Foundation Fighting Blindness

PRPH2 Landscape Document

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Contents

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Abbreviations

AAV – adeno-associated virus

IRD – inherited retinal disease/degeneration/dystrophy

OS – outer segments of rod and cone photoreceptor cells

PRPH2 – when in all capital letters and italicized this refers to the human gene

PRPH2 – when in all capital letters and not italicized this refers to the human and mouse protein

Prph2 – when in lowercase letters and italicized this refers to the mouse gene

An extended glossary can be found at the end of the document.

Executive Summary

This document provides an overview of peripherin-2/PRPH2 at multiple levels: the gene, the protein, its function, and the epidemiology and clinical manifestations of PRPH2-associated disease, as well as potential treatment strategies. It will provide a framework for researchers and companies to identify key gaps and bottlenecks, with the goal of addressing them to advance treatments and therapies for PRPH2-associated disease. This document is largely targeted at a lay audience. Every attempt will be made to cover all important topics, but it will not be comprehensive with regard to all experimental details and/or references. We share our apologies to the authors whose work could not be discussed or included.

Key takeaways:

- PRPH2 is essential for the formation and structure of photoreceptors (rods and cones), the cells that enable us to perceive light.
- *PRPH2* is one of the most commonly mutated inherited retinal degeneration genes.
- Mutations in *PRPH2* lead to multiple retinal diseases, including retinitis pigmentosa and macular dystrophies.
- PRPH2-associated diseases are typically later onset, usually affecting people in their 30s and later.
- PRPH2-associated disease is almost always inherited in an autosomal dominant fashion.
- There are numerous mouse models of PRPH2-associated disease, which have contributed to our understanding of PRPH2 function and dysfunction in disease.
- There are currently no PRPH2-specific therapies in development, though there are other, gene-agnostic approaches that may be beneficial for PRPH2-associated disease.

Challenges and potential bottlenecks

- The cells of the retina are very sensitive to the amount of PRPH2 protein present.
- There is significant variability between patients with PRPH2-associated disease in terms of visual function and disease onset and progression.
- There are no large animal (e.g., dog, pig) models of PRPH2-associated disease, which impedes the ability to test therapeutic strategies.
- Dominant diseases, like PRPH2-associated disease, require different therapeutic approaches than recessive diseases do.

There is a significant body of work around PRPH2 and its associated diseases, including an understanding of its normal and abnormal functions in disease. However, it presents some unique challenges from a therapeutic perspective. Future opportunities include studies that combine information from many patients to clarify which factors influence patient variability and the application of newly developed methodologies to precisely correct gene mutations in *PRPH2*.

Overview

PRPH2 is one of the top five most commonly mutated inherited retinal degeneration (IRD)-causing genes, accounting for 3-5% of all genes containing disease-causing variants in several large-scale IRD cohorts (*1*, *2*). Mutations in *PRPH2* lead to multiple retinal diseases, which are almost exclusively inherited in an autosomal dominant manner. These include retinitis pigmentosa 7 (RP7), pattern macular dystrophy (PMD), vitelliform macular dystrophy (VMD), and central areolar choroidal atrophy (CACD). Patients typically present with changes in visual acuity in the fourth decade of life, though there is significant inter- and intrafamilial variability with respect to age of onset, symptoms, and severity. Research to understand the normal and pathologic function of PRPH2 has been greatly aided by the creation and use of multiple mouse models of PRPH2-associated disease, including the long-standing 'retinal degeneration slow' (rds) mouse. Despite its prevalence, the existence of animal models, its small genomic size

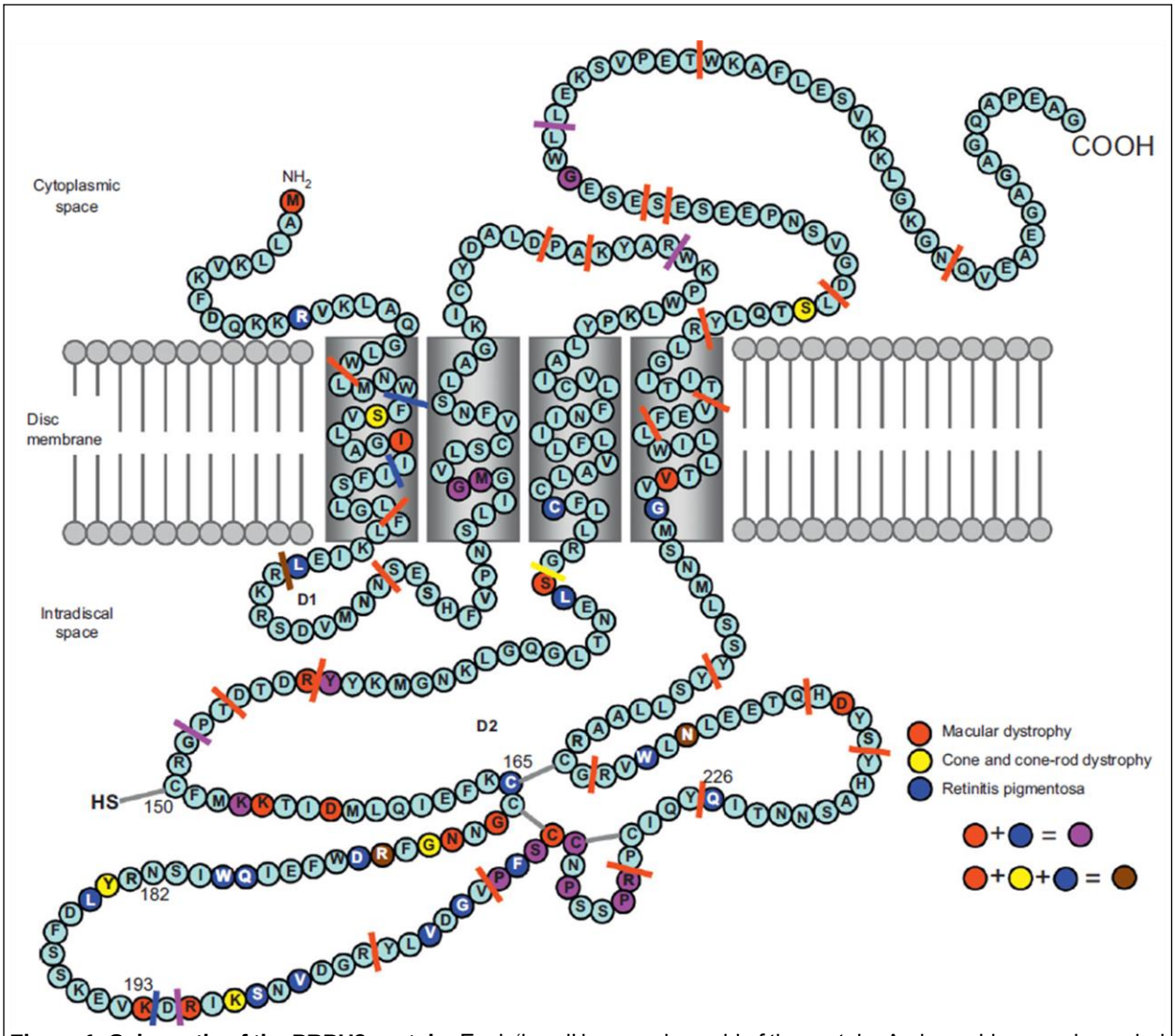

Figure 1: Schematic of the PRPH2 protein. Each 'bead' is an amino acid of the protein. Amino acids are color coded according to diseases that are reported to arise based on mutations at that site. Colored bars represent nonsense or frameshift mutations. Gray bars indicate disulfide bonds between two cysteines, which are important for holding the PPRH2 protein together (e.g., at position 165), and for mediating the formation of protein complexes (e.g., position 150). From *(66)*.

(~1.1kb), which allows it to easily fit into AAV, (the most commonly used vector for retinal gene therapy), and proof of concept studies in mice, no treatments or cures exist for PRPH2-associated disease. This dearth of treatment options may be at least partially attributable to the fact that most PPRH2-associated diseases are inherited in an autosomal dominant manner, which necessitates different therapeutic strategies than autosomal recessive diseases do, and that cells are sensitive to the overall amount of PPRH2 protein present.

Genetics and Genomics: Gene and variants

PRPH2 (*peripherin-2*), which was historically referred to as *peripherin/Rds*, *RDS,* or rd2, was first linked to retinal degeneration over three decades ago, following its identification as the gene responsible for the retinal phenotype in the spontaneously occurring 'retinal degeneration slow' (*rds*) mouse model and its presence in human cases of autosomal dominant retinitis pigmentosa (*3*–*8*). In humans, the *PRPH2* gene is found on chromosome 6, comprising three exons leading to a ~1.1kb cDNA, which will ultimately be translated into the PRPH2 protein. There is strong conservation between mouse and human *PRPH2* (85% DNA sequence identity), and orthologs (the same gene in different species) are found in mammals and lower vertebrates, but not in lower organisms, including *C. elegans* and Drosophila, two widely used research organisms (reviewed in (*9*)).

Table 1: Types of mutations and their relative frequency in PRPH2 as determined for pathogenic mutations in ClinVar (accessed 01.31.2023).

Type	Description	Frequency
Missense	A genetic misspelling where a single DNA base is changed, resulting in a different amino acid being present in the protein	33%
Nonsense	A genetic misspelling that inserts a STOP codon into the protein, ultimately leading to an incorrectly shortened protein or loss of protein	21%
Frameshift	DNA is read into amino acids in groups of three DNA bases (the "frame"). Changes to the protein that alter the "frame" in which the DNA is read, typically due to insertions or deletions, lead to a protein made up of incorrect amino acids and often the incorporation of a STOP codon (called a premature stop codon), leading to a shortened protein or loss of protein	40%
Splice site	DNA is made up of exons and introns. Exons are the parts of the DNA that will ultimately be turned into proteins. At the end and beginning of each exon are short genetic tags that allow cellular machinery to 'splice' together exons while removing the intron. Mutations that change these tags can lead to incorrect parts of introns being included, typically leading to non-functional or dysfunctional proteins	6%

To date, over 200 pathogenic mutations have been identified in *PRPH2* (ClinVar, accessed 1.4.23). There are different classes of mutations, including missense, nonsense, frameshift, and splice site mutations (Table 1), which are observed with different frequency in *PRPH2*. As sequencing methods have improved large genetic rearrangements have also been detected in numerous genes, though to date only two have been reported to affect *PRPH2* (ClinVar, accessed 08.03.2023). Depending on the type of mutation that is present, different therapeutic strategies may be applicable. The ten most common *PRPH2* mutations found in the Leiden Open Variant Database (LOVD) are shown in Table 2. The D2 loop (protein domain, see Figure 1) is the location of a large fraction (69% to >75%) of *PRPH2* mutations (*10*, *11*).

Researchers often try to understand whether there are any correlations between a specific mutation or part of the gene where a mutation occurs and the type of symptoms that it leads to (called genotype-phenotype correlation), as this can inform prognosis and disease progression. However, very few correlations have been established between specific mutations in *PRPH2* and the resulting phenotype. For example, the Tyr141Cys mutation tends to be associated with a late-onset maculopathy with vascular defects that resembles age related macular degeneration (*12*). Our ability to make connections between *PRPH2* mutations and phenotypes is hindered by the fact that the same mutation in the same family may lead to different symptoms (c.f., (*13*)). In an analysis of a large group of individuals with mutations in *PRPH2*, Reeves et al noted that most individuals with a diagnosis of cone-rod dystrophy, retinitis pigmentosa, or Stargardt-like Disease (78%, 50%, and 44%, respectively) had a mutation in exon 1. Conversely, most individuals with a diagnosis of Best Disease or Pattern Dystrophy (56% and 51%, respectively) had a mutation in exon 2 (*10*).

Table 2: The most frequently reported PRPH2 mutations in the Leiden Open Variant Database (LOVD)

DNA Change	Protein Change	Cases Reported	Protein domain affected	Notes
514C>T	Arg172Trp	182	D2 loop	
828+3A>T	Splice site	173	Disrupts D2 loop	Leads to aberrant inclusion of part of intron 2 (*145*)
424C>T	Arg142Trp	158	D2 loop	
422A>G	Tyr141Cys	67	D2 loop	Adds an aberrant cysteine, which may form disulfide bonds
646C>T	Pro216Ser	51	D2 loop	
629C>G	Pro210Arg	44	D2 loop	
136C>T	Arg46Ter	43	D1 loop	Causes the protein to be cut off after amino acid 46
584G>T	Arg195Leu	35	D2 loop	
647C>T	Pro216Leu	34	D2 loop	
866C>T	Ser289Leu	34	C-terminus	Conflicting reports of pathogenicity

Protein: PRPH2 Structure

PRPH2 encodes the 346 amino acid PRPH2 protein, which is a member of the tetraspanin family of proteins. Like other tetraspanin proteins, PRPH2 has four transmembrane domains—coiled regions of the protein that physically sit in and span a cell membrane, a small extracellular loop, and a large extracellular/intradiscal loop (termed D2) (Figure 1). Unlike other tetraspanins, which are found on the outer cell membrane, PRPH2 localizes to the disc membranes of photoreceptors. PRPH2 contains multiple conserved cysteine amino acids, which can form disulfide bonds with other cysteines, an important kind of intra- and intermolecular connection. PRPH2 is also glycosylated (i.e., has a carbohydrate molecule attached to it) (*11*, *14*), which may impact its shape, function, and/or stability. The structure of PRPH2 was not formally determined until 2022 (*11*), but it had already been largely predicted based on similarity to other tetraspanins. Being able to visualize the physical structure of PRPH2 allows scientists to view where a specific mutation sits within the protein and perhaps better understand how it causes disease.

Cellular and Tissue Level: PRPH2 Localization and Function

In the retina, PRPH2 mRNA and protein expression is found only in rod and cone photoreceptors. Cones mediate bright light and color vision, while rods mediate dim-light and peripheral vision. The outer segments (OSs) of photoreceptors are filled with neatly aligned stacks of hundreds to thousands of discs, which are the physical location where light is converted into the electrical signals that are ultimately perceived as vision by the brain. These discs, which contain a high density of proteins that mediate the visual cycle, are constantly being recycled, and must therefore be replaced by new plasma membrane and proteins from the cell body.

PRPH2 is essential for the structure and survival of vertebrate photoreceptors. It plays a critical role in the formation, maintenance, and renewal of OSs. When PRPH2 is completely absent rod OSs fail to form; in contrast, cone OSs—albeit abnormal ones—are still able to form, suggesting different roles for PRPH2 in rods and cones (*15*). When one copy of *Prph2* is present, but the other copy is either nonfunctional or dysfunctional (as is the case in most affected individuals), OSs of both rods and cones are shorter and disorganized, compared to normal photoreceptor OSs (Figures 2 and 3). When PRPH2 is present, the membrane of rods and cones pushes outward (evaginates) and forms neatly stacked discs, but without PRPH2 the membrane pinches off as fluid-filled blobs, called ectosomes (Figure 2). Studies in frogs and mice determined that the C-terminal tail of PRPH2 (see Figure 1 above; the C-terminal tail is the end of the protein that sticks out above the membrane and ends with 'COOH') is responsible for keeping the budding membrane attached to the connecting cilium, and for localizing newly made PRPH2 to the outer segments (*16–18*). After discs are formed, they must flatten to the correct thickness and 'zip' together. PRPH2 is involved in this membrane zipping, and PRPH2 oligomers (multiple PRPH2 molecules joined together, discussed further below) become

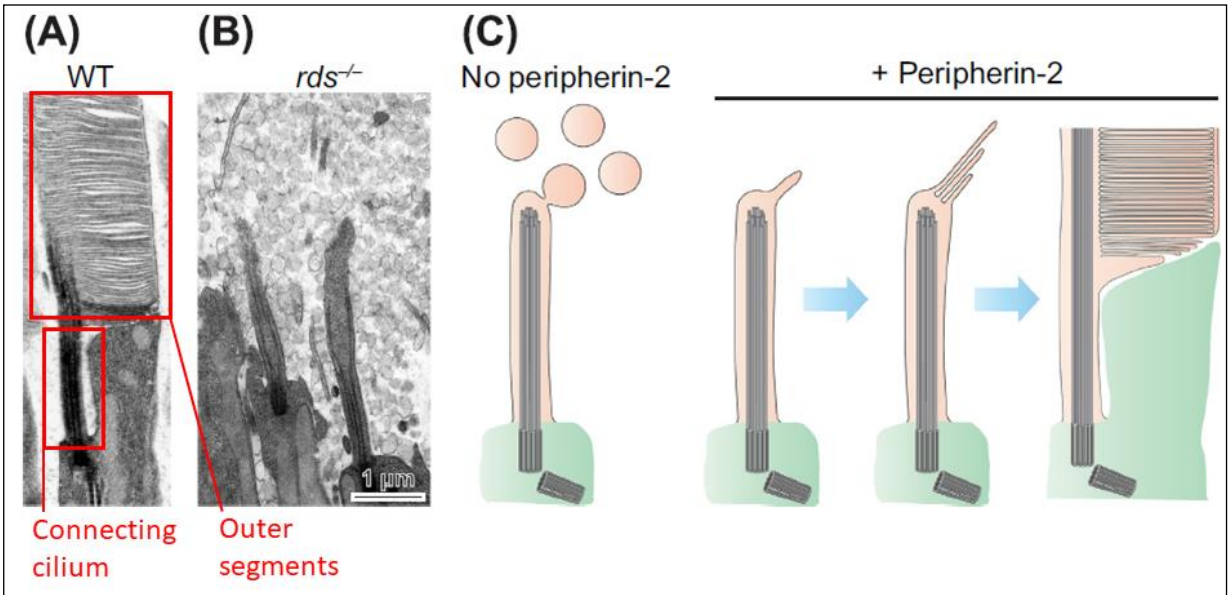

Figure 2: Images and illustrations of the role of PRPH2/peripherin-2 in the formation of disc outer segments. (A) shows an image of a mouse rod. On the top half of the image are the many stacked discs. This is in stark contrast to (B), which shows a rod photoreceptor in a mouse that is missing both copies of *Prph2* (*rds-/-*). The connecting stalk (called the connecting cilium) is present, but no outer segment discs are formed. This is illustrated in (C), where the cell tries to form the outer segments by budding sections of its membrane, but without PRPH2 they pinch off as fluid-filled blobs, called ectosomes. When PRPH2 is present, the membrane pushes outward (evaginates) and forms neatly stacked discs. Figure modified from *(26)*.

concentrated in the disc rim, where this process happens (*19*, *20*). Consistent with a role for PRPH2 in inducing membrane curvature, PRPH2, in combination with its partner ROM1, causes membranes to bend when the two are combined in a test tube (*21*). There are conflicting reports as to whether this function is mediated by the 'tetraspanin core' of PRPH2 (see Figure 1; the part of the protein that sits in the membrane) (*16*) or the C-terminus (*22*, *23*). A recent study demonstrated in cells and frogs that the formation of chains of PRPH2 tetramers is critical for multiple aspects of outer segment generation and structure. Mutations that disrupt the ability of PRPH2 chains to form (e.g., C150S) lead to retinal degeneration (*24*).

In addition to the presence of functional PRPH2, the amount of PRPH2 protein present is also important for disc formation (discussed further below), as mice carrying certain *Prph2* mutations have discs that are incorrect in size and shape (reviewed in (*25*)). The functions of PRPH2, and other proteins, are clearly reviewed in (*26*), and the supplemental videos illustrating disc formation are enlightening (ncbi.nlm.nih.gov/pmc/articles/PMC7584774/).

Importantly, PRPH2 does not work alone in carrying out its function. PRPH2 forms complexes that are made up of regular numbers of itself and its partner protein, ROM1. Interactions with itself and with ROM1 are mediated by the D2 loop of PRPH2 (Figure 1), the site of many *PRPH2* mutations. PRPH2 complexes can take different forms and have different functions in the cell (reviewed in (*27*)). PRPH2 forms homodimers (a working unit made up of two PRPH2 proteins) and hetero-dimers made up of one PRPH2 protein and one ROM1 protein. In addition, these homo- and hetero-dimers can join together, leading to tetramers (4 proteins together), which can further combine to form intermediate complexes, which consist of at least 2 tetramers. Intermediate complexes are held together by both weaker molecular and stronger covalent bonds. These covalent bonds are mediated by the cysteine amino acid at position 150 in the PRPH2 protein (*28*, *29*). Higher order (i.e., > 8 protein molecules together) complexes comprising only PRPH2 are present at the disc rim and mediate its closure (*21*, *30*, *31*). When mice are created that have a mutation at *Prph2* position 150 or 153 (a mutation found in patients), intermediate and higher order complexes are not formed. Moreover, these mice have disrupted rod and cone OSs, highlighting the importance of these intermediate and higher order complexes for the function of PRPH2 (*32*, *33*). Similarly, mutations that lead to the overabundance of incorrect protein complexes can also lead to retinal defects. Mice with a mutation that leads to a cysteine instead of a tyrosine at position 141 (Y141C), which is the fourth most common *PRPH2* mutation reported in the Leiden Open Variant Database (LOVD) (see Table 2), have structural and functional defects in their cone and rod OSs, which form aberrant aggregates of PRPH2 and ROM1 (*34*). Interestingly, mice carrying the *Prph2* Arg172Trp mutation (R172W, most common mutation in LOVD) do not show defects in PRPH2 complex formation, but do have ROM1 aggregates, which are more common in cones than in rods (*35*); this finding is consistent with the fact that the majority of individuals with this mutation have a phenotype that more severely affects cones than rods (c.f., (*36*, *37*)).

Genetic and Biochemical Mechanisms of Disease

Humans have two chromosomal copies of every gene: one copy is inherited from each parent. PRPH2-associated disease is inherited almost exclusively in an autosomal dominant manner. Autosomal refers to chromosomes that do not determine sex, and dominant refers to the fact that a single mutated copy of the gene is sufficient to cause disease. This contrasts with diseases that are inherited in a recessive manner, where two mutated copies of a gene are required to cause symptoms. Individuals who are affected by autosomal dominant diseases have a 50% chance of passing the mutated gene on to each child. As mentioned below, there is

a rare digenic (two gene) form of RP that is caused by a mutation in *PRPH2* and its binding partner *ROM1*. In rare cases, individuals may inherit two mutated *PRPH2* genes—one from each parent. This leads to earlier onset of disease and more severe symptoms.

Even though one only needs a single mutated copy of *PRPH2* to show symptoms, *PRPH2*—like many other dominant genes—shows incomplete penetrance and variable expressivity. Incomplete penetrance means that not everyone who has a mutated copy of *PRPH2* will show disease. This has been documented for numerous *PRPH2* mutations (c.f., (*13*, *38*)). Researchers do not fully understand why this happens and to whom it might happen, but it is likely that other factors in the person's genetic background and perhaps their environment are part of the explanation. Variable expressivity is the formal term for the variability that is observed in the symptoms and disease severity that individuals with *PRPH2* mutations demonstrate. In some families, individuals with the same *PPRH2* mutation can have different visual symptoms and be diagnosed with different retinal diseases. Some studies suggest that some mutations in *ABCA4*, which typically causes autosomal recessive Stargardt Disease, may also modify the disease (*39*, *40*). For example, the R229H mutation in *ROM1*, which is not sufficient to cause disease on its own, causes more severe symptoms in patients who also carry the R172W *PRPH2* mutation. In mice, researchers explored how visual function in different *Prph2* mutant backgrounds changed when one copy of *Rom1* was removed. They discovered that different mutations responded differently—for one *Prph2* mutation, there was a minor improvement in function, whereas for others there was no improvement or worsening in function (*40*). However, for some patients, mutations in *ROM1* have been determined to <u>not</u> play a role in disease variability (*41*, *42*), underscoring our incomplete understanding of the nature of this heterogeneity. Interestingly, subtle changes to the <u>unmutated</u> copy of PRPH2 may themselves account for variation in disease severity. A study of over 60 individuals with the c.838+3A>T mutation found that having three specific non-disease-causing mutations on the second PRPH2 copy resulted in a 7-fold increase that someone would have a severe disease manifestation (RP or CACD) compared to a milder form (pattern dystrophy) (*42*). These genetic differences, which are single nucleotide polymorphisms (SNPs), are present throughout our genomes and contribute to variation between people. In general, people of the same ethnic ancestry will carry many of the same SNPs.

In general, mutations that cause disease can be grouped into two main categories: gain of function (GoF) mutations and loss of function (LoF) mutations (reviewed in (*43*)). Gain of function mutations cause a protein to <u>gain</u> a new function that it didn't have before. In some cases, this new function may have an undesirable (e.g., toxic) effect, which is termed 'dominant negative.' A classic example of a dominant negative effect is when a mutant protein traps another protein in a non-functional complex, inhibiting the ability of that other protein to work. The functional protein that is produced from the unaffected gene copy cannot overcome these dominant negative effects. Mutations that lead to proteins that act in a dominant negative manner require different therapeutic strategies than mutations that cause loss of function mutations, which will be discussed further below.

Loss of function mutations do just what their name implies—they cause a protein to lose a function that it normally has or cause a cell to lose the function of the protein. For PRPH2, since it functions in complex with ROM1, mutations that interfere with its ability to bind to ROM1 would interfere with its ability to carry out its normal roles. There are also mutations that cause the protein to not localize to the correct part of the cell, to become destabilized and degraded by the cell, or to not fold into its proper shape. Some loss of function mutations may lead to a partial reduction in the amount of PRPH2 present, while others lead to a complete absence of

PRPH2 protein from that copy of the gene. For many genes, a loss of function mutation on a single copy of the gene would not cause disease, as the normal protein made by the other copy of the gene would be sufficient to compensate for the non-functional or dysfunctional protein. This is unfortunately not the case with PRPH2. Studies have suggested that photoreceptor cells require at least 60-80% of normal PRPH2 to function properly, however, even at those levels some degeneration is seen (*44*, *45*). When a single copy of a gene is not sufficient to carry out a protein's normal function, that gene is called 'haploinsufficient.' Haploinsufficiency also influences the kinds of therapeutic strategies that can be effective for PRPH2, discussed below.

Understanding the consequence of a specific genetic mutation is important for determining who would be expected to benefit from a given therapy. Decisions around who should be included in a clinical trial, as well as who should be excluded, are based on who is expected to benefit from the trial. For many *PRPH2* mutations we do not know whether they result in a gain or loss of function. This cannot usually be determined from knowing the mutation but must be established experimentally, though the structure of the protein (mentioned above) can inform our predictions of how different mutations might affect PRPH2 function. However, it is generally assumed that mutations that lead to the early incorporation of a stop codon (often marked as X, *, or ter) will prematurely shorten the protein and potentially result in its destruction by the cell. Other mutations can also be predicted to result in a loss of function if they destabilize the protein (c.f., (*46*)).

Further complicating our understanding of the disease is the fact that rods and cones appear to be differentially affected by *PRPH2* mutations; for example, the Arg172Trp mutation seems to preferentially affect cones, whereas mutations in adjacent amino acids can also affect rods (*38*, *47*). It has been proposed that rods are sensitive to the total amount of PRPH2 present, whereas cones are more affected by the presence (or lack) of correctly assembled PRPH2 complexes. To put it a different way, this would suggest that PRPH2-associated diseases that are predominated by rod loss (e.g., RP) are because of haploinsufficiency, whereas cone-dominated diseases (e.g., macular dystrophy) are driven by toxic dominant-negative mutations which render PRPH2 unable to form normal and/or functional complexes. However, there are some exceptions to this theoretical framework, highlighting the complexity of PRPH2 (*46*).

Despite our understanding of some of the cellular consequences of non-functional or dysfunctional PRPH2, we still do not have a complete picture of why outer segment disorganization leads to photoreceptor cell death. Two early studies proposed that mutations in *PRPH2* (and in other retinal degeneration genes) caused cells to die by a programmed cell death mechanism named apoptosis (*48*, *49*). However, a more recent study did not find evidence of activation of a key apoptosis effector protein, suggesting an alternative mechanism. Instead, in mice mutant for *Prph2*, Arango-Gonzalez et al (*50*) found an accumulation of cyclic guanosine monophosphate (cGMP) in photoreceptor cells. Prior work had demonstrated that increased cGMP signaling was associated with photoreceptor cell death, due to the activation of downstream enzymes, including calpain-type proteases, PARP, and HDAC. It remains to be understood how the functional effects of *PRPH2* mutations lead to increased cGMP and why changes in functional vision are not apparent until later in life, despite mutant PRPH2 being present since birth.

Disease Models

As illustrated by the preceding discussion, mouse models that have mutations in *Prph2* have been essential to our understanding of its function. However, while mice can tell us a lot, there

are significant differences between the mouse and human eye. For example, the mouse eye is significantly smaller than the human eye, does not have a macula (the central location of high-acuity vision), and has very few cones. Often, larger animals, such as dog, pig, or non-human primate, are preferred for their more faithful recapitulation of the human eye. Discussed below are some of the available animal and cellular models of PRPH2-associated disease.

Xenopus

A classic research animal model is the African clawed frog, *Xenopus laevis*. Xenopus rods share similarities with human rods, making them useful for studying factors influencing rod structure. Xenopus has three genes that are similar to human *PRPH2*, named xrds38, xrds35, and xrd36, with xrds38 being the most similar. Interestingly, Xenopus does not seem to have a *ROM1* equivalent gene (*51*). Studies in Xenopus have helped to define the functions of different regions of xrds38/PRPH2 protein and to explore how it is trafficked to the outer segment (*18*, *52*, *53*).

Zebrafish

The *PRPH2* gene is conserved in zebrafish, however it has been duplicated, resulting in two genes: *prph2a* and *prph2b*. While strains of zebrafish containing premature stop codons in *prph2a* and *prph2b* were recovered from an ENU mutagenesis screen (ZFIN), there have been no published studies characterizing or utilizing these models.

Mouse

rds – the first mouse model to be described that harbors mutations in *Prph2* was the 'retinal degeneration slow' (*rds*) mouse (*3*, *4*), though it wasn't until over a decade after its discovery that researchers determined that the gene disrupted in the *rds* mice was *Prph2* (*5*, *7*, *8*). In mice carrying two copies of the *Prph2* mutation ($rds^{-/-}$, also referred to as homozygous for *rds/Prph2*), retinal degeneration was seen as early as 2-5 weeks after birth, but complete degeneration was not seen until 7-12 months after birth, leading to its name of 'retinal degeneration slow.' In mice 7-12 months corresponds to approximately mid-life in humans. A later study further demonstrated that mice containing only one mutated copy of *Prph2* ($rds^{+/-}$, also referred to as heterozygous for *rds/Prph2*) also showed retinal degeneration, though to a lesser extent and more slowly than $rds^{-/-}$ mice (*54*). This study demonstrated that the amount of PRPH2 protein is important and that retinal degeneration resulting from mutations in *Prph2* were

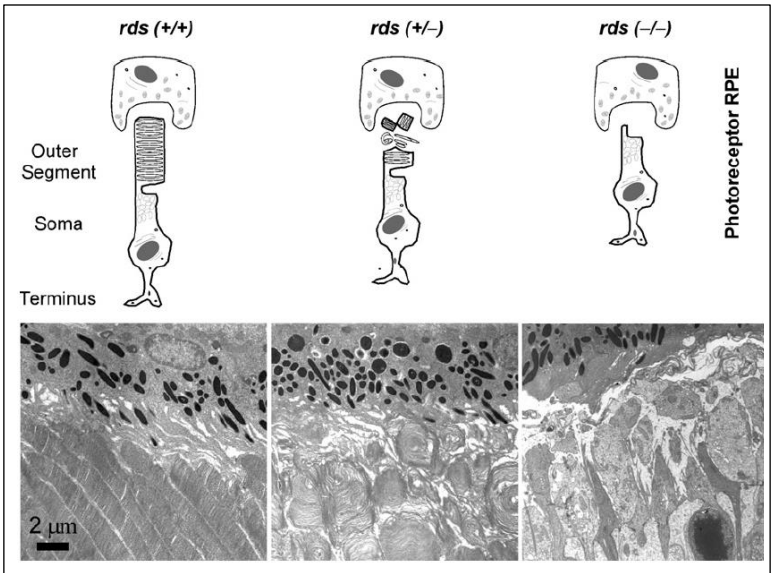

Figure 3 Top row: illustrations showing the morphology of photoreceptor outer segments (OSs) from mice carrying 2 (*rds*(+/+)), 1(rds(+/-)), or 0 (*rds*(-/-)) normal copies of *Prph2*. In all cases a retinal pigmented epithelial cell is shown at the top. Bottom row: transmission electron micrographs from mice carrying 2 (rds(+/+)), 1(rds(+/-)), or 0 (rds(-/-)) normal copies of Prph2, corresponding to the illustrations above. All three show a field of photoreceptors and RPE, which is schematized above. Figure from *(9)*.

inherited in a dominant fashion. In mice carrying no normal copies of *Prph2* (*rds*$^{-/-}$), no photoreceptor outer segments are formed (see Figure 2, 3). In 6-month-old mice with one normal copy of *Prph2* (*rds*$^{+/-}$), an intermediate phenotype is seen, with shortened photoreceptor outer segments and 'whorls' of discs, which are in stark contrast to their normal, orderly arrangement in mice that carry two normal copies of *Prph2* (*rds*$^{+/+}$). Both *rds*$^{+/-}$ and *rds*$^{-/-}$ mice show decreased signals in scotopic and photopic ERG studies (tests that measure the function of rods and cones); *rds*$^{-/-}$ mice have barely detectable ERG signals by one month of age, whereas *rds*$^{+/-}$ mice still have some ERG signal—and therefore some visual function—even at 6 months of age (reviewed in (*27*)). *rds*$^{+/-}$ mice first show a decrease in rod function, followed by a loss of cone function, making them a good model of autosomal dominant retinitis pigmentosa.

Other mouse models – Many additional mouse models of PRPH2-associated disease have been developed, including models of specific human mutations. They have helped to elucidate the role of PRPH2 oligomers and higher order structures, as well as the different function in rods and cones. The different models are nicely reviewed in (*27*).

Large animal models

Whereas *PRPH2* orthologs are found in mammals—including cow, pig, dog, and cat—there have been no published reports of large animal models of PRPH2-associated disease, perhaps in part because mouse models largely recapitulate disease pathophysiology. A large animal model can be a very important tool for evaluating potential therapeutics because dosing, safety, and efficacy can be more accurately assessed in large animals compared to mice. Therefore, consideration should be given to whether the creation of a large animal model for PRPH2 would be a useful endeavor. Given the relatively late age of disease onset in humans and the relatively longer life scales of larger animals, the time and expense to generate, maintain, and fully characterize a PRPH2 large animal could be significant.

Human cellular models

More recently, induced pluripotent stem cells (iPSCs) and their derivatives and retinal organoids have emerged as a human-specific model system for interrogating retinal disease biology in the laboratory. While there has not been a published study that generated *PRPH2*-mutant organoids, Jin et al did create iPSCs from a patient carrying the W316G mutation in *PRPH2* (*55*). These cells were able to differentiate into retinal progenitors and retinal pigment epithelium (RPE) but showed selective loss of rods from differentiation day 120 to day 150. Based on a hypothesis that oxidative stress may be involved in the observed rod death, the authors treated *PRPH2* mutant rod cells with the antioxidants α-tocopherol, ascorbic acid, and β-carotene. Whereas these compounds showed some effect on other RP mutant lines, no improvement in rod survival was observed in the *PRPH2*-mutant cell lines. This study illustrates the usefulness of iPSCs and retinal organoids for modeling human disease and testing potential therapies.

Epidemiology

Mutations in *PRPH2* are among the most common causes of inherited retinal degeneration, accounting for 1-5% of IRD patients worldwide (Table 3). In the United States (among

Table 3: Prevalence of PRPH2-associated disease, as determined by large population-based cohort studies

Country	Frequency	Reference
Australia	3.1%	(*146*)
France	3.4%	(*147*)
Israel	1.0%	(*148*)
Italy	1.0%	(*149*)
United Kingdom	5.2%	(*150*)
United States	4.8%	(*1*)

individuals of primarily Northern European descent), *PRPH2* mutations account for ~8% of all autosomal dominant RP (*56*) and 11% of autosomal dominant cone dystrophy or cone-rod dystrophy (*57*). In contrast, *PRPH2* mutations are relatively rare in adRP in Southern Europe—though not in Spain—and Asia (0-1.4% and 0-1.9%, respectively) (*58–65*). The numbers reported in Table 3 are almost certainly an underestimate of true prevalence, as the studies often look at a single individual or a few members of a family and do not take into account other members of the family who are also affected.

Clinical Manifestations

Diagnosis of a retinal dystrophy is typically made following a combination of genetic testing, retinal imaging, retinal function studies, and patient experiences. Retinal imaging can include fundus images (a photo taken of the back of the eye through a dilated pupil) and OCT (optical coherence tomography, a side view through the retina showing the different layers). Different types of ERGs (electroretinograms) assess the function of different cell types of the retina (e.g., rods or cones).

One of the hallmarks of PRPH2-associated disease is its variability, both within and between families. Correct clinical diagnosis is also challenged by the fact that patients may evolve from one clinical presentation to another over the course of their life. Mutations can lead to multiple autosomal dominant diseases, including retinitis pigmentosa (RP), which initially affects peripheral and night vision, and pattern macular dystrophy (PMD) and central areolar choroidal atrophy (CACD), diseases that affect central vision. Due to its late onset and clinical presentation, mutations in *PRPH2* are sometimes misdiagnosed as age-related macular degeneration (AMD). Patients may also present with a phenotype that is similar to late-onset Stargardt Disease (STDG1, caused by mutations in *ABCA4*), which is termed multifocal pattern dystrophy simulating Stargardt Disease (*66*) or Pseudo-Stargardt Pattern Dystrophy (PSPD). However, despite similarities in the fundus images of patients with mutations in *PRPH2* and *ABCA4*, the inheritance (dominant vs. recessive) and prognosis (mild vision loss vs. more significant vision loss) are different (*67*), highlighting the importance of a correct genetic diagnosis for patient information and genetic counseling.

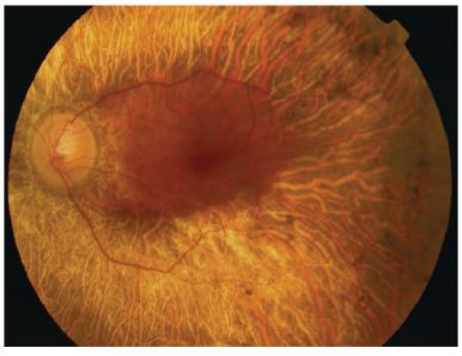

Figure 4 Fundus photograph of a 60-year-old patient with adRP due to a mutation in *PRPH2*. From (*66*).

Retinitis Pigmentosa (RP)

Autosomal dominant retinitis pigmentosa (adRP) was the first retinal dystrophy linked to mutations in *PRPH2* ((*6*) and see Figure 4). RP, which predominantly affects rods, is characterized by initial loss of dim-light vision (i.e., night blindness), followed by loss of peripheral vision leading to constriction of the visual field towards “tunnel” or “pinhole” vision. Disease in PRPH2 adRP patients typically onsets in the 3rd-5th decade of life, but a decrease in visual acuity is not usually seen until the 5th

decade (reviewed in (*66*)). In some cases, PRPH2 adRP may also affect the macula (c.f., (*68*, *69*)). adRP often takes the form of Retinitis Punctata Albescens (RPA), which manifests as night blindness, white pigmented retinal deposits, and which has similar impact on rods and cones. There is also a rare digenic form of RP caused by *PRPH2*, in which the Leu185Pro mutation in *PRPH2* only causes disease when patients also carry a mutation in the *ROM1* gene (*70*, *71*). The digenic form is only thought to account for ~0.5% of adRP (*56*).

Central Areolar Choroidal Dystrophy (CACD)

Central Areolar Choroidal Dystrophy (CACD) affects the retina, the retinal pigment epithelium (RPE), and the choriocapillaris, which is a layer of capillaries that supplies nutrients to the RPE and photoreceptors. CACD patients usually present with mild central visual loss and metamorphopsia (when horizontal and vertical lines appear wavy), sometimes accompanied by mild photophobia (sensitivity to light). CACD disease progression is divided into four stages, and severe loss in visual acuity is not typically seen until the beginning of the final stage, due to death of the foveal area (reviewed in (*66*)). Visual changes onset at different ages depending on the specific mutation, ranging from the 3rd to 6th decade of life. A final visual acuity of 20/200 or less usually occurs in the 5th to 8th decade.

Pattern Macular Dystrophy (PMD)

Pattern dystrophies are autosomal dominant macular dystrophies sub-grouped depending on the pattern of pigmentation seen in fundus images (Figure 5). Mutations in *PRPH2* are associated with butterfly-shaped pigment dystrophy and multifocal pattern dystrophy simulating Stargardt Disease (reviewed in (*66*)). Family members carrying the same *PRPH2* mutation may show different pattern dystrophies, and a single patient may even show different pattern dystrophies in each eye (*66*). In some cases, RP symptoms may also be present in individuals with pattern dystrophies. Age of onset and progression are variable, but most patients with PMDs do not experience symptoms until the 5th decade and some never experience symptoms. Many patients experience relatively mild changes to central visual acuity, but up to 50% may experience severe vision loss after the age of 70 due to the defects in the choroid (blood

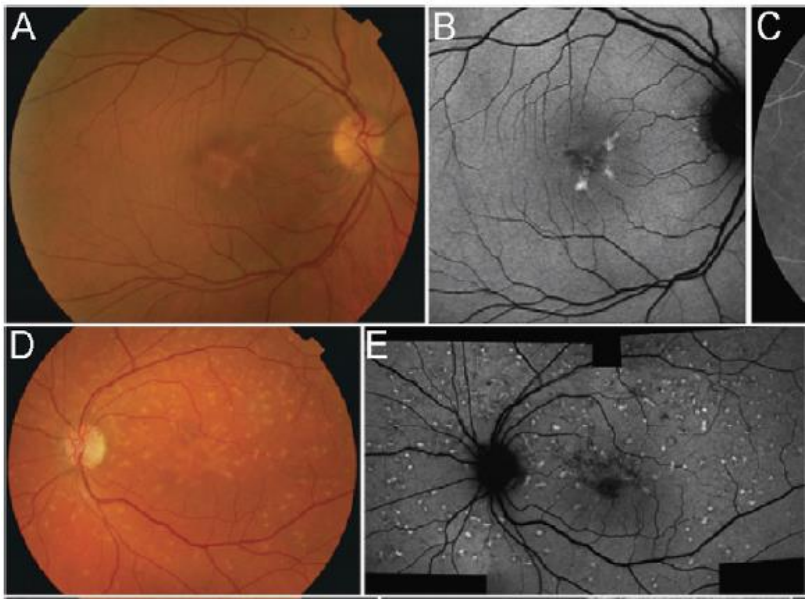

Figure 5 Fundus images of two patients with pattern dystrophies due to mutations in *PRPH2*. A, B, Patient with a butterfly pattern dystrophy. A. Fundus photograph showing a butterfly-shaped lesion in the macula. B. Fundus autofluorescence of the same patient showing some areas of increased fluorescence (white areas) and some areas of decreased fluorescence (dark areas). D, E, Patient with Pseudo-Stargardt Pattern Dystrophy (PSPD). D. Fundus photograph showing irregular yellow-white lesion in central macula with scattered yellow flecks. E. Lesion and flecks viewed on fundus autofluorescence. The lesion shows decreased fluorescence while the flecks show increased fluorescence. From (*66*).

supply to the eye) or neovascularization (growth of new blood vessels). Vitelliform Macular Dystrophy (VMD), also known as adult-onset foveomacular vitelliform dystrophy (AOVD), is another type of pattern macular dystrophy. It was so named for the 'yolk-like' yellow-white, round to oval, slightly elevated subretinal lesion. VMD is clinically and genetically heterogeneous, but various studies find that 2-18% of patients with VMD have a mutation in *PRPH2* (*72–74*).

Leber Congenital Amaurosis (LCA)

Leber Congenital Amaurosis (LCA) is an early-onset (at birth or shortly after birth) form of RP (*75–77*). A diagnosis of LCA due to *PRPH2* mutations is rare and results when an individual inherits two pathogenic mutations. These cases are postulated to represent an autosomal recessive form of PRPH2-associated disease, due to the presence of two pathogenic alleles and the apparent lack of symptoms in the carrier parents (*77*). However, given that PRPH2-associated disease shows incomplete penetrance and variable expressivity, is relatively late onset, and that mutations overlapping the same region are known to be pathogenic in a heterozygous state (as reported in the ClinVar database) and because the family pedigrees often have consanguinity (i.e., marriage among closely related individuals), these are more likely to be a case of incomplete dominance or homozygous dominance, as has been shown for other dominantly inherited diseases (*78*). Mouse models of mutations in *Prph2* further support that *PRPH2* is incompletely dominant (i.e., two mutations are more severe than one mutation) and that the eye is sensitive to *Prph2* gene dose, as retinal phenotypes are exacerbated in homozygous animals compared to heterozygotes (reviewed in (*27*)).

Natural History

Natural history studies are critical observational studies that provide information on the onset, progression, and symptoms of a specific disease. In addition, they can help identify functional and structural metrics that change over time, and which could be used as endpoints in interventional clinical trials to define success of the trial. For example, if best-corrected visual acuity (BCVA) is found to decline significantly in a 2-year natural history study, then improved or stabilized BCVA or slowed rate of BCVA loss could be an endpoint that is measured in response to a treatment. Better understanding of patient genetics and disease progression can also help to segment patients for future treatment trials.

Recently, Heath Jeffery et al published a single-site, retrospective natural history study of 12 patients with pathogenic or likely pathogenic mutations in *PRPH2* (*79*). Over the course of eight years, patients at Lions Eye Institute (Perth, Australia) were tracked and the following measurements were recorded: BCVA and mean macular sensitivity (MMS), as measured by microperimetry, to assess visual function, and fundus autofluorescence-derived total lesion size (TLS), decreased autofluorescence area (DAF), and total macular volume (TMV) to assess structural changes. Two representative figures from the paper are shown (Figure 6, 7). Future natural history studies would be more significant if they were prospective rather than retrospective, included patients from multiple sites, and if more patients were included.

MMS (Mean macula sensitivity)

MMS was measured by microperimetry, which is a visual field test used to create a map of the retina showing which areas can correctly respond to light. Four patients showed a small decrease in MMS, while six showed an increase, perhaps attributable to a learning effect. The authors concluded that MMS warrants further study, and that given their

small sample size and the variability among patients they could not conclude if MMS would be an appropriate clinical trial endpoint.

BCVA (Best Corrected Visual Acuity)

The average change in BCVA was -0.94 and -2.56 letters/year in the right and left eyes, respectively, though two patients demonstrated bilateral improvement (Figure 6). Because the baseline visual acuity of patients may influence the rate of BCVA loss, the authors concluded that BCVA would not be a good clinical trial endpoint.

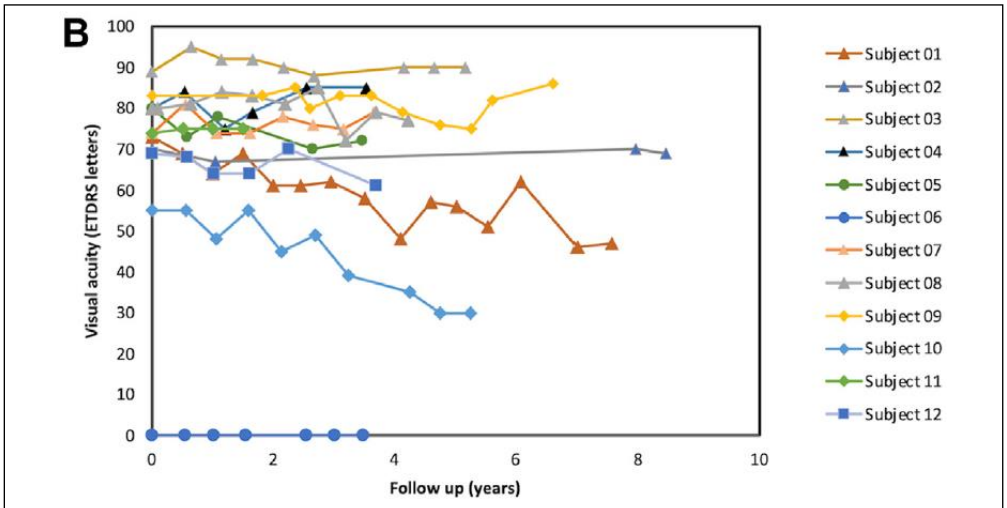

Figure 6 Changes in best corrected visual acuity (as calculated in ETDRS letters) of 12 PRPH2 patients. From (*79*).

TMV (Total macular volume)

TMV changed by -0.07mm^3/year. All patients showed a decline in TMV, and more than half of patients had a TMV more than two standard deviations below the mean of unaffected individuals. The authors concluded that TMV may be a suitable endpoint for clinical trials.

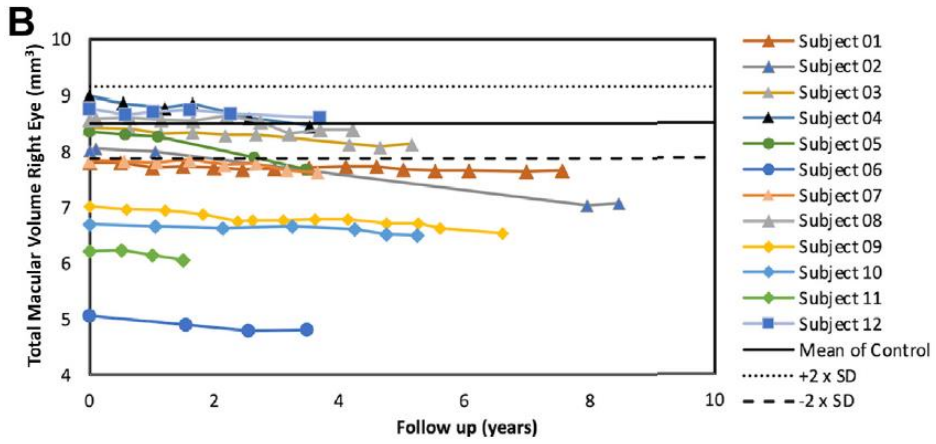

Figure 7 Change in total macular volume of 12 PRPH2 patients. All 12 patients showed a decrease in TMV. From (*79*).

TLS (total lesion size) and DAF (decreased autofluorescence area)

The change in DAF was also slow (0.10mm/year and 0.11mm/year in the right and left eyes, respectively). Given that these measurements both changed relatively slowly, and that they were variable across patients, the authors determined that TLS and DAF would not be effective clinical trial endpoints for trials that followed patients for ≤2 years.

Therapeutic Strategies

As alluded to above, different therapeutic strategies often must be employed for autosomal dominant diseases compared to autosomal recessive diseases. The sections below will cover different possible approaches: *PRPH2*-specific approaches—including gene therapy—as well as gene agnostic approaches like cell-based, neuroprotective, and optogenetic therapies (summarized in Table 4 and Table 5). Gene-specific approaches specifically target the *PRPH2* gene and/or specific mutations within it and therefore address the root cause of disease. Conversely, gene-agnostic approaches can provide benefit across mutations in multiple genes and address the shared secondary effects of gene mutations—for example, protecting photoreceptors from death irrespective of the underlying genetic cause, or replacing cells that have been lost. While there are too many potential therapeutic strategies to mention here, as researchers learn more about PRPH2 disease pathologies, they will be able to identify optimal therapeutic approaches, including those that are in development or use for other forms of IRDs.

In addition to determining which therapies may be effective for PRPH2—and perhaps even which therapy may be effective for a given *PRPH2* mutation—one must also consider what cell types need to be targeted and when a therapy or treatment must be given to be effective, also known as the therapeutic window. Since PRPH2-associated disease is typically late-onset, the therapeutic window is favorable.

PRPH2-Specific Approaches

Gene Replacement Therapy

Gene replacement therapy, sometimes used interchangeably with the terms ‘gene therapy’ and ‘gene augmentation therapy,’ is based on the premise that if we could replace the gene or the gene function that is missing in a cell, we could prevent, slow, or halt disease, and in some cases restore vision. This strategy has been effectively employed for mutations in the *RPE65* gene that cause Leber Congenital Amaurosis Type 2, leading to the creation of the approved drug LUXTURNA (reviewed in (*80*)). Gene therapy has also been demonstrated to be effective in curing or treating blindness in mouse models of many different genes, and clinical trials at various stages are underway for numerous inherited retinal degenerations. Generally, gene replacement is applicable to mutations that result in loss of function. Adding additional protein through traditional gene therapy as described above would not be able to overcome toxic gain of function mutations. Gene replacement therapy for PRPH2 would not generally be expected to restore lost vision, only to prevent the loss of additional vision or slow or stop the subsequent loss of vision.

Gene therapy is generally delivered by an injection of a viral shell that carries the correct gene copy and instructions to make the replacement protein in the appropriate cell type. When injected into the eye, either in the fluid-filled vitreous (intra-vitreal) or under the retina (sub-retinal), the virus finds its way to cells, infects them, but instead of producing viral genes, it

Table 4: Gene-specific therapeutic approaches

Therapy	Advantages	Disadvantages	Expected outcome	Disease stage
AAV gene replacement	Relatively straightforward, mouse proof-of-concept, clinical and regulatory path established, can fix many mutations	Pre-existing AAV immunity, inflammation, ability to achieve correct levels, cost to manufacture, not suitable for toxic GoF mutations	Slowing or stoppage of further degeneration	Early
Nanoparticle gene replacement	Large carrying capacity, mouse proof-of-concept, re-dosing possible, no inflammation, inexpensive manufacturing, can fix many different mutations	Low transduction efficiency, clinical and regulatory path less defined, not suitable for toxic GoF mutations	Slowing or stoppage of further degeneration	Early
Gene knockdown (+/- gene replacement)	Can correct dominant negative and toxic GoF mutations, mouse proof-of-concept, can correct many different mutations	Ability to achieve sufficient levels of PRPH2 protein, too large to fit into a single AAV, siRNA may have off-target effects	Slowing or stoppage of further degeneration	Early
Antisense oligonucleotides	Can correct many types of mutations; mouse and human proof-of-concept	Needs to be combined with replacement therapy, ability to achieve sufficient levels of PRPH2 protein, may have off-target effects	Slowing or stoppage of further degeneration	Early
Genome editing	Precise correction, gene/protein dose maintained, applicable for GoF and LoF mutations	Too large to deliver by AAV, off-target effects, low efficiency, some mutations cannot be targeted	Slowing or stoppage of further degeneration	Early

produces the gene of interest—in this case, *PRPH2*. The most successfully used viral shell (also called the capsid) is that of adeno-associated virus (AAV). AAV gene therapy is generally safe—though there may be some local inflammation—and long lasting. One of the oft-cited challenges with AAV is that it can only hold 4.7 kilobases (kb) worth of DNA; fortunately, this is not a problem for *PRPH2*, which is only 1.1kb and can therefore easily fit into AAV. In addition to delivering *PPRH2*, the gene therapy also needs to produce PRPH2 in the appropriate cells. Researchers use genetic zip codes, called promoters, which provide these instructions. In the case of PRPH2, targeting both rods and cones would be best. Thus photoreceptor-specific or even ubiquitous (i.e., expressed in every cell) promoters could be used. Another important factor to keep in mind with gene therapy is that typically not every rod or cone will be infected with the AAV, meaning that not every cell will be corrected. Researchers are working to generate improved capsids that can better infect the retina (*81–84*) and conducting studies to understand how many photoreceptors must be corrected for visual acuity to be maintained.

Proof of principle has already been demonstrated for *PRPH2* gene replacement therapy in mice (excellently reviewed in (*46*). Using either a rod-specific or rod-and-cone-specific promoter, researchers were able to correct retinal structure and function in *rds*$^{-/-}$ or *rds*$^{+/-}$ mice, and in mice carrying specific *Prph2* mutations (*45*, *85–87*). These studies emphasized that the therapies that generated PRPH2 protein levels close to 100% of normal levels were more effective at reducing symptoms. However, even producing protein levels that were 80% of

normal was not sufficient to fully block retinal degeneration. Thus far, no negative effects have been demonstrated due to overexpression (i.e., more than 100%) of PRPH2. Building on this work, in 2000, the group of Dr. Robin Ali used AAV to deliver *Prph2* to young *rds*$^{-/-}$ mice. Though some improvements were seen, they did not match the functional levels of unaffected or even *rds*$^{+/-}$ mice. Additionally, they found that providing the treatment at later ages was not as effective as earlier timepoints (*88*). An important caveat to this study is the fact that it was done in *rds*$^{-/-}$ mice (loss of both copies of *Prph2*), which have an earlier and more severe degeneration than *rds*$^{+/-}$ mice, instead of in *rds*$^{+/-}$ mice, which are more reflective of human cases (mutation on only one copy of *PRPH2*).

In addition to AAV, there are other carriers that can be used to deliver genes to cells. One of these is nanoparticles. Nanoparticles are small, typically lipid-based balls combined with the DNA to be delivered. Nanoparticles are positively charged, and DNA is negatively charged, so their interaction into a combined nanoparticle is mediated by the fact that opposite charges attract. Unlike AAV, which has a relatively small carrying capacity (~5kb of DNA), nanoparticles can hold upwards of 15kb of DNA, though, as mentioned above, this is not a challenge for *PRPH2* gene replacement therapy. Additionally, unlike AAV, which can typically only be injected once due to concerns of immune responses, nanoparticles can be injected multiple times, increasing the number of cells that can be transduced with *PRPH2* and, if needed, providing a 'booster' of function later. However, nanoparticles typically do not infect as many cells as AAV does. Delivering nanoparticles with either photoreceptor-specific or ubiquitous promoters driving expression of *Prph2*, researchers were able to demonstrate expression of PRPH2 protein in injected *rds*$^{+/-}$ eyes and a modest improvement in photoreceptor structure and function up to 15 months after injection (the latest time point studied). However, improvements were not seen outside the region of the injection (*89*).

Gene Knockdown Therapy

For *PRPH2* cases where the causative mutation leads to a dominant negative effect or generates a toxic gain of function protein, it will likely be necessary to both disable the mutant gene copy (allele) AND restore a normal copy of the gene via gene replacement therapy to overcome the haploinsufficiency of *PRPH2*. An ideal strategy would selectively target only the mutant allele and leave the normal allele untouched and would effectively target all mutant alleles (as opposed to needing to develop a new therapy for each mutant allele). It is difficult to imagine a scenario in which this would be possible; thus, strategies have focused on disabling both the normal and the mutated allele and then replacing both copies with a normal gene that is resistant to subsequent removal. This approach has had some success for mutations in the rhodopsin gene, which are also inherited in an autosomal dominant fashion (*90*), and could be applied to PRPH2.

There are multiple approaches to knockdown or disable genes. One of the most successful platforms is siRNA or shRNA, which is based on the knowledge that short RNA molecules that have a complimentary sequence to a gene target can lead to the destruction of the gene target. Using electroporation (electric shock) to deliver an siRNA targeting *Prph2* and a siRNA-resistant copy of *Prph2*, researchers showed knock-down of existing *Prph2* and replacement with the siRNA-resistant copy (*91*). Another group, using AAV to deliver both a *Prph2* shRNA and a normal *Prph2* gene, were also able to both reduce the amount of *Prph2* and replace it with the shRNA-resistant *Prph2* (*92*). In both cases, the studies were carried out in normal mice as a proof-of-concept and have not yet been applied to mice carrying mutations in *Prph2* (*93*). The CRISPR/Cas system has also been used successfully to knockdown and

replace *Rho*, another gene that leads to autosomal dominant RP (*94*, *95*), though not yet to *Prph2*.

As with gene replacement therapy, a challenge will be delivering and generating sufficient levels of normal PRPH2 to ameliorate disease pathology. Additionally, the shRNA and a rescue copy of *PRPH2* are too big to fit together into a single AAV, so researchers put the shRNA in one AAV and the rescue copy in a second AAV. While this gets around the size limitation of AAV, the efficiency of both viruses getting into all the same cells is low, leading to lower amounts of rescue. As with standard gene replacement therapy, knockdown + gene replacement for PRPH2 would not be expected to restore lost vision but slow or stop further degeneration.

Antisense Oligonucleotides (ASOs or AONs)

Antisense oligonucleotides (ASOs or AONs) are another method to reduce expression of a mutant gene. ASOs are short strings of DNA or RNA that can bind to a genomic region of interest. In doing so, they can interfere with that gene being turned into protein or cause its degradation. Alternatively, ASOs can be designed to bind to a splice site mutation (Table 1), thereby 'hiding' the mutation from the cell's splicing machinery (*96*). This results in that exon not being included in the resulting protein (called 'exon skipping'). In some cases, this can be beneficial, as the skipped exon may have had an incorrect STOP signal in it or be an incorrect exon, called a 'pseudoexon,' and the protein can function reasonably well without that exon. However, if the exon being skipped overlaps with the next exon, then the frame of the protein will be disrupted, likely leading to dysfunctional or absent protein. Like the gene knockdown strategy described above, ASO-mediated therapy would also likely need to be paired with gene replacement to overcome *PRPH2* haploinsufficiency. As with other strategies, the ASO must be correctly delivered to the cells of the retina and must have limited off-target effects. Ongoing studies are aimed at modifying ASOs to optimize both of these aspects. ASOs for inherited retinal diseases are nicely reviewed in (*97*).

ASO-mediated exon skipping in IRDs has been demonstrated for a common splice-site mutation in the *CEP290* gene (*98–101*). This led to a Phase 1b/2 trial, which demonstrated manageable adverse events and some improvement in visual acuity in a post-hoc analysis (*102*). ASO-mediated mutant transcript degradation has been demonstrated for mutant *Rho*, which causes autosomal dominant RP (*103*).

Genome Editing

Genome editing, which is a relatively recent scientific development, is the ability to use specialized proteins to correct genetic 'typos.' There are multiple systems (e.g., zinc finger nucleases, TALENs, CRISPR/Cas) but they are all based on the premise that there are proteins that can cut DNA, and if we can direct them to a place in a gene where there is a mutation, they can cut the faulty DNA and replace it with the correct DNA (actually, they can replace it with whatever genetic information we provide them with, but that is outside the scope of this discussion). This is analogous to moving your computer cursor to a typo in a document, cutting out what is wrong, and pasting in the correct spelling. Theoretically, any of these genome editing approaches could be used to precisely correct either a single mutation or larger stretches of a gene, within the gene itself.

The CRISPR/Cas system is the most flexible and robust, and the scientists who discovered it were awarded the Nobel Prize in Chemistry in 2020. It is an elegant solution that targets the root cause of a disease. For PRPH2, one appealing aspect of genome editing is that because the correction is done directly in the genome (in contrast to gene therapy, where a new

copy of the gene is introduced that functions separate from the rest of the genome), the regulatory systems that dictate how much protein should be made would be maintained, circumventing the protein dose problems that plague other therapeutic strategies. However, it is not without its challenges and drawbacks. One of them is that the protein machinery must find its way to the location to be corrected. This is mediated by short stretches of DNA that the Cas protein recognizes, called a PAM sequence. If there is no PAM sequence near the mutation to be corrected, then no change can be made. Also, there is the possibility of the protein machinery going to incorrect locations in other genes and making unintended, potentially harmful changes to the DNA. Various aspects of the CRISPR/Cas machinery have been and are being optimized to make more sites in the genome 'findable' and to reduce these off-targeting effects. Multiple groups have used computational methods to predict for other IRD genes in which mutations could be corrected by genome editing (*104–107*), but thus far no such report has been published for *PRPH2*. Additionally, the efficiency of the machinery making the correction is low (reviewed in (*108*, *109*)), especially when attempting to correct longer stretches of DNA. Finally, the CRISPR/Cas protein machinery is large, so it is not possible to deliver all components necessary to make the correction in a single AAV molecule. As mentioned above, the machinery can be split into two AAV vectors or other delivery strategies could be used, but this leads to decreased efficiency and subsequently decreased correction. Editas recently conducted a Phase 1/2 clinical trial to use CRISPR/Cas to correct the IVS26 c.2991+1655 A>G mutation in *CEP290*, which is the most common mutation in this inherited retinal degeneration population. Unfortunately, they did not see significant effects in patients who carried one copy of the mutation (in combination with another *CEP290* mutation), though there was some benefit to patients who had two IVS26 c.2991+1655 A>G mutations. However, given the small number of individuals who are homozygous for that mutation and the underwhelming results in heterozygous individuals, Editas has decided not to move forward with additional studies.

More recent adaptations have created two new gene editing platforms, called base editing and prime editing. These are both based on the CRISPR/Cas proteins but have slight modifications. In base editing, instead of cutting the DNA at the site of the typo, the protein instead drives a chemical reaction that directly converts one DNA letter into another. Not all conversions are chemically possible, thus not all mutations can be corrected using this method (currently only C>T, T>C. G>A, and A>G are possible). Similarly, base editing cannot correct insertions or deletions of DNA. Base editing has been successfully used to correct a mutation in *Pde6b* that causes RP in the *rd10* mouse model. Following delivery of the base editing machinery, the authors measured greater than 35% correction of the mutation, leading preservation of rod and cones and their function, though this work has not yet been thoroughly vetted by the scientific community (*110*). Prime editing, on the other hand, can correct all possible single base changes, as well as correct small insertions and deletions. Unlike traditional genome editing, prime editing only makes one cut to DNA and is coupled to the donor DNA to make the genetic correction. It has been successfully applied to the *Pde6b* gene in the *rd10* mouse model of RP. Even though they only saw approximately 5% correction of the mutation, they observed significant improvements in visual function, as measured by ERG (*111*, *112*).

A conceptual and regulatory challenge of genome editing is that precisely correcting each mutation requires the development and separate regulatory approval of each mutation's correction machinery. The time and expense required to do this is untenable. Moreover, for some mutations, there may only be a few affected individuals in the world, making the total population to be treated for an individual drug very small. Unlike some other diseases, *PRPH2*

does not have a single or a few common mutations, making a mutation-specific approach for every *PRPH2* mutation unrealistic. However, methods that use CRISPR/Cas to edit larger stretches of DNA that would correct multiple mutations at once (e.g., twin prime editing) could be utilized for individuals with mutations in *PRPH2*. The use of CRISPR genome editing and base and prime editing in the retina are nicely reviewed in (*109*, *113–115*).

Gene-Agnostic Approaches

Table 5: Gene-agnostic therapeutic approaches

Therapy	Advantages	Disadvantages	Expected outcome	Disease stage
Cell-based therapies	Restorative, gene agnostic, proof-of-concept in mice	Potential need for immunosuppression, limited reports of functional improvement	Restoration of vision, but perhaps no slowing or stoppage of additional vision loss	Mid to late
Neuroprotection	Can treat multiple mutations and diseases (including dominant), proof-of-concept in mice, clinical trials ongoing and imminent	Does not address the root cause of the disease, does not restore function, may not completely stop degeneration	Slowing or stoppage of further degeneration	Mid
Optogenetics	Restorative, gene agnostic, proof-of-concept in mice	Low-acuity vision, does not prevent vision loss	Restoration of low-acuity vision	Late

Cell-Based Therapies

An attractive regenerative medicine-based approach is the replacement of lost retinal cells with new, functional cells. This can be done by direct transplantation of cells into the eye. In 2013, the group of Dr. Rachel Pearson tested the ability of rod precursor cells, which were isolated from other mice, to survive and make connections with existing neurons in the retina (*116*). In addition to other mouse models, the researchers injected these cells into *rds*$^{-/-}$ mice (both copies of *Prph2* are mutated), as well as into *Prph2*$^{+/\Delta 307}$ mice (these mice have one good copy of *Prph2* and one with amino acid 307 deleted) at 6-8 weeks after birth. Because the injected cells were labeled with a visual marker (green fluorescent protein, or GFP), they were able to track these cells over time. In the two *Prph2* mouse models, the researchers found that only half of the transplanted cells made outer segments and that these segments were shorter than the segments made by cells transplanted into unaffected mice. Importantly, however, the segments were longer than those found in the un-injected *Prph2* mice. Similarly, around 50% of the transplanted cells formed structures indicative of connectivity. Interestingly, there were more GFP+ cells found in the *rds*$^{-/-}$ mice than in the unaffected mice. When looking at how disease progression impacted the ability of cells to integrate, the researchers found that this was unchanged in *rds*$^{-/-}$ mice but that the number of cells that were able to integrate into the *Prph2*$^{+/\Delta 307}$ mice increased with disease progression (*116*). The researchers did not look at whether retinal function improved or whether the cells survived past 6 months. Similar results were found when the donor cells were derived from mouse embryonic stem cells and differentiated into rod precursor cells (*117*). Cell transplantation studies have not been conducted in *rds*$^{+/-}$ mice, which would be more reflective of patients.

In 2016, however, two groups reported that the majority of the GFP+ cells that many different researchers had been tracking in the eye were in fact not the cells that had been injected into the eye, but that somehow the injected cells had transferred some of their contents (including the GFP protein) to the resident cells (*118*, *119*). Importantly, the PRPH2 protein was one of the transferred molecules, as it was detected in the photoreceptors of cell-injected *rds*$^{-/-}$ mice, whereas normally there is none (*116*, *117*). Interestingly, whereas in some retinal degeneration models the number of donor-derived cells was only found to be 1% of the total, in *rds*$^{-/-}$ mice that number was closer to 15%. The authors suggested that disruption to the outer limiting membrane in the *rds*$^{-/-}$ mice may enable more cells to integrate (*120*).

Other studies have investigated the transplantation of retinal pigment epithelial (RPE) cells, which provide support for photoreceptors and may be lost during degeneration, as sheets or dissociated cells. Taking a section of unaffected RPE from the periphery of the patient's eye and moving it to an area where RPE has been lost demonstrated proof-of-concept for this therapeutic approach (c.f., (*121*)). Transplantion of RPE cells or sheets has been tested in multiple clinical trials for Stargardt Disease and age-related macular degeneration with no serious adverse effects, but only borderline visual improvements (*122–124*).

Important considerations for the use of cell therapy include identifying the best cell population for transplantation and removing unwanted cells, which may proliferate into tumors once injected into the eye. Moreover, whereas the eye is normally thought to be immune-privileged, this barrier may have broken down in retinal disease, which would necessitate long-term immune suppression to ensure that the host would not reject the transplanted cells. Various groups are exploring immunological tricks to circumvent this problem (for the eye and other tissues), though these studies are ongoing. While the use of a patient's own cells, reprogrammed into induced pluripotent stem cells, converted into the appropriate retinal type, and injected into the eye should avoid the immunological challenges, the transplanted cells would contain the causative mutation, making them a less useful therapy, if one at all.

Neuroprotection

Neuroprotection is based on the premise that, in many different IRDs, regardless of the causative genetic mutation, photoreceptors die in the same way. Understanding the way(s) in which they die and preventing them from dying could therefore help to preserve vision and/or slow or stop subsequent degeneration and vision loss.

Various naturally occurring factors have been explored for their protective abilities. As some of these factors are encoded for by genes, they are often delivered by gene therapy vectors, including AAV. Early studies that investigated whether CNTF (ciliary neurotrophic factor) could provide protection showed promising results. Following injection of a vector encoding CNTF into the retina of *rds*$^{-/-}$ mice, the authors noted there was preservation of the thickness of the outer nuclear layer, where photoreceptors reside, compared to uninjected animals. However, this did not lead to significant improvements in retinal function (*125–127*). Further studies also demonstrated some retinal toxicity following CNTF injection, which has halted additional studies.

Another neuroprotective factor that shows promise is rdCVF, also known as rod-derived cone viability factor (Figure 8). Researchers had noted that in diseases where rods are affected and die, cones would also die once the rods were gone, leading them to hypothesize that rods were secreting a factor that keeps cones alive. In 2004, researchers identified rdCVF, which is the protein produced by the *NXNL1* gene (*128*), and further studies demonstrated that delivering rdCVF by AAV-mediated gene therapy could preserve cones in mice with both recessive and dominant disease (*129*). There have been no published studies investigating rdCVF therapy in a

Prph2 animal model, but there is no known scientific reason why it could not work. In support of this therapeutic strategy, Nour et al showed that increasing PRPH2 expression in the *rds*$^{-/-}$ mouse led to a preservation of rod function and a concomitant maintenance of cone function (*45*). Based on these and other promising studies, Sparing Vision is pursuing AAV-mediated rdCVF delivery as a therapeutic strategy and plans to launch a Phase 1 clinical trial in early 2023. This trial will focus on individuals with RP caused by mutations in *PDE6a*, *PDE6b*, and *RHO*, and evaluate the safety and efficacy of the treatment. rdCVF therapy would not be expected to restore rod-mediated visual function (e.g., dim-light vision) that has already been lost. However, since cones mediate high acuity central vision, their preservation could provide significant quality-of-life improvements.

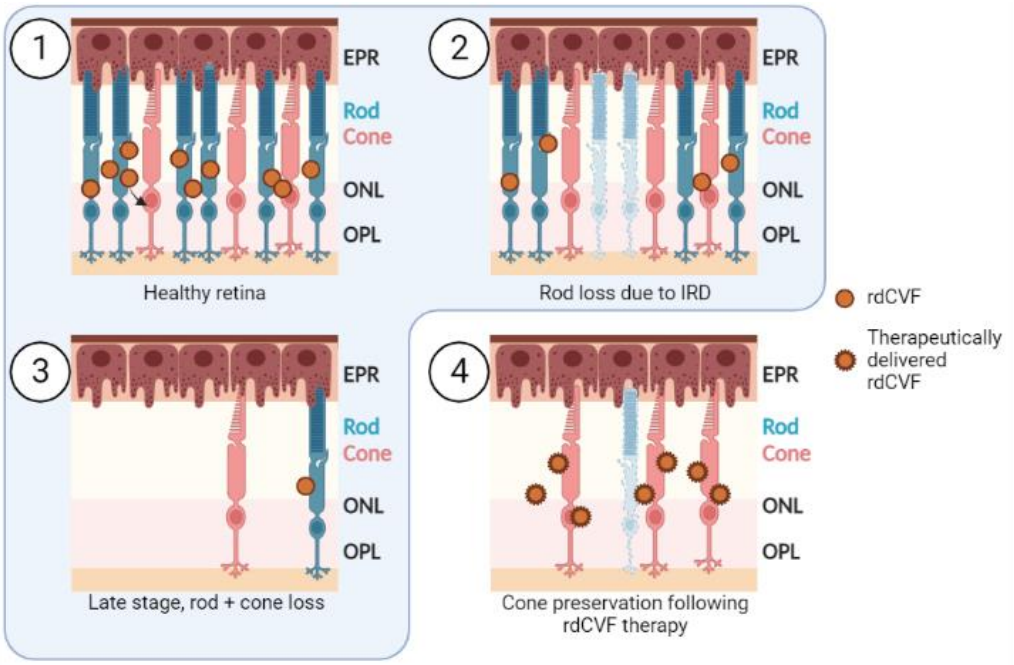

Figure 8 The mechanism of rdCVF. 1) In an unaffected retina, rods secrete rdCVF, which promotes the survival of cones. 2) When rods die, less rdCVF is produced, which ultimately leads to 3) secondary cone death. 4) When rdCVF is supplied by AAV-mediated delivery, cones survive even in the absence of rods. Image created in BioRender.

As mentioned in an earlier section, accumulation of cGMP and its downstream effectors has been observed in mouse models of PRPH2-associated disease (*50*, *130*) and is thought to be an early event in retinal cell degeneration. Researchers are exploring methods to develop small molecules (i.e., drugs) that would interfere in this process to preserve photoreceptors. Proof-of-concept work demonstrated that a cGMP analog, CN03, when injected into the eye of *rds*$^{-/-}$ mice, could decrease photoreceptor cell loss, as compared to no treatment (*131*). This molecule is being further explored for commercialization and advancement to clinical trials. Like cGMP, PARP (Poly (ADP-ribose) polymerase) is also elevated in multiple models of retinal degeneration, including the *rds*$^{-/-}$ mouse. The peak of PARP expression correlates with the time of maximal cell death in *rds*$^{-/-}$ mice. Treating retinal cultures taken from *rds*$^{-/-}$ mice with a PARP inhibitor, PJ34, led to fewer dying photoreceptors and a lengthening of outer segments (*132*). Thus, PARP inhibitors, which are under development for various cancers, could be an attractive therapeutic strategy if repurposed for PRPH2-associated disease.

In addition to the increase in cGMP, oxidative damage is another mechanism that leads to photoreceptor cell death (*133*, *134*). Multiple antioxidants have been demonstrated to promote photoreceptor survival in different retinal degeneration mouse models (*133*, *135*). One molecule in particular has significant potential: n-acetylcysteine (NAC). NAC works by neutralizing reactive oxygen species and by providing a building block for our antioxidant defense system (*136*). Importantly, NAC can be taken in pill form. A Phase 1/2 clinical trial has demonstrated the safety and tolerability of NAC in RP patients and shown a slight increase in BCVA (*137*). A Phase 3 trial will soon begin enrolling patients with RP to determine if long-term usage of NAC can preserve cone survival and function (Clinical Trial Identifier: NCT05537220).

A modified form of NAC, called NACA (n-acetylcysteine amide), is also in a Phase 1/2 clinical trial. The amide modification allows NACA to enter cells more easily than NAC. Like NAC, NACA acts as an antioxidant and is able to preserve retinal function in response to toxic light damage in the *rd10* model of retinal degeneration (*138*, *139*). Nacuity Pharmaceuticals has completed a Phase 2 study in Usher Syndrome patients with RP in Australia and has been given clearance to begin a trial in the United States. While this study will only be open to patients with Usher Syndrome, it will pave the way for further trials. As with rdCVF, there have been no published studies looking at the effect of NAC or NACA on PRPH2 animal models; however, some patients with autosomal dominant RP were included in the Phase 1 NAC trial.

Optogenetics

In late-stage retinal degeneration there is near to complete loss of photoreceptors in sections of, or the entire, retina. This poses a challenge for gene replacement or editing approaches, which need the target cell to be present to receive and express the therapy. Optogenetics, which is a therapeutic strategy that has been rapidly developed over the past 15 years, circumvents this need. Optogenetics ('opto' meaning 'vision' and 'genetics' indicating that it is genetically encoded and/or provided) is based on the concept that remaining, non-photoreceptor cells can be converted to a kind of artificial photoreceptor by providing them with light sensing proteins called opsins (reviewed in (*140*–*142*)). In a landmark paper, the labs of Dr. Pan and Dr. Dizhoor demonstrated that the gene for a rhodopsin present in green algae, *ChR2*, could be delivered by AAV to remaining inner neurons (bipolar cells, amacrine cells, and retinal ganglion cells) in mouse retinae. Significantly, when injected into a mouse model of retinal degeneration after the

Table 6: Optogenetics Clinical Trials

Company	Phase	Stage	Notes
Bionic Sight	1/2	Enrolling	ClinicalTrials.gov identifier number: NCT04278131; advanced RP
GenSight	1/2	On-going	ClinicalTrials.gov identifier number: NCT03326336; early promising results and 1-year safety data; RP
Kiora	1b	On-going	A small molecule approach to optogenetics; small trial initiated in Australia for patients with late-stage RP
Nanoscope	1/2a; 2b	On-going	ClinicalTrials.gov identifier number: NCT04919473; early promising results ClinicalTrials.gov identifier number: NCT04945772
Retrosense	1/2	On-going	ClinicalTrials.gov identifier number: NCT02556736
Sparing Vision	Pre-clinical		Combination strategy that would deliver rdCVF and activate visual function in remaining, but nonfunctional, cones

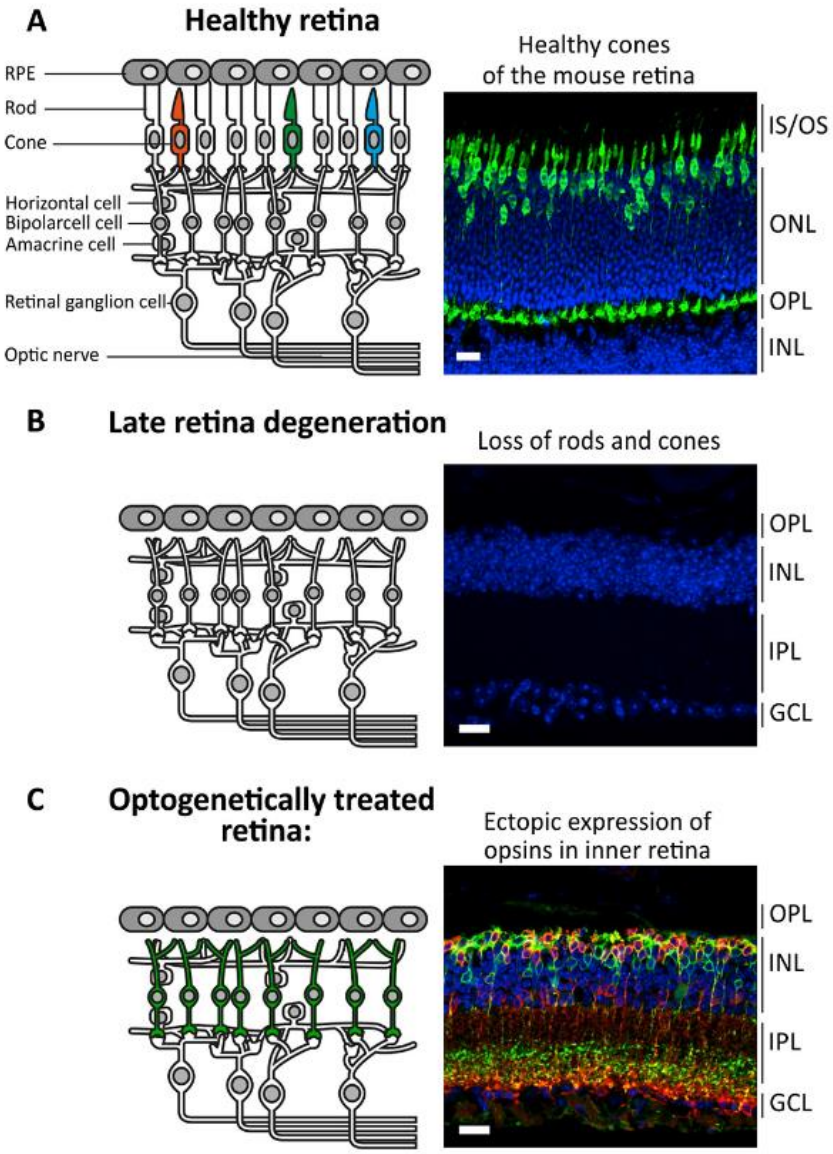

Figure 9 The concept of optogenetics. A. The illustration on the right shows the cell types and layers in a healthy retina. Cones, which mediate high-acuity central vision are colored in red, green, and blue. On the left is a section through a healthy mouse retina showing cones in green. B. In late-stage retinal degeneration, illustrated on the right, rods and cones are lost. Note the absence of green cells in the picture from the mouse retina on the left, and the loss of off the IS/OS and ONL layers. C. Following treatment with an optogenetic therapy, bipolar cells (shown in green in the illustration), which are normally not able to sense light are now endowed with that ability. From (*140*).

photoreceptors had died, this led to light sensitivity, whereas previously there had been none (*143*). Significant work has continued, exploring the best opsin to deliver and the appropriate cell type to target. To date, however, no studies have been published exploring the applicability of optogenetics to PRPH2-associated disease.

There are several companies who are working to develop optogenetic therapies, including Bionic Sight, GenSight, Kiora, Kubota Vision, Nanoscope Therapeutics, Ray Therapeutics, RetroSense Therapeutics (acquired by Allergan), and Vedere Bio (acquired by Novartis) (reviewed in (*140*)). A summary of companies with clinical trials is found in Table 6. GenSight's trial, which is enrolling RP patients (with no requirement for a specific gene mutation), is testing the safety and efficacy of a single injection of an optogenetic gene, combined with special goggles to activate the newly delivered opsin. Early results have been promising, and the first patient to have restored light sensitivity was reported in 2021 (*144*).

Based on its mechanism of action, optogenetics is a gene-agnostic approach; in fact, it doesn't even require knowledge of the causative gene. It is also one of the few therapeutic strategies that is meant for late-stage disease, and which can restore vision after it has been lost. Though there were concerns about an immune response to non-human proteins, no

significant events have been observed. However, optogenetics is not without its limitations. While it can lead to a restoration of vision, this is not the same high-acuity color vision that was lost, as the complex cellular interactions and abilities of the photoreceptors have not been recapitulated.

Concluding remarks

This document has provided an overview of PRPH2 at multiple levels: the gene, the protein, its function, the epidemiology, and clinical manifestations of PRPH2-associated disease, as well as potential treatment strategies. There is a significant body of work that provides researchers with foundational knowledge to drive critical studies moving forward. Outstanding challenges include better understanding the phenotypic variability observed in patients and developing therapies that produce necessary levels of functional protein to slow or prevent degeneration.

Glossary

Adeno-associated virus (AAV) – small viruses that can be engineered to carry and deliver specific genetic cargo for use in gene therapy

Allele – one of the two copies of each gene present in most cells

Anti-sense oligonucleotide (ASO or AON) – a string of RNA that is complimentary (anti-sense) to the messenger RNA (mRNA) of a gene of interest, and can alter the expression of that mRNA

Apoptosis – a controlled process for cell death, triggered by a signal or biochemical reaction, in response to an accumulation of cellular damage

Autosomal dominant – genetic variants that are found on one of the autosomes (non-sex chromosomes) and which require only one copy of the variant to show an effect in the individual

Autosome – any chromosome within the 22 pairs of non-sex (not X or Y) chromosomes inherited by every individual from their biological parents. An autosome is a chromosome (or DNA package) that does not play a primary role in determining a person's sex (i.e., not an X or Y chromosome). Autosomal recessive and autosomal dominant diseases are caused by mutations in genes that reside on one of the 22 paired autosomes

Base editing – a genome editing method that directly generates precise changes in genomic DNA by directly converting one "letter" into another

Best-corrected visual acuity (BCVA) – the ability to distinguish the details of the object and shape at a given distance, after correcting for other eye conditions, such as astigmatism and myopia

Cone cell – a type of photoreceptor that detects light and is responsible for providing fine detail, daylight, and color vision. Although cones are present throughout the retina, they are mainly found in the macula (the central portion of the retina). Cone cells are particularly important for color and day vision and discriminating fine visual detail, like that required for discerning facial features or reading a book. There are three types of cone cells (blue, green and red) that respond to different wavelengths of light to make up the full rainbow of colors

Exon – a segment of a DNA containing the information that will be turned into a protein

Frameshift mutation – DNA is converted into amino acids in groups of three DNA bases (the "frame"). Changes to the protein that alter the "frame" in which the DNA is read, typically due to insertions or deletions, lead to a protein made up of incorrect amino acids and often the incorporation of a stop codon (called a premature stop codon), leading to a shortened protein or loss of protein

Gain of function mutation – a mutation that leads to a protein having a new trait or causing a trait to appear in inappropriate places or times in development

Gene therapy – A therapeutic process that replaces or turns off the "bad" or mutated disease-causing gene and restores some level of normal protein function

Genome editing – a type of genetic engineering in which DNA is inserted, deleted, modified, or replaced in the genome of a living organism

Genotype – the genetic composition of an individual

Genotype-phenotype correlation – a reproducible connection between an individual's genetic composition and their physical symptoms

Haploinsufficient – when one copy of a gene is inactivated or deleted and the remaining functional copy of the gene is not adequate to produce the needed gene product to preserve normal function

Hetero-dimer – a protein complex made up of two different proteins

Homo-dimer – a protein complex made up of two of the same proteins

Incomplete penetrance – when some people with a disease-causing mutation (change) in a gene develop the disease while others do not

Induced pluripotent stem cells (iPSCs) – iPSCs are derived from skin or blood cells that have been reprogrammed ("induced") back into an embryonic-like pluripotent state that enables the development of an unlimited source of any type of human cell needed for therapeutic purposes

Intron – a segment of a gene that does not provide instructions for making a protein

Loss of function mutation – a type of mutation in which the resulting protein lacks the normal function of the unaffected gene

Macular dystrophy – a retinal disease that causes deterioration of the most sensitive part of the central retina (macula), which has the highest concentration of light-sensitive cells (photoreceptors)

Missense mutation – a genetic misspelling where a single DNA base is changed, resulting in a different amino acid being present in the protein

Mutation – a change or "spelling mistake" in the DNA of a gene that can cause a disease or contribute to other physical features

Nanoparticles – small, often fat-based, particles too small to be seen by the naked eye, which can be used to deliver cargo to cells

Neuroprotection – delivering a protein or drug to the eye that prevents the photoreceptors and/or RPE cells from dying, thus saving vision. The 'neuro' prefix refers to the fact that photoreceptors are a type of neuron

Nonsense mutation – A genetic misspelling that inserts a STOP codon into the protein, ultimately leading to an incorrectly shortened protein or loss of protein

Optogenetics – a biological technique to control the activity of neurons or other cell types with light. This is achieved by expression of light-sensitive ion channels, pumps, or enzymes specifically in the target cells. Optogenetics can make cells of the inner retina, which cannot normally perceive light, into light-sensitive artificial photoreceptors

Ortholog – the same gene in a different species (e.g., *PRPH2* in humans and *Prph2* in mice)

Outer segment – the part of a photoreceptor which is pointed toward the back of the eye and contains the light-absorbing materials

Phenotype – Physical symptoms of a retinal degenerative disease that can be clinically defined

Photoreceptor cells – the light sensitive cells in the retina that absorb light and convert it into an electrical signal that is passed to the brain through the optic nerve

Prime editing – a 'search-and-replace' genome editing technology that does not require both strands of DNA to be cut. It can mediate targeted insertions, deletions, and base-to-base conversions

Regenerative medicine – the process of replacing, engineering, or regenerating human or animal cells, tissues, or organs to restore or establish normal function, which may be loss due to genetic mutations, injury, or aging

Retinitis pigmentosa (RP) – a group of inherited diseases causing retinal degeneration. Most forms of RP first cause the breakdown of rod cells. These forms of RP, sometimes called rod-cone dystrophy, usually begin with night blindness. RP is typically diagnosed in adolescents and young adults. It is a progressive disorder. The rate of progression and degree of visual loss varies from person to person. RP can be inherited in a dominant, recessive, or X-linked fashion

Rod cell – a photoreceptor cell that is responsible for black-and-white, peripheral and night vision. In humans, rods are mostly found in the periphery of the retina

shRNA – short hairpin RNA; artificial RNA molecule that can be delivered to cells and processed to form siRNA, which can interfere with the expression of specific genes

siRNA – short interfering RNA; interferes with the expression of genes with complementary nucleotide sequences by degrading their mRNA

Splice-site mutation – DNA is made up of exons and introns. Exons are the parts of the DNA that will ultimately be turned into proteins. At the end and beginning of each exon are short genetic tags that allow cellular machinery to ‘splice’ together exons while removing the intron. Mutations that change these tags can lead to incorrect parts of introns being included, typically leading to non-functional or dysfunctional proteins

Tetraspanin – a family of proteins that have four membrane-spanning domains and 2 extracellular domains. Tetraspanins are involved in a wide range of processes, including cell migration, division, and anchoring

Variable expressivity – when individuals with the same genotype can show different degrees of the same phenotype

Visual cycle – the process in the retina that replenishes the molecule retinal for its use in vision. Retinal is the chromophore of most visual opsins, meaning it captures the photons to begin the phototransduction cascade

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