Insights into Genetic Retinal Diseases

Insights into Genetic Retinal Diseases:

A Collection

Edited by

Saber Imani and Jinjiang Fu

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To my beloved wife, ANNA, and our precious angel, MAGGIE,

In love's embrace, purpose finds its stage;
Your presence, the ink that enriches these pages.
You've shared your hearts, in each word it shows;
My worth translated, a legacy that glows.
With you by my side, this journey's sweet grace;
A legacy crafted, while we both embrace.

With fraternal love,

Saber Imani
(Hangzhou, China; October 1, 2023)

CONTENTS

PREFACE

Welcome to the "Genetic Retinal Insights: A Collection" book on the cutting-edge field of next-generation sequencing (NGS) and the discovery of new mutations for genes responsible for retinal diseases. The world of genetic retinal diseases is rapidly evolving and advancing, with discoveries and innovations emerging every day. To keep up with the latest developments in the field, it is crucial to have a comprehensive and up-to-date resource that covers the most recent research on fundus inherited disorders including retinal diseases. That's where "Genetic Retinal Insights: A Collection" comes in.

With contributions from leading experts in the field, this compendium is the ultimate guide to the latest breakthroughs and innovations in the field of genetic retinal diseases. The book is designed to be accessible to researchers, clinicians, and patients alike, with clear and concise writing that makes even the most complex topics easy to understand.

One of the primary focuses of the book is to bold the power of NGS techniques, eventually, which have revolutionized the field of genetics in recent years. By using targeted NGS techniques and short-tandem-repeat genotypes, researchers can quickly and accurately identify new disease-causing genes, mutations, and variants. This information is critical for developing new diagnostic tools and treatments for patients with genetic retinal diseases.

This book is organized into four parts, each focusing on a different area of research: Inherited Retinal Dystrophies, Choroideremia and Retinal Atrophies, Usher Syndrome, and Other Inherited Disorders and Techniques. Written by a team of expert researchers, this book contains over 18 original research manuscripts that showcase the latest breakthroughs in the field. The manuscripts presented in this book showcase groundbreaking discoveries of previously unknown genetic mutations, as well as novel diagnostic tools and treatments for retinal diseases. This project was a result of cooperation between the Key Laboratory of Epigenetics and Oncology, The Research Center for Preclinical Medicine, Southwest Medical University, Luzhou, Sichuan, China, Department of Ophthalmology, The Affiliated Hospital, Southwest Medical University, Luzhou, Sichuan, China, the Department of Ophthalmology, Bina Eye Hospital Research Center, Tehran, Iran, and Department of Molecular and Human Genetics, Baylor

College of Medicine, Houston, USA, from 2010 to 2022. We thank *Profs. Rui Chen*, *Khosrow Jadidi*, *Marzieh Dehghan Shasaltaneh*, *Songyot Anuchapreeda*, and *Hongbing Lv*, and *Docs. Jingliang Cheng*, *Yumei Li*, *LishaYang*, *Chunli Wei*, *Iqra Ijaz*, Abdolkarim Mobasher-Jannat, and *Qi Zhou* for their contributions. This book will be a valuable resource for anyone interested in advancing their knowledge of the latest research on retinal diseases.

But "Genetic Retinal Insights: A Collection" is much more than just a technical guide to the latest innovations in genetic retinal diseases. It is a powerful tool for patients and their families who are struggling with these debilitating conditions. By providing a comprehensive and up-to-date resource on the latest research and treatments, the book offers hope and encouragement to those who are living with genetic retinal diseases and makes the right picture of the molecular pathogenesis of these unknown diseases.

For researchers and clinicians, "Genetic Retinal Insights: A Collection" is an essential resource that provides in-depth coverage of the latest breakthroughs and innovations. From the discovery of new, disease-causing genes in retinal diseases to the development of innovative diagnostic tools and treatments, the book offers a deep dive into a specific aspect of the field with the latest findings and expert insights.

Professor Fu and Dr. Imani, authors of "Genetic Retinal Insights: A Collection," would like to convey their deepest appreciation to the brilliant minds who contributed to this book. The "Genetic Retinal Insights: A Collection" is a comprehensive and up-to-date resource on the latest research and treatment options for genetic retinal diseases. With contributions from leading experts in the field, this book showcases the latest breakthroughs and innovations in the field, including the use of NGS techniques. Collective efforts of those working in clinical and laboratory settings have yielded invaluable data and groundbreaking discoveries, which we are thrilled to showcase in this stunning volume. Our gratitude knows no bounds for the patients who selflessly shared their stories, entrusted us with their vision, and generously provided us with vital samples, driving our research forward. We must also express our immense thanks to the National Natural Science Foundation of China (82073263, 31701087, 81672887, 81172049, 30371493), the Science and Technology Innovation Team of Colleges and Universities of Sichuan Province (13TD0032), the Joint Research Foundation of Luzhou City and Southwest Medical University (grant no. 2018LZXNYD-YL01), and Southwest Medical University Grants for Postdoctoral Research (2016-BSH0001), whose generous support has been instrumental in this endeavor.

We are deeply indebted to the publisher, Cambridge Scholars Publishing Press publisher, for their rigorous editing, meticulous formatting, publication, marketing, and invaluable assistance in bringing this project to life. Finally, we cannot express enough gratitude to our beloved families, whose unwavering love and encouragement have sustained us throughout the long and often challenging journey of writing this book. Thank you all for your unwavering commitment to advancing our understanding of genetic retinal disease.

Absolutely! "Genetic Retinal Insights: A Collection" is a must-read for anyone looking to deepen their knowledge of genetic retinal diseases. With its comprehensive and up-to-date coverage of the latest advances in the field, this book is an invaluable resource for researchers, clinicians, and patients alike. Whether you're looking to stay abreast of the latest developments in genetic retinal diseases or seeking insights into cutting-edge treatments and innovations, this book is sure to provide you with the latest and most reliable information. So don't wait – pick up a copy of "Genetic Retinal Insights: A Collection" today and discover the latest in this rapidly evolving field!

Best wish
Editors Team

Saber Imani
Assistant Professor,
Shulan International Medical College, Zhejiang Shuren University,
Hangzhou, Zhejiang Province, 310000, P.R. China.

Jinjiang Fu
Full Professor (Level-2 Professor),
Director of the Key Laboratory of Epigenetics and Oncology,
The Research Center for Preclinical Medicine, Southwest Medical,
University, Luzhou, Sichuan Province, 64600, P.R. China.

SPECIAL ACKNOWLEDGMENT

I extend my heartfelt gratitude and offer a special acknowledgment to my esteemed master and doctoral advisor, *Professor Luyun Li*. His unwavering kindness, illuminating guidance, and profound knowledge have left an indelible mark on my journey. It is a privilege to have had the honor of benefiting from his remarkable personality and unwavering diligence, a treasure I will cherish throughout my life. My gratitude towards him knows no bounds.

Professor Luyun Li (May 22, 1937 - April 15, 2020) was a distinguished medical geneticist and a pioneering figure in the field of genetic counseling in China. She dedicated her entire life to the advancement of medical genetics and genetic counseling. Professor Li's legacy includes the establishment of a new discipline in clinical genetics in China. Her contributions have been celebrated with numerous accolades, including 25 national, provincial, and ministerial scientific and technological achievement awards. Notably, she received the National Award for Science and Technology Progress four times in 1985, 1987, 1995, and 1999, respectively.

With deep respect and gratitude,

Jinjiang Fu
Houston, USA; October 1, 2023

PART 1:

INHERITED RETINAL DYSTROPHIES

INTRODUCTION

SABER IMANI[1] AND JINJIANG FU[2]

Welcome to the captivating realm of the human eye, where light meets biology, and vision is born. In this inaugural chapter of our journey, we embark on a profound exploration of 'Inherited Retinal Dystrophies' (IRDs), a remarkable collection of genetic eye disorders that unveil the intricate workings of the ocular universe.

At the heart of this narrative lies the retina, an exquisite tapestry of light-sensitive cells nestled deep within the eye. Here, on this canvas of photoreceptors and sensory neurons, the marvel of human vision takes shape. But alas, for those touched by IRDs, this masterpiece faces a relentless adversary—a cascade of genetic anomalies that set in motion a journey into the shadows.

IRDs, as a collective, defy easy classification. They are a mosaic of clinical symptoms, each painting a unique portrait of visual decline. From the haunting whispers of night blindness to the gradual erosion of visual fields, from the subtle dance of color vision defects to the stark reality of complete blindness, IRDs cast a broad and complex spectrum of challenges.

Yet, the true complexity of IRDs reveals itself in their genetic underpinnings. More than 300 genes have been implicated in the orchestration of these conditions, each playing its role in this intricate genetic ballet. However, this wealth of genetic diversity has also brought forth a formidable challenge—accurate diagnosis and classification. The clinical and genetic heterogeneity of IRDs makes unraveling their mysteries a profound endeavor.

Enter the beacon of modern science: Next-Generation Sequencing (NGS) technologies. With their rapid and cost-effective ability to analyze multiple genes simultaneously, they have ushered in a new era of precision in the diagnosis of IRDs. Through this lens, we delve into the latest discoveries in the field.

[1] Shulan International Medical College, Zhejiang Shuren University, Hangzhou, Zhejiang, China
[2] Key Laboratory of Epigenetics and Oncology, the Research Center for Preclinical Medicine, Southwest Medical University, Luzhou, Sichuan, China

In the first chapter, 'Identification of a novel heterozygous missense mutation in the CACNA1F gene in a Chinese family with retinitis pigmentosa by next-generation sequencing,' we venture into the intricate genetic landscape of X-linked retinitis pigmentosa (XLRP). Here, a novel mutation in the CACNA1F gene surfaces, offering fresh insights into the pathogenesis of XLRP and illuminating potential avenues for diagnosis and treatment (Zhou et al. 2015).

The second chapter, 'Genetic identification and molecular modeling characterization reveal a novel PROM1 mutation in Stargardt4-like macular dystrophy,' delves into Stargardt4-like macular dystrophy. Through genetic identification and molecular modeling, a novel PROM1 mutation is unveiled, shedding light on the genetic basis of this condition (Imani et al. 2018).

Our journey continues in the third chapter, 'A novel, homozygous nonsense variant of the CDHR1 gene in a Chinese family causes autosomal recessive retinal dystrophy by NGS-based genetic diagnosis.' Here, within a Chinese family, a homozygous nonsense variant in the CDHR1 gene emerges, unraveling the genetic foundations of autosomal recessive retinal dystrophy and hinting at innovative treatments (Fu et al. 2018).

The fourth chapter, 'A novel splicing mutation in the PRPH2 gene causes autosomal dominant retinitis pigmentosa in a Chinese pedigree,' takes us into the world of autosomal dominant retinitis pigmentosa within a Chinese pedigree. In this genetic exploration, a novel splicing mutation in the PRPH2 gene is unveiled, contributing to our understanding of the condition (Cheng et al. 2019).

In the fifth chapter, 'Novel compound heterozygous missense variants (c.G955A and c.A1822C) of CACNA2D4 likely causing autosomal recessive retinitis pigmentosa in a Chinese patient,' we delve into autosomal recessive retinitis pigmentosa. Through the discovery of novel compound heterozygous missense variants in the CACNA2D4 gene, we gain insights into the genetic underpinnings of this condition (Cheng et al. 2021).

Lastly, in the sixth chapter, 'Novel Pathogenic CERKL Variant in Iranian Familial with Inherited Retinal Dystrophies: Genotype-Phenotype Correlation,' we explore the genetic landscape of inherited retinal dystrophies within an Iranian familial context. A novel pathogenic CERKL variant emerges, shedding light on the intricate genotype-phenotype correlation within this population (Fu et al. 2023).

Beyond the confines of IRD research, the significance of effective communication skills reverberates in today's world. In an age characterized by globalization, virtual collaboration, and information abundance, the ability to convey ideas effectively transcends boundaries. It is the key to

aligning diverse cultures and backgrounds toward common goals, cutting through the cacophony of information overload, and leaving a lasting impact.

As we navigate these chapters, we invite you to embark on this voyage of genetic retinal insights. May these discoveries not only deepen our comprehension of IRDs but also serve as a testament to the relentless spirit of inquiry and scientific exploration. Together, we set sail into the uncharted waters of ocular genetics, guided by the beacon of knowledge, striving to illuminate the mysteries of these complex disorders.

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1

IDENTIFICATION OF A NOVEL HETEROZYGOUS MISSENSE MUTATION IN THE *CACNA1F* GENE IN A CHINESE FAMILY WITH RETINITIS PIGMENTOSA BY NEXT-GENERATION SEQUENCING

QI ZHOU[1,2,#], JINGLIANG CHENG[2,3,#], WEICHAN YANG[2,#], MOUSUMI TANIA[2], HUI WANG[3], MD. ASADUZZAMAN KHAN[2], CHENGXIA DUAN[1], LI ZHU[2], RUI CHEN[3], HONGBIN LV[1] AND JINJIANG FU[2]

Abstract

Retinitis pigmentosa (RP) is an inherited retinal degenerative disease, that is clinically and genetically heterogeneous, and the inheritance pattern is complex. In this study, we intended to study the possible association of certain genes with X-linked RP (XLRP) in a Chinese family. A Chinese family with RP was recruited, and a total of seven individuals were enrolled in this genetic study. Genomic DNA was isolated from peripheral leukocytes and used for the next-generation sequencing (NGS). The

[1] Department of Ophthalmology, Affiliated Hospital of Luzhou Medical College, Luzhou, Sichuan 646000, China
[2] Key Laboratory of Epigenetics and Oncology, The Research Center for Preclinical Medicine, Luzhou Medical College, Luzhou, Sichuan 646000, China
[3] Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas 77030, USA
# These authors contributed equally to this work.

affected individual presented the clinical signs of XLRP. A heterozygous missense mutation (c.1555C>T, p.R519W) was identified by NGS in exon 13 of the *CACNA1F* gene on the X chromosome and was confirmed by Sanger sequencing. It showed perfect co-segregation with the disease in the family. The mutation at this position in the *CACNA1F* gene of RP was found novel by database searching. By using NGS, we have found a novel heterozygous missense mutation (c.1555C>T, p.R519W) in the CACNA1F gene, which is probably associated with XLRP. The findings might provide new insights into the cause and diagnosis of RP and have implications for genetic counseling and clinical management in this family.

Keywords: CACNA1F, Missense mutation, Next generation sequencing, Retinitis pigmentosa, X-linked disease

1. Introduction

Retinitis pigmentosa (RP; OMIM 268000) is an inherited retinal degenerative disease that causes progressive visual loss, and often complete blindness (Ferrari et al. 2011). RP is caused by the loss of photoreceptors (rods and cones) and abnormalities in the retinal pigment epithelium (RPE) cells, leading to night blindness, development of tunnel vision, and sometimes loss of central vision (Hartong, Berson, and Dryja 2006). The frequency of RP has been reported as 1 in 3,500 people worldwide (Lu et al. 2013). Currently, there is no cure for RP and the visual prognosis is very poor. However the progression of the disease can be reduced by proper vitamin A supplementation (Berson 2007), treating the complications, and helping patients to cope with the social and psychological effects of blindness (Hamel 2006). RP is clinically and genetically heterogeneous, and the inheritance pattern cannot be easily determined because of phenotypic and genetic overlap (Méndez-Vidal et al. 2013). RP can be inherited either in an autosomal dominant (ADRP), autosomal recessive (ARRP), X-linked (XLRP), digenic or mitochondrial mode (Méndez-Vidal et al. 2013, Mansergh et al. 1999). Among RP types, XLRP is particularly severe, typically manifested as night blindness with progressive visual loss causing blindness in affected males (Webb et al. 2012). The molecular characterization and diagnosis of RP is challenging for many patients due to the high number of genes and variants among other factors involved in RP (Anasagasti et al. 2013, Fu et al. 2013). Identification of gene-specific phenotypes is essential for the accurate diagnosis and identification of the cause of frequent genetic defects

underlying heterogeneous retinal dystrophy. So far, mutations in more than 50 genes have been identified to be associated with RP (Fu et al. 2013).

Mutations in the CACNA1F gene have been found to be associated with some retinal diseases and are suspected to have a link with RP. In this study, we intended to study the possible association of certain genes with XLRP disease in a Chinese family. By using next-generation sequencing (NGS) we have found a novel heterozygous missense mutation in the CACNA1F gene probably associated with XLRP.

2. Materials and Methods

Clinical Diagnosis and Sample Collection. A Chinese proband (M067, Figure 1, III:1) suffering from RP was collected from "The Affiliated Hospital of Luzhou Medical College" in Sichuan Province, China. A total of 7 individuals were recruited in this genetic study (Figure 1). All subjects were identified at Luzhou Medical College in Sichuan, China. Full medical and family histories were taken, pedigrees were drawn, and an ophthalmologic examination was performed. Each patient underwent standard ophthalmic examination: best correct visual acuity (BCVA) according to projected Snellen charts, slit-lamp biomicroscopy, dilated indirect ophthalmoscopy, fundus photography, and visual field tests (Carl Zeiss, Germany). Retinal structure was examined by optical coherence tomography (OCT) (Carl Zeiss, Germany). Electroretinograms (ERGs) were performed (RetiPort ERG System; Roland Consult, Wiesbaden, Germany) using corneal ''ERGjet'' contact lens electrodes. The ERG protocol complied with the standards published by the International Society for Clinical Electrophysiology of Vision. The diagnosis of RP was based on the presence of night blindness, fundus findings (retinal pigmentation, vessel attenuation, and various degrees of retinal atrophy), severe loss of peripheral visual field, abnormal ERG findings (dramatic diminution in amplitudes or complete absence of response), and family history. This study received approval from the Ethics Committee of the Luzhou Medical College, China. Written informed consent was obtained from all participating individuals or their guardians. Genomic DNA was isolated from peripheral leukocytes using the previously described method (Fu, Li, and Lu 2002). As controls, 100 unrelated healthy Chinese individuals were recruited and genomic DNA was isolated.

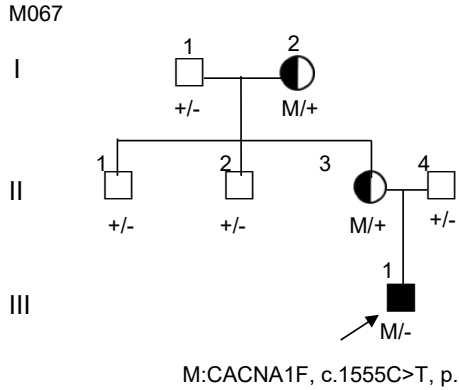

Figure 1. Pedigree M067 structure and segregation of CACNA1F mutation in a Chinese RP family. Normal individuals are shown as clear circles (females) and squares (males), affected individuals are shown as filled symbols, and carriers are shown as half-filled circles. The patient above the arrow indicates the proband. "M" indicates mutant allele of CACNA1F gene, c.1555C>T, p.R519W, "+" indicates c.C1555 normal allele of CACNA1F gene.

Design of Capture Panel. A capture panel of retinal disease genes was described previously (Wang et al. 2014). This capture reagent was manufactured by Agilent (Agilent Technologies, Santa Clara, CA). The probes covered 4405 exons and corresponding splice junctions of 163 known retinal disease genes, with a total of 1176 Mbp in the design region.

Library Preparation and Targeted Sequencing. Illumina paired-end libraries (Illumina, Inc., San Diego, CA) were generated according to the manufacturer's sample preparation protocol for genomic DNA. Briefly, 1μg of each patient's genomic DNA was sheared into fragments of approximately 300 to 500 bp. The DNA fragments were end-repaired using polynucleotide kinase and Klenow fragment (large protein fragment). The 5' ends of the DNA fragments were phosphorylated and a single adenine base was added to the 3' end. Illumina Y-shaped index adaptors were ligated to the repaired ends, then the DNA fragments were amplified by PCR for eight cycles, and fragments of 300 to 500 bp were isolated by purification of beads. The pre-capture libraries were quantified (PicoGreen fluorescence assay kit; Life Technologies, Carlsbad, CA), and their size distributions were determined by a commercial bio-analytical system (Agilent 2100 BioAnalyzer; Agilent Technologies, Santa Clara, CA). For each capture reaction, fifty pre-capture libraries (60 ng/library) were pooled together. Hybridization and wash kits (Agilent Technologies, Santa Clara, CA) were used for the washing and recovery of captured

DNA following the standard manufacturer's protocol. Captured libraries were quantified and sequenced (Illumina HiSeq 2000; Illumina, Inc.) as 100 bp paired-end reads, following the manufacturer's protocols. Illumina sequencing was performed at the BCM-FGI core.

Bioinformatic Analysis of Sequencing Results. Sequence reads were aligned to human genome reference version hg19 by using an aligner (Burrows-Wheeler Aligner, BWA version 0.5.9) (Li and Durbin 2009). After recalibration and local realignment using the Genome Analysis Toolkit (GATK version 1.0.5974) (McKenna et al. 2010), the refined sequencing results were subjected to variant calling using a toolkit (Atlas2) (Challis et al. 2012). Several common variant databases [such as the 1000 genome database [Build 20110521 and 20101123], dbSNP137, NHLBI GO Exome Sequencing Database, NIEHS Exome Sequencing Database, YanHuang Project Database (http://yh.genomics.org.cn/) and an internal control database of 997 exomes] were used to filter out common polymorphisms with an allele frequency higher than 0.5% in any of the above databases (Abecasis et al. 2010, Higbee et al. 2024). Variant annotation was performed using ANNOVAR (Wang, Li, and Hakonarson 2010) to remove synonymous mutations and RefSeq genes were used as references to coordinate the mutations. SIFT, Polyphen2, LRT, MutationTaster, and MutationAssessor were used to make functional prediction of missense variants (Ng and Henikoff 2003). The pathogenicity of novel missense mutations was predicted by dbNSFP (Liu, Jian, and Boerwinkle 2011). The Human Gene Mutation Database (HGMD) was used to search for known pathogenic mutations.

Mutation Validation and Segregation Tests. The putative mutations detected by NGS were validated by Sanger sequencing. For each identified mutation, DNA sequences were obtained from the UCSC Genome Browser. RepeatMasker was used to mask the repetitive regions (Flynn et al. 2020). Primer3 was used to design the primers at least 50 bp upstream and downstream from the mutation (Rozen and Skaletsky 2000), and sequences of primers used for the CACNA1F gene causative variation were as follows:

CACNA1F-L: TGACACCCCTTCTGCCCTTTA and

CACNA1F-R: AGAAGGAATAGGAGGCTGGGG.

After PCR amplification, the amplicons (437 bp) were sequenced on an ABI3500 sequencer (Applied Biosystems Inc., Foster City, CA, USA). The DNA materials of other family members were also sequenced by Sanger sequencing to perform a segregation test.

3. Results

Clinical Phenotypes. The affected individual (III:1, Figure 1) presented the early clinical signs of progression in RP at 1+ years old. The proband (III:1) showed typical fundus features of high myopia, with thinning of the retinal pigment epithelium and the choriocapillaris that resulted in the so-called 'tigroid' or 'tessellated' appearance of the fundus, and pale optic. The fundus features of normal individuals (II:4) and carriers (II:3) were normal (Figure 2). This observation was further confirmed by OCT imaging of the retina showing foveal atrophy of the retina and loss of the normal foveal configuration (Figure 3, a,b,c). Electrooculography (EOG) results showed the Arden Ratio abnormal. ERG results showed severely reduced and delayed a- and b-waves (Figure 3, d,e,f,g,h, i).

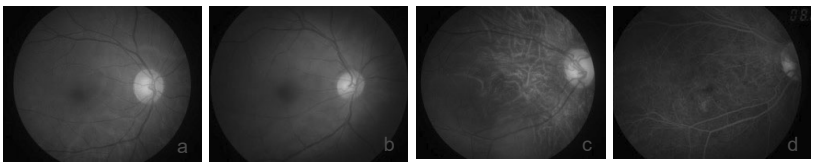

Figure 2. Fundus photograph and fundus autofluorescence of the studied individuals. a, b, and c indicate fundus photographs in II:3 (mother), II:4 (father) and III:1 (proband), respectively. d. fundus autofluorescence in III:1 (proband), showing tigroid or tessellated features and conus pattern of

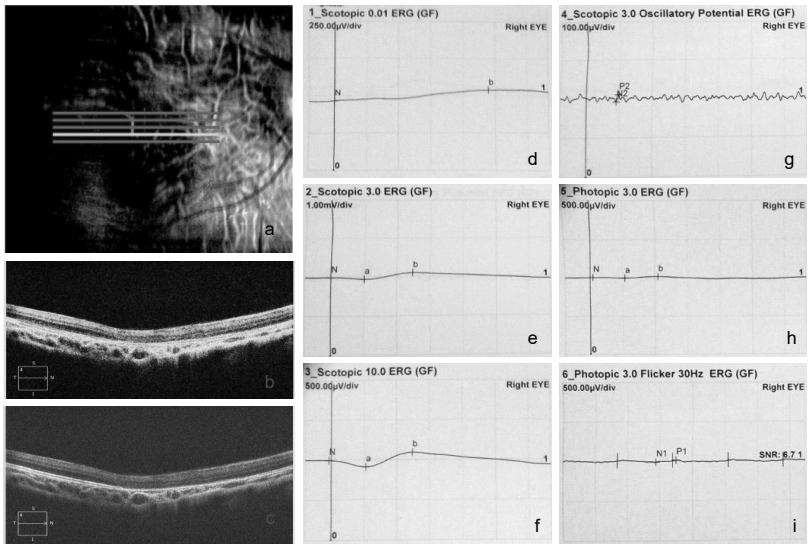

Figure 3. OCT and ERG images of the III:1. The OCT images showed atrophy of the retina at the macula foveal and loss of the fovea (a, b, and c). Full-field ERG characteristics in the right eye (d, e, f, g, h, and i).

Capture Sequencing and Data Processing of Sample. To identify causative mutations in RP patients, we performed targeted capture sequencing of 163 known retinal disease genes using a custom-designed capture panel as described in the "Design of the capture panel" section under "Materials and methods". DNA from the affected member (III:1) was selected, captured, and sequenced. An automatic variant calling, filtering, and annotation pipeline was used to process the capture sequencing data from the sample. We filtered out the common polymorphisms with >0.5 % frequency in any of the variant databases queried, including the 1000 Genome (Build 20110521 and 20101123), dbSNP137, NHLBI GO Exome Sequencing Database, NIEHS Exome Sequencing Database, YanHuang Project Database, and the internal control databases, which were considered too frequent to be pathogenic for RP. Non-pathogenic variations were filtered out by SIFT, Polyphen2, LRT, MutationTaster, MutationAssessor, and dbNSFP. Sequence variants that were not annotated in any of the above public databases were prioritized for further analysis.

Mutation Screening and Validation. A heterozygous missense mutation (c.1555C>T, p.R519W) located in exon 13 of the *CACNA1F* gene (GenBank accession number: NM_005183, NP_005174) on X

chromosome from the proband was detected, and it was confirmed by Sanger sequencing (Figure 1 and Figure 4c), while other known disease-causing gene mutations for RP were excluded. The mutation was not identified in 100 healthy controls. The same heterozygous mutation was subsequently identified in one female carrier (II:3) of this family (Figure 4a), which indicated that the proband (III:1) was inherited from his mother (II:3). Further study showed that his grandmother also has the same mutation, revealed by Sanger sequencing (data not shown), suggesting this variant (III:1) is inherited from his grandmother (I:2), leading to the pathogenic mutation in offspring male, and showed perfect co-segregation with the disease in the family. The variant was searched in the HGMD and found as a novel mutation, as it was not previously reported. The father of proband (II:4) and other members of the family are normal with wild type of *CACNA1F* gene (Figure 1, Figure 4b and data not shown).

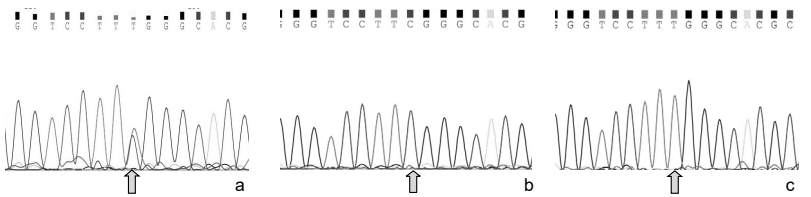

Figure 4 Mutation analysis of CACNA1F gene performed by direct sequencing on genomic DNA. a, b, and c indicate the sequencing results in II:3 (heterozygous type), II:4 (wild type), and III:1 (mutant type), respectively. The arrow indicates the mutation at the nucleotide position c.1555C>T in the CACNA1F gene.

4. Discussion

Genetic sequencing is an important technique that is used to identify genes responsible for a particular phenotype of an organism. It provides important information on genetic function as well as molecular mechanisms. It can also be used to diagnose and potentially develop treatments for genetic diseases (Patton and Zon 2001). However, molecular analysis by using conventional methods such as Sanger sequencing and arrayed primer extension (APEX) is challenging and cannot be offered routinely. These methods are time-consuming and expensive. NGS techniques provide a new approach for a rapid and more efficient way to find disease-causing mutations in affected individuals, and to discover new disease genes (Daiger et al. 2010). In our study, we have applied NGS to find a *CACNA1F* gene mutation causing XLRP in a Chinese family.

The *CACNA1F* gene (OMIM 300110) is located on chromosome Xp11.23 and consists of 48 exons spanning a genomic region of 28 kb. CACNA1F gene encodes a multipass transmembrane protein of 1,977 amino acids which is homologous to L-type calcium channel alpha1-subunits (the CaV1.4 channel) and mediates the influx of calcium ions into the cell (Fisher et al. 1997). CACNA1F is expressed in the inner and outer nuclear layers and the ganglion cell layer of the retina (Naylor, Rancourt, and Bech-Hansen 2000). CACNA1F protein contains four homologous domains (I-IV) and each domain is comprised of six transmembrane helical segments (S1-S6), and forms the pore that permits ions to flow down the electrochemical gradient from the extracellular milieu into the cytoplasm (Yang et al. 1993). Mutation in CACNA1F has been reported to be associated with X-linked congenital stationary night blindness (CSNB), Cone-rod dystrophy-3 (CORDX3), and Aland Island eye disease (AIED) (Bech-Hansen et al. 1998, Jalkanen et al. 2006). In 20 families with incomplete CSNB, Bech-Hansen et al. (Bech-Hansen et al. 1998) identified six different mutations that were all predicted to cause premature protein truncation, and indicated that CACNA1F mutations trigger a novel mechanism of defective retinal neurotransmission in CSNB patients. In 2 affected members of a French family with the incomplete type of X-linked congenital stationary night blindness (CSNB2), Jacobi et al. (Jacobi et al. 2003) identified a 1bp deletion (C) at nucleotide 4548 in the CACNA1F, resulting in a frameshift with a predicted premature termination at codon 1524. Wang et al. (Wang et al. 2012) found a novel mutation c.[1984_1986delCTC;3001G>A], p.[L662del; G1001R] in CACNA1F in one patient with CSNB. In a large Finnish family with CORDX3, Jalkanen et al. (Jalkanen et al. 2006) identified a splice site mutation in the CACNA1F gene which causes premature termination and deletions of the encoded protein, Cav1.4 alpha1 subunit. Hauke et al. (Hauke et al. 2013) analyzed a large family of German origin with CORDX and identified a novel large intragenic in-frame deletion encompassing exons 18 to 26 within the CACNA1F gene. In affected members with AIED, Jalkanen et al. (Jalkanen et al. 2007) identified a novel deletion covering exon 30 and portions of flanking introns of the CACNA1F gene, and this in-frame deletion mutation was predicted to cause a deletion of a trans-membrane segment and an altered membrane topology of the encoded alpha1-subunit of the Cav1.4 calcium channels.

While the mutation in the CACNA1F gene is mostly associated with the pathogenic alterations of CSNB, CORDX3, and AIED, the phenotype observed in this study is most precisely described as RP-like. The OCT and fundus autofluorescence images demonstrated the macular degeneration of

the patient in this study, which is commonly found in RP patients, whereas patients with CSNB show qualitatively normal OCT and Fundus fluorescein angiography (FFA) images (Baumann et al. 2004). Furthermore, EOG results showed an abnormal Arden Ratio, and ERG results also showed severely reduced and delayed a- and b-waves, which are different from CSNB, CORDX3, and AIED. Thus this study indicates that yet another phenotype, XLRP, is also caused by a mutation in the CACNA1F gene. Here, we have identified a single nucleotide change c.1555C>T in exon 13 of the CACNA1F gene leading to the substitution of arginine by tryptophan (p.R519W) in an individual affected with XLRP. Taken together, the same gene mutations leading to different syndromes or diseases with different phenotypes tell us the importance of gene diagnosis, genetic counseling, and clinical management, such as personalized medicine in our medical genetic practice.

The same heterozygous mutation was identified in normal females (I:2, II:3) of this family (Figure 4a), indicating the proband (III:1) inherited the mutation from his grandmother (I:2), and heterozygous females were carriers with X chromosome-linked recessive, which is consistent with previous report (Bech-Hansen et al. 1998).

CACNA1F is important for the functional assembly and/or maintenance and synaptic functions of photoreceptor ribbon synapses. It helps to release neurotransmitters from nerve terminals initiated by calcium influx through presynaptic voltage-dependent calcium channels. It plays a crucial role in the regulation of tonic glutamate release from synaptic terminals of ribbon synapses in retinal photoreceptors and bipolar cells (Baumann et al. 2004). Mutations in CACNA1F cause abnormal electrophysiological response and visual impairments consistent with a retinal neurotransmission defect. Mutation in this gene also causes the developmental failure or loss of photoreceptor ribbon synapses and consequently profound deficits in synaptic transmission from photoreceptor to second-order retinal neurons (Mansergh et al. 2005). Thus mutation (c.1555C>T, p.R519W) in exon 13 of CACNA1F may cause functional abnormality of CACNA1F protein, which is possibly associated with RP development.

5. Conclusions

In this study, we have identified a novel heterozygous missense mutation in the CACNA1F gene (c.1555C>T) in a Chinese RP patient. Currently, the clinical diagnosis of RP is based on the presence of constricted visual fields, night blindness, decreased visual acuity, dark pigmentation in the bone spicules, progressive retinal atrophy, attenuated retinal vessels, and

fine-pigmented vitreous cells, and reduced or absent electroretinogram. Also, the progress of RP is not consistent; some patients exhibit symptoms from infancy while others may not notice symptoms until later in life. Identification of the responsible gene mutation earlier may aid the diagnostic feasibility of RP. Also, this can help in therapeutic research on RP in time. Identification of this mutation (c.1555C>T) in the CACNA1F gene may have a significant contribution to the RP diagnosis, genetic counseling, and clinical management, for example, future treatment strategy in this family.

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