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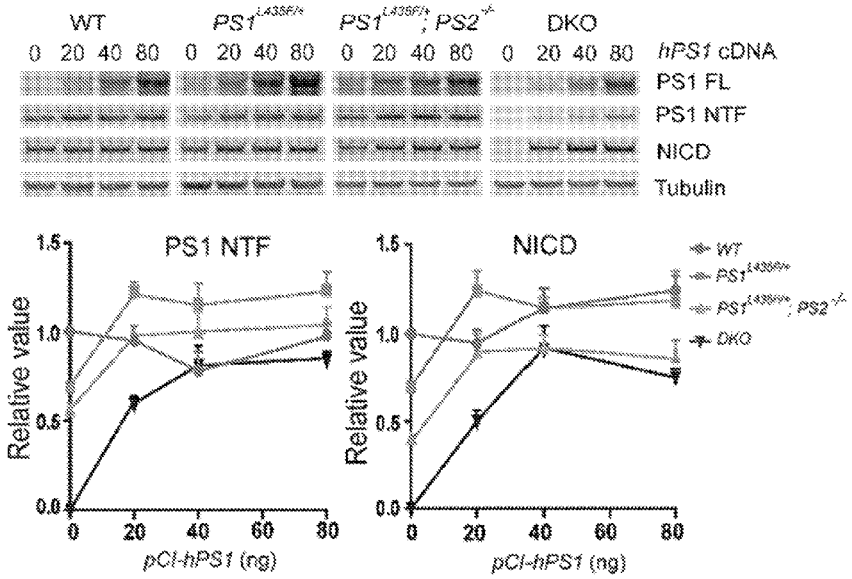

FIG. 3B

(57) **Abstract:** The present disclosure provides, among other things, methods for using presenilin based gene therapy to treat neurodegenerative dementia including, but not limited to Alzheimers disease, frontotemporal dementia, frontotemporal lobar degeneration, Picks disease, Lewy body dementia, memory loss, and cognitive impairment including mild cognitive impairment (MCI).

[Continued on next page]

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GENE THERAPY FOR ALZHEIMER'S DISEASE

CLAIM OF PRIORITY

This application claims the benefit of U.S. Provisional Patent Application Serial No. 62/675,003, filed on May 22, 2018. The entire contents of the foregoing are hereby incorporated by reference.

FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

This invention was made with Government support under Grant Nos. NS041783 and NS075346 awarded by the National Institutes of Health. The Government has certain rights to the invention.

TECHNICAL FIELD

Described herein are, *inter alia,* compositions and methods for using presenilin genetic therapy constructs to treat Alzheimer's disease (AD) and other neurodegenerative diseases.

BACKGROUND

Alzheimer's disease, also known as Alzheimer disease, accounts for majority of neurodegenerative dementia and is the fourth leading cause of death in the United States after heart disease, cancer and stroke. It is characterized by a progressive loss of cognitive function, neurodegeneration, neurofibrillary tangles and amyloid plaques in the brains of patients. Although the progression speed varies in different patients, the average life expectancy following diagnosis is three to nine years. Currently, there is no treatment for Alzheimer's disease.

SUMMARY

Described herein is a novel approach that can be used to treat subjects with Alzheimer's disease (AD) and other neurodegenerative diseases, disorders or conditions. Mutations in the Presenilin genes – PSEN1 and PSEN2 – are highly penetrant and account for ~90% of all mutations identified in familial AD (FAD), highlighting their importance in the pathogenesis of AD. More than 260 distinct mutations in PSEN1 have been reported, and they are dominantly inherited and mostly missense mutations. Pathogenic PSEN1 mutations

act in cis to impair mutant PS1 function and act in trans to inhibit wild-type Presenilin-1 (PS1) function (Heilig et al. J Neurosci 33:11606-717 (2013); Zhou et al. Proc Natl Acad Sci USA 114:12731–12736 (2017). Typically, by their very nature, dominant negative mutations cannot be rescued by expression of wild type protein (Herskowitz, I. Nature, 329:219-222 (1987)). The present disclosure is based, at least in part, on the unexpected discovery that providing a wild-type PSEN1 cDNA into immortalized MEFs carrying heterozygous or homozygous dominant negative Psen1 mutations, a well-established familial Alzheimer's disease model, rescued the impaired γ-secretase activity in these cells. It is known that dominant negative mutations in the PSEN1 and PSEN2 genes are associated with early onset familial Alzheimer's disease. It was generally believed that the PS1 and presenilin-2 (PS2) proteins are part of γ-secretase complex, and that mutations in the PSEN1 and PSEN2 genes contribute to the accumulation of Amyloid beta (Aβ) protein in Alzheimer's disease patients. Thus, the present disclosure provides methods for effective gene therapy based on PSEN1 (to express PS1) and/or PSEN2 (to express PS2) for Alzheimer's disease and other neurodegenerative dementia, representing a significant breakthrough in this disease area.

In a first aspect, provided herein are methods for treating a neurodegenerative disease, disorder or condition comprising administering to a subject in need of treatment a polynucleotide comprising a PSEN1 and/or PSEN2 gene or mRNA, e.g., encoding a PS1 or PS2 protein as described herein. In some embodiments, the neurodegenerative disease, disorder or condition is Alzheimer's disease, e.g., familial Alzheimer's disease, e.g., characterized with one or more mutations in the PSEN1 and/or PSEN2 gene. In some embodiments, the Alzheimer's disease is sporadic Alzheimer's disease. In some embodiments, the Alzheimer's disease is late-onset or early-onset Alzheimer's disease. In some embodiments, the neurodegenerative disease, disorder or condition is frontotemporal dementia, frontotemporal lobar degeneration, Pick's disease, or Lewy body dementia. In some embodiments, the neurodegenerative disease, disorder or condition is memory loss. In some embodiments, the neurodegenerative disease, disorder or condition is cognitive decline or impairment. In some embodiments, the cognitive impairment is mild cognitive impairment (MCI). In some embodiments, the polynucleotide is a vector, e.g., a viral vector.

Also provided herein are polynucleotide sequence encoding a PS1 and/or PS2 protein or therapeutically active fragment thereof, optionally wherein the polynucleotides sequences are in vectors wherein the PS1 and/or PS2 encoding sequence is operably linked to a promotor that drives expression of the PS1 and/or PS2 in the brain. Also provided herein is

the use of the polynucleotide sequences and vectors for use in treating a neurodegenerative disease, disorder or condition as described herein.

In some embodiments, the viral vector is an adeno-associated virus (AAV) vector. In some embodiments, the AAV vector is selected from AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAVrh10, AAV11, AAV12, AAV2/1, AAV2/2, AAV2/5, AAV2/6, AAV2/7, AAV2/8, AAV2/9, AAV2/rh10, AAV2/AAV11, or AAV2/AAV12.

In some embodiments, the viral vector is a lentiviral vector or a retroviral vector.

In some embodiments, the polynucleotide encodes a presenilin 1 (PSEN1) gene or mRNA. In some embodiments, the polynucleotide encodes a presenilin 2 (PSEN2) gene or mRNA. In some embodiments, the polynucleotide encodes a presenilin 1 (PSEN1) and a presenilin 2 (PSEN2) gene or mRNA.

In some embodiments, the polynucleotide sequence encoding PS1 protein, e.g., PSEN1 mRNA, comprises a nucleotide sequence at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of wild-type human presenilin-1 transcript variant 1 mRNA (SEQ ID NO:1) or of wild-type human presenilin-1 transcript variant 2 mRNA (SEQ ID NO:2).

In some embodiments, the polynucleotide sequence encoding PS2 protein, e.g., PSEN2 mRNA, comprises a nucleotide sequence at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of wild-type human presenilin-2 transcript variant 1 mRNA (SEQ ID NO:1) or of wild-type human presenilin-2 transcript variant 2 mRNA (SEQ ID NO:2).

In some embodiments, the polynucleotide sequence encoding a PS1 and/or PS2 protein, e.g., presenilin 1 (PSEN1) and/or presenilin 2 (PSEN2) gene or mRNA, are operably linked to a promoter.

In some embodiments, the promoter is a pan neuronal promoter. In some embodiments, the pan neuronal promoter is a synapsin I promoter. In some embodiments, the pan neuronal promoter is a 1.6-kb hybrid promoter composed of the CMV immediate-early enhancer, and CBA intron 1/exon 1 (commonly called the “CAGGS promoter”).

In some embodiments, the promoter is a neuron subtype-specific promoter, e.g., an alpha-calcium/calmodulin kinase 2A promoter.

In some embodiments, the promoter is a cytomegalovirus (CMV) early enhancer/promoter, a hybrid CMV enhance/chicken β-actin (CBA) promoter, or a promoter comprising a CMV early enhancer element, the first exon and first intron of the chicken β-

actin gene, and the splice acceptor of the rabbit β-globin gene (commonly call the "CAG promoter").

In some embodiments, the polynucleotide sequence encoding a PS1 and/or PS2 protein, e.g., PSEN1 and/or PSEN2 gene or mRNA, is administered to the CNS of the subject in need of treatment.

In some embodiments, the polynucleotide encoding a PS1 and/or PS2 protein, e.g., PSEN1 and/or PSEN2 gene or mRNA, is administered to the CNS via intravenous delivery, intrathecal delivery, intracerebroventricular administration, stereotactic intraparenchymal administration, intracisternal administration, intracerebroventricular delivery, or stereotactic injection(s) into certain areas of brain, e.g., into the cisterna magna, cerebral ventricles, lumbar intrathecal space, direct injection into the hippocampus and/or the neocortex.

In some embodiments, the polynucleotide is associated with (e.g., formulated for delivery using) an exosome or lipid-based nanoparticle (LNP).

Also provided herein is the use of a vector as described herein for treating a neurodegenerative disease, disorder or condition as described herein. In some embodiments, the neurodegenerative disease, disorder or condition is Alzheimer's disease. In some embodiments, the Alzheimer's disease is familial Alzheimer's disease. In some embodiments, the Alzheimer's disease is sporadic Alzheimer's disease. In some embodiments, the Alzheimer's disease is late-onset Alzheimer's disease. In some embodiments, the neurodegenerative disease, disorder or condition is frontotemporal dementia, frontotemporal lobar degeneration, Pick's disease, or Lewy body dementia. In some embodiments, the neurodegenerative disease, disorder or condition is memory loss. In some embodiments, the neurodegenerative disease, disorder or condition is cognitive decline or impairment. In some embodiments, the cognitive impairment is mild cognitive impairment (MCI).

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Methods and materials are described herein for use in the present invention; other, suitable methods and materials known in the art can also be used. The materials, methods, and examples are illustrative only and not intended to be limiting. All publications, patent applications, patents, sequences, database entries, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control.

Other features and advantages of the invention will be apparent from the following detailed description and figures, and from the claims.

DESCRIPTION OF DRAWINGS

FIGs 1A-B. Decreased levels of PS1- and PSEN2-encoding mRNAs in hippocampal pyramidal neurons of sporadic AD brains. A, Representative pictures of human hippocampal CA1 pyramidal neurons before (top) and after (bottom) laser capture microdissection (LCM). B, Quantitative RT-PCR analysis shows a ~30% reduction in PS1, PSEN2, Nicastrin (Nct) mRNAs in CA1 pyramidal neurons of sporadic AD brains (n=9, Braak stage III/IV), compared to control brains (n=8) with normal cognitive ability. Levels of PS1, PSEN2, Nicastrin (Nct) and Pen-2 mRNA are normalized to the RPLP0 mRNA, a ubiquitously expressed ribosomal mRNA, and the values of mRNAs from control brains were set as 100%. All data are expressed as mean ± SEM. Statistical analysis was performed using two-tailed unpaired student's *t*-test. *$p<0.05$; **$p<0.01$; *NS*, Not significant.

FIGs. 2A-B. Decreased γ–secretase activity in Psen1 KI/+, KI/KI and Psen1-/- cells. A, Western blotting using cell lysates from immortalized MEFs derived from embryos carrying various Psen1 genotypes shows reduced γ-secretase activity in PS1 L435F KI/+, KI/KI and $PS1^{-/-}$ cells, as indicated by decreased NICD production. MEFs were transfected with 1.25 μg Hes1-Luc and 5 ng Notch-ΔE. Antibodies specific for the N-terminus of PS1, NICD, and α-tubulin were used. B, Quantification of NICD (left graph) and PS1-NTF (right graph) levels is shown. Protein levels are normalized to α-tubulin, and the protein levels in +/+ cells are expressed as 100%. All data are expressed as mean ± SEM. Statistical analysis was performed using one-way ANOVA with Tukey's post-test. *p<0.05; **p<0.01, ***p<0.001 compared to +/+ cells.

FIGs. 3A-3B. Introduction of WT hPS1 rescues impaired γ-secretase activity in mutant MEFs. A, γ-Secretase activity measured by NICD production is reduced in mutant MEF cells in a PS dosage dependent manner (WT > PS1 heterozygous KI or KO > homozygous PS1 KI or KO > DKO). B, Restoring impaired γ-secretase activity by WT hPS1. Increasing amounts of pCI-hPS1 plasmid DNA, as indicated, are transfected into MEFs of varying genotypes. Western analysis showed that both PS1 NTF and NICD are restored in various PS mutant MEFs. Heterozygous L435F KI cells are labeled as KI/+ or PS1L435F/+. N=3 independent experiments. Data represent mean ± SEM. *p < 0.05; **p < 0.01; ***p < 0.001 (one-way ANOVA with Tukey's post-hoc analysis).

DEFINITIONS

In order for the present disclosure to be more readily understood, certain terms are first defined below. Additional definitions for the following terms and other terms are set forth throughout the specification.

Administration:

As used herein, the term "administration" refers to the delivery or application of a composition to a subject or system. Administration to an animal subject (e.g., to a human) may be by any appropriate route. For example, in some embodiments, administration may be bronchial (including by bronchial instillation), buccal, enteral, interdermal, intra-arterial, intradermal, intragastric, intramedullary, intramuscular, intranasal, intraperitoneal, intrathecal, intravenous, intraventricular, mucosal, nasal, oral, rectal, subcutaneous, sublingual, topical, tracheal (including by intratracheal instillation), transdermal, vaginal and vitreal.

Biologically active:

As used herein, the phrase "biologically active" refers to a characteristic of any substance that has activity in a biological system (e.g., cell culture, organism, etc.). For instance, a substance that, when administered to an organism, has a biological effect on that organism, is considered to be biologically active. Biological activity can also be determined by *in vitro* assays (for example, *in vitro* enzymatic assays). In particular embodiments, where a protein or polypeptide is biologically active, a portion of that protein or polypeptide that shares at least one biological activity of the protein or polypeptide is typically referred to as a "biologically active" portion. In some embodiments, a protein is produced and/or purified from a cell culture system, which displays biologically activity when administered to a subject.

Control:

As used herein, the term "control" has its art-understood meaning of being a standard against which results are compared. Typically, controls are used to augment integrity in experiments by isolating variables in order to make a conclusion about such variables. In some embodiments, a control is a reaction or assay that is performed simultaneously with a test reaction or assay to provide a comparator. In one experiment, the "test" (i.e., the variable being tested) is applied. In the second experiment, the "control," the variable being tested is not applied. In some embodiments, a control is a historical control (i.e., of a test or assay performed previously, or an amount or result that is previously known). In some

embodiments, a control is or comprises a printed or otherwise saved record. A control may be a positive control or a negative control. In some embodiments, the control may be a "reference control", which is a sample used for comparison with a test sample, to look for differences or for the purposes of characterization.

Gene Therapy:

As used herein, the term "gene therapy" refers to any treatment including the direct or indirect administration of a nucleic acid to a subject. In particular instances, a protein of therapeutic value is expressed from an administered nucleic acid.

Identity:

As used herein, the term "identity" refers to the overall relatedness between polymeric molecules, e.g., between nucleic acid molecules (e.g., DNA molecules and/or RNA molecules) and/or between polypeptide molecules. Calculation of the percent identity of two nucleic acid sequences, for example, can be performed by aligning the two sequences for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second nucleic acid sequences for optimal alignment and non-identical sequences can be disregarded for comparison purposes). In certain embodiments, the length of a sequence aligned for comparison purposes is at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or substantially 100% of the length of the reference sequence. The nucleotides at corresponding nucleotide positions are then compared. When a position in the first sequence is occupied by the same nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which needs to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. For example, the percent identity between two nucleotide sequences can be determined using the algorithm of Meyers and Miller (CABIOS, 1989, 4: 11-17), which has been incorporated into the ALIGN program (version 2.0) using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. The percent identity between two nucleotide sequences can, alternatively, be determined using the GAP program in the GCG software package using an NWSgapdna.CMP matrix. Various other sequence alignment programs are available and can be used to determine sequence identity such as, for example, Clustal.

Improve, increase, or reduce:

As used herein, the terms "improve," "increase" or "reduce," or grammatical equivalents, indicate values that are relative to a baseline measurement, such as a measurement in the same individual prior to initiation of the treatment described herein, or a measurement in a control individual (or multiple control individuals) in the absence of the treatment described herein. A "control individual" is an individual afflicted with the same type and approximately the same severity of, e.g., Alzheimer's disease, as the individual being treated, who is about the same age as the individual being treated (to ensure that the stages of the disease in the treated individual and the control individual(s) are comparable).

Neurodegeneration:

As used herein, the term "neurodegeneration" means a process in which one or more neurons are damaged, decrease in function, become dysfunctional, and/or are lost by death. Neurodegeneration encompasses both rapid, gradual, and intermediate forms. Accordingly, a neurodegenerative disease, condition, or symptom is one characterized in that the disease is typically associated with neuronal damage, and/or death.

Subject:

As used herein, the term "subject" refers to a human or any non-human animal (e.g., mouse, rat, rabbit, dog, cat, cattle, swine, sheep, horse or primate). A human includes pre- and post-natal forms. In many embodiments, a subject is a human being. A subject can be a patient, which refers to a human presenting to a medical provider for diagnosis or treatment of a disease. The term "subject" is used herein interchangeably with "individual" or "patient." A subject can be afflicted with or is susceptible to a disease or disorder but may or may not display symptoms of the disease or disorder.

Suffering from:

An individual who is "suffering from" a disease, disorder, and/or condition (e.g., Alzheimer's disease) has been diagnosed with or displays one or more symptoms of the disease, disorder, and/or condition.

Susceptible to:

An individual who is "susceptible to" a disease, disorder, and/or condition has not been diagnosed with and/or may not exhibit symptoms of the disease, disorder, and/or condition. In some embodiments, an individual who is susceptible to a disease, disorder, and/or condition (for example, Alzheimer's disease) may be characterized by one or more of the following: (1) a genetic mutation associated with development of the disease, disorder,

and/or condition; (2) a genetic polymorphism associated with development of the disease, disorder, and/or condition; (3) increased and/or decreased expression and/or activity of a protein associated with the disease, disorder, and/or condition; (4) habits and/or lifestyles associated with development of the disease, disorder, and/or condition; (5) a family history of the disease, disorder, and/or condition; (6) reaction to certain bacteria or viruses; (7) exposure to certain chemicals. In some embodiments, an individual who is susceptible to a disease, disorder, and/or condition will develop the disease, disorder, and/or condition. In some embodiments, an individual who is susceptible to a disease, disorder, and/or condition will not develop the disease, disorder, and/or condition.

Therapeutically effective amount:

As used herein, the term "therapeutically effective amount" refers to an amount of a therapeutic protein which confers a therapeutic effect on the treated subject, at a reasonable benefit/risk ratio applicable to any medical treatment. The therapeutic effect may be objective (i.e., measurable by some test or marker) or subjective (i.e., subject gives an indication of or feels an effect). In particular, the "therapeutically effective amount" refers to an amount of a therapeutic protein or composition effective to treat, ameliorate, or prevent a desired disease or condition, or to exhibit a detectable therapeutic or preventative effect, such as by ameliorating symptoms associated with the disease, preventing or delaying the onset of the disease, and/or also lessening the severity or frequency of symptoms of the disease. A therapeutically effective amount is commonly administered in a dosing regimen that may comprise multiple unit doses. For any particular therapeutic protein, a therapeutically effective amount (and/or an appropriate unit dose within an effective dosing regimen) may vary, for example, depending on route of administration, on combination with other pharmaceutical agents. Also, the specific therapeutically effective amount (and/or unit dose) for any particular patient may depend upon a variety of factors including the disorder being treated and the severity of the disorder; the activity of the specific pharmaceutical agent employed; the specific composition employed; the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and/or rate of excretion or metabolism of the specific fusion protein employed; the duration of the treatment; and like factors as is well known in the medical arts.

Treatment:

As used herein, the term "treatment" (also "treat" or "treating"), in its broadest sense, refers to any administration of a substance (e.g., provided compositions) that partially or

completely alleviates, ameliorates, relives, inhibits, delays onset of, reduces severity of, and/or reduces incidence of one or more symptoms, features, and/or causes of a particular disease, disorder, and/or condition. In some embodiments, such treatment may be administered to a subject who does not exhibit signs of the relevant disease, disorder and/or condition and/or of a subject who exhibits only early signs of the disease, disorder, and/or condition. Alternatively or additionally, in some embodiments, treatment may be administered to a subject who exhibits one or more established signs of the relevant disease, disorder and/or condition. In some embodiments, treatment may be of a subject who has been diagnosed as suffering from the relevant disease, disorder, and/or condition. In some embodiments, treatment may be of a subject known to have one or more susceptibility factors that are statistically correlated with increased risk of development of the relevant disease, disorder, and/or condition.

Although generally speaking "PS1" refers to the presenilin-1 protein, and "PS2" refers to the presenilin-2 protein, in some cases PS1 or PS2 is used to refer to mRNA or gene.

DETAILED DESCRIPTION

The present disclosure provides, among other things, compositions and methods for treating subjects with Alzheimer's disease and other neurodegenerative diseases, disorders and conditions based on delivering functional presenilin-1 (PS1) and/or presenilin-2 (PS2). In particular, the present disclosure contemplates gene therapy by providing a polynucleotide encoding a presenilin-1 (PSEN1) and/or presenilin-2 (PSEN2) gene to a subject in need of treatment. In some embodiments, however, a presenilin-1 (PS1) and/or presenilin-2 (PS2) protein may also be used.

Various aspects of the invention are described in detail in the following sections. The use of sections is not meant to limit the invention. Each section can apply to any aspect of the invention. In this application, the use of "or" means "and/or" unless stated otherwise.

Methods of Treatment

As non-limiting examples, the present methods include gene therapy to express wild-type human Presenilin-1 or Presenilin-2 in a subject suffering from or susceptible to a neurodegenerative disease, e.g., associated with a mutation (e.g., a dominant negative mutation) in PSEN1 or PSEN2, e.g., Alzheimer's disease (e.g., familial AD patients carrying PSEN1 or PSEN2 mutations or sporadic AD patients). The objective of such a gene therapy is, among other things, to enhance expression of PS1 or PS2 in the brains of familial or

sporadic AD patients in order to correct or overcome a deficit in PS1 or PS2 expression and/or activity. In FAD patients, it is expected that a gene therapy method described herein result in increased expression of wild-type PS1 or PS2 in the brain, rescuing the impairment of γ-secretase activity associated with PS1 or PS2 mutations.

Mutations in the Presenilin genes – PSEN1 and PSEN2– are highly penetrant and account for ~90% of all mutations identified in familial AD (FAD), highlighting their importance in the pathogenesis of AD. More than 260 distinct mutations in PSEN1 have been reported, and they are dominantly inherited and mostly missense mutations. Pathogenic PSEN1 mutations act in cis to impair mutant PS1 function and act in trans to inhibit wild-type PS1 function (Heilig et al. J Neurosci 33:11606-717 (2013); Zhou et al. Proc Natl Acad Sci USA 114:12731–12736 (2017). Typically, by their very nature dominant negative mutations cannot be rescued by expression of wild type protein (Herskowitz, I. Nature, 329:219-222 (1987)). However, surprisingly, as shown herein, transfection of hPSEN1 cDNA into immortalized MEFs carrying heterozygous and homozygous PSEN1 mutations rescued the impaired γ-secretase activity in these cells (see Examples, below), indicating that, surprisingly, overexpression of wild-type PS1 protein can overcome the dominant negative effects of the mutant Presenilin protein. Presenilin is the catalytic subunit of the γ-secretase complex, which also includes Nicastrin, APH-1 (anterior pharynx-defective 1) and PEN-2 (presenilin enhancer 2), all of which are required for the assembly, stability and activity of the γ-secretase complex. Surprisingly, and without wishing to be bound by theory, it is believed based on the present data that the wild-type Presenilin protein may be able to replace the mutant Presenilin protein in the γ-secretase complex. Thus, expression of wild type PS1 or PS2 proteins can be used to rescue the impairment of γ-secretase expression and/or activity in AD patients.

The methods and compositions described herein can equally be used to treat other neurodegenerative diseases, disorders or conditions.

Alzheimer's disease

The methods described herein may be used to treat or reduce the risk of developing subjects with all types of Alzheimer's disease including, but not limited to, familial and sporadic Alzheimer's disease, early onset or late onset Alzheimer's disease. In some embodiments, the present methods may be used to treat or reduce the risk of development of

early onset familial form of Alzheimer's disease (AD) that is associated with mutations in presenilin-1 (PS1) and/or presenilin-2 (PS2) (Sherrington, et al., Nature 375:754-760 (1995); Rogaev, et al., Nature 376:775-778 (1995); Levy-Lahad, et al., Science 269:970-973 (1995); Hiltunen, et al., Eur. J. Hum. Genet. 8:259-266 (2000); Jonghe, et al., Hum. Mol. Genet. 8:1529-1540 (1999); Tysoe, et al., Am. J. Hum. Genet. 62:70-76 (1998); Crook, et al., Nat. Med. 4:452-455 (1998), all of which are incorporated by reference herein).

In some embodiments, the present methods may be used to treat a subject that has a mutation in the PSEN1 or PSEN2 allele, e.g., a mutation that has a dominant negative effect on wild-type PS1/PS2 proteins. Exemplary mutations include C410Y, Δex9, G548, D257A, L166P, R278I, L435F, G384A, Y115H, and L392V, as well as N141I, G206A, H163R, A79V, S290C, A260P, A426P, A431E, R269H, L271V, C1410Y, E280G, P264L, E185D, L235V, and M146V mutations (see, e.g., Heilig et al., J. Neurosci., 33(28):11606-11617 (2013); Watanabe et al., J. Neurosci. 32(15):5085–5096 (2012); Brouwers et al., 2008 Ann Med 40 (8): 562–83); Watanabe and Shen, PNAS November 28, 2017 114 (48) 12635-12637; Zhou et al., PNAS November 28, 2017 114 (48) 12731-12736; Hsu et al., Alzheimers Res Ther. 2018 Jul 18;10(1):67). Additional exemplary mutations that may have a dominant negative effect on wild-type PS proteins can include, but are not limited to, in PSEN-1: N32N; R35Q; D40del (delGAC); D40del (delACG); E69D; A79V; V82L; I83_M84del (DelIM, ΔI83/M84, ΔI83/ΔM84); I83T; M84V; L85P; P88L; V89L (G>T); V89L (G>C); C92S; V94M; V96F; V97L; T99A; F105C; F105I; F105L; F105V; R108Q; L113_I114insT (Intron4, InsTAC, p.113+1delG, splice5); L113P; L113Q; Y115C; Y115D; Y115H; T116I; T116N; T116R; P117A; P117L; P117R; P117S; E120D (A>C); E120D (A>T); E120G; E120K; E123K; Q127_R128del(CAGA);InsG(G) (c.379_382delXXXXinsG); H131R; S132A; L134R; N135D; N135S; N135Y; A136G; M139I (G>C); M139I (G>A); M139K; M139L; M139T; M139V; V142F; I143F; I143M; I143N; I143T; I143V; M146I (G>C); M146I (G>T); M146I (G>A); M146L (A>C); M146L (A>T); M146V; T147I; T147P; L150P; L153V; Y154C; Y154N; Y156F; Y156_R157insIY; R157S; H163P; H163R; H163Y; A164V; W165C (G>C); W165C (G>T); W165G; L166H; L166P; L166R; L166V; L166del; I167del (TTAdel); I167del (TATdel); I168T; S169del (ΔS169, Ser169del, ΔS170); S169L; S169P; S170F; S170P; L171P; L173F (G>C); L173F (G>T); L173W; L174del; L174M; L174R; F175S; F176L; F177L; F177S; S178P; G183V; E184D; E184G; V191A; I202F; G206A; G206D; G206S; G206V; G209A; G209E; G209R; G209V; S212Y; I213F; I213L; I213T; H214D; H214N; H214Y; G217D; G217R; L219F; L219P; L219R; R220P; Q222H; Q222P; Q222R;

Q223R; L226F; L226R; I229F; S230I; S230N; S230R; A231P; A231T; A231V; L232P; M233I (G>A); M233I (G>C); M233L (A>T); M233L (A>C); M233T; M233V; L235P; L235R; L235V; F237I; F237L; I238M; K239N; T245P; A246E; A246P; L248P; L248R; L250F; L250S; L250V; Y256S; A260V; V261F; V261L; L262F; L262V; C263F; C263R; P264L; G266S; P267A; P267L; P267S; R269G; R269H; L271V; V272A; E273A; E273G; T274R; A275V; R278I; R278K; R278S; R278T; E280A; (Paisa); E280G; E280K; L282F; L282R; L282V; F283L; P284L; P284S; A285V; L286P; L286V; T291A; T291P; K311R; E318G; D333G; R352C; R352_S353insR; T354I; R358Q; S365A; S365Y; R377M; R377W; G378E; G378V; G378fs; L381F; L381V; G384A; F386I; F386S; F388L; S390I; S390N; V391F; V391G; L392P; L392V; G394V; A396T; N405S; I408T; A409T; C410Y; V412I; I416T; G417S; L418F; L420R; L424F; L424H; L424R; L424V; A426P; A431E; (Jalisco); A431V; A434C; A434T; L435F; P436Q; P436S; I437V; I439S; I439V; T440del; 869-2A>G; 869-22_869-23ins18 (ΔE9, Δ9, deltaE9); I238_K239insI; S290C;T291_S319del (ΔE9Finn, Δ9Finn, Δ9); S290C;T291_S319del (ΔE9, Δ9); S290C;T291_S319del A>G (ΔE9, Δ9); S290C;T291_S319del G>A (ΔE9, Δ9); S290C;T291_S319del G>T (ΔE9, Δ9); or S290W;S291_R377del (Δ9-10 , Delta9-10, p.Ser290_Arg377delinsTrp, g.73671948_73682054del) (mutations are named relative to Uniprot P49768.1/GenBank Ref. No. NM_000021.4), and in PSEN-2,; T18M; R29H; G34S; R62C; R62H; P69A; R71W; K82R; A85V; V101M; K115Efs*; T122P; T122R; P123L; E126fs; E126K; S130L; V139M; N141I (Volga German); N141Y; L143H; V148I; K161R; R163H; H169N; M174V; S175C; G212V; V214L; Q228L; Y231C; I235F; A237V; L238F; L238P; M239I; M239V; A252T; A258T; T301M; K306fs; P334A; P334R; P348L; A377V; V393M; T430M; or D439A mutations are named relative to Uniprot P49810.1/GenBank Ref. No. NP_000438.2). See, e.g., Sun et al., Proc Natl Acad Sci USA. 2017;114:E476–E485; Heilig et al., J Neurosci. 2013 Jul 10; 33(28):11606-17; Zhou et al., PNAS November 28, 2017 114 (48) 12731-12736. In some embodiments, the methods can include determining that a subject has such a mutation, e.g., using methods known in the art.

Typically, increasing forgetfulness or mild confusion are early symptoms of Alzheimer's disease. Gradually, cognitive impairment associated with Alzheimer's disease leads to memory loss, especially recent memories, disorientation and misinterpreting spatial relationships, difficulty in speaking, writing, thinking, reasoning, changes in personality and behavior resulting in depression, anxiety, social withdrawal, mood swings, distrust in others,

irritability and aggressiveness, changes in sleeping habits, wandering, loss of inhibitions, delusions, and eventually death.

Other neurodegenerative diseases, disorders or conditions

In addition to Alzheimer's disease, the present methods may be used to treat other neurodegenerative diseases, disorders or conditions, including frontotemporal dementia, various types of memory loss, cognitive impairment including but not limited to mild cognitive impairment (MCI), or other conditions associated with loss of PS1, e.g., due to a mutation that creates a dominant negative isoform.

Presenilin-1 (PSEN1) and/or presenilin-2 (PSEN2)

A presenilin-1 (PS1) or presenilin-2 (PS2)-encoding polynucleotide suitable for use in the compositions and methods described herein can include a full length gene or a portion or fragment thereof that encodes a protein retaining substantial gamma secretase activity of the wild-type protein, e.g., at least 50% of the gamma secretase activity, or at least 60, 70, 80, 90, or 95% of the activity of the wild-type protein determined by (e.g., in *in vitro* γ-secretase assays including those described in the Examples section, see also Watanabe et al., J. Neurosci. 32(15):5085–5096 (2012)). In some embodiments, a suitable PS1- or PS2-enocoding polynucleotide has a sequence that is at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to the full length wild type genomic or cDNA PSEN1 or PSEN2 sequence, respectively. In some embodiments, a suitable PSEN1 or PSEN2 gene encodes a protein sequence that is at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to the full-length wild type PS1 or PS2 protein sequence, respectively. Exemplary wild type genomic, cDNA, and protein sequences of human PSEN1/PS1 or PSEN2/PS2 are shown in Table 1 and below. Sequences for use in other species are known in the art. PS1 and PS2 are normally cleaved into N- and C-terminal fragments that are the active form. PS-1 is processed to give two fragments: an N-terminal 28 kDa fragment, and a C-terminal 18 kDa fragment; the principal endoproteolytic cleavage occurs at and near Met298 in the proximal portion of the large hydrophilic loop (Podlisny et al., Neurobiol Dis. 1997;3(4):325-37; Marambaud et al., EMBO J. 2002 Apr 15;21(8):1948-56). Sequences comprising or encoding these cleaved forms can also be used in the methods and compositions described herein, e.g., encoding amino acids 1-291, 1-292, 1-293, 1-294, 1-295, 1-296, 1-297, 1-298, or 1-299 of SEQ ID NO:5 or a corresponding fragment of SEQ ID NO:6-8.

Table 1: GenBank Accession Nos.

Isoform	mRNA	Protein	RefSeqGene
presenilin-1 isoform I-467	NM_000021.3 (SEQ ID NO:1)	NP_000012.1 (SEQ ID NO:5)	NG_007386.2 Range 4965 to 92221
presenilin-1 isoform I-463	NM_007318.2 (SEQ ID NO:2)	NP_015557.2 (SEQ ID NO:6)	
presenilin-2 isoform 1	NM_000447.2 (SEQ ID NO:3)	NP_000438.2 (SEQ ID NO:7)	NG_007381.1 Range 5001 to 30532
presenilin-2 isoform 2	NM_012486.2 (SEQ ID NO:4)	NP_036618.2 (SEQ ID NO:8)	

>NM_000021.3 Homo sapiens presenilin 1 (PSEN1), transcript variant 1, mRNA (SEQ ID NO:1)

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>NM_007318.2 Homo sapiens presenilin 1 (PSEN1), transcript variant 2, mRNA (SEQ ID NO:2)

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TGTGTAGCAGGGACGATTTCCCAGAAAGCAATTTTCCTTTTGAAATACGTAATTGTTGAGACTAGGCAGT
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CTTAATTCAGACAGACTGTGAATACACCTTTTTTATAAATACCTTTCAAATTCTTGGTAAGATATAATTT
TGATAGCTGATTGCAGATTTTCTGTATTTGTCAGATTAATAAAGACTGCATGAATCCAAAAAAAAAAAAA
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>NM_000447.2 Homo sapiens presenilin 2 (PSEN2), transcript variant 1, mRNA (SEQ ID NO:3)

GGGGCCTGGGCCGGCGCCGGGTCCGGCCGGGCGCTCAGCCAGCTGCGTAAACTCCGCTGGAGCGCGGCGG
CAGAGCAGGCATTTCCAGCAGTGAGGAGACAGCCAGAAGCAAGCTTTTGGAGCTGAAGGAACCTGAGACA
GAAGCTAGTCCCCCCTCTGAATTTTACTGATGAAGAAACTGAGGCCACAGAGCTAAAGTGACTTTTCCCA
AGGTCGCCCAGCGAGGACGTGGGACTTCTCAGACGTCAGGAGAGTGATGTGAGGGAGCTGTGTGACCATA
GAAAGTGACGTGTTAAAAACCAGCGCTGCCCTCTTTGAAAGCCAGGGAGCATCATTCATTTAGCCTGCTG
AGAAGAAGAAACCAAGTGTCCGGGATTCAGACCTCTCTGCGGCCCCAAGTGTTCGTGGTGCTTCCAGAGG
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CCCAGTGGAGAAGCCAGGAGAACGAGGAGGACGGTGAGGAGGACCCTGACCGCTATGTCTGTAGTGGGGT
TCCCGGGCGGCCGCCAGGCCTGGAGGAAGAGCTGACCCTCAAATACGGAGCGAAGCACGTGATCATGCTG
TTTGTGCCTGTCACTCTGTGCATGATCGTGGTGGTAGCCACCATCAAGTCTGTGCGCTTCTACACAGAGA
AGAATGGACAGCTCATCTACACGCCATTCACTGAGGACACACCCTCGGTGGGCCAGCGCCTCCTCAACTC
CGTGCTGAACACCCTCATCATGATCAGCGTCATCGTGGTTATGACCATCTTCTTGGTGGTGCTCTACAAG
TACCGCTGCTACAAGTTCATCCATGGCTGGTTGATCATGTCTTCACTGATGCTGCTGTTCCTCTTCACCT
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CTCATCATGATCAGTGCGCTCATGGCCCTAGTGTTCATCAAGTACCTCCCAGAGTGGTCCGCGTGGGTCA
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GCCTCCCATCAGCTCTACATCTGAGGGACATGGTGTGCCACAGGCTGCAAGCTGCAGGGAATTTTCATTG

GATGCAGTTGTATAGTTTTACACTCTAGTGCCATATATTTTTAAGACTTTTCTTTCCTTAAAAAATAAAG
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TTGTCCATGATGTCCTTGTTATTTTATTGCCTTTAGAAACTGAGTCCTGTTCTTGTTACGGCAGTCACAC
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>NM_012486.2 Homo sapiens presenilin 2 (PSEN2), transcript variant 2, mRNA (SEQ ID NO:4)

GGGGCCTGGGCCGGCGCCGGGTCCGGCCGGGCGCTCAGCCAGCTGCGTAAACTCCGCTGGAGCGCGGCGG
CAGAGCAGGCATTTCCAGCAGTGAGGAGACAGCCAGAAGCAAGCTTTTGGAGCTGAAGGAACCTGAGACA
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TCGGCTGAGAGCCCCACGCCGCGCTCCTGCCAGGAGGGCAGGCAGGGCCCAGAGGATGGAGAGAACACTG
CCCAGTGGAGAAGCCAGGAGAACGAGGAGGACGGTGAGGAGGACCCTGACCGCTATGTCTGTAGTGGGGT
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TCATTGGCTTGTGTCTGACCCTCCTGCTGCTTGCTGTGTTCAAGAAGGCGCTGCCCGCCCTCCCCATCTC
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CAGATTAGGGCGGGGAGAAGAGCATCCGGCATGAGGGCTGAGATGCGCAAAGAGTGTGCTCGGGAGTGGC
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TCCATGATGTCCTTGTTATTTTATTGCCTTTAGAAACTGAGTCCTGTTCTTGTTACGGCAGTCACACTGC
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>NP_000012.1 presenilin-1 isoform I-467 [Homo sapiens](SEQ ID NO:5)
MTELPAPLSYFQNAQMSEDNHLSNTVRSQNDNRERQEHNDRRSLGHPEPLSNGRPQGNSRQVVEQDEEED
EELTLKYGAKHVIMLFVPVTLCMVVVVATIKSVSFYTRKDGQLIYTPFTEDTETVGQRALHSILNAAIMI
SVIVVMTILLVVLYKYRCYKVIHAWLIISSLLLLFFFSFIYLGEVFKTYNVAVDYITVALLIWNFGVVGM
ISIHWKGPLRLQQAYLIMISALMALVFIKYLPEWTAWLILAVISVYDLVAVLCPKGPLRMLVETAQERNE
TLFPALIYSSTMVWLVNMAEGDPEAQRRVSKNSKYNAESTERESQDTVAENDDGGFSEEWEAQRDSHLGP
HRSTPESRAAVQELSSSILAGEDPEERGVKLGLGDFIFYSVLVGKASATASGDWNTTIACFVAILIGLCL
TLLLLAIFKKALPALPISITFGLVFYFATDYLVQPFMDQLAFHQFYI

>NP_015557.2 presenilin-1 isoform I-463 [Homo sapiens](SEQ ID NO:6)
MTELPAPLSYFQNAQMSEDNHLSNTNDNRERQEHNDRRSLGHPEPLSNGRPQGNSRQVVEQDEEEDEELT
LKYGAKHVIMLFVPVTLCMVVVVATIKSVSFYTRKDGQLIYTPFTEDTETVGQRALHSILNAAIMISVIV
VMTILLVVLYKYRCYKVIHAWLIISSLLLLFFFSFIYLGEVFKTYNVAVDYITVALLIWNFGVVGMISIH
WKGPLRLQQAYLIMISALMALVFIKYLPEWTAWLILAVISVYDLVAVLCPKGPLRMLVETAQERNETLFP
ALIYSSTMVWLVNMAEGDPEAQRRVSKNSKYNAESTERESQDTVAENDDGGFSEEWEAQRDSHLGPHRST
PESRAAVQELSSSILAGEDPEERGVKLGLGDFIFYSVLVGKASATASGDWNTTIACFVAILIGLCLTLLL
LAIFKKALPALPISITFGLVFYFATDYLVQPFMDQLAFHQFYI

>NP_000438.2 presenilin-2 isoform 1 [Homo sapiens] (SEQ ID NO:7)
MLTFMASDSEEEVCDERTSLMSAESPTPRSCQEGRQGPEDGENTAQWRSQENEEDGEEDPDRYVCSGVPG
RPPGLEEELTLKYGAKHVIMLFVPVTLCMIVVVATIKSVRFYTEKNGQLIYTPFTEDTPSVGQRLLNSVL
NTLIMISVIVVMTIFLVVLYKYRCYKFIHGWLIMSSLMLLFLFTYIYLGEVLKTYNVAMDYPTLLLTVWN
FGAVGMVCIHWKGPLVLQQAYLIMISALMALVFIKYLPEWSAWVILGAISVYDLVAVLCPKGPLRMLVET
AQERNEPIFPALIYSSAMVWTVGMAKLDPSSQGALQLPYDPEMEEDSYDSFGEPSYPEVFEPPLTGYPGE
ELEEEEERGVKLGLGDFIFYSVLVGKAAATGSGDWNTTLACFVAILIGLCLTLLLLAVFKKALPALPISI
TFGLIFYFSTDNLVRPFMDTLASHQLYI

>NP_036618.2 presenilin-2 isoform 2 [Homo sapiens] (SEQ ID NO:8)
MLTFMASDSEEEVCDERTSLMSAESPTPRSCQEGRQGPEDGENTAQWRSQENEEDGEEDPDRYVCSGVPG
RPPGLEEELTLKYGAKHVIMLFVPVTLCMIVVVATIKSVRFYTEKNGQLIYTPFTEDTPSVGQRLLNSVL

NTLIMISVIVVMTIFLVVLYKYRCYKFIHGWLIMSSLMLLFLFTYIYLGEVLKTYNVAMDYPTLLLTVWN
FGAVGMVCIHWKGPLVLQQAYLIMISALMALVFIKYLPEWSAWVILGAISVYDLVAVLCPKGPLRMLVET
AQERNEPIFPALIYSSAMVWTVGMAKLDPSSQGALQLPYDPEMEDSYDSFGEPSYPEVFEPPLTGYPGEE
LEEEEERGVKLGLGDFIFYSVLVGKAAATGSGDWNTTLACFVAILIGLCLTLLLLAVFKKALPALPISIT
FGLIFYFSTDNLVRPFMDTLASHQLYI

To determine the percent identity of two amino acid sequences, or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). The length of a reference sequence aligned for comparison purposes is at least 80% of the length of the reference sequence, and in some embodiments is at least 90% or 100%. The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. In another embodiment, the percent identity of two amino acid sequences can be assessed as a function of the conservation of amino acid residues within the same family of amino acids (e.g., positive charge, negative charge, polar and uncharged, hydrophobic) at corresponding positions in both amino acid sequences (e.g., the presence of an alanine residue in place of a valine residue at a specific position in both sequences shows a high level of conservation, but the presence of an arginine residue in place of an aspartate residue at a specific position in both sequences shows a low level of conservation).

For example, the percent identity between two amino acid sequences can be determined using the Needleman and Wunsch ((1970) J. Mol. Biol. 48:444-453) algorithm which has been incorporated into the GAP program in the GCG software package, using a Blossum scoring matrix, e.g., with default values for gap penalty, gap extend penalty of 4, and frameshift gap penalty.

Mutant Presenilin 1

In some embodiments, the PS1 protein contains a mutation. In some embodiments, the mutation is a conservative substitution. Such changes include substituting any of isoleucine (I), valine (V), and leucine (L) for any other of these hydrophobic amino acids; aspartic acid (D) for glutamic acid (E) and vice versa; glutamine (Q) for asparagine (N) and vice versa; and serine (S) for threonine (T) and vice versa. Other substitutions can also be considered conservative, depending on the environment of the particular amino acid and its role in the three-dimensional structure of the protein. For example, glycine (G) and alanine (A) can frequently be interchangeable, as can alanine (A) and valine (V). Methionine (M), which is relatively hydrophobic, can frequently be interchanged with leucine and isoleucine, and sometimes with valine. Lysine (K) and arginine (R) are frequently interchangeable in locations in which the significant feature of the amino acid residue is its charge and the differing pK's of these two amino acid residues are not significant. Still other changes can be considered "conservative" in particular environments (see, e.g. Table III of US20110201052; pages 13-15 "Biochemistry" 2nd ED. Stryer ed (Stanford University); Henikoff et al., PNAS 1992 Vol 89 10915-10919; Lei et al., J Biol Chem 1995 May 19; 270(20):11882-6).

In some embodiments, the methods include introducing one or more additional mutations into the human PS1 sequence (SEQ ID NOs:5 or 6). Thus, in some embodiments, the sequence can be at least 80%, 85%, 90%, 95%, or 99% identical to at least 60%, 70%, 80%, 90%, or 100% of an human PS1. In some embodiments, the methods include introducing one or more additional mutations into the human PS2 sequence (SEQ ID NOs:7 or 8). Thus, in some embodiments, the sequence can be at least 80%, 85%, 90%, 95%, or 99% identical to at least 60%, 70%, 80%, 90%, or 100% of an human PS2.

To determine the percent identity of two amino acid sequences, or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). The length of a reference sequence aligned for comparison purposes is typically at least 80% of the length of the reference sequence, and in some embodiments is at least 90% or 100%. The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or

nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology"). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. In another embodiment, the percent identity of two amino acid sequences can be assessed as a function of the conservation of amino acid residues within the same family of amino acids (e.g., positive charge, negative charge, polar and uncharged, hydrophobic) at corresponding positions in both amino acid sequences (e.g., the presence of an alanine residue in place of a valine residue at a specific position in both sequences shows a high level of conservation, but the presence of an arginine residue in place of an aspartate residue at a specific position in both sequences shows a low level of conservation).

For purposes of the present methods, the comparison of sequences and determination of percent identity between two sequences can be accomplished using a Blossum 62 scoring matrix with a gap penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5.

Delivery Vectors

Nucleic acids encoding a PS1 or PS2 polypeptide or therapeutically active fragment thereof can be incorporated into a gene construct to be used as a part of a gene therapy protocol. For example, described herein are targeted expression vectors for *in vivo* delivery and expression of a polynucleotide that encodes a PS1 or PS2 polypeptide or active fragment thereof in particular cell types, especially cerebral cortical neuronal cells. Expression constructs of such components can be administered in any effective carrier, e.g., any formulation or composition capable of effectively delivering the component gene to cells in vivo. Approaches include insertion of the gene in viral vectors, preferably adeno-associated virus. Viral vectors typically transduce cells directly.

Viral vectors capable of highly efficient transduction of CNS neurons may be employed, including any serotypes of rAAV (e.g., AAV1-AAV12) vectors, recombinant or chimeric AAV vectors, as well as lentivirus or other suitable viral vectors. In some embodiments, a polynucleotide encoding PS1 or PS2 is operably linked to promoter suitable for expression in the CNS. For example, a neuron subtype-specific specific promoter, such as the alpha-calcium/calmodulin kinase 2A promoter may be used to target excitatory neurons. Alternatively, a pan neuronal promoter, such as the synapsin I promoter, may be used to drive PS1 or PS2 expression. Other exemplary promoters include, but are not limited to, a cytomegalovirus (CMV) early enhancer/promoter; a hybrid CMV enhance/chicken β-actin

(CBA) promoter; a promoter comprising the CMV early enhancer element, the first exon and first intron of the chicken β-actin gene, and the splice acceptor of the rabbit β-globin gene (commonly call the "CAG promoter"); or a 1.6-kb hybrid promoter composed of a CMV immediate-early enhancer and CBA intron 1/exon 1 (commonly called the CAGGS promoter; Niwa et al. Gene, 108:193-199 (1991)). The CAGGS promoter (Niwa et al., 1991) has been shown to provide ubiquitous and long-term expression in the brain (Klein et al., Exp. Neurol. 176:66-74 (2002)).A typical approach for *in vivo* introduction of nucleic acid into a cell is by use of a viral vector containing nucleic acid, e.g., a cDNA encoding a PS1 or PS2. Among other things, infection of cells with a viral vector has the advantage that a large proportion of the targeted cells can receive the nucleic acid. Additionally, molecules encoded within the viral vector, e.g., by a cDNA contained in the viral vector, are expressed efficiently in cells that have taken up viral vector nucleic acid.

A viral vector system particularly useful for delivery of nucleic acids is the adeno-associated virus (AAV). Adeno-associated virus is a naturally occurring defective virus that requires another virus, such as an adenovirus or a herpes virus, as a helper virus for efficient replication and a productive life cycle. (For a review see Muzyczka et al., Curr. Topics in Micro and Immunol.158:97-129 (1992)). AAV vectors efficiently transduce various cell types and can produce long-term expression of transgenes *in vivo*. Although AAV vector genomes can persist within cells as episomes, vector integration has been observed (see for example Deyle and Russell, Curr Opin Mol Ther. 2009 Aug; 11(4): 442–447; Asokan et al., Mol Ther. 2012 April; 20(4): 699–708; Flotte et al., Am. J. Respir. Cell. Mol. Biol. 7:349-356 (1992); Samulski et al., J. Virol. 63:3822-3828 (1989); and McLaughlin et al., J. Virol. 62:1963-1973 (1989)). AAV vectors, such as AAV2, have been extensively used for gene augmentation or replacement and have shown therapeutic efficacy in a range of animal models as well as in the clinic; see, e.g., Mingozzi and High, Nature Reviews Genetics 12, 341-355 (2011); Deyle and Russell, Curr Opin Mol Ther. 2009 Aug; 11(4): 442–447; Asokan et al., Mol Ther. 2012 April; 20(4): 699–708. AAV vectors containing as little as 300 base pairs of AAV can be packaged and can produce recombinant protein expression. Protocols for producing recombinant retroviruses and for infecting cells in vitro or in vivo with such viruses are known in the art, e.g, can be found in Ausubel, et al., eds., Current Protocols in Molecular Biology, Greene Publishing Associates, (1989), Sections 9.10-9.14, and other standard laboratory manuals. The use of AAV vectors to deliver constructs for expression in the brain has been described, e.g., in Iwata et al., Sci Rep. 2013;3:1472; Hester et al., Curr

Gene Ther. 2009 Oct;9(5):428-33; Doll et al., Gene Therapy 1996, 3(5):437-447; and Foley et al., J Control Release. 2014 Dec 28;196:71-8.

Thus, in some embodiments, the PS1 or PS2 encoding nucleic acid is present in a vector for gene therapy, such as an AAV vector. In some instances, the AAV vector is selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAVrh10, AAV11, and AAV12.

A vector as described herein can be a pseudotyped vector. Pseudotyping provides a mechanism for modulating a vector's target cell population. For instance, pseudotyped AAV vectors can be utilized in various methods described herein. Pseudotyped vectors are those that contain the genome of one vector, e.g., the genome of one AAV serotype, in the capsid of a second vector, e.g., a second AAV serotype. Methods of pseudotyping are well known in the art. For instance, a vector may be pseudotyped with envelope glycoproteins derived from Rhabdovirus vesicular stomatitis virus (VSV) serotypes (Indiana and Chandipura strains), rabies virus (e.g., various Evelyn–Rokitnicki–Abelseth ERA strains and challenge virus standard (CVS)), Lyssavirus Mokola virus, a rabies-related virus, vesicular stomatitis virus (VSV), Mokola virus (MV), lymphocytic choriomeningitis virus (LCMV), rabies virus glycoprotein (RV-G), glycoprotein B type (FuG-B), a variant of FuG-B (FuG-B2) or Moloney murine leukemia virus (MuLV). A virus may be pseudotyped for transduction of one or more neurons or groups of cells.

Without limitation, illustrative examples of pseudotyped vectors include recombinant AAV2/1, AAV2/2, AAV2/5, AAV2/6, AAV2/7, AAV2/8, AAV9, AAVrh10, AAV11, and AAV12 serotype vectors. It is known in the art that such vectors may be engineered to include a transgene encoding a human protein or other protein. In particular instances, the present disclosures can include a pseudotyped AAV9 or AAVrh10 viral vector including a nucleic acid as disclosed herein. See Viral Vectors for Gene Therapy: Methods and Protocols, ed. Machida, Humana Press, 2003.

In some instances, a particular AAV serotype vector may be selected based upon the intended use, e.g., based upon the intended route of administration.

Various methods for application of AAV vector constructs in gene therapy are known in the art, including methods of modification, purification, and preparation for administration to human subjects (see, e.g., Viral Vectors for Gene Therapy: Methods and Protocols, ed. Machida, Humana Press, 2003). In addition, AAV based gene therapy targeted to cells of the CNS has been described (see, e.g., U.S. patents 6,180,613 and 6,503,888). High titer AAV

preparations can be produced using techniques known in the art, e.g., as described in U.S. Pat. No. 5,658,776

A vector construct refers to a polynucleotide molecule including all or a portion of a viral genome and a transgene. In some instances, gene transfer can be mediated by a DNA viral vector, such as an adenovirus (Ad) or adeno-associated virus (AAV). Other vectors useful in methods of gene therapy are known in the art. For example, a construct as disclosed herein can include an alphavirus, herpesvirus, retrovirus, lentivirus, or vaccinia virus.

Adenoviruses are a relatively well characterized group of viruses, including over 50 serotypes (see, e.g., WO 95/27071, which is herein incorporated by reference). Adenoviruses are tractable through the application of techniques of molecular biology and may not require integration into the host cell genome. Recombinant Ad-derived vectors, including vectors that reduce the potential for recombination and generation of wild-type virus, have been constructed (see, e.g., international patent publications WO 95/00655 and WO 95/11984, which are herein incorporated by reference). Wild-type AAV has high infectivity and is capable of integrating into a host genome with a high degree of specificity (see, e.g. Hermonat and Muzyczka 1984 Proc. Natl. Acad. Sci., USA 81:6466-6470 and Lebkowski et al. 1988 Mol. Cell. Biol. 8:3988-3996).

Non-native regulatory sequences, gene control sequences, promoters, non-coding sequences, introns, or coding sequences can be included in a nucleic acid as disclosed herein. The inclusion of nucleic acid tags or signaling sequences, or nucleic acids encoding protein tags or protein signaling sequences, is further contemplated herein. Typically, the coding region is operably linked with one or more regulatory nucleic acid components.

A promoter included in a nucleic acid as disclosed herein can be a tissue- or cell type-specific promoter, a promoter specific to multiple tissues or cell types, an organ-specific promoter, a promoter specific to multiple organs, a systemic or ubiquitous promoter, or a nearly systemic or ubiquitous promoter. Promoters having stochastic expression, inducible expression, conditional expression, or otherwise discontinuous, inconstant, or unpredictable expression are also included within the scope of the present disclosure. A promoter can include any of the above characteristics or other promoter characteristics known in the art.

In clinical settings, the gene delivery systems for the therapeutic gene can be introduced into a subject by any of a number of methods, each of which is familiar in the art. For instance, a pharmaceutical preparation of the gene delivery system can be introduced systemically, e.g., by intravenous injection, and specific transduction of the protein in the

target cells will occur predominantly from specificity of transfection, provided by the gene delivery vehicle, cell-type or tissue-type expression due to the transcriptional regulatory sequences controlling expression of the receptor gene, or a combination thereof. In other embodiments, initial delivery of the recombinant gene is more limited, with introduction into the subject being quite localized. For example, the gene delivery vehicle can be introduced by catheter (see U.S. Patent 5,328,470) or by stereotactic injection, e.g., optionally into the cisterna magna, cerebral ventricles, lumbar intrathecal space, direct injection into hippocampus (e.g., Chen et al., PNAS USA 91: 3054-3057 (1994)). In some embodiments, delivery methods of Presenilin-expressing virus include intravenous, intrathecal, intracerebroventricular, intracisternal, and stereotactic intraparenchymal administration.

The methods can be further optimized via preclinical testing to achieve the best rescue of neurodegeneration, dementia, synaptic dysfunction and molecular alteration in presenilin conditional double knockout mice and presenilin-1 knockin mice expressing FAD mutations.

The pharmaceutical preparation of the gene therapy construct can consist essentially of the gene delivery system in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is embedded. Alternatively, where the complete gene delivery system can be produced intact from recombinant cells, e.g., retroviral vectors, the pharmaceutical preparation can comprise one or more cells, which produce the gene delivery system.

Delivery Formulations and Pharmaceutical Compositions

In some embodiments, polynucleotides as disclosed herein for delivery to a target tissue in vivo are encapsulated or associated with in a nanoparticle. Methods for nanoparticle packaging are well known in the art, and are described, for example, in Bose S, et al (Role of Nucleolin in Human Parainfluenza Virus Type 3 Infection of Human Lung Epithelial Cells. J. Virol. 78:8146. 2004); Dong Y et al. Poly(d,l-lactide-co-glycolide)/montmorillonite nanoparticles for oral delivery of anticancer drugs. Biomaterials 26:6068. 2005); Lobenberg R. et al (Improved body distribution of 14C-labelled AZT bound to nanoparticles in rats determined by radioluminography. J Drug Target 5:171.1998); Sakuma S R et al (Mucoadhesion of polystyrene nanoparticles having surface hydrophilic polymeric chains in the gastrointestinal tract. Int J Pharm 177:161. 1999); Virovic L et al. Novel delivery methods for treatment of viral hepatitis: an update. Expert Opin Drug Deliv 2:707.2005); and Zimmermann E et al, Electrolyte- and pH-stabilities of aqueous solid lipid nanoparticle (SLN) dispersions in artificial gastrointestinal media. Eur J Pharm Biopharm 52:203. 2001).

In some embodiments, one or more polynucleotides is delivered to a target tissue *in vivo* in a vesicle, e.g. a liposome (see Langer, Science 249:1527-1533 (1990); Treat et al., in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353-365 (1989); Lopez-Berestein, ibid., pp. 317-327; see generally ibid). In some embodiments, lipid-based nanoparticles (LNP) are used; see, e.g., Robinson et al., Mol Ther. 2018 Aug 1;26(8):2034-2046; US9956271B2.

The present methods and compositions can include microvesicles or a preparation thereof, that contains one or more therapeutic molecules – e.g., polynucleotides or RNA – described herein. "Microvesicles", as the term is used herein, refers to membrane-derived microvesicles, which includes a range of extracellular vesicles, including exosomes, microparticles and shed microvesicles secreted by many cell types under both normal physiological and pathological conditions. See, e.g., EP2010663B1. The methods and compositions described herein can be applied to microvesicles of all sizes; in one embodiment, 30 to 200 nm, in one embodiment, 30 to 800 nm, in one embodiment, up to 2 um. The methods and compositions described herein can also be more broadly applied to all extracellular vesicles, a term which encompasses exosomes, shed microvesicles, oncosomes, ectosomes, and retroviral-like particles. Such a microvesicle or preparation is produced by the herein described methods. As the term is used herein, a microvesicle preparation refers to a population of microvesicles obtained/prepared from the same cellular source. Such a preparation is generated, for example, in vitro, by culturing cells expressing the nucleic acid molecule of the instant invention and isolating microvesicles produced by the cells. Methods of isolating such microvesicles are known in the art (Thery et al., Isolation and characterization of exosomes from cell culture supernatants and biological fluids, in Current Protocols Cell Biology, Chapter 3, 322, (John Wiley, 2006); Palmisano et al., (Mol Cell Proteomics. 2012 August; 11(8):230-43) and Waldenström et al., ((2012) PLoS ONE 7(4): e34653.doi: 10.1371/journal.pone.0034653)), some examples of which are described herein. Such techniques for isolating microvesicles from cells in culture include, without limitation, sucrose gradient purification/separation and differential centrifugation, and can be adapted for use in a method or composition described herein. See, e.g., EP2010663B1.

In some embodiments, the microvesicles are isolated by gentle centrifugation (e.g., at about 300 g) of the culture medium of the donor cells for a period of time adequate to separate cells from the medium (e.g., about 15 minutes). This leaves the microvesicles in the supernatant, to thereby yield the microvesicle preparation. In one embodiment, the culture

medium or the supernatant from the gentle centrifugation, is more strongly centrifuged (e.g., at about 16,000 g) for a period of time adequate to precipitate cellular debris (e.g., about 30 minutes). This leaves the microvesicles in the supernatant, to thereby yield the microvesicle preparation. In one embodiment, the culture medium, the gentle centrifuged preparation, or the strongly centrifuged preparation is subjected to filtration (e.g., through a 0.22 um filter or a 0.8 um filter, whereby the microvesicles pass through the filter. In one embodiment, the filtrate is subjected to a final ultracentrifugation (e.g. at about 110,000 g) for a period of time that will adequately precipitate the microvesicles (e.g. for about 80 minutes). The resulting pellet contains the microvesicles and can be resuspended in a volume of buffer that yields a useful concentration for further use, to thereby yield the microvesicle preparation. In one embodiment, the microvesicle preparation is produced by sucrose density gradient purification. In one embodiment, the microvesicles are further treated with DNAse (e.g., DNAse I) and/or RNAse and/or proteinase to eliminate any contaminating DNA, RNA, or protein, respectively, from the exterior. In one embodiment, the microvesicle preparation contains one or more RNAse inhibitors.

The molecules contained within the microvesicle preparation will comprise the therapeutic molecule. Typically the microvesicles in a preparation will be a heterogeneous population, and each microvesicle will contain a complement of molecule that may or may not differ from that of other microvesicles in the preparation. The content of the therapeutic molecules in a microvesicle preparation can be expressed either quantitatively or qualitatively. One such method is to express the content as the percentage of total molecules within the microvesicle preparation. By way of example, if the therapeutic molecule is an mRNA, the content can be expressed as the percentage of total RNA content, or alternatively as the percentage of total mRNA content, of the microvesicle preparation. Similarly, if the therapeutic molecule is a protein, the content can be expressed as the percentage of total protein within the microvesicles. In one embodiment, therapeutic microvesicles, or a preparation thereof, produced by the method described herein contain a detectable, statistically significantly increased amount of the therapeutic molecule as compared to microvesicles obtained from control cells (cells obtained from the same source which have not undergone scientific manipulation to increase expression of the therapeutic molecule). In one embodiment, the therapeutic molecule is present in an amount that is at least about 10%, 20%, 30% 40%, 50%, 60%, 70% 80% or 90%, more than in microvesicles obtained from control cells. Higher levels of enrichment may also be achieved. In one embodiment, the

therapeutic molecule is present in the microvesicle or preparation thereof, at least 2 fold more than control cell microvesicles. Higher fold enrichment may also be obtained (e.g., 3, 4, 5, 6, 7, 8, 9 or 10 fold).

In one embodiment, a relatively high percentage of the microvesicle content is the therapeutic molecule (e.g., achieved through overexpression or specific targeting of the molecule to microvesicles). In one embodiment, the microvesicle content of the therapeutic molecule is at least about 10%, 20%, 30% 40%, 50%, 60%, 70% 80% or 90%, of the total (like) molecule content (e.g., the therapeutic molecule is an mRNA and is about 10% of the total mRNA content of the microvesicle). Higher levels of enrichment may also be achieved. In one embodiment, the therapeutic molecule is present in the microvesicle or preparation thereof, at least 2 fold more than all other such (like) molecules. Higher fold enrichment may also be obtained (e.g., 3, 4, 5, 6, 7, 8, 9 or 10 fold).

EXAMPLES

The invention is further described in the following examples, which do not limit the scope of the invention described in the claims.

Example 1: Pyramidal neurons of sporadic Alzheimer disease (AD) patients exhibit reduced PSEN1 and PSEN2 mRNA levels

Presenilin (PS) protein is the catalytic subunit of γ-secretase, which is required for the production of Aβ peptides. Mutations in the Presenilin-1 (PSEN1) and Presenilin-2 (PSEN2) genes are associated with 90% of cases of familial Alzheimer's disease (FAD). PSEN mutations associated with FAD and frontotemporal dementia cause loss of presenilin expression and presenilin function, leading to reduced γ-secretase activity (Xia et al., Neuron. 2015 Mar 4;85(5):967-81; Watanabe et al., J Neurosci. 2012 Apr 11;32(15):5085-96; Brouwers et al., 2008 Ann Med 40 (8): 562–83).

Individual pyramidal neurons were collected using laser capture microdissection, 300 neurons per human brain were pooled together for RNA preparation (PicoPure RNA Isolation Kit, Life Technologies KIT0204). qRT-PCR was performed using SuperScript III One-Step RT-PCR System with Platinum Taq DNA Polymerase (Life Technologies). The sequence of the primers used are listed in Table 2.

Table 2. List of primers.

UID	Sequence (5'-3')	SEQ ID NO:	Gene Amplified

hPS1-F5	AGACCCGGAAGCTCAAAGGA	9	human Presenilin-1
hPS1-R5	TTCCTCACTGAACCCGCCAT	10	human Presenilin-1
hPS2-F5	AAGTGTCCGGGATTCAGACCTC	11	human Presenilin-2
hPS2-R5	TCGCTGTCAGAGGCCATGAA	12	human Presenilin-2
NCT-104	ACAGGTGGCCTTAAGAACTTCA T	13	human Nicastrin
NCT-204	CCACCTGGTTCCGTACAGAC	14	human Nicastrin
hPen2-F1	TCCTTGTCCCAGCCTACACA	15	human PEN2
hPen2-R1	AGCACTATCACCCAGAAGAGG A	16	human PEN2

PSEN1 and PSEN2 mRNA levels were reduced in laser dissected pyramidal neurons from hippocampal CA1 pyramidal neurons of sporadic AD patients (**FIGs. 1A-B**).

Example 2: Dose-dependent reduction of γ-secretase activity in MEFs carrying heterozygous and homozygous PSEN1 mutations

Methods

Generation of immortalized Psen mutant MEFs

Mouse embryonic fibroblasts (MEFs) carrying various Psen1 genotypes were maintained in media supplemented with 10% FBS and 1% penicillin and streptomycin. In 6-well plates, 300,000 MEFs were immortalized by transfection with 1μg CMV-SV40. MEFs transfected with CMV-SV40 were compared to MEFs transfected with CMV-GFP (1μg), which stopped dividing around passage 7.

Transfection of NdE and hPS1

To determine whether γ-secretase activity is impaired in various immortalized Psen mutant MEFs, CMV-NotchΔE (5ng) was transiently transfected into MEFs in 6-well plates using Lipofectamine LTX (ThermoFisher Scientific 15338030) per manufacturer's instructions.

Roughly 24 hours after transfection, cells were lysed in RIPA buffer: 50 mM Tris-Cl (pH 7.6), 150 mM NaCl, 1% NP40, 0.5% sodium deoxycholate, protease inhibitor cocktail (Sigma), 1mM DTT. Proteins (40μg) were separated in NuPAGE gels (Invitrogen) and transferred to nitrocellulose membranes. Primary antibodies were rabbit anti-cleaved Notch1

Val1744 (Cell Signaling), rabbit anti-PS1 NTF (Calbiochem), which recognizes both mouse and human PS1, mouse anti-cMyc (Sigma) or rabbit anti-tubulin (Cell Signaling). Membranes were then incubated with dye-coupled secondary antibodies (goat anti-rabbit IRdye800, goat anti-mouse IRdye800, or goat anti-rabbit IRdye680 from Licor). Signals were quantified with the Odyssey Infrared Imaging System (LI-COR Bioscience). γ-secretase activity measured by NICD production is reduced in L435F KI/+ MEFs and further reduced in KI/KI and PS1-/- MEFs (**FIG. 3A**).

Example 3: Dose-dependent rescue of γ-secretase activity in MEFs with varying PS genotypes: *PS1*$^{+/+}$, *PS1*$^{L435F/+}$, *PS1*$^{+/-}$, *PS1*$^{L435F/\ L435F}$, *PS1*$^{-/-}$ and *PS1*$^{-/-}$; *PS2*$^{-/-}$

To determine whether reduced γ-secretase activity associated with *PSEN1* mutations can be corrected by introduction of wild-type (WT) hPS1, primary MEFs from embryos carrying varying *PS* genotypes, *PS1*$^{+/+}$, *PS1*$^{L435F/+}$, *PS1*$^{+/-}$, *PS1*$^{L435F/\ L435F}$, *PS1*$^{-/-}$ and *PS1*$^{-/-}$; *PS2*$^{-/-}$ (DKO) were derived. The immortalized MEFs were transiently transfected with *CMV-NdelE*, and γ-secretase activity was evaluated by measuring the levels of NICD and PS1 NTF/CTF. The NICD levels were reduced in a PS1 dosage sensitive manner, and were undetectable in DKO cells (**FIG. 3A**). The NICD levels were reduced but detectable in *PS1*$^{L435F/\ L435F}$ MEFs ("L435F KI/KI" MEFs) and *PS1*$^{-/-}$ MEFs (**FIG. 3A**), whereas *de novo* NICD production was undetectable by *in vitro* γ-secretase assay using L435F KI/KI and *PS1*$^{-/-}$ embryonic brains (Xia et al., Neuron. 2015 Mar 4;85(5):967-81). Without wishing to be bound to a particular theory, applicant submits that this may be due to lower levels of PS2 normally expressed in the embryonic brain relative to MEFs, leading to lower overall PS activity in L435F KI/KI and *PS1*$^{-/-}$ brains, relative to MEFs. To test this hypothesis, the γ-secretase activity was measured in *PS1*$^{L435F/+}$; *PS2*$^{-/-}$ MEFs and compared to *PS1*$^{L435F/+}$ MEFs. The γ-secretase activity was lower in *PS1*$^{L435F/+}$; *PS2*$^{-/-}$ MEFs compared to *PS1*$^{L435F/+}$ MEFs (**FIG. 3B**).

To determine whether the impaired γ-secretase activity in various *PS* mutant MEFs can be rescued by introduction of WT hPS1, varying amounts (0, 20, 40, 80ng) of wild-type *hPS1* cDNA (*pCI-hPS1*) were transfected into MEFs, along with *CMV-NdelE*. Notably, increasing amounts of *pC1-hPS1* transfected into the MEFs resulted in accumulation of PS1 protein and restored levels of PS1 NTF and NICD in mutant (*PS1*$^{L435F/+}$, *PS1*$^{L435F/+}$; *PS2*$^{-/-}$ and DKO) MEFs (**FIG. 3B**). These results indicate that exogenous WT hPS1 can rescue the impaired γ-secretase activity in in various *PS* mutant MEFs.

OTHER EMBODIMENTS

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A method of treating a neurodegenerative disease, disorder, or condition, the method comprising administering to a subject in need of treatment a polynucleotide encoding a presenilin 1 (PS1) and/or presenilin 2 (PS2) protein, wherein the subject has one or more mutations in at least one allele of *PSEN1* and/or *PSEN2* that encodes a dominant negative PS1 or PS2 protein isoform.

2. The method of claim 1, wherein the neurodegenerative disease, disorder or condition is Alzheimer's disease.

3. The method of claim 2, wherein the Alzheimer's disease is familial Alzheimer's disease.

4. The method of claim 3, wherein the subject has a E280A, Y115H, L166P, C410Y, Δex9, G548, D257A, R278I, L435F, G384A, or L392Vmutation in the *PSEN1* gene, or a N141I, G206A, H163R, A79V, S290C, A260P, A426P, A431E, R269H, L271V, C1410Y, E280G, P264L, E185D, L235V, or M146V mutation in the *PSEN1* gene.

5. The method of claim 3, wherein the Alzheimer's disease is sporadic Alzheimer's disease.

6. The method of claim 3, wherein the Alzheimer's disease is late-onset or early-onset Alzheimer's disease.

7. The method of claim 1, wherein the neurodegenerative disease, disorder or condition is frontotemporal dementia.

8. The method of claim 1, wherein the neurodegenerative disease, disorder or condition is memory loss.

9. The method of claim 1, wherein the neurodegenerative disease, disorder or condition is cognitive decline or impairment.

10. The method of claim 9, wherein the cognitive impairment is mild cognitive impairment (MCI).

11. The method of any one of the preceding claims, comprising administering the polynucleotide in a vector, exosome, or lipid-based nanoparticle (LNP).

12. The method of claim 11, wherein the vector is a viral vector.

13. The method of claim 12, wherein the viral vector is an adeno-associated virus (AAV) vector.

14. The method of claim 13, wherein the AAV vector is selected from AAV9, AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAVrh10, AAV11, AAV12, AAV2/1, AAV2/2, AAV2/5, AAV2/6, AAV2/7, AAV2/8, AAV2/9, AAV2/rh10, AAV2/AAV11, or AAV2/AAV12.

15. The method of claim 11, wherein the viral vector is a lentiviral vector.

16. The method of claim 11, wherein the viral vector is a retroviral vector.

17. The method of any one of the preceding claims, wherein the polynucleotide comprises a presenilin 1 (PSEN1) gene or mRNA.

18. The method of any one of the preceding claims, wherein the polynucleotide comprises a presenilin 2 (PSEN2) gene or mRNA.

19. The method of any one of the preceding claims, wherein the polynucleotide comprises a presenilin 1 (PSEN1) gene or mRNA and a presenilin 2 (PSEN2) gene or mRNA.

20. The method of any one of the preceding claims, wherein the polynucleotide encoding PS1 comprises a nucleotide sequence at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO:1 or SEQ ID NO:2.

21. The method of any one of the preceding claims, wherein the polynucleotide encoding PS2 comprises a nucleotide sequence at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO:3 or SEQ ID NO:4.

22. The method of any one of the preceding claims, wherein the polynucleotide encoding a presenilin 1 (PS1) and/or presenilin 2 (PS2) is operably linked to a promoter.

23. The method of claim 22, wherein the promoter is a pan neuronal promoter.

24. The method of claim 23, wherein the pan neuronal promoter is a synapsin I promoter.

25. The method of claim 22, wherein the promoter is a neuron subtype-specific promoter.

26. The method of claim 25, wherein the neuron subtype-specific promoter is an alpha-calcium/calmodulin kinase 2A promoter.

27. The method of any one of the preceding claims, wherein the polynucleotide encoding PS1 and/or PS2 is administered to the CNS of the subject in need of treatment.

28. The method of claim 27, wherein the polynucleotide encoding PS1 and/or PS2 is administered to the CNS via intravenous delivery.

29. The method of claim 27, wherein the polynucleotide encoding PS1 and/or PS2 is administered directly to the CNS via intrathecal delivery.

30. The method of claim 27, wherein the polynucleotide encoding PS1 and/or PS2 is administered directly to the CNS via intracisternal delivery

31. The method of claim 27, wherein the polynucleotide encoding PS1 and/or PS2 is administered directly to the CNS via intracerebroventricular delivery.

32. The method of claim 27, wherein the polynucleotide encoding PS1 and/or PS2 is administered directly to the CNS via stereotactic injection into a certain area of the brain, optionally into the cisterna magna, cerebral ventricles, lumbar intrathecal space, direct injection into hippocampus.

33. A polynucleotide sequence encoding a presenilin 1 (PS1) and/or presenilin 2 (PS2) protein, for use in treating a neurodegenerative disease, disorder or condition in a subject, wherein the subject has one or more mutations in at least one allele of *PSEN1* and/or *PSEN2* that encodes a dominant negative PS1 or PS2 protein isoform.

34. A vector comprising a polynucleotide sequence encoding a presenilin 1 (PS1) and/or presenilin 2 (PS2) protein, wherein the PS1 and/or PS2 encoding sequence is operably linked to a promotor that drives expression of the PS1 and/or PS2 in the brain, for use in treating a neurodegenerative disease, disorder or condition in a subject, wherein the subject has one or more mutations in at least one allele of *PSEN1* and/or *PSEN2* that encodes a dominant negative PS1 or PS2 protein isoform.

35. The vector for the use of claim 34, wherein the vector is a viral vector.

36. The vector for the use of claims 35, wherein the viral vector is an adeno-associated viral (AAV) vector.

37. The vector of for the use claim 36, wherein the AAV vector is selected from AAV9, AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAVrh10, AAV11, AAV12, AAV2/1, AAV2/2, AAV2/5, AAV2/6, AAV2/7, AAV2/8, AAV2/9, AAV2/rh10, AAV2/AAV11, or AAV2/AAV12, preferably wherein the AAV vector is AAV9 or AAVrh10.

38. The vector for the use of claim 35, wherein the viral vector is a lentiviral vector.

39. The vector for the use of claim 35, wherein the viral vector is a retroviral vector.

40. The vector for the use of claim 34, wherein the promoter is a pan neuronal promoter.

41. The vector for the use of claim 40, wherein the pan neuronal promoter is a synapsin I promoter.

42. The vector for the use of claim 34, wherein the promoter is a neuron subtype-specific promoter.

43. The vector for the use of claim 42, wherein the neuron subtype-specific promoter is an alpha-calcium/calmodulin kinase 2A promoter.

44. The polynucleotide for the use of claim 33, or the vector for the use of any one of claims 34-43, wherein the sequence encoding PS1 comprises a nucleotide sequence at least 80%, 85%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO:1 or SEQ ID NO:2.

45. The polynucleotide for the use of claim 33, or the vector for the use of any one of claims 34-43, wherein the sequence encoding PS2 comprises a nucleotide sequence at least 80%, 85%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO:3 or SEQ ID NO:4.

46. The polynucleotide for the use of claim 33, or the vector for the use of any one of claims 34-45, wherein the neurodegenerative disease, disorder or condition is Alzheimer's disease.

47. The polynucleotide or vector for the use of claim 46, wherein the Alzheimer's disease is familial Alzheimer's disease.

48. The polynucleotide or vector for the use of claim 46, wherein the Alzheimer's disease is sporadic Alzheimer's disease.

49. The polynucleotide or vector for the use of claim 46, wherein the Alzheimer's disease is late-onset or early-onset Alzheimer's disease.

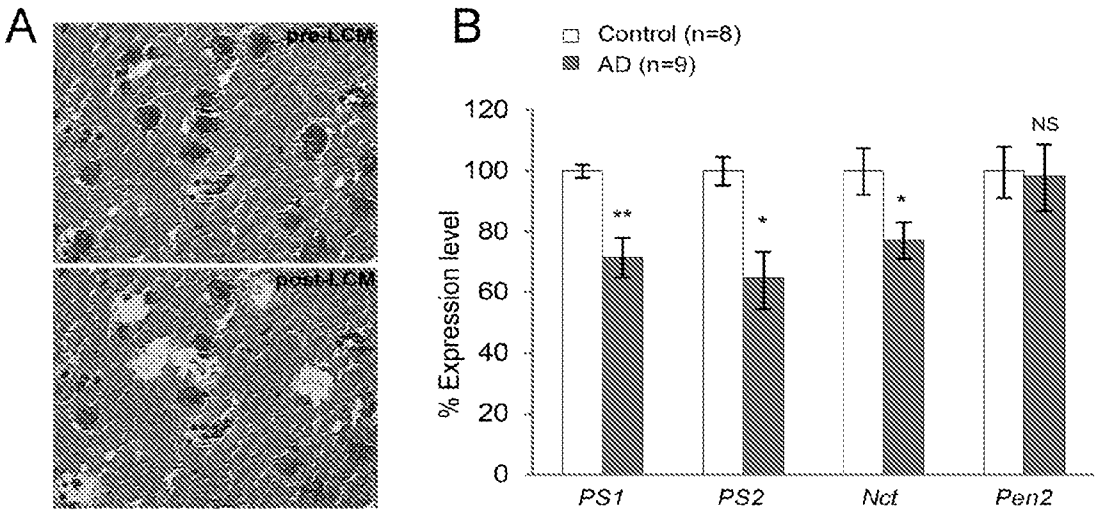
50. The polynucleotide for the use of claim 33, or the vector for the use of any one of claims 34-45, wherein the neurodegenerative disease, disorder or condition is frontotemporal dementia.

51. The polynucleotide for the use of claim 33, or the vector for the use of any one of claims 34-45, wherein the neurodegenerative disease, disorder or condition is memory loss.

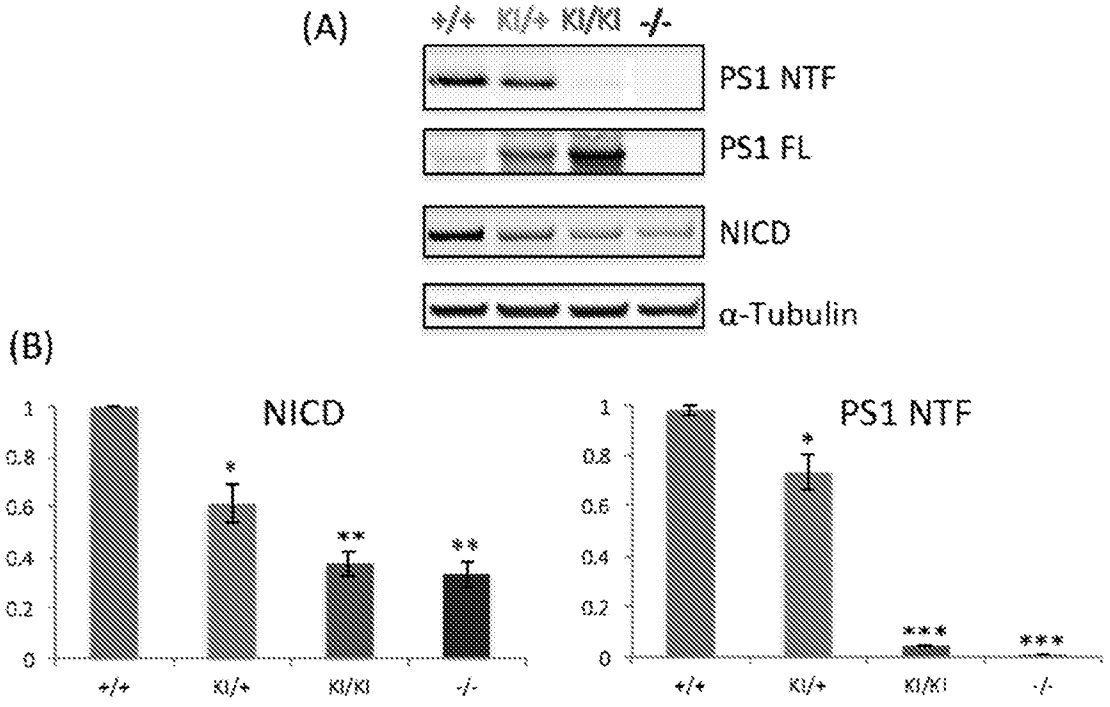
52. The polynucleotide for the use of claim 33, or the vector for the use of any one of claims 34-45, wherein the neurodegenerative disease, disorder or condition is cognitive decline or impairment.

53. The polynucleotide for the use of claim 33, or the vector for the use of any one of claims 34-45, wherein the cognitive impairment is mild cognitive impairment (MCI).

54. The polynucleotide for the use of claims 33 or 44-53, which is associated with or formulated for delivery with an exosome or lipid-based nanoparticle (LNP).



FIGs. 1A-B



FIGs. 2A-B

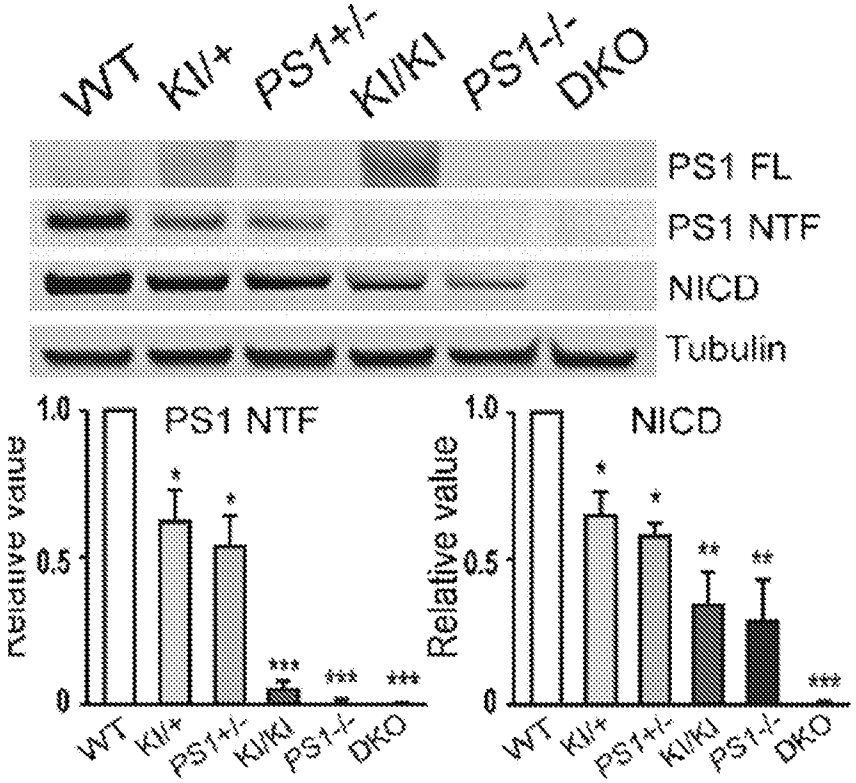

WT
KI/+
PS1+/-
KI/KI
PS1-/-
DKO
PS1 FL
PS1 NTF
NICD
Tubulin
PS1 NTF
NICD
Relative value
1.0
0.5
0
*
**
***

FIG. 3A

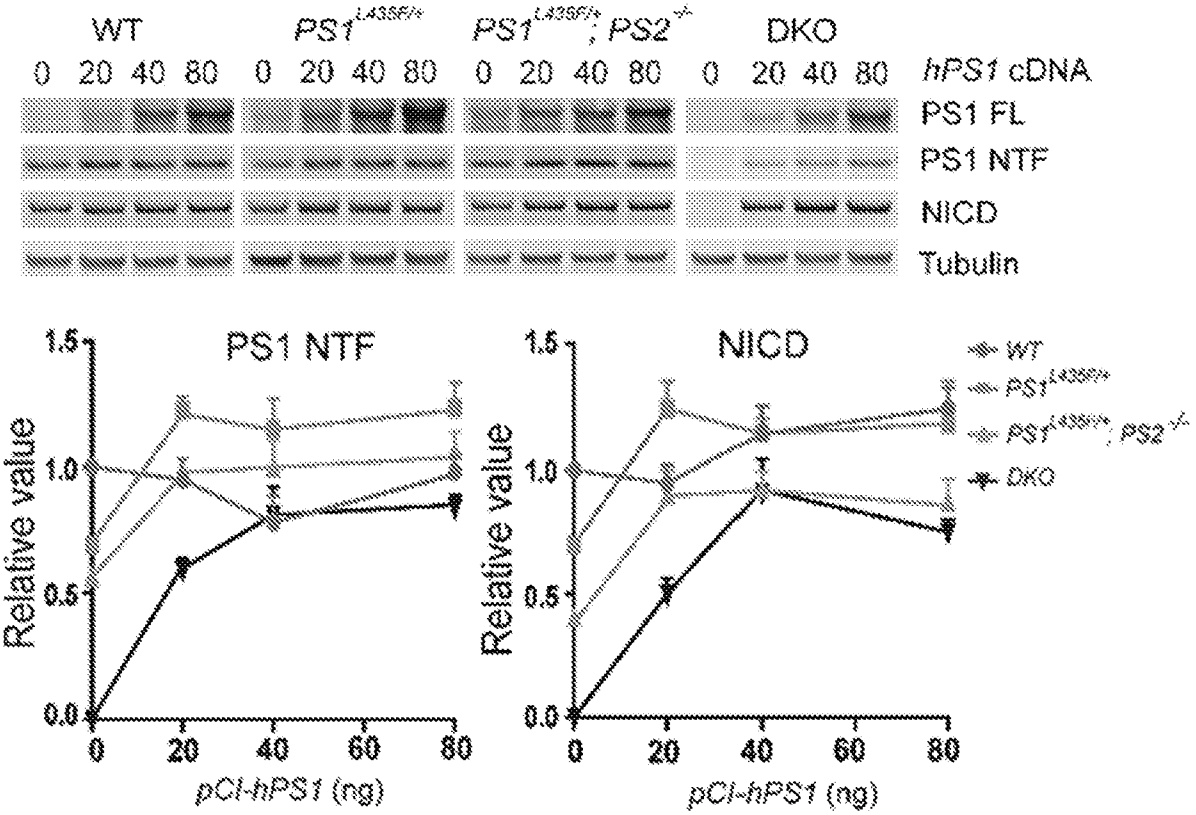

WT
PS1L435F/+
PS1L435F/+; PS2-/-
DKO
0 20 40 80 0 20 40 80 0 20 40 80 0 20 40 80
hPS1 cDNA
PS1 FL
PS1 NTF
NICD
Tubulin
PS1 NTF
NICD
Relative value
1.5
1.0
0.5
0.0
0 20 40 60 80
pCI-hPS1 (ng)
WT
PS1L435F/+
PS1L435F/+; PS2-/-
DKO

FIG. 3B