function (Bainbridge et al. 2008, Maguire et al. 2008, 2009, Hauswirth et al. 2008, Cideciyan et al 2008, 2009) and activity in the visual cortex (Ashtari et al. 2011).

However, in a previous investigation into gene-therapy replacement of RPE65 in the RPE (Bainbridge et al. 2008), the authors reported that though there were improvements to retinal sensitivity and visual-guided mobility in one patient upon treatment, there were no significant improvements in visual acuity and peripheral field vision. Additionally, there were no significant improvements in all measured parameters in the other treated patients. Improvements in electroretinographic responses have yet to be reported in any study. It has also been observed that treating RPE65-/- dogs at 30 months with AAV2/4 vector-mediated therapy did not rescue vision or retinal function (Le Meur et al. 2007).

Therefore, there is a need for improvements in gene-replacement therapies for retinal dystrophies, especially inherited retinal dystrophies, in particular for disorders of the retinal pigment epithelium (RPE) such as Leber congenital amaurosis.

Summary

The present invention is based on the creation of an optimised promoter for expressing genes in the RPE. This optimised promoter is shown in SEQ ID NO: 2. The promoter comprises nucleotides 865 to 1614 of the human RPE65 promoter used in Bainbridge et al. (2008), which is shown in SEQ ID NO:1. The use of this promoter in a vector to drive expression of a control gene in the RPE was effective with an expression level approximately 20x higher than that with the original RPE65 promoter. The optimised RPE65 promoter was both more potent than the original RPE65 promoter and more stringent in driving expression in RPE cells in relation to photoreceptor cells.

In addition, the native coding sequence of RPE65, which is shown in SEQ ID NO: 3, has been optimised to give an optimised sequence which is shown in SEQ ID NO: 4. The optimised RPE65 sequence was tested alongside the original RPE65 sequence *in vitro* in (human) 293T cells to determine the effect on RPE65 protein production levels, after transfection of an AAV2/8 expression plasmid carrying the ubiquitous CMV promoter. *In vitro* protein production in 293T cells after optimisation of the *RPE65* coding sequence showed a seven-fold increase in the amount of RPE65 protein produced from the vector carrying the optimised coding sequence compared to the wild type coding sequence.

The optimised promoter and optimised RPE65 sequence have also been combined in a vector to test their ability to rescue retinal function *in vivo* in RPE65-deficient mice. Efficacy of rescue was compared against the clinical grade vector previously used in Bainbridge et al (2008). Lower vector doses were administered to allow comparison of treatment efficacy under limiting circumstances. b-wave amplitude was used as a measure of rescue. Surprisingly, the b-wave amplitudes from the eyes treated with the optimised vector were as high as or higher than amplitudes from eyes injected with a 300-fold higher dose of the original vector. Optimisation of the promoter and/or the coding sequence according to the invention is therefore highly advantageous compared to using the native sequences.

Accordingly, the invention refers to an expression construct comprising a retinal pigment epithelium (RPE)-specific promoter and an operably linked polynucleotide sequence, wherein the RPE-specific promoter consists of (a) a sequence of no more than 800 contiguous nucleotides from SEQ ID NO:1, wherein the sequence comprises nucleotides 12-761 of SEQ ID NO: 2; or (b) a sequence having at least 90% sequence identity to said sequence of (a), wherein the sequence comprises nucleotides 12-761 of SEQ ID NO: 2, and wherein the operably linked polynucleotide sequence comprises the sequence of SEQ ID NO: 4. In another aspect, the present invention refers to a retinal pigment epithelium (RPE)-specific promotor as defined in the present invention. In yet another aspect, the present invention refers to an expression construct comprising the promoter of the present invention, operably linked to a sequence to be expressed in an RPE-specific manner. In one aspect, the present invention refers to a vector comprising an expression construct according to the present invention or the promotor according to the present invention. In another aspect, the present invention refers to a host cell that contains a vector according to the present invention or produces a viral vector according to the present invention, optionally wherein the host cell is a HEK293 or HEK293T cell. In yet another aspect, the present invention refers to a pharmaceutical composition comprising a vector according to the present invention and a pharmaceutically acceptable carrier. In still another aspect, the present invention refers to the use of a vector according to the present invention in the manufacture of a medicament for the treatment or prevention of retinal dystrophy. In another aspect, the present invention refers to the use of a vector according to the present invention, in the manufacture of a medicament for the treatment or prevention of retinal dystrophy, wherein the retinal dystrophy is Leber

congenital amaurosis (LCA), age-related macular degeneration (AMD), oculo-cutaneous type 1 or Nettleship-Falls type ocular albinism or MERTK deficiency. In yet another aspect, the present invention refers to the use of a vector according to the present invention in the manufacture of a medicament for the treatment or prevention of retinal dystrophy, wherein the vector according to the present invention is to be administered to a patient by direct retinal, subretinal or intravitreal injection, optionally wherein said vector is to be administered directly into the retinal, subretinal space or intravitreal space. Also described herein is a retinal pigment epithelium (RPE)-specific promoter which comprises: (a) a sequence of contiguous nucleotides from SEQ ID NO:1 that confers RPE-specific expression on an operably linked polynucleotide sequence, or (b) a sequence having at least 90% sequence identity to said sequence of (a) and that retains RPE-specific promoter activity.

In another related example, disclosed herein is an expression construct comprising a promoter of the invention, operably linked to a sequence to be expressed in an RPE-specific manner.

In another related example, disclosed herein is a vector comprising a promoter described herein or an expression cassette described herein.

In another related example, disclosed herein is a host cell that contains a vector described herein or produces a viral vector described herein.

In another related example, disclosed herein is a pharmaceutical composition comprising a vector described herein and a pharmaceutically acceptable carrier.

In another related example, disclosed herein is a vector described herein for use in a method of preventing or treating retinal dystrophy.

In another related example, disclosed herein is the use of a vector described herein in the manufacture of a medicament for the treatment or prevention of retinal dystrophy.

In another related example, disclosed herein is a method of treating or preventing retinal dystrophy in a patient in need thereof, comprising administering a therapeutically effective amount of a vector described herein to said patient.

The disclosure also describes expression constructs and vectors comprising the promoters described herein, as well as pharmaceutical compositions comprising such vectors, and the use of such vectors in treatment or prevention of retinal dystrophies, in particular disorders of the retinal pigment epithelium such as Leber congenital amaurosis.

Brief description of the Figures

Figure 1:GFP expression levels and pattern driven by original RPE65 promoter and new RPE65 promoter configurations in murine retinas following subretinal injection. Assessment of promoter activity using quantitative PCR (A) and protein blot (B). (C) Cryosections of eyes 4 weeks following subretinal injection of either AAV-RPE65-eGFP (top) or AAV-NA-eGFP (bottom).

Figure 2:Western blot assessment (A) and subsequent quantification (B) of *in vitro* RPE65 protein production in 293T cells after optimisation of the *RPE65* coding sequence. The white bar shows RPE65 protein production using the unoptimised *RPE65* coding sequence, the black bar shows RPE65 protein production using the optimised *RPE65* coding sequence (SEQ ID NO:4).

Figure 3:Comparison of treatment efficacy of the optimised vector (AAV5-OptimisedRPE65) and the original vector (AAV2-hRPE65, Bainbridge et al 2008), 4 weeks post treatment. The graph shows average scotopic b-wave amplitudes (mean ± SD) at 4 weeks post-treatment, when both vectors had reached peak expression. The optimised vector comprises the new (NA) RPE65 promoter configuration and the optimised *RPE65* coding sequence.

Brief description of the sequences

SEQ ID NO: 1 shows the DNA sequence of the human RPE65 promoter in the form used in Bainbridge et al (2008)

SEQ ID NO: 2 shows the DNA sequence of the optimised RPE65 promoter fragment

CLAIMS

1. An expression construct comprising a retinal pigment epithelium (RPE)-specific promoter and an operably linked polynucleotide sequence, wherein the RPE-specific promoter consists of:

(a) a sequence of no more than 800 contiguous nucleotides from SEQ ID NO:1, wherein the sequence comprises nucleotides 12-761 of SEQ ID NO: 2; or

(b) a sequence having at least 90% sequence identity to said sequence of (a), wherein the sequence comprises nucleotides 12-761 of SEQ ID NO: 2,

and wherein the operably linked polynucleotide sequence comprises the sequence of SEQ ID NO: 4.

2. The expression construct according to claim 1, wherein the RPE-specific promoter consists of a sequence no more than 800 contiguous nucleotides from SEQ ID NO:1, wherein the sequence comprises nucleotides 12-761 of SEQ ID NO: 2.

3. The expression construct of claim 2, wherein the RPE-specific promoter consists of the sequence of SEQ ID NO: 2 or the sequence of nucleotides 12-761 of SEQ ID NO: 2

4. A retinal pigment epithelium (RPE)-specific promotor as defined in any one of claims 1 to 3.

5. An expression construct comprising the promoter of claim 4, operably linked to a sequence to be expressed in an RPE-specific manner.

6. The expression construct according to claim 5, wherein the operably linked sequence is an RPE65, MERTK, LRAT, TYR, GRP143 or MYO7A coding sequence, or a sequence encoding a neurotrophic factor, an anti-angiogenic polypeptide or a polypeptide with anti-apoptotic effects in the RPE.

7. The expression construct according to claim 6, wherein the operably linked sequence comprises a sequence selected from SEQ ID NOs: 3 to 8, or that has at least 80% sequence identity to any one of SEQ ID NOs: 3 to 8 and has the ability to rescue RPE function.

8. A vector comprising an expression construct according to any one of claims 1 to 3 or 5 to 7 or the promotor according to claim 3.

9. The vector according to claim 8, wherein the vector is a viral vector.

10. The vector according to claim 9, wherein the vector is an adeno-associated virus (AAV) vector or comprises an AAV genome or a derivative thereof, optionally wherein said derivative is a chimeric, shuffled or capsid modified derivative.

11. The vector according to claim 10, wherein said AAV genome is from a naturally derived serotype or isolate or clade of AAV, optionally wherein said AAV genome is from AAV serotype 2 (AAV2), AAV serotype 4 (AAV4), AAV serotype 5 (AAV5) or AAV serotype 8 (AAV8) and/or wherein the capsid is derived from AAV5 or AAV8.

12. The vector according to claim 11, wherein the AAV genome is derived from AAV2 and the capsid is derived from AAV5 or AAV8.

13. A host cell that contains a vector of claim 8 or produces a viral vector of any one of claims 9 to 12, optionally wherein the host cell is a HEK293 or HEK293T cell.

14. A pharmaceutical composition comprising a vector of any one of claims 8 to 12 and a pharmaceutically acceptable carrier.

15. The vector according to any one of claims 8 to 12 for use in a method of preventing or treating retinal dystrophy.

16. Use of a vector according to any one of claims 8 to 12 in the manufacture of a medicament for the treatment or prevention of retinal dystrophy.

17. Use of a vector as defined in claim 16, in the manufacture of a medicament for the treatment or prevention of retinal dystrophy, wherein the retinal dystrophy is Leber congenital amaurosis (LCA), age-related macular degeneration (AMD), oculo-cutaneous type 1 or Nettleship-Falls type ocular albinism or MERTK deficiency.

18. Use of a vector as defined in claim 16 or 17 in the manufacture of a medicament for the treatment or prevention of retinal dystrophy, wherein the vector according to any one of claims 8 to 12 is to be administered to a patient by direct retinal, subretinal or intravitreal injection, optionally wherein said vector is to be administered directly into the retinal, subretinal space or intravitreal space.