

CLAIMS

1. A nucleic acid encoding a mutant mammalian RPE65 protein or portion thereof comprising an amino acid sequence having at least 90% identity with at least one of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, and SEQ ID NO: 17, and having an amino acid substitution in at least one of positions 170 and 297; the mutant mammalian RPE65 protein or portion thereof having isomerohydrolase activity.
2. A nucleic acid according to Claim 1:
 - (i) in which the amino acid substitution in position 170 is Lys, Arg, or His; and the amino acid substitution in position 297 is Gly or Ala; or
 - (ii) in which the amino acid substitution in position 170 is Lys, Arg, or His; the amino acid substitution in position 297 is Gly or Ala and in which the mutant mammalian RPE65 protein or portion thereof further comprises an amino acid substitution in at least one of positions 2, 3, and 26, optionally in which the amino acid substitution in position 2 is Tyr, Phe, or His; the amino acid substitution in position 3 is Ser, Thr, or Cys; and the amino acid substitution in position 26 is Val, Ala, or Ile; or
 - (iii) in which the mutant mammalian RPE65 protein or portion thereof comprises amino acid substitutions in positions 2, 3, 26, 170, and 297; or
 - (iv) disposed within a vector; or
 - (v) disposed within a vector, in which the vector comprises: a promoter or enhancer sequence operatively-linked to the nucleic acid; or a stop codon or a poly-A sequence located 3' of the nucleic acid; or
 - (vi) disposed within a vector, in which the vector comprises an RPE65 promoter sequence operatively-linked to the nucleic acid; or
 - (vii) disposed within a vector, in which the vector comprises a CMV enhancer/chicken β -actin promoter sequence operatively-linked to the nucleic acid; or
 - (viii) disposed within a vector, in which the vector is selected from the group consisting of adenovirus, adeno-associated virus (AAV), nanoparticles, plasmids, and lentivirus.
3. An adeno-associated viral (AAV) vector, comprising:

- (i) a nucleic acid encoding a mutant mammalian RPE65 protein or portion thereof comprising an amino acid sequence having at least 90% identity with at least one of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, and SEQ ID NO: 17, and having an amino acid substitution in at least one of positions 170 and 297; the mutant mammalian RPE65 protein or portion thereof having isomerohydrolase activity; or
 - (ii) a nucleic acid according to Claim 1 or 2 (vii).
- 4. The AAV vector of claim 3 (i) in which
 - (i) the amino acid substitution in position 170 is Lys, Arg, or His; and the amino acid substitution in position 297 is Gly or Ala; or
 - (ii) the mutant mammalian RPE65 protein or portion thereof further comprises an amino acid substitution in at least one of positions 2, 3, and 26; or
 - (iii) the mutant mammalian RPE65 protein or portion thereof comprises an amino acid substitution in at least one of positions 2, 3, and 26 and the amino acid substitution in position 2 is Tyr, Phe, or His; the amino acid substitution in position 3 is Ser, Thr, or Cys; and the amino acid substitution in position 26 is Val, Ala, or Ile; or
 - (iv) the mutant mammalian RPE65 protein or portion thereof comprises amino acid substitutions in positions 2, 3, 26, 170, and 297.
- 5. An AAV vector according to Claim 3 or 4:
 - (i) where the vector comprises one or more AAV inverted terminal repeat (ITR) sequences; or
 - (ii) where the vector comprises one or more AAV inverted terminal repeat (ITR) sequences and in which the one or more AAV ITR sequences comprises an ITR sequence of any of: AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, Rh10, Rh74 or AAV-2i8 AAV serotypes, or a mixture of ITR sequences therefrom; or
 - (iii) where the vector comprises one or more AAV inverted terminal repeat (ITR) sequences and in which the AAV vector comprises an AAV capsid sequence, optionally in which the AAV capsid sequence comprises a VP1, VP2 and/or VP3 capsid sequence having at least 90% identity to the VP1, VP2 and/or VP3 sequences of AAV1, AAV2,

AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, Rh10, Rh74 or AAV-2i8.

6. A cell that produces an AAV vector according to any of Claims 3 to 5.

7. A therapeutically-effective amount of a vector comprising a nucleic acid which encodes a mutant mammalian RPE65 protein or portion thereof for use in a gene therapy method of treating a condition related to retinal degeneration in a mammalian subject in need of such treatment, the method comprising:

administering to the subject the therapeutically-effective amount of a vector

comprising a nucleic acid which encodes a mutant mammalian RPE65 protein or portion thereof, in which the mutant mammalian RPE65 protein or portion thereof is expressed *in vivo* in retinal cells of the subject and has isomerohydrolase activity.

8. A therapeutically-effective amount of a vector for use in a gene therapy method according to Claim 7:

(i) in which the mutant mammalian RPE65 protein mutant or portion thereof encoded by the nucleic acid comprises an amino acid sequence having at least 90% identity with at least one of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, and SEQ ID NO: 17, and having an amino acid substitution in at least one of positions 170 and 297; or

(ii) in which the mutant mammalian RPE65 protein mutant or portion thereof encoded by the nucleic acid comprises an amino acid sequence having at least 90% identity with at least one of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, and SEQ ID NO: 17, having an amino acid substitution in at least one of positions 170 and 297 and in which the amino acid substitution in position 170 is Lys, Arg, or His; and the amino acid substitution in position 297 is Gly or Ala; or

(iii) in which the mutant mammalian RPE65 protein mutant or portion thereof encoded by the nucleic acid comprises an amino acid sequence having at least 90% identity with at least one of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, and SEQ ID NO: 17, having an amino acid substitution in at least one of positions 170 and 297 and in which the

mutant mammalian RPE65 protein or portion thereof further comprises an amino acid substitution in at least one of positions 2, 3, and 26; or

(iv) in which the mutant mammalian RPE65 protein mutant or portion thereof encoded by the nucleic acid comprises an amino acid sequence having at least 90% identity with at least one of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, and SEQ ID NO: 17, having an amino acid substitution in at least one of positions 170 and 297 and further comprising an amino acid substitution in position 2 which is Tyr, Phe, or His; an amino acid substitution in position 3 which is Ser, Thr, or Cys; and an amino acid substitution in position 26 which is Val, Ala, or Ile; or

(v) in which the mutant mammalian RPE65 protein or portion thereof has greater isomerohydrolase activity than non-mutated RPE65 protein; or

(vi) in which the mutant mammalian RPE65 protein or portion thereof has greater isomerohydrolase activity than human RPE65 having the amino acid sequence set forth in SEQ ID NO: 1 or bovine RPE65 having the amino acid sequence set forth in SEQ ID NO: 9; or

(vii) in which the vector further comprises a promoter or enhancer sequence operatively-linked to the nucleic acid encoding the mutant mammalian RPE65 protein; or further comprises a stop codon or a poly-A sequence located 3' of the nucleic acid encoding the mutant mammalian RPE65 protein; or

(viii) in which the vector is selected from the group consisting of adenovirus, adeno-associated virus (AAV), nanoparticles, plasmids, and lentivirus; or

(ix) in which the vector comprises an AAV vector which comprises one or more AAV inverted terminal repeat (ITR) sequences, optionally in which the AAV ITR sequence comprises an ITR sequence of any of: AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, Rh10, Rh74 or AAV-2i8 AAV serotypes, or a mixture of ITR sequences therefrom; or

(x) in which the vector comprises an AAV capsid sequence, optionally in which the AAV capsid sequence comprises a VP1, VP2 and/or VP3 capsid sequence having at least 90% identity to the VP1, VP2 and/or VP3 sequences of AAV1, AAV2, AAV3,

AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, Rh10, Rh74 or AAV-2i8; or

(xi) in which the condition related to retinal degeneration is Leber's Congenital Amaurosis or Retinitis pigmentosa.

- 5 9. A therapeutically-effective amount of at least one mutant mammalian RPE65 protein or a portion thereof for use in a method of treating a condition related to retinal degeneration in a subject in need of such treatment, the method comprising:

administering to the subject the therapeutically-effective amount of at least one mutant mammalian RPE65 protein or a portion thereof, thereby mitigating the condition related
10 to retinal degeneration in the subject, the mutant mammalian RPE65 protein or portion thereof having isomerohydrolase activity.

10. A therapeutically-effective amount of at least one mutant mammalian RPE65 protein or portion thereof for use in a method of treating a condition related to retinal degeneration according to Claim 9:

15 (i) in which the mutant mammalian RPE65 protein or portion thereof comprises an amino acid sequence having at least 90% identity with at least one of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, and SEQ ID NO: 17; the mutant mammalian RPE65 protein or portion thereof having an amino acid substitution in at least one of positions
20 170 and 297; or

(ii) in which the mutant mammalian RPE65 protein or portion thereof comprises an amino acid sequence having at least 90% identity with at least one of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, and SEQ ID NO: 17; the mutant mammalian RPE65
25 protein or portion thereof having an amino acid substitution in at least one of positions 170 and 297 and in which the amino acid substitution in position 170 is Lys, Arg, or His; and the amino acid substitution in position 297 is Gly or Ala; or

(iii) in which the mutant mammalian RPE65 protein or portion thereof comprises an amino acid sequence having at least 90% identity with at least one of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, and SEQ ID NO: 17; the mutant mammalian RPE65
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protein or portion thereof having an amino acid substitution in at least one of positions 170 and 297 and in which the mutant mammalian RPE65 protein further comprises an amino acid substitution in at least one of positions 2, 3, and 26; or

(iv) in which the mutant mammalian RPE65 protein or portion thereof comprises an amino acid sequence having at least 90% identity with at least one of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, and SEQ ID NO: 17; the mutant mammalian RPE65 protein or portion thereof having an amino acid substitution in at least one of positions 170 and 297 and further comprising an amino acid substitution in position 2 which is Tyr, Phe, or His; an amino acid substitution in position 3 which is Ser, Thr, or Cys; and an amino acid substitution in position 26 which is Val, Ala, or Ile; or

(v) in which the condition related to retinal degeneration is Leber's Congenital Amaurosis or Retinitis pigmentosa.

11. A therapeutically-effective amount of a vector comprising a nucleic acid which encodes a mutant mammalian RPE65 protein or portion thereof or a therapeutically-effective amount of at least one mutant mammalian RPE65 protein or portion thereof for use in a method according to any of Claims 7 to 10:

(i) in which the subject is human; or

(ii) in which the subject is less than 18 years old, less than 15 years old, less than 12 years old, less than 10 years old, between about 8 to 10 years old, between about 6 to 10 years old, between about 4 to 6 years old, between about 1 to 3 years old or is less than 1 year old or less; or

(iii) in which the subject has or is at risk of having retinal degeneration or dystrophy caused by RPE65 mutation; or

(iv) in which the vector is administered to one or more eyes of the subject; or

(v) in which the vector is administered to the subretinal space and/or suprachoroidal space of one or more eyes of the subject; or

(vi) in which the vector comprises an AAV vector and in which 1×10^8 or more AAV vector genomes are administered to one or more eyes of the subject; or

- (vii) in which the vector comprises an AAV vector and in which about 1×10^8 to about 1×10^{14} AAV vector genomes are administered to one or more eyes of the subject; or
- (viii) in which the vector comprises an AAV vector and in which about 1×10^9 to about 1×10^{13} AAV vector genomes are administered to one or more eyes of the subject; or
- 5 (ix) in which the vector or mutant mammalian RPE65 protein or portion thereof is administered in a volume of about 10 microliters (μ l) to about 1,000 μ l; or
- (x) in which the vector or mutant mammalian RPE65 protein or portion thereof is administered in a volume of about 50 μ l to about 800 μ l; or
- 10 (xi) in which the vector or mutant mammalian RPE65 protein or portion thereof is administered in a volume of about 100 μ l to about 600 μ l; or
- (xii) in which the vector or mutant mammalian RPE65 protein or portion thereof is administered in a volume of about 200 μ l to about 500 μ l; or
- (xiii) in which the vector or mutant mammalian RPE65 protein or portion thereof is delivered or introduce in cells of the retinal pigment epithelium of one or more eyes of the subject.
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- 12. A mutant mammalian RPE65 protein or portion thereof, comprising an amino acid sequence having at least 90% identity with at least one of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, and SEQ ID NO: 17, and having an amino acid substitution in at least one of positions 170 and 297; the mutant mammalian RPE65 protein or portion thereof having isomerohydrolase activity.
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- 13. A mutant mammalian RPE65 protein or portion thereof according to Claim 12, in which the amino acid substitution in position 170 is Lys, Arg, or His; and the amino acid substitution in position 297 is Gly or Ala.
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- 14. A mutant mammalian RPE65 protein or portion thereof according to Claim 12:
 - (i) further comprising an amino acid substitution in at least one of positions 2, 3, and 26; or
 - (ii) in which the mutant mammalian RPE65 protein or portion thereof comprises an amino acid substitution in at least one of positions 2, 3, and 26 and the amino acid

substitution in position 2 is Tyr, Phe, or His; the amino acid substitution in position 3 is Ser, Thr, or Cys; and the amino acid substitution in position 26 is Val, Ala, or Ile; or

(iii) comprising amino acid substitutions in positions 2, 3, 26, 170, and 297; or

5 (iv) in which the mutant mammalian RPE65 protein or portion thereof has greater isomerohydrolase activity than non-mutated RPE65 protein; or

(v) in which the mutant mammalian RPE65 protein or portion thereof has greater isomerohydrolase activity than human RPE65 having the amino acid sequence set forth in SEQ ID NO: 1 or bovine RPE65 having the amino acid sequence set forth in SEQ ID NO: 9.

10 15. A pharmaceutical composition comprising a mutant mammalian RPE65 protein or portion thereof according to Claim 12 disposed in a pharmaceutically-acceptable carrier or vehicle.