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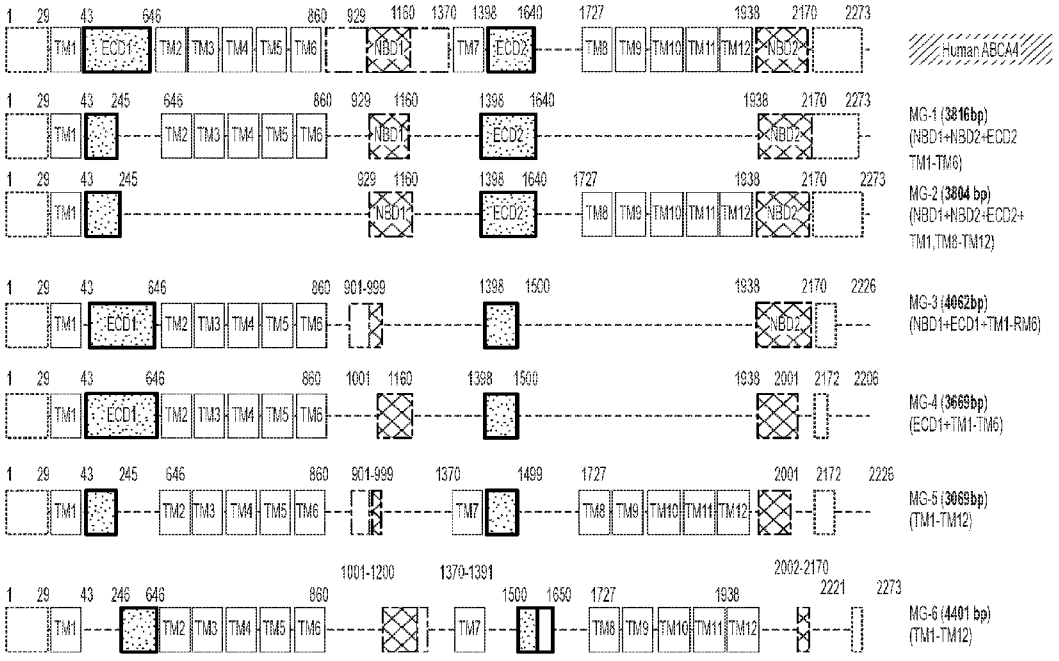

FIG. 1

(57) **Abstract:** Aspects of the disclosure relate to compositions and methods useful for delivering minigenes to a subject. Accordingly, the disclosure is based, in part, on isolated nucleic acids and gene therapy vectors, such as viral (*e.g.*, rAAV) vectors, comprising one or more gene fragments encoding a therapeutic gene product, such as a protein or peptide *(e.g.*, a minigene). In some embodiments, the disclosure relates to gene therapy vectors encoding a ABCA4 protein (*e.g.*, the gene product of *ABCA4* gene) or a portion thereof. In some embodiments, compositions described by the disclosure are useful for treating diseases associated with mutations in the *ABCA4* gene, for example Stargardt disease.

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GENE THERAPIES FOR STARGARDT DISEASE (ABCA4)

RELATED APPLICATION

This Application claims the benefit under 35 U.S.C. § 119(e) of the filing date of U.S. Provisional Application Serial No. 62/836,493, filed April 19, 2019, entitled "GENE THERAPIES FOR STARGARDT DISEASE (ABCA4)", the entire contents of which are incorporated herein by reference.

BACKGROUND

Stargardt Disease is the most common type of autosomal recessive macular degeneration. This disease is caused by mutations in the *ABCA4* gene. The human *ABCA4* gene is ~7 kb long, which exceeds the limit to package into conventional Adeno-associated Viral (AAV) vectors for gene delivery.

SUMMARY

Aspects of the disclosure relate to compositions and methods useful for delivering minigenes to a subject. Accordingly, the disclosure is based, in part, on isolated nucleic acids and gene therapy vectors, such as viral (*e.g.*, rAAV) vectors, comprising one or more gene fragments encoding a therapeutic gene product, such as a protein or peptide (*e.g.*, a minigene). In some embodiments, the disclosure relates to gene therapy vectors encoding a ABCA4 protein (*e.g.*, the gene product of *ABCA4*) or a portion thereof. In some embodiments, compositions described by the disclosure are useful for treating diseases associated with mutations in the *ABCA4* gene, for example Stargardt disease.

Accordingly, in some aspects, the disclosure provides an isolated nucleic acid comprising a transgene encoding a ABCA4 minigene having the sequence set forth in any one of SEQ ID NOs: 3-8 and 15-19.

In some aspects, the disclosure provides an isolated nucleic acid comprising a transgene having a nucleic acid sequence encoding a ABCA4 protein, wherein the ABCA4 protein comprises an amino acid sequence as set forth in any one of SEQ ID NOs: 9-14 and 20-24.

In some embodiments, a transgene further comprises a promoter operably linked to a ABCA4 minigene-encoding sequence. In some embodiments, a promoter is a constitutive promoter, inducible promoter, or a tissue-specific promoter. In some embodiments, the promoter comprises a chicken beta-actin (CBA) promoter. In some embodiments, tissue

specific promoter is a photoreceptor-specific promoter. In some embodiments, a photoreceptor-specific promoter comprises a rhodopsin kinase promoter, such as a human GRK promoter.

In some embodiments, a transgene is flanked by adeno-associated virus (AAV) inverted terminal repeats (ITRs). In some embodiments, at least one of the ITRs flanking a transgene is an AAV2 ITR. In some embodiments, at least one ITR flanking a transgene lacks a terminal resolution site, for example a ΔITR.

In some aspects, the disclosure provides a vector comprising an isolated nucleic acid as described herein. In some embodiments, a vector is a plasmid DNA, or closed-ended DNA, or lipid/DNA nanoparticle, or a viral vector. In some embodiments, a viral vector is an adeno-associated virus (AAV) vector, adenoviral (Ad) vector, lentiviral vector, retroviral vector, or Baculovirus vector.

In some aspects, the disclosure provides a host cell comprising an isolated nucleic acid or a vector as described herein. In some embodiments, a cell is a mammalian (human) cell, bacterial cell, yeast cell, or insect cell.

In some aspects, the disclosure provides a recombinant adeno-associated virus (rAAV) comprising: an isolated nucleic acid as described herein; and an AAV capsid protein.

In some embodiments, a capsid protein has a tropism for ocular cells. In some embodiments, a capsid protein is AAV8 capsid protein.

In some embodiments, an rAAV is formulated for delivery to the eye. In some embodiments, an rAAV is formulated for delivery to photoreceptor cells or retinal pigmented endothelium (RPE).

In some aspects, the disclosure provides a composition comprising an isolated nucleic acid or an rAAV as described herein, and a pharmaceutically acceptable excipient.

In some aspects, the disclosure provides a method for delivering a transgene to a cell, the method comprising administering an isolated nucleic acid or an rAAV as described herein to a cell.

In some embodiments, a cell is in a subject. In some embodiments, a subject is a mammalian subject, such as a human subject. In some embodiments, a cell is an eye cell. In some embodiments, an eye cell is a photoreceptor cell or retinal pigmented epithelium (RPE).

In some aspects, the disclosure provides a method for treating Stargardt disease in a subject in need thereof, the method comprising administering an isolated nucleic acid or an rAAV as described herein to the subject.

In some embodiments, a subject is a mammal. In some embodiments, a subject is a human.

In some embodiments, a subject is characterized by having one or more mutations in a *ABCA4* gene. In some embodiments, a subject has one or more mutations that result in an amino acid substitution selected from G1961E and D2177N corresponding to a wild-type ABCA4 protein (*e.g.*, the amino acid sequence set forth in SEQ ID NO: 2).

In some embodiments, administration is via injection. In some embodiments, the injection is subretinal injection or intravitreal injection or suprachoroidal injection.

In some embodiments, administration is topical administration to the eye of a subject.

BRIEF DESCRIPTION OF DRAWINGS

FIG. 1 is a schematic of several embodiments of MiniABCA4 constructs. MG-1, MG-2, MG-3, MG-4, MG-5, and MG-6, having amino acid sequences corresponding to SEQ ID NOs: 9-14, are shown.

FIG. 2 is a graph showing RPE autofluorescence analysis of wild type *ABCA4* knock-out mice (Abca KO), and *ABCA4* knock-out mice injected with miniABCA4-1 (which corresponds to the MG-4 construct described in FIG. 1) by fluorescence microscopy. ZO-1 was used as cell marker.

FIG. 3 is a graph showing quantification of RPE autofluorescence analysis of uninjected and injected *ABCA4* knock-out mice at different ages. Arrows point to ABCA4 minigene versions that significantly improved autofluorescence. The injections were performed at day P10 or P30. The analyses were performed at the ages indicated with each minigene. Injections at P30 showed highly significant improvement as compared to P10 injections. In this graph, AB-1 corresponds to MG-1, AB-2 corresponds to MG-2, AB-5 corresponds to MG-5.

FIGs. 4 is a schematic of several embodiments of MiniABCA4 constructs. MG-7, MG-8, MG-9, MG-10, and MG-11, having amino acid sequences corresponding to SEQ ID NOs: 20-24, are shown.

FIG. 5 is an immunoblot showing expression of the MiniABCA4 shown in FIG. 4 in HEK cells. Arrows indicate the expected bands. Lipo shows the cells transfected with the vehicle only.

DETAILED DESCRIPTION

In some aspects, the disclosure relates to compositions and methods useful for treating certain genetic diseases, for example Stargardt disease. The disclosure is based, in part, on isolated nucleic acids and gene therapy vectors, such as viral (*e.g.*, rAAV) vectors, comprising one or more gene fragments encoding a therapeutic gene product, such as a MiniABCA4 protein (*e.g.*, the gene product of an *ABCA4* minigene).

A "nucleic acid" sequence refers to a DNA or RNA sequence. In some embodiments, proteins and nucleic acids of the disclosure are isolated. As used herein, the term "isolated" means artificially produced. As used herein with respect to nucleic acids, the term "isolated" means: (i) amplified *in vitro* by, for example, polymerase chain reaction (PCR); (ii) recombinantly produced by cloning; (iii) purified, as by cleavage and gel separation; or (iv) synthesized by, for example, chemical synthesis. An isolated nucleic acid is one which is readily manipulable by recombinant DNA techniques well known in the art. Thus, a nucleotide sequence contained in a vector in which 5' and 3' restriction sites are known or for which polymerase chain reaction (PCR) primer sequences have been disclosed is considered isolated but a nucleic acid sequence existing in its native state in its natural host is not. An isolated nucleic acid may be substantially purified, but need not be. For example, a nucleic acid that is isolated within a cloning or expression vector is not pure in that it may comprise only a tiny percentage of the material in the cell in which it resides. Such a nucleic acid is isolated, however, as the term is used herein because it is readily manipulable by standard techniques known to those of ordinary skill in the art. As used herein with respect to proteins or peptides, the term "isolated" refers to a protein or peptide that has been isolated from its natural environment or artificially produced (*e.g.*, by chemical synthesis, by recombinant DNA technology, *etc.*). In some embodiments, an isolated nucleic acid encodes an ABCA4 protein, such as a MiniABCA4 protein (*e.g.*, a gene product expressed from a *ABCA4* gene or a portion thereof, such as an ABCA4 minigene).

In humans, the *ABCA4* gene (also referred to as *STGD*) encodes ATP-binding cassette, sub-family A (ABC1), member 4 protein, which is localized to outer segment disk edges of rods and cones and may function as an inward-directed flippase. Mutations in *ABCA4* gene have been observed to cause Stargardt disease, which is a form of macular degeneration. In some embodiments, an *ABCA4* gene comprises the nucleic acid sequence set forth in NCBI Reference Sequence Accession Number NM_000350.3 (SEQ ID NO: 1). In some embodiments, an

ABCA4 gene encodes a protein having the amino acid sequence set forth in NCBI Reference Sequence Accession Number NP_000341.2 (SEQ ID NO: 2).

As used herein, "minigene" refers to an isolated nucleic acid sequence encoding a recombinant peptide or protein where one or more non-essential elements of the corresponding gene encoding the naturally-occurring peptide or protein have been removed and where the peptide or protein encoded by the minigene retains function of the corresponding naturally-occurring peptide or protein. A "therapeutic minigene" refers to a minigene encoding a peptide or protein useful for treatment of a genetic disease, for example dystrophin, dysferlin, Factor VIII, Amyloid precursor protein (APP), Tyrosinase (Tyr), *etc.* Minigenes are known in the art and are described, for example by Karpati and Acsadi (1994) *Clin Invest Med* 17(5):499-509; Plantier et al. (2001) *Thromb Haemost.* 86(2):596-603; and Xiao et al. (2007) *World J. Gastroenterol.* 13(2):244-9. In some embodiments, a minigene does not comprise the sequence of the corresponding naturally-occurring peptide or protein.

In some aspects the disclosure relates to isolated nucleic acids encoding an *ABCA4* minigene. Generally, an isolated nucleic acid encoding a minigene (*e.g.*, a therapeutic minigene, such as an ABCA4 minigene) is between about 10% and about 99% (e.g., about 10%, about 15%, about 20%, about 25%, about 30%, about 40% about 50%, about 60%, about 70%, about 75%, about 80%, about 90%, about 99%, etc.) truncated with respect to a nucleic acid sequence encoding the corresponding naturally-occurring wild-type protein (*e.g.*, SEQ ID NO: 1). The truncations may be continuous (*e.g.*, single, continuous truncation of amino acid residues) or discontinuous (*e.g.*, two or more truncations of amino acids, for example truncation of two or more domains, that are separated by one or more peptides). For example, in some embodiments, a minigene encoding a Mini ABCA4 protein is between about 61% and truncated (e.g., comprises about 50% of the nucleic acid sequence) compared to a wild-type *ABCA4* gene (*e.g.*, SEQ ID NO: 1). In some embodiments, an ABCA4 minigene comprises (or consists of) the nucleic acid sequence set forth in any one of SEQ ID NOs: 3-8 and 15-19. In some embodiments, an ABCA4 minigene encodes a protein (referred to as a MiniABCA4 protein) that comprises (or consists of) an amino acid sequence set forth in any one of SEQ ID NOs: 9-14 and 20-24. In some embodiments, a nucleic acid encoding an ABCA4 protein (*e.g.*, a MiniABCA4 protein) comprises a start codon (*e.g.*, the nucleic acid sequence ATG) prior to the nucleic acid sequence encoding the MiniABCA4 protein. In some embodiments a nucleic acid sequence encoding a MiniABCA4 protein is codon-optimized.

An isolated nucleic acid sequence encoding an ABCA4 protein may be operably linked to a promoter. A "promoter" refers to a DNA sequence recognized by the synthetic machinery of the cell, or introduced synthetic machinery, required to initiate the specific transcription of a gene. The phrases "operatively positioned," "under control" or "under transcriptional control" means that the promoter is in the correct location and orientation in relation to the nucleic acid to control RNA polymerase initiation and expression of the gene. A promoter may be a constitutive promoter, inducible promoter, or a tissue-specific promoter.

Examples of constitutive promoters include, without limitation, the retroviral Rous sarcoma virus (RSV) LTR promoter (optionally with the RSV enhancer), the cytomegalovirus (CMV) promoter (optionally with the CMV enhancer) [see, *e.g.*, Boshart et al., Cell, 41:521-530 (1985)], the SV40 promoter, the dihydrofolate reductase promoter, the β-actin promoter, the phosphoglycerol kinase (PGK) promoter, and the EF1α promoter [Invitrogen]. In some embodiments, a promoter comprises a chicken beta-actin (CBA) promoter. In some embodiments, a promoter is an enhanced chicken β-actin promoter. In some embodiments, a promoter is a U6 promoter. In some embodiments, a promoter is a chicken beta-actin (CBA) promoter.

Inducible promoters allow regulation of gene expression and can be regulated by exogenously supplied compounds, environmental factors such as temperature, or the presence of a specific physiological state, *e.g.*, acute phase, a particular differentiation state of the cell, or in replicating cells only. Inducible promoters and inducible systems are available from a variety of commercial sources, including, without limitation, Invitrogen, Clontech and Ariad. Many other systems have been described and can be readily selected by one of skill in the art. Examples of inducible promoters regulated by exogenously supplied promoters include the zinc-inducible sheep metallothionine (MT) promoter, the dexamethasone (Dex)-inducible mouse mammary tumor virus (MMTV) promoter, the T7 polymerase promoter system (WO 98/10088); the ecdysone insect promoter (No et al., Proc. Natl. Acad. Sci. USA, 93:3346-3351 (1996)), the tetracycline-repressible system (Gossen et al., Proc. Natl. Acad. Sci. USA, 89:5547-5551 (1992)), the tetracycline-inducible system (Gossen et al., Science, 268:1766-1769 (1995), see also Harvey et al., Curr. Opin. Chem. Biol., 2:512-518 (1998)), the RU486-inducible system (Wang et al., Nat. Biotech., 15:239-243 (1997) and Wang et al., Gene Ther., 4:432-441 (1997)) and the rapamycin-inducible system (Magari et al., J. Clin. Invest., 100:2865-2872 (1997)). Still other types of inducible promoters which may be useful in this context are those which are

regulated by a specific physiological state, *e.g.*, temperature, acute phase, a particular differentiation state of the cell, or in replicating cells only.

In some embodiments, the regulatory sequences impart tissue-specific gene expression capabilities. In some cases, the tissue-specific regulatory sequences bind tissue-specific transcription factors that induce transcription in a tissue specific manner. Such tissue-specific regulatory sequences (*e.g.*, promoters, enhancers, *etc.*.) are well known in the art. In some embodiments, the tissue-specific promoter is an eye-specific promoter. Examples of eye-specific promoters include but are not limited to a retinoschisin promoter, K12 promoter, a rhodopsin promoter, a rod-specific promoter, a cone-specific promoter, a rhodopsin kinase promoter, a GRK1 promoter, an interphotoreceptor retinoid-binding protein proximal (IRBP) promoter, and an opsin promoter (*e.g.*, a red opsin promoter, a blue opsin promoter, *etc.*).

In some embodiments, a promoter is a RNA polymerase III (pol III) promoter. Non-limiting examples of pol III promoters include U6 and H1 promoter sequences. In some embodiments, a promoter is a RNA polymerase II (pol II) promoter. Non-limiting examples of pol II promoters include T7, T3, SP6, RSV, and cytomegalovirus promoter sequences.

Aspects of the disclosure relate to gene therapy vectors comprising an isolated nucleic acid as described herein. A gene therapy vector may be a viral vector (*e.g.*, a lentiviral vector, an adeno-associated virus vector, an adenoviral (Ad) vector, *etc.*), a plasmid, a closed-ended DNA (*e.g.*, ceDNA), a lipid/DNA nanoparticle, *etc*. In some embodiments, a gene therapy vector is a viral vector. In some embodiments, an expression cassette encoding a minigene is flanked by one or more viral replication sequences, for example lentiviral long terminal repeats (LTRs) or adeno-associated virus (AAV) inverted terminal repeats (ITRs). In some embodiments, a viral vector is a Baculovirus vector.

An isolated nucleic acid described herein may also contain an intron, desirably located between the promoter/enhancer sequence and the transgene. In some embodiments, an intron is a synthetic or artificial (e.g., heterologous) intron. Examples of synthetic introns include an intron sequence derived from SV-40 (referred to as the SV-40 T intron sequence) and intron sequences derived from chicken beta-actin gene. In some embodiments, a transgene described by the disclosure comprises one or more (1, 2, 3, 4, 5, or more) artificial introns. In some embodiments, the one or more artificial introns are positioned between a promoter and a nucleic acid sequence encoding a transgene.

In some embodiments, the rAAV comprises a posttranscriptional response element. As used herein, the term "posttranscriptional response element" refers to a nucleic acid sequence that, when transcribed, adopts a tertiary structure that enhances expression of a gene. Examples of posttranscriptional regulatory elements include, but are not limited to, woodchuck hepatitis virus posttranscriptional regulatory element (WPRE), mouse RNA transport element (RTE), constitutive transport element (CTE) of the simian retrovirus type 1 (SRV-1), the CTE from the Mason-Pfizer monkey virus (MPMV), and the 5′ untranslated region of the human heat shock protein 70 (Hsp70 5′UTR). In some embodiments, the rAAV vector comprises a woodchuck hepatitis virus posttranscriptional regulatory element (WPRE).

In some embodiments, the vector further comprises conventional control elements which are operably linked with elements of the transgene in a manner that permits its transcription, translation and/or expression in a cell transfected with the vector or infected with the virus produced by the disclosure. As used herein, "operably linked" sequences include both expression control sequences that are contiguous with the gene of interest and expression control sequences that act in trans or at a distance to control the gene of interest. Expression control sequences include appropriate transcription initiation, termination, promoter and enhancer sequences; efficient RNA processing signals such as splicing and polyadenylation (polyA) signals; sequences that stabilize cytoplasmic mRNA; sequences that enhance translation efficiency (e.g., Kozak consensus sequence); sequences that enhance protein stability; and when desired, sequences that enhance secretion of the encoded product. A number of expression control sequences, including promoters which are native, constitutive, inducible and/or tissue-specific, are known in the art and may be utilized.

A polyadenylation sequence generally is inserted following the transgene sequences and optionally before a 3' AAV ITR sequence. A rAAV construct useful in the disclosure may also contain an intron, desirably located between the promoter/enhancer sequence and the transgene. One possible intron sequence is derived from SV-40, and is referred to as the SV-40 T intron sequence. Another vector element that may be used is an internal ribosome entry site (IRES). An IRES sequence is used to produce more than one polypeptide from a single gene transcript. An IRES sequence would be used to produce a protein that contain more than one polypeptide chains. Selection of these and other common vector elements are conventional and many such sequences are available [see, e.g., Sambrook et al., and references cited therein at, for example,

pages 3.18 3.26 and 16.17 16.27 and Ausubel et al., Current Protocols in Molecular Biology, John Wiley & Sons, New York, 1989].

The isolated nucleic acids of the disclosure may be recombinant adeno-associated virus (AAV) vectors (rAAV vectors). In some embodiments, an isolated nucleic acid as described by the disclosure comprises a region (*e.g.*, a first region) comprising a first adeno-associated virus (AAV) inverted terminal repeat (ITR), or a variant thereof. The isolated nucleic acid (*e.g.*, the recombinant AAV vector) may be packaged into a capsid protein and administered to a subject and/or delivered to a selected target cell. "Recombinant AAV (rAAV) vectors" are typically composed of, at a minimum, a transgene and its regulatory sequences, and 5' and 3' AAV inverted terminal repeats (ITRs). The transgene may comprise, as disclosed elsewhere herein, one or more regions that encode one or more proteins (*e.g.*, human ABCA4, or a fragment thereof). The transgene may also comprise a region encoding, for example, a miRNA binding site, and/or an expression control sequence (*e.g.*, a poly-A tail).

Generally, ITR sequences are about 145 bp in length. Preferably, substantially the entire sequences encoding the ITRs are used in the molecule, although some degree of minor modification of these sequences is permissible. The ability to modify these ITR sequences is within the skill of the art. (See, *e.g.*, texts such as Sambrook et al., "Molecular Cloning. A Laboratory Manual", 2d ed., Cold Spring Harbor Laboratory, New York (1989); and K. Fisher et al., J Virol., 70:520 532 (1996)). An example of such a molecule employed in the present invention is a "cis-acting" plasmid containing the transgene, in which the selected transgene sequence and associated regulatory elements are flanked by the 5' and 3' AAV ITR sequences. The AAV ITR sequences may be obtained from any known AAV, including presently identified mammalian AAV types. In some embodiments, the isolated nucleic acid (*e.g.*, the rAAV vector) comprises at least one ITR having a serotype selected from AAV1, AAV2, AAV5, AAV6, AAV6.2, AAV7, AAV8, AAV9, AAV10, AAV11, and variants thereof. In some embodiments, the isolated nucleic acid comprises a region (*e.g.*, a first region) encoding an AAV2 ITR.

In some embodiments, the isolated nucleic acid further comprises a region (*e.g.*, a second region, a third region, a fourth region, *etc.*) comprising a second AAV ITR. In some embodiments, the second AAV ITR has a serotype selected from AAV1, AAV2, AAV5, AAV6, AAV6.2, AAV7, AAV8, AAV9, AAV10, AAV11, and variants thereof. In some embodiments, the second AAV ITR is an AAV2 ITR. In some embodiments, the second ITR is a mutant ITR that lacks a functional terminal resolution site (TRS). The term "lacking a terminal resolution

site" can refer to an AAV ITR that comprises a mutation (*e.g.*, a sense mutation such as a non-synonymous mutation, or missense mutation) that abrogates the function of the terminal resolution site (TRS) of the ITR, or to a truncated AAV ITR that lacks a nucleic acid sequence encoding a functional TRS (*e.g.*, a ΔTRS ITR, or ΔITR). Without wishing to be bound by any particular theory, a rAAV vector comprising an ITR lacking a functional TRS produces a self-complementary rAAV vector, for example as described by McCarthy (2008) *Molecular Therapy* 16(10):1648-1656.

Recombinant adeno-associated viruses (rAAVs)

In some aspects, the disclosure provides isolated adeno-associated viruses (AAVs). As used herein with respect to AAVs, the term "isolated" refers to an AAV that has been artificially produced or obtained. Isolated AAVs may be produced using recombinant methods. Such AAVs are referred to herein as "recombinant AAVs". Recombinant AAVs (rAAVs) preferably have tissue-specific targeting capabilities, such that a nuclease and/or transgene of the rAAV will be delivered specifically to one or more predetermined tissue(s). The AAV capsid is an important element in determining these tissue-specific targeting capabilities. Thus, an rAAV having a capsid appropriate for the tissue being targeted can be selected.

Methods for obtaining recombinant AAVs having a desired capsid protein are well known in the art. (See, for example, US 2003/0138772), the contents of which are incorporated herein by reference in their entirety). Typically the methods involve culturing a host cell which contains a nucleic acid sequence encoding an AAV capsid protein; a functional *rep* gene; a recombinant AAV vector composed of, AAV inverted terminal repeats (ITRs) and a transgene; and sufficient helper functions to permit packaging of the recombinant AAV vector into the AAV capsid proteins. In some embodiments, capsid proteins are structural proteins encoded by the cap gene of an AAV. AAVs comprise three capsid proteins, virion proteins 1 to 3 (named VP1, VP2 and VP3), all of which are transcribed from a single cap gene via alternative splicing. In some embodiments, the molecular weights of VP1, VP2 and VP3 are respectively about 87 kDa, about 72 kDa and about 62 kDa. In some embodiments, upon translation, capsid proteins form a spherical 60-mer protein shell around the viral genome. In some embodiments, the functions of the capsid proteins are to protect the viral genome, deliver the genome and interact with the host. In some aspects, capsid proteins deliver the viral genome to a host in a tissue specific manner.

In some embodiments, an AAV capsid protein is of an AAV serotype selected from the group consisting of AAV2, AAV3, AAV4, AAV5, AAV6, AAV8, AAVrh8, AAV9, and AAV10. In some embodiments, an AAV capsid protein is of a serotype derived from a non-human primate, for example AAVrh8 serotype. In some embodiments, the AAV capsid protein is of a serotype that has tropism for the eye of a subject, for example an AAV (*e.g.*, AAV5, AAV6, AAV6.2, AAV7, AAV8, AAV9, AAVrh.8, AAVrh.10, AAVrh.39 and AAVrh.43) that transduces ocular cells of a subject more efficiently than other AAV capsid proteins. In some embodiments, an AAV capsid protein is of an AAV8 serotype.

The components to be cultured in the host cell to package a rAAV vector in an AAV capsid may be provided to the host cell in *trans*. Alternatively, any one or more of the required components (*e.g.*, recombinant AAV vector, rep sequences, cap sequences, and/or helper functions) may be provided by a stable host cell which has been engineered to contain one or more of the required components using methods known to those of skill in the art. Most suitably, such a stable host cell will contain the required component(s) under the control of an inducible promoter. However, the required component(s) may be under the control of a constitutive promoter. Examples of suitable inducible and constitutive promoters are provided herein, in the discussion of regulatory elements suitable for use with the transgene. In still another alternative, a selected stable host cell may contain selected component(s) under the control of a constitutive promoter and other selected component(s) under the control of one or more inducible promoters. For example, a stable host cell may be generated which is derived from 293 cells (which contain E1 helper functions under the control of a constitutive promoter), but which contain the rep and/or cap proteins under the control of inducible promoters. Still other stable host cells may be generated by one of skill in the art.

In some embodiments, the disclosure relates to a host cell containing a nucleic acid that comprises a coding sequence encoding a protein (*e.g.*, a MiniABCA4 protein). In some embodiments, the host cell is a mammalian cell (*e.g.*, HEK293 cell) or an insect cell (*e.g.*, SF9 cell). In some embodiments, the disclosure relates to a composition comprising the host cell described above. In some embodiments, the composition comprising the host cell above further comprises a cryopreservative.

The recombinant AAV vector, rep sequences, cap sequences, and helper functions required for producing the rAAV of the disclosure may be delivered to the packaging host cell using any appropriate genetic element (vector). The selected genetic element may be delivered

by any suitable method, including those described herein. The methods used to construct any embodiment of this disclosure are known to those with skill in nucleic acid manipulation and include genetic engineering, recombinant engineering, and synthetic techniques. See, *e.g.*, Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. Similarly, methods of generating rAAV virions are well known and the selection of a suitable method is not a limitation on the present disclosure. See, *e.g.*, K. Fisher et al., J. Virol., 70:520-532 (1993) and U.S. Pat. No. 5,478,745.

In some embodiments, recombinant AAVs may be produced using the triple transfection method (described in detail in U.S. Pat. No. 6,001,650). Typically, the recombinant AAVs are produced by transfecting a host cell with an recombinant AAV vector (comprising a transgene) to be packaged into AAV particles, an AAV helper function vector, and an accessory function vector. An AAV helper function vector encodes the "AAV helper function" sequences (*i.e.*, rep and cap), which function in *trans* for productive AAV replication and encapsidation. Preferably, the AAV helper function vector supports efficient AAV vector production without generating any detectable wild-type AAV virions (*i.e.*, AAV virions containing functional rep and cap genes). Non-limiting examples of vectors suitable for use with the present disclosure include pHLP19, described in U.S. Pat. No. 6,001,650 and pRep6cap6 vector, described in U.S. Pat. No. 6,156,303, the entirety of both incorporated by reference herein. The accessory function vector encodes nucleotide sequences for non-AAV derived viral and/or cellular functions upon which AAV is dependent for replication (*i.e.*, "accessory functions"). The accessory functions include those functions required for AAV replication, including, without limitation, those moieties involved in activation of AAV gene transcription, stage specific AAV mRNA splicing, AAV DNA replication, synthesis of cap expression products, and AAV capsid assembly. Viral-based accessory functions can be derived from any of the known helper viruses such as adenovirus, herpesvirus (other than herpes simplex virus type-1), and vaccinia virus.

In some aspects, the disclosure provides transfected host cells. The term "transfection" is used to refer to the uptake of foreign DNA by a cell, and a cell has been "transfected" when exogenous DNA has been introduced inside the cell membrane. A number of transfection techniques are generally known in the art. See, *e.g.*, Graham et al. (1973) Virology, 52:456, Sambrook et al. (1989) Molecular Cloning, a laboratory manual, Cold Spring Harbor Laboratories, New York, Davis et al. (1986) Basic Methods in Molecular Biology, Elsevier, and Chu et al. (1981) Gene 13:197. Such techniques can be used to introduce one or more exogenous

nucleic acids, such as a nucleotide integration vector and other nucleic acid molecules, into suitable host cells.

A "host cell" refers to any cell that harbors, or is capable of harboring, a substance of interest. Often a host cell is a mammalian cell. A host cell may be used as a recipient of an AAV helper construct, an AAV minigene plasmid, an accessory function vector, or other transfer DNA associated with the production of recombinant AAVs. The term includes the progeny of the original cell which has been transfected. Thus, a "host cell" as used herein may refer to a cell which has been transfected with an exogenous DNA sequence. It is understood that the progeny of a single parental cell may not necessarily be completely identical in morphology or in genomic or total DNA complement as the original parent, due to natural, accidental, or deliberate mutation.

As used herein, the term "cell line" refers to a population of cells capable of continuous or prolonged growth and division *in vitro*. Often, cell lines are clonal populations derived from a single progenitor cell. It is further known in the art that spontaneous or induced changes can occur in karyotype during storage or transfer of such clonal populations. Therefore, cells derived from the cell line referred to may not be precisely identical to the ancestral cells or cultures, and the cell line referred to includes such variants.

As used herein, the terms "recombinant cell" refers to a cell into which an exogenous DNA segment, such as DNA segment that leads to the transcription of a biologically-active polypeptide or production of a biologically active nucleic acid such as an RNA, has been introduced.

As used herein, the term "vector" includes any genetic element, such as a plasmid, phage, transposon, cosmid, chromosome, artificial chromosome, virus, virion, *etc.*, which is capable of replication when associated with the proper control elements and which can transfer gene sequences between cells. Thus, the term includes cloning and expression vehicles, as well as viral vectors.

Methods

Methods for delivering a transgene to ocular (*e.g.*, photoreceptors, such as rod cells or cone cells, retinal cells, *etc.*) tissue or the ear of a subject are provided herein. The methods typically involve administering to a subject an effective amount of a rAAV comprising a nucleic acid for expressing a transgene (*e.g.*, a MiniABCA4 protein) in the subject. An "effective

amount" of a rAAV is an amount sufficient to infect a sufficient number of cells of a target tissue in a subject. In some embodiments, a target tissue is ocular (*e.g.*, photoreceptor, retinal, *etc.*) tissue. In some embodiments, a transgene is delivered to photoreceptor cells or retinal pigmented epithelium (RPE).

An effective amount of a rAAV may be an amount sufficient to have a therapeutic benefit in a subject, *e.g.*, to improve in the subject one or more symptoms of disease, *e.g.*, a symptom of Stargardt disease (*e.g.*, a disease associated with a deletion or mutation of *ABCA4* gene). Examples of mutations in *ABCA4* gene include mutations resulting in amino acid substitutions G1961E and D2177N in an ABCA4 protein. The effective amount will depend on a variety of factors such as, for example, the species, age, weight, health of the subject, and the ocular tissue to be targeted, and may thus vary among subject and tissue. An effective amount may also depend on the rAAV used.

In certain embodiments, the effective amount of rAAV is 10^{10}, 10^{11}, 10^{12}, 10^{13}, or 10^{14} genome copies per kg. In certain embodiments, the effective amount of rAAV is 10^{10}, 10^{11}, 10^{12}, 10^{13}, 10^{14}, or 10^{15} genome copies per subject.

Aspects of the disclosure relate to methods for treating Stargardt disease in a subject in need thereof. In some embodiments, a subject is a mammal, for example a human, mouse, rat, dog, cat, non-human primate, etc. In some embodiments, a subject is a human.

As used herein, the term "treating" refers to the application or administration of a composition (*e.g.*, an isolated nucleic acid or rAAV as described herein) to a subject who exhibits one or more signs or symptoms of Stargardt disease (*e.g.,* blurry or distorted vision, inability to see in low light, loss of color vision, one or more mutations in an *ABCA4* gene, etc.), with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve, or affect the disorder, the symptom of the disease, or the predisposition toward Stargardt disease.

Alleviating Stargardt disease includes delaying the development or progression of the disease, or reducing disease severity. Alleviating the disease does not necessarily require curative results. As used therein, "delaying" the development of Stargardt disease means to defer, hinder, slow, retard, stabilize, and/or postpone progression of the disease. This delay can be of varying lengths of time, depending on the history of the disease and/or individuals being treated. A method that "delays" or alleviates the development of a disease, or delays the onset of the disease, is a method that reduces probability of developing one or more symptoms of the disease in a given time frame and/or reduces extent of the symptoms in a given time frame,

when compared to not using the method. Such comparisons are typically based on clinical studies, using a number of subjects sufficient to give a statistically significant result.

"Development" or "progression" of a disease means initial manifestations and/or ensuing progression of the disease. Development of the disease can be detectable and assessed using standard clinical techniques as well known in the art. However, development also refers to progression that may be undetectable. For purpose of this disclosure, development or progression refers to the biological course of the symptoms. "Development" includes occurrence, recurrence, and onset.

An effective amount may also depend on the mode of administration. For example, targeting an ocular (*e.g.*, photoreceptor, retinal, *etc.*) tissue by intrastromal administration or subcutaneous injection may require different (*e.g.*, higher or lower) doses, in some cases, than targeting an ocular (*e.g.*, photoreceptor, retinal, *etc.*) tissue by another method (*e.g.*, systemic administration, topical administration). In some embodiments, intrastromal injection (IS) of rAAV having certain serotypes (*e.g.*, AAV5, AAV6, AAV6.2, AAV7, AAV8, AAV9, AAVrh.8, AAVrh.10, AAVrh.39, and AAVrh.43) mediates efficient transduction of ocular (*e.g.*, corneal, photoreceptor, retinal, *etc.*) cells. Thus, in some embodiments, the injection is intrastromal injection (IS). In some embodiments, the administration is via injection, optionally subretinal injection or intravitreal injection or suprachoroidal injection. In some embodiments, the injection is topical administration (*e.g.*, topical administration to an eye). In some cases, multiple doses of a rAAV are administered.

The rAAVs may be delivered to a subject in compositions according to any appropriate methods known in the art. The rAAV, preferably suspended in a physiologically compatible carrier (*i.e.*, in a composition), may be administered to a subject, *i.e.* host animal, such as a human, mouse, rat, cat, dog, sheep, rabbit, horse, cow, goat, pig, guinea pig, hamster, chicken, turkey, or a non-human primate (*e.g.,* Macaque). In some embodiments, a host animal does not include a human.

Delivery of the rAAVs to a mammalian subject may be by, for example, intraocular injection or topical administration (*e.g.*, eye drops). In some embodiments, the intraocular injection comprises intrastromal injection, subconjunctival injection, or intravitreal injection. In some embodiments, the injection is not topical administration. Combinations of administration methods (*e.g.*, topical administration and intrastromal injection) can also be used.

The compositions of the disclosure may comprise an rAAV alone, or in combination with one or more other viruses (*e.g.*, a second rAAV encoding having one or more different transgenes, such as a transgene encoding a different MiniABCA4 protein). In some embodiments, a composition comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more different rAAVs each having one or more different transgenes.

In some embodiments, a composition further comprises a pharmaceutically acceptable carrier. Suitable carriers may be readily selected by one of skill in the art in view of the indication for which the rAAV is directed. For example, one suitable carrier includes saline, which may be formulated with a variety of buffering solutions (*e.g.*, phosphate buffered saline). Other exemplary carriers include sterile saline, lactose, sucrose, calcium phosphate, gelatin, dextran, agar, pectin, peanut oil, sesame oil, and water. The selection of the carrier is not a limitation of the present disclosure.

Optionally, the compositions of the disclosure may contain, in addition to the rAAV and carrier(s), other pharmaceutical ingredients, such as preservatives, or chemical stabilizers. Suitable exemplary preservatives include chlorobutanol, potassium sorbate, sorbic acid, sulfur dioxide, propyl gallate, the parabens, ethyl vanillin, glycerin, phenol, and parachlorophenol. Suitable chemical stabilizers include gelatin and albumin.

The rAAVs are administered in sufficient amounts to transfect the cells of a desired tissue (*e.g.*, ocular tissue, such as photoreceptor, retinal, *etc.,* tissue) and to provide sufficient levels of gene transfer and expression without undue adverse effects. Examples of pharmaceutically acceptable routes of administration include, but are not limited to, direct delivery to the selected organ (*e.g.*, subretinal delivery to the eye), oral, inhalation (including intranasal and intratracheal delivery), intraocular, intravenous, intramuscular, subcutaneous, intradermal, intratumoral, and other parental routes of administration. Routes of administration may be combined, if desired.

The dose of rAAV virions required to achieve a particular "therapeutic effect," *e.g.*, the units of dose in genome copies/per kilogram of body weight (GC/kg), will vary based on several factors including, but not limited to: the route of rAAV virion administration, the level of gene or RNA expression required to achieve a therapeutic effect, the specific disease or disorder being treated, and the stability of the gene or RNA product. One of skill in the art can readily determine a rAAV virion dose range to treat a patient having a particular disease or disorder based on the aforementioned factors, as well as other factors.

An effective amount of an rAAV is an amount sufficient to target infect an animal, target a desired tissue. The effective amount will depend primarily on factors such as the species, age, weight, health of the subject, and the tissue to be targeted, and may thus vary among animal and tissue. For example, an effective amount of the rAAV is generally in the range of from about 1 ml to about 100 ml of solution containing from about 10^9 to 10^{16} genome copies. In some cases, a dosage between about 10^{11} to 10^{13} rAAV genome copies is appropriate. In certain embodiments, 10^9 rAAV genome copies is effective to target ocular tissue (*e.g.*, corneal tissue).

In some embodiments, a dose more concentrated than 10^9 rAAV genome copies is toxic when administered to the eye of a subject. In some embodiments, an effective amount is produced by multiple doses of an rAAV. In some embodiments, a subject is administered one or more immunosuppressive agents (*e.g.*, corticosteroids, methotrexate, cyclosporine A, mycophenolate mofetil, tacrolimus, Rituximab, sirolimus, methylprednisolone, CTLA4Ig, non-depleting CD4 Ab, and T cell-depleting anti-thymocyte gamma-globulin (ATG), etc.) prior to administration of the rAAV.

In some embodiments, a dose of rAAV is administered to a subject no more than once per calendar day (*e.g.*, a 24-hour period). In some embodiments, a dose of rAAV is administered to a subject no more than once per 2, 3, 4, 5, 6, or 7 calendar days. In some embodiments, a dose of rAAV is administered to a subject no more than once per calendar week (*e.g.*, 7 calendar days). In some embodiments, a dose of rAAV is administered to a subject no more than bi-weekly (*e.g.*, once in a two calendar week period). In some embodiments, a dose of rAAV is administered to a subject no more than once per calendar month (*e.g.*, once in 30 calendar days). In some embodiments, a dose of rAAV is administered to a subject no more than once per six calendar months. In some embodiments, a dose of rAAV is administered to a subject no more than once per calendar year (*e.g.*, 365 days or 366 days in a leap year).

In some embodiments, rAAV compositions are formulated to reduce aggregation of AAV particles in the composition, particularly where high rAAV concentrations are present (*e.g.*, ~10^{13} GC/ml or more). Appropriate methods for reducing aggregation of may be used, including, for example, addition of surfactants, pH adjustment, salt concentration adjustment, *etc.* (See, *e.g.*, Wright FR, *et al.*, Molecular Therapy (2005) 12, 171–178, the contents of which are incorporated herein by reference.)

Formulation of pharmaceutically-acceptable excipients and carrier solutions is well-known to those of skill in the art, as is the development of suitable dosing and treatment

regimens for using the particular compositions described herein in a variety of treatment regimens. Typically, these formulations may contain at least about 0.1% of the active compound or more, although the percentage of the active ingredient(s) may, of course, be varied and may conveniently be between about 1 or 2% and about 70% or 80% or more of the weight or volume of the total formulation. Naturally, the amount of active compound in each therapeutically-useful composition may be prepared is such a way that a suitable dosage will be obtained in any given unit dose of the compound. Factors such as solubility, bioavailability, biological half-life, route of administration, product shelf life, as well as other pharmacological considerations will be contemplated by one skilled in the art of preparing such pharmaceutical formulations, and as such, a variety of dosages and treatment regimens may be desirable.

In some embodiments, rAAVs in suitably formulated pharmaceutical compositions disclosed herein are delivered directly to target tissue, *e.g.*, direct to ocular tissue (*e.g.*, photoreceptor, retinal, *etc.,* tissue) However, in certain circumstances it may be desirable to separately or in addition deliver the rAAV-based therapeutic constructs via another route, *e.g.*, subcutaneously, intrapancreatically, intranasally, parenterally, intravenously, intramuscularly, intrathecally, or orally, intraperitoneally, or by inhalation. In some embodiments, the administration modalities as described in U.S. Pat. Nos. 5,543,158; 5,641,515 and 5,399,363 (each specifically incorporated herein by reference in its entirety) may be used to deliver rAAVs. In some embodiments, a preferred mode of administration is by intravitreal injection or subretinal injection.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. Dispersions may also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms. In many cases the form is sterile and fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (*e.g.*, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and/or vegetable oils. Proper fluidity may be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants.

The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

For administration of an injectable aqueous solution, for example, the solution may be suitably buffered, if necessary, and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. In this connection, a suitable sterile aqueous medium may be employed. For example, one dosage may be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermoclysis fluid or injected at the proposed site of infusion, (see for example, "Remington's Pharmaceutical Sciences" 15th Edition, pages 1035-1038 and 1570-1580). Some variation in dosage will necessarily occur depending on the condition of the host. The person responsible for administration will, in any event, determine the appropriate dose for the individual host.

Sterile injectable solutions are prepared by incorporating the active rAAV in the required amount in the appropriate solvent with various of the other ingredients enumerated herein, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

The rAAV compositions disclosed herein may also be formulated in a neutral or salt form. Pharmaceutically-acceptable salts, include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like. Upon formulation, solutions will be administered in a manner compatible with the dosage formulation

and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms such as injectable solutions, drug-release capsules, and the like.

As used herein, "carrier" includes any and all solvents, dispersion media, vehicles, coatings, diluents, antibacterial and antifungal agents, isotonic and absorption delaying agents, buffers, carrier solutions, suspensions, colloids, and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Supplementary active ingredients can also be incorporated into the compositions. The phrase "pharmaceutically-acceptable" refers to molecular entities and compositions that do not produce an allergic or similar untoward reaction when administered to a host.

Delivery vehicles such as liposomes, nanocapsules, microparticles, microspheres, lipid particles, vesicles, and the like, may be used for the introduction of the compositions of the present disclosure into suitable host cells. In particular, the rAAV vector delivered transgenes may be formulated for delivery either encapsulated in a lipid particle, a liposome, a vesicle, a nanosphere, or a nanoparticle or the like.

Such formulations may be preferred for the introduction of pharmaceutically acceptable formulations of the nucleic acids or the rAAV constructs disclosed herein. The formation and use of liposomes is generally known to those of skill in the art. Recently, liposomes were developed with improved serum stability and circulation half-times (U.S. Pat. No. 5,741,516). Further, various methods of liposome and liposome like preparations as potential drug carriers have been described (U.S. Pat. Nos. 5,567,434; 5,552,157; 5,565,213; 5,738,868 and 5,795,587).

Liposomes have been used successfully with a number of cell types that are normally resistant to transfection by other procedures. In addition, liposomes are free of the DNA length constraints that are typical of viral-based delivery systems. Liposomes have been used effectively to introduce genes, drugs, radiotherapeutic agents, viruses, transcription factors and allosteric effectors into a variety of cultured cell lines and animals. In addition, several successful clinical trials examining the effectiveness of liposome-mediated drug delivery have been completed.

Liposomes are formed from phospholipids that are dispersed in an aqueous medium and spontaneously form multilamellar concentric bilayer vesicles (also termed multilamellar vesicles (MLVs). MLVs generally have diameters of from 25 nm to 4 μm. Sonication of MLVs results in the formation of small unilamellar vesicles (SUVs) with diameters in the range of 200 to 500 Å, containing an aqueous solution in the core.

Alternatively, nanocapsule formulations of the rAAV may be used. Nanocapsules can generally entrap substances in a stable and reproducible way. To avoid side effects due to intracellular polymeric overloading, such ultrafine particles (sized around 0.1 μm) should be designed using polymers able to be degraded *in vivo*. Biodegradable polyalkyl-cyanoacrylate nanoparticles that meet these requirements are contemplated for use.

EXAMPLES

Example 1: miniABC4 gene constructs MG-1 to MG-6

This example describes identification and production of AAV vectors (and rAAVs) having one or more domains of ABCA4 (*e.g.*, ABCA4 minigenes and gene products thereof, "MiniABCA4") that retain function (*e.g.*, partial ABCA4 function) in photoreceptors. Expression and stability of miniABCA4 proteins is tested by expressing them as MYC-tagged versions of the proteins in cultured HEK293 cells. The versions that show optimal expression and stability are injected *in vivo* to *Abca4*$^{-/-}$ mice. Viral particles are pseudotyped (*e.g.*, AAV2/8) and expression is driven by promoters that predominantly target photoreceptors. MiniABCA4 constructs were administered using subretinal injection. FIG. 1 and SEQ ID NOs: 3-8 show embodiments of MiniABCA4 constructs MG-1 through MG-6. SEQ ID NOs: 9-14 show embodiments of MiniABCA4 proteins for MG-1 through MG-6.

Reduction of Retinal Pigment Epithelium (RPE) Background Autofluorescence was used as a surrogate assay to test the efficacy of miniABCA4 candidates. *Abca4*$^{-/-}$ mice were injected with Abca4-1 (MG-1) constructs 30 days postnatal, and the RPE autoflorescence was measured by fluorescent microscopy. The RPE cells were stained with a marker, ZO-1. Representative data is shown in FIG. 2. As compared to the wild type control, the *Abca4*$^{-/-}$ mouse showed higher RPE autofluorescence at 488 nM wavelength at 4 months of age. Injection of miniABCA4-1 resulted in a significant decline in the autofluorescence at 488 nM wavelength, indicating that miniABCA4-1 had rescue effects.

Other ABCA4 minigene constructs were tested using the RPE autofluorescence assay, and the results were quantified in FIG. 3. *Abca4*$^{-/-}$ mice were injected with miniABCA4-1 (MG-1), miniABCA4-2 (MG-2), miniABCA4-4 (MG-4), or miniABCA4-5 (MG-5). The injection was done either 10 days or 30 days postnatal (P10 or P30), and RPE autofluorescence was measured at the indicated time of age on the graph. Data indicate that several miniABCA4 constructs significantly reduced RPE autofluorescence compared to *Abca4*$^{-/-}$ mice not receiving

the injections, and that injections at P30 resulted in better improvement of RPE autofluorescence compared to injections at P10.

Example 2: miniABC4 gene constructs MG-7 to MG-11

This example describes additional ABCA4 minigene designs, referred to as MG-7 through MG-11, and production of AAV vector (and rAAVs) expressing these ABCA4 minigenes. Expression and stability of miniABCA4 proteins were tested by expressing them as MYC-tagged versions of the proteins in cultured HEK293 cells (FIG. 5). Tubulin was used as a loading control. FIG. 4 and SEQ ID NOs: 15-19 show embodiments of MiniABCA4 constructs MG-7 through MG-11. SEQ ID NOs: 20-24 show embodiments of MiniABCA4 proteins for MG-7 through MG-11.

SEQUENCES

In some embodiments, an isolated nucleic acid or vector (*e.g.*, rAAV vector) described by the disclosure comprises or consists of a sequence set forth in any one of SEQ ID NOs: 3-8 and 15-19. In some embodiments, an isolated nucleic acid or vector (*e.g.*, rAAV vector) described by the disclosure comprises or consists of a sequence that is complementary (*e.g.*, the complement of) a sequence set forth in any one of SEQ ID NOs: 3-8 and 15-19. In some embodiments, an isolated nucleic acid or vector (*e.g.*, rAAV vector) described by the disclosure comprises or consists of a sequence that is a reverse complement of a sequence set forth in any one of SEQ ID NOs: 3-8 and 15-19. In some embodiments, an isolated nucleic acid or vector (*e.g.*, rAAV vector) described by the disclosure comprises or consists of a portion of a sequence set forth in any one of SEQ ID NOs: 3-8 and 15-19. A portion may comprise at least 25%, 50%, 60%, 70%, 80%, 90%, 95%, or 99% of a sequence set forth in any one of SEQ ID NOs: 3-8 and 15-19. In some embodiments, a nucleic acid sequence described by the disclosure is a nucleic acid sense strand (e.g., 5' to 3' strand), or in the context of a viral sequences a plus (+) strand. In some embodiments, a nucleic acid sequence described by the disclosure is a nucleic acid antisense strand (e.g., 3' to 5' strand), or in the context of viral sequences a minus (-) strand.

EQUIVALENTS

While several embodiments of the present invention have been described and illustrated herein, those of ordinary skill in the art will readily envision a variety of other means and/or structures for performing the functions and/or obtaining the results and/or one or more of the advantages described herein, and each of such variations and/or modifications is deemed to be within the scope of the present invention. More generally, those skilled in the art will readily appreciate that all parameters, dimensions, materials, and configurations described herein are meant to be exemplary and that the actual parameters, dimensions, materials, and/or configurations will depend upon the specific application or applications for which the teachings of the present invention is/are used. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. It is, therefore, to be understood that the foregoing embodiments are presented by way of example only and that, within the scope of the appended claims and equivalents thereto, the invention may be practiced otherwise than as specifically described and claimed. The present invention is directed to each individual feature, system, article, material, and/or method described herein. In addition, any combination of two or more such features, systems, articles, materials, and/or methods, if such features, systems, articles, materials, and/or methods are not mutually inconsistent, is included within the scope of the present invention.

The indefinite articles “a” and “an,” as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to mean “at least one.”

The phrase “and/or,” as used herein in the specification and in the claims, should be understood to mean “either or both” of the elements so conjoined, i.e., elements that are conjunctively present in some cases and disjunctively present in other cases. Other elements may optionally be present other than the elements specifically identified by the “and/or” clause, whether related or unrelated to those elements specifically identified unless clearly indicated to the contrary. Thus, as a non-limiting example, a reference to “A and/or B,” when used in conjunction with open-ended language such as “comprising” can refer, in one embodiment, to A without B (optionally including elements other than B); in another embodiment, to B without A (optionally including elements other than A); in yet another embodiment, to both A and B (optionally including other elements); etc.

As used herein in the specification and in the claims, "or" should be understood to have the same meaning as "and/or" as defined above. For example, when separating items in a list, "or" or "and/or" shall be interpreted as being inclusive, i.e., the inclusion of at least one, but also including more than one, of a number or list of elements, and, optionally, additional unlisted items. Only terms clearly indicated to the contrary, such as "only one of" or "exactly one of," or, when used in the claims, "consisting of," will refer to the inclusion of exactly one element of a number or list of elements. In general, the term "or" as used herein shall only be interpreted as indicating exclusive alternatives (i.e. "one or the other but not both") when preceded by terms of exclusivity, such as "either," "one of," "only one of," or "exactly one of." "Consisting essentially of," when used in the claims, shall have its ordinary meaning as used in the field of patent law.

As used herein in the specification and in the claims, the phrase "at least one," in reference to a list of one or more elements, should be understood to mean at least one element selected from any one or more of the elements in the list of elements, but not necessarily including at least one of each and every element specifically listed within the list of elements and not excluding any combinations of elements in the list of elements. This definition also allows that elements may optionally be present other than the elements specifically identified within the list of elements to which the phrase "at least one" refers, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, "at least one of A and B" (or, equivalently, "at least one of A or B," or, equivalently "at least one of A and/or B") can refer, in one embodiment, to at least one, optionally including more than one, A, with no B present (and optionally including elements other than B); in another embodiment, to at least one, optionally including more than one, B, with no A present (and optionally including elements other than A); in yet another embodiment, to at least one, optionally including more than one, A, and at least one, optionally including more than one, B (and optionally including other elements); etc.

In the claims, as well as in the specification above, all transitional phrases such as "comprising," "including," "carrying," "having," "containing," "involving," "holding," and the like are to be understood to be open-ended, i.e., to mean including but not limited to. Only the transitional phrases "consisting of" and "consisting essentially of" shall be closed or semi-closed transitional phrases, respectively, as set forth in the United States Patent Office Manual of Patent Examining Procedures, Section 2111.03.

Use of ordinal terms such as "first," "second," "third," etc., in the claims to modify a claim element does not by itself connote any priority, precedence, or order of one claim element over another or the temporal order in which acts of a method are performed, but are used merely as labels to distinguish one claim element having a certain name from another element having a same name (but for use of the ordinal term) to distinguish the claim elements.

The terms "about" and "substantially" preceding a numerical value represent ±10% of the recited numerical value.

CLAIMS

What is claimed is:

1. An isolated nucleic acid comprising a transgene encoding a ABCA4 minigene having the sequence set forth in any one of SEQ ID NOs: 3-8 and 15-19.

2. The isolated nucleic acid of claim 1, wherein the transgene further comprises a promoter operably linked to the ABCA4 minigene sequence.

3. The isolated nucleic acid of claim 2, wherein the promoter is a constitutive promoter, inducible promoter, or a tissue-specific promoter, optionally wherein the tissue specific promoter is a photoreceptor-specific promoter.

4. The isolated nucleic acid of any one of claims 1 to 3, wherein the transgene is flanked by adeno-associated virus (AAV) inverted terminal repeats (ITRs).

5. The isolated nucleic acid of claim 4, wherein at least one of the ITRs is an AAV2 ITR.

6. The isolated nucleic acid of claim 4 or 5, wherein at least one ITR lacks a terminal resolution site, optionally wherein the ITR is a ΔITR.

7. An isolated nucleic acid comprising a transgene having a nucleic acid sequence encoding a ABCA4 protein, wherein the ABCA4 protein comprises an amino acid sequence as set forth in any one of SEQ ID NOs: 9-14 and 20-24.

8. The isolated nucleic acid of claim 7, wherein the transgene further comprises a promoter operably linked to the nucleic acid sequence encoding the ABCA4 protein.

9. The isolated nucleic acid of claim 8, wherein the promoter is a constitutive promoter, inducible promoter, or a tissue-specific promoter, optionally wherein the tissue specific promoter is a photoreceptor-specific promoter.

10. The isolated nucleic acid of any one of claims 7 to 9, wherein the transgene is flanked by adeno-associated virus (AAV) inverted terminal repeats (ITRs).

11. The isolated nucleic acid of claim 10, wherein at least one of the ITRs is an AAV2 ITR.

12. The isolated nucleic acid of claim 10 or 11, wherein at least one ITR lacks a terminal resolution site, optionally wherein the ITR is a ΔITR.

13. A vector comprising the isolated nucleic acid of any one of claims 1 to 12.

14. The vector of claim 13, wherein the vector is a plasmid DNA, or closed-ended DNA, or lipid/DNA nanoparticle, or a viral vector.

15. The vector of claim 14, wherein the viral vector is an adeno-associated virus (AAV) vector, adenoviral (Ad) vector, lentiviral vector, retroviral vector, or Baculovirus vector.

16. A host cell comprising the isolated nucleic acid of any one of claims 1 to 12, or the vector of any one of claims 13 to 15.

17. The host cell of claim 16, wherein the cell is a mammalian (human) cell, bacterial cell, yeast cell, or insect cell.

18. A recombinant adeno-associated virus (rAAV) comprising:

(i) the isolated nucleic acid of any one of claims 1 to 12; and

(ii) an AAV capsid protein.

19. The rAAV of claim 18, wherein the capsid protein has a tropism for ocular cells.

20. The rAAV of claim 18 or 19, wherein the capsid protein is AAV8 capsid protein.

21. The rAAV of any one of claims 18 to 20, wherein the rAAV is formulated for delivery to the eye, optionally wherein the rAAV is formulated for delivery to photoreceptor cells or retinal pigmented epithelium (RPE).

22. A composition comprising the isolated nucleic acid of any one of claims 1 to 12, or the rAAV of any one of claims 18 to 21, and a pharmaceutically acceptable excipient.

23. A method for delivering a transgene to a cell, the method comprising administering the isolated nucleic acid of any one of claims 1 to 12, or the rAAV of any one of claims 18 to 21, to a cell.

24. The method of claim 23, wherein the cell is in a subject, optionally a mammalian (human) subject.

25. The method of claim 23 or 24, wherein the cell is an eye cell.

26. The method of claim 25, wherein the eye cell is a photoreceptor cell or retinal pigmented epithelium (RPE).

27. A method for treating Stargardt Disease in a subject in need thereof, the method comprising administering the isolated nucleic acid of any one of claims 1 to 12, or the rAAV of any one of claims 18 to 21 to the subject.

28. The method of claim 27, wherein the subject is a mammal, optionally wherein the subject is a human.

29. The method of claim 27 or 28, wherein the subject is characterized by having one or more mutations in a *ABCA4* gene.

30. The method of any one of claims 27 to 29, wherein the administration is via injection, optionally subretinal injection or intravitreal injection or suprachoroidal injection.

31. The method of any one of claims 27 to 29, wherein the administration is topical administration to the eye of the subject.

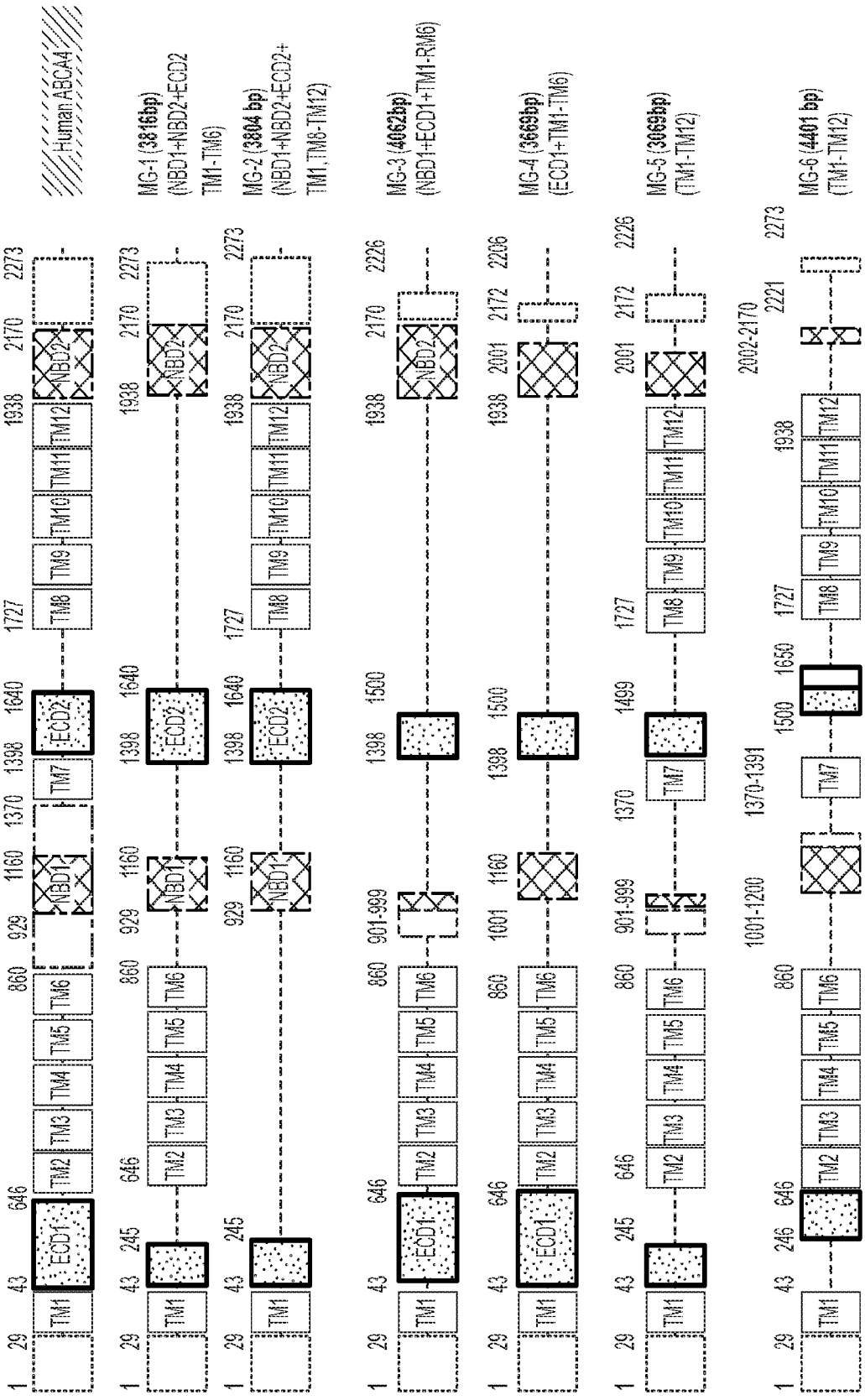
Human ABCA4
MG-1 (3816bp)
(NBD1+NBD2+ECD2
TM1-TM6)
MG-2 (3804 bp)
(NBD1+NBD2+ECD2+
TM1,TM8-TM12)
MG-3 (4062bp)
(NBD1+ECD1+TM1-RM6)
MG-4 (3669bp)
(ECD1+TM1-TM6)
MG-5 (3069bp)
(TM1-TM12)
MG-6 (4401 bp)
(TM1-TM12)
TM1
ECD1
TM2
TM3
TM4
TM5
TM6
NBD1
TM7
ECD2
TM8
TM9
TM10
TM11
TM12
NBD2

FIG. 1

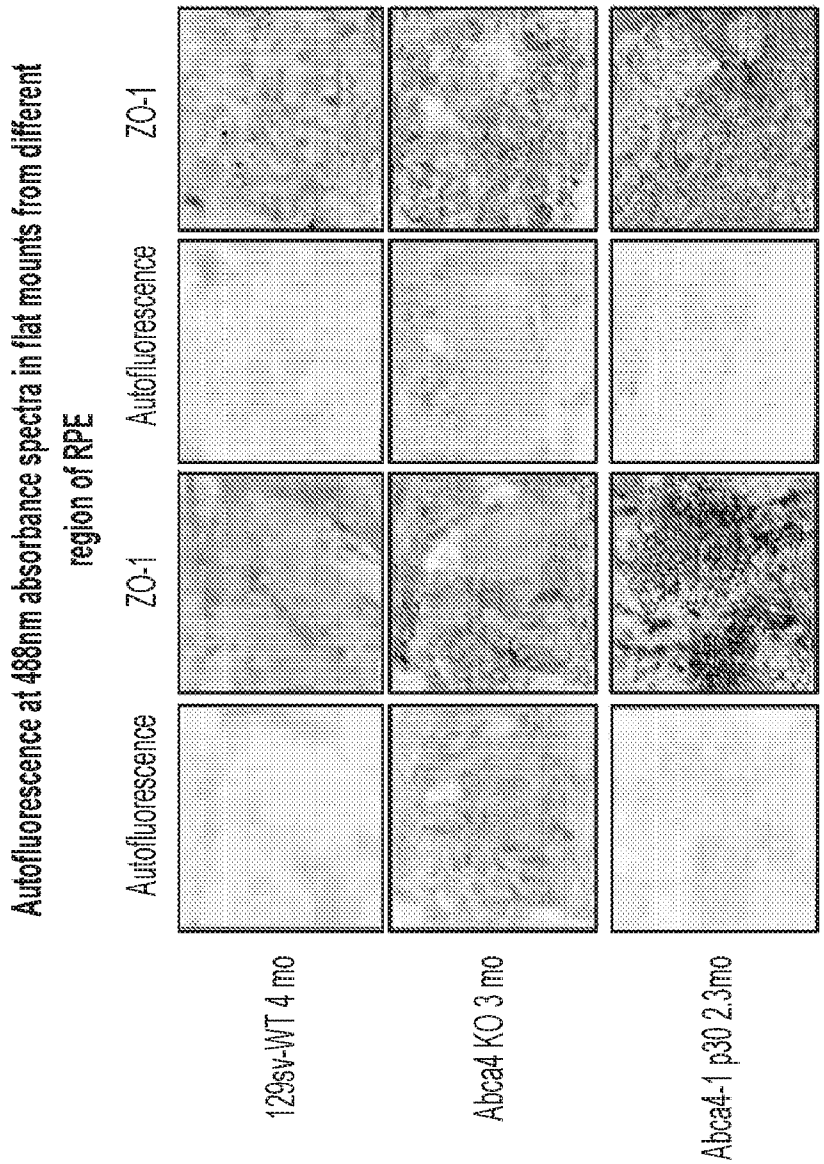
Autofluorescence at 488nm absorbance spectra in flat mounts from different region of RPE
Autofluorescence
ZO-1
Autofluorescence
ZO-1
129sv-WT 4 mo
Abca4 KO 3 mo
Abca4-1 p30 2.3mo

FIG. 2

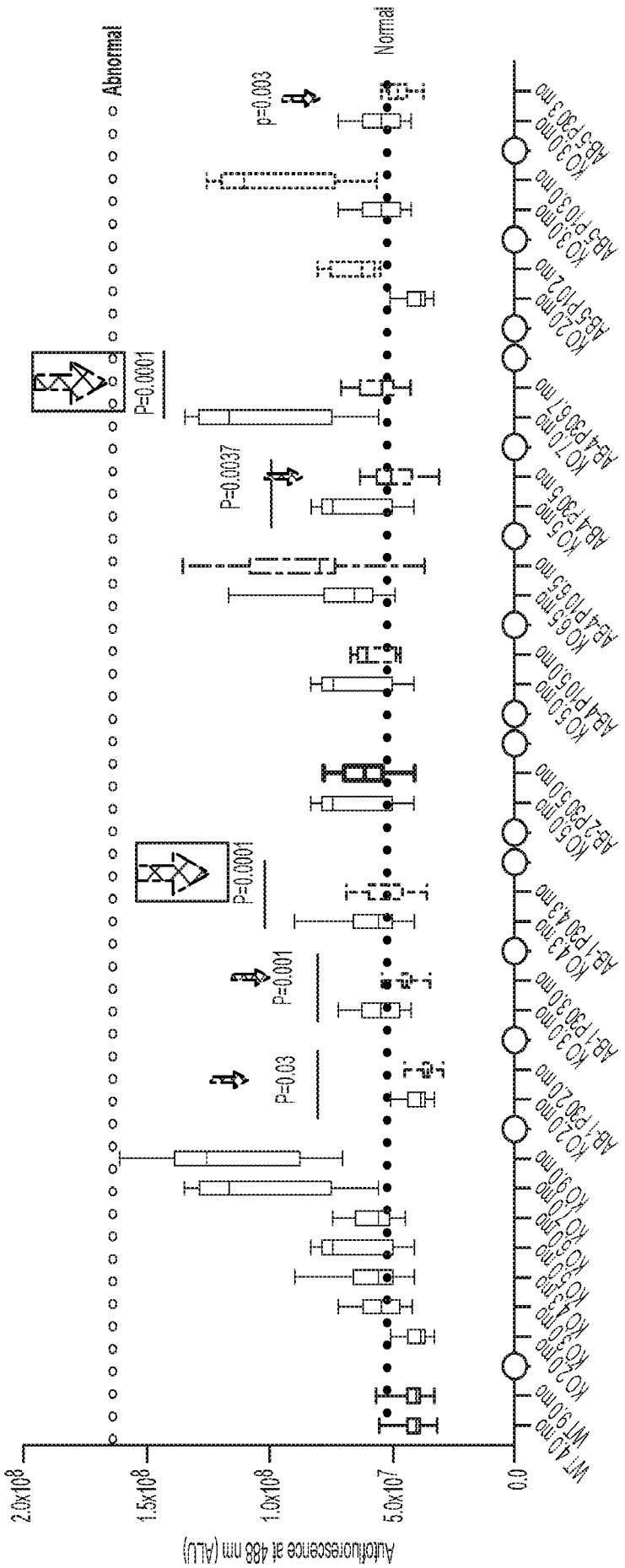
Abnormal
Normal
p=0.003
P=0.0001
P=0.0037
P=0.0001
P=0.001
P=0.03
2.0x10^8
1.5x10^8
1.0x10^8
5.0x10^7
0.0
Autofluorescence at 488 nm (ALU)
WT 4.0mo
WT 9.0mo
KO 0.0mo
KO 4.3mo
KO 5.0mo
KO 6.0mo
KO 7.0mo
KO 8.0mo
KO 9.0mo
KO 2.0mo
KO 3.0mo
KO 6.5mo
KO 7.0mo
KO 5.0mo
KO 2.0mo
KO 3.0mo
KO 3.0mo
KO 3.0mo

FIG. 3

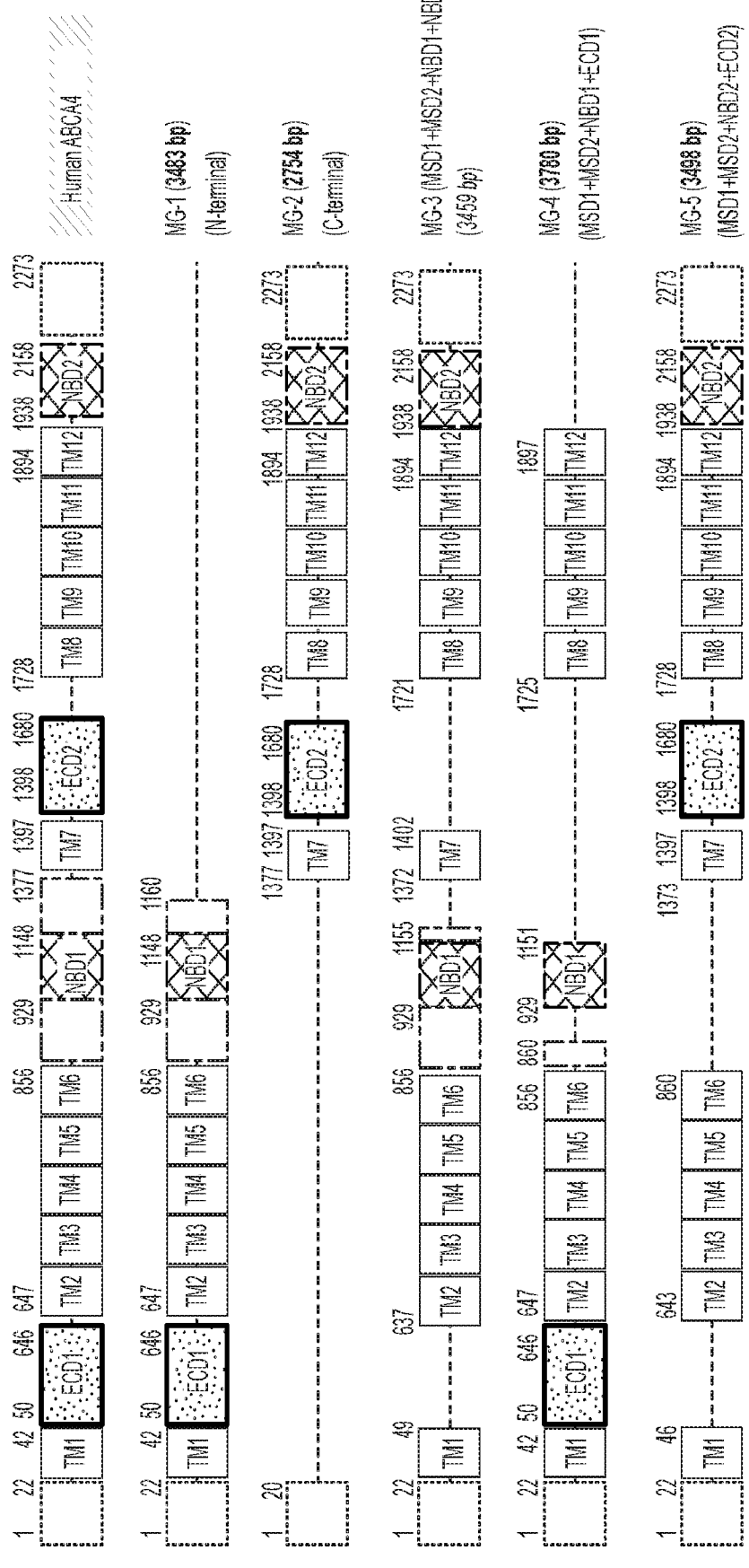
Human ABCA4
MG-1 (3483 bp)
(N-terminal)
MG-2 (2754 bp)
(C-terminal)
MG-3 (MSD1+MSD2+NBD1+NBD2)
(3459 bp)
MG-4 (3780 bp)
(MSD1+MSD2+NBD1+ECD1)
MG-5 (3498 bp)
(MSD1+MSD2+NBD2+ECD2)
TM1
ECD1
TM2
TM3
TM4
TM5
TM6
NBD1
TM7
ECD2
TM8
TM9
TM10
TM11
TM12
NBD2

FIG. 4

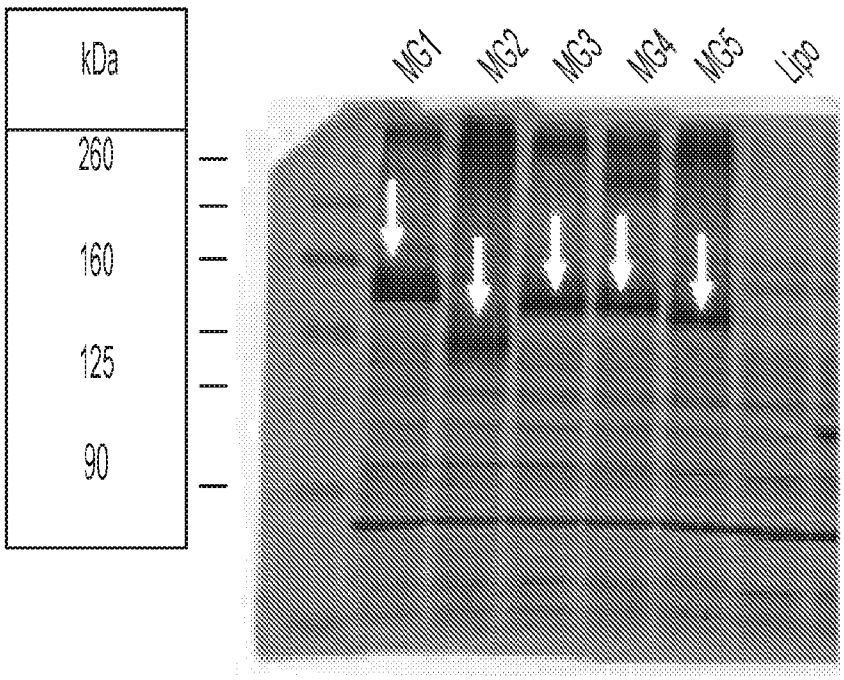
kDa
260
160
125
90
MG1
MG2
MG3
MG4
MG5
Lipo
MYC-MG
Gamma tubulin

FIG. 5