In one example, the present disclosure provides a gene therapy vector for Alzheimer's disease. In one example, a gene therapy vector comprises an AAV expression vector encoding the human APOE2 gene and either in *cis* or in *trans* artificial microRNA(s) that target endogenous APOE4. This vector system silences the expression of detrimental endogenous APOE4 in combination with supplementation of the beneficial APOE2 gene from a gene therapy vector, e.g., an AAV vector. As disclosed herein, exemplary artificial microRNA sequences were designed that target the endogenous APOE4 mRNA for suppression. The microRNAs (miRNAs) may be incorporated in sequences that are 5' to the APOE2 coding sequence, e.g., in a n intron such as the CAG promoter intron, or sequences that are 3' to the APOE2 coding sequence, e.g., sequences that are 5' to the polyA tail of the vector transgene plasmid coding for the human APOE2 coding sequence. Alternatively, the microRNA(s) may be inserted between a PolIII promoter, e.g., a U6 promoter, and a terminator following the polyA site of the APOE2 expression cassette. The vector-derived human APOE2 DNA sequence optionally includes silent nucleotide changes to decrease or inhibit suppression by the microRNAs and in one example may include a tag such as a HA tag for detection, e.g., for pre-clinical detection studies. In one example, the expression construct is packaged into an AAV capsid of a serotype that targets astrocytes and glial cells (for example AAV9) the prominent sites of endogenous APOE expression in the CNS, but can be provided in other vectors, e.g., other viral vectors, plasmids, nanoparticle or liposomes.

In one aspect, the present disclosure refers to a gene therapy vector comprising a first promoter operably linked to a nucleic acid sequence comprising an open reading frame encoding APOE2 and a 3' untranslated region, and an isolated nucleotide sequence comprising one or more RNAi nucleic acid sequences for inhibition of APOE4 mRNA or a composition comprising the gene therapy vector which optionally comprises a pharmaceutically acceptable carrier.

In one example, a gene therapy vector is provided comprising a first promoter operably linked to a nucleic acid sequence comprising an open reading frame encoding APOE2 and a 3' untranslated region, and an isolated nucleotide sequence is provided comprising one or more RNAi nucleic acid sequences for inhibition of APOE4 mRNA. In one example, the vector comprises the nucleotide sequence. In one example, the nucleotide sequence is inserted 5' or 3' to the open reading frame. In one example, the nucleotide sequence is inserted 5' and 3' to the open reading frame. In one example, the nucleotide sequence is on a different vector. In one example, the isolated nucleotide sequence comprises a second promoter operably linked to the one or more RNAi nucleic acid sequences. In one example, the gene therapy vector is a viral

vector. In one example, the different vector is a viral vector. In one example, the viral vector is an AAV, adenovirus, lentivirus, herpesvirus or retrovirus vector. In one example, the AAV is AAV5, AAV9 or AAVrh10. In one example, the APOE4 is human APOE4. In one example, the APOE2 is human APOE2. In one example, the first promoter is a PolI promoter, e.g., a constitutive promoter or a regulatable promoter, for example, an inducible promoter. In one example, the second promoter is a PolIII promoter. In one example,
the isolated nucleotide sequence comprises nucleic acid for one or more miRNA comprising two or more of the RNAi nucleic acid sequences, e.g., one or more RNAi sequences are embedded in a miRNA sequence. In one example, the RNAi comprises siRNA including a plurality of siRNA sequences. In one example, the RNAi comprises shRNA sequences of about 15 to 25 nucleotides in length. In one example, the open reading frame for APOE2 comprises a plurality of silent nucleotide substitutions relative to SEQ ID NO:6, e.g., the open reading frame comprises SEQ ID NO:7 or nucleotide sequence with at least 70%, 75% 80%, 85%, 90%, 95%, 97% or 98% nucleic acid sequence identity to SEQ ID NO:7 and encodes APOE2, or the open reading frame encodes APOE2 and comprises a nucleotide sequence with at least 70%, 75% 80%, 85%, 90%, 95%, 97% or 98% nucleic acid sequence identity to
GAAAGAACTCAAAGCTTATAAGAGCGAGCTGGAGG (SEQ ID NO:13)
but which sequence is not SEQ ID NO:7. In one example, the plurality of the silent nucleotide substitutions in the APOE2 open reading frame are not in the RNAi nucleic acid sequence in the isolated nucleotide sequence, that is the sequence with the nucleotide substitutions differs from the RNAi nucleotide sequence so that the mRNA having the nucleotide substitutions does not bind to, e.g., for a duplex with, the RNAi sequences, e.g., isolated RNAi or RNAi sequences expressed from a vector. In one example, at least 50%, 60%, 70%, 80% or 90% of codons in the open reading frame for APOE2 have a silent nucleotide substitution. In one example, at least 5%, 10%, 20%, 30%, or 40%, of codons in the open reading frame for APOE2 have a silent nucleotide substitution, e.g., in a portion of APOE2 sequences that correspond to the RNAi sequences. That is, the silent nucleotide substitutions in a human APOE2 coding sequence result in a sequence that differs from endogenous human APOE4

sequences and differs from the APOE4 RNAi sequences. In one example, the APOE4 that is inhibited has a sequence having at least 80%, 85%, 90%, 95% or more amino acid sequence identity to a polypeptide encoded by SEQ ID NO:22. In one example, the APOE2 has a sequence having at least 80%, 85%, 90%, 95% or more amino acid sequence identity to a polypeptide encoded by SEQ ID NO:9. In one example, the one or more RNAi nucleic acid sequences have at least 60%, 70%, 80%, 90% or more nucleotide sequence identity to one of SEQ ID Nos. 1-4 or 20-22 or the complement thereof. In one example, the vector has a first PolI promoter operably linked to a nucleic acid sequence comprising an open reading frame encoding human APOE2 and an isolated nucleotide sequence having one or more RNAi nucleic acid sequences for inhibition of human APOE4 mRNA. In one example, the nucleotide sequence is inserted 5' to the open reading frame. In one example, the nucleotide sequence is inserted 3' to the open reading frame. In one example, the nucleotide sequence is inserted 5' and 3' to the open reading frame. In one example, the isolated nucleotide sequence comprises a second promoter operably linked to the one or more RNAi nucleic acid sequences. In one example, the RNAi nucleic acid sequence is about 125 to 500, e.g., about 150 to 175, nucleotides in length. In one example the gene therapy vector may have 2, 3, 4 or more copies of the RNAi nucleic acid sequence which may include miRNA sequences, e.g., miRNA sequences which flank the APOE4 inhibitory sequences.

In one aspect, the present disclosure refers to a composition comprising the gene therapy vector disclosed herein for use to prevent, inhibit or treat Alzheimer's disease in a mammal or to prevent, inhibit or treat a disease associated with APOE4 expression in a mammal.

In one aspect, the present disclosure refers to use of the gene therapy vector disclosed herein in the manufacture of a medicament to prevent, inhibit or treat Alzheimer's disease in a mammal or to prevent, inhibit or treat a disease associated with APOE4 expression in a mammal, wherein optionally the mammal is a E2/E4 heterozygote or a E4/E4 homozygote.

In one example, a method to prevent, inhibit or treat Alzheimer's disease in a mammal is provided comprising: administering to the mammal an effective amount of a composition comprising the gene therapy vector. In one example, the composition comprises nanoparticles comprising the gene therapy vector or the different vector, or both. In one example, the gene therapy vector or the different vector, or both, comprise a viral vector. In one example, the mammal is a E2/E4 heterozygote. In one example, the mammal is a E4/E4 homozygote. In one example, the composition is systemically administered. In one example, the composition is orally administered. In one example, the composition is intravenously administered. In one example, the composition is locally administered. In one example, the composition is injected. In one example, the

composition is administered to the central nervous system. In one example, the composition is administered to the brain. In one example, the composition is a sustained release composition. In one example, the mammal is a human. In one example, the RNAi nucleic acid sequences comprise a plurality of miRNA sequences.

In one example, a method to prevent, inhibit or treat a disease associated with APOE4 expression in a mammal is provided comprising: administering to the mammal an effective amount of a composition comprising the gene therapy vector. In one example, the composition comprises liposomes comprising the gene therapy vector or the different vector, or both. In one example, the composition comprises nanoparticles comprising the gene therapy vector or the different vector, or both. In one example, the gene therapy vector or the different vector, or both, comprise a viral vector. In one example, the mammal is a E2/E4 heterozygote. In one example, the mammal is a E4/E4 homozygote. In one example, the composition is systemically administered. In one example, the composition is orally administered. In one example, the composition is intravenously administered. In one example, the composition is locally administered. In one example, the composition is injected. In one example, the composition is administered to the central nervous system. In one example, the composition is

administered to the brain. In one example, the composition is a sustained release composition. In one example, the mammal is a human. In one example, the RNAi sequences comprise a plurality of miRNA sequences.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1. Production of inhibitory RNAs from an exemplary target transcript template (Boudreau and Davidson. 2012. Methods in Enzymology, Volume 507).

Figure 2. Pathways to inhibit mRNA (Borel et al., 2014. *Mol Ther* 22:692-701).

Figure 3. Exemplary constructs for miRNA insertion(s).

Figure 4. Single vector and dual vector constructs.

Figure 5. Single vector construct with two sites for miRNA sequences.

Figure 6. APOE knock down of expression by four different siRNAs *in*

WHAT IS CLAIMED IS:

1. A gene therapy vector comprising a first promoter operably linked to a nucleic acid sequence comprising an open reading frame encoding APOE2 and a 3' untranslated region, and an isolated nucleotide sequence comprising one or more RNAi nucleic acid sequences for inhibition of APOE4 mRNA or a composition comprising the gene therapy vector which optionally comprises a pharmaceutically acceptable carrier.

2. The vector of claim 1 wherein the vector comprises the nucleotide sequence which optionally is inserted 5' or 3' to the open reading frame or inserted 5' and 3' to the open reading frame.

3. The vector of claim 1 wherein the nucleotide sequence is on a different vector which is optionally a viral vector.

4. The vector of any one of claims 1 to 3 wherein the isolated nucleotide sequence comprises a second promoter operably linked to the one or more RNAi nucleic acid sequences and optionally the second promoter is a PolIII promoter.

5. The vector of any one of claims 1 to 4 wherein the gene therapy vector is a viral vector which is optionally an AAV, adenovirus, lentivirus, herpesvirus or retrovirus vector and optionally the AAV is AAV5, AAV9 or AAVrh10.

6. The vector of any one of claims 1 to 5 wherein the APOE4 is human APOE4 or the APOE2 is human APOE2.

7. The vector of any one of claims 1 to 6 wherein the first promoter is a PolI promoter.

8. The vector of any one of claims 1 to 7 wherein the isolated nucleotide sequence comprises nucleic acid for one or more miRNAs comprising two

or more of the RNAi nucleic acid sequences.

9. The vector of any one of claims 1 to 8 wherein the RNAi comprises siRNA including a plurality of siRNA sequences.

10. The vector of any one of claims 1 to 9 wherein the open reading frame for APOE2 comprises a plurality of silent nucleotide substitutions relative to SEQ ID NO:6.

11. The vector of claim 10 wherein the plurality of the silent nucleotide substitutions in the APOE2 open reading frame are not in the RNAi nucleic acid sequence in the isolated nucleotide sequence.

12. The vector of claim 9, 10 or 11 wherein at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80% or 90% of codons in the open reading frame have a silent nucleotide substitution.

13. The vector of any one of claims 1 to 12 wherein the APOE4 that is inhibited has a sequence having at least 80%, 85%, 90%, 95% or more amino acid sequence identity to a polypeptide comprising SEQ ID NO:10 and/or the APOE2 has a sequence having at least 80%, 85%, 90%, 95% or more amino acid sequence identity to a polypeptide encoded by SEQ ID NO:11.

14. The vector of any one of claims 1 to 13 wherein the one or more RNAi nucleic acid sequences have at least 60%, 70%, 80% 90% or more nucleotide sequence identity to one of SEQ ID Nos. 1-4 or the complement thereof.

15. The vector of claim 1 comprising a first promoter operably linked to a nucleic acid sequence comprising an open reading frame encoding human APOE2 and an isolated nucleotide sequence having one or more RNAi nucleic acid sequences for inhibition of human APOE4 mRNA and optionally the nucleotide sequence is inserted 5' to the open reading frame,

is inserted 3' to the open reading frame or is inserted 5' and 3' to the open reading frame and optionally the isolated nucleotide sequence comprises a second promoter operably linked to the one or more RNAi nucleic acid sequences.

16. A composition comprising the gene therapy vector according to any one of claims 1 to 15 for use to prevent, inhibit or treat Alzheimer's disease in a mammal or to prevent, inhibit or treat a disease associated with APOE4 expression in a mammal.

17. The composition for use of claim 16 which comprises liposomes or nanoparticles comprising the gene therapy vector or the different vector of claim 3, or both, wherein optionally the different vector comprises a PolIII promoter.

18. Use of the gene therapy vector according to any one of claims 1 to 15 in the manufacture of a medicament to prevent, inhibit or treat Alzheimer's disease in a mammal or to prevent, inhibit or treat a disease associated with APOE4 expression in a mammal, wherein optionally the mammal is a E2/E4 heterozygote or a E4/E4 homozygote.

19. The use of claim 18 wherein the gene therapy vector is to be systemically, orally, intravenously or locally administered or injected into the central nervous system or brain.

20. The composition for use of claim 16 or 17 or the use of claim 18 or 19 wherein the RNAi sequences comprise a plurality of miRNA sequences each comprising the one or more RNAi nucleic acid sequences for inhibition of APOE4 mRNA, wherein the RNAi sequences comprise a miRNA sequence comprising the one or more RNAi nucleic acid sequences for inhibition of APOE4 mRNA and optionally wherein the miRNA sequence in the vector is inserted 5' to the open reading frame or wherein the miRNA sequence in the vector is inserted 3' to the open reading frame or wherein one of the miRNA sequences in the vector is

inserted 5’ to the open reading frame and another is inserted 3’ to the open reading frame.