Research Article

A novel missense variant c.G644A (p.G215E) of the RPGR gene in a Chinese family causes X-linked retinitis pigmentosa

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The mutations in patients with X-linked retinitis pigmentosa (XLRP) have not been well described in the Chinese population. In the present study, a five-generation Chinese retinitis pigmentosa (RP) family was recruited; targeted next-generation sequencing (TGS) was used to identify causative genes and Sanger sequencing for co-segregation. RNA-seq data analysis and reverse transcriptional-polymerase chain reaction (RT-PCR) were applied to investigate gene expression patterns of RP GTPase regulator (RPGR) in human and Rpgr in mouse. A novel, hemizygous, deleterious and missense variant: c.G644A (p.G215E) in the RPGR gene (NM_000328.2) exon 7 of X-chromosome was identified in the proband, which was co-segregated with the clinical phenotypes in this family. RNA-seq data showed that RPGR is ubiquitously expressed in 27 human tissues with testis in highest, but no yet tissues data. Then the expressions for Rpgr mRNA in mice including eye tissues were conducted and showed that Rpgr transcript is ubiquitously expressed very highly in retina and testis, and highly in other eye tissues including lens, sclera, and cornea; and expressed highly in the six different developmental times of retinal tissue. Ubiquitous expression in different tissues from eye and very high expression in the retina indicated that RPGR plays a vital role in eye functions, particularly in retina. In conclusion, our study is the first to indicate that the novel missense variant c.G644A (p.G215E) in the RPGR gene might be the disease-causing mutation in this XLRP family, expanding mutation spectrum. These findings facilitate better understanding of the molecular pathogenesis of this disease; provide new insights for genetic counseling and healthcare.

Introduction

Retinitis pigmentosa (RP) (OMIM 268000) is a large genetic heterogeneity of inherited ocular diseases that results in a progressive retinal degeneration affecting 1 in 3000–5000 people [1–3]. Inheritance patterns in RP include autosomal recessive (arRP), autosomal dominant (adRP), and X-linked inheritance (xLRP) [4]. XLRP is a severe form of inherited retinal degeneration that primarily affects the rod photoreceptors with an early onset of night blindness and progressive reduction in the visual field, often causing patients to become legally blind by the age of 30–40 years [5,6].

Hartong et al. [7] estimated that 5–15% of RP is inherited through a model of X linkage. RP2 (OMIM 312600) is caused by mutation in the RP2 gene (OMIM 300757). RP23 (OMIM 300424) is caused by mutation in the ODF1 gene (OMIM 300170). Both RP3 (OMIM 300029) and RP15 (OMIM 300029) is caused by mutation in the RP GTPase regulator (RPGR) gene (OMIM 312610) [8–10]. The RPGR gene was also known as COD1, CORDX1, CRD, orf15, PCDX, RP3, RP15, or XLRP3. Inheritance of RP3 was...
Figure 1. M172 pedigree with xRIP in the proband (IV:9)

Family numbers and disease-causing mutations are presented. Normal individuals are shown as clear circles (females) and squares (males), the affected individual is shown as filled symbol (circles for females and squares for males). The patient above the arrow indicates as a proband (IV: 9), with the hemizygous, missense variant of the RPGR gene: NM_000328.2:c.G644A (p.G215E).

Described as X-linked recessive, while in RP15, both males and carrier females affected presented a wide spectrum of clinical features ranging from asymptomatic to severe RP. RP6 (OMIM 312612) has been mapped to chromosome Xp21.3-p21.2; RP24 (OMIM 300155), to Xq26-q27; and RP34 (OMIM 300605), to Xq28. But genes responsible for RP6, RP24 and RP34 have not been identified yet.

Mutation in the RPGR gene is believed to account for approximately 70% of xRIP and an estimated 11% of all RP patients [11]. In addition, RPGR mutations also caused syndromic RP. Dry et al. (1999) [12] identified an IVS5+1G-T splice site mutation in the RPGR gene in an xRIP family with recurrent respiratory infections. Furthermore, Ayyagari et al. (2002) [13] described a family in which ten males had primarily macular atrophy causing progressive loss of visual acuity with minimal peripheral visual impairment. One additional male showed extensive macular degeneration plus peripheral loss of retinal pigment epithelium and choriocapillaries. Kurata et al. (2019) [14] investigated xRIP from 12 Japanese unrelated families harboring mutations of RPGR or RP2 identified 11 pathogenic mutations with 6 and 5 mutations in RPGR and RP2, respectively, suggesting the possibility that RP2 mutations are highly prevalent in Japanese.

Although mutations in the RPGR gene caused xRIP of Western European ancestry and Japan, RPGR with xRIP and genotype-phenotype correlations in the Chinese population have not been well described. Here, we applied targeted next-generation sequencing (TGS) technology to identify a novel, missense mutation of RPGR gene in a Chinese family with xRIP, expanding its spectrum of mutations.

Materials and methods

Pedigree, clinical assessment, sample collection, and DNA extraction; and ethical statement

This pedigree consisted of a proband (Figure 1, pedigree IV: 9, arrow), five generations and 32-related family members (Figure 1). For clinical diagnosis, a detailed clinical history and ophthalmic examinations were performed in proband, as described in previous studies [4,15]. Fresh peripheral blood was taken and human genomic DNA (gDNA) was isolated using our standard phenol/chloroform method from blood leukocytes of the proband and pedigree members who were accessible [16,17]. Blood samples from 100 RP-unrelated, ethnically matched, and healthy control volunteers were collected. The research had been carried out in accordance with the World Medical Association Declaration of Helsinki, Ethical Committees approval by the Southwest Medical University, and written informed consent was obtained from all subjects.

TGS

The panels of 195 genes for TGS analyses on the DNA sample from the proband (M172) were designed in the Illumina paired-end libraries [18,19]. The capture Agilent probes were used in previously published studies [18–20].
Table 1 The sequences of PCR primers and PCR product sizes

| Primer name   | Left primer   | Sequence (5′–3′)   | Right primer      | Sequence (5′–3′)   | Size | °C |
|---------------|---------------|--------------------|-------------------|--------------------|------|----|
| RPGR-M172     | RPGR-M172L    | Acactgcaggtttggaga| RPGR-M172R        | Gaagcagggacagcaagc| 544  | 60 |
| RT-rpgr       | RT-rpgr-nL    | Gcagcacactaggcaca  | RT-rpgr-nR       | Aggtgtgctcctcagac| 374  | 60 |
| RT-b-actin-m  | RT-b-actin-mL | tgtaccaactggaagcag| RT-b-actin-mL     | tctcaactggtgtgaag | 392  | 60 |

Isolated proband gDNA was randomly sonicated into 300–500 bp fragments, phosphorylated, hybridized, and sequenced on Illumina HiSeq 2000 following the manufacturer’s protocols [20,21]. Then, paired-end sequencing Illumina reads were aligned to the human hg19 reference genome. SNPs and INDELs variations were refined using a toolkit Atlas-SNP2 and Atlas-Indel2 (GATK version 1.0.5974) [22]. The pathogenic variants in all candidate genes were applied to online control databases, CHARGE consortium, 1000 Genome Project, ANNOVAR, ESP-6500, and Exome Aggregation Consortium (ExAC) databases [23].

Primer design, PCR amplification, and Sanger sequencing

For putative mutation verification and co-segregation analysis, PCR amplification and Sanger sequencing of variant was applied to gDNA of all the available individuals [21,24]. Online Primer 3 (http://primer3.ut.ee/) was used to design the primers at least 50 bp upstream and downstream from the mutation. Primer pair (RPGR-M172) was designed by gDNA sequences containing identified RPGR mutation: NM_000328:exon7:c.G644A (Table 1). A product with 544 bp was amplified using gDNA as the template. Then, the PCR products were sequenced on an ABI-3500DX sequencer through the specific primer RPGR-M172L in Table 1. Unrelated controls were sequenced using aforementioned primers of RPGR-M172 (L+R).

Protein structure and bioinformatics analysis

The functional classification of proteins and comparison in different species for RPGR was performed through an online NCBI program (https://www.ncbi.nlm.nih.gov/homologene?Db=homologene&Cmd=Retrieve&list_uids=55459) [15,25]. The RPGR mRNA expression profiles in human normal tissue samples from 95 human individuals representing 27 different tissues were also obtained by RNA-sequencing to determine tissue specificity through an online NCBI database (https://www.ncbi.nlm.nih.gov/gene/6103/?report=expression) [26].

RNA isolation and revere transcriptional-polymerase chain reaction

RNA isolation from mice tissues and semi-quantitative revere transcriptional-polymerase chain reaction (RT-PCR) was performed according to our previously reported standard protocols [4,24]; the β-actin gene of mouse served as an internal control. RT-PCR primer pair, RT-rpgr, targeting the mouse Rpgr gene (GenBank No.: NM_001177950.1) which spanned three introns with 374 bp, was also designed and synthesized (Table 1). Primer pair RT-b-actin-m for mouse β-actin gene with 392 bp was described previously [4]. We performed PCR amplification for the Rpgr gene with 30 cycles and the β-actin gene with 25 cycles, respectively. Each assay was performed thrice.

Results

Pedigree recruitment and clinical characteristics

The proband (Figure 1, IV: 9) was a 39-year-old Chinese male with clinical signs of progression of blindness characteristic of RP. The fundus photographs (FP) and fundus fluorescent photographs (FFP) of the proband in both eyes are shown in the Figure 2. The images of FP displayed attenuated vessels, retinal pigment epithelium (RPE) atrophy (Figure 2,A,B). FFP results showed a hyperautofluorescent ring surrounding a central area of hypoautofluorescence and an atrophic macular region (Figure 2,C,D). Spectral domain-optical coherence tomography (SD-OCT) of proband macula showed a significant reduction in average macular thickness in both eyes (Figure 2,E,F). As a result, the proband in our study was presented with typical RP. The family included 32 members and five generations, all others, except his mother, maternal grandfather, and great grandmother showed similar symptoms, were normal (Figure 1). The pedigree had no consanguineous marriage history based on their genetic and pedigree analyses.
Figure 2. Representative FP and SD-OCT of proband

(A, B) Color FP of proband (right and left, respectively). (C, D). FFP of proband (right and left, respectively). SD-OCT of proband macula for quadrant measuring retinal thickness (between the inner limiting membrane and the retinal pigment epithelium: ILM-RPE) at the right eye (OD, (E)) and the left eye (OS, (F)). Top right: Quadrant measurements of retinal thickness in the eye (between the inner limiting membrane and the retinal pigment epithelium: ILM-RPE). Note the thickness reductions in the macula. Bottom right: The average macular thickness. These are represented in colors that correspond to the normal distribution of macular thickness values. Note that the average macular thickness (cube average thickness) is indicated in the bottom right chart (as well as all of the macular quadrant thicknesses) are represented in red (red denotes values <1% of what would be expected compared with an age-matched reference population), indicating a significant reduction in average macular thickness in both eyes. Abbreviations: ILM, inner limiting membrane; OS, outer segment.

Next-generation sequencing analysis and putative pathogenic mutation screening

Targeted capture high-throughput sequencing of RP-related genes was performed successfully using a capture panel on the gDNA sample of proband (Figure 1, pedigree IV: 9). The causative mutations were identified by automatic variant calling, filtering, and annotation pipeline in the capture sequencing data, and a single nucleotide hemizygous, missense variant (c.G644A) of exon 7 in the RPGR gene (GenBank No.: NM_000328.2) in the proband was identified, leading to an amino acid change from Glycine (Gly, G) to Glutamic acid (Glu, E) at codon 215 of the RPGR protein (p.G215E) (NP_000319.1) (Figure 1 IV: 9, Supplementary Table S1, highlighted in yellow). The deleterious and pathogenic aspect of c.G644A (p.G215E) mutation in the RPGR gene is shown in Table 2. PolyPhen-2 analysis showed probable damage for this change with score 1; MutationTaster revealed the change to be disease causing with score 1; SIFT was deleterious with score 0, which predicted to affect protein function; and I-Mutant2.0 for the free energy change value indicated decrease in stability (DDG = −0.30 kcal/mol, <0). Thus, this missense variant...
Table 2 Characteristics of RPGR variant and analysis of disease-causing effects of proband

| Gene | Exon | Nucleotide* | Protein* | PolyPhen-2 | Mutation Taster | I-Mutant2.0 | SIFT | ExAC |
|------|------|-------------|----------|------------|----------------|-------------|------|------|
| RPGR | 7    | c.G644A     | p.G215E  | Missense   | Hemi          | PD (1)      | DC (1) | DS(-0.30) | D(0) | Novel |

Abbreviations: c, variation at cDNA level; D, deleterious; DC, disease causing; DS, decrease stability; G215E, Glycine (Gly) was substituted by conserved Glutamic acid (Glu) at codon 215; Hemi, hemizygote; p, variation at protein level; PD, probably damaging.

* All nucleotides and amino acids are abbreviated according to the International Union of Pure and Applied Chemistry (IUPAC).

Figure 3. Photogram profiles for validation and segregation by Sanger sequencing

(A–H) Indicate the sequencing results in IV: 9 (mutant hemizygous type), IV: 10 (wild hemizygous type), III:1 (wild hemizygous type), III: 3 (wild hemizygous type), III: 6 (wild homozygous type), III: 10 (wild homozygous type), III: 11 (wild homozygous type), and V:1 (wild homozygous type), respectively. The arrows indicate mutation at the nucleotide position NM_001034853.1: c.G644A in the RPGR gene. ‘N’ indicates the wild-type of RPGR allele.

in the RPGR gene: c.G644A (p.G215E) was pathogenic in this Chinese family. This variant c.G644A (p.G215E) was searched in the ExAC and HGMD databases and found as a novel mutation (Table 2).

Mutation verification of c.G644A (p.G215E) in RPGR and segregation analysis

Albeit deficient, the Sanger sequencing was exploited to confirm the RPGR mutant hemizygous type of c.G644A in proband (pedigree IV: 9; Figure 3A), and to identify mutant heterozygous type in proband’s mother with RP disease (data not shown), wild-types in other family members with normal phenotypes (Figure 3B–H). Thus, this c.G644A (p.G215E) in RPGR was co-segregated with the disease phenotype in all tested family’s members. This mutant was absent from 100 unrelated, normal, ethnically matched controls (data not shown). The proband’s grandfather might have carried the same c.G644A hemizygous type, and great grandmother might have also carried the same c.G644A heterozygous type due to the RP phenotype, but no DNA samples were available because of death. Altogether, these findings showed complete co-segregation in the pedigree for the retinal dystrophy family and pinpoint its role in xLRP pathogenesis.

Functional effects of variant c.G644A (p.G215E) in RPGR

RPGR conservation and position for p.G215E are shown in Figure 4. By orthologous comparison of Homo sapiens PRGR with five other species, including Canis lupus, Mus musculus, Rattus norvegicus, Xenopus tropicalis, and Danio rerio, we found that this protein is highly conserved (Figure 4A), as well as the amino acid Glycine (G) (Figure
Figure 4. RPGR comparison and conserved domains

(A) RPGR comparison and domains. (B) Conserved variant p.G215 in different species.

4B). Altogether, our study revealed that the RPGR hemizygous variant, c.G644A (p.G215E), might cause xLRP disease in this proband.

Expression profiles of RPGR and Rpgr mRNA

RNA-seq data showed that RPGR is ubiquitously expressed in representing 27 different human tissues with testis in highest (reads per kilobase million (RPKM) value: 2.58 ± 0.397) and salivary gland in lowest (RPKM value: 0.196 ± 0.082) (Figure 5A and Table 3). However, no eye tissues data were shown by RNA-seq; then the expressions for Rpgr mRNA in 15 different tissues and 6 different development stages of retina were conducted in mice. The results showed that Rpgr transcript is ubiquitously expressed very highly in retina and testis, as well as highly in other eye tissues including lens, sclera, cornea (Figure 5B); and expressed highly in the six different developmental times of retinal tissue (Figure 5C). Ubiquitous expression in different tissues from eye and very high expression in the retina indicated that RPGR plays a vital role in eye functions, particularly in retina.

Discussion

In the present study, we identified a hemizygous, missense variant c.G644A:p.G215E of the RPGR gene in the proband of a Chinese family, which led to xLRP. By searching the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/gene.php?gene=RPGR) (access date, 30 August 2019), 169 pathogenic variants have been reported, including missense/nonsense (68), splicing (36), small deletions (44), small insertions (9), small indels (2), gross deletions (9), and complex rearrangement (1). It showed that different RPGR mutations caused different clinical correlations of diseases/phenotypes (Table 4). The proband’s mother presented typical RP symptoms in our studies, demonstrating high penetration or likely X-linked dominant. As an X-linked disease, among female carriers from 45 families by retrospective medical records review, Comander et al. (2015) [27] found that those with RPGR ORF15 mutations tended to have worse visual function than those with RPGR exon 1 through 14 mutations [28], demonstrating disease symptoms in the carriers. To the best of our knowledge, RPGR variant c.G644A (p.G215E) is a novel mutation, extending its spectrums of mutations. Thus, these finding shows that the RPGR mutation, c.G644A (p.G215E), likely causes xLRP disease in our studied Chinese pedigree.

Inheritance of RP3 was described as X-linked recessive, while in RP15, affected males and carrier females. With reference to the X-linked dominance of RP15 they stated that ‘since all females with the proposed disease-causing gene are affected, the disease is ‘dominant’ in the traditional sense of the word,’ but they agreed that the terms ‘dominant’ and ‘recessive’ can be misleading, so we called here as xLRP in our studied family.

RPGR plays a vital role as a scaffold protein in the regulation of protein trafficking, thus the cargoes can be transported to the outer segments (OSs) of photoreceptors. This trafficking process is controlled by intraflagellar transport complexes and regulated by the RPGR protein complex [29]. The C-terminus of RPGR that contains prenylated site can interact with PDE6δ, INPP5E, and RPGRIP1L, thus regulates ciliary localization of INPP5E [30,31]. Missense
Figure 5. *RPGR* and *Rpgr* mRNA expression profiles

(A) Expression profiles for *RPGR* mRNA in 27 human tissues. Expression profiles for *Rpgr* mRNA in 15 mouse tissues (B) and in mouse 6 different development stages or times of the retinal tissue (C). Abbreviations: d, day(s); m, month(s); muscle, skeletal muscle; nc, negative control without any template cDNA; w, week(s). Whole eye balls at 12.5 days (12d) and 20.5 days (20d) from embryos in panel (C), respectively.

variations of *RPGR* disrupted those endogenous protein interactions which might be the common feature of *RPGR* causing xLRP [32]. Our missense variant c.G644A (p.G215E) of *RPGR* might disrupt complex formation in this family. But further study should perform to validate the hypothesis in the future. Tauroursodeoxycholic acid (TUDCA) has demonstrated therapeutic potential for *RPGR* patients by suppressing microglial activation and inflammation and preventing photoreceptor degeneration in Rpgr conditional knockout mice [29]. Adeno-associated viral (AAV) vectors were conducted for *RPGR* gene therapy by targeting gene expression to both rods and cones in non-human primates [33–35].

In conclusion, our study is the first to identify that the hemizygous missense variant c.G644A (p.G215E) of the *RPGR* gene in our Chinese proband, which is most likely the disease-causing mutation for xLRP, thereby expanding its spectrum of mutations. These findings facilitate better understanding of the molecular pathogenesis of this disease; provide new insights for genetic counseling and healthcare.

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Ethics Approval

The study has the Ethical Committees approval granted by the Southwest Medical University. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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Table 3 Expression of RPGR mRNA in human different tissues

| Sample          | Numbers | RPKM values | Counts  |
|-----------------|---------|-------------|---------|
| Adrenal         | 3       | 1.167 ± 0.18| 82686   |
| Appendix        | 3       | 1.653 ± 0.501| 101237  |
| Bone marrow     | 4       | 1.882 ± 0.38| 319275  |
| Brain           | 3       | 0.841 ± 0.116| 70353   |
| Colon           | 5       | 1.338 ± 0.063| 246618  |
| Duodenum        | 2       | 0.787 ± 0.179| 33526   |
| Endometrium     | 3       | 1.435 ± 0.159| 118604  |
| Esophagus       | 3       | 1.02 ± 0.361| 122685  |
| Fat             | 3       | 2.139 ± 0.876| 152122  |
| Gall bladder    | 3       | 2.081 ± 0.672| 236257  |
| Heart           | 4       | 0.729 ± 0.232| 121155  |
| Kidney          | 4       | 0.692 ± 0.164| 55366   |
| Liver           | 3       | 0.453 ± 0.082| 96753   |
| Lung            | 5       | 2.335 ± 0.918| 297544  |
| Lymph node      | 5       | 1.324 ± 0.26 | 264618  |
| Ovary           | 2       | 0.792 ± 0.153| 73105   |
| Pancreas        | 2       | 0.294 ± 0.014| 25662   |
| Placenta        | 4       | 1.171 ± 0.277| 191905  |
| Prostate        | 4       | 1.066 ± 0.111| 98785   |
| Salivary gland  | 3       | 0.196 ± 0.082| 29108   |
| Skin            | 3       | 0.849 ± 0.035| 107607  |
| Small intestine | 4       | 0.9 ± 0.128  | 91928   |
| Spleen          | 4       | 0.906 ± 0.114| 120628  |
| Stomach         | 3       | 0.82 ± 0.182  | 79825   |
| Testis          | 7       | 2.58 ± 0.397  | 789604  |
| Thyroid         | 4       | 1.575 ± 0.458| 272805  |
| Urinary bladder | 2       | 1.361 ± 0.27  | 103527  |

Table 4 RPGR mutations and disease relations

| Disease/phenotype                | Number of mutations |
|----------------------------------|---------------------|
| RP, X-linked                     | 157                 |
| RP, X-linked ?                   | 10                  |
| Leber congenital amaurosis       | 1                   |
| Retinal dystrophy                | 1                   |

Author Contribution

Junjiang Fu was incharge of idea, project design and concept of the paper. Jiewen Fu, Jingliang Chen and Chunlei Wei performed DNA isolation, PCR amplification, and sequencing. Junjiang Fu and Hanchun Chen analyzed the data. Hongbin Lv and Qi Zhou recruited the patient and was incharge of clinical assessment. Junjiang Fu wrote and revised the manuscript.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Abbreviations

ExAC, Exome Aggregation Consortium; FFP, fundus fluorescent photograph; FP, fundus photograph; gDNA, genomic DNA; HGMD, The Human Gene Mutation Database; INDEL, Insertion and deletion; OMIM, Online Mendelian Inheritance in Man; RP, retinitis pigmentosa; RPGR, RP GTPase regulator; RPKM, reads per kilobase million; RT-PCR, reverse transcription-polymerase...
chain reaction; SIFT, Sorting Intolerant from Tolerant; SNP, Single Nucleotide Polymorphism; TGS, targeted next-generation sequencing; xRP, X-linked RP.

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