Advances in RPGR gene therapy for X-linked retinitis pigmentosa: From preclinical insights to clinical application (Review)

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Abstract. X-linked retinitis pigmentosa, primarily caused by mutations in the *retinitis pigmentosa GTPase regulator (RPGR)* gene, represents one of the most severe forms of inherited retinal degeneration, with early onset and rapid progression. Conventional interventions, such as vitamin A or docosahexaenoic acid supplementation, offer limited benefits and fail to halt disease progression. By contrast, gene therapy has emerged as a promising approach to alter the disease course. The present review summarizes the clinical phenotypes and pathogenic mechanisms associated with *RPGR* mutations, focusing on their disruption of ciliary transport and metabolic homeostasis. The present review further discusses advances in preclinical models, including mice, dogs, zebrafish and induced pluripotent stem cell-derived organoids, that have facilitated the development of *RPGR*-targeted therapies. Adeno-associated virus-based gene replacement has shown efficacy in restoring retinal structure and function, and several approaches have progressed to early-phase clinical trials. Despite encouraging outcomes, challenges such as RPGR coding sequence instability, vector delivery efficiency and long-term safety remain. The present review integrates current mechanistic understanding and therapeutic progress, providing a translational perspective for precision treatment of *RPGR*-associated retinal diseases.

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Contents

1. Introduction

Retinitis pigmentosa (RP) is an inherited retinal disease (IRD) characterized by progressive degeneration of photoreceptor cells, with a global prevalence of ~1/4,000, and it currently has no effective cure (1-4). X-linked RP (XLRP) is one of the most severe forms of RP. Mutations in the *retinitis pigmentosa GTPase regulator* (*RPGR*) gene are the main cause of XLRP and are also associated with cone-rod dystrophy (CORD) (5-9).

The *RPGR* gene is located on the short arm of the human X chromosome (Xp11.4) (3,10). RPGR proteins interact with other proteins at the connecting cilium (CC) of photoreceptor cells to form a complex regulatory network, which maintains the functional stability of the CC by coordinating key processes such as vesicle transport. Mutations in the *RPGR* gene lead to loss of RPGR function. This disruption impairs the normal protein transport system in photoreceptor cells, which subsequently disturbs metabolic and synthetic homeostasis [such as, renewal of outer segment (OS) disc membranes] and alters light signaling cascades. Together, these changes ultimately lead to photoreceptor damage and retinal structural degeneration (1,11,12).

Although considerable progress has been made in gene therapy research on *RPGR* mutations worldwide and multiple relevant animal models have been established, the key pathogenic mechanisms and pathological features underlying RP caused by *RPGR* mutations remain incompletely elucidated. Moreover, the translation of these findings into clinical applications continues to face key challenges. For patients with

RPGR mutation-associated retinal degeneration, the development of highly sensitive genetic detection technologies (such as long-read sequencing) and functional validation systems is important. A systematic analysis of the population genetic characteristics and the pathogenic mechanisms of *RPGR* gene mutations will provide a theoretical basis for individualized diagnostic stratification and targeted gene therapy. The present review assesses the types of *RPGR* gene mutations, pathogenic mechanisms and their effects on photoreceptor cell function reported in recent years. In the present review, the research progress in cross-species models (such as mouse, dog and zebrafish) is summarized and the effectiveness of existing gene therapy strategies [such as adeno-associated virus (AAV)-mediated gene replacement and CRISPR-Cas9 gene editing] in preclinical models is evaluated. Meanwhile, preclinical studies of RPGR gene therapy are also discussed and recent clinical advances are analyzed.

2. Clinical and genetic features of *RPGR*-associated retinopathies

Based on the inheritance pattern, RP can be categorized into four types: Autosomal recessive (AR) RP, autosomal dominant (AD) RP, X-linked RP and mitochondrial RP (Fig. 1A) (13). Global statistics show that the total number of patients with RP is >1 million (3,10,13,14). Clinical statistics show that AR accounts for ~50% of cases, whereas AD accounts for ~30% (15,16). XLRP has a relatively low prevalence of ~20%, but its patients are found across all age groups (17,18). Individuals with RP show considerable differences in disease severity and progression rates, and this heterogeneity is determined by both the type of causative mutation and the molecular mechanisms that the mutation mediates (3). In addition, >100 causative genes have been identified (2,4,10,12). The majority of patients with RP show an age-related pattern of progression. The disease initially presents with night blindness due to rod dysfunction, followed by rod degeneration that results in peripheral visual field (VF) defects. As the disease progresses, secondary cone damage leads to loss of central vision, with progressive loss of metabolic support from the retinal pigment epithelium (RPE), ultimately leading to blindness (1,11,12).

XLRP is a severe form of retinal ciliopathy, characterized by the progressive degeneration of rod and cone photoreceptor function in the retina (7). XLRP typically has a rapid onset and progression. Some patients first experience nighttime vision loss during adolescence, which progresses to pronounced night blindness in early adulthood and eventually leads to central vision impairment (19). As the disease progresses, patients are observed to have retinal vascular narrowing and bone spicule-like pigmentation on fundus imaging (2,20). The risk of blindness markedly increases in patients >50 years of age (Fig. 1). In patients with XLRP, ~70 to 80% of cases are caused by *RPGR* mutations, whereas *RP2* mutations account for ~10 to 15%. Notably, XLRP resulting from *RPGR* mutations tends to present with a more severe phenotype (20-22). Although both *RPGR* and *RP2* mutations can cause XLRP, the patterns of disease progression are distinct. Patients with *RPGR* mutations typically develop night blindness in early childhood and may experience severe vision loss in their early 20s. In addition, mutations in this gene have been associated with rod-cone dystrophy (RCD), CORD, X-linked cone dystrophy (XLCOD), X-linked macular dystrophy (XLMD) and early-onset RP (1,20,23,24). Mutations in the *RPGR* gene account for 73% of X-linked CORD cases. Affected individuals typically present with reduced visual acuity (VA), color vision deficits, central VF defects and photophobia. They usually have a late onset at ~40 years (25).

Clinical studies have found that vision loss progresses relatively rapidly in these patients, with a decline in best-corrected VA (BCVA) of ~7% per year and >60% exhibit a BCVA of 1 logMAR or worse by the age of 50 years (26,27). There is a notable phenotypic difference between male and female patients with *RPGR*-associated XLRP. Male patients typically present with progressive night blindness and peripheral VF reduction in early childhood, reaching legal blindness by ~40 years of age (3,28,29). A long-term follow-up of 74 male patients with *RPGR* mutations by Talib *et al* (24) demonstrated that the therapeutic window for gene therapy in *RPGR*-associated retinal dystrophies is relatively wide. Another 10-year study evaluating 139 male patients with *RPGR*-associated RP found that binocular VA was highly associated with age, with the difference being more pronounced in patients with worse vision (30). The study found that VA began to decline sharply at an average age of 44 years, with the median age at legal blindness being 45 years, consistent with previous findings. Women who carry XLRP display a more variable phenotype, with differing degrees of vision loss, and often exhibit color vision deficits (31).

Some individuals with a family history of the disease may exhibit X-linked dominant traits, and this phenotypic variability may be associated with random or skewed X-chromosome inactivation (XCI) (18,29,32). Fahim *et al* (33) found that skewed XCI of the *RPGR* allele was positively associated with disease severity in a study of 77 *RPGR* mutation-carrying female patients from 41 family lines, and could serve as an important predictor of disease progression. Notably, the same *RPGR* gene mutation may present with different phenotypes and severities in different patients or even among members of the same family, suggesting that additional factors may influence disease development. Tuekprakhon *et al* (34) reported that an 8-year-old individual who carried XLRP showed only mild early-onset progressive CORD, although they carried the same disease-causing mutation as their affected father. Recent studies have also found that some female patients who carry *RPGR* mutations exhibit increased levels of myopia (31,33,35-40). In a case study, Seliniotaki *et al* (31) described a 4-year-old girl with no family history or other notable medical history, but whole exome sequencing revealed a pathogenic heterozygous stop codon variant c.212C>G (p.Ser71Ter) in the *RPGR* gene, accompanied by XCI. These findings highlight the complexity of phenotypic variation associated with the *RPGR* mutation and the potential role of XCI in modulating disease severity.

The study of *RPGR*-associated XLRP faces two major problems. First, the difficulty of obtaining data from patients at different stages of the disease course limits a detailed understanding of pathogenic mechanisms. Second, the high genetic heterogeneity of *RPGR*-associated XLRP complicates the generalization of the results of individual studies to the entire spectrum of the disease. This poses a challenge for studying the pathogenic mechanisms of *RPGR*-associated XLRP.

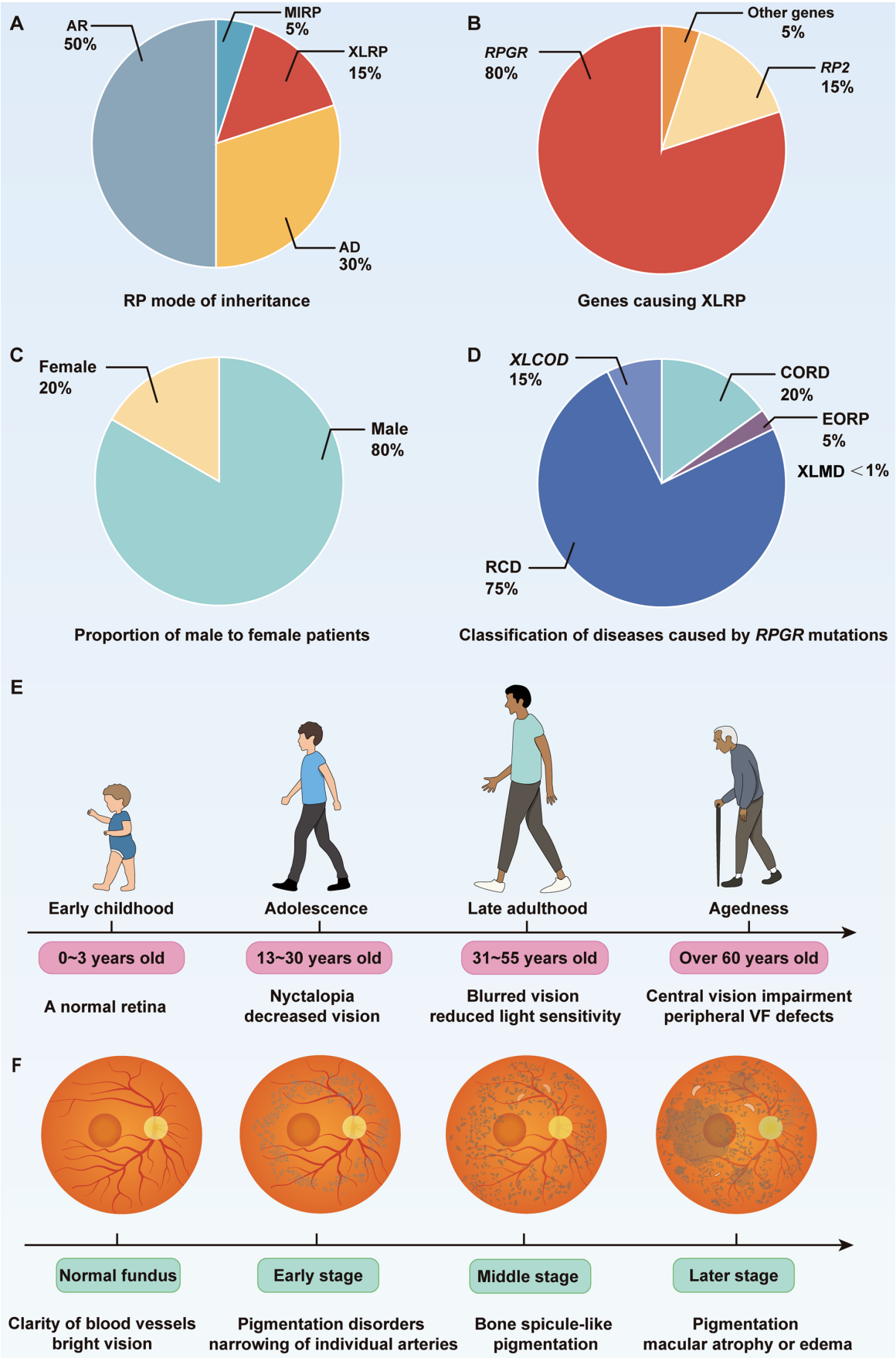

Figure 1. Patterns of inheritance and characteristics of disease progression in RP. (A) RP mode of inheritance. (B) Genes causing XLRP. (C) Proportion of male to female patients. (D) Classification of *RPGR*-associated diseases. (E) Disease course and (F) clinical manifestations. Early stage: Night blindness and reduced light sensitivity; middle stage: Central vision and peripheral VF defects; late stage: bone-spicule-like pigmentation and macular atrophy. Comparison of the diseased retina with the normal retina shows pathological features such as vascular narrowing of the blood vessels and pigmentation abnormalities. RP, retinitis pigmentosa; XLRP, X-linked retinitis pigmentosa; *RPGR*, *retinitis pigmentosa GTPase regulator*; VF, visual field.

3. Molecular features of RPGR and disease associations

The complete sequence of the human *RPGR* gene in the GRCh38 reference genome is 58,347 bp (NC_000023.11) and includes all introns and exons. The gene produces multiple splice variants at the transcriptional stage through extensive alternative splicing, thereby generating functionally distinct isoforms. The *RPGR* gene generates >10 alternative transcripts, of which at least five (for example, $RPGR^{ORF15}$ and $RPGR^{ex1\text{-}19}$) have been shown to encode functional proteins (41). It has been reported that $RPGR^{ex1\text{-}19}$ is widely expressed in a variety of tissues, while the expression level of $RPGR^{ORF15}$ is highest in retinal tissues (10).

The $RPGR^{ex1\text{-}19}$ transcript (NM_000328.3) includes exons 1-19, with a full length of 3,053 bp, encoding an 815-amino-acid protein with a molecular weight of ~90 kDa (41-42). The transcript is expressed in several human tissues, including the testis, kidney, lung, RPE and photoreceptors. In the retina, the primary function of this transcript is to maintain the structural integrity and functional stability of photoreceptor cells. The $RPGR^{ORF15}$ transcript (NM_001034853) consists of exons 1-14 and an open reading frame (ORF15) derived from the variably spliced exon 15 and intron 15. It is 4,377 bp in length, encodes a protein of 1,152 amino acids and is localized primarily to the CC of photoreceptor cells (Fig. 2A) (34,36). The ORF15 region is a specialized exonic region of the *RPGR* gene with a highly repetitive sequence rich in purines [adenine (A) and guanine (G)]. It encodes a region of the protein consisting of low-complexity sequences with high glutamate (Glu) and glycine (Gly) content. The region is ~1 kb and contains 567 amino acids. A probe targeting exons 16-19 detected an 8.9 kb band but failed to detect a 5 kb band for exons 3-10, 14 and 15 (24). Therefore, exon ORF15 may function as a terminal exon or be co-spliced with exons 16-19. However, ORF15 contains an internal stop codon and does not include exons 16-19. To the best of our knowledge, to date, no detailed studies focusing on mutations in exons 16-19 have been reported.

The exon and intron structures and mRNA expression patterns of *RPGR* transcripts from different species differ (Fig. 2B-D). For example, the murine $Rpgr^{ORF15}$ type (NM_001177950) is 3,604 bp in length and encodes 1,001 amino acids, whereas the murine $Rpgr^{ex1\text{-}19}$ type (NM_001177951) is 3,116 bp in length and encodes 934 amino acids. To further assess the evolutionary conservation among species, it is necessary to determine the chromosomal localization of the *RPGR* gene. *Rpgr* is located at 4.62 cM of the mouse X chromosome genetic map, while in rats it maps to Xq12. In zebrafish, two transcripts, *rpgra* and *rpgrb*, are located on chromosomes 9 and 11, respectively. In dogs and rhesus monkeys, the gene is also situated on the X chromosome, the chromosomal position most similar to that of human *RPGR*.

The two major *RPGR* transcripts share an identical N-terminal structure, that is, exons 1-14. Mutations within exons 1-14 are typically associated with earlier disease onset, faster progression and more severe visual impairment. Patients carrying mutations in this region generally exhibit worse visual preservation compared with those harboring ORF15 mutations (43). Statistical analyses show that several individuals with exon 1-14 mutations develop night blindness and VF defects at the age of ~10 and often present with moderate to severe RP. Missense mutations, small insertions or deletions, and nonsense mutations are the most common types observed (Fig. 2E). Mutations within the ORF15 region account for ~66% of XLRP cases, making it the most prevalent pathogenic hotspot in human XLRP (7,18,44). However, ORF15 mutations generally exhibit a milder phenotype, with later onset and slower disease progression (Fig. 2F). In addition to the aforementioned mutation types, ORF15 variants may also introduce aberrant splicing sites, thereby affecting normal mRNA processing (10,32,44-46). Moreover, mutations in this region have been implicated in X-linked CORD and XLMD. These phenotypic differences highlight the complex relationship between gene structure and function. Understanding this structure-function-phenotype association not only provides insight into the molecular pathology of *RPGR*-related diseases but also lays the theoretical foundation for the development of precision therapies targeting specific transcripts or mutation types. The following section will further explore the mechanisms by which *RPGR* mutations lead to photoreceptor dysfunction and retinal degeneration.

4. Mechanistic insights into RPGR-associated retinal degeneration

Photoreceptor architecture and the functional significance of RPGR. The retina is located at the back of the eye as a curved, multilayered structure comprising photoreceptors, bipolar cells and ganglion cells (Fig. 3A and B). From the outside to the inside, the retinal structure includes the RPE, outer nuclear layer (ONL), outer plexiform layer, inner nuclear layer (INL), inner plexiform layer, ganglion cell layer (GCL) and nerve fiber layer, each of which contributes to the capture and processing of light signals (47-49). Photoreceptors are responsible for detecting light and converting it into electrical signals (50). Rods, which are primarily distributed in the peripheral retina, contain rhodopsin and mediate scotopic vision (51). By contrast, cones are concentrated in the central region and contain three distinct opsins that mediate color discrimination and fine VA under bright light conditions (51,52). Both cell types consist of four main components: The synaptic terminal, the inner segment (IS), the OS and the CC linking the IS and OS (Fig. 3C). The OS is a highly specialized primary cilium characterized by densely stacked membranous discs that serve as the central site of phototransduction. The IS contains organelles such as mitochondria, endoplasmic reticulum and Golgi apparatus, which sustain the high metabolic activity required for photoreceptor function. The CC is structurally homologous to the transition zone of the primary cilium and is essential for the efficient trafficking of proteins and lipids. Owing to their exceptionally high metabolic demands, photoreceptors rely on efficient transport of proteins synthesized in the IS through the CC. This transport is essential to sustain their structure and function (7). As a key component of this intersegmental transport system, the CC requires specific proteins to preserve its structural stability and regulate molecular trafficking. Among these proteins, RPGR acts as a ciliary-associated protein, carrying out a central role in sustaining these processes.

The N-terminus of the RPGR protein contains six complete tandem repeats, each comprising 52-54 amino acids (32). It

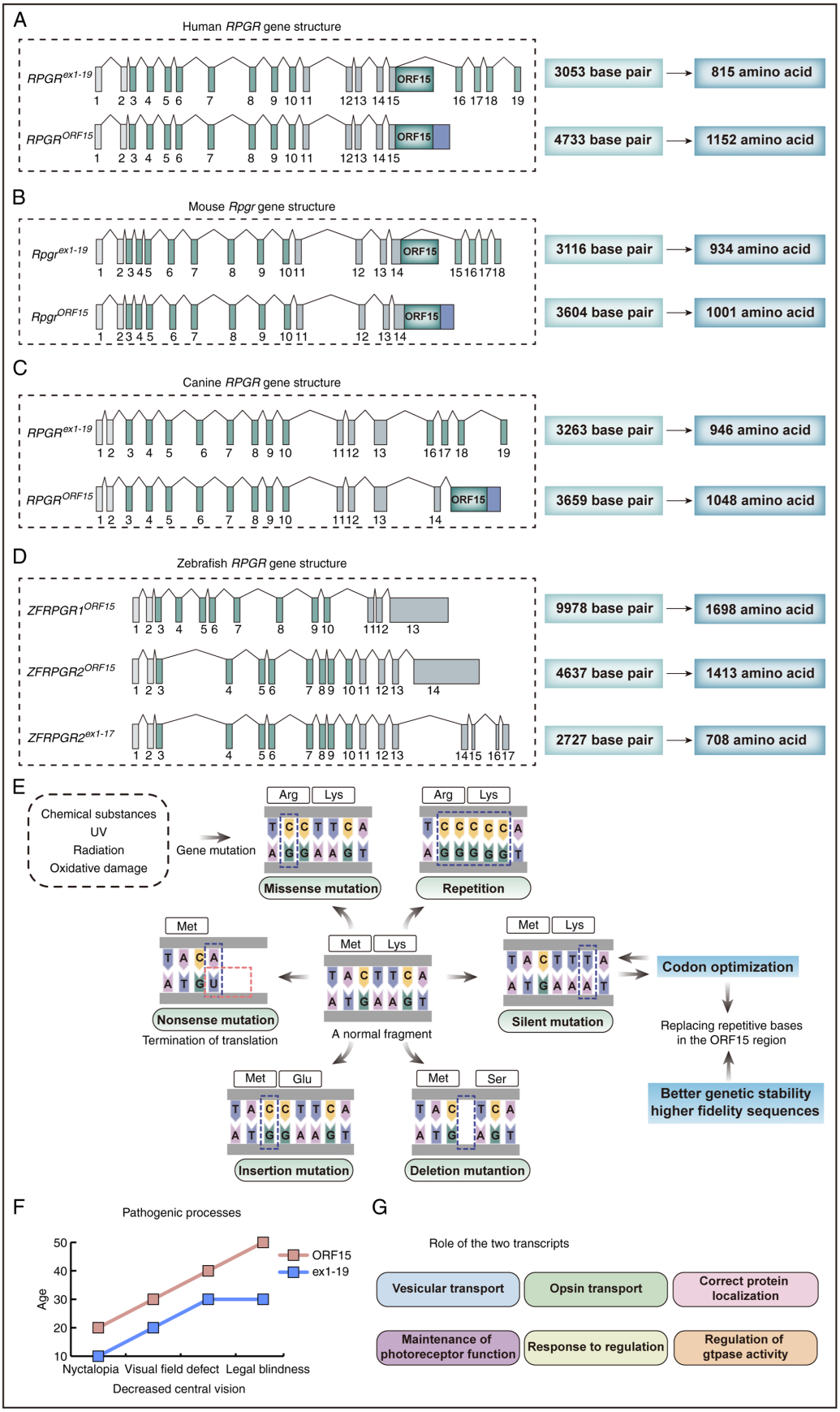

Figure 2. Information on the structure and mutation of *RPGR* genes in different species. Structure of the *RPGR* gene in (A) human, (B) mice, (C) canines and (D) zebrafish, including exon-intron composition, number of base pairs and amino acid length of the different transcripts. (E) Common mutations in RCC1-like domains. (F) Pathogenic processes resulting from mutations in the two transcripts leading to age-dependent phenotypic changes. The horizontal axis represents age, and the vertical axis reflects the severity of ocular symptoms. From left to right, the symptoms progress from night blindness to central vision loss, VF defects, and eventually legal blindness, illustrating the trajectory of disease development under different transcript mutations. (G) Role of the two transcripts in the organism. *RPGR* gene, *retinitis pigmentosa GTPase regulator* gene; VF, visual field.

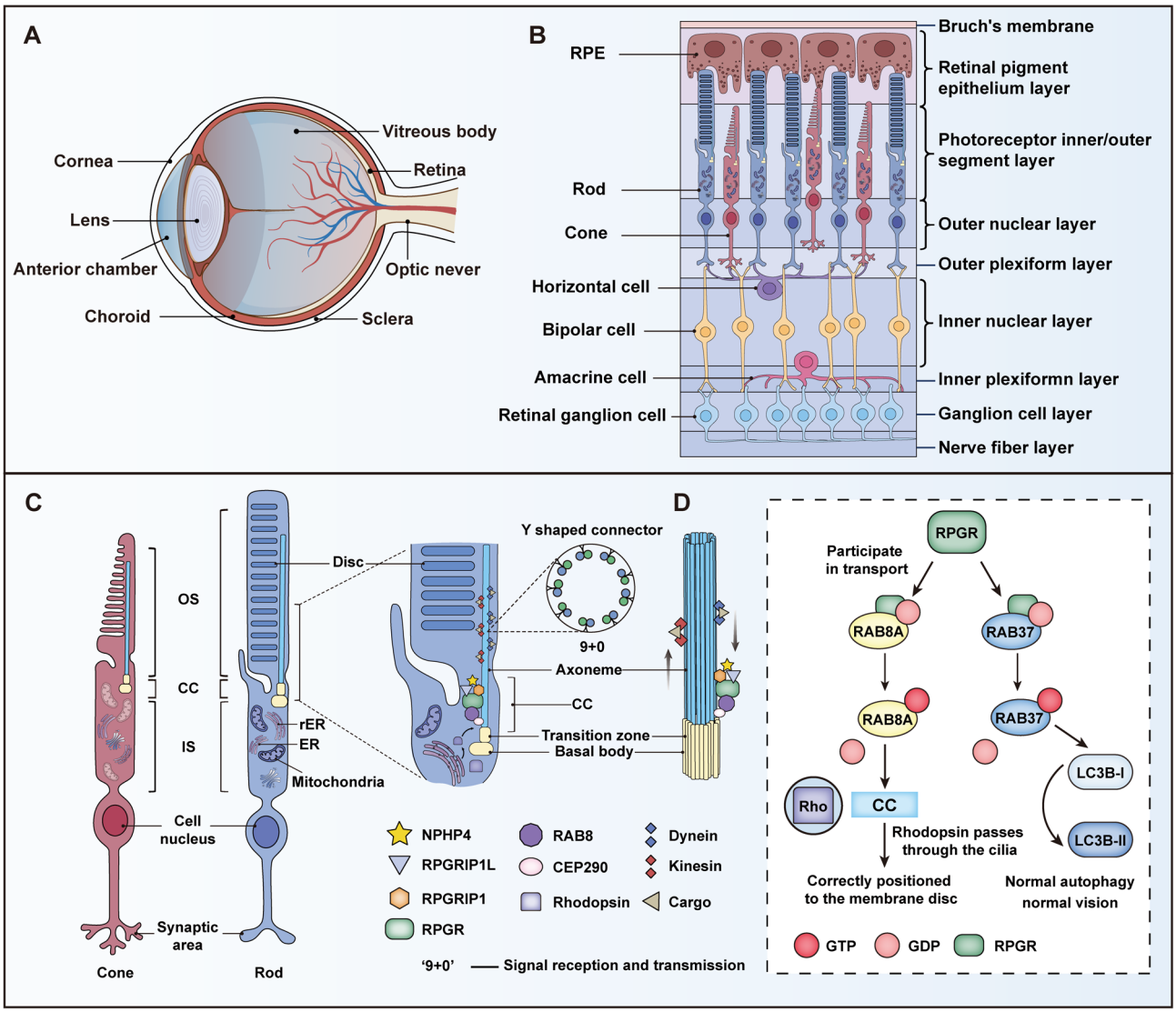

Figure 3. Structure of the eye and the role of RPGR in photoreceptors. (A) Layered structure of the eye and retina. (B) Retinal layers organized into three functional domains: The support layer (BrM; RPE), the light-signal-processing layer (OS, IS and ONL) and the neurointegrative layer (OPL, INL, IPL, GCL and NFL). (C) Photoreceptor subcellular structures and the '9+0' signaling axis, representing the microtubule arrangement pattern unique to non-motile cilia. (D) Mechanisms of internal ciliary transport involving the RPGR complex. IFT: KIF3A and Dynein mediate bi-directional cargo transport. RAB8A participates in vesicle transport and regulates photoreceptor OS disc membrane renewal. Mechanisms involving RPGR, RAB8A and RAB37 in vision-related physiological processes. RPGR participates in the transport processes involving RAB8A and RAB37. These GTPases exert their functions through cycling between GTP-bound and GDP-bound states. RAB8A is involved in the correct localization of RHO to CC, thereby maintaining normal vision. RAB37 facilitates the conversion of LC3B-I to LC3B-II to support normal autophagy, which in turn helps maintain vision. Key regulatory proteins: RPGR (green), RPGRIP1 (orange), NPHP4 (earthy yellow), RAB8A (bright yellow), GTP (red), GDP (light red), RHO (purple), CC (blue), RAB37 (blue), LC3B-I (light blue), LC3B-II (dark blue). BrM, Bruch's membrane; RPE, retinal pigment epithelium; OS, outer segment; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer; NFL, nerve fiber layer. RPGR, retinitis pigmentosa GTPase regulator; RHO, Rhodopsin; RPGRIP1L, RPGRIP1-like protein; RPGRIP1, RPGR-interacting protein 1; RAB8A, RAS-related protein Rab-8A; IFT, intraflagellar transport; KIF3A, kinesin family member 3A; NPHP4, nephrocystin 4; CC, connecting cilium; LC3B-I, microtubule-associated protein 1 light chain 3 β-I; LC3B-II, microtubule-associated protein 1 light chain 3 β-II.

has structural homology with Regulator of Chromosome Condensation 1 (RCC1), forming RCC1-like domains (RLDs) (53). The RLDs possess nucleotide exchange factor (GEF) activity, promoting the conversion of small GTPases from the GDP-bound to GTP-bound forms and thereby carrying out a key role in ciliary function and intracellular transport. RPGR is predominantly localized in the CC between the IS and OS of the photoreceptor (Fig. 3C) (3,8,54,55). In the retina, the *RPGR* gene primarily produces two major isoforms, $RPGR^{ex1\text{-}19}$ and $RPGR^{ORF15}$. Both isoforms contain identical RLDs (exons 1-14) at the N-terminus but differ markedly in their C-terminal sequences. $RPGR^{ex1\text{-}19}$ contains an isoprenylation domain that mediates its localization to the CC. By contrast, $RPGR^{ORF15}$ features a unique C-terminal ORF15 region rich in glutamic acid and glycine residues. Early studies suggested that RPGR might not be essential for photoreceptor development and early function (55-58). However, subsequent studies have confirmed that RPGR is indispensable for protein trafficking, ciliary stability and signal maintenance during photoreceptor maturation. It also serves as a 'longevity factor' key for preserving their structural and functional integrity (7,48).

Disruption of RPGR-mediated protein trafficking and proteostasis leads to photoreceptor degeneration. RPGR carries out a key role in maintaining ciliary function and stability, as well as in regulating opsin transport, vesicular trafficking and proper protein localization. It forms complexes with multiple cilia-associated proteins, including RPGR-interacting protein 1 (RPGRIP1), RPGRIP1-like protein (RPGRIP1L), centrosomal protein 290 (CEP290) and IQ motif containing B1 (IQCB1) (59). Among these, RPGRIP co-localizes with RPGR in the photoreceptor OS and CC, contributing to structural maintenance and protein trafficking (45,56). Distinct RPGR-associated complexes function at different stages of molecular trafficking, and loss of RPGR function disrupts its pivotal role within the ciliary transport network. Under normal conditions, RPGR forms complexes with RPGRIP1 and IQCB1 to mediate rhodopsin trafficking. When the interaction between RPGR and either RPGRIP1 or IQCB1 is disrupted by RPGR mutations, the severity of the associated disease is further exacerbated (18,59). In wild-type photoreceptors, RPGR interacts with CEP290 within the transition zone (60). However, in *RPGR* mutant mice, this interaction is altered, leading to transition zone defects that impair molecular transport. $RPGR^{ORF15}$ interacts with transition zone proteins such as CEP290 to facilitate the transport and renewal of photoreceptor discs in the photoreceptor OS. This interaction helps maintain the integrity of the transition zone and the Y-link structure (Fig. 3C).

In addition to its interactions with RPGRIP1 and IQCB1, RPGR further facilitates molecular transport and signaling in photoreceptors by interacting proteins such as the δ subunit of rod-specific photoreceptor cGMP phosphodiesterase (PDEδ) and ADP-ribosylation factor-like 3 (ARL3). It is also involved in regulating vesicular trafficking and intracellular signaling pathways. Using a yeast two-hybrid screen, Linari *et al* (61) identified that PDEδ binds to the RLDs of RPGR. The researchers therefore proposed that RPGR mutations may cause protein mislocalization and trafficking defects, ultimately leading to retinal degeneration. RPGR also associates with ARL3, linking the cell membrane to the photoreceptor cytoskeleton and participating in specific signaling and vesicular transport processes (62,63). A study published in 2024 reported that RPGR functions as a GEF, facilitating the conversion of RAS-related protein Rab-37 (RAB37) from the GDP-bound to GTP-bound state (Fig. 3D) (64). This activation of RAB37 promotes vesicular trafficking, particularly within the lysosome-autophagy pathway, thereby supporting normal autophagic activity and visual function. The role of RPGR in maintaining photoreceptor function may therefore involve the regulation of intracellular signaling and vesicular transport. Moreover, RPGR helps preserve protein homeostasis and functional stability in mature photoreceptors by modulating proteasome activity and the expression of key transport proteins (65).

Studies have shown that *RPGR* mutations are prevalent in various ciliopathies characterized by severe photoreceptor degeneration. In a 2023 study using *rpgra* mutant zebrafish, the mutation was found to cause downregulation and mislocalization of RAS-related protein Rab-8A (RAB8A) within the cilium, thereby disrupting the trafficking of essential photoreceptor molecules (66). Similarly, a 2010 study showed that *RPGR* knockdown in hTERT-RPE1 cells resulted in shortened primary cilia and mislocalization of RAB8A (67). Proper binding of RPGR to RAB8A ensures the efficient incorporation of rhodopsin into the OS disk membranes for light-signal capture (Fig. 3D). Mutations in *RPGR* lead to abnormal accumulation of rhodopsin in the IS or CC, resulting in disorganization of the OS disk structure (66,67). Multiple *Rpgr* animal models have confirmed that such trafficking defects are a major cause of both rod and cone photoreceptor degeneration (68).

At the molecular level, RPGR encodes a protein essential for the assembly and maintenance of photoreceptor cilia, serving as a key regulator of CC integrity (55). Its N-terminal domain shares homology with RCC1 and catalyze the conversion of Ran guanosine diphosphate (RanGDP) to Ran guanosine triphosphate (RanGTP). Based on this, researchers have proposed that RPGR may regulate intraphotoreceptor protein transport through a RanGTP-dependent mechanism. Under normal conditions, the concentration of RanGTP is increased in the CC compared with the IS and this gradient is considered to underlie the directionality of protein trafficking. Loss of RPGR function may disrupt this gradient, leading to impaired opsin transport. In 2021, Moreno-Leon *et al* (7) reported that imbalanced expression of the two major *RPGR* transcripts may cause XLRP-associated ciliopathy, suggesting that transcriptional dysregulation may contribute to the pathogenesis. Furthermore, some studies have proposed a potential association between *RPGR* mutations and primary ciliary dyskinesia, suggesting that shared molecular mechanisms may underlie different ciliopathies (69-72). These findings provide new insights into the ciliary aspects of XLRP and offer perspectives for developing future therapeutic targets.

Mechanisms of RPGR gene mutations in photoreceptor cells. Mutations in exons 1-14 of the *RPGR* gene usually lead to abnormalities of the RCC1-like domain. By contrast, mutations in the ORF15 region are more complex and can affect multiple aspects, including DNA conformation, protein stability, trafficking and ciliary function (73). As aforementioned, the ORF15 region represents a mutational hotspot of *RPGR* (Fig. 4A), with the majority of pathogenic variants clustered between 949 and 1,047 bp (18,32,74). The majority of these are small deletions of 1-5 bp, typically resulting in truncated or frameshifted proteins (41). Although such truncations may retain a modest role in genomic stability, evidence from the XLPRA2 canine model shows that ORF15 frameshift mutations nonetheless cause severe retinal degeneration (32,75). Subsequent studies have shown that missense mutations disrupt the interaction of RPGR isoforms with their endogenous partners, including Inositol polyphosphate-5-phosphatase E (INPP5E), Phosphodiesterase 6 delta subunit (PDE6D) and RPGRIP1L (65,76-78) The C-terminus of $RPGR^{ex1-19}$ contains a prenylation site that regulates its interaction with PDE6D, INPP5E and RPGRIP1L (Fig. 4C) (76,78). Both *RPGR* isoforms also interact with CEP290 and INPP5E at the genetic and physical levels, and this interaction is important for maintaining the function and survival of the photoreceptor OS (7).

Mutations within exons 1-14 and the proximal ORF15 region are generally associated with RCD, whereas mutations in the distal ORF15 region are more often linked to

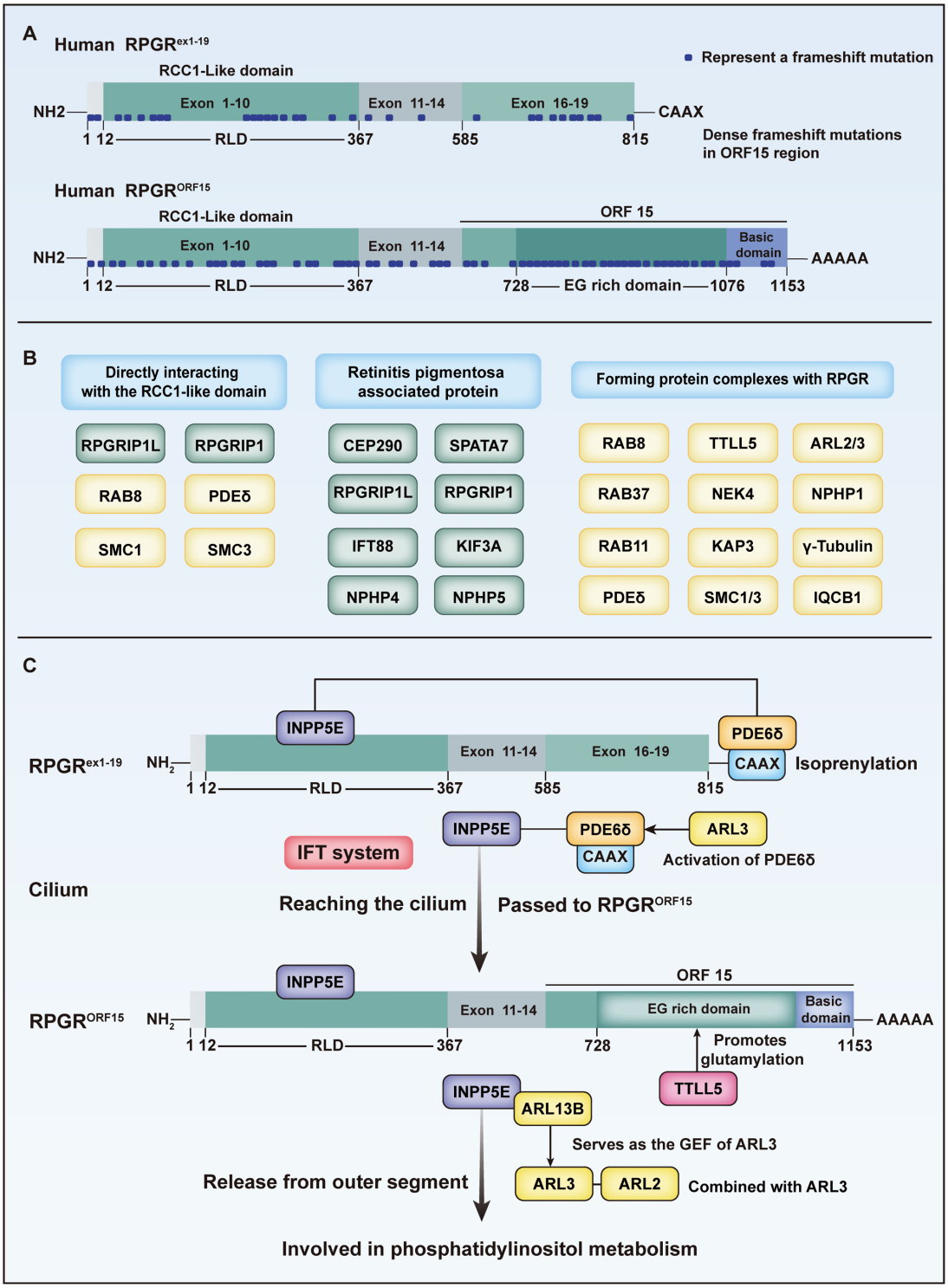

Figure 4. Interaction network of RPGR proteins and their functional mechanism in cilium. (A) Structural domain characterization of RPGR protein isoforms, where blue dots indicate sites of frameshift mutations. *RPGR*$^{ex1\text{-}19}$ contains exons 1-10, 11-14 and 16-19; its N terminus includes the RLD, and its C terminus contains several regions of unknown function. *RPGR*^{ORF15} consists of exons 1-10, 11-14 and ORF15; it contains an acidic, glutamate-rich domain (EG-rich domain) and a basic domain. (B) Interacting protein networks of RPGR. Proteins directly binding to the RLDs: RPGRIP1, RPGRIP1L, RAB8A and PDE6D. Complex-associated proteins: Cilium-transport-related proteins (CEP290, IFT88, KIF3A, RAB11 and γ-Tubulin); signaling-regulation-related [NPHP family proteins (NPHP1/4/5), TTLL5, ARL2/3]; and structure-maintenance-related (SMC1/3, SPATA7). (C) Functional pathways of RPGR in ciliary signaling. RPGR is involved in the regulation of phosphatidylinositol metabolism. INPP5E is isoprenylated through its C-terminal CAAX motif and binds PDE6D to form a complex, which ensures its proper membrane localization. ARL3 promotes the release of PDE6D in the activated state. ARL3, in its activated state, promotes the release of INPP5E from PDE6D and the dissociated INPP5E is translocated to the ciliary membrane via the IFT mechanism. ARL13B ensures the stable localization of INPP5E to the ciliary membrane by binding to INPP5E. RPGR, retinitis pigmentosa GTPase regulator; RLDs, RCC1-like domains; RPGRIP1, RPGR-interacting protein 1; RPGRIP1L, RPGRIP1-like protein; RAB8A, RAS-related protein Rab-8A; PDE6D, Phosphodiesterase 6 δ subunit, CEP290, Centrosomal Protein 290; IFT88, Intraflagellar Transport 88; KIF3A, Kinesin Family Member 3A; RAB11, Ras-Related Protein Rab-11; NPHP1, Nephrocystin 1; NPHP4, Nephrocystin 4; NPHP 5, Nephrocystin 5; TTLL5, Tubulin tyrosine ligase-like family member 5; ARL2, ADP-Ribosylation Factor-Like Protein 2; ARL3, ADP-Ribosylation Factor-Like Protein 3; ARL13B, ADP-Ribosylation Factor-Like Protein 13B; SMC1, Structural maintenance of chromosomes protein 1; SMC3, Structural maintenance of chromosomes protein 3; SPATA7, Spermatogenesis-Associated Protein 7; INPP5E, Inositol polyphosphate-5-phosphatase E.

CORD or XLCOD. The genotype-phenotype association of *RPGR* mutations is largely attributed to glutamylation. This post-translational modification carries out a key role in maintaining the structural stability of the ORF15 domain. Tubulin tyrosine ligase-like 5 (TTLL5) interacts with the basic domain of ORF15 and serves as a key regulator of its glutamylation. Loss of this modification leads to $RPGR^{ORF15}$ dysfunction and may even cause a phenotypic shift from RCD to CORD. In a cohort of 116 male patients, mutations located near the C-terminal ORF15 were found to shift the disease phenotype from rod-dominant to cone-dominant (74). Further investigations revealed that truncations in the distal ORF15 region disrupt its interaction with TTLL5, impair RPGR glutamylation and consequently result in cone photoreceptor degeneration. However, the C-terminal ORF15 domain contains 11 glutamate-rich consensus motifs, and the artificial addition of negatively charged glutamate residues in this region may alter protein folding and stability, thereby affecting protein-protein interactions (22). Therefore, therapeutic strategies involving artificial modification of the ORF15 region should be approached with caution.

Globally, the major types of *RPGR* mutations identified in patients include codon deletions, duplications, nonsense mutations and frameshift mutations (79-85). These variants can lead to abnormal protein synthesis, structural alterations, loss of function and disruption of protein-protein interaction networks (Fig. 4B), thereby compromising ciliary stability and ultimately resulting in retinal degeneration. Studies have also revealed regional differences in the distribution of *RPGR* mutations. Buraczynska *et al* (46) analyzed 80 unrelated patients with XLRP and found that the majority of RPGR mutations were located within the N-terminal RLDs. In another study, Pusch *et al* (45) screened 37 European patients with XLRP and identified mutations in the ORF15 region that resulted in premature translation termination, thereby affecting the structure and function of the RPGR protein (7,45). Similarly, in a cohort of 25 familial XLRP cases, both a previously reported mutation (c.3317) and a novel deletion mutation in the ORF15 region (c.3300_3301del) were identified. These mutations were located at the 3'end of the exon, causing premature termination of translation and the loss of 40-50 amino acids from the RPGR C-terminus (86). All affected individuals exhibited relatively preserved rod photoreceptor function despite these truncations.

Different types of mutations lead to loss of RPGR protein function, resulting in varying degrees of clinical severity. To accurately identify pathogenic variants, the ORF15 region must be analyzed using high-throughput, robust and scalable sequencing approaches (87). Future studies should first aim to precisely identify mutation sites and then develop targeted therapeutic strategies tailored to each mutation type. *RPGR*-XLRP exhibits high clinical phenotypic heterogeneity (53,88). This phenotypic heterogeneity may arise from population differences or environmental factors (89). Therefore, studying the association between different mutations and their corresponding phenotypes is important for understanding the pathogenic mechanisms of RPGR. In addition, analyzing modifier genes that can markedly influence *RPGR* mutant phenotypes is important for developing potential therapeutic strategies (53,71).

Potential involvement of RPGR in photoreceptor metabolic signaling via the mTOR/AMPK axis. Similar to other neurodegenerative diseases, RP exhibits disruptions in core cellular metabolic pathways, with its pathogenesis involving dysregulation of multi-level molecular signaling networks. Abnormalities in multiple signaling pathways have been shown to lead to photoreceptor loss and apoptosis. These abnormalities include transmembrane signaling disruptions, metabolic dysregulation, oxidative stress and apoptotic pathway activation. Given that *RPGR* mutations also contribute to photoreceptor degeneration, we hypothesized that RPGR might be associated with these signaling pathways (Fig. 5D). Metabolic homeostasis carries out a key regulatory role in photoreceptor degeneration (Fig. 5A-C). *RPGR* mutation may disrupt the metabolic homeostasis of photoreceptors through aberrant activation of the mTOR complex 1 (mTORC1) pathway, accelerating their degeneration. mTOR serves as a central regulator of cellular metabolism, primarily controlling lipid synthesis, autophagy and cell growth through the mTORC1 complex (90-92). At the mechanistic level, we hypothesized that the RPGR protein affects phosphatidylinositol metabolism in rods through interactions with PDE6D and INPP5E, leading to the degradation triggered during transport (Fig. 5E). Its deletion results in abnormally elevated levels of phosphatidylinositol (3,4,5)-trisphosphate. This elevation in turn activates the protein kinase B (AKT) signaling pathway, promotes phosphorylation of tuberous sclerosis complex 2 (TSC2) and disrupts Rheb function, resulting in continuous activation of mTORC1. This activation not only promotes anabolic metabolism but also inhibits autophagy and disrupts cellular metabolic homeostasis.

mTORC1 is a key kinase that regulates cell metabolism by balancing demand with supply (93,94). Its activity is modulated by AMPK, a central energy sensor whose impairment may cause photoreceptor degeneration by disrupting cellular energy homeostasis (95). *RPGR*-mutant mice exhibit early autophagic dysfunction, with vesicular structures progressively accumulating in photoreceptor IS (64,96). Proteomic analysis further revealed a notable reduction in mTOR protein expression. Notably, *Pde6b* mutant mice display similar metabolic alterations, including elevated pAKT levels (96). mTORC1 activity is modulated by multiple upstream signals, including AMPK and various growth factor receptors. These signals form a highly intricate feedback regulatory network that highlights its central role in photoreceptor degeneration. *RPGR* mutations likely trigger a cascade of downstream events by disrupting photoreceptor metabolic homeostasis (Fig. 5C). AMPK is an energy sensor that regulates metabolic homeostasis. Upon activation, AMPK phosphorylates TSC2 or Raptor, inhibiting protein translation and fatty acid synthesis to maintain cellular energy balance (97). In age-related macular degeneration (AMD) mouse models, treatment with glucosamine activates AMPK phosphorylation and suppresses mTORC1 phosphorylation via the AMPK/mTOR signaling pathway. This leads to a reduction in lipofuscin-like autofluorescence in the RPE (98). Meanwhile, in a study of retinal autophagy homeostasis in wAMD mice, the treatment targeting the AMPK/mTOR/hypoxia inducible factor-1α/vascular endothelial growth factor (VEGF) and AMPK/reactive oxygen species (ROS)/heme oxygenase-1/VEGF pathways

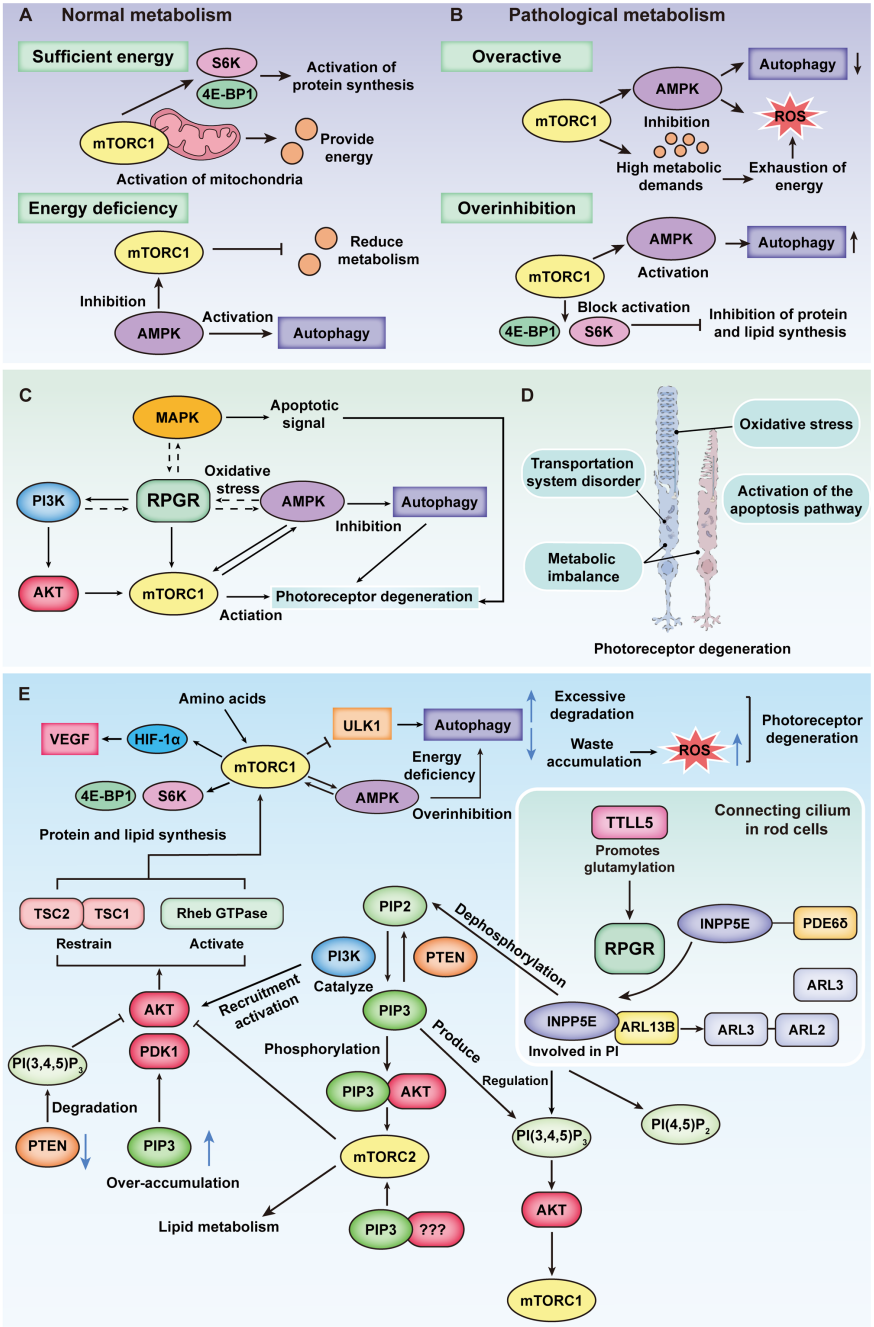

Figure 5. Impact of *RPGR*-associated phosphoinositide signaling on cellular energy homeostasis. (A) Normal metabolic conditions. In retinal cells under normal metabolic conditions, mTORC1 is activated in the presence of sufficient energy, promoting mitochondrial activity to sustain cellular survival. Additionally, mTORC1 activates S6K and 4E-BP1, thereby enhancing protein synthesis. Conversely, during energy depletion, AMPK inhibits mTORC1, reducing metabolic activity while simultaneously activating autophagy to provide an alternative energy source for the cell. (B) Pathological metabolic conditions. In pathological retinal cells, excessive activation of mTORC1 occurs when energy is abundant. Under these conditions, mTORC1 suppresses the AMPK pathway, leading to decreased autophagy and an inability to efficiently clear intracellular waste. AMPK activity also contributes to increased ROS levels. Meanwhile, the heightened metabolic demand results in energy exhaustion, further triggering ROS activation, excessive intracellular waste accumulation and oxidative stress. By contrast, severe energy deprivation leads to excessive inhibition of mTORC1, which blocks the activation of S6K and 4E-BP1, thereby suppressing protein and lipid synthesis and disrupting normal cellular metabolism. This energy-deficient state also activates the AMPK pathway, increasing intracellular autophagy. (C) RPGR may regulate photoreceptor degeneration through multiple signaling pathways. Solid lines indicate pathways supported by existing studies, such as PI3K/AKT/mTORC1 and AMPK in autophagy regulation; dashed lines represent hypothetical mechanisms suggesting potential involvement of MAPK and metabolic stress pathways. (D) RPGR dysfunction leads to structural and functional degeneration of photoreceptors. Key mechanisms include transport defects, metabolic imbalance, oxidative stress and activation of apoptotic pathways. (E) Indirect role of RPGR in phosphoinositide metabolism. mTOR, in complex with mTORC1, serves as a central regulator influenced by multiple factors, including AMPK, the TSC1-TSC2 complex and AKT. These pathways collectively modulate protein and lipid synthesis as well as autophagy. In phosphoinositide metabolism, AKT is activated via the PI3K-PIP_3 pathway, while PTEN dephosphorylates PIP_3 to maintain homeostasis. PTEN dysfunction leads to excessive PIP_3 accumulation. TTLL5 facilitates the proper glutamylation of RPGR, enabling INPP5E to function correctly within the phosphoinositide pathway, thereby indirectly regulating the mTOR pathway and sustaining normal cellular growth and metabolism. mTOR, mechanistic Target of Rapamycin; mTORC1, mTOR Complex 1; S6K, Ribosomal protein S6 kinase; 4E-BP1, Eukaryotic Translation Initiation Factor 4E-Binding Protein 1; AMPK, AMP-activated protein kinase signaling pathway; ROS, Reactive oxygen species; PI3K/AKT, Phosphoinositide 3-kinase/AKT signaling pathway; MAPK, Mitogen-activated protein kinase signaling pathway; PI3K-PIP_3, Phosphoinositide 3-kinase–phosphatidylinositol (3,4,5)-trisphosphate signaling pathway; PTEN, Phosphatase and tensin homolog; TTLL5, Tubulin tyrosine ligase-like family member 5; INPP5E, Inositol polyphosphate-5-phosphatase E; TSC1, Tuberous sclerosis complex 1; TSC2, Tuberous sclerosis complex 2.

reduced retinal damage (99). Although direct evidence of AMPK-mTOR dysregulation in the retinas of *Rpgr*-KO mice is still limited, mitochondrial stress and reduced basal respiration observed in $Rpgr^{Ex3d8}$ retinas indicate altered energy states. This suggests that *RPGR* mutations may trigger activation of the AMPK/mTOR pathway and decreased AMPK activity can compromise mTORC1 inhibition, thereby promoting increased lipid and protein synthesis. This dysregulation also impairs autophagic clearance, exacerbating the accumulation of metabolic waste products such as lipofuscin (Fig. 5B). Moreover, this metabolic imbalance is conserved across species in *RPGR*-deficient models, as lipid droplet accumulation has been found in the RPE of *rpgra*-knockout zebrafish (66). These pathological features suggest that *RPGR* mutations may disrupt retinal metabolic homeostasis by interfering with the AMPK/mTOR pathway. However, in *Rpgr*-KO mice, the activity markers of this pathway (such as p-AMPK, p-S6K and p-4EBP1) and potential functional interventions have not yet been evaluated, which is essential for developing therapies targeting metabolic dysregulation in RP.

In addition to the mTORC1 pathway, other key signaling pathways including PI3K-AKT and MAPK are also implicated in photoreceptor degeneration (96,100). The PI3K-AKT pathway carries out a key role in cell survival and metabolism, and its dysregulation may contribute to photoreceptor apoptosis by affecting downstream targets including mTORC1. Meanwhile, the MAPK pathway, particularly JNK and p38 kinases, is involved in pro-inflammatory responses and stress-induced apoptosis during retinal degeneration (101-104). Although the PI3K-AKT and MAPK pathways both carry out key roles in photoreceptor degeneration, their functional interactions during disease progression in *RPGR*-deficient models remain poorly understood. Emerging evidence indicates that the interplay between these pathways may generate a vicious cycle of metabolic stress, oxidative damage and inflammation, thereby exacerbating photoreceptor cell death. Future studies targeting these signaling cascades may uncover novel therapeutic approaches for *RPGR*-associated retinal degeneration.

Interventional therapy targeting mTOR has emerged as a potential strategy. Rapamycin, an mTOR inhibitor, enhances autophagic activity by upregulating beclin-1 and microtubule-associated protein 1 light chain 3, effectively counteracting z-VAD-induced necroptosis and markedly improving photoreceptor survival (105). Rapamycin markedly increases photoreceptor survival by activating autophagy and inhibiting the ROS-apoptosis-inducing factor pathway in the z-VAD-induced necrotic apoptosis model. However, its therapeutic potential has not yet been systematically evaluated in degeneration models caused by *RPGR* mutations. *RPGR* mutations typically result in impaired protein transport and mitochondrial stress, with hyperactivation of the mTORC1 pathway further exacerbating degeneration. Theoretically, rapamycin could alleviate endoplasmic reticulum stress by inhibiting mTORC1 and restoring autophagic activity, thereby facilitating clearance of mutant RPGR protein aggregates. It also enhances mitochondrial autophagy to reduce ROS production, inhibiting secondary necrotic apoptosis and helping maintain retinal microenvironmental homeostasis. Given the key role of the mTOR signaling axis in retinal degeneration, further exploration of the mechanisms regulating its activity in *RPGR*-associated retinopathy could provide a theoretical basis for the development of new intervention strategies (106).

RPGR, oxidative stress and apoptotic mechanisms in photoreceptors. Photoreceptor cells exhibit extremely high metabolic activity and are highly sensitive to oxidative damage. Early studies in a pig model of RP showed that following rod death, the resulting increase in local retinal oxygen tension induces oxidative stress, ultimately leading to secondary cone cell death (107). Campochiaro and Mir (108) noted that excess oxygen can disrupt the mitochondrial electron transport chain and activate NADPH oxidase. These processes promote the generation of superoxide and peroxynitrite, thereby causing sustained oxidative damage to cone cells. In RP, the gradual degeneration of cone cells following rod cell death is mainly caused by oxidative damage. Based on the metabolic imbalance observed in *RPGR* models, we hypothesize that initial rod cell injury may trigger oxidative stress in cones, thereby accelerating disease progression. Thus, antioxidant therapy shows notable potential in slowing disease progression. ERG assessments by Komeima *et al* (109) demonstrated that systemic administration of antioxidants can markedly delay functional loss in cone cells. In RD10 mice, long-term oral antioxidant treatment using compounds such as naringenin or quercetin reduced intracellular ROS levels, preserved retinal morphology and improved retinal function (110). However, the protective effects of antioxidants have not yet been validated in other mutant models, such as *Rpgr*-KO mice.

Under sustained oxidative stress, photoreceptor cells may undergo programmed cell death. The core regulatory mechanisms involve the coordinated activation of both caspase-dependent and mitochondrial pathways. *RPGR* mutations destabilize the OS disc membranes, impairing normal mitochondrial function. Aberrantly activated caspase-3 and caspase-9 initiate a canonical proteolytic cascade. There is a synergistic effect between mitochondrial damage and caspase activation, and ligand-dependent activation of the death receptor signaling further amplifies the process through the assembly of the death-inducing signaling complex (DISC), triggering downstream effector caspase cascades and ultimately accelerating photoreceptor cell death. Notably, in 2017, Venkatesh *et al* (111) demonstrated through a conditional knockout model that specific deletion of caspase-7 did not notably affect cone survival in the RP model. This suggests that the mechanism of secondary cone death may be independent of the endoplasmic reticulum stress pathway and involves signaling pathways beyond the unfolded protein response. Currently, the specific effects of *RPGR* mutations on mitochondrial homeostasis and apoptotic signaling remain to be further elucidated in animal models.

Regardless of the initial pathogenic mechanism, gene defects eventually lead to photoreceptor cell death. Therefore, investigating the mechanisms underlying photoreceptor cell death is essential. By analyzing the diverse clinical phenotypes resulting from specific site mutations, researchers can further explore the potential mechanisms of interaction and provide a theoretical basis for the development of future therapeutic targets. Additionally, biochemical analyses can help assess the physiological and metabolic status of patients, thereby facilitating the development of targeted therapies. Currently,

the expression pattern of RPGR protein remains incompletely understood, but its functionally distinct isoforms, generated by alternative splicing, are closely associated with specific roles in retinal photoreceptors. Strategies to compensate for the loss of RPGR and its isoforms, as well as to modulate physiological and metabolic processes to mitigate the consequences of RPGR deficiency, may become key directions for future research and therapy.

5. Preclinical research progress in RPGR mutation

Preclinical animal models for RPGR mutation studies. To elucidate the mechanisms underlying *RPGR*-related retinal degeneration and to develop effective gene therapy strategies, a variety of animal models have been established to recapitulate the genetic and phenotypic features of *RPGR*-associated XLRP (Fig. 6A). Mice have a short life cycle, strong reproductive capacity and a high degree of genetic homology with humans, allowing long-term disease progression to be observed within a relatively short period. These characteristics make them valuable for identifying pathogenic factors and associated signaling pathways (13). In *Rpgr* mutation studies, the RD9 mice represent one of the naturally occurring mutants (17,112-114). In addition, researchers have constructed mouse models carrying known mutation sites, including the *Rpgr* knockout (*Rpgr*-KO) and conditional knockout (*Rpgr*-CKO) models, using gene editing techniques to facilitate further studies (17).

The RD9 mouse is a naturally occurring mutant model carrying 32 bp duplication in the ORF15 region, which causes a frameshift mutation and introduces a premature stop codon (17,115). In this model, the $Rpgr^{ORF15}$ transcript can be detected, but the corresponding protein is absent, whereas the $Rpgr^{ex1-19}$ protein remains detectable. At the early stage, a mottled fundus appearance is observed, along with mislocalization of M-opsin to the IS, perinuclear region and synaptic terminals. As the disease progresses to the mid-stage, rhodopsin expression decreases markedly, whereas no mislocalization of S-opsin is observed. In the late stage, severe cell loss is observed in the ONL, with the thickness of the photoreceptor layer reduced by ~50% of its normal thickness (17). ERG recordings show a continuous decline in retinal function starting as early as one month of age. The notably reduced oscillatory potential (OP) amplitudes indicate that both photoreceptors (OP1) and inner retinal neurons (OP2-OP4) are affected by degenerative changes. The retinal degeneration in this model is primarily driven by rod cell death (115). This serves as an important tool for investigating the pathogenic mechanisms of ORF15 mutations in XLRP and for evaluating potential therapeutic strategies.

The *Rpgr*-KO ($Rpgr^{-/-}$) mouse was generated on a C57BL/6 genetic background by replacing exons 4-6 of the *Rpgr* gene with an antibiotic selection cassette. This modification truncates both major transcripts ($Rpgr^{ex1-19}$ and $Rpgr^{ORF15}$) and results in complete loss of RPGR protein expression. Although ERG responses remain normal at postnatal day 20, some proteins that should be localized to the OS are aberrantly distributed within the IS (48). Müller cells exhibit reactive gliosis, which becomes progressively more pronounced over time. Even in the absence of overt degenerative changes, these findings indicate that the retina has already initiated a damage-response process. At mid-to-late stages, ONL thickness is markedly reduced and ERG a-wave and b-wave amplitudes are decreased by ~25 and 31%, respectively (48). Similar findings have been reported in another study of *Rpgr*-KO mice, where rhodopsin was abnormally localized to the CC, accompanied by mistrafficking of proteins to the IS and ONL (116).

The *Rpgr*-CKO model was generated by deleting the proximal promoter and exons 1-3 (3,189 bp). In this model, nephrocystin-4 fails to localize properly to the CC, leading to structural abnormalities in the OS. Consistent with previous observations, mislocalization of rhodopsin and M-opsin is apparent at early stages and is accompanied by progressive photoreceptor degeneration. By late stages, >50% of the ONL is lost. Two new *Rpgr*-KO mouse lines (L1 and L2) have been generated using CRISPR-Cas9 technology (64). Unlike the exons 4-6 deletion model, this version deletes exons 7-13, resulting in the deletion of 502 amino acids.

The zebrafish model is not as homologous to human genes as the mouse model, but it has a faster growth rate and developmental cycle, which helps researchers obtain study samples quickly. Liu *et al* (66) showed that zebrafish *rpgra* possesses a single transcript homologous to human $RPGR^{ORF15}$. In this model, visual dysfunction and early degenerative features can manifest as early as 5 days post-fertilization. With increasing age, photoreceptor OS progressively shorten, the ONL progressively thins and OS architecture becomes disorganized, concomitant with declines in ERG amplitudes. By mid-to-late stages, the cone OS begins to degenerate, accompanied by substantial downregulation of retinal phototransduction genes. Concurrently, the OS disc membranes are disorganized and loosely stacked, with vesicular accumulation around the cilia. Shu *et al* (117) identified two *RPGR* homologs in zebrafish, *ZFRPGR1* and *ZFRPGR2*, with *ZFRPGR2* regarded as the functional homolog of human *RPGR*. Knockdown of *ZFRPGR2* results in abnormal eye development and retinal lamination defects, including failure of the retina to form the normal three-layer structure (GCL, INL and ONL) as well as underdevelopment of the photoreceptor OS, leading to widespread retinal cell apoptosis, a phenotype distinct from that observed in mammalian *Rpgr* mutant models.

Two natural mutations in the ORF15 region were detected in canines that are collectively referred to as progressive retinal atrophy and are homologous to human RP (13). The loss of photoreceptors in the naturally mutated canine model is similar to previous RD9 models, *rpgra* models and *RPGR* ablation in human photoreceptors (112). In XLPRA1, a 5-nucleotide deletion in this region results in a 230-amino acid truncation at the C-terminus, but does not affect normal photoreceptor development (32). Photoreceptors initially develop normally and function properly, but rod degeneration initiates at ~11 months. In XLPRA2, a comparable degeneration pattern is observed, albeit with an earlier onset, beginning at 4 weeks of age (89).

Animal models of *RPGR* mutations have been instrumental in understanding XLRP pathophysiology, identifying early markers of the disease, evaluating the efficacy of genetic and pharmacological treatments and elucidating the mechanisms by which *RPGR* mutations drive the retinal degeneration. A comparative overview of the strengths and limitations of

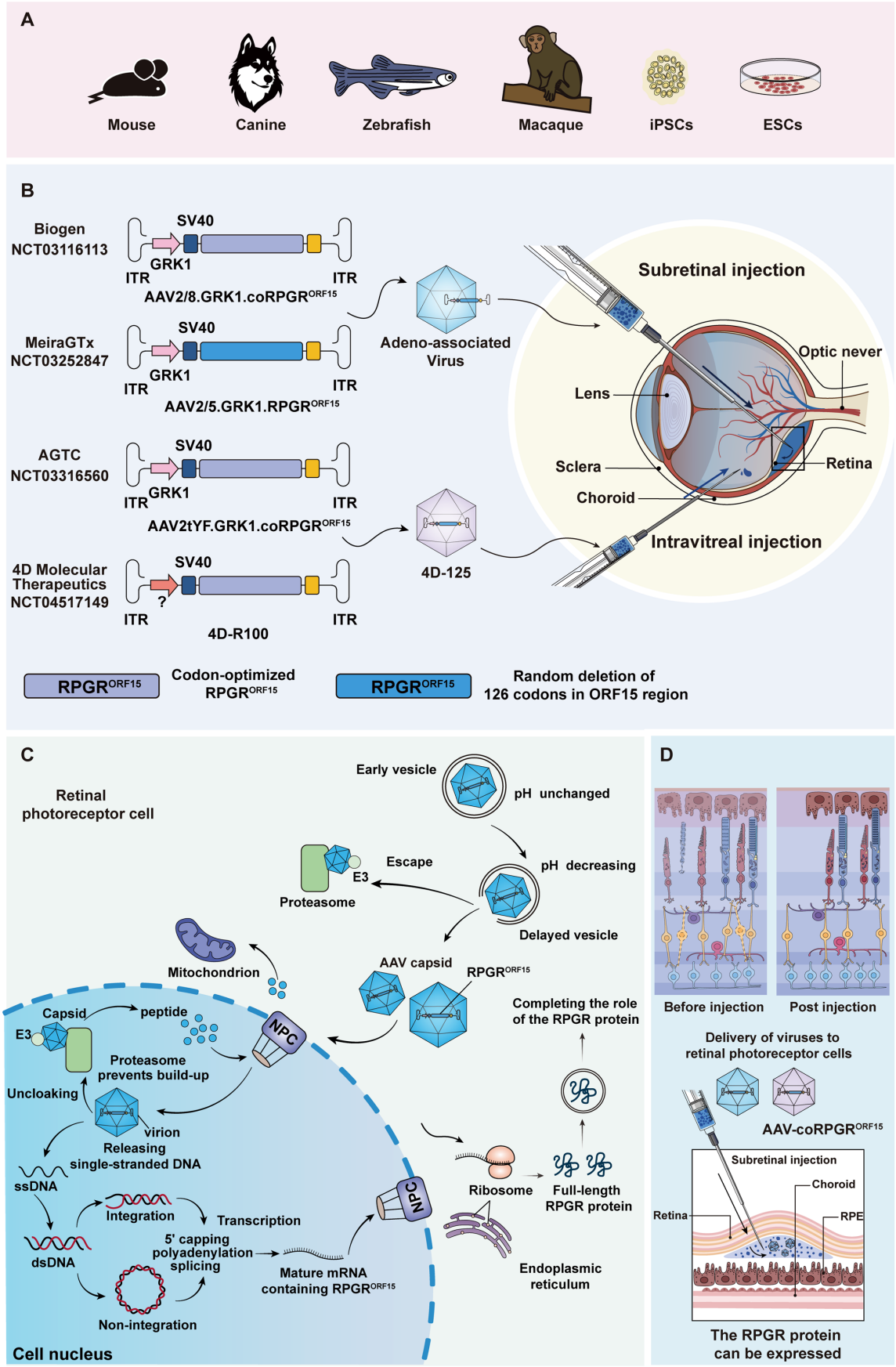

Figure 6. *RPGR* mutant model and gene editing cell therapy. (A) Animal and cell models of *RPGR* mutations. (B) AAV vectors and delivery methods applied in clinical studies targeting four reported *RPGR* mutations. (C) Entry of AAV vectors into the eye of a patient, followed by transduction and subsequent RPGR protein expression. (D) Effects of subretinal injection of RPGR gene therapy vector on retinal photoreceptor cells. Before injection: Rods and cones show extensive degeneration. After injection: Previously damaged rods and cones were partially preserved via subretinal injection delivery of the therapeutic vector. *RPGR* gene, *retinitis pigmentosa GTPase regulator* gene; AAV, adeno-associated virus.

Table I. Comparison of different animal models as treatment models for *RPGR* mutations.

Models	Advantages	Disadvantages	(Refs.)
Mouse	i) The genetic background is clear; ii) nature gene editing technology; iii) low cost; iv) abundant data from existing studies.	i) Retinal structure is very different from humans; ii) slow progression of the *RPGR* mutant phenotype; iii) differences in immune response exist.	(13,17,55,57,118,119)
Zebrafish	i) Transparent embryo; ii) fast reproduction and low cost; iii) easy gene editing; iv) retinal development: Human retina development is highly similar.	i) Simple retinal structure; ii) *RPGR* mutation phenotype may not be as pronounced as mammals; iii) drug metabolism is different from mammals.	(57,66,117,120,121)
Canine	i) Retinal structure is close to that of humans; ii) the *RPGR* mutant canine model exhibits retinal degeneration similar to that of humans; iii) suitable for long-term studies.	i) High cost of breeding and experimentation; ii) difficulty of gene editing; iii) canine models face more ethical controversies compared with other anima models.	(13,57,118,119,122,123)
Hog	i) The structure of the retina is highly similar to that of humans; ii) the *RPGR* mutant pig model exhibits retinal degeneration similar to that of humans; iii) close to human size suitable for surgical eye and gene therapy studies.	i) High breeding and experimental costs; ii) long breeding cycle; iii) difficult for gene editing.	(13,124,125)
Macaque	i) Retinal structure almost identical to humans; ii) disease phenotypes are closest to humans; iii) suitable for preclinical studies, with results of gene therapy and surgical operations directly extrapolated to humans.	i) Extremely high cost; ii) study on apes and monkeys face serious ethical controversies as experimental animals; iii) long breeding cycle.	(126-132)

RPGR gene, *retinitis pigmentosa GTPase regulator* gene.

these models is presented in Table I (13,17,55,57,66,117-132). However, these models still have limitations: The rodent retina is dominated by rods, whereas the human retina contains a higher proportion of cones, especially concentrated in the central concave region, which poses challenges for translating these findings to humans (13,116). Existing mouse models exhibit a slower progression of retinal degeneration, which is not entirely consistent with the more severe and rapidly progressive phenotype of human XLRP. In terms of disease progression, histopathologic features and molecular mechanisms, these models do not fully recapitulate the diverse clinical manifestations observed in humans with *RPGR* mutations. Due to the complexity of alternative splicing of the *RPGR* gene, the expression patterns and functions of *RPGR* isoforms can differ substantially between species, thereby restricting the translational potential of findings obtained from animal models. Existing animal models have limited capacity to accurately replicate the systemic manifestations of *RPGR* mutations, as demonstrated by respiratory infections and hearing loss observed in some patients (41). In translational and therapeutic studies, judicious selection of animal models according to research goals, disease phenotypes and experimental parameters is important. Despite their inherent limitations, these models offer a vital preclinical platform for testing and refining gene therapy approaches for *RPGR*-related retinal degeneration.

Progression of treatment strategies in RPGR-related XLRP. Early therapeutic strategies for *RPGR*-related XLRP primarily focus on nutritional supplementation aimed at slowing photoreceptor degeneration. Vitamin A is converted into 11-cis-retinal, whereas omega-3 polyunsaturated fatty acids (such as docosahexaenoic acid, DHA) contribute to the structural stability of the photoreceptor membrane by integrating into the phospholipid bilayer (133-135). Such supplements are considered to delay photoreceptor degeneration during the early stages of XLRP (136,137). These interventions merely slow visual deterioration and do not repair the retinal structural damage caused by the *RPGR* mutation (138). Moreover, long-term DHA supplementation may lead to side effects such as gastrointestinal discomfort (139). To address the limitations of such supplements, researchers have also explored neuroprotective strategies, including supplementation with brain-derived neurotrophic factor. These factors have shown efficacy in conditions such as macular degeneration and glaucoma by promoting the survival of retinal ganglion cells (RGCs) and reducing microglia-mediated inflammation (140,141). Unlike supplement intervention strategies, neuroprotective strategies target the secondary neurodegenerative processes of XLRP. However, the majority of studies of these neuroprotective strategies have been performed in non-*RPGR* models (142-146), leaving their applicability to XLRP unconfirmed. Importantly, supplements and neuroprotective strategies are not mutually exclusive; rather, they may exert complementary effects by targeting different pathological stages of XLRP. Combination approaches may synergistically delay disease progression through multiple pathways. Future studies using *RPGR* mutation models are needed to systematically evaluate the timing, dosage and interactions of such combined strategies.

Although these interventions may help preserve residual vision, they cannot fundamentally correct the underlying genetic defect. By contrast, gene therapy aims to directly restore normal RPGR function, thereby halting ongoing photoreceptor degeneration at its source and potentially altering the natural course of XLRP. It has emerged as a promising and potentially transformative approach for treating IRDs, particularly those associated with *RPGR* mutations (29,147-150). Because the majority of inherited retinal disorders are monogenic, and the eye possesses immune privilege as well as direct accessibility for observation and intervention, a single gene therapy treatment may achieve long-term, even lifelong, therapeutic benefits (151-153). Among various gene therapy approaches, viral vector-mediated gene replacement represents the primary strategy for treating *RPGR*-related diseases.

Gene therapy studies in Rpgr mutant models. Despite inherent limitations, animal models remain the primary experimental systems for evaluating the safety and efficacy of gene therapy strategies. The therapeutic outcomes largely depend on factors such as promoter choice, vector platform, AAV serotype, capsid modification strategy and host immune response.

In retinal gene therapy, the use of cell type-specific promoters is important for achieving stable and precise transgene expression. Researchers have compared the transcriptional activities of several promoters in rod and cone photoreceptors, including rhodopsin kinase (RK), human interphotoreceptor retinoid-binding protein (hIRBP) and human RK (hGRK1). The hGRK1 promoter exhibited more sustained expression in rod photoreceptors, making it an optimal candidate for *RPGR*-related gene therapy (10,122). In an XLRP canine model, delivery of human $RPGR^{ORF15}$ using an AAV2/5 vector driven by either the hIRBP or hGRK1 promoter successfully preserved photoreceptor nuclei and normal retinal structure. This treatment restored both rod and cone function and corrected rhodopsin mislocalization (122).

In terms of vector design, continuous improvements in recombinant AAV (rAAV) vectors have enabled targeted expression in specific retinal cells and markedly enhanced transduction efficiency (154-156). With a packaging capacity of ~4.8 kb, AAV vectors can readily accommodate the *RPGR* gene (116,156). Because different diseases affect retinal photoreceptors in distinct ways, vector design must be approached with particular caution. Future optimization of AAV vectors should focus on several key aspects: i) Selecting appropriate and cell type-specific promoters; ii) designing safer vectors with minimal off-target effects; iii) simplifying gene expression regulatory mechanisms while enhancing expression stability; iv) improving transduction efficiency alongside scalable production; and v) systematically evaluating the durability and safety of the therapy. Because the ORF15 region undergoes complex post-transcriptional processing with multiple alternatively spliced isoforms and possesses intrinsic sequence instability, maintaining its integrity during vector production and therapeutic application remains a key challenge (157). Fischer *et al* (22) carried out codon optimization of the *Rpgr* sequence and constructed the AAV8-hRK-coRPGR system, which successfully restored retinal function in two animal models ($Rpgr^{-/y}$ and $C57BL/6J^{RD9/Boc}$), markedly ameliorating retinal degeneration phenotypes. Giacalone *et al* (158) used a

bioinformatics approach to introduce synonymous mutations into a highly repetitive region of ORF15, enhancing sequence stability and expression efficiency while maintaining amino acid sequence invariance. Hong *et al* (159) demonstrated earlier that a shortened version of *RPGR*^{ORF15}, with a 654 bp deletion in the repetitive region, effectively alleviated retinal degeneration in *Rpgr*-KO mice. Pawlyk *et al* (75) further compared the effects of varying degrees of linker region truncation and found that moderate truncation (removing ~33% of the sequence) preserved protein function while markedly improving retinal morphology and function in *Rpgr*-KO mice.

Currently, preclinical RPGR gene therapy studies have primarily focused on highly efficient AAV-based delivery systems (8,160-162). AAV is the most widely used viral vector in clinical research on IRDs, with different serotypes exhibiting tropism for specific tissues and cell types. For example, AAV2 shows high affinity for RPE cells and is commonly used for targeted delivery to RGCs (163,164). In animal models of retinal degeneration, AAV2 has successfully restored both retinal structure and function (165-167). AAV8, which is associated with a reduced incidence of neutralizing antibodies and improved immune tolerance, is considered a clinically promising alternative to AAV2. In therapeutic studies using *RPGR*-mutant animal models, AAV2, AAV5 and AAV8 are commonly employed (22,122,123). Different AAV serotypes have distinct capsid protein structures, which influence their transduction efficiency, tropism and immunogenicity. Therefore, gene therapy design requires careful consideration of both serotype selection and administration route to achieve an optimal balance among efficacy, safety and immune tolerance (168,169). The AAV capsid is a key factor in triggering immune responses. Once the capsid proteins are recognized, T cells can be activated, leading to local inflammation. Meanwhile, pre-existing neutralizing antibodies may bind the capsid, thereby blocking vector entry into target cells (170). Song *et al* (123) evaluated the AAV2 vector in which three tyrosine residues of the capsid were substituted with phenylalanine (AAV2tYF). The rAAV2tYF-GRK1-hRPGRco demonstrated excellent transduction efficiency in the *RPGR* mutant canine model and subsequently became a candidate vector for clinical evaluation in the AGTC-501 study. Additionally, the engineered AAV7m8 and modified AAV8 capsids have demonstrated superior transduction efficiency, achieving notably higher delivery rates in developing photoreceptor organoids compared with AAV5 and standard AAV8 (8,171). Pavlou *et al* (172) developed a novel AAV capsid in 2021 that exhibited markedly enhanced delivery efficiency and lower invasiveness across mouse, canine and non-human primate models, confirming its cross-species applicability. These capsid engineering strategies, which modify surface amino acids, provide a foundation for more efficient and targeted gene delivery.

Beyond gene replacement therapy, gene-editing approaches have also demonstrated promising therapeutic potential. In a 2020 study, a single subretinal delivery of CRISPR-Cas9 via AAV in *Rpgr*-KO mice precisely corrected the *RPGR* mutation *in vivo*, protecting photoreceptors for ≥12 months without detectable off-target effects (173). In the RD9 mouse model, CRISPR-Cas9 targeted and excised the ORF15 mutation region, allowing repair via non-homologous end joining and successfully restoring the normal RPGR reading frame and protein expression (113). This markedly improved the retinal pathological phenotype, further confirming the efficacy of gene editing for *in vivo* repair of *RPGR* mutations. Additionally, prime editing has demonstrated a notable effect in treating mice with RP (4,174).

Animal studies have also verified the feasibility of RPGR gene therapy from the perspective of long-term efficacy. Wu *et al* (175) conducted a two-year dose-response study in *Rpgr*-KO mice, delivering full-length *RPGR*^{ORF15} via AAV8 and AAV9 vectors. The results showed that treated mice maintained retinal structure and function over the long term, as evidenced by higher ERG amplitudes, a thicker photoreceptor layer and correct localization of photoreceptor proteins (10,175). While animal models have been invaluable in advancing RPGR gene therapy, emerging organoid models provide promising platforms for therapeutic evaluation and mechanistic insights, as discussed in the following section.

Application of organoid models in RPGR gene therapy. Researchers have utilized human induced pluripotent stem cell (hiPSC) differentiated organoid models to investigate both the simulation and therapeutic targeting of *RPGR* mutations. Organoids are highly organized three-dimensional structures that serve dual roles in disease modeling and therapeutic research. These models can differentiate into retinal tissues containing all major cell types, exhibiting highly refined structural organization and functional characteristics that closely resemble those of native retinal tissue (8,116). Previous studies have investigated the potential of hiPSC-derived organoids as a platform for biomarker screening in *RPGR*-mutant patients (171,176,177). The first study employing CRISPR-Cas9 to correct autologous hiPSC from patients with *RPGR* mutations achieved 13% repair efficiency, and the corrected cells could be used for subsequent transplantation studies, underscoring the potential of precision medicine for *RPGR*-related diseases (178). Similarly, a 2018 study at the *in vitro* stage demonstrated that the application of CRISPR-Cas9 to correct *RPGR* mutations successfully restored the structure and electrophysiological properties of photoreceptors (177). In addition, Sladen *et al* (8) used CRISPR-Cas9 to repair *RPGR*-mutated hiPSC, differentiated them into photoreceptor cells and evaluated the effects of the AAV delivery system, providing preliminary data to support subsequent clinical trials (8). Another study conducted single-cell RNA sequencing on organoids derived from normal and *RPGR*-mutant hiPSCs, providing key insights into the molecular mechanisms of *RPGR*-associated retinal degeneration and informing the development of precision-targeted gene therapy strategies (179).

Although hiPSC-derived organoids show great potential for modeling *RPGR*-related diseases and testing gene therapy strategies, they still face several challenges. The majority of organoids have not yet developed structured photoreceptor OS, making it difficult to fully replicate the pathological process of cilia-associated diseases. Additionally generating organoids is time-consuming and expensive (116,180). Although studies have attempted to introduce antioxidants, lipid supplements and hyaluronic acid to promote photoreceptor OS maturation, the results have been limited (180,181). Overall, organoids

Table II. Comparison of different injection methods for ocular drug delivery.

Injection method	Injection site	Applicable conditions	Advantages	Challenges	(Refs.)
Intravitreal injection	Inside the vitreous cavity	Age-related macular degeneration; diabetic retinopathy.	Fewer procedure-related complications; low risk of immune response; directly into the vitreous cavity; higher concentration of drugs; considerable therapeutic effect; simple to operate and less invasive.	Potential immune response; high accuracy requirements; low delivery efficiency.	(128,169, 182-186)
Subretinal injection	Subretinal (between the retina and retinal pigment epithelium)	Treatment of hereditary retinal diseases.	Direct drug delivery to target area; small injection dose; overall reduction in side effects.	Relatively difficult technique; moderate risk of complications; temporary decrease in visual function; long recovery time; keep pressure low.	(183, 187-191)
Suprachoroidal injection	Above the choroid (the space between the retina and the choroid)	Macular edema; photoreceptor loss; choroidal; neovascularization; retinal detachment; uveitis; glaucoma; uveal melanoma.	Short-term effectiveness; ocular tolerability; wider distribution; higher drug utilization; less impact on the immediate period; lower complication and infection rates; relatively minimally invasive; high targeting; low surgical risk.	Demanding injection techniques; patient stress; limitations of post-injection distribution; need for continuous intraocular monitoring.	(169, 192-197)

cannot yet fully replicate the *in vivo* microenvironment, and their findings should therefore be interpreted cautiously when considering clinical translation.

Delivery routes for ocular RPGR gene therapy. In addition to the aforementioned factors influencing gene therapy, the success of treatment largely depends on the delivery route of the vector. The route of administration not only affects the spatial distribution and cellular uptake efficiency of the therapeutic gene but also directly influences the magnitude of immune responses and the overall precision of treatment. Therefore, the choice of injection modality is important in RPGR gene therapy studies. Currently, the commonly used delivery routes for ocular gene therapy include intravitreal injection, subretinal injection and suprachoroidal injection. Table II summarizes the key features and applicability differences of the three delivery modalities (128,169,182-197). Intravitreal injection delivers the AAV vector carrying the *RPGR* gene directly into the vitreous cavity but may result in reduced therapeutic efficacy and a stronger immune response due to uneven vector distribution. By contrast, subretinal injection administers the vector into the subretinal space between the RPE and photoreceptors, achieving a higher concentration and more efficient transduction in target cells. This approach is particularly suited for targeted and precise RPGR gene therapy (Fig. 6D). Subretinal injection has also become the primary administration route in current clinical trials for patients with *RPGR*-associated RP (Fig. 6B).

Although ocular gene therapy offers advantages such as localized administration, the ability to monitor treatment effects, and using one eye as a control for the other, it still has certain limitations. Currently, only a few gene therapy drugs for incurable eye diseases have been approved worldwide (165). Particularly during subretinal injection applications, several technical challenges remain, including potential damage to the retina and macula, cataract formation, subretinal deposit development and drug reflux into the vitreous cavity (198). In addition, improper technique can lead to more severe complications, including endophthalmitis, choroidal rupture, Bruch's membrane damage, drug misinjection into the sub-scleral cavity, retinal detachment and even irreversible vision loss.

Emerging non-viral delivery strategies for RPGR therapy. Owing to their high efficiency and cell-type specificity, AAV vectors remain the preferred choice for retinal gene delivery; however, their use may pose risks related to inflammation and immune responses. Given these limitations and the potential risks associated with AAV-based therapies, alternative non-viral delivery strategies have gained increasing attention in recent years. Non-viral vectors, with advantages in safety, flexibility and mass production, are gradually becoming an important complement to viral vectors in gene

therapy (199-202). The use of nanoparticles for ocular gene delivery has been continuously explored in recent years. Engineered nanoparticles exhibit enhanced targeting capability and reduced immunogenicity, offering great potential for clinical translation in IRD therapy (203,204). Lipid nanoparticles, polymeric nanoparticles (such as polylactic-co-glycolic acid) and inorganic nanoparticles (such as cerium oxide) have demonstrated efficient drug and gene delivery in ocular diseases such as AMD (202,205,206).

There are no published reports directly applying non-viral vectors in *Rpgr* mutant animal models. Given the therapeutic success of non-viral vectors in AMD, future research should prioritize assessing their delivery efficiency, durability of *RPGR* gene expression, and safety profiles in mutant models (such as RD9, *Rpgr*-KO) to accelerate the translation of preclinical research to clinical application.

6. Clinical progress of RPGR gene therapy

Clinical assessment and trial design of RPGR gene therapy. Comprehensive and accurate assessment tools are essential for detection of RP associated with *RPGR* mutations and for evaluating the efficacy of gene therapy. Currently, commonly used examinations include optical coherence tomography (OCT), fundus autofluorescence (FAF), ERG, visual evoked potential, BCVA and VF testing (37,207,208). With the advancement of RPGR gene therapy clinical trials, the evaluation methodology has been further refined to incorporate multidimensional metrics such as microperimetry, contrast sensitivity function and vision-guided mobility assessment (VMA), thereby enabling a more comprehensive detection of subtle treatment-induced changes (28). The 2024 study highlighted that optimizing subject selection criteria is important for improving the success rate of gene therapy trials (209). In particular, the structural integrity of the ellipsoid zone (EZ) has been identified as a potential biomarker. Patients with *RPGR* mutations who exhibit an EZ width of 600-800 μm and a preserved 1˚ central VF are likely to exhibit improved response to treatment, providing an important basis for accurate patient stratification and efficacy prediction in clinical trials (209,210). These assessment tools are not only suitable for dynamic follow-up across different disease stages, but also facilitate the identification of ideal candidates who retain central vision despite moderate vision impairment. In addition, the stratification and evaluation system for patients with RP also provides important reference value (35,211,212).

Current clinical studies of gene therapy for *RPGR* mutation-associated XLRP primarily focus on AAV vector-based gene replacement approaches (9,73,213). The majority of RPGR gene therapy trials have employed the (RK/GRK1 promoter, as aforementioned in the preclinical model section, the core advantages of this promoter lie in two aspects: Its precise targeting of retinal photoreceptor cells and its compatibility with the packaging capacity limitations of AAV vectors. The main variations among the different studies concern the viral subtypes and the selection of coding sequences (Fig. 6B). To date, four clinical trials have been reported, each sponsored by a different company: i) Biogen Inc. (NCT03116113), AAV2/8.hRK.co*RPGR*^{ORF15} carrying a codon-optimized *RPGR*^{ORF15} cDNA sequence. ii) MeiraGTx (NCT03252847), AAV2/5.hRK.*RPGR*^{ORF15} without codon optimization; iii) AGTC (NCT03316560), AAV2tYF.GRK1.co*RPGR*^{ORF15} employing an AAV2 capsid containing a single tyrosine-to-phenylalanine (Y→F) substitution (10); iv) 4D Molecular Therapeutics (NCT04517149): 4D-R100-based vector carrying codon-optimized human *RPGR*. R100 is an AAV capsid engineered through directed evolution, characterized by highly efficient targeting of retinal photoreceptor cells.

Clinical outcomes and safety of AAV-mediated RPGR gene therapy. AAV-mediated delivery of the RPGR gene has led to substantial advances in the treatment of retinal degenerative diseases such as XLRP (Fig. 6C). The Phase I/II clinical trial AGTC-501 (NCT03316560), initiated in 2017, employed rAAV2tYF-GRK1-RPGR to evaluate the safety and preliminary efficacy of the modified capsid. The codon-optimized human CDS co*RPGR*^{ORF15} sequence overcame the intrinsic instability associated with transgene-based therapies and enhanced gene expression levels in mouse models, while preserving post-translational characteristics (22). In 2020, a Phase I/II dose-escalation trial (NCT03116113) was initiated to evaluate codon-optimized AAV8-RPGR gene therapy for XLRP. The study met its primary safety endpoint, with no dose-limiting toxicities (DLTs) or serious adverse events (SAEs) observed across all dose cohorts. These findings provide initial evidence supporting the safety and feasibility of the codon optimization strategy (73). During the 6-month follow-up, no DLTs or SAEs were observed in the low-dose and intermediate-dose groups (29,61). However, the low-dose cohort showed no improvement in visual function, likely due to insufficient dosing and delayed intervention. The high-dose group exhibited potentially enhanced therapeutic effects, but 77.8% of patients developed mild intraocular inflammation, which was alleviated by local or systemic anti-inflammatory therapy but still affected the treatment experience and overall efficacy. By contrast, the intermediate dose ($5x10^{11}$ gp/ml) provided the best balance between therapeutic efficacy and minimal incidence of SAEs. Efficacy was primarily assessed based on retinal sensitivity responses and the changes over time. In another gene therapy trial for diabetic macular edema, several cases of vision loss were reported due to severe delayed-onset intraocular inflammation. However, pharmacokinetic modeling from the ADVM-022 ocular gene therapy study in monkeys provided useful reference data for fine-tuning dosage regimens (214,215). Based on the aforementioned clinical findings, several considerations should be emphasized in subsequent Phase II/III studies: i) Dose selection strategies: Optimize dosing to maximize efficacy while minimizing inflammation risk; ii) immunosuppressive regimen optimization: Adopt more effective anti-inflammatory strategies to improve patient experience and treatment outcomes; iii) patient enrollment criteria: Focus on patients with early-stage disease to enhance therapeutic benefit.

At the 2023 Annual Meeting of the Association for Research in Vision and Ophthalmology, MeiraGTx in collaboration with Janssen reported on the immunogenicity of the AAV5-RPGR (Botaretigene Sparoparvovec, a gene therapy method using AAV5) gene therapy in the Phase I/II trial (NCT03252847) (41). At baseline, 44% of patients had pre-existing antibodies against AAV5 and 24% had antibodies

against RPGR. Following treatment, only 13.3% of patients developed new anti-RPGR antibodies, and no association between antibody status and dose was observed. In this study, 96.9% of AEs were related to the surgical procedure, and 82.2% of patients experienced at least one treatment-emergent AE (TEAE), with no other immune-related reactions detected. The majority of AEs were procedure-related, including conjunctival hemorrhage (low dose, 72.7%; intermediate dose, 64.7%; high dose, 100.0%) and transient decreases in vision (low dose, 54.5%; intermediate dose, 47.1%; high dose, 50.0%), which negatively affected the patient experience (41). However, these events were generally reversible and resolved over time. Notably, one patient in the low-dose cohort experienced retinal detachment, which was successfully repaired surgically. At the end of the study, another patient still had uveitis (day 33), which may suggest a potential delayed immune response; however, longer-term follow-up is required to determine the incidence and clinical significance of such events.

In 2020, 4D Molecular Therapeutics initiated a Phase I/II clinical trial (NCT04517149) to evaluate the safety and tolerability of intravitreal injection administration of 4D-125 (216). This multicenter study included both an observational natural history cohort and an interventional treatment arm. The therapy utilized a proprietary AAV capsid variant (4D-R100) carrying codon-optimized human *RPGR*^{ORF15}. Updated data presented at the 2021 American Society of Retina Specialists meeting showed that, among the eight enrolled patients, two single-eye dose levels ($3x10^{11}$ and $1x10^{12}$ vg/ml) had been tested in preliminary experiments. The results demonstrated that 4D-125 was well tolerated, with no DLTs or SAEs observed, and with only 25% of patients experiencing mild, transient inflammation. These findings supported the feasibility of administering a single-eye dose of $1x10^{12}$ vg/ml in patients with advanced XLRP. During the intervention phase, TEAEs were generally reversible, including transient intraocular pressure elevation within 24 h post-injection (21%); conjunctival hemorrhage resolving within 1 week (43%); and mild vitreous opacities resolving within 3 months (14%), all of which resolved without medical intervention. Although the small patient population limited the statistical power of these observations, the therapeutic vector and dosing used in NCT04517149 exhibited a favorable safety profile. Further studies in larger patient cohorts are required to validate the true therapeutic potential of this approach.

Results from the AGTC-501 Phase I/II gene therapy trial HORIZON (NCT03316560), published in 2024, showed that 78% of patients receiving central retinal injections exhibited improvements in visual function at 6 months, based on microperimetry and BCVA assessments, providing preliminary validation of the feasibility of the gene replacement strategy. Data from a 2-year follow-up indicated that all participants experienced at least one TEAE, with 93% considered by investigators to be mild and self-limiting, such as transient vitreous opacities (217). Although these SAEs resolved spontaneously or following intervention, procedure-related events such as retinal tears or intraocular pressure elevation could have different implications for patients. Even transient AEs can negatively affect patient experience and potentially impact treatment outcomes. Of the four patients receiving the highest dose ($1.99x10^{12}$ vg/eye), three developed RPE changes, which appeared as progressively depigmented areas on FAF imaging. These changes were observed not only at the surgical incision site but also beyond it, gradually enlarging over the follow-up period. While these changes did not appear to adversely affect improvements in retinal sensitivity for some patients over 24 months, the high incidence (75%) and the uncertainty of potential long-term risks led the study team to select $6.8x10^{11}$ vg/eye as the maximum tolerated dose. At this dose, half of the patients maintained visual sensitivity improvements at a minimum of five retinal test loci (≥7 dB improvement per locus) over 24 months (217). These findings highlight the need to carefully consider the potential RPE toxicity of high-dose AAV vectors in clinical gene therapy development and emphasize the importance of extended follow-up.

In 2024, the MeiraGTx-sponsored AAV5-hRKp.RPGR (NCT03252847) published results from its Phase I/II trial, with primary endpoints divided into assessments of safety and efficacy (41). The core efficacy endpoint focused on functional vision, using VMA as the primary measure, while secondary endpoints included static perimetry mean retinal sensitivity (MRS) within the central 10˚, dark-adapted microperimetry MRS, BCVA and contrast sensitivity. The treatment group showed a notable improvement in MRS in the central 10˚ field of view at week 26 compared with the control group (LS mean difference 1.96 dB). MRS in the dark-adapted microperimetry was also notably improved at Week 26 (LS mean difference 1.06 dB). BCVA in the treatment group remained stable at Week 26, whereas it decreased in the control group and the treatment group continued to maintain stability at Week 52 (LS mean change of 0.40 letters) (218). Overall, the treatment group demonstrated superior visual function improvement at Week 26 and AEs remained manageable, with intraocular inflammation occurring in 22% of patients and no SAEs reported. AAV5-hRKp.RPGR appeared to exhibit good tolerability and a low immunogenic profile. However, the study noted that 52-week follow-up data were insufficient to assess long-term efficacy. Despite this, participation in this trial was not without risk for patients.

In 2022, a Phase II/III study was initiated using AAV8-based gene therapy Cotoretigene Toliparvovec (BIIB112/AAV8-RPGR, NCT03116113). Unfortunately, according to published 12-month evaluations of participating patients, the study did not meet its primary endpoint, as there was no statistically significant difference in microperimetry response rates. Clinical data indicated that low-dose Cotoretigene Toliparvovec reduced the risk of inflammation, but maintained efficacy was limited, with visual improvement declining by ~15% at 52 weeks and a 1.8-fold increased risk of RPE atrophy (28). The primary endpoint was defined based on VF assessments. Specifically, it measured the proportion of patients who showed improvements of ≥7 dB in retinal sensitivity in at least five of the 16 central loci of the study eye at 12 months post-treatment. Results showed no significant differences among the low-dose group (37.5%), high-dose group (25.0%) and untreated group (22.2%). Additionally, microperimetry assessments at 52 weeks did not demonstrate statistically significant improvements in retinal sensitivity in the treatment groups compared with controls. Despite not achieving the primary endpoint, the low-dose group showed trends toward improvement in mean continuous

microperimetry sensitivity and low-luminance VA (LLVA), with ~33% of patients experiencing LLVA gains ≥15 letters, suggesting that the therapy may be effective in some patients.

Limitations of current trials and future directions in clinical RPGR gene therapy. These differences in therapeutic outcomes also reflect the inherent complexity of RPGR gene therapy. Patient type and individual variability can lead to heterogeneous responses, highlighting the need for clearly defined enrollment criteria. Compared with clinical trials using other AAV serotypes, AAV8 exhibits higher immunogenicity and RPE toxicity; high doses are limited by safety concerns, while low doses may provide insufficient efficacy. Attempts to reduce inflammation by lowering the dose reveal the current challenge of balancing dose, efficacy and safety, as simply reducing the dose cannot entirely mitigate the risk of RPE atrophy. Although this study did not meet its primary expectations, its 'failure' provides valuable insights for subsequent vector optimization, dose adjustment, patient selection and treatment strategies. Multiple clinical trials for Leber Congenital Amaurosis, including NCT00999609, NCT02781480, NCT00749957 and NCT00643747, have similarly reported ocular immune-related AEs, such as inflammation, transient increases in AAV neutralizing antibodies and AAV capsid-specific T cell responses (219). These events not only caused temporary declines in visual function but also limited the overall benefit of retinal function improvement. Collectively, these clinical studies demonstrate that patients receiving AAV-mediated ocular gene therapy remain at risk of pronounced immune responses.

Differences in clinical outcomes among vector systems underscore the complexity of vector selection. Patients participating in AAV5-hRKp.RPGR trials exhibited lower rates of inflammation compared with those in AAV8-RPGR (BIIB112) trials. In the BIIB112 high-dose group, the incidence of inflammation reached 77.8%, of which 28% were SAEs, presenting as retinal detachment. By contrast, the high-dose group of AAV5-hRKp.RPGR exhibited a 75% incidence of inflammation without any SAEs. Although AAV8 (XIRIUS) and AAV5 (MeiraGTx) showed similar post-treatment inflammation rates, their efficacy differed markedly, likely reflecting inherent differences in tissue tropism between the vectors. Notably, both AGTC and XIRIUS employed codon-optimized sequences; however, the Phase II/III trial of XIRIUS did not achieve its primary endpoint, suggesting that codon optimization alone may not be decisive for patient outcomes in gene therapy. As previously noted, optimization of AAV vectors extends beyond promoters and codon usage, with capsid engineering and serotype selection being important for enhancing stability, retinal cell targeting, transduction efficiency and minimizing host immune responses to gene therapy.

Regarding endpoint assessments, all clinical trials employed AE, SAE and TEAEs to evaluate safety; however, efficacy endpoints varied among studies. In the Phase I/II trial NCT03116113, efficacy was assessed using BCVA, VF and MRS; NCT03316560 evaluated efficacy with MRS, BCVA and OCT; and NCT03252847 employed VMA, MRS and BCVA. Although safety was achieved at each stage in the Phase I/II trials, the Phase II/III trial of NCT03116113, which focused on VF and MRS, did not meet its primary endpoint. These differences in efficacy assessment led to two key issues: i) Patient outcomes across trials were not directly comparable, making it difficult to objectively evaluate the relative effectiveness of each treatment strategy; ii) the choice of endpoints itself may influence whether a trial achieves its prespecified endpoint, potentially causing treatments that appeared effective in early studies to 'fail' in subsequent trials. Moreover, early-stage patients retain more photoreceptors than late-stage patients, making functional recovery easier to observe. For the clinical development of RPGR gene therapy, establishing a unified and standardized efficacy assessment framework is important to facilitate translation.

A common and key challenge faced by all current RPGR gene therapy trials is the insufficient duration of follow-up. To date, the maximum follow-up period has been only 24 months, which is far from adequate for evaluating the true long-term value of gene therapy. Given that treatment decline has already been observed in the XIRIUS and AGTC-501 trials, the efficacy of these therapies may further diminish over time, and current clinical data remain insufficient to predict patient outcomes beyond this period. Maintaining durable therapeutic effects therefore remains a key concern. Extended follow-up is required to accurately determine the long-term disease trajectory of patients receiving these gene therapy interventions, assess the persistence of dose-dependent efficacy and monitor delayed toxicity risks. Only with such longitudinal data can reliable, comparable and reproducible clinical conclusions be drawn.

Future research should build upon monitoring and optimizing existing gene therapy strategies by further addressing multiple aspects of research and clinical practice. i) Vector and therapeutic development: The creation of next-generation, low-immunogenicity vectors, exploration of non-viral delivery systems or combination therapies integrating anti-inflammatory and neuroprotective strategies is needed to minimize immune responses. Concurrently, improving surgical techniques is essential to reduce treatment-related SAEs arising from procedural issues. ii) Patient selection and management: More comprehensive enrollment criteria should be established, incorporating assessments such as the EZ as part of the screening process. iii) Post-treatment clinical evaluation: A unified and standardized efficacy assessment framework should be implemented to ensure consistent measurement of outcomes. iv) Informed consent and patient counseling: Patients should be fully informed prior to treatment regarding the need for intensive and frequent follow-up, potential systemic side effects from high-dose immunosuppression (for example, prednisone-induced mood changes) and the risks of treatment failure or ocular injury (220). Although challenges remain in optimizing vector and delivery systems and regulatory oversight, the continuous integration of these insights and innovations holds promise for advancing therapeutic outcomes (221). Systematically addressing these issues in RPGR gene therapy clinical research and balancing considerations across these levels is key to translating exploratory trials into routine clinical practice. Table III summarizes both completed clinical trials and ongoing studies with results yet to be reported. Table IV summarizes the main results and efficacy outcomes of completed clinical trials.

Table III. Clinical trials of RPGR gene therapy for retinal degenerative diseases (data was retrieved from ClinicalTrials.gov).

Clinical Trials.gov ID; date	Type and phase	Patient population	Assessment	Sponsors
NCT03252847 (AAV5-hRKp.RPGR); Jul 2017-Nov 2021	Interventional, I/II; participants, 49.	Male patients aged ≥5 years; have X-linked retinitis pigmentosa confirmed by a retinal specialist (co-investigator or principal investigator).	Primary: Incidence of serious adverse events within 9 weeks after administration; secondary: Visual acuity, static visual field, questionnaires (6 months after treatment).	MeiraGTx Limited
NCT03314207; Dec 2017-Feb 2022	Observational; participants: 14.	i) Male patients aged ≥6 years who have mutations in exon ORF15 of the *RPGR* gene; ii) willing and able to perform study procedures. iii) Signed informed consents obtained (child assent where applicable).	Primary: Disease progression; secondary: Visual acuity, perimetry, OCT, electroretinography, national eye institute visual functioning questionnaire-25 (VFQ-25) quality of life questionnaire (Day 0-Month 36).	Beacon Therapeutics
NCT03349242; Dec 2017-Apr 2024	Observational; participants, 140.	i) Males and female patients aged ≥5 years; ii) are able to give informed consent or assent, with the guidance of their parent/guardian where appropriate.	Primary: Retinal structure; secondary: Analysis of retinal structure and function to assess disease progression (6 years); method: Retinal sensitivity, retinal structural detailed phenotyping, fundus autofluorescence, visual field.	MeiraGTx Limited
NCT03116113 (AAV8-RPGR); Mar 2017-Nov 2020	Interventional, I/II; participants: 50.	i) Male patients aged ≥10 years; ii) having XLRP (with a mutation in the *RPGR* gene) and active disease, with obvious lesions observable in the macular area of both eyes.	Primary: Safety ≥24 months after treatment; aim: Dose-limiting toxicities, treatment-emergent adverse events. Secondary: Visual acuity, central retinal thickness, best-corrected visual acuity, low luminance visual acuity, macular integrity assessment microperimetry.	Biogen
NCT04868916; Jul 2021-Apr 2024	Observational; participants: 15.	i) Patients aged ≥5 years; ii) are able to give informed consent or assent, with the guidance of their parent/guardian where appropriate.	Primary: Visual function, retinal structure, retinal function; method: Visual acuity, SD-OCT, static visual field testing.	Japan Pharmaceutical Manufacturers Association (JPMA)
NCT04926129; Sep 2017-Dec 2022	Observational; participants: 201.	i) Patients ages ≥7 years and older; ii) are willing and able to undergo ophthalmic examinations, as required by protocol; ii) have an ETDRS BCVA in ≥1 eye of ≥34 letters (Equivalent to Snellen ≥6/60 or 20/200; decimal 0.1; LogMAR 1.0).	Primary: Changes in retinal sensitivity within the macula; secondary: Change from baseline in contrast sensitivity, low luminance visual acuity, visual function questionnaire score, RP-specific patient-reported outcome (PRO) questionnaire, EuroQol-5 Dimension 5-level (EQ-5D-5L), Health Utilities Index Mark 3 (HUI3), visual field readings, microperimetry readings, multi-luminance mobility	Biogen

Table III. Continued.

Clinical Trials.gov ID; date	Type and phase	Patient population	Assessment	Sponsors
			test (MLMT) readings, SD-OCT, FAF, fundus photography, morphology of eye as assessed by slit-lamp examination.	
NCT03316560 (rAAV2tYF-GRK1-RPGR) Apr 2018-Mar 2025	Interventional, I/II; participants: 29.	i) Male patients aged 6-50 years of age with documented *RPGR* mutations; ii) BCVA ≤78 ETDRS letters; iii) be capable of conducting visual and retinal function tests, evaluated through OCT and have detectable ellipsoid zone lines.	Primary: Number and proportion of AEs, safety and efficiency; secondary: VA, SD-OCT, quality of life questionnaire responses, visual function by light-adapted perimetry, fundus imaging, FST and visual function by dark-adapted full field perimetry (for subjects treated peripherally).	Beacon Therapeutics
NCT06333249 (rAAV2tYF-GRK1-RPGR); Apr 2021-Feb 2027	Interventional, II; participants: 14.	i) Provide written informed consent; ii) male patients ages 8-50 years; iii) 35 letters ≤BCVA≤75 letters; iv) have detectable EZ line in both eyes as assessed by SD-OCT and confirmed by the CRC.	Primary: Efficacy, safety and tolerance. The difference in the proportion of responding eyes between treated and control eyes in the low dose group and high dose group at 12 months, as measured by MAIA microperimetry, where response is defined as a 7dB or more improvement in at least 5 loci. Secondary: VA; ORA-VNC mobility test.	Beacon Therapeutics
NCT06492850 (FT-002); Apr 2024-Feb 2026	Interventional, I/II; participants: 32.	i) Are willing and able to follow study procedures including scheduled visits, treatment plan, and laboratory tests, and sign a written informed consent form; ii) Phase I: Male patients aged 18-45 years. Phase II: Male patients aged 8-45 years old.	Primary: Evaluate the safety and tolerance of FT-002. Incidence and severity of AEs. Secondary: Evaluate the efficacy of FT-002. Changes in visual sensitivity, FST and BCVA.	Frontera Therapeutics, Inc.
NCT05874310 (FT-002); Feb 2023-Nov 2027	Interventional, I; participants: 18.	i) Are able to follow study procedures. Male patients ages 8-45 years. ii) confirmed with variants of *RPGR*. Has not received any other gene therapy products.	Primary: Number and proportion of AEs. Secondary: Change in visual function, retinal structure. Method: Retinal sensitivity within the 30-degree visual field and OCT.	Frontera Therapeutics, Inc.
NCT06275620 (rAAV2tYF-GRK1-RPGR); Nov 2023-Dec 2029	Interventional, II; participants: 24.	i) Male patients aged ≥12 years. ii) Have one eye previously treated with an AAV vector-based gene therapy. iii) Have detectable baseline mean macular sensitivity measured by MAIA microperimetry and have detectable EZ lines. iv) Corticosteroid drugs can be used.	Primary: Incidence of SAEs (Day 0-Month 12). Secondary: Incidence and severity of AEs. Assess photoreceptor function under low light, FST, BCVA, LLVA, EZ area, Ora-VNC mobility test, mobility standardized test-virtual reality (MOST-VR) mobility course test score.	Beacon Therapeutics

Table III. Continued.

Clinical Trials.gov ID; date	Type and phase	Patient population	Assessment	Sponsors
NCT04671433 (AAV5-hRKp.RPG); Dec 2020-Sep 2024	Interventional, III; participants: 97.	i) Patients aged ≥3 years. ii) Has XLRP confirmed by a retinal specialist and has a predicted disease-causing sequence variant in *RPGR* confirmed by an accredited laboratory. iii) Those who have undergone other ocular treatments or eye surgeries are excluded.	Primary: Change from baseline to Week 52 in VMA as measured by the ability of the participant to navigate through a VMA maze. Secondary: Mean retinal sensitivity within the central 10 degrees excluding scotoma, pointwise response in full visual field, pointwise response in worse-seeing eye in the central 30 degrees visual field, mean retinal sensitivity within the full visual field (MRS90) in static perimetry, the modified low luminance questionnaire (mLLQ) extreme lighting domain score, BCVA, LLVA, ocular and non-ocular AEs, abnormalities in laboratory assessments (from baseline to Week 52).	Johnson & Johnson
NCT04312672 (AAV5-hRKp.RPGR); Jul 2017-Nov 2026	Observational; participants: 42.	i) Male patients aged ≥5 years. ii) Received AAV5-hRKp.RPGR injection in MGT009 study.	Primary: Safety. Secondary: Change in functional vision of LLQ domain scores, BCVA, LLVA, MRS10, MRS90, pointwise response in full visual field, functional vision of walk time in VMA.	Johnson & Johnson
NCT04794101 (AAV5-hRKp.RPGR); Dec 2020-Sep 2029	Interventional, III; participants: 97.	i) Patients aged ≥3 years. ii) Have XLRP confirmed by a retinal specialist.	Primary: Incidence of AEs, BCVA, number of participants with abnormalities in laboratory assessments, LLVA.	Johnson & Johnson
NCT03584165 (AAV8-RPGR); Jun 2018-Jun 2026	Interventional, III; participants: 330 (CHM and XLRP participants).	i) Male patients aged ≥18 years. ii) Have received a sub-retinal injection of BIIB112 for XLRP and have exited an antecedent study.	Primary: Incidence of AEs, IOP, abnormal slit lamp examination, lens opacity grading, anterior chamber and vitreous inflammation, indirect ophthalmoscopy (up to 5 years). Secondary: Change from baseline in BCVA, VFQ-25, VF, autofluorescence (AF), fundus photography, SD-OCT, microperimetry.	Biogen
NCT04517149 (4D-125); Jun 2020-May 2029	Interventional, I/II; participants: 21.	i) Male patients aged ≥12 years. ii) ≥1 eye amenable to intravitreal injection and 34 letters ≤ BCVA ≤78 letters.	Primary: Treatment. Incidence and severity of TEAEs and SAEs.	4D Molecular Therapeutics
NCT05926583 (AAV5-hRKp.RPGR); Sep 2023-Feb 2030	Interventional, III; participants: 4.	i) Japanese male or female patients aged ≥5 years. ii) Mean retinal sensitivity of greater than or equal to ≥2 decibel (dB).	Primary: Incidence of AEs, number of participants with abnormalities in clinical laboratory assessments. Secondary: Change from baseline LLVA, BCVA (Baseline-Week 52).	Johnson & Johnson

Table III. Continued.

Clinical Trials.gov ID; date	Type and phase	Patient population	Assessment	Sponsors
NCT06646289 (AAV5-hRKp.RPGR); Oct 2024-Oct 2030	Interventional, II; participants: 42.	i) Male patients ≥5 years. ii) Have been treated with AAV5-hRKp.RPGR in study MGT009 and have completed or is currently enrolled in Study MGT010. iii) Sign an informed consent form. iv) Willing to adhere to the protocol and long-term follow-up.	Primary: Incidence of AEs (from baseline up to 5.5 years). Change from baseline in BCVA, LLVA (baseline, months 12, 24, 36, 48 and 60).	Johnson & Johnson
NCT04850118 (rAAV2tYF-GRK1-hRPGRco); Mar 2024-Oct 2029	Interventional, II/III; participants: 75.	i) Have a parent, guardian or legal representative provide written informed consent. ii) Male patients aged 12-50 year. iii) Follow study instructions, complete study assessments, comply with the protocol. iv) 34 letters ≤ BCVA ≤78 letters, LLVA ≤64 letters, 10< LLD letters. Have detectable EZ line in both eyes as assessed by SD-OCT and confirmed by the CRC.	Primary: The proportion of participants with a ≥15 letter increase from baseline in LLVA. Secondary: Change from baseline in LLVA, mean sensitivity across the whole grid, as measured by MAIA microperimetry, FST. (Day 0-Month 12). Ocular/non-ocular adverse events are collected during the duration of the trial (Day 0-Year 5). Exclusion criteria can be found on the official website.	Beacon Therapeutics

RPGR, *retinitis pigmentosa GTPase regulator*; AE/AEs, adverse event(s); VA, visual acuity; BCVA, best-corrected visual acuity; LLVA, low luminance visual acuity; MAIA, macular integrity assessment; MRS10, macular retinal sensitivity at 10°; MRS90, macular retinal sensitivity at 90°; SD-OCT, spectral-domain optical coherence tomography; OCT, optical coherence tomography; FAF, fundus autofluorescence; VF, visual field; ETDRS, early treatment diabetic retinopathy study (score); FST, full-field stimulus threshold; LLD, low luminance deficit; ORA-VNC, Ora-visual navigation challenge; VMA, vision-guided mobility assessment; VFQ-25, visual function questionnaire; LLQ, low luminance questionnaire domain scores; mLLQ, modified low luminance questionnaire; IOP, intraocular pressure; dB, decibel; EZ, ellipsoid zone; DLTs, dose-limiting toxicities; TEAEs, treatment-emergent adverse events; EQ-5D-5L, EuroQol-5 Dimension 5-level; HUI3, Health Utilities Index Mark 3.

Table IV. Completed clinical trials of RPGR gene therapy: Main results and efficacy outcomes.

Name of gene product	Vector	Primary endpoint	Main clinical results	Limitations
rAAV2tYF-GRK1-hRPGRco (AGTC-501)	i) Employing an AAV2 capsid containing a single tyrosine-to-phenylalanine (Y→F) substitution. ii) Codon-optimized full-length DNA sequence.	i) HORIZON: Incidence and severity of TEAEs and SAEs. ii) VISTA: Proportion of participants achieving a ≥15 letter improvement in LLVA.	i) HORIZON: It has good safety and nodose-limiting inflammation. Early data support the advancement of subsequent experiments. ii) 12-24 months, retinal sensitivity functions are continuously enhanced (≥5 loci with >7 dB improvement). iii) 12 months: MAIA, +1.96 dB (treated), -0.39 dB (control). 24 months: MAIA, +1.63 dB (treated), -1.56 dB (control). iv) The overall response rate was 33% in the treatment group (50% in the highest-dose cohort) and 0% in the control group.	i) In the high-dose cohort, 75% of patients exhibited RPE changes, accompanied by procedure-related complications. ii) Dose-finding studies indicated a narrow optimal dose range. iii) The small number of participants, with only four receiving the high-dose injection, limited the generalizability of the findings.
AAV5-hRKp.RPGR	i) Using AAV5 and hRKp. ii) Carrying a truncated RPGR sequence.	i) MGT009: Incidence of SAEs within 9 weeks after administration. ii) LUMEOS: Change from baseline to Week 52 in VMA (the ability of the participant to navigate through a VMA maze).	i) MGT009: SAEs occurred, including retinal detachment and uveitis. ii) LUMEOS: At Week 52, the primary endpoint VMA data were not statistically significant, but showed a favorable trend. However, improvements were observed in the three secondary endpoints: Retinal function, VA and visual function.	i) Using VMA as an endpoint in RPE65 therapy studies may be less appropriate for XLRP, given differences in visual field and sensitivity. ii) Inaccurate patient stratification: Stratification based on EZ area, age and disease duration may be insufficient, posing a risk of mismatched therapeutic windows. iii) Variations in surgical procedures and immunosuppression regimens further reduce cross-trial comparability.

Table IV. Continued.

Name of gene product	Vector	Primary endpoint	Main clinical results	Limitations
AAV8-RPGR (BIIB112, XIRIUS)	i) Using AAV8 and GRK1/hRKp. ii) Carrying a codon-optimized full-length cDNA sequence.	i) Part 1: Number of participants with DLTs and the number of participants with TEAEs. ii) Part 2: Percentage of study eyes with ≥7 dB improvement from baseline at ≥5 of the 16 central loci on the 10-2 grid and the number of participants with TEAEs.	i) XIRIUS: The predefined primary endpoint was not met, and adose-safety relationship was observed, with higher doses more likely to cause SAEs, including vision loss and noninfectious retinitis. ii) Although the primary endpoint was not met, the secondary endpoint assessments showed favorable trends in visual outcomes. In the mid-dose to high-dose cohorts, both LLVA and microperimetric sensitivity demonstrated sustained improvement over time. iii) 12M: retinal sensitivity, 2.8 dB (treated), 0.1 dB (untreated). iv) 12M: MAIA, + 5.1 dB (treated), -0.9 dB (untreated).	i) Using MAIA microperimetry as an endpoint is susceptible to the effects of fixation instability and locus registration drift. ii) There were differences in disease stage and residual EZ area across patient strata. iii) Patients receiving mid-to high-dose treatment experienced more frequent inflammatory events, which have affected the overall therapeutic outcomes.
4D-125	i) Using 4D-R100; ii) Engineered from AAV2 and having a codon-optimized DNA sequence.	i) The incidence of TEAEs and SAEs. ii) Patients undergoing the intervention received a single-eye injection and were followed for 24 months to monitor safety and tolerability.	i) All 8 patients tolerated the treatment well, with no DLTs or SAEs reported. Only two patients experienced mild and transient inflammation. ii) 9 months: Retinal sensitivity, 1.65 dB (treated), 0.25 dB (untreated).	i) The small sample size limited the data analysis. ii) Participants enrolled in the dose-escalation phase were all in the late clinical stage of XLRP, with residual photoreceptor regions and retinal sensitivity that were limited or even unmeasurable.

HORIZON, NCT03316560, Interventional, I/II, Beacon Therapeutics. VISTA, NCT04850118, Interventional, II/III, Beacon Therapeutics. MGT009, NCT03252847, Interventional, I/II, MeiraGTx UK II Ltd. LUMEOS, NCT04671433, Interventional, III, Janssen Research & Development, LLC. BIIB112 (XIRIUS), NCT03116113, Interventional, I/II, Biogen. NCT03584165, Interventional, III, Biogen. 4D-125, NCT04517149, Interventional, I/II, 4D Molecular Therapeutics. AAV, adeno-associated virus; TEAEs, treatment-emergent adverse events; SAEs, serious adverse events; LLVA, low luminance visual acuity; dB, decibel; RPE, retinal pigment epithelium; XLRP, X-linked retinitis pigmentosa; EZ, ellipsoid zone; VMA, vision-guided mobility assessment; MAIA, macular integrity assessment; DLTs, dose-limiting toxicities.

Current research consensus suggests that the key to XLRP-*RPGR* gene therapy lies in extending the follow-up period, optimizing dose and vector systems, and exploring combination therapy strategies. Future studies should evaluate the long-term efficacy (such as maintenance of visual function for ≥3 years) and delayed risks, such as the mutagenic potential of vector genome integration. Dosing should also be dynamically adjusted based on EZ biomarkers to balance short-term efficacy with long-term safety. Additionally, the combined use of anti-inflammatory agents, including topical corticosteroids, with gene therapy should be investigated. This approach may help reduce inflammation-related complications, alleviate patient anxiety and further improve quality of life.

7. Conclusion and perspective

RPGR-associated RP has attracted increasing attention, and studies on its pathogenic mechanism and gene therapy strategies have become a major focus in the field (9,222-224). This present review summarizes the molecular mechanisms of photoreceptor degeneration triggered by *RPGR* mutations and analyzes the functions of RPGR along with its interacting protein networks. Although the interaction networks of RPGR have been identified, the specific function of individual proteins and the mechanisms of their interaction with RPGR remain to be investigated in depth. RPGR regulates signal transduction and metabolic homeostasis in photoreceptors by maintaining the structural integrity of the CC and its transport functions. Mutations lead to failure of rhodopsin transport, accumulation of metabolic waste and ROS. The survival of photoreceptors depends on the coordinated action of multiple metabolic and signaling regulatory mechanisms and *RPGR* mutations may disrupt these pathways, thereby accelerating photoreceptor degeneration. In addition, an imbalance in the transmembrane signaling systems may further reduce the adaptability of photoreceptors, making them more susceptible to apoptosis under conditions of oxidative stress or limited nutrient availability. Animal models carrying *RPGR* mutations have provided valuable insights into disease mechanisms and carry out an important role in the development of therapeutic strategies. Future research should further investigate the spatiotemporal dynamics and regulatory interactions of *RPGR*-related signaling pathways in animal models. Such studies will help clarify the specific functions of RPGR proteins within these pathways and provide a theoretical basis for the development of targeted therapies.

In recent years, research on *RPGR*-associated XLRP has progressed from efficacy validation in preclinical animal models to the preliminary success of clinical trials. These studies have demonstrated that some patients show pronounced improvements in retinal sensitivity and VF after treatment, offering new hope for gene therapy. However, treatment still faces challenges related to dose control, immune responses and the maintenance of long-term efficacy (219,225). Although codon optimization and long-term immune monitoring have been implemented in clinical studies (175,217,218,223,226), issues remain regarding the balance between dosage and safety, management of postoperative adverse events, delayed immune reactions and the sustained preservation of therapeutic effects. Regarding viral vector optimization, improvements in AAV vectors through capsid engineering are important, and rational functional modification of various viral vectors also represent a key strategy to enhance their clinical utility (227,228). Beyond reducing immunogenicity, it is also necessary to comprehensively assess vector transduction efficiency and safety to improve overall treatment efficacy and precision. At the clinical implementation stage, patient selection, immune monitoring and long-term follow-up systems should be strengthened to balance therapeutic efficacy with safety risks, thereby laying the foundation for the standardization of subsequent gene therapy strategies.

As ocular gene therapy technologies continue to mature, relying solely on a single viral vector for delivery may be insufficient to fully restore retinal function and stabilize the retinal microenvironment. Currently, several neuroprotective agents are undergoing Phase I/II clinical trials (such as, UBX1325, MTP-131 and Metformin) (142). Future research is trending toward combined approaches that integrate gene therapy with neuroprotection. By simultaneously correcting genetic mutations, activating cell survival signaling pathways and upregulating neurotrophic factor expression, such strategies hold the potential to further slow photoreceptor degeneration and maintain microenvironmental stability. These combination therapies enhance treatment stability and long-term efficacy, thereby overcoming the limitations of conventional monotherapies. However, achieving precise temporal control of gene editing and neuroprotective signaling, verifying the safety of co-delivery systems and establishing regulatory evaluation frameworks for combination therapies remain key challenges for future clinical translation.

Meanwhile, non-viral vectors have been advancing toward the development of new platforms with low immunogenicity, strong targeting capabilities and scalable production, including liposomes, solid nanoparticles, polymeric nanoparticles and polyplexes for nucleic acid delivery (229-232). These vectors are being explored for neuroprotective strategies, offering new avenues for multimodal treatment of retinal degenerative diseases. However, nanoparticle-based gene delivery systems share common challenges: Systemic accumulation can induce toxicity, making them unsuitable for long-term use, and the degradation of nanocarriers following nucleic acid release *in vivo* may generate toxic byproducts (229,231,233,234). Injectable retinal nanoimplants combining donor-acceptor polymers with graphene have been developed, capable of restoring light-driven behavior, visual brain activity and retinal function without inducing inflammation, providing an alternative perspective for RP treatment (235). Therefore, delivery strategies using non-viral vectors still require further optimization in terms of material design, biodegradability and long-term safety. In the future, integrated approaches that combine gene therapy, neuroprotective agents and traditional treatments such as DHA may further enhance therapeutic outcomes (Fig. 7A). However, these strategies will require more comprehensive regulatory evaluation frameworks (15,135).

Ethical and regulatory challenges remain prominent in the clinical translation of ocular gene therapies. These challenges encompass not only the evaluation of safety and efficacy at the technical application level but also the protection of patient rights and equitable access to treatment. Current regulatory frameworks primarily focus on safety and efficacy as core

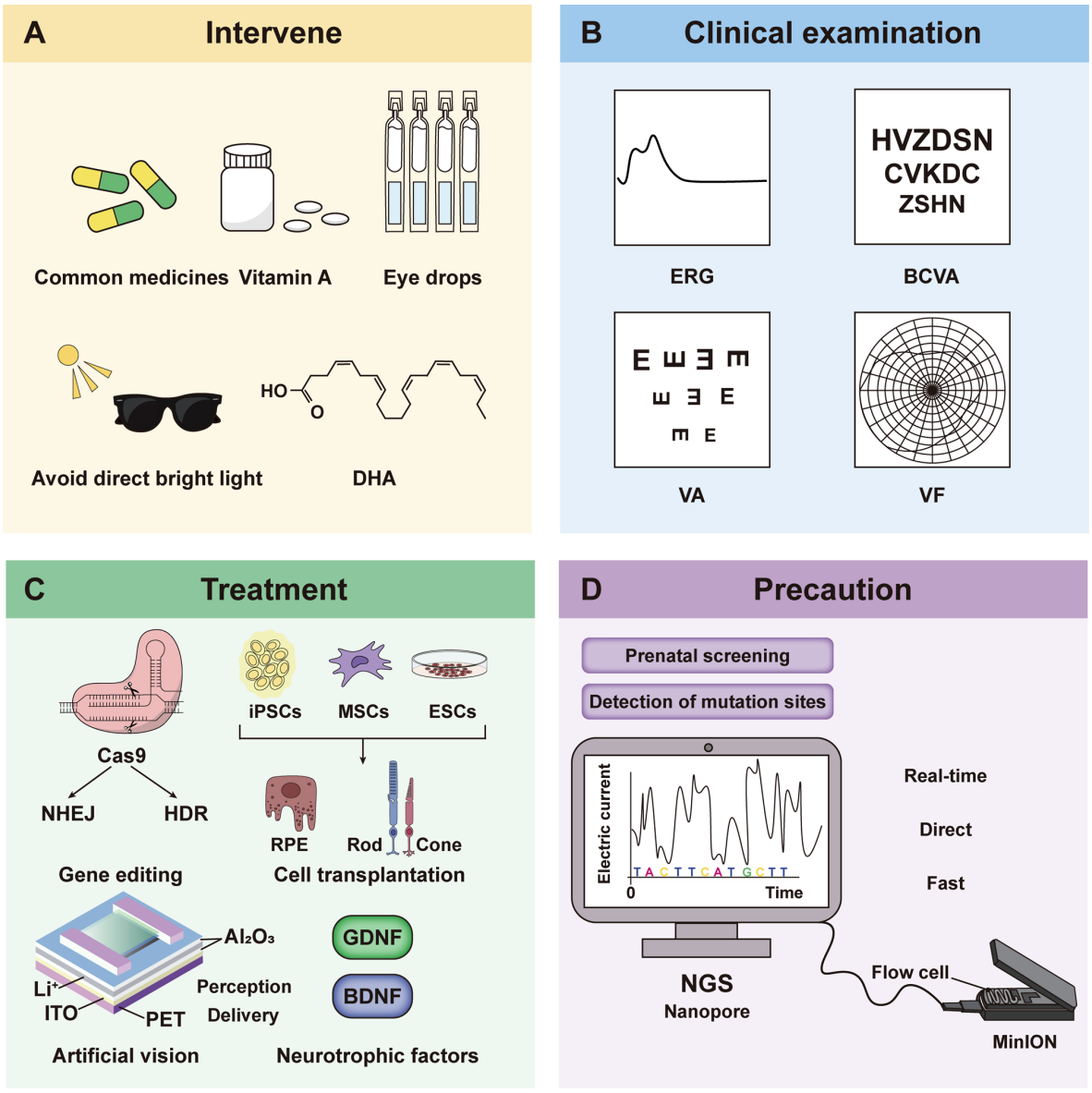

Figure 7. Strategies for integrated management of ocular diseases in patients with mutated *RPGR*. The program encompasses interventions (medications, nutrition and lifestyle modifications), diagnostic tests (such as ERG), therapeutic strategies (genetic and cellular treatments) and preventive screening measures. (A) Interventions: Commonly used medications, vitamin A supplementation, ophthalmic solutions, glare avoidance and DHA intake. (B) Diagnostic assessments: ERG, BCVA, VA and VF testing. (C) Therapeutic approaches: Treatment modalities encompass CRISPR-Cas9-based gene editing (NHEJ and HDR), cell transplantation (iPSCs, MSCs and ESCs), artificial vision technologies and neurotrophic factor therapy (GDNF and BDNF). (D) Preventive measures: Detection of pathogenic variants through NGS and nanopore sequencing. DHA, docosahexaenoic acid; ERG, electroretinography; BCVA, best-corrected visual acuity; VA, visual acuity; VF, visual field; NHEJ, Non-Homologous End Joining; HDR, Homology-Directed Repair; iPSCs, induced pluripotent stem cells; MSCs, mesenchymal stem cells; ESCs, embryonic stem cells; GDNF, Glial cell line-derived neurotrophic factor; BDNF, Brain-derived neurotrophic factor; NGS, next-generation sequencing.

approval criteria; however, specific guidelines addressing potential delayed inflammatory responses following treatment are still under development. Moreover, regulatory systems vary across countries and regions, potentially increasing the costs of clinical translation. Ethical concerns primarily involve patient privacy protection, informed consent and treatment equity. On one hand, a balance must be achieved between safeguarding patient privacy and ensuring transparency of information. On the other hand, the high cost of therapy may exacerbate socioeconomic disparities (236). Therefore, ensuring standardized production of gene therapy products, long-term safety validation, transparent informed consent procedures and equitable access are important prerequisites for the standardized and sustainable clinical translation of ocular gene therapies. Although pronounced progress has been made in RPGR gene therapy, patient phenotype and disease progression pattern vary. Thus, precise stratification of patient populations is essential to implement individualized treatment strategies. Modern technologies allow for more accurate patient assessment and tailored therapeutic planning by analyzing mutation sites in conjunction with genotype-expression associations.

High-precision molecular diagnostics combined with multidimensional analyses are required to characterize the mutation regions of the *RPGR* of patients, overcoming

sequencing limitations and ensuring the reliability of clinical stratification and therapeutic strategies. Integrating early intervention with conventional drugs and emerging approaches such as gene editing, gene replacement and combined neuroprotection, as well as cell transplantation and artificial retinal implantation (Fig. 7B-D), can advance precision medicine for *RPGR* mutations. These strategies are also expected to provide new directions for the treatment of RP.

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Authors' contributions

YL and JQ drafted, revised the manuscript and created figures. WZ assisted with editing. HQ and KY edited the manuscript, supervised the study and provided funding. All authors read and approved the final manuscript. Data authentication is not applicable. Kai Yao-Lead contact.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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