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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 (14q24) 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, were aims to identify the PCG-associate 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 *14q24.3* (*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 *LTPB2* 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 (*D1S1176* /*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*, *D1S1176* 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 exclude 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
KAZN exons (1 to 4) are PCR amplified and sequenced using the given forward and reverse primers.

Target	*PCR amplification primers (Forward and Reverse)*	*Tm (°C)*	*Amplicon size (bp)*
*KAZN*_Exon 1	*GGCGGGGACATTTTCTTGGA* *GGGATAACGCTCCACTTCCC*	60	282
*KAZN*_Exon 2	*CCAGTTGTGACTACTTTTCC* *TCTAATCAGAAATCCAATACTCA*	55	354
*KAZN*_Exon 3	*GACACAACAGGTAGAGCCGG* *ATTTCGCCCCGGTGTGG*	60	423
*KAZN*_Exon 4	*CTAACCTGTGCCCTTCTCC* *GAACTGCTGCTGACTCGTCA*	60	234

PCR conditions were 35 cycles of 1min at 94°C, 1 min at the appropriate Tm for each exon, and 1min at 72C° followed by a final extension at 72C° 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. Genedisteller 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

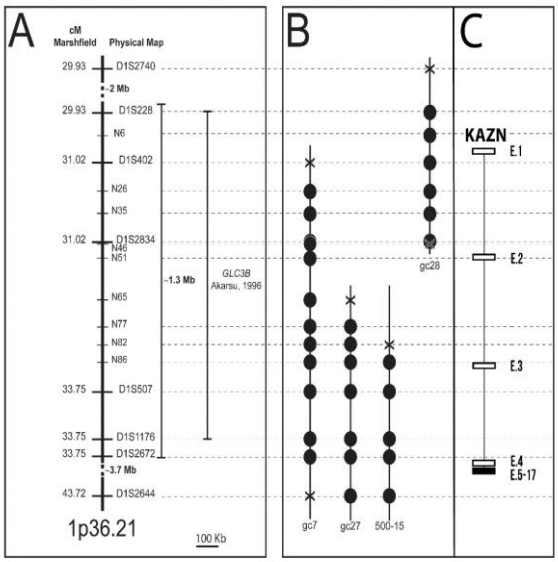

Figure 1: Genetic and physical mapping of the *GLC3B-KAZN* PCG susceptibility region

A: The *GLC3B* susceptibility interval in the *1p36.21* chromosomal region mapped by Akarsu [16].
Positions of STR markers shown are in accordance with the Marshfield card. The physical distances between markers were determined using the human GRCh38.p10 assembly.
B: Homozygosity mapping results of *GLC3B* genotyping within Moroccan PCG patients [15]
Dots indicate homozygote genotypes for each marker tested within the 4 patients identified whose PCG was not correlated with a *CYP1B1* mutation.
C: Kazrin gene structure (Exons 1 to 17) and its position in the *GLC3B* susceptibility interval
Position of the exons shown (KAZN-208) are in accordance to the human GRCh38.p10 assembly. Physical distances are in scale and the size of the exons (rectangle) are not.

4. Discussions

We aimed to evaluate a potential association between the *KAZN* gene and Primary Congenital Glaucoma. In a previous study, we used homozygosity mapping to define within 26 patients not linked to *GLC3A* locus, those linked to *GLC3B* locus. Results showed homozygosity overlapping the *GLC3B* susceptibility region in four patients [16][15] (**Figure1**).

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 contains192 *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.

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7. Declaration of interest and author's Contribution

The authors report no conflicts of interest.

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