Toward Primary Congenital Glaucoma GLC3B Gene Identification: The Case of Kazrin Gene

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Abstract: Primary Congenital Glaucoma (PCG) is an ocular disease that occurs before the age of 3 years, and results from malformation of the anterior eye chamber. To date, three chromosomal loci, GLC3A (2p21), GLC3B (1p36), and GLC3C (1q424) associated with PCG are reported. CYP1B1 and LTBP2 located on GLC3A and GLC3B, respectively, are the genes harboring mutations in PCG, however the PCG-associated gene within GLC3B is still unknown. In a previous study and using homozygosity mapping, we reported that GLC3B locus might be responsible for PCG in 30% of Moroccan patients. Here, we aim to identify the PCG-associated gene within the GLC3B locus. Integrative analysis of genomic databases and using the GeneDistiller software was performed to identify potential PCG-associated genes in the GLC3B locus. Based on these analyses, KAZN gene was identified as a strong candidate gene given its position in the GLC3B susceptibility interval (KAZN exons 1 to 4 out of 17 overlap the GLC3B locus) and its role in morphogenesis of embryonic tissue and cells adhesion. Exons 1-4 of KAZN were amplified, sequenced and analyzed in homozygote patients for the GLC3B locus. No sequence variation was found in the four exons of homozygote patients for the GLC3B locus. This study suggest a priori no involvement of KAZN gene in PCG within Moroccan population, a total sequencing of KAZN gene may removes entirely this probability. Further research are also needed to discover the GLC3B locus causal gene responsible for PCG

Keywords: Congenital glaucoma, Moroccan population, PCG, GLC3B, KAZN.

1. Introduction

Primary congenital glaucoma defines a group of ocular disease [1]. This disease generates high intraocular pressure at the anterior segment of the eye due to anatomical trabecular-meshwork malformation [2], which causes severe eye nerve damage inducing blindness [3]. PCG affects children between birth and 3 years of age. The incidence of PCG is approximately 1/10000 birth and depends on ethnicity [4]. PCG is highly prevalent in inbred population and consanguinity is strongly associated with the disease [5]. To date, three PCG chromosomal loci (GLC3A, GLC3B, and GLC3C) have been identified by linkage analysis in multiple affected families. GLC3A was mapped to 2p21 region (8cM) [6]. CYP1B1 (member of cytochrome P450 gene) has been localized in the GLC3A locus [7]. The percentage of GLC3A linked patient rages between 100 % in Slovakia and 20% in Japan [8][9].In Morocco, CYP1B1 seems to be responsible of 35% to 47% of PCG cases [10][11]. GLC3C maps to 1q424.3 (D14S289-D14S85) interval [12][13], which is partially overlapping the LTBP2 gene encoding beta-transforming growth factor protein 2 [14]. In a previous study, we sequenced LTBP2 gene in PCG Moroccan patients not linked to GLC3A and homozygous for GLC3C region. No mutation has been identified in our patients suggesting the presence of other PCG-associated genes in this region [15].

GLC3B maps to the 1p36 region within a 3 cM region flanked by two groups of tightly linked markers (D1S1579/D1S489/ D1S228) and (D1S176/D1S507/D1S407) with high Lod score Z>4 in (D1S402, D1S2834) region [16]. In a previous study, we employed homozygosity mapping and linkage disequilibrium to evaluate a potential association with GLC3B within 26 patients not linked to GLC3A nor to GLC3C. We genotyped six annotated GLC3B markers (D1S228, D1S402, D1S2834, D1S507, D1S176 and D1S2672), and 8 newly generated markers, based on di- and tri- and tetra-nucleotide repeats, present in the 1p36 region. Results have shown that approximately 30% of patients were homozygous for some of the markers assayed in the reported GLC3B susceptibility region, while none of the individuals in the healthy group have shown homozygosity in this region. Furthermore, the patients were homozygous for an expanding region including the D1S2672 marker. We propose to extended GLC3B susceptibility interval to [15]. Our define GLC3B region and the reported GLC3B region match on a region of 244 kb contains KAZN gene encoding Kazrin: periplakine-interacting protein.

The present study aims to determine if KAZN is the gene responsible of PCG in GLC3B region using direct sequencing.

2. Material and Methods

2.1 Patients

The description of patients recruited for this study is reported in our previous study. Briefly, the study was initiated recruiting 40 unrelated patients at the Pediatric Ophthalmology Department of the “20 Aout hospital” in Casablanca, Morocco after obtaining informed consent according to the declaration of Helsinki protocol. Within the 40 patients, PCG were associated to GLC3A in 14 ones. These 14 patients with confirmed GLC3A associations where excluded from the present study. In the other 26 patients, association with GLC3B were tested by homozygosity mapping as explained in our previous study [15]. Results
returned that only 4 patients were homozygous for GLC3B region. Mutation in KAZN gene was performed in these 4 patients.

2.2. Methods

Ensembl genome browser (www.ensembl.org) and NCBI database (www.ncbi.nlm.nih.gov) were used to identify candidate genes in the GLC3B reported region and retrieve their function and properties. We used GeneDistiller program (http://www.genedistiller.org) to identify the genes located in the GLC3B region that are associated with vision/eye phenotypes. Indeed, GeneDistiller integrates all available information from multiple databases to identify the best gene(s) with the best relationship match between the phenotype and the genes located in the chromosomal region of interest. GeneDistiller identified the KAZN gene as the most likely associated with PCG. Primer 3 software was used to design primers for KAZN exons amplification and sequencing. Primers are listed in Table 1.

Table 1: KAZN sequencing primers

<table>
<thead>
<tr>
<th>Exon</th>
<th>Primer (Forward and Reverse)</th>
<th>Tm °C</th>
<th>Amplicon size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>GCCGCGGACATTTCTTGGAGGGATAAACGTTCC</td>
<td>60</td>
<td>282</td>
</tr>
<tr>
<td>2</td>
<td>CCAGTTGCTGATCACTTTTCCCTTAATCCAGAAGAATTTACTCA</td>
<td>55</td>
<td>354</td>
</tr>
<tr>
<td>3</td>
<td>GACACAACAGGTAGAGCCGGATTCCGCCCGGGTGGG</td>
<td>60</td>
<td>423</td>
</tr>
<tr>
<td>4</td>
<td>TCAACCCTGTGCCCCTTCTCCGAAGTCGCTGACTGGTCGA</td>
<td>60</td>
<td>234</td>
</tr>
</tbody>
</table>

PCR conditions were 35 cycles of 1min at 94°C, 1 min at the appropriate Tm for each exon, and 1min at 72°C followed by a final extension at 72°C for 5 min. sequencing were performed for the 4 patients using standard protocols and Applied Biosystems® Standard dye-labeled primers. Purified PCR products were sequenced in an ABI®3130 genetic analyzer. Nucleotide sequences were analyzed using the BioEdit Software.

3. Results

In silico analysis was performed to identify potential PCG-associated genes located within the GLC3B reported region (1p36). GeneDistiller software identifies candidate genes based on several criteria such as: linkage interval, phenotype, comparison with known gene, expression, cellular localization. The program return KAZN as the best probable associated gene with PCG disease phenotype. GeneDistiller based its prediction on the role of KAZN gene in cell adhesion and in assembly of intermediate filament of desmosomes, which suggest a possible implication in embryonic trabecular meshwork formation.

The KAZN gene is located precisely in the GLC3B region. It have 9 transcripts (KAZN-202, KAZN-201, KAZN-205, KAZN-207, KAZN-204, KAZN-208, KAZN-209, KAZN-202 and KAZN-206). The first four exons of the largest transcript KAZN-208 overlap the GLC3B susceptibility region (Figure 1). KAZN-208 codes for 863 amino acids and contains 17 exons. Based on these analyses, we decided to sequence these four exons in our homozygote patients for the GLC3B region.

Sequencing of the entire first four exons, including intron-exon junction of transcript KAZN-208 in our homozygous patients revealed no variation of sequence

Next, we focused on identifying the PCG-associated gene in this region. First, we used GeneDistiller to identify candidate genes based on the phenotypes and molecular functions associated with PCG. Secondly, we used Ensembl Browser...
to position the markers. Results of in silico study identified KAZN as the most likely gene candidate with the region of interest associated with the GLC3B region.

KAZN codes the Kazrin protein present in different epithelial tissue and implicated in morphogenesis of embryonic tissue, in cell adhesion and in assembly of intermediate filament of desmosomes [17]. The role of Kazrin in tissue junctions suggest its implication in trabecular meshwork formation during embryonic stage. For instance, an interaction between Kazrin and the cadherin protein was reported in Xenopus embryos [18]. Furthermore, the cadherin protein is implicated in cell migration of neural crest in embryonic stage to form iridocorneal angle [19]. These observations indicate an involvement of Kazrin in proliferation and migration of neuronal crest cells, and supports its potential role in trabecular meshwork malformation in GCP patients. However, no direct association between Kazrin and trabecular meshwork formation or PCG was reported.

KAZN gene is located in the telomeric region of the reported GLC3B region. The gene has 9 transcripts. The larger transcript KAZN-208 codes for 863 amino acids and covers all the GLC3B susceptibility region reported by Akarsu and expanded by us [16][15]. While the other transcripts are shorter by at least two exons compared to KAZN-208.

The first exon of KAZN-208 overlaps the interval having high Lod score in the Akarsu study [16], while exon 2 and exon 3 extend over the GLC3B susceptibility region. Finally, the exon 4 exceeds the last marker included in the region of susceptibility (D1S2672) (Figure 1).

According to the Ensembl data base, the first KAZN-208 exon contains 965 pb including the 5’-untranslated region. The second exon contain 158 pb. A stop gained variation in base 10 of the KAZN-208 second exon was annotated in data base. The third exon contains 241 pb. This exon contains a frameshift variation. Finally, the fourth exon contains 192 pb. The most interesting variation is the mutation stop presents in exon two which gives a truncated protein, we looked for this variation in our homozygote patient with no success.

In fact, the results of sequencing of the four first KAZN-208 revealed no variation of sequence in our patients. These results suggest a priori no association between the KAZN-208 gene and congenital glaucoma disease, despite the fact that both the position and function support KAZN as a strong candidate associated with the disease. A total sequencing of all KAZN gene may support our result by rejecting entirely KAZN gene from the list of PCG candidate gene in GLC3B locus. Further research are also needed to discover the GLC3B locus causal gene responsible for PCG.

However, no other gene is present in this region except 2 long intergenic non-coding RNAs (lincRNAs) coding for 764 pb and 282 pb transcripts. lincRNAs which were initially dismissed as "transcriptional noise" are now recognized as crucial elements in biological regulation [20][21]. lincRNAs are a heterogeneous class of RNAs that are non-protein coding transcripts longer than 200 nucleotides. The number and types of known functional non-coding RNAs, short or long in size, has been significantly expanded, as these may be involved in cis or trans regulation of genes located in their vicinity or at distant loci through various mechanisms [22]. It is currently known that lincRNAs are involved in epigenetic regulation (genetic imprinting and chromatin remodeling), transcription, post-transcription (splicing and mRNA decay), and translation [23][24][25].

We thought that these lincRNAs found in GLC3B region may play a role in regulatory of genes implicated in iridocorneal angle morphogenesis pathway. This hypothesis is effectively under investigation.

5. Conclusion

The present results exclude a priori the association between KAZN gene and primary congenital glaucoma in our homozygote patients for GLC3B locus, however a best understanding of lincRNAs roles can challenge the no KAZN involvement in congenital glaucoma. Lastly, further research are needed to light GLC3B involvement in PCG, and to identify the gene associated with the disease within the GLC3B region.

6. Acknowledgements

The authors thank the patients and their families for cooperation, Dr. M. Hamdani (University Hospital Ibn Rochd, Casablanca) for contributing clinical data. 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).

7. Declaration of interest and author’s Contribution

The authors report no conflicts of interest.

References


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Volume 8 Issue 1, January 2019

www.ijsr.net

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Paper ID: ART20193886

10.21275/ART20193886

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