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METHODS AND APOE PHARMACEUTICAL COMPOSITIONS FOR THE TREATMENT AND THE PREVENTION OF ALZHEIMERS DISEASE

CROSS-REFERENCE TO RELATED APPLICATIONS

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[0001] This application claims the benefit of the filing date of application No. 63/496,910, filed on April 18, 2023, application No. 63/592,123, filed on October 20, 2023, and application No. 63/592,469, filed on October 23, 2023, the disclosures of which are incorporated by reference herein.

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SUBMISSION OF SEQUENCE LISTING IN XML FORMAT

[0002] The Sequence Listing XML associated with this application is provided electronically in XML file format and is hereby incorporated by reference into the specification. The name of the XML file containing the Sequence Listing is 1676199WO1.xml. The XML file is 47,801 KB, created on April 17, 2024, and is being submitted electronically via USPTO Patent Center.

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FIELD OF THE INVENTION

[0003] The disclosure relates to a method for preventing or treating Alzheimer's disease in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of an AAV vector which comprises an apolipoprotein (APOE) encoding nucleic acid. Also provided are methods of producing AAV viral vectors encoding an APOE polypeptide.

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BACKGROUND

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[0004] There has been a long-felt but unmet need for therapeutics effective for the treatment or prevention of Alzheimer's disease (AD). Disclosed herein is an AAV vector encoding an apolipoprotein (APOE) variant having at least one mutation that confers a protective role against developing Alzheimer's disease. The AAV vector may be formulated for administration to individuals having Alzheimer's disease or at risk of developing Alzheimer's disease. Also provided are methods of producing APOE AAV vectors. The common APOE alleles (APOE4, APOE3, APOE2) are, by far, the major genetic risk-modifying factors for

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AD, with the APOE4 allele increasing risk and reducing the age of onset and the APOE2 allele decreasing risk and markedly delaying the age of onset. Extensive human genetic data demonstrate that APOE4 and APOE2 are co-dominant. APOE2/APOE4 heterozygotes have the normal risk for AD of APOE3/APOE3 homozygotes, instead of having the 4-fold higher risk for AD of APOE3/APOE4 heterozygotes. APOE4 homozygotes have a markedly increased risk of developing AD (14.5-fold compared to APOE3 homozygotes), as well as an earlier age of onset for developing the disease (approximately 5 years for each APOE4 allele compared to APOE3 homozygotes). Between 45% and 50% of AD patients carry at least one APOE4 allele compared to only 15% of age-matched healthy controls. In contrast, APOE2 is a protective allele, reducing AD risk by approximately 50% (1.8-fold decreased risk) and markedly delaying the age of onset, even in the presence of the APOE4 allele. Thus, roughly equivalent expression of APOE2 cancels out the deleterious effect of the E4 allele in humans.

[0005] Amyloid burden is associated with the neuropathology of AD. Both experimental animal and clinical studies have shown that APOE genotype also predicts the timing and amount of brain A β peptide deposition as well as amyloid burden (APOE4 > APOE3 > APOE2). Amyloid burden is one mechanism by which APOE isoforms exert their effect. Other mechanisms include the tau pathway.

[0006] A further gene variant confers an enhanced risk of developing AD. It has been shown that individuals carrying the dominant PSEN1-E280A have early onset central nervous system (CNS) amyloid-beta (A β) and amyloid accumulation.

[0007] Strikingly, a subject carrying the autosomal dominant PSEN E280A allele was in good cognitive condition at age 70, well past the age when the kindred typically develop cognitive decline. This individual was found to be APOE3 homozygous but carried an R136S mutation in APOE3, called the Christchurch mutation. In vitro experiments showed that APOE3ch behaves like the APOE2 allele; both isoforms bind poorly to heparin, corresponding to the extracellular matrix molecules implicated in the propagation and uptake of toxic forms of tau. Protection against developing AD therefore may correlate with lost binding to heparan sulfate proteoglycans (HSPGs), believed to propagate tau tangles. Accordingly, the APOE Christchurch mutation suggests a protective role against Alzheimer's disease.

[0008] Methods and compositions of the disclosure can be used to provide an APOE polypeptide, such as APOE2, having the R136S Christchurch mutation to delay and/or prevent the onset of AD in individuals carrying at risk of developing AD.

SUMMARY

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[0009] The disclosure provides a recombinant adeno-associated virus (rAAV) vector, wherein the rAAV vector comprises in the 5' to 3' direction: a first AAV ITR sequence comprising SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 23; an enhancer sequence comprising SEQ ID NO: 3; a promoter sequence comprising SEQ ID NO: 4; a chimeric intron comprising SEQ ID NO: 5; a nucleic acid sequence encoding an apolipoprotein 2 (APOE2) polypeptide comprising a Christchurch mutation or an apolipoprotein 3 (APOE3) polypeptide comprising a Christchurch mutation comprising SEQ ID NO: 6 or SEQ ID NO: 20; a polyA sequence comprising SEQ ID NO: 7; and a second ITR sequence comprising SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 23.

[0010] In some aspects, the Christchurch mutation comprises: an R154S mutation in reference to an unprocessed APOE polypeptide; or an R136S mutation in reference to mature APOE polypeptide lacking a signal peptide.

[0011] In some aspects, the rAAV vector comprises the nucleic acid sequence set forth in SEQ ID NO: 8, SEQ ID NO: 21, SEQ ID NO: 22.

[0012] The disclosure provides a recombinant adeno-associated virus (rAAV) vector comprising a nucleic acid sequence encoding an apolipoprotein 2 (APOE2) polypeptide comprising a Christchurch mutation or an apolipoprotein 3 (APOE3) polypeptide comprising a Christchurch mutation, wherein the rAAV vector comprises SEQ ID NO: 8, SEQ ID NO: 21, or SEQ ID NO: 22.

[0013] In some aspects, the rAAV vector is packaged as an rAAV viral vector comprising an AAV capsid protein.

[0014] In some aspects, the AAV capsid protein is an AAV1 capsid protein, an AAV2 capsid protein, an AAV4 capsid protein, an AAV5 capsid protein, an AAV6 capsid protein, an AAV7 capsid protein, an AAV8 capsid protein, an AAV9 capsid protein, an AAV10 capsid protein, an AAV11 capsid protein, an AAV12 capsid protein, an AAV13 capsid protein, an AAVPHP.B capsid protein, an AAVrh74 capsid protein or an AAVrh10 capsid protein. In some aspects, the AAV capsid protein is an AAVrh10 capsid protein.

[0015] The disclosure provides a pharmaceutical composition comprising an AAV viral vector of any embodiment of the disclosure.

[0016] The disclosure provides a method of treating Alzheimer's disease in a human subject comprising administering a therapeutically effective amount of a pharmaceutical composition according to any embodiment of the disclosure.

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[0017] In some aspects, the therapeutically effective amount of the vector is about 1×10^{11} to about 1×10^{16} genome copies.

[0018] In some aspects, the subject is an APOE2/APOE4 heterozygote, an APOE4/APOE4 homozygote or an APOE3/APOE4 heterozygote.

5 [0019] In some aspects, the composition is administered systemically, intracisternally, via intra cisterna magna, or via CI-C2 administration.

[0020] In some aspects, the pharmaceutical composition is administered at a dose of about 5.0×10^9 gc/mL CSF to about 5.0×10^{12} gc/mL CSF. In some aspects, the pharmaceutical composition is administered at a dose of about: i) 1.4×10^{10} gc/mL CSF, ii) 4.4×10^{10} gc/mL CSF, or iii) 1.4×10^{11} gc/mL CSF. In some aspects, the pharmaceutical composition is administered at a dose of about 1.4×10^{14} gc.

[0021] In some aspects, the pharmaceutical composition is administered in a total volume of about 5 mL, about 10 mL, about 15 mL, or about 20 mL.

15 [0022] In some aspects, the subject experiences an at least about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 100% increase in APOE2 Christchurch or APOE3 Christchurch expression.

20 [0023] In some aspects, the APOE2 Christchurch or APOE3 Christchurch expression occurs in the central nervous system. In some aspects, the APOE2 Christchurch or APOE3 Christchurch expression is measured in the cerebral spinal fluid (CSF).

[0024] In some aspects, following administration of the pharmaceutical composition the expression levels of at least one of total tau (T-tau) and phosphorylated tau (P-tau) are reduced in the subject relative to a pre-administration baseline.

25 [0025] In some aspects, the expression levels of T-tau, and/or P-tau are reduced by at least about 5%, at least about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 100%.

30 [0026] In some aspects, following administration of the pharmaceutical composition the amyloid beta 42/amyloid beta 40 ($A\beta_{42/40}$) ratio is increased.

[0027] In some aspects, the $A\beta_{42/40}$ ratio is increased by at least about 5%, at least about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about

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50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 100%.

[0028] In some aspects, prior to treatment with the pharmaceutical composition the subject is administered an immunosuppressant. In some aspects, the immunosuppressant is prednisone.

5 [0029] In some aspects, the prednisone is administered at a dosage of: 40 mg, once daily 1 week prior to AAV viral vector administration; 40 mg once daily for week 1 through week 2 post-AAV viral vector administration; 30 mg once daily for week 3 post-AAV viral vector administration; 20 mg once daily for week 4 post-AAV viral vector administration; 10 mg once daily for week 5 post-AAV viral vector administration; 5 mg once daily for week 6 post-
10 AAV viral vector administration; 2.5 mg once daily for week 7 post-AAV viral vector administration; and 2.5 mg every other day for week 8 post-AAV viral vector administration.

[0030] Any of the above aspects, or any other aspect described herein, can be combined with any other aspect.

15 [0031] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. In the Specification, the singular forms also include the plural unless the context clearly dictates otherwise; as examples, the terms “a,” “an,” and “the” are understood to be singular or plural and the term “or” is understood to be inclusive. By way of example, “an element” means one or more element.

20 [0032] Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. The references cited herein are not admitted to be prior art to the claimed invention. In the case of conflict, the present
25 Specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be limiting. Other features and advantages of the disclosure will be apparent from the following detailed description and claims.

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BRIEF DESCRIPTION OF THE DRAWINGS

[0033] FIG. 1A is a table showing APOE variants. The APOE Alzheimer's disease risk region (amino acids 112-158) includes APOE3 (average risk), APOE4 (high risk) and APOE2 (low risk). Shown also is the sequence of the Columbian APOE3Ch variant and the

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therapeutic gain-of-function APOE2Ch variant to be compared to the APOE2 variant in the amyloid and tau murine models.

[0034] FIG. 1B is a graph depicting heparin binding of APOE variants, APOE2, APOE3, APOE4, APOE2 Christchurch, and APOE3 Christchurch. APOE variant concentration is presented on the left y-axis. Sodium chloride (NaCl) concentration is presented on the right y-axis. The fraction number is presented on the x-axis with lower fraction # correlating to weaker heparin binding and higher fraction number correlating to stronger heparin binding.

[0035] FIG. 1C shows the experimental design for comparison of AAVrh.10hAPOE2 vs AAVrh.10hAPOE2Ch with AAVrh.10Null and PBS controls treatment of APP.PSEN1/TRE4 ("amyloid") and P301S. PSEN1/TRE4 ("tau") mice.

[0036] FIGS. 2A-2E show a series of graphs depicting the levels of viral genome copies (FIGs. 2A and 2B), APOE mRNA (FIG. 2C and 2D) and APOE protein (FIGs. 2E and 2F) following administration of AAVrh10 viral capsids encoding: null (AAV-Rh10-NULL), APOE2 (AAV-Rh10-E2), APOE2 + hemagglutinin (HA) tag (AAV-Rh10-E2HA), APOE2 Christchurch (AAV-Rh10-E2CH), or APOE2 Christchurch + HA tag (AAV-Rh10-E2CHHA) to the hippocampus of amyloid (FIGs. 2A, 2C and 2E) and tau (FIGs. 2B, 2D, and 2F) mice. The y-axis depicts viral genome copies per microgram DNA. Data represented as geometric mean $\pm$ SD. The "n" number of mice for each analysis is below each bar.

[0037] FIGs. 3A-3D show a series of graphs depicting A β 42 (FIG. 3A), A β 40 (FIG. 3B), total tau (FIG. 3C) and total tau (FIG. 3D) levels detected in the hippocampus of amyloid and tau mice treated with AAVrh10 viral capsids encoding: null (AAV-Rh10-NULL), APOE2 (AAV-Rh10-E2), or APOE2 Christchurch (AAV-Rh10-E2CH). The y-axis depicts APOE levels expressed as ng of APOE per microgram of total protein. Data represented as geometric mean $\pm$ SD. The "n" number of mice for each analysis is below each bar.

[0038] FIGs. 4A-4T show histologic parameters β -amyloid, X-34, Iba-1 and GFAP in AAVrh.10hE2 and AAVrh.10hAPOE2Ch treatment groups compared to the AAVrh.10Null and PBS control treatment groups in amyloid mice. FIGs. 4A-4D, 4F-4I, 4K-4N and 4P-4S are representative images. FIGs. 4E (amyloid, A β aggregates), 4J (X-34, amyloid fibrils), 4O (Iba-1, microglial activation) and 4T (GFAP, astroglial activation) are the quantitative analyses of the histochemistry. The analysis was at 3 months after therapy. Data is presented as $\pm$ standard error; the "n" number of mice for each analysis is listed. For β -amyloid, Iba-1, and GFAP, specific staining for neurological markers in the CA-1 region of the hippocampus is shown in red. DAPI-stained nuclei are shown in blue. The dentate gyrus (dense collection of nuclei) is visible at the bottom right corner of each panel. For X34 staining, monochrome

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images of the CA-1 region of the hippocampus are shown. The red color indicates high intensity staining (staining above a fixed intensity threshold). In X34 panels, the nuclei of the dentate gyrus are visible in the lower right corner of the field as stain-negative ovals. Bar = 100 μ m.

- 5 **[0039] FIGs. 5A-5O** show histologic parameters AT8, Iba-1 and GFAP in AAVrh.10hAPOE2 and AAVrh.10hAPOE2Ch treatment groups compared to AAVrh.10Null and PBS control treatment in tau mice. FIGs. 5A-5D, 5F-5I and 5K-5N are representative images. FIGs. 5E (AT8, tau burden and aggregates), 5J (Iba-1, microglial activation) and 5O (GFAP, astroglial activation) are the quantitative analyses of the histochemistry. The analysis was at 3 months after therapy. Data is presented as $\pm$ standard error; the “n” number of mice for each analysis is listed. For AT8, Iba-1, and GFAP, specific staining for neurological markers in the CA-1 region of the hippocampus is shown in red. DAPI-stained nuclei are shown in blue. The dentate gyrus is visible at the bottom right corner of each panel. Bar = 100 μ m.
- 15 **[0040] FIGs. 6A-6H** show the assessment of neurological integrity and hippocampal dependent memory with nesting, Y maze, Novel object recognition and Barnes maze tests in amyloid and tau mice. Assessment of AAVrh.10hAPOE2Ch (E2Ch), AAVrh.10hAPOE2 (E2), AAVrh.10Null and PBS hippocampal treatment of amyloid and tau mice in performance of: Nesting in amyloid mice (FIG. 6A), nesting in tau mice (FIG. 6B), Y maze in amyloid mice (FIG. 6C), Y maze in tau mice (FIG. 6D), novel object recognition in amyloid mice (FIG. 6E), novel object recognition in tau mice (FIG. 6F), Barnes maze in amyloid mice (FIG. 6G) and Barnes maze in tau mice (FIG. 6H). The analysis for the amyloid mice was at age 5.5 to 6 months for the amyloid mice (3 months after therapy) and ages 8.5 to 9 months for the tau mice (3 months after therapy). Data is presented as $\pm$ standard error; the “n” number of mice for each analysis is listed.

- 25 **[0041] FIGs. 7A-7D** show the total number of entries and alternations in the Y Maze assessed in all experimental groups in both amyloid and tau mice. For each analysis, treatment with AAVrh.10hAPOE2Ch (E2Ch) and AAVrh.10hAPOE2 (E2) is compared to AAVrh.10Null (Null) and PBS controls. FIG. 7A shows the total number of entries with amyloid mice; FIG. 7B shows the total numbers of alterations with amyloid mice; FIG. 7C shows the total number of entries with tau mice; and FIG. 7D shows the total numbers of alterations with tau mice. All data represented as mean $\pm$ SEM. The numbers of mice for each analysis are listed.

- 30 **[0042] FIGs. 8A and 8B** show escape latency during training period in Barnes maze test in all experimental groups, including AAVrh.10hAPOE2Ch (E2Ch) and AAVrh.10hAPOE2

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(E2) compared to AAVrh.10Null (Null) and PBS control. FIG. 8A: Barnes maze escape latency over the 7 days training period for amyloid mice; and FIG. 8B: Barnes maze escape latency over the 7-day training period for tau mice. Data is presented as mean $\pm$ SEM.

Improved learning ability were seen for E2 and E2CH in FIG 8A. amyloid mice, while only
5 E2CH treatment followed same pattern in B. tau mice. All data represented as mean $\pm$ SEM.
The numbers of mice are detailed in FIGs. 6A-6H.

[0043] FIGs. 9A and 9B depict quantitative assessment of neurodegeneration by immunohistochemistry in hippocampus of both amyloid and tau mice. FIG. 9A is a graph depicting the ratio of NeuN (neuronal marker) staining to DAPI (Nuclei) represents neuronal cell viability. FIG. 9B is a graph depicting Olig2 (oligodendrocyte marker) staining intensity
10 quantified with QuPath software and is normalized with DAPI staining, this stain provides a measure for myelin integrity/neuronal health. that has shown to be strongly correlated with neurodegeneration. Each dot represents a different animal within the same treatment group and consists of the average of three independent stained sections. Columns and error bars
15 represent mean $\pm$ SEM.

[0044] FIGs. 10A and 10B depict the assessment of neuronal health using spatial transcriptomics. Differentially expressed genes (DEGs) in the hippocampus and cortex was assessed for representative samples from each cohort of amyloid and tau mice using spatial transcriptomics. Neuronal health related transcripts were represented using two
20 classifications, Synaptic/Neuronal integrity markers and neurogenesis markers. Each dot represents mean expression change compared to PBS groups in FIG. 10A (Amyloid mice) and FIG. 10B (Tau mice).

[0045] FIGs. 11A and 11B depict RTqPCR assessment of microglial phenotype. mRNA expression levels of CD68, Clec7a, Trem2, CD163, CD206 and Arg1 in the hippocampus
25 were assessed for from each cohort in amyloid and tau mice. FIG. 11A depict Amyloid mice and FIG. 11B depict Tau mice. Each dot represents a different animal and error bars stand for geometric mean $\pm$ geometric standard deviation.

[0046] FIGs. 12A-12D depict microglial phenotype assessed by spatial transcriptomics in amyloid and tau mice. FIG. 12A (amyloid mice) and FIG. 12B (tau mice) depict relative
30 inflammatory and anti-inflammatory gene expression levels by each of three categories of genes, anti-inflammatory, DAM-activated, and homeostatic. Each dot represents mean expression change compared to PBS groups. FIG. 12C (amyloid mice) and FIG. 12D (tau mice) depict individual genes from FIG. 12A and FIB. 12B that are grouped into similar

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ontologies and the mean expression of these groups for each experimental cohort are shown by the horizontal bars to be up or down regulated.

[0047] FIG. 13A and FIG. 13B depict total cholesterol and triglyceride levels in serum of amyloid and tau mice. Levels were measured in n=4 mice from each treatment group. FIG.

5 13A (Amyloid mice) and FIG. 13B (Tau mice). Reference range indicates homeostatic levels in wild-type C57BL/6 mice. Error bars stand for mean $\pm$ SEM values. P values ANOVA and post-hoc pairwise comparison with Tukey correction for all comparisons except the triglycerides in amyloid mice which did not have a normal distribution and was evaluated by Kruskal Wallis test with post hoc Dunn's test.

10 **[0048] FIG. 14** is a graph depicting sensitivity of APOE ELISA to detect different APOE variants. 293 cells were transfected with pAAV-hAPOE2, pAAV-hAPOE4 and pAAV-hAPOE2Ch and grown in serum-free media for 72 hours before media collection. Triplicate ELISA determinations were performed at three different dilutions. Horizontal line is the mean value for each dilution for each treatment.

15 **[0049] FIGs. 15A-15D** depict the assessment of disease pathology in naïve amyloid and tau mice at the age of vector administration. FIG. 15A depicts immunohistochemical staining for amyloid-beta on sections of amyloid mouse brain showed positive signal in cortex and hippocampus indicated by yellow asterisks at 2.5 months of age at administration and increases by 5.5 months in the control mice; FIG. 15B depicts time dependent change in
20 soluble and insoluble A β 42 and A β 40 by ELISA quantification in hippocampal lysates of control mice; FIG. 15C depicts immunofluorescent staining for tau tangles in hippocampal area of tau mice (Red=AT8, blue=DAPI) shown by yellow asterisk observed at 5.5 month age of administration and increases by 8.5 months in the control mice; FIG. 15D depicts total tau and phospho-tau levels in 5.5 and 8.5 months old control tau mice hippocampal lysates
25 assessed by ELISA showing similar pattern with AT8 staining. Each dot represents different animal in related cohort.

[0050] FIGs. 16A-16C depicts the impact of treatment on lipid-related transcripts in amyloid and tau mice. Spatial transcriptomic analysis was performed for n=1 section from each treatment group in each cohort and transcription of lipid related genes was assessed across the
30 whole section. FIG. 16A is a heatmap of relative expression level for all lipid-related transcripts. Each row represents a different transcript matrixed by experimental group; FIG. 16B (amyloid mice) and FIG. 16C (tau mice) depicts a gene ontology (GO) analysis of differentially expressed lipid-related transcripts in E2 and E2Ch groups compared to PBS.

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Each bar represents related GO-term plotted against enrichment analyzed by Fisher's exact test.

DETAILED DESCRIPTION

[0051] The disclosure provides a method of treating or preventing Alzheimer's Disease in a subject comprising administering an adeno associated virus (AAV) vector comprising an apolipoprotein (APOE) polypeptide having a Christchurch mutation. In some aspects, the subject is an APOE2/APOE4 heterozygote, an APOE4/APOE4 homozygote, an APOE3/APOE3 homozygote, or an APOE3/APOE4 heterozygote. In some aspects, the method comprises administering to the subject a therapeutically effective amount of a recombinant adeno associated virus (rAAV) vector which comprises a nucleic acid sequence encoding an APOE polypeptide or a fragment thereof having a Christchurch mutation. In some aspects, the APOE Christchurch mutation is R136S. In some aspects, the APOE polypeptide having the Christchurch mutation is an APOE2 polypeptide or APOE3 polypeptide. In some aspects, the vector is administered in a therapeutically effective amount at a dose ranging from 5.0×10^9 genome copies (gc)/mL CSF to about 5.0×10^{12} gc/mL CSF. In some aspects, the vector is administered via C1-C2 administration or intracisterna magna (ICM) administration.

AAV Vectors

[0052] In some aspects, an isolated nucleic acid sequence comprising the nucleic acid sequence encoding an apolipoprotein polypeptide having a Christchurch mutation can be a recombinant AAV vector (rAAV vector). In some aspects, an isolated nucleic acid sequence comprising the nucleic acid sequence encoding an apolipoprotein polypeptide having a Christchurch mutation can be a recombinant AAV vector (rAAV vector).

[0053] An "rAAV vector" as used herein is in reference to a vector comprising, consisting essentially of, or consisting of one or more transgene sequences and one or more AAV inverted terminal repeat sequences (ITRs). In some aspects, rAAV vectors contain one or more of an enhancer, a promoter, at least one nucleic acid that may encode at least one protein, an intronic sequence, and a polyA sequence.

[0054] In some aspects, the disclosure relates to an rAAV vector which comprises an apolipoprotein 2 (APOE2) Christchurch mutation encoding nucleic acid for use in the treatment or prevention of Alzheimer's disease in a subject in need thereof.

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[0055] In some aspects, the disclosure relates to an rAAV vector which comprises an APOE2 Christchurch mutation encoding nucleic acid for use in the treatment of Alzheimer's disease in a subject in need thereof.

5 [0056] In some aspects, the disclosure relates to an rAAV vector which comprises an APOE2 Christchurch mutation encoding nucleic acid for reversing or stabilizing symptoms of Alzheimer's disease in a subject in need thereof.

[0057] In some aspects, the disclosure relates to an rAAV vector which comprises an APOE2 Christchurch mutation encoding nucleic acid for improving the symptoms of Alzheimer's disease in a subject in need thereof.

10 [0058] In some aspects, the disclosure relates to an rAAV vector which comprises an apolipoprotein 3 (APOE) Christchurch mutation encoding nucleic acid for use in the treatment or prevention of Alzheimer's disease in a subject in need thereof.

[0059] In some aspects, the disclosure relates to an rAAV vector which comprises an APOE3 Christchurch mutation encoding nucleic acid for use in the treatment of Alzheimer's disease
15 in a subject in need thereof.

[0060] In some aspects, the disclosure relates to an rAAV vector which comprises an APOE3 Christchurch mutation encoding nucleic acid for reversing or stabilizing symptoms of Alzheimer's disease in a subject in need thereof.

[0061] In some aspects, the disclosure relates to an rAAV vector which comprises an APOE3 Christchurch mutation encoding nucleic acid for improving the symptoms of Alzheimer's
20 disease in a subject in need thereof.

[0062] In some aspects, rAAV vectors used in methods of the disclosure comprise in the 5' to 3' direction a first AAV ITR sequence; an enhancer sequence; a promoter sequence; a chimeric intron sequence, a nucleic acid sequence encoding an APOE2 polypeptide
25 comprising a Christchurch mutation (APOE2 Christchurch mutation or APOE2 Christchurch); a polyA sequence; and a second ITR sequence. In some aspects, an APOE2 Christchurch mutation-encoding AAV vector of the disclosure can comprise, consist essentially of, or consist of a nucleic acid sequence at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% (or any percentage in between) identical to SEQ ID NO: 8. In some
30 aspects, an APOE2 Christchurch mutation-encoding AAV vector of the disclosure can comprise, consist essentially of, or consist of a nucleic acid sequence at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% (or any percentage in between) identical to SEQ ID NO: 22.

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[0063] In some aspects, rAAV vectors used in methods of the disclosure comprise in the 5' to 3' direction a first AAV ITR sequence; an enhancer sequence; a promoter sequence; a chimeric intron sequence, a nucleic acid sequence encoding an APOE3 polypeptide comprising a Christchurch mutation (APOE3 Christchurch mutation or APOE3 Christchurch);
5 a polyA sequence; and a second ITR sequence. In some aspects, an APOE3 Christchurch mutation-encoding AAV vector of the disclosure can comprise, consist essentially of, or consist of a nucleic acid sequence at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% (or any percentage in between) identical to SEQ ID NO: 21.

[0064] In some aspects, rAAV vectors used in methods of the disclosure comprise in the 5' to 3' direction a first AAV ITR sequence; an enhancer sequence; a promoter sequence; a chimeric intron sequence, a nucleic acid sequence encoding an APOE3 polypeptide comprising a Christchurch mutation (APOE3 Christchurch mutation or APOE3 Christchurch); a polyA sequence; and a second ITR sequence. In some aspects, an APOE3 Christchurch mutation-encoding AAV vector of the disclosure can comprise, consist essentially of, or
10 consist of a nucleic acid sequence at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% (or any percentage in between) identical to SEQ ID NO: 24 or SEQ ID NO: 25.

[0065] In some aspects, an enhancer sequence can comprise, consist essentially of, or consist of a human cytomegalovirus (CMV) enhancer sequence. A CMV enhancer sequence can
20 comprise, consist essentially of, or consist of a nucleic acid sequence at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% (or any percentage in between) identical to SEQ ID NO: 3.

[0066] In some aspects, a promoter sequence can comprise, consist essentially of, or consist of a chicken β -actin promoter sequence. A chicken β -actin promoter sequence can comprise,
25 consist essentially of, or consist of a nucleic acid sequence at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% (or any percentage in between) identical to SEQ ID NO: 4.

[0067] In some aspects, a chimeric intron sequence can comprise, consist essentially of, or consist of a nucleic acid sequence at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%,
30 98%, 99% or 100% (or any percentage in between) identical to SEQ ID NO: 5.

[0068] In some aspects, a polyA sequence can comprise, consist essentially of, or consist of a β -globin polyA sequence. A β -globin polyA sequence can comprise, consist essentially of, or consist of a nucleic acid sequence at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% (or any percentage in between) identical to SEQ ID NO: 7.

Nucleic acid sequences encoding APOE2 polypeptides

[0069] Apolipoprotein (APOE) is a protein involved in the metabolism of fats in the body. It is a family of proteins that binds fats and interacts with the low density lipoprotein receptor (LDLR) for normal processing of triglyceride rich lipoproteins. In peripheral tissues, APOE is produced by the liver and macrophages, and mediates cholesterol metabolism. In the central nervous system, APOE is produced by astrocytes and transports cholesterol to neurons via APO receptors, which are members of the LDLR family.

[0070] APOE is synthesized as a 317 amino acid protein that is intracellularly processed to cleave an N-terminal 18 amino acid signal peptide (SEQ ID NO: 19) resulting in a mature protein 299 amino acids in length. There are three major variants of APOE: APOE2, APOE3, and APOE4. The three variants differ from one another at two positions: residue 112 (residue 130 inclusive of signal peptide) and residue 158 (residue 176 inclusive of signal peptide). The amino acid differences of the three APOE variants are set forth in Table 1. Table 1 also displays the prevalence in the global population and the relative risk for developing AD.

[0071] APOE2 is characterized in that it has a cysteine at position 112 (residue 130 including the signal peptide) and a cysteine at position 158 (residue 176 including the signal peptide). APOE3 is characterized in that it has a cysteine at position 112 (residue 130 including the signal peptide) and an arginine at position 158 (residue 176 including the signal peptide). APOE4 is characterized in that it has an arginine at position 112 (residue 130 including the signal peptide) and an arginine at position 158 (residue 176 including the signal peptide).

[0072] Previous studies identified a further variant of APOE3 wherein an arginine to serine mutation occurs at position 136 (R136S). This mutation is referred to as the Christchurch mutation. In some aspects, the Christchurch mutation can refer to a mutation of R154S when referring to an unprocessed APOE polypeptide still containing the signal peptide. The Christchurch mutation can be inserted into other APOE variants disclosed herein such as APOE2 or APOE4. In some aspects, an APOE2 polypeptide can be modified herein to also comprise the R136S (R154S) Christchurch mutation.

Table 1: Summary of APOE variants: APOE2, APOE3, and APOE4.

APOE Variant	Amino Acid position mature (Amino Acid position + signal peptide)			Prevalence	Risk for developing AD
	112 (130)	136 (154)	158 (176)		
APOE2	Cys	Arg	Cys	7%	Low
APOE3	Cys	Arg	Arg	79%	Average
APOE4	Arg	Arg	Arg	15-20%	High

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APOE3 Christchurch	Cys	Ser	Arg	13%	Low
APOE2 Christchurch	Cys	Ser	Cys	synthetic	synthetic

- 5 **[0073]** In some aspects, an APOE2 polypeptide comprising the signal peptide can comprise, consist essentially of, or consist of an amino acid sequence at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% (or any percentage in between) identical to SEQ ID NO: 9.
- 10 **[0074]** In some aspects, a mature APOE2 polypeptide lacking the signal peptide can comprise, consist essentially of, or consist of an amino acid sequence at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% (or any percentage in between) identical to SEQ ID NO: 14.
- 15 **[0075]** In some aspects, an APOE3 polypeptide comprising the signal peptide can comprise, consist essentially of, or consist of an amino acid sequence at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% (or any percentage in between) identical to SEQ ID NO: 10.
- 20 **[0076]** In some aspects, a mature APOE3 polypeptide lacking the signal peptide can comprise, consist essentially of, or consist of an amino acid sequence at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% (or any percentage in between) identical to SEQ ID NO: 15.
- 25 **[0077]** In some aspects, an APOE4 polypeptide comprising the signal peptide can comprise, consist essentially of, or consist of an amino acid sequence at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% (or any percentage in between) identical to SEQ ID NO: 13.
- 30 **[0078]** In some aspects, a mature APOE4 polypeptide lacking the signal peptide can comprise, consist essentially of, or consist of an amino acid sequence at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% (or any percentage in between) identical to SEQ ID NO: 16.
- 35 **[0079]** In some aspects, an APOE3 Christchurch mutation polypeptide comprising the signal peptide can comprise, consist essentially of, or consist of an amino acid sequence at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% (or any percentage in between) identical to SEQ ID NO: 12.
- 40 **[0080]** In some aspects, a mature APOE3 Christchurch mutation polypeptide lacking the signal peptide can comprise, consist essentially of, or consist of an amino acid sequence at

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least 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% (or any percentage in between) identical to SEQ ID NO: 18.

[0081] In some aspects, an APOE2 Christchurch mutation polypeptide comprising the signal peptide can comprise, consist essentially of, or consist of an amino acid sequence at least
5 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% (or any percentage in between) identical to SEQ ID NO: 11.

[0082] In some aspects, a mature APOE2 Christchurch mutation polypeptide lacking the signal peptide can comprise, consist essentially of, or consist of an amino acid sequence at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% (or any
10 percentage in between) identical to SEQ ID NO: 17.

[0083] In some aspects, a nucleic acid sequence encoding an apolipoprotein 2 (APOE2) Christchurch mutation polypeptide can comprise, consist essentially of, or consist of a nucleic acid sequence at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% (or any percentage in between) identical to SEQ ID NO: 6.

[0084] In some aspects, a nucleic acid sequence encoding an apolipoprotein 3 (APOE3) Christchurch mutation polypeptide can comprise, consist essentially of, or consist of a nucleic acid sequence at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% (or any percentage in between) identical to SEQ ID NO: 20.

[0085] In some aspects, the disclosure provides a nucleic acid sequence comprising SEQ ID
20 NO: 8 or SEQ ID NO: 21 or a variant thereof for treating Alzheimer's disease.

Inverted Terminal Repeat Sequences

[0086] In some aspects, a first ITR can comprise, consist essentially of, or consist of a nucleic acid sequence at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% (or any percentage in between) identical to SEQ ID NO: 1, or complement thereof. In some
25 aspects, a first ITR can comprise, consist essentially of, or consist of a nucleic acid sequence at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% (or any percentage in between) identical to SEQ ID NO: 2, or complement thereof. In some aspects, a first ITR can comprise, consist essentially of, or consist of a nucleic acid sequence at least
30 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% (or any percentage in between) identical to SEQ ID NO: 23, or complement thereof.

[0087] In some aspects, a second ITR can comprise, consist essentially of, or consist of a nucleic acid sequence at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% (or any percentage in between) identical to SEQ ID NO: 2, or complement thereof.

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[0088] In some aspects, a second ITR can comprise, consist essentially of, or consist of a nucleic acid sequence at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% (or any percentage in between) identical to SEQ ID NO: 1, or complement thereof.

In some aspects, a first ITR can comprise, consist essentially of, or consist of a nucleic acid
5 sequence at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% (or any percentage in between) identical to SEQ ID NO: 23, or complement thereof.

AAV viral vectors

[0089] AAV vectors of the disclosure can be packaged as an AAV viral vector.

[0090] An "rAAV viral vector" refers to a viral particle composed of at least one rAAV
10 capsid protein and an encapsidated polynucleotide AAV vector. Thus, production of an rAAV viral vector necessarily includes production of an rAAV vector. The term "viral capsid" or "capsid" refers to the proteinaceous shell or coat of a viral particle. Capsids function to encapsidate, protect, transport, and release into the host cell a viral genome. Capsids are generally comprised of oligomeric structural subunits of protein ("capsid proteins"). As used
15 herein, the term "encapsidated" means enclosed within a viral capsid. The viral capsid of AAV is composed of a mixture of three viral capsid proteins: VP1, VP2, and VP3.

[0091] rAAV viral vectors useful in the practice of the present disclosure can be constructed utilizing methodologies well known in the art of molecular biology. Typically, AAV viral vectors carrying transgenes are assembled from polynucleotides encoding the transgene,
20 suitable regulatory elements and elements necessary for production of viral proteins which mediate cell transduction.

[0092] The terms "Gene transfer" or "gene delivery" refer to methods or systems for reliably inserting foreign DNA into host cells. Such methods can result in transient expression of non integrated transferred DNA, extrachromosomal replication and expression of transferred
25 replicons (e.g. episomes), or integration of transferred genetic material into the genomic DNA of host cells.

[0093] Examples of viral vectors include but are not limited to adenoviral, retroviral, lentiviral, herpesvirus and adeno-associated virus (AAV) vectors.

[0094] Such recombinant viruses may be produced by techniques known in the art, such as by
30 transfecting packaging cells or by transient transfection with helper plasmids or viruses. Typical examples of virus packaging cells include PA317 cells, PsiCRIP cells, GPenv+ cells, 293 cells, etc. Detailed protocols for producing such replication-defective recombinant viruses

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may be found for instance in WO95/14785, WO96/22378, US5,882,877, US6,013,516, US4,861,719, US5,278,056 and WO94/19478.

[0095] In one embodiment, adeno-associated viral (AAV) vectors are employed.

[0096] In other embodiments, the rAAV vector is AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAVrh10 or any other serotypes of AAV that can infect humans, monkeys or other species.

[0097] In an exemplary embodiment, the rAAV vector is AAVrh10.

[0098] By an "rAAV vector" is meant a vector derived from an adeno-associated virus serotype, including without limitation, AAV-1, AAV-2, AAV-3, AAV-4, AAV-5, AAV6, etc. AAV vectors can have one or more of the AAV wild-type genes deleted in whole or part, e.g., the rep and/or cap genes, but retain functional flanking ITR sequences. Functional ITR sequences are necessary for the rescue, replication and packaging of the AAV virion. Thus, an AAV vector is defined herein to include at least those sequences desired in cis for replication and packaging (e. g., functional ITRs) of the virus. The ITRs need not be the wild-type nucleotide sequences, and may be altered, e. g by the insertion, deletion or substitution of nucleotides, so long as the sequences provide for functional rescue, replication and packaging. AAV expression vectors are constructed using known techniques to at least provide as operatively linked components in the direction of transcription, control elements including a transcriptional initiation region, the DNA of interest (i.e. the APOE2 gene) and a transcriptional termination region.

[0099] The control elements are selected to be functional in a mammalian cell. The resulting construct which contains the operatively linked components is bounded (5' and 3') with functional AAV ITR sequences. By "adeno-associated virus inverted terminal repeats " or "AAV ITRs" is meant the art-recognized regions found at each end of the AAV genome which function together in cis as origins of DNA replication and as packaging signals for the virus. AAV ITRs, together with the AAV rep coding region, provide for the efficient excision and rescue from, and integration of a nucleotide sequence interposed between two flanking ITRs into a mammalian cell genome. In some aspects, ITR sequences of the disclosure may comprise a deletion of one or more nucleotides at one or more positions of the ITR sequence. In some aspects, deleted nucleotides in an ITR sequence can be repaired in vivo or during vector replication. The nucleotide sequences of AAV ITR regions are known. See, e.g., Kotin, 1994; Berns, KI "Parvoviridae and their Replication" in Fundamental Virology, 2nd Edition, (B. N. Fields and D. M. Knipe, eds.) for the AAV2 sequence. As used herein, an "AAV ITR" does not necessarily comprise the wild-type nucleotide sequence, but may be altered, e.g., by

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the insertion, deletion or substitution of nucleotides. Additionally, the AAV ITR may be derived from any of several AAV serotypes, including without limitation, AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, etc. Furthermore, 5' and 3' ITRs which flank a selected nucleotide sequence in an AAV vector need not necessarily be identical or derived from the same AAV serotype or isolate, so long as they function as intended, i.e., to allow for excision and rescue of the sequence of interest from a host cell genome or vector, and to allow integration of the heterologous sequence into the recipient cell genome when AAV Rep gene products are present in the cell. Additionally, AAV ITRs may be derived from any of several AAV serotypes, including without limitation, AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, etc. Furthermore, 5' and 3' ITRs which flank a selected nucleotide sequence in an AAV expression vector need not necessarily be identical or derived from the same AAV serotype or isolate, so long as they function as intended, i. e., to allow for excision and rescue of the sequence of interest from a host cell genome or vector, and to allow integration of the DNA molecule into the recipient cell genome when AAV Rep gene products are present in the cell.

15 [0100] Exemplary vectors are vectors derived from AAV serotypes having tropism for and high transduction efficiencies in cells of the mammalian central nervous system. A review and comparison of transduction efficiencies of different serotypes is provided in Cearley CN et al., 2008. In other non-limiting examples, some vectors include vectors derived from any serotypes like AAV1, AAV2, AAV3, AAV4, AA5, AAV6, AAV7, AAV8, AAV9, or

20 AAVrh10.

[0101] The selected nucleotide sequence is operably linked to control elements that direct the transcription or expression thereof in the subject *in vivo*. Such control elements can comprise control sequences normally associated with the selected gene.

[0102] Alternatively, heterologous control sequences can be employed. Useful heterologous control sequences generally include those derived from sequences encoding mammalian or viral genes. Examples include, but are not limited to, the phosphoglycerate kinase (PKG) promoter, CAG, MCK (muscle creatine kinase), the SV40 early promoter, mouse mammary tumor virus LTR promoter; adenovirus major late promoter (Ad MLP); a herpes simplex virus (HSV) promoter, a cytomegalovirus (CMV) promoter such as the CMV immediate early

25 promoter region (CMVIE), rous sarcoma virus (RSV) promoter, synthetic promoters, hybrid promoters, and the like. The promoters can be of human origin or from other species, including from mice. In addition, sequences derived from nonviral genes, such as the murine metallothionein gene, will also find use herein. Such promoter sequences are commercially available from, e. g. Stratagene (San Diego, CA).

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[0103] Examples of heterologous promoters include the CMV promoter.

[0104] Examples of inducible promoters include DNA responsive elements for ecdysone, tetracycline, hypoxia and aurofin.

[0105] The AAV expression vector which harbors the DNA molecule of interest bounded by
5 AAV ITRs, can be constructed by directly inserting the selected sequence (s) into an AAV
genome which has had the major AAV open reading frames ("ORFs") excised therefrom.
Other portions of the AAV genome can also be deleted, so long as a sufficient portion of the
ITRs remain to allow for replication and packaging functions. Such constructs can be
designed using techniques well known in the art. See, e. g. U. S. Patents Nos. 5,173, 414 and
10 5,139, 941; International Publications Nos. WO 92/01070 (published 23 January 1992) and
WO 93/03769 (published 4 March 1993); Lebkowski et al., 1988 ; Vincent et al., 1990;
Carter, 1992; Muzyczka, 1992 ; Kotin, 1994; Shelling and Smith, 1994 ; and Zhou et al., 1994.
Alternatively, AAV ITRs can be excised from the viral genome or from an AAV vector
containing the same and fused 5' and 3' of a selected nucleic acid construct that is present in
15 another vector using standard ligation techniques. AAV vectors which contain ITRs have
been described in, e. g. U. S. Patent no. 5,139, 941. In particular, several AAV vectors are
described therein which are available from the American Type Culture Collection ("ATCC")
under Accession Numbers 53222, 53223, 53224, 53225 and 53226. Additionally, chimeric
genes can be produced synthetically to include AAV ITR sequences arranged 5' and 3' of one
20 or more selected nucleic acid sequences. Preferred codons for expression of the chimeric gene
sequence in mammalian CNS cells can be used. The complete chimeric sequence is assembled
from overlapping oligonucleotides prepared by standard methods. See, e. g., Edge, 1981 ;
Nambair et al., 1984 ; Jay et al., 1984. In order to produce AAV virions, an AAV expression
vector is introduced into a suitable host cell using known techniques, such as by transfection.
25 A number of transfection techniques are generally known in the art. See, e. g. , Graham et al.,
1973; Sambrook et al. (1989) Molecular Cloning, a laboratory manual, Cold Spring Harbor
Laboratories, New York, Davis et al. (1986) Basic Methods in Molecular Biology, Elsevier,
and Chu et al., 1981. Particularly suitable transfection methods include calcium phosphate co-
precipitation (Graham et al., 1973), direct microinjection into cultured cells (Capecchi, 1980),
30 electroporation (Shigekawa et al., 1988), liposome mediated gene transfer (Mannino et al.,
1988), lipid-mediated transduction (Felgner et al., 1987), and nucleic acid delivery using high-
velocity microprojectiles (Klein et al., 1987).

[0106] AAV viral vectors of the disclosure comprise: i) an AAV vector described herein; and
ii) an AAV capsid protein.

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[0107] In some aspects, an AAV capsid protein can be any AAV capsid protein. In some aspects, the AAV capsid protein is an AAV1 capsid protein, a modified AAV1 capsid protein, an AAV2 capsid protein, a modified AAV2 capsid protein, an AAV4 capsid protein, a modified AAV4 capsid protein, an AAV5 capsid protein, a modified AAV5 capsid protein, an AAV6 capsid protein, a modified AAV6 capsid protein, an AAV7 capsid protein, a modified AAV7 capsid protein, an AAV8 capsid protein, a modified AAV8 capsid protein, an AAV9 capsid protein, a modified AAV9 capsid protein, an AAV10 capsid protein, a modified AAV10 capsid protein, an AAV11 capsid protein, a modified AAV11 capsid protein, an AAV12 capsid protein, a modified AAV12 capsid protein, an AAV13 capsid protein, a modified AAV13 capsid protein, an AAVPHP.B capsid protein, a modified AAVPHP.B capsid protein, an AAVrh74 capsid protein, a modified AAVrh74 capsid protein, a MyoAAV capsid protein, a modified MyoAAV capsid protein, an AAVrh.10 capsid protein, or a modified AAVrh.10 capsid protein. In some aspects, the AAV capsid protein is an AAVrh.10 capsid protein.

15 **Methods of Treatment**

[0108] The disclosure provides a method for treating or preventing Alzheimer's disease (AD) in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of an rAAV vector which comprises a nucleic acid sequence encoding an APOE2 polypeptide having a Christchurch mutation or a fragment thereof or an APOE3 polypeptide having a Christchurch mutation or a fragment thereof.

[0109] The disclosure provides a method for treating Alzheimer's disease in a subject comprising administering a therapeutically effective amount of a pharmaceutical composition comprising an APOE2 Christchurch rAAV viral vector or an APOE3 Christchurch rAAV viral vector.

[0110] The disclosure provides a method for treating Alzheimer's disease in a subject comprising administering a therapeutically effective amount of a pharmaceutical composition comprising an APOE2 Christchurch rAAV viral vector or an APOE3 Christchurch rAAV viral vector, wherein following administration the subject experiences an increase in APOE2 or APOE3 expression. In some aspects, the increased APOE2 or APOE3 expression is an APOE2 Christchurch polypeptide or an APOE3 Christchurch polypeptide.

[0111] Subjects of the disclosure can express any combination of APOE variants including being APOE2 homozygotes, APOE3 homozygotes, APOE4 homozygotes, APOE2/APOE4

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heterozygotes, APOE2/APOE3 heterozygotes, or APOE3/APOE4 heterozygotes. In some aspects, subjects of the disclosure are APOE4 homozygotes.

[0112] Subjects of the disclosure can have a range of cognitive impairment associated with AD including no impairment, mild cognitive impairment (CI), or mild, moderate dementia, or severe dementia. In some aspects, subjects of the disclosure are at least 50 years old. In some aspects, subjects of the disclosure can be any age.

[0113] In some aspects, subjects of the disclosure have CSF biomarkers consistent with Alzheimer's disease. In some aspects, subjects of the disclosure are determined to be positive via amyloid-targeted positron emission tomography (PET).

[0114] In some aspects, the subject experiences an increase in APOE2 Christchurch mutation polypeptide expression relative to a pre-administration baseline. Quantification of APOE2 Christchurch mutation expression can be performed according to any method known in the art. In some aspects the subject experiences an at least about 5%, at least about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 100% increase in APOE2 expression relative to a pre-administration baseline.

[0115] In some aspects, the subject experiences an increase in APOE3 Christchurch mutation polypeptide expression relative to a pre-administration baseline. Quantification of APOE3 Christchurch mutation expression can be performed according to any method known in the art. In some aspects the subject experiences an at least about 5%, at least about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 100% increase in APOE3 expression relative to a pre-administration baseline.

[0116] APOE2 Christchurch mutation or APOE3 Christchurch mutation expression can occur anywhere in the central nervous system including any region of the brain and the cerebral spinal fluid (CSF).

[0117] APOE2, APOE3, and APOE4, with and without the Christchurch mutation, expression can be quantified according to any method known in the art. APOE2, APOE3, and APOE4 quantification can be performed using mass spectrometry (MS), western blot, chromatography, or chromatography-linked mass spectrometry (i.e. LC-MS).

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[0118] In some aspects, APOE2 Christchurch mutation or expression is reflected as a percentage of APOE4 expression and or total APOE expression. In some aspects, APOE2 Christchurch mutation expression is calculated as total APOE2 expression divided by APOE4 expression. This ratio can be multiplied by 100 to produce a percentage. In some aspects, APOE2 Christchurch mutation expression is calculated as total APOE2 Christchurch mutation expression divided by total APOE expression (total expression of APOE2 Christchurch, APOE2, APOE3 and/or APOE4). This ratio can be multiplied by 100 to produce a percentage. Evaluation of APOE expression can be evaluated according to any suitable protein quantification method.

[0119] In some aspects, APOE3 Christchurch mutation or expression is reflected as a percentage of APOE4 expression and or total APOE expression. In some aspects, APOE3 Christchurch mutation expression is calculated as total APOE3 expression divided by APOE4 expression. This ratio can be multiplied by 100 to produce a percentage. In some aspects, APOE3 Christchurch mutation expression is calculated as total APOE3 Christchurch mutation expression divided by total APOE expression (total expression of APOE3 Christchurch, APOE2, APOE3 and/or APOE4). This ratio can be multiplied by 100 to produce a percentage. Evaluation of APOE expression can be evaluated according to any suitable protein quantification method.

[0120] Hippocampal volume declines over time in individuals with AD at the prodromal stage and later. Hippocampal volume is usually correlated with cognitive and functional changes over time, making it a useful marker of disease progression. In some aspects, following treatment with APOE2 Christchurch mutation or APOE3 Christchurch mutation pharmaceutical compositions of the disclosure, a subject experiences an increase in hippocampal volume. In some aspects, following treatment with APOE2 Christchurch mutation or APOE3 Christchurch mutation pharmaceutical compositions of the disclosure, a subject's hippocampal volume stays the same. In some aspects, following treatment with APOE2 Christchurch mutation or APOE3 Christchurch mutation pharmaceutical compositions of the disclosure, a subject experiences a reduced rate of hippocampal volume decline. In some aspects, hippocampal volume decline is assessed by brain MRI.

30 Cognitive Assessment

[0121] Cognitive improvement following administration of APOE2 Christchurch mutation or APOE3 Christchurch mutation pharmaceutical compositions can be assessed according to any method known in the art. Such assessments can include, but are not limited to, Clinical

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Dementia Rating (CDR), Alzheimer's Disease Assessment Scale – Cognitive Assessment (ADAS-Cog 13), or Mini-Mental State Examination.

[0122] The Clinical Dementia Rating (CDR) scale is a clinician-rated dementia staging system that tracks the progression of cognitive impairment in 6 categories (memory, orientation, judgment and problem solving, community affairs, home and hobbies, and personal care). Each category is scored on a 5-point scale in which None=0, Questionable=0.5, Mild=1, Moderate=2, and Severe=3. The global CDR score is established by clinical scoring rules and has values of 0 (no dementia), 0.5 (questionable dementia), 1 (mild dementia), 2 (moderate dementia), and 3 (severe dementia). The CDR-SB is obtained by adding the ratings in each of the 6 categories and ranges from 0 to 18 with higher scores indicative of greater impairment.

[0123] The CDR-SB will be administered at the Screening/Baseline visits before administration of an APOE2 Christchurch mutation or APOE3 Christchurch mutation pharmaceutical composition of the disclosure. These assessments will be used as a measure of clinical effect.

[0124] The Alzheimer's Disease Assessment Scale – Cognitive Subscale (13 items) (ADAS-Cog 13) is a structured scale that evaluates memory, orientation, attention, reasoning, language, and constructional praxis. Higher scores indicate greater impairment.

[0125] The MMSE is a brief 30-point questionnaire used to assess cognitive impairment with lower scores indicating greater impairment. The MMSE assesses 11 categories of cognition including orientation to time, memory, attention, concentration, naming, repetition, comprehension, and the ability to create a sentence and to copy two intersecting polygons.

Biomarker Testing

[0126] APOE2 expression, following administration of an APOE2 Christchurch mutation pharmaceutical composition of the disclosure, can be evaluated in any region of the CNS. In some aspects, APOE2 expression is evaluated in the brain. In some aspects, APOE2 expression is evaluated in the CSF. Amyloid beta ($A\beta$) 42, $A\beta$ 42/40, T-tau, and P-tau, are considered the AD core biomarkers. It is thought that T-tau and P-tau increase after amyloid brain accumulation (amyloid cascade hypothesis). Both amyloid and tau accumulation are associated with inflammation in the brain at different times during disease progression, with amyloid driving inflammation during early accumulation (MCI cases) and tau after amyloid load in the brain is near AD levels (prodromal AD). $A\beta$ 42 is reduced in CSF of AD

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participants, T-tau and P-tau are increased. While not a core CSF biomarker, amyloid beta 40 is also assessed.

[0127] APOE3 expression, following administration of an APOE3 Christchurch mutation pharmaceutical composition of the disclosure, can be evaluated in any region of the CNS. In some aspects, APOE3 expression is evaluated in the brain. In some aspects, APOE3 expression is evaluated in the CSF. Amyloid beta ($A\beta$) 42, $A\beta_{42/40}$, T-tau, and P-tau, are considered the AD core biomarkers. It is thought that T-tau and P-tau increase after amyloid brain accumulation (amyloid cascade hypothesis). Both amyloid and tau accumulation are associated with inflammation in the brain at different times during disease progression, with amyloid driving inflammation during early accumulation (MCI cases) and tau after amyloid load in the brain is near AD levels (prodromal AD). $A\beta_{42}$ is reduced in CSF of AD participants, T-tau and P-tau are increased. While not a core CSF biomarker, amyloid beta 40 is also assessed.

[0128] In some aspects, following administration of the pharmaceutical composition the expression levels of at least one of amyloid beta 42 ($A\beta_{42}$), amyloid beta 40 ($A\beta_{40}$), T-tau, and P-tau are reduced in the subject relative to a pre-administration baseline. In some aspects, following administration of the pharmaceutical composition the expression levels of at least one of amyloid beta 42 ($A\beta_{42}$), amyloid beta 40 ($A\beta_{40}$), T-tau, and P-tau are increased in the subject relative to a pre-administration baseline. In some aspects, following administration of the pharmaceutical composition the expression levels of at least one of amyloid beta 42 ($A\beta_{42}$), amyloid beta 40 ($A\beta_{40}$), T-tau, and P-tau remain constant in the subject relative to a pre-administration baseline.

[0129] In some aspects, the expression levels of $A\beta_{42}$ are reduced by at least about 5%, at least about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 100% relative to a pre-administration baseline.

[0130] In some aspects, the expression levels of $A\beta_{40}$ are reduced by at least about 5%, at least about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 100% relative to a pre-administration baseline.

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[0131] In some aspects, the expression levels of T-tau are reduced by at least about 5%, at least about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 100% relative to a pre-administration baseline.

[0132] In some aspects, the expression levels of P-tau are reduced by at least about 5%, at least about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 100% relative to a pre-administration baseline.

[0133] In some aspects, following administration of the pharmaceutical composition the expression levels of A β ₄₂ is altered. In some aspects, following administration of the pharmaceutical composition, the expression levels of A β ₄₂ is increased. In some aspects, following administration of the pharmaceutical composition, the expression levels of A β ₄₂ is decreased. In some aspects, changes to the expression levels of A β ₄₂ following administration of the pharmaceutical composition is subject-specific.

[0134] In some aspects, following administration of the pharmaceutical composition the amyloid beta 42/amyloid beta 40 (A β _{42/40}) ratio is altered. In some aspects, following administration of the pharmaceutical composition, the A β _{42/40} ratio is increased. In some aspects, following administration of the pharmaceutical composition, the A β _{42/40} ratio is decreased. In some aspects, changes to A β _{42/40} ratio following administration of the pharmaceutical composition is subject-specific.

[0135] In some aspects, the A β _{42/40} ratio is increased by at least about 5%, at least about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 100% relative to a pre-administration baseline.

[0136] In a particular embodiment, the gene encoded by a nucleic acid sequence in an AAV vector is the APOE2 gene having a Christchurch mutation.

[0137] In a particular embodiment, the gene encoded by a nucleic acid sequence in an AAV vector is the APOE3 gene having a Christchurch mutation.

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[0138] As used herein in its broadest meaning, the term “preventing” or “prevention” refers to preventing the disease or condition from occurring in a subject which has not yet been diagnosed as having it or which does not have any clinical symptoms.

[0139] As used herein, the term “treating” or “treatment”, as used herein, means reversing, 5 alleviating, or inhibiting the progress of the disorder or condition to which such term applies, or one or more symptoms of such disorder or condition. A “therapeutically effective amount” is intended for a minimal amount of active agent which is necessary to impart therapeutic benefit to a subject. For example, a “therapeutically effective amount” to a patient is such an amount which induces, ameliorates, stabilises, slows down the progression or otherwise 10 causes an improvement in the pathological symptoms, disease progression or physiological conditions associated with or resistance to succumbing to a disorder.

[0140] As used herein, the term “subject” denotes a mammal, such as a rodent, a feline, a canine, and a primate. In some aspects, a subject according to the disclosure is a human. In the context of the present disclosure, a “subject in need thereof” denotes a subject, e.g., a human, 15 and more particularly a subject with a Alzheimer’s disease.

[0141] As used herein, the term “gene” refers to a polynucleotide containing at least one open reading frame that is capable of encoding a particular polypeptide or protein after being transcribed and translated.

[0142] As used herein, the terms “coding sequence”, “a sequence which encodes a particular protein” or “encoding nucleic acid”, denotes a nucleic acid sequence which is transcribed (in 20 the case of DNA) and translated (in the case of mRNA) into a polypeptide *in vitro* or *in vivo* when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxy) terminus. A coding sequence can include, but is not limited to, 25 cDNA from prokaryotic or eukaryotic mRNA, genomic DNA sequences from prokaryotic or eukaryotic DNA, and even synthetic DNA sequences.

[0143] In a particular embodiment, the disclosure relates to a method for preventing or treating Alzheimer’s disease in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of an AAV vector, AAV viral vector, or 30 pharmaceutical composition which comprises an APOE2 Christchurch mutation encoding nucleic acid.

[0144] In a particular embodiment, the disclosure relates to a method for treating Alzheimer’s disease in a subject in need thereof, comprising administering to said subject a therapeutically

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effective amount of an AAV vector, AAV viral vector, or pharmaceutical composition which comprises an APOE2 Christchurch mutation encoding nucleic acid.

[0145] In a particular embodiment, the disclosure relates to a method for reversing or stabilizing symptoms of Alzheimer's disease in a subject in need thereof, comprising
5 administering to said subject a therapeutically effective amount of an AAV vector, AAV viral vector, or pharmaceutical composition which comprises an APOE2 Christchurch mutation encoding nucleic acid.

[0146] In a particular embodiment, the disclosure relates to a method for preventing or treating Alzheimer's disease in a subject in need thereof, comprising administering to said
10 subject a therapeutically effective amount of an AAV vector, AAV viral vector, or pharmaceutical composition which comprises an APOE3 Christchurch mutation encoding nucleic acid.

[0147] In a particular embodiment, the disclosure relates to a method for treating Alzheimer's disease in a subject in need thereof, comprising administering to said subject a therapeutically
15 effective amount of an AAV vector, AAV viral vector, or pharmaceutical composition which comprises an APOE3 Christchurch mutation encoding nucleic acid.

[0148] In a particular embodiment, the disclosure relates to a method for reversing or stabilizing symptoms of Alzheimer's disease in a subject in need thereof, comprising
20 administering to said subject a therapeutically effective amount of an AAV vector, AAV viral vector, or pharmaceutical composition which comprises an APOE3 Christchurch mutation encoding nucleic acid.

[0149] As used herein, the terms "asymptomatic" or "pre-symptomatic" denotes a subject with the disease (Alzheimer's disease) as defined by a genetic diagnosis but with no detectable clinical cardiac symptom.

25 [0150] As used herein, the terms symptomatic denotes a subject with the disease (Alzheimer's disease) as defined by a genetic diagnosis and with the presence of cognitive impairment including MCI or mild, moderate, or severe dementia.

Delivery of the vectors

[0151] It is herein provided a method for treating Alzheimer's disease in a subject, said
30 method comprising: (a) providing an AAV vector as defined above, which comprises a nucleic acid sequence encoding an APOE2 Christchurch mutation polypeptide or a fragment thereof or an APOE3 Christchurch mutation polypeptide or a fragment thereof; and (b)

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delivering the AAV vector to the subject in need thereof and whereby APOE2 or APOE3 is expressed by the transduced cells at a therapeutically effective level.

[0152] The doses and regimen may be determined by a physician, and depend on the age, sex, weight, of the subject, and the stage of the disease.

5 [0153] In some aspects, the AAV vector or the pharmaceutical composition is administered to the subject orally, rectally, transmucosally, inhalationally, transdermally, parenterally, intravenously, subcutaneously, intradermally, intramuscularly, intrapleurally, intracerebrally, intrathecally, intracerebrally, intraventricularly, intranasally, intra-aurally, intra-ocularly, or peri-ocularly, topically, intralymphatically, intracisternally, intranervally or intravitreally.

10 [0154] In some aspects, the AAV vector or the pharmaceutical composition is administered to the central nervous system (CNS) of the subject. In some aspects, the administration is C1-C2 administration. C1-C2 administration refers to administration of the AAV vector between the first two vertebrae in the cervical spine. In some aspects, the C1-C2 administration is CT-guided. In some aspects, the administration is intracisterna magna (ICM). In some aspects,
15 ICM administration is performed when C1-C2 administration is not feasible. In some aspects, factors that limit C1-C2 administration include aberrant arteries along needle trajectory and/or restricted dorsal subarachnoid space (i.e. < 2mm). In some embodiments, the AAV vector is administered to the hippocampus of the subject, e.g., by intra-hippocampal infusion or intra-hippocampal injection.

20 [0155] In some aspects, the AAV vector or the pharmaceutical composition administration occurs over at least about 1 minute, at least about 2 minutes, at least about 3 minutes, at least about 4 minutes, at least about 5 minutes, at least about 10 minutes, at least about 20 minutes, or at least about 30 minutes. In some aspects, the IV infusion occurs over 60 minutes. In some aspects, C1-C2 administration occurs at a rate of about 1 mL/minute, of about 2 mL/minute,
25 of about 3 mL/minute, of about 4 mL/minute, of about 5 mL/minute, of about 6 mL/minute, of about 7 mL/minute, of about 8 mL/minute, of about 9 mL/minute, or of about 10 mL/minute.

[0156] In some aspects, the volume of AAV vector administered is about 5 mL, about 10 mL, about 15 mL, about 20 mL, or about 25 mL.

[0157] In some aspects, the subject is administered an AAV vector or pharmaceutical
30 composition of the disclosure at a therapeutically effective dosage. In some aspects, the dosage is about 5.0×10^9 genome copies (gc) per milliliter (mL) of cerebral spinal fluid (CSF) (gc/mL) to about 5.0×10^{12} gc/mL CSF. In some aspects, the dosage is about 1.4×10^{10} gc/mL CSF. In some aspects, the dosage is about 4.4×10^{10} gc/mL CSF. In some aspects, the dosage is about 1.4×10^{11} gc/mL CSF.

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[0158] In some aspects, the dosage is about 1.0×10^9 gc/ml CSF, about 1.1×10^9 gc/ml CSF, about 1.2×10^9 gc/ml CSF, about 1.3×10^9 gc/ml CSF, about 1.4×10^9 gc/ml CSF, about 1.5×10^9 gc/ml CSF, about 1.6×10^9 gc/ml CSF, about 1.7×10^9 gc/ml CSF, about 1.8×10^9 gc/ml CSF, about 1.9×10^9 gc/ml CSF, about 2.0×10^9 gc/ml CSF, about 2.1×10^9 gc/ml CSF, about
5 2.2×10^9 gc/ml CSF, about 2.3×10^9 gc/ml CSF, about 2.4×10^9 gc/ml CSF, about 2.5×10^9 gc/ml CSF, about 2.6×10^9 gc/ml CSF, about 2.7×10^9 gc/ml CSF, about 2.8×10^9 gc/ml CSF, about 2.9×10^9 gc/ml CSF, about 3.0×10^9 gc/ml CSF, about 3.1×10^9 gc/ml CSF, about 3.2×10^9 gc/ml CSF, about 3.3×10^9 gc/ml CSF, about 3.4×10^9 gc/ml CSF, about 3.5×10^9 gc/ml CSF, about 3.6×10^9 gc/ml CSF, about 3.7×10^9 gc/ml CSF, about 3.8×10^9 gc/ml
10 CSF, about 3.9×10^9 gc/ml CSF, about 4.0×10^9 gc/ml CSF, about 4.1×10^9 gc/ml CSF, about 4.2×10^9 gc/ml CSF, about 4.3×10^9 gc/ml CSF, about 4.4×10^9 gc/ml CSF, about 4.5×10^9 gc/ml CSF, about 4.6×10^9 gc/ml CSF, about 4.7×10^9 gc/ml CSF, about 4.8×10^9 gc/ml CSF, about 4.9×10^9 gc/ml CSF, about 5.0×10^9 gc/ml CSF, about 5.1×10^9 gc/ml CSF, about 5.2×10^9 gc/ml CSF, about 5.3×10^9 gc/ml CSF, about 5.4×10^9 gc/ml CSF, about 5.5×10^9
15 gc/ml CSF, about 5.6×10^9 gc/ml CSF, about 5.7×10^9 gc/ml CSF, about 5.8×10^9 gc/ml CSF, about 5.9×10^9 gc/ml CSF, about 6.0×10^9 gc/ml CSF, about 6.1×10^9 gc/ml CSF, about 6.2×10^9 gc/ml CSF, about 6.3×10^9 gc/ml CSF, about 6.4×10^9 gc/ml CSF, about 6.5×10^9 gc/ml CSF, about 6.6×10^9 gc/ml CSF, about 6.7×10^9 gc/ml CSF, about 6.8×10^9 gc/ml CSF, about 6.9×10^9 gc/ml CSF, about 7.0×10^9 gc/ml CSF, about 7.1×10^9 gc/ml CSF, about
20 7.2×10^9 gc/ml CSF, about 7.3×10^9 gc/ml CSF, about 7.4×10^9 gc/ml CSF, about 7.5×10^9 gc/ml CSF, about 7.6×10^9 gc/ml CSF, about 7.7×10^9 gc/ml CSF, about 7.8×10^9 gc/ml CSF, about 7.9×10^9 gc/ml CSF, about 8.0×10^9 gc/ml CSF, about 8.1×10^9 gc/ml CSF, about 8.2×10^9 gc/ml CSF, about 8.3×10^9 gc/ml CSF, about 8.4×10^9 gc/ml CSF, about 8.5×10^9 gc/ml CSF, about 8.6×10^9 gc/ml CSF, about 8.7×10^9 gc/ml CSF, about 8.8×10^9 gc/ml
25 CSF, about 8.9×10^9 gc/ml CSF, about 9.0×10^9 gc/ml CSF, about 9.1×10^9 gc/ml CSF, about 9.2×10^9 gc/ml CSF, about 9.3×10^9 gc/ml CSF, about 9.4×10^9 gc/ml CSF, about 9.5×10^9 gc/ml CSF, about 9.6×10^9 gc/ml CSF, about 9.7×10^9 gc/ml CSF, about 9.8×10^9 gc/ml CSF, or about 9.9×10^9 gc/ml CSF or any dosage in between.

[0159] In some aspects, the dosage is about 1.0×10^{10} gc/ml CSF, about 1.1×10^{10} gc/ml CSF, about 1.2×10^{10} gc/ml CSF, about 1.3×10^{10} gc/ml CSF, about 1.4×10^{10} gc/ml CSF, about 1.5×10^{10} gc/ml CSF, about 1.6×10^{10} gc/ml CSF, about 1.7×10^{10} gc/ml CSF, about 1.8×10^{10} gc/ml CSF, about 1.9×10^{10} gc/ml CSF, about 2.0×10^{10} gc/ml CSF, about 2.1×10^{10} gc/ml CSF, about 2.2×10^{10} gc/ml CSF, about 2.3×10^{10} gc/ml CSF, about 2.4×10^{10} gc/ml CSF, about 2.5×10^{10} gc/ml CSF, about 2.6×10^{10} gc/ml CSF, about 2.7×10^{10} gc/ml
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[illegible]

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10¹¹ gc/ml CSF, about 4.5 x 10¹¹ gc/ml CSF, about 4.6 x 10¹¹ gc/ml CSF, about 4.7 x 10¹¹ gc/ml CSF, about 4.8 x 10¹¹ gc/ml CSF, about 4.9 x 10¹¹ gc/ml CSF, about 5.0 x 10¹¹ gc/ml CSF, about 5.1 x 10¹¹ gc/ml CSF, about 5.2 x 10¹¹ gc/ml CSF, about 5.3 x 10¹¹ gc/ml CSF, about 5.4 x 10¹¹ gc/ml CSF, about 5.5 x 10¹¹ gc/ml CSF, about 5.6 x 10¹¹ gc/ml CSF, about 5.7 x 10¹¹ gc/ml CSF, about 5.8 x 10¹¹ gc/ml CSF, about 5.9 x 10¹¹ gc/ml CSF, about 6.0 x 10¹¹ gc/ml CSF, about 6.1 x 10¹¹ gc/ml CSF, about 6.2 x 10¹¹ gc/ml CSF, about 6.3 x 10¹¹ gc/ml CSF, about 6.4 x 10¹¹ gc/ml CSF, about 6.5 x 10¹¹ gc/ml CSF, about 6.6 x 10¹¹ gc/ml CSF, about 6.7 x 10¹¹ gc/ml CSF, about 6.8 x 10¹¹ gc/ml CSF, about 6.9 x 10¹¹ gc/ml CSF, about 7.0 x 10¹¹ gc/ml CSF, about 7.1 x 10¹¹ gc/ml CSF, about 7.2 x 10¹¹ gc/ml CSF, about 7.3 x 10¹¹ gc/ml CSF, about 7.4 x 10¹¹ gc/ml CSF, about 7.5 x 10¹¹ gc/ml CSF, about 7.6 x 10¹¹ gc/ml CSF, about 7.7 x 10¹¹ gc/ml CSF, about 7.8 x 10¹¹ gc/ml CSF, about 7.9 x 10¹¹ gc/ml CSF, about 8.0 x 10¹¹ gc/ml CSF, about 8.1 x 10¹¹ gc/ml CSF, about 8.2 x 10¹¹ gc/ml CSF, about 8.3 x 10¹¹ gc/ml CSF, about 8.4 x 10¹¹ gc/ml CSF, about 8.5 x 10¹¹ gc/ml CSF, about 8.6 x 10¹¹ gc/ml CSF, about 8.7 x 10¹¹ gc/ml CSF, about 8.8 x 10¹¹ gc/ml CSF, about 8.9 x 10¹¹ gc/ml CSF, about 9.0 x 10¹¹ gc/ml CSF, about 9.1 x 10¹¹ gc/ml CSF, about 9.2 x 10¹¹ gc/ml CSF, about 9.3 x 10¹¹ gc/ml CSF, about 9.4 x 10¹¹ gc/ml CSF, about 9.5 x 10¹¹ gc/ml CSF, about 9.6 x 10¹¹ gc/ml CSF, about 9.7 x 10¹¹ gc/ml CSF, about 9.8 x 10¹¹ gc/ml CSF, or about 9.9 x 10¹¹ gc/ml CSF or any dosage in between.

[0161] In some aspects, the dosage is about 1.0 x 10¹² gc/ml CSF, about 1.1 x 10¹² gc/ml CSF, about 1.2 x 10¹² gc/ml CSF, about 1.3 x 10¹² gc/ml CSF, about 1.4 x 10¹² gc/ml CSF, about 1.5 x 10¹² gc/ml CSF, about 1.6 x 10¹² gc/ml CSF, about 1.7 x 10¹² gc/ml CSF, about 1.8 x 10¹² gc/ml CSF, about 1.9 x 10¹² gc/ml CSF, about 2.0 x 10¹² gc/ml CSF, about 2.1 x 10¹² gc/ml CSF, about 2.2 x 10¹² gc/ml CSF, about 2.3 x 10¹² gc/ml CSF, about 2.4 x 10¹² gc/ml CSF, about 2.5 x 10¹² gc/ml CSF, about 2.6 x 10¹² gc/ml CSF, about 2.7 x 10¹² gc/ml CSF, about 2.8 x 10¹² gc/ml CSF, about 2.9 x 10¹² gc/ml CSF, about 3.0 x 10¹² gc/ml CSF, about 3.1 x 10¹² gc/ml CSF, about 3.2 x 10¹² gc/ml CSF, about 3.3 x 10¹² gc/ml CSF, about 3.4 x 10¹² gc/ml CSF, about 3.5 x 10¹² gc/ml CSF, about 3.6 x 10¹² gc/ml CSF, about 3.7 x 10¹² gc/ml CSF, about 3.8 x 10¹² gc/ml CSF, about 3.9 x 10¹² gc/ml CSF, about 4.0 x 10¹² gc/ml CSF, about 4.1 x 10¹² gc/ml CSF, about 4.2 x 10¹² gc/ml CSF, about 4.3 x 10¹² gc/ml CSF, about 4.4 x 10¹² gc/ml CSF, about 4.5 x 10¹² gc/ml CSF, about 4.6 x 10¹² gc/ml CSF, about 4.7 x 10¹² gc/ml CSF, about 4.8 x 10¹² gc/ml CSF, about 4.9 x 10¹² gc/ml CSF, about 5.0 x 10¹² gc/ml CSF, about 5.1 x 10¹² gc/ml CSF, about 5.2 x 10¹² gc/ml CSF, about 5.3 x 10¹² gc/ml CSF, about 5.4 x 10¹² gc/ml CSF, about 5.5 x 10¹² gc/ml CSF, about 5.6 x 10¹² gc/ml CSF, about 5.7 x 10¹² gc/ml CSF, about 5.8 x 10¹² gc/ml CSF, about 5.9 x 10¹² gc/ml

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CSF, about 7.6×10^{13} gc/ml CSF, about 7.7×10^{13} gc/ml CSF, about 7.8×10^{13} gc/ml CSF, about 7.9×10^{13} gc/ml CSF, about 8.0×10^{13} gc/ml CSF, about 8.1×10^{13} gc/ml CSF, about 8.2×10^{13} gc/ml CSF, about 8.3×10^{13} gc/ml CSF, about 8.4×10^{13} gc/ml CSF, about 8.5×10^{13} gc/ml CSF, about 8.6×10^{13} gc/ml CSF, about 8.7×10^{13} gc/ml CSF, about 8.8×10^{13} gc/ml CSF, about 8.9×10^{13} gc/ml CSF, about 9.0×10^{13} gc/ml CSF, about 9.1×10^{13} gc/ml CSF, about 9.2×10^{13} gc/ml CSF, about 9.3×10^{13} gc/ml CSF, about 9.4×10^{13} gc/ml CSF, about 9.5×10^{13} gc/ml CSF, about 9.6×10^{13} gc/ml CSF, about 9.7×10^{13} gc/ml CSF, about 9.8×10^{13} gc/ml CSF, or about 9.9×10^{13} gc/ml CSF or any dosage in between.

[0163] In some aspects, the therapeutically effective dosage of the AAV vector of the disclosure is administered as a fixed dosage. In some aspects a fixed dosage is expressed as a total number of genome copies (gc).

[0164] In some aspects, AAV vectors of the disclosure are administered at a fixed dosage of about 1.0×10^{10} gc to about 1.0×10^{16} gc. In some aspects, AAV vectors of the disclosure are administered at a fixed dosage of about 1.0×10^{13} gc to about 1.0×10^{15} gc.

[0165] In some aspects, AAV vectors of the disclosure are administered at a fixed dosage of about 1.0×10^{10} gc to about 9.0×10^{10} gc. In some aspects, AAV vectors of the disclosure are administered at a fixed dosage of about 1.0×10^{10} gc, about 1.4×10^{10} gc, about 1.5×10^{10} gc, about 2.0×10^{10} gc, about 2.1×10^{10} gc, about 2.2×10^{10} gc, about 2.3×10^{10} gc, about 2.4×10^{10} gc, about 2.5×10^{10} gc, about 2.6×10^{10} gc, about 2.7×10^{10} gc, about 2.8×10^{10} gc, about 2.9×10^{10} gc, about 3.0×10^{10} gc, about 4.0×10^{10} gc, about 5.0×10^{10} gc, about 6.0×10^{10} gc, about 7.0×10^{10} gc, about 8.0×10^{10} gc, or about 9.0×10^{10} gc, or any dosage in between. In some aspects, AAV vectors of the disclosure are administered at a fixed dosage of about 2.0×10^{10} gc.

[0166] In some aspects, AAV vectors of the disclosure are administered at a fixed dosage of about 1.0×10^{11} gc to about 9.0×10^{11} gc. In some aspects, AAV vectors of the disclosure are administered at a fixed dosage of about 1.0×10^{11} gc, about 1.4×10^{11} gc, about 1.5×10^{11} gc, about 2.0×10^{11} gc, about 2.1×10^{11} gc, about 2.2×10^{11} gc, about 2.3×10^{11} gc, about 2.4×10^{11} gc, about 2.5×10^{11} gc, about 2.6×10^{11} gc, about 2.7×10^{11} gc, about 2.8×10^{11} gc, about 2.9×10^{11} gc, about 3.0×10^{11} gc, about 4.0×10^{11} gc, about 5.0×10^{11} gc, about 6.0×10^{11} gc, about 7.0×10^{11} gc, about 8.0×10^{11} gc, or about 9.0×10^{11} gc, or any dosage in between.

[0167] In some aspects, AAV vectors of the disclosure are administered at a fixed dosage of about 1.0×10^{12} gc to about 9.0×10^{12} gc. In some aspects, AAV vectors of the disclosure are administered at a fixed dosage of about 1.0×10^{12} gc, about 1.4×10^{12} gc, about 1.5×10^{12} gc,

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about 2.0×10^{12} gc, about 2.1×10^{12} gc, about 2.2×10^{12} gc, about 2.3×10^{12} gc, about 2.4×10^{12} gc, about 2.5×10^{12} gc, about 2.6×10^{12} gc, about 2.7×10^{12} gc, about 2.8×10^{12} gc, about 2.9×10^{12} gc, about 3.0×10^{12} gc, about 4.0×10^{12} gc, about 5.0×10^{12} gc, about 6.0×10^{12} gc, about 7.0×10^{12} gc, about 8.0×10^{12} gc, or about 9.0×10^{12} gc, or any dosage in

5 between.

[0168] In some aspects, AAV vectors of the disclosure are administered at a fixed dosage of about 1.0×10^{13} gc to about 9.0×10^{13} gc. In some aspects, AAV vectors of the disclosure are administered at a fixed dosage of about 1.0×10^{13} gc, about 1.4×10^{13} gc, about 1.5×10^{13} gc, about 2.0×10^{13} gc, about 2.1×10^{13} gc, about 2.2×10^{13} gc, about 2.3×10^{13} gc, about 2.4×10^{13} gc, about 2.5×10^{13} gc, about 2.6×10^{13} gc, about 2.7×10^{13} gc, about 2.8×10^{13} gc, about 2.9×10^{13} gc, about 3.0×10^{13} gc, about 4.0×10^{13} gc, about 5.0×10^{13} gc, about 6.0×10^{13} gc, about 7.0×10^{13} gc, about 8.0×10^{13} gc, or about 9.0×10^{13} gc, or any dosage in

10 between.

[0169] In some aspects, AAV vectors of the disclosure are administered at a total dosage of about 1.0×10^9 gc, about 1.1×10^9 gc, about 1.2×10^9 gc, about 1.3×10^9 gc, about 1.4×10^9 gc, about 1.5×10^9 gc, about 1.6×10^9 gc, about 1.7×10^9 gc, about 1.8×10^9 gc, about 1.9×10^9 gc, about 2.0×10^9 gc, about 2.1×10^9 gc, about 2.2×10^9 gc, about 2.3×10^9 gc, about 2.4×10^9 gc, about 2.5×10^9 gc, about 2.6×10^9 gc, about 2.7×10^9 gc, about 2.8×10^9 gc, about 2.9×10^9 gc, about 3.0×10^9 gc, about 3.1×10^9 gc, about 3.2×10^9 gc, about 3.3×10^9 gc, about 3.4×10^9 gc, about 3.5×10^9 gc, about 3.6×10^9 gc, about 3.7×10^9 gc, about 3.8×10^9 gc, about 3.9×10^9 gc, about 4.0×10^9 gc, about 4.1×10^9 gc, about 4.2×10^9 gc, about 4.3×10^9 gc, about 4.4×10^9 gc, about 4.5×10^9 gc, about 4.6×10^9 gc, about 4.7×10^9 gc, about 4.8×10^9 gc, about 4.9×10^9 gc, about 5.0×10^9 gc, about 5.1×10^9 gc, about 5.2×10^9 gc, about 5.3×10^9 gc, about 5.4×10^9 gc, about 5.5×10^9 gc, about 5.6×10^9 gc, about 5.7×10^9 gc, about 5.8×10^9 gc, about 5.9×10^9 gc, about 6.0×10^9 gc, about 6.1×10^9 gc, about 6.2×10^9 gc, about 6.3×10^9 gc, about 6.4×10^9 gc, about 6.5×10^9 gc, about 6.6×10^9 gc, about 6.7×10^9 gc, about 6.8×10^9 gc, about 6.9×10^9 gc, about 7.0×10^9 gc, about 7.1×10^9 gc, about 7.2×10^9 gc, about 7.3×10^9 gc, about 7.4×10^9 gc, about 7.5×10^9 gc, about 7.6×10^9 gc, about 7.7×10^9 gc, about 7.8×10^9 gc, about 7.9×10^9 gc, about 8.0×10^9 gc, about 8.1×10^9 gc, about 8.2×10^9 gc, about 8.3×10^9 gc, about 8.4×10^9 gc, about 8.5×10^9 gc, about 8.6×10^9 gc, about 8.7×10^9 gc, about 8.8×10^9 gc, about 8.9×10^9 gc, about 9.0×10^9 gc, about 9.1×10^9 gc, about 9.2×10^9 gc, about 9.3×10^9 gc, about 9.4×10^9 gc, about 9.5×10^9 gc, about 9.6×10^9 gc, about 9.7×10^9 gc, about 9.8×10^9 gc, or about 9.9×10^9 gc or any dosage in

30 between.

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10¹¹ gc, about 6.0 x 10¹¹ gc, about 6.1 x 10¹¹ gc, about 6.2 x 10¹¹ gc, about 6.3 x 10¹¹ gc,
about 6.4 x 10¹¹ gc, about 6.5 x 10¹¹ gc, about 6.6 x 10¹¹ gc, about 6.7 x 10¹¹ gc, about 6.8 x
10¹¹ gc, about 6.9 x 10¹¹ gc, about 7.0 x 10¹¹ gc, about 7.1 x 10¹¹ gc, about 7.2 x 10¹¹ gc,
about 7.3 x 10¹¹ gc, about 7.4 x 10¹¹ gc, about 7.5 x 10¹¹ gc, about 7.6 x 10¹¹ gc, about 7.7 x
5 10¹¹ gc, about 7.8 x 10¹¹ gc, about 7.9 x 10¹¹ gc, about 8.0 x 10¹¹ gc, about 8.1 x 10¹¹ gc,
about 8.2 x 10¹¹ gc, about 8.3 x 10¹¹ gc, about 8.4 x 10¹¹ gc, about 8.5 x 10¹¹ gc, about 8.6 x
10¹¹ gc, about 8.7 x 10¹¹ gc, about 8.8 x 10¹¹ gc, about 8.9 x 10¹¹ gc, about 9.0 x 10¹¹ gc,
about 9.1 x 10¹¹ gc, about 9.2 x 10¹¹ gc, about 9.3 x 10¹¹ gc, about 9.4 x 10¹¹ gc, about 9.5 x
10¹¹ gc, about 9.6 x 10¹¹ gc, about 9.7 x 10¹¹ gc, about 9.8 x 10¹¹ gc, or about 9.9 x 10¹¹ gc or
10 any dosage in between.

[0172] In some aspects, AAV vectors of the disclosure are administered at a total dosage of
about 1.0 x 10¹² gc, about 1.1 x 10¹² gc, about 1.2 x 10¹² gc, about 1.3 x 10¹² gc, about 1.4 x
10¹² gc, about 1.5 x 10¹² gc, about 1.6 x 10¹² gc, about 1.7 x 10¹² gc, about 1.8 x 10¹² gc,
about 1.9 x 10¹² gc, about 2.0 x 10¹² gc, about 2.1 x 10¹² gc, about 2.2 x 10¹² gc, about 2.3 x
15 10¹² gc, about 2.4 x 10¹² gc, about 2.5 x 10¹² gc, about 2.6 x 10¹² gc, about 2.7 x 10¹² gc,
about 2.8 x 10¹² gc, about 2.9 x 10¹² gc, about 3.0 x 10¹² gc, about 3.1 x 10¹² gc, about 3.2 x
10¹² gc, about 3.3 x 10¹² gc, about 3.4 x 10¹² gc, about 3.5 x 10¹² gc, about 3.6 x 10¹² gc,
about 3.7 x 10¹² gc, about 3.8 x 10¹² gc, about 3.9 x 10¹² gc, about 4.0 x 10¹² gc, about 4.1 x
10¹² gc, about 4.2 x 10¹² gc, about 4.3 x 10¹² gc, about 4.4 x 10¹² gc, about 4.5 x 10¹² gc,
20 about 4.6 x 10¹² gc, about 4.7 x 10¹² gc, about 4.8 x 10¹² gc, about 4.9 x 10¹² gc, about 5.0 x
10¹² gc, about 5.1 x 10¹² gc, about 5.2 x 10¹² gc, about 5.3 x 10¹² gc, about 5.4 x 10¹² gc,
about 5.5 x 10¹² gc, about 5.6 x 10¹² gc, about 5.7 x 10¹² gc, about 5.8 x 10¹² gc, about 5.9 x
10¹² gc, about 6.0 x 10¹² gc, about 6.1 x 10¹² gc, about 6.2 x 10¹² gc, about 6.3 x 10¹² gc,
about 6.4 x 10¹² gc, about 6.5 x 10¹² gc, about 6.6 x 10¹² gc, about 6.7 x 10¹² gc, about 6.8 x
25 10¹² gc, about 6.9 x 10¹² gc, about 7.0 x 10¹² gc, about 7.1 x 10¹² gc, about 7.2 x 10¹² gc,
about 7.3 x 10¹² gc, about 7.4 x 10¹² gc, about 7.5 x 10¹² gc, about 7.6 x 10¹² gc, about 7.7 x
10¹² gc, about 7.8 x 10¹² gc, about 7.9 x 10¹² gc, about 8.0 x 10¹² gc, about 8.1 x 10¹² gc,
about 8.2 x 10¹² gc, about 8.3 x 10¹² gc, about 8.4 x 10¹² gc, about 8.5 x 10¹² gc, about 8.6 x
10¹² gc, about 8.7 x 10¹² gc, about 8.8 x 10¹² gc, about 8.9 x 10¹² gc, about 9.0 x 10¹² gc,
30 about 9.1 x 10¹² gc, about 9.2 x 10¹² gc, about 9.3 x 10¹² gc, about 9.4 x 10¹² gc, about 9.5 x
10¹² gc, about 9.6 x 10¹² gc, about 9.7 x 10¹² gc, about 9.8 x 10¹² gc, or about 9.9 x 10¹² gc or
any dosage in between.

[0173] In some aspects, AAV vectors of the disclosure are administered at a total dosage of
about 1.0 x 10¹³ gc, about 1.1 x 10¹³ gc, about 1.2 x 10¹³ gc, about 1.3 x 10¹³ gc, about 1.4 x

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10¹³ gc, about 1.5 x 10¹³ gc, about 1.6 x 10¹³ gc, about 1.7 x 10¹³ gc, about 1.8 x 10¹³ gc,
about 1.9 x 10¹³ gc, about 2.0 x 10¹³ gc, about 2.1 x 10¹³ gc, about 2.2 x 10¹³ gc, about 2.3 x
10¹³ gc, about 2.4 x 10¹³ gc, about 2.5 x 10¹³ gc, about 2.6 x 10¹³ gc, about 2.7 x 10¹³ gc,
about 2.8 x 10¹³ gc, about 2.9 x 10¹³ gc, about 3.0 x 10¹³ gc, about 3.1 x 10¹³ gc, about 3.2 x
5 10¹³ gc, about 3.3 x 10¹³ gc, about 3.4 x 10¹³ gc, about 3.5 x 10¹³ gc, about 3.6 x 10¹³ gc,
about 3.7 x 10¹³ gc, about 3.8 x 10¹³ gc, about 3.9 x 10¹³ gc, about 4.0 x 10¹³ gc, about 4.1 x
10¹³ gc, about 4.2 x 10¹³ gc, about 4.3 x 10¹³ gc, about 4.4 x 10¹³ gc, about 4.5 x 10¹³ gc,
about 4.6 x 10¹³ gc, about 4.7 x 10¹³ gc, about 4.8 x 10¹³ gc, about 4.9 x 10¹³ gc, about 5.0 x
10¹³ gc, about 5.1 x 10¹³ gc, about 5.2 x 10¹³ gc, about 5.3 x 10¹³ gc, about 5.4 x 10¹³ gc,
10 about 5.5 x 10¹³ gc, about 5.6 x 10¹³ gc, about 5.7 x 10¹³ gc, about 5.8 x 10¹³ gc, about 5.9 x
10¹³ gc, about 6.0 x 10¹³ gc, about 6.1 x 10¹³ gc, about 6.2 x 10¹³ gc, about 6.3 x 10¹³ gc,
about 6.4 x 10¹³ gc, about 6.5 x 10¹³ gc, about 6.6 x 10¹³ gc, about 6.7 x 10¹³ gc, about 6.8 x
10¹³ gc, about 6.9 x 10¹³ gc, about 7.0 x 10¹³ gc, about 7.1 x 10¹³ gc, about 7.2 x 10¹³ gc,
about 7.3 x 10¹³ gc, about 7.4 x 10¹³ gc, about 7.5 x 10¹³ gc, about 7.6 x 10¹³ gc, about 7.7 x
15 10¹³ gc, about 7.8 x 10¹³ gc, about 7.9 x 10¹³ gc, about 8.0 x 10¹³ gc, about 8.1 x 10¹³ gc,
about 8.2 x 10¹³ gc, about 8.3 x 10¹³ gc, about 8.4 x 10¹³ gc, about 8.5 x 10¹³ gc, about 8.6 x
10¹³ gc, about 8.7 x 10¹³ gc, about 8.8 x 10¹³ gc, about 8.9 x 10¹³ gc, about 9.0 x 10¹³ gc,
about 9.1 x 10¹³ gc, about 9.2 x 10¹³ gc, about 9.3 x 10¹³ gc, about 9.4 x 10¹³ gc, about 9.5 x
10¹³ gc, about 9.6 x 10¹³ gc, about 9.7 x 10¹³ gc, about 9.8 x 10¹³ gc, or about 9.9 x 10¹³ gc or
20 any dosage in between.

[0174] In some aspects, AAV vectors of the disclosure are administered at a total dosage of
about 1.0 x 10¹⁴ gc, about 1.1 x 10¹⁴ gc, about 1.2 x 10¹⁴ gc, about 1.3 x 10¹⁴ gc, about 1.4 x
10¹⁴ gc, about 1.5 x 10¹⁴ gc, about 1.6 x 10¹⁴ gc, about 1.7 x 10¹⁴ gc, about 1.8 x 10¹⁴ gc,
about 1.9 x 10¹⁴ gc, about 2.0 x 10¹⁴ gc, about 2.1 x 10¹⁴ gc, about 2.2 x 10¹⁴ gc, about 2.3 x
25 10¹⁴ gc, about 2.4 x 10¹⁴ gc, about 2.5 x 10¹⁴ gc, about 2.6 x 10¹⁴ gc, about 2.7 x 10¹⁴ gc,
about 2.8 x 10¹⁴ gc, about 2.9 x 10¹⁴ gc, about 3.0 x 10¹⁴ gc, about 3.1 x 10¹⁴ gc, about 3.2 x
10¹⁴ gc, about 3.3 x 10¹⁴ gc, about 3.4 x 10¹⁴ gc, about 3.5 x 10¹⁴ gc, about 3.6 x 10¹⁴ gc,
about 3.7 x 10¹⁴ gc, about 3.8 x 10¹⁴ gc, about 3.9 x 10¹⁴ gc, about 4.0 x 10¹⁴ gc, about 4.1 x
10¹⁴ gc, about 4.2 x 10¹⁴ gc, about 4.3 x 10¹⁴ gc, about 4.4 x 10¹⁴ gc, about 4.5 x 10¹⁴ gc,
30 about 4.6 x 10¹⁴ gc, about 4.7 x 10¹⁴ gc, about 4.8 x 10¹⁴ gc, about 4.9 x 10¹⁴ gc, about 5.0 x
10¹⁴ gc, about 5.1 x 10¹⁴ gc, about 5.2 x 10¹⁴ gc, about 5.3 x 10¹⁴ gc, about 5.4 x 10¹⁴ gc,
about 5.5 x 10¹⁴ gc, about 5.6 x 10¹⁴ gc, about 5.7 x 10¹⁴ gc, about 5.8 x 10¹⁴ gc, about 5.9 x
10¹⁴ gc, about 6.0 x 10¹⁴ gc, about 6.1 x 10¹⁴ gc, about 6.2 x 10¹⁴ gc, about 6.3 x 10¹⁴ gc,
about 6.4 x 10¹⁴ gc, about 6.5 x 10¹⁴ gc, about 6.6 x 10¹⁴ gc, about 6.7 x 10¹⁴ gc, about 6.8 x

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10¹⁴ gc, about 6.9 x 10¹⁴ gc, about 7.0 x 10¹⁴ gc, about 7.1 x 10¹⁴ gc, about 7.2 x 10¹⁴ gc,
about 7.3 x 10¹⁴ gc, about 7.4 x 10¹⁴ gc, about 7.5 x 10¹⁴ gc, about 7.6 x 10¹⁴ gc, about 7.7 x
10¹⁴ gc, about 7.8 x 10¹⁴ gc, about 7.9 x 10¹⁴ gc, about 8.0 x 10¹⁴ gc, about 8.1 x 10¹⁴ gc,
about 8.2 x 10¹⁴ gc, about 8.3 x 10¹⁴ gc, about 8.4 x 10¹⁴ gc, about 8.5 x 10¹⁴ gc, about 8.6 x
5 10¹⁴ gc, about 8.7 x 10¹⁴ gc, about 8.8 x 10¹⁴ gc, about 8.9 x 10¹⁴ gc, about 9.0 x 10¹⁴ gc,
about 9.1 x 10¹⁴ gc, about 9.2 x 10¹⁴ gc, about 9.3 x 10¹⁴ gc, about 9.4 x 10¹⁴ gc, about 9.5 x
10¹⁴ gc, about 9.6 x 10¹⁴ gc, about 9.7 x 10¹⁴ gc, about 9.8 x 10¹⁴ gc, or about 9.9 x 10¹⁴ gc or
any dosage in between.

[0175] In some aspects, AAV vectors of the disclosure are administered at a total dosage of
10 about 1.0 x 10¹⁵ gc, about 1.1 x 10¹⁵ gc, about 1.2 x 10¹⁵ gc, about 1.3 x 10¹⁵ gc, about 1.4 x
10¹⁵ gc, about 1.5 x 10¹⁵ gc, about 1.6 x 10¹⁵ gc, about 1.7 x 10¹⁵ gc, about 1.8 x 10¹⁵ gc,
about 1.9 x 10¹⁵ gc, about 2.0 x 10¹⁵ gc, about 2.1 x 10¹⁵ gc, about 2.2 x 10¹⁵ gc, about 2.3 x
10¹⁵ gc, about 2.4 x 10¹⁵ gc, about 2.5 x 10¹⁵ gc, about 2.6 x 10¹⁵ gc, about 2.7 x 10¹⁵ gc,
about 2.8 x 10¹⁵ gc, about 2.9 x 10¹⁵ gc, about 3.0 x 10¹⁵ gc, about 3.1 x 10¹⁵ gc, about 3.2 x
15 10¹⁵ gc, about 3.3 x 10¹⁵ gc, about 3.4 x 10¹⁵ gc, about 3.5 x 10¹⁵ gc, about 3.6 x 10¹⁵ gc,
about 3.7 x 10¹⁵ gc, about 3.8 x 10¹⁵ gc, about 3.9 x 10¹⁵ gc, about 4.0 x 10¹⁵ gc, about 4.1 x
10¹⁵ gc, about 4.2 x 10¹⁵ gc, about 4.3 x 10¹⁵ gc, about 4.4 x 10¹⁵ gc, about 4.5 x 10¹⁵ gc,
about 4.6 x 10¹⁵ gc, about 4.7 x 10¹⁵ gc, about 4.8 x 10¹⁵ gc, about 4.9 x 10¹⁵ gc, about 5.0 x
10¹⁵ gc, about 5.1 x 10¹⁵ gc, about 5.2 x 10¹⁵ gc, about 5.3 x 10¹⁵ gc, about 5.4 x 10¹⁵ gc,
20 about 5.5 x 10¹⁵ gc, about 5.6 x 10¹⁵ gc, about 5.7 x 10¹⁵ gc, about 5.8 x 10¹⁵ gc, about 5.9 x
10¹⁵ gc, about 6.0 x 10¹⁵ gc, about 6.1 x 10¹⁵ gc, about 6.2 x 10¹⁵ gc, about 6.3 x 10¹⁵ gc,
about 6.4 x 10¹⁵ gc, about 6.5 x 10¹⁵ gc, about 6.6 x 10¹⁵ gc, about 6.7 x 10¹⁵ gc, about 6.8 x
10¹⁵ gc, about 6.9 x 10¹⁵ gc, about 7.0 x 10¹⁵ gc, about 7.1 x 10¹⁵ gc, about 7.2 x 10¹⁵ gc,
about 7.3 x 10¹⁵ gc, about 7.4 x 10¹⁵ gc, about 7.5 x 10¹⁵ gc, about 7.6 x 10¹⁵ gc, about 7.7 x
25 10¹⁵ gc, about 7.8 x 10¹⁵ gc, about 7.9 x 10¹⁵ gc, about 8.0 x 10¹⁵ gc, about 8.1 x 10¹⁵ gc,
about 8.2 x 10¹⁵ gc, about 8.3 x 10¹⁵ gc, about 8.4 x 10¹⁵ gc, about 8.5 x 10¹⁵ gc, about 8.6 x
10¹⁵ gc, about 8.7 x 10¹⁵ gc, about 8.8 x 10¹⁵ gc, about 8.9 x 10¹⁵ gc, about 9.0 x 10¹⁵ gc,
about 9.1 x 10¹⁵ gc, about 9.2 x 10¹⁵ gc, about 9.3 x 10¹⁵ gc, about 9.4 x 10¹⁵ gc, about 9.5 x
10¹⁵ gc, about 9.6 x 10¹⁵ gc, about 9.7 x 10¹⁵ gc, about 9.8 x 10¹⁵ gc, or about 9.9 x 10¹⁵ gc or
30 any dosage in between.

[0176] In some aspects, AAV vectors of the disclosure are administered at a fixed dosage of
about 1.0 x 10¹⁴ gc to about 9.0 x 10¹⁴ gc. In some aspects, AAV vectors of the disclosure are
administered at a fixed dosage of about 1.0 x 10¹⁴ gc, about 1.4 x 10¹⁴ gc, about 1.5 x 10¹⁴ gc,
about 2.0 x 10¹⁴ gc, about 2.1 x 10¹⁴ gc, about 2.2 x 10¹⁴ gc, about 2.3 x 10¹⁴ gc, about 2.4 x

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10¹⁴ gc, about 2.5 x 10¹⁴ gc, about 2.6 x 10¹⁴ gc, about 2.7 x 10¹⁴ gc, about 2.8 x 10¹⁴ gc, about 2.9 x 10¹⁴ gc, about 3.0 x 10¹⁴ gc, about 4.0 x 10¹⁴ gc, about 5.0 x 10¹⁴ gc, about 6.0 x 10¹⁴ gc, about 7.0 x 10¹⁴ gc, about 8.0 x 10¹⁴ gc, or about 9.0 x 10¹⁴ gc, or any dosage in between. In some aspects, AAV vectors of the disclosure are administered at a fixed dosage of about 1.4 x 10¹⁴ gc.

5 [0177] Any dosage form or method of calculating said dosage form of the AAV vectors of the disclosure is contemplated herein. In some aspects, dosages are based on the mass and/or volume of the brain. In some aspects, dosages are based on the weight of the subject. In some aspects, dosages are calculated using a qPCR titer method. In some aspects, dosages are
10 calculated using a ddPCR titer method.

[0178] In some aspects, the therapeutically effective dosage can be tailored for each AAV capsid serotype. In some aspects, the therapeutically effective dosage is tailored to account for differences in cardiac tropism for distinct AAV capsid serotypes.

15 [0179] In some aspects, the subject is administered a single dose of AAV vector. In some aspects, the subject is further administered a second, third, fourth, or fifth dosage of the AAV vector. In some aspects, second and subsequent administrations of AAV vector can be at a different dosage from the first dosage.

[0180] In some aspects, the dosage is measured by quantitative polymerase chain reaction (qPCR) titer. In some aspects, the dosage is measured by droplet digital polymerase chain
20 reaction (ddPCR) titer.

[0181] In some aspects, the subject is further administered an immunosuppressant along with AAV vector administration. The immunosuppressant can be any immunosuppressant and/or corticosteroid known in the art. Immunosuppressants can be dosed at any amount at any schedule or interval. Immunosuppressants may be administered to improve patient safety,
25 minimize host immune response to AAV-based therapies, and/or enhance therapeutic efficacy of AAV-based therapies. In some aspects, the subject is further administered prednisone along with AAV vector administration.

[0182] In some aspects, the prednisone is administered at a dosage of:

[0183] 40 mg, once daily 1 week prior to AAV viral vector administration;

30 [0184] 40 mg once daily for week 1 through week 2 post-AAV viral vector administration;

[0185] 30 mg once daily for week 3 post-AAV viral vector administration;

[0186] 20 mg once daily for week 4 post-AAV viral vector administration;

[0187] 10 mg once daily for week 5 post-AAV viral vector administration;

[0188] 5 mg once daily for week 6 post-AAV viral vector administration;

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[0189] 2.5 mg once daily for week 7 post-AAV viral vector administration; and

[0190] 2.5 mg every other day for week 8 post-AAV viral vector administration.

[0191] In some aspects, if evidence of host immune response is observed following AAV vector administration, the dose of immunosuppressant can be held constant (i.e. not tapered as described above) or increased. In some aspects, a maximum dose of prednisone is about 60 mg. Once evidence of host immune response subsides or decreases, tapering of the immunosuppressant can be initiated.

[0192] In some aspects, the subject is further administered a proton-pump inhibitor during prednisone use.

10 [0193] In some aspects, the disclosure relates to a vector which comprises an APOE2 encoding nucleic acid for use in treatment or prevention of Alzheimer's disease in a subject wherein the AAV vector is delivering the subject in need thereof and wherein APOE2 is expressed by the transduced cells at a therapeutically effective level.

15 [0194] In a particular embodiment, the disclosure relates to a vector which comprises an APOE2 encoding nucleic acid for reversing symptoms of Alzheimer's disease in a subject in need thereof wherein the AAV vector is delivering the subject in need thereof and wherein APOE2 is expressed by the transduced cells at a therapeutically effective level.

Non-viral vectors

20 [0195] In a particular embodiment, the vector use according to the disclosure is a non viral vector. Typically, the non-viral vector may be a plasmid which includes nucleic acid sequences encoding the APOE2 gene, or variants thereof, as described above.

Pharmaceutical compositions

25 [0196] In some aspects, the disclosure concerns a pharmaceutical composition for preventing or treating Alzheimer's disease in a subject in need thereof, which comprises a therapeutically effective amount of an AAV vector which comprises an APOE2 Christchurch mutation encoding nucleic acid.

[0197] The disclosure provides a pharmaceutical composition comprising APOE2 Christchurch mutation rAAV viral particles; wherein the rAAV viral particle comprises an AAVrh10 capsid protein and an APOE2 Christchurch mutation rAAV vector.

30 [0198] The disclosure provides a pharmaceutical composition comprising APOE3 Christchurch mutation rAAV viral particles; wherein the rAAV viral particle comprises an AAVrh10 capsid protein and an APOE3 Christchurch mutation rAAV vector.

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[0199] In some aspects, the pharmaceutical composition comprises at least about 1.5×10^9 gc/mL to about 1.5×10^{11} gc/mL. In some aspects, the pharmaceutical composition comprises at least about 1.5×10^{10} gc/mL. 1.5×10^{11} gc/mL to about 1.5×10^{15} gc/mL. In some aspects, the pharmaceutical composition comprises at least about 1.5×10^{13} gc/mL.

5 [0200] In some aspects, the pharmaceutical composition comprises less than about 30% empty rAAV capsids.

[0201] In some aspects, a pharmaceutical composition disclosed herein comprises less than about 30% empty rAAV capsids. In some aspects, a pharmaceutical composition disclosed herein comprises less than about 25% empty rAAV capsids, less than about 20% empty rAAV
10 capsids, or less than about 15% empty rAAV capsids. In some aspects, a pharmaceutical composition comprises less than about 10%, less than about 8% empty rAAV capsids, less than 7%, less than about 5%, less than about 3%, or less than about 1% empty rAAV capsids. In some aspects, a pharmaceutical composition comprises from about 1% to about 10% empty rAAV capsids. In some aspects, a pharmaceutical composition comprises from about 2% to
15 about 30% empty rAAV capsids, about 2% to about 25% empty rAAV capsids, about 2% to about 20% empty rAAV capsids, about 2% to about 15% empty rAAV capsids, about 2% to about 10% empty rAAV capsids. In some aspects, a pharmaceutical composition comprises from about 2% to about 8% empty rAAV capsids. In some aspects, a pharmaceutical composition comprises less than or equal to about 6% empty rAAV capsids, about 5% empty
20 rAAV capsids, about 4% empty rAAV capsids, about 3% empty rAAV capsids, about 2% empty rAAV capsids, or about 1% empty rAAV capsids. In some aspects, the number of empty rAAV capsids is below the limit of detection.

[0202] In some aspects, the percentage of empty rAAV capsids is determined as a percentage of total rAAV capsids, *e.g.*, using analytical ultracentrifugation (AUC). In some aspects, the
25 AUC is sedimentation velocity AUV (SV-AUC). In some aspects, these low percentages of empty rAAV particles improve efficacy of treatment and/or reduce adverse events (*e.g.*, inflammatory responses, liver injury) after administration to a subject, *e.g.*, as compared to administering compositions having higher percentage empty rAAV particles. In some aspects, the methods of preparing rAAV compositions disclosed herein provide these low
30 percentages of empty rAAV particles, as compared to the levels of empty rAAV particles produced in other methods, *e.g.*, those not using the production and/or the purification methods described herein. In some aspects, a pharmaceutical composition disclosed herein comprises at least 80% full rAAV particles. In some aspects, a pharmaceutical composition

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comprises at least 85% full rAAV particles, at least 90% full rAAV particles, or at least 95% full rAAV particles.

[0203] By a “therapeutically effective amount” is meant a sufficient amount of the AAV vector of the disclosure to treat Alzheimer’s disease at a reasonable benefit/risk ratio

5 applicable to any medical treatment.

[0204] It will be understood that the single dosage or the total daily dosage of the compounds and compositions of the present disclosure will be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective dose level for any particular patient will depend upon a variety of factors including the disorder being treated

10 and the severity of the disorder; activity of the specific compound employed; the specific composition employed, the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the specific polypeptide employed; and like factors well known in the medical arts. For example,
15 it is well within the skill of the art to start doses of the compound at levels lower than those to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved. However, the daily dosage of the products may be varied over a wide range per adult per day. The therapeutically effective amount of the vector according to the disclosure that should be administered, as well as the dosage for the treatment of a
20 pathological condition with the number of viral or non-viral particles and/or pharmaceutical compositions of the disclosure, will depend on numerous factors, including the age and condition of the patient, the severity of the disturbance or disorder, the method and frequency of administration and the particular peptide to be used.

[0205] The presentation of the pharmaceutical compositions that contain the AAV vector
25 according to the disclosure may be in any form that is suitable for the selected mode of administration, for example, C1-C2 administration or ICM administration.

[0206] In the pharmaceutical compositions of the present disclosure for administration to the CNS, the active principle, alone or in combination with another active principle, can be administered in a unit administration form, as a mixture with conventional pharmaceutical
30 supports, to animals and human beings.

[0207] In one aspect, the pharmaceutical compositions contain vehicles which are pharmaceutically acceptable for a formulation capable of being injected. These may be in particular isotonic, sterile, saline solutions (monosodium or disodium phosphate, sodium, potassium, calcium or magnesium chloride and the like or mixtures of such salts), or dry,

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especially freeze-dried compositions which upon addition, depending on the case, of sterilized water or physiological saline, permit the constitution of injectable solutions.

[0208] The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions; formulations including sesame oil, peanut oil or aqueous propylene glycol; and
5 sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi.

[0209] Solutions comprising compounds of the disclosure as free base or pharmacologically
10 acceptable salts can be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

[0210] The AAV vector according to the disclosure can be formulated into a composition in a
15 neutral or salt form. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric
20 hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like.

[0211] The carrier can also be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained,
25 for example, by the use of a coating, such as lecithin, by the maintenance of the particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it may be preferable to include isotonic agents, for example, sugars or sodium chloride.
30 Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminium monostearate and gelatin.

[0212] Sterile injectable solutions are prepared by incorporating the active polypeptides in the amount in the appropriate solvent with several of the other ingredients enumerated above, as

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desired, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, exemplary methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[0213] Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms, such as the type of injectable solutions described above, but drug release capsules and the like can also be employed.

[0214] Multiple doses can also be administered.

[0215] In some aspects, pharmaceutical compositions comprising rAAV viral vectors of the disclosure are formulated with more or more excipients suitable for administration to a subject in need thereof by any suitable method of administration. In some aspects, the one or more excipients include a phosphate buffer and a salt. In some aspects, the one or more excipients include a Tris buffer and a salt.

[0216] In some aspects, the Tris buffer is used at a concentration of about 0.01 mM to about 100 mM. In some aspects, the Tris buffer is used at a concentration of about 1 mM, about 5 mM, about 10 mM, about 15 mM, about 20 mM, about 25 mM, about 30 mM, about 35 mM, about 40 mM, about 45 mM, about 50 mM, about 55 mM, about 60 mM, about 65 mM, about 70 mM, about 75 mM, about 80 mM, about 85 mM, about 90 mM, about 95 mM, or about 100 mM, or any concentration in between.

[0217] In some aspects, the phosphate buffer comprises potassium phosphate monobasic and sodium phosphate dibasic. In some aspects, the potassium phosphate monobasic is used at a concentration of about 0.01 mM to about 100 mM. In some aspects, the potassium phosphate monobasic at a concentration of about 0.1 mM, about 0.25 mM, about 0.5 mM, about 0.75 mM, about 1 mM, about 1.25 mM, about 1.5 mM, about 2 mM, about 2.5 mM, about 3 mM, about 3.5 mM, about 4 mM, about 4.5 mM, about 5 mM, about 6 mM, about 7 mM, about 8 mM, about 9 mM, about 10 mM, about 15 mM, about 20 mM, or about 30 mM, or any concentration in between. In some aspects, the potassium phosphate monobasic is used at a concentration of about 1 mM. In some aspects, the sodium phosphate dibasic is used at a concentration of about 0.01 mM to about 100 mM. In some aspects, the sodium phosphate dibasic at a concentration of about 0.5 mM, about 1 mM, about 1.5 mM, about 2 mM, about

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2.5 mM, about 3 mM, about 3.5 mM, about 4 mM, about 4.5 mM, about 5 mM, about 6 mM, about 7 mM, about 8 mM, about 9 mM, about 10 mM, about 15 mM, about 20 mM, or about 30 mM, or any concentration in between. In some aspects, the sodium phosphate dibasic is used at a concentration of about 3 mM.

5 [0218] In some aspects, the salt is sodium chloride. In some aspects, the sodium chloride is at a concentration of about 0.01 mM to 1 M. In some aspects, the sodium chloride is at a concentration of about 25 mM to 300 mM. In some aspects, the sodium chloride is at a concentration of about 100 mM, about 110 mM, about 120 mM, about 130 mM, about 140 mM, about 150 mM, about 155 mM, about 160 mM, about 170 mM, about 180 mM, about
10 190 mM, about 200 mM, about 210 mM, about 220 mM, about 230 mM, about 240 mM, about 250 mM, about 260 mM, about 270 mM, about 280 mM, about 290 mM, or about 300 mM. In some aspects, the sodium chloride is at a concentration of about 200 mM.

[0219] In some aspects, pharmaceutical compositions are formulated at a pH suitable for administration to a subject. In some aspects, the pH of the pharmaceutical composition is at
15 about 6.0 to about 9.0. In some aspects, the pH of the pharmaceutical composition is about 7.0, about 7.1, about 7.2, about 7.3, about 7.4, about 7.5, about 7.6, about 7.7, about 7.8, about 7.9, or about 8.0. In some aspects, the pH of the pharmaceutical composition is about 7.4. In some aspects, the pH of the pharmaceutical composition is about 7.6.

[0220] In some aspects, the formulation further comprises sucrose. In some aspects, the
20 formulation comprises between about 0.1% sucrose to 5% sucrose, or any concentration in between. In some aspects, the formulation comprises about 0.2%, about 0.3%, about 0.4%, about 0.5%, about 0.6%, about 0.7%, about 0.8%, about 0.9%, about 1.0 %, about 1.1%, about 1.2%, about 1.3%, about 1.4%, about 1.5%, about 1.6%, about 1.7%, about 1.8%, about 1.9%, or about 2.0 % sucrose. In some aspects, the formulation comprises about 1% sucrose.

25 [0221] In some aspects, the formulation further comprises magnesium chloride (MgCl_2). In some aspects, the magnesium chloride is at a concentration of about 0.5 mM, about 1 mM, about 1.5 mM, about 2 mM, about 2.5 mM, about 3 mM, about 3.5 mM, about 4 mM, about 4.5 mM, about 5 mM, about 6 mM, about 7 mM, about 8 mM, about 9 mM, about 10 mM, about 15 mM, about 20 mM, or about 30 mM, or any concentration in between. In some
30 aspects, the magnesium chloride is at a concentration of about 1 mM.

[0222] In some aspects, the formulation further comprises poloxamer. In some aspects, the poloxamer is poloxamer 188. In some aspects, the poloxamer 188 is at a concentration of about 0.001% to about 1%, or any concentration in between. In some aspects, the poloxamer 188 is at a concentration of about 0.001%, about 0.002%, about 0.003%, about 0.004%, about

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0.005%, about 0.006%, about 0.007%, about 0.008%, about 0.009%, about 0.01%, about 0.02%, about 0.03%, about 0.04%, about 0.05%, about 0.06%, about 0.07%, about 0.08%, about 0.09%, or about 0.1%.

[0223] In some aspects, the formulation comprises about 20 mM Tris, about 0.01%

5 poloxamer 188, about 1% sucrose, about 200mM NaCl, and about 1mM MgCl₂ at a pH of about 7.6.

[0224] Pharmaceutical compositions of the disclosure can be administered to a subject in any volume deemed appropriate by an attending physician and can vary depending on the specific needs of each individual subject. In some aspects, pharmaceutical compositions of the

10 disclosure including pre-packaged pharmaceutical compositions can be diluted in an appropriate volume of fluid prior to administration. In some aspects, if the pharmaceutical composition of the disclosure is diluted, the volume of the diluted pharmaceutical composition administered to the subject is equivalent to the dosages of rAAV viral particles provided herein. In other words, if the volume of pharmaceutical composition is increased,

15 thereby diluting the concentration of rAAV viral particles, the patient will still be administered the same total number of either genome copies of rAAV vector or number of rAAV viral capsids as specified by the dosages disclosed herein.

[0225] In some aspects, the volume of the subject's CSF determines the final volume of the pharmaceutical composition. In some aspects, CSF volume is measured by magnetic

20 resonance image (MRI).

[0226] In some aspects, the pharmaceutical composition is administered in a total volume of about 0.5 mL, about 1 mL, about 2 mL, about 5 mL, about 10 mL, about 15 mL, about 20 mL, about 25 mL, about 30 mL, about 35 mL, about 40 mL, about 45 mL, or about 50 mL, or any volume in between.

25 **Manufacture of APOE2 AAV viral vectors**

[0227] The disclosure provides methods of producing rAAV vectors and rAAV viral vectors encoding an APOE2 polypeptide comprising a Christchurch mutation. In some aspects, rAAV viral vectors are derived from a cellular lysate produced by cell culture and purification methods described herein.

30 [0228] The disclosure provides methods of producing rAAV vectors and rAAV viral vectors encoding an APOE3 polypeptide comprising a Christchurch mutation. In some aspects, rAAV viral vectors are derived from a cellular lysate produced by cell culture and purification methods described herein.

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[0229] The disclosure provides a method of producing a cellular lysate comprising rAAV viral vectors, the method comprising: (i) obtaining a culture vessel comprising HEK293T cells in a culture medium; (ii) transfecting the HEK293T cells in a transfection medium with a first plasmid encoding an APOE2 Christchurch mutation AAV vector or an APOE3 Christchurch mutation AAV vector and a second plasmid encoding AAV Rep proteins and AAV Cap proteins, wherein the ratio of second plasmid to first plasmid is 2:1; (iii) culturing the transfected HEK293T cells in the culture medium under conditions which the transfected HEK293T cells produce recombinant adeno-associated virus (rAAV) viral vectors encoding an APOE2 polypeptide; (iv) harvesting the transfected HEK293T cells; (v) lysing the transfected HEK293T cells to produce a cellular lysate comprising the rAAV viral vectors.

[0230] Culture media of the disclosure can be any culture media capable of culturing mammalian cells including but not limited to HEK293T cells. In some aspects, the culture medium comprises Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum (FBS). Cells can be expanded for any length of time necessary to produce a desirable number of cells. In some aspects, the HEK293T cells are obtained after an expansion culture over about two to about five days.

[0231] Transfection can be performed in any suitable media. In some aspects, the transfection medium comprises serum-free DMEM and polyethylenimine (PEI).

[0232] The transfection of the two plasmids can occur in any order. In some aspects, the transfection of the first plasmid and the second plasmid occur simultaneously.

[0233] In some aspects, prior to transfection, the HEK293T cells are present in the culture vessel at a density of between about 2.0×10^4 to about 2.0×10^6 cells/cm². In some aspects, the HEK293T cells are present at a density of about 2.0×10^5 cells/cm².

[0234] Following transfection, cells can be cultured for any suitable length of time to produce a desired quantity of APOE2 rAAV viral vectors or APOE3 rAAV viral vectors. In some aspects, transfected cells are cultured for about 1 day to about 7 days. In some aspects, transfected cells are cultured for about 3 days.

[0235] Following harvest of transfected cells, cells comprising APOE2 Christchurch mutation rAAV viral vectors or APOE3 Christchurch mutation rAAV viral vectors are lysed to produce a cellular lysate comprising APOE2 Christchurch mutation rAAV viral vectors or APOE3 Christchurch mutation rAAV viral vectors. Cell lysis can be performed by any suitable method including freeze-thaw, mechanical lysis, and lysis by chemical agents such as a

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detergent. In some aspects, the HEK293T cells are lysed via at least about 4 sequential freeze-thaw cycles to produce the cellular lysate.

[0236] Cellular lysates of the disclosure can be treated to remove any residual nonencapsidated viral or cellular DNA. In some aspects, the cellular lysate is further treated with a recombinant nuclease to digest any viral DNA or cellular DNA. In some aspects, the recombinant nuclease is benzonase. In some aspects, DNA digestion is performed in the presence of magnesium chloride.

[0237] Following DNA digestion, the lysate comprising APOE2 Christchurch mutation rAAV viral vectors or APOE3 Christchurch mutation rAAV viral vectors is clarified. Clarification can be performed according to any method known in the art. In some aspects, clarification is performed by centrifugation or ultracentrifugation. Clarification of the cellular lysate removes cell debris and unbroken cells.

[0238] Following clarification, the number of rAAV vectors or rAAV viral vectors in the cellular lysate can be quantified. In some aspects, the cellular lysate comprises from about 1.0×10^9 to about 5.0×10^{14} genome copies (gc) per milliliter.

[0239] The disclosure further provides a method for producing an APOE2 Christchurch mutation rAAV pharmaceutical composition or an APOE3 Christchurch mutation rAAV pharmaceutical composition, the method comprising: (i) obtaining a cellular lysate comprising rAAV viral vectors encoding an APOE2 Christchurch mutation polypeptide or an APOE3 Christchurch mutation polypeptide; (ii) contacting a density gradient with the cellular lysate comprising rAAV viral vectors encoding APOE2 Christchurch mutation polypeptides or APOE2 Christchurch mutation polypeptides and subjecting the density gradient to centrifugation; (iii) contacting a chromatography column with the cellular lysate comprising rAAV viral vectors encoding APOE2 Christchurch mutation polypeptides or APOE3 Christchurch mutation polypeptides; (iv) eluting the rAAV viral particles from the column; (v) concentrating the eluted rAAV viral particles via ultrafiltration into a formulation buffer thereby producing an APOE2 Christchurch mutation rAAV pharmaceutical composition or an APOE2 Christchurch mutation rAAV pharmaceutical composition.

[0240] Density gradients of the disclosure can be any suitable density gradient. Density gradients provide a means of separating components in a mixture by their size and or molecular weight. Density gradient purification can be used to separate empty AAV capsids from AAV capsids comprising APOE2 Christchurch mutation rAAV vectors or APOE3

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Christchurch mutation rAAV vectors. In some aspects, the density gradient is an iodixanol density gradient.

[0241] In some aspects, the iodixanol gradient comprises a step-wise density gradient comprising: (i) an about 10% to about 20% iodixanol solution; (ii) an about 20% to about 50% iodixanol solution; (iii) an about 40% to about 50% iodixanol solution; and (iv) an about 50% to about 60% iodixanol solution. In some aspects, the iodixanol gradient comprises a step-wise density gradient comprising: (i) an about 15% iodixanol solution; (ii) an about 25% iodixanol solution; (iii) an about 40% iodixanol solution; and (iv) an about 54% iodixanol solution.

[0242] In some aspects, alternative means of purification can be used in place of density gradient purification. In some aspects, chromatography can be used. In some aspects, ion exchange chromatography can be used.

[0243] Chromatography can be used to purify rAAV viral vectors following density gradient purification. In some aspects, the chromatography is anion exchange chromatography. In some aspects, the anion exchange column is a Q sepharose high performance strong quaternary ammonium anion exchange resin column.

[0244] Following chromatographic purification, APOE2 Christchurch mutation rAAV viral vectors or APOE3 Christchurch mutation rAAV viral vectors are buffer exchanged into a formulation buffer via ultrafiltration thereby producing a pharmaceutical composition of the disclosure. In some aspects, the formulation buffer comprises phosphate buffered saline (PBS).

[0245] In some aspects, the pharmaceutical composition, following ultracentrifugation, comprises about 1.0×10^{10} to about 5.0×10^{13} genome copies (viral genomes) per milliliter. In some aspects, the pharmaceutical composition, following ultracentrifugation, comprises about 1.5×10^{13} genome copies (viral genomes) per milliliter.

[0246] The disclosure provides methods of producing rAAV vectors and rAAV viral vectors encoding an APOE2 or APOE3 polypeptide comprising a Christchurch mutation. In some aspects, rAAV vectors and rAAV viral vectors encoding an APOE2 or APOE3 polypeptide comprising a Christchurch mutation can be produced in a baculoviral system using a viral expression construct and a payload construct vector. In certain embodiments, the baculoviral system includes Baculovirus expression vectors (BEVs) and/or baculovirus infected insect cells (BIICs). In certain embodiments, the baculoviral system includes Baculovirus expression vectors (BEVs) and/or baculovirus infected Sf9 insect cells. In certain embodiments, a viral expression construct or a payload construct of the present disclosure can be a bacmid, also

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known as a baculovirus plasmid or recombinant baculovirus genome. In certain embodiments, a viral expression construct or a payload construct of the present disclosure can be polynucleotide incorporated by homologous recombination (transposon donor/acceptor system) into a bacmid by standard molecular biology techniques known and performed by a person skilled in the art. Transfection of separate viral replication cell populations produces two or more groups (e.g. two, three) of baculoviruses (BEVs), one or more group which can include the viral expression construct (Expression BEV), and one or more group which can include the payload construct (Payload BEV). The baculoviruses may be used to infect a viral production cell for production of AAV particles or viral vector.

[0247] Baculoviruses are made of several essential proteins which are essential for the function and replication of the Baculovirus, such as replication proteins, envelope proteins and capsid proteins. The Baculovirus genome thus includes several essential-gene nucleotide sequences encoding the essential proteins. As a non-limiting example, the genome can include an essential-gene region which includes an essential-gene nucleotide sequence encoding an essential protein for the Baculovirus construct. The essential protein can include: GP64 baculovirus envelope protein, VP39 baculovirus capsid protein, or other similar essential proteins for the Baculovirus construct.

[0248] Baculovirus expression vectors (BEV) for producing AAV particles in insect cells, including but not limited to *Spodoptera frugiperda* (Sf9) cells, provide high titers of viral vector product. Recombinant baculovirus encoding the viral expression construct and payload construct initiates a productive infection of viral vector replicating cells. Infectious baculovirus particles released from the primary infection secondarily infect additional cells in the culture, exponentially infecting the entire cell culture population in a number of infection cycles that is a function of the initial multiplicity of infection, see Urabe, M. et al. J Virol. 2006 Feb;80(4): 1874-85, the contents of which are herein incorporated by reference in their entirety as related to the production and use of BEVs and viral particles. Production of AAV particles with baculovirus in an insect cell system may address known baculovirus genetic and physical instability.

ENUMERATED EMBODIMENTS

[0249] Enumerated Embodiment 1. A recombinant adeno-associated virus (rAAV) vector, wherein the rAAV vector comprises in the 5' to 3' direction:
a first AAV ITR sequence comprising SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 23;
an enhancer sequence comprising SEQ ID NO: 3;

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a promoter sequence comprising SEQ ID NO: 4;
a chimeric intron comprising SEQ ID NO: 5;
a nucleic acid sequence encoding an apolipoprotein 2 (APOE2) polypeptide
comprising a Christchurch mutation or an apolipoprotein 3 (APOE3) polypeptide
5 comprising a Christchurch mutation comprising SEQ ID NO: 6 or SEQ ID NO: 20;
a polyA sequence comprising SEQ ID NO: 7; and
a second ITR sequence comprising SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 23.

[0250] Enumerated Embodiment 2. The rAAV vector of embodiment 1, wherein the Christchurch mutation comprises:

- 10 an R154S mutation in reference to an unprocessed APOE polypeptide; or
an R136S mutation in reference to mature APOE polypeptide lacking a signal peptide.

[0251] Enumerated Embodiment 3. The rAAV vector of any one of the preceding embodiments, wherein the rAAV vector comprises the nucleic acid sequence set forth in SEQ ID NO: 8, SEQ ID NO: 21, SEQ ID NO: 22.

- 15 **[0252]** Enumerated Embodiment 4. The rAAV vector of any one of the preceding embodiments, wherein the rAAV vector is packaged as an rAAV viral vector comprising an AAV capsid protein.

- [0253]** Enumerated Embodiment 5. The rAAV vector of any one of the preceding embodiments, wherein the AAV capsid protein is an AAV1 capsid protein, an AAV2 capsid protein, an AAV4 capsid protein, an AAV5 capsid protein, an AAV6 capsid protein, an
20 AAV7 capsid protein, an AAV8 capsid protein, an AAV9 capsid protein, an AAV10 capsid protein, an AAV11 capsid protein, an AAV12 capsid protein, an AAV13 capsid protein, an AAVPHP.B capsid protein, an AAVrh74 capsid protein or an AAVrh10 capsid protein.

- [0254]** Enumerated Embodiment 6. The rAAV vector of any one of the preceding
25 embodiments, wherein the AAV capsid protein is an AAVrh10 capsid protein.

[0255] Enumerated Embodiment 7. A pharmaceutical composition comprising the rAAV viral vector of embodiment 4.

- [0256]** Enumerated Embodiment 8. A method of treating Alzheimer's disease in a human subject comprising administering a therapeutically effective amount of a pharmaceutical
30 composition according to embodiment 7.

[0257] Enumerated Embodiment 9. The method of embodiment 8, wherein the therapeutically effective amount of the vector is about 1×10^{10} to about 1×10^{16} genome copies.

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[0258] Enumerated Embodiment 10. The method of embodiment 8, wherein the subject is an APOE2/APOE4 heterozygote, an APOE4/APOE4 homozygote or an APOE3/APOE4 heterozygote.

5 [0259] Enumerated Embodiment 11. The method of embodiment 8, wherein the composition is administered systemically, intracisternally, via intra cisterna magna, or via CI-C2 administration.

[0260] Enumerated Embodiment 12. The method of embodiment 8, wherein the pharmaceutical composition is administered at a dose of about 5.0×10^9 gc/mL CSF to about 5.0×10^{12} gc/mL CSF.

10 [0261] Enumerated Embodiment 13. The method of embodiment 8, wherein the pharmaceutical composition is administered at a dose of about:

- i) 1.4×10^{10} gc/mL CSF,
- ii) 4.4×10^{10} gc/mL CSF, or
- iii) 1.4×10^{11} gc/mL CSF.

15 [0262] Enumerated Embodiment 14. The method of embodiment 8, wherein the pharmaceutical composition is administered at a dose of about 1.4×10^{14} gc.

[0263] Enumerated Embodiment 15. The method of embodiment 8, wherein the pharmaceutical composition is administered in a total volume of about 5 mL, about 10 mL, about 15 mL, or about 20 mL.

20 [0264] Enumerated Embodiment 16. The method of embodiment 8, wherein the subject experiences an at least about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 100% increase in APOE2
25 Christchurch or APOE3 Christchurch expression.

[0265] Enumerated Embodiment 17. The method of embodiment 8, wherein the APOE2 Christchurch or APOE3 Christchurch expression occurs in the central nervous system.

[0266] Enumerated Embodiment 18. The method of embodiment 8, wherein the APOE2 Christchurch or APOE3 Christchurch expression is measured in the cerebral spinal fluid
30 (CSF).

[0267] Enumerated Embodiment 19. The method of embodiment 8, wherein following administration of the pharmaceutical composition the expression levels of at least one of T-tau, and P-tau are reduced in the subject relative to a pre-administration baseline.

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- [0268] Enumerated Embodiment 20. The method of embodiment 19, wherein the expression levels of T-tau, and/or P-tau are reduced by at least about 5%, at least about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 100%.
- [0269] Enumerated Embodiment 21. The method of embodiment 8, wherein following administration of the pharmaceutical composition the amyloid beta 42/amyloid beta 40 ($A\beta_{42/40}$) ratio is increased.
- [0270] Enumerated Embodiment 22. The method of embodiment 21, wherein the $A\beta_{42/40}$ ratio is increased by at least about 5%, at least about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 100%.
- [0271] Enumerated Embodiment 23. The method of embodiment 8, wherein prior to treatment with the pharmaceutical composition the subject is administered an immunosuppressant.
- [0272] Enumerated Embodiment 24. The method of embodiment 23, wherein the immunosuppressant is prednisone.
- [0273] Enumerated Embodiment 25. The method of embodiment 24, wherein the prednisone is administered at a dosage of:
- 40 mg, once daily 1 week prior to AAV viral vector administration;
 - 40 mg once daily for week 1 through week 2 post-AAV viral vector administration;
 - 30 mg once daily for week 3 post-AAV viral vector administration;
 - 20 mg once daily for week 4 post-AAV viral vector administration;
 - 10 mg once daily for week 5 post-AAV viral vector administration;
 - 5 mg once daily for week 6 post-AAV viral vector administration;
 - 2.5 mg once daily for week 7 post-AAV viral vector administration; and
 - 2.5 mg every other day for week 8 post-AAV viral vector administration.

30 EXAMPLES

[0274] The invention will be further illustrated by the following figures and examples. However, these examples and figures should not be interpreted in any way as limiting the scope of the present disclosure.

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[0275] FIG. 1C shows the experimental design for the studies described in Examples 1 and 2.

Example 1: Heparin binding of APOE variants

[0276] Heparan sulfate proteoglycans (HSPG) have been linked to tau pathology; it has been suggested that HSPG allow tau fibrils to attach to neurons, facilitating uptake and propagation of toxic forms of tau. It has been demonstrated that APOE variants differentially bind heparin, with APOE4 binding heparin the tightest, followed by APOE3, and finally APOE2. Previous studies demonstrated that APOE3 contain the Christchurch mutation bound heparin even more weakly than APOE2. Protection of the APOE3 Christchurch variant therefore may correlate with lost binding to heparan sulfate proteoglycans (HSPGs), believed to propagate tau tangles.

Methods

[0277] The assessment of heparin binding of the APOE isoforms was carried out as described by Futamura *et al.* (J Biol Chem 2005;280(7):5414-22) Hemagglutinin (HA) 3' tagged human (h) APOE variants, including hAPOE2, hAPOE2Ch, hAPOE3, hAPOE3Ch and hAPOE4 were generated in HEK293T cells by plasmid transfection with plasmids/PEI (Polyplus, France) complex in serum free-DMEM. After 72 hours, cell media was collected every 24 hours and refreshed with fresh serum-free DMEM 3 times. The pooled transfected cell medium was centrifuged at 1500xg to remove cell debris. APOE levels in each sample were quantified by hAPOE-specific ELISA (ab108813, Abcam, UK). To assess heparin binding of each isoform, APOE-containing media were diluted with binding buffer (5 times in 20 mM Tris-HCl, pH 7.5) and loaded into heparin-binding columns (7040703, Cytiva, Marlborough, MA) maintained at 23°C for 1 hour before use. The columns were washed with 5 ml of 20 mM Tris-HCl, pH 7.5 buffer. Each APOE variant (1 ml, 50 µg/ml) in 20 mM Tris-HCl, pH 7.5 was recycled through the column 5 times. The column was then washed 5 times using the same buffer. An increasing step gradient of NaCl in 20 mM Tris-HCl (0.025–1 M, 1 ml per each gradient step) was passed through the column, and 1 ml fractions collected and analyzed for APOE with the human APOE-specific ELISA. Three independent experiments were performed for each hAPOE isoform. Background APOE levels in each fraction were subtracted based on the non-transfected cells in the same transfection conditions. The quantified binding characteristics of each APOE isoform from the 3 experiments were averaged and plotted as a function of NaCl concentration at which each was eluted from the column.

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AAV Vectors

[0278] All vectors were based on the clade E nonhuman primate AAVrh.10 serotype, including AAVrh.10hAPOE2 (coding for the human APOE2 variant), AAVrh.10hAPOE2Ch (coding for human APOE2Christchurch) and AAVrh.10Null (as a control, identical but with no translatable sequence). The expression cassette for all the vectors in the study use the cytomegalovirus-enhancer fused to the chicken beta-actin promoter (CAG) promoter. For some experiments, a hemagglutinin (HA) tag was added to the 3' end of the coding sequence. The AAVrh.10 vectors were produced, purified, and characterized as previously described in Rosenberg *et al.*, (Hum Gene Ther Clin Dev 2018;29(1):24-47). HEK293T cells were transfected with expression cassette plasmid (pAAV-hAPOE2, pAAV-hAPOE2Ch, pAAV-Null) and adenovirus/AAVrh.10 helper plasmid (pPAK-MArh.10), expressing the AAVrh.10 cap gene and AAV2 rep gene necessary for viral reproduction and capsid production. Cells were maintained in Dulbecco's modified Eagle's medium, supplemented with 5% fetal bovine serum, 100 U/mL penicillin, and 100 mg/mL streptomycin, and maintained at 37°C with 5% CO₂. Cells were seeded into the CellSTACKS (Corning, Tewksbury, MA) for 24 hours at 70-80% confluence and transfected with PEIpro lipid reagent (Polyplus, France). Following incubation at 37°C for 72 hours, the cells were harvested and lysed by five freeze/thaw cycles. The cell lysate was treated with 50 U/mL of benzonase (E1014, Millipore, Billerica, MA) at 37°C for 30 minutes to remove any contaminant genomic DNA. The crude viral lysate was centrifuged at 3300xg and then purified by discontinuous iodixanol density gradient followed by QHP ion-exchange chromatography. The vectors were concentrated in spin columns (Millipore) at 13,000xg for 15 minutes in phosphate-buffered saline, pH 7.4 (PBS). The purified vectors were sterile filtered and tested for bacterial and fungal contamination and tested for endotoxin. Vector genome titers were determined by TaqMan qPCR using a CAG promoter-specific primer-probe set (Applied Biosystems, Foster City, CA). The purified AAVrh.10 vectors were digested with proteinase K in the presence of 0.5% sodium dodecyl sulfate and 25 mM ethylenediaminetetraacetate at 70°C for 1 hour, followed by inactivation of the protease at 95° C for 15 min. The vector was then used as a template for TaqMan analysis using an AAV plasmid DNA standard of known copy number to generate a standard curve. Prior to use, all vectors were tested for expression in an *in vivo* potency assay as previously described in De *et al.* (Hum Gene Ther Methods 2018;29(3):146-155).

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Results

[0279] APOE predominantly binds and interacts with heparin through residues 136-150 (Figure 1A). Of the common APOE variants, the APOE4 has the highest affinity for heparin, APOE3 intermediate and APOE2 the lowest affinity (see Arboleda-Velasquez *et al.*, Med 5 2019;25(11):1680-1683; Futamura *et al.* J Biol Chem 2005;280(7):5414-22). Arboleda-Velasquez *et al.* demonstrated that the Christchurch variant with the APOE3 variant (APOE3Ch) has decreased heparin binding compared to APOE2, resulting from the change of amino acid at position 136 with arginine switched to serine. Here, this APOE variant heparin-binding data (APOE4>E3>E2>E3Ch) was reproduced in comparison to the therapeutic E2Ch 10 variant. Interestingly, it was observed that the E2Ch variant had lower binding affinity than all other isoforms, including E3Ch (Figure 1B).

[0280] The APOE2 Christchurch variant was found to bind even more weakly to heparin than the APOE3 Christchurch variant. This suggests that an APOE2 Christchurch variant can have an even greater protective effect against tau formation and Alzheimer's disease.

15 Example 2: In vivo studies of AAV vectors encoding APOE2 Christchurch polypeptides

[0281] The hypothesis that AAVrh.10-mediated expression of the combined human APOE2 allele with the Christchurch mutation (AAVrh.10hAPOE2Ch), will effectively protect against the development of Alzheimer's disease was evaluated.

Methods

20 Mouse Models

[0282] APOE4 targeted replacement mouse (TRE4; mouse ApoE4 knockout, human APOE4 knock in) was obtained from Duke University Medical Center. Both the APP.PSEN1 (amyloid, B6C3-Tg/APPswe, PSEN1dE9/85#004462/034829 MMRRC), and P301S (tau, B6; C3-Tg/Prnp-MAPT*P301S/PS19Vle/J #008169) were obtained from Jackson Laboratories 25 (Bar Harbor, ME). Both strains were crossbred with TRE4 mice to generate the APP.PSEN1/TRE4 (humanized APOE4, amyloid mice) and P301S/TRE4 (humanized APOE4, tau mice) strains. Each mouse colony was maintained and expanded at the Belfer Gene Therapy Core Facility. Genotypes were confirmed through analysis of tail snips or ear punches (Transnetyx, Cordova, TN). The APP.PSEN1/TRE4 mice were treated at 2.5 months 30 and assessed at 5.5 to 6 months. The P301/PSEN1/TRE4 mice were treated at 5.5 months and assessed at 8.5 to 9 months.

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Vector Administration and Experimental Groups

[0283] Intrahippocampal injection of AAV vectors was performed by stereotaxic surgery as previously described (see Zhao *et al.*, Neurobiol Aging 2016;44(159-172)). Animals were anesthetized with isoflurane and placed on a stereotaxic frame (Harvard Apparatus, Holliston, MA). Following a skin incision, burr holes the size of the injection needle were drilled using a high-speed drill. The vectors (2×10^{10} gc in 2 μ l, each administration) were bilaterally administered to the hippocampus using a 33-gauge needle (Hamilton, Reno NV) and a syringe pump (KD Scientific, Holliston, MA) at a rate of 0.2 μ l/min for 10 minutes to administer 2 μ l (stereotactic coordinates, 1.7 mm anteroposterior from bregma; 1.2 mm mediolateral from bregma, and 1.7 mm dorsoventral below the dura). The injection needle was left in place for 4 minutes to minimize backflow and slowly withdrawn. After surgery, the mouse was housed in a separate cage for three months and monitored daily for general health conditions and weight during the experimental period.

[0284] Amyloid mice were treated at 2.5 months old and tau mice at 5.5 months old. Three months after treatment, all mice were assessed for behavior over 2 weeks (see below for details) and then were sacrificed via CO₂ gas asphyxiation and transcardially perfused with 0.3% heparinized saline (2500 IU/mL). The brains were rapidly collected, and each divided along the sagittal plane with one hemisphere processed for histologic and immunohistochemistry analyses. Isolated hemispheres were microdissected under a stereomicroscope. Meninges were removed, and midbrain tissue extracted to visualize the hippocampus. Based on the gross morphology, the hippocampus was carefully removed without stripping any cortex and then rapidly frozen on dry ice and stored at -80 °C for biochemical analyses.

[0285] Amyloid mice were administered AAV vectors expressing E2-HA (n=13, 8M/5F), E2CH-HA (n=13, 7M/6F), E2 (n=22, 12M/10F), E2Ch (n=24, 12M/12F), Null (n=27, 17M/10F) or PBS (n=34, 14M/20F). Tau mice were administered AAV vectors expressing E2-HA (n=11, 6M/5F), E2CH-HA (n=9, 5M/4F), E2 (n=19, 10M/9F), E2CH (n=23, 14M/9F), Null (n=20, 10M/10F) or PBS (n=20, 10M/10F). To avoid injection-side-related bias, different hemispheres were randomly used for histologic and other analyses.

Quantification of Vector Genome Copies, Transgene Expression, and APOE Protein Levels

[0286] To quantify vector-related parameters, dissected brains were thawed and homogenized in ice-cold sterile Tris-buffered saline, pH 7.4 (TBS). Three equal volumes of homogenate

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were used for DNA, RNA, and protein isolation. DNA isolation was performed with a DNeasy tissue/blood DNA isolation kit, following the manufacturer's instructions (Qiagen, Valencia, CA). RNA isolation was performed via Qiazol (Qiagen, Valencia, CA), and the final RNA concentrated with RNeasy mini elute columns (Qiagen, Valencia, CA). DNA and RNA concentrations were determined with a Nanodrop (ThermoFisher Scientific, Waltham, MA). All RNA samples were converted to cDNA with a high-capacity reverse transcription kit (ThermoFisher Scientific). hAPOE mRNA levels were quantified using the primer/probes: forward 5'-GTGGAGAAGGTGCAGGCT-3', reverse 5'-GATTGTCGCTGGGCACAGGG-3' by RT-PCR. Total protein content was determined by the Bradford assay (see Kielkopf *et al.* Cold Spring Harb Protoc 2020;2020(4):102269). APOE levels were measured with a human APOE-specific ELISA (ab108813, Abcam, UK) and normalized to total protein.

Quantification of Insoluble and Soluble A β 42, A β 40 Levels

[0287] A sequential extraction method was used for the quantification of insoluble and soluble A β 42 and A β 40 peptides. Hippocampal extracts homogenized in TBS were centrifuged at 100,000xg, 1 hr, 4°C. TBS soluble supernatants were aliquoted into separate 0.6 ml tubes before freezing in liquid nitrogen and storage at -80°C. The pellet was washed with 200 μ l TBS buffer centrifuged at 14,000 x g for 5 min at 4°C, and the wash was discarded. Pellets were resuspended in 15 volumes (weight/volume of tissue) of TBS buffer containing 1% Triton X-100 (TBSTX) and mixed gently by rotation at 4°C for 30 min, followed by a second centrifugation at 100,000xg, 1 hr, 4°C. The TBSTX soluble supernatants were aliquoted into separate 0.6 ml tubes before freezing in liquid nitrogen and storage at -80 °C. The pellet was washed with TBSTX buffer that was discarded. The TBSTX-insoluble pellet was resuspended in 400 μ l of 5 M guanidine HCl, mixed by rotation at 23°C, 6 hr, and centrifuged at 16,000 x g for 30 min. The guanidine HCl resuspended peptide (insoluble fraction) was aliquoted and frozen. The total protein content in the TBS fraction was quantified with the Bradford assay (see Kielkopf *et al.* Cold Spring Harb Protoc 2020;2020(4):102269). Due to the interference of Triton X-100 and the final buffer of the insoluble fraction in 1% sodium dodecyl sulfate (SDS) a transition metal-based protein assay evaluated at 660 nm (polyhydroxybenzenesulfonephthalein-type dye, 22660, ThermoFisher Scientific) was used to quantify total protein (see Antharavally *et al.*, Anal Biochem 2009;385(2):342-5). A β 42 (KHB3441, ThermoFisher Scientific), A β 40 (KHB3481, ThermoFisher Scientific), levels in soluble and insoluble fractions were quantified using commercially available ELISA kits following the manufacturer's instructions. The total

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amount of A β 42 and A β 40 were normalized to total protein content. TBSTX and guanidine HCl fractions together were considered as the insoluble fraction and the TBS fraction was considered the soluble fraction.

Quantification of Insoluble and Soluble Total Tau and Phospho-tau levels

5 [0288] Increased levels of phospho-tau, phosphorylated at residues such as Thr181, Thr231 and Ser396, are associated with neurofibrillary tangle formation and neuronal dysfunction (see Shi *et al.*, Nature 2017;549(7673):523-527). Total tau levels reflect the overall tau burden and serves as a marker of neurodegeneration, as does tau solubility (Limorenko *et al.*, Chem Soc Rev 2022;51(2):513-565). A sequential extraction method was used for the quantification
10 of insoluble and soluble tau. TBS homogenized hippocampal extracts were centrifuged at 100,000 x g, 1 hr, 4°C. TBS soluble supernatants were aliquoted into separate 0.6 ml tubes before freezing in liquid nitrogen and storage at -80°C. The pellet was washed with 200 μ l TBS buffer and centrifuged at 14,000 x g for 5 min at 4°C. Pellets were resuspended in 15 volumes (weight/volume of tissue) of TBS buffer containing 1% Triton X-100 (TBSTX)
15 and mixed gently at 4°C for 30 min, followed by a second centrifugation at 100,000 x g, 1 hr, 4°C. TBSTX soluble supernatants were aliquoted into separate 0.6 ml tubes before freezing in liquid nitrogen and stored at -80°C. The pellet was washed with TBSTX buffer. The pellet was resuspended in 400 μ l of 5 M guanidine HCl, gently mixed at 23°C, 6 hr, and centrifuged at 16,000 x g for 30 min. The guanidine HCl resuspended fraction (insoluble fraction), was
20 aliquoted and frozen. The proteins in the insoluble fraction precipitated with ethanol-chloroform precipitation (Wessel *et al.*, Anal Biochem 1984;138(1):141-3). The total protein content in the TBS fraction was quantified with the Bradford assay (Kielkopf *et al.* Cold Spring Harb Protoc 2020;2020(4):102269). Due to the interference of Triton X-100 and the final buffer of the insoluble fraction in 1% SDS, a transition metal-based protein assay
25 evaluated at 660 nm (polyhydroxybenzenesulfonephthalein-type dye, 22660, ThermoFisher Scientific) was used to quantify total protein (Antharavally *et al.*, Anal Biochem 2009;385(2):342-5). Total tau and phospho-tau levels in the soluble and insoluble fractions were assayed using ELISA kits following the manufacturer's instructions (KHB0041 and KHB7031, respectively, ThermoFisher Scientific). The total amount of total tau, and p-tau
30 were normalized to total protein content. TBSTX and guanidine HCl fractions together were considered as the insoluble fraction and the TBS fraction was considered as the soluble fraction.

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Histologic Assessments

- [0289] Following necropsy, one hemisphere of the mouse brain was fixed immediately in 4% paraformaldehyde in PBS, processed for embedding in paraffin, and serially sectioned at 5 μ m in the sagittal plane (Histoserv, Germantown, MD). Sections identified by the gross morphology as the hippocampus were deparaffinized using xylene and a series of graded ethanol washes followed by treatment with a low pH antigen retrieval solution (00-4955-58, ThermoFisher Scientific) at 88°C for 20 min. The sections were then blocked using the SuperBlock Buffer (37515, ThermoFisher Scientific) overnight in a humid chamber at 4°C. The sections were then incubated at room temperature for 2 hours with the primary and secondary antibodies as described below.
- [0290] **MOAB-2.** MOAB-2 antibody which specifically binds to the N-terminal of beta-amyloid peptides was used to assess beta-amyloid burden in amyloid mice (Youmans *et al.*. Mol Neurodegener 2012;7(8)). Staining was performed with MOAB-2 antibody (NBP2-13075, MOAB-2, Novus Biologicals). After incubation with the antibody, the sections were washed four times in Tris buffered saline/0.01% tween 20 (TBS-T) and incubated for 30 min with goat anti-mouse IgG Alexa Flour 488 conjugated antibody (A28175, ThermoFisher Scientific), diluted 1:1,000 in 20% SuperBlock in TBS, using 4',6-diamidino-2-phenylindole (DAPI) solution for counterstaining. The slides were covered with coverslips using EMS-Mount Mounting Medium (Electron Microscopy Sciences,) and imaged using a EVOS fluorescence microscope (ThermoFisher Scientific).
- [0291] **Glial fibrillary acidic protein (GFAP).** GFAP is an intermediate filament protein highly expressed in astrocytes, providing structural support and stability to astrocytic processes (Parhizkar *et al.* Semin Immunol 2022;59:101594). Because GFAP staining specifically targets astrocytes, it was used for visualization and quantification of astrogliosis in amyloid and tau mice. After incubation with the primary chicken anti-GFAP antibody (ab4674, Abcam), the sections were washed four times in TBS-T and incubated for 30 min with goat anti-chicken IgY H&L (Alexa Fluor 488, ab150169, Abcam, Waltham MA), diluted 1:1,000 in 20% SuperBlock in TBS, using DAPI for counterstaining. The slides were covered with coverslips using the EMS Shield Mount mounting medium with Dabco (Electron Microscopy Sciences) and imaged using an EVOS fluorescence microscope (ThermoFisher Scientific).
- [0292] **Iba-1.** Iba-1 is a calcium-binding protein that is highly expressed by microglia, is localized in the cytoplasm and processes (Moser *et al.*. iScience 2021;24(11):103238). Iba-1 staining was used for visualization and quantification of microglial activation in amyloid and

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tau mice. After incubation with the primary rabbit anti-Iba-1 antibody (019-19741, Fujifilm, Santa Clara, CA), the sections were washed four times in TBS-T and incubated for 30 min with goat anti-rabbit IgG conjugated to Alexa Flour 564 (A11035, ThermoFisher Scientific), diluted 1:1,000 in 20% SuperBlock in TBS, using DAPI for counterstaining. The slides were covered with coverslips using the EMS Shield Mountmounting medium (Electron Microscopy Science) and imaged using an EVOS fluorescence microscope (ThermoFisher Scientific).

5 [0293] **AT8.** Total-tau burden and the presence of neurofibrillary tangles (intracellular aggregates composed of abnormal tau protein), were assessed with AT8 antibody in tau mice (Koutsodendris *et al.*, Nat Aging 2023;3(3):275-296). The AT8 antibody specifically targets a phosphorylated epitope at amino acid residues Ser202 and Thr205 of the tau protein
10 (MN1020, AT8, ThermoFisher Scientific). After incubation with the primary antibodies, the sections were washed four times in TBS-T and incubated for 30 min with goat anti mouse IgG conjugated to Alexa Flour 488 (A28175, ThermoFisher Scientific), diluted 1:1,000 in 20% SuperBlock in TBS, using DAPI for counterstaining. The slides were covered with coverslips
15 using the EMS-Mount Mounting Medium and imaged using an EVOS fluorescence microscope.

[0294] **X-34.** X-34 (SML1954, Sigma Aldrich) is a small molecule dye, a fluorescence derivative of Congo red, that has an affinity to A β aggregates, resulting in a distinct fluorescence signal (Ulrich *et al.*, J Exp Med 2018;215(4):1047-1058). X-34 staining was
20 used to assess the total amyloid burden quantifying amyloid aggregates in amyloid mice (Styren *et al.* J Histochem Cytochem 2000;48(9):1223-32). Following serial ethanol washes, slides were incubated in PBS for 5 min. The sections were placed into a solution of X-34 in 40% ethanol/60% distilled H₂O (adjusted to pH 10 by the addition of 1 N NaOH) at the concentration of 1 mM for 5 minutes. The sections were then washed with PBS and briefly
25 dipped 5 times in tap water and transferred to 0.2 g% NaOH in 80% for 2 min. Sections were cover slipped with DPX mounting medium (Electron Microscopy Science).

Quantification of Histologic Parameters

[0295] To quantify histologic parameters, sections for histology from tissue blocks that contained the hippocampus were selected. All were used but randomly assigned to the
30 histological stain to avoid bias. The individuals performing the staining were blinded to the experimental conditions. Following staining, immunopositivity assessment was fully automated using Qupath software, with predefined settings and parameters to identify and quantify immunopositive cells. Staining intensity in X34 was measured with Haralick's

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texture features to assess the co-occurrence matrix in black background (Löfstedt *et al.*, PLoS One 2019;14(2):e0212110). Utilizing grid-based sampling, starting points within the hippocampus were randomly selected and picture areas systematically sampled at regular intervals. This selection approach prevented bias and provided representative sampling across the region of interest (Zhao *et al.*, Nat Commun 2020;11(1):4275).

Behavioral Assessments

[0296] Behavior was assessed over a period of 2 weeks in the amyloid mice ages 5.5 to 6 months, 3 to 3.5 months after therapy, and in the tau mice at age 8.5 to 9 months, 3 to 3.5 months after therapy. Neurological screening and behavioral deficits were evaluated with four behavioral tests during the experimental period, including nest building, Y maze, novel object recognition and Barnes maze. All behavior equipment were cleaned with 70% ethanol between animals to avoid any olfactory cues in all behavioral tests except nesting. Behavior assays were performed on animals with blinded identification and data analysis utilizing fully automated software (AnyMaze, v7.0, 2022, Stoelting, San Diego, CA) to ensure unbiased evaluation without subjective input (Bailoo *et al.*, J Neurosci Methods 2010;188(1):45-52). Nesting assays were an exception to the automated method, and nesting behavior assessment was scored by 3 or 4 blinded observers.

[0297] **Nest Building:** Nest building is an innate behavior in mice and is considered an early indicator of behavioral deficits (Dorninger *et al.*, Bio Protoc 2020;10(24)). Two weeks before necropsies, animal bedding was changed with cotton squares and evaluated after 24 hours, by 3 or 4 blinded observers (Samaey *et al.*, Front Aging Neurosci 2019;11:335). Animal nesting behavior was quantified by how the nesting material was used (using a 5-point scale), scored as follows: no nest-building behavior (score of 1), minimal engagement and organization in manipulating the nesting material and constructing the nest structure (score of 2), partial engagement and organization in manipulating the nesting material and constructing the nest structure (score of 3), near-complete nest structure (score of 4) and excellent performance characterized by meticulous gathering and arrangement resulting in a well-organized and stable nest structure (score of 5) (Samaey *et al.*, Front Aging Neurosci 2019;11:335).

[0298] **Y Maze:** The Y maze measures spatial working memory and tests animals' innate curiosity to explore new environments and ability to differentiate between familiar and novel spatial cues (Kraeuter *et al.*, Pre-Clinical Models: Techniques and Protocols. (Guest PC. ed.) Springer New York: New York, NY; 2019; pp. 105-111). Animals were placed on the Y maze platform (Stoelting, IL) and recorded for 5 minutes. Consecutive novel arm entries were

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considered as alternations. Total arm entries and the number of alternations as assessed by the AnyMaze software were used to calculate the alternation ratio, a measure of novel exploratory behavior. In addition, to avoid possible effect of significant immobile time on alternation behavior, total number of entries and number of alternations was also assessed.

5 [0299] Novel Object Recognition: The novel object recognition task assesses working memory and is based on the spontaneous tendency of mice to explore a novel object over a familiar one (Lueptow *et al.*, J Vis Exp 2017;126). The novel object recognition task was performed on a novel object recognition test platform (Stoelting, IL) over three days, each pertaining to a unique phase. On Day 1, the habituation phase, each animal was allowed to
10 freely explore an open arena (40 cm x 40 cm x 30 cm tall white plastic box) for two 5 minute trials with a 5 minute inter-trial interval. On Day 2, the familiarization phase, each animal was returned to the same arena for two 5 minute sessions with two identical objects in opposite corners. After a 24 hr retention period (the period to forget or remember object novelty), the testing phase was initiated on Day 3, with the animal returning to the arena with two objects
15 placed in identical positions as the previous day but with one replaced with a novel object. Mice were consistently placed in the arena facing the posterior wall to prevent object preference and allowed to explore for 5 minutes. The time spent exploring the novel objects divided by the time spent exploring both objects was calculated as the discrimination index. The criteria for exploration by the AnyMaze software were defined as the mouse sniffing,
20 climbing on or interacting while facing the novel object within 3 cm.

[0300] Barnes Maze: The Barnes maze tests hippocampal-dependent spatial memory, capitalizing on the natural aversion of rodents to open spaces and their instinctual tendency to seek shelter (Pitts *et al.*, Bio Protoc 2018;8(5)). The apparatus contains 20 circular holes (50 mm diameter) evenly spaced along the perimeter of the maze (Stoelting, IL). Only one
25 hole provides access to an enclosed escape chamber (108 mm x 55 mm x 55 mm) beneath the maze floor. Testing procedures and distal visual cues were modified to enhance visuo-spatial learning and memory. Bright overhead lighting (10.8 x 102 lux) and white noise were used to drive escape motivation. The animals were trained for seven days to use distal visual cues to locate the escape hole. The trial was terminated when the mouse had escaped through the hole
30 or 180 sec had elapsed. Latency to escape the maze by entering the appropriate hole was calculated to assess spatial learning. Escape latency was evaluated in training days and probe trials. Data was recorded in a final trial performed 24 hours after the last training day by closing the escape hole and recording latency using the 10 to 15 mm diameter setting in the ANY-maze software.

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Statistical Analyses

[0301] All experimental data were collected and analyzed using GraphPad Prism (GraphPad Software, La Jolla, CA). Linear quantitative data are presented as the mean, $\pm$ standard error of means. Logarithmic scale data are presented as geometric mean $\pm$ geometric standard deviation. Data normality was assessed by Shapiro-Wilk's test. Based on data distribution, one-way ANOVA and Kruskal-Wallis tests were used. Multiple comparisons were performed with Dunn's and Bonferroni's post hoc analysis. Some experiments were carried out with HA-tagged vectors and some without the HA-tag. Statistical differences between HA/non-HA and male/female results were analyzed by independent t-test and Mann-Whitney U tests. There were no differences in the assessments with and without the tag, so the data was combined. There were no differences in the studies carried out with males vs females, so data from both sexes was combined. For all assessments p values < 0.05 were considered significant.

Results

AAV Expression In Vitro

[0302] To assure that the detection of the vector mediated APOE isoforms reliably detects each with equal sensitivity, the manufacturer provided data that this ELISA kit detected APOE2, APOE4 with equal sensitivity. It was also established that the kit equally detected APOE2Ch as APOE4 and APOE2 by assaying APOE in the media of 293 cells transfected with equal amounts of otherwise identical plasmids (FIG. 14).

AAV Expression In Vivo

[0303] The studies were designed to treat the amyloid and tau murine models with early evidence of disease as shown by histological and biochemical assessment (FIG. 15A, FIG. 15B, FIG. 15C, and FIG. 15D). Amyloid mice (APP.PSEN1/TRE4) were treated at 2.5 months; at this time, these mice have evidence of amyloid deposits, and the p-tau mice (P301S/TRE4) were treated at 5.5 months; at this time, these mice have evidence of p-tau aggregates. In this context, early disease was modeled.

[0304] To assure all experimental groups had comparable AAV transduction, vector genome copies, APOE mRNA and APOE protein were assayed in all study cohorts controls in the hippocampus of both the amyloid and tau mice (FIG. 2A-2F). No vector genome copies were detected in the PBS cohort in either the amyloid or tau mice (FIGs. 2A and 2B) and there were no significant differences in vector copies in Null, E2 and E2Ch treated amyloid ($p > 0.1$, FIG. 2A) or tau mice ($p > 0.2$, FIG. 2B). Human APOE transgene mRNA expression was quantified with specific primer/probes for hAPOE to measure the background levels in control

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mice and levels above background in treated mice (FIGs. 2C and 2D). No significant difference in APOE mRNA levels were observed between PBS and Null groups in amyloid ($p>0.5$, FIG. 2C) or tau mice ($p>0.9$, FIG. 2D), i.e., both had similar background levels. Treatment with E2 and E2CH each had significantly greater mRNA levels in both amyloid and tau mice compared to PBS ($p<0.01$, FIG. 2C) and Null ($p<0.01$, FIG. 2D) cohorts. PBS and Null groups had similar background levels of hAPOE protein in amyloid and tau mice ($p>0.9$, FIGs. 2E and 2F). The E2 and E2CH treated mice had comparable ($p>0.9$) 3- to 5-fold higher hAPOE protein levels in amyloid and tau mice ($p<0.01$, FIGs. 2E and 2F) than background.

10 *Effect of Treatment on the Amyloid Burden in Amyloid Mice*

[0305] The effects of treatment on amyloid burden were investigated with quantification of hippocampus A β 42 and A β 40 levels in insoluble and soluble fractions (FIGs. 3A and 3B). In amyloid mice, no significant difference between PBS and Null groups were observed in insoluble or soluble fractions of A β 42 ($p>0.3$ insoluble, $p>0.9$, soluble, FIG. 3A) or A β 40 ($p>0.3$ insoluble, $p>0.9$, soluble, FIG. 3B). E2 treatment significantly suppressed insoluble and soluble A β 42 levels by 79.7% ($p<0.01$) and 80.0% ($p<0.01$), respectively and A β 40 levels by 68.0% ($p<0.01$) and 68.1% ($p<0.01$), respectively (FIGs. 3A and 3B) compared to PBS and Null groups. Additionally, E2Ch significantly decreased insoluble and soluble A β 42 levels by 80.5% ($p<0.01$), 81.6% ($p<0.01$) and A β 40 levels by 66.6% ($p<0.01$) and 71.0% ($p<0.01$) compared to PBS and Null groups, respectively. No significant difference was observed between E2 and E2CH treatment in both A β 42 and A β 40 levels in insoluble and soluble fractions ($p>0.8$ all comparisons (FIGs. 3A and 3B)).

Effect of Treatment on Tau Burden in Tau Mice

[0306] Sequential extraction from hippocampal tissue was used to quantify total tau and p-tau in each cohort (FIGs. 3C and 3D). The levels of total tau ($p>0.3$ insoluble, $p>0.5$, soluble, FIG. 3C) and p-tau ($p>0.9$ insoluble, $p>0.5$, soluble, FIG. 3D) were similar in the PBS and Null groups. In contrast, total tau in the insoluble fraction was significantly lower in E2 group, ($p<0.01$ compared to PBS, but not compared to Null $p>0.1$, FIG. 3C), but not with soluble total tau ($p>0.9$ compared to PBS, $p>0.1$ compared to Null, FIG. 3C) or in insoluble and soluble fractions of p-tau ($p>0.9$ compared to PBS in both fractions, $p>0.4$ and $p>0.3$ compared to Null; FIG. 3D). In contrast, administration of E2Ch decreased total tau levels in insoluble fraction by 40.5% and 68.6% in the soluble fraction compared to PBS ($p<0.01$ both

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insoluble and soluble fractions) and compared to Null ($p < 0.01$ insoluble, $p < 0.05$ soluble; FIG. 3C). Additionally, E2Ch significantly decreased p-tau levels by 36.2% in insoluble and 44.5% in soluble fractions compared to PBS group ($p < 0.01$ insoluble, $p < 0.05$ soluble, FIG. 3D) and compared to Null ($p < 0.01$ insoluble, $p < 0.01$ soluble, FIG. 3D) cohort. Importantly, a significant difference was observed between the E2CH and E2 treatments for assessed amounts of soluble total tau ($p < 0.01$, FIG. 3C), p-tau soluble ($p < 0.05$, FIG. 3D) and insoluble p-tau ($p < 0.05$, FIG. 3D), indicating a pronounced effect of the E2CH above E2 on reducing tau pathology.

Effects of Treatment on Lipid Profile

- 10 [0307] Over-expression of APOE2, may result in serum hyperlipidemia, therefore serum cholesterol and triglyceride levels as a function of treatment cohort in both the tau and amyloid mice was assessed. There were no treatment-dependent deviations from the baseline mild hyperlipidemia of the TRE4 mouse strain (FIG. 13A and FIG. 13B).
- 15 [0308] For measures of lipid efflux, which might be a mechanism of ApoE2 protection, lipid metabolism-related transcripts in hippocampal-cells was investigated using spatial transcriptomics. For example, SGMS2 (Sphingomyelin Synthase 2), a key factor in control of sphingomyelin and diacylglycerol metabolism, is typically decreased in AD but SGMS2 was increased in both E2 and E2Ch treatment. In addition, reduction of LRP2 (LDL Receptor Related Protein 2) is thought to be a causal factor in Alzheimer's disease (AD). The E2Ch cohort had an increase in LRP2 expression (FIG. 16A, FIG. 16B, FIG. 16C, and FIG. 16D).
- 20

Effect on Amyloid Aggregation, Neuronal Degeneration and Microglial/Astroglial Activation in Amyloid Mice

- [0309] Amyloid beta staining as detected by MOAB-2 was used to examine the accumulation of amyloid beta plaques (see Youmans *et al.*, Mol Neurodegener 2012;7(8)). The PBS and Null groups showed similar levels of amyloid beta staining ($p > 0.07$, FIGs. 4A, 4B and 4E). Both E2 (FIG. 4C) and E2CH (FIG. 4D) treatments led to a significant decrease in MOAB-2 detectable amyloid beta ($p < 0.01$ for E2 and $p < 0.01$ for E2Ch compared to PBS and to Null ($p < 0.01$ for both E2 and E2Ch compared to Null), FIG. 4E). No significant difference was observed between treatment with E2 and E2Ch ($p > 0.9$, FIG. 4E).
- 25
- 30 [0310] X34 staining was used to evaluate the presence of amyloid aggregates (see Styren *et al.* J Histochem Cytochem 2000;48(9):1223-32). Similar to the MOAB-2 staining, the PBS (FIG. 4F) and Null (FIG. 4G) groups showed comparable X34 staining profiles ($p > 0.05$, FIG. 4J). Treatment with both E2 (FIG. 4H) and E2Ch (FIG. 4I) resulted in a decrease of X34

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staining ($p < 0.01$ for both E2 and E2Ch, compared to PBS), demonstrating that both E2 and E2Ch mediated suppression of the accumulation of amyloid aggregates in amyloid mice ($p > 0.4$, FIG. 4J).

[0311] Iba-1 immunostaining provides a measure of microglial activation (see Moser *et al.*.

- 5 iScience 2021;24(11):103238). Both the PBS (FIG. 4K) and Null (FIG. 4L) groups displayed strong staining profiles with Iba-1 indicating similar levels of microglial activation. In contrast, treatment with both E2 (FIG. 4M) and E2CH (FIG. 4N) significantly inhibited the immunopositivity of Iba-1 ($p < 0.01$ for E2 and E2CH, FIG. 4O) when compared to the PBS and Null groups. There was no difference between the E2 and E2Ch groups ($p > 0.9$, FIG. 4O).
- 10 These results suggest that both E2 and E2Ch have similar capacity to suppress microglial-related neuroinflammation, associated with amyloid pathology.

[0312] GFAP immunostaining provides an additional measure of neuroinflammation via

astroglia activation (see Parhizkar *et al.* Semin Immunol 2022;59). Both the PBS (4P) and Null (Figure 4Q) groups displayed strong staining with GFAP indicating similar levels of

- 15 astroglia activation. However, treatment with both E2 (Figure 4R) and E2CH (Figure 4S) significantly inhibited the immunopositivity of GFAP ($p < 0.01$ for E2 and E2CH, Figure 4T) when compared to the PBS and Null groups. There was no significant difference between E2 and E2CH groups ($p > 0.7$, Figure 4T), suggesting that both E2 and E2CH treatments have the capacity to suppress amyloid-associated astroglia activation.

- 20 *Effect on Phosphorylated Tau, Microglial and Astroglial Activation in Tau Mice*

[0313] AT8 staining detects phosphorylated tau (see Koutsodendris *et al.*, Nat Aging

2023;3(3):275-296). There was a similar level of staining in both the PBS (FIG. 5A) and Null (FIG. 5B) groups ($p > 0.9$, FIG. 5E). E2 did not induce any significant changes in AT8 staining (FIG. 5C) compared to the control groups ($p > 0.9$, FIG. 5E). However, E2CH (FIG. 5D)

- 25 significantly suppressed AT8 staining when compared to the PBS, Null, and E2 groups ($p < 0.01$, $p < 0.01$, $p < 0.05$, respectively, FIG. 5E).

[0314] Both the PBS (FIG. 5F) and Null (FIG. 5G) groups had strong staining profiles in the tau mice with Iba-1 indicating similar levels of microglial activation. However, treatment with E2CH (FIG. 5I) significantly inhibited the immunopositivity of Iba-1 when compared to the

- 30 E2 ($p < 0.01$ FIGs. 5H and 5O), PBS and Null groups (both $p < 0.01$ Figure 5J), supporting the conclusion that E2Ch treatment is efficacious for mitigating tau pathology-related neuronal inflammation whereas E2 is not.

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[0315] Both the PBS (FIG. 5K) and Null (FIG. 5L) groups displayed strong staining with GFAP indicating similar levels of astroglia activation. However, treatment with E2CH (FIG. 5N) significantly inhibited the immunopositivity of GFAP when compared to the E2 (p<0.01 FIGs. 5M and 5O), PBS and Null groups (both p<0.01 FIG. 5O), i.e., that E2CH treatment is efficacious for mitigating tau pathology-related astroglia activation whereas E2 is not.

Effects on Behavior in Amyloid and Tau Mice

[0316] The nesting test reflects general well-being, motor coordination, and cognitive function and impairments in nesting behavior have been observed in animal models of Alzheimer's disease (see Torres-Lista *et al.*, Behav Brain Res 2013;247(153-7)). A significant difference was observed between E2 and PBS treatments in amyloid mice (p<0.03), but not between E2 and Null, E2Ch and the controls, and E2 and E2Ch (all comparisons p> 0.9, FIG. 6A). In the nesting test for tau mice, E2Ch, but not E2 treatment resulted in improved nesting score compared to the PBS and Null controls (p<0.01), and the E2Ch group outperformed the E2 group (p<0.04, FIG. 6B).

[0317] The Y maze test assesses spatial working memory and spontaneous alternation behavior, measuring the ability of the animals to remember and navigate through a series of arm choices in a maze (see Kraeuter *et al.*, Pre-Clinical Models: Techniques and Protocols. (Guest PC. ed.) Springer New York: New York, NY; 2019; pp. 105-111). Impairments in spontaneous alternation have been linked to memory deficits and cognitive decline (see Lalonde *et al.*, Neurosci Biobehav Rev 2002;26(1):91-104). To assure that the Y maze performance was valid, all groups were tested for total number of entries; no significant difference was observed (p>0.9 all comparisons among the amyloid mouse groups, FIG. 7A; p>0.06 all comparisons among the tau mice groups, FIG. 7C). In the Y maze test for amyloid mice, both E2 and E2Ch treatments improved alternation ratios compared to the PBS (p<0.01 for E2 and E2CH, FIG. 6C) and Null groups (p<0.01 for E2 and E2Ch), but no significant difference was observed in the amyloid mice between E2 and E2Ch treatments (p>0.9, FIG. 6C). In contrast, in the Y maze test for tau mice, E2Ch showed significantly higher alternation ratios compared to all other groups (p<0.01 compared to all groups, FIG. 6D). Similarly, the assay was valid for tau mice as all groups had indistinguishable number of entries (FIG. 7C), but tau mice were significantly different from E2 (p<0.04), PBS (p<0.02) and Null (p<0.03) whereas there were no differences between E2 and controls (PBS p>0.2, Null p>0.1, FIG. 7D).

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[0318] The novel object recognition test is employed to evaluate memory and the ability to discriminate between familiar and novel objects (Antunes *et al.*, Cogn Process 2012;13(2):93-110). This test assesses the animal's innate preference for novelty and helps to assess memory deficits and impairment recognition. For the novel object recognition test in amyloid mice, both E2 and E2Ch treatments showed significantly higher discrimination indices compared to PBS ($p<0.01$, $p<0.04$, respectively), with no significant difference between E2 and E2Ch ($p>0.8$, FIG. 6E). However, in the tau mice, E2CH treatment had significantly higher discrimination indices compared to PBS and Null (both $p<0.05$) and was significantly more efficacious compared to treatment with E2 ($p<0.01$, FIG. 6F).

For the Barnes maze, animals were trained for seven days for identification of visual cues to locate the escape hole. After 7 days of training, the last 24 hours of data were assayed as a measure of performance (FIGs. 8A and 8B). In the amyloid mice, the Barnes maze test demonstrated that both E2 and E2CH significantly decreased escape latency compared to PBS and Null (both $p<0.01$ for E2 and E2CH, FIG. 6G), indicating enhanced spatial learning and memory retention (see Gawel *et al.*, Naunyn Schmiedebergs Arch Pharmacol 2019;392(1):1-18). In the amyloid mice, there was no significant difference between E2 and E2CH treatments ($p>0.9$, Figure 6G). In tau mice, no significant effect was observed in E2 groups compared to PBS and Null groups ($p>0.9$). In contrast, in the tau mice E2Ch treatment significantly reduced escape latency compared to PBS, Null and E2 (all $p<0.01$, FIG. 6H), and E2Ch was sufficiently better than E2 ($p<0.01$), consistent with the concept that relevant to tau pathology E2Ch treatment is efficacious for enhanced spatial learning and memory retention whereas E2 is not.

Effects of treatment on Neuronal Loss in Amyloid and Tau Mice

[0319] In addition to the accumulation of amyloid plaque and tau tangles, mouse models of Alzheimer's disease are also marked by neuronal loss and therefore for effective therapy these must be preserved. It was evaluated if E2 therapy led to the rescue of neurons by quantitative imaging of staining of hippocampus sections with NeuN (neurons) and Olig2 (myelin), both stains showed decreased neurodegeneration (FIG. 9). In the amyloid mice, both E2 and E2Ch cohort showed significantly higher NeuN staining ($p<0.01$) compared to PBS and Null controls but were no different from each other ($p>0.3$) (FIG. 9A). In contrast, only the E2Ch cohort showed significantly higher NeuN staining ($p<0.01$ compared to all other cohorts) in tau mice. Similar results were seen for Olig2 thereby confirming that these therapies protect against neuronal loss (FIG. 9B).

Effects of Treatment on Neuronal Health in Amyloid and Tau Mice

[0320] For quantitative measure of gene expression at the cellular level a pilot study was performed using spatial transcriptomics on a coronal section from each cohort with a total of 800 million reads from each sample. Brain regions and cell types were determined by standard methods. The data provided an indication of the effects of treatment on neuronal health.

5 Neurons were identified by the standard marker genes and the expression ratios were determined for each gene in each cohort relative to the corresponding PBS control (FIG. 10A and FIG. 10B). This impact of gene transfer was greater in the tau mouse model than in the amyloid mouse model.

Effects of Treatment on Microglia Inflammation

10 [0321] As an independent assessment of the benefits of APOE gene therapy using E2 and E2Ch vectors in both amyloid and tau models the microglia inflammation as indicated by the expression level of inflammatory was assesses and anti-inflammatory markers were chosen based on the data of Lu et al. *Cell Rep* **42**, 112785 (2023), including Arg-1, CD163, CD68, CLEC7A, TREM2 and CD206 by RTqPCR. From the data generated with these new

15 inflammatory and anti-inflammatory markers it was clear that the glial cells were less activated in the amyloid E2 and E2Ch cohorts and the tau E2Ch cohorts (Supplemental Figure 8). In addition to assessing mRNA levels of additional inflammatory and anti-inflammatory markers, we also used spatial transcriptomics with additional markers of microglial activation to compare genome-wide gene expression in animals treated with E2 and E2Ch compared to

20 controls FIG. 11A and FIG. 11B). The data show a consistent pattern of reduced damage-associated microglia (FIG. 12A, FIG. 12B, FIG. 12C, and FIG. 12D). As an example of the results, a list of 14 genes that reflect damage-associated microglia (DAM) and homeostatic microglia, it was evident E2 and E2Ch gene transfer suppressed the DAM phenotype and promoted the homeostatic phenotype. This impact of gene transfer was greater in the tau

25 mouse model than in the amyloid mouse model.

Table 2

LEXE-014			
SEQ ID NO	description	Type	Species
1	5' ITR	DNA	artificial
cctgcaggcagctgcgcgctcgctcactgaggccgcccgggcaaagcccgggctcgggcgaccttggtcgcccggcctcagt gagcgagcgagcgcgagagagggagtgccaactccatcactaggggttct			
2	3' ITR	DNA	artificial
aggaaccctagtgatggagttggccactccctctctgcgcgctcgctcactgaggccgggacccaaaggtgccccgacgccc ggctttgccggggcgccctcagtgagcgagcgagcgcgagctgcctgcagg			
3	CMV enhancer	DNA	artificial
gacattgattattgactagtattaatagtaataattacggggtcattagttcatagcccatatatggagttccggttacataacttacggtaa atggcccgcctggctgaccgcccacgacccccgccattgacgtcaataatgacgtatgttcccatagtaacgccaatagggacttcc attgacgtcaatgggtggagtatttacggtaaactcccacttggcagtcacatcaagtgtatcatatgccaaagtacgccccctattgacgtca			

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atgacggtaaatggccgcctggcattatgccagtcacatgacctatgggactttcctacttggcagtcacatctacGTATTAGTCATCGCTATTACCATG			
4	Chicken B-actin promoter	DNA	artificial
TCGAGGTGAGCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCCTCCCCACCCCCAATT TTGTATTTATTTATTTTAAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGGGGGGCG CGCGCCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCG GCGGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGGCGGGCGG GGCGGCCCTATAAAAAGCGAAGCGCGCGGGGCGG			
5	chimeric intron	DNA	artificial
GGAGTCGCTGCGCGCTGCCTTCGCCCCGTGCCCGCTCCGCCCGCCGCTCGCGCCGCCCG CCCCGGCTCTGACTGACCGCGTTACTCCACAGGTGAGCGGGCGGGACGGCCCTTCTCCTC CGGGCTGTAATTAGCGCTTGGTTAATGACGGCTTGTTCCTTTCTGTGGCTGCGTGAAAGCC TTGAGGGGCTCCGGGAGGGCCCTTTGTGCGGGGGAGCGGCTCGGGGGGTGCGTGCGTGT GTGTGTGCGTGCGGAGCGCCGCGTGCGGCTCCGCGCTGCCCGCGGCTGTGAGCGCTGCG GGCGCGGCGCGGGGCTTTGTGCGCTCCGCAGTGTGCGCGAGGGGAGCGCGGCGGGGGGCG GGTGCCCCGCGTGCGGGGGGGCTGCGAGGGGAACAAAGGCTGCGTGCGGGGTGTGTG CGTGGGGGGGTGAGCAGGGGGTGTTGGGCGCGTGCGTGGGCTGCAACCCCCCTGCACCC CCCTCCCCGAGTTGCTGAGCACGGCCCGGCTTCGGGTGCGGGGCTCCGTACGGGGCGTGG CGCGGGGCTCGCCGTGCCGGGCGGGGGGTGGCGGCAGGTGGGGGTGCCGGGCGGGGCG GGGCCGCTCGGGCCGGGAGGGCTCGGGGAGGGGCGCGGCGGCCCGGAGCGCGG GCGGCTGTGCGAGGCGCGGCGAGCCGAGCCATTGCCTTTTATGGTAATCGTGCGAGAGGGC GCAGGGACTTCCTTTGTCCTAAATCTGTGCGGAGCCGAAATCTGGGAGGCGCCGCCGACCC CCCTCTAGCGGGCGCGGGGCGAAGCGGTGCGGCGCGCGGCGAGGAAGGAAATGGGCGGGGA GGGCCTTCGTGCGTGCGCGCGCGCGGCTCCCTTCTCCCTCTCCAGCCTCGGGGCTGTCCG CGGGGGGACGGCTGCCTTCGGGGGGGACGGGGCAGGGCGGGGTTGCGCTTCGGCGTGT GACCGGCGGCTCTAGAGCCTCTGCTAACCATGTTTCATGCCTTCTTCTTTTCTACAG			
6	APOE2 christchurch DNA	DNA	artificial
ATGAAGTTCTGTGGGCTGCGTTGCTGGTCACATTCCTGGCAGGATGCCAGGCCAAGGTGG AGCAAGCGGTGGAGACAGAGCCGGAGCCCGAGCTGCGCCAGCAGACCGAGTGCCAGAGCG GCCAGCGCTGGGAACCTGGCACTGGGTGCTTTTGGGATTACCTGCGCTGGGTGCAGACACT GTCTGAGCAGGTGCAGGAGGAGCTGCTCAGCTCCAGGTACCCAGGAAGTGAAGGCGCTG ATGGACGAGACCATGAAGGAGTTGAAGGCCTACAAATCGGAAGTGGAGGAACAAGTACCCCC GGTGGCGGAGGAGACGCGGGCACGGCTGTCCAAGGAGCTGCAGGCGGCGCAGGCCCGGC TGGGCGCGGACATGGAGGACGTGTGCGGCCGCTGGTGCAGTACCGCGGCGAGGTGCAGG CCATGCTCGGGCAGAGCACCGAGGAGCTGCGGGTGAGCCTCGCCTCCACCTGCGCAAGCT GCGTAAGCGGCTCCTCCGCGATGCCGATGACCTGCAGAAAGTGCCTGGCAGTGTACCAGGCC GGGGCCCGCGAGGGGCGCGAGCGCGGCTCAGCGCCATCCGCGAGCGCCTGGGGCCCCCT GGTGGAACAGGGCCGCGTGCGGGCCGCCACTGTGGGCTCCCTGGCCGGCCAGCCGCTACA GGAGCGGGGCCAGGCCTGGGGCGAGCGGCTGCGCGCGCGGATGGAGGAGATTGGGACAGCC GGACCCGCGACCGCCTGGACGAGGTGAAGGAGCAGGTGGCGGAGGTGCGCGCCAAGCTGG AGGAGCAGGCCAGCAGATACGCTGCAGGCCGAGGCCTTCCAGGCCCGCCTCAAGAGCT GGTTCGAGCCCCCTGGTGGAAGACATGCAGCGCCAGTGGGCGGGGCTGGTGGAAGGTGC AGGCTGCCGTGGGCACCGCGCCGCCCTGTGCCAGCGACAATCACTGA			
7	B-globin polyA	DNA	artificial
AATAAAGGAAATTTATTTTCAATTGCAATAGTGTGTTGGAATTTTTGTGTCTCTCA			
8	APOE2 Ch ITR to ITR	DNA	homo sapiens
cctgcaggcagctgcgcgtcgtcactgagggcgccgggcaagccccggcgctcgggcgacctttggcgcccgccctcagt gagcgagcgagcgcgagagagggagtgcccaactccatcactaggggttctgcggcgctctagaactagtcgacattgattatga ctagttattaatagtaataaattacgggtcattagttcatagccatataatggagttccggttacataacttacggtaaatggccgcctggc tgaccgccaacgacccccgccattgacgtcaataatgacgtatgttccatagtaacgccaatagggactttccattgacgtcaatgggt ggagatttacggtaaaactgcccacttggcagtcacatcaagtgtatcatatgccaaagtacccccctattgacgtcaatgacggtaaatggc ccgcctggcattatgccagtcacatgacctatgggactttcctacttggcagtcacatctacGTATTAGTCATCGCTATTACCA TGGTCGAGGTGAGCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCCTCCCCACCCCCA ATTTTGTATTTATTTATTTTAAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGGGGG CGCGCGCCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTG CGGCGGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGCGGCG GCGGCGGCCCTATAAAAAGCGAAGCGCGCGGGGCGGGGAGTCGCTGCGCGCTGCCTTCG CCCCGTGCCCCGCTCCGCCGCCGCTCGCGCGCGCGCCCCCGGCTCTGACTGACCGCGTT			

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MKVLWAALLVTFLAGCQAKVEQAVETEPEPELRQQTEWQSGQRWELALGRFWDYLRWVQTLSE QVQEELLSSQVTQELRALMDETMKELKAYKSELEEQLTPVAEETRARLSKELQAAQARLGADME DVCGRVLVQYRGEVQAMLGQSTEELRVSLASHLRKLRKLLRDADDLQKCLAVYQAGAREGAER GLSAIRERLGPLVEQGRVRAATVGSAGQPLQERAQAWGERLRARMEEMGSRTDRDLDEVKEQ VAEVRAKLEEQAQQIRLQAEAFQARLKSWFEPLVEDMQRQWAGLVEKVQAAVGTSAAPVPSDN H			
12	APOE3 christchurch PRT	PRT	homo sapiens
MKVLWAALLVTFLAGCQAKVEQAVETEPEPELRQQTEWQSGQRWELALGRFWDYLRWVQTLSE QVQEELLSSQVTQELRALMDETMKELKAYKSELEEQLTPVAEETRARLSKELQAAQARLGADME DVCGRVLVQYRGEVQAMLGQSTEELRVSLASHLRKLRKLLRDADDLQKCLAVYQAGAREGAER GLSAIRERLGPLVEQGRVRAATVGSAGQPLQERAQAWGERLRARMEEMGSRTDRDLDEVKEQ VAEVRAKLEEQAQQIRLQAEAFQARLKSWFEPLVEDMQRQWAGLVEKVQAAVGTSAAPVPSDN H			
13	APOE4 PRT	PRT	homo sapiens
MKVLWAALLVTFLAGCQAKVEQAVETEPEPELRQQTEWQSGQRWELALGRFWDYLRWVQTLSE QVQEELLSSQVTQELRALMDETMKELKAYKSELEEQLTPVAEETRARLSKELQAAQARLGADME DVCGRVLVQYRGEVQAMLGQSTEELRVSLASHLRKLRKLLRDADDLQKCLAVYQAGAREGAER GLSAIRERLGPLVEQGRVRAATVGSAGQPLQERAQAWGERLRARMEEMGSRTDRDLDEVKEQ VAEVRAKLEEQAQQIRLQAEAFQARLKSWFEPLVEDMQRQWAGLVEKVQAAVGTSAAPVPSDN H			
14	APOE2 PRT minus signal peptide	PRT	homo sapiens
QAMLGQSTEELRVSLASHLRKLRKLLRDADDLQKCLAVYQAGAREGAERGLSAIRERLGPLVEQ GRVRAATVGSAGQPLQERAQAWGERLRARMEEMGSRTDRDLDEVKEQVAEVRAKLEEQAQQI RLQAEAFQARLKSWFEPLVEDMQRQWAGLVEKVQAAVGTSAAPVPSDNH			
15	APOE3 PRT minus signal peptide	PRT	homo sapiens
KVEQAVETEPEPELRQQTEWQSGQRWELALGRFWDYLRWVQTLSEQVQEELLSSQVTQELRAL MDETMKELKAYKSELEEQLTPVAEETRARLSKELQAAQARLGADMEDVCGRVLVQYRGEVQAML GQSTEELRVSLASHLRKLRKLLRDADDLQKCLAVYQAGAREGAERGLSAIRERLGPLVEQGRVR AATVGSAGQPLQERAQAWGERLRARMEEMGSRTDRDLDEVKEQVAEVRAKLEEQAQQIRLQA EAFQARLKSWFEPLVEDMQRQWAGLVEKVQAAVGTSAAPVPSDNH			
16	APOE4 PRT minus signal peptide	PRT	homo sapiens
KVEQAVETEPEPELRQQTEWQSGQRWELALGRFWDYLRWVQTLSEQVQEELLSSQVTQELRAL MDETMKELKAYKSELEEQLTPVAEETRARLSKELQAAQARLGADMEDVCGRVLVQYRGEVQAML GQSTEELRVSLASHLRKLRKLLRDADDLQKCLAVYQAGAREGAERGLSAIRERLGPLVEQGRVR AATVGSAGQPLQERAQAWGERLRARMEEMGSRTDRDLDEVKEQVAEVRAKLEEQAQQIRLQA EAFQARLKSWFEPLVEDMQRQWAGLVEKVQAAVGTSAAPVPSDNH			
17	APOE2 christchurch PRT minus signal peptide	PRT	homo sapiens
KVEQAVETEPEPELRQQTEWQSGQRWELALGRFWDYLRWVQTLSEQVQEELLSSQVTQELRAL MDETMKELKAYKSELEEQLTPVAEETRARLSKELQAAQARLGADMEDVCGRVLVQYRGEVQAML GQSTEELRVSLASHLRKLRKLLRDADDLQKCLAVYQAGAREGAERGLSAIRERLGPLVEQGRVR AATVGSAGQPLQERAQAWGERLRARMEEMGSRTDRDLDEVKEQVAEVRAKLEEQAQQIRLQA EAFQARLKSWFEPLVEDMQRQWAGLVEKVQAAVGTSAAPVPSDNH			
18	APOE3 christchurch PRT minus signal peptide	PRT	homo sapiens
KVEQAVETEPEPELRQQTEWQSGQRWELALGRFWDYLRWVQTLSEQVQEELLSSQVTQELRAL MDETMKELKAYKSELEEQLTPVAEETRARLSKELQAAQARLGADMEDVCGRVLVQYRGEVQAML GQSTEELRVSLASHLRKLRKLLRDADDLQKCLAVYQAGAREGAERGLSAIRERLGPLVEQGRVR AATVGSAGQPLQERAQAWGERLRARMEEMGSRTDRDLDEVKEQVAEVRAKLEEQAQQIRLQA EAFQARLKSWFEPLVEDMQRQWAGLVEKVQAAVGTSAAPVPSDNH			
19	APOE signal peptide	PRT	homo sapiens
MKVLWAALLVTFLAGCQA			
20	APOE3 christchurch DNA	DNA	artificial
ATGAAGGTTCTGTGGGCTGCGTTGCTGGTCACATTCCTGGCAGGATGCCAGGCCAAGGTGG AGCAAGCGGTGGAGACAGAGCCGGAGCCCGAGCTGCGCCAGCAGACCGAGTGGCAGAGCG			

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GCCAGCGCTGGGAACTGGCACTGGGTCGCTTTTGGGATTACCTGCGCTGGGTGCAGACACT GTCTGAGCAGGTGCAGGAGGAGCTGCTCAGCTCCCAGGTACCCAGGAACTGAGGGCGCTG ATGGACGAGACCATGAAGGAGTTGAAGGCCTACAAATCGGAACTGGAGGAACAACCTGACCCC GGTGGCGGAGGAGACGCGGGCACGGCTGTCCAAGGAGCTGCAGGCGGCGCAGGCCCGGGC TGGGCGCGGACATGGAGGACGTGTGCGGCCGCTGGTGCAGTACCGCGGCGAGGTGCAGG CCATGCTCGGCCAGAGCACCGAGGAGCTGCGGGTGAGCCTCGCCTCCACCTGCGCAAGCT GCGTAAGCGGCTCCTCCGCGATGCCGATGACCTGCAGAAGCGCCTGGCAGTGTACCAGGCC GGGGCCCCGCGAGGGCGCGGAGCGCGGCTCAGCGCCATCCGCGAGCGCCTGGGGCCCCCT GGTGGAACAGGGCCGCGTGCGGGCCGCCACTGTGGGCTCCTGGCCGGCCAGCCGCTACA GGAGCGGGCCAGGCCTGGGGCGAGCGGCTGCGCGCGCGGATGGAGGAGATGGGCGAGCC GGACCCGCGACCGCCTGGACGAGGTGAAGGAGCAGGTGGCGGAGGTGCGCGCCAAGCTGG AGGAGCAGGCCAGCAGATACGCCTGCAGGCCGAGGCCTTCCAGGCCCGCCTCAAGAGCT GGTTCGAGCCCCCTGGTGGAAGACATGCAGCGCCAGTGGGCCGGGCTGGTGAGAAGGTGC AGGCTGCCGTGGGCACCGCGCCGCCCTGTGCCAGCGACAATCACTGA			
21	APOE3 Ch ITR to ITR	DNA	artificial
cctgcaggcagctgcgcgctgcctgcctcactgagccgccccgggcaaaagccccggcgctcgggcgacacattggcgccccgctcagt gagcgagcgagcgcgagagagggagtgcccaactccatcactaggggttctgcggcgctctagaactagtcgacattgattattga ctagttattaatagtaataaattacgggtcattagttcatagcccatataggagttccggttacataactacggtaaatggccgctggc tgaccgccaacgacccccgcccattgacgtcaataatgacgtatgttcccatagtaacgccaatagggaacttccattgacgtcaatgggt ggagttattacggtaaactgccacttggcagttacatcaagtgtatcatatgccaaagtagccccctattgacgtcaatgacgtcaatggc ccgctggcattatgccagttacatgacattatgggacttctacttggcagttacatctacGTATTAGTCATCGCTATTACCA TGGTCGAGGTGAGCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCCTCCCCACCCCCA ATTTTGTATTTATTTATTTTAAATTTTGTGTCAGCGATGGGGCGGGGGGGGGGGGGGGGG CGCGCGCCAGGCGGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTG CGGCGGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCTTTTATGGCGAGGCGGCGGCG GCGGCGGCCCTATAAAAAGCGAAGCGCGCGGGCGGGGAGTGCCTGCGCGCTGCCTTCG CCCCGTGCCCGCTCCGCGCGCGCTCGCGCGCGCGCGCGCGCGGCTCTGACTGACCGCGTT ACTCCACAGGTGAGCGGGCGGGACGGCCCTTCTCCTCCGGGCTGTAATTAGCGCTTGGTT TAATGACGGCTTGTCTTTCTTTCTGTGGCTGCGTGAAAGCCTTGAGGGGCTCCGGGAGGGCCC TTTGTGCGGGGGAGCGGCTCGGGGGGTGCGTGCGTGTGTGTGCGTGGGGAGCGCCGC GTGCGGCTCCGCGCTGCCCGCGGCTGTGAGCGCTGCGGGCGCGGCGCGGGGCTTTGTG CGTCCGCAGTGTGCGCGAGGGGAGCGCGCGGGGGCGGTGCCCCGCGTGCAGGGGG GGCTGCGAGGGGAACAAAGGCTGCGTGCGGGGTGTGTGCGTGGGGGGGTGAGCAGGGG TGTGGGCGCGTGGTGGGCTGCAACCCCCCTGCACCCCCCTCCCCGAGTTGCTGAGCAC GGCCCCGCTTCGGGTGCGGGGCTCCGTACGGGGCGTGGCGCGGGGCTCGCCGTGCCGGG CGGGGGGTGGCGGCAGGTGGGGGTGCCGGGCGGGGCGGGGCGCCCTCGGGCCGGGGAG GGCTCGGGGAGGGGCGCGGCGGCCCGGAGCGCGCGGCTGTGAGGCGCGGCGA GCCGAGCCATTGCCTTTATGGTAATCGTGCGAGAGGGCGCAGGGACTTCCTTTGTCCAA ATCTGTGCGGAGCCGAAATCTGGGAGGCGCGCGCCGACCCCCCTAGCGGGCGCGGGGCG AAGCGGTGCGGCGCGGCGAGGAAGGAAATGGGCGGGGAGGGCCTTCGTGCGTGCCTCG CCCGCTCCCCTTCTCCCTCTCCAGCCTCGGGGCTGTCCGCGGGGGGACGGCTGCCTTCGG GGGGGACGGGCGAGGGCGGGGTTCCGGCTTCTGGCGTGTGACCGGCGGCTCTAGAGCCTCT GCTAACCATGTTTCATGCCTTCTTCTTTTCTACAGCTCCTGGGCAACGTGCTGGTTATTGTG CTGTCTCATATTTTGGCAAAGAATTCGCCCTTAGCCGCCACCATGAAGGTTCTGTGGGCTG CGTTGCTGCTCACATTCTGGCAGGATGCCAGGCCAAGGTGGAGCAAGCGGTGGAGACAGA CCCGGAGCCCCGAGCTGCGCCAGCAGACCGAGTGGCAGAGCGGCCAGCGCTGGGAAGTGGC ACTGGTGCCTTTTGGGATTACCTGCGCTGGGTGCAGACACTGTCTGAGCAGGTGCAGGAG GAGCTGCTCAGCTCCCAGGTACCCAGGAACTGAGGGCGCTGATGGACGAGACCATGAAGG AGTTGAAGGCCTACAAATCGGAACTGGAGGAACAACAGACCCCGGTGGCGGAGGAGACGCG GGCACGGCTGTCCAAGGAGCTGCAGGCGGCGCAGGCCCGGCTGGGCGCGGACATGGAGG ACGTGTGCGGCCGCTGGTGCAGTACCGCGGCGAGGTGCAGGCCATGCTCGGCCAGAGCA CCGAGGAGCTGCGGGTGAAGCTCGCCTCCACCTGCGCAAGCTGCGTAAGCGGCTCCTCCG CGATGCCGATGACCTGCAGAAGCGCTGGCAGTGTACCAGGCCGGGGCCCGGAGGGCGC CGAGCGCGGCTCAGCGCCATCCGCGAGCGCCTGGGGCCCTGGTGGAACAGGGCCGCGT GCGGGCCGCCACTGTGGGCTCCCTGGCCGGCCAGCCGCTACAGGAGCGGGCCAGGCCTG GGGCGAGCGGCTGCGCGCGCGGATGGAGGAGATGGGCGAGCCGACCCGCGACCGCTGG ACGAGGTGAAGGAGCAGGTGGCGGAGGTGCGCGCCAAGCTGGAGGAGCAGGCCAGCAGA TACGCTCGAGGCCGAGGCTTCCAGGCCGCTCAAGAGCTGTTTCGAGCCCTGGTGA AGACATGACGCGCAGTGGGCCGGGCTGGTGGAGAAGGTGCAGGCTGCCGTGGGCACCA CGCCGCCCTGTGCCAGCGACAATCACTGAATCTGTTGAAGGGCGAATCACTCCTCAGGT			

[0322] All publications, patents and patent applications are incorporated herein by reference. While in the foregoing specification, this invention has been described in relation to certain preferred embodiments thereof, and many details have been set forth for purposes of 5 illustration, it will be apparent to those skilled in the art that the invention is susceptible to

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additional embodiments and that certain of the details herein may be varied considerably without departing from the basic principles of the invention.

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CLAIMS

We Claim:

1. A recombinant adeno-associated virus (rAAV) vector comprising a nucleic acid
5 sequence encoding an apolipoprotein 2 (APOE2) polypeptide comprising a Christchurch
mutation or an apolipoprotein 3 (APOE3) polypeptide comprising a Christchurch mutation,
wherein the rAAV vector comprises SEQ ID NO: 8, SEQ ID NO: 21, or SEQ ID NO: 22.
2. The rAAV vector of claim 1, wherein the Christchurch mutation comprises:
10 an R154S mutation in reference to an unprocessed APOE polypeptide; or
an R136S mutation in reference to mature APOE polypeptide lacking a signal peptide.
3. The rAAV vector of any one of the preceding claims, wherein the rAAV vector is
packaged as an rAAV viral vector comprising an AAV capsid protein.
15
4. The rAAV vector of any one of the preceding claims, wherein the AAV capsid protein
is an AAV1 capsid protein, an AAV2 capsid protein, an AAV4 capsid protein, an AAV5
capsid protein, an AAV6 capsid protein, an AAV7 capsid protein, an AAV8 capsid protein, an
AAV9 capsid protein, an AAV10 capsid protein, an AAV11 capsid protein, an AAV12 capsid
20 protein, an AAV13 capsid protein, an AAVPHP.B capsid protein, an AAVrh74 capsid protein
or an AAVrh10 capsid protein.
5. The rAAV vector of any one of the preceding claims, wherein the AAV capsid protein
is an AAVrh10 capsid protein.
25
6. A pharmaceutical composition comprising the AAV viral vector of claim 3.
7. A method of treating Alzheimer's disease in a human subject comprising
administering a therapeutically effective amount of a pharmaceutical composition according
30 to claim 6.
8. The method of claim 7, wherein the therapeutically effective amount of the vector is
about 1×10^{10} to about 1×10^{16} genome copies.

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9. The method of claim 7, wherein the subject is an APOE2/APOE4 heterozygote, an APOE4/APOE4 homozygote or an APOE3/APOE4 heterozygote.
10. The method of claim 7, wherein the composition is administered systemically,
5 intracisternally, via intra cisterna magna, or via CI-C2 administration.
11. The method of claim 7, wherein the pharmaceutical composition is administered at a dose of about 5.0×10^9 gc/mL CSF to about 5.0×10^{12} gc/mL CSF.
- 10 12. The method of claim 7, wherein the pharmaceutical composition is administered at a dose of about:
i) 1.4×10^{10} gc/mL CSF,
ii) 4.4×10^{10} gc/mL CSF, or
iii) 1.4×10^{11} gc/mL CSF.
- 15 13. The method of claim 7, wherein the pharmaceutical composition is administered at a fixed dose of about 1.4×10^{14} gc.
14. The method of claim 7, wherein the pharmaceutical composition is administered in a
20 total volume of about 5 mL, about 10 mL, about 15 mL, or about 20 mL.
15. The method of claim 7, wherein the subject experiences an at least about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%,
25 about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 100% increase in APOE2 Christchurch or APOE3 Christchurch expression.
16. The method of claim 7, wherein the APOE2 Christchurch or APOE3 Christchurch
30 expression occurs in the central nervous system.
17. The method of claim 7, wherein the APOE2 Christchurch or APOE3 Christchurch expression is measured in the cerebral spinal fluid (CSF).

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18. The method of claim 7, wherein following administration of the pharmaceutical composition the expression levels of at least one of T-tau, and P-tau are reduced in the subject relative to a pre-administration baseline.

5 19. The method of claim 18, wherein the expression levels of T-tau, and/or P-tau are reduced by at least about 5%, at least about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 100%.

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20. The method of claim 7, wherein following administration of the pharmaceutical composition the amyloid beta 42/amyloid beta 40 ($A\beta_{42/40}$) ratio is increased.

21. The method of claim 20, wherein the $A\beta_{42/40}$ ratio is increased by at least about 5%, at
15 least about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 100%.

20 22. The method of claim 7, wherein prior to treatment with the pharmaceutical composition the subject is administered an immunosuppressant.

23. The method of claim 22, wherein the immunosuppressant is prednisone.

25 24. The method of claim 23, wherein the prednisone is administered at a dosage of:
40 mg, once daily 1 week prior to AAV viral vector administration;
40 mg once daily for week 1 through week 2 post-AAV viral vector administration;
30 mg once daily for week 3 post-AAV viral vector administration;
20 mg once daily for week 4 post-AAV viral vector administration;
30 10 mg once daily for week 5 post-AAV viral vector administration;
5 mg once daily for week 6 post-AAV viral vector administration;
2.5 mg once daily for week 7 post-AAV viral vector administration; and
2.5 mg every other day for week 8 post-AAV viral vector administration.

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