Methods and compositions are provided for intravitreally delivering a polynucleotide to cone photoreceptors. Examples of the methods include injecting a recombinant adeno-associated virus comprising a polynucleotide of interest into the vitreous of the eye. These methods and compositions find particular use in treating ocular disorders associated with cone dysfunction and/or death. In a first aspect, the present disclosure refers to use of a recombinant adeno-associated virus serotype 2 (rAAV2) variant comprising a therapeutic polynucleotide in the manufacture of a medicament for treating or preventing a cone-associated retinal disorder in a primate subject having or at risk for developing the cone-associated retinal disorder, wherein:

I) said rAAV2 variant comprises a variant AAV2 capsid protein comprising the amino acid sequence LGETTRP (SEQ ID NO:11) inserted into the GH loop of the VP1 capsid protein; and

II) the therapeutic polynucleotide comprises a MNTC regulatory cassette operably linked to a polynucleotide encoding a therapeutic protein,

wherein the MNTC regulatory cassette comprises a human L/M opsin Locus Control Region ("LCR") enhancer and a truncated M opsin promoter comprising about 140 nucleotides upstream of the transcriptional start site;

and wherein the medicament is to be administered intravitreally in an amount effective to treat or prevent the cone-associated retinal disorder.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is best understood from the following detailed description when read in conjunction with the accompanying drawings. It is emphasized that, according to common practice, the various features of the drawings are not to-scale. On the contrary, the dimensions of the various features are arbitrarily expanded or reduced for clarity. Included in the drawings are the following figures.

Figure 1 illustrates how intravitreally-delivered AAV2 variant AAV2-7m8 transduces retinal cells in the fovea centralis and parafovea of primates more efficiently than intravitreally-delivered AAV2. 5×10^{11} vector genomes of AAV2.CMV.GFP (upper left); AAV-2.5T.CMV.GFP (upper right) (Excoffon K. J., et al. 2009. Proc. Natl. Acad. Sci. U. S. A. 106:3865–3870); (lower left) AAV2-7.8.CMV.GFP (Dalkara D, et al. Sci Transl Med. 2013 Jun 12;5(189):189ra76); or AAV-ShH10.CMV.GFP (lower right) (Klimczak RR et al. PLoS One. 2009 Oct 14;4(10):e7467) was injected into the vitreous of an African green monkey in a volume of 50 uL, and GFP expression was observed 8 weeks later by OCT fluorescence imaging in vivo.

Figure 2 illustrates how the robustly the AAV2-7m8 capsid transduces foveal cones of primates. (a-b) AAV2-7m8.MNTC.GFP was injected into the central vitreous of a baboon and expression was observed (a) 5 weeks and (b) 8 weeks later by fundus fluorescence. (c and d) Natural GFP fluorescence within a 15 micron section of the fovea at approximately 6 months after injection with AAV2-7m8.MNTC.GFP at low magnification (c) and high magnification (d).

CLAIMS

That which is claimed is:

1. Use of a recombinant adeno-associated virus serotype 2 (rAAV2) variant comprising a therapeutic polynucleotide in the manufacture of a medicament for treating or preventing a cone-associated retinal disorder in a primate subject having or at risk for developing the cone-associated retinal disorder, wherein:

I) said rAAV2 variant comprises a variant AAV2 capsid protein comprising the amino acid sequence LGETTRP (SEQ ID NO:11) inserted into the GH loop of the VP1 capsid protein; and

II) the therapeutic polynucleotide comprises a MNTC regulatory cassette operably linked to a polynucleotide encoding a therapeutic protein,

wherein the MNTC regulatory cassette comprises a human L/M opsin Locus Control Region ("LCR") enhancer and a truncated M opsin promoter comprising about 140 nucleotides upstream of the transcriptional start site;

and wherein the medicament is to be administered intravitreally in an amount effective to treat or prevent the cone-associated retinal disorder.

2. The use according to claim 1, wherein the variant AAV2 capsid protein comprises a VP1 protein having a sequence identity of at least 90% to the polypeptide of SEQ ID NO: 19, and wherein the variant AAV2 capsid protein comprises the amino acid sequence LGETTRP (SEQ ID NO:11) inserted into the GH loop of the VP1 capsid protein.

3. The use according to claim 2, wherein the variant AAV2 capsid protein comprises a VP1 protein having a sequence identity of at least 95% to the polypeptide of SEQ ID NO: 19, and wherein the variant AAV2 capsid protein comprises the amino acid sequence LGETTRP (SEQ ID NO:11) inserted into the GH loop of the VP1 capsid protein.

4. The use according to claim 2, wherein the variant AAV2 capsid protein comprises a VP1 protein of SEQ ID NO: 19.

5. The use according to claim 1, wherein the human L/M opsin LCR enhancer comprises: GACTTGATCTTCTGTTAGCCCTAATCATCAATTAGC.

6. The use according to claim 1, wherein the human L/M opsin LCR enhancer comprises:
CCTACAGCAGCCAGGGTGAGATTATGAGGCTGAGCTGAGAATATCAAGACTGTACCGAGT
AGGGGGCCTTGGCAAGTGTGGAGAGCCCGGCAGCTGGGGCAGAGGGCGGAGTACGGTG
TGCGTTTACGGACCTCTTCAAACGAGGTAGGAAGGTCAGAAGTCAAAAAGGGAACAAATGA
TGTTTAACCACACAAAAATGAAAATCCAATGGTTGGATATCCATTCCAAATACACAAAGGCA
ACGGATAAGTGATCCGGGCCAGGCACAGAAGGCCATGCACCCGTAGGATTGCACTCAGA
GCTCCCAAATGCATAGGAATAGAAGGGTGGGTGCAGGAGGCTGAGGGGTGGGGAAAGGG
CATGGGTGTTTCATGAGGACAGAGCTTCCGTTTCATGCAATGAAAAGAGTTTGGAGACGGA
TGGTGGTGACTGGACTATACACTTACACACGGTAGCGATGGTACACTTTGTATTATGTATAT
TTTACCACGATCTTTTTAAAGTGTCAAAGGCAAATGGCCAAATGGTTCCTTGTCCTATAGCT
GTAGCAGCCATCGGCTGTTAGTGACAAAGCCCCTGAGTCAAGATGACAGCAGCCCCCATA
ACTCCTAATCGGCTCTCCCGCGTGGAGTCATTTAGGAGTAGTCGCATTAGAGACAAGTCCA
ACATCTAATCTTCCACCCTGGCCAGGGCCCCAGCTGGCAGCGAGGGTGGGAGACTCCGG
GCAGAGCAGAGGGCGCTGACATTGGGGCCCGGCCTGGCTTGGGTCCCTCTGGCCTTTCC
CCAGGGGCCCTCTTTCCTTGGGGCTTTCTTGGGCCGCCACTGCTCCCGCTCCTCTCCCCC
CATCCCACCCCCTCACCCCCTCGTTCTTCATATCCTTCTCTAGTGCTCCCTCCACTTTCATC
CACCCTTCTGCAAGAGTGTGGGACCACAAATGAGTTTTCACCTGGCCTGGGGACACACGT
GCCCCCACAGGTGCTGAGTGACTTTCTAGGACAGTAATCTGCTTTAGGCTAAAATGGGACT
TGATCTTCTGTTAGCCCTAATCATCAATTAGCAGAGCCGGTGAAGGTGCAGAACCTACCGC
CTTTCCAGGCCTCCTCCCACCTCTGCCACCTCCACTCTCCTTCCTGGGATGTGGGGGCTG
GCACACGTGTGGCCCAGGGCATTGGTGGGATTGCACTGAGCTGGGTCATTAGCGTAATCC
TGGACAAGGGCAGACAGGGCGAGCGGAGGGCCAGCTCCGGGGCTCAGGCAAGGCTGGG
GGCTTCCCCCAGACACCCCACTCCTCCTCTGCTGGACCCCCACTTCATAGGGCACTTCGT
GTTCTCAAAGGGCTTCCAAATAGCATGGTGGCCTTGGATGCCCAGGGAAGCCTCAGAGTT
GCTTATCTCCCTCTAGACAGAAGGGGAATCTCGGTCAAGAGGGAGAGGTCGCCCTGTTCA
AGGCCACCCAGCCAGCTCATGGCGGTAATGGGACAAGGCTGGCCAGCCATCCCACCCTC
AGAAGGGACCCGGTGGGGCAGGTGATCTCAGAGGAGGCTCACTTCTGGGTCTCACATTCT
T.

7. The use according to claim 1, wherein the truncated M opsin promoter comprises:
CCAGCAAATCCCTCTGAGCCGCCCCCGGGGGCTCGCCTCAGGAGCAAGGAAGCAAGGGG
TGGGAGGAGGAGGTCTAAGTCCCAGGCCCAATTAAGAGATCAGATGGTGTAGGATTTGGG
AGCTTTTAAGGTGAAGAGGCCCGGGCTGAT.

8. The use according to claim 1, wherein the MNTC regulatory cassette comprises:
CCTACAGCAGCCAGGGTGAGATTATGAGGCTGAGCTGAGAATATCAAGACTGTACCGAGT
AGGGGGCCTTGGCAAGTGTGGAGAGCCCGGCAGCTGGGGCAGAGGGCGGAGTACGGTG
TGCGTTTACGGACCTCTTCAAACGAGGTAGGAAGGTCAGAAGTCAAAAAGGGAACAAATGA
TGTTTAACCACACAAAAATGAAAATCCAATGGTTGGATATCCATTCCAAATACACAAAGGCA
ACGGATAAGTGATCCGGGCCAGGCACAGAAGGCCATGCACCCGTAGGATTGCACTCAGA
GCTCCCAAATGCATAGGAATAGAAGGGTGGGTGCAGGAGGCTGAGGGGTGGGGAAAGGG
CATGGGTGTTTCATGAGGACAGAGCTTCCGTTTCATGCAATGAAAAGAGTTTGGAGACGGA
TGGTGGTGACTGGACTATACACTTACACACGGTAGCGATGGTACACTTTGTATTATGTATAT
TTTACCACGATCTTTTTAAAGTGTCAAAGGCAAATGGCCAAATGGTTCCTTGTCCTATAGCT
GTAGCAGCCATCGGCTGTTAGTGACAAAGCCCCTGAGTCAAGATGACAGCAGCCCCCATA
ACTCCTAATCGGCTCTCCCGCGTGGAGTCATTTAGGAGTAGTCGCATTAGAGACAAGTCCA
ACATCTAATCTTCCACCCTGGCCAGGGCCCCAGCTGGCAGCGAGGGTGGGAGACTCCGG
GCAGAGCAGAGGGCGCTGACATTGGGGCCCGGCCTGGCTTGGGTCCCTCTGGCCTTTCC
CCAGGGGCCCTCTTTCCTTGGGGCTTTCTTGGGCCGCCACTGCTCCCGCTCCTCTCCCCC
CATCCCACCCCCTCACCCCCTCGTTCTTCATATCCTTCTCTAGTGCTCCCTCCACTTTCATC
CACCCTTCTGCAAGAGTGTGGGACCACAAATGAGTTTTCACCTGGCCTGGGGACACACGT
GCCCCCACAGGTGCTGAGTGACTTTCTAGGACAGTAATCTGCTTTAGGCTAAAATGGGACT
TGATCTTCTGTTAGCCCTAATCATCAATTAGCAGAGCCGGTGAAGGTGCAGAACCTACCGC
CTTTCCAGGCCTCCTCCCACCTCTGCCACCTCCACTCTCCTTCCTGGGATGTGGGGGCTG
GCACACGTGTGGCCCAGGGCATTGGTGGGATTGCACTGAGCTGGGTCATTAGCGTAATCC
TGGACAAGGGCAGACAGGGCGAGCGGAGGGCCAGCTCCGGGGCTCAGGCAAGGCTGGG
GGCTTCCCCCAGACACCCCACTCCTCCTCTGCTGGACCCCCACTTCATAGGGCACTTCGT
GTTCTCAAAGGGCTTCCAAATAGCATGGTGGCCTTGGATGCCCAGGGAAGCCTCAGAGTT
GCTTATCTCCCTCTAGACAGAAGGGGAATCTCGGTCAAGAGGGAGAGGTCGCCCTGTTCA
AGGCCACCCAGCCAGCTCATGGCGGTAATGGGACAAGGCTGGCCAGCCATCCCACCCTC
AGAAGGGACCCGGTGGGGCAGGTGATCTCAGAGGAGGCTCACTTCTGGGTCTCACATTCT

TCCAGCAAATCCCTCTGAGCCGCCCCCGGGGGCTCGCCTCAGGAGCAAGGAAGCAAGGG
GTGGGAGGAGGAGGTCTAAGTCCCAGGCCCAATTAAGAGATCAGATGGTGTAGGATTTGG
GAGCTTTTAAGGTGAAGAGGCCCGGGCTGATCCCACTGGCCGGTATAAAGCACCGTGACC
CTCAGGTGACGCACCAGGGCCGGCTGCCGTCGGGGACAGGGCTTTCCATAGCCCAGGTA
AGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAAACTGGGCTTGTCGAGACAGA
GAAGACTCTTGCGTTTCTGATAGGCACCTATTGGTCTTACTGACATCCACTTTGCCTTTCTC
TCCACAGGCCCAGAGAGGAGACAGGCCGCCACC.

9. The use according to claim 1, wherein the cone-associated retinal disorder is selected from the group consisting of: rod-cone dystrophy; cone-rod dystrophy; progressive cone dystrophy; retinitis pigmentosa (RP); Stargardt Disease; macular telangiectasia, Leber hereditary optic neuropathy, Best's disease; adult vitelliform macular dystrophy; X-linked retinoschisis; a color vision disorder; age-related macular degeneration; wet age-related macular degeneration; geographic atrophy; diabetic retinopathy; a retinal vein occlusion; retinal ischemia; Familial Exudative Vitreoretinopathy (FEVR); COATs disease; and Sorsby's fundus dystrophy.

10. The use according to claim 1, wherein the cone-associated retinal disorder is a color vision disorder.

11. The use according to claim 10, wherein the color vision disorder is blue cone monochromacy.

12. The use according to claim 1, wherein the color vision disorder is color vision deficiency.