REVIEW ARTICLE

RPGR-containing protein complexes in syndromic and non-syndromic retinal degeneration due to ciliary dysfunction

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Abstract

Dysfunction of primary cilia due to mutations in cilia-centrosomal proteins is associated with pleiotropic disorders. The primary (or sensory) cilium of photoreceptors mediates polarized trafficking of proteins for efficient phototransduction. Retinitis pigmentosa GTPase regulator (RPGR) is a cilia-centrosomal protein mutated in >70% of X-linked RP cases and 10%–20% of simplex RP males. Accumulating evidence indicates that RPGR may facilitate the orchestration of multiple ciliary protein complexes. Disruption of these complexes due to mutations in component proteins is an underlying cause of associated photoreceptor degeneration. Here, we highlight the recent developments in understanding the mechanism of cilia-dependent photoreceptor degeneration due to mutations in RPGR and RPGR-interacting proteins in severe genetic diseases, including retinitis pigmentosa, Leber congenital amaurosis (LCA), Joubert syndrome, and Senior–Loken syndrome, and explore the physiological relevance of photoreceptor ciliary protein complexes.

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Introduction

The cilium is an extension of the cell membrane formed by nucleation of microtubules. The primary cilium is present in almost all cell types and has diverse functions (Pazour and Witman 2003; Davenport and Yoder 2005; Scholey and Anderson 2006); it contains a central core structure (the axoneme), which comprises of nine outer doublet microtubules with no central microtubule pair (9+0) (Pazour and Witman 2003). The ciliary subunits are assembled at the basal body or mother centriole of post-mitotic cells. This process involves coordinated action of centrosomal proteins and small GTPases that control the switch between cytokinesis and ciliogenesis (Doxsey 2001; Spektor *et al*. 2007; Tsang *et al*. 2008). During ciliogenesis, protein complexes are transported distally for growth of the axoneme using an elaborate mechanism called intraflagellar transport (IFT) (Rosenbaum *et al*. 1999). According to the current model of IFT, protein and membrane cargo are transported bidirectionally along the axoneme by coordinated action of kinesin (anterograde; KIF family members) and dynein (retrograde) motors (Besharse *et al*. 2003; Follit *et al*. 2006).

Given their near-ubiquitous presence, cilia are involved in diverse cellular processes, including establishment of left–right asymmetry, sonic hedgehog signalling, mechanosensation, olfaction, chemosensation, and phototransduction (Gerdes *et al*. 2009). Commensurate with this, defects in primary cilia result in severe developmental and lethal disorders, such as altered embryonic patterning, renal cystic diseases, mental retardation and photoreceptor degeneration (Gerdes *et al*. 2009).

Photoreceptor sensory cilium

Photoreceptor inner segment (having the metabolic machinery) and the outer segment (membranous disks containing phototransduction proteins) are linked by a connecting cil-

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ium, which is a modified primary cilium (Young 1968) (figure 1). Approximately 10% of outer segments are turned over each day, with new discs being formed proximally and shed distally. About 2000 opsin molecules are transported per minute to maintain the function/integrity of each rod outer segment (Besharse 1986); these molecules are synthesized in the inner segment, sorted at the post-Golgi vesicles and transported to the base of the connecting cilium, where they probably associate with transport proteins for trafficking to the outer segment (Chuang and Sung 1998; Deretic *et al*. 1998). The polarized post-Golgi trafficking and docking of rhodopsin at the basal bodies involve the activity of small GTPases, including Rab8 and ARF4 (Deretic *et al*. 1995; Moritz *et al*. 2001) and is also probably mediated by a FYVE-domain containing protein SARA, phosphatidylinositol 1-phosphate (PI3P), and syntaxin-3 (Chuang *et al*. 2007; Mazelova *et al*. 2009). Perturbation of rhodopsin transport leads to RP (Sung *et al*. 1994; Colley *et al*. 1995).

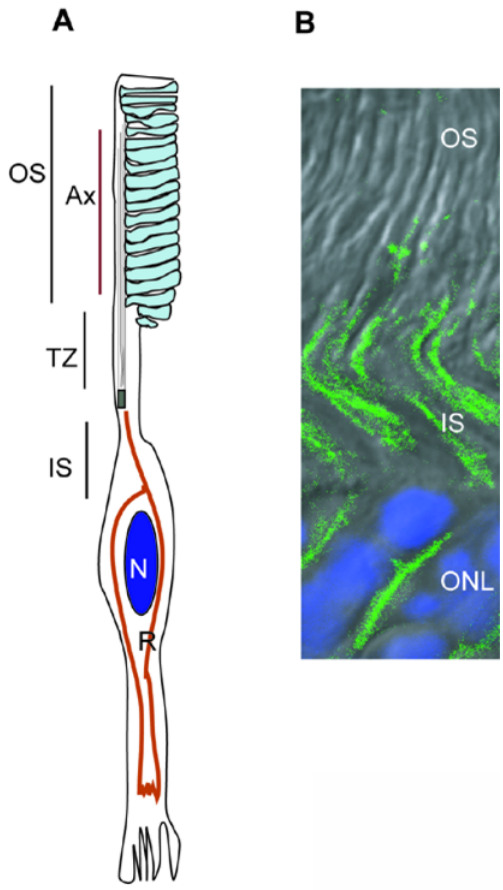

Figure 1. (A) Schematic of a rod photoreceptor cell showing the sensory cilium axoneme (Ax); TZ, transition zone; R, the rootlet; IS, inner segment; OS, outer segment connected by the TZ; N, nucleus. (B) Immunofluorescence image of photoreceptor layer of mouse retina stained with anti-acetylated a-tubulin antibody, a ciliary marker (green); ONL, outer nuclear layer.

The transition zone (TZ) of photoreceptor sensory cilium serves as a 'transport corridor' for bidirectional trafficking of macromolecular complexes along the microtubule network (figure 1). IFT particle proteins, including Tg737/Polaris/IFT88, localize at the basal body and the axoneme of photoreceptor cilium (Pazour *et al*. 2002). Disruption of IFT in *Kif3a* conditional knockout mice (Marszalek *et al*. 2000) and in *Tg737*^{orpk} mice (Pazour *et al*. 2002) results in opsin accumulation in inner segments and consequently photoreceptor degeneration. The phototransduction proteins, transducin, arrestin, and recoverin undergo light-dependent reversible translocation between outer and inner segments (Sokolov *et al*. 2002; Strissel *et al*. 2005, 2006). Transport of arrestin is, at least in part, mediated by simple diffusion and anchoring through protein–protein interactions (Nair *et al*. 2005).

Photoreceptor ciliopathies

Retinal degeneration due to ciliary dysfunction appears as part of a spectrum of diseases, where one end are some forms of Leber congenital amaurosis (LCA) (MIM 204000), characterized at birth or during early childhood. On the other side of the spectrum resides RP (MIM 268000), wherein a majority of patients exhibit early signs of night blindness in the early stages (due to rod photoreceptor dysfunction) progressing to decreased visual fields and culminating in complete blindness usually in the later stages of life. Depending upon the gene, age of onset phenotype can vary. RP is inherited in autosomal dominant (~30% of cases), autosomal recessive (~20%) as well as X-linked manner (Daiger *et al*. 2007).

X-linked RP (XLRP)

XLRP is one of the severe forms of RP associated with considerable clinical and genetic heterogeneity and accounts for 10%–20% of inherited nonsyndromic RP (Fishman 1978; Jay 1982; Breuer *et al*. 2002). Most affected males exhibit early-onset visual symptoms with night blindness in the first decade and rapid progression towards blindness by age 40 (Bird 1975; Fishman *et al*. 1988; Daiger *et al*. 2007; Shu *et al*. 2007). Heterozygous carrier females can show electroretinographic (ERG) abnormalities and tapetal-like reflex (Bird 1975; Fishman *et al*. 1986; Cideciyan and Jacobson 1994; Sieving 1995). Some XLRP patients have abnormal sperm phenotype (Hunter *et al*. 1988) or hearing defects (Zito *et al*. 2003; Iannaccone *et al*. 2004). To date, six genetic loci have been identified of which, two causative genes *RP2* and *RP3* (RPGR) have been cloned (Wright *et al*. 1991; McGuire *et al*. 1995; Fujita *et al*. 1996; Meindl *et al*. 1996; Roepman *et al*. 1996; Gieser *et al*. 1998; Schwahn *et al*. 1998; Hardcastle *et al*. 2000; Melamud *et al*. 2006).

RP2

Mutations in *RP2* account for approximately 10% of XLRP (Hardcastle *et al*. 1999; Mears *et al*. 1999; Sharon *et al*. 2000, 2003; Breuer *et al*. 2002). The *RP2* gene encodes a putative protein of 350 amino acids (Schwahn *et al*. 1998; Chapple *et al*. 2000). The crystal structure of the RP2 protein reveals an amino-terminal β-helix that is structurally and functionally homologous to the tubulin-specific chaperone, cofactor C; most disease-causing missense mutations are present in this domain (Bartolini *et al*. 2002; Grayson *et al*. 2002; Kuhnel *et al*. 2006). RP2 appears to be targeted predominantly to the plasma membrane (Chapple *et al*. 2000; Grayson *et al*. 2002). It interacts with ADP-ribosylation factor-like 3 (ARL3) (Grayson *et al*. 2002; Kuhnel *et al*. 2006), a microtubule-associated small GTP-binding protein (Kahn *et al*. 2005), which localizes to the connecting cilium of photoreceptors (Grayson *et al*. 2002; Schrick *et al*. 2006).

Retinitis pigmentosa GTPase regulator (RPGR)

Mutations in the retinitis pigmentosa GTPase regulator (*RPGR*) gene are associated with more than 70% of the patients with X-linked retinitis pigmentosa (XLRP) and 10%–20% of simplex RP males (Meindl *et al*. 1996; Roepman *et al*. 1996; Breuer *et al*. 2002; Vervoort and Wright 2002; Sharon *et al*. 2003). These data indicate that RPGR mutations are one of the most common causes of retinal degeneration in addition to rhodopsin. In addition, some patients with mutations in RPGR may also display syndromic features like primary cilia dyskinesia or hearing loss (van Dorp *et al*. 1992; Iannaccone *et al*. 2003, 2004; Koenekoop *et al*. 2003; Zito *et al*. 2003; Moore *et al*. 2006). RPGR is predominantly located at the primary cilium of photoreceptors and multiple different isoforms are recognized (Hong *et al*. 2003; Khanna *et al*. 2005; He *et al*. 2008); however two major ones are recognized: $RPGR^{ORF15}$ and $RPGR^{EX1-19}$ (Vervoort *et al*. 2000; Breuer *et al*. 2002).

Expression and localization of RPGR protein isoforms

Complex-splicing patterns are reported for *RPGR* though the physiological relevance of these transcripts is unclear (Yan *et al*. 1998; Kirschner *et al*. 1999; Vervoort *et al*. 2000; Hong and Li 2002; Ferreira 2005). Purine-rich exon splicing enhancers in ORF15 may also modify the efficiency of splicing (Hong and Li 2002). Multiple immunoreactive bands are observed using isoform-specific RPGR antibodies (Yan *et al*. 1998; Hong and Li 2002; Mavlyutov *et al*. 2002; He *et al*. 2008; Khanna *et al*. 2005; Otto *et al*. 2005; Shu *et al*. 2005; Chang *et al*. 2006). The constitutive $RPGR^{EX1-19}$ isoform is isoprenylated and localizes to golgi in transfected cells (Yan *et al*. 1998). The amino-terminal region of RPGR encoded by exons 1–15 encompasses a common RCC1-like domain (Meindl *et al*. 1996; Vervoort *et al*. 2000) (figure 2). Although RCC1 is a guanine nucleotide exchange factor (GEF) for Ran GTPases (Meindl *et al*. 1996; Renault *et al*. 2001; Sazer *et al*. 2005), no such activity has yet been reported for RPGR. The $RPGR^{ORF15}$ isoforms that include a C-terminal acidic domain rich in Glu–Gly repeats (Vervoort *et al*. 2000) are reported to have distinct subcellular localizations (Mavlyutov *et al*. 2002; Hong *et al*. 2003). In addition, multiple predicted homology domains can also be detected in the primary structure of the RPGR protein. $RPGR^{ORF15}$ localizes predominantly to the cilia and basal bodies of both mouse and human photoreceptors though some additional labelling

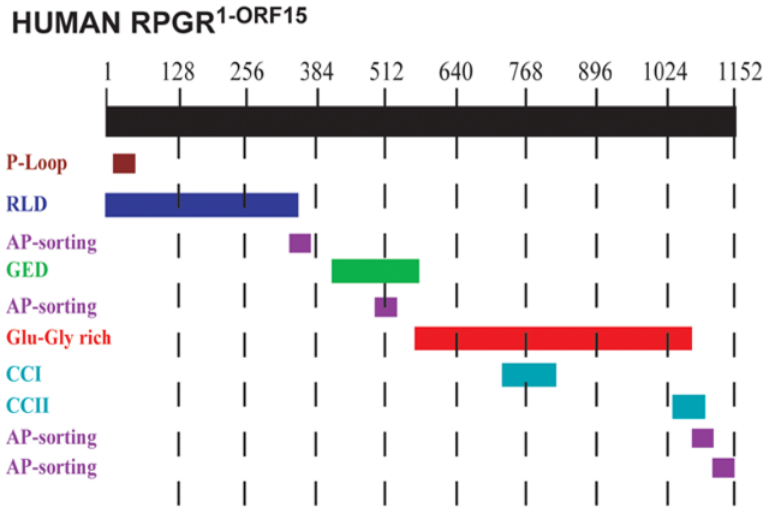

Figure 2. Schematic representation of the predicted domain organization of human RPGR protein. P-Loop, ATP/GTP binding loop; RLD, RCC1-like domain; AP-sorting, adaptor protein sorting domain; Glu-Gly, glutamic acid and glycine rich domain; CC, coiled-coil domain.

can be found in the inner and outer segments (Khanna *et al*. 2005; Shu *et al*. 2005). In proliferating cells, centrosomes and midbody are labelled with anti-RPGR antibodies (**?**Chang *et al*. 2006; Shu *et al*. 2005). Our analysis of RPGR using isoform-specific antibodies revealed that the $RPGR^{EX1-19}$ and $RPGR^{ORF15}$ isoforms localize to overlapping as well as distinct subcellular compartments in the retina (He *et al*. 2008). Moreover, the $RPGR^{EX1-19}$isoforms exist in overlapping protein complexes with $RPGR^{ORF15}$.

Animal models of RPGR

A *Rpgr*-knockout (ko) mouse with deletion of exons 4–6 of *Rpgr* is reported to show late-onset cone-rod degeneration (Hong *et al*. 2000); however, this *Rpgr*-ko mouse is not a complete null and expresses some $RPGR^{ORF15}$ isoforms that localize to the photoreceptor cilium (Khanna *et al*. 2005). The phenotype of this mouse can be partially rescued by an ORF15-variant (Hong *et al*. 2005). However, the same variant in transgenic mice may result in dominant gain-of-function phenotype exhibiting rapid disease progression (Hong *et al*. 2004). Attempts to generate a complete *Rpgr* null mutation in mice showing no RPGR isoforms have so far been futile. $RPGR^{ORF15}$ frameshift mutations have been identified in two naturally occurring canine mutants; the XLPRA2 dog exhibits relatively rapid photoreceptor degeneration and severe ERG abnormalities, whereas the XLPRA1 mutant shows a milder phenotype (Zhang *et al*. 2002; Beltran *et al*. 2006).

RPGR-interactome in photoreceptors

Until 2005, two RPGR-interacting proteins were identified by Y2H analysis using the RLD bait: RPGRIP1, which is localized to the sensory cilium and mutated in patients with LCA (Boylan and Wright 2000; Dryja *et al*. 2001; Hong *et al*. 2001); and PDE6d, a prenyl-binding protein involved in the retrieval of PDE from rod outer segment membranes by interacting with the GTPase Rab13 (Linari *et al*. 1999; Zhang *et al*. 2004). Two chromosome-associated proteins, SMC1 and SMC3 and a centrosomal protein, nucleophosmin (NPM) (Shu *et al*. 2005) directly interact with RPGR (Khanna *et al*. 2005; Hirano 2006). Later IP studies identified intraflagellar transport protein, Tg737/Polaris/IFT88, which is involved in ciliogenesis and photoreceptor outer segment maintenance in mice (Pazour *et al*. 2002; Davenport and Yoder 2005) and with several microtubule transport proteins, including cytoplasmic dynein and kinesin-II as part of RPGR containing protein complexes in the retina (Khanna *et al*. 2005). Studies on the analysis of selected RPGR-interacting ciliary disease proteins are described below.

RPGR-interacting ciliary disease proteins

RPGRIP1

The RPGR interacting protein 1 (RPGRIP1) was first described as an interactor of RPGR after screening a bovine retina library by yeast two-hybrid analysis (Boylan and Wright 2000; Roepman *et al*. 2000). Initial analysis showed that RPGR and RPGRIP1 can co-localize from the connecting cilium towards the outer segment of bovine and human photoreceptors, and disease associated mutations in *RPGR* can disrupt this interaction. Subsequent studies revealed that mutations in RPGRIP1 are associated with LCA (Dryja *et al*. 2001; Gerber *et al*. 2001). RPGRIP1 is predominantly localized at the connecting cilium of mouse photoreceptors (Hong *et al*. 2001), and the *Rpgrip1* ko mice features early-onset retinal degeneration (Zhao *et al*. 2003). Analysis of the $Rpgrip1^{-/-}$ mice revealed redistribution of RPGR in the inner segments as opposed to the sensory cilium of photoreceptors, suggesting that RPGRIP1 functions as an anchoring protein for RPGR (Hong *et al*. 2001; Zhao *et al*. 2003).

NPHP5

Nephronophthisis (NPHP) is characterized by progressive loss of renal function when presented with RP is termed Senior–Loken syndrome (SLSN). Nine NPHP genes (NPHP1–9) have been identified; all are implicated in ciliary function (Hildebrandt and Otto 2005; Chang *et al*. 2006; Khanna *et al*. 2009). All patients with *NPHP5* mutations develop RP (Otto *et al*. 2005). We have shown that NPHP5 localizes to photoreceptor cilium and interacts with RPGR, highlighting their functional interaction in ciliary transport (Otto *et al*. 2005).

CEP290/NPHP6

CEP290 is a cilia-centrosomal protein that was initially identified as a tumour antigen (Guo *et al*. 2004). Centrosomes are the major microtubule-organizing centre for the cell (Doxsey *et al*. 2005) and mutations in centrosomal proteins are associated with syndromic diseases like Alstrom syndrome (MIM 203800) or complex pathological processes like cancer (Andersen *et al*. 2003; Badano *et al*. 2005). CEP290 is not the exception; mutations in the *CEP290/NPHP6* are associated with syndromic disorders, including Joubert syndrome, Meckel–Gruber syndrome, and BBS (Sayer *et al*. 2006; Valente *et al*. 2006; Baala *et al*. 2007; Leitch *et al*. 2008). We showed that a hypomorphic allele of the *Cep290* gene in the *rd16* (retinal degeneration 16) mouse model of autosomal recessive early-onset retinal degeneration and olfactory dysfunction (Chang *et al*. 2006). The mutant CEP290 protein exhibits increased association with RPGR and likely results in the mislocalization of RPGR in photoreceptors (Chang *et al*. 2006).

Based on these studies, den Hollander *et al*. and others screened LCA patients for mutations in the *CEP290* gene and reported predicted hypomorphic alleles as a frequent cause of LCA (den Hollander *et al*. 2006; Helou *et al*. 2007; Perrault *et al*. 2007). We later showed that hypomorphic *CEP290* alleles are also associated with olfactory dysfunction in patients (McEwen *et al*. 2007). Taken together, we suggest that loss of function mutations in CEP290 can cause pleiotropic

and developmental disorders while a moderately functional CEP290 protein primarily results in sensory deficits.

RPGRIP1L/NPHP8

Mutations in RPGR interacting protein 1-like (RPGRIP1L/NPHP8) are associated with Joubert syndrome, Meckel–Gruber syndrome and Bardet–Biedl syndrome (Arts *et al*. 2007; Delous *et al*. 2007; Wolf *et al*. 2007; Brancati *et al*. 2008; Doherty *et al*. 2009). RPGRIP1L regulates sonic hedgehog signalling pathway and mediates left–right asymmetry as well as limb patterning. Interestingly, a missense variant of RPGRIP1L and A229T is frequently associated with retinal degeneration in ciliopathy patients (Khanna *et al*. 2009). RPGRIP1L can physically interact with RPGR, and the A229T in RPGRIP1L variation severely compromises this interaction (Khanna *et al*. 2009). These data suggest that variations in NPHP8 can act as modifier alleles that affect the penetrance and expressivity of the retinopathy phenotype, likely through its interaction with RPGR.

Conclusions and future directions

Over 200 different RPGR mutations have so far been reported in patients with X-linked retinopathies of diverse clinical phenotypes. A vast majority of these are nonsense mutations or deletions/insertions resulting in frameshift, which are predicted to cause premature truncation of the RPGR^{ORF15} protein. While mutations in the ORF15 exon are generally associated with a milder disease, mutations in RPGR exons 1–14 result in a more severe disease. Initially, most human RPGR mutations were hypothesized to have a null phenotype in males; however, wide variations in clinical phenotype of males and carrier females, and complexities associated with RPGR transcripts and protein isoforms strongly indicate that several disease alleles may in fact be hypomorphs (with partial function).

What is the role of RPGR as part of distinct multiprotein complexes at the cilium and how do mutations in RPGR cause photoreceptor degeneration? Though basic components associated with ciliary transport have been discovered (Rosenbaum *et al*. 1999; Rosenbaum 2002), the mechanisms of cargo sorting and assembly of protein complexes, and their regulation by signalling pathways in photoreceptors have not been elucidated. Periodic turnover of photoreceptor outer segments demands efficient functioning of the ciliary transport process (Besharse *et al*. 2003). We hypothesize that RPGR facilitates the assembly of transport protein complexes by interacting with distinct ciliary—basal body—centrosome (CBC) proteins, and that RPGR's localization in the photoreceptor cilia is necessary for efficient intersegmental transport (figure 3).

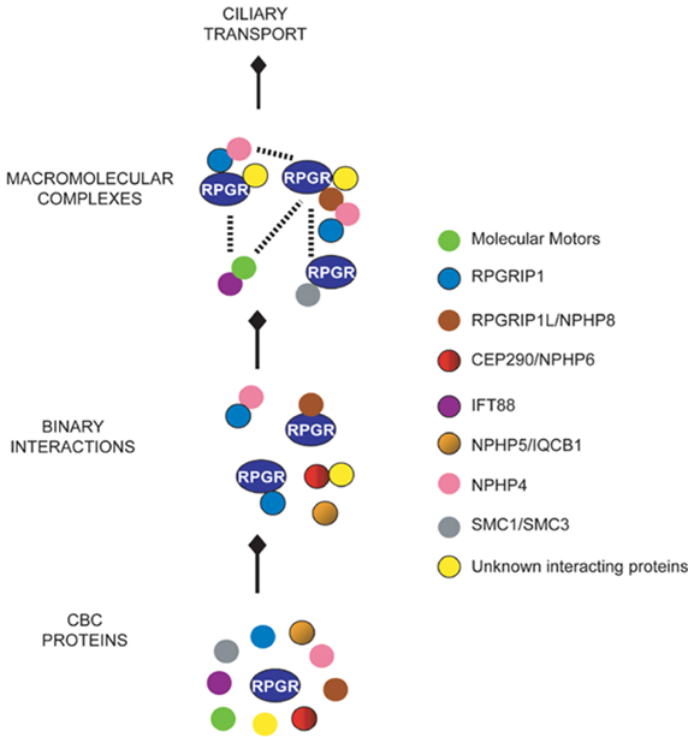

Figure 3. Schematic representation of the RPGR and ciliarybasal body-centrosomal (CBC) protein complexes in photoreceptors; binary interactions and macromolecular protein complexes facilitate microtubule-based ciliary transport. Dashed lines indicate interactions as part of complexes.

Owing to the associated clinical heterogeneity, a detailed genotype–phenotype correlation analysis is critical to understand the progression and pathogenesis of RPGR-associated disease. These studies will benefit from characterization of additional animal model systems representing RPGR mutations as well as identification of components of the RPGR-interactome in photoreceptors. For example, characterization of photoreceptor dysfunction and degeneration in knock-in mouse mutants of *Rpgr* can assist in understanding associated disease pathogenesis. In addition, functional analysis of the disease-causing mutations of RPGR, such as the effect on RPGR localization, integrity of the interactome and effect on cilia-dependent development are required to delineate the mechanism of heterogenic phenotype observed in patients. These investigations should assist in designing rational therapeutic paradigms for XLRP as well as associated ciliary disorders.

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