Retinitis pigmentosa GTPase regulator-related retinopathy and gene therapy

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Abstract:

Retinitis pigmentosa GTPase regulator *(RPGR)*-related retinopathy is a retinal dystrophy inherited in a X-linked recessive manner that typically causes progressive visual loss starting in childhood with severe visual impairment by the fourth decade of life. It manifests as an early onset and severe form of retinitis pigmentosa. There are currently no effective treatments for *RPGR*-related retinopathy; however, there are multiple clinical trials in progress exploring gene augmentation therapy aimed at slowing down or halting the progression of disease and possibly restoring visual function. This review focuses on the molecular biology, clinical manifestations, and the recent progress of gene therapy clinical trials.

Keywords:

Gene therapy, retinitis pigmentosa GTPase regulator, X-linked retinitis pigmentosa

Introduction

Retinitis pigmentosa (RP) is an inherited retinal dystrophy (IRD) that causes progressive vision impairment, potentially leading to legal blindness, with an estimated worldwide prevalence of about 1:4000.[1] Nonsyndromic RP, which accounts for approximately 65% of all RP cases, can be classified according to its inheritance pattern. Autosomal dominant RP represents 20%–25% of all RP, while autosomal recessive (AR) and X-linked (XL) RP account for about 15%–20% and 5%–15% of cases, respectively. The remaining 40%–50% are designated as simplex RP, with no identifiable family history.[2,3]

XLRP is characterized by childhood onset with progression to severe vision impairment by the fourth decade of life, resulting in greater morbidity compared to other RP genotypes. The prevalence of XLRP has been estimated to be 4.0–5.2/100,000 males in the United States (US), Europe, and Australia.[4] Pathogenic variants in RP GTPase regulator (*RPGR*) gene has been shown to be responsible for 70%–80% of all XLRP cases. The remaining 10%–20% of cases are mostly caused by pathogenic variants in the *RP2* gene. Rarely, variants in *OFD1* have been identified as a cause of XLRP.[5-9]

In the past, there have been no treatments for these inherited retinal diseases. However, in recent years, numerous preclinical and clinical trials have been developed to investigate a wide range of treatment strategies. These recent advancements have brought newfound hope for IRD patients, offering the possibility of stabilizing or even improving their vision. In this review, we describe the characteristics of *RPGR*-related retinopathy, focus on the molecular biology, clinical manifestations, and recent advances in the development of gene therapy.

Gene and Molecular Biology

RPGR is located on the short arm of the X-chromosome at position Xp21.1. The gene comprised 19 exons. Alternative splicing results in more than ten isoforms.[10,11] The two most common isoforms are the full length or constitutive $RPGR_{Exon1\text{-}19}$ isoform and the $RPGR_{ORF15}$ isoform [Figure 1]. The $RPGR_{Exon1\text{-}19}$ isoform, widely expressed in various tissues,

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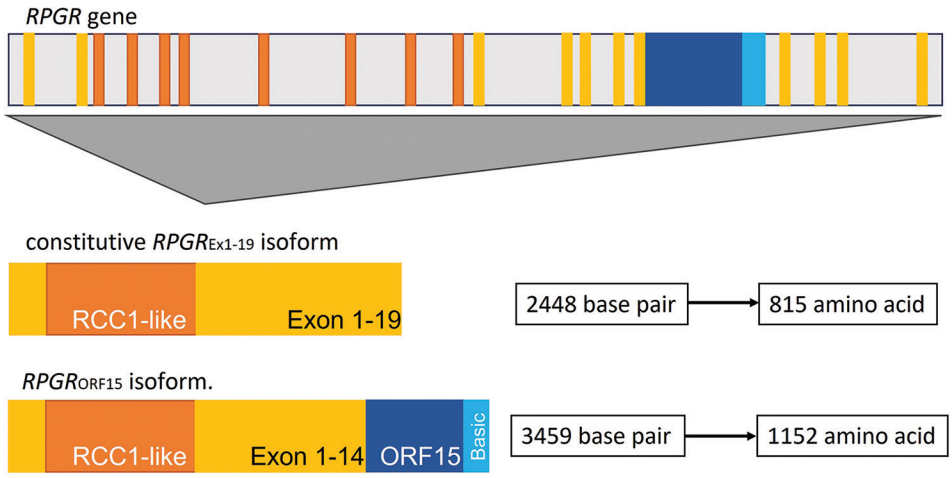

Figure 1: Structural diagram of retinitis pigmentosa GTPase regulator gene and two common splicing isoforms

comprises 19 exons with a length of 2448 base pairs (bp), encoding 815 amino acids.[12] The $RPGR_{ORF15}$ isoform, highly expressed in retinal photoreceptors, has a length of 3459 bp, and generates a 1152 amino acid sequence. It encompasses exons 1 through 14 and open reading frame (ORF) 15 which is derived from an alternatively spliced exon 15 with part of intron 15. All isoforms have a regulator of chromosome condensation 1 (RCC1)-like domain at the N-terminus which comprises exons 3–10.[13] The coding segment within the ORF15 exon region is characterized by a repetitive sequence rich in purines, mostly adenine (A) and guanine (G) nucleotides, followed by the basic domain, a region with abundance of basic amino acid residues at the C-terminus. Approximately 80% of variants in *RPGR* are located in the ORF15 region,[14] whereas the remaining 20% are found in exons 1–14. Interestingly, no disease-causing mutations have been reported in exons 16–19.[10] This concludes that the $RPGR_{ORF15}$ isoform is unable to be compensated for by the constitutive $RPGR_{Exon1-19}$ isoform in rescuing the phenotype in the retina.

The most common cause of pathogenic variants are small deletions leading to frameshifts and protein truncation.[14,15] Pathogenic variants in *RPGR* exons 1–14 can cause abnormalities in the RCC1-like domain, implicated in protein stability and interactions,[16] and results in loss of function, eventually leading to progressive retinal degeneration. The ORF15 region contains a repetitive sequence rich in glutamic acid and glycine, which causes abnormal deoxyribonucleic acid (DNA) double helix or triplex conformations, and results in poor stability of the genome during DNA replication and transcription.[11,14,15] Pathogenic variants in ORF15 disrupt the production and stability of the *RPGR* protein, resulting in impaired protein transport across the connecting cilium and can lead to photoreceptor cell death.[17] Most ORF15 mutations are microdeletions of 1–5 bp that are predicted to produce truncated proteins with novel amino acid sequences. Missense mutations and small deletions or insertions are also reported in the ORF15 region; however, in-frame alterations are generally considered to be nonpathogenic.[17-19] Large deletions in the ORF15 region can impact *RPGR* function by altering the degree of *RPGR* glutamylation and its capability to form associations with other ciliary proteins.[20]

RPGR is expressed broadly throughout the body including the eye, brain, testis, lung, and kidney.[21] Two major isoforms are highly expressed in the retina, and primarily localize to the photoreceptor connecting cilium, inner segments and outer segments.[22] *RPGR* regulates the activity of GTPases and plays a vital role in a diverse array of cellular processes including signal transduction, protein transport, and cytoskeletal organization. In particular, opsin and other proteins involved in the phototransduction cascade rely on *RPGR* for proper localization and transportation across the photoreceptor connecting cilium.[23-25]

Disruption of the normal function of *RPGR* leads to the degeneration and death of photoreceptor cells. The RCC1-like region interacts with small GTPases such as RPGR interacting protein 1, phosphodiesterase 6D, Ras associated protein (RAB8A), structural maintenance of chromosome (SMC1 and SMC3), nephronocystin 5 (NPHP5), and centrosomal protein 290 (CEP290).[22,23,26-30] The ORF15 region is known to interact with at least two proteins: nucleophosmin and whirlin. Nucleophosmin is a chaperone protein which contributes to the functioning and arrangement of metaphase centrosomes during cell division. Whirlin serves as a scaffold protein responsible for the preservation of ciliary structures within the eye and ear.[31,32] Tubulin tyrosine ligase-like (TTLL5) enzyme also interacts with the basic domain of the ORF15 region and helps to stabilize the $RPGR_{ORF15}$ protein.[33,34] Loss-of-function mutations in the TTLL5 enzyme can result in an *RPGR*-like phenotype by interrupting the glutamylation process.[20,34] This reinforces the vital role of glutamylation in facilitating normal $RPGR_{ORF15}$ function.

Clinical Manifestations

RPGR pathogenic variants manifest in a range of phenotypes including rod-cone dystrophy (70%), cone-rod dystrophy (6%–23%), and cone dystrophy (7%).[5,35] A small number of patients

may exhibit extra-ocular manifestations, such as hearing loss or recurrent sinorespiratory infections.[36,37] Several studies identified that variants in exons 1–14 and at the 5'end of ORF15 are associated with rod-cone dystrophies, whereas variants located towards the 3'end of ORF15 are more often associated with cone/cone-rod dystrophies.[3,38-42] However, conflicting reports of genotype–phenotype correlation exists. Some reported variants in exon 1–14 were associated with more severe disease phenotypes than ORF15 variants,[5,43,44] while others report the opposite.[35,45,46] It has also been proposed that patients harboring ORF15 variants were associated with greater variability in disease severity.[5,43] Indeed, intra- and interfamilial phenotypic heterogeneity has been described in subjects harboring the same disease-causing variant, such as the coexistence of rod-cone dystrophy and cone-rod dystrophy patterns within the same family.[47] This suggests the influence of epigenetics, genetic modifiers, and/or environmental factors as modulators of disease phenotype.

When *RPGR*-related retinopathy presents as a rod-cone dystrophy, the first symptoms are typically nyctalopia and impaired dark adaptation, followed by constriction of peripheral vision, and ultimately central visual loss. Onset is often in the first decade (median age of onset 5 years). Visual acuity deteriorates more rapidly compared to other forms of RP, around 4%–5% each year,[48,49] and most patients reach legal blindness at a median age of approximately 45 years old.[48] Fundus examination shows retinal atrophy starting in the midperiphery, intraretinal pigmentation (bone spicules), optic disc pallor, and vascular attenuation.[45] High myopia is common with a prevalence of up to 80% and has been associated with more rapid visual acuity decline.[35,45] Clinical characteristics and multimodal imaging of male *RPGR*-related XLRP are demonstrated in Figures 2 and 3.

When the presentation is cone-rod or cone dystrophy, symptoms typically manifest in the second to third decades of life with reduced central visual acuity, color vision abnormalities, or central scotomas followed by nyctalopia then peripheral visual field loss. These patterns are also associated with myopia. The age range and phenotypic expression can vary widely, and visual decline is typically rapid, often resulting in legal blindness by the age range of 40–50.[35]

Short-wave fundus autofluorescence (FAF) is a valuable tool for evaluating lipofuscin in retinal pigment epithelium (RPE). Hyperautofluorescent signal on FAF imaging can indicate accumulation of lipofuscin within a metabolically stressed RPE or reflect the unmasking of RPE fluorescence from photoreceptor outer segment loss. With disease progression, hypoautofluorescent signals may be observed in atrophic areas of RPE.[50] Notably, parafoveal hyperautofluorescent rings on FAF images can serve as a distinct boundary between healthy and degenerative retina.[51] These rings have been observed in the macular region of more than one-half of male patients with different phenotypes related to *RPGR* pathogenic variants. With rod-cone patterns, the hyperfluorescent ring typically becomes constricted over time, whereas in cone or cone-rod dystrophies, the macular area is often affected initially with progressively enlarging hyperautofluorescent rings. The rate of ring area constriction was approximately 1.3–1.5 mm^2/year with the greatest rates of progression seen in younger subjects.[52]

Optical coherence tomography (OCT) is especially useful for characterizing the phenotype in *RPGR*-related retinopathy.[53] Ellipsoid zone (EZ) anatomy and integrity is an important predictor of central retinal function and visual acuity and can help monitor disease progression. The transition zone in OCT is the area between the healthy retina and the affected peripheral retina[54] and corresponds to the hyperautofluorescent ring in FAF. For rod-cone dystrophies, the lesion progresses from the periphery to the foveal center. Changes in the transition zone begin with photoreceptor outer segment thinning, followed by thinning of the outer nuclear layer (ONL). As the disease progresses, extensive loss of the EZ appears,

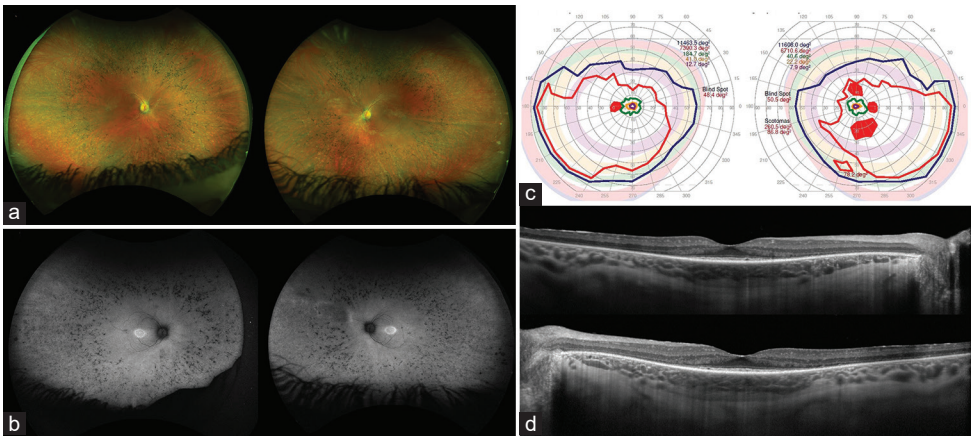

Figure 2: Images of a 21-year-old male patient with retinitis pigmentosa (RP) GTPase regulator related RP. (a) Pseudocolour images showed sparse pigmentary changes across fundus, (b) autofluorescence imaging demonstrated areas of patchy mid-peripheral hypoautofluorescence, and hyperfluorescence ring within the macula region. (c) kinetic visual field revealed peripheral constriction and scattered scotoma around mid-periphery area and (d) optical coherence tomography images of right and left eyes demonstrated outer nuclear layer and ellipsoid zone attenuation sparing central fovea

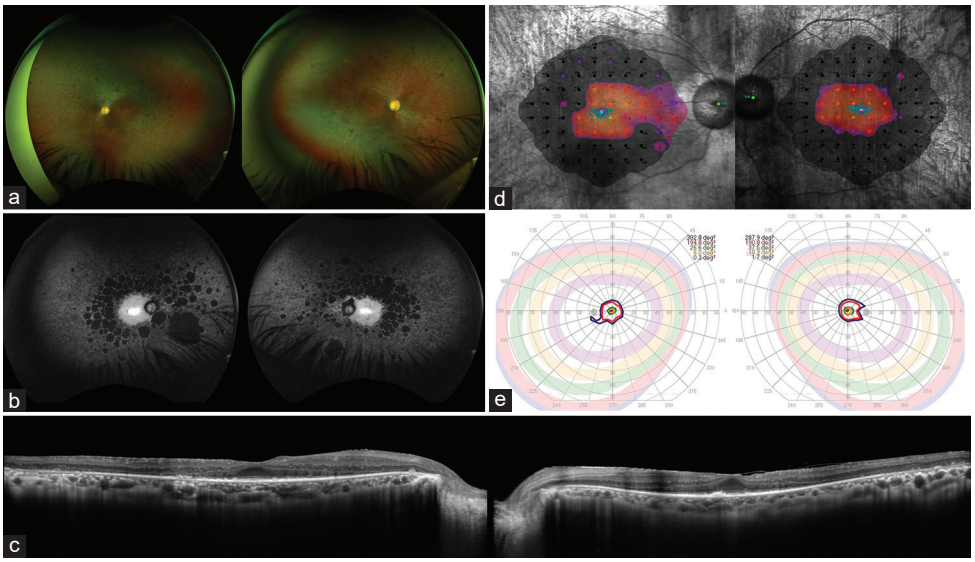

Figure 3: Images of a 53-year-old male patient with advance stage of retinitis pigmentosa (RP) secondary to a variant in RPGTPase regulator. (a) Pseudocolour images demonstrated extensive bone spicule pigmentation and macular atrophy. (b) autofluorescence demonstrated widespread areas of hypoautofluorescence, and hyperfluorescence within the macula region. (c) Optical coherence tomography (OCT) images revealed diffuse attenuation of outer retina, ellipsoid zone (EZ) and retinal pigment epithelium. (d) Macular integrity assessment microperimetry measured marked reduction in central retinal sensitivity with small residual area in the center correlated with the residual of EZ in the OCT. (e) Kinetic visual field revealed tubular field preserved in the central area <20°

followed by complete loss of ONL, and finally, disruption of RPE. Patients with XLRP exhibit a mean annual decrease in the width of EZ up to 7% (173–248 μm/year),[55-57] which is equivalent to a mean rate of change of 13% for the functioning area in the retina and consistent with the rate of change in visual function observed by visual field and full-field electroretinography (ERG). In addition, the transition zone shows a faster decline in visual field sensitivity annually than other parts of the retina.[58] The peripapillary retinal nerve fiber layer (RNFL), another biomarker for *RPGR*-associated RP, was observed to be thickened in both male and female carriers.[59,60] This phenomenon is not well understood. It may reflect a neuronal-glial cell remodeling response to photoreceptor cell stress or loss. However, as the disease progresses along with increasing age RNFL thickening may become less prominent.

ERGs, if still recordable, are useful for distinguishing between patterns of rod-cone degeneration versus cone-rod degeneration. These abnormalities are evident in both dark-adapted (DA) and light-adapted (LA) responses early in childhood. Early macular dysfunction has also been reported, as evidenced by a reduction in the P50 component of the pattern ERG. In contrast, cone/cone-rod dystrophy phenotypes have a LA ERG more delayed and/or reduced than seen in the DA ERG, where early and severe macular involvement is typically observed.

Adaptive optics (AO) imaging may be useful in early detection of structural damage in *RPGR*-related RP retinopathy. Even in cases where patients, including female carriers, exhibited normal visual acuity and unremarkable spectral domain-OCT results, lower cone density and reduced sensitivity are measured by AO microperimetry.[61,62] This highlights the potential of gene therapy to potentially enhance the visual function of the remaining cones.

Characteristics of Female Carriers

Female carriers of *RPGR* pathogenic variants show high phenotypic variability and asymmetry between affected eyes.[63] The presentation can range from an asymptomatic or a mild phenotype to severe disease indistinguishable from male phenotypes.[64] Random inactivation of the X-chromosome is thought to underlie this variability. X-chromosome inactivation is a natural process that occurs in females to compensate for the presence of two X chromosomes. A nontranslated region of RNA in the X-inactivation center is responsible for choosing which X chromosome should be translated and maintained.[63,65] In cases of affected female *RPGR* carriers with a more severe phenotype, X chromosome inactivation may be "skewed," wherein the normal allele of *RPGR* is silenced more often than the allele with a pathogenic variant. This leads to a higher proportion of cells with reduced or absent *RPGR* protein function.[66] The degree of skewing varies widely among individuals, different tissues, and is not reliably predictable based on the type or location of the *RPGR* pathogenic variant. Other genetic and environmental factors can also influence disease progression and severity.

Four patterns of retinal findings and FAF are reported in *RPGR* carriers: A normal or near normal fundus and FAF appearance, a radial tapetal reflex without pigmentary retinopathy, focal pigmentary retinopathy limited to a quadrant or hemisphere, and bone-spicule pigmentation or atrophy similar to the male phenotype.[63,67]

The tapetal reflex appears as a bright, glowing reflection that emanates from the retina [Figure 4]. The characteristic radial pattern of the tapetal reflex may be explained by the random process of X-inactivation during early embryogenesis, which is then maintained in all daughter cells, as well as the centrifugal radial growth pattern of the developing neuroretina.[68] FAF may similarly show a radial pattern of hyperautofluoresence and helpful in identifying subclinical cases.[68,69]

A vast majority of female carriers have mild phenotypes and maintain good visual acuity, mean visual acuity of each eye was 20/27.[67] Only the bottom fifth percentile demonstrated a visual acuity of 20/200 or worse. Fundus appearance is a strong indicator of visual function. Patients who exhibit a normal fundus or tapetal reflex are more likely to maintain their visual acuity.[67] Myopia was proposed to be aggravating factor for decreased visual acuity which was observed in high proportions up to 73%.[70] Interestingly, female carriers with $RPGR_{ORF15}$ pathogenic variants generally exhibited poorer visual function compared to those carrying *RPGR* exon 1–14 pathogenic variants, as indicated by measures such as dark adaptation thresholds, 0.5 Hertz (Hz) and 30 Hz ERG amplitudes.[67] In a female carrier, the average amplitude of the ERG response to 30 Hz flashes was found to be approximately half of normal, and the rate of amplitude loss over time was half of that compared to XLRP males.(3.7%/year vs. 7.4%/year, respectively).

Diagnostics

The molecular diagnosis of *RPGR*-related retinopathy traditionally utilizes next-generation sequencing methods to detect mutations and exclude other pathogenic XL genes, such as *RP2, PRPS1,* and *OFD1*. However, the ORF15 region of *RPGR* and its repetitive nature makes it a challenge to sequence accurately. Small mutations, including single nucleotide variants and small insertions and deletions (indels), can be mistaken for artifacts, leading to inaccurate results. There are several strategies that can be employed to sequence the ORF15 region, such as ORF15 Amplification, Sanger Sequencing and long-read sequencing technologies that generate reads capable of spanning the repetitive region.[71] Careful interpretation of

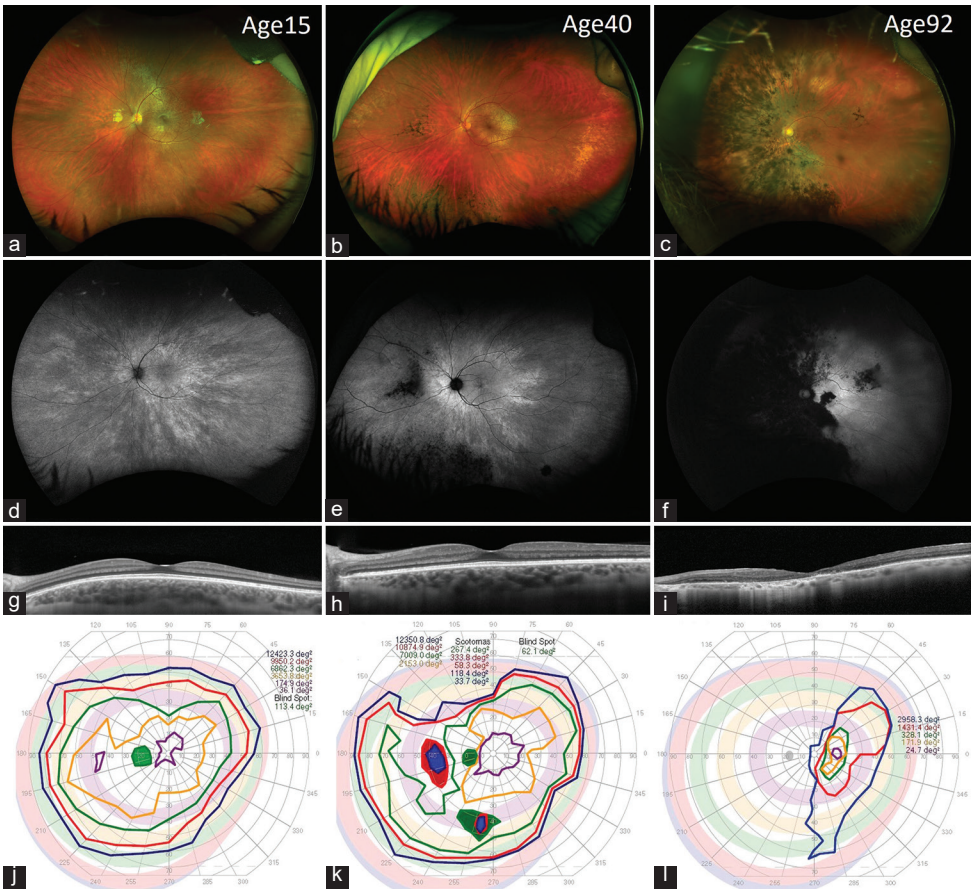

Figure 4: Left eye Images of female carriers of retinitis pigmentosa GTPase regulator pathogenic variant at different ages. (a-c) Pseudocolour, (d-f) autofluorescence and (g-i) Optical coherence tomography (OCT) images and (j-l) Kinetic visual field, pseudocolour images illustrating range of fundus appearance in female carrier from a mild radial tapetal reflex (a), which is more evident on autofluorescence imaging (d), patchy pigmentary and atrophic changes in periphery (b) and male fundus pattern (c). Autofluorescence imaging demonstrated areas of abnormal hypoautofluorescence, corresponding to pigmentary and atrophic change in the fundus (e and f), OCT images normal in mild disease (g) then demonstrated increased ellipsoid zone disruption and outer nuclear layer attenuation with advanced disease (h and i). Progressive constriction of kinetic visual field related to fundus changes were observed (j-l)

Table 1: Summary of clinical trials for retinitis pigmentosa GTPase regulator related X-linked retinitis pigmentosa

Sponsor	Type of gene product	Name of gene product	Route of delivery	NCT number	Design	Number of participants	Trial status
Nightstar Therapeutics (now Biogen Inc), UK	Codon-optimized full-length DNA sequence	AAV2/8. GRK1. co*RPGR*$_{ORF15}$	Subretinal	NCT03116113 (BIIB112)	Phase 1 dose escalation and phase 2/3 dose expansion trial (XIRIUS)	Phase 1: 18 participants/phase 2: 32 participants	Completed (paper published)
MeiraGTx, UK, Janssen Research and Development, LLC	Truncated *RPGR* sequence	AAV2/5. GRK1. *RPGR*$_{ORF15}$	Subretinal	NCT03252847	Phase 1/2 dose escalation trial	36 participants	completed
			Subretinal	NCT04671433	Phase 3 randomized, controlled trial (lumeos)	96 participants	Enrolling
AGTC (Now Beacon Therapeutics), USA	Codon-optimized full-length DNA sequence	rAAV2tYF. GRK1. co*RPGR*$_{ORF15}$	Subretinal	NCT03316560	Phase 1/2 dose escalation study, with a phase 2 randomized, controlled expansion trial (skyline)	42 participants	Enrolling
			Subretinal	NCT04850118	Phase 2/3, randomized, controlled trial (Vista)	-	not yet enrolling
4D Molecular Therapeutics	Codon-optimized DNA sequence	4D–125	Intravitreous	NCT04517149	Phase 1/2 trial	43 participants	Enrolling
Frontera Therapeutics	No data	FT–002	Intravitreous	NCT05874310	Phase 1 dose escalation trial	18 participants	Enrolling

DNA: Deoxyribonucleic acid, AGTC: Applied Genetic Technologies Corporation, *RPGR*: Retinitis Pigmentosa GTPase Regulator, NCT: National clinical trial

molecular analysis in conjunction with consideration of the XL inheritance pattern and clinical presentation are necessary for accurate diagnosis of *RPGR*-related retinopathies.

Gene Therapy

Adeno-associated virus (AAV) is a small, nonpathogenic virus that can be engineered as a vector to efficiently deliver episomal DNA to target retinal cells which are capable of long-term expression and at a low risk of inducing an immune response. Gene replacement therapy using AAV vectors is a promising approach to treat XLRP caused by pathogenic variants in the *RPGR* for multiple reasons. First, XLRP is a significant contributor to disease burden characterized by a relatively high prevalence, rapid progression, and more severe phenotype compared with other RP variants. Secondly, the disease phenotype is caused by pathological loss of function. Finally, the coding sequence size (3.5 kb) is small enough to fit within the AAV carrying capacity, making it a feasible option for gene replacement therapy.

Whilst mutations in *RPGR*$_{ORF15}$ account for up to 80% of *RPGR*-related retinopathy, the repetitive purine-rich sequence found in ORF15 poses challenges in the manufacturing of therapeutic vectors. The intrinsic instability of the sequence within this particular region poses difficulties in generating the full-length *RPGR* transgene without introducing spontaneous mutations. Additionally, it is difficult to preserve the entire sequence of the gene.[72,73] To address these issues, a truncated form of *RPGR*$_{ORF15}$ and codon optimization of the transgene were used.[74,75] The objective of optimizing codons in the *RPGR*$_{ORF15}$ sequence is to enhance stability, eliminate cryptic splice sites and boost expression levels of the therapeutic transgene.

Animal models in mice and canines have provided proof of concept that mutations in the ORF15 region can be treated with gene replacement therapy.[12,76]

Truncated forms of *RPGR*$_{ORF15}$ have also been utilized to rescue the *RPGR* phenotype in the Rpgr-Knock Out mouse model. A longer form of the *RPGR*$_{ORF15}$ (deletion of 126 codons) demonstrated partial rescue in the disease phenotype, while the short form (deletion of 314 codons) showed no rescue in the disease phenotype.[74] The therapeutic potential of this truncated *RPGR*$_{ORF15}$ form has not been investigated in XL RP canine models.

Some groups have hypothesized that the length of the ORF15 linker region is not critical for function. Moderate shortening of its length has been proposed to preserve its function by adding stability. However, treatment with an abbreviated or truncated *RPGR*$_{ORF15}$ form has demonstrated new mutations in purine repetitive regions, resulting in toxic effects[77] and raises safety concerns for human applications. To improve the stability of the *RPGR*$_{ORF15}$ sequence and enhance its safety profile while maintaining efficacy, the correct full-length *RPGR*$_{ORF15}$ coding sequence in AAV vector was also generated and appears to be more stable *in vitro*.[72]

Other groups have developed alternative strategies for delivering the *RPGR*$_{ORF15}$ transgene. Fischer *et al*. generated a full-length, codon-optimized version of *RPGR*$_{ORF15}$ to stabilize the coding sequence. This codon optimization does not impact glutamylation or posttranslational modification.[75] The study demonstrated that this codon-optimized version could rescue the disease phenotype in two mouse models of XLRP (Rpgr-/y and Rd9). Moreover, in canine studies, the codon-optimized *RPGR* vector, under the control of a human G Protein-coupled Receptor Kinase 1 (GRK1) promoter, exhibited excellent stability. Delivering the transgene to RPGR mutant dogs at the early-disease stage preserved the ONL structure along with efficient rod and cone transduction.[78]

The five retinal gene therapy clinical trials for RPGR associated retinopathy are sponsored by Nightstar/Biogen, Meira GTx, Applied Genetic Technologies Corporation (AGTC)/ BEACON, 4D Molecular Therapeutics, and Frontera Therapeutics. The summary of clinical trials were presented in Table 1.

Both NCT03252847 and NCT04671433. sponsored by Meira GTx, deliver AAV2/5. GRK1. RPGR$_{ORF15}$ (botaretigene

sparoparvovec) subretinally. The vector utilizes a wild-type AAV2/5 capsid containing a truncated *RPGR* sequence driven by the GRK1 promoter. The truncated form of $RPGR_{ORF15}$ involves the removal of approximately one-third of the ORF15 region. This deletion enhances stability and minimizes the likelihood of recombination errors and potential mutations.

The phase 1/2 dose-escalation trial from Meira GTx,[79] conducted at five sites in the US and United Kingdom (UK), showed that treated eyes demonstrated improvement in retinal sensitivity and functional vision compared to randomized controls at 6 months. The low and intermediate doses treated groups showed a concurrent control difference of 1.06 decibels (dB) in microperimetry least square mean change *(*$P < 0.05$) and 1.96 dB in static perimetry least square mean change *(*$P < 0.05$) based on sensitivity analysis. The main adverse event observed was ocular inflammation, which was anticipated and manageable with the addition of a subtenon injected steroid at the time of gene therapy surgery. The three serious adverse events (SAEs),-retinal tear, panuveitis, and increased intraocular pressure-were reported but resolved with treatment. These findings will likely be applied as criteria for the Phase 3 Lumeos study of AAV5-RPGR (NCT04671433), which is now enrolling participants in 2023. The primary outcome measure is the change from baseline to week 52 in vision-guided mobility assessment (VMA) as measured by the ability of the participant to navigate through a VMA maze.

Nightstar Therapeutics (since acquired by Biogen Inc), sponsored a gene therapy trial using subretinally injected AAV2/8. GRK1. $coRPGR_{ORF15}$ (cotoretigene toliparvovec), which is a wild-type AAV8 capsid containing human GRK1 promoter and a codon-optimized full-length DNA sequence of $RPGR_{ORF15.}$ The XIRIUS trial (NCT03116113) phase 1 is a dose-escalation study conducted with 18 patients diagnosed with *RPGR*-associated XLRP in the UK and the US. It was a trial involving participants aged 18 and above, recruiting six cohorts of three patients receiving an increasing concentration of vector from 5×10^{10} genomic particles (gp)/ml to 5×10^{12} gp/ml. The results of this study were published in 2020[80] and reported no significant dose limiting toxicities. Seven out of nine cases of intraocular inflammation in the high dose cohort required additional oral corticosteroids and inflammation resolved in all cases by 6 months. Seven patients from mid-dose to high-dose cohorts demonstrated visual gains in treated eyes (gain in retinal sensitivity and reversal of some of the visual field loss) beginning at month 1 that were sustained at month 6 follow-up.

A Phase 2/3 dose-expansion study was carried out with 32 randomized patients aged 10 years and above. This study encompassed three arms: a low dose arm, a high dose arm determined through a benefit/risk assessment conducted in Phase 1/2, and a third arm comprised of an untreated group serving as control. The trial assessed the clinical outcome of cotoretigene toliparvovec gene therapy at 12 months, and the results were compared to the study eye of patients randomized to the untreated group. The study did not achieve the primary endpoint requirement, which was a statistically significant improvement in the retinal sensitivity assessed by macular integrity assessment microperimetry. However, the study has reported positive trends in other prespecified clinically relevant endpoints, such as improvement in low luminance visual acuity.[81] A *post hoc* analysis from phase 1 XIRIUS trial was published in 2023;[82] comparison of participants to the untreated group in the Natural History of the progression of X-linked retinitis pigmentosa (XOLARIS) trial (NCT04926129) revealed sustained improvements in visual function. Notably, these improvements were observed in patients who received the four highest therapy doses during the XIRIUS trial, and no dose-limiting toxicities were reported.

AGTC, now Beacon Therapeutics, also utilized the codon optimization strategy in their clinical trial to subretinally deliver rAAV2tYF. $GRK1.coRPGR_{ORF15}$. This employs a modified AAV2 capsid harboring tyrosine to phenylalanine (YF) mutations and is packaged with full-length, codon optimized human $RPGR_{ORF15}$. The sequence is regulated by a GRK1 promoter.

The proof of concept for this gene product has been demonstrated in both mice and canine models.[83,84] In mice model, two doses (4×10^8 or 4×10^9 vector genome (vg)/eye) were tested. Vector injections were well tolerated, with no systemic toxicity. The canine study revealed the rescue of photoreceptor function and structure with absence of ocular toxicity observed in the low dose (1.2×10^{11} vg/mL) and mid dose (6×10^{11} vg/mL) treatment groups, while the high-dose group (3×10^{12} vg/mL) showed evidence of both photoreceptor rescue and posterior segment toxicity.

In the phase 1/2 study (NCT03316560), 29 participants were recruited from five sites in the US[85] and sequentially assigned to one of five dose groups (4.0×10^{10}, 1.2×10^{11}, 3.6×10^{11}, 1.1×10^{12}, and 3.2×10^{12} vg/mL). There were promising outcomes in terms of anatomical recovery in the treated eyes of the patients enrolled in the study. Notably, nine out of 13 (69%) patients who had residual macular EZ visible on OCT showed EZ recovery in the treated eye at 3–6 months follow-up, and 67% of these patients subsequently showed EZ improvement compared to baseline, that was sustained through 18 months of follow-up.[86] Interestingly, central foveal thickness was more stable postoperatively and correlated with EZ recovery in the treated eye. Treated eyes with EZ improvement had improved mean macular sensitivity (microperimetry) compared to the fellow eye ($P = 0.003$). The phase 2 randomized masked study (Skyline) showed that 3 months after dosing,[87] there was a significant difference in response rate between the two masked doses: 62.5% in dose group B and 25% in dose group A, wherein response was defined by improved macular function by microperimetry of at least 7 dB in at least five loci. Overall, there were no clinically significant safety events related to gene therapy reported in the studies. These findings

suggest a positive outcome in terms of both anatomical and functional recovery in the treated eyes of the patients enrolled in the study. Plans for a randomized masked Phase 2/3 clinical trial are currently pending (NCT04850118).

In 2020, a phase 1/2 clinical trial (NCT04517149) was initiated by 4D Molecular Therapeutics to evaluate the safety and tolerability of an intravitreally delivered vector, 4D-125, which carries a codon-optimized version of the human $RPGR_{ORF15}$ and utilizes an AAV capsid variant known as 4D-R100.[88] As of the last press release, the study has enrolled eight patients, and the preliminary clinical data indicates that 4D-125 is well-tolerated, with only 25% of patients experiencing mild, transient inflammation and no dose-limiting toxicities or serious adverse events observed.

In February 2023, Frontera Therapeutics also introduced FT-002, a recombinant AAV virus containing the *RPGR* gene product.[89] It was administered to the first patient with XLRP in China. However, details regarding the gene product and clinical trial have not yet been announced (NCT05874310).

In recent years, there have been an increasing number of novel experimental therapies for XLRP. Alternative strategies for treating XLRP are being considered, such as antisense oligonucleotides, which are small molecules capable of modifying RNA expression. An antisense RNA-based therapeutic approach called specific U1 antisense snRNAs (U1_asRNAs) skipping of exon E9a was developed by Covello *et al*.[90] The objective of this approach was to correct the splicing defect of RPGR caused by a nucleotide substitution in intron 9. *In vitro* testing demonstrated that U1_snRNA molecules can efficiently be used to correct the splicing of *RPGR* transcripts.[90] Additionally, Peking University has also proposed the use of clustered regularly interspaced short palindromic repeats (CRISPR) gene editing technique, along with the endonuclease cas-9 for treatment of a specific pathogenic variant in *RPGR*.[91] They used a genetically engineered mouse model with a 5-bp deletion at exon eight of Rpgr−/yCas9+/WT male mice. The mice were treated with subretinal injection of the AAV2/8-donor and AAV2/8-sgRNA. After 12 months of treatment, significant rescue of photoreceptors was observed, characterized by a thicker ONL and improved expression of markers in the treated region of the retinas. These findings provide evidence that CRISPR/Cas9-mediated *RPGR* gene editing therapy prevented photoreceptor degeneration and exhibited long lasting effects, providing valuable insights for potential clinical trials in this field.

Conclusion

The successful approval of gene therapy for Leber Congenital Amaurosis associated with RPE65 has opened doors for treating other retinal dystrophies. *RPGR* related retinal dystrophy is a promising candidate for gene therapy due to its relatively high prevalence, severity, and small size gene capacity. Significant progress has been made in developing a gene therapy approach to deliver a healthy *RPGR* gene copy into photoreceptors cells in recent years. However, the main challenge lies in maintaining the stability of the $RPGR_{ORF15}$ sequence, which is prone to spontaneous mutation. Recent advancements in vector manufacturing have addressed this challenge by utilizing the truncated form of *RPGR* and optimizing codons within the vectors. These strategies aim to enhance transduction efficiencies and improve transgene expression. Phase 1/2 gene replacement trials conducted by several companies have demonstrated promising results, paving the way for further progression into phase 3 trials. These advancements have brought us closer to the successful approval of gene therapy for *RPGR*-related retinal dystrophy.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

Mark E. Pennesi MD, PhD has been a consultant for AGTC, Biogen, and Janssen companies. Paul Yang MD, PhD has been a consultant for 4D Molecular Therapeutics and MieraGTx companies and received support from 4D Molecular Therapeutics, Beacon Therapeutics and Biogen companies. Casey Eye Institute, Oregon Health and Science University had received support for the AGTC trial and Biogen trials.

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