voltage-gated channel modifier subfamily V member 2 protein. Mutations in *KCNV2* are associated with cone dystrophy with supernormal rod electroretinogram (ERG), or retinal cone dystrophy type 3B, an autosomal recessive disorder that causes lifelong visual loss combined with a supernormal ERG response to a bright flash of light. The *PDE6H* gene encodes the inhibitory (gamma) subunit of the cone-specific cGMP phosphodiesterase. Mutations in this gene are associated with retinal cone dystrophy type 3A (RCD3A). The *GNAT2* gene encodes the cone-specific alpha subunit of transducin. Mutations in the gene can result in infantile onset cone dystrophy. The *CACNA2D4* gene encodes the calcium channel, voltage-dependent, alpha-2/delta subunit 4. Mutations in the gene can cause non-progressive cone dysfunction (retinal cone dystrophy 4, RCD4).

In age-related macular degeneration (AMD), visual impairment is caused primarily by degeneration of the cone-rich fovea in the central macula. Thus patients lose central vision and acuity, but often have relatively well preserved peripheral macula and thus have some useful residual vision that is limited by the paucity of cones outside the fovea.

There is a need to develop therapies that can improve cone survival and function, in order to treat or prevent retinal disorders such as cone-rod dystrophies.

Summary

The disclosure provides nucleic acids, transcriptional control units (TCUs), optimized gene sequences, expression constructs, and vectors for expressing genes in cone photoreceptors.

The TCUs disclosed herein comprise an M-opsin promoter or a fragment thereof under control of the M/L-opsin Locus Control Region (LCR) and are useful for driving high levels of expression in all three human cone types.

Also provided are expression constructs, comprising a human *CNGA3* gene under control of a TCU optimized for expressing genes in cone photoreceptors, wherein the TCU comprises an M-opsin promoter or fragment thereof under control of the M/L-opsin Locus Control Region.

In some examples, the TCUs and expression constructs contain a mutation of 6 bp immediately downstream of the transcription start site in the M-opsin promoter or fragment thereof (mutation "M8"), wherein the mutation may increase the treatment effect of vectors and expression constructs containing this mutation over time.

Further provided is a codon-optimized sequence of the CNGA3 gene, which is provided as SEQ ID NO:8.

Also provided are vectors, such as viral vectors, comprising the expression constructs disclosed herein. The expression construct is preferably delivered using a vector derived from adenovirus serotype 8 (AAV8) or an alternative strong AAV serotype.

The disclosure also provides methods of using the nucleic acids, transcriptional control units (TCUs), optimized gene sequences, expression constructs, and vectors for the treatment and/or prevention of retinal disorders or dystrophies, including but not limited to cone dystrophies such as Achromatopsia.

Accordingly, in one aspect the disclosure provides:

a transcriptional control unit (TCU) of up to 2500 nucleotides in length comprising in a 5' to 3' direction:

(a) a Locus Control Region (LCR) comprising

(i) SEQ ID NO 1; or

(ii) a sequence having at least 90% sequence identity to said sequence (a)(i); and

(b) a promoter element comprising

(i) at least 200 nucleotides of either SEQ ID NO: 2 or SEQ ID NO: 17; or

(ii) a sequence having at least 90% sequence identity to said sequence (b)(i);

said TCU exhibiting cone photoreceptor-specific promoter activity.

In one example, promoter element (b) may optionally comprise at least the last 200 or the last 500 nucleotides of either SEQ ID NO: 2 or SEQ ID NO: 17, or a sequence having at least 90% sequence identity to the last 200 or the last 500 nucleotides of either SEQ ID NO: 2 or SEQ ID NO: 17.

According to any one of the above examples, promoter element (b) may comprise at least 200 nucleotides of SEQ ID NO: 3, optionally wherein promoter element (b) also comprises a sequence of at least 10 contiguous nucleotides selected from nucleotides 1 to 35 of SEQ ID NO:3, or a sequence comprising at least 10 contiguous nucleotides selected from a sequence having at least 90% sequence identity to nucleotides 1 to 35 of SEQ ID NO:3.

According to any one of the above examples, promoter element (b) may comprise SEQ ID NO: 3 [529 bp promoter element in hG1.7] or SEQ ID NO:5 [247 bp promoter element in hG1.4] or a sequence having at least 90% sequence identity to SEQ ID NO:3 or SEQ ID NO: 5.

In any one of the above examples, the promoter element may further comprise SEQ ID NO:16 [the M8 mutation]. For example, according to any one of the above examples, nucleotides corresponding to nucleotides 1934 to 1939 (GGGCCG) of SEQ ID NO:2 may be replaced by SEQ ID NO:16.

According to one example, the TCU comprises SEQ ID NO: 4 [variant of the hG1.7 promoter construct, four nucleotides missing], SEQ ID NO: 6 [hG1.4 construct] or SEQ ID NO:15 [hG1.7 promoter construct in product].

In another aspect, the disclosure also provides an expression construct comprising a TCU described herein, wherein the TCU is operably linked to a sequence to be expressed in a cone photoreceptor-specific manner. In one example, the sequence operably linked to the TCU comprises a gene encoding CNGA3, CNGB3, PDE6C, PDE6H, GNAT2, KCNV2 or CACNA2D4. In some examples, the operably linked sequence comprises SEQ ID NO: 7, 8, 9, 10, 11, 12, 13 or 14, or that has at least 80% sequence identity to SEQ ID NO: 7, 8, 9, 10, 11, 12, 13 or 14 and has the ability to rescue cone photoreceptor function. In one example, the operably linked sequence comprises SEQ ID NO: 8 [CNGA3 codon optimized sequence], or that has at least 80% sequence identity to SEQ ID NO: 8 and has the ability to rescue cone photoreceptor function.

In yet another aspect, the disclosure refers to a vector comprising the TCU as described herein or an expression construct as described herein. The disclosure also provides vectors comprising any of the nucleic acids, TCUs, promoter fragments, codon optimized genes, and/or expression constructs described herein. In some examples, the vector is a viral vector.

In some examples, the vector is an AAV vector and/or comprises an AAV genome or a derivative thereof. In one example, the derivative is a chimeric, shuffled or capsid modified derivative. In one example, the AAV genome is from a naturally derived serotype or isolate or clade of AAV. In one example, the AAV genome is from AAV serotype 2 (AAV2), AAV serotype 4 (AAV4), or AAV serotype 8 (AAV8), and/or the AAV capsid is derived from AAV8. In a preferred example, the genome is derived from AAV2 and the capsid is derived from AAV8. In one example, AAV vector carries a gene encoding CNGA3.

In yet another aspect, the present disclosure refers to a host cell that contains a vector as described herein or produces a viral vector as described herein. The disclosure further provides host cells containing a nucleic acid or vector disclosed herein, as well as host cells that produce a

nucleic acid or viral vector as disclosed herein. In one example, the host cell is a HEK293 or HEK293T cell.

In yet another aspect, the present disclosure refers to a pharmaceutical composition comprising a vector as described herein and a pharmaceutically acceptable carrier. Also provided are pharmaceutical compositions comprising a nucleic acid or vector described herein and a pharmaceutically acceptable carrier.

In a further aspect, the present disclosure refers to the vector as described herein for use in preventing or treating retinal disorders. In a further aspect, the present disclosure refers to use of a vector as described herein in the manufacture of a medicament for the treatment or prevention of retinal disorders. In a further aspect, the present disclosure refers to use of a vector as described herein in the manufacture of a medicament for the treatment or prevention of retinal disorders in a patient in need thereof, comprising that a therapeutically effective amount of the vector is to be administered to said patient.

The disclosure further provides methods of using the nucleic acids, vectors, optimized gene sequences, and/or expression constructs described herein in a method of preventing or treating retinal disorders. In one example, the nucleic acids, vectors, optimized gene sequences, and/or expression constructs described herein are used in the manufacture of a medicament for the treatment or prevention of retinal disorders. Also provided is a method of treating or preventing retinal disorders in a patient in need thereof, comprising administering a therapeutically effective amount of a vector disclosed herein. In one example, the retinal disorder is Achromatopsia. In some examples, the vector is administered to a patient by direct retinal, subretinal, or intravitreal injection.

Brief description of the Figures

Figure 1 shows the results of an *in vivo* reporter gene expression study in transgenic mice in order to assess the position effect of the Locus Control Region (LCR) on the green (M) opsin core promoter. Top left: The normal human red and green pigment gene array showing the locations of the LCR, transcription units, and exons. Lower left: Modified visual pigment gene

arrays shown at a 10-fold enlarged scale. The start sites and direction of transcription are shown by arrows. P_{red} = human red pigment gene promoter. P_{green} = human green pigment gene promoter. AP, human placental alkaline phosphatase. lacZ, *E. coli* β-galactosidase. Right: Pie charts showing the fraction of transgene-expressing cells that express only AP (red), only lacZ (dark green), both AP and lacZ (yellow), or lacZ>>AP (light green) in chimeric or germ-line-transmitted mice for the constructs shown on the left. Cell counts from different mice derived from the same ES cell line were pooled to produce a single pie chart. Cell counts for those mouse lines from which the PGK-neo marker was excised by crossing to germ-line cre mice are shown immediately to the right of the pie chart for the corresponding parental line.

Figure 2 shows a schematic diagram of the chromosomal arrangement of the red (L-) and green (M-) opsin promoters (boxed, top). Schematic diagrams of previously developed transcriptional units (pR2.1 and PR1.7), as well as the engineered transcriptional control units disclosed in this study. LCR = Locus Control Region.

Figure 3 illustrates the transduction pattern of cone cells transduced with an AAVssh10 vector expressing green fluorescent protein (GFP) under the control of the hG1.4 TCU. Shown

CLAIMS

1. A transcriptional control unit (TCU) of up to 2500 nucleotides in length comprising in a 5' to 3' direction:

(a) a Locus Control Region (LCR) comprising

(i) SEQ ID NO 1; or

(ii) a sequence having at least 90% sequence identity to said sequence (a)(i); and

(b) a promoter element comprising

(i) at least 200 nucleotides of either SEQ ID NO: 2 or SEQ ID NO: 17; or

(ii) a sequence having at least 90% sequence identity to said sequence (b)(i);

said TCU exhibiting cone photoreceptor-specific promoter activity.

2. The TCU of claim 1, wherein (b) comprises:

(i) at least the last 200 nucleotides of either SEQ ID NO: 2 or SEQ ID NO: 17, or

(ii) a sequence having at least 90% sequence identity to said sequence (i);

said TCU exhibiting cone photoreceptor-specific promoter activity.

3. The TCU of claim 2, wherein (b) comprises:

(i) at least the last 500 nucleotides of either SEQ ID NO: 2 or SEQ ID NO: 17, or

(ii) a sequence having at least 90% sequence identity to said sequence (i);

said TCU exhibiting cone photoreceptor-specific promoter activity.

4. The TCU of any one of the preceding claims, wherein (b) comprises at least 200 nucleotides of SEQ ID NO: 3.

5. The TCU of any one of the preceding claims, wherein (b) comprises a sequence of at least 10 contiguous nucleotides selected from nucleotides 1 to 35 of SEQ ID NO:3, or a sequence comprising at least 10 contiguous nucleotides selected from a sequence having at least 90% sequence identity to nucleotides 1 to 35 of SEQ ID NO:3.

6. The TCU according to any one of the preceding claims, wherein (b) comprises:

(i) SEQ ID NO: 3; or

(ii) a sequence having at least 90% sequence identity to said sequence (i);

said TCU exhibiting cone photoreceptor-specific promoter activity.

7. The TCU of claim 6, wherein nucleotides 1934 to 1939 (GGGCCG) of SEQ ID NO:2 are replaced by SEQ ID NO:16.

8. The TCU of claim 6, wherein the TCU comprises SEQ ID NO: 4 or SEQ ID NO:15.

9. The TCU of any one of claims 1 to 4, wherein the promoter element comprises SEQ ID NO: 5.

10. The TCU of claim 9, wherein the TCU comprises SEQ ID NO: 6.

11. An expression construct comprising the TCU of any one of the preceding claims, operably linked to a sequence to be expressed in a cone photoreceptor-specific manner.

12. The expression construct according to claim 11, wherein the operably linked sequence is *CNGA3, CNGB3, PDE6C, PDE6H, GNAT2*, *KCNV2* or *CACNA2D4*.

13. The expression construct according to claim 12, wherein the operably linked sequence comprises SEQ ID NO: 7, 8, 9, 10, 11, 12, 13 or 14, or that has at least 80% sequence identity to SEQ ID NO: 7, 8, 9, 10, 11, 12, 13 or 14 and has the ability to rescue cone photoreceptor function.

14. The expression construct according to claim 12 or 13, wherein the operably linked sequence comprises SEQ ID NO: 8, or that has at least 80% sequence identity to SEQ ID NO: 8 and has the ability to rescue cone photoreceptor function.

15. A vector comprising the TCU according to any one of claims 1 to 10 or an expression construct according to any one of claims 11 to 14.

16. The vector according to claim 15 which is a viral vector.

17. The vector according to claims 16, which is an adeno-associated virus (AAV) vector.

18. The vector according to claim 17, wherein the vector comprises an AAV genome or a derivative thereof.

19. The vector according to claim 18, wherein the vector comprises an AAV genome or a derivative thereof, and wherein the AAV capsid is derived from AAV8.

20. The vector according to claim 19, wherein the AAV capsid is derived from AAV8 and the expression construct is defined according to any one of claims 11 to 14.

21. The vector according to claim 20, wherein the operably linked sequence is *CNGA3*.

22. The vector according to any one of claims 18 to 21, wherein said derivative is a chimeric, shuffled or capsid modified derivative.

23. The vector according to any of claims 18 to 22, wherein said AAV genome is from a naturally derived serotype or isolate or clade of AAV.

24. The vector according to claim 23, wherein said AAV genome is from AAV serotype 2 (AAV2), AAV serotype 4 (AAV4), or AAV serotype 8 (AAV8) and/or wherein the capsid is derived from AAV8.

25. The vector according to claim 24, wherein the genome is derived from AAV2 and the capsid is derived from AAV8.

26. A host cell that contains a vector of any one of claims 15 to 25 or produces a viral vector of any one of claims 15 to 25.

27. The cell according to claim 26 that is a HEK293 or HEK293T cell.

28. A pharmaceutical composition comprising a vector of any one of claims 15 to 25 and a pharmaceutically acceptable carrier.

29. The vector according to any one of claims 15 to 25 for use in preventing or treating retinal disorders.

30. Use of a vector according to any one of claims 15 to 25 in the manufacture of a medicament for the treatment or prevention of retinal disorders.

31. Use of a vector according to any one of claims 15 to 25 in the manufacture of a medicament for the treatment or prevention of retinal disorders in a patient in need thereof, comprising that a therapeutically effective amount of the vector is to be administered to said patient.

32. The vector according to claim 29, or the use of claim 30 or 31, wherein the retinal disorder is Achromatopsia.

33. The vector according to claim 29 or 32, or the use of any one of claims 30 to 32, wherein the treatment or prevention is by administration of the vector according to any one of claims 15 to 25 to a patient by direct retinal, subretinal or intravitreal injection.

34. The vector, use or method according to claim 33, wherein said vector is to be administered directly into the retinal, subretinal space or intravitreal space.