# AAV -VECTORS FOR USE IN GENE THERAPY OF CHOROIDEREMIA

## Field of the Invention

The present invention relates to gene therapy for treatment or prevention of choroideremia.

## Background of the Invention

Choroideremia is a rare X-linked progressive degeneration of the choroid, retinal pigment epithelium and photoreceptors of the eye. The typical natural history in afflicted males is onset of nightblindness during teenage years, and then progressive loss of peripheral vision during the 20's and 30's leading to complete blindness in the 40's. Female carriers have mild symptoms most notably nightblindness but may occasionally have a more severe phenotype.

The disease is caused by mutations in the REP1 gene, (Rab escort protein 1), which is located on the X chromosome 21q region. In most cells in the body, the REP2 protein, which is 75% homologous to REP1, compensates for the REP1 deficiency. In the eye, however, for reasons that are not yet clear, REP2 is unable to compensate for the REP1 deficiency. Hence in the eye, REP polypeptide activity is insufficient to maintain normal prenylation of the target proteins (Rab GTPases) leading to cellular dysfunction and ultimate death, primarily affecting the outer retina and choroid.

There is no treatment for choroideremia, and there is a lack of models to assess therapeutic strategies. There is a need for provision of such a therapy.

## Summary

The present disclosure relates to a vector which can be used for gene therapy of choroideremia, and methods of preventing or treating this disease using the vector. The disclosure also relates to the use of the vector in methods of preventing or treating choroideremia.

The vector of the disclosure is a viral vector, specifically based on the genome of adeno-associated virus (AAV). The vector comprises a sequence which encodes REP1 or a variant thereof, thus allowing for the expression of REP1 function in a target cell. The methods and uses of the disclosure specifically involve the administration of the vector to a patient by direct retinal, subretinal or intravitreal injection to treat or prevent choroideremia.

Accordingly, the disclosure provides a vector, which comprises an adeno-associated virus

(AAV) genome or a derivative thereof and a polynucleotide sequence encoding REP1 or a variant thereof. The disclosure further provides a method of treating or preventing choroideremia in a patient in need thereof, comprising administering a therapeutically effective amount of a vector according to any one of the preceding claims to said patient by direct retinal, subretinal or intravitreal injection, and thereby treating or preventing choroideremia in said patient. The disclosure additionally provides a vector of the disclosure for use in a method of treating or preventing choroideremia by administering said vector to a patient by direct retinal, subretinal or intravitreal injection.

In one aspect, there is provided the use of an adeno-associated virus serotype 2 (AAV2) vector particle which comprises an AAV2 genome or a derivative thereof and a polynucleotide sequence encoding REP1 or a variant thereof having at least 90% homology to SEQ ID NO:3 in the manufacture of a medicament for the treatment or prevention of choroideremia.

**Brief Description of Figures**

Figure 1 shows that the AAV.REP1 (AAV-CAG-REP1) vector can transduce human fibroblasts isolated from a patient with choroideremia (Chm) efficiently. The relative levels of expression of human REP1 protein (hREP1) are compared by Western blot, allowing quantification of AAV2.REP1 vector activity by comparing the amount of hREP1 in different concentrations of cell lysate. With regard to the labelling, CAG is the Chicken beta Actin with CMV enhancer promoter sequence - interchangeably referred to as 'CBA' in various publications and parts of this document.

Western blots are shown in the left panels for REP1 (top panel) and alpha-tubulin (bottom panel) as a loading control. Lane 1: 40 μg cell lysate from control wildtype (WT) fibroblasts. Lane 2: 40 μg cell lysate from Chm fibroblasts. Lanes 3-6: 40, 20, 10 and 5μg cell lysate from Chm fibroblasts transduced with AAV2.REP1 vector. Lane 7: human REP1 recombinant protein. Since the 5 μg lysate hREP1 band has a similar density to 40 μg of the WT fibroblast lysate, the level of hREP1 achieved with the AAV2.REP1 vector can achieve at least 8 times (40/5) the normal wild type levels under these conditions.

As a positive control for the promoter and other non-REP1 sequences in this assay, results from a control AAV vector expressing green fluorescent protein (GFP) in place of REP1 (AAV-CAG-GFP) are also shown.

Western blots are shown in the right panels for GFP (top panel) and alpha-tubulin (bottom panel) as a loading control. Lane 1: 40 μg cell lysate from wildtype (WT) fibroblasts. Lane 2: 40 μg cell lysate from Chm fibroblasts transduced with AAV2.GFP vector (AAV-CAG-REP1). The high levels of GFP shown confirm the efficiency of this vector expression cassette in transducing human cells that are deficient of REP1 activity, as would be the case in patients with