

# Genetic analysis of Chinese families reveals a novel truncation allele of the retinitis pigmentosa GTPase regulator gene

Fang Hu<sup>1</sup>, Xiang-Yun Zeng<sup>1</sup>, Lin-Lin Liu<sup>1</sup>, Yao-Ling Luo<sup>1</sup>, Yi-Ping Jiang<sup>1</sup>, Hui Wang<sup>1</sup>, Jing Xie<sup>1</sup>, Cheng-Quan Hu<sup>1</sup>, Lin Gan<sup>2</sup>, Liang Huang<sup>1,2</sup>

<sup>1</sup>Department of Ophthalmology, the First Affiliated Hospital, Gannan Medical University, Ganzhou 341000, Jiangxi Province, China

<sup>2</sup>Flaum Eye Institute and Department of Ophthalmology, School of Medicine and Dentistry, University of Rochester, New York 14642, USA

**Co-first authors:** Fang Hu and Xiang-Yun Zeng

**Correspondence to:** Liang Huang and Lin Gan. Department of Ophthalmology, the First Affiliated Hospital, Gannan Medical University, Ganzhou 341000, Jiangxi Province, China. hliang169@163.com; lin\_gan@urmc.rochester.edu  
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## Abstract

• **AIM:** To make comprehensive molecular diagnosis for retinitis pigmentosa (RP) patients in a consanguineous Han Chinese family using next generation sequencing based Capture-NGS screen technology.

• **METHODS:** A five-generation Han Chinese family diagnosed as non-syndromic X-linked recessive RP (XLRP) was recruited, including four affected males, four obligate female carriers and eleven unaffected family members. Capture-NGS was performed using a custom designed capture panel covers 163 known retinal disease genes including 47 RP genes, followed by the validation of detected mutation using Sanger sequencing in all recruited family members.

• **RESULTS:** Capture-NGS in one affected 47-year-old male reveals a novel mutation, c.2417\_2418insG:p.E806fs, in exon ORF15 of RP GTPase regulator (*RPGR*) gene results in a frameshift change that results in a premature stop codon and a truncated protein product. The mutation was further validated in three of four affected males and two of four female carriers but not in the other unaffected family members.

• **CONCLUSION:** We have identified a novel mutation, c.2417\_2418insG:p.E806fs, in a Han Chinese family with XLRP. Our findings expand the mutation spectrum of *RPGR* and the phenotypic spectrum of XLRP in Han Chinese families, and confirms Capture-NGS could be an

**effective and economic approach for the comprehensive molecular diagnosis of RP.**

• **KEYWORDS:** retinitis pigmentosa GTPase regulator; retinitis pigmentosa; next-generation sequencing; genetic diagnosis

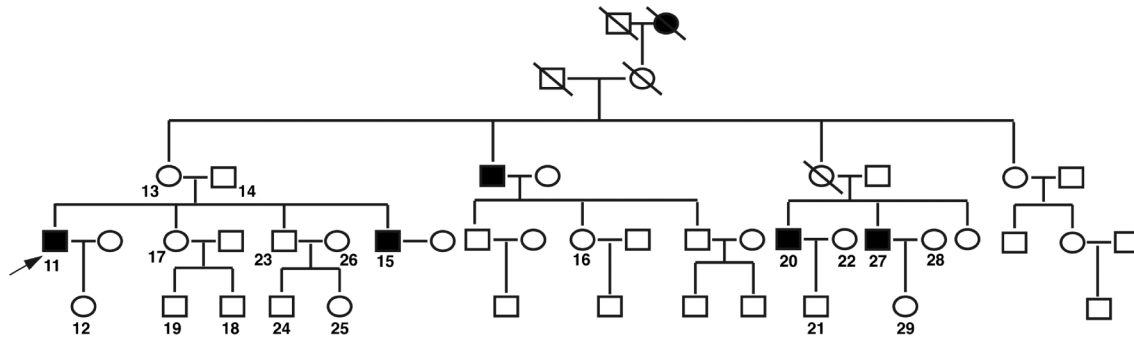
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## INTRODUCTION

Retinitis pigmentosa (RP), the most common inherited retinal degeneration disease, affects millions of people worldwide<sup>[1]</sup>. RP can be categorized into dominant, recessive and X-linked forms based on the inheritance, as well as a portion of isolated cases<sup>[2,3]</sup>. RP is a highly variable disorder, the typical symptoms of RP initiates from night blindness and dark adaption defects, followed by the loss of mid-peripheral vision field due to the dysfunction and loss of rod photoreceptors. As the disease progresses, patients lose their peripheral vision and then central vision subsequently and ultimately develop tubular visual field or complete blindness around the age of 60<sup>[3]</sup>. The diagnosis of RP mainly based on the characteristic fundus changes including attenuation of the retinal arterioles, bone spicule-like pigment deposit and waxy yellow appearance of the optic disc, as well as the vision field defects and abnormal electroretinograms (ERG) recordings<sup>[3,4]</sup>. Currently there is no effective treatment for RP and clinical diagnosed RP cases are most in advanced stages, since the inconspicuous early symptoms such as night blindness and vision field loss may not be noticed until late stages<sup>[3]</sup>.

Genetic diagnosis provides an effective approach for the diagnosis of affected RP patients and the risk evaluation of their descendants. Currently, there are over 50 genes causing non-syndromic RP, in which 23 genes account for autosomal-dominant RP, 36 genes lead to recessive RP, and 3 genes cause X-linked recessive RP (XLRP)<sup>[5]</sup>. However, most of these causative genes and mutations are reported in



**Figure 1** Pedigree of a Han Chinese family with XLRP. Squares and circles are males and females respectively. Filled squares are affected males. A diagonal line through a symbol indicates a deceased family member. The arrow indicates the proband.

European Caucasians with only a few associated with Chinese RP families [6–10]. RP is highly heterogeneous as not only the causative genes and mutations can vary among different ethnic groups but also the same mutation may lead to different outcomes among the members in the same family. Since reported mutation spectra of RP genes in Chinese population are dramatically different from those among European Caucasians, identification of causative genes and mutations of RP in Chinese population is of great importance [11–15]. Now there are at least 340 000 RP patients in China based on the reported prevalence in the 80s, and the high heterogeneity of RP makes it challenging to make a comprehensive genetic diagnosis using the traditional Sanger sequencing due to the high cost of reagent and labor [16]. Capture-Next generation sequencing (Capture-NGS), a method that coupled NGS with DNA capture technology, provides an effective alternative for its ability to parallel sequence a large panel of known retinal disease genes rapidly and economically. In this study, we report a novel mutation detected by Capture-NGS in a five-generation Han Chinese family with diagnosis of XLRP. In this family, affected males exhibit typical early onset and severe RP phenotypes at the time of diagnosis, while female carriers reveal normal ophthalmic examination results. Capture-NGS in one affected male identified a novel mutation, c.2417\_2418insG:p.E806fs, in the exon ORF15 of RP GTPase regulator (*RPGR*) gene. The mutation was further validated in three of four affected males and two of four female carriers by Sanger sequencing. This mutation results in a premature stop codon due to the frameshift change and ultimately leads to a truncated protein product.

## SUBJECTS AND METHODS

**Participants and Clinical Diagnosis** A five-generation Han Chinese consanguineous family with non-syndromic XLRP was recruited. All subjects were identified at the First Affiliated Hospital of Gannan Medical University (GMU, Ganzhou, Jiangxi, China). This research was approved by the ethical review board of GMU in accordance with the Declaration of Helsinki and the Guidance on Sample Collection of Human Genetic Diseases by the Ministry of

Public Health of China. Written informed consent was obtained from all patients and their relatives participating in this study. The individual in this manuscript has given written informed consent (as outlined in PLOS consent form) to publish these case details. All recruited family members underwent comprehensive ophthalmologic examination including best correct visual acuity (BCVA), slit-lamp biomicroscopy, fundus photography, visual field tests and electroretinograms. Genomic DNA was isolated from peripheral blood using a commercial kit (TIANamp Blood DNA kit, Cat#DP318, TIANGEN, Beijing, China) according to the manufacturer's protocol. The diagnosis of RP was based on the family history and presence of night blindness, retinal pigmentation, vessel attenuation, visual field loss and abnormal ERG recordings.

**Genetic Analysis** Capture-NGS was performed as previously described [17,18]. Briefly, a capture panel that covers 163 known retinal disease genes including 47 RP genes was designed to synthesize the hybridization probes. Patient genomic DNA sample (1  $\mu$ g) was sheared into 300–500 bp fragments, followed by the end-repair and insertion of an extra "adenine" base to the 3' end. Eight cycles of PCR amplification were applied to each sample after ligation of Illumina Y-shape index adapters to the ends of the DNA fragments, followed by the quantification of DNA libraries using the PicoGreen assay (Invitrogen). Fifty precapture libraries were pooled together for one capture reaction. After Hybridization and washing, captured libraries were sequenced as 100-bp paired-end reads on the Illumina HiSeq 2000 sequencer. Bioinformatics analysis of sequencing results was performed as previously described for variants calling, filtering and annotation [17]. To validate the mutations detected by capture-NGS, Sanger sequencing was performed as previously described on Applied BioSystems (ABI) 3730x1 capillary sequencer [18].

## RESULTS

**Clinical Evaluation** There are 46 members in this five-generation pedigree, which was compiled and revealed X-linked recessive inheritance (Figure 1). Four individuals in the family passed away, among which one female had night

**Table 1 Clinical findings of all affected males**

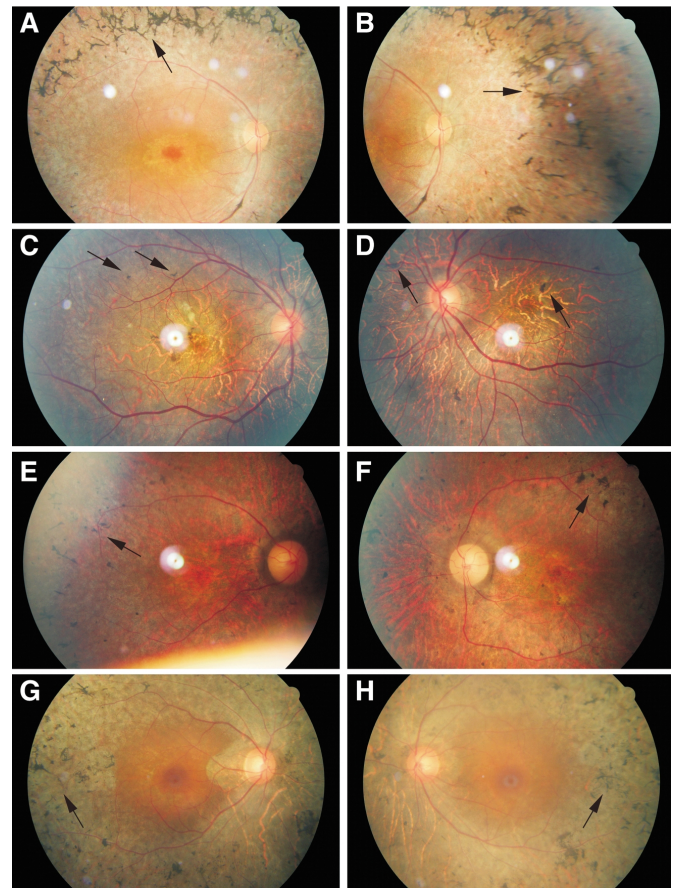
Patient No.	Age of onset (a)	Age of diagnosis (a)	BCVA OD/OS	Fundus change	Visual field	ERG recordings
11	13	53	HM/HM	Light yellow appearance of optic disk; pigment deposit; retinal vessel attenuation.	Tubular visual field	Reduced
15	15	40	0.02/0.02	Tigroid fundus; light yellow appearance of optic disk; pigment deposit; Retinal vessel attenuation.	Tubular visual field	Reduced
20	12	42	0.04/0.04	Light yellow appearance of optic disk; pigment deposit; retinal vessel attenuation.	Tubular visual field	Reduced
27	13	47	0.01/0.01	Light yellow appearance of optic disk; pigment deposit; retinal vessel attenuation.	Tubular visual field	Reduced

BCVA: Best correct visual acuity; OD: Right eye; OS: Left eye; HM: Hand motion; ERG: Electroretinograms.

blindness and visual impairment from mid-age as described by her grandchildren. Nineteen family members agreed to participate in our study including four affected males, four obligate female carriers and eleven unaffected family members. All recruited family members were subjected to ophthalmic examination by the Ophthalmologists in the Department of Ophthalmology, the First Affiliated Hospital of GMU. Four affected males were diagnosed as XLRP.

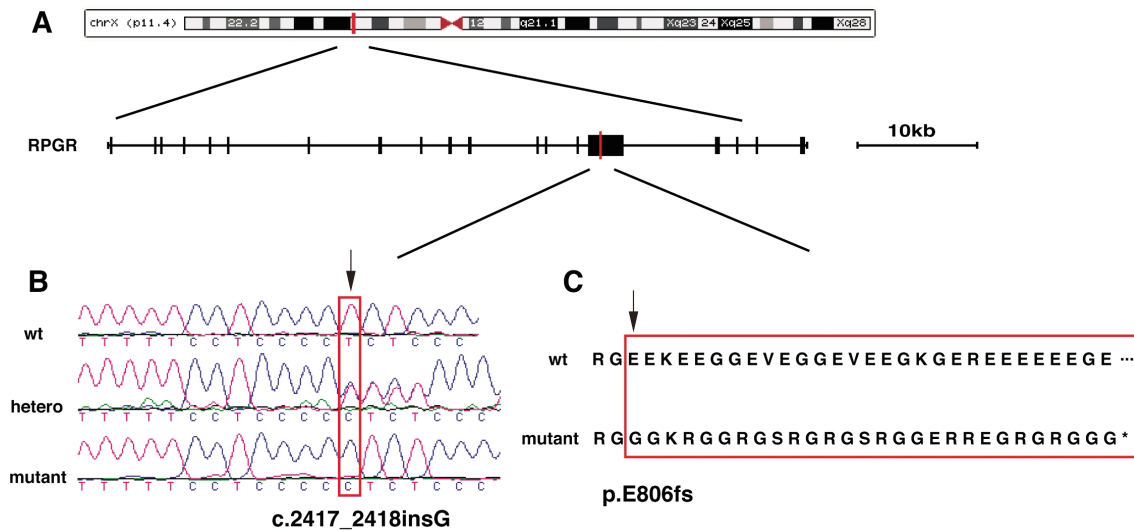
The phenotypes of all affected males were described in details in Table 1. Patient No.11, the index patient, is a 53-year-old male who had an onset of night blindness at age of 13. The proband's brother (No.15) and two younger cousins (No.20 and 27) had their onset of night blindness at the age of 15, 12 and 13, respectively. All affected males advanced rapidly into severe RP phenotypes. At the time of diagnosis, the BCVA of four affected males ranged from hand motion (HM) to 0.04. Fundus examination revealed vessel attenuation and pigment deposit in the mid-peripheral retina in all affected males (Figure 2). Patients No.11, 15 and 20 showed light yellow appearance of the optic disk and patient No.20 and 27 showed tigroid fundus. Octopus perimetry showed tunnel vision in all affected males, as well as both rod and cone ERG amplitudes were below the normal thresholds. The other six unaffected male family members displayed no abnormality in ophthalmic examinations. All female carriers in this pedigree showed normal visual field with BCVA 20/20 in both eyes, as well as the normal ERG responses. Fundus observation of female carriers found no spicule formation in the peripheral retina.

**Genetic Analysis** One affected male (No.27) was screened by Capture-NGS. Sequence reads were mapped to hg19 human genome reference using Burrows-Wheeler Aligner (BWA version 0.5.9) and variants were called using Atlas toolkit [19,20]. Raw variants were further filtered against several variants database including 1000 genome, dbSNP, ESP6500 and 997 internal control database using a cutoff of 0.5% [17,21]. The remaining rare variants were annotated using ANNOVAR software [22]. Finally a novel mutation, c. 2417\_2418insG : p. E806fs, in exon ORF15 of *RPGR* was identified. The exon ORF15 was then PCR amplified using primers previously described [23] and validated in all



**Figure 2 Representative fundus images of affected males** All affected males in this family exhibit characteristic fundus changes. Characteristic fundus image of patient No.11 (A-B), No.15 (C-D), No.20 (E-F) and No.27 (G-H) are present. All affected males revealed pigment deposit (arrows), retinal vessel attenuation and light yellow appearance of optic disk. Patient No.15 showed a tigroid fundus.

recruited family members using Sanger sequencing. The novel mutation was found in three of four affected males (No.15, 20 and 27) and two of four female carriers (No.13 and 16), but not in other unaffected family members. The insertion of guanine between codons 2417 and 2418 results in the significant change of translational products and brings in a premature stop codon (Figure 3). The mutation in one affected male (No.11) and two female carriers (No.12 and 29) could not be cleanly validated by Sanger sequencing due to the high repetitive feature of exon ORF15.



**Figure 3 Identification of c.2417\_2418insG:p.E806fs mutation in the RPGR gene** A: Structure of RPGR gene and its location in the X chromosome. Red line indicates the location of novel mutation in exon ORF15; B: Sanger sequencing reveals the insertion of Guanine between codons 2417 and 2418 (arrow); C: The frameshift change (p.E806fs) (arrow) caused by the c.2417\_2418insG mutation results in a premature stop codon and a truncated protein product.

**DISCUSSION**

To date, more than 3000 mutations have been reported in identified RP genes [5]. Due to the limited resources, only a small set of genes have been accessed by Sanger sequencing, the traditional main approach for RP molecular diagnosis. With the emergence of Next-generation Sequencing and DNA capture technology, Capture-NGS provides a promising alternative for the molecular diagnosis of RP with an approximately diagnostic rate above 50% [17,24-26]. In this study, we reported a novel mutation, c.2417\_2418insG:p.E806fs, in exon ORF15 of *RPGR* by Capture-NGS technology in a Han Chinese family. After screening of one affected male using Capture-NGS, the mutation was identified and then further validated in three of four affected males and two of four female carriers but not in unaffected family members by Sanger sequencing.

XLRP is the most severe form of RP for its early age of onset and rapid progression. Affected males always exhibit night blindness and severely abnormal ERG recordings in early adolescence, followed by the profound loss of peripheral visual fields in the second decade and ultimately tubular visual field or complete blindness in the fourth or fifth decade. Affected males usually exhibit much more severe phenotypes than the affected females. While female carriers may experience variable symptoms and visual impairment in the mid-age [3,4,27,28]. In this study, all four affected males suffered severe RP phenotypes that initiated around the age of 12 to 15 and progressed rapidly. At the time of diagnosis, all affected males showed tubular visual field with barely left central vision, accompany with the severe abnormal ERG recordings (Table 1 and Figure 2). Whereas, there is no ophthalmic abnormality observed in four female carriers.

Most XLRP cases are reported caused by the mutations in *RPGR* or retinitis pigmentosa 2 gene (*RP2*), which covers approximately up to 75% and 25% of XLRP cases respectively [29-32]. Meanwhile, *RPGR* mutations are suggested be responsible for up to 20% of all RP cases, higher than any other single genetic locus [33]. *RP2* is composed of five exons encoding a ubiquitously expressed polypeptide of 350 amino acids [34]. *RP2* suggested being a membrane- and/or tubulin-associated chaperone or signaling protein and most causative mutations are localized in its cofactor C homology domain in the N-terminus [33]. *RPGR* locates at chromosome Xp21.1. It contains 19 originally identified exons and is thought to encode a cilia-centrosomal protein essential for cilia function and protein trafficking along the photoreceptor cilium [35-38]. Alterations of *RPGR* protein are believed to interrupt the normal protein interactions and leads to photoreceptor degeneration. A number of alternatively spliced isoforms of *RPGR* have been identified including *RPGR*<sup>ex1-19</sup> and *RPGR*<sup>ORF15</sup> [39-41]. *RPGR*<sup>ex1-19</sup> was derived from initially identified exon 1 to 19 and mutations within *RPGR*<sup>ex1-19</sup> was found in only up to 20% of the families with XLRP [8]. *RPGR*<sup>ORF15</sup>, which preferentially expressed in retina, was derived from exon 1 to 14 of *RPGR*<sup>ex1-19</sup> and a large terminal exon ORF15 that encodes a 567 amino acid polypeptide with a repetitive domain rich in glutamic acid residues [41]. Exon ORF15 is regarded as a mutation hot spot, in which clustered over 60% mutations of *RPGR*<sup>ORF15</sup> in XLRP families [33,40,43-44]. Most mutations in exon ORF15 are frameshift or missense mutations, both of which lead to early translational termination and result in truncated protein products [45,46]. In this study, we have identified a novel mutation, c.2417\_2418insG:p.E806fs, located in the highly

repetitive region of exon ORF15. The mutation leads to a frameshift change and results in a truncated protein product lost due to the premature stop codon. To date, more than 100 mutations have been identified within exon ORF15, but only a few are reported in Han Chinese population, and the precise role of ORF15 remains unknown<sup>[6,9,10,46-48]</sup>.

In conclusion, our study is the first attempt of comprehensive genetic diagnosis of XLRP in Chinese population using Capture-NGS technology. The identification of novel mutation, c.2417\_2418insG:p.E806fs, in exon ORF15 of *RPGR* associated with XLRP in this Han Chinese family expands the mutation spectrum of *RPGR* and the phenotypic spectrum of XLRP in Chinese families. Our finding is important for exploring the genotype-phenotype correlation and for elucidating the distribution and contribution of the ORF15 mutations and will be useful for genetic consultation and diagnosis. Further investigation on the mechanism through which this mutation acts will require further study of *RPGR* protein in both normal and mutant retinas.

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