Genetic Heterogeneity in Moroccan Primary Congenital Glaucoma Patients

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Abstract: Purpose: Primary Congenital Glaucoma (PCG) is an autosomal recessive, infantile ocular disorder resulting from malformation of the anterior eye chamber. Three loci associated with PCG are known, namely GLC3A (2p21), GLC3B (1p36), and GLC3C (14q24). The CYP1B1 and LTBP2 genes associated with PCG are localized, respectively, to the GLC3A and GLC3C loci, while the associated gene within GLC3B is still unknown. The GLC3A locus is implicated in approximately 35% of Moroccan PCG patients. This study aimed to evaluate the genetic heterogeneity in a Moroccan PCG population and to assess the involvement of the GLC3B and GLC3C-LTBP2 loci in mediating the disease. Methods: We recruited 26 unrelated patients lacking mutations in the GLC3A-CYP1B1 gene, 13 of which were born from first-degree consanguineous parents, and 50 healthy controls. Homozygosity mapping and linkage disequilibrium analysis were used to define linkages to the GLC3B or GLC3C loci. The LTBP2 gene was screened for mutations in patients that were homozygous for GLC3C. Results: Linkages to GLC3B and GLC3C were excluded in 46% of the PCG patients studied. GLC3B homozygosity profile analysis suggested that the causative gene is likely localized between the D1S2834 and D1S2672 markers. This region contains a newly mapped gene (KAZN) gene that mediates cellular adhesion and apoptosis. Finally, LTBP2 sequencing did not reveal any causative mutations. Conclusions: These results revealed a high genetic heterogeneity within the Moroccan PCG population, suggesting the possible involvement of unknown loci mediating PCG development.


Keywords: Primary congenital glaucoma, Moroccan population, CYP1B1, GLC3A, GLC3B, GLC3C, GLC3D, LTBP2

1. Introduction

Primary congenital glaucoma (PCG, OMIM 231300) or infantile glaucoma comprises a rare group of ocular disorders that occurs between the first and third years of life [1]. PCG is characterized by anatomical trabecular meshwork malformation (trabeculodysgenesis), causing obstruction of aqueous outflow from the anterior segment of the eye and inducing high intraocular pressure (IOP) [2]. Increased IOP causes irreversible optic nerve damage and leads to blindness [3]. The incidence of PCG is geographically and ethnically variable, with a high prevalence occurring in consanguineous populations. The frequency of occurrence is estimated at 1:10,000 individuals in western countries [4], 1:3300 in southern India [5], 1:2500 in Saudi Arabia [6], and 1:1250 in a gypsy population in Slovakia [7]. PCG is generally inherited in an autosomal recessive manner, with variable penetrance [8].

To date, 3 PCG loci have been identified by linkage analysis in multiply affected families. The first locus (GLC3A) was mapped to the 2p21 region (a region of 8 cM) when studying a group of PCG families with multiply affected subjects [9]. The CYP1B1 gene is a member of the cytochrome P450 superfamily of genes that has been localized to GLC3A [8]. Numerous studies with different ethnical groups have identified CYP1B1 mutations and have reported the predominance of this locus in individuals with PCG [10-12]. Indeed, over 100 CYP1B1 mutations are reported in the Human Gene Mutation Database [13-16]. Although the percent of PCG patients with CYP1B1 mutations is variable, it is estimated as 100% in Slovakia, 78% in Oman, and 20% in Japan [17-19]. In a previous study, we reported that CYP1B1 mutations are responsible for approximately 35% of Moroccans PCG cases [20]. Results from a recent investigation showed that CYP1B1 mutations occur in ~47% of Moroccan individuals with PCG [21]. A second locus (GLC3B) maps to 1p36 and was described in a study including 8 families that were unlinked to GLC3A locus. GLC3B was mapped to within a 3-cM region that is flanked by 2 groups of tightly linked markers (D1S1579/D1S489/D1S228) and (D1S1176/D1S507/D1S407) [22]. However, only 4 of these families showed linkage to chromosome 1, indicating the presence of at least 1 more locus.
involved in primary congenital glaucoma disorder [22]. To date, no direct attribution of a gene within the GLC3B locus has been reported. The third locus identified (GLC3C) was originally mapped to the chromosome 14q24.3 between the D14S61 and D14S1000 markers [23] and was subsequently localized to a 6.5-cM region between the D14S289 and D14S85 markers [24]. These regions are only partially overlapping. By function-position studies, LTBP2, the gene encoding beta-transforming growth factor protein 2 (MIM 602091), was identified as the gene causing PCG in 4 Pakistani consanguineous families and in gypsy patients [25]. Mutations in the LTBP2 gene are linked to severe PCG cases [25, 26]. However, it is unclear if LTBP2 gene corresponds to the GLC3C locus or to an adjacent locus (GLC3D) [27]. Investigations with numerous populations have not reported LTBP2 mutations in PCG cases that were unlinked to GLC3A, suggesting the presence of at least 1 additional PCG-associated locus [28-30].

In the present study, we aimed to characterize the involvement of the GLC3B and GLC3C/LTBP2 loci in PCG and to investigate heterogeneity in relevant loci within a Moroccan PCG population.

2. Material and Methods

Patients:

Unrelated PCG patients were recruited at the Pediatric Ophthalmology Department of the hospital (20 August, 1953, Casablanca, Morocco), after providing informed consent according to the Declaration of Helsinki. All patients underwent surgical therapy following definitive diagnosis of congenital glaucoma, showing symptoms of corneal edema and elevated IOP. Patients with associated ocular or extraocular malformations were excluded.

In a previous study, we screened a cohort of 40 PCG patients for CYP1B1 mutations [20] and identified 26 patients (8 females and 18 males) in the cohort that lacked mutations in this gene. These patients are suspected to have mutations in the GLC3B or GLC3C/LTBP2 loci.

The patient groups tested in this study were composed of 13 unrelated patients born of first-degree consanguineous marriages and 13 unrelated patients with no parental consanguineous relationships. DNA was extracted from peripheral blood samples by using a salting-out procedure with phenol-chloroform extraction, as described [31]. A control group of 50 healthy persons, randomly selected from the general population and with no glaucoma diagnosis after examination by ophthalmologists, was included.

Methods:

Homozygosity mapping and linkage disequilibrium studies were performed to evaluate potential associations of the GLC3B and GLC3C loci with PCG disease within a Moroccan population. Homozygosity mapping is an efficient method for highlighting human genes that may be involved in rare recessive diseases in inbred populations [32]. Because of the high rate of consanguinity in Morocco [33], we used a homozygosity mapping strategy to map genes associated with PCG at the GLC3B and GLC3C loci within PCG patients born of first-degree consanguineous marriages. Variant genes within homozygosity-mapped intervals were considered as candidate genetic aberrations underlying PCG disease.

We genotyped 6 annotated GLC3B markers (D1S228, D1S402, D1S2834, D1S507, D1S1176, and D1S2672) and 7 GLC3C markers (D14S606, D14S74, D14S59, D14S61, D14S1045, D14S43, and D14S71) that were deposited in the UniSTS database (National Center for Biotechnology Information) and spanned these loci. We also analyzed the human genome for additional microsatellite repetitions by using Ensembl Human Genome Sequence Assembly GRCh37 to refine susceptibility intervals and homozygosity localizations (Density of 1 marker by 100 kb). Using the Artemis and Primer3Plus software packages [34, 35], we identified 10 highly informative, novel GLC3B markers (N6, N26, N35, N46, N51, N59, N65, N77, N82, and N86), which are fully characterized in Table 1 and Figures 1A and 2A.

In parallel to homozygosity mapping studies, allelic frequencies were compared between patients and control groups. A common ancestral mutation was searched by linkage disequilibrium analysis in patients born from non-consanguineous marriages (13 cases). Statistical analyses were performed using the Genepop 4.2 [36] and Arlequin 3.5.1.3 [37] software programs.

Direct sequencing of the LTBP2 gene in homozygous patients was used to study PCG-associated mutations. The sequences of primers used for LTBP2 sequencing are indicated in Table 2.

PCR assays for genotyping and sequencing were performed using standard reaction mixtures and Applied Biosystems® Standard dye-labeled primers. Purified PCR products were directly sequenced in an ABI® 3130 Genetic analyzer. Genotypes were determined using ABI® Peak Scanner software v1.0. Nucleotide sequences were compared with a reference DNA sequence of the LTBP2 gene (HGNC: 6715) by using BioEdit software.
### Table 1. Description and molecular characterization of newly generated GLC3B microsatellite markers

<table>
<thead>
<tr>
<th>Name</th>
<th>Location a</th>
<th>Repeat a</th>
<th>PCR amplification primers (Forward, Reverse)</th>
<th>Size a (bp)</th>
<th>Alleles (N; Range in bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N6</td>
<td>14068076-14068296</td>
<td>(CA)22</td>
<td>CTTGATTTGGGGTTTGCTCGTGCTGGAATTACAC</td>
<td>221</td>
<td>13; 209-239</td>
</tr>
<tr>
<td>N26</td>
<td>14272115-14272389</td>
<td>(CA)15/CT)13</td>
<td>CATTAGGAGCCTCCTCGGGATG CAGGAATTTACACACACCTGTTTCC</td>
<td>275</td>
<td>10; 267-287</td>
</tr>
<tr>
<td>N35</td>
<td>14357106-14357298</td>
<td>(CA)20</td>
<td>CACCCCTGTTTAAAGTCTGGAATGAAAAGTTGCCATGACAC</td>
<td>193</td>
<td>10; 177-205</td>
</tr>
<tr>
<td>N46</td>
<td>14466353-14466575</td>
<td>(CA)20</td>
<td>GAACACCTGCTTCTACAGTCTG GGTCTTTTGATATACGAGGAAG</td>
<td>223</td>
<td>9; 221-243</td>
</tr>
<tr>
<td>N51</td>
<td>14521965-14522143</td>
<td>(CA)16</td>
<td>TTTATCTGCTAACTATTTTCAGCC GAGAAAATCATCCATCAGGG</td>
<td>179</td>
<td>7; 171-189</td>
</tr>
<tr>
<td>N65</td>
<td>14681053-14681331</td>
<td>(CA)21</td>
<td>GGTGCCTGACATGTCAGTGG GCTGGGTCATAGGATAGCTG</td>
<td>279</td>
<td>13; 263-293</td>
</tr>
<tr>
<td>N77</td>
<td>14785618-14785866</td>
<td>(CA)11</td>
<td>GACCCATGCTACTGCTGACTG GAGAAATCGTCCTGACGT</td>
<td>249</td>
<td>4; 247-253</td>
</tr>
<tr>
<td>N82</td>
<td>14854435-14854600</td>
<td>(GATA)13</td>
<td>CATTCACTTGTCTGGTGCTG CAGGCTGGAGTTCTGGTCTC</td>
<td>166</td>
<td>6; 152-176</td>
</tr>
<tr>
<td>N86</td>
<td>14917813-14917968</td>
<td>(CA)15</td>
<td>TTCCCTCCTTGAATCTTTCG TGTGGTTGTTTTTCCCTGTCAC</td>
<td>156</td>
<td>5; 154-168</td>
</tr>
</tbody>
</table>

For each microsatellite marker, location repeat and size are given according to the Ensembl Human genome sequence GRCh37 assembly, version 75.37 (GCA_000001405.14) (*). STR’s are PCR amplified using the given primers (forward and reverse). The total number of alleles (N) and the size of the PCR product are also given.

### Table 2. LTBP2 Sequencing primers

<table>
<thead>
<tr>
<th>Exon</th>
<th>Forward and Reverse sequence primers</th>
<th>Product size (bp)</th>
<th>Exon</th>
<th>Forward and Reverse sequence primers</th>
<th>Product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>GCGCCCCCTTGGTGCTGCTGGAGAA GAGTGGCTCTTCCGGGTTCTGCTGGAGAACCGCCCTGCTGAGACCCCCCTTGCAGTACCTCCCTGAG</td>
<td>482</td>
<td>17</td>
<td>GGCTGACTTTATGGCTTCCA GAGGCTGGAGTTCTGGTCTC</td>
<td>457</td>
</tr>
<tr>
<td>1b</td>
<td>GCGCCCCCTTGGTGCTGCTGGAGAA GAGTGGCTCTTCCGGGTTCTGCTGGAGAACCGCCCTGCTGAGACCCCCCTTGCAGTACCTCCCTGAG</td>
<td>482</td>
<td>17</td>
<td>GGCTGACTTTATGGCTTCCA GAGGCTGGAGTTCTGGTCTC</td>
<td>457</td>
</tr>
<tr>
<td>2</td>
<td>GCAAGCAGACCCAGCATITTCCTAACCTCAGCCGCTGCTGAGACCCCCCTTGCAGTACCTCCCTGAG</td>
<td>482</td>
<td>17</td>
<td>GGCTGACTTTATGGCTTCCA GAGGCTGGAGTTCTGGTCTC</td>
<td>457</td>
</tr>
<tr>
<td>3</td>
<td>GCAAGCAGACCCAGCATITTCCTAACCTCAGCCGCTGCTGAGACCCCCCTTGCAGTACCTCCCTGAG</td>
<td>482</td>
<td>17</td>
<td>GGCTGACTTTATGGCTTCCA GAGGCTGGAGTTCTGGTCTC</td>
<td>457</td>
</tr>
<tr>
<td>4</td>
<td>GCAAGCAGACCCAGCATITTCCTAACCTCAGCCGCTGCTGAGACCCCCCTTGCAGTACCTCCCTGAG</td>
<td>482</td>
<td>17</td>
<td>GGCTGACTTTATGGCTTCCA GAGGCTGGAGTTCTGGTCTC</td>
<td>457</td>
</tr>
<tr>
<td>5</td>
<td>GCAAGCAGACCCAGCATITTCCTAACCTCAGCCGCTGCTGAGACCCCCCTTGCAGTACCTCCCTGAG</td>
<td>482</td>
<td>17</td>
<td>GGCTGACTTTATGGCTTCCA GAGGCTGGAGTTCTGGTCTC</td>
<td>457</td>
</tr>
<tr>
<td>6</td>
<td>GCAAGCAGACCCAGCATITTCCTAACCTCAGCCGCTGCTGAGACCCCCCTTGCAGTACCTCCCTGAG</td>
<td>482</td>
<td>17</td>
<td>GGCTGACTTTATGGCTTCCA GAGGCTGGAGTTCTGGTCTC</td>
<td>457</td>
</tr>
<tr>
<td>7</td>
<td>GCAAGCAGACCCAGCATITTCCTAACCTCAGCCGCTGCTGAGACCCCCCTTGCAGTACCTCCCTGAG</td>
<td>482</td>
<td>17</td>
<td>GGCTGACTTTATGGCTTCCA GAGGCTGGAGTTCTGGTCTC</td>
<td>457</td>
</tr>
<tr>
<td>8</td>
<td>GCAAGCAGACCCAGCATITTCCTAACCTCAGCCGCTGCTGAGACCCCCCTTGCAGTACCTCCCTGAG</td>
<td>482</td>
<td>17</td>
<td>GGCTGACTTTATGGCTTCCA GAGGCTGGAGTTCTGGTCTC</td>
<td>457</td>
</tr>
<tr>
<td>9</td>
<td>GCAAGCAGACCCAGCATITTCCTAACCTCAGCCGCTGCTGAGACCCCCCTTGCAGTACCTCCCTGAG</td>
<td>482</td>
<td>17</td>
<td>GGCTGACTTTATGGCTTCCA GAGGCTGGAGTTCTGGTCTC</td>
<td>457</td>
</tr>
</tbody>
</table>

**LTBP2** exons (1 to 36) are PCR amplified and sequenced using the given forward and reverse primers. The longest exon 1 is PCR amplified as two amplicons (1a and 1b).

### 3. Results

GLC3B and GLC3C STR (Short tandem repeat) markers were genotyped in 26 Moroccan patients lacking GLC3A mutations and in a healthy control group to determine the involvement of these loci in PCG. The data obtained was analyzed to calculate Hardy-Weinberg equilibrium (HWE)
values. We found that all STR markers were in HWE, except for \(D1S2834\) and \(D1S1176\) \((p < 0.01)\).

**GLC3B locus:**

Approximately 30\% \((4/13)\) of patients born from consanguineous parents were homozygous for markers in the GLC3B region between the \(D1S228\) and \(D1S1176\) markers, corresponding to the GLC3B susceptibility region reported by Akarsu [22]. While none of the 50 control subjects or the 13 patients born from non-consanguineous parents showed any homozygote profile, note that inspection of the genotypes in homozygous patients did not reveal any shared or common haplotype. Three of these patients \((gc7, gc27, \text{and} 500-15)\) were homozygous in the centromeric region of the Akarsu interval and had an extended homozygosity region of 250 kb between the \(D1S507\) and \(D1S2672\) markers, while a fourth patient \((gc28)\) was homozygous for the telomeric region of the considered interval (Figure 1B).

![Figure 1. Genetic and physical mapping of the GLC3B PCG susceptibility region](image)

**Figure 1.** Genetic and physical mapping of the GLC3B PCG susceptibility region

A: The GLC3B susceptibility interval in the 1p36.21 chromosomal region mapped by Akarsu [22]

Positions of STR markers shown are in accordance with the Marshfield card. The physical distances between markers were determined using the human GRCh37 assembly.

B: Homozygosity mapping results of GLC3B genotyping within Moroccan PCG patients

Dots indicate homozygote genotypes for each marker tested within the 4 patients identified whose PCG status was not correlated with a \(CYP1B1\) mutation.

These results suggested that the GLC3B susceptibility region should be expanded to include the centromeric region spanning from the \(D1S507\)-\(D1S1176\) markers to the \(D1S2672\) marker (Figure 1B). Allelic frequency comparisons between patients and controls subjects showed a strong association of the \(D1S2672\) marker with PCG \((p < 0.001)\), further indicating that GLC3B PCG susceptibility interval extends to the \(D1S2672\) marker region. Note that we did not found any linkage disequilibrium between the tested markers. The additional proposed region is gene-poor, containing 1 gene named \(KAZN\), which encodes the kazrin, periplakin-interacting protein.

**GLC3C locus:**

Three patients in the consanguineous group and no patients in the non-consanguineous or control groups were homozygous for the markers studied in the GLC3C susceptibility region (Figure 2B).

![Figure 2. Genetic and physical mapping of the GLC3C-LTBP2 PCG susceptibility region](image)

**Figure 2.** Genetic and physical mapping of the GLC3C-LTBP2 PCG susceptibility region

A: The GLC3C susceptibility interval \([23, 24]\), including the relative position of the \(LTBP2\) gene \([25]\)

Positions of STR markers are represented according to the Marshfield card. Physical distances between markers were determined, based on the human GRCh37 assembly. Note that \(D14S85\) and \(D14S1000\) are not physically mapped (dashed line).

B: Homozygosity mapping results of GLC3C genotyping within Moroccan PCG patients

Dots indicate homozygote genotypes for each marker tested within the 3 patients identified whose PCG status was not linked to a \(CYP1B1\) mutation.

One patient \((gc28)\) was homozygous for all the studied markers, while 2 patients \((gc15 \text{ and} gc40)\) were respectively homozygous for the first GLC3C region described by Stoilov et al. \([23]\) and the second GLC3C region reported by Firast et al. \([24]\). This latter region contains the \(LTBP2\) gene, whose involvement in PCG disease has been reported \([25]\). Some investigators believe that the PCG-associated gene in the GLC3C locus corresponds to the second region containing the \(LTBP2\) gene. Others investigators has suggested that the \(LTBP2\) gene corresponds to a different locus (GLC3D \([27]\)), while the gene within the GLC3C locus is still unidentified.
Statistical analyses of allelic frequencies and linkage disequilibrium did not reveal any significant association.

**LTBP2 gene:**
Sequencing of the 36 LTBP2 exons (exon-intron junctions included) in 2 patients (gc28 and gc40) homozygous for the markers corresponding to this region identified 3 annotated polymorphisms: rs699374 A/G in exon 14, rs862031 A/G in exon 15, and rs7145480 G/A in exon 17. Three other intronic variations were identified between exons 16–17, exons 18–19, and 19–20. No causative mutation was identified in the patients studied.

### 4. Discussions

We aimed to evaluate the genetic heterogeneity of PCG in a Moroccan population. In a previous study involving 40 unrelated Moroccan PCG patients, we reported that CYP1B1 mutations occur in about 35% of patients (14/40 patients) [20]. Two CYP1B1 mutations were identified, namely, the 4339delG mutation specific to the Moroccan population and the G61E mutation reported in a Saudi Arabian population study [10]. In studies with other populations, the involvement of CYP1B1 in PCG varies from ~20% to 100% [38]. For example, the frequency of CYP1B1 mutations in PCG patients is estimated at 14.9% in United States [30], 40–70% in Iran [13, 39], 78% in Oman and Saudi Arabia [18, 29], and 100% in Pakistan [40]. These percentages imply the presence of other loci involved in PCG. In fact, studies in consanguineous Turkish families with multiple affected individuals have reported the involvement of the GLC3B locus in the 1p36.2-36.1 region [22] and of GLC3C in the 1q42.3 region [23]. In addition, studies with Pakistani and Iranian families [25, 38] have reported the implication of the LTBP2 gene in PCG. LTBP2 is localized 1.3 Mb away from the 1q42.3 region and was the first gene implicated in PCG that resided in the GLC3C PCG susceptibility region [23]. In the present study, homozygosity mapping revealed a contribution of the GLC3B and GLC3C loci to PCG in 13 Moroccan patients born from consanguineous parents that did not have linked CYP1B1 mutations. This approach enables the identification by homozygosity within patients not linked to CYP1B1 those potentially linked to the explored loci, with the possibility to reduce the susceptibility intervals previously described. As previously indicated, linkage disequilibrium analysis has also been applied to Moroccan patients born from non-consanguineous parents to identify a possible linkage disequilibrium, indicative of a founder-effect mutation in the population. Concerning LTBP2, we explored its involvement in PCG by directly sequencing each of its 36 exons (exon-intron junctions included) in patients born from consanguineous parents having a homozygous haplotype in the region corresponding to the LTBP2 interval.

Homozygosity-mapping tool applied to the study of GLC3B and GLC3C within the cohort of 13 PCG patients (unlinked to GLC3A) born of first-degree consanguineous marriages showed distinct homozygosity profiles in 4 patients with GLC3B and 3 patients with GLC3C. Factoring in patients born from consanguineous parents and the PCG autosomal recessive transmission model, the linkage to GLC3B and GLC3C may be ruled out in 6 of 13 PCG patients (~46%). Similar results were obtained in a recent study with Iranian families where linkages to GLC3B and GLC3C were excluded in 47% of unlinked GLC3A families [39].

Inspecting GLC3B homozygosity profiles has enabled confirmation of Akarsu’s GLC3B susceptibility interval [22] with a possible shift of the centromeric D1S507-D1S1176 terminal to the D1S2672 marker that was found strongly associated with the PCG (p < 0.001). The newly proposed interval contains the recently mapped KAZN gene (encoding the kazrin, periplakin interacting protein) that spans the telomeric region of the GLC3B susceptibility interval (D1S2834 to D1S2672). According to the human GRCh37 assembly annotation, KAZN is expressed as 6 alternatively spliced transcripts with the longest one containing 15 exons and coding for a protein containing 775 residues. Kazrin localizes to the nucleus, desmosomes, cell membrane, and cortical actin-based structures and regulates keratinocyte cytoskeletal networks, intercellular junctions, and differentiation [41, 42]. Kazrin may also play a role in regulating cellular apoptosis [43]. It was reported in Xenopus embryos that kazrin interacts with cadherin [44], which was found to be implicated in the migration and differentiation of neural crest cells [45]. Considering that neural crest cells are responsible for eye formation at embryonic stages, we suggest a possible contribution of Kazrin in primary congenital glaucoma.

GLC3C homozygosity mapping profiles of the 3 identified patients overlap partially (2 patients) or completely (1 patient) with the previously reported two GLC3C susceptibility regions that were localized by a distance of 1.3 Mb from each other (Figure 2) maintaining the ambiguity of GLC3C localization [23, 24, 38]. In recent studies, the LTBP2 gene was mapped to the second GLC3C susceptibility region [24] and was found to be mutated within primary or secondary glaucoma patients from numerous populations (Table 3).
Table 3. LTBP2 mutations worldwide reported

<table>
<thead>
<tr>
<th>Nucleotidic variation</th>
<th>Amino-acid variation</th>
<th>Exon</th>
<th>Ethnicity</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.331C&gt;T</td>
<td>p.Q111X</td>
<td>1</td>
<td>Pakistan</td>
<td>PCG [25]</td>
</tr>
<tr>
<td>c.1243-1256del</td>
<td>p.E415RfsX596</td>
<td>6</td>
<td>Morocco</td>
<td>Marfan Syndrome [48]</td>
</tr>
<tr>
<td>c.1796_1797insC</td>
<td>p.val1600GlyfsX2</td>
<td>9</td>
<td>Morocco</td>
<td>Marfan Syndrome [48]</td>
</tr>
<tr>
<td>c.537delE</td>
<td>p.Tyr1793fsX55</td>
<td>36</td>
<td>Iran</td>
<td>PCG [38]</td>
</tr>
<tr>
<td>c.1415delE</td>
<td>p.Ser472fsX3</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c.1287G&gt;A</td>
<td>p.Leu429Leu</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c.2966C&gt;G</td>
<td>p.Pro989Arg</td>
<td>19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c.544dupG</td>
<td>p.H1816PfsX28</td>
<td>36</td>
<td>India</td>
<td>Microspherophakia [47]</td>
</tr>
<tr>
<td>C&gt;G</td>
<td>g.75070493</td>
<td>Intron 6_7</td>
<td>Saudi Arabia</td>
<td>Secondary glaucoma [47]</td>
</tr>
<tr>
<td>c.1012delT</td>
<td>p.S338pfsX4</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c.4855C&gt;T</td>
<td>p.Q1619X</td>
<td>33</td>
<td></td>
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</tr>
<tr>
<td>c.4313G&gt;A</td>
<td>p.C1438Y</td>
<td>29</td>
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<tr>
<td>c.895C&gt;T</td>
<td>p.Arg299X</td>
<td>4</td>
<td>Macedonia</td>
<td>Marfan Syndrome [46, 48]</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Gypsy</td>
<td>PCG [25]</td>
</tr>
</tbody>
</table>

Reported LTBP2 variations (nucleotidic and amino-acid) are listed with the associated phenotype and the ethnic origin.

However, screening of the LTBP2 within GLC3C homozygous patients did not show any notable variations, apart from some polymorphisms in exonic and intronic regions. These results suggest that the homozygosity observed in these 3 patients is due to the patients’ parental consanguinity, which induced the presence of conserved regions in parents’ genome. This possibility may significantly reduce the estimated involvement of GLC3C in PCG within Moroccan patients. The presence of variations in intronic splice regulation domains or in transcription regulatory elements is also a possibility worth considering. Indeed, recently an intronic variation (rs3742793) causing a C=G inversion between exons 6 and 7 was associated with PCG. This polymorphism was found in 18 of 54 patients (30%), while it was absent in 50 controls [28].

The involvement of LTBP2 in PCG does not occur in other populations. Indeed, in Saudi Arabia, no LTBP2 mutation has been reported among 74 patients from 54 families [29]. Sequencing of LTBP2 in 54 Indian patients also revealed no mutations. In the literature, all LTBP2 mutations have been observed in severe types of glaucoma associated with megalocornea or in secondary glaucoma [28, 46-48]. These observations raise the possibility that LTBP2 is involved especially in severe types of glaucoma, with minimal involvement in most populations.

Finally, linkage disequilibrium analysis in the 13 patients with no parental consanguineous relationship fails to identify any PCG founder effect association.

Our investigation provides evidence of genetic heterogeneity occurring within a Moroccan PCG population. Our results indicate that GLC3A is still the most frequently implicated locus in PCG, with an occurrence of 35–47%. The association of GLC3B, GLC3C, and LTBP2 with PCG development was ruled out in at least 46% of the GLC3A-unlinked patients born from consanguineous parents, which strongly suggests the presence of at least 1 additional PCG-causing locus other than GLC3A, GLC3B, GLC3C, and LTBP2. Recent advances in genomics such as “exome sequencing” and “whole genome sequencing” may accelerate the discovery of PCG genes enhancing the understanding of PCG aetiology and the genetic interactions between PCG genes [49].

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