

A novel *CRX* frameshift mutation causing cone-rod dystrophy in a Chinese family

A case report

Lihua Wang, MD^a, Anhui Qi, MS^{b,c}, Hong Pan, MS^{b,c}, Beihong Liu, MS^{b,c}, Jingjing Feng, MS^a, Wei Chen, MD^{a,*}, Binbin Wang, PhD^{b,c,*}

Abstract

Background: Cone-rod dystrophy (CORD) is an inherited, progressive retinal disorder with genetic and phenotypic heterogeneity. Here, we aimed to identify the pathogenic mutation in affected individuals in a Chinese family with autosomal dominant cone-rod dystrophy (adCORD).

Methods: Genomic DNA and clinical examination results were collected from a Chinese family presenting with adCORD. The candidate disease-causing mutations were screened with whole-exome sequencing (WES) and bioinformatics analyses. Sanger sequencing was used for validation and cosegregation analysis.

Results: A novel frameshift mutation (NM_000554.4; c.538dupG;p.Val180fs) in exon 4 of the *CRX* gene was identified in all affected individuals in the Chinese family with adCORD. Cosegregation analysis confirmed that this mutation was cosegregated with the disease. This variant, which results in premature termination of the protein, was absent from all public variant databases or internal exome databases.

Conclusions: We used whole-exome sequencing to identify a novel *CRX* mutation causing adCORD in a Chinese family. This study broadens the known pathogenic mutation spectrum of the *CRX* gene and shows the potential of WES in identifying the pathogenic mutations of CORD disease.

Abbreviations: AD = autosomal dominant, adCORD = autosomal dominant cone-rod dystrophy, AR = autosomal recessive, CORD = cone-rod dystrophy, ERG = Electroretinogram, F-ERG = full field electroretinography, FFA = fluorescein fundus angiography, HD = homeodomain, IS/OS = inner segment/outer segment, LCA = Leber congenital amaurosis, MAF = minor allele frequency, OCT = optical coherence tomography, RP = retinitis pigmentosa, RPE = retinal pigment epithelium, SNVs = single nucleotide variants, WES = whole-exome sequencing, XL = X-linked.

Keywords: cone-rod dystrophy, cosegregation analysis, *CRX* mutation, sanger sequencing, whole-exome sequencing

1. Introduction

Cone-rod dystrophy (CORD) is an inherited, progressive retinal disorder with a prevalence of approximately 1 in 40,000.^[1] The

Editor: Suleyman Demircan.

LW and AQ have contributed equally to this work.

This study was supported by National Science and Technology Basic Work (2014FY130100), National Infrastructure of Chinese Genetic Resources (YCZYPT [2017]01-6), and the National Key Research and Development Program of China (2016YFC1000307-6).

The authors have no competing interests to declare.

Supplemental Digital Content is available for this article.

^a Department of Ophthalmology, Beijing Haidian Maternal and Child Health Hospital, ^b Graduate School of Peking Union Medical College, ^c Center for Genetics, National Research Institute for Family Planning, Beijing, China.

* Correspondence: Wei Chen, Department of Ophthalmology, Beijing Haidian Maternal and Child Health Hospital, 33 Haidian South Road, Haidian, Beijing 100080, China (e-mail: chenweidg@163.com); Binbin Wang, Center for Genetics, National Research Institute for Family Planning, 12 Dahuisi Road, Haidian, Beijing 100081, China (e-mail: wbbahu@163.com).

Copyright © 2018 the Author(s). Published by Wolters Kluwer Health, Inc. This is an open access article distributed under the terms of the Creative Commons Attribution-Non Commercial-No Derivatives License 4.0 (CCBY-NC-ND), where it is permissible to download and share the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.

Medicine (2018) 97:32(e11499)

Received: 29 March 2018 / Accepted: 20 June 2018

<http://dx.doi.org/10.1097/MD.0000000000011499>

predominant symptoms include progressively declining visual acuity, photophobia, color vision disturbances and night blindness, and nystagmus is often present. Electroretinogram (ERG) shows cone photoreceptor dysfunction or loss at an early stage, later followed by rod photoreceptor disorder. Fundus examination reveals retinal pigmentation and various degrees of retinal atrophy.^[2,3] CORD is genetically and phenotypically heterogeneous and is transmitted through 3 forms of inheritance: autosomal dominant (AD), autosomal recessive (AR), and X-linked (XL) inheritance.^[4–7] At present, >30 genes are reported to be associated with cone and cone-rod dystrophy in the RetNet database (<http://www.sph.uth.tmc.edu/retnet/>). No therapies are yet available to treat these diseases.

Here, we report a 4-generation Chinese family of 8 patients with adCORD. We used whole-exome sequencing (WES) to identify the pathogenic mutations in these affected individuals and discovered a novel mutation in the *CRX* gene.

2. Methods

2.1. Subjects and examinations

This research project was approved by the ethics committee of the National Research Institute for Family Planning. Written informed consent adhered to the tenets of the Declaration of Helsinki and the ARVO statement on the use of human subjects.

A 4-generation Chinese family including 8 affected individuals was enrolled in this study (Fig. 1). All ophthalmic examinations,

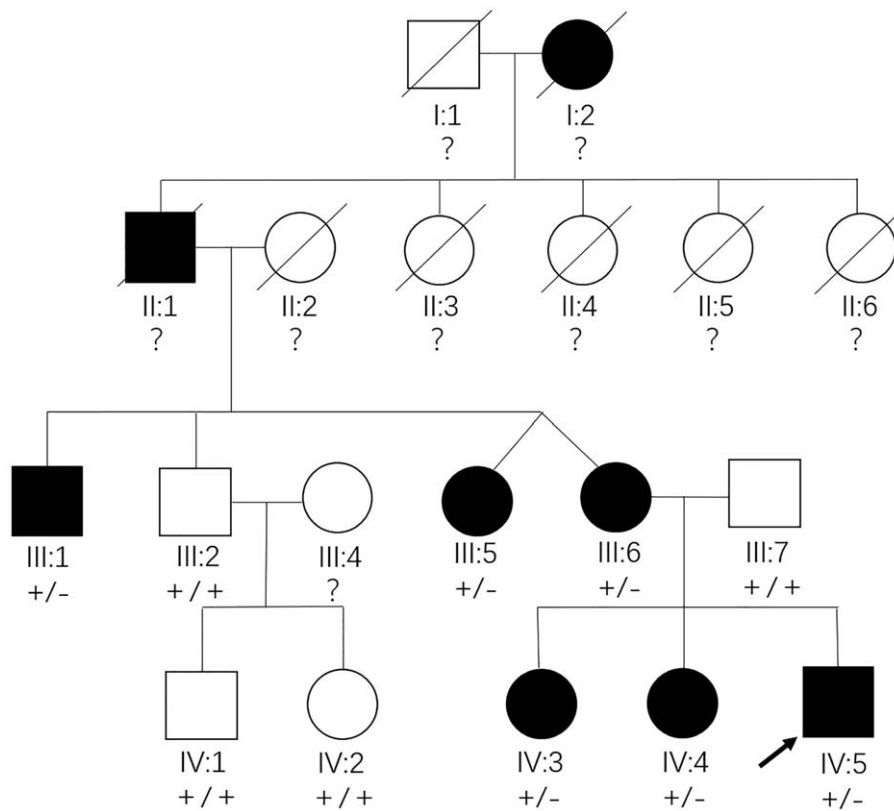


Figure 1. Pedigree of a 4-generation Chinese family with autosomal dominant cone-rod dystrophy. Square: male, circle: female, filled symbol: patient, unfilled symbol: unaffected member, slash: deceased, black arrow: the proband, plus: normal allele, minus: mutant allele, question marks: members whose genotype could not be examined.

including visual acuity, fundus examination, fluorescein fundus angiography (FFA), optical coherence tomography (OCT), visual field examination, and full field electroretinography (F-ERG), were performed at the Maternal and Child Health Hospital, Haidian, Beijing, where the diagnosis was made.

2.2. Whole-exome sequencing and variant analyses

Peripheral venous blood was collected from 10 participants and genomic DNA was extracted with the QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany). The DNA concentrations were measured with a NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA). WES was performed by a commercial company, MacroGen (Shenzhen, China). Briefly, all exons were captured with the SureSelect Human All Exon V5 Kit (Agilent, Santa Clara, CA) and the enriched DNA fragments were sequenced with a HiSeq 4000 sequencer (Illumina, San Diego, CA). The Burrows-Wheeler Alignment (BWA, <http://bio-bwa.sourceforge.net/bwa.shtml>) algorithm was used to align the sequencing reads against the human reference genome. The Genome Analysis Toolkit (GATK, <https://www.broadinstitute.org/gatk/>) was used to call variants such as single nucleotide variants (SNVs) and indels. SnpEff (<http://snpeff.sourceforge.net/SnpEff.html>) was then used to annotate the variants and predict their functional effects.

The candidate pathogenic variants were filtered as follows: the exonic non-synonymous mutations in known retinal disease-causing genes in the RetNet database were extracted from the WES results, including the missense, nonsense, frameshift, and splice-site mutations; and mutations with a minor allele

frequency (MAF) ≥ 0.01 in the 1000 Genomes (<https://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/>), ESP6500 (<http://evs.gs.washington.edu/EVS/>), and ExAC (<http://exac.broadinstitute.org/>) databases were excluded. The impact of the filtered candidate mutations on the proteins was predicted with 4 online programs: PolyPhen 2 (<http://genetics.bwh.harvard.edu/pph2/>), SIFT (<http://sift.jcvi.org/>), MutationTaster (<http://www.mutationtaster.org/>), and SNPs&GO (<http://snps.biofold.org/snps-and-go/>).

2.3. Sanger sequencing

Sanger sequencing was used to validate the presence of the filtered mutations for the proband. The forward and reverse primers for the *BBS4*, *CRX*, and *RAX2* genes were as follows: 5'-ATGCTCTTATTCCCAGTG-3', 5'-CTTAGGGTACAAGGTG TCT-3' (*BBS4*); 5'-CCCCATCTCCGCTCTTAT-3', 5'-TCTGA AACTTCCAGGCACTC-3' (*CRX*); and 5'-TGGGCTTAGG-GAACGCTTGG-3', 5'-GGTCACCTCCTGCCTGACACT-3' (*RAX2*). Cosegregation analysis of the mutations and disease phenotype was performed with Sanger sequencing for all available pedigree members.

3. Results

3.1. Clinical manifestations

The proband (Fig. 1, IV:5), a 19-year-old man, first presented 10 years ago with binocular vision loss, photophobia, and impaired

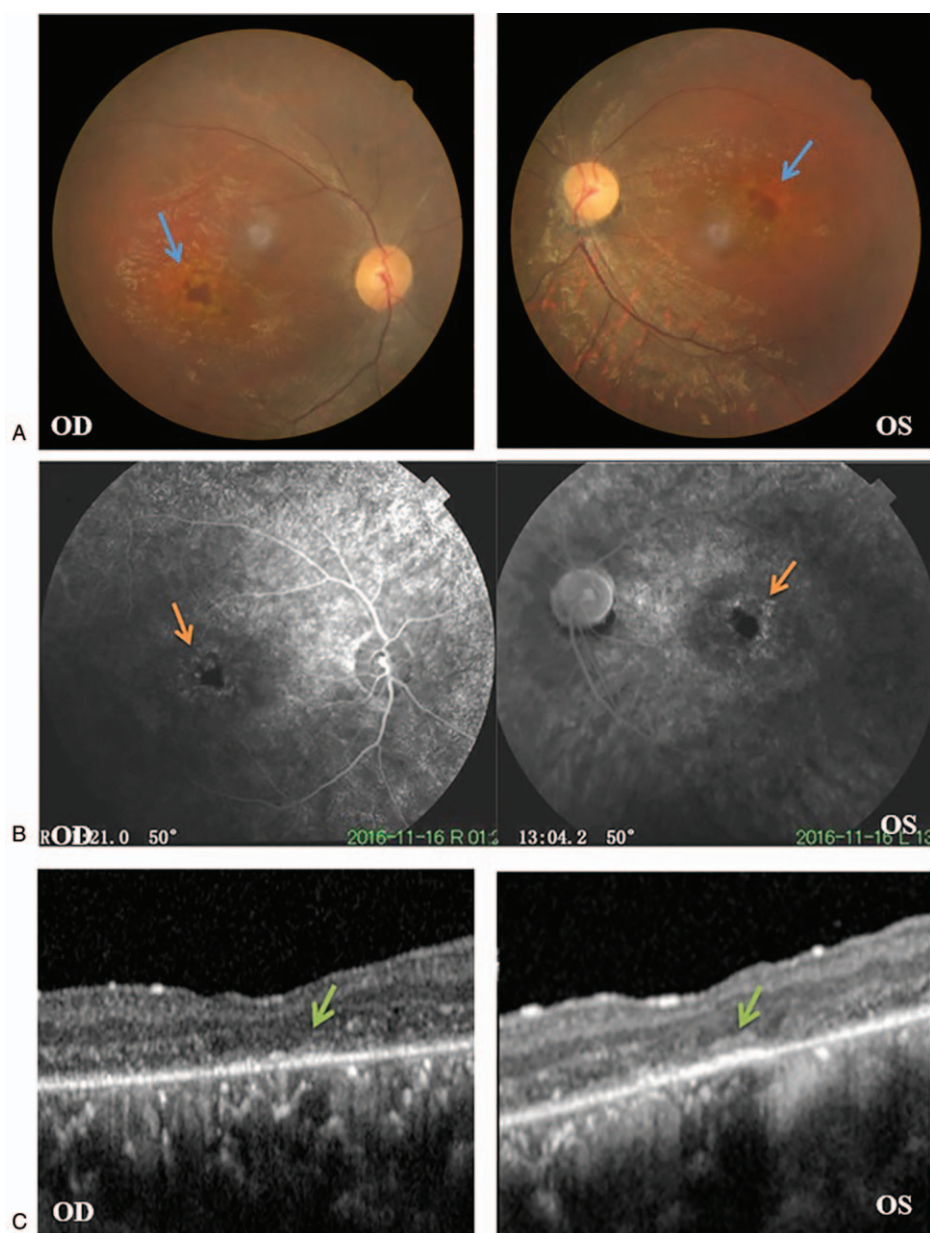


Figure 2. Ophthalmic examination results of the proband. A: Fundus images showed retinal pigment epithelium atrophy and gold foil-like reflection in the macular area. Blue arrow: geographic atrophy foci. B: Fluorescein fundus angiography showed diffuse transmitted fluorescence and geographic hypofluorescence in the macular area (orange arrow). C: Optical coherence tomography showed an absent fovea centralis and an undefined IS/OS layer (green arrow). OD: right eye, OS: left eye.

vision in the dark without any obvious cause. The uncorrected visual acuity of each eye was 0.5 and no significant improvement was observed with spectacles. Since then, the proband's visual acuity has decreased progressively. Over the past 4 years, binocular vision has decreased significantly and the night blindness has become severe. The ocular examination results showed that uncorrected visual acuity was 0.15 (right eye) and 0.1 (left eye), with no improvement observed with correction. We found no abnormality in the anterior segment. Fundus examination showed a clear optic disc border, slightly light color, a thinning artery, mid-peripheral diffuse retinal pigment epithelium (RPE) atrophy, gold foil-like reflection, and geographic pigment epithelium atrophy foci in each macular area (Fig. 2A). FFA showed delayed retinal arterial filling, diffuse

transmitted fluorescence, and irregularly geographic hypofluorescence in the macular area (Fig. 2B). OCT revealed a thinning retinal nerve epithelium layer in the binocular macular area, a significantly thinning outer nuclear layer of the fovea centralis, and an absent fovea centralis, with the structure of the inner segment/outer segment (IS/OS) layer being difficult to distinguish (Fig. 2C). Visual field examination revealed quadrant defects in the binocular nasal field (Fig. 3A). F-ERG showed the binocular rod cell response amplitude and maximum response amplitude were both decreased, and both the cone cell reaction and 30 Hz scintillation reaction indicated the absence of an effective waveform (Fig. 3B).

The proband's sisters (IV:3, IV:4), mother (III:6), maternal aunt (III:5), maternal uncle (III:1), and maternal grandfather (II:1) all

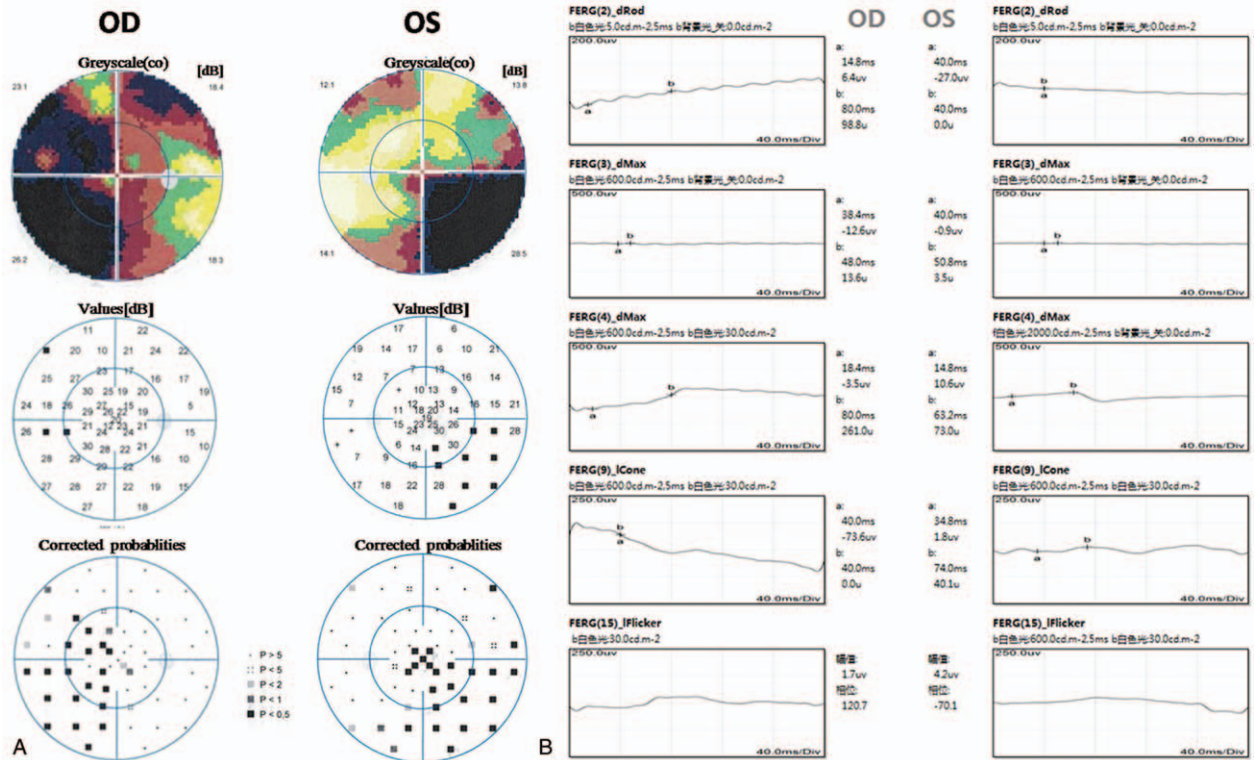


Figure 3. Further ophthalmic examination results of the proband. A: Visual field examination showed quadrant defects in the binocular nasal field. B: Full field electroretinography showed the binocular rod cell response amplitude and maximum response amplitude were both decreased, and both the cone cell reaction and 30Hz scintillation reaction indicated the absence of an effective waveform. OD: right eye, OS: left eye.

have a similar history of eye disease. His sister (IV:3) started to experience progressive binocular vision loss and photophobia at the age of 15, followed by night blindness symptoms. She was diagnosed with CORD at the Beijing Tongren Hospital in 2013. The results of her ocular examinations were an uncorrected visual acuity of 0.2 (right eye) and 0.05 (left eye). Fundus examination showed diffuse RPE atrophy and geographic atrophy foci in the macular area (Supplemental Figure 1A, <http://links.lww.com/MD/C382>). FFA showed hypofluorescence in the fovea centralis of the macular area surrounded by punctate hyperfluorescence, with no fluorescence leakage (Supplemental Figure 1B, <http://links.lww.com/MD/C382>). OCT revealed an undefined IS/OS layer, and a significantly thinning outer nuclear layer of the fovea centralis (Supplemental Figure 1C, <http://links.lww.com/MD/C382>). Visual field examination revealed quadrant defects (Supplemental Figure 2A, <http://links.lww.com/MD/C382>). And the sister's FERG results were similar to those of the proband (Supplemental Figure 2B, <http://links.lww.com/MD/C382>).

3.2. Candidate pathogenic variants

A total of 78,471 SNPs and 9078 indels were identified in the proband (IV:5) by WES. After applying the first 2 filtering steps described in the Methods, 3 candidate mutations including 2 missense mutations and 1 frameshift mutation in 3 known retinal disease-causing genes were obtained. These mutations were c.233G>T in *BBS4* (Gene ID: 585, OMIM: 600374), c.538dupG in *CRX* (Gene ID: 1406, OMIM: 602225), and c.235C>T in *RAX2* (Gene ID: 84839, OMIM: 610362). These mutations were all absent from or were present at an extremely low frequency in the 1000 Genomes, ESP6500, and ExAC databases. The functional effects of these mutations, predicted with the online tools PolyPhen2, SIFT, MutationTaster, and SNPs&GO, indicated a deleterious impact for all 3 candidate mutations on their respective proteins (Table 1). Subsequent Sanger sequencing results validated the presence of these filtered mutations in the proband. Cosegregation analysis among all available individuals in the proband's family showed that only

Table 1
Candidate mutations in known retinal disease-causing genes identified by whole-exome sequencing.

Gene	Mutation	1000G (%)	ESP6500 (%)	ExAC* (%)	Online prediction				Cosegregation
					PolyPhen2	SIFT	MutationTaster	SNPs&GO	
BBS4	NM_033028.4:c.233G>T:p.Arg78Leu	0.01997	0	0.01164	Probably damaging	Damaging	Disease causing	Neutral	No
CRX	NM_000554.4:c.538dupG:p.Val180fs	0	0	0	NA	NA	Disease causing	NA	Yes
RAX2	NM_032753.3:c.235C>T:p.Arg79Trp	0	0	0	Probably damaging	Damaging	Disease causing	Disease	No

NA = not applicable.
* Only lists the East Asian population frequencies.

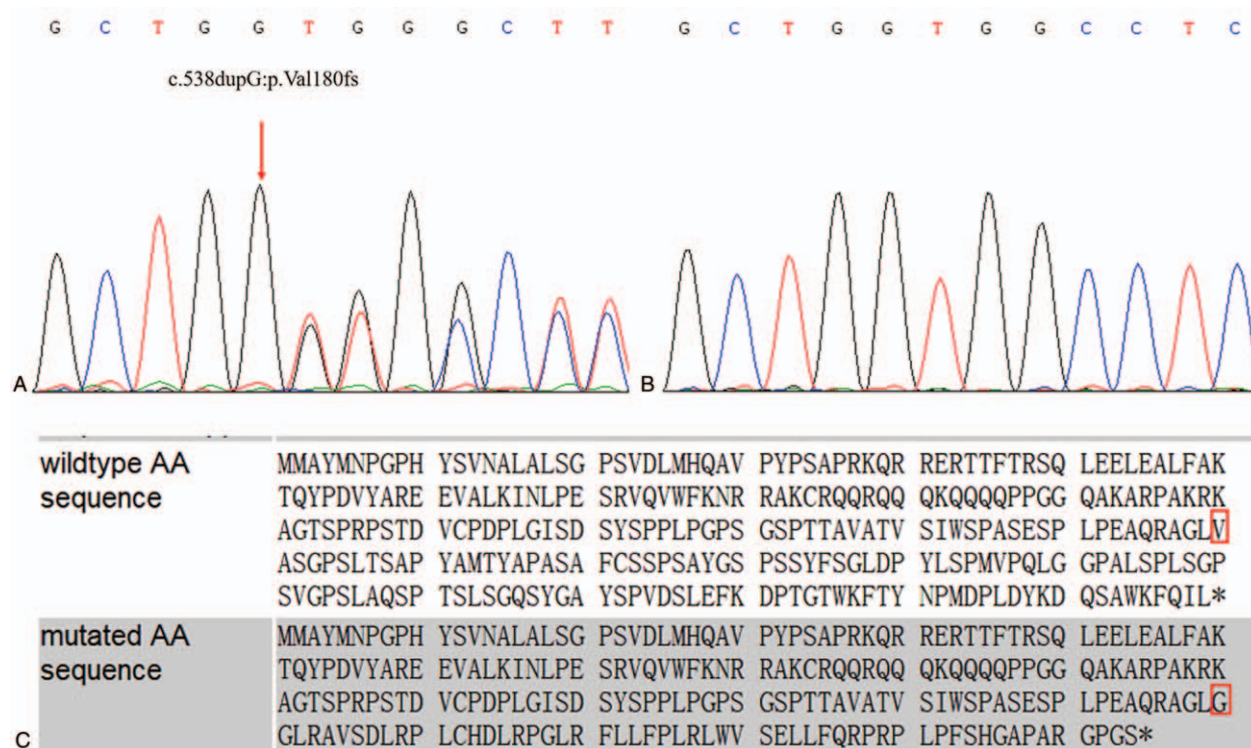


Figure 4. Sanger sequencing results and *CRX* mutation prediction. A: The sequencing result for affected members. B: The sequencing result for unaffected members. C: The amino acid sequence in the presence and absence of the *CRX* mutation. Red square: the mutation site.

the *CRX* gene mutation (c.538dupG:p.Val180fs) was present in all affected members but not in any of the unaffected members (Fig. 4A, B); that is, only the *CRX* gene mutation was observed to cosegregate with the family disease phenotype. *CRX* is one of the pathogenic genes of cone-rod dystrophy.^[8] Therefore, this mutation was identified as the pathogenic variant in this Chinese family with cone-rod dystrophy.

The frameshift mutation we identified in the *CRX* gene in this family (c.538dupG:p.Val180fs) was absent in all public databases. It was predicted by MutationTaster to result in a frameshift and premature termination. The 1bp guanine duplication at position 538 in the open reading frame alters the reading frame, resulting in the valine at position 180 changing to a glycine thereby causing premature protein termination at codon 235 (Fig. 4C). Based on the ACMG standards and guidelines for the interpretation of sequence variants, this mutation satisfies 5 different pathogenic evidence including PVS1, PM2, PM4, PP1, and PP3, and it is classified as pathogenic.^[9]

4. Discussion

Retinal diseases exhibit extensive genetic and phenotypic diversity, with links to more than 250 genes (<https://sph.uth.edu/retnet/>). This poses a challenge for specific diagnosis based on clinical manifestations and for using Sanger sequencing to identify the pathogenic mutations. Thus, we narrowed our search by selecting all known retinal disease-causing genes only, and by using WES as the sequencing analysis tool in this study. Three candidate pathogenic mutations in 3 retinal disease-causing genes were identified as a result of the initial WES of the proband. Cosegregation analysis excluded 2 of these mutations, as they did not cosegregate with the disease in the proband's family. The

remaining *CRX* variant was therefore identified as the disease-causing mutation in this Chinese adCORD family. This mutation was predicted to result in a frameshift and premature termination that would deleteriously impact normal *CRX* protein function.

The human cone-rod homeobox gene *CRX* is located on 19q13.33 and contains 4 exons encoding a 299-amino acid homeodomain transcription factor.^[8] Animal experiments have shown that this gene is preferentially expressed in vertebrate photoreceptor cells of the retina and pinealocytes of the pineal gland.^[10,11] The *CRX* protein plays an essential role in regulating the expression of specific genes in mammalian rod and cone photoreceptors to maintain the normal functioning of the cones and rods.^[12] In *Crx*^{-/-} mice, which lack photoreceptor outer segments, ERG shows the absence of normal rod and cone activity.^[12]

Mutations of the human *CRX* gene have been reported to cause not only adCORD but also autosomal dominant inherited retinitis pigmentosa (RP) and Leber congenital amaurosis (LCA), which have different severities and ages of onset.^[8,13,14] Many *CRX* frameshift mutations are known retinal disease-causing variants.^[4,15,16] Huang et al^[17] reported that the 49 reported *CRX* mutations are composed of 38.78% missense, 4.08% nonsense, 36.73% deletion, 16.33% insertion, and 4.08% indel. Mammalian *CRX* protein includes 5 conserved functional domains, comprising (from the N- to the C-terminus) the homeodomain (HD) and glutamine-rich (Gln) domain, and the basic, WSP, and OTX-tail motifs. The HD is associated with DNA binding while the C-terminal region of the protein, with the basic to OTX-tail domains, is associated with transcriptional regulation.^[18] The reported missense mutations that cosegregate with disease phenotype lie within the HD domain, while frameshift and nonsense mutations are restricted to the C-

terminal region of the protein.^[19] Thus, the frameshift mutation observed in this Chinese family (c.538dupG:p.Val180fs) would impact normal CRX protein function by effecting the transcriptional regulation of photoreceptor-specific genes.

In conclusion, with the use of WES and Sanger sequencing, we revealed a novel frameshift mutation (NM_000554.4:c.538dupG:p.Val180fs) in exon 4 of the *CRX* gene in a Chinese family with adCORD. This study broadens the known pathogenic mutation spectrum of the *CRX* gene and contributes to improved genetic counseling for adCORD patients. In addition, this work showed that whole-exome sequencing is an efficient tool for identifying the pathogenic variants of a hereditary disease associated with multiple pathogenic genes.

Acknowledgments

The authors are grateful to all the study participants.

Author contributions

Data curation: Lihua Wang, Jingjing Feng.

Investigation: Lihua Wang.

Writing – original draft: Lihua Wang, Anhui Qi.

Formal analysis: Anhui Qi.

Methodology: Anhui Qi, Hong Pan.

Supervision: Hong Pan, Beihong Liu, Jingjing Feng.

Visualization: Beihong Liu.

Project administration: Wei Chen, Binbin Wang.

Validation: Wei Chen, Binbin Wang.

Resources: Binbin Wang.

References

- [1] Thiadens AA, Phan TM, Zekveld-Vroon RC, et al. Clinical course, genetic etiology, and visual outcome in cone and cone-rod dystrophy. *Ophthalmology* 2012;119:819–26.
- [2] Oishi M, Oishi A, Gotoh N, et al. Next-generation sequencing-based comprehensive molecular analysis of 43 Japanese patients with cone and cone-rod dystrophies. *Mol Vis* 2016;22:150–60.
- [3] Hamel C. Cone rod dystrophies. *Orphanet J Rare Dis* 2007;2:7.
- [4] Kitiratschky V, Nagy D, Zabel T, et al. Cone and cone-rod dystrophy segregating in the same pedigree due to the same novel *CRX* gene mutation. *Br J Ophthalmol* 2008;92:1086–91.
- [5] Huang L, Zhang Q, Li S, et al. Exome sequencing of 47 Chinese families with cone-rod dystrophy: mutations in 25 known causative genes. *PLoS ONE* 2013;8:e65546.
- [6] Cremers F, van de Pol D, van Driel M, et al. Autosomal recessive retinitis pigmentosa and cone-rod dystrophy caused by splice site mutations in the Stargardt's disease gene *ABCR*. *Hum Mol Genet* 1998;7:355–62.
- [7] Demirci F, Rigatti B, Wen G, et al. X-linked cone-rod dystrophy (locus COD1): identification of mutations in RPGR exon ORF15. *Am J Hum Genet* 2002;70:1049–53.
- [8] Freund C, Gregory-Evans C, Furukawa T, et al. Cone-rod dystrophy due to mutations in a novel photoreceptor-specific homeobox gene (*CRX*) essential for maintenance of the photoreceptor. *Cell* 1997;91:543–53.
- [9] Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015;17:405–24.
- [10] Chen S, Wang Q, Nie Z, et al. *Crx*, a novel *Otx*-like paired-homeodomain protein, binds to and transactivates photoreceptor cell-specific genes. *Neuron* 1997;19:1017–30.
- [11] Furukawa T, Morrow E, Cepko C. *Crx*, a novel *otx*-like homeobox gene, shows photoreceptor-specific expression and regulates photoreceptor differentiation. *Cell* 1997;91:531–41.
- [12] Furukawa T, Morrow E, Li T, et al. Retinopathy and attenuated circadian entrainment in *Crx*-deficient mice. *Nat Genet* 1999;23:466–70.
- [13] Sohocki M, Daiger S, Bowne S, et al. Prevalence of mutations causing retinitis pigmentosa and other inherited retinopathies. *Hum Mutat* 2001;17:42–51.
- [14] Freund C, Wang Q, Chen S, et al. De novo mutations in the *CRX* homeobox gene associated with Leber congenital amaurosis. *Nat Genet* 1998;18:311–2.
- [15] Rivolta C, Peck N, Fulton A, et al. Novel frameshift mutations in *CRX* associated with Leber congenital amaurosis. *Hum Mutat* 2001;18:550–1.
- [16] Lines M, Hébert M, McTaggart K, et al. Electrophysiologic and phenotypic features of an autosomal cone-rod dystrophy caused by a novel *CRX* mutation. *Ophthalmology* 2002;109:1862–70.
- [17] Huang L, Xiao X, Li S, et al. *CRX* variants in cone-rod dystrophy and mutation overview. *Biochem Biophys Res Commun* 2012;426:498–503.
- [18] Chau K, Chen S, Zack D, et al. Functional domains of the cone-rod homeobox (*CRX*) transcription factor. *J Biol Chem* 2000;275:37264–70.
- [19] Tran N, Chen S. Mechanisms of blindness: animal models provide insight into distinct *CRX*-associated retinopathies. *Dev Dyn* 2014;243:1153–66.