Medical Sciences

Insights from Genetic Model Systems of Retinal Degeneration: Role of Epsins in Retinal Angiogenesis and VEGFR2 Signaling

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The retina is a light sensitive tissue that contains specialized photoreceptor cells called rods and cones which process visual signals. These signals are relayed to the brain through interneurons and the fibers of the optic nerve. The retina is susceptible to a variety of degenerative diseases, including age-related macular degeneration (AMD), diabetic retinopathy (DR), retinitis pigmentosa (RP) and other inherited retinal degenerations. In order to reveal the mechanism underlying these diseases and to find methods for the prevention/treatment of retinal degeneration, animal models have been generated to mimic human eye diseases. In this paper, several well-characterized and commonly used animal models are reviewed. Of particular interest are the contributions of these models to our understanding of the mechanisms of retinal degeneration and thereby providing novel treatment options including gene therapy, stem cell therapy, nanomedicine, and CRISPR/Cas9 genome editing. Role of newly-identified adaptor protein epsins from our laboratory is discussed in retinal angiogenesis and VEGFR2 signaling.

Retinal Degeneration | CRISPR/Cas9 | Genome Editing Technology | Epsins | VEGFR2 Signaling

Introduction

It is estimated that more than 15 million people in the world suffer from vision loss due to inherited retinal diseases. Although the wide variety of the causes (mutation of genes and variants leading to vast differences in phenotype), photoreceptor death is a common outcome. While inherited retinal degenerations are currently incurable, animal models provide significantly useful tools to investigate the mechanisms underlying the diseases and thereby produce potential treatments for vision loss.

Two strategies have been commonly used to study the mechanisms of human ocular diseases in animal models: phenotype-driven and genotype-driven. Phenotype-driven studies start with an animal model exhibiting a phenotype of interest and consequently identify the gene(s) responsible for the phenotype. Genotype-driven studies take advantage of genetic engineering approaches, such as the creation of transgenics, knockouts and knockins, to mutate genes known to cause human disease and thereafter examine the associated phenotype [*1*]. Animal models for retinal degeneration can be categorized into two types: naturally occurring and genetically modified. The former category includes the *rodless* mouse model (termed *rd1*) with a defect subsequently identified in the *Pde6b* (rod cGMP-specific 3',5'-cyclic phosphodiesterase subunit beta) gene [*2*]; *rd2* with a defect in retinal degeneration slow ($Rds^{-/-}$) [*3*], *rd3* [*4*], *rd4* [*5*], *rd5* with a defect in *tubby* gene ($Tub^{-/-}$) [*6*], *rd6* [*7*], *rd7* ($Nr2e3^{-/-}$, nuclear receptor subfamily 2, group E, member 3) [*8*]. Examples of genetically engineered models for inherited retinal degenerations include transgenic mice expressing mutant human rhodopsin (P23H) [*9*], transgenic mice with the P23H, V20G and P27L mutations (VPP) in the rhodopsin gene [*10*], knockout mice that lack the neural retina leucine zipper gene ($Nrl^{-/-}$) [*11*], the ATP-binding cassette transporter ($Abcr^{-/-}$) [*12*], and the cone-rod homeobox gene ($Crx^{-/-}$) [*13*]. The remainder of this review will examine these disease models in more depth.

1. Retinitis pigmentosa (RP)

RP is a heterogeneous group of diseases characterized by loss of night vision followed by progressive loss of peripheral vision (most severely affecting rods). Cones degeneration and loss of central vision usually occurs after the death of most rods [*14*]. The disease symptoms, electroretinographic (ERG) evaluation and genetic assessment in animal models have been reviewed [*15-17*]. The three types of RP are categorized by inheritance: autosomal recessive, autosomal dominant, and recessive/ dominant.

1.1. Animal models of recessive RP

1.1.1. *Pde6b*: PDE6b is a subunit of the rod-specific phosphodiesterase (PDE). PDEs selectively catalyze the hydrolysis of 3' cyclic phosphate bonds in guanine 3', 5' cyclic monophosphate (cGMP). Multiple transcript variants encoding different isoforms have been found for this gene. Mutations in the PDE6b gene result in human RP and are inherited in an autosomal recessive manner. A naturally occurring mutant mouse model (later determined to have a PDE6b mutation) was first described by Keeler in 1924 [*2*]. The mutant allele carries a nonsense mutation in the *Pde6b* gene caused by a viral insertion in intron 1 or nonsense mutation in exon 7 C->A transversion in codon 347 (the gene symbol for the mutant is $Pde6b^{rd1}$, formerly referred to as *rd1* or *rd)* [*18-20*]. Degeneration of the outer retina in this model starts at postnatal day (P) 8 to 10 and processes rapidly with complete loss of rods by P36 [*21*]. But ¾ cones remains at P17 (whereas only 2% of rods left), and still 1.5% of cones present at 18 months of age [21]. Photoreceptor apoptosis involves co-activation of apoptotic pathways from the mitochondrion and endoplasmic reticulum (ER) and apoptosis-inducing factor (AIF), and caspase 12 and increasing Ca^{2+} concentration also contribute to this process [*22, 23*].

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1.1.2. *Rpe65*: RPE65, retinal pigment epithelium-specific 65 kDa protein, is an important component of the visual cycle responsible for regeneration of visual pigment (11-*cis*-retinal). It is an isomerohydrolase which converts all-*trans*-retinal to 11-*cis*-retinol [*24-26*]. RPE65 has two forms: a soluble form (sRPE65) and a palmitoylated, membrane-bound form (mRPE65). mRPE65 and sRPE65 have different binding specificity and serve as regulatory proteins to control retinoid recycling [*27*]. Mutation in *RPE65* gene causes Leber's congenital amaurosis (LCA), a severe form of autosomal recessive RP (ARRP) in humans [*28*]. *Rpe65*$^{-/-}$ knockout mice were created by replacing the 5' flanking region (1.1 kb) containing exons 1, 2 and 3, intervening introns and 0.5 kb of intron c with the PGK (phosphoglycerate kinase I gene promoter)-neo gene cassette [*25*]. The knockout mice develop a slow retinal degeneration. Retinal anatomy is normal at 7 weeks of age, but some small lipid-like droplets appear in the RPE cells. By 15 weeks of age, rod outer segment (OS) length is shortened [*25*]. Photoreceptor degeneration is age-dependent [*25*] and peaks at 18-24 months [*29*]. This mouse has nearly undetectable rod ERG responses at a very early age while the cone function remains up to 4 months of age [*25*]. *Rpe65*$^{-/-}$ retinas have no 11-*cis*-retinal and therefore unable to synthesis and recycle rhodopsin; they also lack 11-*cis*-retinyl esters, and accumulate all-*trans*-retinyl esters in the RPE cells [*25*]. A naturally occurring mouse model containing a nonsense mutation in exon of the *RPE65* gene (*Rpe65*^{rd12}) was also identified. The rd12 mutant allele has a C -> T transition, creating a premature stop codon [*30*]. The retinal phenotype of rd12 mice is similar to *Rpe65*$^{-/-}$ mice but exhibits small white dots spread throughout the fundus at 5-9 months of age [*30*]. By 7 months, the OS becomes shorter and the nuclear number of photoreceptors (in the outer nuclear layer-ONL) is reduced to about 30% of wild type [*30*]. The naturally occurring Swedish Briard dog model contains a 4-nucleotide (AAGA) deletion in the *Rpe65* gene at position 487-490 bp resulting in a frameshift and premature termination in exon 5 [*31*]. The mutant dog exhibits slow progressive retinal degeneration and abnormal ERG (no detectable rod responses and very low cone responses) at 5 weeks of age. At older ages (2.5 years old), large lipid-like inclusions become apparent in the RPE cells, the OSs are disorganized and the number of photoreceptors declines from the periphery to the central retina, although the fundus is normal [*32*]. Recently, an interesting publication indicated that suppressing thyroid hormone signaling reduces cone death in this mouse [*33*].

1.1.3. *Nr2e3*: Nr2e3 is an orphan nuclear receptor that is a ligand-dependent transcription factor and has dual regulatory function: it activates rod development and suppresses cone development. *NR2E3*-associated disease in humans is characterized by S cone hyperfunction (enhanced S-cone syndrome), thickened retinal layers, but an otherwise normally structured retina. Lamination is coarse, however, and retinas exhibit a strikingly thick and bulging appearance, localized to an annulus encircling the central fovea [*34*]. A naturally occurring mutant mouse model, rd7, was determined to have a 380 bp deletion in exons 4 and 5 of the *Nr2e3* gene resulting in a frameshift and a premature stop codon. The fundus of these mice exhibits discrete white spots over the entire retina [*8*]. At early ages, the retinas of rd7 mice contain whorls in OS structures and rosettes in the ONL. The retina has an excess S-cone with slow rod degeneration although normal rod function is detected in young mice. Eventually the mutation causes blindness [*35*].

1.1.4. *tubby:* TUB belongs to the Tubby family of bipartite transcription factors. A splicing mutation in the *tub* gene caused a G->T transversion at the 3' end of exon 11, which results in the replacement of a 44 amino acids at the C-terminal end with an intron-encoded 24 amino acids [*36*]. This mouse exhibits retinal and cochlear degeneration, maturity onset obesity, insulin resistance and reduced fertilities [*37*]. It previously was a phenotypic model for human Usher syndrome, but a human ocular disease results from mutation of the *TUB* gene exhibits the same phenotype seen in the mouse [*38*]. It has been shown that rapid photoreceptor degeneration starts at P14 [*39*] and peaks at P19, and at P28, almost half of the photoreceptors are gone [*39*]. TUB protein is involved in protein trafficking [*40*], mutation of TUB protein results in mislocalization of opsins in the retina [*39-41*]. Cai et al reported that ER stress was correlated with retinal degeneration during *tubby* retinal development, and the UPR (unfolded protein response) was dysregulated [*42*]. Moreover, prolonged ER stress condition caused heat shock protein family 70 deficiency, together with the impairment of transportation of the structural proteins to the proper site for building up photoreceptors, caused the failure of the OS formation [*42*].

1.2. Animal models of dominant RP

1.2.1. *Rds*: Retinal degeneration slow (RDS, peripherin/rds or peripherin 2) is a tetraspanin glycoprotein required for the morphogenesis and structural stability of photoreceptors. It is also necessary for proper OS orientation and disc renewal. Mutations in this gene cause dominant RP and dominant macular degeneration (MD) in humans. The naturally occurring *rds* mutant mouse contains an insertion of 9.5 kb of foreign DNA into exon 2 of the *Rds* gene and results in large transcripts but no functional RDS protein [*43*]. The phenotype of *rds*$^{+/-}$ is a typical autosomal dominant RP (ADRP) while the phenotype of *rds*$^{-/-}$ is a much severe form of RP. The *rds*$^{-/-}$ homozygous mouse completely lacks OS, has no detectable ERG, accompanying by slow photoreceptor degeneration [*3*]. The heterozygous mouse exhibits a haploinsufficiency RP phenotype characterized by progressive, early onset rod degeneration followed by late onset cone degeneration [*44, 45*]. Photoreceptors start dying at P14, and the complete loss of the cells takes almost a year. There are several other mouse models with mutation in *Rds*. The P216L mutation causes ADRP in humans, and transgenic mice carrying P216L-RDS exhibit faster photoreceptor degeneration than non-transgenic littermates and its phenotype is similar to that seen in patients [*46*]. Transgenic mice carrying an ADRP-causing mutation (C214S) and one copy of wild type RDS exhibit a phenotype similar to the *rds*$^{+/-}$ mice [*47*]. Nystuen *et al* reported that a novel *Rds* gene mutation, nmf193 mutation, was caused by a single base change that causes aberrant splicing between exons 1 and 2, and exhibited OS defects and progressive retinal degeneration. Its histological features of photoreceptor degeneration were similar to that of Nr2e3 (*rd7/rd7*) [*48*].

1.2.2. *Nrl*: Nrl (neural retinal leucine zipper), a basic motif-leucine zipper transcription factor, is a critical intrinsic regulator of photoreceptor development and function. Mutations in this gene have been identified associated with dominant RP [*49, 50*]. The lack of NRL in the genetically engineered knockout mice leads to a complete absence of rods [*11*], and conversion of rods to S-cones [*11, 51*]. The retina exhibits progressive degeneration in adulthood. Cone function is supernormal at 1 month of age and decreases by more than two-thirds between 2 to 7 months of age [*52*]. The adult retina contains rosettes throughout the entire ONL [*52*].

1.2.3. *Crx*: Crx (cone-rod homeobox) is a photoreceptor-specific transcription factor which plays a critical role in the differentiation of photoreceptor cells. This homeodomain protein is necessary for the differentiation and maintenance of normal cones and rods. Mutations in human *Crx* gene are associated with RP and LCA [*53*]. *Crx* knockout mice were created by the replacement of exon 3 and part of exon 4 with the PGK-neo cassette [*13*]. At all ages, $Crx^{-/-}$ retinas lack OSs thus abrogates the phototransduction pathway in photoreceptors. Degeneration of photoreceptor cells starts at P21 and is almost complete at 6 months. The expression of photoreceptor specific genes is also reduced [*13*]. Transmission and scanning electron microscopy observation of the photoreceptor development demonstrated that numerous small vesicles were scattered in the "future OS" area. Structural analysis indicates that OS morphogenesis was blocked at the elongation stage, likely due to decreased expression of required genes [*54*].

1.3. Animal models of recessive/dominant RP

1.3.1. *Rho*: Rhodopsin, a G-protein coupled receptor, is the protein required for the formation of functional retinal pigment (rhodopsin + 11-*cis*-retinal) in rods. Rhodopsin-mediated signaling starts the phototransduction cascade and is required for rod-based vision and the OS formation. Mutations in this gene cause ADRP and ARRP in humans. One of the most common dominant rhodopsin-disease causing mutations is P23H. Another most commonly used mouse (the VPP transgenic model) was created by introducing three amino acid substitutions (G->V, H->P & L->P) at the N-terminus of mouse opsin gene [*10*]. *Rho* knockout mouse develops a normal complement of rods but without OS and lost photoreceptor cells within 3 months. The visual function was mediated by cones only at the age of 8 weeks [*55*]. ERG assessment of VPP mice demonstrated that the rod amplitude declined by 1 month and gradually decreased to 30% of normal ERG [*10*]. Machida *et al* [*56*] created P23H transgenic rat models carrying mutant P23H mouse opsin which exhibited different degeneration rate and progressive shortening rod OS and photoreceptor cell loss with age. S334ter, another rhodopsin mutation associated with RP in patients is caused by a premature termination codon at residue 334. Transgenic mice, expressing S334ter truncated mutant protein lacking the last 15 amino acids of the C-terminal, exhibit mislocalization of the truncated opsin to inner segment and ONL besides the OS, and progressive retinal degeneration [*57, 58*]. Recently, ER stress induced by aggregation and mislocalization of mutant protein is suggested to be involved in photoreceptor degeneration in P23H and S334ter mice [*59, 60*].

Taken together, RP, a genetically heterogeneous group of disorders, is classically characterized by 1) impaired rod function/night vision defects, 2) progressive retinal degeneration beginning in the midperiphery, and 3) accumulation of retinal deposits. RP usually spares the central retina in the early stages, but eventually most RP patients lose both rod and cone function.

2. Age related macular degeneration (AMD)

AMD is another heterogeneous set of diseases characterized by the presence of soft and hard drusen, hyperpigmentation and hypopigmentation of the RPE, RPE atrophy, geographic atrophy, choroidal neovascularization (CNV) and later fibrous scarring. AMD is the major cause of blindness among the elderly in the developed country (review [*61*]). AMD is usually classified as two forms: "dry" AMD which involves the accumulation of debris and deposits in the outer retina, and "wet" AMD which involves angiogenesis and neovascular changes (see **3. Retinal neovascularization**). Because of the complicated etiology which involves genetic, environmental, and unknown factors, there is no animal models that truly mimic AMD phenotype, although several animal models have been generated that shed light on various aspects of macular degenerative processes. Models with features of dry AMD include $Abcr^{-/-}$ and $ELOVL4^{-/-}$ (elongation of very long-chain fatty acid-like 4). Both $Abcr^{-/-}$ and $ELOVL4^{-/-}$ cause Stargardt macular dystrophy (STGD) and share some pathological features with AMD, but $ELOVL4^{-/-}$ occurs at younger age than $Abcr^{-/-}$. The common biomarker for both AMD and STGD is accumulation of high levels of lysosomal bodies (lipofuscin) in the RPE cells, which precedes RPE atrophy and degeneration of the photoreceptors in the macula (review [*61*]). Models with features of wet AMD can be generated by either surgical or genetic (such as endothelial growth factor (VEGF) overexpression) and exhibit CNV, and ultimately functional impairment occurs.

2.1. *Abcr*: ABCR, ATP-binding cassette transporter sub-family A member 4 (ABCA4), is a multi-spanning membrane-bound protein exclusively localized in the rim region of OS discs. ABCR is hypothesized to function as an outward-directed flippase for removing all-*trans*-RAL and its derivatives from the OS disc lumen to the cytoplasmic side [*12, 62*]. Mutations in the *Abcr* gene cause a wide spectrum of retinal degenerative diseases including AMD and recessive RP in humans. The $Abcr^{-/-}$ mouse was created by replacing 4.0 kb of the promoter region plus exon 1 by a PGK-neo cassette [*12*]. These mice exhibit normal photoreceptor structure with very slow degeneration [*63*]. $Abcr^{-/-}$ have delayed rod recovery and abnormal clearance of all-*trans*-retinal from the OS, which results in the accumulation of *N*-retinylidene phosphatidylethanolamine (N-ret-PE) and all-*trans*-RAL in the OSs [*12*]. It is hypothesized that accumulation of these abnormal retinoid compounds leads to the eventual formation of high levels of small, electron dense lipofuscin granules (at 44 weeks of age), lipofusion fluorophores, A_2E and A_2PE-H2, in RPE cells [*12*].

2.2. *ELOVL4*: ELOVL4, a membrane-bound protein residing on the ER of the retina, participates in the biosynthesis of very long-chain fatty acids and docosahexaenoic acid (DHA) [*64*]. Mutations in this gene are associated with Stargardt-like macular dystrophy (STGD3) and autosomal dominant Stargardt-like macular dystrophy (ADMD), also referred to as autosomal dominant atrophic macular degeneration in humans. The transgenic mice were created by introducing 5-bp deletion of nucleotides (deletion of AACTT at 790–794 bp) of the human wild type *ELOVL4* gene [*65*]. In this mouse model, high A_2E levels (compared to wild type) in the RPE were detected at 2 months of age even in lines that express very low amounts of transgenic protein and lipofuscin accumulates in the RPE at 7 months of age [*64*]. In lines that express higher levels of the transgene, photoreceptor cells fail to form normal OS structures. The loss of 50% of photoreceptors occurs at 6 weeks, 16 weeks and 18 months in higher, middle, and lower *ELOVL4*-expressing lines, respectively [*65*]. Decline in retinal function correlates with the expression level of mutant ELOVL4 [*65*]. ELOVL 4 belongs to the ELO (elongation of long chain fatty acid) family and possesses biochemical features and therefore participation in reduction reactions during fatty acid elongation [*66*]. Further studies demonstrated that ELOVL4 is required for the synthesis of C28 and C30 saturated fatty acids (VLC-FA) and of C28-C38

very long chain polyunsaturated fatty acids (VLC-PUFA). Mutation of this gene causes the failure of protein target to the ER, the site of fatty acid biosynthesis [*67*].

2.3. *Cathepsin D*: Cathepsin D is a lysosomal aspartyl protease which is a member of the peptidase C1 family. Mutations in this gene cause early childhood blindness and progressive psychomotor disabilities [*68*]**.** Transgenic mice expressing a mutant form of cathepsin D (*mcd*) in RPE cells manifest many features of "dry" AMD [*69, 70*], including increased levels of autofluorescent debris and increased basal laminar deposits (BLD, is the accumulation of granular material with wide-spaced collagen located between the plasma membrane and the basal lamina of the RPE). *Mcd* mice exhibit photoreceptor degeneration, OS shortening, and accumulation of immunoreactive photoreceptor breakdown products in RPE cells. Accelerated photoreceptor cell death was detected in 12-month-old *mcd/mcd* mice [*69*].

2.4. *ApoE*: Apolipoprotein E deficiency produced hypercholesterol and also developed thickened lipid-rich Bruch's membrane and increased levels of autofluorescent debris [*71*]. BLD appears in the *ApoE*$^{-/-}$ mice at 2 months of age [*71*]. Besides developing a multiple pathological characters associated with human AMD, ApoE4 deficient mice also develop marked CNV [*72*].

2.5. *XLJR*: X-linked juvenile retinoschisis is an early-onset and a common form of macular degeneration in male. It is caused by a mutation in the retinoschisin (*RS1)* gene [*73*]. The retinoschisin-null mice, *Rsh1* (homolog of human *RS1* gene) deficient mice, exhibit normal scotopic a-wave but scotopic b-wave and photopic b-wave are significantly reduced. The disease is a panretinal disorder which effects the organization of the retinal cell layers and the retinal synapse structure [*74*].

2.6. *Rds*: One form of *Rds* mutation (R172W) also causes AMD. R172W transgenic mouse model exhibits significantly diminished structure and function of both cones and rods in wild type background, indicating a dominant-negative, cone–rod defect. Whereas in the *rds*$^{+/-}$ background, the mice maintained the normal cone function at early ages but cone responses were diminished to 41% of the wild type level. Conversely a significant rescue of rod function and improvement of rod OS structure were shown signifying a preferential damaging effect of the mutation on cones. Although *rds*$^{-/-}$ mice have no detectable rod or cone responses, R172W animals in *rds*$^{-/-}$ background retained 30% of wild type structure and rod function, but no significant rescue of cone function was detected at 1 month of age [*75*].

2.7. *Ccl2/Ccr2*: CCL2 (chemokine (C-C motif) ligand 2), also known as monocyte chemotactic protein-1 (MCP-1), is a small cytokine belonging to the CC chemokine family. CCL2 recruits monocytes, memory T cells, and dendritic cells to sites of tissue injury and infection. CCL2 and the cell surface receptor that binds CCL2, CCR2, are associated with macrophage mobilization. It has been reported that single nucleotide polymorphisms in Ccl2/Cx3Cr1 are associated with AMD [*76*], but no mutations have been identified in human eyes. Ccl2 and Ccr2 knockout mice were generated by targeted gene disruption and exhibit defects in monocyte recruitment to sites of inflammation [*77, 78*]. This model exhibits features of both "dry" and "wet" AMD, including drusen, lipofuscin (start at 9 months of age), Bruch's membrane thickening and disruption, RPE degeneration, ONL loss, as well as CNV and blood vessel leakage [*79*].

In summary, AMD is the leading cause of vision loss and blindness among adults over age 65 years old. Macular degeneration is diagnosed as either dry (non-neovascular) or wet (neovascular) form. AMD begins with characteristic yellow deposits (drusen) in the macula. People with drusen could develop advanced AMD.

3. Retinal neovascularization

Retinal neovascularization (RNV) is characterized with a disturbed vascular bed, formation of capillary microaneurysms, increased vascular permeability, and proliferation of the new vessels and fibrous tissues [*80, 81*]. Neovascularization (NV) is associated with a variety of conditions including vascular occlusion, sickle cell diseases, and sarcoidosis. The animal models used to study NV include models of DR, retinopathy of prematurity (ROP) and exudative (wet) AMD, in which growth of new capillaries from preexisting blood vessels in the eye causes CNV or RNV. Studies suggest that angiogenic and angiostatic factors such as VEGF [*82*] and Insulin-like growth factor (IGF) [*83*] play pivotal roles in the pathogenesis of ocular neovascularization. Animal models for NV include *vldlr*$^{-/-}$ (the very low density lipoprotein receptor knockout), laser photocoagulation-induced CNV, oxygen induced retinopathy (OIR) (also termed as ROP) [*84*]. Here we only focus on the animal models caused by gene defects.

3.1. ***Ins2* (*Akita* mice)**: Insulin is a hormone that is central to regulating the energy and glucose metabolism in the body. Mutations in the insulin gene lead to diabetic retinopathy in human beings. A naturally occurring diabetic mouse model for type I diabetes (Ins2^{Akita}) has a point mutation (a single amino acid substitution) in the Insulin 2 gene causing misfolding of the insulin protein and leading to rapid onset of hyperglycemia and hypoinsulinemia in heterozygous mice by ~4 weeks of age [*85*]. Increased retinal vascular permeability occurred at ~16 weeks, an increase in cellular capillaries, decrease in the thickness of the whole retina and the inner and outer nuclear layers were also observed at 3 months of age [*86-88*]. In addition, severe impairment of synaptic connectivity in the outer plexiform layer (OPL) and significant loss of amacrine and ganglion cells were reported in this mouse model [*87, 88*].

3.2. *Vldlr*: VLDLR is a lipoprotein receptor which plays an important role in VLDL-triglyceride metabolism and the reelin signaling pathway. Mutations in this gene cause VLDLR-associated cerebellar hypoplasia. Genetic association analysis has linked VLDLR to AMD [*89*]. The VLDLR knockout mouse (*vldlr*$^{-/-}$) is characterized with progressive NV [*90, 91*], in which new blood vessels originate from the inner retina (the OPL) and extend through the ONL to the subretinal space and choroid, and caused RPE disruption, exposure of Bruch's membrane, photoreceptor death, and significant fibrosis [*92*]. This model represents a distinct form of AMD, called RAP (retinal angiomatous proliferation). At two months of age, the *vldlr*$^{-/-}$ mice exhibit reduced mRNA levels for genes required for the phototransduction cascade (rhodopsin and cone-opsin) [*93*], but retinal degeneration and ERG decline occurred very late (4 months to 1 year) [*91*]. Recent studies suggest that inflammation, Wnt and the ASK1-P38/JNK-NF-κB signaling pathway play a pathogenic role in *vldlr* $^{-/-}$ mouse [*91, 94, 95*].

3.3. VEGF: Overexpression of VEGF in the eye often stimulates NV, which originates from the vascular bed closest to the ganglion cell layer. Genetic association analysis suggests excessive VEGF level is linked to AMD [*89*]. Several transgenic mouse models were generated by introducing different VEGF isoforms such as human $VEGF_{165}$ [*96, 97*] or murine VEGF [*98*] to the animals. Almost all the animal models display extensive and rapid NV which results in severe retinal damage [*99*].

3.4. IGF-1: IGF-1 is also known as somatomedin C or mechano growth factor. IGF-1 is a hormone similar in molecular structure to insulin. It plays an important role in childhood growth and continues to have anabolic effects in adults. Transgenic mice overexpressing IGF-1 in the retina develop vascular alterations such as pericyte loss, thickened capillary basement membrane, intraretinal microvascular abnormalities, as well as proliferative retinopathy and retinal detachment [*83*].

In summary, NV is a significant complication of several ocular disorders. Common to all the proliferative retinopathies is the initial development of retinal vascular closure. Diabetic retinopathy induces most NV events. Animal models open new avenues to investigate the underlying pathogenesis of NV and methods of controlling this important ocular complication.

Perspectives of therapeutic treatment of retinal degenerative diseases

Animal models have been widely used in preclinical drug screening for human diseases, including eye diseases. Historically, gene therapies are virus-based and exhibit high transduction efficiency (review [*100-102*]). In recent years, rAAV (recombinant adeno-associated virus) has been safely and successfully employed to deliver *RPE65* gene to animal models including *Rpe65*$^{-/-}$ mice, *Rpe65*^{rd12} mice, and *Rpe65*$^{-/-}$ dogs (with functional rescue last 1 year and half [*103*]). Moreover, the present of subretinally delivered rAAV vectors in the retinas of dogs and primates last for several years [*104*]. These experiments have been sufficiently successful in terms of restoration of visual function, improvements in chromophore and rhodopsin levels, retardation of the photoreceptor degeneration, and visual acuity [*105-107*]. These breakthroughs have prompted the successful initiation of multiple human clinical trials (review [*108*]). Clinical trials involving 12 patients (NCT00643747) [*109, 110*], 15 patients (NCT00481546) [*111*], and 5 patients (NCT-00516477) [*112, 113*] showed improvement of retinal sensitivity up to 3 years. Significant improvement in visual function, visual acuity, and visual field up to 3 years was documented [*111, 113*]. Since many degenerative eye diseases are caused by gain-of-function mutations, treatments often have to include both a knockdown and a gene replacement component. For example, RNA interference–based suppression of mutant rhodopsin, together with gene replacement significant improved retinal function in a *Rho*-adRP mouse model [*114*]. For loss-of-function mutations, gene replacement strategies have been the most common. Several gene replacement studies using RDS have been undertaken using AAV vector [*115, 116*]. After delivery of AAV containing RDS, rods form stable OS discs [*115*] and retinal function is improved [*116*]. Rescue of rd1 mice is difficult because of too fast retinal degeneration. So far, only partial structural preservation and minimal function rescue were achieved in this mouse model. Ali group reported that AAV-mediated gene replacement therapy with the removal of *Gpr179* mutation results in robust structural (80% of wild type), functional (60% of wild type) and behavioral rescue (appears as normal visual acuity) in rd1 mice [*117*]. Combinatorial delivery of the neurotrophic factor GDNF (glial cell line-derived neurotrophic factor) and *Rds* gene by AAV to the *rds*$^{-/-}$ retina, resulted in significantly improved retinal function when compared to a *Rds*-replacement therapy alone [*118*]. In spite of these successes, rAAV-based treatments have traditionally been limited by the capacity of the vector. However, a recent study demonstrated that modified rAAV 2/5 can package the 6.8 kb mouse *Abcr* gene (much larger than the initial AAV capacity of 4.8 kb) [*119*] and when delivered to the *Abcr*$^{-/-}$ mouse retina resulted in reduced A_2E levels in RPE cells and increased ABCR protein expression. In an AAV-mediated secretion gene therapy (AAV.sFlt-1) in diseased mice and monkey, the expression of the transgene lasted for up to 8 months and the reduction of CNV in all treated eyes was observed [*96*]. Furthermore, rAAV.sFLT-1 clinical trial for treatment of "wet" AMD patient was carried out and 9 patients were enrolled for the subretinal injection (NTC01494805). Clinical assessment demonstrated that rAAV.sFLT-1 was safe and well-tolerated [*120*]. In addition, AAV has also been employed to deliver neuroprotective factors alone such as PEDF (pigment epithelium-derived factor) to a mouse CNV model [*121*], GDNF to S334ter rats with rhodopsin mutation [*122*] and CNTF (ciliary neurotrophic factor) to *rds*$^{-/-}$ mice [*123*], P23H and S334ter rats [*124*], and rd1 mice [*125*], and resulted in effective inhibition of CNV, retardation of photoreceptor apoptosis and delayed retinal degeneration.

Nanomedicine arises as a new attractive approach for treatment of retinal degenerative diseases in the last decade. Using polyethylene glycol (PEG) compacted DNA nanoparticles, Cai et al successfully achieved significantly structural and functional improvement of the *rds*$^{+/-}$ mouse model after subretinal delivery of the wild type *Rds* gene at P5 and P22 [*126, 127*]. In addition, Cai et al using catalytic and direct antioxidants cerium oxide nanoparticles (nanoceria) to treat *tubby* and *vldlr*$^{-/-}$ young adult mice at P28 by intravitreal injection resulted in sustained protection against retinal degeneration in *tubby* mice [*41*] and regression of existing neovascularization in *vldlr*$^{-/-}$ mice [*95*].

In recent years, the use of stem cells (SCs) and regenerative medicine have attracted great attention in both the scientific community and the public. Regenerative therapy for retinal degeneration in animal models holds a promise to restore vision in the diseased eye. Induced pluripotent SCs (iPSCs), with capacity to differentiate into specialized cells, are the most promising candidates for clinical application [*128*]. Despite encouraging results, iPSCs will likely need to be used in combination with up-to-date tissue engineering to achieve ultimate success [*128*]. Several recent reports claim successful improvement of vision in different animal models such as *Rpe65*$^{-/-}$ mice [*129*], Royal College Surgeons rat [*130*] and *Crx*$^{-/-}$ mice [*131*]. Human embryonic SCs (HESCs)-derived retina tissues were transplanted into two primate models of photoreceptor degeneration, a range of retinal cell types was differentiated with rods and cones developed structured ONL [*132*]. Two clinical trials enrollment of 9 patients each (NCT01345006 for Stargardt's macular dystrophy, and NCT01344993 for "dry" AMD) for subretinal transplantation of hESC-derived RPE, up to 3 year follow up demonstrated best-correction of visual acuity which was significantly improved and remained stable for 3 years. In addition, increased subretinal pigment was present at the border of the atrophic area, suggesting successful cellular engraftment. No adverse side effects were observed [*133, 134*]. Although much progress has been made, plentiful challenges remain to be solved. A major obstacle to developing stem cell-based therapies for degenerative retinal disorders is the poor integration and

differentiation of retinal stem cells transplanted into the recipient retinas. The current challenge is to understand the developmental processes that guide embryonic or adult stem cells towards to differentiation to photoreceptor, so that large numbers of these cells might be transplanted at the optimal stage. Future advances in reproductive cloning technology could lead to the successful generation of stem cells from adult somatic cells, thereby facilitating auto-transplantation of genetically identical cells in patients requiring photoreceptor replacement [*135*]. For additional information, readers are referred to these excellent review articles [*135-141*].

Epsins are adaptor proteins in endocytosis for ubiquitylated receptors on plasma membrane [*142*]. In animal models, we have identified the functions of epsins in embryogenesis via Notch signaling was by severely reducing Notch primary target genes in DKO embryos [*143*], in vascular angiogenesis, lymphatic angiogenesis [*144*] and tumor angiogenesis [*145-148*] via VEGFR2 or VEGFR3 signaling, in colon cancer via Wnt signaling [*149*]. Treatment of pups (P3-P6) of wild type C57BL/6 by an epsin ubiquitin interacting motif (UIM) memetic peptide UPI leads to overwhelming vessels in the retina [*146*]. Loss of endothelial epsins (EC-iDKO) in mice produced heightening VEGFR2 signaling and increased postnatal retinal angiogenesis [*147*], strongly suggesting that epsins are critical for physiological retinal angiogenesis. Interestingly, physiological angiogenesis is increased by administration of UIM peptide, but not UIM $E^{3, 4, 5A}$ mutant peptide [*147*]. Whether epsins play a role in retinal degeneration and diabetic retinopathy warrants further investigation. Under pathological conditions, administration of siRNA of epsins by liposome or UPI peptide may have therapeutic outcomes.

CRISPR/Cas9 genome editing technology is emerging and develops very quickly in recent years [*150-156*]. Using this new technology, several groups have obtained promising results to correct (or repair) mutant gene sequences from patient-derived iPSCs to treat inherited ocular diseases including RP, AMD, and other retinal diseases (review [*157*]). Recently, CRISPR/Cas9 system was used to identify the disease-causative gene in the *rd1* mice and genome editing-generated CRISPR-repaired mice showed robust rescue of the neurofunction [*158*]. Bakondi B. et al reported that disruption of a dominantly genetic mutation S334ter of rhodopsin gene (Rho^{S334}) by CRISPR/Cas9 in a rat ADRP model prevent retinal degeneration and improve visual function [*159*]. Furthermore, Bassuk AG et al generated iPSCs from a patient with a mutation in the retinitis pigmentosa GTPase regulator (RPGR) gene, which causes an aggressive, X-linked variant of RP (XLRP) [*160*]. About 13% of RPGR gene copies showed mutation correction and conversion to the wild type allele. This is the first report using CRISPR to correct a pathogenic mutation in iPSCs derived from a patient with photoreceptor degeneration. This important proof-of-concept finding supports the development of personalized iPSC-based transplantation therapies for retinal diseases [*160*]. These reports strongly support that CRISPR/Cas9 genome editing technology holds great promising to correct genetic mutations of retinal degeneration.

Conclusion

The extensive search for naturally occurring retinal degenerative models has been long but successful. Genetic alteration of genes has significantly increased the pool of animal models. The study of physiology and pathology in these animal models has accumulated abundant knowledge about the genetics, biochemistry, signaling, metabolism, as well as structure and function underlying the pathology of retinal degenerations, especially through inflammation-mediated signaling pathways. These animal models provide a platform for scientists to uncover the underlying molecular mechanisms and hold promise in preclinical drug screening and potential gene therapy or stem cell therapy for the treatment of human ocular diseases. However, it should be noted that each animal model could not completely reproduce human retinal degeneration diseases. Drug screening from animal models needs further validation using other approach. Besides, animal model of retinal degeneration *per se* may contain other complications. For example, *tubby* mice are obese and hard to reproduce offspring. Therefore, each animal model has its limitations in the identification of molecular mechanism or in preclinical drug screening. Targeting retinal degeneration-associated proteins including epsins by siRNA-liposome technology or by CRISPR/Cas9 genome editing is a perspective to correct or repair genetic mutation-induced retinal degeneration.

Abbreviation

ABCR: ATP-binding cassette transporter; ADRP: autosomal dominant retinitis pigmentosa; AMD: age-related macular degeneration; ADMD: autosomal dominant Stargardt-like macular dystrophy; ARRP: autosomal recessive retinitis pigmentosa; ARVO: The Association for Research in Vision and Ophthalmology; BLD: basal laminar deposit; Cas-9: CRISPR associated protein 9; ccl2: CC-cytokin ligand 2; ccr2: CC-cytokine receptor 2; CNTF: ciliary neurotrophic factor; CNV: choroidal neovascularization; CRISPR: Clustered Regularly Interspaced Short Palindromic Repeat; Crx: cone–rod homeobox; DHA: docosahexaenoic acid; DR: diabetic retinopathy; ELO: elongation of long chain fatty acid; ELOVL4: elongation of very long chain fatty acid protein 4; ER: endoplasmic reticulum; ERG: Electroretinography; GDNF: glial cell line-derived neurotrophic factor; HESCs: human embryonic stem cells; IGF: Insulin-like growth factor; iPSCs: induced pluripotent stem cells; LCA: Leber's congenital amaurosis; Mcd: mutation form of cathepsin D; Nrl: neural retina leucine zipper; Nr2e3: nuclear receptor subfamily 2, group E, member 3; OIR: oxygen induced retinopathy; ONL: outer nuclear layer; OPL: outer plexiform layer; OS: outer segment; Pde6b: rod cGMP-specific 3',5'-cyclic phosphodiesterase subunit beta; PEDF: pigment epithelium-derived factor; PGK: phosphoglycerate kinase I; rAAV: recombinant adeno-associated virus; RAP: retinal angiomatous proliferation; rd: retinal degeneration; Rds: retinal degeneration slow; Rho: rhodopsin; RNV: retinal neovascularization; ROP: retinopathy of prematurity; RP: retinitis pigmentosa; RPE: retinal pigment epithelium; RPE65: Retinal pigment epithelium-specific 65 kDa protein; RPGR: retinitis pigmentosa GTPase regulator; *RS1:* retinoschisin; SCs: Stem cells; STGD: Stargardt macular dystrophy; VEGF: vascular endothelial growth factor; VEGFR2: vascular endothelial growth factor receptor 2; VLDLr: very low density lipoprotein receptor; XLJR: linked juvenile retinoschisis.

Authors' contributions

All authors read and approved the manuscript. YD and XC initiated the topic and co-wrote the manuscript. YW, YL, LD, and HC contributed to discuss and edit the article.

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[1] K. Paigen, "Understanding the human condition: experimental strategies in mammalian genetics", *Institute for Laboratory Animal Research,* vol. *43*, no. 3, pp. 123-135, 2002.

[2] C. E. Keeler, "The Inheritance of a Retinal Abnormality in White Mice", *Proceedings of the National Academy of Sciences of the United States of America,* vol. *10*, no. 7, pp. 329-333, 1924.

[3] S. Sanyal, and H. G. Jansen, "Absence of receptor outer segments in the retina of rds mutant mice", *Neuroscience Letter,* vol. *21*, no. 1, pp. 23-26, 1981.

[4] B. Chang, J. R. Heckenlively, N. L. Hawes, and T. H. Roderick, "New mouse primary retinal degeneration (rd-3)", *Genomics,* vol. *16*, no. 1, pp. 45-49, 1993.

[5] T. H. Roderick, B. Chang, N. L. Hawes, and J. R. Heckenlively, "A new dominant retinal degeneration (Rd4) associated with a chromosomal inversion in the mouse", *Genomics,* vol. *42*, no. 3, pp. 393-396, 1997.

[6] K. Noben-Trauth, J. K. Naggert, M. A. North, and P. M. Nishina, "A candidate gene for the mouse mutation tubby", *Nature,* vol. *380*, no. 6574, pp. 534-538, 1996.

[7] N. L. Hawes, B. Chang, G. S. Hageman, S. Nusinowitz, P. M. Nishina, B. S. Schneider, R. S. Smith, T. H. Roderick, M. T. Davisson, and J. R. Heckenlively, "Retinal degeneration 6 (rd6): a new mouse model for human retinitis punctata albescens", *Investigative ophthalmology & visual science,* vol. *41*, no. 10, pp. 3149-3157, 2000.

[8] N. B. Akhmedov, N. I. Piriev, B. Chang, A. L. Rapoport, N. L. Hawes, P. M. Nishina, S. Nusinowitz, J. R. Heckenlively, T. H. Roderick, C. A. Kozak, M. Danciger, M. T. Davisson, and D. B. Farber, "A deletion in a photoreceptor-specific nuclear receptor mRNA causes retinal degeneration in the rd7 mouse", *Proceedings of the National Academy of Sciences of the United States of America,* vol. *97*, no. 10, pp. 5551-5556, 2000.

[9] J. E. Olsson, J. W. Gordon, B. S. Pawlyk, D. Roof, A. Hayes, R. S. Molday, S. Mukai, G. S. Cowley, E. L. Berson, and T. P. Dryja, "Transgenic mice with a rhodopsin mutation (Pro23His): a mouse model of autosomal dominant retinitis pigmentosa", *Neuron,* vol. *9*, no. 5, pp. 815-830, 1992.

[10] M. I. Naash, J. G. Hollyfield, M. R. Al-Ubaidi, and W. Baehr, "Simulation of human autosomal dominant retinitis pigmentosa in transgenic mice expressing a mutated murine opsin gene", *Proceedings of the National Academy of Sciences of the United States of America,* vol. *90*, no. 12, pp. 5499-5503, 1993.

[11] A. J. Mears, M. Kondo, P. K. Swain, Y. Takada, R. A. Bush, T. L. Saunders, P. A. Sieving, and A. Swaroop, "Nrl is required for rod photoreceptor development", *Nature Genetics,* vol. *29*, no. 4, pp. 447-452, 2001.

[12] J. Weng, N. L. Mata, S. M. Azarian, R. T. Tzekov, D. G. Birch, and G. H. Travis, "Insights into the function of Rim protein in photoreceptors and etiology of Stargardt's disease from the phenotype in abcr knockout mice", *Cell,* vol. *98*, no. 1, pp. 13-23, 1999.

[13] T. Furukawa, E. M. Morrow, T. Li, F. C. Davis, and C. L. Cepko, "Retinopathy and attenuated circadian entrainment in Crx-deficient mice", *Nature Genetics,* vol. *23*, no. 4, pp. 466-470, 1999.

[14] H. R. Krauss, and J. R. Heckenlively, "Visual field changes in cone-rod degenerations", *Archives of Ophthalmology,* vol. *100*, no. 11, pp. 1784-1790, 1982.

[15] J. K. Phelan, and D. Bok, "A brief review of retinitis pigmentosa and the identified retinitis pigmentosa genes", *Molecular Vision,* vol. *6*, no. pp. 116-124, 2000.

[16] W. Baehr, and J. M. Frederick, "Naturally occurring animal models with outer retina phenotypes", *Vision Research,* vol. *49*, no. 22, pp. 2636-2652, 2009.

[17] C. Dalke, and J. Graw, "Mouse mutants as models for congenital retinal disorders", *Exp Eye Res,* vol. *81*, no. 5, pp. 503-512, 2005.

[18] C. Bowes, T. Li, M. Danciger, L. C. Baxter, M. L. Applebury, and D. B. Farber, "Retinal degeneration in the rd mouse is caused by a defect in the beta subunit of rod cGMP-phosphodiesterase", *Nature,* vol. *347*, no. 6294, pp. 677-680, 1990.

[19] S. J. Pittler, and W. Baehr, "Identification of a nonsense mutation in the rod photoreceptor cGMP phosphodiesterase beta-subunit gene of the rd mouse", *Proceedings of the National Academy of Sciences of the United States of America,* vol. *88*, no. 19, pp. 8322-8326, 1991.

[20] C. Bowes, T. Li, W. N. Frankel, M. Danciger, J. M. Coffin, M. L. Applebury, and D. B. Farber, "Localization of a retroviral element within the rd gene coding for the beta subunit of cGMP phosphodiesterase", *Proceedings of the National Academy of Sciences of the United States of America,* vol. *90*, no. 7, pp. 2955-2959, 1993.

[21] L. D. Carter-Dawson, M. M. Lavail, and R. L. Sidman, "Differential effect of the rd mutation on rods and cones in the mouse retina", *Investigative ophthalmology & visual science,* vol. *17*, no. 6, pp. 489-498, 1978.

[22] D. Sanges, A. Comitato, R. Tammaro, and V. Marigo, "Apoptosis in retinal degeneration involves cross-talk between apoptosis-inducing factor (AIF) and caspase-12 and is blocked by calpain inhibitors", *Proc Natl Acad Sci U S A,* vol. *103*, no. 46, pp. 17366-17371, 2006.

[23] L. E. Johnson, T. Van Veen, and P. A. Ekstrom, "Differential Akt activation in the photoreceptors of normal and rd1 mice", *Cell Tissue Res,* vol. *320*, no. 2, pp. 213-222, 2005.

[24] M. Jin, S. Li, W. N. Moghrabi, H. Sun, and G. H. Travis, "Rpe65 is the retinoid isomerase in bovine retinal pigment epithelium", *Cell,* vol. *122*, no. 3, pp. 449-459, 2005.

[25] T. M. Redmond, S. Yu, E. Lee, D. Bok, D. Hamasaki, N. Chen, P. Goletz, J. X. Ma, R. K. Crouch, and K. Pfeifer, "Rpe65 is necessary for production of 11-cis-vitamin A in the retinal visual cycle", *Nature Genetics,* vol. *20*, no. 4, pp. 344-351, 1998.

[26] G. Moiseyev, Y. Chen, Y. Takahashi, B. X. Wu, and J. X. Ma, "RPE65 is the isomerohydrolase in the retinoid visual cycle", *Proceedings of the National Academy of Sciences of the United States of America,* vol. *102*, no. 35, pp. 12413-12418, 2005.

[27] L. Xue, D. R. Gollapalli, P. Maiti, W. J. Jahng, and R. R. Rando, "A palmitoylation switch mechanism in the regulation of the visual cycle", *Cell,* vol. *117*, no. 6, pp. 761-771, 2004.

[28] F. Marlhens, C. Bareil, J. M. Griffoin, E. Zrenner, P. Amalric, C. Eliaou, S. Y. Liu, E. Harris, T. M. Redmond, B. Arnaud, M. Claustres, and C. P. Hamel, "Mutations in RPE65 cause Leber's congenital amaurosis", *Nature Genetics,* vol. *17*, no. 2, pp. 139-141, 1997.

[29] B. Rohrer, P. Goletz, S. Znoiko, Z. Ablonczy, J. X. Ma, T. M. Redmond, and R. K. Crouch, "Correlation of regenerable opsin with rod ERG signal in Rpe65-/- mice during development and aging", *Investigative ophthalmology & visual science,* vol. *44*, no. 1, pp. 310-315, 2003.

[30] J. J. Pang, B. Chang, N. L. Hawes, R. E. Hurd, M. T. Davisson, J. Li, S. M. Noorwez, R. Malhotra, J. H. Mcdowell, S. Kaushal, W. W. Hauswirth, S. Nusinowitz, D. A. Thompson, and J. R. Heckenlively, "Retinal degeneration 12 (rd12): a new, spontaneously arising mouse model for human Leber congenital

amaurosis (LCA)", *Molecular Vision,* vol. *11*, no. pp. 152-162, 2005.

[31] A. Veske, S. E. Nilsson, K. Narfstrom, and A. Gal, "Retinal dystrophy of Swedish briard/briard-beagle dogs is due to a 4-bp deletion in RPE65", *Genomics,* vol. *57*, no. 1, pp. 57-61, 1999.

[32] K. Narfstrom, M. L. Katz, R. Bragadottir, M. Seeliger, A. Boulanger, T. M. Redmond, L. Caro, C. M. Lai, and P. E. Rakoczy, "Functional and structural recovery of the retina after gene therapy in the RPE65 null mutation dog", *Investigative ophthalmology & visual science,* vol. *44*, no. 4, pp. 1663-1672, 2003.

[33] H. Ma, A. Thapa, L. Morris, T. M. Redmond, W. Baehr, and X. Q. Ding, "Suppressing thyroid hormone signaling preserves cone photoreceptors in mouse models of retinal degeneration", *Proc Natl Acad Sci U S A,* vol. *111*, no. 9, pp. 3602-3607, 2014.

[34] S. G. Jacobson, A. Sumaroka, T. S. Aleman, A. V. Cideciyan, S. B. Schwartz, A. J. Roman, R. R. Mcinnes, V. C. Sheffield, E. M. Stone, A. Swaroop, and A. F. Wright, "Nuclear receptor NR2E3 gene mutations distort human retinal laminar architecture and cause an unusual degeneration", *Hum Mol Genet,* vol. *13*, no. 17, pp. 1893-1902, 2004.

[35] N. B. Haider, J. K. Naggert, and P. M. Nishina, "Excess cone cell proliferation due to lack of a functional NR2E3 causes retinal dysplasia and degeneration in rd7/rd7 mice", *Human Molecular Genetics,* vol. *10*, no. 16, pp. 1619-1626, 2001.

[36] P. W. Kleyn, W. Fan, S. G. Kovats, J. J. Lee, J. C. Pulido, Y. Wu, L. R. Berkemeier, D. J. Misumi, L. Holmgren, O. Charlat, E. A. Woolf, O. Tayber, T. Brody, P. Shu, F. Hawkins, B. Kennedy, L. Baldini, C. Ebeling, G. D. Alperin, J. Deeds, N. D. Lakey, J. Culpepper, H. Chen, M. A. Glucksmann-Kuis, G. A. Carlson, G. M. Duyk, and K. J. Moore, "Identification and characterization of the mouse obesity gene tubby: a member of a novel gene family", *Cell,* vol. *85*, no. 2, pp. 281-290, 1996.

[37] J. R. Heckenlively, B. Chang, L. C. Erway, C. Peng, N. L. Hawes, G. S. Hageman, and T. H. Roderick, "Mouse model for Usher syndrome: linkage mapping suggests homology to Usher type I reported at human chromosome 11p15", *Proc Natl Acad Sci U S A,* vol. *92*, no. 24, pp. 11100-11104, 1995.

[38] A. D. Borman, L. R. Pearce, D. S. Mackay, K. Nagel-Wolfrum, A. E. Davidson, R. Henderson, S. Garg, N. H. Waseem, A. R. Webster, V. Plagnol, U. Wolfrum, I. S. Farooqi, and A. T. Moore, "A homozygous mutation in the TUB gene associated with retinal dystrophy and obesity", *Hum Mutat,* vol. *35*, no. 3, pp. 289-293, 2014.

[39] L. Kong, F. Li, C. E. Soleman, S. Li, R. V. Elias, X. Zhou, D. A. Lewis, J. F. Mcginnis, and W. Cao, "Bright cyclic light accelerates photoreceptor cell degeneration in tubby mice", *Neurobiol Dis,* vol. *21*, no. 3, pp. 468-477, 2006.

[40] X. Sun, J. Haley, O. V. Bulgakov, X. Cai, J. Mcginnis, and T. Li, "Tubby is required for trafficking G protein-coupled receptors to neuronal cilia", *Cilia,* vol. *1*, no. 1, pp. 21, 2012.

[41] X. Cai, S. A. Sezate, S. Seal, and J. F. Mcginnis, "Sustained protection against photoreceptor degeneration in tubby mice by intravitreal injection of nanoceria", *Biomaterials,* vol. *33*, no. 34, pp. 8771-8781, 2012.

[42] X. Cai, L. Chen, and J. F. Mcginnis, "Correlation of ER stress and retinal degeneration in tubby mice", *Exp Eye Res,* vol. *140*, no. pp. 130-138, 2015.

[43] G. H. Travis, M. B. Brennan, P. E. Danielson, C. A. Kozak, and J. G. Sutcliffe, "Identification of a photoreceptor-specific mRNA encoded by the gene responsible for retinal degeneration slow (rds)", *Nature,* vol. *338*, no. 6210, pp. 70-73, 1989.

[44] J. H. Reuter, and S. Sanyal, "Development and degeneration of retina in rds mutant mice: the electroretinogram", *Neuroscience Letter,* vol. *48*, no. 2, pp. 231-237, 1984.

[45] T. Cheng, N. S. Peachey, S. Li, Y. Goto, Y. Cao, and M. I. Naash, "The effect of peripherin/rds haploinsufficiency on rod and cone photoreceptors", *Journal of Neuroscience,* vol. *17*, no. 21, pp. 8118-8128, 1997.

[46] W. Kedzierski, M. Lloyd, D. G. Birch, D. Bok, and G. H. Travis, "Generation and analysis of transgenic mice expressing P216L-substituted rds/peripherin in rod photoreceptors", *Investigative ophthalmology & visual science,* vol. *38*, no. 2, pp. 498-509, 1997.

[47] H. M. Stricker, X. Q. Ding, A. Quiambao, S. J. Fliesler, and M. I. Naash, "The Cys214-->Ser mutation in peripherin/rds causes a loss-of-function phenotype in transgenic mice", *Biochemistry Journal,* vol. *388*, no. Pt 2, pp. 605-613, 2005.

[48] A. M. Nystuen, A. J. Sachs, Y. Yuan, L. Heuermann, and N. B. Haider, "A novel mutation in Prph2, a gene regulated by Nr2e3, causes retinal degeneration and outer-segment defects similar to Nr2e3 (rd7/rd7) retinas", *Mamm Genome,* vol. *19*, no. 9, pp. 623-633, 2008.

[49] D. A. Bessant, A. M. Payne, K. P. Mitton, Q. L. Wang, P. K. Swain, C. Plant, A. C. Bird, D. J. Zack, A. Swaroop, and S. S. Bhattacharya, "A mutation in NRL is associated with autosomal dominant retinitis pigmentosa", *Nat Genet,* vol. *21*, no. 4, pp. 355-356, 1999.

[50] M. M. Deangelis, J. L. Grimsby, M. A. Sandberg, E. L. Berson, and T. P. Dryja, "Novel mutations in the NRL gene and associated clinical findings in patients with dominant retinitis pigmentosa", *Arch Ophthalmol,* vol. *120*, no. 3, pp. 369-375, 2002.

[51] L. L. Daniele, C. Lillo, A. L. Lyubarsky, S. S. Nikonov, N. Philp, A. J. Mears, A. Swaroop, D. S. Williams, and E. N. Pugh, Jr., "Cone-like morphological, molecular, and electrophysiological features of the photoreceptors of the Nrl knockout mouse", *Investigative ophthalmology & visual science,* vol. *46*, no. 6, pp. 2156-2167, 2005.

[52] A. Wenzel, J. Von Lintig, V. Oberhauser, N. Tanimoto, C. Grimm, and M. W. Seeliger, "RPE65 is essential for the function of cone photoreceptors in NRL-deficient mice", *Investigative ophthalmology & visual science,* vol. *48*, no. 2, pp. 534-542, 2007.

[53] S. G. Foxman, J. R. Heckenlively, J. B. Bateman, and J. D. Wirtschafter, "Classification of congenital and early onset retinitis pigmentosa", *Arch Ophthalmol,* vol. *103*, no. 10, pp. 1502-1506, 1985.

[54] E. M. Morrow, T. Furukawa, E. Raviola, and C. L. Cepko, "Synaptogenesis and outer segment formation are perturbed in the neural retina of Crx mutant mice", *BMC Neuroscience,* vol. *6*, no. pp. 5, 2005.

[55] M. M. Humphries, D. Rancourt, G. J. Farrar, P. Kenna, M. Hazel, R. A. Bush, P. A. Sieving, D. M. Sheils, N. Mcnally, P. Creighton, A. Erven, A. Boros, K. Gulya, M. R. Capecchi, and P. Humphries, "Retinopathy induced in mice by targeted disruption of the rhodopsin gene", *Nature Genetics,* vol. *15*, no. 2, pp. 216-219, 1997.

[56] S. Machida, M. Kondo, J. A. Jamison, N. W. Khan, L. T. Kononen, T. Sugawara, R. A. Bush, and P. A. Sieving, "P23H rhodopsin transgenic rat: correlation of retinal function with histopathology", *Investigative ophthalmology & visual science,* vol. *41*, no. 10, pp. 3200-3209, 2000.

[57] C. H. Sung, B. G. Schneider, N. Agarwal, D. S. Papermaster, and J. Nathans, "Functional heterogeneity of mutant rhodopsins responsible for autosomal dominant retinitis pigmentosa", *Proceedings of the National Academy of Sciences of the United States of America,* vol. *88*, no. 19, pp. 8840-8844, 1991.

[58] F. Concepcion, A. Mendez, and J. Chen, "The carboxyl-terminal domain is essential for rhodopsin transport in rod photoreceptors", *Vision Res,* vol. *42*, no. 4, pp. 417-426, 2002.

[59] M. S. Gorbatyuk, A. Shabashvili, W. Chen, C. Meyers, L. F. Sullivan, M. Salganik, J. H. Lin, A. S. Lewin, N. Muzyczka, and O. S. Gorbatyuk, "Glucose regulated protein 78 diminishes alpha-synuclein neurotoxicity in a rat model of Parkinson disease", *Mol Ther,* vol. *20*, no. 7, pp. 1327-1337, 2012.

[60] V. M. Shinde, O. S. Sizova, J. H. Lin, M. M. Lavail, and M. S. Gorbatyuk, "ER stress in retinal degeneration in S334ter Rho rats", *PLoS One,* vol. *7*, no. 3, pp. e33266, 2012.

[61] P. Elizabeth Rakoczy, M. J. Yu, S. Nusinowitz, B. Chang, and J. R. Heckenlively, "Mouse models of age-related macular degeneration", *Exp Eye Res,* vol. *82*, no. 5, pp. 741-752, 2006.

[62] N. L. Mata, J. Weng, and G. H. Travis, "Biosynthesis of a major lipofuscin fluorophore in mice and humans with ABCR-mediated retinal and macular degeneration", *Proc Natl Acad Sci U S A,* vol. *97*, no. 13, pp. 7154-7159, 2000.

[63] N. L. Mata, R. T. Tzekov, X. Liu, J. Weng, D. G. Birch, and G. H. Travis, "Delayed dark-adaptation and lipofuscin accumulation in

abcr+/- mice: implications for involvement of ABCR in age-related macular degeneration", *Invest Ophthalmol Vis Sci,* vol. *42*, no. 8, pp. 1685-1690, 2001.

[64] G. Karan, C. Lillo, Z. Yang, D. J. Cameron, K. G. Locke, Y. Zhao, S. Thirumalaichary, C. Li, D. G. Birch, H. R. Vollmer-Snarr, D. S. Williams, and K. Zhang, "Lipofuscin accumulation, abnormal electrophysiology, and photoreceptor degeneration in mutant ELOVL4 transgenic mice: a model for macular degeneration", *Proc Natl Acad Sci U S A,* vol. *102*, no. 11, pp. 4164-4169, 2005.

[65] K. Zhang, M. Kniazeva, M. Han, W. Li, Z. Yu, Z. Yang, Y. Li, M. L. Metzker, R. Allikmets, D. J. Zack, L. E. Kakuk, P. S. Lagali, P. W. Wong, I. M. Macdonald, P. A. Sieving, D. J. Figueroa, C. P. Austin, R. J. Gould, R. Ayyagari, and K. Petrukhin, "A 5-bp deletion in ELOVL4 is associated with two related forms of autosomal dominant macular dystrophy", *Nature Genetics,* vol. *27*, no. 1, pp. 89-93, 2001.

[66] P. Tvrdik, R. Westerberg, S. Silve, A. Asadi, A. Jakobsson, B. Cannon, G. Loison, and A. Jacobsson, "Role of a new mammalian gene family in the biosynthesis of very long chain fatty acids and sphingolipids", *The Journal of cell biology,* vol. *149*, no. 3, pp. 707-718, 2000.

[67] M. P. Agbaga, M. N. Mandal, and R. E. Anderson, "Retinal very long-chain PUFAs: new insights from studies on ELOVL4 protein", *J Lipid Res,* vol. *51*, no. 7, pp. 1624-1642, 2010.

[68] R. Steinfeld, K. Reinhardt, K. Schreiber, M. Hillebrand, R. Kraetzner, W. Bruck, P. Saftig, and J. Gartner, "Cathepsin D deficiency is associated with a human neurodegenerative disorder", *American Journal of Human Genetics,* vol. *78*, no. 6, pp. 988-998, 2006.

[69] P. E. Rakoczy, D. Zhang, T. Robertson, N. L. Barnett, J. Papadimitriou, I. J. Constable, and C. M. Lai, "Progressive age-related changes similar to age-related macular degeneration in a transgenic mouse model", *American Journal of Pathology,* vol. *161*, no. 4, pp. 1515-1524, 2002.

[70] D. Zhang, M. Brankov, M. T. Makhija, T. Robertson, E. Helmerhorst, J. M. Papadimitriou, and P. E. Rakoczy, "Correlation between inactive cathepsin D expression and retinal changes in mcd2/mcd2 transgenic mice", *Investigative ophthalmology & visual science,* vol. *46*, no. 9, pp. 3031-3038, 2005.

[71] S. Dithmar, C. A. Curcio, N. A. Le, S. Brown, and H. E. Grossniklaus, "Ultrastructural changes in Bruch's membrane of apolipoprotein E-deficient mice", *Invest Ophthalmol Vis Sci,* vol. *41*, no. 8, pp. 2035-2042, 2000.

[72] G. Malek, L. V. Johnson, B. E. Mace, P. Saloupis, D. E. Schmechel, D. W. Rickman, C. A. Toth, P. M. Sullivan, and C. Bowes Rickman, "Apolipoprotein E allele-dependent pathogenesis: a model for age-related retinal degeneration", *Proc Natl Acad Sci U S A,* vol. *102*, no. 33, pp. 11900-11905, 2005.

[73] C. G. Sauer, A. Gehrig, R. Warneke-Wittstock, A. Marquardt, C. C. Ewing, A. Gibson, B. Lorenz, B. Jurklies, and B. H. Weber, "Positional cloning of the gene associated with X-linked juvenile retinoschisis", *Nat Genet,* vol. *17*, no. 2, pp. 164-170, 1997.

[74] B. H. Weber, H. Schrewe, L. L. Molday, A. Gehrig, K. L. White, M. W. Seeliger, G. B. Jaissle, C. Friedburg, E. Tamm, and R. S. Molday, "Inactivation of the murine X-linked juvenile retinoschisis gene, Rs1h, suggests a role of retinoschisin in retinal cell layer organization and synaptic structure", *Proc Natl Acad Sci U S A,* vol. *99*, no. 9, pp. 6222-6227, 2002.

[75] X. Q. Ding, M. Nour, L. M. Ritter, A. F. Goldberg, S. J. Fliesler, and M. I. Naash, "The R172W mutation in peripherin/rds causes a cone-rod dystrophy in transgenic mice", *Human Molecular Genetics,* vol. *13*, no. 18, pp. 2075-2087, 2004.

[76] J. Tuo, B. C. Smith, C. M. Bojanowski, A. D. Meleth, I. Gery, K. G. Csaky, E. Y. Chew, and C. C. Chan, "The involvement of sequence variation and expression of CX3CR1 in the pathogenesis of age-related macular degeneration", *FASEB J,* vol. *18*, no. 11, pp. 1297-1299, 2004.

[77] W. A. Kuziel, S. J. Morgan, T. C. Dawson, S. Griffin, O. Smithies, K. Ley, and N. Maeda, "Severe reduction in leukocyte adhesion and monocyte extravasation in mice deficient in CC chemokine receptor 2", *Proceedings of the National Academy of Sciences of the United States of America,* vol. *94*, no. 22, pp. 12053-12058, 1997.

[78] B. Lu, B. J. Rutledge, L. Gu, J. Fiorillo, N. W. Lukacs, S. L. Kunkel, R. North, C. Gerard, and B. J. Rollins, "Abnormalities in monocyte recruitment and cytokine expression in monocyte chemoattractant protein 1-deficient mice", *Journal of Experimental Medicine,* vol. *187*, no. 4, pp. 601-608, 1998.

[79] J. Ambati, A. Anand, S. Fernandez, E. Sakurai, B. C. Lynn, W. A. Kuziel, B. J. Rollins, and B. K. Ambati, "An animal model of age-related macular degeneration in senescent Ccl-2- or Ccr-2-deficient mice", *Nature Medicine,* vol. *9*, no. 11, pp. 1390-1397, 2003.

[80] D. A. Antonetti, E. Lieth, A. J. Barber, and T. W. Gardner, "Molecular mechanisms of vascular permeability in diabetic retinopathy", *Semin Ophthalmol,* vol. *14*, no. 4, pp. 240-248, 1999.

[81] T. A. Ciulla, A. Harris, P. Latkany, H. C. Piper, O. Arend, H. Garzozi, and B. Martin, "Ocular perfusion abnormalities in diabetes", *Acta Ophthalmol Scand,* vol. *80*, no. 5, pp. 468-477, 2002.

[82] M. J. Tolentino, J. W. Miller, E. S. Gragoudas, F. A. Jakobiec, E. Flynn, K. Chatzistefanou, N. Ferrara, and A. P. Adamis, "Intravitreous injections of vascular endothelial growth factor produce retinal ischemia and microangiopathy in an adult primate", *Ophthalmology,* vol. *103*, no. 11, pp. 1820-1828, 1996.

[83] J. Ruberte, E. Ayuso, M. Navarro, A. Carretero, V. Nacher, V. Haurigot, M. George, C. Llombart, A. Casellas, C. Costa, A. Bosch, and F. Bosch, "Increased ocular levels of IGF-1 in transgenic mice lead to diabetes-like eye disease", *Journal of Clinical Investigation,* vol. *113*, no. 8, pp. 1149-1157, 2004.

[84] A. O. Edwards, and G. Malek, "Molecular genetics of AMD and current animal models", *Angiogenesis,* vol. *10*, no. 2, pp. 119-132, 2007.

[85] M. Yoshioka, T. Kayo, T. Ikeda, and A. Koizumi, "A novel locus, Mody4, distal to D7Mit189 on chromosome 7 determines early-onset NIDDM in nonobese C57BL/6 (Akita) mutant mice", *Diabetes,* vol. *46*, no. 5, pp. 887-894, 1997.

[86] A. J. Barber, D. A. Antonetti, T. S. Kern, C. E. Reiter, R. S. Soans, J. K. Krady, S. W. Levison, T. W. Gardner, and S. K. Bronson, "The Ins2Akita mouse as a model of early retinal complications in diabetes", *Investigative ophthalmology & visual science,* vol. *46*, no. 6, pp. 2210-2218, 2005.

[87] M. J. Gastinger, A. R. Kunselman, E. E. Conboy, S. K. Bronson, and A. J. Barber, "Dendrite remodeling and other abnormalities in the retinal ganglion cells of Ins2 Akita diabetic mice", *Invest Ophthalmol Vis Sci,* vol. *49*, no. 6, pp. 2635-2642, 2008.

[88] J. R. Hombrebueno, M. Chen, R. G. Penalva, and H. Xu, "Loss of synaptic connectivity, particularly in second order neurons is a key feature of diabetic retinal neuropathy in the Ins2Akita mouse", *PLoS One,* vol. *9*, no. 5, pp. e97970, 2014.

[89] J. L. Haines, N. Schnetz-Boutaud, S. Schmidt, W. K. Scott, A. Agarwal, E. A. Postel, L. Olson, S. J. Kenealy, M. Hauser, J. R. Gilbert, and M. A. Pericak-Vance, "Functional candidate genes in age-related macular degeneration: significant association with VEGF, VLDLR, and LRP6", *Investigative ophthalmology & visual science,* vol. *47*, no. 1, pp. 329-335, 2006.

[90] J. R. Heckenlively, N. L. Hawes, M. Friedlander, S. Nusinowitz, R. Hurd, M. Davisson, and B. Chang, "Mouse model of subretinal neovascularization with choroidal anastomosis", *Retina,* vol. *23*, no. 4, pp. 518-522, 2003.

[91] Y. Chen, Y. Hu, K. Lu, J. G. Flannery, and J. X. Ma, "Very low density lipoprotein receptor, a negative regulator of the wnt signaling pathway and choroidal neovascularization", *Journal of Biological Chemeistry,* vol. *282*, no. 47, pp. 34420-34428, 2007.

[92] W. Hu, A. Jiang, J. Liang, H. Meng, B. Chang, H. Gao, and X. Qiao, "Expression of VLDLR in the retina and evolution of subretinal neovascularization in the knockout mouse model's retinal angiomatous proliferation", *Investigative ophthalmology & visual science,* vol. *49*, no. 1, pp. 407-415, 2008.

[93] M. I. Dorrell, E. Aguilar, R. Jacobson, O. Yanes, R. Gariano, J. Heckenlively, E. Banin, G. A. Ramirez, M. Gasmi, A. Bird, G. Siuzdak, and M. Friedlander, "Antioxidant or neurotrophic factor treatment preserves function in a mouse model of neovascularization-associated oxidative stress", *J Clin Invest,* vol. *119*, no. 3, pp. 611-623, 2009.

[94] Y. Chen, Y. Hu, T. Zhou, K. K. Zhou, R. Mott, M. Wu, M. Boulton, T. J. Lyons, G. Gao, and J. X. Ma, "Activation of the Wnt pathway plays a pathogenic role in diabetic retinopathy in humans and animal models", *Am J Pathol,* vol. *175*, no. 6, pp. 2676-2685, 2009.

[95] X. Cai, S. Seal, and J. F. Mcginnis, "Sustained inhibition of neovascularization in vldlr-/- mice following intravitreal injection of cerium oxide nanoparticles and the role of the ASK1-P38/JNK-NF-kappaB pathway", *Biomaterials,* vol. *35*, no. 1, pp. 249-258, 2014.

[96] C. M. Lai, W. Y. Shen, M. Brankov, Y. K. Lai, N. L. Barnett, S. Y. Lee, I. Y. Yeo, R. Mathur, J. E. Ho, P. Pineda, A. Barathi, C. L. Ang, I. J. Constable, and E. P. Rakoczy, "Long-term evaluation of AAV-mediated sFlt-1 gene therapy for ocular neovascularization in mice and monkeys", *Molecular Therapy,* vol. *12*, no. 4, pp. 659-668, 2005.

[97] F. Wang, K. G. Rendahl, W. C. Manning, D. Quiroz, M. Coyne, and S. S. Miller, "AAV-mediated expression of vascular endothelial growth factor induces choroidal neovascularization in rat", *Investigative ophthalmology & visual science,* vol. *44*, no. 2, pp. 781-790, 2003.

[98] C. Schwesinger, C. Yee, R. M. Rohan, A. M. Joussen, A. Fernandez, T. N. Meyer, V. Poulaki, J. J. Ma, T. M. Redmond, S. Liu, A. P. Adamis, and R. J. D'amato, "Intrachoroidal neovascularization in transgenic mice overexpressing vascular endothelial growth factor in the retinal pigment epithelium", *American Journal of Pathology,* vol. *158*, no. 3, pp. 1161-1172, 2001.

[99] K. Ohno-Matsui, A. Hirose, S. Yamamoto, J. Saikia, N. Okamoto, P. Gehlbach, E. J. Duh, S. Hackett, M. Chang, D. Bok, D. J. Zack, and P. A. Campochiaro, "Inducible expression of vascular endothelial growth factor in adult mice causes severe proliferative retinopathy and retinal detachment", *American Journal of Pathology,* vol. *160*, no. 2, pp. 711-719, 2002.

[100] P. K. Buch, J. W. Bainbridge, and R. R. Ali, "AAV-mediated gene therapy for retinal disorders: from mouse to man", *Gene Therapy,* vol. *15*, no. 11, pp. 849-857, 2008.

[101] J. W. Bainbridge, M. H. Tan, and R. R. Ali, "Gene therapy progress and prospects: the eye", *Gene Therapy,* vol. *13*, no. 16, pp. 1191-1197, 2006.

[102] A. Dinculescu, L. Glushakova, S. H. Min, and W. W. Hauswirth, "Adeno-associated virus-vectored gene therapy for retinal disease", *Human Gene Therapy,* vol. *16*, no. 6, pp. 649-663, 2005.

[103] F. Simonelli, A. M. Maguire, F. Testa, E. A. Pierce, F. Mingozzi, J. L. Bennicelli, S. Rossi, K. Marshall, S. Banfi, E. M. Surace, J. Sun, T. M. Redmond, X. Zhu, K. S. Shindler, G. S. Ying, C. Ziviello, C. Acerra, J. F. Wright, J. W. Mcdonnell, K. A. High, J. Bennett, and A. Auricchio, "Gene therapy for Leber's congenital amaurosis is safe and effective through 1.5 years after vector administration", *Molecular Therapy,* vol. *18*, no. 3, pp. 643-650, 2010.

[104] K. Stieger, J. Schroeder, N. Provost, A. Mendes-Madeira, B. Belbellaa, G. Le Meur, M. Weber, J. Y. Deschamps, B. Lorenz, P. Moullier, and F. Rolling, "Detection of intact rAAV particles up to 6 years after successful gene transfer in the retina of dogs and primates", *Molecular Therapy,* vol. *17*, no. 3, pp. 516-523, 2009.

[105] J. Bennicelli, J. F. Wright, A. Komaromy, J. B. Jacobs, B. Hauck, O. Zelenaia, F. Mingozzi, D. Hui, D. Chung, T. S. Rex, Z. Wei, G. Qu, S. Zhou, C. Zeiss, V. R. Arruda, G. M. Acland, L. F. Dell'osso, K. A. High, A. M. Maguire, and J. Bennett, "Reversal of blindness in animal models of leber congenital amaurosis using optimized AAV2-mediated gene transfer", *Molecular Therapy,* vol. *16*, no. 3, pp. 458-465, 2008.

[106] J. W. Bainbridge, A. J. Smith, S. S. Barker, S. Robbie, R. Henderson, K. Balaggan, A. Viswanathan, G. E. Holder, A. Stockman, N. Tyler, S. Petersen-Jones, S. S. Bhattacharya, A. J. Thrasher, F. W. Fitzke, B. J. Carter, G. S. Rubin, A. T. Moore, and R. R. Ali, "Effect of gene therapy on visual function in Leber's congenital amaurosis", *The New England Journal of Medicine,* vol. *358*, no. 21, pp. 2231-2239, 2008.

[107] A. M. Maguire, F. Simonelli, E. A. Pierce, E. N. Pugh, Jr., F. Mingozzi, J. Bennicelli, S. Banfi, K. A. Marshall, F. Testa, E. M. Surace, S. Rossi, A. Lyubarsky, V. R. Arruda, B. Konkle, E. Stone, J. Sun, J. Jacobs, L. Dell'osso, R. Hertle, J. X. Ma, T. M. Redmond, X. Zhu, B. Hauck, O. Zelenaia, K. S. Shindler, M. G. Maguire, J. F. Wright, N. J. Volpe, J. W. Mcdonnell, A. Auricchio, K. A. High, and J. Bennett, "Safety and efficacy of gene transfer for Leber's congenital amaurosis", *The New England Journal of Medicine,* vol. *358*, no. 21, pp. 2240-2248, 2008.

[108] S. G. Jacobson, A. V. Cideciyan, G. D. Aguirre, A. J. Roman, A. Sumaroka, W. W. Hauswirth, and K. Palczewski, "Improvement in vision: a new goal for treatment of hereditary retinal degenerations", *Expert Opin Orphan Drugs,* vol. *3*, no. 5, pp. 563-575, 2015.

[109] J. W. Bainbridge, M. S. Mehat, V. Sundaram, S. J. Robbie, S. E. Barker, C. Ripamonti, A. Georgiadis, F. M. Mowat, S. G. Beattie, P. J. Gardner, K. L. Feathers, V. A. Luong, S. Yzer, K. Balaggan, A. Viswanathan, T. J. De Ravel, I. Casteels, G. E. Holder, N. Tyler, F. W. Fitzke, R. G. Weleber, M. Nardini, A. T. Moore, D. A. Thompson, S. M. Petersen-Jones, M. Michaelides, L. I. Van Den Born, A. Stockman, A. J. Smith, G. Rubin, and R. R. Ali, "Long-term effect of gene therapy on Leber's congenital amaurosis", *N Engl J Med,* vol. *372*, no. 20, pp. 1887-1897, 2015.

[110] C. Ripamonti, G. B. Henning, S. J. Robbie, V. Sundaram, L. I. Van Den Born, I. Casteels, T. J. De Ravel, A. T. Moore, A. J. Smith, J. W. Bainbridge, R. R. Ali, and A. Stockman, "Spectral sensitivity measurements reveal partial success in restoring missing rod function with gene therapy", *J Vis,* vol. *15*, no. 15, pp. 20, 2015.

[111] S. G. Jacobson, A. V. Cideciyan, R. Ratnakaram, E. Heon, S. B. Schwartz, A. J. Roman, M. C. Peden, T. S. Aleman, S. L. Boye, A. Sumaroka, T. J. Conlon, R. Calcedo, J. J. Pang, K. E. Erger, M. B. Olivares, C. L. Mullins, M. Swider, S. Kaushal, W. J. Feuer, A. Iannaccone, G. A. Fishman, E. M. Stone, B. J. Byrne, and W. W. Hauswirth, "Gene therapy for leber congenital amaurosis caused by RPE65 mutations: safety and efficacy in 15 children and adults followed up to 3 years", *Arch Ophthalmol,* vol. *130*, no. 1, pp. 9-24, 2012.

[112] M. Ashtari, L. L. Cyckowski, J. F. Monroe, K. A. Marshall, D. C. Chung, A. Auricchio, F. Simonelli, B. P. Leroy, A. M. Maguire, K. S. Shindler, and J. Bennett, "The human visual cortex responds to gene therapy-mediated recovery of retinal function", *J Clin Invest,* vol. *121*, no. 6, pp. 2160-2168, 2011.

[113] F. Testa, A. M. Maguire, S. Rossi, E. A. Pierce, P. Melillo, K. Marshall, S. Banfi, E. M. Surace, J. Sun, C. Acerra, J. F. Wright, J. Wellman, K. A. High, A. Auricchio, J. Bennett, and F. Simonelli, "Three-year follow-up after unilateral subretinal delivery of adeno-associated virus in patients with Leber congenital Amaurosis type 2", *Ophthalmology,* vol. *120*, no. 6, pp. 1283-1291, 2013.

[114] N. Chadderton, S. Millington-Ward, A. Palfi, M. O'reilly, G. Tuohy, M. M. Humphries, T. Li, P. Humphries, P. F. Kenna, and G. J. Farrar, "Improved retinal function in a mouse model of dominant retinitis pigmentosa following AAV-delivered gene therapy", *Molecular Therapy,* vol. *17*, no. 4, pp. 593-599, 2009.

[115] G. M. Sarra, C. Stephens, M. De Alwis, J. W. Bainbridge, A. J. Smith, A. J. Thrasher, and R. R. Ali, "Gene replacement therapy in the retinal degeneration slow (rds) mouse: the effect on retinal degeneration following partial transduction of the retina", *Human Molecular Genetics,* vol. *10*, no. 21, pp. 2353-2361, 2001.

[116] F. C. Schlichtenbrede, L. Da Cruz, C. Stephens, A. J. Smith, A. Georgiadis, A. J. Thrasher, J. W. Bainbridge, M. W. Seeliger, and R. R. Ali, "Long-term evaluation of retinal function in Prph2Rd2/Rd2 mice following AAV-mediated gene replacement therapy", *J Gene Med,* vol. *5*, no. 9, pp. 757-764, 2003.

[117] K. M. Nishiguchi, L. S. Carvalho, M. Rizzi, K. Powell, S. M. Holthaus, S. A. Azam, Y. Duran, J. Ribeiro, U. F. Luhmann, J. W. Bainbridge, A. J. Smith, and R. R. Ali, "Gene therapy restores vision in rd1 mice after removal of a confounding mutation in Gpr179", *Nat Commun,* vol. *6*, no. pp. 6006, 2015.

[118] P. K. Buch, R. E. Maclaren, Y. Duran, K. S. Balaggan, A. Macneil, F. C. Schlichtenbrede, A. J. Smith, and R. R. Ali, "In contrast to AAV-mediated Cntf expression, AAV-mediated Gdnf expression enhances gene replacement therapy in rodent models of retinal degeneration", *Molecular Therapy,* vol. *14*, no. 5, pp. 700-709, 2006.

[119] M. Allocca, M. Doria, M. Petrillo, P. Colella, M. Garcia-Hoyos, D. Gibbs, S. R. Kim, A. Maguire, T. S. Rex, U. Di Vicino, L. Cutillo, J. R. Sparrow, D. S. Williams, J. Bennett, and A. Auricchio,

"Serotype-dependent packaging of large genes in adeno-associated viral vectors results in effective gene delivery in mice", *Journal of Clinical Investigation,* vol. *118*, no. 5, pp. 1955-1964, 2008.

[120] E. P. Rakoczy, C. M. Lai, A. L. Magno, M. E. Wikstrom, M. A. French, C. M. Pierce, S. D. Schwartz, M. S. Blumenkranz, T. W. Chalberg, M. A. Degli-Esposti, and I. J. Constable, "Gene therapy with recombinant adeno-associated vectors for neovascular age-related macular degeneration: 1 year follow-up of a phase 1 randomised clinical trial", *Lancet,* vol. *386*, no. 10011, pp. 2395-2403, 2015.

[121] K. Mori, P. Gehlbach, S. Yamamoto, E. Duh, D. J. Zack, Q. Li, K. I. Berns, B. J. Raisler, W. W. Hauswirth, and P. A. Campochiaro, "AAV-mediated gene transfer of pigment epithelium-derived factor inhibits choroidal neovascularization", *Investigative ophthalmology & visual science,* vol. *43*, no. 6, pp. 1994-2000, 2002.

[122] L. H. Mcgee Sanftner, H. Abel, W. W. Hauswirth, and J. G. Flannery, "Glial cell line derived neurotrophic factor delays photoreceptor degeneration in a transgenic rat model of retinitis pigmentosa", *Molecular Therapy,* vol. *4*, no. 6, pp. 622-629, 2001.

[123] F. C. Schlichtenbrede, A. Macneil, J. W. Bainbridge, M. Tschernutter, A. J. Thrasher, A. J. Smith, and R. R. Ali, "Intraocular gene delivery of ciliary neurotrophic factor results in significant loss of retinal function in normal mice and in the Prph2Rd2/Rd2 model of retinal degeneration", *Gene Therapy,* vol. *10*, no. 6, pp. 523-527, 2003.

[124] F. Q. Liang, T. S. Aleman, N. S. Dejneka, L. Dudus, K. J. Fisher, A. M. Maguire, S. G. Jacobson, and J. Bennett, "Long-term protection of retinal structure but not function using RAAV.CNTF in animal models of retinitis pigmentosa", *Molecular Therapy,* vol. *4*, no. 5, pp. 461-472, 2001.

[125] M. Cayouette, and C. Gravel, "Adenovirus-mediated gene transfer of ciliary neurotrophic factor can prevent photoreceptor degeneration in the retinal degeneration (rd) mouse", *Hum Gene Ther,* vol. *8*, no. 4, pp. 423-430, 1997.

[126] X. Cai, S. M. Conley, Z. Nash, S. J. Fliesler, M. J. Cooper, and M. I. Naash, "Gene delivery to mitotic and postmitotic photoreceptors via compacted DNA nanoparticles results in improved phenotype in a mouse model of retinitis pigmentosa", *FASEB J,* vol. *24*, no. 4, pp. 1178-1191, 2010.

[127] X. Cai, Z. Nash, S. M. Conley, S. J. Fliesler, M. J. Cooper, and M. I. Naash, "A partial structural and functional rescue of a retinitis pigmentosa model with compacted DNA nanoparticles", *PLoS One,* vol. *4*, no. 4, pp. e5290, 2009.

[128] V. Enzmann, E. Yolcu, H. J. Kaplan, and S. T. Ildstad, "Stem cells as tools in regenerative therapy for retinal degeneration", *Arch Ophthalmol,* vol. *127*, no. 4, pp. 563-571, 2009.

[129] N. K. Wang, J. Tosi, J. M. Kasanuki, C. L. Chou, J. Kong, N. Parmalee, K. J. Wert, R. Allikmets, C. C. Lai, C. L. Chien, T. Nagasaki, C. S. Lin, and S. H. Tsang, "Transplantation of reprogrammed embryonic stem cells improves visual function in a mouse model for retinitis pigmentosa", *Transplantation,* vol. *89*, no. 8, pp. 911-919, 2010.

[130] S. Wang, B. Lu, S. Girman, J. Duan, T. Mcfarland, Q. S. Zhang, M. Grompe, G. Adamus, B. Appukuttan, and R. Lund, "Non-invasive stem cell therapy in a rat model for retinal degeneration and vascular pathology", *PLoS One,* vol. *5*, no. 2, pp. e9200, 2010.

[131] D. A. Lamba, J. Gust, and T. A. Reh, "Transplantation of human embryonic stem cell-derived photoreceptors restores some visual function in Crx-deficient mice", *Cell Stem Cell,* vol. *4*, no. 1, pp. 73-79, 2009.

[132] H. Shirai, M. Mandai, K. Matsushita, A. Kuwahara, S. Yonemura, T. Nakano, J. Assawachananont, T. Kimura, K. Saito, H. Terasaki, M. Eiraku, Y. Sasai, and M. Takahashi, "Transplantation of human embryonic stem cell-derived retinal tissue in two primate models of retinal degeneration", *Proc Natl Acad Sci U S A,* vol. *113*, no. 1, pp. E81-90, 2016.

[133] S. D. Schwartz, C. D. Regillo, B. L. Lam, D. Eliott, P. J. Rosenfeld, N. Z. Gregori, J. P. Hubschman, J. L. Davis, G. Heilwell, M. Spirn, J. Maguire, R. Gay, J. Bateman, R. M. Ostrick, D. Morris, M. Vincent, E. Anglade, L. V. Del Priore, and R. Lanza, "Human embryonic stem cell-derived retinal pigment epithelium in patients with age-related macular degeneration and Stargardt's macular dystrophy: follow-up of two open-label phase 1/2 studies", *Lancet,* vol. *385*, no. 9967, pp. 509-516, 2015.

[134] S. D. Schwartz, G. Tan, H. Hosseini, and A. Nagiel, "Subretinal Transplantation of Embryonic Stem Cell-Derived Retinal Pigment Epithelium for the Treatment of Macular Degeneration: An Assessment at 4 Years", *Invest Ophthalmol Vis Sci,* vol. *57*, no. 5, pp. ORSFc1-9, 2016.

[135] R. E. Maclaren, and R. A. Pearson, "Stem cell therapy and the retina", *Eye (Lond),* vol. *21*, no. 10, pp. 1352-1359, 2007.

[136] Z. B. Jin, S. Okamoto, M. Mandai, and M. Takahashi, "Induced pluripotent stem cells for retinal degenerative diseases: a new perspective on the challenges", *Journal of Genetics,* vol. *88*, no. 4, pp. 417-424, 2009.

[137] O. Comyn, E. Lee, and R. E. Maclaren, "Induced pluripotent stem cell therapies for retinal disease", *Currient Opinion Neurololy,* vol. *23*, no. 1, pp. 4-9, 2010.

[138] I. Mooney, and J. Lamotte, "A review of the potential to restore vision with stem cells", *Clin Exp Optom,* vol. *91*, no. 1, pp. 78-84, 2008.

[139] M. W. Djojosubroto, and Y. Arsenijevic, "Retinal stem cells: promising candidates for retina transplantation", *Cell Tissue Res,* vol. *331*, no. 1, pp. 347-357, 2008.

[140] F. Gaillard, and Y. Sauve, "Cell-based therapy for retina degeneration: the promise of a cure", *Vision Research,* vol. *47*, no. 22, pp. 2815-2824, 2007.

[141] L. Da Cruz, F. K. Chen, A. Ahmado, J. Greenwood, and P. Coffey, "RPE transplantation and its role in retinal disease", *Prog Retin Eye Res,* vol. *26*, no. 6, pp. 598-635, 2007.

[142] H. Chen, S. Fre, V. I. Slepnev, M. R. Capua, K. Takei, M. H. Butler, P. P. Di Fiore, and P. De Camilli, "Epsin is an EH-domain-binding protein implicated in clathrin-mediated endocytosis", *Nature,* vol. *394*, no. 6695, pp. 793-797, 1998.

[143] H. Chen, G. Ko, A. Zatti, G. Di Giacomo, L. Liu, E. Raiteri, E. Perucco, C. Collesi, W. Min, C. Zeiss, P. De Camilli, and O. Cremona, "Embryonic arrest at midgestation and disruption of Notch signaling produced by the absence of both epsin 1 and epsin 2 in mice", *Proceedings of the National Academy of Sciences of the United States of America,* vol. *106*, no. 33, pp. 13838-13843, 2009.

[144] X. Liu, S. Pasula, H. Song, K. L. Tessneer, Y. Dong, S. Hahn, T. Yago, M. L. Brophy, B. Chang, X. Cai, H. Wu, J. Mcmanus, H. Ichise, C. Georgescu, J. D. Wren, C. Griffin, L. Xia, R. S. Srinivasan, and H. Chen, "Temporal and spatial regulation of epsin abundance and VEGFR3 signaling are required for lymphatic valve formation and function", *Science signaling,* vol. *7*, no. 347, pp. ra97, 2014.

[145] S. Pasula, X. Cai, Y. Dong, M. Messa, J. Mcmanus, B. Chang, X. Liu, H. Zhu, R. S. Mansat, S. J. Yoon, S. Hahn, J. Keeling, D. Saunders, G. Ko, J. Knight, G. Newton, F. Luscinskas, X. Sun, R. Towner, F. Lupu, L. Xia, O. Cremona, P. De Camilli, W. Min, and H. Chen, "Endothelial epsin deficiency decreases tumor growth by enhancing VEGF signaling", *The Journal of clinical investigation,* vol. *122*, no. 12, pp. 4424-4438, 2012.

[146] Y. Dong, H. Wu, H. N. Rahman, Y. Liu, S. Pasula, K. L. Tessneer, X. Cai, X. Liu, B. Chang, J. Mcmanus, S. Hahn, J. Dong, M. L. Brophy, L. Yu, K. Song, R. Silasi-Mansat, D. Saunders, C. Njoku, H. Song, P. Mehta-D'souza, R. Towner, F. Lupu, R. P. Mcever, L. Xia, D. Boerboom, R. S. Srinivasan, and H. Chen, "Motif mimetic of epsin perturbs tumor growth and metastasis", *The Journal of clinical investigation,* vol. *125*, no. 12, pp. 4349-4364, 2015.

[147] H. N. Rahman, H. Wu, Y. Dong, S. Pasula, A. Wen, Y. Sun, M. L. Brophy, K. L. Tessneer, X. Cai, J. Mcmanus, B. Chang, S. Kwak, N. S. Rahman, W. Xu, C. Fernandes, J. M. Mcdaniel, L. Xia, L. Smith, R. S. Srinivasan, and H. Chen, "Selective Targeting of a Novel Epsin-VEGFR2 Interaction Promotes VEGF-Mediated Angiogenesis", *Circulation research,* vol. *118*, no. 6, pp. 957-969, 2016.

[148] K. L. Tessneer, S. Pasula, X. Cai, Y. Dong, J. Mcmanus, X. Liu, L. Yu, S. Hahn, B. Chang, Y. Chen, C. Griffin, L. Xia, R. H. Adams, and H. Chen, "Genetic reduction of vascular endothelial growth factor receptor 2 rescues aberrant angiogenesis caused by epsin

deficiency", *Arteriosclerosis, thrombosis, and vascular biology,* vol. *34*, no. 2, pp. 331-337, 2014.

[149] B. Chang, K. L. Tessneer, J. Mcmanus, X. Liu, S. Hahn, S. Pasula, H. Wu, H. Song, Y. Chen, X. Cai, Y. Dong, M. L. Brophy, R. Rahman, J. X. Ma, L. Xia, and H. Chen, "Epsin is required for Dishevelled stability and Wnt signalling activation in colon cancer development", *Nature communications,* vol. *6*, no. pp. 6380, 2015.

[150] O. Parnas, M. Jovanovic, T. M. Eisenhaure, R. H. Herbst, A. Dixit, C. J. Ye, D. Przybylski, R. J. Platt, I. Tirosh, N. E. Sanjana, O. Shalem, R. Satija, R. Raychowdhury, P. Mertins, S. A. Carr, F. Zhang, N. Hacohen, and A. Regev, "A Genome-wide CRISPR Screen in Primary Immune Cells to Dissect Regulatory Networks", *Cell,* vol. *162*, no. 3, pp. 675-686, 2015.

[151] L. Cong, and F. Zhang, "Genome engineering using CRISPR-Cas9 system", *Methods in molecular biology,* vol. *1239*, no. pp. 197-217, 2015.

[152] X. Wu, D. A. Scott, A. J. Kriz, A. C. Chiu, P. D. Hsu, D. B. Dadon, A. W. Cheng, A. E. Trevino, S. Konermann, S. Chen, R. Jaenisch, F. Zhang, and P. A. Sharp, "Genome-wide binding of the CRISPR endonuclease Cas9 in mammalian cells", *Nature biotechnology,* vol. *32*, no. 7, pp. 670-676, 2014.

[153] F. A. Ran, P. D. Hsu, J. Wright, V. Agarwala, D. A. Scott, and F. Zhang, "Genome engineering using the CRISPR-Cas9 system", *Nature protocols,* vol. *8*, no. 11, pp. 2281-2308, 2013.

[154] D. W. Taylor, Y. Zhu, R. H. Staals, J. E. Kornfeld, A. Shinkai, J. Van Der Oost, E. Nogales, and J. A. Doudna, "Structural biology. Structures of the CRISPR-Cmr complex reveal mode of RNA target positioning", *Science,* vol. *348*, no. 6234, pp. 581-585, 2015.

[155] K. S. Makarova, Y. I. Wolf, O. S. Alkhnbashi, F. Costa, S. A. Shah, S. J. Saunders, R. Barrangou, S. J. Brouns, E. Charpentier, D. H. Haft, P. Horvath, S. Moineau, F. J. Mojica, R. M. Terns, M. P. Terns, M. F. White, A. F. Yakunin, R. A. Garrett, J. Van Der Oost, R. Backofen, and E. V. Koonin, "An updated evolutionary classification of CRISPR-Cas systems", *Nature reviews. Microbiology,* vol. *13*, no. 11, pp. 722-736, 2015.

[156] D. Heckl, and E. Charpentier, "Toward Whole-Transcriptome Editing with CRISPR-Cas9", *Molecular cell,* vol. *58*, no. 4, pp. 560-562, 2015.

[157] X. Cai, and J. F. Mcginnis, "Diabetic Retinopathy: Animal Models, Therapies, and Perspectives", *Journal of diabetes research,* vol. *2016*, no. pp. 3789217, 2016.

[158] W. H. Wu, Y. T. Tsai, S. Justus, T. T. Lee, L. Zhang, C. S. Lin, A. G. Bassuk, V. B. Mahajan, and S. H. Tsang, "CRISPR Repair Reveals Causative Mutation in a Preclinical Model of Retinitis Pigmentosa", *Molecular Therapy,* vol., no. pp. 2016.

[159] B. Bakondi, W. Lv, B. Lu, M. K. Jones, Y. Tsai, K. J. Kim, R. Levy, A. A. Akhtar, J. J. Breunig, C. N. Svendsen, and S. Wang, "In Vivo CRISPR/Cas9 Gene Editing Corrects Retinal Dystrophy in the S334ter-3 Rat Model of Autosomal Dominant Retinitis Pigmentosa", *Molecular Therapy,* vol. *24*, no. 3, pp. 556-563, 2016.

[160] A. G. Bassuk, A. Zheng, Y. Li, S. H. Tsang, and V. B. Mahajan, "Precision Medicine: Genetic Repair of Retinitis Pigmentosa in Patient-Derived Stem Cells", *Scientific reports,* vol. *6*, no. pp. 19969, 2016.