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(54) Benævnelse: **ADENO-ASSOCIEREDE VIRUS-VIRIONER MED VARIANT KAPSID OG FREMGANGSMÅDER TIL ANVENDELSE HERAF**

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HILDA PETRS-SILVA ET AL: "High-efficiency Transduction of the Mouse Retina by Tyrosine-mutant AAV Serotype Vectors", MOLECULAR THERAPY, vol. 17, no. 3, March 2009 (2009-03-01), pages 463 - 471, XP055076954, ISSN: 1525-0016, DOI: 10.1038/mt.2008.269
T K PARK ET AL: "Intravitreal delivery of AAV8 retinoschisin results in cell type-specific gene expression and retinal rescue in the Rs1-KO mouse", GENE THERAPY, vol. 16, no. 7, July 2009 (2009-07-01), pages 916 - 926, XP055113217, ISSN: 0969-7128, DOI: 10.1038/gt.2009.61

Fortsættes ...

DESCRIPTION

Description

BACKGROUND

[0001] Photoreceptors are the first neurons in the retina to receive and process visual information, converting visible electromagnetic radiation into hyperpolarized responses through phototransduction. The overwhelming majority of inherited retinal diseases result in the loss of these cells, either directly, such as in dominant mutations that affect rhodopsin protein folding, or indirectly, such as in recessive mutations that affect retinal recycling pathways in the retinal pigment epithelium (RPE).

[0002] AAV belongs to the *Parvoviridae* family and Dependovirus genus, whose members require co-infection with a helper virus such as adenovirus to promote replication, and AAV establishes a latent infection in the absence of a helper. Virions are composed of a 25 nm icosahedral capsid encompassing a 4.9 kb single-stranded DNA genome with two open reading frames: *rep* and *cap.* The non-structural *rep* gene encodes four regulatory proteins essential for viral replication, whereas *cap* encodes three structural proteins (VP1-3) that assemble into a 60-mer capsid shell. This viral capsid mediates the ability of AAV vectors to overcome many of the biological barriers of viral transduction-including cell surface receptor binding, endocytosis, intracellular trafficking, and unpackaging in the nucleus.

[0003] WO2010093784A2 discloses AAV capsid proteins (VP1, VP2 and/or VP3) comprising a modification in the amino acid sequence in the three-fold axis loop 4 and virus capsids and virus vectors comprising the modified AAV capsid protein..

[0004] Petrs-Silva, et al. "High-efficiency Transduction of the Mouse Retina by Tyrosine-mutant AAV Serotype Vectors", Molecular Therapy vol. 17 no.3, 463-471 discloses a study evaluating the intraocular transduction characteristics of vectors containing point mutations in surface-exposed capsid tyrosine residues in AAV serotypes 2, 8 and 9.

[0005] Park, et al. "Intravitreal delivery of AAV8 retinoschisin results in cell type-specific gene expression and retinal rescue in the Rs1-KO mouse", Gene Therapy vol. 16 no.7, 916-926 discloses that all layers of the retinoschisin knockout (*Rs1*-KO) mouse retina can be efficiently transduced with AAV type 8 (AAV8) vectors administered by simple vitreous injection.

Literature

[0006] U.S. Patent Publication No. 2005/0053922; U.S. Patent Publication No. 2009/0202490; Allocca et al. (2007) J. Virol. 81:11372; Boucas et al. (2009) J. Gene Med. 11:1103.

SUMMARY OF THE INVENTION

[0007] The present disclosure provides adeno-associated virus (AAV) virions with altered capsid protein, where the AAV virions exhibit greater infectivity of a retinal cell, when administered via intravitreal injection, compared to wild-type AAV. The present disclosure further provides methods of delivering a gene product to a retinal cell in an individual, and methods of treating ocular disease.

[0008] According to the invention, there is provided a recombinant adeno-associated virus (rAAV) virion, or a pharmaceutical composition comprising said virion, for use in a method of treating an ocular disease , wherein the composition further comprises a pharmaceutically acceptable excipient, and wherein the recombinant adeno-associated virus (rAAV) virion comprises:

1. a) a variant AAV capsid protein, wherein the variant AAV capsid protein comprises amino acid sequence having at least 95% sequence identity to SEQ ID NO: 1, and an insertion of a peptide in the capsid protein GH loop relative to SEQ ID NO: 1, wherein the peptide comprises the amino acid sequence LALGETTRPA (SEQ ID NO:45); and
2. b) a heterologous nucleic acid comprising a nucleotide sequence encoding a gene product;

wherein the variant AAV capsid protein confers increased infectivity of a retinal cell by the rAAV virion, compared to the infectivity of the retinal cell by an AAV virion comprising a wild-type capsid protein having the amino acid sequence set forth in SEQ ID NO: 1.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009]

Figure 1 provides a representative three-dimensional model of AAV2 containing a random heptamer following amino acid 587.

Figure 2 depicts greater levels of intravitreal transduction by AAV2 7M8 variant (right), relative to AAV2 (left).

Figure 3 provides representative fluorescence images of retinal cryoslices showing green fluorescent protein (GFP) expression resulting from 7M8 carrying the GFP gene under the control of the ubiquitous CAG promoter (left) or a photoreceptor-specific Rho promoter (right).

Figure 4 depicts GFP^+ photoreceptor cells per million retinal cells as counted by flow

cytometry, following transduction by 7M8 or by 7M8 bearing 4 tyrosine mutations (7m8.4YF).

Figure 5 provides an amino acid sequence of AAV2 VP1 (SEQ ID NO:1).

Figure 6 provides amino acid sequences corresponding to amino acids 570-610 of AAV2 (Figure 5) of AAV capsid protein VP1 of various AAV serotypes.

Figures 7A-I depict structural improvements in the *Rslh-/-* mouse retina after gene transfer.

Figures 8A-D depict functional rescue of the electroretinogram A and B waves following RS 1 gene delivery.

Figures 9A-E depict sustained improvements in retinal thickness measured at 10 months post 7m8-rho-RS1 treatment.

Figure 10 provides an amino acid sequence of retinoschisin.

Figure 11 provides an amino acid sequence of brain derived neurotrophic factor.

Figure 12 provides an amino acid sequence of RPE65.

Figures 13A-C provide the nucleotide sequence of the 7m8-rho-RS 1 construct.

Figure 14 provides an amino acid sequence of peripherin-2.

Figure 15 provides an amino acid sequence of peripherin.

Figure 16 provides an amino acid sequence of retinitis pigmentosa GTPase regulator-interacting protein-1.

Figures 17A-C provide an alignment of amino acid sequences of AAV capsid protein loop IV (GH loop) regions. Insertion sites are shown in bold and underlining.

Figures 18A-C provide an alignment of amino acid sequences of AAV capsid protein GH loop regions, with heterologous peptide insertions.

Figure 19 provides a fluorescence fundus image showing GFP expression in central primate retina 9 weeks after administration of 7m8 carrying GFP under the control of a connexin36 promoter.

DEFINITIONS

[0010] The term "retinal cell" can refer herein to any of the cell types that comprise the retina, such as retinal ganglion cells, amacrine cells, horizontal cells, bipolar cells, and photoreceptor cells including rods and cones, Müller glial cells, and retinal pigmented epithelium.

[0011] "AAV" is an abbreviation for adeno-associated virus, and may be used to refer to the virus itself or derivatives thereof. The term covers all subtypes and both naturally occurring and recombinant forms, except where required otherwise. The abbreviation "rAAV" refers to recombinant adeno-associated virus, also referred to as a recombinant AAV vector (or "rAAV vector"). The term "AAV" includes AAV type 1 (AAV-1), AAV type 2 (AAV-2), AAV type 3 (AAV-3), AAV type 4 (AAV-4), AAV type 5 (AAV-5), AAV type 6 (AAV-6), AAV type 7 (AAV-7), AAV type 8 (AAV-8), avian AAV, bovine AAV, canine AAV, equine AAV, primate AAV, non-primate AAV, and ovine AAV. "Primate AAV" refers to AAV that infect primates, "non-primate AAV" refers to AAV that infect non-primate mammals, "bovine AAV" refers to AAV that infect bovine mammals, etc.

[0012] The genomic sequences of various serotypes of AAV, as well as the sequences of the native terminal repeats (TRs), Rep proteins, and capsid subunits are known in the art. Such sequences may be found in the literature or in public databases such as GenBank. See, e.g., GenBank Accession Numbers NC_002077 (AAV-1), AF063497 (AAV-1), NC_001401 (AAV-2), AF043303 (AAV-2), NC_001729 (AAV-3), NC_001829 (AAV-4), U89790 (AAV-4), NC_006152 (AAV-5), AF513851 (AAV-7), AF513852 (AAV-8), and NC_006261 (AAV-8); the disclosures of which are incorporated by reference herein for teaching AAV nucleic acid and amino acid sequences. See also, e.g., Srivistava et al. (1983) J. Virology 45:555; Chiorini et al. (1998) J. Virology 71:6823; Chiorini et al. (1999) J. Virology 73: 1309; Bantel-Schaal et al. (1999) J. Virology 73:939; Xiao et al. (1999) J. Virology 73:3994; Muramatsu et al. (1996) Virology 221:208; Shade et al.,(1986) J. Virol. 58:921; Gao et al. (2002) Proc. Nat. Acad. Sci. USA 99: 11854; Moris et al. (2004) Virology 33:375-383; international patent publications WO 00/28061, WO 99/61601, WO 98/11244; and U.S. Pat. No. 6,156,303.

[0013] An "rAAV vector" as used herein refers to an AAV vector comprising a polynucleotide sequence not of AAV origin (i.e., a polynucleotide heterologous to AAV), typically a sequence of interest for the genetic transformation of a cell. In general, the heterologous polynucleotide is flanked by at least one, and generally by two, AAV inverted terminal repeat sequences (ITRs). The term rAAV vector encompasses both rAAV vector particles and rAAV vector plasmids. An rAAV vector may either be single-stranded (ssAAV) or self-complementary (scAAV).

[0014] An "AAV virus" or "AAV viral particle" or "rAAV vector particle" refers to a viral particle composed of at least one AAV capsid protein (typically by all of the capsid proteins of a wild-type AAV) and an encapsidated polynucleotide rAAV vector. If the particle comprises a heterologous polynucleotide (i.e. a polynucleotide other than a wild-type AAV genome such as a transgene to be delivered to a mammalian cell), it is typically referred to as an "rAAV vector particle" or simply an "rAAV vector". Thus, production of rAAV particle necessarily includes production of rAAV vector, as such a vector is contained within an rAAV particle.

[0015] "Packaging" refers to a series of intracellular events that result in the assembly and encapsidation of an AAV particle.

[0016] AAV "rep" and "cap" genes refer to polynucleotide sequences encoding replication and encapsidation proteins of adeno-associated virus. AAV rep and cap are referred to herein as

AAV "packaging genes."

[0017] A "helper virus" for AAV refers to a virus that allows AAV (e.g. wild-type AAV) to be replicated and packaged by a mammalian cell. A variety of such helper viruses for AAV are known in the art, including adenoviruses, herpesviruses and poxviruses such as vaccinia. The adenoviruses encompass a number of different subgroups, although Adenovirus type 5 of subgroup C is most commonly used. Numerous adenoviruses of human, non-human mammalian and avian origin are known and available from depositories such as the ATCC. Viruses of the herpes family include, for example, herpes simplex viruses (HSV) and Epstein-Barr viruses (EBV), as well as cytomegaloviruses (CMV) and pseudorabies viruses (PRV); which are also available from depositories such as ATCC.

[0018] "Helper virus function(s)" refers to function(s) encoded in a helper virus genome which allow AAV replication and packaging (in conjunction with other requirements for replication and packaging described herein). As described herein, "helper virus function" may be provided in a number of ways, including by providing helper virus or providing, for example, polynucleotide sequences encoding the requisite function(s) to a producer cell in trans. For example, a plasmid or other expression vector comprising nucleotide sequences encoding one or more adenoviral proteins is transfected into a producer cell along with an rAAV vector.

[0019] An "infectious" virus or viral particle is one that comprises a competently assembled viral capsid and is capable of delivering a polynucleotide component into a cell for which the viral species is tropic. The term does not necessarily imply any replication capacity of the virus. Assays for counting infectious viral particles are described elsewhere in this disclosure and in the art. Viral infectivity can be expressed as the ratio of infectious viral particles to total viral particles. Methods of determining the ratio of infectious viral particle to total viral particle are known in the art. See, e.g., Grainger et al. (2005) Mol. Ther. 11:S337 (describing a TCID50 infectious titer assay); and Zolotukhin et al. (1999) Gene Ther. 6:973. See also the Examples.

[0020] A "replication-competent" virus (e.g. a replication-competent AAV) refers to a phenotypically wild-type virus that is infectious, and is also capable of being replicated in an infected cell (i.e. in the presence of a helper virus or helper virus functions). In the case of AAV, replication competence generally requires the presence of functional AAV packaging genes. In general, rAAV vectors as described herein are replication-incompetent in mammalian cells (especially in human cells) by virtue of the lack of one or more AAV packaging genes. Typically, such rAAV vectors lack any AAV packaging gene sequences in order to minimize the possibility that replication competent AAV are generated by recombination between AAV packaging genes and an incoming rAAV vector. In many embodiments, rAAV vector preparations as described herein are those which contain few if any replication competent AAV (rcAAV, also referred to as RCA) (e.g., less than about 1 rcAAV per 10^2 rAAV particles, less than about 1 rcAAV per 10^4 rAAV particles, less than about 1 rcAAV per 10^8 rAAV particles, less than about 1 rcAAV per 10^{12} rAAV particles, or no rcAAV).

[0021] The term "polynucleotide" refers to a polymeric form of nucleotides of any length, including deoxyribonucleotides or ribonucleotides, or analogs thereof. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs, and may be interrupted by non-nucleotide components. If present, modifications to the nucleotide structure may be imparted before or after assembly of the polymer. The term polynucleotide, as used herein, refers interchangeably to double- and single-stranded molecules. Unless otherwise specified or required, any embodiment of the invention described herein that is a polynucleotide encompasses both the double-stranded form and each of two complementary single-stranded forms known or predicted to make up the double-stranded form.

[0022] Nucleic acid hybridization reactions can be performed under conditions of different "stringency". Conditions that increase stringency of a hybridization reaction of widely known and published in the art. See, e.g., Sambrook et al. Molecular Cloning, A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989. For example, see page 7.52 of Sambrook et al. Examples of relevant conditions include (in order of increasing stringency): incubation temperatures of 25°C, 37°C, 50°C and 68°C; buffer concentrations of 10 × SSC, 6 × SSC, 1 × SSC, 0.1 × SSC (where 1 × SSC is 0.15 M NaCl and 15 mM citrate buffer) and their equivalents using other buffer systems; formamide concentrations of 0%, 25%, 50%, and 75%; incubation times from 5 minutes to 24 hours; 1, 2, or more washing steps; wash incubation times of 1, 2, or 15 minutes; and wash solutions of 6 × SSC, 1 × SSC, 0.1 × SSC, or deionized water. An example of stringent hybridization conditions is hybridization at 50°C or higher and 0.1×SSC (15 mM sodium chloride/1.5 mM sodium citrate). Another example of stringent hybridization conditions is overnight incubation at 42°C in a solution: 50% formamide, 1 × SSC (150 mM NaCl, 15 mM sodium citrate), 50 mM sodium phosphate (pH 7.6), 5 × Denhardt's solution, 10% dextran sulfate, and 20 µg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1 × SSC at about 65°C. As another example, stringent hybridization conditions comprise: prehybridization for 8 hours to overnight at 65° C in a solution comprising 6X single strength citrate (SSC) (1X SSC is 0.15 M NaCl, 0.015 M Na citrate; pH 7.0), 5X Denhardt's solution, 0.05% sodium pyrophosphate and 100 µg/ml herring sperm DNA; hybridization for 18-20 hours at 65° C in a solution containing 6X SSC, 1X Denhardt's solution, 100 µg/ml yeast tRNA and 0.05% sodium pyrophosphate; and washing of filters at 65° C for 1 h in a solution containing 0.2X SSC and 0.1% SDS (sodium dodecyl sulfate).

[0023] Stringent hybridization conditions are hybridization conditions that are at least as stringent as the above representative conditions. Other stringent hybridization conditions are known in the art and may also be employed to identify nucleic acids of this particular embodiment of the invention.

[0024] "T_m" is the temperature in degrees Celsius at which 50% of a polynucleotide duplex made of complementary strands hydrogen bonded in anti-parallel direction by Watson-Crick base pairing dissociates into single strands under conditions of the experiment. T_m may be predicted according to a standard formula, such as:

$$T_m = 81.5 + 16.6 \log[X^+] + 0.41 (\%G/C) - 0.61 (\%F) - 600/L$$

where $[X^+]$ is the cation concentration (usually sodium ion, Na^+) in mol/L; (%G/C) is the number of G and C residues as a percentage of total residues in the duplex; (%F) is the percent formamide in solution (wt/vol); and L is the number of nucleotides in each strand of the duplex.

[0025] A polynucleotide or polypeptide has a certain percent "sequence identity" to another polynucleotide or polypeptide, meaning that, when aligned, that percentage of bases or amino acids are the same when comparing the two sequences. Sequence similarity can be determined in a number of different manners. To determine sequence identity, sequences can be aligned using the methods and computer programs, including BLAST, available over the world wide web at ncbi.nlm.nih.gov/BLAST/. Another alignment algorithm is FASTA, available in the Genetics Computing Group (GCG) package, from Madison, Wisconsin, USA, a wholly owned subsidiary of Oxford Molecular Group, Inc. Other techniques for alignment are described in Methods in Enzymology, vol. 266: Computer Methods for Macromolecular Sequence Analysis (1996), ed. Doolittle, Academic Press, Inc., a division of Harcourt Brace & Co., San Diego, California, USA. Of particular interest are alignment programs that permit gaps in the sequence. The Smith-Waterman is one type of algorithm that permits gaps in sequence alignments. See Meth. Mol. Biol. 70: 173-187 (1997). Also, the GAP program using the Needleman and Wunsch alignment method can be utilized to align sequences. See J. Mol. Biol. 48: 443-453 (1970)

[0026] Of interest is the BestFit program using the local homology algorithm of Smith and Waterman (Advances in Applied Mathematics 2: 482-489 (1981) to determine sequence identity. The gap generation penalty will generally range from 1 to 5, usually 2 to 4 and in many embodiments will be 3. The gap extension penalty will generally range from about 0.01 to 0.20 and in many instances will be 0.10. The program has default parameters determined by the sequences inputted to be compared. Preferably, the sequence identity is determined using the default parameters determined by the program. This program is available also from Genetics Computing Group (GCG) package, from Madison, Wisconsin, USA.

[0027] Another program of interest is the FastDB algorithm. FastDB is described in Current Methods in Sequence Comparison and Analysis, Macromolecule Sequencing and Synthesis, Selected Methods and Applications, pp. 127-149, 1988, Alan R. Liss, Inc. Percent sequence identity is calculated by FastDB based upon the following parameters:

Mismatch Penalty:	1.00;
Gap Penalty:	1.00;
Gap Size Penalty:	0.33; and
Joining Penalty:	30.0.

[0028] A "gene" refers to a polynucleotide containing at least one open reading frame that is capable of encoding a particular protein after being transcribed and translated.

[0029] A "gene product" is a molecule resulting from expression of a particular gene. Gene products include, e.g., a polypeptide, an aptamer, an interfering RNA, an mRNA, and the like.

[0030] A "small interfering" or "short interfering RNA" or siRNA is a RNA duplex of nucleotides that is targeted to a gene interest (a "target gene"). An "RNA duplex" refers to the structure formed by the complementary pairing between two regions of a RNA molecule. siRNA is "targeted" to a gene in that the nucleotide sequence of the duplex portion of the siRNA is complementary to a nucleotide sequence of the targeted gene. In some embodiments, the length of the duplex of siRNAs is less than 30 nucleotides. In some embodiments, the duplex can be 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11 or 10 nucleotides in length. In some embodiments, the length of the duplex is 19-25 nucleotides in length. The RNA duplex portion of the siRNA can be part of a hairpin structure. In addition to the duplex portion, the hairpin structure may contain a loop portion positioned between the two sequences that form the duplex. The loop can vary in length. In some embodiments the loop is 5, 6, 7, 8, 9, 10, 11, 12 or 13 nucleotides in length. The hairpin structure can also contain 3' or 5' overhang portions. In some embodiments, the overhang is a 3' or a 5' overhang 0, 1, 2, 3, 4 or 5 nucleotides in length.

[0031] A "short hairpin RNA," or shRNA, is a polynucleotide construct that can be made to express an interfering RNA such as siRNA.

[0032] "Recombinant," as applied to a polynucleotide means that the polynucleotide is the product of various combinations of cloning, restriction or ligation steps, and other procedures that result in a construct that is distinct from a polynucleotide found in nature. A recombinant virus is a viral particle comprising a recombinant polynucleotide. The terms respectively include replicates of the original polynucleotide construct and progeny of the original virus construct.

[0033] A "control element" or "control sequence" is a nucleotide sequence involved in an interaction of molecules that contributes to the functional regulation of a polynucleotide, including replication, duplication, transcription, splicing, translation, or degradation of the polynucleotide. The regulation may affect the frequency, speed, or specificity of the process, and may be enhancing or inhibitory in nature. Control elements known in the art include, for example, transcriptional regulatory sequences such as promoters and enhancers. A promoter is a DNA region capable under certain conditions of binding RNA polymerase and initiating transcription of a coding region usually located downstream (in the 3' direction) from the promoter.

[0034] "Operatively linked" or "operably linked" refers to a juxtaposition of genetic elements, wherein the elements are in a relationship permitting them to operate in the expected manner. For instance, a promoter is operatively linked to a coding region if the promoter helps initiate transcription of the coding sequence. There may be intervening residues between the promoter and coding region so long as this functional relationship is maintained.

[0035] An "expression vector" is a vector comprising a region which encodes a polypeptide of interest, and is used for effecting the expression of the protein in an intended target cell. An expression vector also comprises control elements operatively linked to the encoding region to facilitate expression of the protein in the target. The combination of control elements and a gene or genes to which they are operably linked for expression is sometimes referred to as an "expression cassette," a large number of which are known and available in the art or can be readily constructed from components that are available in the art.

[0036] "Heterologous" means derived from a genotypically distinct entity from that of the rest of the entity to which it is being compared. For example, a polynucleotide introduced by genetic engineering techniques into a plasmid or vector derived from a different species is a heterologous polynucleotide. A promoter removed from its native coding sequence and operatively linked to a coding sequence with which it is not naturally found linked is a heterologous promoter. Thus, for example, an rAAV that includes a heterologous nucleic acid encoding a heterologous gene product is an rAAV that includes a nucleic acid not normally included in a naturally-occurring, wild-type AAV, and the encoded heterologous gene product is a gene product not normally encoded by a naturally-occurring, wild-type AAV.

[0037] The terms "genetic alteration" and "genetic modification" (and grammatical variants thereof), are used interchangeably herein to refer to a process wherein a genetic element (e.g., a polynucleotide) is introduced into a cell other than by mitosis or meiosis. The element may be heterologous to the cell, or it may be an additional copy or improved version of an element already present in the cell. Genetic alteration may be effected, for example, by transfecting a cell with a recombinant plasmid or other polynucleotide through any process known in the art, such as electroporation, calcium phosphate precipitation, or contacting with a polynucleotide-liposome complex. Genetic alteration may also be effected, for example, by transduction or infection with a DNA or RNA virus or viral vector. Generally, the genetic element is introduced into a chromosome or mini-chromosome in the cell; but any alteration that changes the phenotype and/or genotype of the cell and its progeny is included in this term.

[0038] A cell is said to be "stably" altered, transduced, genetically modified, or transformed with a genetic sequence if the sequence is available to perform its function during extended culture of the cell in vitro. Generally, such a cell is "heritably" altered (genetically modified) in that a genetic alteration is introduced which is also inheritable by progeny of the altered cell.

[0039] The terms "polypeptide," "peptide," and "protein" are used interchangeably herein to refer to polymers of amino acids of any length. The terms also encompass an amino acid polymer that has been modified; for example, disulfide bond formation, glycosylation, lipidation, phosphorylation, or conjugation with a labeling component. Polypeptides such as anti-angiogenic polypeptides, neuroprotective polypeptides, and the like, when discussed in the context of delivering a gene product to a mammalian subject, and compositions therefor, refer to the respective intact polypeptide, or any fragment or genetically engineered derivative thereof, which retains the desired biochemical function of the intact protein. Similarly, references to nucleic acids encoding anti-angiogenic polypeptides, nucleic acids encoding

neuroprotective polypeptides, and other such nucleic acids for use in delivery of a gene product to a mammalian subject (which may be referred to as "transgenes" to be delivered to a recipient cell), include polynucleotides encoding the intact polypeptide or any fragment or genetically engineered derivative possessing the desired biochemical function.

[0040] An "isolated" plasmid, nucleic acid, vector, virus, virion, host cell, or other substance refers to a preparation of the substance devoid of at least some of the other components that may also be present where the substance or a similar substance naturally occurs or is initially prepared from. Thus, for example, an isolated substance may be prepared by using a purification technique to enrich it from a source mixture. Enrichment can be measured on an absolute basis, such as weight per volume of solution, or it can be measured in relation to a second, potentially interfering substance present in the source mixture. Increasing enrichments of the embodiments of this disclosure are increasingly more isolated. An isolated plasmid, nucleic acid, vector, virus, host cell, or other substance is in some embodiments purified, e.g., from about 80% to about 90% pure, at least about 90% pure, at least about 95% pure, at least about 98% pure, or at least about 99%, or more, pure.

[0041] As used herein, the terms "treatment," "treating," and the like, refer to obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of a partial or complete cure for a disease and/or adverse effect attributable to the disease. "Treatment," as used herein, covers any treatment of a disease in a mammal, particularly in a human, and includes: (a) preventing the disease from occurring in a subject which may be predisposed to the disease or at risk of acquiring the disease but has not yet been diagnosed as having it; (b) inhibiting the disease, i.e., arresting its development; and (c) relieving the disease, i.e., causing regression of the disease.

[0042] The terms "individual," "host," "subject," and "patient" are used interchangeably herein, and refer to a mammal, including, but not limited to, human and non-human primates, including simians and humans; mammalian sport animals (e.g., horses); mammalian farm animals (e.g., sheep, goats, etc.); mammalian pets (dogs, cats, etc.); and rodents (e.g., mice, rats, etc.).

[0043] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

[0044] Unless defined otherwise, 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. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention, the preferred methods and materials are now described.

[0045] It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a recombinant AAV virion" includes a plurality of such virions and reference to "the photoreceptor cell" includes reference to one or more photoreceptor cells and equivalents thereof known to those skilled in the art, and so forth.

DETAILED DESCRIPTION

[0046] The present disclosure provides adeno-associated virus (AAV) virions with altered capsid protein, where the AAV virions exhibit greater infectivity of a retinal cell, when administered via intravitreal injection, compared to wild-type AAV when administered via intravitreal injection. The present disclosure further provides said virions for use in a method of treating an ocular disease.

[0047] The retinal cell can be a photoreceptor (e.g., rods; cones), a retinal ganglion cell (RGC), a Müller cell (a Müller glial cell), a bipolar cell, an amacrine cell, a horizontal cell, or a retinal pigmented epithelium (RPE) cell.

VARIANT AAV CAPSID POLYPEPTIDES

[0048] The present disclosure provides a variant AAV capsid protein, where the variant AAV capsid protein comprises an insertion of from about 5 amino acids to about 11 amino acids in an insertion site in the capsid protein GH loop, relative to a corresponding parental AAV capsid protein, and where the variant capsid protein, when present in an AAV virion, confers increased infectivity of a retinal cell compared to the infectivity of the retinal cell by an AAV virion comprising the corresponding parental AAV capsid protein. In some cases, the retinal cell is a photoreceptor cell (e.g., rods; cones). In other cases, the retinal cell is an RGC. In other cases, the retinal cell is an RPE cell. In other cases, the retinal cell is a Müller cell. Other retinal cells include amacrine cells, bipolar cells, and horizontal cells. An "insertion of from about 5 amino acids to about 11 amino acids" is also referred to herein as a "peptide insertion" (e.g., a heterologous peptide insertion). A "corresponding parental AAV capsid protein" refers to an AAV capsid protein of the same AAV serotype, without the peptide insertion.

[0049] The insertion site is in the GH loop of the AAV capsid protein. For the GH loop/loop IV of AAV capsid, see, e.g., van Vliet et al. (2006) Mol. Ther. 14:809; Padron et al. (2005) J. Virol. 79:5047; and Shen et al. (2007) Mol. Ther. 15: 1955. For example, the insertion site can be within amino acids 411-650 of an AAV capsid protein, as depicted in Figures 17A and 17B. For

example, the insertion site can be within amino acids 570-611 of AAV2, within amino acids 571-612 of AAV1, within amino acids 560-601 of AAV5, within amino acids 571 to 612 of AAV6, within amino acids 572 to 613 of AAV7, within amino acids 573 to 614 of AAV8, within amino acids 571 to 612 of AAV9, or within amino acids 573 to 614 of AAV10, as depicted in Figure 6.

[0050] In some cases, from about 5 amino acids to about 11 amino acids are inserted in an insertion site in the GH loop of the capsid protein relative to a corresponding parental AAV capsid protein. For example, the insertion site can be between amino acids 587 and 588 of AAV2, or the corresponding positions of the capsid subunit of another AAV serotype. It should be noted that the insertion site 587/588 is based on an AAV2 capsid protein. From about 5 amino acids to about 11 amino acids can be inserted in a corresponding site in an AAV serotype other than AAV2 (e.g., AAV8, AAV9, etc.). Those skilled in the art would know, based on a comparison of the amino acid sequences of capsid proteins of various AAV serotypes, where an insertion site "corresponding to amino acids 587-588 of AAV2" would be in a capsid protein of any given AAV serotype. Sequences corresponding to amino acids 570-611 of capsid protein VP1 of AAV2 (see Figure 5) in various AAV serotypes are shown in Figure 6. See, e.g., GenBank Accession No. NP_049542 for AAV1; GenBank Accession No. AAD13756 for AAV5; GenBank Accession No. AAB95459 for AAV6; GenBank Accession No. YP_077178 for AAV7; GenBank Accession No. YP_077180 for AAV8; GenBank Accession No. AAS99264 for AAV9 and GenBank Accession No. AAT46337 for AAV10.

[0051] The insertion site may be a single insertion site between two adjacent amino acids located between amino acids 570-614 of VP1 of any AAV serotype, e.g., the insertion site is between two adjacent amino acids located in amino acids 570-610, amino acids 580-600, amino acids 570-575, amino acids 575-580, amino acids 580-585, amino acids 585-590, amino acids 590-600, or amino acids 600-614, of VP1 of any AAV serotype or variant. For example, the insertion site can be between amino acids 580 and 581, amino acids 581 and 582, amino acids 583 and 584, amino acids 584 and 585, amino acids 585 and 586, amino acids 586 and 587, amino acids 587 and 588, amino acids 588 and 589, or amino acids 589 and 590. The insertion site can be between amino acids 575 and 576, amino acids 576 and 577, amino acids 577 and 578, amino acids 578 and 579, or amino acids 579 and 580. The insertion site can be between amino acids 590 and 591, amino acids 591 and 592, amino acids 592 and 593, amino acids 593 and 594, amino acids 594 and 595, amino acids 595 and 596, amino acids 596 and 597, amino acids 597 and 598, amino acids 598 and 599, or amino acids 599 and 600.

[0052] For example, the insertion site can be between amino acids 587 and 588 of AAV2, between amino acids 590 and 591 of AAV1, between amino acids 575 and 576 of AAV5, between amino acids 590 and 591 of AAV6, between amino acids 589 and 590 of AAV7, between amino acids 590 and 591 of AAV8, between amino acids 588 and 589 of AAV9, or between amino acids 588 and 589 of AAV10.

[0053] As another example, the insertion site can be between amino acids 450 and 460 of an AAV capsid protein, as shown in Figure 17A. For example, the insertion site can be at (e.g.,

immediately N-terminal to) amino acid 453 of AAV2, at amino acid 454 of AAV1, at amino acid 454 of AAV6, at amino acid 456 of AAV7, at amino acid 456 of AAV8, at amino acid 454 of AAV9, or at amino acid 456 of AAV10, as shown in Figure 17A.

[0054] A subject capsid protein may include a GH loop comprising an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to an amino acid sequence set forth in Figure 18A-C.

[0055] Specifically, according to the invention, the variant AAV capsid protein comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 1, and an insertion of a peptide in the capsid protein GH loop relative to SEQ ID NO: 1, wherein the peptide comprises the amino acid sequence LALGETTRPA (SEQ ID NO:45)

Insertion peptides

[0056] As noted above, a peptide of from about 5 amino acids to about 11 amino acids in length is inserted into the GH loop of an AAV capsid. The insertion peptide has a length 10 amino acids, or 11 amino acids.

[0057] The insertion peptide comprises the following amino acid sequence: LALGETTRPA (SEQ ID NO:45

[0058] In some examples, a subject variant AAV capsid does not include any other amino acid substitutions, insertions, or deletions, other than an insertion of from about 5 amino acids to about 11 amino acids in the GH loop relative to a corresponding parental AAV capsid protein. In other examples, a subject variant AAV capsid includes from 1 to about 25 amino acid insertions, deletions, or substitutions, compared to the parental AAV capsid protein, in addition to an insertion of from about 5 amino acids to about 11 amino acids in the GH loop relative to a corresponding parental AAV capsid protein. For example, in some examples , a subject variant AAV capsid includes from 1 to about 5, from about 5 to about 10, from about 10 to about 15, from about 15 to about 20, or from about 20 to about 25 amino acid insertions, deletions, or substitutions, compared to the parental AAV capsid protein, in addition to an insertion of from about 5 amino acids to about 11 amino acids in the GH loop or loop IV relative to a corresponding parental AAV capsid protein.

[0059] In some examples, a subject variant capsid polypeptide does not include one, two, three, or four, of the following amino acid substitutions: Y273F, Y444F, Y500F, and Y730F.

[0060] In some examples, a subject variant capsid polypeptide comprises, in addition to an insertion peptide as described above, one, two, three, or four, of the following amino acid substitutions: Y273F, Y444F, Y500F, and Y730F.

[0061] In some examples , a variant AAV capsid polypeptide is a chimeric capsid, e.g., the capsid comprises a portion of an AAV capsid of a first AAV serotype and a portion of an AAV capsid of a second serotype; and comprises an insertion of from about 5 amino acids to about 11 amino acids in the GH loop relative to a corresponding parental AAV capsid protein.

[0062] A subject variant capsid protein comprises an amino acid sequence having at least 95%, at least about 98%, or at least about 99%, amino acid sequence identity to the amino acid sequence of SEQ ID NO: 1; and an insertion of from about 5 amino acids to about 11 amino acids in the GH loop relative to a corresponding parental AAV capsid protein.

[0063] In some examples, a subject variant capsid protein is isolated, e.g., purified. In some cases, a subject variant capsid protein is included in an AAV vector, which is also provided. As described in detail below, a subject variant capsid protein can be included in a recombinant AAV virion.

RECOMBINANT AAV VIRION

[0064] The present disclosure provides a recombinant adeno-associated virus (rAAV) virion comprising: a) a variant AAV capsid according to the invention; and b) a heterologous nucleic acid comprising a nucleotide sequence encoding a gene product. In some cases, the retinal cell is a photoreceptor cell (e.g., rods and/or cones). In other cases, the retinal cell is an RGC cell. In other cases, the retinal cell is an RPE cell. In other cases, the retinal cell is a Müller cell. In other cases, retinal cells may include amacrine cells, bipolar cells, and horizontal cells.

[0065] A subject rAAV virion may exhibit at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 50-fold, or more than 50-fold, increased infectivity of a retinal cell, compared to the infectivity of the retinal cell by an AAV virion comprising the corresponding parental AAV capsid protein.

[0066] In some cases, a subject rAAV virion exhibits at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 50-fold, or more than 50-fold, increased infectivity of a retinal cell, when administered via intravitreal injection, compared to the infectivity of the retinal cell by an AAV virion comprising the corresponding parental AAV capsid protein, when administered via intravitreal injection.

[0067] In some embodiments, a subject rAAV virion exhibits at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 50-fold, or more than 50-fold, increased infectivity of a photoreceptor (rod or cone) cell, compared to the infectivity of the photoreceptor cell by an AAV virion comprising the corresponding parental AAV capsid protein.

[0068] In some embodiments, a subject rAAV virion exhibits at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 50-fold, or more than 50-fold, increased infectivity of a photoreceptor (rod or cone) cell, when administered via intravitreal injection, compared to the

infectivity of the photoreceptor cell by an AAV virion comprising the corresponding parental AAV capsid protein, when administered via intravitreal injection.

[0069] In some embodiments, a subject rAAV virion exhibits at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 50-fold, or more than 50-fold, increased infectivity of an RGC, compared to the infectivity of the RGC by an AAV virion comprising the corresponding parental AAV capsid protein.

[0070] In some embodiments, a subject rAAV virion exhibits at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 50-fold, or more than 50-fold, increased infectivity of an RGC, when administered via intravitreal injection, compared to the infectivity of the RGC by an AAV virion comprising the corresponding parental AAV capsid protein, when administered via intravitreal injection.

[0071] In some embodiments, a subject rAAV virion exhibits at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 50-fold, or more than 50-fold, increased infectivity of an RPE cell, compared to the infectivity of the RPE cell by an AAV virion comprising the corresponding parental AAV capsid protein.

[0072] In some embodiments, a subject rAAV virion exhibits at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 50-fold, or more than 50-fold, increased infectivity of an RPE cell, when administered via intravitreal injection, compared to the infectivity of the RPE cell by an AAV virion comprising the corresponding parental AAV capsid protein, when administered via intravitreal injection.

[0073] In some embodiments, a subject rAAV virion exhibits at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 50-fold, or more than 50-fold, increased infectivity of a Müller cell, compared to the infectivity of the Müller cell by an AAV virion comprising the corresponding parental AAV capsid protein.

[0074] In some embodiments, a subject rAAV virion exhibits at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 50-fold, or more than 50-fold, increased infectivity of a Müller cell, when administered via intravitreal injection, compared to the infectivity of the Müller cell by an AAV virion comprising the corresponding parental AAV capsid protein, when administered via intravitreal injection.

[0075] In some embodiments, a subject rAAV virion exhibits at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 50-fold, or more than 50-fold, increased infectivity of a bipolar cell, compared to the infectivity of the bipolar cell by an AAV virion comprising the corresponding parental AAV capsid protein.

[0076] In some embodiments, a subject rAAV virion exhibits at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 50-fold, or more than 50-fold, increased infectivity of a bipolar cell, when administered via intravitreal injection, compared to the infectivity of the

bipolar cell by an AAV virion comprising the corresponding parental AAV capsid protein, when administered via intravitreal injection.

[0077] In some embodiments, a subject rAAV virion exhibits at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 50-fold, or more than 50-fold, increased infectivity of an amacrine cell, compared to the infectivity of the amacrine cell by an AAV virion comprising the corresponding parental AAV capsid protein.

[0078] In some embodiments, a subject rAAV virion exhibits at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 50-fold, or more than 50-fold, increased infectivity of an amacrine cell, when administered via intravitreal injection, compared to the infectivity of the amacrine cell by an AAV virion comprising the corresponding parental AAV capsid protein, when administered via intravitreal injection.

[0079] In some embodiments, a subject rAAV virion exhibits at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 50-fold, or more than 50-fold, increased infectivity of a horizontal cell, compared to the infectivity of the horizontal cell by an AAV virion comprising the corresponding parental AAV capsid protein.

[0080] In some embodiments, a subject rAAV virion exhibits at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 50-fold, or more than 50-fold, increased infectivity of a horizontal cell, when administered via intravitreal injection, compared to the infectivity of the horizontal cell by an AAV virion comprising the corresponding parental AAV capsid protein, when administered via intravitreal injection.

[0081] In some cases, a subject rAAV virion exhibits at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 50-fold, or more than 50-fold, increased ability to cross the internal limiting membrane (ILM), compared to the ability of an AAV virion comprising the corresponding parental AAV capsid protein to cross the ILM.

[0082] In some cases, a subject rAAV virion exhibits at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 50-fold, or more than 50-fold, increased ability, when administered via intravitreal injection, to cross the internal limiting membrane (ILM), compared to the ability of an AAV virion comprising the corresponding parental AAV capsid protein to cross the ILM when administered via intravitreal injection.

[0083] A subject rAAV virion can cross the ILM, and can also traverse cell layers, including Müller cells, amacrine cells, etc., to reach the photoreceptor cells and or RPE cells. For example, a subject rAAV virion, when administered via intravitreal injection, can cross the ILM, and can also traverse cell layers, including Müller cells, amacrine cells, etc., to reach the photoreceptor cells and or RPE cells.

[0084] In some embodiments, a subject rAAV virion selectively infects a retinal cell, e.g., a subject rAAV virion infects a retinal cell with 10-fold, 15-fold, 20-fold, 25-fold, 50-fold, or more

than 50-fold, specificity than a non-retinal cell, e.g., a cell outside the eye. For example, in some embodiments, a subject rAAV virion selectively infects a retinal cell, e.g., a subject rAAV virion infects a photoreceptor cell with 10-fold, 15-fold, 20-fold, 25-fold, 50-fold, or more than 50-fold, specificity than a non-retinal cell, e.g., a cell outside the eye.

[0085] In some embodiments, a subject rAAV virion selectively infects a photoreceptor cell, e.g., a subject rAAV virion infects a photoreceptor cell with 10-fold, 15-fold, 20-fold, 25-fold, 50-fold, or more than 50-fold, specificity than a non-photoreceptor cell present in the eye, e.g., a retinal ganglion cell, a Müller cell, etc.

[0086] In some embodiments, a subject rAAV virion exhibits at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 50-fold, or more than 50-fold, increased infectivity of a photoreceptor cell, when administered via intravitreal injection, compared to the infectivity of the photoreceptor cell by an AAV virion comprising the corresponding parental AAV capsid protein, when administered via intravitreal injection.

Gene products

[0087] A subject rAAV virion comprises a heterologous nucleic acid comprising a nucleotide sequence encoding a gene product. In some embodiments, the gene product is an interfering RNA. In some embodiments, the gene product is an aptamer. In some embodiments, the gene product is a polypeptide. In some embodiments, the gene product is a site-specific nuclease that provide for site-specific knock-down of gene function.

Interfering RNA

[0088] Where the gene product is an interfering RNA (RNAi), suitable RNAi include RNAi that decrease the level of an apoptotic or angiogenic factor in a cell. For example, an RNAi can be an shRNA or siRNA that reduces the level of a gene product that induces or promotes apoptosis in a cell. Genes whose gene products induce or promote apoptosis are referred to herein as "pro-apoptotic genes" and the products of those genes (mRNA; protein) are referred to as "pro-apoptotic gene products." Pro-apoptotic gene products include, e.g., *Bax, Bid, Bak,* and *Bad* gene products. See, e.g., U.S. Patent No. 7,846,730.

[0089] Interfering RNAs could also be against an angiogenic product, for example VEGF (e.g., Cand5; see, e.g., U.S. Patent Publication No. 2011/0143400; U.S. Patent Publication No. 2008/0188437; and Reich et al. (2003) Mol. Vis. 9:210), VEGFR1 (e.g., Sirna-027; see, e.g., Kaiser et al. (2010) Am. J. Ophthalmol. 150:33; and Shen et al. (2006) Gene Ther. 13:225), or VEGFR2 (Kou et al. (2005) Biochem. 44:15064). See also, U.S. Patent Nos. 6,649,596, 6,399,586, 5,661,135, 5,639,872, and 5,639,736; and U.S. Patent Nos. 7,947,659 and 7,919,473.

Aptamers

[0090] Where the gene product is an aptamer, exemplary aptamers of interest include an aptamer against vascular endothelial growth factor (VEGF). See, e.g., Ng et al. (2006) Nat. Rev. Drug Discovery 5:123; and Lee et al. (2005) Proc. Natl. Acad. Sci. USA 102:18902. For example, a VEGF aptamer can comprise the nucleotide sequence 5'-cgcaaucagugaaugcuuauacauccg-3' (SEQ ID NO:17). Also suitable for use is a PDGF-specific aptamer, e.g., E10030; see, e.g., Ni and Hui (2009) Ophthalmologica 223:401; and Akiyama et al. (2006) J. Cell Physiol. 207:407).

Polypeptides

[0091] Where the gene product is a polypeptide, the polypeptide is generally a polypeptide that enhances function of a retinal cell, e.g., the function of a rod or cone photoreceptor cell, a retinal ganglion cell, a Müller cell, a bipolar cell, an amacrine cell, a horizontal cell, or a retinal pigmented epithelial cell. Exemplary polypeptides include neuroprotective polypeptides (e.g., GDNF, CNTF, NT4, NGF, and NTN); anti-angiogenic polypeptides (e.g., a soluble vascular endothelial growth factor (VEGF) receptor; a VEGF-binding antibody; a VEGF-binding antibody fragment (e.g., a single chain anti-VEGF antibody); endostatin; tumstatin; angiostatin; a soluble Flt polypeptide (Lai et al. (2005) Mol. Ther. 12:659); an Fc fusion protein comprising a soluble Flt polypeptide (see, e.g., Pechan et al. (2009) Gene Ther. 16:10); pigment epithelium-derived factor (PEDF); a soluble Tie-2 receptor; etc.); tissue inhibitor of metalloproteinases-3 (TIMP-3); a light-responsive opsin, e.g., a rhodopsin; anti-apoptotic polypeptides (e.g., Bcl-2, Bcl-Xl); and the like. Suitable polypeptides include, but are not limited to, glial derived neurotrophic factor (GDNF); fibroblast growth factor 2; neurturin (NTN); ciliary neurotrophic factor (CNTF); nerve growth factor (NGF); neurotrophin-4 (NT4); brain derived neurotrophic factor (BDNF; e.g., a polypeptide comprising an amino acid sequence having at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 200 amino acids to 247 amino acids of the amino acid sequence depicted in Figure 11 (SEQ ID NO: 1 1)); epidermal growth factor; rhodopsin; X-linked inhibitor of apoptosis; and Sonic hedgehog.

[0092] Suitable light-responsive opsins include, e.g., a light-responsive opsin as described in U.S. Patent Publication No. 2007/0261127 (e.g., ChR2; Chop2); U.S. Patent Publication No. 2001/0086421; U.S. Patent Publication No. 2010/0015095; and Diester et al. (2011) Nat. Neurosci. 14:387.

[0093] Suitable polypeptides also include retinoschisin (e.g., a polypeptide comprising an amino acid sequence having at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 200 amino acids to 224 amino acids of the amino acid sequence depicted in Figure 10 (SEQ ID

NO:10). Suitable polypeptides include, e.g., retinitis pigmentosa GTPase regulator (RGPR)-interacting protein-1 (see, e.g., GenBank Accession Nos. Q96KN7, Q9EPQ2, and Q9GLM3) (e.g., a polypeptide comprising an amino acid sequence having at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 1150 amino acids to about 1200 amino acids, or from about 1200 amino acids to 1286 amino acids, of the amino acid sequence depicted in Figure 16 (SEQ ID NO:21); peripherin-2 (Prph2) (see, e.g., GenBank Accession No. NP_000313 (e.g., a polypeptide comprising an amino acid sequence having at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 300 amino acids to 346 amino acids of the amino acid sequence depicted in Figure 14 (SEQ ID NO:19); and Travis et al. (1991) Genomics 10:733); peripherin (e.g., a polypeptide comprising an amino acid sequence having at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 400 amino acids to about 470 amino acids of the amino acid sequence depicted in Figure 15 (SEQ ID NO:20); a retinal pigment epithelium-specific protein (RPE65), (e.g., a polypeptide comprising an amino acid sequence having at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 200 amino acids to 247 amino acids of the amino acid sequence depicted in Figure 12 (SEQ ID NO:12)) (see, e.g., GenBank AAC39660; and Morimura et al. (1998) Proc. Natl. Acad. Sci. USA 95:3088); and the like.

[0094] Suitable polypeptides also include: CHM (choroidermia (Rab escort protein 1)), a polypeptide that, when defective or missing, causes choroideremia (see, e.g., Donnelly et al. (1994) Hum. Mol. Genet. 3:1017; and van Bokhoven et al. (1994) Hum. Mol. Genet. 3:1041); and Crumbs homolog 1 (CRB1), a polypeptide that, when defective or missing, causes Leber congenital amaurosis and retinitis pigmentosa (see, e.g., den Hollander et al. (1999) Nat. Genet. 23:217; and GenBank Accession No. CAM23328).

[0095] Suitable polypeptides also include polypeptides that, when defective or missing, lead to achromotopsia, where such polypeptides include, e.g., cone photoreceptor cGMP-gated channel subunit alpha (CNGA3) (see, e.g., GenBank Accession No. NP_001289; and Booij et al. (2011) Ophthalmology 118:160-167); cone photoreceptor cGMP-gated cation channel beta-subunit (CNGB3) (see, e.g., Kohl et al.(2005) Eur J Hum Genet. 13(3):302); guanine nucleotide binding protein (G protein), alpha transducing activity polypeptide 2 (GNAT2) (ACHM4); and ACHM5; and polypeptides that, when defective or lacking, lead to various forms of color blindness (e.g., L-opsin, M-opsin, and S-opsin). See Mancuso et al. (2009) Nature 461(7265):784-787.

Site-specific endonucleases

[0096] In some cases, a gene product of interest is a site-specific endonuclease that provide for site-specific knock-down of gene function, e.g., where the endonuclease knocks out an allele associated with a retinal disease. For example, where a dominant allele encodes a

defective copy of a gene that, when wild-type, is a retinal structural protein and/or provides for normal retinal function, a site-specific endonuclease can be targeted to the defective allele and knock out the defective allele.

[0097] In addition to knocking out a defective allele, a site-specific nuclease can also be used to stimulate homologous recombination with a donor DNA that encodes a functional copy of the protein encoded by the defective allele. Thus, e.g., a subject rAAV virion can be used to deliver both a site-specific endonuclease that knocks out a defective allele, and can be used to deliver a functional copy of the defective allele, resulting in repair of the defective allele, thereby providing for production of a functional retinal protein (e.g., functional retinoschisin, functional RPE65, functional peripherin, etc.). See, e.g., Li et al. (2011) Nature 475:217. In some embodiments, a subject rAAV virion comprises a heterologous nucleotide sequence that encodes a site-specific endonuclease; and a heterologous nucleotide sequence that encodes a functional copy of a defective allele, where the functional copy encodes a functional retinal protein. Functional retinal proteins include, e.g., retinoschisin, RPE65, retinitis pigmentosa GTPase regulator (RGPR)-interacting protein-1, peripherin, peripherin-2, and the like.

[0098] Site-specific endonucleases that are suitable for use include, e.g., zinc finger nucleases (ZFNs); and transcription activator-like effector nucleases (TALENs), where such site-specific endonucleases are non-naturally occurring and are modified to target a specific gene. Such site-specific nucleases can be engineered to cut specific locations within a genome, and non-homologous end joining can then repair the break while inserting or deleting several nucleotides. Such site-specific endonucleases (also referred to as "INDELs") then throw the protein out of frame and effectively knock out the gene. See, e.g., U.S. Patent Publication No. 2011/0301073.

Regulatory sequences

[0099] In some embodiments, a nucleotide sequence encoding a gene product of interest is operably linked to a constitutive promoter. In other embodiments, a nucleotide sequence encoding a gene product of interest is operably linked to an inducible promoter. In some instances, a nucleotide sequence encoding a gene product of interest is operably linked to a tissue-specific or cell type-specific regulatory element. For example, in some instances, a nucleotide sequence encoding a gene product of interest is operably linked to a photoreceptor-specific regulatory element (e.g., a photoreceptor-specific promoter), e.g., a regulatory element that confers selective expression of the operably linked gene in a photoreceptor cell. Suitable photoreceptor-specific regulatory elements include, e.g., a rhodopsin promoter; a rhodopsin kinase promoter (Young et al. (2003) Ophthalmol. Vis. Sci. 44:4076); a beta phosphodiesterase gene promoter (Nicoud et al. (2007) J. Gene Med. 9: 1015); a retinitis pigmentosa gene promoter (Nicoud et al. (2007) *supra*); an interphotoreceptor retinoid-binding protein (IRBP) gene enhancer (Nicoud et al. (2007) *supra*); an IRBP gene promoter (Yokoyama et al. (1992) Exp Eye Res. 55:225).

PHARMACEUTICAL COMPOSITIONS

[0100] The present disclosure provides a pharmaceutical composition comprising: a) a subject rAAV virion, as described above; and b) a pharmaceutically acceptable carrier, diluent, excipient, or buffer. In some embodiments, the pharmaceutically acceptable carrier, diluent, excipient, or buffer is suitable for use in a human.

[0101] Such excipients, carriers, diluents, and buffers include any pharmaceutical agent that can be administered without undue toxicity. Pharmaceutically acceptable excipients include, but are not limited to, liquids such as water, saline, glycerol and ethanol. Pharmaceutically acceptable salts can be included therein, for example, mineral acid salts such as hydrochlorides, hydrobromides, phosphates, sulfates, and the like; and the salts of organic acids such as acetates, propionates, malonates, benzoates, and the like. Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present in such vehicles. A wide variety of pharmaceutically acceptable excipients are known in the art and need not be discussed in detail herein. Pharmaceutically acceptable excipients have been amply described in a variety of publications, including, for example, A. Gennaro (2000) "Remington: The Science and Practice of Pharmacy," 20th edition, Lippincott, Williams, & Wilkins; Pharmaceutical Dosage Forms and Drug Delivery Systems (1999) H.C. Ansel et al., eds., 7th ed., Lippincott, Williams, & Wilkins; and Handbook of Pharmaceutical Excipients (2000) A.H. Kibbe et al., eds., 3rd ed. Amer. Pharmaceutical Assoc.

METHODS OF DELIVERING A GENE PRODUCT TO A RETINAL CELL AND TREATMENT METHODS

[0102] The references to methods of treatment in the following paragraphs of this description are to be interpreted as references to the compounds, pharmaceutical compositions and medicaments of the present invention for use in a method for treatment of the human or animal body by therapy. The present disclosure provides a method of delivering a gene product to a retinal cell in an individual, the method comprising administering to the individual a subject rAAV virion as described above. The gene product can be a polypeptide or an interfering RNA (e.g., an shRNA, an siRNA, and the like), an aptamer, or a site-specific endonuclease, as described above. Delivering a gene product to a retinal cell can provide for treatment of a retinal disease. The retinal cell can be a photoreceptor, a retinal ganglion cell, a Müller cell, a bipolar cell, an amacrine cell, a horizontal cell, or a retinal pigmented epithelial cell. In some cases, the retinal cell is a photoreceptor cell, e.g., a rod or cone cell.

[0103] The present disclosure provides a method of treating a retinal disease, the method comprising administering to an individual in need thereof an effective amount of a subject rAAV virion as described above. A subject rAAV virion can be administered via intraocular injection, by intravitreal injection, or by any other convenient mode or route of administration. Other

convenient modes or routes of administration include, e.g., intravenous, intranasal, etc.

[0104] A "therapeutically effective amount" will fall in a relatively broad range that can be determined through experimentation and/or clinical trials. For example, for in vivo injection, i.e., injection directly into the eye, a therapeutically effective dose will be on the order of from about 10^6 to about 10^{15} of the rAAV virions, e.g., from about 10^8 to 10^{12} rAAV virions. For *in vitro* transduction, an effective amount of rAAV virions to be delivered to cells will be on the order of from about 10^8 to about 10^{13} of the rAAV virions. Other effective dosages can be readily established by one of ordinary skill in the art through routine trials establishing dose response curves.

[0105] In some embodiments, more than one administration (e.g., two, three, four or more administrations) may be employed to achieve the desired level of gene expression over a period of various intervals, e.g., daily, weekly, monthly, yearly, etc.

[0106] Ocular diseases that can be treated using a subject method include, but are not limited to, acute macular neuroretinopathy; Behcet's disease; choroidal neovascularization; diabetic uveitis; histoplasmosis; macular degeneration, such as acute macular degeneration, non-exudative age related macular degeneration and exudative age related macular degeneration; edema, such as macular edema, cystoid macular edema and diabetic macular edema; multifocal choroiditis; ocular trauma which affects a posterior ocular site or location; ocular tumors; retinal disorders, such as central retinal vein occlusion, diabetic retinopathy (including proliferative diabetic retinopathy), proliferative vitreoretinopathy (PVR), retinal arterial occlusive disease, retinal detachment, uveitic retinal disease; sympathetic opthalmia; Vogt Koyanagi-Harada (VKH) syndrome; uveal diffusion; a posterior ocular condition caused by or influenced by an ocular laser treatment; posterior ocular conditions caused by or influenced by a photodynamic therapy; photocoagulation, radiation retinopathy; epiretinal membrane disorders; branch retinal vein occlusion; anterior ischemic optic neuropathy; non-retinopathy diabetic retinal dysfunction; retinoschisis; retinitis pigmentosa; glaucoma; Usher syndrome, cone-rod dystrophy; Stargardt disease (fundus flavimaculatus); inherited macular degeneration; chorioretinal degeneration; Leber congenital amaurosis; congenital stationary night blindness; choroideremia; Bardet-Biedl syndrome; macular telangiectasia; Leber's hereditary optic neuropathy; retinopathy of prematurity; and disorders of color vision, including achromatopsia, protanopia, deuteranopia, and tritanopia.

NUCLEIC ACIDS AND HOST CELLS

[0107] The present disclosure provides an isolated nucleic acid comprising a nucleotide sequence that encodes a subject variant adeno-associated virus (AAV) capsid protein as described above, where the variant AAV capsid protein comprises an insertion of from about 5 amino acids to about 11 amino acids in the GH loop relative to a corresponding parental AAV capsid protein, and where the variant capsid protein, when present in an AAV virion, provides

for increased infectivity of a retinal cell compared to the infectivity of the retinal cell by an AAV virion comprising the corresponding parental AAV capsid protein. A subject isolated nucleic acid can be an AAV vector, e.g., a recombinant AAV vector.

[0108] A subject recombinant AAV vector can be used to generate a subject recombinant AAV virion, as described above. Thus, the present disclosure provides a recombinant AAV vector that, when introduced into a suitable cell, can provide for production of a subject recombinant AAV virion.

[0109] There may be provided host cells, e.g., isolated (genetically modified) host cells, comprising a subject nucleic acid. A subject host cell can be an isolated cell, e.g., a cell in *in vitro* culture. A subject host cell is useful for producing a subject rAAV virion, as described below. Where a subject host cell is used to produce a subject rAAV virion, it is referred to as a "packaging cell." A subject host cell may be stably genetically modified with a subject nucleic acid. A subject host cell may be transiently genetically modified with a subject nucleic acid.

[0110] A subject nucleic acid is introduced stably or transiently into a host cell, using established techniques, including, but not limited to, electroporation, calcium phosphate precipitation, liposome-mediated transfection, and the like. For stable transformation, a subject nucleic acid will generally further include a selectable marker, e.g., any of several well-known selectable markers such as neomycin resistance, and the like.

[0111] A subject host cell is generated by introducing a subject nucleic acid into any of a variety of cells, e.g., mammalian cells, including, e.g., murine cells, and primate cells (e.g., human cells). Suitable mammalian cells include, but are not limited to, primary cells and cell lines, where suitable cell lines include, but are not limited to, 293 cells, COS cells, HeLa cells, Vero cells, 3T3 mouse fibroblasts, C3H10T1/2 fibroblasts, CHO cells, and the like. Non-limiting examples of suitable host cells include, e.g., HeLa cells (e.g., American Type Culture Collection (ATCC) No. CCL-2), CHO cells (e.g., ATCC Nos. CRL9618, CCL61, CRL9096), 293 cells (e.g., ATCC No. CRL-1573), Vero cells, NIH 3T3 cells (e.g., ATCC No. CRL-1658), Huh-7 cells, BHK cells (e.g., ATCC No. CCL10), PC12 cells (ATCC No. CRL1721), COS cells, COS-7 cells (ATCC No. CRL1651), RAT1 cells, mouse L cells (ATCC No. CCLI.3), human embryonic kidney (HEK) cells (ATCC No. CRL1573), HLHepG2 cells, and the like. A subject host cell can also be made using a baculovirus to infect insect cells such as Sf9 cells, which produce AAV (see, e.g., U.S. Patent No. 7,271,002; US patent application 12/297,958)

[0112] In some examples, a subject genetically modified host cell includes, in addition to a nucleic acid comprising a nucleotide sequence encoding a variant AAV capsid protein, as described above, a nucleic acid that comprises a nucleotide sequence encoding one or more AAV rep proteins. In other examples, a subject host cell further comprises an rAAV vector. An rAAV virion can be generated using a subject host cell. Methods of generating an rAAV virion are described in, e.g., U.S. Patent Publication No. 2005/0053922 and U.S. Patent Publication No. 2009/0202490.

EXAMPLES

[0113] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g. amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Celsius, and pressure is at or near atmospheric. Standard abbreviations may be used, e.g., bp, base pair(s); kb, kilobase(s); pl, picoliter(s); s or sec, second(s); min, minute(s); h or hr, hour(s); aa, amino acid(s); kb, kilobase(s); bp, base pair(s); nt, nucleotide(s); i.m., intramuscular(ly); i.p., intraperitoneal(ly); s.c., subcutaneous(ly); and the like.

Example 1: AAV variant with enhanced transduction of retinal cells

[0114] The approach used was to create a peptide display library by introducing a unique AvrII site into the wild type AAV2 genome between amino acid 587 and 588 by polymerase chain reaction (PCR) mutagenesis. A random 21 nucleotide insert, 7mer For, was used to synthesize dsDNA inserts, along with the antisense primer 7mer Rev. The resulting dsDNA inserts were cloned into the AvrII site of the genome after digestion with *Nhe*I, producing a diverse 7mer display library which was then packaged (Perabo et al., 2003; Muller et al., 2003). The virus was generated such that each viral genome was packaged or encapsidated within the capsid protein variant that that genome encoded. In this respect, functional improvements identified through selection can be linked to the genome sequence encoding this improved function contained within the viral capsid.

[0115] This library was subjected to positive selection within rho-GFP mice (Wensel et al. (2005) Vision Res. 45:3445). Briefly, in one round of selection, adult rho-GFP mice were intravitreally injected with 2µL of phosphate buffered saline (PBS)-dialyzed, iodixanol-purified library with a genomic titer of approximately 1×10^{12} viral genomes (vg)/mL. An ultrafine 30 1/2-gauge disposable needle was passed through the sclera of the animal's eye, at the equator and next to the limbus, into the vitreous cavity. Injection of 2µl of virus was made with direct observation of the needle in the center of the vitreous cavity. One week post-injection, eyes were enucleated and retinas dissociated using a light, papain protease treatment, followed by fluorescence activated cell sorter (FACS) isolation of photoreceptor populations. Successful virions were then PCR amplified from subsequent genomic extractions and further cloned and repackaged for injection.

[0116] Further iterations of this selection were performed, narrowing the pool of variants to a

subset with the most permissive mutations. After three iterations, a round of error-prone PCR was performed to create a further generation of variants for selection. In total, this process was repeated for two generations. In this respect, this process of directed evolution created photoreceptor-permissive AAV variants through the application of positive selection and induced mutagenesis, similar to the process of natural evolution.

[0117] Subsequently, the cap genes of fifty variants were sequenced to determine the most prominent and successful variants to have permissive mutations for intravitreal photoreceptor transduction. Of the 50 clones, 46 gave readable sequences of a 7mer insert. Remarkably, nearly two thirds of clones contained the same distinct 7mer motif (~^{588}LGETTRP~; SEQ ID NO: 13). Interestingly, the next most prominent variant (~^{588}NETITRP~; SEQ ID NO: 14) also contained a similar flanking motif consisting of a positively-charged arginine residue in between a polar threonine and a nonpolar proline residue (TRP).

Table 1

Clone	Appr. Frequency (%)	Frequency
~^{588}LGETTRP~ (SEQ ID NO:13)	64	31
~^{588}NETITRP~ (SEQ ID NO:14)	12	5
~^{588}KAGQANN~ (SEQ ID NO:15)	6	3
~^{588}KDPKTTN~ (SEQ ID NO:16)	4	2
~^{588}KDTDTTR (SEQ ID NO:57)		2
~^{588}RAGGSVG (SEQ ID NO:58)		1
~^{588}AVDTTKF (SEQ ID NO:59)		1
~^{588}STGKVPN (SEQ ID NO:60)		1

[0118] *Table 1* Sequencing of isolated variants from directed evolution reveals a high degree of convergence in viral libraries. All variants derived from the AAV2 7mer library, with approximately 64% of variants containing the same 7mer motif (~^{588}LGETTRP~ (SEQ ID NO:13)).

[0119] Among the 7mer insert sequences, there were moderate preferences at particular positions, e.g., a positively charged amino acid at position 1; a negatively charged amino acid at position 2; an alcohol (e.g., an amino acid having an alcohol group (a free hydroxyl group), such as Thr or Ser) at position 5.

[0120] The 7mer inserts were flanked by spacers, as shown in Table 2:

Clone	Frequency
~^{588}LALGETTRPA~ (SEQ ID NO:45)	31

Clone	Frequency
~^{588}LANETITRPA~ (SEQ ID NO:46)	5
~^{588}LAKAGQANNA~ (SEQ ID NO:47)	3
~^{588}LAKDPKTTNA~ (SEQ ID NO:48)	2
~^{588}LAKDTDTTRA~ (SEQ ID NO:61)	2
~^{588}LARAGGSVGA~ (SEQ ID NO:62)	1
~^{588}LAAVDTTKFA~ (SEQ ID NO:63)	1
~^{588}LASTGKVPNA~ (SEQ ID NO:64)	1

[0121] *Figure 1.* Representative three-dimensional capsid model of AAV2 containing a random heptamer (shown in orange) following amino acid 587. This area of the AAV2 capsid likely participates in cell-surface receptor binding.

[0122] In light of the high degree of library convergence from the above-described selection, a recombinant form of AAV2 ~^{588}LGETTRP~ (SEQ ID NO:13; nick named 7M8) was cloned and packaged the vector with a scCAG-GFP transgene to visualize its transduction profile. Three weeks following intravitreal injection in adult mice, robust expression in numerous cell types, including retinal ganglion cells (RGCs) and Müller cells, was observed. Importantly, transduction of photoreceptors in retinas injected with 7M8, as seen by GFP expression in outer nuclear layer (ONL) nuclei (red arrows) and in outer segments (Figure 2, blue arrow), was observed, whereas AAV2 showed no discernable photoreceptor expression.

[0123] *Figure 2* AAV2 7M8 variant (right) demonstrates greater levels of intravitreal photoreceptor transduction relative to AAV2 (left). Confocal microscopy of transverse retinal sections three weeks after intravitreal injection of 2µL of 1×10^{12} vg/mL of AAV2 7M8 and AAV2 scCAG GFP in adult mice. Red arrows (top) denote photoreceptor nuclei and blue arrow (top) denote photoreceptor outer segments.

[0124] In light of these gains in retinal cell transduction, an attempt was made to increase specificity in expression through the use of a ssRho-eGFP transgene containing a photoreceptor-specific rhodopsin promoter to better resolve transduction efficiencies specifically in photoreceptors (Figure 3). Indeed the use of a photoreceptor specific Rho promoter limited the GFP expression to the photoreceptors. An attempt was made to improve 7M8 transduction efficiency by combining a rational design approach to the previous directed evolution approach. Therefore, four surface exposed tyrosine residues were mutagenized to phenylalanines on the 7M8 capsid (Y273F, Y444F, Y500F, and Y730F) which has previous been shown to increase photoreceptor infectivity (Petrs-Silva et al., 2009). Interestingly, the addition of mutations decreased number of photoreceptors transduced compared to the original virus as show by FACs sorting of the GFP(+) photoreceptors from 7m8 or 7m8-4YF

infected retinas (Figure 4).

[0125] *Figure 3.* Representative fluorescence images of retinal cryoslices showing GFP expression resulting from 7m8 carrying the GFP gene under the control of the ubiquitous CAG promoter (left) or a photoreceptor specific Rho promoter (right).

[0126] *Figure 4.* GFP(+) photoreceptor cells per million retinal cells as counted by flow cytometry. 7m8 transduces more than 2x the amount of photoreceptors compared 7m8 bearing 4 tyrosine mutations (top).

Example 2: Treatment of retinoschisis

[0127] Using the expression construct 7m8-rho-RS 1, a functional retinoschisin (RS1) protein was delivered to retinoschisin-deficient mice (Rs1h-deficient mice; Rs1h is the mouse homolog of human RS 1). The vector comprises a nucleotide sequence encoding a functional retinoschisin protein under transcriptional control of a rhodopsin promoter. See Figures 13A-C, where the bold and underlined nucleotide sequence (nucleotides 4013-4851) are the rhodopsin promoter; and nucleotides 4866-5540 (with the start atg and stop tga sequences shown in bold) encode a human RS 1 protein.

[0128] The 7m8-rho-RS1 construct was administered intravitreally to Rs1h-/- mice at P15. Rs1h-/- mice were generated through targeted disruption of exon 3 of the Rs1h gene, as described (Weber et al. (2002) Proc. Natl. Acad. Sci. USA 99:6222). The Rs1h-/- mice are deficient in the Rs1h protein product, have an electronegative ERG (e.g., a reduced b-wave with relative preservation of the a-wave) and splitting of the layers of the retina, similar to what is seen in human retinoschisis patients. Injection of the 7m8-rho-RS1 vector into the Rs1h-/- led to high levels of panretinal RS 1 expression from photoreceptors in the retina. RS 1 expression led to improved retinal morphology with a decrease in the number and size of cavities in the retina as seen in spectral-domain optical coherence tomography (SD-OCT) imaging (Figures 7A-I), a rescue of the ERG b-wave (Figures 8A-D), and long-term structural preservation of the retina (Figures 9A-E).

[0129] Figures 7A-I. Representative high-resolution SD-OCT images of retinas injected with 7m8-rho-GFP (left column), 7m8-rho-RS 1 (middle column), or uninjected WT animals (right column). Fundus images were taken through the inner nuclear layer of the superior retina and exclude other layers (a-c). Transverse images of the superior (d-f) and inferior (g-i) retina were taken using the optic nerve head as a landmark.

[0130] The untreated RS 1 retina increases in overall thickness when measured from the inner limiting membrane (ILM) to the photoreceptors, as the pathology progresses due to the schisis splitting the inner retina. This process is distinct from that observed in most retinal degenerative diseases (RDD) which do not form schisis, but exhibit progressive photoreceptor cell death in the INL and concomitant retinal thinning and loss of ERG amplitude. In RS 1, the

ONL thins as photoreceptors die from the disease, but this is distinct from the overall retinal thickness change. It is generally thought that a successful therapy for RS 1 would return the overall retinal thickness to the wildtype and ameliorate the photoreceptor loss in the ONL. In most RDD other than Rs1, the loss of photoreceptors, marked by ONL thinning, is paralleled by a decrease in retinal physiological output as measured by the ERG amplitude. RS 1 is one of the very few examples of a retinal disease in which the pathology increases the retinal thickness with concomitant erg amplitude loss. In summary, restoring the RS1 gene product, an extracellular retinal "glue; - thins the retina back to the wildtype thickness and the erg amplitude returns to near normal levels as the schisis resolves.

[0131] Figure 8a shows a comparison of functional rescue of untreated Rs1-/- eyes to AAV2-rho-RS1, 7m8-rho-GFP, and 7m8-rho-RS1 injected eyes both one month (left) and 4 months (right) after injection. One month post-injection, 7m8-rho-RS1 led to considerable rescue of the ERG b-wave amplitude, whereas AAV2-rho.RS 1 was statistically indistinguishable from untreated eyes.

[0132] After 4 months, the 7m8-rho-RS 1 amplitude further increases toward the wild-type amplitude (right). Figure 8b shows representative ERG traces from 7m8-rho-RS1-injected eyes show improved amplitude of the a-wave and b-wave and a waveform closer to wild-type eyes, compared to 7m8-rho-GFP-injected eyes. Figure 8c shows the amplitude of the full-field scotopic b-wave resulting from a high intensity (1 log cd × s/m2) stimulus was recorded on a monthly basis beginning one month after injection at P15 for each condition. Three responses were recorded and averaged for each eye at each time point.

[0133] Mean ERG b-wave amplitudes were plotted as a function of time post-injection. n=7 was used for both conditions. Figure 8d shows an analysis of ERG responses under scotopic (upper traces, stimulus range from -3 to 1 log cd × s/m2) and photopic (lower traces, range from -0.9 to 1.4 log cd × s/m2) conditions indicates improved rod and cone function over a range of stimuli intensities.

[0134] Figures 9A-E. Sustained improvements in retinal thickness measured at 10 months post 7m8-rho-RS1 treatment. Representative transverse SD-OCT images of a) 7m8-rho-RS1 or b) or 7m8-rho-GFP treated retinas 10 months post-injection centered on the optic nerve head. Measurements of c) retinal thickness, d) ONL thickness, and e) and inner and outer segment thickness are plotted as a function of distance from the optic nerve head.

[0135] Example 3: AAV variant used to deliver a protein to retinal cells in the macaque A recombinant AAV2 virion (7m8 carrying GFP under the control of a connexin36 promoter) was generated. The recombinant AAV2 virion included an AAV2 capsid variant with an insertion of LALGETTRPA peptide between amino acids 587 and 588 of AAV2 capsid, and GFP under transcriptional control of a connexin36 promoter, which is expressed in interneurons. The rAAV2 virion was injected intravitreally into the eye of a macaque. The data are shown in Figure 18.

[0136] Figure 18 provides a fluorescence fundus image showing GFP expression at the back of the retina 9 weeks after administration of 7m8 carrying GFP under the control of a connexin36 promoter. Compared to the parental AAV2 serotype (Yin et al, IOVS 52(5); 2775), a higher level of expression was seen in the foveal ring, and visible fluorescence was seen in the central retina outside the fovea.

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PATENTKRAV

1. rAAV-(Recombinant Adeno-associeret Virus)-virion, eller en farmaceutisk sammensætning der omfatter virionet, til anvendelse i en fremgangsmåde til behandling af en øjensygdom, hvilken sammensætning yderligere omfatter en farmaceutisk acceptabel excipiens, og hvor det rAAV-(Recombinant Adeno-associeret Virus)-virion omfatter:

a) et variant-AAV-kapsidprotein, hvilket variant-AAV-kapsidprotein omfatter en aminosyresekvens med mindst 95 % sekvensidentitet med SEQ ID NO:1 og en insertion af et peptid i kapsidproteinets GH-løkke i forhold til SEQ ID NO:1, hvilket peptid omfatter aminosyresekvensen LALGETTRPA (SEQ ID NO:45); og

b) en heterolog nukleinsyre der omfatter en nukleotidsekvens, som koder for et genprodukt,

hvor variant-AAV-kapsidproteinet bibringer øget infektivitet af en nethindecelle af rAAV-virionet sammenlignet med infektiviteten af nethindecellen af et AAV-virion, der omfatter et vildtype-kapsidprotein med aminosyresekvensen, der er angivet i SEQ ID NO:1 .

2. rAAV-virion eller farmaceutisk sammensætning til anvendelse ifølge krav 1, hvori øjensygdommen er diabetisk makulært ødem, exudativ-aldersrelateret makuladegeneration eller diabetisk retinopati.

3. rAAV-virion eller farmaceutisk sammensætning til anvendelse ifølge krav 1, hvor genproduktet er et anti-angiogent polypeptid.

4. rAAV-virion eller farmaceutisk sammensætning til anvendelse ifølge et hvilket som helst af kravene 1-3, hvor insertionsstedet er inden for aminosyrerne 570-611 i forhold til SEQ ID NO:1.

5. rAAV-virion eller farmaceutisk sammensætning til anvendelse ifølge et hvilket som helst af kravene 1-3, hvor insertionsstedet er mellem aminosyrerne 587 og 588 i forhold til SEQ ID NO:1.

6. rAAV-virion eller farmaceutisk sammensætning til anvendelse ifølge et hvilket som helst af kravene 1-5, hvor variant-AAV-kapsidproteinet omfatter en aminosyresekvens med mindst 99 % sekvensidentitet med SEQ ID NO:1.

7. rAAV-virion eller farmaceutisk sammensætning til anvendelse ifølge krav 3 eller et hvilket som helst krav afhængigt af krav 3, hvor det anti-angiogene polypeptid er en opløselig VEGF-(Vascular Endothelial Growth Factor)-receptor.

8. rAAV-virion eller farmaceutisk sammensætning til anvendelse ifølge krav 3 eller et hvilket som helst krav afhængigt af krav 3, hvor det anti-angiogene polypeptid er et VEGF-bindende antistof.

9. rAAV-virion eller farmaceutisk sammensætning til anvendelse ifølge krav 3 eller et hvilket som helst krav afhængigt af krav 3, hvor det anti-angiogene polypeptid er et Fc-fusionspolypeptid, der omfatter et opløseligt Flt-polypeptid.

10. rAAV-virion eller farmaceutisk sammensætning til anvendelse ifølge et hvilket som helst af kravene 1-9, hvor 10^6 til 10^{15} rAAV-virioner administreres.

11. rAAV-virion eller farmaceutisk sammensætning til anvendelse ifølge et hvilket som helst af kravene 1-9, hvor 10^8 til 10^{12} rAAV-virioner administreres.

12. rAAV-virion eller farmaceutisk sammensætning til anvendelse ifølge et hvilket som helst af kravene 1-9, hvor fremgangsmåden omfatter intravitreal injektion.

13. rAAV-(Recombinant Adeno-associated Virus)-Virion der omfatter:

a) et variant-AAV-kapsidprotein, hvilket variant-AAV-kapsidprotein omfatter en aminosyresekvens med mindst 95 % sekvensidentitet med SEQ ID NO:1 og en insertion af et peptid i GH-løkken af kapsidproteinet i forhold til SEQ ID NO:1, hvilket peptid omfatter aminosyresekvensen LALGETTRPA (SEQ ID NO:45); og

b) en heterolog nukleinsyre der omfatter en nukleotidsekvens, som koder for et genprodukt,

hvilket variant-AAV-kapsidprotein bibringer øget infektivitet af en nethindecelle af rAAV-virionet sammenlignet med infektiviteten af nethindecellen af et AAV-virion, der omfatter et vildtype-kapsidprotein med aminosyresekvensen, som er angivet i SEQ ID NO:1 .

14. rAAV ifølge krav 13, hvor genproduktet er et anti-angiogent polypeptid.

15. rAAV ifølge krav 13, hvor insertionsstedet er inden for 570-611 i forhold til SEQ ID NO:1 eller mellem aminosyrerne 587 og 588 i forhold til SEQ ID NO:1.

DRAWINGS

Drawing

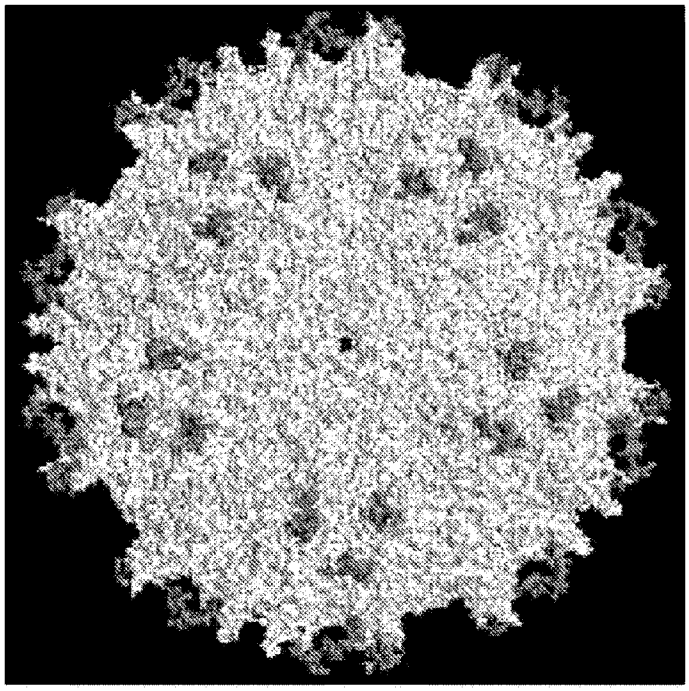
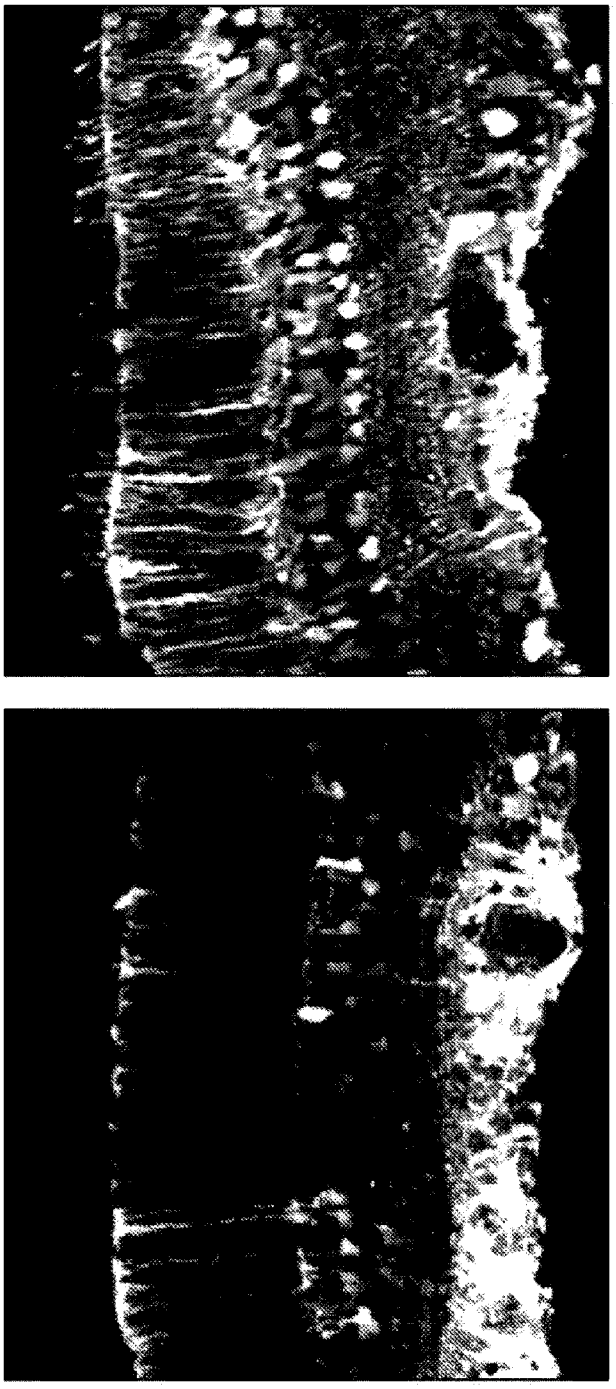


FIG. 1



7m8.CAG.GFP
(variant enhanced for PR transduction by directed evolution)

AAV2.CAG.GFP
(parental serotype)

FIG. 2

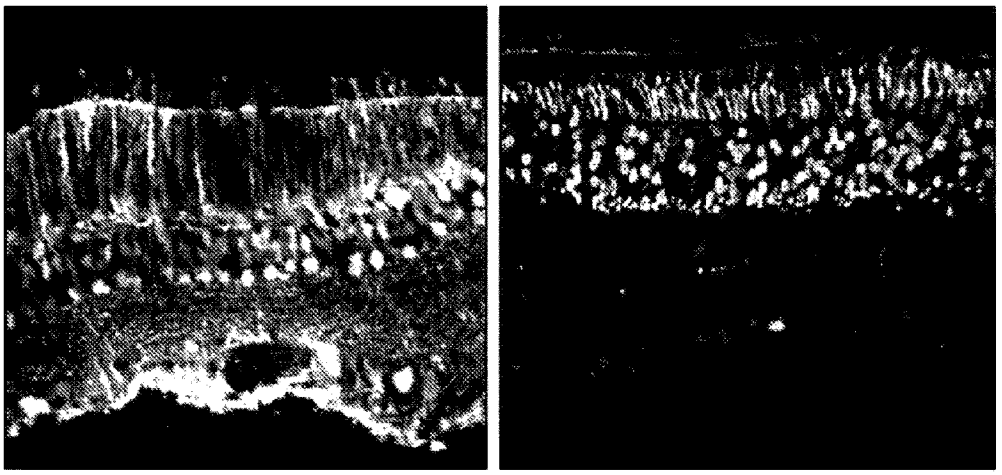

7m8.CAG.GFP
7m8.Rho.GFP

FIG. 3

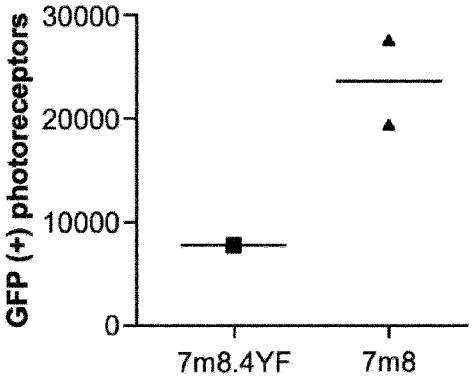

GFP (+) photoreceptors
30000
20000
10000
0
7m8.4YF
7m8

FIG. 4

AAV2 VP1 1 MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPPKPAERHKDDSRGLVLPGYKYLGPFNGLD

AAV2 VP1 61 KGEPVNEADAAALEHDKAYDRQLDSGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQ

AAV2 VP1 121 AKKRVLEPLGLVEEPVKTAPGKKRPVEHSPVEPDSSSGTGKAGQQPARKRLNFGQTGDAD

AAV2 VP1 181 SVPDPQPLGQPPAAPSGLGTNTMATGSGAPMADNNEGADGVGNSSGNWHCDSTWMGDRVI

AAV2 VP1 241 TTSTRTWALPTYNNHLYKQISSQSGASNDNHYFGYSTPWGYFDFNRFHCHFSPRDWQRLI

AAV2 VP1 301 NNNWGFRPKRLNFKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTDSEYQLPYVLGSAHQG

AAV2 VP1 361 CLPPFPADVFMVPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFTFSYTFEDVPF

AAV2 VP1 421 HSSYAHSQSLDRLMNPLIDQYLYYLSRTNTPSGTTTQSRLQFSQAGASDIRDQSRNWLPG

AAV2 VP1 481 PCYRQQRVSKTSADNNNSEYSWTGATKYHLNGRDSLVNPGPAMASHKDDEEKFFPQSGVL

AAV2 VP1 541 IFGKQGSEKTNVDIEKVMITDEEEIRTTNPVATEQYGSVSTNLQRG**NR**QAATADVNTQGV

AAV2 VP1 601 LPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKHPPPQILIKNTPVPANPSTT

AAV2 VP1 661 FSAAKFASFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNYNKSVNVDFTVDTNGVY

AAV2 VP1 721 SEPRPIGTRYLTR (SEQ ID NO:1)

FIG. 5

AAV-2	570	PVATEQYGSVSTNLQRG**NR**QAATADVNTQGVLPGMVWQDRDV	611	(SEQ ID NO:2)
AAV-1	571	PVATERFGTVAVNFQSSST**DP**ATGDVHAMGALPGMVWQDRDV	612	(SEQ ID NO:3)
AAV-5	560	RVAYNVGGQMATNNQ**SS**TTAPATGTYNLQEIVPGSVWMERDV	601	(SEQ ID NO:4)
AAV-6	571	PVATERFGTVAVNLQSSST**DP**ATGDVHVMGALPGMVWQDRDV	612	(SEQ ID NO:5)
AAV-7	572	PVATEEYGIVSSNLQAA**NT**AAQTQVVNNQGALPGMVWQNRDV	613	(SEQ ID NO:6)
AAV-8	573	PVATEEYGIVADNLQQQ**NT**APQIGTVNSQGALPGMVWQNRDV	614	(SEQ ID NO:7)
AAV-9	571	PVATESYGQVATNHQSA**QA**QAQTGWVQNQGILPGMVWQDRDV	612	(SEQ ID NO:8)
AAV-10	573	PVATEQYGVVADNLQ**QA**NTGPIVGNVNSQGALPGMVWQNRDV	614	(SEQ ID NO:9)

FIG. 6

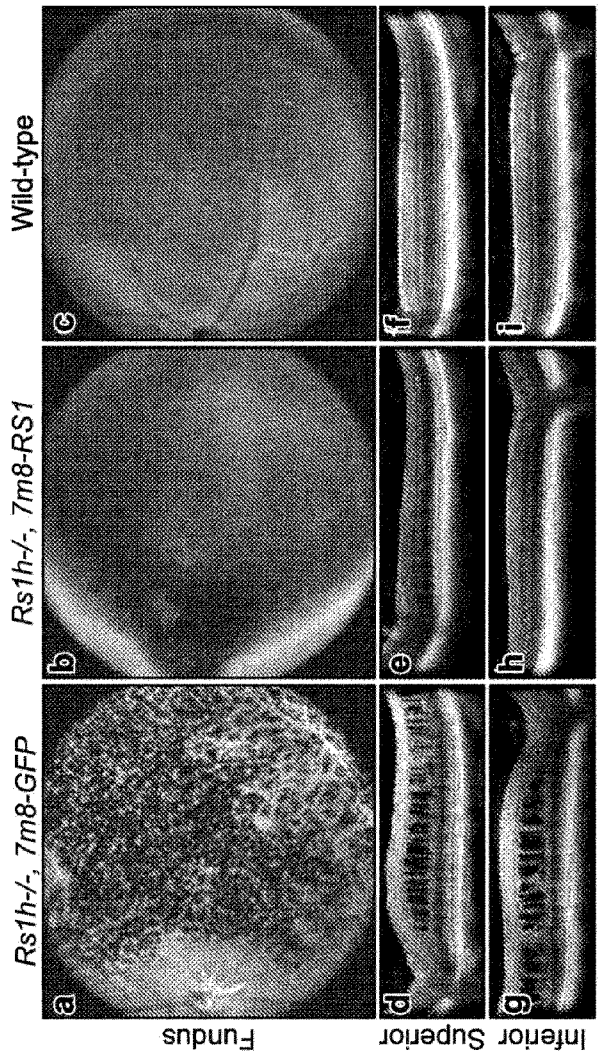

Rs1h-/-, 7m8-GFP
Rs1h-/-, 7m8-RS1
Wild-type
a
b
c
d
e
f
g
h
i
Fundus
Superior
Inferior

FIG. 7

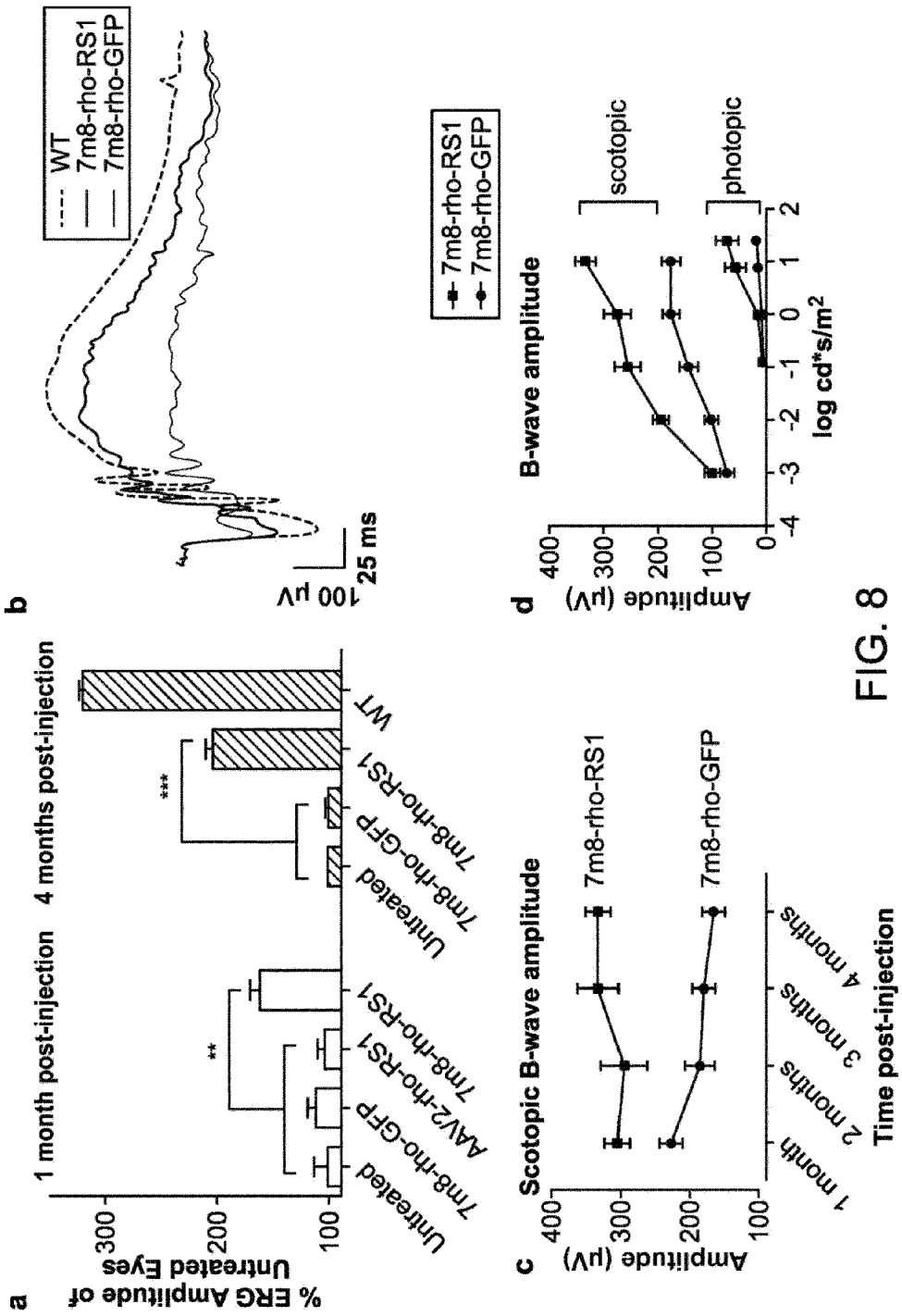

a
% ERG Amplitude of Untreated Eyes
1 month post-injection
4 months post-injection
Untreated
7m8-rho-GFP
AAV2-rho-RS1
7m8-rho-RS1
Untreated
7m8-rho-GFP
7m8-rho-RS1
WT
**
***
b
WT
7m8-rho-RS1
7m8-rho-GFP
100 µV
25 ms
c
Scotopic B-wave amplitude
Amplitude (µV)
7m8-rho-RS1
7m8-rho-GFP
1 month
2 months
3 months
4 months
Time post-injection
d
7m8-rho-RS1
7m8-rho-GFP
B-wave amplitude
scotopic
photopic
Amplitude (µV)
log cd*s/m2

FIG. 8

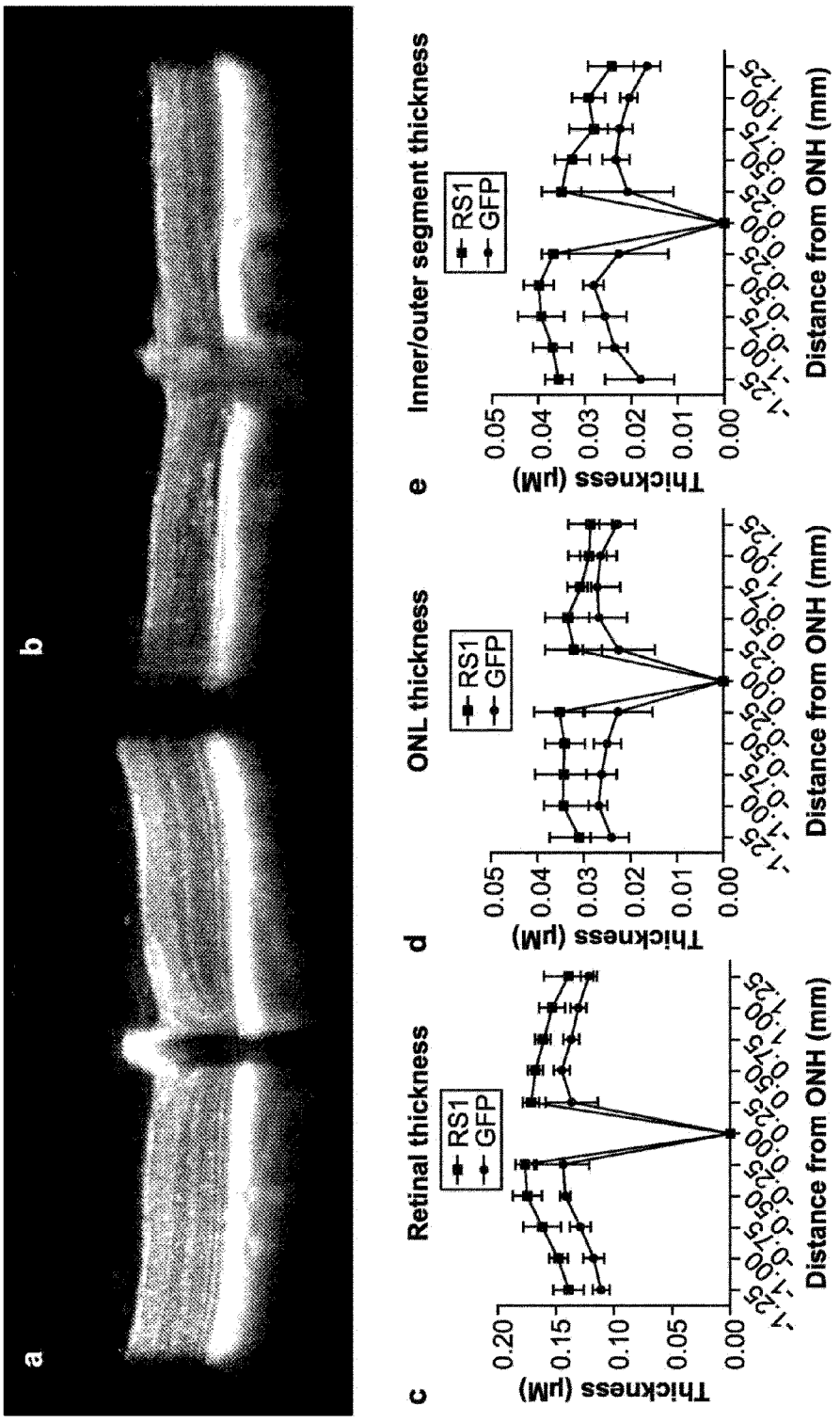

a
b
c
Retinal thickness
RS1
GFP
Thickness (μM)
0.20
0.15
0.10
0.05
0.00
Distance from ONH (mm)
d
ONL thickness
RS1
GFP
Thickness (μM)
0.05
0.04
0.03
0.02
0.01
0.00
Distance from ONH (mm)
e
Inner/outer segment thickness
RS1
GFP
Thickness (μM)
0.05
0.04
0.03
0.02
0.01
0.00
Distance from ONH (mm)

FIG. 9

Retinoschisin-1

Homo sapiens

GenBank CAI42483

```
  1 msrkiegfll lllfgyeatl glsstedege dpwyqkackc dcqggpnalw sagatsldci
 61 pecpyhkplg fesgevtpdq itcsnpeqyv gwysswtank arlnsqgfgc awlskfqdss
121 qwlqidlkei kvisgiltqg rcdidewmtk ysvqyrtder lnwiyykdqt gnnrvfygns
181 drtstvqnll rppiisrfir liplgwhvri airmellecv skca (SEQ ID NO:10)
```

FIG. 10

Brain-derived neurotrophic factor

Homo sapiens

GenBank CAA62632

```
  1 mtilfltmvi syfgcmkaap mkeanirgqg glaypgvrth gtlesvngpk agsrgltsla
 61 dtfehvieel ldedhkvrpn eennkdadly tsrvmlssqv pleppllfll eeyknyldaa
121 nmsmmvlrhs dparrgelsv cdsisewvta adkktavdms ggtvtvlekv pvskgqlkqy
181 fyetkcnpmg ytkegcrgid krhwnsqcrt tqsyvraltm dskkrigwrf iridtscvct
241 ltikrgr (SEQ ID NO:11)
```

FIG. 11

RPE65

Homo sapiens

GenBank AAC39660

```
  1 msiqvehpag gykklfetve elsspltahv tgriplwltg sllrcgpglf evgsepfyhl
 61 fdgqallhkf dfkeghvtyh rrfirtdayv ramtekrivi tefgtcafpd pcknifsrff
121 syfrgvevtd nalvnvypvg edyyactetn fitkinpetl etikqvdlcn yvsvngatah
181 phiendgtvy nigncfgknf siaynivkip plqadkedpi skseivvqfp csdrfkpsyv
241 hsfgltpnyi vfvetpvkin lfkflsswsl wganymdcfe snetmgvwlh iadkkrkkyl
301 nnkyrtspfn lfhhintyed ngflivdlcc wkgfefvyny lylanlrenw eevkknarka
361 pqpevrryvl plnidkadtg knlvtlpntt atailcsdet iwlepevlfs gprqafefpq
421 inyqkycgkp ytyayglgln hfvpdrlckl nvktketwvw qepdsypsep ifvshpdale
481 eddgvvlsvv vspgagqkpa yllilnakdl sevaraevei nipvtfhglf kks (SEQ ID NO:12)
```

FIG. 12

```
   1 agcttggatc caatcaacct ctggattaca aaatttgtga aagattgact ggtattctta
  61 actatgttgc tccttttacg ctatgtggat acgctgcttt aatgcctttg tatcatgcta
 121 ttgcttcccg tatggctttc atttctcctt cctgtataaa atcctggttg ctgtctcttt
 181 atgaggagtt gtggcccgtt gtcaggcaac gtggcgtggt gtgcactgtg tttgctgacg
 241 caacccccac tggttggggc attgccacca cctgtcagct cctttccggg actttcgctt
 301 tcccctcccc tattgccacg gcggaactca tcgccgcctg ccttgcccgc tgctggacag
 361 gggctcggct gttgggcact gacaattccg tggtgttgtc ggggaagctg acgtcctttc
 421 catggctgct cgcctgtgtt gccacctgga ttctgcgcgg gacgtccttc tgctacgtcc
 481 cttcggccct caatccagcg gaccttcctt cccgcggcct gctgccggct ctgcggcctc
 541 ttccgcgtct tcgagatctg cctcgactgt gccttctagt tgccagccat ctgttgttig
 601 cccctccccc gtgccttcct tgaccctgga aggtgccact cccactgtcc tttcctaata
 661 aaatgaggaa attgcatcgc attgtctgag taggtgtcat tctattctgg ggggtggggt
 721 ggggcaggac agcaaggggg aggattggga agacaatagc aggcatgctg gggactcgag
 781 ttaagggcga attcccgatt aggatcttcc tagagcatgg ctacgtagat aagtagcatg
 841 gcgggttaat cattaactac aaggaacccc tagtgatgga gttggccact ccctctctgc
 901 gcgctcgctc gctcactgag gccgggcgac caaaggtcgc ccgacgcccg ggctttgccc
 961 gggcggcctc agtgagcgag cgagcgcgca gccttaatta acctaattca ctggccgtcg
1021 tttacaacg tcgtgactgg gaaaaccctg gcgttaccca acttaatcgc cttgcagcac
1081 atccccctt cgccagctgg cgtaatagcg aagaggcccg caccgatcgc ccttcccaac
1141 agttgcgcag cctgaatggc gaatgggacg cgccctgtag cggcgcatta agcgcggcgg
1201 gtgtggtggt tacgcgcagc gtgaccgcta cacttgccag cgccctagcg cccgctcctt
1261 tcgctttctt ccttcctttt ctcgccacgt tcgccggctt tccccgtcaa gctctaaatc
1321 ggggctccc tttagggttc cgatttagtg ctttacggca cctcgacccc aaaaaacttg
1381 attagggtga tggttcacgt agtgggccat cgccccgata gacggtttt cgccctttga
1441 cgctggagtt cacgttcctc aatagtggac tcttgttcca aactggaaca acactcaacc
1501 ctatctcggt ctattctttt gatttataag ggatttttcc gatttcggcc tattggttaa
1561 aaaatgagct gatttaacaa aaatttaacg cgaatttaa caaaatatta acgtttataa
1621 ttcaggtgg catcttcgg ggaaatgtgc gcggaacccc tatttgttta ttttctaaa
1681 tacattcaaa tatgtatccg ctcatgagac aataaccctg ataaatgctt caataatatt
1741 gaaaaaggaa gagtatgagt attcaacatt tccgtgtcgc cttattccc ttttttgcgg
1801 catttgcct tcctgttttt gctcacccag aaacgctggt gaaagtaaaa gatgctgaag
```

FIG. 13A

```
1861 atcagttggg tgcacgagtg ggttacatcg aactggatct caatagtggt aagatccttg
1921 agagttttcg ccccgaagaa cgttttccaa tgatgagcac ttttaaagtt ctgctatgtg
1981 gcgcggtatt atcccgtatt gacgccgggc aagagcaact cggtcgccgc atacactatt
2041 ctcagaatga cttggttgag tactcaccag tcacagaaaa gcatcttacg gatggcatga
2101 cagtaagaga attatgcagt gctgccataa ccatgagtga taacactgcg gccaacttac
2161 ttctgacaac gatcggagga ccgaaggagc taaccgcttt tttgcacaac atgggggatc
2221 atgtaactcg ccttgatcgt tgggaaccgg agctgaatga agccatacca aacgacgagc
2281 gtgacaccac gatgcctgta gtaatggtaa caacgttgcg caaactatta actggcgaac
2341 tacttactct agcttcccgg caacaattaa tagactggat ggaggcggat aaagttgcag
2401 gaccacttct gcgctcggcc cttccggctg gctggtttat tgctgataaa tctggagccg
2461 gtgagcgtgg gtctcgcggt atcattgcag cactggggcc agatggtaag ccctcccgta
2521 tcgtagttat ctacacgacg gggagtcagg caactatgga tgaacgaaat agacagatcg
2581 ctgagatagg tgcctcactg attaagcatt ggtaactgtc agaccaagtt tactcatata
2641 tactttagat tgatttaaaa cttcattttt aatttaaaag gatctaggtg aagatccttt
2701 ttgataatct catgaccaaa atcccttaac gtgagtttc gttccactga gcgtcagacc
2761 ccgtagaaaa gatcaaagga tcttcttgag atccttttt tctgcgcgta atctgctgct
2821 tgcaaacaaa aaaaccaccg ctaccagcgg tggtttgttt gccggatcaa gagctaccaa
2881 ctcttttcc gaaggtaact ggcttcagca gagcgcagat accaaatact gtccttctag
2941 tgtagccgta gttaggccac cacttcaaga actctgtagc accgcctaca tacctcgctc
3001 tgctaatcct gttaccagtg gctgctgcca gtggcgataa gtcgtgtctt accgggttgg
3061 actcaagacg atagttaccg gataaggcgc agcggtcggg ctgaacgggg ggttcgtgca
3121 cacagcccag cttggagcga acgacctaca ccgaactgag atacctacag cgtgagctat
3181 gagaaagcgc cacgcttccc gaagggagaa aggcggacag gtatccggta agcggcaggg
3241 tcggaacagg agagcgcacg agggagcttc cagggggaaa cgcctggtat ctttatagtc
3301 ctgtcgggtt tcgccacctc tgacttgagc gtcgattttt gtgatgctcg tcaggggggc
3361 ggagcctatg gaaaaacgcc agcaacgcgg cctttttacg gttcctggcc ttttgctgcg
3421 gttttgctca catgttcttt cctgcgttat cccctgattc tgtggataac cgtattaccg
3481 cctttgagtg agctgatacc gctcgccgca gccgaacgac cgagcgcagc gagtcagtga
3541 gcgaggaagc ggaagagcgc ccaatacgca aaccgcctct ccccgcgcgt tggccgattc
3601 attaatgcag ctggcacgac aggtttcccg actggaaagc gggcagtgag cgcaacgcaa
3661 ttaatgtgag ttagctcact cattaggcac cccaggcttt acactttatg cttccggctc
```

FIG. 13B

3721 gtatgttgtg tggaattgtg agcggataac aatttcacac aggaaacagc tatgaccatg
3781 attacgccag atttaattaa ggctgcgcgc tcgctcgctc actgaggccg cccgggcaaa
3841 gcccgggcgt cgggcgacct ttggtcgccc ggcctcagtg agcgagcgag cgcgcagaga
3901 gggagtggcc aactccatca ctaggggttc cttgtagtta atgattaacc cgccatgcta
3961 cttatctacg tagccatgct ctaggaagat cggaattcgc ccttaagcta gc**agatcttc**
4021 **cccacctagc cacctggcaa actgctcctt ctctcaaagg cccaaacatg gcctcccaga**
4081 **ctgcaacccc caggcagtca ggccctgtct ccacaacctc acagccaccc tggacggaat**
4141 **ctgcttcttc ccacatttga gtcctcctca gcccctgagc tcctctgggc agggctgttt**
4201 **ctttccatct ttgtattccc aggggcctgc aaataaatgt ttaatgaacg aacaagagag**
4261 **tgaattccaa ttccatgcaa caaggattgg gctcctgggc cctaggctat gtgtctggca**
4321 **ccagaaacgg aagctgcagg ttgcagcccc tgccctcatg gagctcctcc tgtcagagga**
4381 **gtgtggggac tggatgactc cagaggtaac ttgtgggggа acgaacaggt aaggggctgt**
4441 **gtgacgagat gagagactgg gagaataaac cagaaagtct ctagctgtcc agaggacata**
4501 **gcacagaggc ccatggtccc tatttcaaac ccaggccacc agactgagct gggaccttgg**
4561 **gacagacaag tcatgcagaa gttaggggac cttctcctcc cttttcctgg atggatcctg**
4621 **agtaccttct cctccctgac ctcaggcttc ctcctagtgt caccttggcc cctcttagaa**
4681 **gccaattagg ccctcagttt ctgcagcggg gattaatatg attatgaaca cccccaatct**
4741 **cccagatgct gattcagcca ggagcttagg agggggaggt cactttataa gggtctgggg**
4801 **gggtcagaac ccagagtcat cccctgaatt ctgcagatat ccatcacact g**gcggccgcg
4861 ccacc**atg**tc acgcaagata gaaggctttt tgttattact tctctttggc tatgaagcca
4921 cattgggatt atcgtctacc gaggatgaag gcgaggaccc ctggtaccaa aaagcatgca
4981 agtgcgattg ccaaggagga cccaatgctc tgtggtctgc aggtgccacc tccttggact
5041 gtataccaga atgcccatat cacaagcctc tgggtttcga gtcaggggag gtcacaccgg
5101 accagatcac ctgctctaac ccggagcagt atgtgggctg gtattcttcg tggactgcaa
5161 acaaggcccg gctcaacagt caaggctttg ggtgtgcctg gctctccaag ttccaggaca
5221 gtagccagtg gttacagata gatctgaagg agatcaaagt gatttcaggg atcctcaccc
5281 aggggcgctg tgacatcgat gagtggatga ccaagtacag cgtgcagtac aggaccgatg
5341 agcgcctgaa ctggatttac tacaaggacc agactggaaa caaccgggtc ttctatggca
5401 actcggaccg cacctccacg gttcagaacc tgctgcggcc ccccatcatc tcccgcttca
5461 tccgcctcat cccgctgggc tggcacgtcc gcattgccat ccggatggag ctgctggagt
5521 gcgtcagcaa gtgtgcc**tga** a (SEQ ID NO:18)

FIG. 13C

Peripherin-2

```
  1 mallkvkfdq kkrvklaqgl wlmnwfsvla giiifslglf lkielrkrsd vmnnseshfv
 61 pnsligmgvl scvfnslagk icydaldpak yarwkpwlkp ylaicvlfni ilflvalccf
121 llrgslentl gqglkngmky yrdtdtpgrc fmkktidmlq iefkccgnng frdwfeiqwi
181 snryldfssk evkdriksnv dgrylvdgvp fsccnpsspr pciqyqitnn sahysydhqt
241 eelnlwvrgc raallsyyss lmnsmgvvtl liwlfevtit iglrylqtsl dgvsnpeese
301 sesqgwller svpetwkafl esvkklgkgn qveaegadag qapeag (SEQ ID NO:19)
```

FIG. 14

Peripherin

```
  1 mshhpsglra gfsstsyrrt fgpppslspg afsysssrf sssrllgsas psssvrlgsf
 61 rspragagal lrlpserldf smaealnqef latrsnekqe lqelndrfan fiekvrfleq
121 qnaalrgels qargqepara dqlcqqelre lrrelellgr erdrvqverd glaedlaalk
181 qrleeetrkr edaehnlvlf rkdvddatls rlelerkies lmdeieflkk lheeelrdlq
241 vsvesqqvqq veveatvkpe ltaalrdira qyesiaaknl qeaeewyksk yadlsdaanr
301 nhealrqakq emnesrrqiq sltcevdglr gtneallrql releeqfale aggyqagaar
361 leeelrqlke emarhlreyq ellnvkmald ieiatyrkll egeesrisvp vhsfaslnik
421 ttvpeveppq dshsrktvli ktietrngev vtesqkeqrs eldkssahsy (SEQ ID NO:20)
```

FIG. 15

DK/EP 4005603 T3

RPGR-interacting protein-1

```
   1 mshlvdptsg dlpvrdidai plvlpaskgk nmktqpplsr mnreeledsf frlredhmlv
  61 kelswkqqde ikrlrttllr ltaagrdlrv aeeaaplset arrgqkagwr qrlsmhqrpq
 121 mhrlqghfhc vgpasprraq prvqvghrql htagapvpek pkrgprdrls ytappsfkeh
 181 atnenrgeva skpselvsgs nsiisfssvi smakpiglcm pnsahimasn tmqveeppks
 241 pekmwpkden feqrssleca qkaaelrasi kekvelirlk kllhernasl vmtkaqltev
 301 qeayetllqk nqgilsaahe allkqvnelr aelkeeskka vslksqledv silqmtlkef
 361 qervedleke rkllndnydk llesmldssd sssqphwsne liaeqlqqqv sqlqdqldae
 421 ledkrkvlle lsrekaqned lklevtnilq khkqevellq naatisqppd rqsepathpa
 481 vlqentqiep sepknqeekk lsqvlnelqv shaettlele ktrdmlilqr kinvcyqeel
 541 eammtkadnd nrdhkekler ltrlldlknn rikqlegilr shdlptseql kdvaygtrpl
 601 slcletlpah gdedkvdisl lhqgenlfel hihqafltsa alaqagdtqp ttfctysfyd
 661 fethctplsv gpqplydfts qyvmetdslf lhylqeasar ldihqamase hstlaagwic
 721 fdrvletvek vhglatliga ggeefgvley wmrlrfpikp slqacnkrkk aqvylstdvl
 781 ggrkaqeeef rseswepqne lwieitkccg lrsrwlgtqp spyavyrfft fsdhdtaiip
 841 asnnpyfrdq arfpvlvtsd ldhylrreal sihvfddedl epgsylgrar vpllplakne
 901 sikgdfnltd paekpngsiq vqldwkfpyi ppesflkpea qtkgkdtkds skisseeeka
 961 sfpsqdqmas pevpieagqy rskrkpphgg erkekehqvv sysrrkhgkr igvqgknrme
1021 ylslnilngn tpeqvnytew kfsetnsfig dgfknqheee emtlshsalk qkeplhpvnd
1081 kesseqgsev seaqttdsdd vivppmsqky pkadsekmci eivslafype aevmsdenik
1141 qvyveykfyd lplsetetpv slrkpragee ihfhfskvid ldpqeqqgrr rflfdmlngq
1201 dpdqghlkft vvsdpldeek keceevgyay lqlwqilesg rdileqeldi vspedlatpi
1261 grlkvslqaa avlhaiykem tedlfs (SEQ ID NO:21)
```

FIG. 16

```
AAV1          --TFSYTFEEVPFHSSYAHSQSLDRLMNPLIDQYLYYLNRTQ-NQSGSAQNKDLLFSRGS 467
AAV6          --TFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLNRTQ-NQSGSAQNKDLLFSRGS 467
AAV3          ---FSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLNRTQGTTSGTTNQSRLLFSQAG 467
AAV2          ---FSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTN-TPSGTTTQSRLQFSQAG 466
AAV8          NFQFTYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQTT-GGTANTQTLGFSQGG 469
AAV8.1        NFQFTYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQTT-GGTANTQTLGFSQGG 469
AAV8 rh8       FQFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLVRTQTTGTGGTQTLAFSQAGPS 469
AAV10         NFEFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQST-GGTQGTQQLLFSQAG 469
AAV7          -FEFSYSFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLARTQSNPGGTAGNRELQFYQGG 469
AAV9          -FQFSYEFENVPFHSSYAHSQSLDRLMNPLIDQYLYYLSKTI--NGSGQNQQTLKFSVAG 467
AAV9.1        -FQFSYEFENVPFHSSYAHSQSLDRLMNPLIDQYLYYLSKTI--NGSGQNQQTLKFSVAG 467
AAV5          NFEFTYNFEEVPFHSSFAPSQNLFKLANPLVDQYLYRFVSTN-------NTGGVQFNKNL 453
                 *:* **:******:* **.* :* ***:***** :  *           : *

AAV1          PAGMSVQPKNWLPGPCYRQQRVSKTKTDNNNSNFTWTGASKYNLNGRESIINPGTAMASH 527
AAV6          PAGMSVQPKNWLPGPCYRQQRVSKTKTDNNNSNFTWTGASKYNLNGRESIINPGTAMASH 527
AAV3          PQSMSLQARNWLPGPCYRQQRLSKTANDNNNSNFPWTAASKYHLNGRDSLVNPGPAMASH 527
AAV2          ASDIRDQSRNWLPGPCYRQQRVSKTSADNNNSEYSWTGATKYHLNGRDSLVNPGPAMASH 526
AAV8          PNTMANQAKNWLPGPCYRQQRVSTTTGQNNNSNFAWTAGTKYHLNGRNSLANPGIAMATH 529
AAV8.1        PNTMANQAKNWLPGPCYRQQRVSTTTGQNNNSNFAWTAGTKYHLNGRNSLANPGIAMATH 529
AAV8 rh8      S--MANQARNWVPGPCYRQQRVSTTTNQNNNSNFAWTGAAKFKLNGRDSLMNPGVAMASH 527
AAV10         PANMSAQAKNWLPGPCYRQQRVSTTLSQNNNSNFAWTGATKYHLNGRDSLVNPGVAMATH 529
AAV7          PSTMAEQAKNWLPGPCFRQQRVSKTLDQNNNSNFAWTGATKYHLNGRNSLVNPGVAMATH 529
AAV9          PSNMAVQGRNYIPGPSYRQQRVSTTVTQNNNSEFAWPGASSWALNGRNSLMNPGPAMASH 527
AAV9.1        PSNMAVQGRNYIPGPSYRQQRVSTTVTQNNNSEFAWPGASSWALNGRNSLMNPGPAMASH 527
AAV5          AGRYANTYKNWFPGPMGRTQGWNLGSGVNRASVSAFATTNRMELEGASYQVPPQPNGMTN 513
              .      :*::***  * *  .    *. *  .:.  .  *:* .   *    ::
```

FIG. 17A

FIG. 17B

```
AAV1          PQILIK- 650 (SEQ ID NO:22)
AAV6          PQILIK- 650 (SEQ ID NO:23)
AAV3          PQIMIK- 650 (SEQ ID NO:24)
AAV2          PQILIKN 650 (SEQ ID NO:25)
AAV8          PQILIKN 653 (SEQ ID NO:26)
AAV8.1        PQILIKN 653 (SEQ ID NO:27)
AAV8 rh8      PQILIKN 651 (SEQ ID NO:28)
AAV10         PQILIKN 653 (SEQ ID NO:29)
AAV7          PQILIKN 652 (SEQ ID NO:30)
AAV9          PQILIK- 650 (SEQ ID NO:31)
AAV9.1        PQILIK- 650 (SEQ ID NO:32)
AAV5          PMMLIKN 640 (SEQ ID NO:33)
              * ::**
```

FIG. 17C

```
AAV1          --TFSYTFEEVPFHSSYAHSQSLDRLMNPLIDQYLYYLNRTQ-NQSGSAQNKDLLFSRGS 467
AAV6          --TFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLNRTQ-NQSGSAQNKDLLFSRGS 467
AAV3          ---FSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLNRTQGTTSGTTNQSRLLFSQAG 467
AAV2          ---FSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTN-TPSGTTTQSRLQFSQAG 466
AAV8          NFQFTYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQTT-GGTANTQTLGFSQGG 469
AAV8.1        NFQFTYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQTT-GGTANTQTLGFSQGG 469
AAV8 rh8       FQFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLVRTQTTGTGGTQTLAFSQAGPS 469
AAV10         NFEFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQST-GGTQGTQQLLFSQAG 469
AAV7          -FEFSYSFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLARTQSNPGGTAGNRELQFYQGG 469
AAV9          -FQFSYEFENVPFHSSYAHSQSLDRLMNPLIDQYLYYLSKTI--NGSGQNQQTLKFSVAG 467
AAV9.1        -FQFSYEFENVPFHSSYAHSQSLDRLMNPLIDQYLYYLSKTI--NGSGQNQQTLKFSVAG 467
AAV5          NFEFTYNFEEVPFHSSFAPSQNLFKLANPLVDQYLYRFVSTN-------NTGGVQFNKNL 453

AAV1          PAGMSVQPKNWLPGPCYRQQRVSKTKTDNNNSNFTWTGASKYNLNGRESIINPGTAMASH 527
AAV6          PAGMSVQPKNWLPGPCYRQQRVSKTKTDNNNSNFTWTGASKYNLNGRESIINPGTAMASH 527
AAV3          PQSMSLQARNWLPGPCYRQQRLSKTANDNNNSNFPWTAASKYHLNGRDSLVNPGPAMASH 527
AAV2          ASDIRDQSRNWLPGPCYRQQRVSKTSADNNNSEYSWTGATKYHLNGRDSLVNPGPAMASH 526
AAV8          PNTMANQAKNWLPGPCYRQQRVSTTTGQNNNSNFAWTAGTKYHLNGRNSLANPGIAMATH 529
AAV8.1        PNTMANQAKNWLPGPCYRQQRVSTTTGQNNNSNFAWTAGTKYHLNGRNSLANPGIAMATH 529
AAV8 rh8      S--MANQARNWVPGPCYRQQRVSTTTNQNNNSNFAWTGAAKFKLNGRDSLMNPGVAMASH 527
AAV10         PANMSAQAKNWLPGPCYRQQRVSTTLSQNNNSNFAWTGATKYHLNGRDSLVNPGVAMATH 529
AAV7          PSTMAEQAKNWLPGPCFRQQRVSKTLDQNNNSNFAWTGATKYHLNGRNSLVNPGVAMATH 529
AAV9          PSNMAVQGRNYIPGPSYRQQRVSTTVTQNNNSEFAWPGASSWALNGRNSLMNPGPAMASH 527
AAV9.1        PSNMAVQGRNYIPGPSYRQQRVSTTVTQNNNSEFAWPGASSWALNGRNSLMNPGPAMASH 527
AAV5          AGRYANTYKNWFPGPMGRTQGWNLGSGVNRASVSAFATTNRMELEGASYQVPPQPNGMTN 513
```

FIG. 18A

```
AAV1          KDDEDKFFPMSGVMIFGK--ESAGASNTALD-NVMITDEEEIKATNPVATERFGTVAVNF 584
AAV6          KDDKDKFFPMSGVMIFGK--ESAGASNTALD-NVMITDEEEIKATNPVATERFGTVAVNL 584
AAV3          KDDEEKFFPMHGNLIFGK--EGTTASNAELD-NVMITDEEEIRTTNPVATEQYGTVANNL 584
AAV2          KDDEEKFFPQSGVLIFGK--QGSEKTNVDIE-KVMITDEEEIRTTNPVATEQYGSVSTNL 583
AAV8          KDDEERFFPSNGILIFGK--QNAARDNADYS-DVMLTSEEEIKTTNPVATEEYGIVADNL 586
AAV8.1        KDDEERFFPSNGILIFGK--QNAARDNADYS-DVMLTSEEEIKTTNPVATEEYGIVADNL 586
AAV8 rh8      KDDDDRFFPSSGVLIFGK--QGAGNDGVDYS-QVLITDEEEIKATNPVATEEYGAVAINN 584
AAV10         KDDEERFFPSSGVLMFGK--QGAGRDNVDYS-SVMLTSEEEIKTTNPVATEQYGVVADNL 586
AAV7          KDDEDRFFPSSGVLIFGK--TGAT-NKTTLE-NVLMTNEEEIRPTNPVATEEYGIVSSNL 585
AAV9          KEGEDRFFPLSGSLIFGK--QGTGRDNVDAD-KVMITNEEEIKTTNPVATESYGQVATNH 584
AAV9.1        KEGEDRFFPLSGSLIFGK--QGTGRDNVDAD-KVMITNEEEIKTTNPVATESYGQVATNH 584
AAV5          NLQGSNTYALENTMIFNSQPANPGTTATYLEGNMLITSESETQPVNRVAYNVGGQMATNN 573

AAV1          QSSSTDLALGETTRPAPATGDVHAMGALPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKNPP
AAV6          QSSSTDLALGETTRPAPATGDVHVMGALPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKHPP
AAV3          QSSNTAPTTGTVNHQGALPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKHPP 644
AAV2          QRGNLALGETTRPARQAATADVNTQGVLPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKHPP
AAV8          QQQNLALGETTRPATAPQIGTVNSQGALPGMVWQNRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGLKHPP
AAV8.1        QGQRGLGETTRPAQAAQIGTVNSQGALPGMVWQNRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGLKHPP
AAV8 rh8      QAANLALGETTRPATQAQTGLVHNQGVIPGMVWQNRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGLKHPP
AAV10         QQLALGETTRPAANTGPIVGNVNSQGALPGMVWQNRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGLKHPP
AAV7          QAANLALGETTRPATAAQTQVVNNQGALPGMVWQNRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGLKHPP
AAV9          QSAQLALGETTRPAQAQTGWVQNQGILPGMVWQDRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGMKHPP
AAV9.1        QSGQAALGETTRPAQAATGWVQNQGILPGMVWQDRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGMKHPP
AAV5          QSLALGETTRPASTTAPATGTYNLQEIVPGSVWMERDVYLQGPIWAKIPETGAHFHPSPAMGGFGLKHPP
```

FIG. 18B

AAV1	PQILIK- (SEQ ID NO:34)
AAV6	PQILIK- (SEQ ID NO:35)
AAV3	PQIMIK- (SEQ ID NO:24)
AAV2	PQILIKN (SEQ ID NO:36)
AAV8	PQILIKN (SEQ ID NO:37)
AAV8.1	PQILIKN (SEQ ID NO:38)
AAV8 rh8	PQILIKN (SEQ ID NO:39)
AAV10	PQILIKN (SEQ ID NO:40)
AAV7	PQILIKN (SEQ ID NO:41)
AAV9	PQILIK- (SEQ ID NO:42)
AAV9.1	PQILIK- (SEQ ID NO:43)
AAV5	PMMLIKN (SEQ ID NO:44)

FIG. 18C

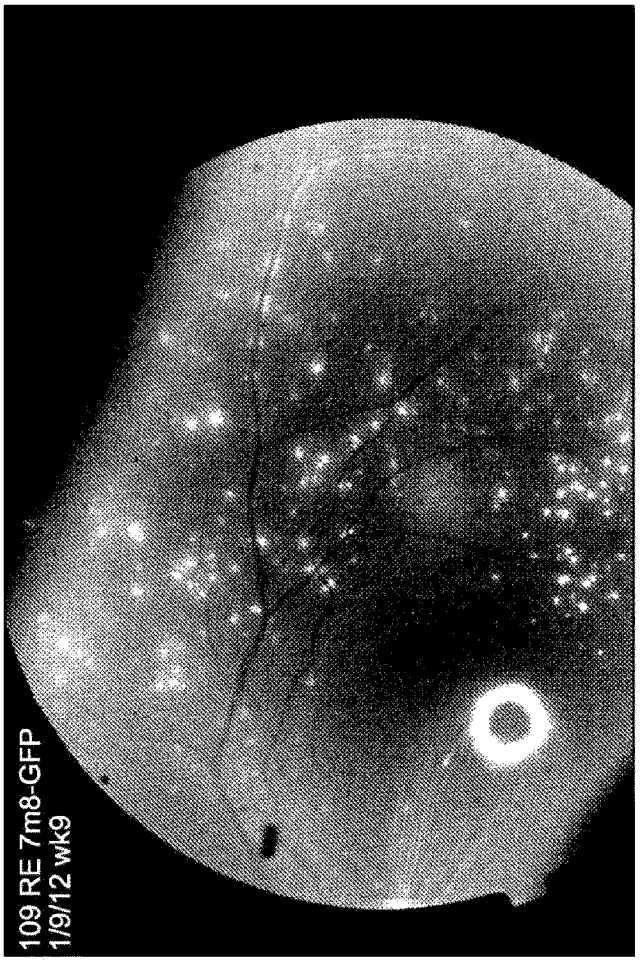


FIG. 19