Identification of a Novel Non-Stop Mutation in PDE6C Gene in an Iranian Family With Con-Rod Dystrophy

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Abstract- Cone-rod dystrophy (CORD) is one of the most common genetic eye disorders. Recent genetic studies have demonstrated that it is a genetically heterogeneous disease among patients. Molecular genetic analysis of the 22 genes was performed in a family with Cone-rod dystrophy. Bioinformatics was applied for Next Generation Sequencing, and the variants were confirmed by Sanger sequencing. In this study, the nonstop mutation in the PDE6C gene (a normal stop codon is 859th codon of PDE6C located in exon 22 TAA (Stop) --> CAA (Gln) = Stop859Q) leads to a termination-site change and run-on into the 3' untranslated region (UTR) that predicts an extended protein which was found in the family. This mutation has not been described in patients with the CORD phenotype. Also, this is the first study indicating that a nonstop mutation in the homozygous state in PDE6C is responsible for the congenital recessive CORD phenotype.

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Introduction

Cone-rod dystrophies (CORD) are inherited retinal degenerations distinguished by cone degeneration which precedes the rod degeneration. The estimated prevalence of CORD is to be 1 in 40,000 (1-2). Also, CORD is characterized by the early loss of cone receptors or sometimes by concomitant loss of both cone and rod receptors that explain the predominant symptoms of CORD, which includes: photoaversion, abnormalities of color vision, decreased visual acuity with or without nystagmus and decreased sensitivity of the central visual field (3).

The disease shows phenotypical heterogeneity with all types of Mendelian inheritance, and recent genetic research has involved some different gene loci in its etiology (4).

CORD may be syndromic and non-syndromic, the latter of which is more common (5-6). To date, thirty-one genes have been identified in the pathogenesis of non-syndromic CORD.

This study examined a consanguineous family of Iranian origin, including two siblings with CORD. To recognize the disease-causing mutation in the family, we performed Next Generation Sequencing (NGS) of candidate genes involved in the structural development of the cone photoreceptors or with previous association with CORD and direct sequencing of PDE6C. We recognized a novel homozygous nonstop mutation in the PDE6C gene, which producesa novel protein-coding transcript, which might explain the CORD phenotype in the family.

Case Report

A consanguineous Iranian family (Figure 1A) with CORD was presented to our study for molecular investigation. The affected and healthy family members agreed to a genetic test, and for the purpose of this study, informed consent was obtained from legal guardians and patients and the study was, according to the guidelines the Ethics Committee of Iran's Ministry of Health and Medical Education.

In the two index patients, a detailed clinical

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examination was performed that included family history and a full ophthalmologic exam. Full-field electroretinogram (ERG) is the key test to make a definitive diagnosis for CORD. Patients were examined with ERG that indicated a shift in implicit time of cone responses, with the progressive abnormality of rod responses appearing later.

Genomic DNA was extracted from peripheral leukocytes of the two patients, unaffected sister and their parents using the standard salting-out method (7). DNA concentration of samples was quantified using a nano-drop and for targeted NGS.

The sequencing analysis is performed by using a custom-designed Nimblegen chip capturing of 22 candidate genes that involved in the pathogenesis of CORD followed by NGS approach (BGI-Shenzhen, Guangdong, China). After NGS sequencing, the sequence reads were mapped to the reference human genomic DNA (UCSC/hg19) using Burrows-Wheeler Alignment software for the subsequent variant analysis. The 1000 Genomes database and HapMap samples were also used for comparison. Moreover, online tools such as PolyPhen-2 (8) and SIFT (9) were applied to evaluate the pathogenicity of the identified SNP variants.

PCR-based Sanger sequencing was performed to analyze the potential mutation identified by NGS in the patients. The specific PCR primers were used for the amplification of the target gene site that previously reported (10). The sequences were compared with the annotated PDE6C gene reference sequence (NM_006204) to confirm the candidate variant.

The proband was a 16-year-old female (II-1), the first child of consanguineous parents from southwest Iran. She was diagnosed with cone-rod dystrophy at the age of 14. Her parents reported her and her very poor color vision soon after. She had normal hearing.

Next-Generation Sequencing analysis of the patient, after comparison with PDE6C reference sequence in 1000 Genomes database, demonstrated one novel mutation, a homozygous nonstop mutation that exchanges a normal stop codon 859 of the gene in exon 22 [TAA (Stop) --> CAA (Gln)=Stop859Q) to an amino acid in the PDE6C (Figure 1B,1C). Sequence analysis of the other 21 genes failed to identify any disease-causing sequence variants in the patient. The mutation was confirmed by using PCR. The region of mutation was amplified, and then the PCR product was sequenced (The sequencing of the mutation was repeated with the second sample of DNA). Then, we genotyped all members of the family (the two patients, unaffected sister, and their parents) and found this mutation co-segregated with the disease phenotype, and the mutation was not identified in unaffected family members.

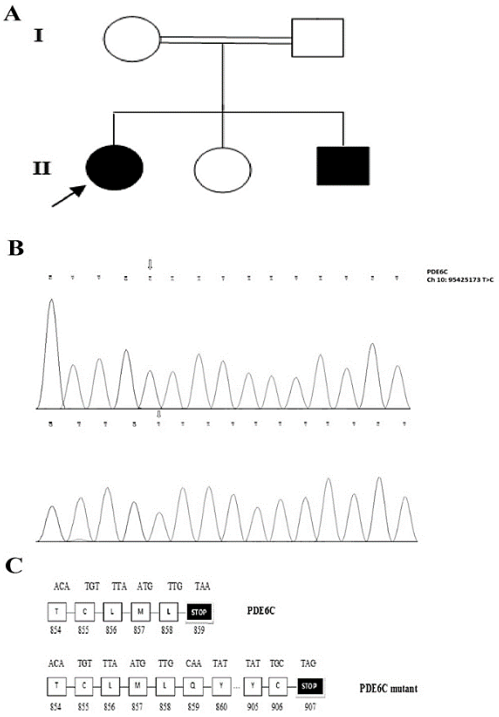

Figure 1. Combined figure. (A) Pedigree of a family with CORD. The patients are denoted in black. (B) Chromatogram of the variant chr10:95425173T.C from a patient is compared with the sequence from a control. (C) Zoomed-in view of the region containing the variant, including the amino acid sequences of protein-coding isoform and the mutated sequence caused by the variant

Discussion

In the present study, 22 genes involved in CORD were evaluated to determine possible changes by using the NGS method. We have found one novel mutation in the PDE6C gene in the Iranian consanguineous family that so far has not been reported in any of the information banks. To date, four mutations responsible for CORD have been recognized in the PDE6C gene based on Human Gene Mutation Database (HGMD). Most of them are missense mutations that modify only one amino acid in the highly conserved sequence. In this study, the change in PDE6C gene is a nonstop mutation in the normal stop codon 859 of the gene in exon 22 that leads to a termination-site change and run-on into the 3' untranslated region (UTR) identified from two alleles of two patients.

'Non-stop' mutations are nucleotide substitutions, deletions, or insertions that happen within translational termination (stop) codons, and that can result in the

continued and improper translation of the mRNA into the 3'UTR. This type of mutation is very unusual. Up to 2008, there was>60000 nonsense, and missense mutations (in nearly 4000 different genes) listed in the HGMD database that is known to cause, or to is related to, human inherited disorder; only 119 mutations (in 87 different genes) happened within stop codons (11). For a nonstop mutation, a number of authors implicitly suppose that the normal open reading frame is developed until the next in-frame stop codon is encountered; though, very few human nonstop mutations have been identified to allow any general conclusions to be drawn as to their possible phenotypic consequences, at either the mRNA or the protein level (11). Although rare, nonstop mutations are reported in some cases involving different diseases (12-13).

The Stop859Q mutation resulted in an open reading frame and a mutant-type (MT) PDE6C protein, which included 906 amino acid residues, compared with the 858 amino acid residues of wild-type (WT) protein. A nonstop mutation in the PDE6C gene (c.2575 T>C: p.Ter859Q; Figure 1B) causes disruption of the functional ochre stop codon (UAA) at the 3′ end of PDE6C. The next available stop codon is 141 bp downstream (in the 3′ UTR), predicting the addition of 48 amino acid residues to the C-terminus of PDE6C.

PDE6C encodes the cone alpha subunit of cyclic guanosine monophosphate (cGMP-specific) phosphodiesterase, an enzyme consisting of two alpha and two gamma subunits that are important in the cone phototransduction cascade. It changes the second messenger cGMP to 5′-GMP during light exposure. This leads to closure of the cGMP-gated ion channel in the cone outer segment plasma membrane, resulting in hyperpolarization of the cell (14). Based on the OMIM database, the PDE6C gene has previously been shown to be involved in disorders of the retina, including Cone Dystrophy 4 and Achromatopisia type 4 and 5.

This mutation has previously not been reported in mutation databases, and this is the first report of mutations of the PDE6C gene in patients affected by CORD. The following pieces of evidence prove that this mutation can lead to CORD: 1-Next generation sequencing only identified this mutation to be the main cause of CORD in the patients. 2- As can be seen in Figure1b, direct Sanger sequencing proved the mutation in the proband and affected members of the family, and based on recognized heterozygote mutations in their parents, the pattern of inheritance must be an autosomal recessive for PDE6C gene. 3- Bioinformatics software such as SIFT and PolyPhen software are predicted that these variants will be damaging. 4. Also, a nonstop mutation in exon 22 of the PDE6C gene in the C-terminal of the protein, which is predicted to can lead to the continued and inappropriate translation of the mRNA into the 3'UTR, can create major problems in the PDE6C protein. 5- On the other hand, because this modify was not present in the healthy controls, it cannot be ruled out that it has an effect on the phenotype of these patients. Thus, this mutation inthe PDE6C gene is pathogenic in our patients with CORD.

In future studies, the evaluation of the mRNA level related to a nonstop mutation in the PDE6C gene causing CORD is a need. Our findings will progress the diagnosis of patients and their families and characterize one more step toward solving the etiology of CORD.

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