Case Report

The Identification and Stereochemistry Analysis of a Novel Mutation p.(D367Tfs*61) in the CYP1B1 Gene: A Case Report

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Abstract

Purpose: To investigate the presence of a probable genetic defect(s) that may cause primary congenital glaucoma (PCG) in a seven-year-old female patient.

Methods: A seven-year-old female patient and her family received genetic counseling and underwent full clinical examinations by an expert ophthalmologist. The patient's genomic DNA was subjected to the targeted gene capture and next-generation sequencing (NGS) along with Sanger sequencing method. The 3D structure prediction and stereochemistry analysis were performed for both mutant and wild-type forms of the CYP1B1 protein.

Results: The clinical examinations indicated that the diagnosis of PCG was correctly made. We identified a novel homozygous deletion in which a "C" nucleotide was deleted from the final exon of the Cytochrome P450 Family 1 Subfamily B Member 1 (CYP1B1) gene. The 3D molecular modeling of the CYP1B1 protein predicted significant structural changes could occur in this protein as a result of the mutation mentioned earlier. The stereochemistry analysis revealed mutant features of the protein, as well as significant misfolding and possible malfunctions in the mutant form of the CYP1B1 protein.

Conclusions: This mutation might cause a frameshift in the translation process, leading to the malfunction of the CYP1B1 protein and development of glaucoma. This newly-identified mutation could be regarded as potential deletion mutation in genetic counseling and molecular examination for the detection of PCG disease in Iran.

Keywords: Cytochrome P450 family 1 subfamily B member 1, CYP1B1, Deletion mutation, Iran, Primary congenital glaucoma

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Introduction

Congenital glaucoma (CG) is the most common form of infantile glaucoma, also known as primary congenital glaucoma (PCG).[1] The disease occurs in one of every 27800 live births in Europe, and 70–80% of cases are classified as bilateral glaucoma (www.orpha.net). The diagnosis of PCG is made according to the predefined clinical criteria, including elevated intraocular pressure (IOP) in a child (especially in the first year of life), enlargement of the globe (buphthalmos), increased corneal diameter, cloudy corneas, cracks in Descemet's membrane, thinning of the anterior sclera and iris atrophy, photophobia, excessive tearing, anomalously deep anterior chamber, and structurally normal posterior segment except for progressive glaucomatous optic atrophy.[2-4] Most cases of PCG are sporadic, but 40% of cases are inherited in

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an autosomal recessive manner with incomplete penetrance.[5] To date, five loci have been identified to be associated with PCG, namely: GLC3A (2p22.2),[6,7] GLC3B (1p36),[8] GLC3C (14q24.3),[9] GLC3D (14q24),[10] and GLC3E (9p21.2).[11] Mutations that occur in the Cytochrome P450 Family 1 Subfamily B Member 1 (CYP1B1) gene explain most PCG cases, and its mutations mainly exhibit variable expressivity rather than nonpenetrance.[12] Approximately, 70% of PCG cases diagnosed in the Iranian population stem from mutations in the CYP1B1 gene,[13] while more than 130 pathogenic mutations have been so far characterized, showing that different ethnic groups are prone to develop PCG (http://www. hgmd.cf.ac.uk/ac/index.php). This gene encodes Cytochrome P450 B1 (CYP1B1), a polycyclic aromatic hydrocarbon (PAH) protein that metabolizes Cytochrome P450 (CYP).[14] In this study, we identified a single base pair deletion in exon 3 of the CYP1B1 gene in an Iranian female patient with PCG. We also evaluated the potential consequences of the mutation in the resultant protein encoded by the CYP1B1 gene to predict its anomalous effects on the protein structure, function, and disease development.

Case Report

A seven-year-old female patient with clinical manifestations of PCG referred to us [Table 1] to receive genetic counseling and molecular testing.

The subject was born of consanguineous marriage [Figure 1a]. All family members received genetic counseling, and informed consent was obtained from her parents for participation in the study and publishing the results. We conducted the targeted sequence analysis for the genes associated with PCG (ASB10,[15,16] CYP1B1,[6] FOXC1,[17] LTBP2,[18] TEK,[11] MYOC,[19] NTF4,[20] OPA1,[21] OPTN,[22] TBK1,[23] and WDR36[24,25]). Accordingly, we identified a homozygous single base pair deletion in exon 3 of the CYP1B1 gene in reverse strand of genomic position as follows:

Table 1: Clinical findings of the patient

Sing and symptoms	
IOP (mmHg)	16
Enlargement of globe	++
Increased corneal diameter	+
Cloudy corneas	+
Cracks in Descemet's membrane	+
Thinning of the anterior sclera	+
Iris atrophy	-
Photophobia	-
Excessive tearing	++
Anomalously deep anterior chamber	+
Structurally normal posterior segment	++
Glaucomatous optic atrophy	-
Corneal edema	++
Optic nerve exam (cup-to-disc ratio)	0.6

IOP: Intraocular pressure

(chr2:38298398_38298398delC) (NM_00010 4(CYP1B1):c.1099_1099delG), p.(D367Tfs*61) [Figure 1b].

This variant is theoretically predicted as a disease-causing factor by Sorting Intolerant From Tolerant (SIFT) (https://sift.bii.a-star.edu.sg/), Protein Variation Effect Analyzer.

(PROVEAN) (http://provean.jcvi.org/index.php), and MutationTaster (http://www.mutationtaster.org/) software and classified as very strong pathogenic (PVS1) null variant according to the ACMG guidelines and standards.[26] This variant is also absent from the 1000 genomes (http://browser.1000genomes.org/index.html), ExAC browser (http://exac.broadinstitute.org/), Exome Variant Server (EVS) (http://evs.gs.washington. edu/EVS/), and Iranome databases (http://www.iranome.com/). This deletion variant may lead to a frameshift in the translation process, causing the conversion of an amino acid at the position of 367 into the threonine residue. This procedure is probably followed by the emergence of a stop-codon located at 61 codons downstream of the mutation position p.(Asp367Thrfs*61). The Sanger sequencing and segregation analysis confirmed homozygosity of the variant in the patient, while her parents were heterozygote [Figure 1a and b].

The prediction of the 3D structure of the mutated form of the CYP1B1 protein was conducted by the Phyre 2 web portal.

(http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index) and then visualized by the Yet Another Scientific Artificial Reality Application (YASARA) view software (http://www.yasara.org/viewdl.html). The results of the 3D structure prediction for both mutant and wild-type forms of the CYP1B1 protein revealed that some features might be affected in the mutant form of the protein [Figure 2a and b].

It was also demonstrated that several protein features of the mutant form of CYP1B1 would be lost in each output pairwise alignment when compared with its reference alignment [Figure 2c]. These data were consistent with the stereochemistry analysis obtained from the COACH server, COFACTOR server, and PSIPRED workbench [Table 2].

Discussion

The female patient who presented the clinical manifestations of PCG was accurately diagnosed with the hierarchical medical diagnosis [Table 1]. The determination of causative mutation(s) seems necessary concerning the prenatal diagnosis (PND) request from her parents; therefore, the targeted sequence analysis was employed for the identification of the gene mutation. We consequently characterized a new homozygous deletion (chr2:38298398_38298398delC) in the CYP1B1 gene which appears to be responsible for the development of PCG [Figure 1a and b].

Homozygous or compound heterozygous mutations that occur in the CYP1B1 gene can cause autosomal recessive PCG, which is assigned to the GLC3A locus. The PCG-associated CYP1B1 gene encodes a mono-oxygenase enzyme, which is a member

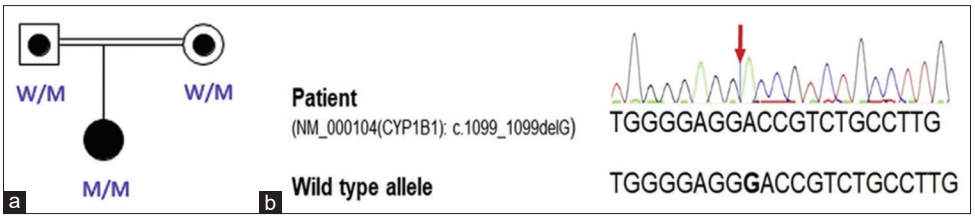

Figure 1: Pedigree and identification of the mutation in the family. (a) The segregation and genotyping analyses of the family members concerning the (NM_000104 (CYP1B1):c.1099_1099delG) mutation. (b) The Sanger sequencing method confirmed the mutation c.1099_1099delG in the patient and her parents. The deleted nucleotide was designated in the bold and red font within the reference sequence (wild-type) and also pointed by an arrow in the electropherogram. The Sanger sequencing was performed by the reverse primer; then the base-calling errors appeared next to the mutation position and represent different reads in heterozygous parents

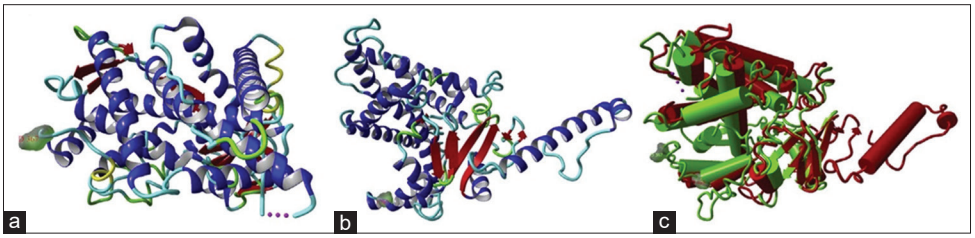

Figure 2: The three-dimensional structure prediction and pairwise alignment of the protein in the mutant and wild-type forms. (a) The three-dimensional structure prediction of the wild-type form of the cytochrome P450 family 1 subfamily B member 1 (CYP1B1) protein (right view), while aspartic acid, at the position 367, is located at the protein surface and colored green. (b) The three-dimensional structure prediction of the mutant form of the CYP1B1 protein (right view) in which threonine, at the position 367, is located at the protein surface and colored green. (c) The wild-type protein was colored as green, while the mutant one was colored red. The dashed red circle encompassed the un-aligned features of the two forms of the CYP1B1 protein (wild-type vs. mutant) and represented the structural changes caused by p.(D367Tfs*61) mutation

Table 2: Brief description of stereochemistry analysis results and mutation effects on cytochrome P450 family 1 subfamily B member 1 (CYP1B1) mutant protein

Services[a]	Change in cluster size	Change in predicted ligand	Change in consensus binding residues	Protein features
COACH analysis	+	+	+	NA
TM site analysis	+	+	+	NA
COFACTOR server	NA	+	+	NA
PSIPRED analysis	NA	NA	NA	Loss of six helices, seven strands, and one turn

[a]All services are available via Zhang Lab server (https://zhanglab.ccmb.med.umich.edu/). NA: Not applicable

of the CYP superfamily that is in charge of the metabolism and synthesis of cholesterol, steroids, and other lipids.[27,28] The CYP1B1 gene has been previously reported as a candidate gene for the causation of primary open angle glaucoma (POAG),[29] while it is considered a leading cause of PCG.

The presence and involvement of GLC3A, GLC3B, GLC3C, GLC3D, and GLC3E loci in PCG indicates the genetic heterogeneity of the disease. The CYP1B1 mutations account for 20–100% of PCG cases in different ethnic groups as 147 various mutations have so far been reported in the CYP1B1 gene.[30] According to the literature, about 70% of Iranian PCG sufferers are the carriers of four types of CYP1B1 mutations, namely G61E, R368H, R390H, and R469W. These mutations are the most significant mutations which are critical to be checked in Iranian patients with PCG.[13] This study was in agreement with previous reports, indicating that the CYP1B1 gene is regarded as a high-priority gene in molecular diagnosis of PCG in Iran.[31]

Our in silico findings were in line with the results obtained from the loss of function (LOF) paradigm, as this variant was considered a disease-causing factor and confirmed by the PROVEAN and MutationTaster tools. Moreover, this deletion variant was also evaluated as a "null" variant according to the American College of Medical Genetics and Genomics (ACMG) standards and guideline.[26] The Combined Annotation Dependent Depletion (CADD) Phred score was estimated as 25.1 for NM_000104 (CYP1B1):c.1099_1099delG that denotes this variant could be ranked as a rare variant in top 0.1–1% of rare variants. Also, the variant (NM_000104 (CYP1B1):c 1099_1099delG) was absent in the ExAC, 1000G, and Iranome databases. The Iranome database provides suitable ethnicity-matched control groups for this study to consider the variant a mutation with more confidence.

The newly-identified variant is predicted to be functionally incompetent when evaluated by the MutationTaster online

tool (http://www.mutationtaster.org/), while the mutated amino acid is conserved across humans, rhesus monkeys, mice, elephants, and lampreys. However, the amino acid aspartate at the mutation position has been altered in dogs and zebrafish. We postulated that aspartic acid at the position 367 of the CYP1B1 protein has a critical role in the Homo sapiens specie; thus, the modification of aspartic acid at the position 367 can result in the malfunction of the CYP1B1 protein. It has been reported that mutations occurring in the CYP1B1 gene contribute to the development of PCG, and its enzymatic product can produce a signaling molecule, possibly a steroid, that might be involved in eye development.[32] Therefore, the dysfunction of the CYP1B1 protein could lead to the perturbation of vision and eventually blindness. According to the mentioned information, the variant (CYP1B1):c. 1099_1099delG might be regarded as a mutation in the CYP1B1 gene. This newly-identified truncating mutation is located in the last exon of the CYP1B1 gene, eliminating more than 10% of the protein sequence and producing a truncated protein [Figure 2c]. Some strand and helix features might be vanished in the mutated form of the protein [Table 2], causing impairment in the functionality of the CYP1B1 protein. The wildtype form of the CYP1B1 protein has a metal-binding domain at the proximity of the 470th amino acid[33] that could be destructed upon p.(Asp367Thrfs*61) mutation. It has been predicted that a group of structural changes can occur in the consensus ligand-binding domain in the mutant form of the CYP1B1 protein when analyzed by the COACH and TM-site tools [Table 2].

Additionally, some modifications predicted emerge in the ligand and ligand-binding site domain when the mutant form of CYP1B1 was compared with the wildtype one via the COFACTOR server [Table 2]. These devastating alterations influence the proper function and interactions of the protein, leading to the interference with eye development and consequently glaucoma.

Moreover, the impairment in the nonsense-mediated mRNA decay (NMD) is another reason for the pathogenesis of PCG in the female patient. These two scenarios provide sufficient evidence for the clinical manifestations of the patient. Recently, a homozygous mutation p.(R368H) has been reported at the next position of the p.(D367Tfs*61) mutation, while its correlation with a reduction of the activity of estradiol metabolism has been proven in patients with PCG.[28] Likewise, in the p.(R368H) mutation, the null activity of retinol metabolism has been previously addressed. As a result, the lowered rate of retinoic acid metabolism is capable of affecting the eye development, and it is considered a pathogenic event concerning the p.(R368H) mutation.[34]

Regarding the information provided above, we concluded that the mutation (NM_000104 (CYP1B1):c.1099_1099delG) is a new mutation in the CYP1B1 gene that might cause a frameshift in the translational process of the protein synthesis, resulting in a production of a truncated protein. Some critical properties of the CYP1B1 protein might be lost since this mutation affects the appropriate function of the CYP1B1 protein in the eye development and cellular signaling cascades. Considering that this mutation (NM_000104 (CYP1B1):c.1099_1099delG) is a deletion mutation, this study highlights the significance of the CYP1B1 gene screening in Iranian patients with PCG. The presence of CYP1B1 variants in PCG, POAG, and juvenile open-angle glaucoma (JOAG) have been extensively studied in previous research,[35,36] and mutations occurring in this gene can lead to the LOF in the CYP1B1 protein as shown in the pathogenesis of PCG.[37,38] Herein, we reported a newly-identified truncating mutation p.(Asp367Thrfs*61) at a position that is immediately located adjacent to a previously reported pathogenic variant. Therefore, it would be plausible that the same clinical manifestations, observed for the p.(R368H) mutation are expected for this newly-identified mutation. This new variant p.(Asp367Thrfs*61) might be a truncating mutation and could be applied for PND and genetic counseling, especially in Iran.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the legal parents has given her consent for images and other clinical information to be reported in the journal. The parents understands that names and initials will not be published and due efforts will be made to conceal patient identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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