RPGR related retinopathy: Clinical features, molecular genetics and gene replacement therapy.

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Abstract

Retinitis pigmentosa GTPase regulator (*RPGR*) gene variants are the predominant cause of X-linked retinitis pigmentosa (XLRP) and a common cause of cone-rod dystrophy (CORD). XLRP presents as early as the first decade of life, with impaired night vision and constriction of peripheral visual field, rapid progression, and eventually leading to blindness. In this chapter we present *RPGR* gene structure and function, molecular genetics, animal models, *RPGR*-associated phenotypes and highlight emerging potential treatments such as gene replacement therapy.

Keywords

Retinitis pigmentosa, GTPase regulator, *RPGR,* XLRP, CORD, clinical features, gene therapy, OCT, FAF

Introduction

Retinitis pigmentosa GTPase regulator (*RPGR*) gene variants are the predominant cause of X-linked retinitis pigmentosa (XLRP), accounting for 70-80% of cases (Tee et al. 2016). The other less common causes of XLRP are retinitis pigmentosa 2 (*RP2*) and 23 (*RP23* or *OFD1*).(Branham et al. 2012; Webb et al. 2012). *RPGR* XLRP is one of the most severe forms of rod-cone dystrophy (RCD), in terms of the early age of onset and rate of degeneration. Typically, symptoms include abnormal night vision and peripheral visual field constriction, leading to visual impairment by the fourth decade of life.

In male patients, the retinal degeneration is particularly severe and shows rapid progression, while the phenotype of *RPGR* female carriers can be variable, ranging from early onset severe disease to most commonly being asymptomatic or mildly symptomatic in later life.(Georgiou et al. 2021a) Skewed inactivation of the X-chromosome in females is suggested to be the molecular basis of the variable phenotype,(Wu et al. 2010). Female carries usually have a radial pattern tapetal-like reflex originating from the fovea, often more visible on fundus autofluorescence imaging (FAF) (Figure X).

More rarely, *RPGR* variants can cause cone-rod dystrophy (CORD), which presents in the second to fourth decade of life and is characterized by early central degeneration of the cones, reduced visual acuity, photophobia, and myopia.(Thiadens et al. 2011)

Molecular Genetics

The *RPGR* gene is located on the short arm of the X chromosome and is responsible for the expression of at least 21 alternative transcripts. This gene consists of 19 exons and encodes a protein product of 90 kDa. Exons 2–11 coding for the N-terminus, which is highly similar to regulator of chromosome condensation 1 (RCC1)(Shu et al. 2007). RCC1 is a well characterized protein that plays a role in nucleocytoplasmic transport and cell division regulation.(Hadjebi et al. 2008) All proteins known to directly interact with RPGR do so through the Regulator of Chromosome and Condensation-like domain (RLD), and all known splice-site variants of *RPGR* contain the RLD domain (Georgiou et al. 2021b).

To date, more than 300 *RPGR* variants have been identified with the majority present in the guanine-cytosine-rich mutational hotspot open reading frame15 (ORF15). *RPGR* isoforms are formed from post-translational modification (He et al. 2008) and alternative splicing.(Kirschner et al. 1999; Yan et al. 1998) *RPGR* isoforms are expressed in multiple tissues including the kidney, testis, lung and retina, with *RPGR* OFR15 being the major isoform expressed in the retina.(Vervoort et al. 2000). The majority of pathogenic variants affecting the retina are identified in the *RPGR* OFR15 isoform.(Sharon et al. 2000)

RPGR structure and function

In the retina, RPGR is typically found in the photoreceptor connecting cilium (Hong et al. 2003) and consists of an RCC1-like domain at the N-terminus, and the ORF15 at the C-terminal which still has no known predicted function. Previous studies have identified an

RPGR protein interaction network, (Hadjebi et al. 2008; Vervoort and Wright 2002; Murga-Zamalloa et al. 2010; Hong et al. 2001) and suggested that RPGR interacting protein (RPGRIP1) contributed to RPGR localization at the connecting cilia. (Hong et al. 2001) Regulation of intercellular protein transport between the photoreceptor inner and outer segments (Hong et al. 2001), as well as maintaining correct location and concentration of opsin, is achieved when RPGR is bound to the connecting cilia. Multiple other proteins interact with RPGR, such as whirlin, maintenance of chromosomes 1 and 3 proteins, rod cyclic guanosine monophosphate phosphodiesterase (subunit δ), nephrocystin-5 and structural GTPase Rab8A.(Hong et al. 2000; Beltran et al. 2006; Nemet et al. 2015; Tee et al. 2016)

In a murine RPGR knockout model, mislocalization of opsin with ensuing degeneration was reported.(Hong et al. 2000) An RPGR-deficient mouse model showed that the connecting cilium is structurally not affected. (Shu et al. 2005; Khanna et al. 2005) More recently, an important post-translational modification has been identified, namely glutamylation, which is necessary to enable RPGR function as a regulator of photoreceptor ciliary transport.(Kapetanovic et al. 2019b)

RPGR **Animal models**

The Siberian husky canine breed has two naturally occurring *RPGR* ORF15 disease-causing variants that result in distinct phenotypes. A 5-nucleotide deletion in *RPGR* ORF15 (del1028-1032) leads to a premature stop codon and truncation of 230 residues, resulting in X-linked progressive retinal atrophy 1 (XLPRA1) secondary to loss of RPGR

function.(Zhang et al. 2002) This phenotype is characterised by gradual post-developmental photoreceptor degeneration, affecting rods more than cones, which is in keeping with human RP, but with slower progression. Optical coherence tomography (OCT) shows normal outer nuclear layer (ONL) thickness up to 28 weeks of age, while from 56 weeks of age, ONL thickness starts to thin in the inferior retina, while initially remaining preserved at the visual streak.(Beltran et al. 2012)

The more severe phenotype, XLPRA2, is caused by a 2-nucleotide deletion in ORF15 (del1084-1085), downstream to the first, resulting in frameshift and the inclusion of 34 basic amino acids with truncation of 161 residues.(Zhang et al. 2002) A generalised decrease in ONL thickness is shown on OCT, that is more profound in the center than the periphery.(Beltran et al. 2012) In XLRPA2, an accumulation of abnormal protein product in the endoplasmic reticulum was suggested to cause the rapid course and early disease onset from around 5 weeks, affecting rods and cones.(Beltran et al. 2006; Zhang et al. 2002)

Three mouse models of RPGR deficiency exist, one mouse model was designed from the deletion of RPGR exons 4-6.(Hong et al. 2000) This model, 20 days after birth, has showed reduced rhodopsin levels in rods and mislocalisation of cone opsin to inner segments, nuclear and synaptic regions. However, electroretinography (ERG) was within normal limits. After 6 months, degeneration was documented with photoreceptor loss, but the connecting cilia remained distinguishable.(Hong et al. 2000)

A naturally occurring RPGR deficient murine model is the retinal degeneration 9 (Rd9) strain of mice. RPGRORF15 levels are undetectable in the retina of affected male mice, which is caused by duplication of a 32-base pair in ORF15 that leads to a truncated

protein product.(Thompson et al. 2012) Deletion of exon 1 has resulted in another RPGR deficient model, (Huang et al. 2012), with a similar phenotype to the aforementioned murine model.

Transgenic mice have been generated with mutant RPGR ORF15 into RPGR knockout backgrounds and wild type,(Hong et al. 2004) which are very similar to XLPRA2, but differ from the RPGR null mice in being more severe (Hong and Li 2002)

Photoreceptor rescue has been reported in both murine and canine models with adeno-associated virus (AAV) gene augmentation. (Pawlyk et al. 2016; Beltran et al. 2012(Hong et al. 2005)) In a canine model, late retinal degeneration arrest was documented which suggested a wide therapeutic window.(Beltran et al. 2015) ORF15 DNA sequence variations were noticed in this AAV vector and are likely caused by the repetitive purine nucleotides.(Deng et al. 2015) This inherent mutability has been addressed with codon-optimisation(Fischer et al. 2017) and repetitive sequence abbreviation.(Pawlyk et al. 2016; Hong et al. 2005)

Structural and functional defects were detected in transgenic mice that were engineered with multiple copies of *RPGR* in their genome, where the copies of *RPGR* were proportionate to the disease severity.(Brunner et al. 2008) Mice that carried 8-10 copies had no sperm flagella detected, while other mice with 4-5 copies had lower sperm levels. These observations are relevant when considering the potential deleterious effects of *RPGR* overexpression.(Georgiou et al. 2021b)

RPGR Phenotypes and Clinical features

A range of phenotypes are caused by *RPGR* pathogenic variants, which includes RCD (also known as RPGR associated RP), early-onset severe retinal dystrophy (EOSRD), CORD, cone dystrophy (COD), macular atrophy, and syndromic XLRP. The most common presenting conditions, RCD and CORD, are described below.

***RPGR*-associated Rod-Cone Dystrophy**

XLRP is the most common phenotype associated with *RPGR*. It is also one of the most severe forms, in which patients present with nyctalopia as early as in the first decade and progress to blindness in their thirties or forties.(Sandberg et al. 2007) Electrophysiological changes are detected in childhood, as well as myopia and retinal abnormalities.(Flaxel et al. 1999)

On retinal examination, affected males have an accumulation of pigment that reflect the byproduct of photoreceptor outer segment and retinal pigment epithelium (RPE) degeneration (**Figure 1**). Changes in the photoreceptor layer are readily documented and monitored over time with OCT, in the form of disruption in the ellipsoid zone (EZ) (**Figure 1**). This disruption starts typically in the rod dense region at the retinal periphery and progresses gradually towards the center of the fovea. A mean rate of EZ decline of 0.67 mm^2 was documented in 38 RPGR RP patients using *en face* images of the macular OCT volume scans.(Tee et al. 2019)

Functional disease progression using retinal sensitivity decline has been reported with static perimetry.(Tee et al. 2018) Microperimetry (MP) testing has also documented objective assessment of retinal sensitivity change over time, and may in addition to

static perimetry, be valuable in assessing gene therapy outcomes. In a recent report, MP testing was performed in 76 individuals (53 adults, 23 children) with *RPGR* retinopathy, who were followed for 2.8 years. Strong correlation of the baseline best corrected visual acuity (BCVA) and contrast sensitivity (CS) with the mean sensitivity (MS) and volumetric indices was statistically significant, while the rate of progression in the ORF15 genotype subgroup was comparable to that of the subgroup with disease-causing variants in exons 1 to 14. Most patients investigated in the study lost retinal sensitivity rapidly during their second and third decades of life.(Anikina et al. 2022) A faster progression rate in younger affected males was also documented by following up the constriction of the parafoveal hyperautofluorescent ring, which is reported in the majority of *RPGR* RP patients.(Tee et al. 2019)

Adaptive optics scanning light ophthalmoscopy (AOSLO) imaging enables in vivo noninvasive visualization of the cone mosaic.(Georgiou et al. 2018) With the good repeatability previously reported in *RPGR*-XLRP, (Tanna et al. 2017) AOSLO may provide new insights into possible mechanisms of cone vision loss in patients with retinal degeneration and a more rapid trial readout.

***RPGR*-associated COD and CORD**

RPGR variants are responsible for 1-2 % of all cases of COD and CORD phenotypes.(Gill et al. 2019) These are progressive phenotypes, that present in the second to fourth decade of life and primarily affect cones, albeit in the first instance. Patients initially experience symptoms of central visual field defects, reduced visual

acuity and colour vision, and photophobia.(Ebenezer et al. 2005; Vervoort et al. 2000) Although ophthalmoscopic findings are often most evident at the macula, symptoms (and signs) of rod involvement occur in the majority of patients over time.(Michaelides et al. 2006) Patients with XLCORD/COD typically also present with moderate to high myopia. A correlation between higher rates of vision loss and greater degrees of myopia have been previously described.(Talib et al. 2019)

XLCORD patients classically harbor *RPGR* ORF15 variants that are frequently located 3' to the highly repetitive region; while OFR15 variants that are located 5' typically lead to RCD (RP). (Michaelides et al. 2006; Branham et al. 2012) However, exceptions have been reported, including in an *RPGR* XLCORD pedigree with a frameshift insertion detected 5' to the repetitive region of ORF15.(Mears et al. 2000)

On fundoscopy, a range of macular changes are observed, from mild disturbance of the RPE to severe chorioretinal atrophy. Fundus autofluorescence imaging may identify a parafoveal hyperautofluorescent ring, which unlike those in RP, increase in size with disease progression, associated with worsening amplitude of pattern electroretinogram P50. (Robson et al. 2008b, 2008a)

OCT typically shows early foveal EZ disruption, which is usually followed by increasing gradual disruption extending into the periphery (**Figure 2**).

Female Carriers

RPGR-carriers are frequently asymptomatic or mildly affected.(Ebenezer et al. 2005) Only a minority exhibit severe RP/EOSRD or CORD.(Georgiou et al. 2021a) However,

on examination, 40% of female carriers have the typical XLRP macular tapetal-like reflex, which is best seen with FAF, but can also be seen clinically. It appears in the posterior pole as a hyperreflective radial spoke-like pattern with a golden sheen.(Talib et al. 2018, 2019) (**Figure 3**) Cellular imaging of the Tapetal-Like Reflex (TLR) areas in carriers of *RPGR*-associated retinopathy showed increased rod photoreceptor reflectivity on confocal AOSLO and reduced cone photoreceptor densities. Moreover, increased reflectivity of the outer retinal bands was documented in the parafoveal TLR areas on OCT (Inner Segment Ellipsoid Zone and Outer Segment Interdigitation Zone).(Kalitzeos et al. 2019) It was suggested that mosaicism, to an extent, could be responsible for the heterogeneity seen in carriers.(Talib et al. 2018)

***RPGR* associated syndromic ciliopathy**

Ciliopathies are a group of genetic conditions caused by defects in cilia, which is currently an established cause of retinal dystrophy due to the important role of retinal proteins in cilia maintenance and function.(Wheway et al. 2014) Clinical evidence of *RPGR*'s role in cilia function is highlighted by certain disease-causing variants resulting in patients with *RPGR* XLRP also having hearing impairment, respiratory tract infection and bronchiectasis. (Zito et al. 2003) It is believed that ORF15 may not be involved in extraocular *RPGR* phenotypes, as all variants reported to date are in exons 1-14.(Tee et al. 2016)

Treatment Principles

The management of *RPGR* related retinopathy remains supportive, with treatment being available only for complications, such as cataract and cystoid macular oedema.

Different treatment approaches have been explored aiming to improve vision or halt/slow disease progression. Neuroprotection and dietary supplements of high doses of vitamin A and docosahexaenoic acid (DHA) were investigated in previous trials and neither modality showed visual therapeutic benefits.(Birch et al. 2013; Hoffman et al. 2004; Berson et al. 2004, 1993) Gene replacement therapy is becoming a promising treatment option, which is currently being investigated in phase 1/2 and phase 3 trials following success in multiple animal models.(Beltran et al. 2015, 2012a; Pawlyk et al. 2016)

Retinal prosthesis, such as The Argus II retinal system (Second Sight Medical Products) is another approach that has been explored to aid blind or severely visually impaired patients with RP (Ho et al. 2015). Argus II retinal prosthesis, also called 'Bionic eye' was FDA approved in 2013(Greenemeier 2013) to be used by RP patients aged above 25 years, with vision level of light perception or no light perception in both eyes with previous history of vision; although it has now been discontinued. Other approaches for advanced visual loss include optogenetics and stem cell therapies (please see Chapiters X and Y)

Gene Replacement therapy

With the evolution in genetics over the last 20 years, gene replacement trials are now significantly facilitated by the application of engineered viruses as vectors introducing

functional genetic material. Adeno-associated virus (AAV) is the vector of choice for retinal gene therapy given its small size, non-pathogenic in humans, weaker post-injection immune response and being simple and amendable to engineering.(Naso et al. 2017)

Delivery of subretinal AAV gene therapy has been performed in three phase 1/2 clinical trials (NCT03316560, NCT03252847, and NCT03116113), as well as in an on-going phase 3 clinical trial (NCT04671433). (**Table 1**) Whereas NCT04517149, is a phase 1/2 trial of a single intravitreal injection of AAV-RPGR.

NCT03116113 consisted of a dose escalation phase where eighteen patients received subretinal AAV8 encoding codon-optimized human RPGR (AAV-coRPGR), and were followed for 6 months. The early findings included subretinal inflammation in some patients, which was steroid responsive; with the study achieving the prespecified safety endpoint. Retinal sensitivity improvement on mesopic MP was recorded in 6 patients at 1 month, which was variably sustained throughout the follow up period.(Dufour et al. 2020)

Safety and efficacy of AAV5-RPGR is investigated in NCT03252847. The primary endpoint of this clinical trial was to accomplish absence of safety events, while the secondary measures are the improvement of vision, retinal sensitivity, vision guided mobility and better quality of life, assessed using quality-of-life (QOL) questionnaires. The one-year results from the dose escalation phase were presented (n=10, AAO 2020 and ARVO 2022) and described well tolerated AAV5-RPGR, static perimetry and MP improvements in addition to enhanced vision-guided mobility.(Georgiou et al. 2021b)

The number of patients to acquire clinically relevant hematology and chemistry adverse events is the primary outcome of NCT03316560 which investigates rAAV2tYF-GRK1-RPGR. The change from baseline in vision by ETDRS, perimetry, retinal structure and QOL questionnaire are the secondary outcome measures of the trial. The reported results of the dose escalation phase identified improvements in retinal sensitivity on MP.

In NCT04517149, safety and efficacy are investigated by a single intravitreal injection of AAV-RPGR at 2 dose levels.(Georgiou et al. 2021c)

The favorable results observed at the low and intermediate doses in term of both safety and efficacy are being further investigated in a randomized controlled phase 3 clinical trial (NCT04671433), which raises the anticipation and hopes for establishing a possible treatment in the near future.

Concluding remarks and future prospects

Inherited retinal diseases carry undeniable disease burden (Liew et al. 2014; Galvin et al. 2020). Recent advances in both genetic engineering and retinal imaging are contributing to the rapid progress in the field of inherited retinal disease. *RPGR*-associated disease is at the severe end of the phenotypic spectrum, leading to progressive vision loss and eventual blindness. Multiple novel approaches are under investigation aiming for vision restoration and to halt/slow degeneration.

Gene editing and post-transcriptional regulation as in Clustered regularly interspaced short palindromic repeats (CRISPR) and their associated enzyme (Cas), antisense oligonucleotides (AON), stem cell therapies, retinal implants and optogenetics are all

currently being investigated in the search for a cure for RP. The promising positive safety and efficacy results of gene therapy in phase 1/2 have supported the advancement of an *RPGR* XLRP gene replacement therapy, raising the anticipation for on-going and upcoming phase 3 clinical trials, hoping to result in the long awaited approved treatment for *RPGR* RP.

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Figure Legends

Figure 1: *RPGR*-associated rod-cone dystrophy

Colour fundus photograph of the right eye of a 29-year-old patient with *RPGR*-associated retinitis pigmentosa showing extensive peripheral retinal bone spicule pigmentation and atrophy (top); Optical coherence tomography (bottom) showing macular thinning and loss of outer retinal architecture peripherally, with relative preservation centrally.

Figure 2: *RPGR*-associated cone-rod dystrophy

Fundus autofluorescence image (top) showing parafoveal hyperautofluorescent ring with corresponding ellipsoid zone disruption on optical coherence tomography (below).

Figure 3: Retinal imaging in a female *RPGR*-retinopathy carrier

Fundus autofluorescence image of the left eye of an asymptomatic 45-year-old female carrier showing a radial pattern 'tapetal' reflex. Optical coherence tomography of the right eye shows preserved retinal lamination (below).

Tables:

Clinicaltrials.gov Identifier	Intervention	Transgene details	Phase
NCT03252847	AAV5-RPGR	Shortened ORF15	I/II
NCT03116113	AAV8.GRK1.RPGR	Codon-optimised	I/II
NCT03316560	AAV2tYF.GRK1.RPGR	Codon-optimised	I/II
NCT04517149	AAV-RPGR (4D-125)	Codon-optimized	I/II
NCT04671433	AAV5-RPGR	Shortened ORF15	III

Table 1: RPGR Gene Therapy Clinical Trials