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(54) Benævnelse: **VIRALE VEKTORER OMFATTENDE RDH12-KODENDE OMRÅDER OG FREMGANGSMÅDER TIL BEHANDLING AF NETHINDEDYSTROFIER**

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DESCRIPTION

Description

FIELD

[0001] The invention relates to an adeno-associated viral vector for use in a method of treating a human subject who has an ophthamological condition due to one or more loss-of-function mutations in the gene encoding the Retinol Dehydrogenase 12 (RDH12) protein, an adeno-associated viral vector, and an isolated host cell comprising the adeno-associated viral vector provided herein.

BACKGROUND

[0002] Inherited retinal disease (IRD) is a leading cause of legal blindness in children. Leber congenital amaurosis (LCA) and early-onset severe retinal dystrophy (EOSRD) result in severe visual impairment beginning at birth to a few years of age, and together account for 5% or more of all IRD (Koenekoop et al. 2004). LCA/EOSRD is associated with autosomal dominant and autosomal recessive modes of inheritance, involving the retinal pigment epithelium and the rod and cone photoreceptors as primary targets (Weleber et al. 2013). Approximately 10% of LCA/EOSRD is caused by mutations in the gene encoding RDH12 (Kumaran et al. 2017). Given the role of RDH12 in the visual cycle that provides chromophore to the photoreceptor cells (Haeseleer et al. 2002; Chen et al. 2012), and which constitutes a critical therapeutic target, *RDH12* is one of the most important LCA genes.

[0003] Despite the prevalence of ophthalmological conditions such as inherited retinal disease in humans and non-human mammals, and knowledge of genes encoding retinol dehydrogenases as well as the effect some mutations in those genes have, there is no known treatment for LCA caused by one or more mutations in *RDH12.* Accordingly, a need continues to exist in the art for materials and methods useful in treating retinal dystrophies such as LCA as well as materials and methods useful in correcting genetic anomalies that can lead to such dystrophies.

[0004] The disclosure relates to medical treatment methods, such as methods for treating a human subject with an ophthalmological condition, e.g., Leber congenital amaurosis, due to at least one loss-of-function mutation in the gene encoding the Retinol Dehydrogenase 12 protein (RDH12), the method comprising administering to the subject an effective amount of a nucleic acid comprising an adeno-associated viral vector comprising a human *RDH12* complementary

DNA (cDNA).

[0005] Thompson et al discloses AAV-mediated expression of human Rdh12 in mouse retina (Thompson et al, ARVO Annual Meeting Abstract, March 2012, URL: https://iovs.arvojournals.org/article.aspx?articleid=2351889). WO2016001693 discloses RPGR-ORF 15 variant gene in the treatment of retinitis pigmentosa.

[0006] WO2011/034947 discloses reagents and methods for modulating cone photoreceptor activity.

[0007] Fingert et al discloses the association of a novel mutation in the Retinol Dehydrogenase 12 (RDH12) gene with autosomal dominant Retinitis Pigmentosa (Fingert et al, Ophthalmic Molecular Genentics, 1 September 2008, vol. 126 (NO. 9)).

SUMMARY

[0008] The presently claimed invention is as defined in the claims.

[0009] The disclsoure provides an adeno-associated virus (AAV) vector comprising a coding region for the gene product of the *RDH12* gene, a gene encoding a retinol dehydrogenase enzyme. The AAV vector comprising *RDH12* is useful in treating retinal dystrophy disorders such as Leber Congenital Amaurosis (LCA) by providing a recombinant construct in which an *RDH12* coding region is placed under the control of a regulable, or controllable, promoter, such as a heterologous promoter, to provide complementing retinol dehydrogenase to subjects lacking wild-type levels of RDH12 activity, such as would result from mutations in *RDH12.* Despite the advantages of relatively small genome size, relatively low tendency to integrate into host DNA, and relatively low immunogenic profile, a surprising finding has been that some AAV serotype combinations, or pseudotypes, yield expression levels for encoded RDH12 product that is not therapeutically effective and/or that exhibits undesirable toxicity in the context of administration to subjects to treat retinal dystrophies. Coincident with that finding is the finding that certain pseudotypes, such as the AAV2/5 pseudotype, demonstrate unexpected and surprisingly effective expression levels and toxicity profiles compatible with therapeutic use to treat retinal dystrophies. Subjects who can be treated by methods disclosed herein can include those who have loss of visual function (*e.g.*, impaired response on electroretinogram (ERG) testing), but who retain some photoreceptor cells as determined by optical coherence tomography (OCT). Thus, in one instance, the disclosure provides a method of treating a human subject who has an ophthalmological condition, such as Leber Congenital Amaurosis, or LCA, or another clinically defined ophthalmological condition, due to one or more loss-of-function mutations in the gene encoding the Retinol Dehydrogenase12 (RDH12) protein. More particularly, one aspect of the presently claimed invention provides an adeno-associated viral vector as defined in the claims for use in a method of treating a human subject who has an ophthalmological condition due to one or more loss-of-function mutations in the gene encoding the Retinol Dehydrogenase12 (RDH12) protein, the method comprising administering to at

least one eye of the subject the adeno-associated viral vector, wherein the adeno-associated viral vector comprises a nucleic acid, wherein the nucleic acid comprises human *RDH12* DNA, *e.g.,* human *RDH12* cDNA and wherein the human *RDH12* DNA encodes a protein that is at least 70%, 80%, 90%, 95%, or 99% identical to the full length of SEQ ID NO:2. The ophthalmological condition is Leber Congenital Amaurosis (LCA).

[0010] The *RDH12* DNA, *e.g., RDH12* cDNA, is under the expression control of a human rhodopsin kinase 1 (hGRK1) promoter, such as wherein the hGRK1 promoter comprises or consists essentially of SEQ ID NO:3. The adeno-associated viral vector is AAV-2, serotype-5 (AAV2/5).

[0011] In some embodiments, the *RDH12* DNA, *e.g., RDH12* cDNA, comprises a sequence that is at least 60% or 70% identical to SEQ ID NO:1. In some embodiments, the nucleic acid is administered at a titer of about 2×10^{10} viral genomes per milliliter (vg/mL) to about 2×10^{12} vg/mL, *e.g.,* a titer of about 2×10^{10} viral genomes per milliliter (vg/mL) to about 2×10^{12} vg/mL, such as about 2×10^{11} vg/mL or about 2×10^{12} vg/mL. In some embodiments, the nucleic acid is administered into the subretinal space, *e.g.*, wherein a micro injection cannula is inserted into the subretinal space.

[0012] An instance of the disclosure is drawn to a nucleic acid encoding a human *RDH12* DNA, *e.g.,* a human *RDH12* cDNA, wherein the *RDH12* DNA (*e.g.,* human *RDH12* DNA such as cDNA) encodes a protein that is at least 70%, 80%, 90%, 95%, or 99% identical to the full length of SEQ ID NO:2, wherein the *RDH12* DNA is under the control of a human rhodopsin kinase 1 (hGRK1) promoter. In some embodiments, the hGRK1 promoter comprises or consists essentially of SEQ ID NO:3. In some embodiments, the human *RDH12* DNA, *e.g.,* human *RDH12* cDNA, encodes a protein comprising SEQ ID NO:2. In some embodiments, the human *RDH12* DNA, *e.g.*, human *RDH12* cDNA, is at least 60% or 70% identical to the full length of SEQ ID NO: 1.

[0013] Yet another instance of the disclosure is a nucleic acid as disclosed herein for use in treating a human subject who has an ophthalmological condition due to one or more loss-of-function mutations in the gene encoding the Retinol Dehydrogenase 12 (RDH12) protein. In some embodiments, the ophthalmological condition is Leber Congenital Amaurosis (LCA).

[0014] Another aspect of the presently claimed invention provides a viral vector comprising a nucleic acid encoding RDH12 as defined in the claims. The viral vector is an adeno-associated viral vector. The adeno-associated viral vector is AAV-2, serotype-5 (AAV2/5).

[0015] Another instance of the disclosure is a viral vector as disclosed herein for use in treating a human subject who has an ophthalmological condition due to one or more loss-of-function mutations in the gene encoding the Retinol Dehydrogenase 12 (RDH12) protein. In some embodiments, the ophthalmological condition is Leber Congenital Amaurosis (LCA).

[0016] Another aspect of the presently claimed invention is directed to an isolated host cell comprising a viral vector as defined in the claims. In some embodiments, the isolated host cell expresses a human RDH12 protein.

[0017] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Methods and materials are described herein for use in the present invention. In case of conflict, the present specification, including definitions, will control.

[0018] Other features and advantages of the invention will be apparent from the following detailed description and figures, and from the claims.

BRIEF DESCRIPTION OF THE DRAWING

[0019] The presently claimed invention is as defined in the claims. Any of the following Figures that do not fall within the scope of the claims do not form part of the presently claimed invention and are provided for comparative purposes only.

Figure 1. RDH activity in the visual cycle and photoreceptor cells. (A) The visual cycle converts vitamin A to *11-cis* retinal, the chromophore of the visual pigments, and recycles *all-trans* retinal released after bleaching. (B) Retinoid flow shown for a RPE-photoreceptor cell pair. RDH8 in the outer segment can reduce *all-trans* retinal. RDH12 in the inner segment can reduce all-*trans* retinal, *11 -cis* retinal, and other toxic short chain aldehydes. Abbreviations: 11cRAL, 11-*cis* retinal; 11cROL, 11-*cis* retinol; AtRAL, all-*trans* retinal; AtROL, all-*trans* retinol; RCHO, short-chain aldehyde; RCHOH, short-chain alcohol; Rh, rhodopsin; MRh, metarhodopsin.

Figure 2. Expression and localization of recombinant RDH12 in *AAV2/5-hGRK1p.hRDH12* injected mice. (A) Schematic of the *AAV2/5-hGRK1p.hRDH12* gene-therapy construct in which a human *RDH12* cDNA is cloned downstream of a human rhodopsin kinase promoter, between inverted terminal repeat sequences derived from the AAV2 genome. (B, C) Expression of human RDH12 protein in mouse retinas at 6 weeks following sub-retinal injection of *AAV2/5-hGRK1p.hRDH12* (1.3×10^9 vg) or PBS, evaluated using antibodies specific for mouse Rdh12 or human RDH12. (B) Western analysis of retinal lysates from C57BL/6J mice, PBS injected *Rdh12*$^{-/-}$ mice, and *AAV2/5-hGRK1p.hRDH12*-injected *Rdh12*$^{-/-}$ mice. (C) Immunohistochemical analysis shows localization of native mouse Rdh12 (dark gray) to the IS, ONL, and OPL of the retina in C57BL/6J mice but not in *Rdh12*$^{-/-}$ mice, whereas recombinant human RDH12 (light gray) resulting from injection of *AAV2/5-hGRK1p.hRDH12* shows similar localization both C57BL/6J and *Rdh12*$^{-/-}$ mice. Phase contrast images (left). Abbreviations: ITR, inverted terminal repeat; hGRK1, human rhodopsin kinase promoter; SD/SA, Simian virus 40 splice donor/splice acceptor site; hRDH12, human RDH12 cDNA; polyA, Simian virus 40 polyadenylation signal; RPE, retinal pigment epithelium; OS, outer segments; IS, inner

segments; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer.

Figure 3. *AAV2/5-hGRK1p.*hRDH12 gene-replacement therapy restores RDH12 function in *Rdh12*-deficent mice. (A) HPLC analysis of retinal reductase activity in retinas from C57BL/6J and *Rdh12*$^{-/-}$ mice injected with *AAV2/5-hGRK1p.hRDH12* (1.3 × 10^9 vg) or PBS, or non-injected. At 6 weeks post-injection, all-*trans* retinol formation was quantitated in assays with all-*trans* retinal as a substrate. Each data point represents the mean ± standard error for a minimum of 5 independent experiments where retinas from 3 to 5 mice were pooled and assayed in triplicate. ● C57BL/6J ; △ *Rdh12*$^{-/-}$; ◊ *Rdh12*$^{-/-}$ PBS-injected;
▨
Rdh12$^{-/-}$ AAV-injected. (B) Immunohistochemistry of recombinant RDH12 expression in an *Rdh12*$^{-/-}$ mouse whole-retina section evaluated 16 weeks post-injection of *AAV2/5-hGRK1p.hRDH12.* Human RDH12 (light gray) localizes to the IS, ONL, and OPL of the injected region of the retina (right-hand side of the image).

Figure 4. *AAV2/5-hGRK1p.*hRDH12 gene-replacement therapy reduces light-damage in albino *Rdh12*-deficent mice. ERG analysis was performed, one week before and one week after exposure to 5,000 lux for 2 hours, on mice that were injected in one eye with *AAV2/5-hGRK1p.hRDH12* and were uninjected in the contralateral eye. Scotopic (rod-isolated and combined rod-cone) responses were quantified for groups of 10-13 mice, and the percentage of the initial ERG response remaining after light damage was calculated. Averaged outcomes with standard errors are shown, as well as the significance of the differences between injected and uninjected eyes calculated using two-tailed paired t-test analysis.

Figure 5. *AAV2/5-hGRK1p.hRDH12* does not significantly affect steady-state levels of 11-cis retinal in the retina. Mice received *AAV2/5-hGRK1p.hRDH12* (1.3 × 10^9 vg) or PBS via sub-retinal injection, or were non-injected. Following overnight dark adaptation, retinoids were extracted under dim-red light, and quantified by HPLC analysis. (A) Representative chromatograms from each treatment condition; peaks for syn-11-*cis* retinal oxime, anti-11-*cis* retinal oxime, and syn-all-*trans* retinal oxime are indicated. Total retinal levels of (B) 11-*cis* retinal and (C) all-*trans* retinal for each treatment condition ± standard error (■ C57BL/6J non-injected;
▩
Rdh12$^{-/-}$ non-injected;
▨
Rdh12$^{-/-}$ AAV injected; □ *Rdh12*$^{-/-}$ PBS injected).

Figure 6. Retinal function is not adversely affected by *AAV2/5-hGRK1p.hRDH12.* Scotopic (rod-isolated and combined rod-cone) and photopic (cone-mediated) electroretinogram (ERG) responses recorded at 6 weeks post treatment from C57BL/6J, non-injected; *Rdh12*$^{-/-}$, non-injected; and *Rdh12*$^{-/-}$ *AAV2/5-hGRK1p.hRDH12* injected mice (up to 2 × 10^9 vg). ERGs from a representative animal in each treatment group measured at 6 weeks post-injection are shown.

Figure 7. Visual pigment localization is not perturbed by *AAV2/5-hGRK1p.hRDH12.* Immunohistochemical localization of rhodopsin and cone opsin in non-injected and injected (1.3×10^9 vg) *Rdh12*$^{-/-}$ mice evaluated at 16 weeks post treatment. Human RDH12 protein expression in IS and ONL (light gray). Rhodopsin and red/green opsin (dark gray) in *AAV2/5-hGRK1p.hRDH12* injected eyes. Abbreviations are as described for Figure 2.

Figure 8. Retinal structure is not damaged by long-term expression of *AAV2/5-hGRK1p.hRDH12.* Optical coherence tomography (OCT) analysis of eyes of C57BL/6J and *Rdh12*$^{-/-}$ mice evaluated at 54 weeks post injection of *AAV2/5-hGRK1p.hRDH12* (up to 2×10^9 vg).

Figure 9. Infiltrating CD68+ macrophages and RDH12 expression in AAV2/8-*hGRK1p.hRDH12* injected retinas. *Rdh12*$^{-/-}$ and C57BL/6J mice evaluated by immunohistochemical analysis 8 weeks following subretinal injection of AAV2/8-*hGRK1p.hRDH12* (2×10^9 vg). Human RDH12 expression in IS, ONL, and OPL (light gray) and CD68 labeling of macrophages (white). Abbreviations are as described for Figure 2.

DETAILED DESCRIPTION

[0020] The presently claimed invention is as defined in the claims. Methods of treatment as such do not form part of the claimed invention but are herein discussed as related subject matter.

[0021] Inherited retinal degeneration is a rare cause of profound vision loss that is a focus of current efforts to develop targeted gene-therapy. Viral vector-mediated somatic gene therapy has shown great promise in treating animal models of human retinal degenerative disease. To date, there have been a number of successful studies using adeno-associated virus (AAV)-mediated gene delivery to rescue photoreceptor degeneration in small animal models (Ali et al. 2000; Pang et al. 2012; Pawlyk et al. 2010; Pawlyk et al. 2005; Tan et al. 2009) and large animal models (Acland et al. 2001; Alexander et al. 2007; Beltran et al. 2012; Komaromy et al. 2010; Lheriteau et al. 2009). In these cases, the retinal pigment epithelium (RPE) or photoreceptors have been the primary targets for transgene expression. In addition, phase I clinical trials involving gene therapy for patients with Leber Congenital Amaurosis (LCA) targeting the RPE (Bainbridge et al. 2008; Cideciyan et al. 2008; Maguire et al. 2008) and more recently choroideremia (Maclaren et al. 2014), have already met with some success. There are currently no clinical trials using AAV-mediated gene replacement therapy for the treatment of patients with inherited retinal degeneration caused by mutations in *RDH12.*

[0022] With an interest in developing AAV-mediated gene therapy for the treatment of individuals with mutations in *RDH12,* we generated adeno-associated virus vectors carrying human *RDH12* cDNA in which expression is under the control of a rhodopsin-kinase promoter.

In the disclosed study of vectors with capsids derived from AAV serotype 5 or AAV serotype 8, we have shown that subretinal delivery of *AAV2/5-hGRK1p.RDH12* in Rdh12-deficient (*Rdh12*$^{-/-}$) mice results in expression of recombinant human RDH12 that is stable, correctly localized, reconstitutes retinal reductase activity, reduces light-damage susceptibility, and does not cause the retinal toxicity seen with *AAV2/8-hGRK1p.hRDH12.* This *AAV2/5-hGRK1p.RDH12* construct provides a product for *RDH12* gene-replacement therapy.

RDH12

[0023] Mutations in the gene encoding retinol dehydrogenase 12 (RDH12) cause severe early-onset retinal degeneration most often diagnosed as Leber congenital amaurosis (LCA) or early-onset severe retinal dystrophy (EOSRD). A member of the family of short-chain dehydrogenases/reductases, RDH12 is essential for reducing retinaldehydes that are generated by the activity of the vitamin A visual cycle that is integral to the light response of the photoreceptor cells. When visual pigments containing the 11-*cis* retinal chromophore absorb a photon of light, 11-*cis* retinal is isomerized to all-*trans* retinal, thus initiating a signal transduction cascade that regulates synaptic signaling. Visual pigment inactivation involves release of all-*trans* retinal, its reduction to all-*trans* retinol and return to the retinal pigment epithelium (RPE) for regeneration of the 11-*cis* retinal chromophore (Figure 1A). When these recycling reactions are inefficient or disrupted, *e.g.*, by aging or inherited disease, retinaldehydes and retinaldehyde-condensation products accumulate in the photoreceptors and retinal pigment epithelium, resulting in profound damage to the outer retina (Ben-Shabat et al. 2001; Thompson et al. 2003; Sparrow, 2010; Chen et al. 2012).

[0024] To protect against toxicity, a number of retinoid binding proteins and enzymes are expressed in the retina. RDH12 is a member of the family of short-chain dehydrogenases/reductases that uses NADPH to reduce a broad range of substrates, including *cis*- and *trans*-retinaldehydes (Haeseleer et al. 2002), C9 aldehydes generated as a result of lipid photo-oxidation (Belyaeva et al. 2005; Lee et al. 2008; Marchette et al. 2010), and steroid substrates (Keller et al. 2007). Individuals with loss-of-function mutations in the *RDH12* gene exhibit a severe retinal degeneration phenotype often diagnosed as Leber congenital amaurosis (LCA) (Janecke et al. 2004; Thompson et al. 2005; Perrault et al. 2004; den Hollander et al. 2008; Mackay et al. 2011), for which there is currently no treatments or cures. RDH12 localizes to the inner segments of rod and cone photoreceptor cells (Haeseleer et al. 2002; Maeda et al. 2006) where it protects against light-induced damage caused, at least in part, by reactive retinaldehydes (Maeda et al. 2006). All-*trans* retinal generated after light exposure has been shown to leak from photoreceptor outer segments to inner segments, and its effective clearance from photoreceptor cells requires the activity of both RDH8 present in the outer segments, and RDH12 present in the inner segments (Chen et al. 2012) (Figure 1B). As such, RDH12 also has the potential to play an important role in reducing 11-*cis* retinal present in excess of that required for opsin biosynthesis, which can enter the inner segment from the subretinal space (Chen et al. 2012).

[0025] To investigate the potential of gene replacement therapy for LCA/EOSRD due to *RDH12* mutations, we generated adeno-associated virus vectors carrying a human *RDH12* cDNA, in which expression is under the control of a rhodopsin-kinase promoter that directs photoreceptor-cell specific expression (Khani et al. 2007; Sun et al. 2010; Young et al. 2003). The DNA construct was packaged with a capsid derived from AAV serotype 8 that mediates efficient and robust transduction of photoreceptor cells (Allocca et al. 2007; Natkunarajah et al. 2008; Vandenberghe et al. 2011; Vandenberghe et al. 2013), or with a AAV serotype 5 capsid that mediates photoreceptor transduction, but with slower kinetics and less robust expression compared to the AAV serotype 8 capsid (Yang et al. 2002; Lotery et al. 2003; Allocca et al. 2007; Lebherz et al. 2008).

[0026] In comparative studies performed in a mouse model of Rdh12-deficiency (*Rdh12*$^{-/-}$) (Kurth et al. 2007), our work has shown that the two vectors have significantly different safety profiles, with *AAV2/5-hGRK1p.hRDH12* demonstrating unexpected and surprisingly effective expression levels and toxicity profiles compatible with therapeutic use to treat retinal dystrophies.

Human RDH12 sequence

[0027] Sequence of an exemplary human RDH12 cDNA, consisting of nucleotides -10 to +980 relative to the translation initiation site, encoding all seven translated RDH12 exons (GenBank # NM_152443) is provided in SEQ ID NO:1.

[0028] The full-length human RDH12 protein sequence is provided in SEQ ID NO:2.

Rhodopsin kinase promoter (hGRK1p)

[0029] In some embodiments of the methods described herein, a replacement gene construct is used in which a human RDH12 cDNA as described herein is placed under the control of a human rhodopsin kinase (hGRK1) promoter. In some embodiments, the hGRK1 promoter is approximately 200 base pairs (bp) in length containing a short promoter derived from the rhodopsin kinase (RK) hGRK1 gene, which has been shown to drive cell-specific expression in rods and cones (Khani et al. 2007; Sun et al. 2010; Young et al. 2003). An exemplary hGRK1 promoter sequence contains nucleotides -112 to +87 of SEQ ID NO:3 (Khani et al. 2007).

Viral Delivery Vector

[0030] The abbreviated human *RDH12* cDNA, as described above, is packaged into a delivery vector, i.e. a AAV2/5 vector.

[0031] Replacement genes (cDNA) can be administered in any effective carrier, *e.g.,* any formulation or composition capable of effectively delivering the component gene to cells *in vivo.* Approaches include insertion of the gene into non-pathogenic, non-replicating viral vectors, including recombinant retroviruses, adenovirus, adeno-associated virus, lentivirus, and herpes simplex virus-1, or recombinant bacterial or eukaryotic plasmids. Viral vectors transfect cells directly; plasmid DNA can be delivered naked or with the help of, for example, cationic liposomes (lipofectamine) or derivatized (*e.g.*, antibody conjugated), poly lysine conjugates, gramacidin S, artificial viral envelopes or other such intracellular carriers, as well as direct injection of the gene construct or $Ca_3(PO_4)_2$ precipitation carried out *in vivo.*

[0032] A preferred approach for *in vivo* introduction of nucleic acid into a cell is by use of a viral vector containing nucleic acid, *e.g.,* a cDNA. Infection of cells with a viral vector has the advantage that a large proportion of the targeted cells can receive the nucleic acid. Additionally, molecules encoded within the viral vector, *e.g.,* by a cDNA contained in the viral vector, are expressed efficiently in cells that have taken up viral vector nucleic acid. Retrovirus vectors and adenovirus derived vectors can be used as a recombinant gene delivery system for the transfer of exogenous genes *in vivo,* particularly into humans, in a number of cell types. However, they do not transduce the photoreceptor cells with sufficient efficiency to make them useful for this application.

[0033] Yet another viral vector system useful for delivery of nucleic acids is the adeno-associated virus (AAV). Adeno-associated virus is a naturally occurring defective virus that requires another virus, such as an adenovirus or a herpes virus, as a helper virus for efficient replication and a productive life cycle (Muzyczka et al. 1992). It is also one of the few viruses that may integrate its DNA into non-dividing cells, and exhibits a high frequency of stable integration (see, for example, Flotte et al. 1992; Samulski et al. 1989; and McLaughlin et al. 1988). Vectors containing as little as 300 base pairs of AAV can be packaged and can integrate. Space for exogenous DNA is limited to about 4.5 kb. An AAV vector such as that described in Tratschin et al., 1985 can be used to introduce DNA into cells. A variety of nucleic acids have been introduced into different cell types using AAV vectors (see, for example, Hermonat et al. 1984; Tratschin et al. 1984a; Tratschin et al. 1984b; Wondisford et al. 1988; and Flotte et al. 1993).

[0034] The viral delivery vector is a recombinant AAV2/5 virus. Prior to administration, the final product can undergo a series of ultrapurification steps to meet clinical grade criteria.

Subject selection

[0035] Subjects who are candidates for the claimed treatments include those who have a diagnosis of LCA caused by mutations in the gene encoding RDH12. Subjects suffering from other ophthalmological clinically-defined conditions caused by mutations in the gene encoding RDH12, *e.g.*, early-onset retinitis pigmentosa, can also be treated using the methods described

herein. A diagnosis of LCA or another ophthalmological condition caused by mutations in the gene encoding RDH12 can be made using methods known in the art.

[0036] The methods described herein can include identifying a subject, *e.g.,* a child, adolescent, or young adult subject, who has LCA or another ophthalmological condition caused by one or more mutations in the gene encoding RDH12, or who is suspected of having LCA or another ophthalmological condition caused by one or more mutations in the gene encoding RDH12 (*e.g.*, based on the presence of symptoms of the condition and no other obvious cause), and obtaining a sample comprising genomic DNA from the subject, detecting the presence of mutations in *RDH12* using known molecular biological methods, and selecting a patient who has mutations in both *RDH12* alleles that cause LCA or another condition. Symptoms of the condition include macular atrophy, foveal thinning and disruption of laminar architecture, resulting in early central vision loss and progression to LP vision. Visual fields are constricted at the earliest age measured, and ERG responses become unrecordable by early adulthood. Detecting mutations in *RDH12* can include sequencing all or part of the *RDH12* gene in a subject, and comparing the sequence to a reference sequence (*e.g.*, GenBank Accession No. NG_008321.1) to detect a mutation. Frameshift mutations, truncation mutations, mutations that alter a conserved amino acid, mutations that affect transcript splicing, or mutations that affect a regulatory (*e.g.*, promoter) region are considered to be mutations that can cause LCA or another ophthalmological condition as described herein; an alteration in function can be confirmed by expressing the mutant *in vitro* (*e.g.,* in cultured cells), and assaying, *e.g.,* enzymatic function. Exemplary mutations in the homozygous state include: Glu127X, Gln189X, Tyr226Cys, Ala269GlyfsX1, and Leu274Pro (all position references refer to the RDH12 protein sequence of SEQ ID NO:2). Exemplary mutations in the compound heterozygous state include: Thr49Met/Arg62X; Arg65X/Ala269GlyfsX1; His151D/Thr155Ile; His151D/Arg269GlyfsX1 (Janecke et al. 2004; Schuster et al. 2007). (Positions refer to the protein sequence of SEQ ID NO:2.)

[0037] Patients with LCA or another ophthalmological condition due to at least one *RDH12* mutation that can be treated using a method described herein preferably retain some photoreceptors and visual function, *e.g.*, as measured by standard visual function or field tests and/or Optical Coherence Tomography (OCT, *e.g.,* Spectral Domain-OCT (SD-OCT)). The methods described herein can include identifying subjects who have been diagnosed with LCA or another ophthalmological condition due to at least one *RDH12* mutation, who have at least one confirmed mutation in *RDH12* that causes their condition, and testing their visual ability and detecting the presence of residual central photoreceptors.

EXAMPLES

[0038] As described elsewhere herein, the presently claimed invention is as defined in the claims. Any of the following Examples that do not fall within the scope of the claims do not form part of the presently claimed invention and are provided for comparative purposes only.

[0039] The disclosed and claimed subject matter is further described in the following examples, which do not limit the scope of the invention described in the claims.

Materials and Methods

[0040] The following materials and methods were used in the experiments disclosed in the Examples set forth below.

Animals

[0041] The generation and analysis of *Rdh12*$^{-/-}$ mice have been described previously (Kurth et al. 2007). The *Rdh12*$^{-/-}$ mice used in this study were bred from sibling mating among nullizygous males and females maintained in our institutional animal facility. WT mice used in the study were C57BL/6 from The Jackson Laboratory (Wilmington, MA).

[0042] Transgenic mice of the following genotypes were used for the studies disclosed herein: *Rdh12*$^{-/-}$ mice on C57BL/6J background homozygous for the Rpe65-Met450 (M/M) variant (Kurth, 2007), and albino *Rdh12*$^{-/-}$ mice on BALB/c background homozygous for the Rpe65-Leu450 (L/L) variant (Chrispell, 2009), that were obtained by breeding. Mice were reared in a 12-hour (light)/12-hour (dark) cycle and were euthanized by CO_2 inhalation followed by bilateral pneumothorax.

Plasmid construction and production of recombinant AAV2/5 and AAV2/8

[0043] Human *RDH12* cDNA were amplified from human retinal cDNA by PCR using primers designed to encompass the entire RDH12 coding region, cloned, and sequenced to verify fidelity, as described previously (Janecke et al. 2004). To construct the AAV vectors, *RDH12* cDNAs were inserted into the multiple cloning site of the parental *pAAV- hGRK1-hrGFP* vector. The resulting *pAAV- hGRK1-Rdh12* vector was packaged into AAV. AAV2/5 and AAV2/8 pseudotyped vectors were generated by bipartite transfection: (1) AAV vector plasmid encoding the gene of interest, (2) AAV helper plasmid encoding AAV Rep proteins from serotype 2 and Cap proteins from either serotype 5 or serotype 8, and adenovirus helper functions into 293T cells. The transfection and purification were performed using a protocol as published (Nishiguchi et al 2015). Two days after transfection, cells were lysed by repeated freeze and thaw cycles. After initial clearing of cell debris, the nucleic acid component of the virus producer cells was removed by Benzonase treatment. The recombinant AAV vector particles were purified by affinity chromatography using a AVB matrix, washed in 1x PBS and concentrated to a volume of 100-150 ml using Vivaspin 4 (10 kDa) concentrators. Vectors were titered by qPCR amplification.

Subretinal injections

[0044] Cohorts of mice at approximately 4 weeks of age were placed under general anesthesia with an intraperitoneal injection of ketamine (90 mg/kg)/xylazine (9 mg/kg). A 0.5% proparacaine solution was applied to the cornea as a topical anesthetic. Pupils were dilated with topical application of tropicamide (0.5%). Under an ophthalmic surgical microscope, a small incision was made through the cornea adjacent to the limbus using a 30-gauge needle. A 34-gauge blunt needle fitted to a Hamilton syringe was inserted through the incision behind the lens and pushed through the retina. All injections were made subretinally in a location within the nasal quadrant of the retina. Each eye received up to 2×10^9 vg *AAV2/5-(hGRK1)-hRDH12* in up to a 2 µL volume. RDH12-encoding vector was administered separately to one eye of each mouse receiving treatment, and the contralateral eyes were uninjected. Fundus examination following the injection found more than 30% of the retina detached in most cases, confirming successful subretinal delivery.

Antibodies

[0045] Primary antibodies used were: a rabbit anti-Rdh12 polyclonal antibody (CSP) specific for the mouse protein (against 252SPFFKSTSQGAQ263, SEQ ID NO:4), and a mouse anti-RDH12 monoclonal antibody (2C9) specific for the human protein (against C-284DCKRTWVSPRARNNKT299; SEQ ID NO:5) (Kurth et al. 2007); a mouse anti-RHO monoclonal antibody (1D4) (MacKenzie et al. 1984); a rabbit anti-RHO polyclonal antibody generated against the denatured protein; and a rabbit anti-red/green cone opsin polyclonal antibody (Millipore cat# AB5405).

Immunoblotting analysis

[0046] Proteins in retina homogenates were separated by SDS-PAGE, transferred onto nitrocellulose membranes that were then blocked, incubated with primary antibody overnight, washed, incubated with alkaline phosphatase-conjugated secondary antibody, and developed using 5-bromo-4-chloro-3'-indolylphosphate p-toluidine and nitro-blue tetrazolium chloride.

Histology and Immunofluorescence

[0047] Mice were euthanized, eyes scored for orientation, then enucleated. For cryosections, lens and anterior segments were removed, eyes briefly fixed with 4% paraformaldehyde, washed with PBS, transitioned to sucrose/OCT, flash-frozen, and sectioned at a thickness of 10 µm. For freeze-substitution preparation, whole eyes were flash-frozen in dry-ice-cooled

isopentane for 30 seconds, and then transferred to dry- ice-cooled methanol containing 3% glacial acetic acid. Eyes were incubated at 80°C for 48 hours, then overnight at -20°C, embedded in paraffin, and sectioned at a thickness of 6 µm. Paraffin sections were de-paraffinized and antigens retrieved by incubating in 1mM EDTA, 0.05% Tween 20, pH8.0, at 90°C for 30 minutes prior to immune labeling as follows. Briefly, retinal cross sections were washed with PBS and permeabilized with PBS-T (0.3% Triton X-100); blocked with 1% bovine serum albumin, 10% normal goat serum, and 0.3% Triton X-100; and incubated with primary antibodies overnight at 4°C, washed, then incubated with fluorophore-conjugated secondary antibodies for 1 hour at room temperature. Sections were cover-slipped using ProLong Gold gel mount containing 4',6-diamidino-2-phenylindole (DAPI; Invitrogen), and imaged using a Leica DM6000 fluorescence microscope.

ERG recording

[0048] ERGs were performed as described previously (Thompson, 2012) using the Espion e2 recording system (Diagnosys, Lowell, MA). Briefly, mice were dark-adapted overnight and anesthetized with an intra-peritoneal injection of Ketamine (93 mg/kg) and Xylazine (8 mg/kg). Pupils were dilated with topical tropicamide (0.5%). Body temperature was maintained at 37°C with a heating pad. Corneal ERGs were recorded from both eyes using gold wire loops with 0.5% tetracaine topical anesthesia and a drop of 2% methylcellulose for corneal hydration. A gold wire loop placed in the mouth was used as reference, and a ground electrode was on the tail. The ERG protocol consisted of recording dark-adapted (scotopic) responses to brief white flashes (-2.31 log cd.s.m^{-2} for rod isolated B-waves; 1.09 log cd.s.m^{-2} for rod-cone combined A-and B-waves). Light-adapted (photopic) ERGs were recorded after 10 minutes of adaptation to a white 32 cd.m^{-2} rod-suppressing background in response to 1.09 log cd.s.m^{-2} intensity flashes (for cone isolated B-waves). Responses were amplified at 1,000 gain at 1.25 to 1000 Hz, and digitized at a rate of 2000 Hz. A notch filter was used to remove 60 Hz line noise. Responses were computer-averaged and recorded at 3- to 60-second intervals depending upon the stimulus intensity. For statistical analysis, paired t-tests were used to determine if ERG amplitudes in treated eyes were significantly different from untreated eyes.

Analysis of light-induced damage

[0049] Albino *Rdh12*$^{-/-}$ mice were injected in one eye with *AAV2/5-hGRK1p*.hRDH12 (1.3 × 10^{9} vg), or with an equal volume of PBS, and contralateral eyes were uninjected. At 6 weeks post-injection, ERG analysis was performed and scotopic responses were quantified as described above. One week later, mice were dark-adapted overnight, their pupils were dilated with tropicamide (0.5%), and then were placed in a light-box in individual clear trays. The mice were exposed to 5,000 lux for 2 hours, and then were returned to vivarium housing (12-hour dark/12-hour light (<20 lux)) for 7 days, after which ERG analysis was repeated. The percent of the original ERG response remaining after light damage was calculated for each eye, and the

averages plotted with standard errors shown. Two-tailed paired t-tests were used to determine if the ERG amplitudes in treated eyes were significantly different from untreated eyes.

Optical Coherence Tomography

[0050] Mice were anesthetized and pupils dilated with 0.5% tropicamide. A spectral domain optical coherence tomography (OCT) system (Bioptigen Envisu R2200 SD-OCT system (Durham, NC, USA)), with a volume analysis size of 1.4 × 1.4 mm, was centered on the optic nerve head. Systane (Alcon) lubricating drops were used throughout the imaging process.

Analysis of retinoid content

[0051] *All-trans* retinal and 11-*cis* retinal in mouse eyes were extracted using a modification of a previously described method (Bligh and Dyer, 1959). Six-week, post-injection mice were dark-adapted overnight, then under dim-red light, euthanized via CO_2 overdose, and eyes enucleated and frozen in liquid N_2. Under dim red light and on ice, each eye was homogenized in 1 mL chloroform: methanol: hydroxylamine (2 M) (3:6:1) and incubated at room temperature for 2 minutes. Next, 200 µL chloroform and 240 µL water were added, and each sample was vortexed and centrifuged at 14,000 rpm for 5 minutes. The lower phase was collected, the solvent was evaporated under nitrogen, and the sample was dissolved in hexane. Retinoids in the extracts were identified and quantified by high-performance liquid chromatography (HPLC) analysis, using a Waters Alliance separation module and photodiode array detector with a Supelcosil LC-31 column (25 cm by 4.6 mm by 3 µm) developed with 5% 1,4-dioxane in hexane. Peak identification was done by comparison to retention times of standard compounds and evaluation of wavelength maxima. Quantitative analysis was done by comparison of peak areas at 347 and 351 nm for syn- and anti-11-*cis* retinal oxime, respectively, and at 357 and 361 nm for syn- and anti-all-*trans* retinal oxime, respectively (Kurth et al. 2007).

Assay of retinal reductase activity

[0052] Mouse retina homogenates were assayed for retinal reductase activity at 6 weeks post-injection. Light-adapted mice were euthanized, and each retina was homogenized individually in 125 µL of 0.25 M sucrose, 25 mM Tris-acetate, pH 7, 1 mM dithiothreitol. The homogenates were centrifuged at 1000 × g for 5 minutes to remove unbroken cells, and then the supernates were sonicated with a microtip probe (30 times for 1 second each) on ice. Protein concentrations were determined by a modification of the Lowry procedure (Peterson et al. 1977), and levels of RDH12 were evaluated by western blot. Like samples were pooled and 20 µg of each pooled lysate was assayed (in triplicate) in buffer containing 200 µM all-*trans* retinal and 200 µM NADPH in HEPES buffer (pH 8); reactions were incubated for 0-45 minutes in a 37°C water bath (a reaction temperature that minimized thermal isomerization of the retinoid

substrates, as well as enzyme inactivation). All-*trans* retinol formation was quantitated using normal-phase HPLC analysis with comparison to known standards (Chrispell et al. 2009).

Example 1

AAV-mediated Expression of human RDH12

[0053] A variety of vectors for RDH12-replacement therapy were developed and tested. The optimal RDH12 vector construct is shown in Figure 2A. It comprises the human *RDH12* cDNA under control of a human Rhodopsin Kinase (GRK1) promoter fragment. The construct is packaged in an AAV2/5 serotype. The AAV serotype 5 capsid has been shown to mediate photoreceptor transduction, but with slower kinetics and less robust expression compared to the AAV8 capsid (Yang et al. 2002; Lotery et al. 2003; Allocca et al. 2007; Lebherz et al. 2008). Human RDH12 protein expression in mouse retinas 6 weeks following sub-retinal injection of *AAV2/5-hGRK1p.hRDH12* (1.3×10^9 vg) was evaluated using antibodies specific for the mouse Rdh12 or the human RDH12 proteins. Vector-delivered levels of human RDH12 appear to be roughly comparable to the amount of mouse Rdh12 (Figure 2B). Indirect immunofluorescence imaging on retinal sections was used to evaluate native mouse Rdh12 and recombinant human RDH12, using species-specific antibodies. Localization of endogenous and recombinant RDH12 appears to be identical, indicating that the protein is being processed normally (Figure 2C).

[0054] The capacity of retinas of *Rdh12*$^{-/-}$ mice to reduce exogenous retinaldehydes is significantly reduced compared to wild-type mice (Chrispell et al. 2009). Six weeks after subretinal injection of 1.3×10^9 vg *AAV2/5-hGRK1p.hRDH12* into Rdh12$^{-/-}$ mice, retinal homogenates were assayed for *in vitro* retinal reductase activity. All-*trans* retinol formation was quantitated using normal-phase HPLC analysis. Each data point represents the mean ± standard error for a minimum of 5 independent experiments where retinas from 3 to 5 mice were pooled and assayed in triplicate. Initial rates of all-*trans* retinol formation in retinas from uninjected *Rdh12*$^{-/-}$ and C57BL/6J mice were 0.013 pmol minute^{-1} µg protein^{-1}, and 0.071 pmol minute^{-1} µg protein^{-1}, respectively (Figure 3A). The residual activity present in Rdh12$^{-/-}$ mice reflects the presence of other RDH isoforms that are capable of reducing all-*trans* retinal (Rattner et al. 2000; Haeseleer et al. 2002). In *Rdh12*$^{-/-}$ mice injected with AAV2/5-hGRK1p.hRDH12, the rate of all-*trans* retinol formation of 0.046 pmol minute^{-1} µg protein^{-1} was significantly greater than for PBS injected *Rdh12*$^{-/-}$ mice (0.016 pmol minute^{-1} µg protein^{-1}). The recovery of retinal reductase activity (about 50%) is consistent with the partial transduction of the retina (about 30%) and indicated that the vector restored normal levels of activity in transduced photoreceptors. The transgene was expressed for over 12 months (longest time point examined). Immunofluorescence labelling showed the extent of retinal

transduction and recombinant RDH12 expression in an *Rdh12*$^{-/-}$ mouse evaluated at 16 weeks post-injection covering approximately one-third of the retinal surface (Figure 3B).

[0055] The effect of *AAV2/5-hGRK1p*.hRDH12 on susceptibility to light-induced damage was evaluated in albino *Rdh12*$^{-/-}$ mice that were injected in one eye with 1.3×10^9 vg or an equal volume of PBS, and received no treatment in the contralateral eye. ERG analysis of scotopic retinal activity (rod-isolated and combined rod-cone) was performed 1 week before, and 1 week after, subjecting the mice to light levels that cause significant retinal damage in albino animals (5,000 lux for 2 hours). The percent retinal activity remaining in vector-treated eyes was significantly greater than that remaining in untreated eyes ($p \leq 0.0168$) (Figure 4). In contrast, in control animals injected with PBS, the percent of scotopic retinal activity remaining in uninjected eyes was not significantly greater than in untreated eyes ($p \geq 0.2255$). These findings are consistent with *AAV2/5-hGRK1p.hRDH12*-mediated protection against increased susceptibility to light-induced damage associated with RDH12 deficiency.

[0056] Toxicity of the vector was assessed through the direct effect of human RDH12 activity on retinoid metabolism, and for indirect effects on retinal structure and retinal function, as described in the following passage.

[0057] The broad substrate specificity of RDH12 that enables the reduction of both 11-*cis* retinal and all-*trans* retinal created the potential for overexpression or mislocalization of recombinant protein to negatively impact visual cycle function. HPLC analysis of retinoid content was used to evaluate visual-cycle activity in eyes from *Rdh12*$^{-/-}$ mice injected with *AAV2/5-hGRK1p.hRDH12,* or with PBS, or from uninjected C57BL/6J controls. Analysis of retinoids at 6 weeks post-subretinal-injection of *AAV2/5-hGRK1p.hRDH12* (1.3×10^9 vg) into *Rdh12*$^{-/-}$ mice showed that hRDH12 expression did not significantly affect the steady state 11-cis retinal levels in the retina (Figure 5A). A representative chromatogram shows various controls with elution times for various retinoids indicated. Total retinal levels of 11-*cis* retinal and all-*trans* retinal represent average values ± standard error for a minimum of 5 independent experiments (Figures 5B, C). ERG responses of retinal activity were measured in C57BL/6J and *Rdh12*$^{-/-}$ mice that received 1.3×10^9 vg of *AAV2/5-hGRK1p.hRDH12* and were maintained in vivarium housing (12-hour dark/12-hour light (<20 lux)). Scotopic (rod-isolated -2.3 log cd.s.m^{-2} stimulus), combined rod-cone (1.09 log cd.s.m^{-2} stimulus), and photopic (cone-mediated responses, 1.09 log cd.s.m^{-2} stimulus) ERGs from a representative animal in each treatment group measured at 6 weeks post-injection showed no significant effect of human RDH12 expression on retinal function (Figure 6).

[0058] Mislocalization of rhodopsin and cone opsin is a well-characterized indicator of decreased photoreceptor cell viability (Adamian et al. 2006; Turney et al. 2007; Brunner et al. 2010; Lopes et al. 2010). Immunohistochemical localization of rhodopsin and red/green opsin was evaluated in *Rdh12*$^{-/-}$ mice that were non-injected or *AAV2/5-hGRK1p.hRDH12*-injected (1.3×10^9 vg). In mice exhibiting robust transgene expression, no decrease in opsin

expression levels or evidence of rod and cone opsin mislocalization were observed at 16 weeks post-treatment; retinal structure remained intact at 54 weeks post-injection (Figure 7).

[0059] Optical coherence tomography (OCT) analysis of C57BL/6J and *Rdh12*$^{-/-}$ mice evaluated at 54 weeks post-injection of *AAV2/5-hGRK1p.hRDH12* (2 × 10^9 vg) showed no gross alterations in retinal lamination across a wide area including both sides of the optic nerve. Retinal structure was stable for at least one year post-injection when compared to uninjected contralateral eyes (Figure 8).

EXAMPLE 2. Retinal damage caused by AAV2/8

Retinal damage in AAV2/8-hGRK1p.hRDH12 injected mice.

[0060] A capsid derived from AAV serotype 8 has been shown to mediate efficient and robust transduction of photoreceptor cells (Allocca et al. 2007; Natkunarajah et al. 2008; Vandenberghe et al. 2011; Vandenberghe et al. 2013). Initial studies were performed with a AAV2/8 serotype carrying the vector construct described above. *Rdh12*$^{-/-}$ mice treated by subretinal injection of *AAV2/8-hGRK1p.hRDH12* at doses of 10^8-10^9 viral genomes (vg) resulted in robust expression of recombinant human RDH12 protein. However, the injected eyes developed significant retinal damage starting as early as 3 weeks post-injection, and in almost all cases by 6 weeks post-injection. Macrophage infiltration was observed in the majority of cases by the presence of CD68$^+$ cells (Figure 9). Retinal damage was not mitigated by systemic administration of cyclosporin (Borel et al. 1976) to *Rdh12*$^{-/-}$ mice continuously from the time of weaning to the time of sacrifice, indicating that immune responses against the vector or the transgene were not the cause of damage. This view is consistent with outcomes obtained in wild-type C57BL/6J mice injected with constructs encoding either human RDH12 (*AAV2/8-hGRKlp.hRDH12*) or mouse Rdh12 (*AAV2/8-hGRK1p.mRdh12*), in which significant retinal thinning and macrophage infiltration also occurred by 6 weeks post-injection.

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PATENTKRAV

1. Adenoassocieret, viral vektor omfattende en nukleinsyre, hvor nukleinsyren omfatter humant RDH12-DNA, og hvor det humane RDH12-DNA koder for et protein, der er mindst 70 %, 80 %, 90 %, 95 %, eller 99 % identisk med fuldlængden af SEQ ID NO: 2, til anvendelse i en fremgangsmåde til behandling af et menneske, der har en oftalmologisk tilstand grundet én eller flere funktionstabsmutationer i det gen, der koder for retinoldehydrogenase 12- (RDH12) proteinet, hvilken fremgangsmåde omfatter administration af den adenoassocierede, virale vektor i mindst ét af menneskets øjne, hvor den adenoassocierede, virale vektor er AAV-2, serotype-5 (AAV2/5), hvor RDH12-DNA'et er under ekspressionskontrol af en human rhodopsinkinase 1- (hGRK1) promotor, og hvor den oftalmologiske tilstand er Lebers kongenitale amaurose (LCA).

2. Adenoassocieret, viral vektor til anvendelse ifølge krav 1, hvor hGRK1-promotoren omfatter SEQ ID NO: 3, eller
hvor RDH12-DNA'et omfatter en sekvens, der er mindst 60 % eller 70 % identisk med SEQ ID NO: 1, eller
som omfatter administration af nukleinsyren ved en titer på ca. 2×10^{10} virale genomer pr. milliliter (vg/mL) til en titer på ca. 2×10^{12} vg/mL, eller
hvor nukleinsyren administreres i det subretinale rum.

3. Adenoassocieret, viral vektor til anvendelse ifølge krav 2, hvor nukleinsyren administreres i det subretinale rum med en mikroinjektionskanyle, der indføres i det subretinale rum.

4. Adenoassocieret, viral vektor til anvendelse ifølge krav 1, hvor det humane RDH12-DNA koder for et protein omfattende SEQ ID NO: 2, og hvor hGRK1-promotoren består af SEQ ID NO: 3.

5. Adenoassocieret, viral vektor, der omfatter en nukleinsyre, der koder for et humant RDH12-DNA, hvor det humane RDH12-DNA koder for et protein, der er mindst 70 %, 80 %, 90 %, 95 % eller 99 % identisk med fuldlængden af SEQ ID NO: 2, hvor RDH12-DNA'et er under kontrol af en human rhodopsinkinase 1- (hGRK1) promotor, og hvor den adenoassocierede, virale vektor er AAV-2, serotype-5 (AAV2/5).

6. Adenoassocieret, viral vektor ifølge krav 5, hvor hGRK1-promotoren omfatter SEQ ID NO: 3, eller hvor det humane RDH12-DNA koder for et protein omfattende SEQ ID NO: 2.

7. Adenoassocieret, viral vektor ifølge krav 5 eller krav 6, hvor hGRK1-promotoren består af SEQ ID NO: 3.

8. Adenoassocieret, viral vektor ifølge krav 5, hvor det humane RDH12-DNA er mindst 60 % eller 70 % identisk med fuldlængden af SEQ ID NO: 1.

9. Isoleret værtscelle, der omfatter den adenoassocierede, virale vektor ifølge et hvilket som helst af krav 5 til 8.

10. Isoleret værtscelle ifølge krav 9, hvor cellen udtrykker et humant RDH12-protein.

DRAWINGS

Drawing

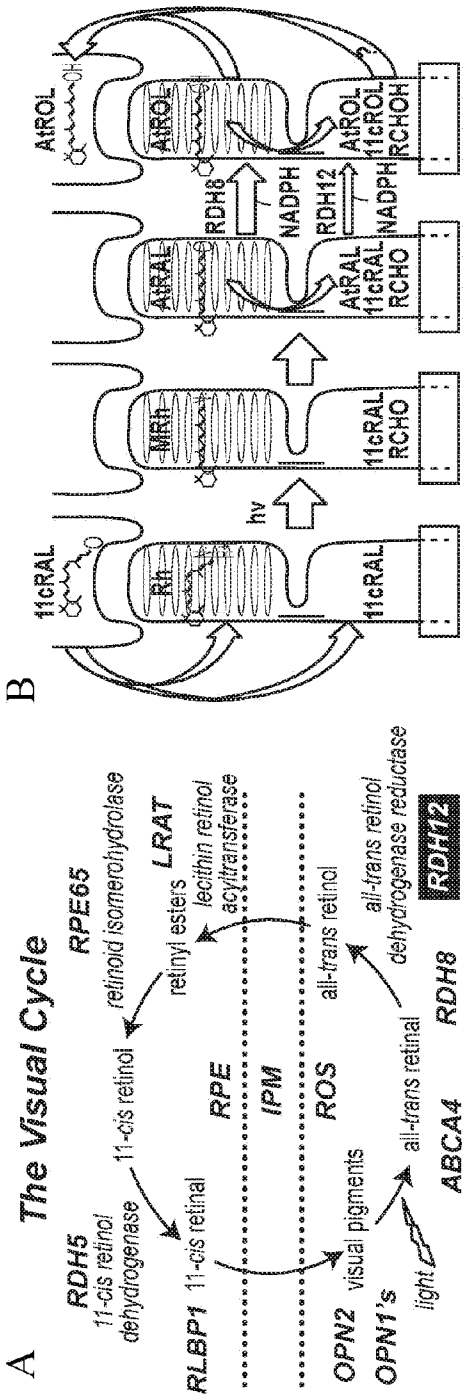

Figure 1

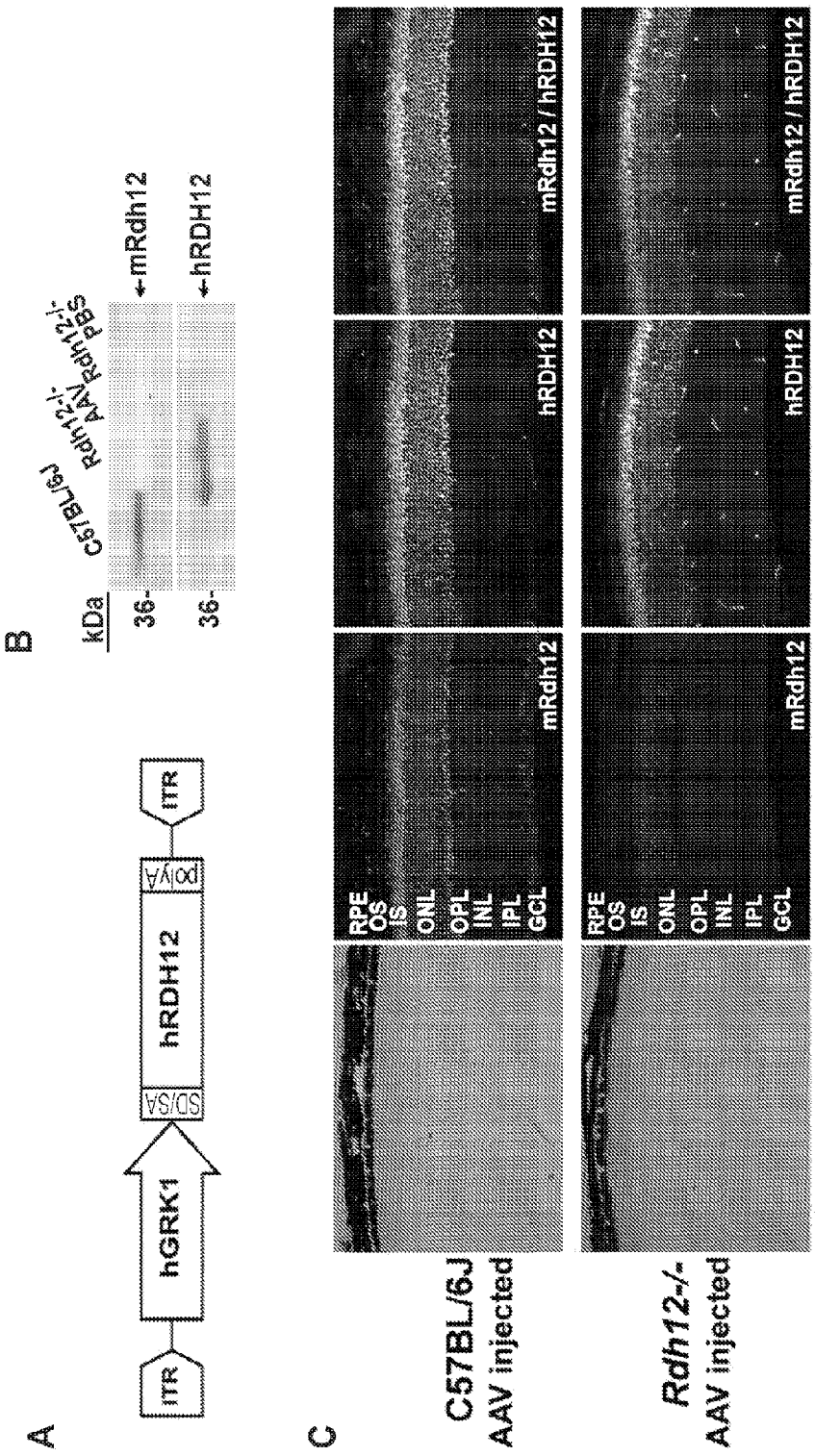
A
ITR
hGRK1
SD/SA
hRDH12
polyA
ITR
B
kDa
C57BL/6J
Rdh12-/-
Rdh12-/- AAV PBS
36-
36-
←mRdh12
←hRDH12
C
C57BL/6J
AAV injected
Rdh12-/-
AAV injected
RPE
OS
IS
ONL
OPL
INL
IPL
GCL
mRdh12
hRDH12
mRdh12 / hRDH12

Figure 2

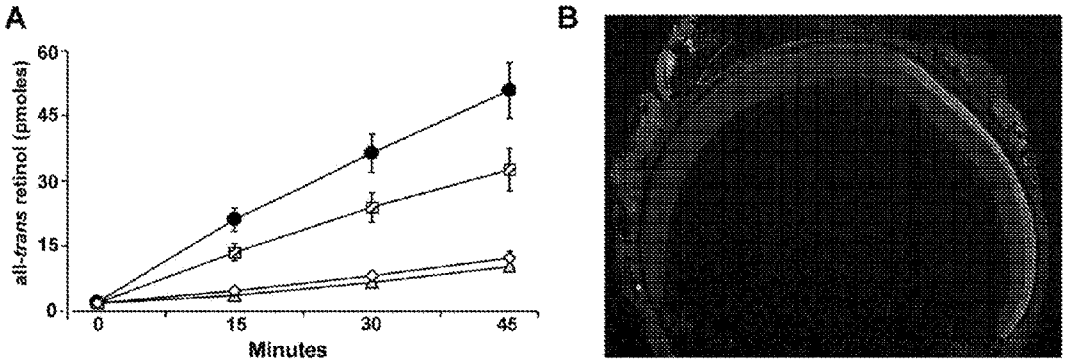

Figure 3

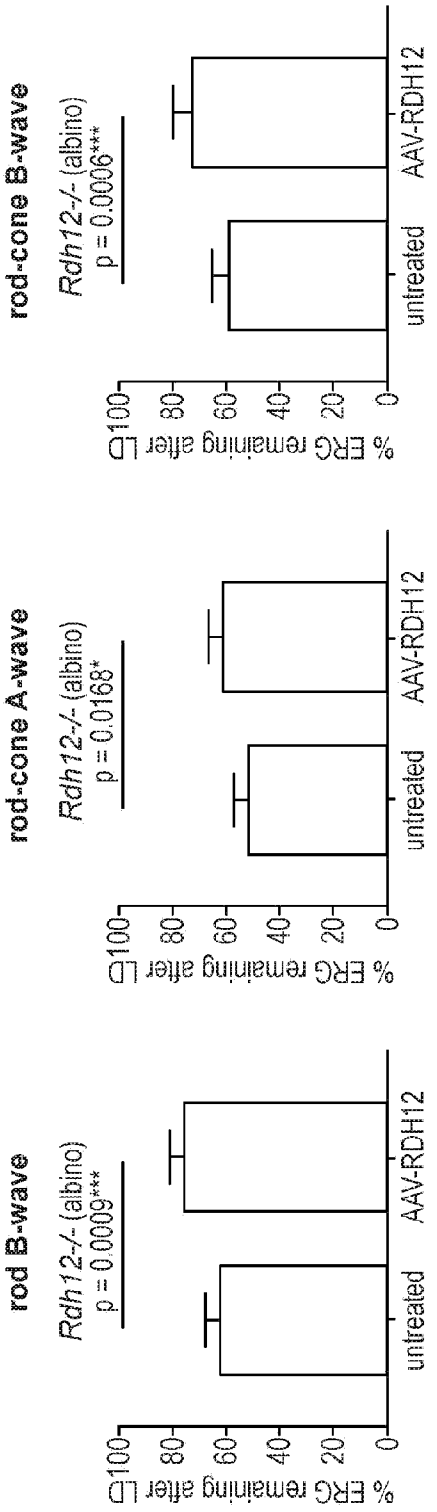
rod B-wave
Rdh12-/- (albino)
p = 0.0009***
% ERG remaining after LD
100
80
60
40
20
0
untreated
AAV-RDH12
rod-cone A-wave
Rdh12-/- (albino)
p = 0.0168*
% ERG remaining after LD
100
80
60
40
20
0
untreated
AAV-RDH12
rod-cone B-wave
Rdh12-/- (albino)
p = 0.0006***
% ERG remaining after LD
100
80
60
40
20
0
untreated
AAV-RDH12

Figure 4

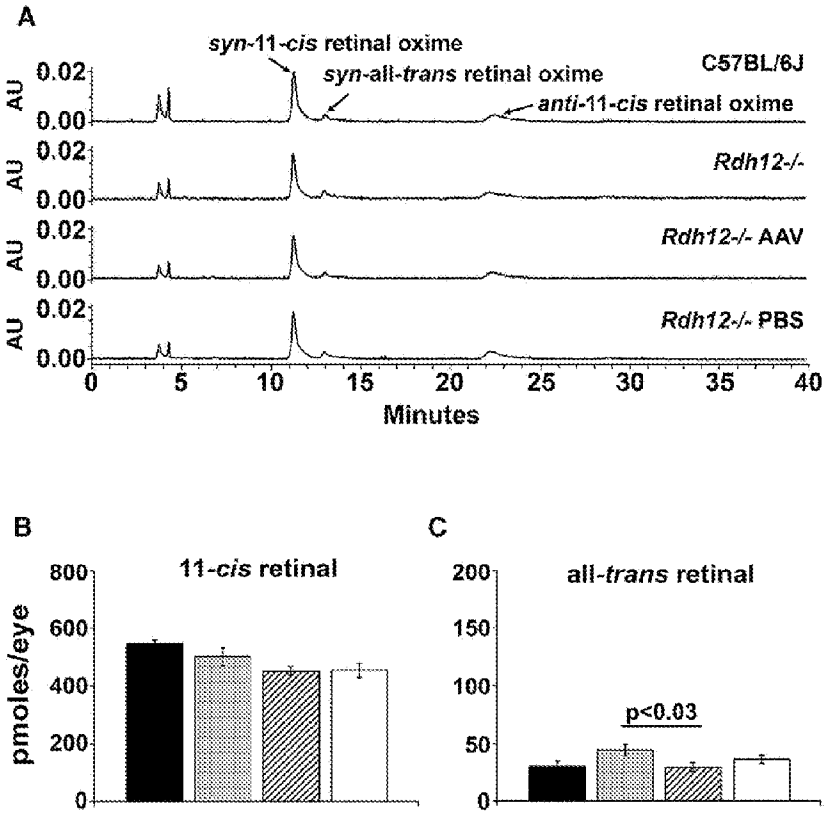
A
syn-11-cis retinal oxime
syn-all-trans retinal oxime
anti-11-cis retinal oxime
C57BL/6J
Rdh12-/-
Rdh12-/- AAV
Rdh12-/- PBS
AU
0.02
0.00
0
5
10
15
20
25
30
35
40
Minutes
B
11-cis retinal
pmoles/eye
800
600
400
200
0
C
all-trans retinal
200
150
100
50
0
p<0.03

Figure 5

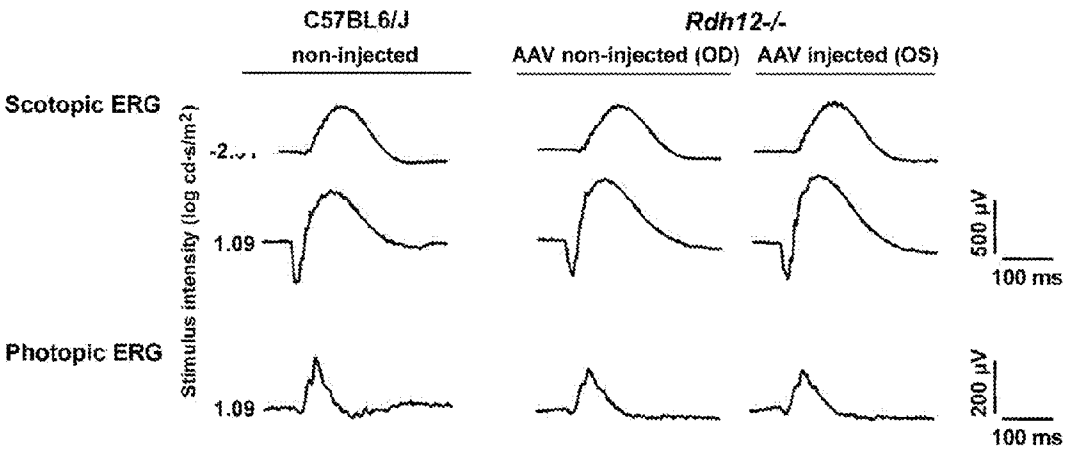
C57BL6/J
non-injected
Rdh12-/-
AAV non-injected (OD)
AAV injected (OS)
Scotopic ERG
Photopic ERG
Stimulus intensity (log cd-s/m2)
1.09
1.09
500 µV
100 ms
200 µV
100 ms

Figure 6

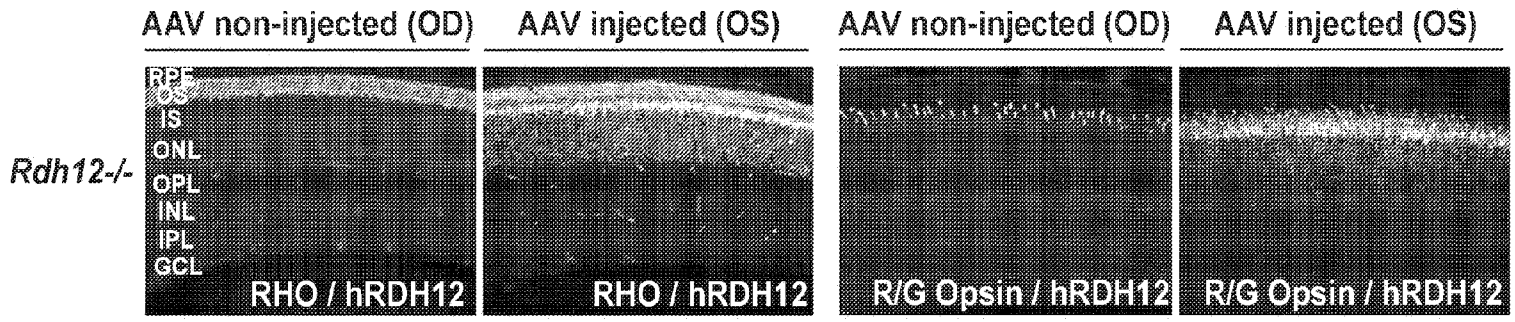

Figure 7

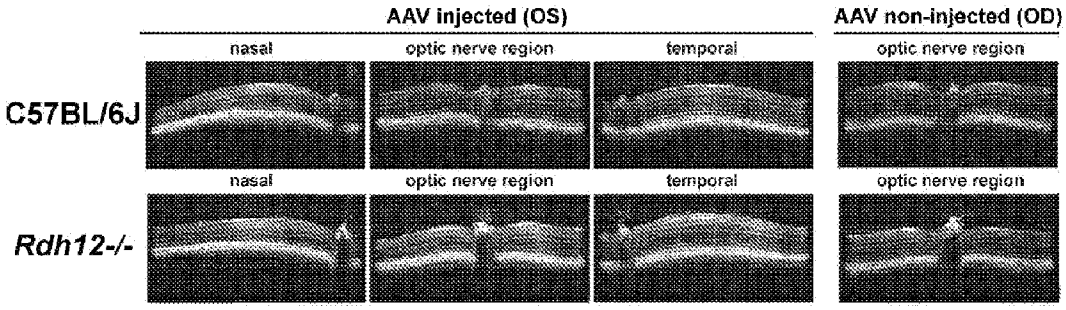
AAV injected (OS)
AAV non-injected (OD)
nasal
optic nerve region
temporal
optic nerve region
C57BL/6J
nasal
optic nerve region
temporal
optic nerve region
Rdh12-/-

Figure 8

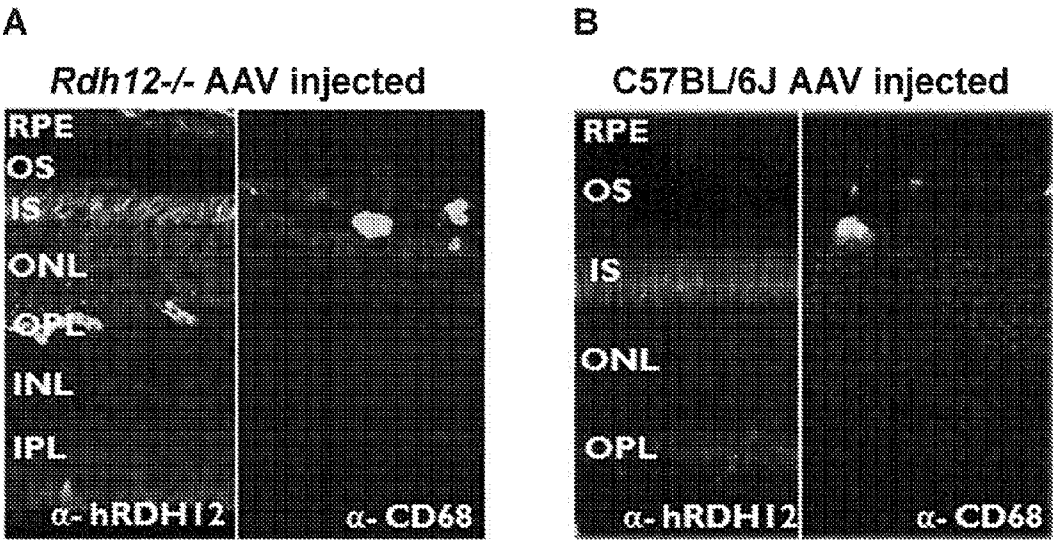
A
Rdh12-/- AAV injected
RPE
OS
IS
ONL
OPL
INL
IPL
α- hRDH12
α- CD68
B
C57BL/6J AAV injected
RPE
OS
IS
ONL
OPL
α- hRDH12
α- CD68

Figure 9

SEKVENSLISTE

Sekvenslisten er udeladt af skriftet og kan hentes fra det Europæiske Patent Register.

The Sequence Listing was omitted from the document and can be downloaded from the European Patent Register.