

Genetic Heterogeneity in Moroccan Primary Congenital Glaucoma PatientsSoumaya El Akil¹, Rahma Melki^{1,2}, Ahmed Belmouden¹¹ Laboratoire de Biologie Cellulaire et Génétique Moléculaire, Faculté des Sciences, Université Ibnou Zohr, Agadir, Morocco² Present address: Laboratoire de génétique et biotechnologies, Faculté des Sciences, Université Mohammed I, Oujda, Morocco
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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 *DIS2834* and *DIS2672* 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.

[El Akil S, Melki R, Belmouden A. **Genetic Heterogeneity in Moroccan Primary Congenital Glaucoma Patients.** *Life Sci J* 2014;11(11):890-898]. (ISSN:1097-8135). <http://www.lifesciencesite.com>. 159

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 (*DIS1579/DIS489/DIS228*) and (*DIS1176/DIS507/DIS407*) [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 (*DIS228*, *DIS402*, *DIS2834*, *DIS507*, *DIS1176*, and *DIS2672*) 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

Name	Location ^a	Repeat ^a	PCR amplification primers (Forward, Reverse)	Size ^a (bp)	Alleles (N; Range in bp)
N6	14068076-14068296	(CA)22	CTATTTAGGTTTGTTCGCCCG TTTGGGTGCTGCTGAATACCAC	221	13; 209-239
N26	14272115-14272389	(CA)15(CT)13	CATAGGAGCCATCTCGGATG GGGAATTTAACACAGCTGTTC	275	10; 267-287
N35	14357106-14357298	(CA)20	CACCCGTGCTTAAGTCGTTAG GAAAAAATTGGCAATGTACACAG	193	10; 177-205
N46	14466353-14466575	(CA)20	GACACCTGCCTCTCTACAGTTC GGCTTTTGATAAAGCATGGAG	223	9; 221-243
N51	14521965-14522143	(CA)16	TTTATCTGCTAACTATTTTCTCAG CCAGAAAATCATCCATCAGGG	179	7; 171-189
N65	14681053-14681331	(CA)21	GGTCTCAACGTCAGTAGTC GCTGGTCATAGGATAGCTG	279	13; 263-293
N77	14785618-14785866	(CA)11	GAGCCTTAGCTCACTGTGGAG GACACCACTGAACACGCAGGC	249	4; 247-253
N82	14854435-14854600	(GATA)13	CATTACTTGTCTGGTGCCTG CCACATGGAGAGGCTGCATAG	166	6; 152-176
N86	14917813-14917968	(CA)15	TTCCCTCCTTGAATCTTCTTG CTGTGGTTGTTTTCCCTGTCAC	156	5; 154-168

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) (^a). 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

Exon	Forward and Reverse sequence primers	Product size (bp)	Exon	Forward and Reverse sequence primers	Product size (bp)
1a	GGCCCCCTAGACTCAGAGAA GAGTGCTTCTCCGGGTCTG	482	17	GGCTGACTTTATGGCTTCCA CAGGCTGGAGTTCTGGTCTC	457
1b	TGCAGCCAAGGTGTACAGTC CCCCTCTGTACCCTCCAAAC	342	18	ATCCTTTGTCCTTGGCCTCT GAATGCACTGAGGGGATGG	406
2	GATGTGCAGAGAATGGCAGA GCGGAGTGTCTGCTACTGGT	286	19	AGGGACAAGGATTTGCTGTG ACCTCTTCCCTTCCGTTG	287
3	AGAGTGGCTTCTGTCTGAG CTTACCAAACGGTCCAAAG	476	20	CCCTGGCCTCATAACTGAGA GGATGTGTTGGGTCAGTGTG	350
4	GCAGCCAGAGAGCATTTTTC AACTCAGCCCCCTGTGAGA	403	21-22	CTGCAGAGTCCCACACAGAA TATTCTGTCCCCTTCCACCA	202
5	AATGCCCTGAGATGAATGC CTGGCTCTCTGGCCATCTAC	349	23	TCCCAAGTTCAGAGTGAAG AGCTTGTGAGCGACTCTTGG	466
6	GCCTGTTTCTCTGTGGTGGT CAGCTTCCCTATCCCTGTCA	386	24	GGAGACTTCCCCCTTGACTC GCTGGCTTCCCATGCTCCTG	475
7	TGGTGGATACCCTTCAGAGG GAGGAGGAGAAGGGCAGACT	441	25	GCCCAGAGGAAGCTACACAG GAGCTAAGGACCAGGCAGTG	273
8	AATGTGGGGAGTGAAGTCTG AAGGCAGGTCTGGGAAGTCT	351	26	GGAAATCGTCCCTGACCTTGA TGAAAAGCAGCCTCTCAACC	492
9	AGGTGGGCTGAGAGGAGTCT TAGTCCCCTGGAATCAGCAG	415	27	CTGCATGCGTGTGAGAGAGT CAGGACCAGTTGAGGAGGAG	298
10	CGGGCACTTGGTCACTCCT GTGTCAGGTCTGGGAAAAA	262	28-29	AAGGCCTAGCCTGCTTCTTT CCTGTAGCTCCTGGTTTTGC	447
11	GCTCCAAACTTCCCAACTGA GGTTGGGATAAGCACGTGAG	444	30	CCTTAGAGGGTTCATGAACAGA CCCACTCAGGTGAAGGAGTT	489
12	TCACGTGCTTATCCCAACCT AGGGACCCAGGATTAACACC	447	31-32	CCCTCATACTGCCTCTCACC TCTGGGGACAATCTCTGAC	356
13	CCCTGAGCTCACGCTGTTAC TCCCTCCCTCATGAGACACT	150	33	TGATAGGCAAAACACCCTTCC CTGCAGGGTATCCCCTTGG	499
14	CAGAGGAGCCAAAAGTGACC TCCTTCTCACCTCCTCTGA	206	34	CAGAGGGTACCAGTCCCTTGC CCTGCAGGGTATCCCCTTGG	455
15	GTCTGAGCACCAGGGAAGAG GAGGGACCCTGTGTTCTTGG	370	35	AGTCTGGACACAGCCCTCAG TCTTCCAGCCTTCTGAGTT	279
16	CACCCGTCCATAACCTCTGT TGCTTGGACCTTCTGCTTCT	271	36	CTTGGGCATGGTATGAGCTT AACCTTGGCCTGATGTAC	450

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 *DIS2834* and *DIS1176* ($p < 0.01$).

GLC3B locus:

Approximately 30% (4/13) of patients born from consanguineous parents were homozygous for markers in the *GLC3B* region between the *DIS228* and *DIS1176* 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, and 500-15) were homozygous in the centromeric region of the Akarsu interval and had an extended homozygosity region of 250 kb between the *DIS507* and *DIS2672* markers, while a fourth patient (gc28) was homozygous for the telomeric region of the considered interval (Figure 1B).

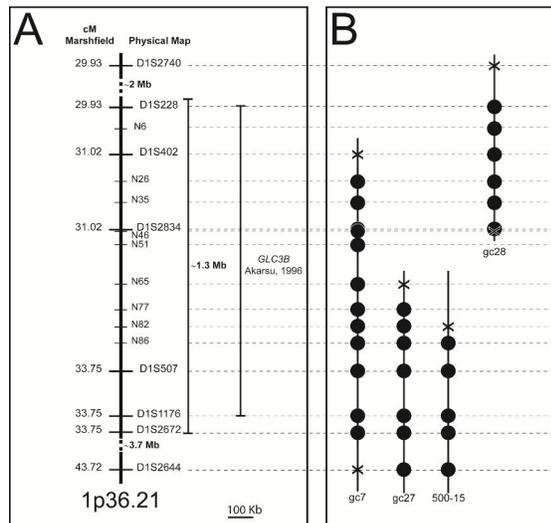


Figure 1. Genetic and physical mapping of the *GLC3B* PCG susceptibility region

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 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 *DIS507*-*DIS1176* markers to the *DIS2672* marker (Figure 1B). Allelic frequency comparisons between patients

and controls subjects showed a strong association of the *DIS2672* marker with PCG ($p < 0.001$), further indicating that *GLC3B* PCG susceptibility interval extends to the *DIS2672* marker region. Note that we did not find 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).

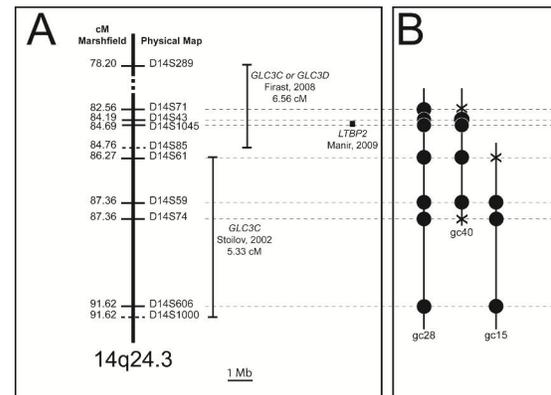


Figure 2. Genetic and physical mapping of the *GLC3C-LTBP2* PCG susceptibility region

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 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 is 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 *14q24.3* 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 *14q24.3* 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 *DIS507-DIS1176* terminal to the *DIS2672* 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 (*DIS2834* to *DIS2672*). 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

	Nucleotidic variation	Amino-acid variation	Exon	Ethnicity	Phenotype
1	c.331C>T	p.Q111X	1	Pakistan	PCG [25]
2	c.412delG	p.A138PfsX278	1		
3	c.1243-1256del	p.E415RfsX596	6		
4	c.1796_1797incC	p.val600GlyfsX2	9	Morocco	Marfan Syndrome [48]
5	c.5376delC	p.Tyr1793fsX55	36	Iran	PCG [38]
6	c.1415delC	p.Ser472fsX3	7		
7	c.1287G>A	p.Leu429Leu	6		
8	c.2966C>G	p.Pro989Arg	19		
9	c.4808G>A	p.Arg1603His	33	India	Microspherophakia [47]
10	c.544dupG	p.H1816PfsX28	36		PCG [28]
11	C>G	g.75070493	Intron 6 7	Saudi Arabia	Secondary glaucoma [47]
12	c.1012delT	p.S338pfsX4	4		
13	c.4855C>T	p.Q1619X	33		
14	c.4313G>A	p.C1438Y	29		
15	c.895C>T	p.Arg299X	4	Macedonia	Marfan Syndrome [46, 48]
				Gypsy	PCG [25]

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].

Acknowledgements:

The authors thank the patients and their families for cooperation, Dr. M. Hamdani (University Hospital Ibn Rochd, Casablanca) for contributing clinical data. We would like to thank Dr. H.J. Garchon (INSERM U1016, Cochin Hospital, Paris, France) for helpful discussion and part of technical support. Support for this study was provided by grants from the Ibnou Zohr University (14/2012) and the CNRST of Morocco (PROTARS III D14/56 and genotyping-sequencing facilities at the UATRS).

S. El Akil was supported by a fellowship from CNRST (E6/007).

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Declaration of interest and author's contribution:

The authors report no conflicts of interest.
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11/9/2014