

CRISPR-MEDIATED MANIPULATION OF APP EXPRESSION AS A THERAPEUTIC APPROACH FOR AMYLOID PATHOLOGIES

RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application No.

5 63/272,689, filed October 28, 2021, and U.S. Provisional Patent Application No. 63/334,650, filed April 25, 2022, the contents of both of which are hereby incorporated by reference in the entirety for all purposes.

BACKGROUND OF THE INVENTION

[0002] Brain diseases such as neurodegenerative diseases and neuroinflammatory disorders
10 are devastating conditions that affect a large subset of the population. Many of such diseases are incurable, highly debilitating, and often result in progressive deterioration of the brain structure and function over time. Disease prevalence is also increasing rapidly due to growing aging populations worldwide, since the elderly are at high risk for developing these conditions. Currently, many neurodegenerative diseases and neuroinflammatory disorders
15 are difficult to diagnose due to limited understanding of the pathophysiology of these diseases. Meanwhile, current treatments are ineffective and do not meet market demand; demand that is significantly increasing each year due to the ever-growing aging populations. For example, Alzheimer's disease (AD) is marked by gradual but progressive decline in learning and memory, and a leading cause of mortality in the elderly. Increasing prevalence
20 of AD is driving the need and demand for better and earlier diagnostics. According to Alzheimer's Disease International, the disease currently affects 46.8 million people globally, but the number of cases is projected to triple in the coming three decades. One of the countries with the fastest elderly population growth is China. Based on population projections, by 2030 one in four individuals will be over the age of 60, which will place a
25 vast proportion at risk of developing AD. In fact, the number of AD cases in China doubled from 3.7 million to 9.2 million from 1990-2010, and the country is projected to have 22.5 million cases by 2050. Hong Kong's population is also aging quickly. It is estimated that the elderly aged 65+ will make up 24% of the population by 2025, and 39.3% of the population by 2050. The number of AD cases is projected to rise to 332,688 by 2039. As such, there
30 exists an urgent need for developing new methods for effectively treating AD patients who suffer from this devastating condition. This invention fulfills this and other related needs by

disclosing novel compositions and methods useful for effective treatment, and potentially providing a cure, of the disease.

BRIEF SUMMARY OF THE INVENTION

[0003] In a first aspect, this invention provides a method for treating an amyloid pathology (such as Alzheimer's Disease) in a person or reducing the person's risk for later developing the pathology. The claimed method comprises the step of administering to the person an effective amount of a composition disrupting a genomic sequence encompassing the APP gene coding sequence or transcript.

[0004] In some embodiments, the claimed method comprises, prior to the administering step, sequencing at least a portion of the person's genome. In some embodiments, the person's APP genomic sequence comprises at least one mutation, for example, a Swedish mutation (K670N/M671L), a Florida mutation (I716V), a London mutation (V717I), or any combination thereof, as well as other genes harboring mutations or allelic variants relevant to amyloid plaque formation. In some embodiments, the mutation comprises a Swedish mutation (K670N/M671L). In some embodiments, the amyloid pathology is Alzheimer's Disease (AD, including both familial and sporadic AD), cerebral amyloid angiopathy, or Down Syndrome. In some embodiments, the person has been diagnosed with AD, whereas in other embodiments, the person is not yet diagnosed with AD but has known risk factors for AD, such as harboring certain genetic mutations known to increase one's risk for AD or having a family history of AD, especially early onset AD (*e.g.*, before 60 or 70 years of age). In some embodiments, the method of this invention is practiced using a composition comprising an siRNA, a microRNA, a miniRNA, a lncRNA, or an antisense oligonucleotide targeting the APP genomic sequence. In some embodiments, the method utilizes a CRISPR composition comprising one or more vectors encoding (1) an endonuclease guided by a small guide RNA (sgRNA) targeting a location within the APP genomic sequence; and (2) the sgRNA. In some embodiments, the sgRNA targets a region within the APP genomic sequence comprising a known mutation (*e.g.*, the Swedish mutation) or a β - or γ -secretase cleavage site, for example, the targeted endonuclease cleavage site is within 2, 3, 5, 7, 8, 9, 10, 11, 12, 15 nucleotides upstream or downstream from the mutation or secretase cleavage site. In some embodiments, the composition comprises one vector encoding a Cas9 nuclease and one sgRNA, *e.g.*, a *Streptococcus pyogenes* Cas9 nuclease (SpCas9). In some embodiments, the Cas9 and sgRNA are operably linked to and under the transcription control of a tissue-specific promoter, such as a neuronal cell-specific promoter known in the

technical field or disclosed herein. In some embodiments, each of the one or more vectors is one viral vector, preferably an adeno-associated virus (AAV) vector. In some embodiments, the composition is administered by subcutaneous, intramuscular, intravenous, intraperitoneal, or intracranial injection or by oral or nasal administration. In some embodiments, the
5 composition is administered in the form of a solution, a suspension, a powder, a paste, a tablet, or a capsule.

[0005] In a second aspect, the present invention provides a composition comprising an effective amount of one or more agents that disrupt the APP genomic sequence plus one or more physiologically acceptable excipient.

10 **[0006]** In some embodiments, the composition comprising an siRNA, a microRNA, a miniRNA, a lncRNA, or an antisense oligonucleotide targeting the APP genomic sequence. In some embodiments, the method utilizes a CRISPR composition comprising one or more vectors encoding (1) an endonuclease guided by a small guide RNA (sgRNA) targeting a location within the APP genomic sequence; and (2) the sgRNA. In some embodiments, the
15 sgRNA targets a region within the APP genomic sequence comprising a known mutation (e.g., the Swedish mutation) or a β - or γ -secretase cleavage site, for example, the targeted endonuclease cleavage site is within 2, 3, 5, 7, 8, 9, 10, 11, 12, 15 nucleotides upstream or downstream from the mutation or secretase cleavage site. In some embodiments, the composition comprises one vector encoding a Cas9 nuclease and one sgRNA, e.g., a
20 *Streptococcus pyogenes* Cas9 nuclease (SpCas9). In some embodiments, the Cas9 and sgRNA are operably linked to and under the transcription control of a tissue-specific promoter, such as a neuronal cell-specific promoter known in the technical field or disclosed herein. In some embodiments, each of the one or more vectors is one viral vector, preferably an adeno-associated virus (AAV) vector. In some embodiments, the composition is
25 administered by subcutaneous, intramuscular, intravenous, intraperitoneal, or intracranial injection or by oral or nasal administration. In some embodiments, the composition is administered in the form of a solution, a suspension, a powder, a paste, a tablet, or a capsule.

[0007] In a third aspect, the present invention provides a kit for treating an amyloid pathology (such as Alzheimer's Disease) in a person or for reducing the person's risk of later
30 developing the disease/condition. The kit comprises a first container containing a composition disrupting APP genomic sequence.

- [0008] In some embodiments, the composition is formulated for subcutaneous, intramuscular, intravenous, intraperitoneal, or intracranial injection, or for oral or nasal administration. In some embodiments, the composition comprising an siRNA, a microRNA, a miniRNA, a lncRNA, or an antisense oligonucleotide targeting the APP genomic sequence.
- 5 In some embodiments, the composition comprises (1) one or more vectors encoding an endonuclease guided by a small guide RNA (sgRNA) and (2) one sgRNA (or two sgRNAs) targeting one (or two locations) within the APP genomic sequence, for example, in a small region in close proximity to a pre-selected mutation (*e.g.*, the Swedish mutation) or a β - or γ -secretase cleavage site within the APP genomic sequence, so that the intended endonuclease
- 10 cleavage site is within 2, 3, 5, 7, 8, 9, 10, 11, 12, 15 nucleotides upstream or downstream from the mutation or secretase cleavage site. In some embodiments, the kit contains a composition comprising one single vector encoding a Cas9 nuclease, for example, a *Streptococcus pyogenes* Cas9 nuclease (SpCas9), and one or more sgRNAs. In some embodiments, the endonuclease and the sgRNA(s) may be encoded in two separate vectors.
- 15 In some embodiments, the vector is a viral vector, such as one constructed based on an adenovirus or an adeno-associated virus (AAV). In some embodiments, the composition is administered by subcutaneous, intramuscular, intravenous, intraperitoneal, or intracranial injection or by oral or nasal administration. In some embodiments, the kit further contains a second container containing agents for sequencing at least a portion of the person's genome
- 20 (*e.g.*, the APP gene as well as other genes that might harbor mutations or variant known to play a role in amyloid pathogenesis such as presenilin 1 (PS1) and presenilin 2 (PS2), and APOE- ϵ 4). Optionally, the kit also includes an instruction manual for administration of the composition.
- [0009] Related to this aspect of the present invention, a use of one or more agents
- 25 disrupting APP genomic sequence is further provided in accordance with the disclosure herein for the manufacturing of (1) a medicament for treating an amyloid pathology (*e.g.*, Alzheimer's Disease); and/or (2) a kit containing the medicament for treating the amyloid pathology (*e.g.*, Alzheimer's Disease).

BRIEF DESCRIPTION OF THE DRAWINGS

- 30 [0010] **Figure 1: Design and validation of CRISPR/Cas9-mediated genome editing to disrupt the mutant *APP_{swe}* allele.** a, The APP Swedish (*APP_{swe}*) double-base mutation (KM670/671NL) is close to the β -secretase site. Two single-guide RNAs (sgRNAs)—SW1 and SW2—were designed to target the *APP_{swe}* mutation using *Staphylococcus aureus* Cas9

(SaCas9). PAM, protospacer-adjacent motif. The arrowhead indicates the SaCas9 cleavage site. **b**, Schematic diagram of the EGxxFP system used to analyze the editing efficiency of the CRISPR/Cas9 system *in vitro*. The APP_{WT} and APP_{swe} sequences were separately inserted between EGFP fragments with overlapping arms. SaCas9 (blue) editing resulted in

5 homology-directed repair (HDR)-mediated DNA repair and reconstitution of the EGFP expression cassette, which yielded GFP signals. **c**, *In vitro* validation of allele-specific editing using the EGxxFP system. Cas9-SW1 and Cas9-SW2 were co-transfected with EGxxFP reporter plasmids containing APP_{WT} or APP_{swe} (EGxxFP- APP_{WT} or EGxxFP- APP_{swe}), respectively, into HEK 293T cells. Scale bar: 100 μ m. **d**, A single adeno-associated

10 virus (AAV) vector, EFS::Cas9-SW1, was constructed to express HA-tagged SaCas9 and sgRNA SW1 for intrahippocampal injection. EFS, elongation factor 1-alpha short promoter; pA, poly(A) tail; U6, human U6 promoter; ITR, inverted terminal repeat. **e**, T7 endonuclease I assay for AAV-mediated Cas9-SW1 editing in virus-transduced hippocampi from 5XFAD mice. Arrowheads indicate the CRISPR/Cas9-edited DNA fragments. Con, Untransduced

15 hippocampal region of 5XFAD mice. **f**, Percentage of insertion/deletion (indel) mutations. CRISPR/Cas9 editing led to the formation of indel mutations. The indel percentage indicates the editing efficiency. Values are mean $\pm$ SEM ($n = 3$ mice per group; *** $P < 0.001$; unpaired two-tailed t -test).

[0011] Figure 2: Intrahippocampal AAV-mediated Cas9-SW1 editing decreases amyloid plaque burden in 5XFAD mice. Intrahippocampal injection of AAV-mediated Cas9-SW1 in 3-month-old 5XFAD mice decreased amyloid-beta ($A\beta$) levels and deposition. **a-c**, Soluble and insoluble $A\beta$ contents in the dorsal hippocampal homogenates of 6-month-old 5XFAD mice after intrahippocampal injection of Cas9-SW1. **a**, Representative western blots of soluble and insoluble $A\beta$ with GAPDH as a loading control ($n = 3$ mice per group). **b**,

25 **c**, Quantitative analysis of soluble and insoluble $A\beta_{x-40}$ and $A\beta_{x-42}$ by ELISA. Values are mean $\pm$ SEM ($n = 5$ mice per group; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; unpaired two-tailed t -test). **d-k**, Amyloid plaque deposition decreased in the hippocampal regions in 5XFAD mice that expressed AAV-mediated Cas9-SW1. Immunohistochemistry for $A\beta$ (4G8; green), HA (red), and nuclei (blue). Representative images of distinct hippocampal regions

30 (i.e., the cornu ammonis and subiculum) of 5XFAD mice at 6 months (**d**, **e**) and 9 months (**h**, **i**). HA tag expression indicates Cas9 expression. Scale bars: 50 μ m. Quantification of $A\beta$ -positive areas in the cornu ammonis (**f**, **j**) and subiculum (**g**, **k**). Values are mean $\pm$ SEM ($n = 4$ mice per group; * $P < 0.05$, ** $P < 0.01$; paired two-tailed t -test).

- [0012] Figure 3: Intrahippocampal AAV-mediated Cas9-SW1 editing decreases gliosis in 5XFAD mice.** **a, b**, AAV-mediated Cas9-SW1 editing decreased microgliosis in the hippocampus of 5XFAD mice. Immunohistochemistry for Iba1 (red), amyloid-beta (A β , 4G8; green), and nuclei (blue) in 6-month-old 5XFAD mice. **a**, Representative images of the subiculum. Scale bars: 50 μ m (left), 10 μ m (right). **b**, Quantification of Iba1-positive cells. Values are mean $\pm$ SEM ($n = 3$ mice per group; $*P < 0.05$; paired two-tailed t -test). **c–e**, AAV-mediated Cas9-SW1 editing decreased the activation of astrocytes in the hippocampus in 5XFAD mice. Immunohistochemistry for GFAP (red), A β (4G8; green), and nuclei (blue) of 6-month-old 5XFAD mice. **c**, Representative images of the subiculum. Scale bars: 50 μ m (left), 10 μ m (right). **d, e**, AAV-mediated Cas9-SW1 editing in 5XFAD mice did not change the number of astrocytes but decreased astrocyte activation. **d**, Quantification of GFAP-positive cells. Values are mean $\pm$ SEM ($n = 4$ mice per group; paired two-tailed t -test). **e**, Percentages of GFAP-positive areas. Values are mean $\pm$ SEM ($n = 4$ mice per group; $*P < 0.05$; paired two-tailed t -test).
- [0013] Figure 4: Intrahippocampal AAV-mediated Cas9-SW1 editing improves neuronal functions in 5XFAD mice.** **a, b**, AAV-mediated Cas9-SW1 editing reversed the decrease of long-term potentiation (LTP) in 6-month-old 5XFAD mice. LTP in the hippocampal CA1 region was induced by 4 trains of theta-burst stimulation. WT, wild type. **a**, Averaged slopes of baseline normalized field excitatory postsynaptic potential (fEPSP; mean $\pm$ SEM). (*Inset*) Examples of fEPSPs recorded 5 min before (1, gray) and 55 min after (2, black) LTP induction. **b**, Quantification of mean fEPSP slopes during the last 10 min of recording after LTP induction. Values are mean $\pm$ SEM (WT Con $n = 9$ slices from 6 mice; 5XFAD Con $n = 9$ slices from 4 mice; 5XFAD SW1 $n = 9$ slices from 5 mice; $**P < 0.01$, $***P < 0.001$; unpaired two-tailed t -test). **c–e**, AAV-mediated Cas9-SW1 injection reduced the decrease of excitatory synapses (stained with PSD-95 [green] and synaptophysin [red]) in the subiculum in 9-month-old 5XFAD mice. **c**, Representative images. Scale bar: 5 μ m. **d, e**, Quantification of PSD-95 puncta, synaptophysin puncta, and synaptophysin–PSD-95 co-localized puncta in the subiculum. Values are mean $\pm$ SEM ($n = 4$ mice per group; $*P < 0.05$; paired two-tailed t -test). **f–i**, AAV-mediated Cas9-SW1 editing decreased neurite dystrophy and neuronal loss in the subiculum in 5XFAD mice. Co-staining with amyloid-beta (A β , 4G8; green), nuclei (blue), and LAMP1 (a marker of neurite dystrophy; red) in 6-month-old 5XFAD mice (**f**) or NeuN (red) in 9-month-old 5XFAD mice (**g**). Scale bars: 50 μ m (**f**), 200 μ m (**g**). **h**, Quantification of LAMP1-positive dystrophic neurites. Values are mean $\pm$ SEM

($n = 3$ mice per group; paired two-tailed t -test). **i**, Quantification of NeuN-positive cell density. Values are mean $\pm$ SEM ($n = 4$ mice per group; $*P < 0.05$; paired two-tailed t -test).

[0014] Figure 5: Intrahippocampal AAV-mediated delivery of Cas9-SW1 decreases Alzheimer's disease-associated pathologies in APP/PS1 mice. Intrahippocampal injection of AAV-mediated Cas9-SW1 in 9-month-old APP/PS1 mice decreased amyloid-beta ($A\beta$) deposition and gliosis. **a–d**, Amyloid plaque deposition decreased in the hippocampal regions in APP/PS1 mice that expressed AAV-mediated Cas9-SW1. **a, b**, Immunohistochemistry for $A\beta$ (4G8; green), HA (red), and nuclei (blue) in 15-month-old APP/PS1 mice. Scale bar: 50 μ m. Quantification of $A\beta$ -positive plaque areas in the cornu ammonis (**c**) and subiculum (**d**). Values are mean $\pm$ SEM ($n = 4$ mice per group; $*P < 0.05$, $**P < 0.01$; paired two-tailed t -test). **e–h**, AAV-mediated Cas9-SW1 editing decreased gliosis in the subiculum in APP/PS1 mice. **e**, Immunohistochemistry for microglial marker Iba1 (red), $A\beta$ (4G8; green), and nuclei (blue). Scale bars: 50 μ m (left), 10 μ m (right). **f**, Quantification of Iba1-positive cells. Values are mean $\pm$ SEM ($n = 4$ mice per group; $*P < 0.05$; paired two-tailed t -test). **g**, Immunohistochemistry for the astrocyte marker GFAP (red), $A\beta$ (4G8; green), and nuclei (blue) in APP/PS1 mice. Scale bars: 50 μ m (left), 10 μ m (right). **h**, Quantification of GFAP-positive cells. Values are mean $\pm$ SEM ($n = 4$ mice per group; $*P < 0.05$; paired two-tailed t -test). **i**, Percentages of GFAP-positive areas. Values are mean $\pm$ SEM ($n = 4$ mice per group; $*P < 0.05$; paired two-tailed t -test).

[0015] Figure 6: Systemic delivery of AAV-PHP.eB-mediated Cas9-SW1 globally decreases amyloid plaque burden in 5XFAD mice. **a**, Diagram of brain-wide APP_{swe} editing. A single AAV vector, designated SW1-Syn::Cas9-mWPRE, was designed to express HA-tagged SaCas9 and sgRNA SW1 for systemic administration using an AAV-PHP.eB capsid. ITR, inverted terminal repeat; U6, human U6 promoter; Syn, human synapsin 1 promoter; mWPRE, truncated form of Woodchuck Hepatitis Virus (WHP) Posttranscriptional Regulatory Element (WPRE); pA, poly(A) tail. **b–d**, Systemic injection of AAV-PHP.eB-mediated Cas9-SW1 into 3-month-old 5XFAD mice globally decreased the amyloid-beta ($A\beta$)-positive plaque area in the brain. **b**, Representative images of amyloid plaque (i.e., 4G8) staining in 6-month-old 5XFAD mice after AAV-PHP.eB:SW1-Syn::Cas9-mWPRE injection. Quantification of amyloid plaque areas in the cortex (**c**) and hippocampus (**d**). Values are mean $\pm$ SEM ($n = 4$ mice per group; $*P < 0.05$, $**P < 0.01$; unpaired two-tailed t -test). **e–l**, The decrease in X34-labeled amyloid plaque burden was associated with Cas9 expression in the brain. **e, f, i, j**, Immunohistochemistry for X34 (green), HA (red), and nuclei (blue) in the

cornu ammonis (e), subiculum (f), cortex (i), and medulla (j) in 6-month-old 5XFAD mice. HA tag expression indicates Cas9 expression. Scale bar: 50 μ m. **g, h, k, l**, Quantification of X34-labeled plaque areas. Values are mean $\pm$ SEM ($n = 4$ mice per group; $*P < 0.05$, $**P < 0.01$; unpaired two-tailed t -test).

- 5 **[0016] Figure 7: Systemic delivery of AAV-PHP.eB-mediated Cas9-SW1 decreases microgliosis and neurite dystrophy, and improves cognitive performance in 5XFAD mice.** **a, b**, Systemic delivery of AAV-PHP.eB-mediated Cas9-SW1 in 3-month-old 5XFAD mice decreased microgliosis in the subiculum. **a**, Immunohistochemistry for Iba1 (red), amyloid-beta ($A\beta$, 4G8; green), and nuclei (blue) in 6-month-old 5XFAD mice. Scale bars: 50 μ m (left), 10 μ m (right). **b**, Quantification of Iba1-positive microglia. Values are mean $\pm$ SEM ($n = 4$ mice per group; $*P < 0.05$; unpaired two-tailed t -test). **c, d**, AAV-PHP.eB-mediated Cas9-SW1 editing decreased neurite dystrophy in 5XFAD mice. **c**, Representative images showing staining for LAMP1 (a marker of neurite dystrophy; red), $A\beta$ (4G8; green), and nuclei (blue) in the subiculum in 6-month-old 5XFAD mice. Scale bar: 10 μ m. **d**, Quantification of LAMP1-positive dystrophic neurites. Values are mean $\pm$ SEM ($n = 4$ mice per group; $**P < 0.01$; unpaired two-tailed t -test). **e–g**, Systemic delivery of AAV-PHP.eB-mediated Cas9-SW1 improved the cognitive performance of 5XFAD mice. **e**, Systemic delivery of AAV-PHP.eB-mediated Cas9-SW1 improved habituation in the exploratory open field (OF) test. Percentage change in the distance traveled relative to that traveled on the first day of training. Values are mean $\pm$ SEM ($n = 12$ mice per group; $*P < 0.05$, $**P < 0.01$; two-way repeated-measures ANOVA). **f**, Systemic delivery of AAV-PHP.eB-mediated Cas9-SW1 improved spatial working memory in the spontaneous alteration Y-maze test. Values are mean $\pm$ SEM ($n = 12$ mice per group; $*P < 0.05$, $**P < 0.01$; unpaired two-tailed t -test). **g**, Upon systemic delivery of AAV-PHP.eB-Cas9-SW1, the 5XFAD mice spent significantly less time in the open arms. Values are mean $\pm$ SEM ($n = 12$ mice per group; $*P < 0.05$; unpaired two-tailed t -test).

- [0017] Figure 8: Validation of allele-specific editing in HEK 293T cells and 5XFAD mice.** **a, b**, Cas9-SW1 and Cas9-SW2 did not target APP_{WT} . **a**, T7 endonuclease I mismatch assay for CRISPR/Cas9-mediated genome editing in HEK 293T cells carrying APP_{WT} alleles. WT1 and WT2 are single-guide RNAs (sgRNAs) that target the APP_{WT} allele at the same genetic loci as those of SW1 and SW2, respectively. **b**, Insertion/deletion (indel) rates were examined by targeted deep sequencing. Values are mean $\pm$ SEM ($n = 5$ replicates per group; $***P < 0.001$; unpaired Student's t -test). **c, d**, Off-target analysis of Cas9-SW1. **c**,

Sequences of the top 5 predicted off-target sites in the human genome. **d**, T7EI assay for the top 5 predicted off-target sites in HEK 293T cells. The lack of cleavage bands by T7EI indicates that the off-target activities of Cas9-SW1 were less than 1%. **e–g**, Validation of AAV-mediated Cas9-SW1 editing *in vivo*. **e**, The 10 most abundant *APP_{swe}* sequences from Cas9-SW1-injected 5XFAD mouse brains determined by targeted deep sequencing. Mutation sites are shown in blue text. Percentages are the frequencies of the modified *APP_{swe}* sequences. Editing events are labeled with “#.” **f**, Number of somatic mutations detected by whole-genome sequencing. The virus-transduced hippocampal regions (SW1) and untransduced brain regions (Con) of the same mouse were subjected to whole-genome sequencing to detect somatic mutations. Values are mean $\pm$ SEM ($n = 2$ mice). **g**, Workflow for identification of potential off-target mutations..

[0018] Figure 9: Intrahippocampal AAV-mediated Cas9-SW1 editing in 5XFAD transgenic mice decreases amyloid plaque deposition in Cas9-expressing brain regions. **a, b**, Immunohistochemistry for A β (4G8; green), HA (red), and nuclei (blue) in 6- or 9-month-old 5XFAD mice after hippocampal injection of AAV-mediated Cas9-SW1 (EFS::SaCas9-SW1) at 3 months of age. HA staining indicates Cas9 expression. **a**, Representative images of sagittal sections from control (Con) and virus-injected (SW1) 5XFAD mouse brains. Scale bar: 1 mm. **b**, Representative images of the hippocampus, cornu ammonis (CA), and subiculum in 6- and 9-month-old mice, respectively. Scale bar: 200 μ m..

[0019] Figure 10: Intrahippocampal AAV-mediated Cas9-SW1 editing decreases gliosis in the subiculum in 5XFAD mice. **a, b**, Immunohistochemical analysis of amyloid plaque deposition in microglia (**a**) and astrocytes (**b**). Staining for A β (4G8; green) and nuclei (blue) together with Iba1 (red; **a**) or GFAP (red; **b**), respectively, in 6-month-old mice (3 months after virus injection). Scale bars: 100 μ m. WT, wild type.

[0020] Figure 11: Intrahippocampal AAV-mediated Cas9-SW1 editing decreases neurite dystrophy in 5XFAD mice. **a, b**, Immunohistochemistry for LAMP1 (red), A β (4G8; green), and nuclei (blue) in the subiculum in 5XFAD mice at 6 months (**a**) and 9 months (**b**). Scale bar: 200 μ m.

[0021] Figure 12: Intrahippocampal AAV-mediated Cas9-SW1 editing decreases amyloid plaque deposition in the Cas9-expressing brain regions in APP/PS1 transgenic mice. **a**, T7 endonuclease I assay for AAV-mediated Cas9-SW1 editing in the virus-

transduced hippocampi from APP/PS1 mice. Arrowheads indicate the CRISPR/Cas9-edited DNA fragments. Con, Untransduced hippocampal regions of APP/PS1 mice. **b**, Percentage of insertion/deletion (indel) mutations. Values are mean $\pm$ SEM ($n = 4$ mice per group; *** $P < 0.001$; unpaired two-tailed t -test). **c, d**, Immunohistochemistry for A β (4G8; green), HA (red), and nuclei (blue) in 15-month-old APP/PS1 mice (6 months after virus injection). HA staining indicates Cas9 expression. **c**, Representative images of sagittal sections from APP/PS1 mouse brains. Scale bar: 1 mm. **d**, Representative images of the hippocampus, cornu ammonis (CA), and subiculum from APP/PS1 mouse brains. Scale bar: 200 μ m

[0022] Figure 13: Optimization of the AAV construct for enhanced gene transfer and

robust expression in the central nervous system. **a**, Schematic comparison of 2 AAV vectors for systemic delivery (with or without the insertion of the mWPRES element). ITR, inverted terminal repeat; U6, human U6 promoter; Syn, human synapsin 1 promoter; mWPRES, a truncated form of Woodchuck Hepatitis Virus (WHP) Posttranscriptional Regulatory Element (WPRES); pA, poly(A) tail. **b**, Immunohistochemistry for HA (green) indicates Cas9 expression in C57 mice injected with SW1-Syn::SaCas9 (–mWPRES) or SW1-Syn::SaCas9-mWPRES (+mWPRES) at a dose of 1×10^{12} vg/mouse. Scale bars: 300 μ m.

[0023] Figure 14: Systemic delivery of AAV-PHP.eB-mediated Cas9-SW1 globally decreases amyloid plaque burden in 5XFAD mice. **a**, T7 endonuclease I assay for AAV-PHP.eB-mediated Cas9-SW1 editing in multiple brain regions in 5XFAD mice. Arrowheads indicate the CRISPR/Cas9-edited DNA fragments. HP, hippocampus; Sub, subiculum; CA, cornu ammonis; DG, dentate gyrus; CX, cortex. **b**, Percentage of insertion/deletion (indel) mutations. CRISPR/Cas9 editing led to the formation of indel mutations. Indel percentage indicates editing efficiency. Values are mean $\pm$ SEM ($n = 3$ mice per group). **c, d**, Immunohistochemistry for X34 (green), HA (red), and nuclei (blue) in 6-month-old 5XFAD mice after systemic administration of Cas9-SW1 (SW1-Syn::SaCas9-mWPRES). HA staining indicates Cas9 expression. **c**, Representative images of sagittal sections of 5XFAD mouse brains 3 months after virus injection. Scale bar: 1 mm. **d**, Representative images of the hippocampus, cornu ammonis (CA), subiculum, and dentate gyrus (DG) from 5XFAD mouse brains. Scale bars: 200 μ m.

[0024] Figure 15: Systemic delivery of AAV-PHP.eB-mediated Cas9-Con does not induce adverse effects in WT or 5XFAD mice. **a–c**, Systemic delivery of AAV-PHP.eB-mediated Cas9-Con did not induce adverse behavioral effects in wild-type (WT) mice. Uninject, uninjected. **a**, The locomotor activity of mice did not change in the exploratory

open field test upon systemic delivery of AAV-PHP.eB-mediated Cas9-Con. Values are mean $\pm$ SEM ($n = 12$ mice per group; unpaired two-tailed t -test). **b**, Systemic delivery of AAV-PHP.eB-mediated Cas9-Con did not alter habituation in the exploratory open field (OF) test. The percentage change in the distance traveled relative to that traveled on the first day of training. Values are mean $\pm$ SEM ($n = 12$ mice per group; unpaired two-tailed t -test). **c**, Systemic delivery of AAV-PHP.eB-mediated Cas9-Con did not affect spatial working memory in the spontaneous alternation Y-maze test. Values are mean $\pm$ SEM ($n = 12$ mice per group; unpaired two-tailed t -test). **d–f**, Systemic delivery of AAV-PHP.eB-mediated Cas9-Con did not induce gliosis in WT mice. **d**, Immunohistochemistry for HA (red, *Left panels*), Iba1 (red, *Middle panels*), GFAP (red, *Right panels*), and nuclei (blue) in 6-month-old WT mice. Scale bars: 50 μ m. **e**, Quantification of Iba1-positive cells. Values are mean $\pm$ SEM ($n = 4$ mice per group; unpaired two-tailed t -test). **f**, Quantification of GFAP-positive cells. Values are mean $\pm$ SEM ($n = 4$ mice per group). **g, h**, Immunohistochemistry for X34 (green), HA (red), and nuclei (blue) in 6-month-old 5XFAD mice after systemic administration of Cas9-Con (Con-Syn::SaCas9-mWPRE). HA tag expression indicates Cas9 expression. Scale bars: 1 mm (**g**), 200 μ m (**h**). **i**, Quantification of X34-labeled plaque areas in the hippocampus of 6-month-old 5XFAD mice. Values are mean $\pm$ SEM ($n = 3$ mice per group; unpaired two-tailed t -test).

[0025] Figure 16: CRISPR-targeting range of APP. The Cas9-sgRNA complex specifically disrupts the cleavage sites in exons 14 to 17 of APP. Figure is adopted from Alzforum. 3' UTR, three prime untranslated region; AD, Alzheimer's disease; APP, amyloid precursor protein; Ex, exon.

[0026] Figure 17: Efficient deletion of APP in HEK293T cells. **a**, T7 endonuclease I mismatch assay for CRISPR/Cas9-mediated genome editing in HEK293T cells carrying APP_{WT} alleles. WT1 and WT2 are single-guide RNAs (sgRNAs) that target the APP_{WT} allele. SW1 and SW2 are sgRNAs that target the APP Swedish mutation (serve as sgRNA controls; irrelevant to this invention). **b**, Insertion/deletion (indel) rates were examined by targeted deep sequencing. Values are mean $\pm$ SEM ($n = 5$ replicates per group; *** $P < 0.001$; unpaired Student's t -test). APP, amyloid precursor protein; Con, control.

[0027] Figure 18: CRISPR/Cas9-mediated deletion of β -secretase site reduces APP expression and A β production in HEK293T cells. Representative western blots of APP with β -actin as a loading control. HEK293T cells were co-transfected with APP-expressing

construct and Cas9/gRNA constructs. L, molecular size ladder; con, control; unt, untransfected.

DEFINITIONS

[0028] The term “**APP**” refers to amyloid precursor protein or the gene encoding this protein. The human APP gene is localized to human chromosome 21q11.2–q21 and encodes a protein having the exemplary amino acid sequence set forth herein as SEQ ID NO:2.

[0029] As used herein, the term “**an amyloid pathology**” refers to a disease or condition that is caused or exacerbated by an accumulation of excessive quantity of amyloid plaque in the pertinent tissues and anatomic sites of a person, *e.g.*, the nervous system (such as brain), blood vessel wall, and various organs. Examples of “an amyloid pathology” include, but are not limited to, Alzheimer’s disease (AD) of both the familial and sporadic types, cerebral amyloid angiopathy, Down Syndrome, as well as various amyloidosis.

[0030] The term “**nucleic acid**” or “**polynucleotide**” refers to deoxyribonucleic acids (DNA) or ribonucleic acids (RNA) and polymers thereof in either single- or double-stranded form. Unless specifically limited, the term encompasses nucleic acids containing known analogues of natural nucleotides that have similar binding properties as the reference nucleic acid and are metabolized in a manner similar to naturally occurring nucleotides. Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (*e.g.*, degenerate codon substitutions), alleles, orthologs, SNPs, and complementary sequences as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer *et al.*, *Nucleic Acid Res.* **19**:5081 (1991); Ohtsuka *et al.*, *J. Biol. Chem.* **260**:2605-2608 (1985); and Rossolini *et al.*, *Mol. Cell. Probes* **8**:91-98 (1994)). The term nucleic acid is used interchangeably with gene, cDNA, and mRNA encoded by a gene.

[0031] The term “**gene**” means the segment of DNA involved in producing a polypeptide chain. It may include regions preceding and following the coding region (leader and trailer) as well as intervening sequences (introns) between individual coding segments (exons).

[0032] The term “**amino acid**” refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the

genetic code, as well as those amino acids that are later modified, *e.g.*, hydroxyproline, γ -carboxyglutamate, and O-phosphoserine. Amino acid analogs refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, *i.e.*, an α carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, *e.g.*, homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (*e.g.*, norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. “Amino acid mimetics” refers to chemical compounds having a structure that is different from the general chemical structure of an amino acid, but that functions in a manner similar to a naturally occurring amino acid.

- 10 [0033] There are various known methods in the art that permit the incorporation of an unnatural amino acid derivative or analog into a polypeptide chain in a site-specific manner, *see, e.g.*, WO 02/086075.

- [0034] Amino acids may be referred to herein by either the commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes.

- [0035] “**Polypeptide**,” “**peptide**,” and “**protein**” are used interchangeably herein to refer to a polymer of amino acid residues. All three terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymers. As used herein, the terms encompass amino acid chains of any length, including full-length proteins, wherein the amino acid residues are linked by covalent peptide bonds.

- [0036] As used herein, a “**composition disrupting APP genomic sequence**” refers to any composition comprising one or more agents capable of suppressing or eliminating the transcription or translation of the genomic sequence encoding the amyloid precursor protein (APP), which may be achieved by the direct deletion or alternation of at least a portion of the APP genomic sequence (*e.g.*, by genomic editing technique such as the clustered regularly interspaced short palindromic repeat (CRISPR) system or the like) or may be achieved via reduction or elimination of the mRNA transcribed from the genomic sequence through the action of small inhibitory DNA or RNA molecules or other enzymes (*e.g.*, antisense oligonucleotides, small inhibitory RNAs such as siRNA or shRNA, and ribozymes *etc.*) so as

to ultimately achieve the result of reduction of APP expression up to elimination of its protein expression.

[0037] The term “**targeting**,” when used in the context of describing an inhibitory oligonucleotide (such as a small inhibitory RNA or an antisense oligonucleotide) or an sgRNA in relation to a genomic sequence that the inhibitory oligonucleotide or gene editing system is used to negatively regulate, refers to a sufficient sequence complementarity between at least a portion of the oligonucleotide or sgRNA and the genomic sequence, *e.g.*, at least 80, 85, 90, 95% or higher percentage of nucleotide sequence complementarity based on the Watson-Crick base-pairing principle, so as to allow specific hybridization between the sgRNA or oligonucleotide and the genomic sequence or its mRNA transcript, which subsequently leads to the cleavage of the genomic sequence at a pre-determined location (*e.g.*, at a location near a pre-selected mutation or a β - or γ -secretase cleavage site within the APP genomic sequence, for example, within 3, 5, 7, 8, 10, 12, 15, or 20 nucleotides upstream or downstream from the mutation or β - or γ -secretase cleavage site) or the destruction of its mRNA transcript.

[0038] The term “**recombinant**” when used with reference, *e.g.*, to a cell, or a nucleic acid, protein, or vector, indicates that the cell, nucleic acid, protein or vector, has been modified by the introduction of a heterologous nucleic acid or protein or the alteration of a native nucleic acid or protein, or that the cell is derived from a cell so modified. Thus, for example, recombinant cells express genes that are not found within the native (non-recombinant) form of the cell or express native genes that are otherwise abnormally expressed, under expressed or not expressed at all.

[0039] A “**promoter**” is defined as an array of nucleic acid control sequences that direct transcription of a polynucleotide sequence. As used herein, a promoter includes necessary polynucleotide sequences near the start site of transcription, such as, in the case of a polymerase II type promoter, a TATA element. A promoter also optionally includes distal enhancer or repressor elements, which can be located as much as several thousand base pairs from the start site of transcription. A “constitutive” promoter is a promoter that is active under most environmental and developmental conditions. An “inducible” promoter is a promoter that is active under environmental or developmental regulation. The term “operably linked” refers to a functional linkage between a polynucleotide expression control sequence (such as a promoter, or array of transcription factor binding sites) and a second

polynucleotide sequence, wherein the expression control sequence directs transcription of the polynucleotide sequence corresponding to the second sequence.

[0040] An “**expression cassette**” is a nucleic acid construct, generated recombinantly or synthetically, with a series of specified polynucleotide elements that permit transcription of a particular polynucleotide sequence in a host cell. An expression cassette may be part of a plasmid, viral genome, or nucleic acid fragment. Typically, an expression cassette includes a polynucleotide to be transcribed, operably linked to a promoter.

[0041] The term “**heterologous**” as used in the context of describing the relative location of two elements, refers to the two elements such as polynucleotide sequences (*e.g.*, a promoter and an mRNA- or a protein/polypeptide-encoding sequence) or polypeptide sequences (*e.g.*, two peptides as fusion partners within a fusion protein) that are not naturally found in the same relative positions. Thus, a “heterologous promoter” of a coding sequence refers to a promoter that is not naturally operably linked to that coding sequence. Similarly, a “heterologous polypeptide” or “heterologous polynucleotide” to a particular protein or its coding sequence is one derived from an origin that is different from that particular protein, or if derived from the same origin but not naturally connected to that particular protein or its coding sequence in the same fashion. The fusion of one polypeptide (or its coding sequence) with a heterologous polypeptide (or polynucleotide sequence) does not result in a longer polypeptide or polynucleotide sequence that can be found in nature.

[0042] The phrase “**specifically hybridize(s) to**” refers to the binding, duplexing, or hybridization of one polynucleotide sequence to another polynucleotide sequence based on Watson-Crick nucleotide base-pairing under stringent hybridization conditions when that sequences are present in a complex mixture (*e.g.*, total cellular or library DNA or RNA). The phrase “stringent hybridization conditions” refers to conditions under which a nucleic acid (*e.g.*, a polynucleotide probe) will hybridize to its target nucleotide sequence, typically in a complex mixture of nucleic acids, but to no other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. An extensive guide to the hybridization of nucleic acids is found in Tijssen, *Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Probes*, “Overview of principles of hybridization and the strategy of nucleic acid assays” (1993). Generally, stringent conditions are selected to be about 5-10°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength pH. The T_m is the temperature (under defined ionic strength, pH, and nucleic

concentration) at which 50% of the probes complementary to the target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at T_m , 50% of the probes are occupied at equilibrium). Stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (*e.g.*, 10 to 50 nucleotides) and at least about 60°C for long probes (*e.g.*, greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. For high stringency hybridization, a positive signal is at least two times background, preferably 10 times background hybridization. Exemplary high stringency hybridization conditions include: 50% formamide, 5x SSC and 1% SDS incubated at 42° C or 5x SSC and 1% SDS incubated at 65° C, with a wash in 0.2x SSC and 0.1% SDS at 65° C.

[0043] By “**host cell**” is meant a cell that contains an expression vector and supports the replication or expression of the expression vector. Host cells may be prokaryotic cells such as *E. coli*, or eukaryotic cells such as yeast, insect, amphibian, or mammalian cells such as CHO, HeLa and the like, *e.g.*, cultured cells, explants, and cells *in vivo*.

[0044] The term “**inhibiting**” or “**inhibition**,” as used herein, refers to any detectable negative effect an inhibitory agent has on a target biological process, such as APP protein expression, formation of amyloid β ($A\beta$) plaques in an AD patient’s brain, an AD patient’s cognitive decline, protein phosphorylation, cellular signal transduction, protein synthesis, cell proliferation, tumorigenicity, and metastatic potential *etc.* Typically, an inhibition is reflected in a decrease of at least 10%, 20%, 30%, 40%, or 50% in target process (*e.g.*, APP protein expression or $A\beta$ plaque accumulation), or any one of the downstream parameters mentioned above, when compared to a control not exposed to the inhibitory agent. In a similar fashion, the term “increasing” or “increase” is used to describe any detectable positive effect an enhancing agent has on a target biological process, such as a positive change of at least 25%, 50%, 75%, 100%, or as high as 2, 3, 4, 5 or up to 10 or 20 fold, when compared to a control in the absence of the enhancer. Conversely, the term “substantially unchanged” describes a state in which the positive or negative changes are less than 10%, 5%, 2%, 1% or lower.

[0045] The term “**effective amount**,” as used herein, refers to an amount that is sufficient to produces an intended effect for which a substance is administered. The effect may include a desirable change in a biological process (*e.g.*, a detectable decrease of APP expression,

reduction in A β plaque formation, or slowing of cognitive decline in an AD patient) as well as the prevention, correction, or inhibition of progression of the symptoms of a disease/condition and related complications to any detectable extent. The exact amount “effective” for achieving a desired effect will depend on the nature of the therapeutic agent, the manner of administration, and the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques (*see, e.g.,* Lieberman, *Pharmaceutical Dosage Forms* (vols. 1-3, 1992); Lloyd, *The Art, Science and Technology of Pharmaceutical Compounding* (1999); and Pickar, *Dosage Calculations* (1999)).

[0046] As used herein, the term “**treatment**” or “**treating**” includes both therapeutic and preventative measures taken to address the presence of a disease or condition or the risk of developing such disease or condition at a later time. It encompasses therapeutic or preventive measures for alleviating ongoing symptoms, inhibiting or slowing disease progression, delaying of onset of symptoms, or eliminating or reducing side-effects caused by such disease or condition. A preventive measure in this context and its variations do not require 100% elimination of the occurrence of an event; rather, they refer to a suppression or reduction in the likelihood or severity of such occurrence or a delay in such occurrence.

[0047] A “**pharmaceutically acceptable**” or “**pharmacologically acceptable**” excipient is a substance that is not biologically harmful or otherwise undesirable, *i.e.*, the excipient may be administered to an individual along with a bioactive agent without causing any undesirable biological effects. Neither would the excipient interact in a deleterious manner with any of the components of the composition in which it is contained.

[0048] The term “**excipient**” refers to any essentially accessory substance that may be present in the finished dosage form of the composition of this invention. For example, the term “excipient” includes vehicles, binders, disintegrants, fillers (diluents), lubricants, glidants (flow enhancers), compression aids, colors, sweeteners, preservatives, suspending/dispersing agents, film formers/coatings, flavors and printing inks.

[0049] The term “**consisting essentially of**,” when used in the context of describing a composition containing an active ingredient or multiple active ingredients, refer to the fact that the composition does not contain other ingredients possessing any similar or relevant biological activity of the active ingredient(s) or capable of enhancing or suppressing the activity, whereas one or more inactive ingredients such as physiological or pharmaceutically acceptable excipients may be present in the composition. For example, a composition

consisting essentially of active agent(s) effective for disrupting the APP genomic sequence or for suppressing mRNA transcribed from the genomic sequence in a subject is a composition that does not contain any other agents that may have any detectable positive or negative effect on the same target process or that may increase or decrease to any measurable extent of the disease occurrence or symptoms among the receiving subjects.

[0050] The term “**about**” denotes a range of +/- 10% of a pre-determined value. For example, “about 10” sets a range of 90% to 110% of 10, *i.e.*, 9 to 11.

DETAILED DESCRIPTION OF THE INVENTION

I. Introduction

[0051] It has long been observed and understood the correlation between abnormal amyloid plaque accumulation and various pathologies such as Alzheimer’s Disease (AD). The present inventors for the first time ever achieved successful suppression of amyloid plaque build-up by using CRISPR-based genomic editing to target one or more specific sub-regions within the amyloid precursor protein (APP) genomic sequence (*e.g.*, within close proximity to a pre-elected mutation site or a β - or γ -secretase cleavage site), while little off-target editing was detected. It is therefore demonstrated that such disruption of the APP genomic sequence provides therapeutic benefits in the treatment of patients suffering excessive build-up of amyloid plaque in the brain and other organs in a variety of pathologies in which amyloid plaque plays a critical role in the onset and progression of the disease (such as AD) as well as prophylactic benefits in the prevention or risk reduction of such disease in individuals who are not yet diagnosed of the disease but may have known risks such as family history of AD.

II. General Recombinant Technology

[0052] Basic texts disclosing general methods and techniques in the field of recombinant genetics include Sambrook and Russell, *Molecular Cloning, A Laboratory Manual* (3rd ed. 2001); Kriegler, *Gene Transfer and Expression: A Laboratory Manual* (1990); and Ausubel *et al.*, eds., *Current Protocols in Molecular Biology* (1994).

[0053] For nucleic acids, sizes are given in either kilobases (kb) or base pairs (bp). These are estimates derived from agarose or acrylamide gel electrophoresis, from sequenced nucleic acids, or from published DNA sequences. For proteins, sizes are given in kilodaltons (kDa) or amino acid residue numbers. Proteins sizes are estimated from gel electrophoresis, from sequenced proteins, from derived amino acid sequences, or from published protein sequences.

[0054] Oligonucleotides that are not commercially available can be chemically synthesized, *e.g.*, according to the solid phase phosphoramidite triester method first described by Beaucage & Caruthers, *Tetrahedron Lett.* **22**: 1859-1862 (1981), using an automated synthesizer, as described in Van Devanter *et. al.*, *Nucleic Acids Res.* **12**: 6159-6168 (1984).

5 Purification of oligonucleotides is performed using any art-recognized strategy, *e.g.*, native acrylamide gel electrophoresis or anion-exchange HPLC as described in Pearson & Reanier, *J. Chrom.* **255**: 137-149 (1983).

[0055] The sequence of a gene of interest, a polynucleotide encoding a polypeptide of interest, and synthetic oligonucleotides can be verified after cloning or subcloning using, *e.g.*,
10 the chain termination method for sequencing double-stranded templates of Wallace *et al.*, *Gene* **16**: 21-26 (1981).

II. Composition Disrupting a Genomic Sequence

[0056] Earlier work by the present inventors as well as others illustrated the involvement of APP and its mutations in the development of Alzheimer's Disease. Their latest discovery
15 reveals that, by disrupting the APP genomic sequence especially in the close proximity of a pre-selected mutation (*e.g.*, Swedish mutation) or a β - or γ -secretase cleavage site, APP expression and A β plaque accumulation in the brain can be effectively and significantly reduced. This revelation leads to the therapeutic and prophylactic use of compositions disrupting this genomic sequence, which may act at the level of intact genomic sequence at or
20 immediately surrounding the pre-chosen the genomic locus of a specific mutation or a β - or γ -secretase cleavage site, or which may act at the level of mRNA transcribed from the genomic sequence, for treating Alzheimer's Disease in patients already diagnosed with the disease and preventing/reducing risk of later developing Alzheimer's Disease in individuals who have not yet received a diagnosis but are at heightened risk for the disease, *e.g.*, due to
25 family history or known genetic background (for instance, carrier of one or two *APOE* $\epsilon 4$ alleles, point mutation in the genomic sequence encoding amyloid precursor protein (APP) on chromosome 21, point mutation in the genomic sequence encoding Presenilin 1 (PSEN1) on chromosome 14, and point mutation in the genomic sequence encoding Presenilin 2 (PSEN2) on chromosome 1). Various categories of possible agents acting through different
30 mechanisms (*e.g.*, by genomic editing or mRNA suppression) are useful in formulating such compositions for the disruption of the APP genomic sequence and are discussed below.

A. Antisense Oligonucleotides

[0057] In some embodiments, the agent useful for disrupting the APP genomic sequence is an antisense oligonucleotide. Antisense oligonucleotides are relatively short nucleic acids that are complementary (or antisense) to the coding strand (sense strand) of the RNA

5 transcribed from the genomic sequence encompassing the APP gene. Although antisense oligonucleotides are typically RNA-based, they can also be DNA-based. Also, antisense oligonucleotides are often modified to increase their stability.

[0058] Without being bound by theory, the binding of these relatively short oligonucleotides to the mRNA is believed to induce stretches of double-stranded RNA that
10 trigger degradation of the messages by endogenous RNases. Additionally, sometimes the oligonucleotides are specifically designed to bind near the promoter of the coding sequence, and under these circumstances, the antisense oligonucleotides may additionally interfere with translation of the mRNA. Regardless of the specific mechanism by which antisense oligonucleotides function, their administration to a cell or tissue allows the degradation of the
15 RNA transcribed from the genomic sequence encompassing the APP gene. Accordingly, antisense oligonucleotides decrease the expression and/or activity of encoded product from the genomic sequence.

[0059] The oligonucleotides can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be
20 modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, *etc.* The oligonucleotide may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors), or agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger *et al.*, 1989, *Proc. Natl. Acad. Sci. U.S.A.* 86:6553-6556; Lemaitre *et al.*, 1987, *Proc. Natl. Acad. Sci.* 84:648-652; WO 88/09810) or
25 the blood-brain barrier (see, *e.g.*, WO 89/10134), hybridization-triggered cleavage agents (See, *e.g.*, Krol *et al.*, 1988, *BioTechniques* 6:958-976) or intercalating agents (see, *e.g.*, Zon, 1988, *Pharm. Res.* 5:539-549). To this end, the oligonucleotide can be conjugated to another molecule.

[0060] Oligonucleotides of the invention may be synthesized by standard methods known
30 in the art, *e.g.*, by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, *etc.*). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein *et al.* (1988, *Nucl. Acids Res.* 16:3209),

methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin *et al.*, 1988, *Proc. Natl. Acad. Sci. U.S.A.* 85:7448-7451) *etc.*

[0061] A number of methods have been developed for delivering antisense DNA or RNA to cells, *e.g.*, antisense molecules can be injected directly into the target anatomic site, or
5 modified antisense molecules, designed to target the desired cells (*e.g.*, antisense linked to peptides or antibodies that specifically bind receptors or antigens expressed on the target cell surface) can be administered systematically.

[0062] It may be difficult to achieve intracellular concentrations of the antisense sufficient to suppress translation on endogenous mRNAs in certain instances. Therefore, another
10 approach utilizes a recombinant DNA construct in which the antisense oligonucleotide is placed under the control of a strong pol III or pol II promoter. For example, a vector can be introduced *in vivo* such that it is taken up by a cell and directs the transcription of an antisense RNA. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be
15 constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in mammalian cells. Expression of the sequence encoding the antisense RNA can be by any promoter known in the art to act in mammalian, preferably human cells. Such promoters can be inducible or constitutive. Such promoters include but are not limited to: the SV40 early
20 promoter region (Bernoist and Chambon, 1981, *Nature* 290:304-310), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto *et al.*, 1980, *Cell* 22:787-797), the herpes thymidine kinase promoter (Wagner *et al.*, 1981, *Proc. Natl. Acad. Sci. U.S.A.* 78:1441-1445), the regulatory sequences of the metallothionein gene (Brinster *et al.*, 1982, *Nature* 296:39-42), *etc.* Any type of plasmid, cosmid, YAC or viral vector can be used
25 to prepare the recombinant DNA construct that can be introduced directly into the target tissue site. Alternatively, viral vectors can be used which selectively infect the desired tissue, in which case administration may be accomplished by another route (*e.g.*, systematically).

B. Small Interfering RNA

[0063] In some embodiments, the agent is a small interfering RNA (siRNA or RNAi)
30 molecule. RNAi constructs comprise double stranded RNA that can specifically block expression of a target gene. "RNA interference" or "RNAi" is a term initially applied to a phenomenon where double-stranded RNA (dsRNA) blocks gene expression in a specific and

post-transcriptional manner. RNAi provides a useful method of inhibiting gene expression *in vitro* or *in vivo*. RNAi constructs can include small interfering RNAs (siRNAs), short hairpin RNAs (shRNAs), and other RNA species that can be cleaved *in vivo* to form siRNAs. RNAi constructs herein also include expression vectors ("RNAi expression vectors") capable of
5 giving rise to transcripts which form dsRNAs or hairpin RNAs in cells, and/or transcripts which can produce siRNAs *in vivo*.

[0064] RNAi expression vectors express (transcribe) RNA which produces siRNA moieties in the cell in which the construct is expressed. Such vectors include a transcriptional unit comprising an assembly of (1) genetic element(s) having a regulatory role in gene expression,
10 for example, promoters, operators, or enhancers, operatively linked to (2) a "coding" sequence which is transcribed to produce a double-stranded RNA (two RNA moieties that anneal in the cell to form an siRNA, or a single hairpin RNA, which can be processed to an siRNA), and (3) appropriate transcription initiation and termination sequences. The choice of promoter and other regulatory elements generally varies according to the intended host cell.

[0065] The RNAi constructs contain a nucleotide sequence that hybridizes under physiologic conditions of the cell to the nucleotide sequence of at least a portion of the mRNA transcript for the gene to be inhibited (*i.e.*, an RNA transcribed from a genomic sequence encompassing the APP gene). The double-stranded RNA need only be sufficiently similar to natural RNA that it has the ability to mediate RNAi. Thus, the invention has the
20 advantage of being able to tolerate sequence variations that might be expected due to genetic mutation, strain polymorphism or evolutionary divergence. The number of tolerated nucleotide mismatches between the target sequence and the RNAi construct sequence is no more than 1 in 5 basepairs, or 1 in 10 basepairs, or 1 in 20 basepairs, or 1 in 50 basepairs. Mismatches in the center of the siRNA duplex are most critical and may essentially abolish
25 cleavage of the target RNA. In contrast, nucleotides at the 3' end of the siRNA strand that is complementary to the target RNA do not significantly contribute to specificity of the target recognition.

[0066] Production of RNAi constructs can be carried out by chemical synthetic methods or by recombinant nucleic acid techniques. Endogenous RNA polymerase of the treated cell
30 may mediate transcription *in vivo*, or cloned RNA polymerase can be used for transcription *in vitro*. The RNAi constructs may include modifications to either the phosphate-sugar backbone or the nucleoside, *e.g.*, to reduce susceptibility to cellular nucleases, improve bioavailability, improve formulation characteristics, and/or change other pharmacokinetic

properties. For example, the phosphodiester linkages of natural RNA may be modified to include at least one of a nitrogen or sulfur heteroatom. Modifications in RNA structure may be tailored to allow specific genetic inhibition while avoiding a general response to dsRNA. Likewise, bases may be modified to block the activity of adenosine deaminase. The RNAi construct may be produced enzymatically or by partial/total organic synthesis, any modified ribonucleotide can be introduced by *in vitro* enzymatic or organic synthesis.

[0067] In certain embodiments, the subject RNAi constructs are "small interfering RNAs" or "siRNAs." These nucleic acids are around 19-30 nucleotides in length, and even more preferably 21-23 nucleotides in length, *e.g.*, corresponding in length to the fragments generated by nuclease "dicing" of longer double-stranded RNAs. The siRNAs are understood to recruit nuclease complexes and guide the complexes to the target mRNA by pairing to the specific sequences. As a result, the target mRNA is degraded by the nucleases in the protein complex. In a particular embodiment, the 21-23 nucleotides siRNA molecules comprise a 3' hydroxyl group.

[0068] In certain embodiments, the RNAi construct is in the form of a short hairpin structure (named as shRNA). The shRNAs can be synthesized exogenously or can be formed by transcribing from RNA polymerase III promoters *in vivo*. Examples of making and using such hairpin RNAs for gene silencing in mammalian cells are described in, for example, Paddison *et al.*, *Genes Dev*, 2002, 16:948-58; McCaffrey *et al.*, *Nature*, 2002, 418:38-9; Yu *et al.*, *Proc Natl Acad Sci USA*, 2002, 99:6047-52). Often, such shRNAs are engineered in cells or in an animal to ensure continuous and stable suppression of a desired gene. It is known in the art that siRNAs can be produced by processing a hairpin RNA in the cell.

[0069] A plasmid can be used to deliver the double-stranded RNA, *e.g.*, as a transcriptional product. In such embodiments, the plasmid is designed to include a "coding sequence" for each of the sense and antisense strands of the RNAi construct. The coding sequences can be the same sequence, *e.g.*, flanked by inverted promoters, or can be two separate sequences each under transcriptional control of separate promoters. After the coding sequence is transcribed, the complementary RNA transcripts base-pair to form the double-stranded RNA.

C. Ribozymes

[0070] In some embodiments, the agent is a ribozyme. Ribozymes molecules designed to catalytically cleave an mRNA transcript are also used to disrupt and prevent the downstream effects of the mRNA (See, *e.g.*, WO 90/11364; Sarver *et al.*, 1990, *Science* 247:1222-1225).

and U.S. Pat. No. 5,093,246). While ribozymes that cleave mRNA at site-specific recognition sequences can be used to destroy particular mRNAs, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole
5 requirement is that the target mRNA have the following sequence of two bases: 5'-UG-3'. The construction and production of hammerhead ribozymes is well known in the art and is described more fully in Haseloff and Gerlach, 1988, *Nature*, 334:585-591.

[0071] The ribozymes for use in this invention may also include RNA endoribonucleases (hereinafter "Cech-type ribozymes") such as the one that occurs naturally in *Tetrahymena*
10 *thermophila* (known as the IVS, or L-19 IVS RNA) and has been extensively described by Thomas Cech and collaborators (Zaug, *et al.*, 1984, *Science*, 224:574-578; Zaug and Cech, 1986, *Science*, 231:470-475; Zaug, *et al.*, 1986, *Nature*, 324:429-433; WO 88/04300; Been and Cech, 1986, *Cell*, 47:207-216). The Cech-type ribozymes have an 8-basepair active site that hybridizes to a target RNA sequence whereafter cleavage of the target RNA takes place.
15 The invention encompasses those Cech-type ribozymes that target 8-basepair active site sequences.

[0072] As in the antisense approach, the ribozymes can be composed of modified oligonucleotides (*e.g.*, for improved stability, targeting, *etc.*) and can be delivered to cells *in vitro* or *in vivo*. A preferred method of delivery involves using a DNA construct "encoding"
20 the ribozyme under the control of a strong constitutive pol III or pol II promoter, so that transfected cells will produce sufficient quantities of the ribozyme to destroy targeted mRNA and inhibit its effect. Because ribozymes unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficiency.

[0073] There are currently two basic types of DNA enzymes, and both of these were
25 identified by Santoro and Joyce (see, *e.g.*, U.S. Pat. No. 6,110,462). The 10-23 DNA enzyme comprises a loop structure which connect two arms. The two arms provide specificity by recognizing the particular target nucleic acid sequence while the loop structure provides catalytic function under physiological conditions.

[0074] Briefly, to design an ideal DNA enzyme that specifically recognizes and cleaves a
30 target nucleic acid, one of skill in the art must first identify the unique target sequence. This can be done using the same approach as outlined for antisense oligonucleotides. Preferably, the unique or substantially sequence is a G/C rich of approximately 18 to 22 nucleotides.

High G/C content helps insure a stronger interaction between the DNA enzyme and the target sequence.

[0075] When synthesizing the DNA enzyme, the specific antisense recognition sequence that will target the enzyme to the message is divided so that it comprises the two arms of the DNA enzyme, and the DNA enzyme loop is placed between the two specific arms.

[0076] Methods of making and administering DNA enzymes can be found, for example, in U.S. Pat. No. 6,110,462. Similarly, methods of delivery DNA ribozymes *in vitro* or *in vivo* include methods of delivery RNA ribozyme, as outlined in detail above. Additionally, one of skill in the art will recognize that, like antisense oligonucleotide, DNA enzymes can be optionally modified to improve stability and improve resistance to degradation.

D. Genomic Editing

[0077] The inhibition of APP expression can be achieved by way of disruption of the genetic sequence encompassing the coding sequence for this protein. One effective means of targeted gene cleavage is the CRISPR system.

[0078] The term CRISPR, abbreviation for Clustered Regularly Interspaced Short Palindromic Repeats, was originally coined in reference to segments of prokaryotic DNA that contain short, repetitive base sequences, initially found in bacteria and archaea. In a palindromic repeat, the sequence of nucleotides is the same in both directions. Each repetition is followed by short segments of spacer DNA from previous exposures to foreign DNA (*e.g.*, DNA of a virus). Small clusters of *Cas* (CRISPR-associated) genes are located next to CRISPR sequences. It was later recognized that the CRISPR/*Cas* system is a prokaryotic immune system that confers resistance to foreign genetic elements especially those of viral origin and thereby provides a form of acquired immunity. RNA harboring the spacer sequence helps *Cas* (CRISPR-associated) proteins recognize and cut exogenous DNA. Other RNA-guided *Cas* proteins cut foreign RNA. CRISPRs are found in approximately 50% of sequenced bacterial genomes and nearly 90% of sequenced archaea, and recently the CRISPR/*Cas* system have been adapted for use in targeted gene editing in eukaryotic cells. See, *e.g.*, Ledford (2016), *Nature* 531 (7593):156–9.

[0079] A simple version of the CRISPR/*Cas* system, CRISPR/*Cas9*, has been modified to edit genomes. By delivering the *Cas9* nuclease complexed with one or more synthetic guide RNA (gRNA) into a cell, typically by transfecting the cell with one or more expression vectors encoding for the *Cas9* nuclease and the gRNA(s), the cell's genome can be cut at one

or more pre-selected location, allowing a target gene (*e.g.*, the genomic sequence harboring rs1921622) to be removed and/or substituted by a new sequence.

[0080] In the instant case, an expression vector (for example, a viral vector such as an adeno-associated virus or AAV vector) carrying the coding sequence for one or more gRNA
5 specific for a targeted sub-region of the APP genomic sequence can be introduced into a cell in which the endogenous APP genomic sequence is to be knocked out (for example, in neuronal cells). The same expression vector optionally also carries the coding sequence for the CRISPR/Cas9 nuclease or equivalent. In the alternative, a separate expression vector may be used to introduce the CRISPR/Cas9 nuclease coding sequence for its expression in
10 the target cells. In some cases, more than one (*e.g.*, two) distinct gRNAs are used to ensure removal and/or replacement of a target genomic sequence (*e.g.*, one that encompasses the APP coding sequence).

[0081] Additional gene editing systems that can be used for practicing the present invention include TALENs (Transcription activator-like effector nucleases), ZFNs (Zinc-finger
15 nucleases), and base editing, as well as newly developed techniques such as homing endonucleases and meganucleases (MegNs) (which target and cleave DNA sequences) and prime editing (which generates RNA templates for gene alteration).

III. Pharmaceutical Compositions and Administration

[0082] The present invention also provides pharmaceutical compositions or physiological
20 compositions comprising an effective amount of one or more agents useful in the methods of the present invention in both prophylactic and therapeutic applications. Such pharmaceutical or physiological compositions also include one or more pharmaceutically or physiologically acceptable excipients or carriers. For instance, one exemplary composition of this invention comprises or consists essentially of one or more expression vectors encoding a CRISPR
25 system (*e.g.*, a Cas9 nuclease or equivalent and one or two sgRNAs) plus one or more physiologically acceptable excipients or carriers. In another exemplary composition of this invention comprises or consists essentially of one or more expression vectors encoding one or more inhibitory oligonucleotides (*e.g.*, a small inhibitory RNA molecule or an antisense DNA or RNA oligonucleotide) plus one or more physiologically acceptable excipients or carriers.
30 Pharmaceutical compositions of the invention are suitable for use in a variety of drug delivery systems. Suitable formulations for use in the present invention are found in *Remington's*

Pharmaceutical Sciences, Mack Publishing Company, Philadelphia, PA, 17th ed. (1985). For a brief review of methods for drug delivery, *see*, Langer, *Science* **249**: 1527-1533 (1990).

[0083] The pharmaceutical compositions of the present invention can be administered by various routes, *e.g.*, oral, subcutaneous, transdermal, intramuscular, intravenous, or
5 intracranial. The preferred routes of administering the pharmaceutical compositions are local delivery to a relevant organ or tissue to the target disease in a recipient at a pre-determined daily dose. The appropriate dose may be administered in a single daily dose or as divided doses presented at appropriate intervals, for example as two, three, four, or more subdoses per day.

10 [0084] For preparing pharmaceutical compositions containing one or more active agents of this invention, inert and pharmaceutically acceptable carriers are also used. Typically, the pharmaceutical carrier can be either solid or liquid. Solid form preparations include, for example, powders, tablets, dispersible granules, capsules, cachets, and suppositories. A solid carrier can be one or more substances that can also act as diluents, flavoring agents,
15 solubilizers, lubricants, suspending agents, binders, or tablet disintegrating agents; it can also be an encapsulating material.

[0085] In powders, the carrier is generally a finely divided solid that is in a mixture with the finely divided active component. In tablets, the active ingredient is mixed with the carrier having the necessary binding properties in suitable proportions and compacted in the shape
20 and size desired.

[0086] For preparing pharmaceutical compositions in the form of suppositories, a low-melting wax such as a mixture of fatty acid glycerides and cocoa butter is first melted and the active ingredient is dispersed therein by, for example, stirring. The molten homogeneous mixture is then poured into convenient-sized molds and allowed to cool and solidify.

25 [0087] Powders and tablets preferably contain between about 5% to about 70% by weight of the active ingredient. Suitable carriers include, for example, magnesium carbonate, magnesium stearate, talc, lactose, sugar, pectin, dextrin, starch, tragacanth, methyl cellulose, sodium carboxymethyl cellulose, a low-melting wax, cocoa butter, and the like.

[0088] The pharmaceutical compositions can include the formulation of the active agent(s)
30 with encapsulating material as a carrier providing a capsule in which the agent or agents (with or without other carriers) is/are surrounded by the carrier, such that the carrier is thus in association with the agent(s). In a similar manner, cachets can also be included. Tablets,

powders, cachets, and capsules can be used as solid dosage forms suitable for oral administration.

[0089] Liquid pharmaceutical compositions include, for example, solutions suitable for oral or parenteral administration, suspensions, and emulsions suitable for oral administration.

5 Sterile water solutions of the active component (s) or sterile solutions of the active component(s) in solvents comprising water, buffered water, saline, PBS, ethanol, or propylene glycol are examples of liquid compositions suitable for parenteral administration. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as pH adjusting and buffering agents, tonicity
10 adjusting agents, wetting agents, detergents, and the like.

[0090] Sterile solutions can be prepared by dissolving the active component(s) in the desired solvent system, and then passing the resulting solution through a membrane filter to sterilize it or, alternatively, by dissolving the sterile component(s) in a previously sterilized solvent under sterile conditions. The resulting aqueous solutions may be packaged for use as
15 is, or lyophilized, the lyophilized preparation being combined with a sterile aqueous carrier prior to administration. The pH of the preparations typically will be between 3 and 11, more preferably from 5 to 9, and most preferably from 7 to 8.

[0091] The pharmaceutical compositions containing one or more active agents can be administered for prophylactic and/or therapeutic treatments. In therapeutic applications,
20 compositions are administered to a patient already suffering from Alzheimer's Disease in an amount sufficient to prevent, cure, reverse, or at least partially slow or arrest the symptoms of the disease and its complications, such as the onset, progression, duration, and severity of the disease. An amount adequate to accomplish this is defined as a "therapeutically effective dose." Amounts effective for this use will depend on the severity of the disease, the weight
25 and general state of the patient, as well as the nature of the active agent(s).

[0092] In prophylactic applications, pharmaceutical compositions containing one or more active agents are administered to a patient susceptible to or otherwise at risk of developing Alzheimer's Disease in an amount sufficient to delay or prevent the onset of the symptoms. Such an amount is defined to be a "prophylactically effective dose." In this use, the precise
30 amounts of the active agent(s) again depend on the patient's state of health and weight, as well as the nature of the active agent(s).

[0093] Single or multiple administrations of the compositions can be carried out with dose levels and pattern being selected by the treating physician. In any event, the pharmaceutical formulations should provide a quantity of agent(s) sufficient to effectively suppress APP expression and A β plaque formation in the patient, either therapeutically or prophylactically.

5 IV. Therapeutic Applications Using Nucleic Acids

[0094] A variety of conditions can be treated by therapeutic approaches that involve introducing a nucleic acid encoding one or more agents disrupting APP genomic sequence or inhibiting mRNA encoded by the APP genomic sequence (such as antisense or miRNA or Cas9 nuclease and sgRNAs) into a cell such that the coding sequence is transcribed and the
10 polypeptide or oligonucleotide agent is produced in the cell. For discussions on the application of gene therapy towards the treatment of genetic as well as acquired diseases, *see*, Miller *Nature* **357**:455-460 (1992); and Mulligan *Science* **260**:926-932 (1993).

A. Vectors for Gene Delivery

[0095] For delivery to a cell or organism, a polynucleotide encoding one or more active
15 agents can be incorporated into a vector. Examples of vectors used for such purposes include expression plasmids capable of directing the expression of the nucleic acids in the target cell. In other instances, the vector is a viral vector system wherein the polynucleotide is incorporated into a viral genome that is capable of transfecting the target cell. In one embodiment, the encoding polynucleotide can be operably linked to expression and control
20 sequences that can direct expression of the polypeptide or oligonucleotide in the desired target host cells. Often, the encoding sequence is transcribed under the control of a promoter and optionally at least one transcription enhancer element. Such a promoter may be constitutive (*i.e.*, always active for directing transcription of the coding sequence) or inducible (*i.e.*, becoming active for directing transcription of the coding sequence upon a
25 specific signal) in nature, and it is preferably a promoter specifically directing transcription in the particularly tissue type or cell type of the delivery target. For instance, a neuronal cell-specific promoter is preferably used for a vector intended for delivery into an anatomic site within the central nervous system, *e.g.*, brain. Thus, one can achieve expression of the polypeptide or oligonucleotide inhibitor under appropriate conditions in the target cells.

30 B. Gene Delivery Systems

[0096] Viral vector systems useful in the expression of a polypeptide or oligonucleotide disrupting a genomic sequence encompassing the APP coding sequence include, for example,

naturally occurring or recombinant viral vector systems. Depending upon the particular application, suitable viral vectors include replication competent, replication deficient, and conditionally replicating viral vectors. For example, viral vectors can be derived from the genome of human or bovine adenoviruses, vaccinia virus, herpes virus, adeno-associated virus (AAV), minute virus of mice (MVM), HIV, sindbis virus, and retroviruses (including but not limited to Rous sarcoma virus and lentivirus), and MoMLV. Typically, the coding sequence of interest (*e.g.*, one encoding for a polypeptide or oligonucleotide active agent of the present invention) are inserted into such vectors to allow packaging of the gene construct, typically with accompanying viral DNA, followed by infection of a sensitive host cell and expression of the coding sequence of interest.

[0097] As used herein, “gene delivery system” refers to any means for the delivery of a polynucleotide sequence of the interest to a target cell. In some embodiments of the invention, nucleic acids are conjugated to a cell receptor ligand for facilitated uptake (*e.g.*, invagination of coated pits and internalization of the endosome) through an appropriate linking moiety, such as a DNA linking moiety (Wu *et al.*, *J. Biol. Chem.* **263**:14621-14624 (1988); WO 92/06180), or by ultrasound-microbubble delivery system (Lan HY *et al.*, *J. Am Soc. Nephrol.* **14**:1535-1548). For example, nucleic acids can be linked through a polylysine moiety to asialo-oromucoid, which is a ligand for the asialoglycoprotein receptor of hepatocytes.

[0098] Similarly, viral envelopes used for packaging gene constructs that include the nucleic acids of the interest can be modified by the addition of receptor ligands or antibodies specific for a receptor to permit receptor-mediated endocytosis into specific cells (*see, e.g.*, WO 93/20221, WO 93/14188, and WO 94/06923). In some embodiments of the invention, the DNA constructs of the invention are linked to viral proteins, such as adenovirus particles, to facilitate endocytosis (Curiel *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **88**:8850-8854 (1991)). In other embodiments, the active agents of the instant invention can include microtubule inhibitors (WO/9406922), synthetic peptides mimicking influenza virus hemagglutinin (Plank *et al.*, *J. Biol. Chem.* **269**:12918-12924 (1994)), and nuclear localization signals such as SV40 T antigen (WO93/19768).

[0099] Retroviral vectors may also be useful for introducing the coding sequence of a polypeptide or oligonucleotide active agent of the invention into target cells or tissues. Retroviral vectors are produced by genetically manipulating retroviruses. The viral genome of retroviruses is RNA. Upon infection, this genomic RNA is reverse transcribed into a DNA

copy which is integrated into the chromosomal DNA of transduced cells with a high degree of stability and efficiency. The integrated DNA copy is referred to as a provirus and is inherited by daughter cells as is any other gene. The wild-type retroviral genome and the proviral DNA have three genes: the *gag*, the *pol* and the *env* genes, which are flanked by two long terminal repeat (LTR) sequences. The *gag* gene encodes the internal structural (nucleocapsid) proteins; the *pol* gene encodes the RNA directed DNA polymerase (reverse transcriptase); and the *env* gene encodes viral envelope glycoproteins. The 5' and 3' LTRs serve to promote transcription and polyadenylation of virion RNAs. Adjacent to the 5' LTR are sequences necessary for reverse transcription of the genome (the tRNA primer binding site) and for efficient encapsulation of viral RNA into particles (the Psi site) (*see*, Mulligan, In: *Experimental Manipulation of Gene Expression*, Inouye (ed), 155-173 (1983); Mann *et al.*, *Cell* **33**:153-159 (1983); Cone and Mulligan, *Proceedings of the National Academy of Sciences, U.S.A.*, **81**:6349-6353 (1984)).

[0100] The design of retroviral vectors is well known to those of ordinary skill in the art. In brief, if the sequences necessary for encapsidation (or packaging of retroviral RNA into infectious virions) are missing from the viral genome, the result is a *cis* acting defect which prevents encapsidation of genomic RNA. However, the resulting mutant is still capable of directing the synthesis of all virion proteins. Retroviral genomes from which these sequences have been deleted, as well as cell lines containing the mutant genome stably integrated into the chromosome are well known in the art and are used to construct retroviral vectors. Preparation of retroviral vectors and their uses are described in many publications including, *e.g.*, European Patent Application EPA 0 178 220; U.S. Patent No. 4,405,712; Gilboa *Biotechniques* **4**:504-512 (1986); Mann *et al.*, *Cell* **33**:153-159 (1983); Cone and Mulligan *Proc. Natl. Acad. Sci. USA* **81**:6349-6353 (1984); Eglitis *et al.* *Biotechniques* **6**:608-614 (1988); Miller *et al.* *Biotechniques* **7**:981-990 (1989); Miller (1992) *supra*; Mulligan (1993), *supra*; and WO 92/07943.

[0101] The retroviral vector particles are prepared by recombinantly inserting the desired nucleotide sequence into a retrovirus vector and packaging the vector with retroviral capsid proteins by use of a packaging cell line. The resultant retroviral vector particle is incapable of replication in the host cell but is capable of integrating into the host cell genome as a proviral sequence containing the desired nucleotide sequence. As a result, the patient is capable of producing, for example, a polypeptide or polynucleotide active agent useful in the

methods of the invention and thus restore the target cells (*e.g.*, brain endothelial cells) to a normal phenotype.

[0102] Packaging cell lines that are used to prepare the retroviral vector particles are typically recombinant mammalian tissue culture cell lines that produce the necessary viral structural proteins required for packaging, but which are incapable of producing infectious virions. The defective retroviral vectors that are used, on the other hand, lack these structural genes but encode the remaining proteins necessary for packaging. To prepare a packaging cell line, one can construct an infectious clone of a desired retrovirus in which the packaging site has been deleted. Cells comprising this construct will express all structural viral proteins, but the introduced DNA will be incapable of being packaged. Alternatively, packaging cell lines can be produced by transforming a cell line with one or more expression plasmids encoding the appropriate core and envelope proteins. In these cells, the *gag*, *pol*, and *env* genes can be derived from the same or different retroviruses.

[0103] A number of packaging cell lines suitable for the present invention are also available in the prior art. Examples of these cell lines include Crip, GPE86, PA317 and PG13 (*see Miller et al., J. Virol.* **65**:2220-2224 (1991)). Examples of other packaging cell lines are described in Cone and Mulligan *Proceedings of the National Academy of Sciences, USA*, **81**:6349-6353 (1984); Danos and Mulligan *Proceedings of the National Academy of Sciences, USA*, **85**:6460-6464 (1988); Eglitis *et al.* (1988), *supra*; and Miller (1990), *supra*.

[0104] Packaging cell lines capable of producing retroviral vector particles with chimeric envelope proteins may be used. Alternatively, amphotropic or xenotropic envelope proteins, such as those produced by PA317 and GPX packaging cell lines may be used to package the retroviral vectors.

C. Pharmaceutical formulations

[0105] When used for pharmaceutical purposes, the nucleic acid encoding a polypeptide or oligonucleotide active agent is generally formulated in a suitable buffer, which can be any pharmaceutically acceptable buffer, such as phosphate buffered saline or sodium phosphate/sodium sulfate, Tris buffer, glycine buffer, sterile water, and other buffers known to the ordinarily skilled artisan such as those described by Good *et al. Biochemistry* **5**:467 (1966).

[0106] The compositions can additionally include a stabilizer, enhancer or other pharmaceutically acceptable carriers or vehicles. A pharmaceutically acceptable carrier can

contain a physiologically acceptable compound that acts, for example, to stabilize the nucleic acids of the invention and any associated vector. A physiologically acceptable compound can include, for example, carbohydrates, such as glucose, sucrose or dextrans, antioxidants, such as ascorbic acid or glutathione, chelating agents, low molecular weight proteins or other
5 stabilizers or excipients. Other physiologically acceptable compounds include wetting agents, emulsifying agents, dispersing agents or preservatives, which are particularly useful for preventing the growth or action of microorganisms. Various preservatives are well known and include, for example, phenol and ascorbic acid. Examples of carriers, stabilizers or adjuvants can be found in Remington's *Pharmaceutical Sciences*, Mack Publishing Company,
10 Philadelphia, PA, 17th ed. (1985).

D. Administration of Formulations

[0107] The formulations containing a polynucleotide sequence encoding a polypeptide or oligonucleotide active agent can be delivered to target tissue or organ using any delivery method known to the ordinarily skilled artisan. In some embodiments of the invention, the
15 encoding polynucleotide sequences are formulated for subcutaneous, intramuscular, intravenous, or intraperitoneal injection, or for oral ingestion/nasal inhalation, or for topical application.

[0108] The formulations containing the nucleic acid of interest are typically directly administered to a cell. The cell can be provided as part of a tissue, such as red blood cells as
20 a part of the circulatory system, or as an isolated cell, such as in tissue culture. The cell can be provided *in vivo*, *ex vivo*, or *in vitro*.

[0109] The formulations can be introduced into the tissue of interest *in vivo* or *ex vivo* by a variety of methods. In some embodiments of the invention, the nucleic acids of interest are introduced into cells by such methods as microinjection, calcium phosphate precipitation,
25 liposome fusion, ultrasound, electroporation, or biolistics. In further embodiments, the nucleic acids are taken up directly by the target tissue or organ relevant to the disease or condition being treated, for example, when the targeted cells are the brain endothelial cells intracranial injection is appropriate.

[0110] In some embodiments of the invention, the nucleic acids of interest are administered
30 *ex vivo* to cells or tissues explanted from a patient, then returned to the patient. Examples of *ex vivo* administration of therapeutic gene constructs include Nolta *et al.*, *Proc Natl. Acad. Sci. USA* **93**(6):2414-9 (1996); Koc *et al.*, *Seminars in Oncology* **23**(1):46-65 (1996); Raper

et al., *Annals of Surgery* **223**(2):116-26 (1996); Dalesandro *et al.*, *J. Thorac. Cardi. Surg.*, **11**(2):416-22 (1996); and Makarov *et al.*, *Proc. Natl. Acad. Sci. USA* **93**(1):402-6 (1996).

[0111] Effective dosage of the formulations will vary depending on many different factors, including means of administration, target site, physiological state of the patient, and other medicines administered. Thus, treatment dosages will need to be titrated to optimize safety and efficacy. In determining the effective amount of the vector to be administered, the physician should evaluate the particular nucleic acid used, the disease state being diagnosed; the age, weight, and overall condition of the patient, circulating plasma levels, vector toxicities, progression of the disease, and the production of anti-vector antibodies. The size of the dose also will be determined by the existence, nature, and extent of any adverse side-effects that accompany the administration of a particular vector. For example, an antisense oligonucleotide in the amount of 1-1000, 10-200, or 20-100 mg can be delivered to a patient via intravenous injection at a frequency of weekly, bi-weekly, or monthly administration over at least one to three months or a longer time period. For CRISPR editing targeting the APP genomic region, as another example, each 5×10^5 cells (*e.g.*, HEK 293T cells or cells of a neuronal origin) are transfected with 0.5-50 μ g; 1-20 μ g; or 2-10 μ g of a vector carrying genes encoding Cas9 together with a pair of sgRNAs. For CRISPR editing targeting the APP genomic region in human patients, the dose for lipid nanoparticles carrying sgRNAs and mRNA encoding Cas9 is in the range of 0.01-2; 0.02-1.0; 0.05-0.5; or 0.10-0.30 mg/kg of body weight delivered by *i.v.* injection 1-3 times over a period of 1-4 weeks.

V. KITS

[0112] The invention also provides kits for treating or reducing risk of an amyloid pathology (such as Alzheimer's Disease) in a person in need thereof according to the method of the present invention. The kits typically include a container that contains (1) a pharmaceutical composition having an effective amount of one or more active agent capable of disrupting a genomic sequence encompassing APP coding sequence and/or suppressing mRNA transcribed from the genomic sequence; and (2) informational material containing instructions on how to dispense the pharmaceutical composition, including description of the type of patients who may be treated (*e.g.*, human patients suffering from Alzheimer's Disease or at increased risk for the disease), the schedule (*e.g.*, dose and frequency) and route of administration, and the like. In some cases, two or more containers are included in the kit to provide multiple pharmaceutical compositions each comprising an effective amount of at least one active agent, such as vector or vectors encoding components of a CRISPR system

(e.g., a Cas9 nuclease or equivalent and one or more sgRNAs) or encoding an siRNA, a microRNA, a miniRNA, a lncRNA, or an antisense oligonucleotide targeting the genomic sequence encompassing APP coding sequence. Optionally, the kit may further comprise one or more additional containers, each containing at least one agent useful for sequencing at least a portion of the person's genome, especially the APP genomic sequence encompassing subregion(s) of interest, e.g., encompassing one or more sites of known mutations such as the Swedish mutation.

EXAMPLES

[0113] The following examples are provided by way of illustration only and not by way of limitation. Those of skill in the art will readily recognize a variety of non-critical parameters that could be changed or modified to yield essentially similar results.

EXAMPLE 1: CRISPR-Mediated Disruption of APP Targeting the *Swedish* Mutation

[0114] Familial Alzheimer's disease is caused by dominant mutations in the genes that encode amyloid precursor protein (APP), presenilin 1, and presenilin 2. Pathological features including extracellular amyloid plaques and intracellular neurofibrillary tangles are present in multiple brain regions. Although targeting mutations using CRISPR/Cas9-mediated genome editing is a promising disease-modifying treatment strategy, brain-wide amelioration of Alzheimer's disease phenotypes using this approach has not been demonstrated. Here, we show that CRISPR/Cas9-mediated genome editing by a single administration of adeno-associated virus (AAV) efficiently and selectively disrupts mutated *APP* in transgenic mouse models that carry the human *APP* Swedish mutation, resulting in the alleviation of amyloid-beta-associated pathologies for at least 6 months post-injection. Importantly, intravenous delivery of a modified AAV-Cas9 that can cross the blood-brain barrier decreases the amyloid-beta-associated brain pathologies and the impairment of cognitive performance. Thus, our work on brain-wide gene editing provides a novel approach toward developing a noninvasive, single-administration, disease-modifying treatment for familial Alzheimer's disease and other monogenic diseases that affect multiple brain regions.

INTRODUCTION

[0115] Alzheimer's disease (AD), one of the most prevalent neurodegenerative diseases, is characterized by the deposition of amyloid-beta (A β) peptides and the presence of neurofibrillary tangles composed of hyperphosphorylated tau protein. While the inherited forms of AD, termed familial AD, account for 3–5% of AD cases^{1, 2, 3}, their prevalence is

comparable to that of other neurological diseases such as Huntington's disease⁴ and amyotrophic lateral sclerosis⁵. Familial AD is caused by fully penetrant and autosomal dominant mutations in the genes encoding amyloid precursor protein (APP) as well as the catalytic components of the γ -secretase complex: presenilin 1 (PS1) and presenilin 2 (PS2)².
 5 Dysregulated APP processing by γ -secretase causes the accumulation of A β peptides, which is a major factor in the disease pathogenesis⁶. Despite considerable advances in our understanding of the genetic causes of familial AD, there are currently no effective disease-modifying treatments.

[0116] Clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9-mediated
 10 genome editing is a powerful tool potentially capable of targeting specific mutations⁷. However, the development of CRISPR/Cas9 as a targeted genome-editing approach for disease-modifying familial AD therapies requires addressing the needs of widespread genome editing in the brain. Like most brain disorders, AD affects multiple brain regions including the cortex and hippocampus^{8, 9}. Current genome-editing strategies for brain disorders are
 15 delivered via intraparenchymal injection¹⁰, which affects limited brain regions with restricted beneficial outcomes. Therefore, an efficient and global genome-editing method that can be used in the adult brain is urgently needed. Modified adeno-associated virus (AAV) variants that can cross the blood-brain barrier (BBB) have recently been developed, enabling widespread central nervous system transduction after intravenous delivery^{11, 12, 13, 14}.
 20 Concordantly, brain-wide overexpression of a protein delivered via a BBB-crossing virus has been shown to promote recovery after ischemic stroke in aged mice¹⁴. Therefore, modifying the expression of disease-modifier gene(s) in the central nervous system by administration of a BBB-crossing virus has potential for development as a therapeutic strategy for diseases such as familial AD. However, the ability of such a virus to deliver CRISPR/Cas9 genome-
 25 editing components to ameliorate the disease phenotypes has not yet been tested *in vivo*.

[0117] Given that most cases of familial AD are caused by monogenic mutations, CRISPR/Cas9-mediated genome editing has been demonstrated for its ability to disrupt or correct familial AD mutations and decrease A β production *in vitro*^{15, 16}. Most patients with familial AD have a heterozygous disease-causing mutation², and humans that naturally carry
 30 one copy of *APP*, *PSEN1*, or *PSEN2* do not exhibit obvious neurological symptoms^{17, 18}. Thus, disruption of the disease-causing allele in familial AD patients at the adult stage is a rational and feasible disease-modifying treatment strategy for familial AD. Among the familial *APP* mutations, the *APP* Swedish (*APP*_{sw}) mutation (KM670/671) is located at the

sequence encoding the β -secretase cleavage site of APP and it makes the mutant APP more accessible for enzymatic cleavage, resulting in higher A β production^{19, 20, 21}. While a recent study reports the feasibility of disrupting the *APP_{swe}* mutation in *APP* mutation-carrying transgenic mice by genome editing, the low genome-editing efficiency of less than 3% prohibits the evaluation of the potential disease-modifying effect of disrupting this mutation¹⁶.

[0118] In the present study, we show that the AAV-mediated delivery of Cas9 and sgRNA specifically targeting the *APP_{swe}* mutation into the adult hippocampus generates efficient genome editing and alleviates multiple A β -associated pathologies in transgenic mouse models of A β deposition. Notably, this CRISPR-mediated beneficial effect persists for at least 6 months in the transgenic mice. Furthermore, a single systemic administration of a BBB-crossing AAV-Cas9 vector targeting the *APP_{swe}* mutation ameliorates A β pathologies throughout the brain. Thus, our results demonstrate that systemic AAV administered in a single dose is a promising approach for the development of disease-modifying therapeutic interventions for familial AD. Moreover, such a strategy could be applicable to other central nervous system disorders caused by autosomal dominant mutations that affect multiple brain regions.

RESULTS

CRISPR/Cas9-mediated genome editing specifically disrupts APP_{swe} allele

[0119] We first targeted the *APP_{swe}* double-base mutation (KM670/671NL) by designing 2 sgRNAs—SW1 and SW2—that place the mutation site within the first 8 nucleotides immediately adjacent to the protospacer-adjacent motif (PAM) sequence (5'-NNGRRT-3') of Cas9 from *Staphylococcus aureus* (SaCas9) (Fig. 1a); this region is considered the “seed sequence” region important for Cas9-sgRNA recognition and Cas9 nuclease activity²². Because mismatches between the sgRNAs and the wild-type (WT) sequence located in the seed sequence are poorly tolerated by Cas9²³, we expected SW1 and SW2 to distinguish the mutant *APP* sequence from the WT sequence. We validated the targeting specificities of the sgRNAs by using an EGxxFP reporter system²⁴ in HEK 293T cells (Fig. 1b). The expression of enhanced green fluorescent protein (GFP) indicated that both SW1 and SW2 cleaved the *APP_{swe}* sequence in the EGxxFP-*APP_{swe}* reporter and not the *APP_{WT}* sequence in the EGxxFP-*APP_{WT}* reporter (Fig. 1c). This indicates that both SW1 and SW2 conferred high allele specificity to the *APP_{swe}* mutation. Given that SW1 exhibits higher genome-editing efficiency in the EGxxFP-*APP_{swe}* reporter and that SW1-mediated genome editing would disrupt the β -secretase cleavage site of APP, we chose SW1 for the subsequent genome-

editing experiments. To confirm that the allele specificities of SW1 and SW2 were not due to their inability to edit the *APP_{WT}* allele, we generated 2 other sgRNAs—WT1 and WT2—to target the *APP_{WT}* allele at the same genetic loci corresponding to SW1 and SW2, respectively. The genome-editing efficiencies of WT1 and WT2 exceeded 50%, whereas neither SW1 nor

5 SW2 induced any observable genome editing in HEK 293T cells carrying *APP_{WT}* alleles (Fig. 8a, b), indicating that SW1 and SW2 specifically targeted the *APP_{swe}* mutation. Moreover, examination of potential off-target effects by T7 endonuclease I mismatch assay among the top 5 computationally predicted off-target sites detected no off-target activity in HEK 293T cells (Fig. 8c, d).

10 **[0120]** To investigate the ability of the CRISPR/Cas9 system to disrupt the *APP_{swe}* mutation *in vivo*, we packaged sgRNA SW1 together with Cas9 driven by the elongation factor 1-alpha short (EFS) promoter into a single AAV9 vector—EFS::Cas9-SW1—and injected the virus into the hippocampus of 3-month-old 5XFAD mice (Fig. 1d). These 5XFAD mice harbor human *APP* cDNA with K670N/M671L [Swedish], I716V [Florida],

15 and V717I [London] mutations. The CRISPR-mediated genome editing of the *APP* Swedish mutation can abolish the expression of full-length mutated APP and disrupt the effects of *APP* mutations. Four weeks post-injection, we collected the mouse brains for genomic analysis. AAV-mediated *in vivo* delivery of Cas9-SW1 efficiently edited the *APP_{swe}* mutation in the virus-transduced brain regions (27% genome-editing efficiency; Fig. 1e, f &

20 Fig. 8e). This suggests that AAV-mediated administration of Cas9-SW1 efficiently disrupted the *APP_{swe}* mutation *in vivo*.

[0121] We then examined the potential off-target events induced by Cas9-SW1 genome editing by performing whole-genome sequencing. A similar number of mutations (~35,000) was detected in both the virus-transduced and untransduced regions in individual mouse

25 brains ($n = 2$; Fig. 8f & Table 1), suggesting that off-target events induced by Cas9-SW1 editing are rare. We subsequently identified 257 somatic mutations residing in 46 genes as potential off-target events (Fig. 8g). In particular, we identified 17 genes marked by 23 somatic mutations in exons or untranslated regions that might affect the regulation of gene expression and hence function (Table 2). Of note, the cell-type-specific transcriptome

30 analysis suggested that only 3 genes—*Sp110*, *Sp140*, and *Abcg2*—were expressed in the mouse brain²⁵. Nonetheless, as these 3 genes were not prominently expressed in neurons²⁵, the identified mutations are unlikely to affect biological functions in the mouse brain.

Cas9-SW1 editing decreases amyloid plaque burden in 5XFAD mice

[0122] To examine whether the disruption of the *APP_{swe}* mutation in 5XFAD mice can decrease A β -associated pathologies, we delivered EFS::Cas9-SW1 via AAV injection into the hippocampus of 3-month-old 5XFAD mice, when A β plaques start to accumulate without causing any apparent functional deficits²⁶. By 6 months, 5XFAD mice develop severe A β -associated pathologies characterized by elevated A β levels, A β plaque deposition, gliosis, and neuronal dysfunction (Fig. 2–4)^{26, 27, 28, 29}. Three months after Cas9-SW1-mediated gene editing, the contents of both diethylamine-extracted soluble and formic acid-extracted insoluble A β in the dorsal hippocampal regions decreased (Fig. 2a) and the levels of both A β_{x-40} and A β_{x-42} species decreased by more than 60% (Fig. 2b, c).

[0123] We subsequently investigated whether the AAV-mediated expression of Cas9-SW1 affects amyloid plaque deposition. Three months after injection of Cas9-SW1, 6-month-old 5XFAD mice exhibited significantly lower A β plaque load in the virus-transduced hippocampal subregions when compared to the corresponding subregions of the uninjected side. Upon genome editing, the A β -positive areas decreased by 72.9% in the cornu ammonis (CA) region (Fig. 2d, f & Fig. 9) and by 77.9% in the subiculum (Fig. 2e, g & Fig. 9). Amyloid plaque burden remained lower in 9-month-old 5XFAD mice (i.e., 6 months post-injection); the A β -positive area decreased by 74.3% in the CA region (Fig. 2h, j & Fig. 9) and by 83.6% in the subiculum (Fig. 2i, k & Fig. 9). These results demonstrate that the effect of Cas9-SW1-mediated editing on amyloid pathology persisted for at least 6 months after a single administration (Fig. 2h–k & Fig. 9).

Cas9-SW1 editing decreases gliosis in 5XFAD mice

[0124] In 5XFAD mice, A β pathology is accompanied by gliosis, which is characterized by increased numbers of activated microglia and astrocytes surrounding amyloid plaques²⁶. Therefore, we examined whether the lower amyloid plaque burden observed in 5XFAD mice following genome editing is associated with decreased gliosis. In the subiculum of 5XFAD mice, which exhibits the most severe amyloid plaque deposition (Fig. 9), AAV-mediated Cas9-SW1 editing led to decreases of microgliosis and astrogliosis. Specifically, in 5XFAD mice, disruption of the *APP_{swe}* allele decreased the proportion of microglia labeled by ionized calcium-binding adapter molecule 1 (Iba1) to 64.9% (Fig. 3a, b & Fig. 10a). While AAV-mediated Cas9-SW1 editing did not change the number of astrocytes positive for glial fibrillary acidic protein (GFAP) (Fig. 3c, d & Fig. 10b), we observed a decrease in the GFAP-positive area (Fig. 3c, e & Fig. 10b). These findings collectively suggest that concurrent with

the decreases in A β levels and deposition, CRISPR/Cas9-mediated genome editing of familial AD mutations resulted in a decrease of gliosis, as indicated by fewer microglia and decreased activation of astrocytes.

Cas9-SW1 editing improves neuronal functions in 5XFAD mice

- 5 [0125] To determine whether a single injection of AAV-mediated Cas9-SW1 can reverse the hippocampal synaptic plasticity impairment in 5XFAD mice, we measured the formation of long-term potentiation (LTP). Compared to WT mice, LTP at Schaffer collateral–CA1 synapses was significantly impaired in 6-month-old 5XFAD mice; AAV-mediated Cas9-SW1 editing reversed the decrease of LTP by 90% (Fig. 4a, b). Furthermore, we
- 10 demonstrated that AAV-mediated Cas9-SW1 editing increased the number of excitatory synapses in the subiculum of 5XFAD mice by 43.9%, as evidenced by co-staining for the presynaptic marker synaptophysin and postsynaptic marker postsynaptic density 95 (PSD-95) (Fig. 4c–e). These results indicate that the genome editing decreased synaptic loss in the transgenic mice.
- 15 [0126] Dystrophic neurites, which are swollen neuritic processes that surround amyloid plaques, also contribute to synaptic impairment in AD. Specifically, the aggregation of lysosomes in these neurites, which can be labeled by the lysosomal protein LAMP1, alters axonal transports and synaptic release properties, consequently disrupting synaptic communication^{30, 31, 32}. Accordingly, AAV-mediated Cas9-SW1 editing in 5XFAD mice
- 20 decreased LAMP1-positive dystrophic neurites by approximately 50% (Fig. 4f, h & Fig. 11) and increased the number of NeuN-positive neurons in the subiculum (Fig. 4g, i), suggesting that genome editing decreased neuronal loss.

Cas9-SW1 editing decreases A β pathologies in APP/PS1 mice

- [0127] To examine the efficacy of the CRISPR-mediated disruption of the *APP_{swe}* mutation
- 25 in transgenic mice with advanced disease status, we injected AAV-EFS::Cas9-SW1 into the hippocampus of APP/PS1 mice, a transgenic mouse model of A β pathology with a temporally different onset of disease phenotypes compared to 5XFAD mice. In the APP/PS1 mouse model, A β plaque deposition and gliosis start to appear at approximately 4 months^{33, 34}; LTP impairment and cognitive deficits are observed at 6 and 12 months, respectively^{35, 36}.
- 30 Accordingly, we injected AAV-EFS::Cas9-SW1 at 9 months of age (when the A β -associated pathologies and hippocampal synaptic dysfunctions are well developed) and collected the brains 6 months later. Genome-editing efficiency was 24% in APP/PS1 mice (Fig. 12a, b),

which is comparable to 27% observed in 5XFAD mice. Moreover, after Cas9-SW1 administration, amyloid plaque burden was lower in the virus-transduced hippocampal regions of APP/PS1 mice than in the untransduced regions. Specifically, the amyloid plaque burden in the CA and subiculum in APP/PS1 mice decreased by 31.7% and 49.2%, respectively (Fig. 5a–d & Fig. 12c, d). Concurrent with the decreased A β deposition, Cas9-SW1 editing approximately halved the number of Iba1-labeled microglia (Fig. 5e, f). We also observed fewer GFAP-labeled astrocytes (Fig. 5g, h) and a smaller GFAP-positive area (Fig. 5g, i). These results collectively suggest that CRISPR/Cas9-mediated genome editing decreased amyloid deposition and gliosis in transgenic mice with well-developed disease-associated phenotypes.

Systemic delivery of Cas9-SW1 globally decreases A β pathologies in 5XFAD mice

[0128] Given that hippocampal injection of AAV-EFS::Cas9-SW1 decreased A β -related pathologies in the virus-transduced regions (Fig. 2–4), we investigated the possibility of achieving Cas9-SW1-mediated genome editing throughout the brain. The recent development of a BBB-crossing AAV9 variant, AAV-PHP.eB¹¹, has made it possible to efficiently deliver Cas9-SW1 into the adult brain via systemic administration. Accordingly, we generated a BBB-crossing AAV-Cas9 vector driven by a neuron-specific human synapsin 1 (Syn) promoter, SW1-Syn::Cas9 (Fig. 13a). Given that systemic administration of this AAV vector only resulted in limited expression of Cas9 in the mouse brain (Fig. 13b), we generated a virus construct that would result in enhanced expression of Cas9 *in vivo*. Specifically, inserting a truncated form of the Woodchuck Hepatitis Virus (WHP) Posttranscriptional Regulatory Element (WPRE)³⁷ into the AAV construct (SW1-Syn::Cas9-mWPRE; Fig. 6a) dramatically increased Cas9 expression in the hippocampus and cortex; it also kept the size of the construct within the ~5-kb package capacity of the AAV³⁸ (Fig. 13a). With these optimizations, the new BBB-crossing, neuron-specific, genome-editing system achieved a genome-editing efficiency in the brain comparable to that by intrahippocampal injection (Fig. 14a, b).

[0129] Systemic delivery of Cas9-SW1 into 5XFAD mice by AAV-PHP.eB led to widespread expression of HA-tagged Cas9 throughout the brain (Fig. 14c) and a decrease of amyloid plaque deposition in various brain regions (Fig. 6b–d). In particular, amyloid plaque burden was not only decreased in the CA and subiculum in the hippocampus (Fig. 6e–h & Fig. 14d), but also in the cortex (Fig. 6i, k) and other brain regions including the medulla of the brainstem (Fig. 6j, l & Fig. 14c). Meanwhile, systemic injection of Cas9 with a

scrambled sgRNA (i.e., AAV-PHP.eB:Con-Syn::Cas9-mWPRE) as a control did not cause any apparent adverse effects in either WT or 5XFAD mice. Specifically, the control virus induced neither gliosis nor behavioral deficits in WT mice (Fig. 15a–f). Moreover, the control virus did not affect the amyloid plaque burden in 5XFAD mice (Fig. 15g–i). Thus, our results collectively demonstrate that systemic administration of Cas9-SW1 decreased A β pathologies in multiple brain regions of 5XFAD mice.

Systemic delivery of Cas9-SW1 decreases microgliosis and neurite dystrophy, and improves cognitive performance in 5XFAD mice

[0130] After systemic Cas9-SW1 editing, 5XFAD mice exhibited fewer Iba1-labeled microglia (Fig. 7a, b) and LAMP1-positive dystrophic neurites in the subiculum (Fig. 7d, e), suggesting that systemic delivery of Cas9-SW1 globally decreased A β -associated phenotypes including microgliosis and neurite dystrophy in 5XFAD mice.

[0131] Previous studies report that transgenic mouse models of A β deposition exhibit impaired habituation and working memory as well as increased risk-taking behavior^{26, 35, 39, 40}. Given that systemic delivery of Cas9-SW1 globally decreased amyloid-associated pathologies, we assessed its beneficial effects on habituation ability, working memory, and risk-taking behavior of 5XFAD mice by using the exploratory open field test, Y-maze spontaneous alternation test, and elevated plus maze test, respectively.

In the open field test, the 5XFAD mice exhibited significantly slower habituation to the novel testing environment than the WT mice during the 3-day training course. In contrast to the uninjected 5XFAD mice, the mice that received Cas9-SW1 exhibited significantly improved habituation to the testing environment (Fig. 7e).

[0132] The Y-maze test measures the working memory of mice by allowing them to explore 3 arms of the maze. The ability of mice to alternate the arms into which they enter requires intact working memory (to remember which arm they previously visited). Compared to the WT mice, 5XFAD mice exhibited a significantly smaller percentage of spontaneous alternation among all entries (Fig. 7f). Meanwhile, 5XFAD mice that received Cas9-SW1 exhibited a significantly greater percentage of spontaneous alternation than those that did not receive Cas9-SW1 (Fig. 7f).

[0133] Meanwhile, the elevated plus maze test examines the risk-taking behavior and explorative drive of mice by evaluating their exploration of open anxiogenic areas^{39, 40}. The 5XFAD mice spent more time in the open arms than the WT mice, while the 5XFAD mice

that received Cas9-SW1 spent significantly less time in the open arms, compared to uninjected 5XFAD mice (Fig. 7g). These results collectively suggest that systemic delivery of Cas9-SW1 improved the cognitive performance of the 5XFAD mice.

DISCUSSION

- 5 [0134] In this study, we show that a single injection of AAV-Cas9 vector selectively and efficiently edited the human *APP_{swe}* allele *in vivo*, alleviating A β -associated pathologies in transgenic mouse models of A β deposition. By utilizing an improved BBB-crossing AAV vector, AAV-PHP.eB¹¹, systemic administration of this CRISPR/Cas9 system in transgenic mice achieves efficient genome editing in multiple brain regions and exerts a global
- 10 beneficial effect, ameliorating A β deposition, microgliosis, and neurite dystrophy as well as improving cognitive functions. To our knowledge, this is the first demonstration of systemic AAV delivery targeting a disease-causing mutation in the adult stage that resulted in efficient brain-wide genome editing, consequently alleviating disease phenotypes throughout the brain.
- [0135] Among the familial AD mutations, the *APP_{swe}* mutation is a double-point mutation
- 15 located in the “seed sequence” of SaCas9, which enables selective target recognition; this also makes it an ideal candidate for allele-specific editing, because the mismatches between the sgRNA and genomic DNA in the “seed sequence” region are poorly tolerated by Cas9 nuclease²². While selective disruption of the mutated allele of *APP_{swe}* has been achieved in the hippocampus in a transgenic mouse model of A β deposition by utilizing 2 AAVs that
- 20 express an *APP_{swe}*-specific sgRNA and Cas9 nuclease from *Streptococcus pyogenes* (SpCas9, 1,368 amino acids), respectively, the genome-editing rate of that CRISPR/Cas9 system on the familial AD mutant allele was less than 3%¹⁶. Therefore, to increase Cas9 editing efficiency, we utilized a CRISPR/Cas9 system that can be packaged into an all-in-one AAV vector with the smaller SaCas9 (1,053 amino acids) and its sgRNA instead of 2 vectors for SpCas9⁴¹.
- 25 Accordingly, we successfully improved the *in vivo* editing efficiency of the CRISPR/Cas9 system to around 30%. While the *APP_{swe}* mutation accounts for only a small proportion of familial AD cases, other familial AD mutations such as *PSEN1* L166P are also amenable to allele-specific gene disruption. Over 200 dominant disease-causing mutations in *APP*, *PSEN1*, and *PSEN2* have been identified to date⁴². Moreover, the development of the SaCas9
- 30 variant, which possesses alternative PAM compatibility⁴³, has expanded the targeting range of the CRISPR/Cas9 system, thereby broadening the candidate nucleotide sequences in familial AD mutations for allele-specific disruption. Although familial AD only accounts for

approximately 3–5% of the total AD cases^{1, 2, 3}, this CRISPR/Cas9-mediated gene approach still has immense potential for therapeutic development for familial AD.

[0136] While two recent studies have demonstrated that CRISPR/Cas9-based strategies can ameliorate A β -associated phenotypes in transgenic mouse models, their clinical translatability might incur challenges. In one study, decreasing *APP* expression in the germline of *APP*-knock-in mice using a CRISPR/Cas9 system alleviated amyloid plaque burden⁴⁴. However, it might not be possible to translate germline editing to an intervention strategy for AD. The other study used CRISPR/Cas9-mediated genome editing to knock out β -secretase 1 (BACE1) in the hippocampus of adult transgenic mice to alleviate A β -associated pathologies⁴⁵.

BACE1 is not a good target for clinical applications, however, because its inhibition can cause detrimental effects such as impairment of synaptic function and cognitive behaviors^{46, 47, 48}. In this study, we show that CRISPR/Cas9-mediated disruption of *APP*_{*swe*} allele ameliorates not only amyloid plaque deposition but also gliosis, synaptic dysfunctions, and cognitive impairment in transgenic mouse models of A β deposition. Thus, disruption of the disease-causing allele in familial AD patients in the adult stage is a promising disease-modifying strategy for familial AD.

[0137] Our study also effectively addresses one major challenge of developing CRISPR/Cas9 systems for AD therapy: the need for brain-wide genome editing. AD affects multiple brain regions, but current delivery methods into the adult brain (both viral and nonviral) require intraparenchymal injection¹⁰, which limits the Cas9-edited region. Single-site hippocampal injection of AAV9 only infects part of the hippocampus. Thus, the insufficient delivery of the CRISPR/Cas9 system may negate its potential beneficial effects. Therefore, efficient brain-wide delivery of genome-editing components is urgently needed for the therapeutic development of the CRISPR/Cas9 system for familial AD. We modified the AAV-PHP.eB¹¹ BBB-crossing virus with the CRISPR/Cas9 system to edit the *APP* gene *in vivo*. Importantly, we used a neuron-specific promoter to drive the expression of Cas9 to achieve neuron-specific genome editing, because A β is mainly produced by APP-expressing neurons. This system only disrupts the mutated *APP* in neurons, which mitigates the potential safety issue caused by Cas9 editing, because post-mitotic neurons cannot divide. With the optimization of the Cas9 expression construct (i.e., the insertion of a truncated WPRE element into the AAV vector construct to enhance Cas9 expression), our PHP.eB-mediated CRISPR/Cas9 system achieved a global editing rate in the brain comparable to that by local intrahippocampal injection. Although AAV-PHP.eB is limited to certain strains of

mice, there are emerging engineered vectors that can transduce previously resistant strains such as BALB/c and other species such as marmosets^{49, 50}. Given these ongoing developments, our work is a proof-of-concept towards genome-editing therapy using systemic delivery vectors.

5 [0138] To bolster the therapeutic development of CRISPR-mediated genome editing, it is important to evaluate the potential off-target events induced by genome editing. Herein, comparison of the whole-genome sequencing data of virus-transduced and untransduced brain regions within an individual animal suggested that off-target events induced by Cas9-SW1 editing are rare, as the number of the mutations in virus-transduced regions was close to
10 the basal number in the untransduced regions. After prioritizing the mutations according to their genomic locations and the brain expression of corresponding genes, we narrowed the potential off-target events of Cas9-SW1 to 3 genes expressed in the brain—*Sp110*, *Sp140* and *Abcg2*—whose expression levels might be affected. In particular, *Sp110* and *Sp140* belong to the nuclear body protein family, which potentially regulate gene transcription⁵¹, whereas
15 *Abcg2* belongs to the ATP-binding cassette (ABC) transporter superfamily, which serves as a xenobiotic exporter⁵². Notably, these genes are not prominently expressed in neurons²⁵. As the synapsin-driven Cas9 system can only target the neurons, these mutations are unlikely to affect the expression and hence functions of these genes in neurons. Therefore, the biological effects of these potential off-target mutations are likely to be minimal. Furthermore, our
20 findings also underscore the importance of an unbiased and comprehensive analysis of potential off-target events, such as whole-genome sequencing, for the development of CRISPR/Cas9-mediated gene therapy.

[0139] In summary, we applied a BBB-crossing, AAV-mediated, CRISPR/Cas9-based strategy to selectively and efficiently edit a disease-causing allele *in vivo*. Our proof-of-
25 concept study demonstrates that the brain-wide editing of the mutated *APP_{swe}* allele led to persistent beneficial effects *in vivo*, including alleviated A β -associated pathologies, which makes it a promising strategy for further development. Hence, our AAV-mediated, single-dose, noninvasive CRISPR/Cas9 system represents a promising approach to address the lack of therapeutic options for familial AD as well as other forms of brain diseases caused by
30 dominant mutations that affect multiple brain regions.

METHODS

Molecular cloning and GFP reporter assay

[0140] We adapted the AAV packaging construct from pX601-AAV-CMV::NLS-SaCas9-NLS-3xHA-bGHpA;U6::BsaI-sgRNA (Zhang Lab; Addgene plasmid #61591)⁴¹. For intrahippocampal injection, we replaced the cytomegalovirus (CMV) promoter with the promoter of EFS. For systemic injection, we used a modified pX601 construct in which the promoter was replaced with the human synapsin 1 (hSyn) gene promoter and a truncated form of WPRE cassette (Addgene plasmid #61463) was added to enhance Cas9 expression³⁷. Moreover, we replaced bovine growth hormone (BGH) poly A with synthetic poly(A) (spA). We inserted the sgRNAs into the construct by BsaI sites. The sequences of the sgRNAs are listed in Table 3.

[0141] Masahito Ikawa generously provided the GFP reporter construct, pCAG-EGxxFP (Addgene plasmid #50716)²⁴, to evaluate the genome-editing efficiency of Cas9-sgRNA. We inserted the *APP_{swc}* and *APP_{WT}* alleles by ligating oligos into BamHI and EcoRI sites. We mixed 1 µg Cas9-sgRNA with 1 µg pCAG-EGxxFP-APP and transfected the mixture into HEK 293T cells using Lipofectamine 3000 (Thermo Fisher Scientific). We examined the resultant GFP expression by fluorescence microscopy 12 h post-transfection.

Mice

[0142] We obtained 5XFAD transgenic mice, which harbor 5 familial AD mutations (*APP* K670N/M671L [Swedish], I716V [Florida], V717I [London], and *PSEN1* M146L and L286V)²⁶, and APP/PS1 transgenic mice, which harbor the *APP* K670N/M671L (Swedish) mutation and *PSEN1* exon 9 deletion³³ from the Jackson Laboratory (stock no. 008730 and 004462, respectively). All experiments were conducted using male 5XFAD mice or female APP/PS1 mice. All mice were housed in the Animal and Plant Care Facility at the Hong Kong University of Science and Technology. The Animal Ethics Committee of the Hong Kong University of Science and Technology approved all animal experiments.

Intrahippocampal and systemic injection of AAV

[0143] For AAV used in intrahippocampal injections, AAV9-Cas9-SW1 was generated by the Vector Core at the University of North Carolina at Chapel Hill and titered by dot blot. We anesthetized 3-month-old mice with isoflurane and injected 6×10^{10} vector genomes (vg) virus per animal (2 µL of 3×10^{13} vg/mL). We used the following coordinates (relative to the bregma) to target the CA1 region of the hippocampus: anteroposterior (AP), -2.0 mm;

mediolateral (ML), -1.7 mm; dorsoventral (DV), -1.4 mm⁵³. Given that intrahippocampal injection of empty AAV9 vector does not generate adverse effects in mice^{54, 55, 56}, the uninjected side of the same mouse served as the control. We sacrificed the mice 3 or 6 months after virus injection.

- 5 [0144] For AAV used in systemic delivery, we generated AAV-PHP.eB: Cas9-Con and AAV-PHP.eB:Cas9-SW1 as previously described⁵⁷ and titrated it by quantitative polymerase chain reaction (qPCR). We anesthetized 3-month-old mice and retro-orbitally injected the virus at a dose of 1×10^{13} vg/animal (100 μ L of 1×10^{14} vg/mL). We sacrificed the mice at 6 or 9 months of age to analyze A β -associated pathologies.

10 ***T7 endonuclease I assay***

- [0145] We performed the T7 endonuclease I mismatch assay to analyze editing efficiency. Briefly, we dissected the subiculum and cornu ammonis regions from 300- μ m-thick hippocampal slices and extracted the genomic DNA using QuickExtract DNA Extraction Solution (Epicentre Biotechnologies, QE09050). Amplified DNA was denatured and
15 reannealed from 95°C to room temperature. We subsequently added T7 endonuclease I (New England Biolabs, M0302) and incubated the mixture at 37°C for 15 min. We analyzed the products by 2.5% agarose *gel electrophoresis* for visualization. Next, we quantified the intensity of enzyme-cut and enzyme-uncut bands using ImageJ (NIH) software. Finally, we calculated the editing efficiency as follows:

$$20 \text{ Editing efficiency \%} = 1 - \sqrt{1 - \frac{\text{cut bands}}{(\text{uncut band} + \text{cut bands})}} \times 100$$

Genome sequencing and bioinformatics analysis

- [0146] We extracted genomic DNA using the E.Z.N.A. Tissue DNA Kit (Omega Bio-tek) and subjected it to the Illumina NovaSeq (Novogene) for whole-genome sequencing (50 $\times$). We subjected raw reads (150 bp paired-end) to the Trimmomatic (version 0.32) for the
25 trimming and filtering of low-quality reads. The reads that passed quality control were mapped to a modified version of the mouse reference genome (UCSC mm10) with the further inclusion of sequences for human *APP* and *PSEN1* using BWA mem (version 0.7.12-r1039). Germline and somatic mutations were genotyped following the Genome Analysis Toolkit (GATK) Best Practices (Version 4.1.2.0) using the default setting. Specifically, the PCR
30 duplicated reads were marked, and Base Quality Score Recalibration (BQSR) was carried out

during the processing of BAM files. We subjected the final BAM files obtained by merging reads from multiple lanes to variant calling. We detected germline and somatic mutations using HaplotypeCaller (version 4.1.2.0) and MuTect2 (version 4.1.2.0), respectively.

[0147] We considered mutations in the virus-transduced regions of both mice that were not detected in the untransduced regions of either mouse as having greater potential to be induced by genome editing. To detect germline mutations, we applied Base Quality Score Recalibration (BQSR) using single nucleotide polymorphism (SNP) and insertion/deletion (INDEL) calls from version 3 of the Mouse Genome Project, which comprises genomic variants detected from 18 mouse strains⁵⁸. We estimated the frequencies of editing events at corresponding sites for each genomic DNA sample on the basis of the read counts of the modified and unmodified alleles stored in the VCF files. We used R programming (R studio; version 1.3.1056) to filter mutations that resided in the repetitive elements (UCSC repeatmasker mm10) and mutations that overlapped with the germline mutations. We annotated the presence of the genetic variants in the gene body from the Ensemble database obtained from the BioMart database (<http://asia.ensembl.org/biomart>). We further designed somatic mutations that resided in exons and untranslated regions. We subsequently examined the expression levels of associated genes in brain cells by querying the cell-type-specific transcriptome dataset (Brain RNA-Seq)²⁵. We considered genes to be expressed in the brain if FPKM was greater than 1 in at least one brain cell type.

20 *Aβ extraction, western blot analysis, and ELISA*

[0148] We sequentially extracted Aβ peptides from the soluble and insoluble fractions of the dorsal hippocampi³⁵. We first homogenized frozen hippocampal tissues in tissue homogenization buffer (20 mM Tris · HCl [pH 7.4], 250 mM sucrose, 1 mM EDTA, and 1 mM EGTA) with protease inhibitor cocktail (Sigma-Aldrich). We sequentially extracted soluble and insoluble Aβ by using diethylamine and formic acid, respectively. We analyzed the levels of soluble and insoluble Aβ by western blot analysis. Moreover, we analyzed soluble Aβ_{x-40} and Aβ_{x-42} by using the V-PLEX Aβ Peptide Panel 1 (6E10) Kit (Meso Scale Discovery).

Immunohistochemistry

30 [0149] We anesthetized the mice with pentobarbital and perfused them transcardially with Dulbecco's PBS (DPBS). We dissected the mouse brains, post-fixed them in 4% paraformaldehyde, and sectioned them into 30-μm sagittal brain slides by using a free-

floating vibratome (VT1000S, Leica) for immunohistochemistry. For 4G8 immunostaining, we performed antigen retrieval with 70% formic acid. We then washed the brain sections 3 times with DPBS, blocked them with 2% goat serum and 0.3% Triton X-100 in DPBS⁵⁹ for 60 min at room temperature, and then incubated them with primary antibodies overnight at 4°C.

[0150] We used the following primary antibodies: anti-HA tag antibody (1:50; #3724, Cell Signaling Technology); anti-A β , 17-24 antibody (1:1,000, clone 4G8, 800701, BioLegend); anti-Iba1 antibody (1:500, 019-19741, Wako); anti-GFAP antibody (1:5,000, #3670, Cell Signaling Technology); anti-PSD-95 antibody (1:500, ab2723, Abcam); anti-synaptophysin 1 antibody (1:500, 101011, Synaptic Systems); anti-LAMP-1 antibody (1:500, 1D4B, Developmental Studies Hybridoma Bank); and anti-NeuN antibody (1:100, MAB377, Millipore).

[0151] After we incubated the sections with primary antibodies, we washed them 3 times with DPBS and 0.3% Triton X-100 (DPBST). We then incubated the sections with secondary antibodies (i.e., anti-rabbit, anti-mouse, or anti-rat Alexa Fluor 488, 546, 568, or 647; Thermo Fisher Scientific) in blocking buffer for 120 min at room temperature. After applying the corresponding secondary antibodies, we stained the brain sections with nuclear staining dye (4',6-diamidin-2-phenylindol [DAPI, 5 μ g/mL] or SYTOX Green [1:30,000, S7020, Thermo Fisher Scientific]) before mounting them onto slides.

[0152] For X34 amyloid plaque staining, we incubated the brain sections in 1 μ M X34 in X34 staining buffer (40% ethanol/60% DPBS mix [pH adjusted to 10]) for 10 min at room temperature and then washed them 3 times with X34 staining buffer⁶⁰.

[0153] We performed imaging using a Leica DM6000 B compound microscope system and a Leica TCS SP8 confocal system.

Electrophysiology

[0154] We sacrificed the mice, dissected their brains, and immediately transferred the brains to ice-cold oxygenated (95% O₂/5% CO₂) artificial cerebrospinal fluid buffer. We subsequently prepared 300- μ m brain slices by using a vibratome (HM650V; Thermo Fisher Scientific) and recovered them in artificial cerebrospinal fluid at 32°C for at least 1 h. We recorded the CA1 field excitatory postsynaptic potentials (fEPSPs) by using MED-P210A probes (Panasonic International) with a 100- μ m interelectrode distance. After recording the baseline for 30 min, we induced LTP by 4 trains of theta-burst stimulation (10 brief bursts

consisting of 4 pulses at 100 Hz)^{61, 62}. We continued to record the fEPSPs for 60 min. Finally, we quantified the magnitude of LTP as the percentage change in the average slope of the fEPSP from 50–60 min after LTP induction.

Behavioral tests

5 [0155] For animal behavioral testing, we submitted the mice to the open field test and Y-maze spontaneous alternation test, then let them rest for 2 months, and finally submitted them to the elevated plus maze test.

[0156] For the open field test, we recorded the locomotor activity of the mice using a photobeam activity system and software (San Diego Instruments)³⁵. We placed each mouse
10 in the center of an open-top chamber (41 × 41 × 38 cm) and allowed the mouse to explore the chamber for 15 min each day for 3 consecutive days. The distance moved was recorded by the photobeams.

[0157] The spontaneous alternation Y-maze test examines the spontaneous alternation performance of mice using a symmetrical Y-maze (30 cm long × 20 cm high × 8 cm wide)⁶³.
15 Each mouse was habituated to the Y-maze environment 1 day before the test. On the test day, we placed each mouse into the center of the Y-maze and allowed it to explore for 8 min. The sequence and total number of arms entered were recorded using EthoVision XT7 (Noldus). We calculated the percentage of alternation by dividing the number of consecutive entries into all 3 arms by the total number of arms entered minus 2. We did not count reentry into
20 the same arm for analysis.

[0158] Finally, the elevated plus maze test measures risk-taking behavior using a plus-shaped maze elevated 60 cm above the floor. Four arms (30 cm long × 5 cm wide), including 2 open arms and 2 closed arms with 15-cm-high walls, extend from the central platform. We placed each mouse on the central platform facing an open arm and allowed them to freely
25 explore the maze for 5 min³⁹. The time the mice spent in each zone (i.e., closed arms, open arms, or the central area) was recorded using EthoVision XT7 (Noldus).

Statistical analysis

[0159] All data are presented as the mean ± SEM, and *n*-values indicate the number of individual experiments or mice. All statistical analyses were performed using GraphPad
30 Prism (version 6.01). The significance of differences was assessed by the paired or unpaired two-tailed Student's *t*-test and two-way repeated-measures ANOVA as indicated in the figure legends. We used the paired two-tailed *t*-test to analyze the intrahippocampal injections,

because the comparison was between the uninjected and injected sides of the hippocampus within a single mouse. Two-way, repeated-measures ANOVA was performed to analyze habituation in the open field test as a group comparison; the unpaired two-tailed *t*-test was performed in other instances. Intergroup differences were considered statistically significant at $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$. No statistical methods were used to predetermine sample size. We randomly assigned the mice to different experimental groups. The investigators who performed the immunohistochemical, electrophysiological, and the behavioral tests were blinded to the genotypes of the mice and injection conditions.

Table 1: Mutations detected by whole-genome sequencing in the virus-transduced and untransduced brain regions

Sample	Untransduced region	Virus-transduced region	Shared in virus-transduced and untransduced regions	Uniquely identified in untransduced region (%)	Uniquely identified in transduced region (%)
Mouse 1	35,053	33,727	23,412	11,641 (33.2%)	10,315 (30.6%)
Mouse 2	35,193	34,458	23,459	11,734 (33.3%)	10,656 (30.9%)

Table 2: Summary of potential off-target sites and consequences

Genomic location	Number of mutations	Number of corresponding genes*	Number of genes expressed in brain cells
Intergenic	131	N/A	N/A
Intronic	103	37	14
Exonic	13	13	1
3' UTR	7	2	0
5' UTR	3	3	2

* A given gene may be counted more than once owing to the presence of multiple somatic mutations residing in different locations.

Table 3: Sequences of sgRNAs

Name	Sequence	PAM
SW1	TCTCTGAAGTGAATCTGGATG	CAGAAT
SW2	GAGGAGATCTCTGAAGTGAAT	CTGGAT
WT1	TCTCTGAAGTGAAGATGGATG	CAGAAT
WT2	GAGGAGATCTCTGAAGTGAAG	ATGGAT
CON	GGAGACCACGGCAGGTCTCA	-

PAM, protospacer-adjacent motif.

EXAMPLE 2: Disruption of APP Targeting the β - and γ -Secretase Cleavage Sites**INTRODUCTION**

- [0160] Alzheimer's disease (AD), one of the most prevalent neurodegenerative diseases, is characterized by the deposition of amyloid-beta ($A\beta$) peptides and the presence of neurofibrillary tangles comprised of hyperphosphorylated Tau protein. APP (amyloid precursor protein) is the precursor protein for $A\beta$, one of the hallmarks of AD. Missense mutations or copy number duplication in APP gene is sufficient to cause early-onset AD. $A\beta$ is mainly generated upon cleavage of APP in neurons; hence, decreasing the APP gene, which would lower $A\beta$ generation, could be a potential therapeutic strategy for AD treatment.
- APP has a physiological role during neural development, including neuronal migration and synaptic plasticity. Thus, neuronal-specific modulation of APP in the adult stage may be a beneficial approach for treating AD with minimal adverse effects.
- [0161] APP is the precursor protein of $A\beta$, the main constituent of amyloid plaques. APP can undergo amyloidogenic and non-amyloidogenic proteolytic pathways. In amyloidogenic pathway, APP is first cleaved by β -secretase, forming a soluble secreted form of APP (sAPP β) and a C-terminal fragment (β APP-CTF). β APP-CTF is sequentially cleaved by γ -secretase, resulting in the generation of the APP intracellular domain (AICD) and $A\beta$ peptides of varying lengths, including synaptotoxic 40-residue peptide ($A\beta_{1-40}$) and 42-residue peptide ($A\beta_{1-42}$). The non-amyloidogenic pathway, *i.e.*, the α -secretase-mediated cleavage pathway, inhibits generation of $A\beta$. In this pathway, cleavage of APP by α -secretase is within the $A\beta$ sequence, which prevents $A\beta$ formation.
- [0162] Most familial AD-causing mutations cluster at or near the cleavage sites of α -, β -, and γ -secretases, promoting $A\beta$ formation by favoring the proteolytic processing of APP by β - and γ -secretases. This results in increased total $A\beta$ or $A\beta_{42}$ levels and promotes the self-aggregation of $A\beta$ into amyloid plaques. Therefore, disrupting the cleavage sites of β - and γ -secretases in APP can inhibit the amyloidogenic proteolytic pathway of APP and prevent the generation of toxic $A\beta$. Microbial clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9-mediated genome editing is a powerful tool that disrupts the genome sequence that encodes both β - and γ -secretases and has great potential for AD treatment.
- [0163] In this invention, we designed single-guide RNAs (sgRNAs) to specifically target exons 14–17 of the APP gene (**Figure 16 & Tables A, B**) using a neuron-specific promoter (**Table C**) and analyzed the editing efficiency *in vitro* (**Figure 17**). Compared to that targets specific mutation site (*i.e.*, APP Swedish mutation), CRISPR/Cas9-mediated deletion of β -

secretase cleavage site of APP can be used to decrease APP expression in wild-type APP as well as in APP with most of the familial APP mutations (**Figure 18, Table D**). Thus, this approach can be developed for the treatment of most of familial AD cases. We demonstrated that CRSIPR/Cas9-mediated deletion of β -secretase cleavage site of APP significantly
 5 reduced A β secretion (**Figure 18**). These data collectively indicate that targeting β - and γ -secretase cleavage sites of APP is a feasible approach to reduce A β production and could be applied to the treatment for most of patients with familial AD mutations.

METHODS

Plasmids

10 [0164] We inserted sgRNA into AAV-EFS::NLS-SaCas9-NLS-3xHA-bGHpA;U6::sgRNA via the BsaI site (New England Biolabs). We then transformed the ligation product into NEB Stable Competent *E. coli* (New England Biolabs) for molecular cloning. We prepared plasmid DNAs using the E.Z.N.A. Plasmid DNA Mini Kit I (Omega Bio-tek) according to the manufacturer's instructions, and prepared large-scale plasmids in endotoxin-
 15 free conditions using the Qiagen Plasmid Maxi Kit (Qiagen).

Cell culture

[0165] We incubated HEK 293T cells in Dulbecco's modified Eagle medium (DMEM, Invitrogen) with 10% heat-inactivated fetal bovine serum (v/v, GIBCO) plus 1% penicillin/streptomycin. We transfected HEK 293T cells using Lipofectamine 3000 Reagent
 20 (Thermo Fisher Scientific) according to the manufacturer's instructions. We harvested cells 48 h post transfection and collected medium and cell pellets.

Genomic DNA extraction and PCR amplification

[0166] We extracted genomic DNA from cell pellets using QuickExtract™ DNA Extraction Solution (Lucigen) according to the manufacturer's instructions. We used KAPA
 25 HiFi HotStart ReadyMix (Roche) to amplify the genomic DNA of 293T cells that were cotransfected with the gRNA/Cas9 plasmid and pcDNA3-APP plasmid. PCR amplification was as follows: denaturation at 98° C for 30 sec. followed by (98° C 20 sec, 65° C 20 sec, and 72° C 30 sec) for 30 cycles; and 72° C for 2 minutes as a final elongation cycle.

T7 endonuclease I assay

30 [0167] We performed the T7 endonuclease I mismatch assay to analyze editing efficiency. We added T7 endonuclease I (New England Biolabs, M0302) into the PCR products and

incubated the mixture at 37°C for 15 min. We analyzed the products by 2.5% agarose gel electrophoresis for visualization. Next, we quantified the intensity of enzyme-cut and enzyme-uncut bands using ImageJ (NIH) software. Finally, we calculated the editing efficiency as follows:

$$\text{Editing efficiency \%} = 1 - \sqrt{1 - \frac{\text{cut bands}}{(\text{uncut band} + \text{cut bands})}} \times 100$$

5

Quantification of secreted A β

[0168] We quantified secreted A β by using MSD Human (6E10) Abeta3-Plex Kits (Meso Scale Discovery) according to the manufacturer's instructions. We normalized secreted A β levels to total protein levels determined by BCA assay (Thermo Fisher Scientific).

10 **Table A: Sequence of exons 14–17 in APP**

Exon	Sequence
4	Atgagctgcttcagaaagagcaaaactattcagatgacgtcttgccaacatgattagtgaaccaaggat cagttacggaaacgatgctctcatgccatcttgaccgaaacgaaaaccaccgtggagctcctcccgtga atggagagttcagcctggacgatctccagccgtggcattctttggggctgactctgtgccagccaacac agaaaacgaag
5	ttgagcctgttgatgcccgcctgctgccgaccgaggactgaccactcgaccag
6	Gttctgggttgacaaatatcaagacggaggagatctctgaagtgaagatggatgcagaattccgacatg actcaggatatgaagttcatcatcaaaaattg
7	Gtgttcttgcagaagatgtgggttcaacaaaggtgcaatcattggactcatggtgggcggtgtgtgat agcgacagtgatcgatcatcaccttggtgatgctgaagaagaaacagtacacatccattcatcatggtgtg gtggag

Table B: Sequence for deletion of APP in exons 14–17

Name	Cas9 & variants	Sequence	PAM
APP1	SaCas9	tcatgttggccaagacgtcat	ctgaat
APP2	SaCas9	ggccaacatgattagtgaacc	aaggat
APP3	SaCas9	accaccgtggagctcctccc	gtgaat
APP4	SaCas9	tggagctcctcccgtgaatg	gagagt
APP5	SaCas9	ctggcacagagtcagcccaa	aagaat
APP6	SaCas9	cgtttctgtgttgctggca	cagagt
APP7	SaCas9	gaggagatctctgaagtgaag	atggat

APP8	SaCas9	tctctgaagtgaagatggatg	cagaat
APP9	SaCas9	acttcatacctgagtcattg	cggaat
APP10	SaCas9	tttgatgatgaacttcatac	ctgagt
APP11	SaCas9	atgcagaattccgacatgact	caggat
APP12	SaCas9	gctatgacaacaccgcccacc	atgagt
APP13	SaCas9KKH	cagatgacgtcttgccaaca	aatagt
APP14	SaCas9KKH	gacgtcttgccaacatgatt	tcagat
APP15	SaCas9KKH	ttcagatgacgtcttgccaa	catgat
APP16	SaCas9KKH	gatgacgtcttgccaacatg	attagt
APP17	SaCas9KKH	gcatcggttccgtaactgac	cttggt
APP18	SaCas9KKH	aacatgattagtgaaccaagg	atcagt
APP19	SaCas9KKH	gaaccaaggatcagttacgga	aacgat
APP20	SaCas9KKH	acgggtggttttcgttcggtc	aaagat
APP21	SaCas9KKH	gagctccacgggtggtttcgt	ttcgggt
APP22	SaCas9KKH	attcacgggaaggagctccac	ggtggt
APP23	SaCas9KKH	tccattcacgggaaggagctc	cacgggt
APP24	SaCas9KKH	gtgaatggagagttcagcctg	gacgat
APP25	SaCas9KKH	agccgtggcattctttgggg	ggagat
APP26	SaCas9KKH	acggaggagatctctgaagtg	cttgat
APP27	SaCas9KKH	gttgacaaatatcaagacgga	ggagat
APP28	SaCas9KKH	gaagtgaagatggatgcagaa	agagat
APP29	SaCas9KKH	tatcaagacggaggagatctc	tgaagt
APP30	SaCas9KKH	gacggaggagatctctgaagt	gaagat
APP31	SaCas9KKH	attccgacatgactcaggata	tgaagt
APP32	SaCas9KKH	caggatatgaagttcatcatc	aaaaat
APP33	SaCas9KKH	gcagaagatgtgggtcaaac	aaagggt
APP34	SaCas9KKH	agatgtgggttcaacaaagg	tgcaat
APP35	SaCas9KKH	aacaccgcccaccatgagtc	aatgat
APP36	SaCas9KKH	gacaacaccgcccaccatgag	tccaat
APP37	SaCas9KKH	caaaggtgcaatcattggact	catggt
APP38	SaCas9KKH	gcaatcattggactcatggtg	ggcgggt
APP39	SaCas9KKH	ggtgggcgggtgtgtcatagc	gacagt
APP40	SaCas9KKH	gggcgggtgtgtcatagcgac	agtgat
APP41	SaCas9KKH	cttcagcatcaccaagggtgat	gacgat
APP42	SaCas9KKH	tttcttctcagcatcaccaa	ggtgat
APP43	SaCas9KKH	ctgttcttctcagcatcac	caagggt
APP44	SaCas9KKH	agcgacagtgtcgtcatcac	cttggt
APP45	SaCas9KKH	gacagtgtcgtcatcacctt	ggtgat
APP46	SaCas9KKH	ccttggtgatgctgaagaaga	aacagt
APP47	SaCas9KKH	aaacagtacatccattcat	catggt
APP48	SaCas9KKH	gtacacatccattcatcatgg	tgtggt
APP49	CjCas9	tttgggctgactctgtgcca	gccaacac

APP50	CjCas9	ttgaaccacatcttctgca	aagaacac
APP51	CjCas9	accgcccaccatgagtccaat	gattgcac
APP52	CjCas9	gatgacgatcactgtcgctat	gacaacac
APP53	CjCas9	ccttggtgatgctgaagaaga	aacagtac
APP54	CjCas9	ttggtgatgctgaagaagaaa	cagtacac
APP55	CjCas9	ccaccacaccatgatgaatgg	atgtgtac

APP, amyloid precursor protein; PAM, protospacer adjacent motif.

Table C: Promoters for neuron-specific deletion

Promoter	Features
Human synapsin 1	Neuron
Thy1	Neuron
PrP	Expression in the brain and spinal cord
CamKII	Excitatory neuron
mGAD65	Inhibitory neuron
SST	Somatostatin-expressing neurons
TH	Tyrosine hydroxylase (TH) - expressing neurons
PV	Parvalbumin (PV)-expressing interneurons

5

Table D: Mutations in *APP* gene

Mutation	Genomic Region	Mutation Type	Codon Change
A201V	Exon 5	Point, Missense	GCG to GTG
A235V	Exon 6	Point, Missense	GCT to GTT
D243N	Exon 6	Point, Missense	GAT to AAT
E246K	Exon 6	Point, Missense	GAG to AAG
E296K	Exon 7	Point, Missense	GAG to AAG
P299L	Exon 7	Point, Missense	CCG to CTG
R468H	Exon 11	Point, Missense	CGC to CAC
A479S	Exon 11	Point, Missense	GCT to TCT
K496Q	Exon 12	Point, Missense	AAG to CAG
A500T	Exon 12	Point, Missense	GCA to ACA
Y538H	Exon 13	Point, Missense	TAT to CAT
V562I	Exon 13	Point, Missense	GTT to ATT
E599K	Exon 14	Point, Missense	GAA to AAA
T600M	Exon 14	Point, Missense	ACG to ATG
P620A	Exon 14	Point, Missense	CCG to GCG
P620L	Exon 14	Point, Missense	CCG to CTG
T663M	Exon 16	Point, Missense	ACG to ATG

E665D		Exon 16	Point, Missense	GAG to GAC
KM670/671NL	Swedish	Exon 16	Point, Double	AAG.ATG to AAT.CTG
A673T	Icelandic	Exon 16	Point, Missense	GCA to ACA
A673V		Exon 16	Point, Missense	GCA to GTA
H677R	English	Exon 16	Point, Missense	CAT to CGT
D678H	Taiwanese	Exon 16	Point, Missense	GAC to CAC
D678N	Tottori	Exon 16	Point, Missense	GAC to AAC
E682K	Leuven	Exon 16	Point, Missense	GAA to AAA
K687N		Exon 16	Point, Missense	AAA to AAT
A692G	Flemish	Exon 17	Point, Missense	GCA to GGA
E693del	Osaka, E693Δ, E693delta	Exon 17	Deletion	GAA to ---
E693G	Arctic, E22G	Exon 17	Point, Missense	GAA to GGA
D694N	Iowa	Exon 17	Point, Missense	GAT to AAT
G708G		Exon 17	Point, Silent	GGC to GGT
G709S		Exon 17	Point, Missense	GGT to AGT
A713T		Exon 17	Point, Missense	GCG to ACG
A713V		Exon 17	Point, Missense	GCG to GTG
T714A	Iranian	Exon 17	Point, Missense	ACA to GCA
T714I	Austrian	Exon 17	Point, Missense	ACA to ATA
V715A	German	Exon 17	Point, Missense	GTG to GCG
V715M	French	Exon 17	Point, Missense	GTG to ATG
I716F	Iberian	Exon 17	Point, Missense	ATC to TTC
I716M		Exon 17	Point, Missense	ATC to ATG
I716T		Exon 17	Point, Missense	ATC to ACC
I716V	Florida	Exon 17	Point, Missense	ATC to GTC
V717F	Indiana	Exon 17	Point, Missense	GTC to TTC
V717G		Exon 17	Point, Missense	GTC to GGC
V717I	London	Exon 17	Point, Missense	GTC to ATC
V717L		Exon 17	Point, Missense	GTC to CTC
T719P		Exon 17	Point, Missense	ACC to CCC
M722K		Exon 17	Point, Missense	ATG to AAG
L723P	Australian	Exon 17	Point, Missense	CTG to CCG
K724N	Belgian	Exon 17	Point, Missense	AAG to AAC
H733P		Exon 17	Point, Missense	CAT to CCT
IVS17 83-88delAAGTAT		Exon 17	Deletion	\

DISCUSSIONS

[0169] In this study, generation of amyloid-beta is first decreased by disrupting the cleavage sites in APP that involve the amyloidogenic proteolytic pathway. Abolishing the cleavage sites of β - and γ -secretases in APP (amyloid precursor protein) results in reduced

generation of amyloid-beta ($A\beta$), one of the hallmarks of Alzheimer's disease (AD). This approach can be generally applied to all familial AD cases with mutations in APP, PSEN1 and PSEN2 and to the people with sporadic AD that are associated with increased $A\beta$ generation.

5 [0170] APP expression is manipulated at the adult stage. APP has physiological roles in animal development; for example, in neuronal migration and synaptic plasticity. Thus, specific manipulation of APP expression in the brain at adult stage exhibits beneficial outcome and reduces potential side effects (due to the off-target effects of the genome-editing tool).

10 [0171] Neuron- or neuron subtype-specific manipulation of APP is performed. $A\beta$ is generated from the cleavage of APP in neurons. Thus, specific manipulation of APP expression in neurons by promoter-specific-driven genome editing could avoid the potential off-target effects produced in other cell types. Furthermore, genome-editing can be conducted in specific neuronal subpopulations (*i.e.*, inhibitory neurons, excitatory neurons) to
15 reduce or eliminate any potential side effects.

[0172] the effects of brain-wide genome editing are studied. Noninvasive delivery of the genome-editing components are performed via intravenous administration. Whole-genome sequencing is performed and shows that Cas9-sgRNA does not introduce off-target events.

[0173] All patents, patent applications, and other publications, including GenBank
20 Accession Numbers, cited in this application are incorporated by reference in the entirety for all purposes.

REFERENCES

1. Cai, Y., An, S.S. & Kim, S. Mutations in presenilin 2 and its implications in Alzheimer's disease and other dementia-associated disorders. *Clinical interventions in aging*
25 **10**, 1163-1172 (2015).
2. Campion, D., *et al.* Early-onset autosomal dominant Alzheimer disease: prevalence, genetic heterogeneity, and mutation spectrum. *American journal of human genetics* **65**, 664-670 (1999).
3. Cacace, R., Sleegers, K. & Van Broeckhoven, C. Molecular genetics of early-onset
30 Alzheimer's disease revisited. *Alzheimer's & dementia : the journal of the Alzheimer's Association* **12**, 733-748 (2016).
4. Harper, P.S. The epidemiology of Huntington's disease. *Human genetics* **89**, 365-376 (1992).
5. Chio, A., *et al.* Global epidemiology of amyotrophic lateral sclerosis: a systematic
35 review of the published literature. *Neuroepidemiology* **41**, 118-130 (2013).
6. Selkoe, D.J. & Hardy, J. The amyloid hypothesis of Alzheimer's disease at 25 years.

- EMBO molecular medicine* **8**, 595-608 (2016).
7. Ran, F.A., *et al.* Genome engineering using the CRISPR-Cas9 system. *Nature protocols* **8**, 2281-2308 (2013).
 8. Serrano-Pozo, A., Frosch, M.P., Masliah, E. & Hyman, B.T. Neuropathological alterations in Alzheimer disease. *Cold Spring Harbor perspectives in medicine* **1**, a006189 (2011).
 9. Braak, H. & Braak, E. Frequency of stages of Alzheimer-related lesions in different age categories. *Neurobiology of aging* **18**, 351-357 (1997).
 10. Ingusci, S., Verlengia, G., Soukupova, M., Zucchini, S. & Simonato, M. Gene Therapy Tools for Brain Diseases. *Frontiers in pharmacology* **10**, 724 (2019).
 11. Chan, K.Y., *et al.* Engineered AAVs for efficient noninvasive gene delivery to the central and peripheral nervous systems. *Nature neuroscience* **20**, 1172-1179 (2017).
 12. Deverman, B.E., *et al.* Cre-dependent selection yields AAV variants for widespread gene transfer to the adult brain. *Nature biotechnology* **34**, 204-209 (2016).
 13. Bedbrook, C.N., Deverman, B.E. & Gradinaru, V. Viral Strategies for Targeting the Central and Peripheral Nervous Systems. *Annual review of neuroscience* **41**, 323-348 (2018).
 14. Zeng, J., *et al.* TRIM9-Mediated Resolution of Neuroinflammation Confers Neuroprotection upon Ischemic Stroke in Mice. *Cell reports* **27**, 549-560 e546 (2019).
 15. Ortiz-Virumbrales, M., *et al.* CRISPR/Cas9-Correctable mutation-related molecular and physiological phenotypes in iPSC-derived Alzheimer's PSEN2 (N141I) neurons. *Acta neuropathologica communications* **5**, 77 (2017).
 16. Gyorgy, B., *et al.* CRISPR/Cas9 Mediated Disruption of the Swedish APP Allele as a Therapeutic Approach for Early-Onset Alzheimer's Disease. *Molecular therapy. Nucleic acids* **11**, 429-440 (2018).
 17. Karczewski, K.J., *et al.* The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature* **581**, 434-443 (2020).
 18. Wang, B., *et al.* Gamma-secretase gene mutations in familial acne inversa. *Science* **330**, 1065 (2010).
 19. Mullan, M., *et al.* A pathogenic mutation for probable Alzheimer's disease in the APP gene at the N-terminus of beta-amyloid. *Nature genetics* **1**, 345-347 (1992).
 20. Citron, M., *et al.* Excessive production of amyloid beta-protein by peripheral cells of symptomatic and presymptomatic patients carrying the Swedish familial Alzheimer disease mutation. *Proceedings of the National Academy of Sciences of the United States of America* **91**, 11993-11997 (1994).
 21. Johnston, J.A., *et al.* Increased beta-amyloid release and levels of amyloid precursor protein (APP) in fibroblast cell lines from family members with the Swedish Alzheimer's disease APP670/671 mutation. *FEBS letters* **354**, 274-278 (1994).
 22. Wiedenheft, B., *et al.* RNA-guided complex from a bacterial immune system enhances target recognition through seed sequence interactions. *Proceedings of the National Academy of Sciences of the United States of America* **108**, 10092-10097 (2011).
 23. Gao, X., *et al.* Treatment of autosomal dominant hearing loss by in vivo delivery of genome editing agents. *Nature* **553**, 217-221 (2018).
 24. Mashiko, D., *et al.* Generation of mutant mice by pronuclear injection of circular plasmid expressing Cas9 and single guided RNA. *Scientific reports* **3**, 3355 (2013).
 25. Zhang, Y., *et al.* An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. *J Neurosci* **34**, 11929-11947 (2014).
 26. Oakley, H., *et al.* Intraneuronal beta-amyloid aggregates, neurodegeneration, and neuron loss in transgenic mice with five familial Alzheimer's disease mutations: potential factors in amyloid plaque formation. *J Neurosci* **26**, 10129-10140 (2006).
 27. Kimura, R. & Ohno, M. Impairments in remote memory stabilization precede

- hippocampal synaptic and cognitive failures in 5XFAD Alzheimer mouse model. *Neurobiology of disease* **33**, 229-235 (2009).
28. Shao, C.Y., Mirra, S.S., Sait, H.B., Sacktor, T.C. & Sigurdsson, E.M. Postsynaptic degeneration as revealed by PSD-95 reduction occurs after advanced Abeta and tau pathology in transgenic mouse models of Alzheimer's disease. *Acta neuropathologica* **122**, 285-292 (2011).
29. Gowrishankar, S., *et al.* Massive accumulation of luminal protease-deficient axonal lysosomes at Alzheimer's disease amyloid plaques. *Proceedings of the National Academy of Sciences of the United States of America* **112**, E3699-3708 (2015).
30. Sharoar, M.G., Hu, X., Ma, X.M., Zhu, X. & Yan, R. Sequential formation of different layers of dystrophic neurites in Alzheimer's brains. *Molecular psychiatry* **24**, 1369-1382 (2019).
31. Spires-Jones, T.L. & Hyman, B.T. The intersection of amyloid beta and tau at synapses in Alzheimer's disease. *Neuron* **82**, 756-771 (2014).
32. Woodhouse, A., West, A.K., Chuckowree, J.A., Vickers, J.C. & Dickson, T.C. Does beta-amyloid plaque formation cause structural injury to neuronal processes? *Neurotoxicity research* **7**, 5-15 (2005).
33. Jankowsky, J.L., *et al.* Mutant presenilins specifically elevate the levels of the 42 residue beta-amyloid peptide in vivo: evidence for augmentation of a 42-specific gamma secretase. *Human molecular genetics* **13**, 159-170 (2004).
34. Garcia-Alloza, M., *et al.* Characterization of amyloid deposition in the APP^{swe}/PS1^{dE9} mouse model of Alzheimer disease. *Neurobiology of disease* **24**, 516-524 (2006).
35. Fu, A.K., *et al.* IL-33 ameliorates Alzheimer's disease-like pathology and cognitive decline. *Proceedings of the National Academy of Sciences of the United States of America* **113**, E2705-2713 (2016).
36. Lalonde, R., Kim, H.D., Maxwell, J.A. & Fukuchi, K. Exploratory activity and spatial learning in 12-month-old APP(695)SWE/co+PS1/DeltaE9 mice with amyloid plaques. *Neuroscience letters* **390**, 87-92 (2005).
37. Choi, J.H., *et al.* Optimization of AAV expression cassettes to improve packaging capacity and transgene expression in neurons. *Mol Brain* **7**, 17 (2014).
38. Patricio, M.I., Barnard, A.R., Orleans, H.O., McClements, M.E. & MacLaren, R.E. Inclusion of the Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element Enhances AAV2-Driven Transduction of Mouse and Human Retina. *Molecular therapy. Nucleic acids* **6**, 198-208 (2017).
39. Jawhar, S., Trawicka, A., Jenneckens, C., Bayer, T.A. & Wirths, O. Motor deficits, neuron loss, and reduced anxiety coinciding with axonal degeneration and intraneuronal Abeta aggregation in the 5XFAD mouse model of Alzheimer's disease. *Neurobiology of aging* **33**, 196 e129-140 (2012).
40. Tibble, M., *et al.* PKR knockout in the 5xFAD model of Alzheimer's disease reveals beneficial effects on spatial memory and brain lesions. *Aging cell* **18**, e12887 (2019).
41. Ran, F.A., *et al.* In vivo genome editing using Staphylococcus aureus Cas9. *Nature* **520**, 186-191 (2015).
42. Cruts, M., Theuns, J. & Van Broeckhoven, C. Locus-specific mutation databases for neurodegenerative brain diseases. *Human mutation* **33**, 1340-1344 (2012).
43. Kleinstiver, B.P., *et al.* Broadening the targeting range of Staphylococcus aureus CRISPR-Cas9 by modifying PAM recognition. *Nature biotechnology* **33**, 1293-1298 (2015).
44. Nagata, K., *et al.* Generation of App knock-in mice reveals deletion mutations protective against Alzheimer's disease-like pathology. *Nature communications* **9**, 1800 (2018).
45. Park, H., *et al.* In vivo neuronal gene editing via CRISPR-Cas9 amphiphilic

- nanocomplexes alleviates deficits in mouse models of Alzheimer's disease. *Nature neuroscience* **22**, 524-528 (2019).
46. Filser, S., *et al.* Pharmacological inhibition of BACE1 impairs synaptic plasticity and cognitive functions. *Biological psychiatry* **77**, 729-739 (2015).
- 5 47. Ou-Yang, M.H., *et al.* Axonal organization defects in the hippocampus of adult conditional BACE1 knockout mice. *Science translational medicine* **10** (2018).
48. Blume, T., *et al.* BACE1 Inhibitor MK-8931 Alters Formation but Not Stability of Dendritic Spines. *Frontiers in aging neuroscience* **10**, 229 (2018).
49. Ravindra Kumar, S., *et al.* Multiplexed Cre-dependent selection yields systemic AAVs for targeting distinct brain cell types. *Nature methods* **17**, 541-550 (2020).
- 10 50. Flytzanis, N.C., *et al.* Broad gene expression throughout the mouse and marmoset brain after intravenous delivery of engineered AAV capsids. *BioRxiv*, 2020.2006.2016.152975 (2020).
51. Bloch, D.B., *et al.* Sp110 localizes to the PML-Sp100 nuclear body and may function as a nuclear hormone receptor transcriptional coactivator. *Mol Cell Biol* **20**, 6138-6146 (2000).
- 15 52. Mo, W. & Zhang, J.T. Human ABCG2: structure, function, and its role in multidrug resistance. *Int J Biochem Mol Biol* **3**, 1-27 (2012).
53. Liang, Z., *et al.* The pseudokinase CaMKv is required for the activity-dependent maintenance of dendritic spines. *Nature communications* **7**, 13282 (2016).
- 20 54. Sun, J., *et al.* CRISPR/Cas9 editing of APP C-terminus attenuates beta-cleavage and promotes alpha-cleavage. *Nature communications* **10**, 53 (2019).
55. Hocquemiller, M., Giersch, L., Audrain, M., Parker, S. & Cartier, N. Adeno-Associated Virus-Based Gene Therapy for CNS Diseases. *Human gene therapy* **27**, 478-496 (2016).
- 25 56. Hudry, E. & Vandenberghe, L.H. Therapeutic AAV Gene Transfer to the Nervous System: A Clinical Reality. *Neuron* **101**, 839-862 (2019).
57. Challis, R.C., *et al.* Systemic AAV vectors for widespread and targeted gene delivery in rodents. *Nature protocols* **14**, 379-414 (2019).
- 30 58. Keane, T.M., *et al.* Mouse genomic variation and its effect on phenotypes and gene regulation. *Nature* **477**, 289-294 (2011).
59. Wojtowicz, J.M. & Kee, N. BrdU assay for neurogenesis in rodents. *Nature protocols* **1**, 1399-1405 (2006).
60. Styren, S.D., Hamilton, R.L., Styren, G.C. & Klunk, W.E. X-34, a fluorescent derivative of Congo red: a novel histochemical stain for Alzheimer's disease pathology. *The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society* **48**, 1223-1232 (2000).
- 35 61. Seo, J., *et al.* Activity-dependent p25 generation regulates synaptic plasticity and Abeta-induced cognitive impairment. *Cell* **157**, 486-498 (2014).
- 40 62. Lee, K., *et al.* Replenishment of microRNA-188-5p restores the synaptic and cognitive deficits in 5XFAD Mouse Model of Alzheimer's Disease. *Scientific reports* **6**, 34433 (2016).
63. Devi, L. & Ohno, M. Mitochondrial dysfunction and accumulation of the beta-secretase-cleaved C-terminal fragment of APP in Alzheimer's disease transgenic mice. *Neurobiology of disease* **45**, 417-424 (2012).
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SEQUENCE LISTING

SEQ ID NO:1 nucleotide sequence of the sgRNA-Syn::Cas9-mWPRE construct ITR-U6-SW1-Syn-SaCas9-HA-mWPRE-pA-ITR)

CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCGGGCGTCGGGCG
ACCTTTGGTCGCCCCGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGCC
AACTCCATCACTAGGGGTTCCTGCGGCCTCTAGAGAGGGCCTATTTCCCATGATT
CCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAATTGGAATTAATT
TGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAAT
TTCTTGGGTAGTTTGCAGTTTTTAAAATTATGTTTTTAAAATGGACTATCATATGCTT
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 AGAATCTGGATGTGATCAAAAAAGAAAAGTACTACGAAGTGAATAGCAAGTGCT
 ATGAGGAAGCTAAGAAGCTGAAGAAGATCAGCAACCAGGCCGAGTTTATCGCCT
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 TCGCCGCTGCCTTGCCCGCTGCTGGACAGGGGCTCGGCTGTTGGGCACTGACAA
 TTCCGTGGTGTATTTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATCT
 AGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTTATAAGCTG
 CAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTTCAGGGG
 GAGATGTGGGAGGTTTTTTAAAGCGGCCGCAGGAACCCCTAGTGATGGAGTTGG
 CCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGC
 CCGACGCCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGCTG
 CCTGCAGG

SEQ ID NO:2 APP amino acid sequence (the α secretase site in **bold**, the β secretase site underlined, and the γ secretase site double underlined)

MLPGLALLLLAAWTARALEVPTDGNAGLLAEPQIAMFCGRLNMHMNVQNGKWD
 DPSGKTCTIDTKEGILQYCQEVYPELQITNVVEANQPVTIQNWCKRGRKQCKTHPHF
 VIPYRCLVGEFVSDALLVPDKCKFLHQERMDVCETHLHWHTVAKETCSEKSTNLHD

YGMLPCGIDKFRGVEFVCCPLAEESDNVDSADAEEDDSVWWGGADTDYADGSE
DKVVEVAEEEEVAEVEEEEADDDDEDDEDGDEVVEEEAEEPYEEATERTTSIATTTTTT
TESVEEVVRVPTTAASTPDAVDKYLETPGDENEHAHFQKAKERLEAKHRERMSQVM
REWEEAERQAKNLPKADKKAVIQHFQEKVESLEQEAANERQQLVETHMARVEAML
NDRRRLALENYITALQAVPPRPRHVFNMLKKYVRAEQKDRQHTLKHFEHVRMVDP
KKAAQIRSQVMTHLRVIYERMNQSLSLYNVPAVAEEIQDEVDELLQKEQNYSDDV
LANMISEPRISYGNDALMPSLTETKTTVELLPVNGEFSLDDLQPWHSFGADSVPANTE
NEVEPVDARPAADRGLTTRPGSGLTNIKTEEISEVKMDAEFRHDSGYEVHHQKLVFF
AEDVGSNKGAIIGLMVGGVVIATVIVITLVMLKKKQYTSIHGVEVDAAVTPEERH
LSKMQQNGYENPTYKFFEQMQN

WHAT IS CLAIMED IS:

1. A method for treating or reducing risk of an amyloid pathology in a person in need thereof, comprising the step of administering to the person an effective amount of a composition disrupting amyloid precursor protein (APP) genomic sequence.
2. The method of claim 1, further comprising, prior to the administering step, sequencing at least a portion of the person's genome.
3. The method of claim 1, wherein the person's APP genomic sequence comprises at least one mutation.
4. The method of claim 3, wherein the at least one mutation comprises a Swedish mutation (K670N/M671L), a Florida mutation (I716V), a London mutation (V717I), or any combination thereof.
5. The method of claim 4, wherein the at least one mutation comprises a Swedish mutation (K670N/M671L).
6. The method of claim 1, wherein the amyloid pathology is Alzheimer's Disease (AD), cerebral amyloid angiopathy, or Down Syndrome.
7. The method of claim 6, wherein the AD is familial AD or sporadic AD.
8. The method of claim 1, wherein the person has been diagnosed with AD, or the person is not yet diagnosed with AD but has known risk factors for AD.
9. The method of claim 1, wherein the composition comprising an siRNA, a microRNA, a miniRNA, a lncRNA, or an antisense oligonucleotide targeting the APP genomic sequence.
10. The method of claim 1, wherein the composition comprising one or more vectors encoding (1) an endonuclease guided by a small guide RNA (sgRNA) targeting a location within the APP genomic sequence; and (2) the sgRNA.

11. The method of claim 1, wherein the sgRNA targets a region within the APP genomic sequence comprising the at least one mutation or a β - or γ -secretase cleavage site.
12. The method of claim 10, wherein the composition comprises one vector encoding a Cas9 nuclease and one sgRNA, preferably a *Streptococcus pyogenes* Cas9 nuclease (SpCas9).
13. The method of claim 10 or 11, wherein the one or more vectors are one viral vector, preferably an adeno-associated virus (AAV) vector.
14. The method of claim 1, wherein the composition is administered by subcutaneous, intramuscular, intravenous, intraperitoneal, or intracranial injection or by oral or nasal administration.
15. The method of claim 13, wherein the composition is administered in the form of a solution, a suspension, a powder, a paste, a tablet, or a capsule.
16. A kit for treating or reducing risk of an amyloid pathology in a person in need thereof, comprising a container containing a composition disrupting APP genomic sequence.
17. The kit of claim 16, wherein the composition is formulated for subcutaneous, intramuscular, intravenous, intraperitoneal, or intracranial injection, or for oral or nasal administration.
18. The kit of claim 16, wherein the composition comprising an siRNA, a microRNA, a miniRNA, a lncRNA, or an antisense oligonucleotide targeting the APP genomic sequence.
19. The kit of claim 16, wherein the composition comprising one or more vectors encoding (1) an endonuclease guided by a small guide RNA (sgRNA) targeting one location within the APP genomic sequence; and (2) the sgRNA.
20. The kit of claim 19, wherein the sgRNA targets a region within the APP genomic sequence comprising the at least one mutation or a β - or γ -secretase cleavage site.

21. The kit of claim 16, wherein the composition comprises one vector encoding a Cas9 nuclease and one sgRNA, preferably a *Streptococcus pyogenes* Cas9 nuclease (SpCas9).
22. The kit of any one of claims 19-21, wherein the one or more vectors are one or more viral vectors, preferably an adeno-associated virus (AAV) vector.
23. The kit of claim 16, wherein the composition is administered by subcutaneous, intramuscular, intravenous, intraperitoneal, or intracranial injection or by oral or nasal administration.
24. The kit of claim 16, further comprising a second container containing agents for sequencing at least a portion of the person's genome.
25. The kit of claim 16, further comprising an instruction manual for administration of the composition.