A novel missense variant in *CYP1B1* in an Egyptian patient with Primary Congenital Glaucoma

Original Article

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ABSTRACT

Background: Primary Congenital Glaucoma (PCG) is a critical disease that can lead to blindness if left untreated. It is considered the most common type among pediatric glaucoma cases. Mutations in *CYP1B1* gene are the predominant cause for the disease in most cases especially in the Middle East and North Africa regions where high consanguinity rates are reported.
Purpose: Reporting a new PCG case from Egypt harboring a novel variant in *CYP1B1*.
Patients and Methods: The patient underwent a full clinical examination, reporting visible symptoms, and measuring both eyes' IOP and corneal diameter. Genetic testing of *CYP1B1* was performed using Sanger sequencing.
Results: The patient was found to carry compound heterozygous missense variants: c.1310C>G (p.P437R) and c.1320T>G (p.F440L). Of them, the c.1310C>G (p.P437R) was not reported before.
Conclusions: We detected a new variant in *CYP1B1* expanding the mutational spectrum of this rare disorder. Further, identifying an additional case with biallelic *CYP1B1* variants strongly supports the critical role this gene possesses to PCG phenotype.

Key Words: *CYP1B1* gene, Egyptian patient, novel variant, primary congenital glaucoma.

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INTRODUCTION

Primary Congenital Glaucoma (PCG) is the most common type of glaucoma amongst pediatric glaucomas (**Papadopoulos *et al*., 2020**). It is a critical disorder affecting the anterior segment of the eye, in specific it affects the trabecular meshwork (TM); the region responsible for draining the eye's aqueous humor back to the lymphatic system. Obstruction in the TM results in elevating the eye's intraocular pressure (IOP) due to the abnormal accumulation of the aqueous humor (**Sowden, 2007**). PCG affects males in a slightly higher rate than females reaching around 65%, majority of cases manifest the disease bilaterally; around two-third of cases (**Cantor *et al*., 2019**). Most cases (around 80%) present their first symptoms within the first few years of life (**Berlin *et al*., 2009**).

PCG is commonly inherited in an autosomal recessive mode (**François, 1980**), hence its incidence is higher in closed populations and in populations with high consanguinity rate. The highest incidence was reported in the Slovakian Gypsies (1 in 1,250) and Saudi Arabians (1 in 3,030) (**Genĉík, 1989; Alanazi *et al*., 2013**). On the contrary, the incidence is very low reaching 0.07/100,000 in the United States (**Aponte *et al*., 2010**). The most common causative gene for recessive PCG is *CYP1B1* followed by LTBP2. Mutations in the two genes have been described in patients from diverse populations (**Chakrabarti *et al*., 2006; Narooie-Nejad *et al*., 2009**). Herein, we describe the clinical and genetic data of a new patient with *CYP1B1*-related PCG from Egypt.

PATIENTS AND METHODS:

Patient:

An Egyptian male patient, 3 years old, presented to the Outpatient Clinic of Clinical Genetics Department at the National Research Centre (NRC) suffering from congenital glaucoma. He was the first child born to non-consanguineous parents and there were no other similarly affected family members (Figure 1A). The pregnancy and delivery histories were unremarkable.

Clinical examination did not reveal any dysmorphic features or abnormality in the proband other than the symptoms of the left eye manifesting corneal edema,

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buphthalmos, and photophobia with totally normal right eye. Corneal diameter was 14 mm and IOP measured 25 mmHg. Examination of the parents' eyes were did not reveal any abnormalities concerning either PCG or any other optical dysmorphologies.

Methods:

Genomic DNA was extracted from peripheral blood lymphocytes after having a signed informed consent according to the guidelines of the Medical Research Ethics Committee of the NRC. DNA was extracted using a standard salting out procedure. The entire coding region of the *CYP1B1* gene was amplified using specific primers designed by Primer3 SOFTWARE (Table 1). The coding region and exon/intron boundaries of approximately 50 bp sequence were investigated to identify any splice site variants as well. Our standard PCR cycling conditions were: initial denaturation at 96°C for 5 min; 30 cycles of denaturation at 96°C for 30 sec; annealing at 62°C for 30 sec; extension at 72°C for 30 min, and a final extension at 72°C for 5 min. PCR products were checked for successful amplification by running on 2% agarose gel (Figure 1B) then purified using Exo-SAP PCR Clean-up kit (Fermentas, Germany) and sequenced in both directions using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and analyzed on the ABI Prism 3500 Genetic Analyzer (Applied Biosystems) according to manufacturer's instructions. The sequence data of *CYP1B1* gene was compared with the reference genomic and cDNA sequence of the gene (NM_000104.3). The variants identified were inspected in dbSNP141, gnomAD, Exome Variant Server and 1000 Genomes databases. Furthermore, the effect of the variants was predicted using MutationTaster, PolyPhen2 and SIFT software.

Table 1: The sequence of primers used in amplification of *CYP1B1* gene and size of PCR products.

CYP1B1 Exon	Forward Primer Reverse Primer	Amplicon size (bp)
Exon 2-1	5'-TCTCCAGAGAGTCAGCTCCG-3' 5'-GGGTCGTCGTGGCTGTAG-3'	786
Exon 2-2	5'-ATGGCTTTCGGCCACTACT-3' 5'-GATCTTGGTTTTGAGGGGTG-3'	787
Exon 3-1	5'-AGTGAGAAATTAGGAAGCTGTTTTAGA-3' 5'-GCCAGGATGGAGATGAAGAG-3'	594
Exon 3-2	5'-CCCAAGGACACTGTGGTTTT-3' 5'-AACGCTAATTGAGAAGCAGCA-3'	498

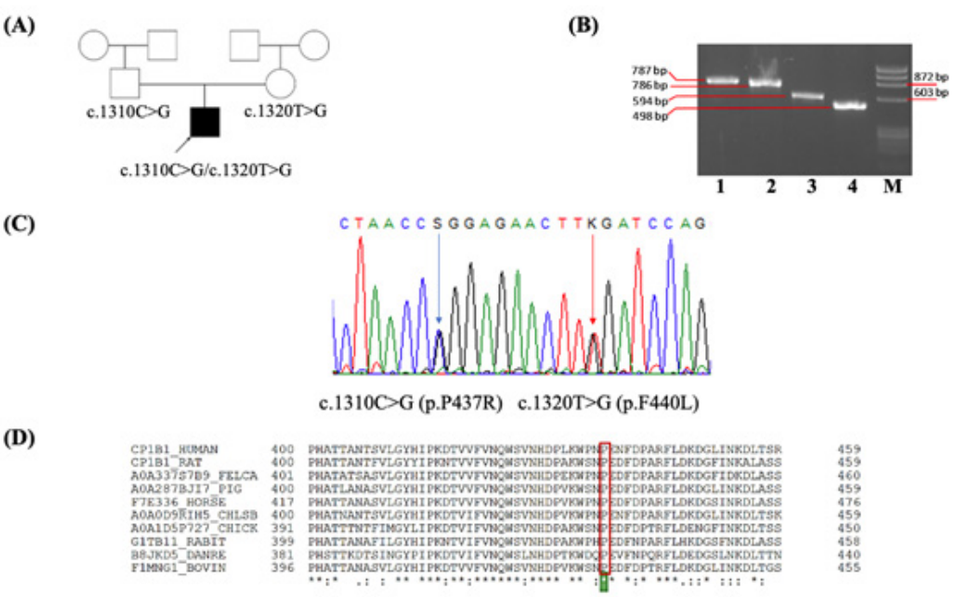

Fig. 1: (A) Pedigree of the family. (B) A 2% Agarose gel showing the amplification of the four fragments of *CYP1B1* gene in a single patient. Lanes 1-4: PCR products of exon 2 (Fragment 2-1 and 2-2) and exon 3 (Fragment 3-1 and 3-2), M: Size marker (PhiX174 DNA/HaeIII digest) (C) Portion of the sequencing electropherogram showing the two *CYP1B1* variants identified in our patient. The arrow indicates the site of mutation. (D) The conservation of p.P437 among different species.

RESULTS:

Mutational analysis of the *CYP1B1* gene revealed two heterozygous missense variants: c.1310C>G (p.P437R) and c.1320T>G (p.F440L) (Figure 1C). Segregation analysis revealed that the c.1310C>G (p.P437R) was inherited from the father while the c.1320T>G (p.F440L) was maternally inherited. The c.1320T>G (p.F440L) is a known disease-causing variant while c.1310C>G (p.P437R) was not described before. The new variant c.1310C>G (p.P437R) was not found in the dbSNP, 1000G and gnomAD databases and was predicted to be disease causing by various bioinformatics tools. It is located in the meander region, a highly conserved segment amongst several cytochromes (Figure 1D). Regarding the PremPS mutation tool (Chen *et al*., 2020) the variant is predicted to be destabilizing, PremPS scores depend on the ΔΔG; change in the unfolding free energy of the mutation, scores higher than 0 indicate destabilization of the mutated protein, while scores lower than 0 indicate preserved stability. This variant scored ΔΔG of 1.61 meaning a high protein destabilization occurred. The resultant variant caused the formation of a new polar bond with tryptophan at position 434 that was not present in the wildtype form (Figure 2).

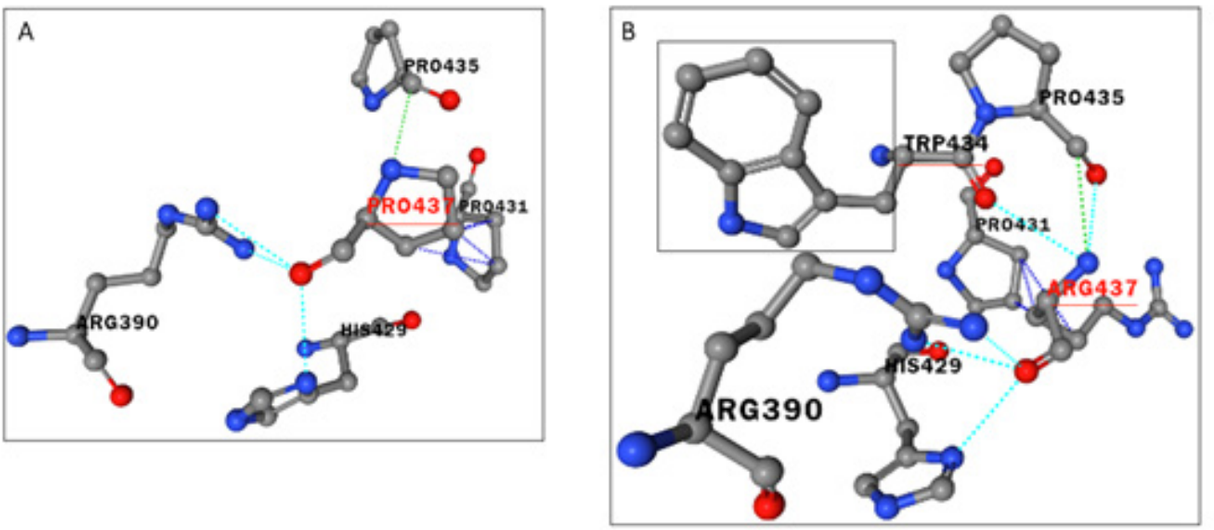

Fig. 2: (A) Normal amino acids' interactions with Proline at position 437. (B) The extra bonded Tryptophan at position 434 with the mutated Arginine at position 437.

DISCUSSION

In this study, we report an Egyptian patient with early onset primary congenital glaucoma. Genetic testing identified two missense variants in the *CYP1B1* gene including a novel one (c.1310C>G, p.P437R) segregating with the phenotype in the family. The new c.1310C>G variant represents a change from Proline into Arginine at position 437. Interestingly, mutations affecting the same position have been reported before in patients from diverse ethnic background, for example, Proline was mutated into Leucine (p.P437L) in PCG patients from Turkey (**Stoilov *et al*., 1998**), Brazil (**Stoilov *et al*., 2002**), Pakistan (**Rashid *et al*., 2019**), Siberia (**Ivanoshchuk *et al*., 2020**) and China as well (**Cai *et al*., 2021**). In the same context, Proline was also mutated into Alanine (p.P437A) in a Tunisian patient (**Bouyacoub *et al*., 2014**). Proline at this position is located at the surface of the protein, presumably providing structural firmness resulting from the rigid properties of Proline, hence mutations at this position could affect the normal structural configuration (**Rashid *et al*., 2019**).

The second missense variant identified in our patient (p.F440L) is a very rare known pathogenic *CYP1B1* variant. It has been reported before in in the heterozygous state in two unrelated Italian patients (**Giuffre *et al*., 2007; Giuffre, 2011**). The p.F440L changed the phenylalanine; an aromatic amino acid into leucine; an aliphatic amino acid, hence recording score of pathogenicity reaching 1 according to the polyphen-2 models. Generally, this variant has an extremely low allele frequency in gnomAD (only 1 heterozygous carrier with minor allele frequency of 0.00000397). The presence of this variant in Egypt and Italy only so far may be related to the geographical region of the two countries, being located on the Mediterranean Sea. However, it remains unclear whether this variant arose sporadically or due to a founder event. However, proving the later needs the identification of more patients carrying this variant and haplotype analyses.

CONCLUSION

In conclusion, we described a new patient with early onset primary congenital glaucoma from Egypt carrying

two missense variants in the *CYP1B1* gene. Our results expand the mutational spectrum of the disorder and clearly confirm critical role this gene possesses to PCG phenotype.

CONFLICT OF INTEREST

There are no conflicts of interest.

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