vary widely in different ethnic populations. The spectrum of CYP1B1 mutations causing PCG in the Chinese population is not yet well understood so far. A few studies on CYP1B1 gene in Chinese sporadic PCG cases were reported, but few family studies were reported in Chinese population. The aim of this study is to analyze mutations of CYP1B1 in a Chinese pedigree of PCG.

Case report

A three-generation Chinese Han family with primary congenital glaucoma (PCG) which includes 19 family members was collected. The study followed the tenets of the Declaration of Helsinki with written informed consent obtained from all family members or from their parents if their age was less than 18. After undergoing a complete eye examination including slit-lamp biomicroscopy, gonioscopy, optic nerve examination, visual field test with Humphrey automated perimetry and measurement of IOP by Goldmann tonometry, all enrolled subjects were evaluated the state of affected with PCG or unaffected. PCG was defined according to the following criteria: age of onset less than three years; IOP greater than 21 mmHg without any treatment; characteristic glaucomatous optic disc damage and/or visual field loss, rupture of Descemet's membrane, or horizontal corneal diameter greater than 12 mm with or without corneal edema. Patients older than three years without secondary causes but with buphthalmos were considered to have PCG.
CYP1B1 mutations in a Chinese pedigree with PCG

The pedigree structure is described in Figure 1. The mutation p.E173K was cosegregated with the disorder within the family. The individual II: 8 (unaffected) had AA haplotype suggested incomplete penetrance in this pedigree.

Table 1. Ocular manifestations in PCG patients in this family

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age (y)</th>
<th>Visual acuity</th>
<th>IOP (mmHg)</th>
<th>C/D</th>
<th>Visual field loss</th>
<th>Nystagmus</th>
<th>Underwent trabeculectomy</th>
</tr>
</thead>
<tbody>
<tr>
<td>II: 4</td>
<td>M</td>
<td>43</td>
<td>NLP/2/20</td>
<td>50/25</td>
<td>1.0/1.0</td>
<td>Bilateral late-stage glaucomatous</td>
<td>Bilateral</td>
<td>Bilateral</td>
</tr>
<tr>
<td>II: 6</td>
<td>M</td>
<td>39</td>
<td>1/20/1/20</td>
<td>53/17</td>
<td>1.0/1.0</td>
<td>Bilateral late-stage glaucomatous</td>
<td>Bilateral</td>
<td>Bilateral</td>
</tr>
<tr>
<td>II: 9</td>
<td>M</td>
<td>34</td>
<td>HM/4/20</td>
<td>25/18</td>
<td>0.9/0.5</td>
<td>Late-stage glaucomatous/normal</td>
<td>Bilateral</td>
<td>Bilateral</td>
</tr>
</tbody>
</table>

Note: Abbreviations: C/D, cup-to-disc ratio; IOP, increased intraocular pressure; NLP, no light perception; HM, hand moving.

Table 2. Three sets of primers flanking the exons II and III of CYP1B1 gene

<table>
<thead>
<tr>
<th>Exon</th>
<th>Primer</th>
<th>Sequence</th>
<th>Amplicon size (bp)</th>
<th>Melting temp (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>II</td>
<td>CYP1B1-2a</td>
<td>Forward GGCCATTTCCTCCAGAGTC</td>
<td>830</td>
<td>58.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse GAACTCTTGGTGCTGTA</td>
<td></td>
<td>59</td>
</tr>
<tr>
<td>II</td>
<td>CYP1B1-2b</td>
<td>Forward ATGATGCGCACTTCTTCAC</td>
<td>754</td>
<td>58.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse CACGTGATGCTCCTTCAGC</td>
<td></td>
<td>58.2</td>
</tr>
<tr>
<td>III</td>
<td>CYP1B1-3</td>
<td>Forward AGCCATTATAGAAAAAGTGGA</td>
<td>798</td>
<td>56.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse AATTGAGAAGCAGGCACAAA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: Exon II of CYP1B1 gene was amplified by PCR with two sets of primers CYP1B1-2a and CYP1B1-2b. Exon III of CYP1B1 gene was amplified by PCR with primer CYP1B1-3.

The pedigree structure is described in Figure 1. The clinical characters of patients in this pedigree were summarized in Table 1. Twenty ethnically matched normal individuals without any ocular disorders were recruited as controls.

Blood samples were taken from all the subjects. Genomic DNA was extracted from the whole blood using a Genomic DNA Extraction Kit (QIAGEN genomic DNA and RNA Kit, QIAGEN, Hilden, Germany). Three sets of primers flanking the exons II and III (see Table 2) were designed based on gene sequences of CYP1B1 in GenBank (Gene ID: 1545) for amplification by polymerase chain reaction (PCR). The 50 μl PCR system contained 5×PrimerSTAR Buffer (Mg⁺⁺ plus) 10 μl, dNTP Mixture 4 μl (2.5 mM each), PrimerSTAR HS DNA polymerase 0.5 μl (2.5 U/ul), (TaKaRa Biotechnology Dalian Co., Ltd., Dalian, China), genomic DNA 2 μl (50 ng), 0.5 μl (25 μM) of each forward and reverse primer, and double distilled water (ddH₂O) 32.5 μl. The PCR protocol was as follows: initial denaturation at 98°C for 10 s, followed by annealing at 55°C for 15 s, extension at 72°C for 2 min for 35 cycles. PCR products were directly sequenced with forward and reverse primers using BigDye Terminator v3.1 Kit.
CYP1B1 mutations in a Chinese pedigree with PCG

(Applied Biosystems, Foster City, California, USA) in a 3730XL capillary sequencer (Applied Biosystems).

Seven reported variations of CYP1B1 gene were identified in this pedigree, which including 3 mutations: c.319 C>G p.L107V, c.517 G>A p.E173K (see Figure 2) and c.592 G>A p.V198I, and 4 SNPs: rs10012, rs1056827, rs1056836 and rs105683. For p.L107V, heterozygous mutation genotype CG was detected in only one unaffected subjects (II: 8), and genotype CC in all the other unaffected and affected members and all the controls. For p.V198I, heterozygous mutation genotype GA was detected in only one unaffected subjects (II: 5), and genotype GG in all the other unaffected and affected members and all the controls. For p.E173K, homozygous mutation genotype AA was detected in all the affected individuals (II: 4, II: 6, II: 9) and not in all the unaffected ones except one individual (II: 8) Homozygous GG genotype was detected in all the normal controls. Double bidirectional sequencing reactions were performed to rule out for sequencing artifact.

Discussion

Three mutations were found in this study, which were p.L107V, p.E173K and p.V198I. Heterozygous mutations CG for c.319 C>G p. L107V and GA for c.592 G>A p.V198I were found in one unaffected subject separately and not in all the affected subjects. These two mutations were not associated with disease in this family. Homozygous mutation AA for c.517 G>A p.E173K was found in all the affected subjects and not in unaffected subjects and controls. The mutation of c.517 G>A p.E173K cosegregated with the disorder within this family, which means the mutation was associated with disease causing in this pedigree.

The mutation of p.E173K was first identified in an Egyptian family and was reported later in Iranian patients with PCG [2, 3]. It was an uncommon mutation of CYP1B1 with percentage of only 1.9% among the CYP1B1 mutations in the Iranian patients. This variation located at non conservative area of exon II of CYP1B1 gene and the effect of the mutation was unclear.

The spectra of CYP1B1 mutations causing PCG vary widely in different ethnic populations, the spectrum of CYP1B1 mutations causing PCG in the Chinese population is not yet well understood so far. Mutation p.E173K was found in this family study, mutations R390H, R469W and some novel mutations were found in some sporadic PCG cases [4-8]. Further study in Chinese population in large sample size is to be performed.

In this Chinese PCG family, homozygous mutation p.E173K was found in one unaffected individual (II: 8, who was the sister of the proband) except the three affected individuals. The possible explain is incomplete penetrance which was first confirmed in the Saudi families with PCG, in which apparently unaffected individuals had mutations and haplotypes identical to their affected sibs [9]. It suggested the presence of a dominant modifier locus that is not linked genetically to CYP1B1. Further study of possible regulation from other factors or other genes (e.g. LTBP2) is to be performed to explore the pathogenesis of PCG in this Chinese family.
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Disclosure of conflict of interest

None.

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References


