related macular degeneration, macular telangiectasia, retinitis pigmentosa, diabetic retinopathy, retinal vein occlusions, glaucoma, Sorsby's fundus dystrophy, adult vitelliform macular dystrophy, Best's disease, and X-linked retinoschisis. It is expected that these cone cell disorders may be treated by delivering to cone photoreceptors a therapeutic gene that, when expressed by the cone photoreceptors, complements the deficiency and "rescues" the cone cell viability and/or function.

The highest density of cone photoreceptors exist at the 1.5mm depression located in the center of the macula of the retina. This region, called the "fovea centralis" or "foveal pit", is responsible for sharp central vision (also called foveal vision), which is necessary in humans for activities where visual detail is of primary importance, such as reading and driving. The fovea centralis consists of two sub-regions: the foveola, a 0.35mm diameter rod-free region of retina at the center of the pit; and the fovea, a 1.5mm-diameter cone-enriched region of retina that surrounds the foveola and forms the slopes of the pit. Surrounding the fovea centralis is the parafovea, which forms the lip of the depression and is comprised of all cells of the retina, cone photoreceptors being represented in reduced numbers relative to in the fovea centralis. Beyond the parafovea is the perifovea, a region of retina which contains an even more diminished density of cones. Because cone cells of the fovea constitute the vast majority of cone photoreceptors in the retina, these cells are ideal target recipients of therapeutic genes delivered for the treatment of cone-associated disorders (Oster 1935).

Some success at delivering genes to cells of the retina has been achieved by employing viral vectors such as adeno-associated virus (AAV) or lentivirus. However, these vectors must be administered by subretinal injection, a procedure that disrupts the structure of the retina and carries with it a risk of creating additional damage to retinal tissue that is often already damaged by the disorder being treated. One alternative is to deliver the viral vector to the retina intravitreally, i.e., by injecting the vector into the vitreous of the eye and hoping that the vector permeates the retina and transduces the retinal cells. However, as demonstrated by the art, foveal cone cells are notoriously resistant to transduction by viral vectors delivered intravitreally to the retina.

Thus, there is a need in the art for viral vectors that transduce cone cells with high efficiency when delivered from the vitreous of the eye. The present invention addresses these issues.

SUMMARY

Methods and compositions are provided for intravitreally delivering a polynucleotide to cone photoreceptors. Embodiments 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, there is provided use of a recombinant adeno-associated virus (rAAV) 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 rAAV variant comprises a variant AAV capsid protein comprising 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;

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 (rAAV) 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 rAAV variant comprises a variant AAV capsid protein comprising 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;

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 rAAV variant comprises a VP1 protein having a sequence identity of at least 80% to the polypeptide of SEQ ID NO: 19.

3. The use according to claim 2, wherein the rAAV variant comprises a VP1 protein having a sequence identity of at least 95% to the polypeptide of SEQ ID NO: 19.

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

5. The use according to claim 1, wherein the cone-associated 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.

6. The use according to claim 1, wherein the use further comprises identification of the subject

as having a cone-associated disorder.

7. The use according to claim 1, wherein the use further comprises detection of an improvement in vision following the administration step.

8. The use according to claim 5, wherein the color vision disorder is blue cone monochromacy.

9. The use according to claim 5, wherein the color vision disorder is color vision deficiency.