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Research Opportunities

Current and Future Directions in Developing Effective Treatments for *PRPH2*-Associated Retinal Diseases: A Workshop Report

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Purpose and Methods: A workshop of affected individuals and their families, clinicians, researchers, and industry representatives was convened in March 2023 to define the knowledge landscape of peripherin 2 (PRPH2) biology and identify challenges and opportunities towards developing *PRPH2*-associated inherited retinal disease (IRD) treatments.

Results: The results of an online survey and presentations from affected individuals and their family members revealed disease characteristics and impacts on daily living. Scientific sessions highlighted the significant heterogeneity in clinical presentation of *PRPH2*-related retinopathy; PRPH2's crucial function in rod and cone outer segment formation and maintenance; the usefulness of existing animal and cellular models for understanding disease pathophysiology; and possible therapeutic approaches for autosomal dominant *PRPH2*-associated IRDs, including gene-specific therapies and gene-agnostic approaches. Priority gaps identified by the workshop included having a more complete understanding of PRPH2's fundamental biology and factors contributing to *PRPH2*-related disease phenotypic diversity, establishing genotype–phenotype correlations, and creating additional models to probe the functional consequences of *PRPH2* variants and to test therapies. Additionally, a natural history study involving a large number of participants is required to more fully characterize *PRPH2*-related disease progression, aiding in interventional clinical trial design.

Conclusions: Because *PRPH2*-associated IRDs are rare, maximizing opportunities for communication and collaboration among stakeholders, such as that provided by the workshop, is crucial to overcome the challenges to developing effective treatments and improve the lives of affected individuals.

Translational Relevance: Fostering communication among stakeholders to identify knowledge gaps, therapeutic challenges, and potential opportunities toward developing effective treatments for *PRPH2*-related IRDs.

Introduction

The peripherin 2 (*PRPH2*) gene encodes the PRPH2 protein, which is essential for the formation and structure of the photoreceptor outer segment (OS).[1,2] *PRPH2* is one of the most commonly mutated inherited retinal disease (IRD) genes, accounting for 3% to 5% of pathogenic variants in several large-scale IRD cohort studies,[3,4] which translates to an estimated 6000 to 22,000 people in the United States and up to 200,000 worldwide with a *PRPH2*-associated IRD. Pathogenic variants in *PRPH2* are associated with multiple clinical phenotypes, which typically follow an autosomal dominant inheritance pattern. These include retinitis

pigmentosa (RP) and various macular dystrophies, including butterfly-shaped pattern dystrophy (BPD), adult-onset foveomacular vitelliform dystrophy (AOFMD), central areolar choroidal dystrophy (CACD), Stargardt-like fundus flavimaculatus–type dystrophy, and cone and cone-rod dystrophies, all of which typically onset in the third to fifth decade of life.[5] In addition, *PRPH2*-associated IRDs show significant intra- and interfamilial heterogeneity.[5] *PRPH2*-associated IRD cases are frequently misdiagnosed as having age-related macular degeneration. *PRPH2*-associated IRDs are also often considered to be relatively benign conditions, which are associated with minimal visual deficit and impact on quality of life. However, recent large cohort studies of molecularly confirmed cases have confirmed that *PRPH2*-associated IRDs commonly lead to severe visual deficits which are often as severe as that associated with late-stage age-related macular degeneration.[6] At present, there are no US Food and Drug Administration (FDA)-approved treatments, but new therapeutic approaches are being explored, including gene-specific (e.g. gene augmentation therapy, gene editing therapy, and RNA-based therapies) and gene-agnostic (e.g. neuroprotective agents and optogenetics) approaches.

On March 29 to 31, 2023, the Foundation Fighting Blindness, Columbia, Maryland, in partnership with the Nixon Visions Foundation, Rancho Santa Fe, California, and the Shiley Eye Institute of the University of California, San Diego, California, hosted a workshop in La Jolla, California that brought together >120 stakeholders, including clinicians, researchers, industry representatives, advocates, and affected individuals and their families, to discuss challenges and opportunities associated with *PRPH2*-associated IRDs. Participants were invited to share their experiences, present findings from their research, and engage in dialogue to determine gaps in understanding and a path forward for developing effective treatments. The workshop was co-chaired by Claire Gelfman, PhD, of the Foundation Fighting Blindness, and Radha Ayyagari, PhD, and Shyamanga Borooah, MBBS, PhD, both of the Shiley Eye Institute. The workshop interwove patient perspectives and scientific sessions with active engagement of attendees through question-and-answer sessions. Initial sessions focused on clinical presentation and management, followed by a discussion of PRPH2 fundamental science and experimental models. Subsequent sessions discussed therapeutic approaches and considerations for treating *PRPH2*-associated IRDs. Topics included:

- Patient and caregiver perspectives on *PRPH2*-associated diseases
- Genetics, retinal biology, and the fundamental science of *PRPH2*-associated diseases
- Clinical perspectives on diagnosing and managing *PRPH2*-associated diseases
- Current and potential future therapeutic strategies for *PRPH2*-associated diseases

This paper presents highlights from the workshop sessions, as well as identified gaps and next steps.

Patient Perspectives

Understanding the needs, challenges, and expectations of individuals living with *PRPH2*-associated disease and appreciating how the disease impacts their daily functioning and wellbeing are key considerations when determining appropriate goals for a treatment. To provide workshop participants with a perspective on living with a *PRPH2*-associated IRD, Todd Durham, PhD, of the Foundation Fighting Blindness, presented the results of a Foundation-conducted survey of individuals with a self-reported *PRPH2*-associated IRD. Participants were recruited via the My Retina Tracker Registry (Foundation Fighting Blindness; Columbia, MD, USA) and contact lists from Retina International, Dublin, Ireland, and the University of Melbourne in Australia (see the Table). The anonymous, self-administered, online survey of 304 participants collected 117 responses over a 2-month period (January–March 2023), primarily from individuals in the United States (78%), but also from those in Switzerland, Mexico, India, Australia, and the United Kingdom. The mean (standard deviation [SD]) age of respondents was 57.8 (12.4) years, and 57% were women. The survey revealed that respondents typically started experiencing *PRPH2*-associated IRD symptoms in adulthood (mean [SD] age = 35.9 [15.4] years) but were not clinically diagnosed until several years later (mean [SD] age = 44.2 [13.6] years). The majority of survey participants (n = 93/117; 79%) had >1 family member affected with a *PRPH2*-associated IRD, consistent with its autosomal dominant inheritance pattern. Of the respondents who reported knowing their current best corrected visual acuity (BCVA) in the better seeing eye (n = 63), 83% had BCVA of 20/63 or better (age range = 22–88 years). Most respondents (approximately 50%–95%) reported that performing daily tasks, such as taking medication, finding food on the plate, using a computer or tablet, and navigating familiar and unfamiliar places, were very or somewhat easy. However, although 80% (n = 20/25) of respondents aged ≤50 years reported they continued to

Table. Results of a Web-Based Survey of Individuals Affected by *PRPH2*-Associated Disease*

Parameter	No. of Respondents	Result
Current age, mean years (SD)	113	57.8 (12.4)
Gender, *n* (%)	117	
Female		67 (57)
Male		50 (43)
Age of first visual symptoms, mean years (SD)	117	35.9 (15.4)
Age of first clinical diagnosis, mean years (SD)	116	44.2 (13.6)
Age of first genetic diagnosis, mean years (SD)	116	51.1 (14.1)
BCVA in better seeing eye, *n* (%)	63	
20/16		3 (5)
20/20		22 (35)
20/25		6 (10)
20/32		6 (10)
20/40		10 (16)
20/50		2 (3)
20/63		3 (5)
20/160		1 (2)
20/200		4 (6)
20/400		2 (3)
Hand motion		2 (3)
Light perception		2 (3)
Clinical diagnosis, *n* (%)	116	
Pattern dystrophy		35 (30)
Retinitis pigmentosa		27 (23)
I don't know		17 (15)
Cone-rod dystrophy		12 (10)
Macular dystrophy		9 (8)
Stargardt disease		7 (6)
Other		6 (6)
Macular degeneration		2 (2)
CACD		1 (1)
Affected family members, *n* (%)	117	
1 (respondent only)		24 (21)
2		31 (26)
3		21 (18)
4		14 (12)
>4		27 (23)
Have driven a car/automobile, *n* (%)	116	114 (98)
Still driving by age category, *n* (%)	111	
≤50 y	25	20 (80)
51–60 y	37	22 (59)
61–70 y	32	21 (66)
>70 y	17	7 (41)
Most significant impacts of retinal disease on your life, *n* (%)	81	
Inability or difficulty driving		31 (38)
Reading		26 (32)
Night blindness		16 (20)
Recognizing faces		10 (12)
Light sensitivity		10 (12)
Loss of central vision		9 (11)
Loss of peripheral vision		8 (10)
Blind spots		7 (9)
Work		6 (7)
Using computers		6 (7)
Floater, streaks, other visual phenomena		6 (7)
Important activities that are limited because of your retinal disease, *n* (%)	69	
Sports and other outdoor recreation		30 (43)
Reading print		29 (42)
Driving		25 (36)
Hobbies and crafting		12 (17)
Social activities		10 (14)
Work limitations		6 (9)
Computer work		4 (6)
Watching television and films		4 (6)
Shopping		3 (4)
Traveling		3 (4)
Hoped-for treatment benefits (short of a complete cure and restoration of your lost vision), *n* (%)	99	
Slow or stop disease progression		62 (63)
Improvement in some aspect of vision		21 (21)
Maintain ability to read		8 (8)
Continue to drive		6 (6)
I'm still hoping for a cure		4 (4)
A treatment for future affected individuals		4 (4)
Maintain ability to travel		3 (3)
Stem cell therapy		3 (3)

*Self-administered, on-line survey in English and German conducted from January 12 to March 3, 2023.

N = 117 participants diagnosed with IRD associated with *PRPH2*. BCVA, best corrected visual acuity; CACD, central areolar choroidal dystrophy; SD, standard deviation.

drive a vehicle at least some of the time, the proportion dropped to 41% ($n = 7/17$) in respondents aged >70 years. The most commonly reported significant impacts to daily life were driving (38%; $n = 31/81$) and reading (32%; $n = 26/81$). Out of the 99 survey participants who responded to the open-ended question, "Short of a complete cure and restoration of your lost vision, what treatment benefits would you hope for yourself?," 63% mentioned slowing or stopping progression, 21% mentioned an improvement in some aspect of vision, 8% mentioned maintaining the ability to read, 6% expressed their desire to continue to drive, and 4% mentioned a cure for *PRPH2*-associated IRDs.

Additional perspectives of affected individuals and family members were interspersed among the scientific presentations, providing poignant reminders of the importance and urgency of research progress. One workshop participant, a male, said he first noticed blind spots in his vision at the age of 43 years, but it was not until 4 years later that he was diagnosed with an IRD due to a *PRPH2* mutation. Over the last 1.5 years, his central vision has deteriorated to the point that now, at age 50 years, he has approximately 5% central vision in his right eye and 30% to 40% in his left eye. Despite experiencing sadness and frustration, he said he tries not to worry about the things he cannot control and, instead, focuses on those he can, like being the best father for his children and nurturing his son's natural baseball skills. He concluded by expressing gratitude from himself and the community of those affected by *PRPH2*-associated diseases to the doctors and scientists working on developing a treatment and cure.

Another male workshop participant, whose vision is affected by a mutation in *PRPH2*, discussed watching his father struggle with *PRPH2*-associated disease as he aged, as well as his own experiences after being diagnosed at age 38 years. His vision loss is primarily peripheral, with about 7 degrees of vision in both eyes, but his BCVA remains 20/20, and his color vision unaffected. He described his appreciation for the PRPH2 advocacy community and its ability to help people with a *PRPH2*-associated IRD become accustomed to their changing circumstances. He noted that, because he witnessed his father's experience, unlike others who are affected, he knew what to expect as he aged. This foreknowledge allowed him to better anticipate changes in his vision. He encouraged all people with a clinical diagnosis of an IRD to undergo genetic testing and believes it is imperative that other people with a *PRPH2* mutation have information available to them at all stages of disease progression.

Disease Presentation and PRPH2 Biology

PRPH2-Associated IRD Clinical Presentation

PRPH2-associated IRDs almost always are inherited in an autosomal dominant fashion, although rare cases of recessive and digenic inheritance have been documented.[5–8] The clinical spectrum of *PRPH2*-related disease comprises RP; cone and cone–rod dystrophies; macular dystrophies, including pattern dystrophies and CACD; and, to a more limited extent, Leber's congenital amaurosis (LCA). *PRPH2*-related disease also may be misdiagnosed as age-related macular degeneration, Stargardt disease, or Best vitelliform macular dystrophy (BVMD).[5,9]

Over 200 pathogenic variants have been identified in *PRPH2*.[10] As discussed by Claire Gelfman, PhD, of the Foundation Fighting Blindness, and Radha Ayyagari, PhD, of the Shiley Eye Institute, *PRPH2*-associated diseases demonstrate significant intrafamilial and interfamilial variation, including variability in severity, age of onset, and disease progression. Strikingly, the same *PRPH2* mutation can present as a variety of different phenotypes, even among members of the same family, some of whom may never develop symptoms.[5,9,11,12] Although this variability argues for the influence of genetic and/or environmental disease modifiers, only a few genetic modifiers have been suggested,[13] with significant variability remaining to be explained. For example, as discussed by Shannon Conley, PhD, MPH, of the University of Oklahoma Health Science Center, Oklahoma City, Oklahoma, in some instances, *ROM1*/*Rom1* has been shown to play a role in regulating phenotypic heterogeneity in *PRPH2*-associated disease, whereas, in other cases, its contribution has been excluded.[13–16]

Shyamanga Borooah, MBBS, PhD, of the Shiley Eye Institute, who regularly sees patients with *PRPH2*-associated disease in his IRD clinic, discussed the importance of various retina imaging techniques, including optical coherence tomography (OCT) and microperimetry, in tracking disease progression, providing visual examples of each. *ABCA4*-associated Stargardt disease and *PRPH2*-associated retinopathy look very similar when viewed via fundus autofluorescence (FAF) imaging, and it can be difficult for clinicians to distinguish between the two diseases.[5] Rachael Heath Jeffery, MChD, MPH, from the Royal Victorian Eye and Ear Hospital in Melbourne, Australia, and her team used OCT to compare the outer retinal bands in people diagnosed with *ABCA4*-associated Stargardt

disease with those in people diagnosed with *PRPH2*-associated disease.[17] The integrity of the outer retinal bands correlated with visual function, and calculating the ratio of band 2 to band 4 thickness allowed them to determine whether vision loss was caused by mutations in *ABCA4* or *PRPH2*. Ultimately, the band 2–band 4 ratio may have clinical utility in discriminating overlapping *PRPH2*- from *ABCA4*-associated disease, which could aid in diagnosis in cases where genetic testing is not available.[17]

Longitudinal studies using the imaging techniques described by Dr. Borooah are needed to identify reliable, objective markers of disease progression for clinical trials. Measurements for clinical trials of *PRPH2*-associated disease treatments may include patient-reported outcomes (PROs), which are subjective measurements of visual function, and measurements of structural changes, which are objective measurements. Fred Chen, MBBS, PhD, from the Lions Eye Institute in Nedlands, Australia, presented the results of a longitudinal study (mean follow-up = 4.7 years) that collected retrospective and prospective data on retinal function and structural changes to track the rate of *PRPH2*-associated disease progression in 12 individuals.[18] Outcome measures in the study included BCVA, total macular volume (TMV), mean macular sensitivity (MMS), FAF-derived total lesion size (TLS), and decreased autofluorescence area. The study found that BCVA and MMS were unreliable as end points for *PRPH2*-associated retinal diseases. This finding is significant, given that a change in BCVA of ≥15 letters from baseline is one of the few end points approved by the FDA for evaluating the clinical benefit of therapies for retinal disorders,[19] and supports the need for additional end points specific to *PRPH2*-associated disease and for IRDs in general. Although the study found a mean TLS expansion rate, which provides an estimate of how long it would take before a patient starts to lose central vision, of 0.16 mm in radius per year, the investigators suggested the change rate was likely too slow to be of use as an outcome measure in shorter duration (1–2 years) clinical trials. However, the investigators found that TMV, an indication of the number of cells remaining in the retina that is derived by summing the OCT-measured thicknesses across several retina slices, declined markedly over the study (–0.071 mm^3/year) and at a rated that exceeded that of healthy controls. Therefore, TMV may be an effective objective clinical endpoint for measuring *PRPH2*-associated disease progression.[18] Despite these findings, additional studies that include larger, more diverse populations are needed to fully establish the natural history of the multiple retinal diseases caused by mutations in *PRPH2* and to identify robust and reproducible measures of disease progression for use in future interventional clinical trials.

PRPH2 Biology

Muna Naash, PhD, from the University of Houston, Houston, Texas, and the keynote speaker for the meeting, provided an overview of the role of PRPH2 in retinal health and disease. The *PRPH2* gene is found on chromosome 6, encodes a 346–amino acid protein, and is essential for the formation and structure of both rod and cone OSs.[5,20,21] The PRPH2 protein is a member of the tetraspanin family of proteins and localizes in the rim area of the photoreceptor OS, where light signals are received and converted to an electrical signal. PRPH2 has 4 transmembrane domains, 2 conserved extracellular matrix loops (D1 and D2), and conserved cysteines that participate in intramolecular and intermolecular disulfide bonds.[5,21,22] The C-terminal region of PRPH2 is responsible for OS targeting, membrane fusion, and membrane curvature, but approximately 70% of disease-causing mutations are found in the D2 loop, a region of the protein important for mediating higher order protein–protein interactions.[2,5,23,24] *PRPH2* is haploinsufficient, and photoreceptors are sensitive to the total amount of functional PRPH2 present.[25] In mice lacking functional *Prph2*, rod OSs fail to form; in contrast, cone OSs still form, albeit abnormally, arguing for different requirements for PRPH2 in rods and cones.[26] When one copy of *Prph2* is present but the other copy is either nonfunctional or dysfunctional, OSs of both rods and cones are shorter and disorganized.[25]

James Birtley, PhD, of Epsilogen Ltd., London, United Kingdom, discussed a recent publication by El Mazouni and colleagues[2] deciphering the atomic structure of PRPH2 and how visualization of every amino acid within the protein can provide insights into the role that mutations play in disease pathology. He described potential mechanisms by which common *PRPH2* mutations may impact protein stability, shape, and/or function. For example, loss of disulfide bonds in p. Cys165Tyr and p. Cys213Tyr mutations likely lead to PRPH2 proteins with reduced stability, whereas the p. Arg172Trp mutation may lead to protein aggregation and/or disrupt protein-protein interactions.

Dr. Naash also discussed the roles of PRPH2 homodimers; heterodimers with a related tetraspanin protein, ROM1; and higher order molecular species in disease pathology. The presence and ratios of different PRPH2-PRPH2 and PRPH2-ROM1 complexes are differentially affected by specific mutations in *PRPH2* and can lead to different cellular phenotypes. For

example, the *PRPH2*-Tyr141Cys (Y141C) mutation in humans has been observed in individuals affected by a variety of pattern dystrophies (including BPD and AOFMD) characterized by accumulation of lipofuscin in the retinal pigment epithelium (RPE). Clinical findings in these individuals include hypofluorescent or hyperfluorescent spots in the macula, yellow deposits at the macula, reduced visual acuity, and choroidal neovascularization and leakage.[27,28] Data from mouse models indicate *ROM1* could act as a disease modifier for the Y141C mutation, contributing to the variability in *PRPH2*-associated disease phenotypes. When *Rom1* was eliminated from mice with a *Prph2*-Y141C background, the disease phenotype shifted from a pattern dystrophy to RP.[29]

Vadim Arshavsky, PhD, of the Duke University Eye Center in Durham, North Carolina, discussed the role of PRPH2 in regulating OS structure. Photoreceptor OSs are modified cilia and share many biological processes with primary cilia, including the production of ectosomes, which are bioactive vesicles released from the membrane. In photoreceptors, PRPH2 suppresses release of these ectosomes, enabling their retention and incorporation into the flattened discs comprising the OS. When PRPH2 is defective or missing, these discs cannot properly form in the photoreceptor OS, resulting in a loss of OS structure.[30] Ectosome retention is mediated by the C-terminus of PRPH2 whereas the tetraspanin PRPH2 core also supports the structure of disc edges, which may help to explain the variability of disease phenotypes associated with different PRPH2 mutations.[30]

Models for PRPH2 Biology and Pathophysiology

Animal models are powerful tools to understand normal gene/protein function and disease pathophysiology and to test potential novel therapies. Numerous mouse models, including the spontaneous, *Prph2*^{Rd2} mouse (historically known as the *rds* mouse) and multiple mutation knock-in models , have provided valuable information.[31–35] Currently, no large animal models (e.g. dog, pig, and nonhuman primate) of *PRPH2*-associated disease have been described.

Dr. Conley discussed how the use of human point mutation knock-in mouse lines has enabled her laboratory and others to disentangle the cell-type specific effects of *PRPH2* mutations and the role of *ROM1* as a modifier. She described how mice with a *Prph2*-K153del mutation, which is associated with a wide degree of disease variability in humans,[12] experienced improved rod function when *Rom1* expression was reduced. Cone function was not improved in these mice; however, age-related loss of cone function was slowed.[14] Similarly, in a separate model, the removal of *Rom1* from mice with the *Prph2*-Y141C mutation resulted in reduced rod function, but did not compromise cone function, as measured by electroretinography (ERG).[29] However, altering *Rom1* did not alter disease pathophysiology in the context of some other *Prph2* mutations studied in mouse models in her laboratory.[14] From these studies, Dr. Conley concluded that the effects of ROM1 reduction on rod function and structure in the context of different *Prph2* variants differ based whether the result is to stabilize or destabilized Prph2. However, why changing *Rom1* has different effects on different *Prph2* mutations remains a question for further research.

Deepak Lamba, MBBS, PhD, of the University of California, San Francisco, California, detailed a method of using patient-derived induced pluripotent stem cells to create three-dimensional retinal organoids, or "mini retinas," for modeling retinal degeneration. Because human stem cell models develop more slowly than mouse systems, a drawback of this research model is the long-time scales required. As Dr. Lamba explained, "Our eyes take nine months of embryonic development in the womb to form a retina, so it takes that long in the lab to create a mini retina." Retinal organoids can help scientists and clinicians understand why and how a patient develops disease and delineate the effect(s) of their mutation, as well as test therapeutic interventions and refine genome editing approaches.[36]

Ongoing and Potential Therapeutic Approaches

Despite its prevalence, its small genomic size, the existence of animal models, and proof-of-concept studies in mice, no treatments or cures exist for *PRPH2*-associated disease. At the workshop, presenters discussed potential paths forward for developing gene-specific and -agnostic *PRPH2*-associated disease therapies, including potentially applicable on-going clinical trials for gene-agnostic approaches.

Gene-Specific Approaches

Angela Bowman, PhD, of the Foundation Fighting Blindness, reviewed how the different types and impact of variants associated with autosomal dominant disorders like *PRPH2*-associated disease can compli-

cate treatment development. Loss-of-function (LOF) mutations, which often are associated with autosomal recessive disorders, cause cells or proteins to lose the ability to carry out their normal processes, potentially via protein destabilization or mislocalization. However, autosomal dominant diseases often are caused by gain-of-function (GOF) mutations, which result in more heterogenous effects on proteins than LOF variants.[37] Although a systematic interrogation of the functional consequences of all *PRPH2* mutations has not been conducted, it has been shown that mutations in *PRPH2* can act either via LOF or GOF mechanisms, requiring different therapeutic approaches.

Strategies for treating disease arising from LOF *PRPH2* mutations generally involve delivering a wild-type (WT) *PRPH2* gene copy into the eye via a viral vector or nonviral particles.[38] Proof-of-concept studies in disease models have demonstrated some correction of retinal structure and function is possible following *Prph2* gene augmentation.[38–41] These studies also emphasized the sensitivity of photoreceptors to the amount of PRPH2 protein present: a dose-dependent response was seen relative to the amount of WT protein produced, although even achieving 80% of WT protein levels in mice was not sufficient to fully rescue all degeneration.[39] *PRPH2* is relatively small (approximately 1.1 kb), and thus able to easily fit into adeno-associated viral (AAV) vectors, a commonly used delivery vehicle for gene augmentation therapy.[38] A study using recombinant AAV as a delivery vehicle for gene-replacement therapy in a mouse model of LOF *Prph2*-associated disease found some improvements in ERG amplitudes and OS structure, but no reduction in the rate of photoreceptor cell loss.[38,41]

Dr. Naash discussed developing gene replacement therapies for the treatment of *PRPH2*-associated retinal diseases that do not use viral vectors, including engineered DNA vectors and DNA nanoparticles, which can be delivered via intravitreal injection. Key challenges of intravitreal delivery, including dilution in the vitreous, therapy degradation, and lack of retinal penetration, potentially can be addressed by encapsulating therapeutic material in hyaluronic acid–fabricated nanospheres (HA-NS) to promote protection, pan-retinal distribution, and controlled release and/or by codelivery of sulfotyrosine to improve retinal penetration through the inner limiting membrane (ILM). She and her colleagues have shown that intravitreal co-injection of sulfotyrosine and HA-NS allows nanoparticles to pass the ILM and accumulate between the photoreceptors and the RPE with minimal dilution and little-to-no toxicity.[42] She also mentioned the potential for a nanowafer-based, noninvasive, controlled gene delivery platform developed by Acharya and colleagues[43] to deliver genomic elements that can enhance gene expression and allow them to persist as long as possible in cells without becoming integrated, potentially via HA-NS.

In contrast to disorders arising from LOF variants, treating diseases arising from GOF mutations is more involved. Ash Jayagopal, PhD, from Opus Genetics in Durham, North Carolina, discussed a theoretical "knockdown and replace" strategy for disease arising from *PRPH2* GOF mutations, a 2-step process that (1) eliminates the mutant *PRPH2* allele and then (2) reintroduces a WT copy of *PRPH2* to overcome *PRPH2* haploinsufficiency,[44] that could be implemented in the future. He described his company's unique approach to developing therapeutics for IRDs as "a lean, efficient machine to quickly develop and manufacture gene therapies for inherited retinal diseases." Although the company currently is not developing a therapy for *PRPH2*-associated disease, Opus has partnered with the Foundation Fighting Blindness to access appropriate patient registries and scientific and clinical networks to help facilitate clinical trials. Rob Collin, PhD, of Radboud University Medical Center in Nijmegen, The Netherlands, discussed an antisense oligonucleotide (ASO) approach to treating mutations in *PRPH2* that, when paired with gene augmentation, also could be a promising approach for developing GOF mutation-specific therapies. Short, synthetic, single-stranded oligonucleotides, ASOs can bind to RNA and reduce, restore, or modify protein expression.[45] Dr. Collins' team was able to achieve ASO-directed ribonuclease H-mediated degradation of *PRPH2*-R142W mutant RNA, while leaving the WT allele unaffected (unpublished data).

Finally, Peter Quinn, PhD, from Columbia University, New York, New York, discussed how his laboratory develops prime editing strategies to treat IRDs resulting from GOF and LOF variants by precisely correcting mutations at the DNA level. As Dr. Quinn described it, "Prime editing is like a word processor with a search and replace function that can swap one set of DNA letters for another." Prime editing is highly flexible, allowing many different types of DNA letter swaps, can work anywhere in the genome, and is highly efficient.[46,47] A recent study using a mouse model of *Pde6b*-associated RP showed that prime editing via AAV delivery can correct some aspects of structure and function in the mouse retina.[48] Dr. Quinn and his colleagues are currently working to correct *PRPH2* mutations via prime editing in retinal organoids (Quinn PMJ, et al. IOVS 2023;64:ARVO E-Abstract 3860).

Gene-Agnostic Approaches

Gene-agnostic therapeutic strategies for IRDs, which could be applicable for developing interventions to treat *PRPH2* regardless of whether the mutation type is LOF or GOF, include cell replacement, which involves transplanting exogenously derived cells into the eye to replace those that have been lost or are dysfunctional; neuroprotection, which can prevent photoreceptor cell death; and optogenetics, which endows non-degenerating cells that are light insensitive with light sensitivity.[49]

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.[49,50] Francois Paquet-Durand, PhD, of the Eberhard Karl University of Tübingen in Tübingen, Germany, discussed a form of neuroprotection involving inhibiting cyclic guanosine-monophosphate (cGMP)-dependent protein kinase G (PKG) to delay mouse photoreceptor degeneration and preserve retinal function.[50] cGMP is a signaling molecule used by most cells in the human body and is particularly important for regulating phototransduction. Accumulation of cGMP and/or overactivation of the cGMP effector PKG can cause photoreceptor cell death.[50] The Drugs for Retinal Degeneration project that Dr. Paquet-Durand leads focuses on the development of cGMP analogs that will inhibit cGMP signaling. Dr. Paquet-Durand described how, in mouse models of different IRDs, two cGMP analogs developed by the project inhibited PKG to protect photoreceptors, preserving their viability and function, regardless of causative gene, with potential applicability for treating *PRPH2*-associated disease.[50,51] Additional work is needed to further develop these molecules for deployment in clinical trials.

Daniel Chung, DO, from SparingVision in Paris, France, discussed two potential IRD therapies based on the concept of neuroprotection in development by his company: (1) rod-derived cone viability factor (RdCVF)–based therapy and (2) g-protein inwardly rectifying potassium channel (GIRK) gene therapy, which both target mid-stage retinal disease. Healthy rod photoreceptors secrete RdCVF. When they degenerate, as they do in an individual with RP, cone photoreceptors undergo secondary degeneration.[52] Thus, providing exogenous RdCVF may help preserve cones to maintain central vision for as long as possible. A clinical trial for this strategy is recruiting and treating participants with genetic variants in the rhodopsin and phosphodiesterase β and α genes. Although this trial currently is not open to patients with *PRPH2*-associated disease, the company hopes to enroll more genotypes in later clinical trials. In a second program, Dr. Chung and his team are planning to use gene therapy to deliver GIRK, a channel protein that plays a critical role in maintaining extracellular potassium concentrations, to dormant cones that have stopped functioning but are still present in the retina. This approach would allow for phototransduction in otherwise non-functional cones. The method has shown promise in mouse models of RP,[53] and Dr. Chung and his team hope to start clinical trials of this therapy within a year.

Aaron Osborne, MD, MBBS, of Nanoscope Therapeutics, Inc., Dallas, Texas, discussed developing novel optogenetic therapies for vision restoration. Optogenetic therapy is not gene- or mutation-specific and can potentially work in individuals in whom photoreceptors have already degenerated by converting healthy retinal cells into photosensitive cells.[54] The company's lead candidate is MCO-010, an AAV2-delivered multicharacteristic opsin that codes for a light sensitive ion channel and is delivered by intravitreal injection to bipolar cells. Results from phase I/II and phase IIb studies in participants with RP have been promising. In the phase IIb study, participants experienced improvements in vision-guided mobility and other vision function tests, and MCO-010 demonstrated a favorable safety profile (Boyer DS, et al. IOVS 2023;64:ARVO E-Abstract 5443).

Gaps and Next Steps

The presentations and discussions at the workshop brought into focus several gaps and challenges for therapeutic development for *PRPH2*-associated diseases, including:

- Gaining a more complete understanding of fundamental PRPH2 biology, including identifying additional binding partners, and conducting studies to probe the differential function of PRPH2 in rod and cone photoreceptors,
- Creating and characterizing additional cellular and animal models to probe the functional consequences of *PRPH2* mutations and to test therapies,
- Further exploring the factors contributing to *PRPH2*-associated disease heterogeneity and elucidating genotype-phenotype correlations,

- Conducting a large (>100 participants), prospective natural history study for *PRPH2*-associated diseases to facilitate interventional clinical trial design,
- Leveraging knowledge gained to date to explore therapeutic strategies specifically for *PRPH2*-associated diseases.

To begin to address these gaps, Amy Laster, PhD, of the Foundation Fighting Blindness concluded the workshop by announcing a newly created funding program specifically targeted at supporting research for *PRPH2*-associated retinal diseases. This program, which is a collaboration between the Foundation Fighting Blindness and the Nixon Visions Foundation, will award six 3-year awards over 3 years, with the first awards planned for 2024.

Summary

Although there are no approved therapies and no companies actively developing treatments specific for *PRPH2*-associated diseases, the presentations and discussions at this workshop suggest promise for the future. Researchers have made significant progress toward addressing the challenges inherent with developing treatments, work continues toward determining gene-specific and gene-agnostic approaches, and strategies being explored for other IRDs may be applicable to forms of *PRPH2*-associated disease. Because *PRPH2*-associated IRDs are rare, the amount of collaboration among stakeholders likely will correlate with how much time it will take for treatment research and development efforts to be successful. Initiatives like this workshop are important to facilitate communication among affected individuals and their families and clinicians, researchers, and industry representatives, all of whom work in support of achieving the shared goal of treating and curing *PRPH2*-associated diseases.

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