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Case Report

Deletion of *OPN1LW* exons 2-5 results in red-green color blindness: a case report

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Abstract: Red-green color blindness is a common X-linked recessive genetic disease, which is caused by pathogenic variants in the *OPN1LW* or *OPN1MW* (opsin 1, long or middle wave sensitive) genes encoding long- and middle-wavelength-sensitive cone opsins. In this study, peripheral blood samples were collected from a multigenerational family exhibiting red-green color blindness, including two affected males and one female carrier. Whole exome sequencing was performed on the proband's DNA. The locus control region and exons 2, 4, and 5 of *OPN1LW* were analyzed by polymerase chain reaction with the whole family DNA. A novel mutation, deletion of exons 2-5 of *OPN1LW,* was found in the affected family members. This is the first description of the deletion of exons 2-5 in *OPN1LW*, which leads to red-green color blindness.

Keywords: *OPN1LW*, *OPN1MW*, red-green color blindness, exons 2-5, deletion

Introduction

In humans, three opsin proteins named long (OPN1LW)-, middle (OPN1MW)-, and short-wave sensitive opsins, are responsible for red, green, and blue spectral discrimination, respectively [1]. Among these genes, *OPN1LW* and *OPN1MW* are present as a tandem array within the Xq28 telomeric region of the X chromosome [2]. This gene cluster typically consists of a single *OPN1LW* copy followed by one or multiple *OPN1MW* gene copies. Although each gene copy has a direct upstream promoter, the expression of the genes within the gene cluster is regulated by an upstream locus control region (LCR), which is a conserved 600-bp cis-regulatory sequence located upstream of the *OPN1LW/MW* gene cluster. LCR drives gene expression in a distance dependent manner, with only the first two genes within the cluster expressed [3, 4]. Abnormalities in the cluster are associated with X-linked red-green color vision blindness, blue cone monochromacy, and Bornholm eye disease [1]. In this study, we described a novel mutation, a deletion of exons 2-5 in *OPN1LW* found by whole exome sequencing and confirmed by normal *PCR*, in a red-green color blindness family.

Materials and methods

Subjects

Peripheral blood samples were obtained from a family with red-green color blindness (two affected, one carrier, and eight unaffected members, listed in **Figure 1A**). We followed the Declaration of Helsinki protocols. This study was approved by the Ethics Committee of Shengjing Hospital of China Medical University. All participants were fully informed of the study with written consent obtained from each participant.

Whole exome sequencing and polymerase chain reaction (PCR) methodology

Genomic DNA was extracted with the QIAamp DNA Mini Kit (Qiagen, China). A DNA library was prepared according to the kit's instructions (Illumina, USA). Amplified DNA was captured using a whole exome capture kit (MyGenostics GenCap Enrichment Technologies, China). The captured DNA was eluted and amplified, and the PCR products were purified with SPRI beads (Beckman, USA). The enriched libraries were sequenced for paired-end reads of 150 bp with

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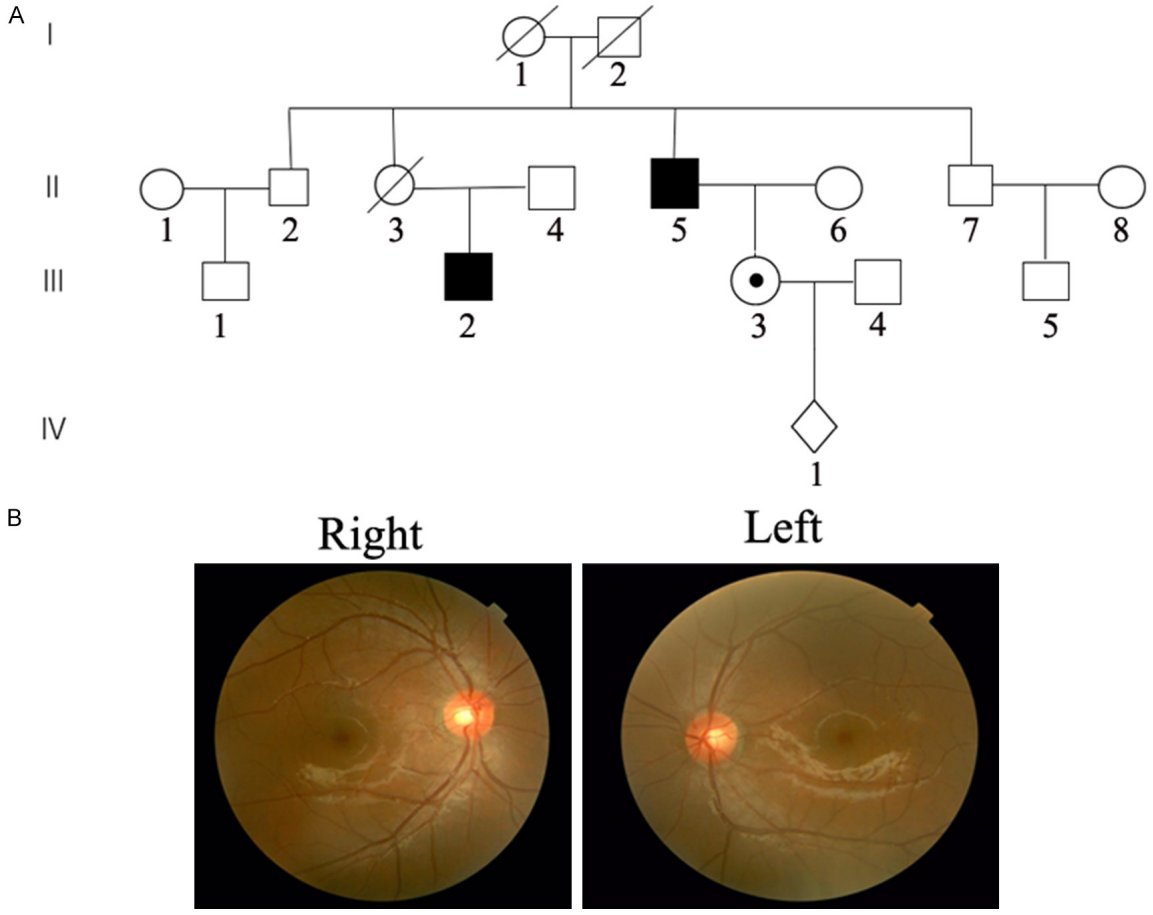

Figure 1. Genetic and clinical characterization of the family with red-green color blindness. A: Pedigree of the red-green blindness family, the carrier III3 underwent preimplantation genetic diagnosis to obtain a normal embryo. B: No abnormality was shown in the eye fundus image of the patient, II5. ■: patient; ⊙: carrier.

an Illumina HiSeq X Ten platform. Raw data were saved in FASTQ format. Illumina sequencing adapters and low-quality reads (< 80 bp) were filtered by Cutadapt. Clean reads were aligned to the UCSC hg19 human reference genome using the Burrows-Wheeler Alignment tool. Duplicated reads were removed with Picard (http://broadinstitute.github.io/picard). For the X chromosome, the average of exome reads in normal females was normalized as two, and males was normalized as one. The relative read number for each exome was compared with the respective gender. Small insertions, deletions, and single nucleotide polymorphisms (SNPs) were detected and filtered with the Genome Analysis Toolkit. The identified variants were annotated using ANNOVAR and assessed with the following databases: 1,000 Genomes, Exome Aggregation Consortium, Human Gene Mutation Database, Mutation Taster (MT), Sorting Intolerant From Tolerant (SIFT), PolyPhen-2 (PP2), and Genomic Evolutionary Rate Profiling (GERP++). Sites of variation were identified through a comparison of DNA sequences with the corresponding GenBank reference sequences using Mutation Surveyor software. The pathogenicity of mutations was assessed in accordance with the American College of Medical Genetics and Genomics Guidelines (ACMG). PCR of the LCR and exons 2, 4, and 5 of *OPN1LW* was performed. Primers are listed in **Table 1**.

Results

Subject information

Subject III3 requested pregnancy genetic counseling. Her father (II5) and her cousin (III2) had red and green color blindness, which is a recessive X-linked recessive inheritable disease. The eye fundus image of the two patients seemed

Table 1. Primers for PCR

Name	Forward primer (5'-3')	Reverse primer (5'-3')
exon 2	TGGATGATCTTTGTGGTCACT	CCCAGCACGAAGTAGCCAGA
exon 4	CACGGCCTGAAGACTTCATG	GAGGTAGCAGAGCATGATGATAGC
exon 5	ATGGTGGTGGTGATGATCTTTG	GATAGTGGCACTTTTGGCAAAGTA
LCR	AAGTGTCAAAGGCAAATGGC	ATCCAAGAATGTGAGACC

Note: PCR: polymerase chain reaction. LCR: locus control region.

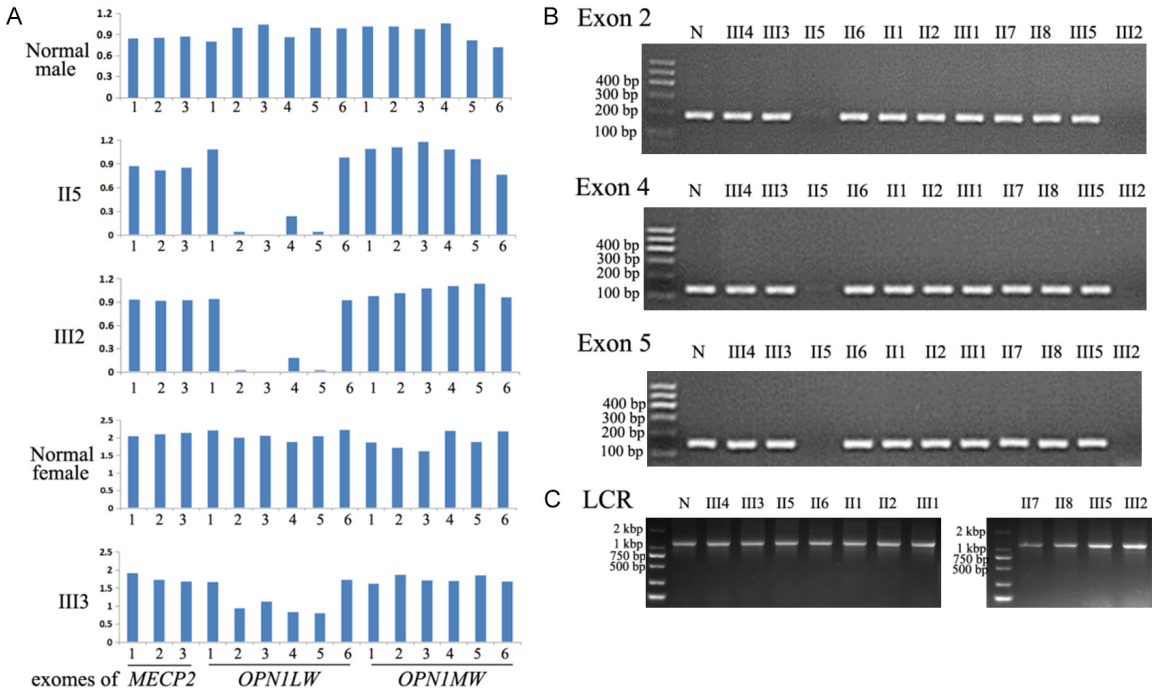

Figure 2. Mutation in the red-green blindness family. A: *OPN1LW* exons 2-5 deletion was identified in the family by whole exome sequencing, compared with respective normal control. B: *OPN1LW* exons 2/4/5 tested by PCR, suggesting exons 2/4/5 in II5 and III2 were deleted. C: LCR measured by PCR, showing that no LCR deletion were found in family members. N: Normal control. LCR: locus control region. PCR: polymerase chain reaction.

to be normal, shown in **Figure 1**. Their vision was also normal. The two affected subjects of the immediate family shared similar clinical symptoms and could not distinguish red or green colors. No other ocular or systemic abnormalities were found in the family. Diagnosis of color blindness was by color vision tests.

Mutations within the family

Whole exome sequencing was performed to identify mutations within the family. None of the clinically significant single nucleotide variants (SNVs) or small fragment variations (< 100 bp) were found in the pedigree. However, the relative reads number of *OPN1LW* exons 2-5 almost disappeared in the affected male II5/III2 and decreased to half in the female carrier III3, compared with respective controls (shown in **Figure 2A**). This meant that II5, III2 and III3 existed with deletions of exons 2-5 of *OPN1LW*. PCR was used to assess exons 2, 4, and 5. (Exon 3 of *OPN1LW* has the same sequence as exon 3 of *OPN1MW* and was not assessed). The PCR results confirmed deletions of exons 2, 4, and 5 of *OPN1LW* for II5 and III2, shown in **Figure 2B**. The LCR region (**Figure 2C**) was demonstrated to be present.

In addition, SNP haplotype analysis was constructed for the linkage analysis, shown in **Figure 3**. Both 15 upstream and 6 downstream SNP sites of the *OPN1LW* gene were selected. III3 underwent preimplantation genetic testing for monogenic disorders (PGT-M), which identi-

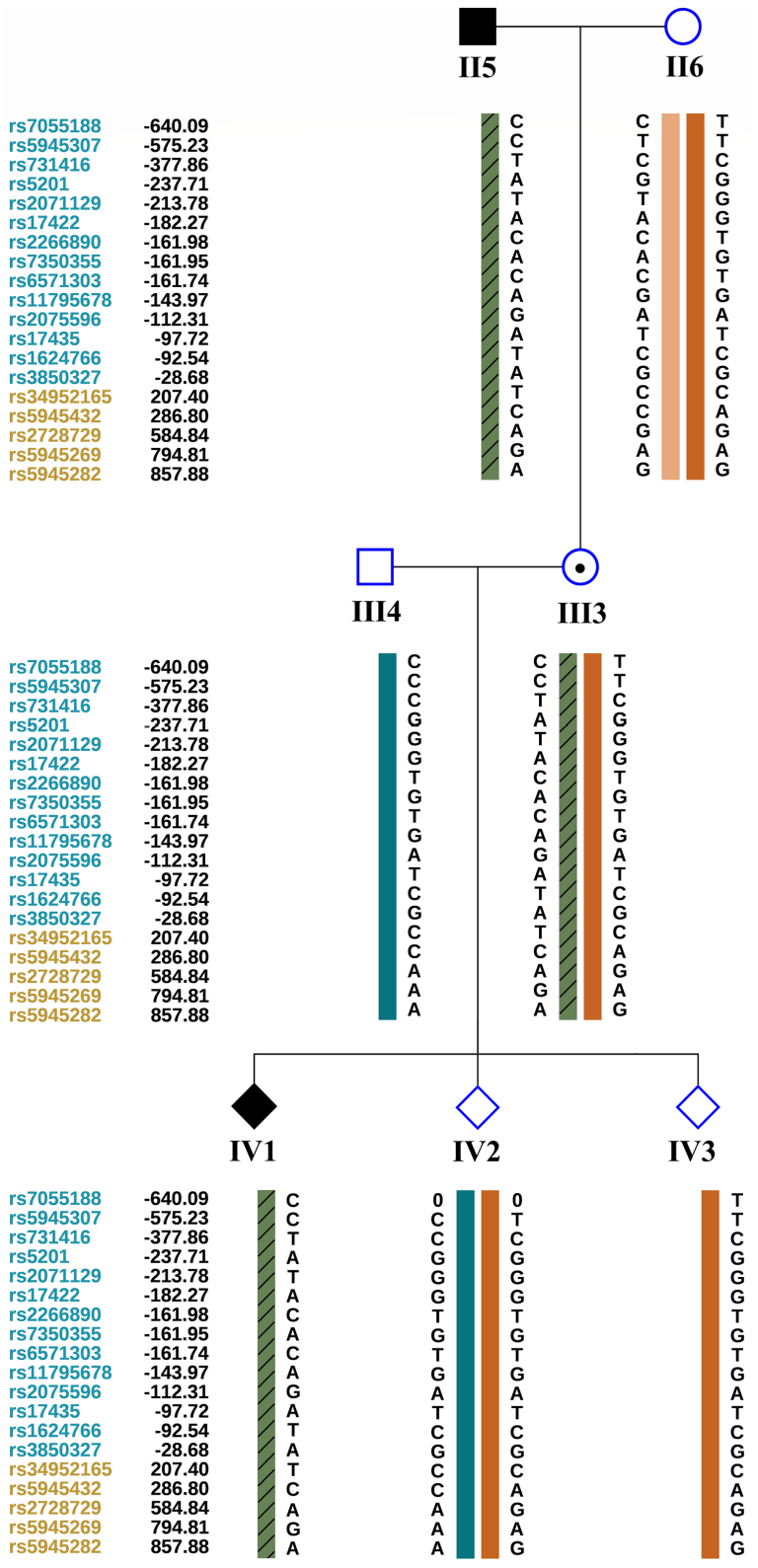

Figure 3. Single nucleotide polymorphism haplotype linkage analysis for the embryo and family. The number represents the relative distance from upstream (blue) and downstream (orange) of the *OPN1LW* gene (unit: kb).

fied two euploid embryos without the *OPN1LW* exons 2-5 deletion. A single embryo was transferred, resulting in a successful singleton pregnancy, and amniocentesis at 18 weeks of gestation confirmed concordance with the PGT-M result. Now, the baby is three and half years old and has normal color vision.

Discussion

In this study, we found deletion of exons 2-5 of *OPN1LW* to be associated with red-green color blindness. Red-green color blindness is a common X-linked recessive genetic disorder. The prevalence rate is 2-8% in males and 0.4-1.7% in females, with regional differences [5]. Stable red-green color vision deficiency with normal visual acuity is common. This typically occurs because of a complete or partial deletion of either the *OPN1LW* or *OPN1MW* genes, resulting in the expression of only a single functional gene in all cones. Hence, it is difficult for the affected individuals to differentiate color within the red/green spectrum [1, 6]. *OPN1LW* is located at chrX: 153,409,698-153,424,507 (GRCh37/hg19). The full-length DNA sequence of *OPN1LW* contains 14,810 bases, and its mRNA is 1,242 bases (NM_020061) with six exons. The coding sequence (CDS) of *OPN1LW* exhibits 96% sequence identity with that of *OPN1MW*.

In this study, deletion of exons 2-5 in *OPN1LW* was found in this family. Based on ACMG guidelines, such a mutation

was classified as likely pathogenic. The evidence of evaluation was listed as PVS1 + PM2. PVS1: The mutation is a null mutation (exon deletion), which is predicted to result in complete loss of gene function. PM2: The variant exhibits a low frequency in a normal population. Similar mutations have been previously reported in *OPN1LW*. Exon 4 deletions [7], exons 2-3 deletions [8], or exons 2-6 deletions [3] in *OPN1LW* were found in blue cone monochromacy, a condition characterized by red-green blindness, reduced visual acuity, pendular nystagmus, and photophobia.

In addition to causing red-green blindness, mutations within the *OPN1LW/MW* gene cluster also result in color vision disorders associated with loss of visual acuity, including blue cone monochromacy and Bornholm eye disease [1]. Three types of causative mutations associated with *OPN1LW/OPN1MW*-related diseases have been identified and classified according to their mechanism of action. 1) Deletion of LCR ablates expression of both genes [9, 10]. 2) Inactivating mutations of both *OPN1LW* and *OPN1MW* genes, like C203R, P307L, R247X [11], and the LIAVA haplotype in exon 3 [12], which affects splicing. 3) Deleterious mutations within a single-gene array, such as exon 2, exons 2-3, exon 4, and exons 2-6. For the family described herein, neither of the first two mutation types was found. The LCR was not missing (shown in **Figure 2C**). The haplotype for exon 3 (p. 153; 171; 174; 178; 180) of *OPN1MW* was MVVVA (data not shown), a normal haplotype. Moreover, no deleterious point mutations were detected in *OPN1MW* or other genes within this family. The exact mutation was a deletion of exons 2-5 in *OPN1MW*. The mutation was identified through both whole exome sequencing and PCR analysis of exons 2, 4, and 5. In summary, we found a novel mutation, deletion of exons 2-5 of *OPN1LW*, in a family with red-green color blindness. With this knowledge it will be possible to prevent transmission of the mutation to the next generation.

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Disclosure of conflict of interest

None.

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