Genetic Treatment for autosomal dominant inherited retinal dystrophies: approaches, challenges, and targeted genotypes

Malena Daich Varela,[1,2] Tassos Georgiadis,[3] Michel Michaelides[1,2]

1 UCL Institute of Ophthalmology, University College London, London, United Kingdom.

2 Moorfields Eye Hospital, London, United Kingdom.

3 MeiraGTx Ltd.

Corresponding author:

Professor Michel Michaelides,

UCL Institute of Ophthalmology, 11-43 Bath St, EC1V 9EL. London, United Kingdom.

michel.michaelides@ucl.ac.uk

Tel: 0207 608 6864. Fax: 0207 608 6903.

Word count: 3447 words

Abstract

Inherited retinal diseases (IRD) have been in the frontline of gene therapy development for the last decade, providing a useful platform to test novel therapeutic approaches. More than 40 clinical trials have been completed or are on-going, tackling autosomal recessive and X-linked conditions, mostly through adeno-associated viral (AAV) vector delivery of a normal copy of the disease-causing gene. However, only recently has autosomal dominant disease (ad/AD) been targeted, with the commencement of a trial for rhodopsin (*RHO*) associated retinitis pigmentosa (RP), implementing antisense oligonucleotide (AON) therapy, with promising preliminary results (NCT04123626).

Autosomal dominant RP represents 15 to 25% of all RP, with *RHO* accounting for 20-30% of these cases. Autosomal dominant macular and cone-rod dystrophies (MD/CRD) correspond to approximately 7.5% of all IRDs, and approximately 35% of all MD/CRD cases, with the main causative gene being *BEST1*. Autosomal dominant IRDs are not only less frequent than recessive, but also tend to be less severe and later onset; e.g. an individual with *RHO*-adRP typically would be severely visually impaired at an age 2 to 3 times older than in X-linked *RPGR*-RP.

Gain-of-function and dominant negative aetiologies are frequently seen in the prevalent adRP genes *RHO*, *RP1*, and *PRPF31* among others, which would not be effectively addressed by gene supplementation alone and need creative, novel approaches. Zinc fingers, RNA interference, AON, translational read-through therapy, and gene editing by CRISPR/Cas are some of the strategies that are currently under investigation and will be discussed herein.

Introduction

The complex group of inherited retinal dystrophies (IRDs) has been under the spotlight for the last two decades.[1,2] The accessible ocular anatomy, relative immune privilege, lack of photoreceptor mitosis, state-of-the-art instruments to evaluate the retina, nearly-exclusive monogenic aetiology, and small volume of the eye, have made IRDs a promising field for the development of cutting-edge gene therapies.

Autosomal recessive and X-linked IRDs have been the main therapeutic target, with gene supplementation being the leading technique.[3] Over 40 clinical trials have been completed or are on-going, using mostly adeno-associated viral (AAV) vectors to supply a normal copy of the disease-causing gene and create a normal, fully functioning protein. In 2019, the first gene-specific nucleic acid therapeutic approach phase 1/2 trial for an autosomal dominant (ad/AD) IRD started, recruiting individuals with *RHO* P23H-related retinitis pigmentosa (RP; NCT04123626). Preliminary results of improvements in best corrected visual acuity (BCVA) and retinal sensitivity are promising (https://www.progr.com/files/2021-11/Analyst-Event-2021_FOR-DOWNLOAD_OK.pdf). It is anticipated that this will be the first of a new wave of clinical trials for the large unmet need of treatments for AD IRDs.

Herein, we discuss the current clinical and preclinical landscape of the therapeutic approaches for ad-IRD, and prioritise the most investigated genotypes and most likely to be translated to clinical trial(s).

Dominant IRD and potential therapeutic approaches

Autosomal dominant RP accounts for approximately 15 to 22% of all RP.[4–6] The most common causative gene is rhodopsin (*RHO*), found in 20-30% of cases.[7–9] The missense p.(P23H) is the most common variant, as well as the first point mutation identified to cause adRP in humans.[7,10] Rhodopsin is followed in frequency by *PRPF31* (8-10%),[11] *RP1* (8-

10%),[8,12] *PRPH2* (10%),[13] *IMPDH1* (5-10%),[14] *NR2E3* (1-3.5%, with p.(G56R) being the second most commonly associated variant with adRP),[15] *SNRNP200* (1.5-2.3%),[9,16] and *CRX* (1%).[9,16]

AD macular and cone-rod dystrophies (MD/CRD) account for approximately 7.5% of IRD, and 34% of MD/CRD cases in total.[17,18] The main causative gene is *BEST1* (3.5%), followed by *PRPH2* (2%), and then *EFEMP1*, *TIMP3*, *GUCA1A*, *GUCY2D*, *PRDM13*, *ELOVL4* and *PROM1*, each with less than 1% frequency.[17]

Dominant conditions are not only less frequent than recessive, but also tend to be less severe. Individuals with *RHO*-related RP are reported to reach legal blindness at a mean age of 79 years old.[19] Whilst in patients with recessive *USH2A*-RP, this occurs at a median of 58 years old,[20] and in X-linked *RPGR*-RP, by the third to fourth decade of life.[21] Patients with Best disease (*BEST1*) can maintain good BCVA over time, often showing no significant differences between baseline and follow up acuity in longitudinal studies.[22,23] On the other hand, individuals with recessive *ABCA4*-related Stargardt disease, often lose three or more ETDRS lines over 10 years.[24]

A challenge that AD conditions face is that haploinsufficiency is rarely their mechanism of disease. Gain-of-function and dominant negative aetiologies are frequently seen in the most prevalent AD genes: *RHO*, *RP1*, and *PRPF31*, among others.[25] These cannot be treated by gene supplementation alone and need creative, novel approaches that are in the early stages of first in man testing (Table 1). These methodologies (Figure 1) include:

- Zinc fingers (ZFs), are proteins that bind promoters and function as artificial transcription factors, enhancing/supressing transcription;[26]
- Antisense oligonucleotides (AONs), single-stranded RNA or DNA molecules that bind pre-mRNA or mRNA, and alter its splicing, and/or block translation;[27]
- RNA interference (RNAi), a naturally occurring pathway that identifies viral RNA and prevents their translation through: (i) short-interfering RNA (siRNA), highly selective double-stranded complex that binds and cleaves mRNA;

(ii) microRNA (miRNA), single-stranded RNA molecules that commonly bind to the 3' untranslated region and block mRNA translation;[27] and (iii) short-hairpin RNA (shRNA), double-stranded RNA sequences linked by a short loop, capable of DNA integration, are subsequently transformed into siRNA in the cytosol.[28]

- Translational read-through therapy, is an approach applicable for nonsense point mutations where drugs bind to ribosomes and force translation beyond the erroneous stop codon, leading to a full-length protein;[29,30]
- And CRISPR (clustered regularly interspaced short palindromic repeats)/Cas genome editing system, correcting disease-causing variants in native alleles.[31]

Prioritised Disease-Causing Genes

RHO

RHO encodes rhodopsin, a G protein–coupled receptor located in the disc membrane of rod outer segments, which is the first component of the phototransduction cascade.[32] *RHO*-related retinopathy is common, with a well understood molecular basis. It can be classified either according to the genotype or phenotype. Sung *et al*. divided the causative variants into two classes according to their biochemical properties: Class I - accumulating in the plasma membrane and resembling the wild-type regarding regeneration of 11-cis-retinal; and Class II - with variable regeneration of the chromophore and accumulation in the endoplasmic reticulum.[33] Cideciyan *et al*. classified on the basis of disease severity, where class A presents with severe, widespread loss of rods, and class B corresponds to sector RP, often involving the inferior retina.[34] Genotype-phenotype correlations have been attempted, with a relationship observed between rhodopsin destabilization and phenotype severity. However, disease often presents with markedly variable severity, even within families, indicating possible epigenetic interactions.[35,36]

RHO-adRP is characterized by a slow rate of progression (particularly Class B), posing a challenge when determining clinical endpoints. It has been suggested that a vertical foveal photoreceptor and retinal pigment epithelium (RPE) band thickness and ellipsoid zone (EZ) width may be possible outcome measures,[37] as well as the hyperautofluorescent ring diameter seen on short wavelength-autofluorescence (SW-AF).[37–39]

The most common disease-causing variants in *RHO* are gain-of-function and have a dominant negative effect.[40,41] This means that the defective protein is retained intracellularly, inducing the unfolded protein response and the degradation of both the abnormal and wild-type protein. Animal models resembling the human disease have been successfully achieved in mice,[42,43] setting the basis for testing novel preclinical therapeutic approaches.[44] A natural history study for *RHO*-RP is currently active and taking place in USA and France (NCT04285398, Table 2).

Many techniques have been explored to treat *RHO*-retinopathy. Price *et al*. have used the somewhat classical technique of AAV-associated gene supplementation in P23H mice, and found that the retinal degeneration persisted, suggesting that excessive amounts of rhodopsin alone cannot rescue photoreceptors.[25,45] AAV-delivered ZFs have also been employed, targeting the *RHO* promoter, and were associated with mutation-unspecific decreased translation and improved disease in a mouse model.[26] Another method to interfere with promoter function that has been tested is through AAV–mediated ectopic expression of a transcription factor capable of silencing *RHO* (KLF15), with structural and functional protection observed in mouse models.[46]

Post-transcriptional protein knockdown has also been attempted through hammerhead and hairpin ribozymes designed to target and cleave P23H, with good specificity in vitro.[47] In addition, a dual-approach to both suppress the mutated gene and supplement a wild type gene is being actively developed. Suppression has been implemented via RNA silencing (e.g. RNAi and siRNA)[48–50] and CRISPR/Cas9 [51], combined with gene supplementation (RNAi-resistant where applicable), leading to visual function improvement in mouse models. This was assessed by electrophysiology, where rod-isolated

responses improved significantly post-treatment, and by histology, with preservation of the outer nuclear layer (ONL) and the outer segments of photoreceptors.[48–51] Different groups have tried allele specific CRISPR/Cas9 editing alone, with VA and retinal function improvement in Rho^{S334} and $Rho^{+/P23H}$ mouse models.[52–54] RNA knockdown alone has also shown significant improvement in retinal function and structure in P23H rats and mice.[55] The latter has led to a phase I/II clinical trial of AON and targets patients with P23H *RHO*-RP (NCT04123626), with favourable preliminary results.

Translational read-through drugs have also been tested in *RHO* S334ter rat models, with an increased number of surviving photoreceptors and improved electroretinography (ERG) recordings.[56] Gregory-Evans *et al.* tested the use of a read-through drug combined with neuroprotection in the same rats, and found indistinguishable histology from unaffected controls.[57]

Neuroprotection has also been investigated with a subretinal injection of an AAV vector expressing a glial cell line derived neurotrophic factor (GDNF), which was shown to result in preservation of ONL thickness and increased ERG responses in mouse models.[58] Lastly, Yao *et al*. found that reducing autophagy in P23H photoreceptors through hydroxychloroquine oral treatment and/or deletion of the autophagic gene *ATG5*, decreased cell death in mice and had a protective effect.[59] This led to another ongoing clinical trial (NCT04120883), which uses oral hydroxychloroquine to alter the autophagy pathway in P23H-*RHO* photoreceptors.

In summary, individuals will likely need detailed genetic characterization to determine the most suitable therapeutic approach. Allele-specific approaches may lead to fewer eligible patients, small cohorts, and conclusions with limited external validity. Nevertheless, the breadth of treatment avenues being explored in *RHO*-retinopathy has resulted in the first on-going dominant IRD gene therapy clinical trial, with several more approaches anticipated to be in early phase trials in the near future.

PRPF31

PRPF31 encodes one of the core components of spliceosomes and has a key function in RNA splicing processes and in modulating alternative splicing.[60,61] Most variants in *PRPF31* are loss-of-function and cause decreased splicing efficiency and mis-splicing.[62] Haploinsufficiency with dominant negative effect has been proposed as the pathogenic mechanism, given the milder presentation in patients with large deletions versus in those with point mutations.[11,63] This gene affects ciliogenesis in the retina,[64] and therefore *PRPF31* could be considered a 'ciliopathy gene'.[65]

Individuals carrying a heterozygous disease-causing variant in *PRPF31* can develop RP, however marked intrafamilial variability and incomplete penetrance is one of the hallmarks of this gene.[66] Age of onset is also highly variable, reported between 6 and 71 years of age.[66] *PRPF31* non-penetrance has been associated with the co-inheritance of a 4-copy MSR1 repeat, with the complete underlying basis of variable expressivity remaining unclear.[67] Genotype-phenotype correlations have been investigated, with an earlier age of onset observed in those with null versus missense variants.[11]

An exponential yearly decline in kinetic visual field, cone ERG responses, and EZ area, has been reported.[66] However, others have identified heterogeneous disease progression.[68]

Mouse models with late-onset RP,[69] and induced pluripotent stem cells (iPSC) have been developed from a patient with *PRPF31*-RP and a related non-penetrant subject, to improve our understanding.[70] The latter have been used to create iPSC-RPE cells *PRPF31*$^{+/-}$ and conduct a proof of concept AAV-mediated gene augmentation. Brydon *et al*. reported a rescue in ciliogenesis, phagocytosis, and cell morphology.[71]

A natural history study for individuals with *PRPF31*-RP and non-penetrant subjects is currently ongoing (NCT04805658, Table 2), which will likely be informative in terms of clinical endpoints. Developing a treatment trial for *PRPF31* will likely require further preclinical work of different approaches that take into account its dominant negative basis, potentially

regulating interacting genes such as *MSR1*, and possibly considering alternative disease models which better recapitulate human disease.

RP1

RP1 protein is located in the connecting cilia of photoreceptors,[72] and is thought to have a role in the stacking of outer segment discs.[73] It can cause adRP and autosomal recessive (ar) RP, early onset severe retinal dystrophy (EOSRD), MD and CORD.[74,75] Genotype-phenotype correlations have been described, where truncations affecting the middle portion of the gene were associated with adRP (Arg677Ter being the third most common adRP variant described), while those in the N- and C-terminals caused arRP.[76,77] adRP1 has a similar phenotype to *RHO*-RP, and also often presents with wide phenotypic variability, with asymptomatic carriers described.[8]

The disease mechanism is reported to be dominant negative, where the truncated RP1 competes with the wild type protein for binding to axonemal microtubules.[78] Mouse models of heterozygous *RP1* damaging variants had half the normal protein concentration, but did not show significant retinal structural or functional abnormality.[78] Gene supplementation has been tested in the aforementioned mouse models and proven successful in biallelic *RP1* disease, but no preclinical work towards treating ad*RP1*-retinopathy, which is by far the most common mode of inheritance, is present in the literature to date.[78]

PRPH2

PRPH2 has great phenotypic variability, being associated with adRP, MD, pattern dystrophy, central areolar choroidal dystrophy, and EOSRD.[75,79–81] Despite the noteworthy inter- and intrafamilial variation and even incomplete penetrance,

genotype-phenotype correlations have been developed, where Arg142Trp and Arg172Trp generally result in MD, and variants between Pro210 and Pro216, in adRP.[82] Patients with pattern dystrophy tend to remain asymptomatic until the fifth decade of life, while the majority of individuals with adRP have symptoms between the third and fifth decade.[82]

PRPH2 encodes a tetraspanin transmembrane protein, key in the formation and stabilization of outer segment discs.[83] Homozygous and heterozygous mouse models have been developed, with similar phenotype to their human counterparts.[84] No outer segments were noticed in *Prph2*$^{-/-}$ mice,[85] while disorganised yet present discs were found in *Prph2*$^{+/-}$, suggesting a dose-dependent variation in phenotypic expression.[86] Loss-of-function,[87] dominant negative,[88] a combination of the two,[89] and gain-of-function have been described as the pathophysiology of *PRPH2*-associated diseases.[90] However, it is thought that rod-dominant RP generally occurs due to haploinsufficiency, while cone-dominant MD and pattern dystrophy are secondary to dominant-negative effect.[91]

Nour *et al*. had good structural results when supplementing a wild-type copy of *PRPH2* in a loss-of-function transgenic mouse model of RP, but failed in a gain-of-function, CORD model.[92] Compacted DNA nanoparticles (NP) injection caused sustained gene expression, and long term, yet circumscribed, structural and functional improvement in a heterozygote mouse model.[93] Although over-expressing *PRPH2* appears to be well tolerated by the retina,[94] complete, widespread, longstanding rescue has not been accomplished thus far through gene supplementation alone.[91]

Subretinal injections of siRNA and siRNA-resistant *PRPH2* has shown efficacy in a mouse model and mouse retinal explants, with preserved ERG responses and decreased Prph2 mRNA and protein expression, becoming a promising mutation-independent approach for this gene.[95,96] Georgiadis *et al*. also used AAV-mediated subretinal injections of miRNA-adapted shRNA in mice, finding silencing of *PRPH2* as early as three weeks post-injection.[97] AAV was also used to deliver ciliary neurotrophic factor into the subretinal space, showing long-term rescue of photoreceptors, however with panretinal rod photoreceptor nuclear changes that require further investigation.[98,99]

PRPH2 has well-characterized animal models and a small size (~1.1 kb coding region). However, the large phenotypic variability, the multiple postulated disease mechanisms, and often relatively good prognosis till later adult age, makes therapy development challenging.[91] Gene augmentation could indeed work for loss-of-function alleles, and gene knockdown combined with supplementation may have a positive effect on gain-of-function alleles.

IMPDH1

Disease-associated variants in inosine monophosphate dehydrogenase 1 (*IMPDH1*) are known to cause adRP and, less frequently, EOSRD.[100] *IMPDH1*-RP has been characterised as having a relatively rapid rate of progression, with early decreased VA.[101] Significantly decreased VA and visual fields usually occurs within the second decade of life.[102] The D226N allele accounts for about 1% of all adRP cases,[103] and families with incomplete penetrance have also been reported.[104]

IMPDH proteins form homotetramers and are key in the synthesis of guanine nucleotide, having a direct effect on the intracellular concentration of GMP, GDP, and GTP.[100] Although having ubiquitous expression, *IMPDH1* transcripts have a high concentration in the retina, particularly in the periphery.[105] Alternative splicing-resulting transcripts are also expressed solely in the retina.[105] Given that disease-associated variants in *IMPDH1* cause protein misfolding and aggregation, with preserved enzymatic activity, it is likely that the disease mechanism is due to a dominant-negative effect exerted by the abnormal protein.[106]

Mouse models have been developed through AAV inoculation of the mutant allele. Double knock-out mice models and mice with an additionally inoculated copy of *IMPDH1* displayed only minimal retinopathy, proving that both scenarios are well tolerated.[102,106] Tam *et al*. used an AAV-mediated RNAi suppression strategy in vitro and in vivo (mice), and found

effective and sequence-specific suppression of IMPDH1 mRNA and protein, and preserved retinal structure.[102] It appears that by suppressing both normal and mutant *IMPDH1* alleles, the dominant negative effect exerted by the mutant protein might be abolished and the retinal degeneration slowed. This strategy, with the possible inclusion of an RNAi-resistant *IMPDH1* transgene, holds substantial promise, however characterisation studies are not yet in place and preclinical work still needs to show extensive conclusive data.

BEST1

BEST1 encodes a transmembrane, calcium-activated chloride channel that is located in the RPE.[107] Autosomal dominant disease-associated variants lead to Best Disease (BD) and adult vitelliform macular dystrophy, the latter with a later disease onset.[81,108] These two conditions are characterized by an excess of lipofuscin within the RPE cells and the formation of subretinal vitelliform lesions.[109] *BEST1* can also cause vitreoretinochoroidopathy (ADVIRC) and Bestrophinopathy, both affecting the retina in a broader, more severe fashion.

BD can have a variable age of onset and progression rate, even among family members.[109] Although this phenotypic heterogeneity makes VA prediction challenging, visual impairment occurs mostly in adulthood. The disease-causing mechanisms of BD entail loss-of-function in a dominant-negative manner in most cases, particularly in the alleles associated with the chloride and calcium binding sites.[110,111] Variants linked to the channel gate/neck, outside the neck, and also some at the calcium binding sites, appear to have a gain-of-function mechanism.[111,112]

Animal models for *BEST1*-associated diseases have naturally occurred in dogs (recessive models),[113] and have also been developed in mice (dominant).[114] In vitro models have been generated from patient samples, iPSC-RPE emulating both the ad and ar forms.[115] Different treatment approaches have been tested in these models. Lentivirus- and AAV2-mediated gene augmentation increased wild-type protein transduction and improved retinal detachments both in biallelic

models of BD in vitro and in vivo.[116,117] Lentivirus gene augmentation was also tested in BD in vitro models and the result depended on the variant affected.[117] Arg218Cys and Asn296His were fully responsive, with a functioning calcium channel and preserved voltage, while Ala146Lys did not show any changes. Sinha *et al*. have attempted gene editing through CRISPR-Cas9 in these three heterozygous variants, demonstrating efficient editing and high in vitro allele specificity in all.[117]

BD is certainly an attractive target, with strengths such as a significant prevalence, wide window of opportunity, extensive preclinical data, and multiple approaches showing promise, however, the not insignificant challenges include patient selection given often the relatively good prognosis.

Gene-independent approaches

Novel approaches that could apply to many genes by targeting cellular metabolomics, proteomics, and oxidative stress are currently under development. Although not specific and with possibly dose-dependent toxicity, they could slow down progression until a long-term treatment was administered.[118]

The insulin/mammalian target of rapamycin (mTOR) pathway has been found to be neuroprotective in mouse models.[119–121] Adenosine monophosphate activated protein kinase (AMPK) regulates mTOR and is activated by metformin. Treating mice with metformin has shown a positive effect on photoreceptors, preserving their function and structure, possibly by reducing oxidative stress.[122] Metformin has also been tested in iPSC-derived RPE from patients with late onset retinal dystrophy, alleviating the disease cellular phenotype.[123] A clinical trial of metformin in individuals with *ABCA4*-retinopathy is currently ongoing (NCT04545736).

N-acetylcysteine (NAC), a commonly used mucolytic, also serves as an enhancer of the formation of glutathione, a powerful neuronal antioxidant.[124] An active phase I clinical trial (NCT03999021) is assessing the effects of oral NAC in patients with RP, with promising early results.[125] A phase 3 NAC trial is planned.

Conclusions

RHO-RP is the most advanced ad IRD with respect to potential therapy, with multiple mechanisms tested and various animal models developed. *BEST1* and *PRPF31* are the next likely targets, with extensive pre-clinical data and various approaches under investigation (Table 1). Key factors in the design of an IRD treatment trial include the determination of (i) eligibility criteria, (ii) endpoints for the evaluation of clinical efficacy, (iii) a window of opportunity, and (iv) the suitability of the contralateral eye as the control (symmetry between eyes). The phenotypic heterogeneity and wide range of severity of ad IRD,[126,127] usually not associated with age and often slow progression or relative stability, may thereby be challenging. The ability to predict participants who will have a poor prognosis would be valuable.

Nevertheless, cutting edge techniques are being developed, showing promising results at a cellular level. Dominant-negative mechanisms, in which the abnormal protein competes with the wild-type, are potentially amenable through gene augmentation therapy. Gain-of-function variants, on the other hand, will require gene or RNA editing/knockdown to suppress the mutant allele and prevent toxic protein production.[78] Promising results in mouse models have been seen by delivering these components through AAV vectors or NP.[128]

However, modulation of these silencing therapeutics will be key to their success, aiming for optimal protein concentrations that can lead to photoreceptor survival, also importantly avoiding off target effects.[26] Regulatory agencies closely overseeing the safety of novel therapeutic approaches such as CRISPR-mediated DNA (Cas9) and RNA (CasRx)[129]

editing will be necessary. Gene regulation of certain novel approaches, including the RNA editors, might also aid their safety profile.

Although the development of mutation-specific therapies may not be time or economically efficient at present, personalized medicine is increasingly being championed, and may become more feasible with technological advancements e.g. through faster and cheaper personalized iPSCs.[130] There is no doubt that IRD will continue to be on the frontline of novel therapies for the next decade, with dominant diseases at the heart of these developments.

Legend Figure 1: Disease mechanisms and therapeutic approaches for autosomal dominant inherited retinal dystrophies (IRDs).

A) Normal. We see the normal process of DNA transcription to messenger RNA (mRNA), and then RNA translation to protein. The wild type gene is depicted in yellow and its promoter in darker shade. Normal proteins are seen in pink, two of them bound to their receptor.

B) Loss-of-function (LOF), where the gene is seen in red. In this case, the mRNA transcript is shorter due to a null disease-causing variant prematurely stopping transcription, consequently halting translation and leading to truncated/absent protein. The yellow squares represent the therapeutic approaches under development to treat LOF IRDs, at the location where they have their therapeutic effect. The * corresponds to the only mechanism currently approved to treat LOF *RPE65*-associated retinal dystrophy.

C) Gain-of-function (GOF), with the gene in blue. We see abnormal protein formed (light blue), toxic to the cell, and the yellow squares representing therapeutic avenues.

D) Dominant negative effect (DNE), with a light green gene. In this situation, abnormal proteins (green) compete with the wild type for binding receptors. In yellow, therapeutic mechanisms.

AON: antisense oligonucleotides; CRISPR: clustered regularly interspaced short palindromic repeats.

Contributors: All authors contributed to the design of this review article, literature review, manuscript preparation and review. The authors were responsible for all content and editorial decision.

Declaration of interest: The authors alone are responsible for the content and writing of this article. MM consults for MeiraGTx Ltd and TG is MeiraGTx Ltd staff.

Funding: This work was supported by grants from The Wellcome Trust [099173/Z/12/Z], the National Institute for Health Research Biomedical Research Centre at Moorfields Eye Hospital NHS Foundation Trust and UCL Institute of Ophthalmology, Moorfields Eye Charity, Retina UK and the Foundation Fighting Blindness (no specific grant/award number for the latter). The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health.

1. Daich Varela M, Guimaraes T, Georgiou M, et al. Leber Congenital Amaurosis/Early-Onset Severe Retinal Dystrophy: Current Management and Clinical Trials. *Br J Ophthalmol*. Published online 2021.

2. Bucher K, Rodríguez-Bocanegra E, Dauletbekov D, Fischer MD. Immune responses to retinal gene therapy using adeno-associated viral vectors - Implications for treatment success and safety. *Prog Retin Eye Res*. 2021;83:100915. doi:10.1016/j.preteyeres.2020.100915

3. Kumaran N, Michaelides M, Smith AJ, et al. Retinal gene therapy. *Br Med Bull*. 2018;126(1):13-25. doi:10.1093/bmb/ldy005

4. Boughman JA, Fishman GA. A genetic analysis of retinitis pigmentosa. *Br J Ophthalmol*. 1983;67(7):449-454. doi:10.1136/bjo.67.7.449

5. Coco-Martin RM, Diego-Alonso M, Orduz-Montaña WA, et al. Descriptive Study of a Cohort of 488 Patients with Inherited Retinal Dystrophies. *Clin Ophthalmol*. 2021;15:1075-1084. doi:10.2147/OPTH.S293381

6. Huang L, Zhang Q, Huang X, et al. Mutation screening in genes known to be responsible for Retinitis Pigmentosa in 98 Small Han Chinese Families. *Sci Rep*. 2017;7(1):1948. doi:10.1038/s41598-017-00963-6

7. Sullivan LS, Bowne SJ, Reeves MJ, et al. Prevalence of mutations in eyeGENE probands with a diagnosis of autosomal dominant retinitis pigmentosa. *Invest Ophthalmol Vis Sci*. 2013;54(9):6255-6261. doi:10.1167/iovs.13-12605

8. Berson EL, Grimsby JL, Adams SM, et al. Clinical features and mutations in patients with dominant retinitis pigmentosa-1 (RP1). *Invest Ophthalmol Vis Sci*. 2001;42(10):2217-2224.

9. Daiger SP, Bowne SJ, Sullivan LS. Genes and Mutations Causing Autosomal Dominant Retinitis Pigmentosa. *Cold*

Spring Harb Perspect Med. 2014;5(10). doi:10.1101/cshperspect.a017129

10. Dryja TP, McGee TL, Reichel E, et al. A point mutation of the rhodopsin gene in one form of retinitis pigmentosa. *Nature*. 1990;343(6256):364-366. doi:10.1038/343364a0

11. Wheway G, Douglas A, Baralle D, et al. Mutation spectrum of PRPF31, genotype-phenotype correlation in retinitis pigmentosa, and opportunities for therapy. *Exp Eye Res*. 2020;192:107950. doi:10.1016/j.exer.2020.107950

12. Audo I, Mohand-Saïd S, Dhaenens C-M, et al. RP1 and autosomal dominant rod-cone dystrophy: novel mutations, a review of published variants, and genotype-phenotype correlation. *Hum Mutat*. 2012;33(1):73-80. doi:10.1002/humu.21640

13. Manes G, Guillaumie T, Vos WL, et al. High prevalence of PRPH2 in autosomal dominant retinitis pigmentosa in france and characterization of biochemical and clinical features. *Am J Ophthalmol*. 2015;159(2):302-314. doi:10.1016/j.ajo.2014.10.033

14. Bowne SJ, Sullivan LS, Blanton SH, et al. Mutations in the inosine monophosphate dehydrogenase 1 gene (IMPDH1) cause the RP10 form of autosomal dominant retinitis pigmentosa. *Hum Mol Genet*. 2002;11(5):559-568. doi:10.1093/hmg/11.5.559

15. Blanco-Kelly F, García Hoyos M, Lopez Martinez MA, et al. Dominant Retinitis Pigmentosa, p.Gly56Arg Mutation in NR2E3: Phenotype in a Large Cohort of 24 Cases. *PLoS One*. 2016;11(2):e0149473. doi:10.1371/journal.pone.0149473

16. Martin-Merida I, Aguilera-Garcia D, Jose PF-S, et al. Toward the Mutational Landscape of Autosomal Dominant Retinitis Pigmentosa: A Comprehensive Analysis of 258 Spanish Families. *Invest Ophthalmol Vis Sci*. 2018;59(6):2345-2354. doi:10.1167/iovs.18-23854

17. Stone EM, Andorf JL, Whitmore SS, et al. Clinically Focused Molecular Investigation of 1000 Consecutive Families with Inherited Retinal Disease. *Ophthalmology*. 2017;124(9):1314-1331. doi:10.1016/j.ophtha.2017.04.008

18. Birtel J, Eisenberger T, Gliem M, et al. Clinical and genetic characteristics of 251 consecutive patients with macular and cone/cone-rod dystrophy. *Sci Rep*. 2018;8(1):4824. doi:10.1038/s41598-018-22096-0

19. Nguyen X-T-A, Talib M, van Cauwenbergh C, et al. CLINICAL CHARACTERISTICS AND NATURAL HISTORY OF RHO-ASSOCIATED RETINITIS PIGMENTOSA: A Long-Term Follow-Up Study. *Retina*. 2021;41(1):213-223. doi:10.1097/IAE.0000000000002808

20. Sandberg MA, Rosner B, Weigel-DiFranco C, et al. Disease course in patients with autosomal recessive retinitis pigmentosa due to the USH2A gene. *Invest Ophthalmol Vis Sci*. 2008;49(12):5532-5539. doi:10.1167/iovs.08-2009

21. Flaxel CJ, Jay M, Thiselton DL, et al. Difference between RP2 and RP3 phenotypes in X linked retinitis pigmentosa. *Br J Ophthalmol*. 1999;83(10):1144-1148. doi:10.1136/bjo.83.10.1144

22. Querques G, Zerbib J, Santacroce R, et al. Functional and clinical data of Best vitelliform macular dystrophy patients with mutations in the BEST1 gene. *Mol Vis*. 2009;15:2960.

23. Nowomiejska K, Nasser F, Stingl K, et al. Disease expression caused by different variants in the BEST1 gene: genotype and phenotype findings in bestrophinopathies. *Acta Ophthalmol*. 2021;n/a(n/a). doi:https://doi.org/10.1111/aos.14958

24. Fujinami K, Lois N, Davidson AE, et al. A longitudinal study of stargardt disease: clinical and electrophysiologic assessment, progression, and genotype correlations. *Am J Ophthalmol*. 2013;155(6):1075-1088.e13. doi:10.1016/j.ajo.2013.01.018

25. Diakatou M, Manes G, Bocquet B, et al. Genome Editing as a Treatment for the Most Prevalent Causative Genes of Autosomal Dominant Retinitis Pigmentosa. *Int J Mol Sci*. 2019;20(10). doi:10.3390/ijms20102542

26. Mussolino C, Sanges D, Marrocco E, et al. Zinc-finger-based transcriptional repression of rhodopsin in a model of dominant retinitis pigmentosa. *EMBO Mol Med*. 2011;3(3):118-128. doi:10.1002/emmm.201000119

27. Bajan S, Hutvagner G. RNA-Based Therapeutics: From Antisense Oligonucleotides to miRNAs. *Cells*. 2020;9(1). doi:10.3390/cells9010137

28. Taxman DJ, Moore CB, Guthrie EH, et al. Short Hairpin RNA (shRNA): Design, Delivery, and Assessment of Gene Knockdown BT - RNA Therapeutics: Function, Design, and Delivery. In: Sioud M, ed. Humana Press; 2010:139-156. doi:10.1007/978-1-60761-657-3_10

29. Nagel-Wolfrum K, Möller F, Penner I, et al. Targeting Nonsense Mutations in Diseases with Translational Read-Through-Inducing Drugs (TRIDs). *BioDrugs*. 2016;30(2):49-74. doi:10.1007/s40259-016-0157-6

30. Roy B, Leszyk JD, Mangus DA, Jacobson A. Nonsense suppression by near-cognate tRNAs employs alternative base pairing at codon positions 1 and 3. *Proc Natl Acad Sci U S A*. 2015;112(10):3038-3043. doi:10.1073/pnas.1424127112

31. Cong L, Ran FA, Cox D, et al. Multiplex genome engineering using CRISPR/Cas systems. *Science*. 2013;339(6121):819-823. doi:10.1126/science.1231143

32. Suda K, Filipek S, Palczewski K, et al. The supramolecular structure of the GPCR rhodopsin in solution and native disc membranes. *Mol Membr Biol*. 2004;21(6):435-446. doi:10.1080/09687860400020291

33. Sung CH, Davenport CM, Nathans J. Rhodopsin mutations responsible for autosomal dominant retinitis

pigmentosa. Clustering of functional classes along the polypeptide chain. *J Biol Chem*. 1993;268(35):26645-26649.

34. Cideciyan A V, Hood DC, Huang Y, et al. Disease sequence from mutant rhodopsin allele to rod and cone photoreceptor degeneration in man. *Proc Natl Acad Sci U S A*. 1998;95(12):7103-7108. doi:10.1073/pnas.95.12.7103

35. McKeone R, Wikstrom M, Kiel C, et al. Assessing the correlation between mutant rhodopsin stability and the severity of retinitis pigmentosa. *Mol Vis*. 2014;20:183-199.

36. Gal A, Apfelstedt-Sylla E, Janecke AR, et al. Rhodopsin mutations in inherited retinal dystrophies and dysfunctions. *Prog Retin Eye Res*. 1997;16(1):51-79.

37. Sumaroka A, Cideciyan A V, Charng J, et al. Autosomal Dominant Retinitis Pigmentosa Due to Class B Rhodopsin Mutations: An Objective Outcome for Future Treatment Trials. *Int J Mol Sci*. 2019;20(21). doi:10.3390/ijms20215344

38. Takahashi VKL, Takiuti JT, Carvalho-Jr JRL, et al. Fundus autofluorescence and ellipsoid zone (EZ) line width can be an outcome measurement in RHO-associated autosomal dominant retinitis pigmentosa. *Graefe's Arch Clin Exp Ophthalmol = Albr von Graefes Arch fur Klin und Exp Ophthalmol*. 2019;257(4):725-731. doi:10.1007/s00417-018-04234-6

39. Jacobson SG, McGuigan DB 3rd, Sumaroka A, et al. Complexity of the Class B Phenotype in Autosomal Dominant Retinitis Pigmentosa Due to Rhodopsin Mutations. *Invest Ophthalmol Vis Sci*. 2016;57(11):4847-4858. doi:10.1167/iovs.16-19890

40. Mendes HF, van der Spuy J, Chapple JP, et al. Mechanisms of cell death in rhodopsin retinitis pigmentosa: implications for therapy. *Trends Mol Med*. 2005;11(4):177-185. doi:10.1016/j.molmed.2005.02.007

41. Mendes HF, Cheetham ME. Pharmacological manipulation of gain-of-function and dominant-negative mechanisms in rhodopsin retinitis pigmentosa. *Hum Mol Genet*. 2008;17(19):3043-3054. doi:10.1093/hmg/ddn202

42. Sakami S, Kolesnikov A V, Kefalov VJ, et al. P23H opsin knock-in mice reveal a novel step in retinal rod disc morphogenesis. *Hum Mol Genet*. 2014;23(7):1723-1741. doi:10.1093/hmg/ddt561

43. Sancho-Pelluz J, Tosi J, Hsu C-W, et al. Mice with a D190N mutation in the gene encoding rhodopsin: a model for human autosomal-dominant retinitis pigmentosa. *Mol Med*. 2012;18(1):549-555. doi:10.2119/molmed.2011.00475

44. Massengill MT, Lewin AS. Gene Therapy for Rhodopsin-associated Autosomal Dominant Retinitis Pigmentosa. *Int Ophthalmol Clin*. 2021;61(4):79-96. doi:10.1097/IIO.0000000000000383

45. Price BA, Sandoval IM, Chan F, et al. Rhodopsin gene expression determines rod outer segment size and rod cell resistance to a dominant-negative neurodegeneration mutant. *PLoS One*. 2012;7(11):e49889. doi:10.1371/journal.pone.0049889

46. Botta S, de Prisco N, Marrocco E, et al. Targeting and silencing of rhodopsin by ectopic expression of the transcription factor KLF15. *JCI insight*. 2017;2(24). doi:10.1172/jci.insight.96560

47. Chakraborty D, Whalen P, Lewin AS, et al. In vitro analysis of ribozyme-mediated knockdown of an ADRP associated rhodopsin mutation. *Adv Exp Med Biol*. 2008;613:97-106. doi:10.1007/978-0-387-74904-4_10

48. Millington-Ward S, Chadderton N, O'Reilly M, et al. Suppression and Replacement Gene Therapy for Autosomal Dominant Disease in a Murine Model of Dominant Retinitis Pigmentosa. *Mol Ther*. 2011;19(4):642-649. doi:10.1038/mt.2010.293

49. Mao H, Gorbatyuk MS, Rossmiller B, et al. Long-Term Rescue of Retinal Structure and Function by Rhodopsin RNA

Replacement with a Single Adeno-Associated Viral Vector in P23H RHO Transgenic Mice. *Hum Gene Ther*. 2012;23(4):356-366. doi:10.1089/hum.2011.213

50. Greenwald DL, Cashman SM, Kumar-Singh R. Mutation-independent rescue of a novel mouse model of Retinitis Pigmentosa. *Gene Ther*. 2013;20(4):425-434. doi:10.1038/gt.2012.53

51. Tsai Y-T, Wu W-H, Lee T-T, et al. Clustered Regularly Interspaced Short Palindromic Repeats-Based Genome Surgery for the Treatment of Autosomal Dominant Retinitis Pigmentosa. *Ophthalmology*. 2018;125(9):1421-1430. doi:10.1016/j.ophtha.2018.04.001

52. Bakondi B, Lv W, Lu B, et al. In Vivo CRISPR/Cas9 Gene Editing Corrects Retinal Dystrophy in the S334ter-3 Rat Model of Autosomal Dominant Retinitis Pigmentosa. *Mol Ther*. 2016;24(3):556-563. doi:10.1038/mt.2015.220

53. Giannelli SG, Luoni M, Castoldi V, et al. Cas9/sgRNA selective targeting of the P23H Rhodopsin mutant allele for treating retinitis pigmentosa by intravitreal AAV9.PHP.B-based delivery. *Hum Mol Genet*. 2018;27(5):761-779. doi:10.1093/hmg/ddx438

54. Patrizi C, Llado M, Benati D, et al. Allele-specific editing ameliorates dominant retinitis pigmentosa in a transgenic mouse model. *Am J Hum Genet*. 2021;108(2):295-308. doi:10.1016/j.ajhg.2021.01.006

55. Murray SF, Jazayeri A, Matthes MT, et al. Allele-Specific Inhibition of Rhodopsin With an Antisense Oligonucleotide Slows Photoreceptor Cell Degeneration. *Invest Ophthalmol Vis Sci*. 2015;56(11):6362-6375. doi:10.1167/iovs.15-16400

56. Guerin K, Gregory-Evans CY, Hodges MD, et al. Systemic aminoglycoside treatment in rodent models of retinitis pigmentosa. *Exp Eye Res*. 2008;87(3):197-207. doi:10.1016/j.exer.2008.05.016

57. Gregory-Evans K, Po K, Chang F, et al. Pharmacological enhancement of ex vivo gene therapy neuroprotection in a rodent model of retinal degeneration. *Ophthalmic Res*. 2012;47(1):32-38. doi:10.1159/000325730

58. McGee Sanftner LH, Abel H, Hauswirth WW, et al. Glial cell line derived neurotrophic factor delays photoreceptor degeneration in a transgenic rat model of retinitis pigmentosa. *Mol Ther*. 2001;4(6):622-629. doi:10.1006/mthe.2001.0498

59. Yao J, Qiu Y, Frontera E, et al. Inhibiting autophagy reduces retinal degeneration caused by protein misfolding. *Autophagy*. 2018;14(7):1226-1238. doi:10.1080/15548627.2018.1463121

60. Tanackovic G, Ransijn A, Thibault P, et al. PRPF mutations are associated with generalized defects in spliceosome formation and pre-mRNA splicing in patients with retinitis pigmentosa. *Hum Mol Genet*. 2011;20(11):2116-2130. doi:10.1093/hmg/ddr094

61. Li J, Liu F, Lv Y, et al. Prpf31 is essential for the survival and differentiation of retinal progenitor cells by modulating alternative splicing. *Nucleic Acids Res*. 2021;49(4):2027-2043. doi:10.1093/nar/gkab003

62. Azizzadeh Pormehr L, Ahmadian S, Daftarian N, et al. PRPF31 reduction causes mis-splicing of the phototransduction genes in human organotypic retinal culture. *Eur J Hum Genet*. 2020;28(4):491-498. doi:10.1038/s41431-019-0531-1

63. Valdés-Sánchez L, Calado SM, de la Cerda B, et al. Retinal pigment epithelium degeneration caused by aggregation of PRPF31 and the role of HSP70 family of proteins. *Mol Med*. 2019;26(1):1. doi:10.1186/s10020-019-0124-z

64. Buskin A, Zhu L, Chichagova V, et al. Disrupted alternative splicing for genes implicated in splicing and ciliogenesis causes PRPF31 retinitis pigmentosa. *Nat Commun*. 2018;9(1):4234. doi:10.1038/s41467-018-06448-y

65. Wheway G, Schmidts M, Mans DA, et al. An siRNA-based functional genomics screen for the identification of regulators of ciliogenesis and ciliopathy genes. *Nat Cell Biol*. 2015;17(8):1074-1087. doi:10.1038/ncb3201

66. Kiser K, Webb-Jones KD, Bowne SJ, et al. Time Course of Disease Progression of PRPF31-mediated Retinitis Pigmentosa. *Am J Ophthalmol*. 2019;200:76-84. doi:10.1016/j.ajo.2018.12.009

67. McLenachan S, Zhang D, Grainok J, et al. Determinants of Disease Penetrance in PRPF31-Associated Retinopathy. *Genes (Basel)*. 2021;12(10). doi:10.3390/genes12101542

68. Hafler BP, Comander J, Weigel DiFranco C, et al. Course of Ocular Function in PRPF31 Retinitis Pigmentosa. *Semin Ophthalmol*. 2016;31(1-2):49-52. doi:10.3109/08820538.2015.1114856

69. Farkas MH, Lew DS, Sousa ME, et al. Mutations in pre-mRNA processing factors 3, 8, and 31 cause dysfunction of the retinal pigment epithelium. *Am J Pathol*. 2014;184(10):2641-2652. doi:10.1016/j.ajpath.2014.06.026

70. McLenachan S, Zhang D, Zhang X, et al. Generation of two induced pluripotent stem cell lines from a patient with dominant PRPF31 mutation and a related non-penetrant carrier. *Stem Cell Res*. 2019;34:101357. doi:10.1016/j.scr.2018.11.018

71. Brydon EM, Bronstein R, Buskin A, et al. AAV-Mediated Gene Augmentation Therapy Restores Critical Functions in Mutant PRPF31(+/-) iPSC-Derived RPE Cells. *Mol Ther Methods Clin Dev*. 2019;15:392-402. doi:10.1016/j.omtm.2019.10.014

72. Liu Q, Zhou J, Daiger SP, et al. Identification and subcellular localization of the RP1 protein in human and mouse photoreceptors. *Invest Ophthalmol Vis Sci*. 2002;43(1):22-32.

73. Liu Q, Lyubarsky A, Skalet JH, et al. RP1 is required for the correct stacking of outer segment discs. *Invest*

Ophthalmol Vis Sci. 2003;44(10):4171-4183. doi:10.1167/iovs.03-0410

74. Verbakel SK, van Huet RAC, den Hollander AI, et al. Macular Dystrophy and Cone-Rod Dystrophy Caused by Mutations in the RP1 Gene: Extending the RP1 Disease Spectrum. *Invest Ophthalmol Vis Sci*. 2019;60(4):1192-1203. doi:10.1167/iovs.18-26084

75. Georgiou M, Ali N, Yang E, et al. Extending the phenotypic spectrum of PRPF8, PRPH2, RP1 and RPGR, and the genotypic spectrum of early-onset severe retinal dystrophy. *Orphanet J Rare Dis*. 2021;16(1):128. doi:10.1186/s13023-021-01759-8

76. Khaliq S, Abid A, Ismail M, et al. Novel association of RP1 gene mutations with autosomal recessive retinitis pigmentosa. *J Med Genet*. 2005;42(5):436-438. doi:10.1136/jmg.2004.024281

77. Wang J, Xiao X, Li S, et al. Dominant RP in the Middle While Recessive in Both the N- and C-Terminals Due to RP1 Truncations: Confirmation, Refinement, and Questions. *Front cell Dev Biol*. 2021;9:634478. doi:10.3389/fcell.2021.634478

78. Liu Q, Collin RWJ, Cremers FPM, et al. Expression of wild-type Rp1 protein in Rp1 knock-in mice rescues the retinal degeneration phenotype. *PLoS One*. 2012;7(8):e43251. doi:10.1371/journal.pone.0043251

79. Wells J, Wroblewski J, Keen J, et al. Mutations in the human retinal degeneration slow (RDS) gene can cause either retinitis pigmentosa or macular dystrophy. *Nat Genet*. 1993;3(3):213-218. doi:10.1038/ng0393-213

80. Gill JS, Georgiou M, Kalitzeos A, et al. Progressive cone and cone-rod dystrophies: clinical features, molecular genetics and prospects for therapy. *Br J Ophthalmol*. 2019;103(5):711-720. doi:10.1136/bjophthalmol-2018-313278

81. Rahman N, Georgiou M, Khan KN, et al. Macular dystrophies: clinical and imaging features, molecular genetics and

therapeutic options. *Br J Ophthalmol*. 2020;104(4):451-460. doi:10.1136/bjophthalmol-2019-315086

82. Boon CJF, den Hollander AI, Hoyng CB, et al. The spectrum of retinal dystrophies caused by mutations in the peripherin/RDS gene. *Prog Retin Eye Res*. 2008;27(2):213-235. doi:10.1016/j.preteyeres.2008.01.002

83. Travis GH, Sutcliffe JG, Bok D. The retinal degeneration slow (rds) gene product is a photoreceptor disc membrane-associated glycoprotein. *Neuron*. 1991;6(1):61-70. doi:10.1016/0896-6273(91)90122-g

84. Cai X, Conley SM, Naash MI. Gene therapy in the Retinal Degeneration Slow model of retinitis pigmentosa. *Adv Exp Med Biol*. 2010;664:611-619. doi:10.1007/978-1-4419-1399-9_70

85. Sanyal S, Jansen HG. Absence of receptor outer segments in the retina of rds mutant mice. *Neurosci Lett*. 1981;21(1):23-26. doi:10.1016/0304-3940(81)90051-3

86. Hawkins RK, Jansen HG, Sanyal S. Development and degeneration of retina in rds mutant mice: photoreceptor abnormalities in the heterozygotes. *Exp Eye Res*. 1985;41(6):701-720. doi:10.1016/0014-4835(85)90179-4

87. Stricker HM, Ding X-Q, Quiambao A, et al. The Cys214-->Ser mutation in peripherin/rds causes a loss-of-function phenotype in transgenic mice. *Biochem J*. 2005;388(Pt 2):605-613. doi:10.1042/BJ20041960

88. Loewen CJR, Moritz OL, Tam BM, et al. The Role of Subunit Assembly in Peripherin-2 Targeting to Rod Photoreceptor Disk Membranes and Retinitis Pigmentosa. *Mol Biol Cell*. 2003;14(8):3400-3413. doi:10.1091/mbc.e03-02-0077

89. McNally N, Kenna PF, Rancourt D, et al. Murine model of autosomal dominant retinitis pigmentosa generated by targeted deletion at codon 307 of the rds–peripherin gene. *Hum Mol Genet*. 2002;11(9):1005-1016. doi:10.1093/hmg/11.9.1005

90. Chakraborty D, Strayve DG, Makia MS, et al. Novel molecular mechanisms for Prph2-associated pattern dystrophy. *FASEB J Off Publ Fed Am Soc Exp Biol*. 2020;34(1):1211-1230. doi:10.1096/fj.201901888R

91. Conley SM, Naash MI. Gene therapy for PRPH2-associated ocular disease: challenges and prospects. *Cold Spring Harb Perspect Med*. 2014;4(11):a017376. doi:10.1101/cshperspect.a017376

92. Nour M, Fliesler SJ, Naash MI. Genetic supplementation of RDS alleviates a loss-of-function phenotype in C214S model of retinitis pigmentosa. *Adv Exp Med Biol*. 2008;613:129-138. doi:10.1007/978-0-387-74904-4_14

93. Cai X, Nash Z, Conley SM, et al. A partial structural and functional rescue of a retinitis pigmentosa model with compacted DNA nanoparticles. *PLoS One*. 2009;4(4):e5290. doi:10.1371/journal.pone.0005290

94. Nour M, Ding X-Q, Stricker H, et al. Modulating expression of peripherin/rds in transgenic mice: critical levels and the effect of overexpression. *Invest Ophthalmol Vis Sci*. 2004;45(8):2514-2521. doi:10.1167/iovs.04-0065

95. Petrs-Silva H, Yasumura D, Matthes MT, et al. Suppression of rds expression by siRNA and gene replacement strategies for gene therapy using rAAV vector. *Adv Exp Med Biol*. 2012;723:215-223. doi:10.1007/978-1-4614-0631-0_29

96. Palfi A, Ader M, Kiang A-S, et al. RNAi-based suppression and replacement of rds-peripherin in retinal organotypic culture. *Hum Mutat*. 2006;27(3):260-268. doi:10.1002/humu.20287

97. Georgiadis A, Tschernutter M, Bainbridge JWB, et al. AAV-mediated knockdown of peripherin-2 in vivo using miRNA-based hairpins. *Gene Ther*. 2010;17(4):486-493. doi:10.1038/gt.2009.162

98. Bok D, Yasumura D, Matthes MT, et al. Effects of adeno-associated virus-vectored ciliary neurotrophic factor on retinal structure and function in mice with a P216L rds/peripherin mutation. *Exp Eye Res*. 2002;74(6):719-735.

doi:10.1006/exer.2002.1176

99. Rhee K Do, Ruiz A, Duncan JL, et al. Molecular and cellular alterations induced by sustained expression of ciliary neurotrophic factor in a mouse model of retinitis pigmentosa. *Invest Ophthalmol Vis Sci*. 2007;48(3):1389-1400. doi:10.1167/iovs.06-0677

100. Bowne SJ, Sullivan LS, Mortimer SE, et al. Spectrum and frequency of mutations in IMPDH1 associated with autosomal dominant retinitis pigmentosa and leber congenital amaurosis. *Invest Ophthalmol Vis Sci*. 2006;47(1):34-42. doi:10.1167/iovs.05-0868

101. Bennett LD, Klein M, John FT, et al. Disease Progression in Patients with Autosomal Dominant Retinitis Pigmentosa due to a Mutation in Inosine Monophosphate Dehydrogenase 1 (IMPDH1). *Transl Vis Sci Technol*. 2020;9(5):14. doi:10.1167/tvst.9.5.14

102. Tam LCS, Kiang A-S, Kennan A, et al. Therapeutic benefit derived from RNAi-mediated ablation of IMPDH1 transcripts in a murine model of autosomal dominant retinitis pigmentosa (RP10). *Hum Mol Genet*. 2008;17(14):2084-2100. doi:10.1093/hmg/ddn107

103. Wada Y, Sandberg MA, McGee TL, et al. Screen of the IMPDH1 Gene among Patients with Dominant Retinitis Pigmentosa and Clinical Features Associated with the Most Common Mutation, Asp226Asn. *Invest Ophthalmol Vis Sci*. 2005;46(5):1735-1741. doi:10.1167/iovs.04-1197

104. Ali S, Khan SY, Naeem MA, et al. Phenotypic variability associated with the D226N allele of IMPDH1. *Ophthalmology*. 2015;122(2):429-431. doi:10.1016/j.ophtha.2014.07.057

105. Bowne SJ, Liu Q, Sullivan LS, et al. Why do mutations in the ubiquitously expressed housekeeping gene IMPDH1 cause retina-specific photoreceptor degeneration? *Invest Ophthalmol Vis Sci*. 2006;47(9):3754-3765.

doi:10.1167/iovs.06-0207

106. Aherne A, Kennan A, Kenna PF, et al. On the molecular pathology of neurodegeneration in IMPDH1-based retinitis pigmentosa. *Hum Mol Genet*. 2004;13(6):641-650. doi:10.1093/hmg/ddh061

107. Rosenthal R, Bakall B, Kinnick T, et al. Expression of bestrophin-1, the product of the VMD2 gene, modulates voltage-dependent Ca2+ channels in retinal pigment epithelial cells. *FASEB J Off Publ Fed Am Soc Exp Biol*. 2006;20(1):178-180. doi:10.1096/fj.05-4495fje

108. Krämer F, White K, Pauleikhoff D, et al. Mutations in the VMD2 gene are associated with juvenile-onset vitelliform macular dystrophy (Best disease) and adult vitelliform macular dystrophy but not age-related macular degeneration. *Eur J Hum Genet*. 2000;8(4):286-292. doi:10.1038/sj.ejhg.5200447

109. Guziewicz KE, Sinha D, Gómez NM, et al. Bestrophinopathy: An RPE-photoreceptor interface disease. *Prog Retin Eye Res*. 2017;58:70-88. doi:10.1016/j.preteyeres.2017.01.005

110. Ji C, Li Y, Kittredge A, et al. Investigation and Restoration of BEST1 Activity in Patient-derived RPEs with Dominant Mutations. *Sci Rep*. 2019;9(1):19026. doi:10.1038/s41598-019-54892-7

111. Zhao Q, Kong Y, Kittredge A, et al. Distinct expression requirements and rescue strategies for BEST1 loss- and gain-of-function mutations. *Elife*. 2021;10. doi:10.7554/eLife.67622

112. Ji C, Kittredge A, Hopiavuori A, et al. Dual Ca(2+)-dependent gates in human Bestrophin1 underlie disease-causing mechanisms of gain-of-function mutations. *Commun Biol*. 2019;2:240. doi:10.1038/s42003-019-0433-3

113. Guziewicz KE, Zangerl B, Lindauer SJ, et al. Bestrophin gene mutations cause canine multifocal retinopathy: a novel animal model for best disease. *Invest Ophthalmol Vis Sci*. 2007;48(5):1959-1967. doi:10.1167/iovs.06-1374

114. Milenkovic A, Schmied D, Tanimoto N, et al. The Y227N mutation affects bestrophin-1 protein stability and impairs sperm function in a mouse model of Best vitelliform macular dystrophy. *Biol Open*. 2019;8(7). doi:10.1242/bio.041335

115. Lee JH, Oh JO, Lee CS. Induced Pluripotent Stem Cell Modeling of Best Disease and Autosomal Recessive Bestrophinopathy. *Yonsei Med J*. 2020;61(9):816-825. doi:10.3349/ymj.2020.61.9.816

116. Guziewicz KE, Cideciyan A V, Beltran WA, et al. BEST1 gene therapy corrects a diffuse retina-wide microdetachment modulated by light exposure. *Proc Natl Acad Sci U S A*. 2018;115(12):E2839-E2848. doi:10.1073/pnas.1720662115

117. Sinha D, Steyer B, Shahi PK, et al. Human iPSC Modeling Reveals Mutation-Specific Responses to Gene Therapy in a Genotypically Diverse Dominant Maculopathy. *Am J Hum Genet*. 2020;107(2):278-292. doi:10.1016/j.ajhg.2020.06.011

118. Rossmiller B, Mao H, Lewin AS. Gene therapy in animal models of autosomal dominant retinitis pigmentosa. *Mol Vis*. 2012;18:2479-2496.

119. Iadevaia V, Huo Y, Zhang Z, et al. Roles of the mammalian target of rapamycin, mTOR, in controlling ribosome biogenesis and protein synthesis. *Biochem Soc Trans*. 2012;40(1):168-172. doi:10.1042/BST20110682

120. Park KS, Xu CL, Cui X, et al. Reprogramming the metabolome rescues retinal degeneration. *Cell Mol Life Sci*. 2018;75(9):1559-1566. doi:10.1007/s00018-018-2744-9

121. Zhang L, Justus S, Xu Y, et al. Reprogramming towards anabolism impedes degeneration in a preclinical model of retinitis pigmentosa. *Hum Mol Genet*. 2016;25(19):4244-4255. doi:10.1093/hmg/ddw256

122. Xu L, Kong L, Wang J, et al. Stimulation of AMPK prevents degeneration of photoreceptors and the retinal pigment epithelium. *Proc Natl Acad Sci U S A*. 2018;115(41):10475-10480. doi:10.1073/pnas.1802724115

123. Miyagishima KJ, Sharma R, Nimmagadda M, et al. AMPK modulation ameliorates dominant disease phenotypes of CTRP5 variant in retinal degeneration. *Commun Biol*. 2021;4(1):1360. doi:10.1038/s42003-021-02872-x

124. Arakawa M, Ito Y. N-acetylcysteine and neurodegenerative diseases: basic and clinical pharmacology. *Cerebellum*. 2007;6(4):308-314. doi:10.1080/14734220601142878

125. Campochiaro PA, Iftikhar M, Hafiz G, et al. Oral N-acetylcysteine improves cone function in retinitis pigmentosa patients in phase I trial. *J Clin Invest*. 2020;130(3):1527-1541. doi:10.1172/JCI132990

126. Talib M, van Schooneveld MJ, Thiadens AA, et al. CLINICAL AND GENETIC CHARACTERISTICS OF MALE PATIENTS WITH RPGR-ASSOCIATED RETINAL DYSTROPHIES: A Long-Term Follow-up Study. *Retina*. 2019;39(6):1186-1199. doi:10.1097/IAE.0000000000002125

127. Talib M, van Schooneveld MJ, van Genderen MM, et al. Genotypic and Phenotypic Characteristics of CRB1-Associated Retinal Dystrophies: A Long-Term Follow-up Study. *Ophthalmology*. 2017;124(6):884-895. doi:10.1016/j.ophtha.2017.01.047

128. Han Z, Banworth MJ, Makkia R, et al. Genomic DNA nanoparticles rescue rhodopsin-associated retinitis pigmentosa phenotype. *FASEB J Off Publ Fed Am Soc Exp Biol*. 2015;29(6):2535-2544. doi:10.1096/fj.15-270363

129. Chuang Y-F, Wang P-Y, Kumar S, et al. Methods for in vitro CRISPR/CasRx-Mediated RNA Editing. *Front cell Dev Biol*. 2021;9:667879. doi:10.3389/fcell.2021.667879

130. Singh R, Kuai D, Guziewicz KE, et al. Pharmacological Modulation of Photoreceptor Outer Segment Degradation in

a Human iPS Cell Model of Inherited Macular Degeneration. *Mol Ther*. 2015;23(11):1700-1711. doi:10.1038/mt.2015.141

622 Table 1: Gene therapy strategies currently being investigated for autosomal dominant inherited retinal dystrophies

623

Gene	Variant	Mechanism (drug)	Route of delivery	Status
RHO	P23H	Antisense oligonucleotide (QR-1123)	Intravitreal	Phase I/II CT (NCT04123626; ProQR Therapeutics)
RHO	P23H	Autophagy reduction (Hydroxychloroquine)	Oral	Phase I/II CT (NCT04120883; University of Michigan)
RHO	P23H	Transgenic gene supplementation	-	Mouse models[45]
RHO	Unspecific	Zinc Fingers	Subretinal	Mouse models[26]
RHO	Unspecific	Ectopic silencing transcription factor (KLF15)	Subretinal	Mouse models[46]
RHO	P347S	Coadministration of two AAV containing RNAi and a codon-modified gene replacement	Subretinal	Mouse models[48]
RHO	P23H	Administration of one AAV containing both siRNA and a codon-modified gene replacement	Subretinal	Mouse models[49]
RHO	P347S	Administration of shRNA-expressing AAV and an AAV expressing shRNA-resistant rhodopsin	Subretinal	Mouse models[50]
RHO	P23H & D190N	Ablate-and-replace strategy, with dual AAV injection of CRISPR/Cas9 and gene replacement	Subretinal	Mouse models[51]
RHO	S334	Allele-specific ablation using CRISPR/Cas9 with targeting-guide RNA constructs	Subretinal	Mouse models[52]
RHO	P23H	AAV delivered CRISPR/Cas9 with short guide RNA	Intravitreal	Mouse models and human cells[53]
RHO	P23H	AAV delivered CRISPR/Cas9 with short guide RNA	Subretinal	Mouse models[54]
RHO	P23H	Antisense oligonucleotide	Intravitreal	Mouse and rat models[55]

RHO	S334	Aminoglycoside read-through (gentamicin or geneticin)	Subcutaneous	Rat model[56]
PRPF31	Unspecific	AAV-mediated gene augmentation	-	Induced pluripotent stem cells - RPE cells[71]
PRPH2	Unspecific	Nanoparticles containing wild-type *PRPH2*	Subretinal	Mouse models[93]
PRPH2	Unspecific	AAV-delivered siRNAs and resistant *PRPH2*	Subretinal	Mouse models[95]
PRPH2	Unspecific	si/shRNAs and resistant *PRPH2*	-	Retinal organotypic culture[96]
PRPH2	Unspecific	AAV-delivered shRNAs	Subretinal	Mouse models[97]
IMPDH1	Unspecific	AAV-delivered shRNA and resistant *IMPDH1*	Subretinal	Mouse models[102]
BEST1	R218H, 234P, A243T, 293K, & D302A	AAV-mediated gene augmentation	-	iPSC-RPEs[110]
BEST1	D203A, I205T, & Y236C	Baculovirus-based silencing vector delivery of CRISPR/Cas9 and resistant *BEST1*	-	iPSC-RPE cells[111]
BEST1	R218C & N296H	Lentivirus mediated gene augmentation	-	iPSC-RPE[117]
BEST1	R218C, N296H, & A146K	Lentivirus construct delivery of CRISPR/Cas9	-	iPSC-RPE[117]

Abbreviations: CT: clinical trial; AAV: adeno-associated virus; RNAi: RNA interference; siRNA: short-interfering RNA; shRNA: short-hairpin RNA; CRISPR: clustered regularly interspaced short palindromic repeats; RPE: retinal pigment epithelium; iPSC: induced pluripotent stem cells.

Table 2: Ongoing natural history studies being conducted on autosomal dominant IRD genes.

Gene	ClinicalTrials.gov Identifier	Status	Location	Estimated completion date	Sponsor
RHO	NCT04285398	Active, not recruiting	USA and France	June 2026	SparingVision
PRPF31	NCT04805658	Recruiting	Norway	February 2025	Oslo University Hospital