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RPE65: Role in the visual cycle, human retinal disease, and gene therapy

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Abstract

RPE65 is an isomerohydrolase expressed in retinal pigment epithelium. It is critical for the regeneration of the visual pigment necessary for both rod and cone-mediated vision. Mutations in human *RPE65* cause Leber's congenital amaurosis and other forms of autosomal recessive retinitis pigmentosa which are associated with early-onset blindness. Several RPE65 animal models including two different mouse models and a naturally occurring canine model have been thoroughly characterized to determine the mechanisms that underlie RPE65 associated retinal dystrophies. More recently, substantial effort has gone into designing gene therapies for these diseases. Based on several encouraging reports from animal models, at least three clinical trials are currently underway for the treatment of LCA using modified AAV vectors carrying the *RPE65* cDNA and have reported positive preliminary results.

Keywords

RPE65; retinal pigment epithelium; retina; LCA; gene therapy

Introduction

The visual cycle (or retinoid cycle) is the process by which 11-*cis*-retinal is regenerated from all-*trans*-retinal after a photoisomerization event. This cycle takes place in the photoreceptors and retinal pigment epithelium (RPE) and involves a series of enzymatic reactions[1]. RPE65 is a key isomerase in this process and is responsible for converting all-*trans*-retinyl ester to 11-*cis*-retinol. Without RPE65, 11-*cis*-retinal levels are substantially reduced and retinyl esters accumulate in RPE. RPE65 is an evolutionarily conserved 61-kDa membrane associated protein found in the smooth endoplasmic reticulum of the RPE cells[2–5]. Mutations in *RPE65* are associated with recessive blinding diseases and models of RPE65 deficiency have been a common target for gene therapy studies over the past several years.

Gene cloning and structure

RPE65 was first described in 1993[6]. Initial studies by Hamel *et al.* using the RPE9 monoclonal antibody suggested that the protein was 65 kDa in size and partitioned in the microsomal membrane fraction[6]. The same group also first isolated *RPE65* cDNA in 1993[4] (although other groups were also studying the cDNA[7]) and expression studies in *E. coli* showed a protein size of 61 kDa. In 1994, the *RPE65* gene was mapped to human chromosome 1 (mouse chromosome 3), and was refined to 1p31 by fluorescence *in situ* hybridization[5,8]. In 1995, Nicoletti et al.

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described the human gene structure[5]. The *RPE65* gene contains 14 coding exons[5,8] spanning 20 kb and encodes a protein of 533 amino acids[5,8–10]. In the coding region, the canine *RPE65* gene shares 88–89% nucleotide sequence identity with the human and bovine sequence and 83% identity with the rat[9]. Sequence analysis combined with the observed tissue-specific expression, high abundance, evolutionary conservation, and developmental regulation, suggest that this protein is functionally important and its expression is tightly regulated[5,10]. Due to its functional importance in vision (discussed below), several groups have tried to study RPE65 *in vitro*, but transcripts are rapidly lost from RPE cells established in culture. It has been suggested that this downregulation may occur posttranscriptionally due to regions in the 3′ UTR that may target the RNA for rapid degradation in the absence of other environmental cues[4,5].

RPE65 function

RPE65 expression is developmentally regulated[6]. In rat, *Rpe65* message is detectable as early as embryonic day (E) 18 and levels peak twice during retinal development. From postnatal day (P) 2 to P4, *Rpe65* message stabilizes at levels about 10 fold higher than those seen at E18. The second peak (from P10-P12) is substantially larger, transcript levels are approximately 200 times higher than those seen at P2-4[10]. The detection of RPE65 protein in the RPE (at P4-5) coincides with the earliest appearance of photoreceptor outer segment (OS) membranes[11,12], and protein is continuously detectable throughout life[10]. These observations suggest that developmental expression of RPE65 may be coordinated with expression of some photoreceptor-specific proteins[10].

RPE65 has two forms; a soluble form called sRPE65, and a palmitoylated, membrane-bound form known as mRPE65. Although it was proposed in 2004 that the palmitoylation state of RPE65 could serve as a molecular switch mechanism to help regulate retinoid recycling and transport[13] (as a result of the differential binding affinity of the two forms of RPE65 for different retinoids), subsequent experiments have shown that this is not likely to be the case[14].

It was originally thought that the role of RPE65 was restricted to the binding and mobilization of all-*trans*-retinyl esters for processing by the isomerohydrolase responsible for the critical conversion of all-*trans*- to 11-*cis*- retinoids[15]. However, more recently, three independent groups simultaneously published evidence that RPE65 is the isomerohydrolase[16–18]. Moiseyev et al. showed that RPE65 protein expression correlates linearly with isomerohydrolase activity and its enzymatic activity can be reconstituted *in vitro* after transfection with *RPE65* cDNA[17]. The retinal isomerohydrolase activity is iron dependent, with Fe^{2+} being the critical species, and purified RPE65 does contain iron[19]. Travis' group conducted a cDNA expression screen using bovine RPE[16]. They isolated a single clone with high isomerohydrolase activity which, when sequenced, proved to be *RPE65*[16]. The third line of independent evidence came from the Redmond group which reconstituted the visual cycle *in vitro*. Only when RPE65 was expressed with other visual cycle proteins did retinoid isomerization occur[18]. The role of iron in this enzymatic process was confirmed by their observation that *RPE65* constructs carrying mutations in the residues thought to be necessary for iron coordination (H180, H241, H313, H527) had little or no enzymatic activity.[18]

Although RPE65 was originally identified only in RPE, it is also expressed in both amphibian and mammalian cone photoreceptors but not rods[20]. The role of RPE65 in cones is unknown, but this observation (and others) provides support for the hypothesis that mammalian cones may utilize a different retinoid processing cycle in addition to the traditional one used by rods[20–23]. If this is the case, retinoid regeneration in cones is likely still RPE65 dependent. When mice lacking RPE65 were crossed into the "cone-only" $Nrl^{-/-}$ or $Rho^{-/-}$ backgrounds,

11-*cis*-retinal was undetectable and retinyl esters and total retinoids were increased[24,25]. Consistent with the absence of pigment, retinal sensitivity dropped by a factor of one thousand and cone OSs degenerated[24,25]. Taken together, these data suggest that RPE65 is not only essential for rod function but it is also indispensable for cone function.

RPE65 associated diseases

Over 60 different mutations in the *RPE65* gene have been associated with a heterogeneous group of inherited retinal dystrophies including Leber's congenital amaurosis (LCA) and autosomal recessive retinitis pigmentosa (RP). These diseases are usually associated with blindness from birth or early childhood (reviewed in[26] and summarized in http://www.retina-international.com/sci-news/rpe65mut.htm). Mutations in the *RPE65* gene account for approximately 2% of cases of recessive RP and approximately 16% of cases of LCA in humans[27,28].

In vivo assessments of human outer nuclear layer (ONL) thickness by high-resolution optical cross-sectional imaging indicate that in spite of early-onset blindness, the retinas of LCA patients retain normal retinal laminae with a definable photoreceptor layer into adulthood[21,29] supporting the possibility that gene replacement therapy may be a viable treatment option. Furthermore, many patients have normal optic nerve diameter, anatomic structure including subcortical structure (in the lateral geniculate nucleus), and both gray and white matter; however, cortical responses are markedly diminished as shown by functional magnetic resonance imaging[29].

Recent findings showed that many point mutations in *RPE65*, including those associated with LCA, have different effects on protein function. For example, *in vivo* and *in vitro* experiments have shown that Y144D, P363T, and Y368H RPE65 mutant proteins are significantly less stable than wild-type RPE65[30,31]. In addition to effects on protein levels, different point mutations cause different deficiencies in RPE65 function, and study of specific mutations has helped explain the heterogeneity observed in disease phenotypes. While Y144D and P363T RPE65 mutant proteins have no detectable isomerohydrolase activity (acting as virtual null alleles)[31], other mutations cause a milder phenotype. For example, R91W[32], P25L[33], and L22P[34] (and others) cause a slightly milder form of LCA with later onset deficits in vision than other mutations. These mutant proteins have been shown to retain more functional capacity, measured by 11-*cis* retinal levels and capacity for rhodopsin regeneration in the case of R91W[32] and by *in vitro* isomerase activity in the case of P25L and L22P[33], than mutations resulting in a null allele[32].

RPE65 animal models

Several animal models with RPE65 mutations have been characterized. Among them the naturally occurring canine[35] and murine (*Rpe65*^{rd12})[36] models and the genetically engineered *Rpe65*$^{-/-}$ knockout have been widely used for pathological, biochemical, genetic, structural, functional, and therapeutic studies. *Rpe65*$^{-/-}$ knockout mice develop a slow retinal degeneration with relatively normal retinal anatomy at 7 weeks with normal OS structure and ONL thickness[3,37], but at 15 weeks the OS length starts to decrease and inclusions start to appear in the RPE[3]. By 12 months, 30% of photoreceptor nuclei are lost, with even more severe photoreceptor loss at 18–24 months[37]. *Rpe65*$^{-/-}$ mice lack 11-*cis* retinal and 11-*cis*-retinyl esters, and accumulate excessive levels of all-*trans* retinyl esters in the RPE[3] providing further support for the idea that the RPE65 protein is essential for the isomerization of all-*trans* retinyl esters[3,38]. Based on microarray analysis, mice lacking RPE65 have altered expression of many genes including some involved in phototransduction, apoptosis, cytoskeletal organization, and extracellular matrix regulation[39]. The knockout mice have nearly undetectable dark-adapted ERG responses[3], and undetectable levels of functional rhodopsin even though the opsin

apoprotein is structurally intact[3,38]. There was some confusion about the status of cone function in these mice[3,38], but critical experiments by Seeliger et al. shed some light on this issue[23]. They crossed *Rpe65*$^{-/-}$ mice into either the *Cnga3*$^{-/-}$ background which lacks cone function or the *Rho*$^{-/-}$ which lacks rod function[23], and demonstrated that the small ERG signal detected in *Rpe65*$^{-/-}$ mice comes from the remaining functional rods and not cones. Furthermore, they suggest that the dramatic decrease in the presence of functional chromophore in *Rpe65*$^{-/-}$ rods enables them to respond under photopic conditions which would normally saturate rods[23]. This hypothesis is supported by the observation that the *Rpe65*$^{-/-}$ has significantly decreased levels of cone-specific phototransduction genes but no changes in rod phototransduction genes, suggesting an early loss of cones[39–42]. This conclusion was confirmed by the structural observation that cone degeneration started as early as 2 weeks of age with massive cone loss at 4 weeks[40].

The naturally occurring mutant model, *Rpe65*^{rd12}, is caused by a nonsense mutation in exon 3 in which a C to T transition creates a premature stop codon, R44X, resulting in loss-of-function due to truncation of the protein and mRNA degradation[36]. The phenotype is similar to the *Rpe65*$^{-/-}$ knockout; rod ERG response was profoundly diminished and small lipid-like droplets were deposited in RPE cells at 3 weeks. With time, droplet accumulation persists and is accompanied by slow retinal degeneration; at 6 weeks, the retina appears mostly normal with voids occasionally appearing in the OSs, but by 7 months, the OSs are obviously shorter and there is an approximately 30% reduction in the number of photoreceptor nuclei compared to wild-type[36,43]. No RPE65 expression, 11-*cis* retinal, or rhodopsin is detected in the retinas and retinyl esters accumulate in the RPE[36,43]. The only notable difference between the *Rpe65*$^{-/-}$ and the *Rpe65*^{rd12} models is that under ophthalmoscopic examination the latter model exhibits small white dots spread evenly throughout the retina at 5 to 9 months[36].

The final commonly used RPE65 animal model is the Swedish Briard dog. This dog has very poor vision with severely depressed dark- and light-adapted ERG responses and serves as a naturally occurring large-animal model of LCA. Molecular analysis revealed a 4-nucleotide (AAGA) deletion in the *RPE65* gene resulting in a frameshift and a premature stop codon which truncates the protein and causes the observed hereditary retinal dystrophy[11,44]. This deletion in the canine *RPE65* sequence (487–490) corresponds to nucleotides 340–343 of human exon 5[9]. Affected dogs have a normal fundus appearance until 3 years of age, however at 5 weeks of age, ERGs are abnormal with barely detectable scotopic responses and very low photopic amplitudes[35]. The disease is slowly progressive, with advanced age large lipid-like inclusions appear in the RPE, the OSs are disorganized, and rods and cones are lost starting from the peripheral retina and proceeding to the center[45].

Gene therapy

Canine and mouse models of LCA caused by defects in the *RPE65* gene are very attractive for gene therapy for a variety of reasons. RPE65-associated LCA is monogenic, and is caused by a recessive, loss-of-function mutation giving gene replacement therapy good prospects for attaining measurable improvements in visual function. Furthermore, the Briard dog model has a phenotype strikingly similar to that in human LCA patients[46].

Rpe65$^{-/-}$ animal models (dogs and mice) have been treated with gene replacement therapy with positive outcomes[47], although one prerequisite for success with these models appears to be the presence of an intact photoreceptor layer[21]. RPE is readily transduced by a variety of viruses[48]. Most recently, lentivirus has been used to transfer the mouse cDNA to the five day old *Rpe65*$^{-/-}$ mouse leading to long-lasting transgene expression in the RPE cells and maintenance of normal ERG function and cone number until at least 4 months[49,50].. Untreated

mice demonstrated the typical deficits in ERG function and degeneration of almost all cones by that time[49,50].

Currently, recombinant adeno-associated virus (rAAV), a nonpathogenic parvovirus which contains no viral coding regions and has low cytotoxicity is the most popular vector used for therapeutic gene delivery. It elicits minimal immune response and induces efficient, long-term transgene expression. rAAV is also the only viral vector to efficiently transduce both RPE and photoreceptors[48].

Rpe65$^{-/-}$ and *Rpe65*^{rd12} mice have been treated with rAAV vectors containing the *RPE65* cDNA at different times including embryonic day 14[51], postnatal day 14[42,43], postnatal day 18[52], 1–2.5 months[51,53], and 6 & 13 months[52]. Treatment at all ages resulted in efficient RPE transduction, with transferred RPE65 protein expression detectable up to 7 months post-injection by immunohistochemistry[43,51]. Rhodopsin and retinyl-ester levels in treated mice were nearly normal[43,51], 11-*cis*-retinal levels were significantly increased[42], and retinal morphology was close to normal[43,53]. Treated animals exhibited pronounced functional rescue as well; ERG responses were significantly improved (approaching normal) at 4–7 months after treatment[43,51,53,54] with consequent improvements in visual acuity[53] or visual guided behavior[43]. It was reported that treatment age affected treatment success; a smaller portion of animals treated at 17–26 months of age exhibited rescue compared to those treated at younger ages[21].

Successful RPE65 gene replacement therapy has also been shown using the Swedish Briard dog model. rAAV was subretinally injected at a variety of ages from 1 month to 4 years and resulted in significantly improved visual function (ERG), retinoid content, and visual behavior[29,47,53,55–58]. Functional recovery was evident within 2 weeks of treatment[53,57] and peaked at 3 months post injection[57,59,60] but substantial rescue persisted for more than 7 years[53]. Interestingly, improvement in photopic ERG responses was also seen in the contralateral, untreated control eye in the treated dogs[58]. As with the rodent models, it was reported that functional recovery is better when treatment occurs earlier (8 months) rather than later (30 months)[57,60]. Although most studies in dogs and rodents report improvements in both scotopic and photopic visual function, some cases observed that improvements were limited to cones[42,54]. It has been reported that photopic ERG amplitudes were increased, that cone numbers were increased, and that in some cases cone rescue appeared earlier and persisted longer than improvements in rods[42,52,59].

Various groups have worked to optimize rAAV vectors for *RPE65* gene transfer, expression, and rescue. It was reported that inclusion of a modified Kozak sequence at the translational start site and the incorporation of a lengthy stuffer sequence improve gene expression. Neither varying the rAAV2 serotype nor the promoter had a significant impact on gene expression, but the site of injection proved critical. While subretinal injections led to RPE65 expression in the RPE and functional rescue, neither was observed following intravitreal injection. After subretinal injection, gene expression was usually limited to RPE cells surrounding the injection area but in some cases the expression was more widely distributed[55]. As a final step before beginning clinical trials, toxicity studies were undertaken in rodents, dogs and primates. Generally treatment was well-tolerated with no significant adverse effects[45,53,55]. Occasionally inflammation presented soon after treatment, but resolved by three months post-injection in dogs[61] and by one week post-injection in monkeys[62]. Overall, RPE65 gene replacement therapy using rAAV vectors has been successful by multiple standards; the treatment is safe and effective, and rescue is persistent in multiple animal models. This body of work has paved the way for the exciting clinical trials now ongoing for people suffering from LCA2.

Recently, very encouraging reports were published by three different groups who are treating LCA2 patients as part of Phase 1 clinical trials[63–65]. Although the purpose of these initial studies was merely to test the safety of the vector, all three groups do report modest improvements in visual acuity. Maguire et al. subretinally injected rAAV containing human *RPE65* cDNA under the control of the ubiquitous chicken β-actin promoter into the eyes of three young adults. They report that 3/3 patients showed evidence of improvement in retinal function based on testing of visual acuity and pupillometry (for papillary light reflex). After treatment, the sensitivity of papillary response to light in the treated eyes was three times higher than the baseline[65]. Visual acuity improved and the visual field was enlarged 2 weeks after treatment[65]. The second group headed by Robin Ali used an rAAV vector containing human *RPE65* cDNA and the human *RPE65* promoter[63]. Of the three initial patients treated, one showed evidence of improvement in retinal function by microperimetry, dark-adapted perimetry, and visual mobility[63]. The third group reported increased light sensitivity and expanded visual fields in treated patients as early as PI-30[64]. Although treatment was limited to the area of the injection, patients reported substantial improvements[64]. Importantly, none of the groups observed any significant intraocular inflammation or immune responses.

As a result of the groundbreaking positive reports, these three trials (NCT00516477, NCT00643747, NCT00481546 www.clinicaltrials.gov) are ongoing and expanding their patient population to further examine treatment safety and efficacy. The success of RPE65 gene therapy in both animal models and humans provides great promise for the treatment of blinding LCA2. In addition, results from all these studies will be relevant to the larger gene therapy research community in terms of study design and model selection, vector optimization and delivery, and outcome assessment.

References

1. Travis GH, Golczak M, Moise AR, Palczewski K. Diseases caused by defects in the visual cycle: retinoids as potential therapeutic agents. Annual review of pharmacology and toxicology 2007;47:469.
2. Bavik CO, Busch C, Eriksson U. Characterization of a plasma retinol-binding protein membrane receptor expressed in the retinal pigment epithelium. The Journal of biological chemistry 1992;267 (32):23035. [PubMed: 1331074]
3. Redmond TM, et al. Rpe65 is necessary for production of 11-cis-vitamin A in the retinal visual cycle. Nature genetics 1998;20 (4):344. [PubMed: 9843205]
4. Hamel CP, et al. Molecular cloning and expression of RPE65, a novel retinal pigment epithelium-specific microsomal protein that is post-transcriptionally regulated in vitro. The Journal of biological chemistry 1993;268 (21):15751. [PubMed: 8340400]
5. Nicoletti A, et al. Molecular characterization of the human gene encoding an abundant 61 kDa protein specific to the retinal pigment epithelium. Human molecular genetics 1995;4 (4):641. [PubMed: 7633413]
6. Hamel CP, et al. A developmentally regulated microsomal protein specific for the pigment epithelium of the vertebrate retina. Journal of neuroscience research 1993;34 (4):414. [PubMed: 8474143]
7. Bavik CO, et al. The retinal pigment epithelial membrane receptor for plasma retinol-binding protein. Isolation and cDNA cloning of the 63-kDa protein. The Journal of biological chemistry 1993;268 (27): 20540. [PubMed: 8397208]
8. Hamel CP, et al. The gene for the retinal pigment epithelium-specific protein RPE65 is localized to human 1p31 and mouse 3. Genomics 1994;20 (3):509. [PubMed: 8034329]
9. Aguirre GD, et al. Congenital stationary night blindness in the dog: common mutation in the RPE65 gene indicates founder effect. Molecular vision 1998;4:23. [PubMed: 9808841]
10. Manes G, et al. Rat messenger RNA for the retinal pigment epithelium-specific protein RPE65 gradually accumulates in two weeks from late embryonic days. FEBS letters 1998;423 (2):133. [PubMed: 9512345]

11. Gal A, et al. Mutation spectrum of PRE65 in autosomal recessive childhood-onset severe retinal destrophy. Investigative ophthalmology & visual science 1998;39:S901.
12. Galbavy ES, Olson MD. Morphogenesis of rod cells in the retina of the albino rat: a scanning electron microscopic study. The Anatomical record 1979;195 (4):707. [PubMed: 525833]
13. Xue L, et al. A palmitoylation switch mechanism in the regulation of the visual cycle. Cell 2004;117 (6):761. [PubMed: 15186777]
14. Takahashi Y, Moiseyev G, Chen Y, Ma JX. The roles of three palmitoylation sites of RPE65 in its membrane association and isomerohydrolase activity. Investigative ophthalmology & visual science 2006;47 (12):5191. [PubMed: 17122102]
15. Jin M, Yuan Q, Li S, Travis GH. Role of LRAT on the retinoid isomerase activity and membrane association of Rpe65. The Journal of biological chemistry 2007;282 (29):20915. [PubMed: 17504753]
16. Jin M, et al. Rpe65 is the retinoid isomerase in bovine retinal pigment epithelium. Cell 2005;122 (3): 449. [PubMed: 16096063]
17. Moiseyev G, et al. RPE65 is the isomerohydrolase in the retinoid visual cycle. Proceedings of the National Academy of Sciences of the United States of America 2005;102 (35):12413. [PubMed: 16116091]
18. Redmond TM, et al. Mutation of key residues of RPE65 abolishes its enzymatic role as isomerohydrolase in the visual cycle. Proceedings of the National Academy of Sciences of the United States of America 2005;102 (38):13658. [PubMed: 16150724]
19. Moiseyev G, et al. RPE65 is an iron(II)-dependent isomerohydrolase in the retinoid visual cycle. The Journal of biological chemistry 2006;281 (5):2835. [PubMed: 16319067]
20. Znoiko SL, Crouch RK, Moiseyev G, Ma JX. Identification of the RPE65 protein in mammalian cone photoreceptors. Investigative ophthalmology & visual science 2002;43 (5):1604. [PubMed: 11980880]
21. Jacobson SG, et al. Identifying photoreceptors in blind eyes caused by RPE65 mutations: Prerequisite for human gene therapy success. Proceedings of the National Academy of Sciences of the United States of America 2005;102 (17):6177. [PubMed: 15837919]
22. Mata NL, et al. Rpe65 is a retinyl ester binding protein that presents insoluble substrate to the isomerase in retinal pigment epithelial cells. The Journal of biological chemistry 2004;279 (1):635. [PubMed: 14532273]
23. Seeliger MW, et al. New views on RPE65 deficiency: the rod system is the source of vision in a mouse model of Leber congenital amaurosis. Nature genetics 2001;29 (1):70. [PubMed: 11528395]
24. Feathers KL, et al. Nrl-knockout mice deficient in Rpe65 fail to synthesize 11-cis retinal and cone outer segments. Investigative ophthalmology & visual science 2008;49 (3):1126. [PubMed: 18326740]
25. Wenzel A, et al. RPE65 is essential for the function of cone photoreceptors in NRL-deficient mice. Investigative ophthalmology & visual science 2007;48 (2):534. [PubMed: 17251447]
26. Thompson DA, Gal A. Vitamin A metabolism in the retinal pigment epithelium: genes, mutations, and diseases. Prog Retin Eye Res 2003;22 (5):683. [PubMed: 12892646]
27. Morimura H, et al. Mutations in the RPE65 gene in patients with autosomal recessive retinitis pigmentosa or leber congenital amaurosis. Proceedings of the National Academy of Sciences of the United States of America 1998;95 (6):3088. [PubMed: 9501220]
28. Gu SM, et al. Mutations in RPE65 cause autosomal recessive childhood-onset severe retinal dystrophy. Nature genetics 1997;17 (2):194. [PubMed: 9326941]
29. Aguirre GK, et al. Canine and human visual cortex intact and responsive despite early retinal blindness from RPE65 mutation. PLoS medicine 2007;4 (6):e230. [PubMed: 17594175]
30. Chen Y, Moiseyev G, Takahashi Y, Ma JX. Impacts of two point mutations of RPE65 from Leber's congenital amaurosis on the stability, subcellular localization and isomerohydrolase activity of RPE65. FEBS letters 2006;580 (17):4200. [PubMed: 16828753]
31. Takahashi Y, Chen Y, Moiseyev G, Ma JX. Two point mutations of RPE65 from patients with retinal dystrophies decrease the stability of RPE65 protein and abolish its isomerohydrolase activity. The Journal of biological chemistry 2006;281 (31):21820. [PubMed: 16754667]

32. Samardzija M, et al. R91W mutation in Rpe65 leads to milder early-onset retinal dystrophy due to the generation of low levels of 11-cis-retinal. Human molecular genetics 2008;17 (2):281. [PubMed: 17933883]
33. Lorenz, B., et al. Investigative ophthalmology & visual science. 2008. A novel RPE65 hypomorph expands the clinical phenotype of RPE65 mutations. A comprehensive clinical and biochemical functional study.
34. Marlhens F, et al. Autosomal recessive retinal dystrophy associated with two novel mutations in the RPE65 gene. Eur J Hum Genet 1998;6 (5):527. [PubMed: 9801879]
35. Nilsson SE, Wrigstad A, Narfstrom K. Changes in the DC electroretinogram in Briard dogs with hereditary congenital night blindness and partial day blindness. Experimental eye research 1992;54 (2):291. [PubMed: 1559557]
36. Pang JJ, et al. Retinal degeneration 12 (rd12): a new, spontaneously arising mouse model for human Leber congenital amaurosis (LCA). Molecular vision 2005;11:152. [PubMed: 15765048]
37. Rohrer B, et al. Correlation of regenerable opsin with rod ERG signal in Rpe65−/− mice during development and aging. Investigative ophthalmology & visual science 2003;44 (1):310. [PubMed: 12506090]
38. Ablonczy Z, et al. 11-cis-retinal reduces constitutive opsin phosphorylation and improves quantum catch in retinoid-deficient mouse rod photoreceptors. The Journal of biological chemistry 2002;277 (43):40491. [PubMed: 12176991]
39. Cottet S, et al. Biological characterization of gene response in Rpe65−/− mouse model of Leber's congenital amaurosis during progression of the disease. Faseb J 2006;20 (12):2036. [PubMed: 17012256]
40. Znoiko SL, et al. Downregulation of cone-specific gene expression and degeneration of cone photoreceptors in the Rpe65−/− mouse at early ages. Investigative ophthalmology & visual science 2005;46 (4):1473. [PubMed: 15790918]
41. Ekesten B, Gouras P, Salchow DJ. Ultraviolet and middle wavelength sensitive cone responses in the electroretinogram (ERG) of normal and Rpe65 −/−mice. Vision research 2001;41 (19):2425. [PubMed: 11483174]
42. Chen Y, Moiseyev G, Takahashi Y, Ma JX. RPE65 gene delivery restores isomerohydrolase activity and prevents early cone loss in Rpe65−/− mice. Investigative ophthalmology & visual science 2006;47 (3):1177. [PubMed: 16505056]
43. Pang JJ, et al. Gene therapy restores vision-dependent behavior as well as retinal structure and function in a mouse model of RPE65 Leber congenital amaurosis. Mol Ther 2006;13 (3):565. [PubMed: 16223604]
44. Veske A, Nilsson SE, Narfstrom K, Gal A. Retinal dystrophy of Swedish briard/briard-beagle dogs is due to a 4-bp deletion in RPE65. Genomics 1999;57 (1):57. [PubMed: 10191083]
45. Narfstrom K, et al. Functional and structural recovery of the retina after gene therapy in the RPE65 null mutation dog. Investigative ophthalmology & visual science 2003;44 (4):1663. [PubMed: 12657607]
46. Wrigstad A. Hereditary dystrophy of the retinna and the retinal pigment epithelium in a strain of Briard dogs: a clinical, morphological and electrophysiological study. Linkoping Univ Med Dissertations 1994;423:1.
47. Acland GM, et al. Gene therapy restores vision in a canine model of childhood blindness. Nature genetics 2001;28 (1):92. [PubMed: 11326284]
48. Buch PK, Bainbridge JW, Ali RR. AAV-mediated gene therapy for retinal disorders: from mouse to man. Gene therapy 2008;15 (11):849. [PubMed: 18418417]
49. Bemelmans AP, et al. Lentiviral gene transfer-mediated cone vision restoration in RPE65 knockout mice. Advances in experimental medicine and biology 2008;613:89. [PubMed: 18188932]
50. Bemelmans AP, et al. Lentiviral gene transfer of RPE65 rescues survival and function of cones in a mouse model of Leber congenital amaurosis. PLoS medicine 2006;3 (10):e347. [PubMed: 17032058]
51. Dejneka NS, et al. In utero gene therapy rescues vision in a murine model of congenital blindness. Mol Ther 2004;9 (2):182. [PubMed: 14759802]

52. Nusinowitz S, et al. Cortical visual function in the rd12 mouse model of Leber Congenital Amarousis (LCA) after gene replacement therapy to restore retinal function. Vision research 2006;46 (22):3926. [PubMed: 16814838]

53. Bennicelli J, et al. Reversal of blindness in animal models of leber congenital amaurosis using optimized AAV2-mediated gene transfer. Mol Ther 2008;16 (3):458. [PubMed: 18209734]

54. Lai CM, et al. Recombinant adeno-associated virus type 2-mediated gene delivery into the Rpe65−/− knockout mouse eye results in limited rescue. Genetic vaccines and therapy 2004;2 (1):3. [PubMed: 15109394]

55. Acland GM, et al. Long-term restoration of rod and cone vision by single dose rAAV-mediated gene transfer to the retina in a canine model of childhood blindness. Mol Ther 2005;12 (6):1072. [PubMed: 16226919]

56. Jacobs JB, et al. Eye movement recordings as an effectiveness indicator of gene therapy in RPE65-deficient canines: implications for the ocular motor system. Investigative ophthalmology & visual science 2006;47 (7):2865. [PubMed: 16799026]

57. Le Meur G, et al. Restoration of vision in RPE65-deficient Briard dogs using an AAV serotype 4 vector that specifically targets the retinal pigmented epithelium. Gene therapy 2007;14 (4):292. [PubMed: 17024105]

58. Narfstrom K, et al. In vivo gene therapy in young and adult RPE65−/− dogs produces long-term visual improvement. The Journal of heredity 2003;94 (1):31. [PubMed: 12692160]

59. Narfstrom K, et al. Assessment of structure and function over a 3-year period after gene transfer in RPE65−/− dogs. Documenta ophthalmologica 2005;111 (1):39. [PubMed: 16502306]

60. Rolling F, et al. Gene therapeutic prospects in early onset of severe retinal dystrophy: restoration of vision in RPE65 Briard dogs using an AAV serotype 4 vector that specifically targets the retinal pigmented epithelium. Bulletin et memoires de l'Academie royale de medecine de Belgique 2006;161 (10–12):497. [PubMed: 17503728]

61. Jacobson SG, et al. Safety of recombinant adeno-associated virus type 2-RPE65 vector delivered by ocular subretinal injection. Mol Ther 2006;13 (6):1074. [PubMed: 16644289]

62. Jacobson SG, et al. Safety in nonhuman primates of ocular AAV2-RPE65, a candidate treatment for blindness in Leber congenital amaurosis. Human gene therapy 2006;17 (8):845. [PubMed: 16942444]

63. Bainbridge JW, et al. Effect of gene therapy on visual function in Leber's congenital amaurosis. The New England journal of medicine 2008;358 (21):2231. [PubMed: 18441371]

64. Cideciyan AV, et al. Human gene therapy for RPE65 isomerase deficiency activates the retinoid cycle of vision but with slow rod kinetics. Proceedings of the National Academy of Sciences of the United States of America 2008;105 (39):15112. [PubMed: 18809924]

65. Maguire AM, et al. Safety and efficacy of gene transfer for Leber's congenital amaurosis. The New England journal of medicine 2008;358 (21):2240. [PubMed: 18441370]