Gene therapy in bestrophinopathies: Insights from preclinical studies in preparation for clinical trials

Alessia Amato, Nida Wongchaisuwat, Andrew Lamborn, Ryan Schmidt, Lesley Everett, Paul Yang, Mark E. Pennesi

Access this article online

Quick Response Code:

Website: www.saudijophthalmol.org

DOI: 10.4103/sjopt.sjopt_175_23

Abstract:

The *BEST1* gene encodes bestrophin-1, a homopentameric ion channel expressed in the retinal pigment epithelium (RPE), where it localizes to the basolateral plasma membrane. Pathogenic variants in this gene can cause different autosomal dominant and recessive inherited retinal diseases (IRDs), collectively named "bestrophinopathies." These disorders share a number of clinical and molecular features that make them an appealing target for gene therapy. Clinically, bestrophinopathies are often slowly progressive with a wide window of opportunity, and the presence of subretinal material (vitelliform deposits and/or fluid) as a hallmark of these conditions provides an easily quantifiable endpoint in view of future clinical trials. From a molecular standpoint, most *BEST1* pathogenic variants have been shown to cause either loss of function (LOF) of the protein or a dominant-negative (DN) effect, with a smaller subset causing a toxic gain of function (GOF). Both LOF and DN mutations may be amenable to gene augmentation alone. On the other hand, individuals harboring GOF variants would require a combination of gene silencing and gene augmentation, which has been shown to be effective in RPE cells derived from patients with Best disease. In this article, we review the current knowledge of *BEST1*-related IRDs and we discuss how their molecular and clinical features are being used to design novel and promising therapeutic strategies.

Keywords:

Best disease, *BEST1*, bestrophinopathies, gene therapy

Introduction

Pathogenic variants in *BEST1* are associated with a broad group of dominantly and recessively inherited retinal diseases (IRDs), collectively referred to as the "bestrophinopathies," which account for 3.5% of all IRDs.[1] Even though most IRDs currently lack a regulatory body-approved treatment, many novel therapeutic strategies have been explored in preclinical and clinical settings. Moreover, the marketing of Luxturna® (voretigene neparvovec-rzyl), the first approved retinal gene-therapy product, contributed to increase interest in this field.

Most recent clinical trials have targeted autosomal recessive (AR) and X-linked IRDs, caused by loss-of-function (LOF) mechanisms and amenable to gene augmentation. While some autosomal dominant (AD) disorders result from haploinsufficiency or dominant-negative (DN) mutations, others result from gain of function (GOF), which require more complex editing strategies.[2] In this context, *BEST1* stands out as an optimal candidate for gene therapy for a number of reasons. First, AR bestrophinopathy (ARB) has a natural canine model that recapitulates the phenotype of its human disease counterpart.[3-5] Second, dominantly inherited Best vitelliform macular dystrophy (BVMD), unlike many other AD IRDs, has the potential for gene replacement, due to the DN effect underlying many of its causative mutations.[6] Moreover, recent evidence suggests that a combination of gene augmentation and gene editing could provide a universal treatment strategy for all bestrophinopathies, regardless of the inheritance pattern.[7]

Herein, we review the structural and functional characteristics of the *BEST1* gene, as well as the

Department of Ophthalmic Genetics, Casey Eye Institute, Oregon Health and Science University, Portland, Oregon, United States of America

Address for correspondence:
Prof. Mark E. Pennesi, Department of Ophthalmic Genetics, Casey Eye Institute, Oregon Health and Science University, Portland, Oregon, United States of America.
E-mail: pennesim@ohsu.edu

Submitted: 02-Aug-2023
Revised: 15-Aug-2023
Accepted: 17-Aug-2023
Published: 01-Dec-2023

How to cite this article: Amato A, Wongchaisuwat N, Lamborn A, Schmidt R, Everett L, Yang P, *et al*. Gene therapy in bestrophinopathies: Insights from preclinical studies in preparation for clinical trials. Saudi J Ophthalmol 2023;37:287-95.

phenotypic spectrum of bestrophinopathies, and discuss how these molecular and clinical features are being used to design novel promising therapies.

The *Best1* Gene

BEST1 is located on chromosome 11q13 and encodes bestrophin-1, a 68 kDa protein with 585 amino acids, the first 350 of which are highly conserved among species.[8] In the human eye, this gene is only expressed in retinal pigment epithelium (RPE), where it localizes to the basolateral plasma membrane[9,10] and on the endoplasmic reticulum membrane.[11,12] Bestrophin-1 functions as both a pentameric calcium-activated chloride channel[13-15] and a regulator of intracellular Ca^{2+} signaling.[16-18] The crystal structure of two bestrophin-1 homologs (*Klebsiella pneumoniae* and chicken) has recently been solved and provides critical insights into the pathophysiology of this protein.[19,20] Both structures are homopentameric (i.e., formed by five identical protomers) with a central funnel-shaped pore. This ion permeation pathway has two restriction sites that confer a "flower-vase shape" [Figure 1].[21] The first narrowing forms the neck region and is due to hydrophobic amino acids Ile 76, Phe 80, and Phe 84, which are highly conserved in human bestrophin-1. After this restriction, the pore opens into a larger inner cavity that represents its positively charged cytosolic portion which attracts intracellular anions. Lying below the inner cavity is a second restriction, which in human bestrophin-1 is due to the residue Ile 205. Each protomer has a calcium-binding site, called the Ca^{2+} clasp,[19] located within its intracellular portion in close proximity to the neck region. These five sites (formed by amino acids Pro 297, Glu 300, and Asp 301-304) assemble to form a belt-like structure around the central section of the channel and control the closing and opening of the neck through calcium-induced conformational changes of the protein. The Ca^{2+} clasps are therefore critical regions and mutations affecting their corresponding amino acid residues can decrease or alter the function of the channel. Indeed, while loss of function mutations act by destabilizing the protein and can occur throughout the gene, many dominantly acting variants have more specific effects and have been found to cluster in or around the first restriction and the Ca^{2+} clasp.[22]

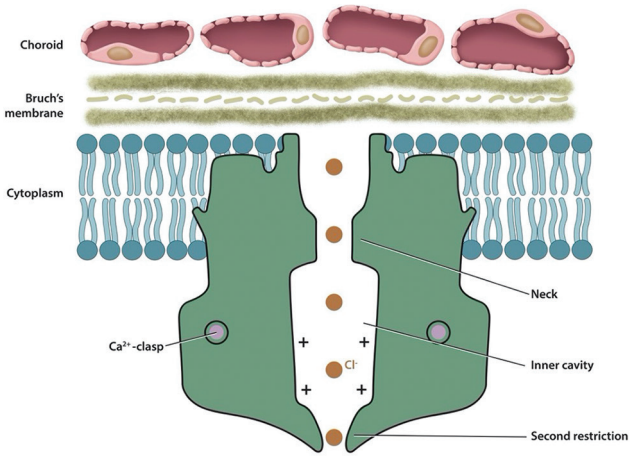

Figure 1: Schematic representation of the bestrophin-1 protein, an anion channel that localizes to the basolateral membrane of the retinal pigment epithelium (RPE) cells

The Clinical Spectrum of Bestrophinopathies

Pathogenic variants in the *BEST1* gene have been associated with at least five different phenotypic presentations: BVMD, adult-onset foveomacular vitelliform dystrophy (AOFVD), AD vitreoretinochoroidopathy (ADVIRC), retinitis pigmentosa (RP), and ARB, the latter being the only recessively inherited bestrophinopathy. Since the RPE is the source of the standing potential of the eye, a reduced light peak (LP) on the electrooculogram (EOG), which indirectly measures the amplitude of this potential, is a hallmark of all bestrophinopathies.[23] However, several cases of *BEST1*-associated IRDs presenting with a normal EOG have been described and an Arden ratio >1.65 was measured in 8% of cases in a case series of 113 patients with AD or AR bestrophinopathies.[24] Importantly, most bestrophinopathies exhibit a slow rate of decline and central photoreceptors (PRs) usually remain viable for decades despite the presence of subretinal vitelliform material and/or fluid.[21] This feature provides a long therapeutic window for novel treatment options, and makes *BEST1* a compelling target for gene- and cell-based therapies.

Best vitelliform macular dystrophy

BVMD, also known as Best disease, inherited in an AD fashion, is the most common bestrophinopathy. BVMD is characterized by highly variable expressivity, both among and within families,[25,26] with many asymptomatic patients lacking fundus lesions on examination but often showing subtle changes on optical coherence tomography (OCT) or fundus autofluorescence (FAF), as described below. Five stages have been described based on ophthalmoscopy, OCT, and FAF, although these stages do not always occur in all patients or might be missed by episodic examinations.[27,28]

In stage 1 (previtelliform), the fundus is unremarkable, but imaging modalities are able to detect early signs of disease. OCT can show a thicker and more reflective appearance of the interdigitation zone (the layer between the RPE and the outer segments [OSs]).[28,29] Short-wavelength FAF is normal, while near-infrared FAF is abnormal with loss of central hyperautofluorescence.[30] In stage 2 (vitelliform), a yellow, well-demarcated vitelliform lesion develops in the central macula, appearing as a dome-shaped subretinal hyperreflective structure on OCT and displaying hyperautofluorescence on FAF [Figure 2]. In stage 3 (pseudohypopyon), the subretinal material partially liquifies and can gravitate inferiorly, resulting in a lesion that resembles anterior chamber hypopyon [Figure 3]. Over time the egg yolk vitelliform material is resorbed, producing a "scrambled-egg" appearance on ophthalmoscopy and clumping of hyperreflective material

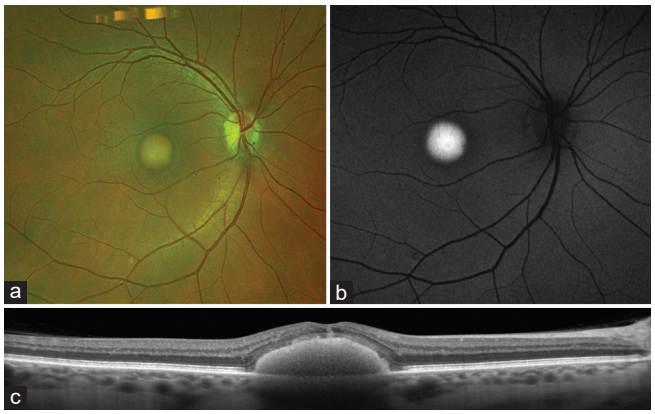

Figure 2: Multimodal imaging of the vitelliform stage of Best disease. (a) Pseudocolor fundus photo shows a round vitelliform lesion centered on the fovea. (b) Fundus autofluorescence (FAF) shows homogeneous hyperautofluorescence of the macular vitelliform lesion. (c) On optical coherence tomography (OCT), the vitelliform material appears as a dome-shaped subretinal hyperreflective lesion

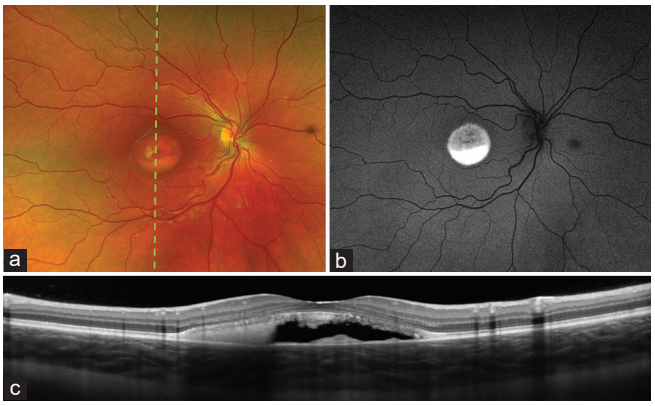

Figure 3: Multimodal imaging of the pseudohypopyon stage of Best disease. (a) Pseudocolor fundus photo showing the yellow material that accumulates inferiorly to form a pseudohypopyon appearance. (b) Fundus autofluorescence better highlights the level between the inferior vitelliform deposit and the resorbed portion of the lesion. (c) Optical coherence tomography (OCT) B-scan passing vertically through the lesion (as shown by the green dotted line in panel A) shows the two different zones that characterize the pseudohypopyon lesion: in the upper part, OCT shows a hyporeflective area with clumping of hyperreflective material on the posterior retinal surface; in the lower part, the inferiorly gravitated vitelliform material appears as a subretinal hyperreflective lesion

mainly on the posterior retinal surface on OCT that is typical of stage 4 (vitelliruptive). Finally, the disease can result in macular atrophy or fibroatrophic lesions, which characterize stage 5, as shown in Figure 4. Choroidal neovascularizations (CNVs) can be detected in up to 65% of patients by OCT angiography.[31] More specifically, nonexudative CNVs have been reported to be present in the vast majority of eyes (up to 96%) at the vitelliruptive or fibroatrophic stage, and they are usually not treated.[31] CNVs are more rare in patients with stage 2–3 disease (up to 13%), and they are frequently exudative, often requiring intravitreal injections of antivascular endothelial growth factor agents.[31] While typical Best disease is bilateral and unifocal, it can also display atypical features, with unilateral,[32,33] multifocal,[34] and asymmetrical presentations.

Adult-onset foveomacular vitelliform macular dystrophy

AOFVD is characterized by subretinal vitelliform macular lesions and is usually diagnosed after the age of 40 years. These lesions can increase and decrease in size and eventually leave an area of central atrophy, resulting in decreased visual acuity (VA).[35] While a minority of patients with AOFVD are found to have pathogenic variants in the *BEST1*, *PRPH 2*, *IMPG1*, or *IMPG2* genes, many cases are idiopathic.[35] Although AOFVD was initially thought to be a clinically distinct entity from BVMD, some authors have proposed that individuals with AOFVD carrying *BEST1* pathogenic variants should be reclassified as a milder form of BVMD.[8]

Autosomal dominant vitreoretinochoroidopathy

ADVIRC is a rare condition characterized by a peripheral retinal circumferential hyperpigmented band, punctate white retinal opacities, fibrillar condensation of the vitreous, vascular abnormalities, and neovascularization.[36] ADVIRC can also cause anterior segment manifestations, including narrow angles and early-onset cataracts.[8] Interestingly, this bestrophinopathy has been shown to result from *BEST1* pathogenic variants that cause exon skipping, thus leading to the production of shortened and internally deleted isoforms.[37] Microcornea, rod-cone dystrophy, cataract, and posterior staphyloma (MRCS) is a rare *BEST1*-related phenotype, which like ADVIRC seems to be caused by splicing-altering pathogenic variants.[27] The few published reports described an entity with near-identical presentation to that ADVIRC,[38,39] and the two conditions have been hypothesized to be the same disease.[8]

Retinitis pigmentosa

The association between RP and *BEST1* variants was first reported in five unrelated families; three out of four missense pathogenic variants appeared to be AD, while one of them was AR.[40] It has subsequently been suggested that RP associated with *BEST1* pathogenic variants actually represents misdiagnosed ADVIRC,[41] while a more recent report described the case of a patient with a heterozygous 10 kbp deletion in the *BEST1* gene who also had several other single pathogenic variants in known RP genes, leading the authors to hypothesize a multigenic inheritance for *BEST1*-associated RP.[42]

Autosomal recessive bestrophinopathy

ARB is the only bestrophinopathy caused by biallelic pathogenic variants in *BEST1*.[16,43] It typically presents with multifocal vitelliform material along the arcades and subretinal fluid (SRF) on OCT, but ARB has a broad phenotypic spectrum which includes mid-peripheral patches of RPE atrophy, subretinal drusen-like deposits, as well as anterior segment manifestations, such as iridocorneal abnormalities, reduced axial length, and a shallow anterior chamber with increased risk of angle-closure glaucoma.[22] OCT can show intraretinal fluid, OS elongation,

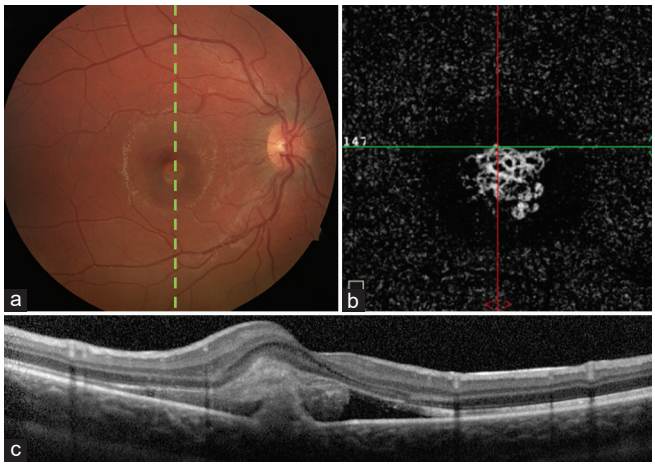

Figure 4: Multimodal imaging of a choroidal neovascularization (CNV) in best disease. (a) Fundus photo shows the central vitelliform lesion associated with subretinal blood. (b) Optical coherence tomography (OCT) angiography highlights the neovascular network. (c) OCT B-scan passing through the lesion (as indicated by the green dotted line in panel A) shows the presence of three components in the subretinal space: a dome-shaped hyperreflective lesion corresponding to the CNV, an overlying ill-defined hyperreflective material corresponding to blood, and a hyporeflective space containing fluid

SRF, subretinal deposits, subretinal fibrosis, and shallow RPE detachments often associated with focal choroidal excavation, as shown in Figure 5.[22] Choroidal thickening on enhanced depth imaging-OCT is another frequently reported feature, often leading to misdiagnosis of chronic central serous chorioretinopathy.[44]

Although ARB has been thought to represent the human "null" phenotype for *BEST1*,[16,45] this theory is still controversial. Evidence in favor of the "null phenotype" hypothesis includes several ARB patients who are homozygous for truncating mutations,[16] as well as the naturally occurring canine *BEST1* knockout model, which shows clinical features similar to those seen in human ARB.[3] However, the variants that have been associated with ARB vary from missense to truncations to single base changes in introns, and this mutation spectrum seems to correspond to a clinical spectrum of retinal dysfunction. Casalino *et al*. observed that ARB patients carrying null alleles had an earlier onset of disease and a faster decline in VA compared to noncarriers, although these differences did not reach statistical significance.[22] This led the authors to speculate that, rather than ARB representing the null phenotype, affected individuals have a significant reduction in *BEST1* activity, which can range from total absence in truly nullizygous patients to partial functional preservation in those with at least one hypomorphic, usually missense, variant.[22]

Inheritance Patterns and Molecular Mechanisms of Monogenic Diseases

To better understand the challenges of gene-based therapies in

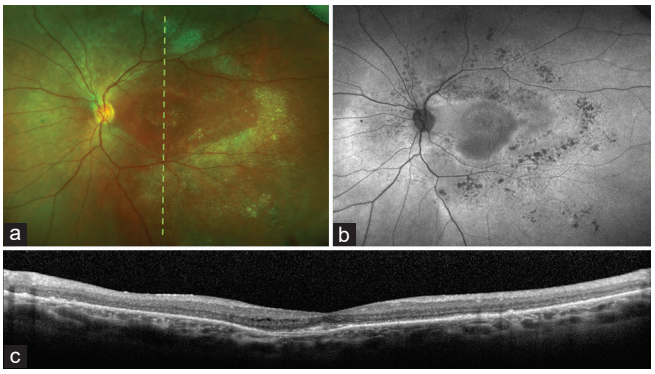

Figure 5: Multimodal imaging of autosomal recessive bestrophinopathy (ARB). (a) Pseudocolor fundus photo shows multifocal vitelliform lesions and drusen-like deposits along the arcades. (b) Fundus autofluorescence better highlights the presence of patchy areas of atrophy. (c) Optical coherence tomography B-scan passing vertically through the lesion (as indicated by the green dotted line in panel A) shows some of the typical features of ARB, including intraretinal fluid, focal choroidal excavation, and subretinal deposits

BEST1-related retinopathies, it is fundamental to be familiar with the basic terminology and concepts used in Mendelian genetics.

Inheritance patterns show the transmission of phenotypes to offspring. When a gene is located on an autosomal chromosome (like *BEST1*), there are two possible types of inheritance:

- AD: The disease manifests in individuals who are heterozygotes for a disease-causing variant;
- AR: Both alleles of a given gene need to have a change in their DNA sequence for the subject to be affected.

Inheritance patterns of monogenic diseases, however, should not be confused with their molecular mechanisms, that is, the effect of a mutation at the protein level. Disease-causing variants can act via three main mechanisms, which may present different patterns of inheritance [Figure 6]:[46]

- LOF: These mutations are usually recessively inherited and act by reducing (hypomorphic) or (null or amorphic) the normal biological activity of a protein. LOF mutations, however, can also be dominantly inherited: this scenario, referred to as haploinsufficiency, occurs when a gene's function is dosage-sensitive and both alleles are required to express a sufficient amount of protein
- GOF: These mutations cause disease by increasing protein activity (hypermorphic) or introducing a completely new function (neomorphic). The vast majority of GOF mutations are dominant, although there are some rare examples of recessive GOFs, none of which pertain to the field of ocular genetics[46]
- DN: These dominantly inherited mutations are considered antimorphic, characterized by the mutant protein blocking the normal biological function of the wild-type (WT) protein. DN effect can occur via competition-based mechanisms, in which there is no direct interaction between the WT and mutant proteins. However, more often these DN mutations act by the poisoning of macromolecular

complexes. The latter mechanism is typical of genes encoding polymeric proteins, such as *BEST1*, which rely on the ability of the gene product to coassemble into a complex with WT subunits. The resulting hybrid WT: mutant complex can then be nonfunctional, mislocated, or subject to enhanced degradation.

Distinguishing among these molecular mechanisms is a key factor for the design of effective treatment strategies. Both AD and AR LOF mutations can be successfully rescued by gene replacement alone. DN mutations have the potential to be treated by simply increasing normal protein levels, although higher augmentation dosages may be necessary for variants with a greater dominant effect. GOF mutations result in an aberrant product that is damaging to the cell that expresses it, thus necessitating gene editing or suppression as opposed to supplementation alone.

Molecular mechanisms of *BEST1* mutations

To date, over 250 distinct *BEST1* pathogenic variants have been identified in bestrophinopathy patients, but their pathological mechanisms often remain unclear.[7] The majority of *BEST1* pathogenic variants are dominantly inherited.[8] While the inheritance pattern of a mutation can be an indicator of its molecular mechanism, these concepts should not be used interchangeably (e.g., LOF as a synonym for recessive and GOF as a synonym for dominant) for two main reasons. First, as described above, although uncommon in the setting of IRDs, there are cases in which LOF mutations are dominantly inherited (haploinsufficiency). Second, disease-causing variants that are dominant at a clinical level (i.e., patients only have one mutated allele) can sometimes behave recessively at a molecular and cellular level that is when expressed at a 1:1 ratio with the WT protein *in vitro*.[7] This scenario, known as allelic expression imbalance (AEI), is characterized by a higher transcription level of the mutant allele, and it has been previously observed at the *BEST1* locus in human RPE,[7,47] with important therapeutic implications. A recent study quantitatively examined the functional influence of nine dominantly inherited patient-derived mutations on the channel activity when the mutant and WT *BEST1* were coexpressed at various ratios in HEK293 cells.[7]

Interestingly, six of these mutations were found to actually behave recessively *in vitro* at a 1:1 ratio with the WT *BEST1* and required a superior 4:1 ratio to exhibit the mutant phenotype. This indicates that AEI of *BEST1* transcription contributes to determine a dominant-negative effect in patients harboring these variants, and provides preclinical evidence that many dominantly inherited mutations can be rescued by gene augmentation.[6] Moreover, this allele-specific epigenetic control provides an explanation for incomplete penetrance and variable clinical expressivity in patients bearing the same variant.[7]

However, the same study found that three of the clinically dominant pathogenic variants turned out to also behave dominantly at a cellular level, even at an inferior 1:4 ratio with the WT *BEST1*. This type of variant cannot be rescued by gene supplementation and requires other approaches. A combination of gene editing via CRISPR/Cas9-mediated silencing of endogenous *BEST1* with gene replacement was shown to effectively overcome the strong dominant effect of GOF mutations *in vitro*,[7] potentially providing a universal strategy for the treatment of all bestrophinopathies, regardless of their mutation type. Recessive LOF mutations responsible for ARB have been shown to reduce (hypomorph) or abolish (null) the function of the channel *in vitro,*[48] and preclinical studies demonstrated that these types of patient-derived mutations are rescuable with viral gene supplementation,[7,49,50] providing proof of concept of the efficacy of this strategy in ARB.

Gene Therapy in Bestrophinopathies

The human eye is an ideal site for gene therapy for a number

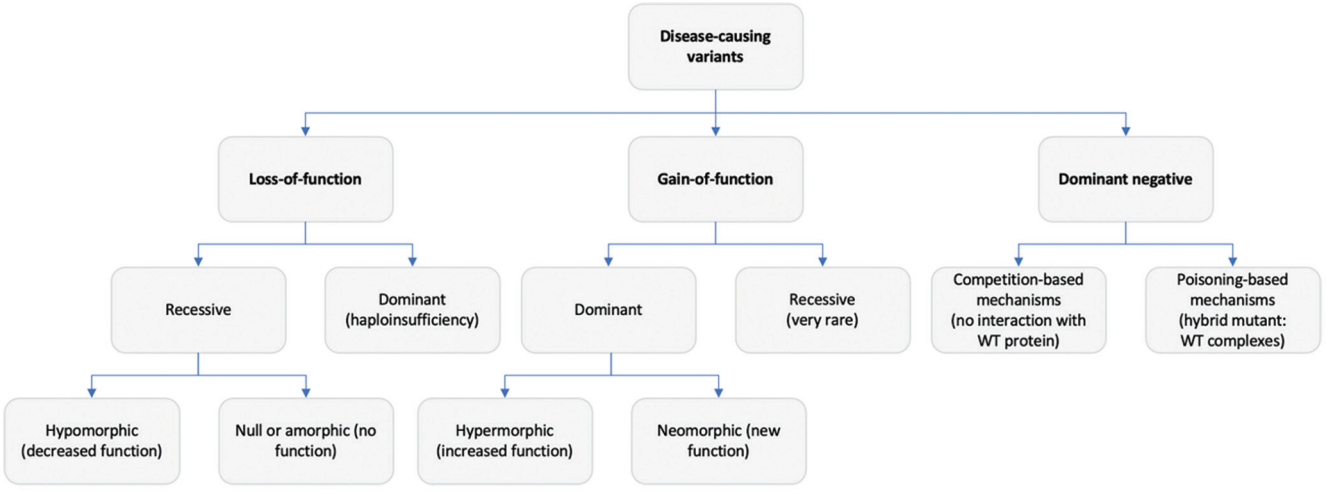

Figure 6: Diagram showing the three main molecular mechanisms through which pathogenic variants can cause diseases and their possible inheritance patterns. WT: Wild-type

of reasons including the relative immune privilege conferred by the blood–retinal barrier, the possibility to use fellow eyes as controls to assess efficacy and safety in early phase studies, and the accessibility which allows for noninvasive studies to be performed before and after treatment.[2]

BEST1-related IRDs share some features that make them a compelling target for gene therapy approaches.[21]

First, *BEST1* is a relatively small gene which is exclusively expressed at the level of the RPE in the human eye, making it an appealing candidate for adeno-associated virus (AAV)-mediated gene therapy, since numerous serotypes exhibit a good tropism for these cells.[51]

Moreover, while bestrophinopathies are progressive, the rate of decline is typically slow and allows a wider therapeutic window, as central PRs remain viable for decades and many patients maintain an excellent VA until their fifth decade of life. However, this also means that a mindful assessment of the risk–benefit ratio is required before exposing subjects with preserved vision to potentially sight-threatening surgical procedures.[52]

In addition, the presence of subretinal vitelliform material and/or fluid as a hallmark of these diseases provides easily quantifiable endpoints. Relying on OCT thickness to establish efficacy, rather than on outcome measures aimed at assessing cell preservation, offers the potential for utilization of smaller sample sizes for future clinical trials[21] and could even reduce the gap between early-phase interventional studies and approval of a treatment by regulatory bodies.

Although there are currently no active trials for bestrophinopathies, many promising preclinical studies have provided proof of concept of the translational potential of gene therapy for future clinical use.

Insights from animal models

Canine multifocal retinopathy (*cmr*) is a spontaneous early-onset disease caused by biallelic mutations in the *BEST1* dog ortholog (*cBEST1*), which were found to recapitulate the full spectrum of clinical, molecular, and histological features of the human counterpart.[3] The *cmr* disorder can result from any of three distinct mutations identified to date in *cBEST1*, which spontaneously occur in 11 dog breeds: R25X, an early stop mutation resulting in *BEST1* null phenotype; G161D, a missense variant causing protein misfolding and mistrafficking; and P464fs, a frameshift mutation truncating the bestrophin-1 C-terminus.[53]

Guziewicz *et al*. demonstrated that the earliest abnormality in *cmr* is a retina-wide RPE-PR interface alteration,[50] caused by impaired calcium signaling. The anatomical apposition and sustained interaction between RPE apical microvilli (MVs) and PR OSs (POSs) are considered crucial for normal vision.[50] MV extensions expand the functional surface of a single RPE cell by 20- to 30-fold in the central retina,[54] and by approximately 50-fold for the small RPE cells in the macular region.[55] Therefore, the underdevelopment of these apical projections in *cBEST1*-mutant eyes results in a decreased number of transport and signaling molecules and, ultimately, in a reduced adhesiveness with POS.[56] This RPE-PR interface disruption manifests on OCT as a hyporeflective layer located distal to the outer nuclear layer (ONL). These diffuse microdetachments, which have been detectable as early as 11 weeks of age, well before any other ophthalmoscopic lesion, were shown to expand with light exposure *in vivo*.[50] From this subclinical stage, *cmr* progresses to form a macrodetachment limited to the canine fovea and surrounded by microdetachments. The advanced stages were characterized by a partial resorption and dispersion of the vitelliform material within the central lesion, associated with significant thinning of the ONL.

Because of its similarity to human bestrophinopathy, *cmr* is particularly suited for carrying out mechanistic studies, as well as for the development and testing of therapeutic strategies, such as recombinant AAV-based gene augmentation therapy. AAV-mediated subretinal injection of canine ortholog (*cBEST1*) or human *BEST1* (*hBEST1*) transgene in 22 *cBEST1* eyes with different genotypes has yielded promising results. Both *cBEST1* and *hBEST1* treatments determined an early (4–12 weeks postinjection) lesion reversal with a sustained long-term effect (up to 245 weeks postinjection), and neither was associated with inflammatory responses.[50] Gene replacement therapy also corrected the light-modulated microdetachments, as demonstrated by the substantial reduction of the distance between the inner segment (IS)/OS band and the RPE-tapetum interface. These results suggest that this approach can restore the cytoarchitecture of the RPE-PR interface at a molecular level, indicating a potential for treatment of both early and advanced stages of AR disease.

While *cmr* is well suited for the development of treatment strategies, the utility of other animals as disease models is less clear. *BEST1*-/-knockout mice do not exhibit a phenotype reminiscent of bestrophinopathy, and no differences in chloride currents were found between *BEST1* knockout and WT mice.[57] In contrast, knock-in mice carrying the BVMD-causing W93C mutation, common in human patients, show a phenotype that is similar to BVMD, including a dominant inheritance and incomplete penetrance,[58] as well as a reduced LP on EOG[59] and the development of ophthalmoscopically evident serous retinal detachments.[8]

Insights from *In vitro* studies

An alternative to animal models, stem cells can be derived from patients' fibroblasts to obtain the so-called induced pluripotent stem cells (iPSCs), which can then be differentiated into RPE (iPSC-RPEs). iPSC-RPEs provide a useful experimental system to study bestrophinopathies and to assess the efficacy of gene therapy on specific patient-derived mutations. Li *et al*. reported that impaired Cl − current in RPE derived from an ARB patient was rescuable by baculovirus (BV)-mediated supplementation of the WT *BEST1* gene.[49] While these results provided proof of concept for treating recessive variants by

gene replacement, most *BEST1* mutations are dominantly inherited. As canines do not have *BEST1* dominant mutation genotypes and *BEST1* knockout mice do not show any retinal phenotype or Cl − current abnormality, iPSCs stand out as a promising model for testing the rescue of *BEST1* dominant mutations.[6]

Determining the disease-causing mechanism of dominant variants is crucial to assess the therapeutic potential of gene replacement, as well as to determine the need for suppression of the mutant allele as part of the treatment strategy. Many of the dominantly inherited *BEST1* pathogenic variants have been shown to determine a complete or partial deficiency of channel activity,[6] mostly resulting from the interaction between mutant and WT protomers, consistently with a DN effect favored by AEI of *BEST1* transcription.[7] Therefore, at least in principle, as long as the mutation does not result in a toxic GOF of the bestrophin-1 protein, it should be possible to overwhelm the mutant *BEST1* by delivering an excess of WT *BEST1*. Importantly, overexpression of WT *hBEST1* has been shown to be well tolerated in a canine model.[53]

Patient-derived RPEs of dominantly inherited *BEST1* mutations result in defective channel activity at a molecular level and were successfully rescued by gene augmentation via BV, AAV,[6] and lentivirus vectors.[60] For non-GOF variants, supplementation restores the diminished Cl − currents in iPSC-RPEs with the same dose and time dependency regardless of the mutation type (dominant vs. recessive) or deficiency level (null vs. partial).[6]

The only pathogenic variants that are not amenable to gene replacement alone are those acting through a GOF molecular mechanism. These are the mutations that display a DN behavior when co-expressed at a 1:1 (or even lower) ratio with WT *BEST1*. There are two strategies to overcome the DN effect of GOF mutations: specific silencing of the mutant allele or nonselective silencing of both endogenous alleles and simultaneous supplementation of an exogenous WT gene. A CRISPR/Cas9-based gene silencing vector (BVSi) was used in three iPSC-RPEs of GOF mutations to suppress the endogenous *BEST1* expression and successfully coupled with BV-mediated augmentation.[7] The restoration of Cl − currents to WT levels for all three mutations provides evidence that the silencing plus augmentation strategy may be feasible for the treatment of all bestrophinopathies.[7]

Conclusion

The retina has been the frontier of translational gene therapy for over two decades. Although there is yet to be an active clinical trial for any of the bestrophinopathies, *BEST1* is one of the most frequently mutated genes in the IRD population[1] and the associated genotype has a number of characteristics that make it suited for gene therapy. Bestrophinopathies are slowly progressive diseases with a wide window of opportunity for clinical trial success, including clear and quantifiable endpoints, broad treatment window, and relatively highly affected population.[21] At a molecular level, *BEST1* mutations have been shown to mostly act through a LOF or a DN mechanism,[6,21] both of which can be effectively treated by gene augmentation.[6,7,50] In addition, the combination of gene silencing and gene supplementation holds promise as an effective strategy for treating the smaller subset of patients harboring GOF variants.[7]

In conclusion, over the last decade, a conspicuous body of literature has supported the efficacy of gene therapy approaches in all types of *BEST1* mutations, providing hope for clinical translation in the near future.

Financial support and sponsorship

This work was supported by the National Institutes of Health (Bethesda, MD) P30 EY010572 core grant, the Malcolm M. Marquis, MD Endowed Fund for Innovation, and an unrestricted grant from Research to Prevent Blindness (New York, NY) to Casey Eye Institute, Oregon Health and Science University and the Foundation Fighting Blindness TRAP1 Award TA-NMT-0521-0803-OHSU-TRAP (Columbia, MD) (PY).

Conflicts of interest

There are no conflicts of interest.

References

1. Daich Varela M, Georgiadis A, Michaelides M. Genetic treatment for autosomal dominant inherited retinal dystrophies: approaches, challenges and targeted genotypes. Br J Ophthalmol 2023;107:1223-30. doi:10.1136/bjo-2022-321903.
2. Amato A, Arrigo A, Aragona E, Manitto MP, Saladino A, Bandello F, *et al.* Gene therapy in inherited retinal diseases: An update on current state of the art. Front Med (Lausanne) 2021;8:750586.
3. Guziewicz KE, Zangerl B, Lindauer SJ, Mullins RF, Sandmeyer LS, Grahn BH, *et al.* Bestrophin gene mutations cause canine multifocal retinopathy: A novel animal model for best disease. Invest Ophthalmol Vis Sci 2007;48:1959-67.
4. Hoffmann I, Guziewicz KE, Zangerl B, Aguirre GD, Mardin CY. Canine multifocal retinopathy in the Australian Shepherd: A case report. Vet Ophthalmol 2012;15 Suppl 2:134-8.
5. Zangerl B, Wickström K, Slavik J, Lindauer SJ, Ahonen S, Schelling C, *et al.* Assessment of canine BEST1 variations identifies new mutations and establishes an independent bestrophinopathy model (*cmr3*). Mol Vis 2010;16:2791-804.
6. Ji C, Li Y, Kittredge A, Hopiavuori A, Ward N, Yao P, *et al.* Investigation and restoration of BEST1 activity in patient-derived RPEs with dominant mutations. Sci Rep 2019;9:19026.
7. Zhao Q, Kong Y, Kittredge A, Li Y, Shen Y, Zhang Y, *et al.* Distinct expression requirements and rescue strategies for BEST1 loss- and gain-of-function mutations. Elife 2021;10:e67622.
8. Johnson AA, Guziewicz KE, Lee CJ, Kalathur RC, Pulido JS, Marmorstein LY, *et al.* Bestrophin 1 and retinal disease. Prog Retin Eye Res 2017;58:45-69.
9. Marmorstein AD, Marmorstein LY, Rayborn M, Wang X, Hollyfield JG, Petrukhin K. Bestrophin, the product of the best vitelliform macular dystrophy gene (VMD2), localizes to the basolateral plasma membrane of the retinal pigment epithelium. Proc Natl Acad Sci U S A 2000;97:12758-63.
10. Milenkovic VM, Röhrl E, Weber BH, Strauss O. Disease-associated missense mutations in bestrophin-1 affect cellular trafficking and anion conductance. J Cell Sci 2011;124:2988-96.
11. Barro-Soria R, Aldehni F, Almaça J, Witzgall R, Schreiber R, Kunzelmann K. ER-localized bestrophin 1 activates Ca2+-dependent

ion channels TMEM16A and SK4 possibly by acting as a counterion channel. Pflugers Arch 2010;459:485-97.
12. Gomez NM, Tamm ER, Straubeta O. Role of bestrophin-1 in store-operated calcium entry in retinal pigment epithelium. Pflugers Arch 2013;465:481-95.
13. Marmorstein AD, Kinnick TR, Stanton JB, Johnson AA, Lynch RM, Marmorstein LY. Bestrophin-1 influences transepithelial electrical properties and Ca2+signaling in human retinal pigment epithelium. Mol Vis 2015;21:347-59.
14. Milenkovic A, Brandl C, Milenkovic VM, Jendryke T, Sirianant L, Wanitchakool P, *et al.* Bestrophin 1 is indispensable for volume regulation in human retinal pigment epithelium cells. Proc Natl Acad Sci U S A 2015;112:E2630-9.
15. Moshfegh Y, Velez G, Li Y, Bassuk AG, Mahajan VB, Tsang SH. BESTROPHIN1 mutations cause defective chloride conductance in patient stem cell-derived RPE. Hum Mol Genet 2016;25:2672-80.
16. Burgess R, Millar ID, Leroy BP, Urquhart JE, Fearon IM, De Baere E, *et al.* Biallelic mutation of BEST1 causes a distinct retinopathy in humans. Am J Hum Genet 2008;82:19-31.
17. Rosenthal R, Bakall B, Kinnick T, Peachey N, Wimmers S, Wadelius C, *et al.* Expression of bestrophin-1, the product of the VMD2 gene, modulates voltage-dependent Ca2+channels in retinal pigment epithelial cells. FASEB J 2006;20:178-80.
18. Yu K, Xiao Q, Cui G, Lee A, Hartzell HC. The best disease-linked Cl- channel *hBest1* regulates Ca V 1 (L-type) $Ca2^{+}$ channels via src-homology-binding domains. J Neurosci 2008;28:5660-70.
19. Kane Dickson V, Pedi L, Long SB. Structure and insights into the function of a Ca (2+)-activated Cl(-) channel. Nature 2014;516:213-8.
20. Yang T, Liu Q, Kloss B, Bruni R, Kalathur RC, Guo Y, *et al.* Structure and selectivity in bestrophin ion channels. Science 2014;346:355-9.
21. Yang T, Justus S, Li Y, Tsang SH. BEST1: The best target for gene and cell therapies. Mol Ther 2015;23:1805-9.
22. Casalino G, Khan KN, Armengol M, Wright G, Pontikos N, Georgiou M, *et al.* Autosomal recessive bestrophinopathy: Clinical features, natural history, and genetic findings in preparation for clinical trials. Ophthalmology 2021;128:706-18.
23. Schachat A, Wilkinson CP, Hinton DR, Sadda SR. Ryan's Retina.Ch. 10, 269. Elsevier, Amsterdam, 2018.
24. Khan KN, Islam F, Holder GE, Robson A, Webster AR, Moore AT, *et al.* Normal electrooculography in best disease and autosomal recessive bestrophinopathy. Retina 2018;38:379-86.
25. Lacassagne E, Dhuez A, Rigaudière F, Dansault A, Vêtu C, Bigot K, *et al.* Phenotypic variability in a French family with a novel mutation in the BEST1 gene causing multifocal best vitelliform macular dystrophy. Mol Vis 2011;17:309-22.
26. Walter P, Brunner R, Heimann K. Atypical presentations of best's vitelliform macular degeneration: Clinical findings in seven cases. Ger J Ophthalmol 1994;3:440-4.4).
27. Boon CJ, Klevering BJ, Leroy BP, Hoyng CB, Keunen JE, den Hollander AI. The spectrum of ocular phenotypes caused by mutations in the BEST1 gene. Prog Retin Eye Res 2009;28:187-205.
28. Querques G, Zerbib J, Georges A, Massamba N, Forte R, Querques L, *et al.* Multimodal analysis of the progression of best vitelliform macular dystrophy. Mol Vis 2014;20:575-92.
29. Querques G, Regenbogen M, Quijano C, Delphin N, Soubrane G, Souied EH. High-definition optical coherence tomography features in vitelliform macular dystrophy. Am J Ophthalmol 2008;146:501-7.
30. Parodi MB, Iacono P, Del Turco C, Bandello F. Near-infrared fundus autofluorescence in subclinical best vitelliform macular dystrophy. Am J Ophthalmol 2014;158:1247-52.e2.
31. Parodi MB, Arrigo A, Bandello F. Optical coherence tomography angiography quantitative assessment of macular neovascularization in best vitelliform macular dystrophy. Invest Ophthalmol Vis Sci 2020;61:61.
32. Arora R, Khan K, Kasilian ML, Strauss RW, Holder GE, Robson AG, *et al.* Unilateral BEST1-associated retinopathy. Am J Ophthalmol 2016;169:24-32.
33. Kaden TR, Tan AC, Feiner L, Freund KB. Unilateral best disease: A case report. Retin Cases Brief Rep 2017;11 Suppl 1:S191-6.
34. Querques G, Regenbogen M, Soubrane G, Souied EH. High-resolution spectral domain optical coherence tomography findings in multifocal vitelliform macular dystrophy. Surv Ophthalmol 2009;54:311-6.
35. Chowers I, Tiosano L, Audo I, Grunin M, Boon CJ. Adult-onset foveomacular vitelliform dystrophy: A fresh perspective. Prog Retin Eye Res 2015;47:64-85.
36. Kaufman SJ, Goldberg MF, Orth DH, Fishman GA, Tessler H, Mizuno K. Autosomal dominant vitreoretinochoroidopathy. Arch Ophthalmol 1982;100:272-8.
37. Burgess R, MacLaren RE, Davidson AE, Urquhart JE, Holder GE, Robson AG, *et al.* ADVIRC is caused by distinct mutations in BEST1 that alter pre-mRNA splicing. J Med Genet 2009;46:620-5.
38. Reddy MA, Francis PJ, Berry V, Bradshaw K, Patel RJ, Maher ER, *et al*. A clinical and molecular genetic study of a rare dominantly inherited syndrome (MRCS) comprising of microcornea, rod-cone dystrophy, cataract, and posterior staphyloma. Br J Ophthalmol 2003;87:197-202.
39. Yardley J, Leroy BP, Hart-Holden N, Lafaut BA, Loeys B, Messiaen LM, *et al.* Mutations of VMD2 splicing regulators cause nanophthalmos and autosomal dominant vitreoretinochoroidopathy (ADVIRC). Invest Ophthalmol Vis Sci 2004;45:3683-9.
40. Davidson AE, Millar ID, Urquhart JE, Burgess-Mullan R, Shweikh Y, Parry N, *et al.* Missense mutations in a retinal pigment epithelium protein, bestrophin-1, cause retinitis pigmentosa. Am J Hum Genet 2009;85:581-92.
41. raboulsi, E. Genetic diseases of the eye (2 edn). Oxford University Press, Oxford, 2012.
42. Dalvin LA, Pulido JS, Marmorstein AD. Vitelliform dystrophies: Prevalence in Olmsted County, Minnesota, United States. Ophthalmic Genet 2017;38:143-7.
43. Schatz P, Klar J, Andréasson S, Ponjavic V, Dahl N. Variant phenotype of best vitelliform macular dystrophy associated with compound heterozygous mutations in VMD2. Ophthalmic Genet 2006;27:51-6.
44. Khojasteh H, Azarmina M, Ebrahimiadib N, Daftarian N, Riazi-Esfahani H, Naraghi H, *et al.* Autosomal Recessive Bestrophinopathy: Clinical and Genetic Characteristics of Twenty-Four Cases. J Ophthalmol 2021:6674290. doi:10.1155/2021/6674290.
45. Pomares E, Burés-Jelstrup A, Ruiz-Nogales S, Corcóstegui B, González-Duarte R, Navarro R. Nonsense-mediated decay as the molecular cause for autosomal recessive bestrophinopathy in two unrelated families. Invest Ophthalmol Vis Sci 2012;53:532-7.
46. Backwell L, Marsh JA. Diverse molecular mechanisms underlying pathogenic protein mutations: Beyond the loss-of-function paradigm. Annu Rev Genomics Hum Genet 2022;23:475-98.
47. Llavona P, Pinelli M, Mutarelli M, Marwah VS, Schimpf-Linzenbold S, Thaler S, *et al.* Allelic expression imbalance in the human retinal transcriptome and potential impact on inherited retinal diseases. Genes (Basel) 2017;8:283.
48. Lee JH, Oh JO, Lee CS. Induced pluripotent stem cell modeling of best disease and autosomal recessive bestrophinopathy. Yonsei Med J 2020;61:816-25.
49. Li Y, Zhang Y, Xu Y, Kittredge A, Ward N, Chen S, *et al.* Patient-specific mutations impair BESTROPHIN1's essential role in mediating Ca (2+)-dependent Cl(-) currents in human RPE. Elife 2017;6:e29914.
50. Guziewicz KE, Cideciyan AV, Beltran WA, Komáromy AM, Dufour VL, Swider M, *et al.* BEST1 gene therapy corrects a diffuse retina-wide microdetachment modulated by light exposure. Proc Natl Acad Sci U S A 2018;115:E2839-48.
51. Castle MJ, Turunen HT, Vandenberghe LH, Wolfe JH. Controlling AAV tropism in the nervous system with natural and engineered capsids. Methods Mol Biol 2016;1382:133-49.
52. Duncker T, Greenberg JP, Ramachandran R, Hood DC, Smith RT, Hirose T, *et al.* Quantitative fundus autofluorescence and optical coherence tomography in best vitelliform macular dystrophy. Invest Ophthalmol Vis Sci 2014;55:1471-82.
53. Guziewicz KE, Zangerl B, Komáromy AM, Iwabe S, Chiodo VA, Boye SL, *et al.* Recombinant AAV-mediated BEST1 transfer to the retinal pigment epithelium: Analysis of serotype-dependent retinal

effects. PLoS One 2013;8:e75666.
54. Miller SS, Steinberg RH. Passive ionic properties of frog retinal pigment epithelium. J Membr Biol 1977;36:337-72.
55. Volland S, Esteve-Rudd J, Hoo J, Yee C, Williams DS. A comparison of some organizational characteristics of the mouse central retina and the human macula. PLoS One 2015;10:e0125631.
56. Guziewicz KE, Sinha D, Gómez NM, Zorych K, Dutrow EV, Dhingra A, *et al.* Bestrophinopathy: An RPE-photoreceptor interface disease. Prog Retin Eye Res 2017;58:70-88.
57. Marmorstein LY, Wu J, McLaughlin P, Yocom J, Karl MO, Neussert R, *et al.* The light peak of the electroretinogram is dependent on voltage-gated calcium channels and antagonized by bestrophin (best-1). J Gen Physiol 2006;127:577-89.
58. Zhang Y, Stanton JB, Wu J, Yu K, Hartzell HC, Peachey NS, *et al.* Suppression of Ca2+signaling in a mouse model of Best disease. Hum Mol Genet 2010;19:1108-18.
59. Stanton JB, Wu J, McLaughlin PJ, Marmorstein N, Peachey N, Marmorstein AD. A Comparison of the Phenotypes of Bestrophin Knock-In and Knock-Out Mice: Implications for the Pathogenesis of best Vitelliform Macular Degeneration. ARVO Annual Meeting Abstract (2008).
60. Sinha D, Steyer B, Shahi PK, Mueller KP, Valiauga R, Edwards KL, *et al.* Human iPSC modeling reveals mutation-specific responses to gene therapy in a genotypically diverse dominant maculopathy. Am J Hum Genet 2020;107:278-92.