Description

Title of Invention: METHOD FOR TREATING ALZHEIMER'S DISEASE BY TARGETING MAPT GENE

Technical Field

[0001] The present invention relates to methods for treating Alzheimer's disease by targeting the human microtuble-associated protein tau (MAPT) gene, and the like. More particularly, the present invention relates to methods and agents for treating or preventing Alzheimer's disease by suppressing expression of human MAPT gene by using a guide RNA targeting a particular sequence of human MAPT gene and a fusion protein of a transcription inhibitor and a CRISPR effector protein, and the like.

Background Art

[0002] Tau protein is a microtubule-binding protein that is mainly expressed in the nervous system. It promotes polymerization of tubulin, stabilizes microtubules, and contributes to the construction and maintenance of nerve axons. Tau protein is a product of a single gene named MAPT (microtubule-associated protein tau) located on chromosome 17 in humans, and six kinds of isoforms are expressed in the human brain by alternative splicing. All of these isoforms are known to lose binding ability to microtubule and self-aggregate when excessively phosphorylated. Self-assembly of Tau protein is involved in pathologies such as Alzheimer's disease (hereinafter to be referred to as AD) and frontotemporal dementia with Parkinsonism linked to chromosome (hereinafter to be referred to as FTDP-17). Aggregates of phosphorylated tau are generated in nerve cells in the brain and contribute to many neurodegenerative diseases.

[0003] Thus, a neurodegenerative disease accompanied by aggregation and intracellular accumulation of tau and considered to involve tau aggregation process in the onset of the disease is called Tauopathy.

[0004] A plurality of therapeutic strategies has been proposed to treat AD (non-patent document 1), and the gene therapy approach has been attracting attention as one of the strategies.

[0005] As a gene therapy targeting MAPT, for example, WO2018/102665 A1 discloses an invention directed to a genetic modulator of a MAPT gene, comprising a DNA-binding domain that binds to a target site of at least 12 nucleotides in the MAPT gene; and a transcriptional regulatory domain or nuclease domain.

[0006] On the other hand, a system using a combination of Cas9 with deactivated nuclease activity (dCas9) and a transcription activation domain or transcription repression domain has been developed in recent years, in which expression of a target gene is

controlled through targeting of the protein to the gene by using guide RNA and without cleaving DNA sequence of the gene (patent document 1, which is incorporated herein by reference in its entirety). Its clinical application is expected (non-patent document 2, which is incorporated herein by reference in its entirety). However, a problem exists in that a sequence encoding a complex of dCas9, guide RNA and a co-transcription repressor exceeds the capacity of the most common viral vectors (e.g., AAV), which represent the most promising method for gene delivery in vivo (non-patent document 3, which is incorporated herein by reference in its entirety).

Citation List

Patent Literature

[0007] [PTL 1] WO2013/176772

Non Patent Literature

[0008] [NPL 1] Ballard C. et al., Lancet 2011; 377:1019-31

[NPL 2] Dominguez A. et al., Nat Rev Mol Cell Biol. 2016 Jan; 17(1): 5-15

[NPL 3] Liao H. et al., Cell. 2017 Dec 14; 171(7): 1495-507

Summary of Invention

Technical Problem

[0009] Accordingly, it is one object of the present invention to provide novel therapeutic approaches to tauopathy (particularly, AD).

[0010] This and other objects, which will become apparent during the following detailed description, have been achieved by the inventors' discovery that the expression of human MAPT gene (Gene ID:4137) can be strongly suppressed by using a guide RNA targeting a particular sequence of human MAPT gene and a fusion protein of a transcription repressor and a nuclease-deficient CRISPR effector protein.

Solution to Problem

[0011] The present inventors have found that the expression of human MAPT gene can be strongly suppressed by a single AAV vector carrying a base sequence encoding the fusion protein and a base sequence encoding the guide RNA, using a compact nuclease-deficient CRISPR effector protein and a compact transcription repressor.

Thus, the present invention provides:

[0012] [1] A polynucleotide, comprising the following base sequences:

(a) a base sequence encoding a fusion protein of a nuclease-deficient CRISPR effector protein and a transcription repressor, and

(b) a base sequence encoding a guide RNA targeting a continuous region of 18 to 24 nucleotides in length in a region set forth in SEQ ID NO: 54, 55, 56, 57, 68, 153 or 97 in the expression regulatory region of human MAPT gene.

[2] The polynucleotide of [1], wherein the base sequence encoding the guide RNA

comprises the base sequence set forth in SEQ ID NO: 54, 55, 56, 57, 68, 153 or 97, or the base sequence set forth in SEQ ID NO: 54, 55, 56, 57, 68, 153 or 97 in which 1 to 3 bases are deleted, substituted, inserted, and/or added.

[3] The polynucleotide of [1] or [2], comprising at least two different base sequences encoding the guide RNA.

[4] The polynucleotide of any of [1] to [3], wherein the transcriptional repressor is selected from the group KRAB, MeCP2, SIN3A, HDT1, MBD2B, NIPP1, and HP1A.

[5] The polynucleotide of [4], wherein the transcriptional repressor is KRAB.

[6] The polynucleotide of any of [1] to [5], wherein the nuclease-deficient CRISPR effector protein is dCas9.

[7] The polynucleotide of [6], wherein the dCas9 is derived from Staphylococcus aureus.

[8] The polynucleotide of any of [1] to [7], further comprising a promoter sequence for the base sequence encoding the guide RNA and/or a promoter sequence for the base sequence encoding the fusion protein of the nuclease-deficient CRISPR effector protein and the transcriptional repressor.

[9] The polynucleotide of [8], wherein the promoter sequence for the base sequence encoding the guide RNA is selected from the group U6 promoter, SNR6 promoter, SNR52 promoter, SCR1 promoter, RPR1 promoter, U3 promoter, and H1 promoter.

[10] The polynucleotide of [9], wherein the promoter sequence for the base sequence encoding the guide RNA is U6 promoter.

[11] The polynucleotide of any of [8] to [10], wherein the promoter sequence for the base sequence encoding the fusion protein of the nuclease-deficient CRISPR effector protein and the transcriptional repressor is a ubiquitous promoter or a neuron specific promoter.

[12] The polynucleotide of [11], wherein the ubiquitous promoter is selected from the group EFS promoter, CMV promoter and CAG promoter.

[13] A vector comprising a polynucleotide of any of [1] to [12].

[14] The vector of [13], wherein the vector is a plasmid vector or a viral vector.

[15] The vector of [14], wherein the viral vector is selected from the group adeno-associated virus (AAV) vector, adenovirus vector, and lentivirus vector.

[16] The vector of [15], wherein the AAV vector is selected from the group AAV1, AAV2, AAV6, AAV7, AAV8, AAV9, Anc80, $AAV_{587}MTP$, $AAV_{588}MTP$, AAV-B1, AAVM41, and AAVrh74.

[17] The vector of [16], wherein the AAV vector is AAV9.

[18] A pharmaceutical composition comprising a polynucleotide of any of [1] to [12] or a vector of any of [13] to [17].

[19] The pharmaceutical composition of [18] for treating or preventing Alzheimer's

disease.

[20] A method for treating or preventing Alzheimer's disease, comprising administering a polynucleotide of any of [1] to [12], or a vector of any of [13] to [17], to a subject in need thereof.

Advantageous Effects of Invention

[0013] According to the present invention, the expression of the human MAPT gene can be suppressed and, consequently, the present invention is expected to be able to treat tauopathy including AD.

[0014] A more complete appreciation of the invention and many of the attendant advantages thereof will be readily obtained as the same become better understood by reference to the following detailed description when considered in connection with the accompanying drawings.

Brief Description of Drawings

[0015] [Fig. 1] Fig. 1 shows the relative sa sgRNA location to 'UCSC Genome Browser on Human Dec. 2013 (GRCh38/hg38) Assembly; chromosome 17: 45,887,381-45,962,898'.

[Fig. 2] Fig. 2 shows the results of evaluating the sa sgRNA for reducing MAPT mRNA levels between chromosome 17: 45,887,381- 45,962,898 (UCSC Genome Browser on Human Dec. 2013 (GRCh38/hg38) Assembly), within regions defined in Fig. 1.

[Fig. 3] Fig. 3 shows the results of evaluating the sa sgRNA efficacy for reducing MAPT mRNA levels between chromosome 17: 45,887,381- 45,962,898 (UCSC Genome Browser on Human Dec. 2013 (GRCh38/hg38) Assembly), within regions defined in Fig.1.

Description of Embodiments

[0016] The embodiments of the present invention are explained in detail below.

[0017] 1. Polynucleotide

The present invention provides a polynucleotide comprising the following base sequences (hereinafter sometimes to be also referred to as "the polynucleotide of the present invention"):

(a) a base sequence encoding a fusion protein of a nuclease-deficient CRISPR effector protein and a transcription repressor, and

(b) a base sequence encoding a guide RNA targeting a continuous region of 18 to 24 nucleotides (i.e., 18 to 24 contiguous nucleotides) in length in a region set forth in SEQ ID NO: 54, 55, 56, 57, 68 or 153, or 97 in the expression regulatory region of human MAPT gene. The region set forth in SEQ ID NO: 97 (CAGCTCCGGCACCAACAGCAGCGCCGCTGCCACCGCCCACCTTCTGCCGC

CGCCACCACAGCCACCTTCTCCTCCTCCGCTGTCCTCTCCCGTCCTCGCCTC
TGTCGACTATCAGGTAAGCGCCGCGGCTCCGAAATCTGCCTCGCCGTCCGC
CTCTGTGCACCCCTGCGCCGCCGCCCCTCGCCCTCCCTCTCCGCAGACTGGG
GCTTCGTGCGCCGGGCATCGGTCGGGGCCACCGCAGGGCCCCTCCCTGCCT
CCCCTGCTCGGGGGCTGGGGCCAGGGCGGCCTGGAAAGGGACCTGAGCAA
GGGATGCACGCACGC) comprises the regions set forth in SEQ ID NOs: 54, 55, 56 and 57.

[0018] The polynucleotide of the present invention is introduced into a desired cell and transcribed to produce a fusion protein of a nuclease-deficient CRISPR effector protein and a transcription repressor, and a guide RNA targeting a particular region of the expression regulatory region of the human MAPT gene. These fusion protein and guide RNA form a complex (hereinafter the complex is sometimes referred to as "ribonucleoprotein; RNP") and cooperatively act on the aforementioned particular region, thus suppressing transcription of the human MAPT gene. In one embodiment of the present invention, the expression of the human MAPT gene can be suppressed by, for example, not less than about 40%, not less than about 50%, not less than about 60%, not less than about 70%, not less than about 75%, not less than about 80%, not less than about 85%, not less than about 90%, not less than about 95%, or about 100%.

[0019] (1) Definition

In the present specification, "the expression regulatory region of human microtubule-associated protein tau (MAPT) gene" means any region in which the expression of human MAPT gene can be suppressed by binding RNP to that region. That is, the expression regulatory region of human MAPT gene may exist in any region such as the promoter region, enhancer region, intron, and exon of the human MAPT gene, as long as the expression of the human MAPT gene is suppressed by the binding of RNP. In the present specification, when the expression regulatory region is shown by the particular sequence, the expression regulatory region includes both the sense strand sequence and the antisense strand sequence conceptually.

[0020] In the present invention, a fusion protein of a nuclease-deficient CRISPR effector protein and a transcription repressor is recruited by a guide RNA into a particular region in the expression regulatory region of the human MAPT gene. In the present specification, the "guide RNA targeting ..." means a "guide RNA recruiting a fusion protein into ...".

[0021] In the present specification, the "guide RNA (to be also referred to as 'gRNA')" is an RNA comprising a genome specific CRISPR-RNA (to be referred to as "crRNA"). crRNA is an RNA that binds to a complementary sequence of a targeting sequence (described later). When Cpf1 is used as the CRISPR effector protein, the "guide RNA" refers to an RNA comprising an RNA consisting of crRNA and a specific sequence

attached to its 5'-terminal (for example, an RNA sequence set forth in SEQ ID NO: 101 in the case of FnCpf 1). When Cas9 is used as the CRISPR effector protein, the "guide RNA" refers to chimera RNA (to be referred to as "single guide RNA (sgRNA)") comprising crRNA and trans-activating crRNA attached to its 3'-terminal (to be referred to as "tracrRNA") (see, for example, Zhang F. et al., Hum Mol Genet. 2014 Sep 15; 23(R1): R40-6 and Zetsche B. et al., Cell. 2015 Oct 22; 163(3): 759-71, which are incorporated herein by reference in their entireties).

[0022] In the present specification, a sequence complementary to the sequence to which crRNA is bound in the expression regulatory region of the human MAPT gene is referred to as a "targeting sequence". That is, in the present specification, the "targeting sequence" is a DNA sequence present in the expression regulatory region of the human MAPT gene and adjacent to PAM (protospacer adjacent motif). PAM is adjacent to the 5'-side of the targeting sequence when Cpf1 is used as the CRISPR effector protein. PAM is adjacent to the 3'-side of the targeting sequence when Cas9 is used as the CRISPR effector protein. The targeting sequence may be present on either the sense strand sequence side or the antisense strand sequence side of the expression regulatory region of the human MAPT gene (see, for example, the aforementioned Zhang F. et al., Hum Mol Genet. 2014 Sep 15; 23(R1): R40-6 and Zetsche B. et al., Cell. 2015 Oct 22; 163(3): 759-71, which are incorporated herein by reference in their entireties).

[0023] (2) Nuclease-deficient CRISPR effector protein

In the present invention, using a nuclease-deficient CRISPR effector protein, a transcriptional repressor fused thereto is recruited to the expression regulatory region of the human MAPT gene. The nuclease-deficient CRISPR effector protein (hereinafter to be simply referred to as "CRISPR effector protein") to be used in the present invention is not particularly limited as long as it forms a complex with gRNA and is recruited to the expression regulatory region of the human MAPT gene. For example, nuclease-deficient Cas9 (hereinafter sometimes to be also referred to as "dCas9") or nuclease-deficient Cpf1 (hereinafter sometimes to be also referred to as "dCpf1") can be included.

[0024] Examples of the above-mentioned dCas9 include, but are not limited to, a nuclease-deficient variant of Streptococcus pyogenes-derived Cas9 (SpCas9; PAM sequence: NGG (N is A, G, T or C. hereinafter the same)), Streptococcus thermophilus-derived Cas9 (StCas9; PAM sequence: NNAGAAW (W is A or T. hereinafter the same)), Neisseria meningitidis-derived Cas9 (NmCas9; PAM sequence: NNNNGATT), or Staphylococcus aureus-derived Cas9 (SaCas9; PAM sequence: NNGRRT (R is A or G. hereinafter the same)) and the like (see, for example, Nishimasu et al., Cell. 2014 Feb 27; 156(5): 935-49, Esvelt KM et al., Nat Methods. 2013 Nov; 10(11):1116-21, Zhang

Y. Mol Cell. 2015 Oct 15; 60(2):242-55, and Friedland AE et al., Genome Biol. 2015 Nov 24; 16:257, which are incorporated herein by reference in their entireties). For example, in the case of SpCas9, a double mutant in which the 10th Asp residue is converted to Ala residue and the 840th His residue is converted to Ala residue (sometimes referred to as "dSpCas9") can be used (see, for example, the aforementioned Nishimasu et al., Cell. 2014). Alternatively, in the case of SaCas9, a double mutant in which the 10th Asp residue is converted to Ala residue and the 580th Asn residue is converted to Ala residue (SEQ ID NO: 102), or a double mutant in which the 10th Asp residue is converted to Ala residue and the 557th His residue is converted to Ala residue (SEQ ID NO: 103) (hereinafter any of these double mutants is sometimes to be referred to as "dSaCas9") can be used (see, for example, the aforementioned Friedland AE et al., Genome Biol. 2015, which is incorporated herein by reference in its entirety).

[0025] In addition, in one embodiment of the present invention, as dCas9, a variant obtained by modifying a part of the amino acid sequence of the aforementioned dCas9, which forms a complex with gRNA and is recruited to the expression regulatory region of the human MAPT gene, may also be used. Examples of such variants include a truncated variant with a partly deleted amino acid sequence. In one embodiment of the present invention, as dCas9, variants disclosed in WO2019/235627 and WO2020/085441, which are incorporated herein by reference in their entireties, can be used. Specifically, dSaCas9 obtained by deleting the 721st to 745th amino acids from dSaCas9 that is a double mutant in which the 10th Asp residue is converted to Ala residue and the 580th Asn residue is converted to Ala residue (SEQ ID NO: 104), or dSaCas9 in which the deleted part is substituted by a peptide linker (e.g., one in which the deleted part is substituted by GGSGGS linker (SEQ ID NO: 105) is set forth in SEQ ID NO: 106, and one in which the deleted part is substituted by SGGGS linker (SEQ ID NO: 107) is set forth in SEQ ID NO: 108, etc.) (hereinafter any of these double mutants is sometimes to be referred to as "dSaCas9[-25]"), or dSaCas9 obtained by deleting the 482nd to 648th amino acids from dSaCas9 that is the aforementioned double mutant (SEQ ID NO: 109), or dSaCas9 in which the deleted part is substituted by a peptide linker (one in which the deleted part is substituted by GGSGGS linker is set forth in SEQ ID NO: 110) may also be used.

[0026] Examples of the above-mentioned dCpf1 include, but are not limited to, a nuclease-deficient variant of Francisella novicida-derived Cpf1 (FnCpf1; PAM sequence: NTT), Acidaminococcus sp.-derived Cpf1 (AsCpf1; PAM sequence: NTTT), or Lachnospiraceae bacterium-derived Cpf1 (LbCpf1; PAM sequence: NTTT) and the like (see, for example, Zetsche B. et al., Cell. 2015 Oct 22; 163(3):759-71, Yamano T et al., Cell. 2016 May 5; 165(4):949-62, and Yamano T et al., Mol Cell. 2017 Aug 17;

67(4):633-45, which are incorporated herein by reference in their entireties). For example, in the case of FnCpf1, a double mutant in which the 917th Asp residue is converted to Ala residue and the 1006th Glu residue is converted to Ala residue can be used (see, for example, the aforementioned Zetsche B et al., Cell. 2015, which is incorporated herein by reference in its entirety). In one embodiment of the present invention, as dCpf1, a variant obtained by modifying a part of the amino acid sequence of the aforementioned dCpf1, which forms a complex with gRNA and is recruited to the expression regulatory region of the human MAPT gene, may also be used.

[0027] In one embodiment of the present invention, dCas9 is used as the nuclease-deficient CRISPR effector protein. In one embodiment, the dCas9 is dSaCas9, and, in a particular embodiment, the dSaCas9 is dSaCas9[-25].

[0028] A polynucleotide comprising a base sequence encoding a CRISPR effector protein can be cloned by, for example, synthesizing an oligoDNA primer covering a region encoding a desired part of the protein based on the cDNA sequence information thereof, and amplifying the polynucleotide by PCR method using total RNA or mRNA fraction prepared from the cells producing the protein as a template. In addition, a polynucleotide comprising a base sequence encoding a nuclease-deficient CRISPR effector protein can be obtained by introducing a mutation into a nucleotide sequence encoding a cloned CRISPR effector protein by a known site-directed mutagenesis method to convert the amino acid residues (e.g., 10th Asp residue, 557th His residue, and 580th Asn residue in the case of SaCas9; 917th Asp residue and 1006th Glu residue in the case of FnCpf1, and the like can be included, but are not limited to these) at a site important for DNA cleavage activity to other amino acids.

[0029] Alternatively, a polynucleotide comprising a base sequence encoding nuclease-deficient CRISPR effector protein can be obtained by chemical synthesis or a combination of chemical synthesis and PCR method or Gibson Assembly method, based on the cDNA sequence information thereof, and can also be further constructed as a base sequence that underwent codon optimization to give codons suitable for expression in human.

[0030] (3) Transcriptional repressor

In the present invention, human MAPT gene expression is repressed by the action of the transcriptional repressor fused with the nuclease-deficient CRISPR effector protein. In the present specification, the "transcriptional repressor" means a protein having the ability to repress gene transcription of human MAPT gene or a peptide fragment retaining the function thereof. The transcriptional repressor to be used in the present invention is not particularly limited as long as it can repress expression of human MAPT gene. It includes, for example, Kruppel-associated box (KRAB), MBD2B, v-ErbA, SID (including chain state of SID (SID4X)), MBD2, MBD3, DNMT family

(e.g., DNMT1, DNMT3A, DNMT3B), Rb, MeCP2, ROM2, LSD1, AtHD2A, SET1, HDAC11, SETD8, EZH2, SUV39H1, PHF19, SALI, NUE, SUVR4, KYP, DIM5, HDAC8, SIRT3, SIRT6, MESOLO4, SET8, HST2, COBB, SET-TAF1B, NCOR, SIN3A, HDT1, NIPP1, HP1A, ERF repressor domain (ERD), and variants thereof having transcriptional repression ability, fusions thereof and the like. In one embodiment of the present invention, KRAB is used as the transcriptional repressor.

[0031] A polynucleotide comprising a base sequence encoding a transcriptional repressor can be constructed by chemical synthesis or a combination of chemical synthesis and PCR method or Gibson Assembly method. Furthermore, a polynucleotide comprising a base sequence encoding a transcriptional repressor can also be constructed as a codon-optimized DNA sequence to be codons suitable for expression in human.

[0032] A polynucleotide comprising a base sequence encoding a fusion protein of a transcriptional repressor and a nuclease-deficient CRISPR effector protein can be prepared by ligating a base sequence encoding the CRISPR effector protein to a base sequence encoding the transcriptional repressor directly or after adding a base sequence encoding a linker, NLS (nuclear localization signal)(for example, a base sequence set forth in SEQ ID NO: 111 or SEQ ID NO: 112), a tag and/or others. In the present invention, the transcriptional repressor may be fused with either N-terminal or C-terminal of the nuclease-deficient CRISPR effector protein. As the linker, a linker with an amino acid number of about 2 to 50 can be used, and specific examples thereof include, but are not limited to, a G-S-G-S linker in which glycine (G) and serine (S) are alternately linked and the like. In one embodiment of the present invention, as the polynucleotide comprising a base sequence encoding a fusion protein of a nuclease-deficient CRISPR effector protein and a transcriptional repressor, the base sequence set forth in SEQ ID NO: 113, which encodes SV40 NLS, dSaCas9, NLS and KRAB as a fused protein, can be used.

[0033] (4) Guide RNA

In the present invention, a fusion protein of nuclease-deficient CRISPR effector protein and transcription repressor can be recruited to the expression regulatory region of the human MAPT gene by guide RNA. As described in the aforementioned "(1) Definition", guide RNA comprises crRNA, and the crRNA binds to a complementary sequence of the targeting sequence. crRNA may not be completely complementary to the complementary sequence of the targeting sequence as long as the guide RNA can recruit the fusion protein to the target region, and may comprise a base sequence of the targeting sequence in which at least 1 to 3 bases are deleted, substituted, inserted and/or added.

[0034] When dCas9 is used as the nuclease-deficient CRISPR effector protein, for example, the targeting sequence can be determined using a published gRNA design web site

(CRISPR Design Tool, CRISPR direct, etc.). To be specific, from the sequence of the target gene (i.e., human MAPT gene), candidate targeting sequences of about 20 nucleotides in length for which PAM (e.g., NNGRRT in the case of SaCas9) is adjacent to the 3'-side thereof are listed, and one having a small number of off-target sites in human genome from among these candidate targeting sequences can be used as the targeting sequence. The base length of the targeting sequence is 18 to 24 nucleotides in length, preferably 20 to 23 nucleotides in length, more preferably 21 to 23 nucleotides in length. As a primary screening for the prediction of the off-target site number, a number of bioinformatic tools are known and publicly available, and can be used to predict the targeting sequence with the lowest off-target effect. Examples thereof include bioinformatics tools such as Benchling (https://benchling.com), and COSMID (CRISPR Off-target Sites with Mismatches, Insertions, and Deletions) (Available on https://crispr.bme.gatech.edu on the internet). Using these, the similarity to the base sequence targeted by gRNA can be summarized. When the gRNA design software to be used does not have a function to search for off-target site of the target genome, for example, the off-target site can be searched for by subjecting the target genome to Blast search with respect to 8 to 12 nucleotides on the 3'-side of the candidate targeting sequence (seed sequence with high discrimination ability of targeted nucleotide sequence).

[0035] In one embodiment of the present invention, in the region existing in the GRCh38/hg38 of human chromosome 17 (Chr 17), the region of "45,887,381-45,962,898" can be the expression regulatory region of the human MAPT gene. Therefore, in one embodiment of the present invention, the targeting sequence can be 18 to 24 nucleotides in length, preferably 20 to 23 nucleotides in length, more preferably 21 to 23 nucleotides in length, in the regions of "45,887,381-45,962,898" existing in the GRCh38/hg38 of human chromosome 17 (Chr 17).

[0036] In one embodiment of the present invention, a base sequence encoding crRNA may be the same base sequence as the targeting sequence. For example, when the targeting sequence set forth in SEQ ID NO: 57 (GAGCAAGGGATGCACGCACG) is introduced into the cell as a base sequence encoding crRNA, crRNA transcribed from the sequence is GAGCAAGGGAUGCACGCACG (SEQ ID NO:114) and is bound to CGTGCGTGCATCCCTTGCTC (SEQ ID NO: 115), which is a sequence complementary to the base sequence set forth in SEQ ID NO: 57 and is present in the expression regulatory region of the human MAPT gene. In another embodiment, a base sequence which is a targeting sequence in which at least 1 to 3 bases are deleted, substituted, inserted and/or added can be used as the base sequence encoding crRNA as long as guide RNA can recruit a fusion protein to the target region. Therefore, in one embodiment of the present invention, as a base sequence encoding crRNA, the base

sequence set forth in SEQ ID NO: 54, 55, 56, 57, 68, 153 or 97, or the base sequence set forth in SEQ ID NO: 54, 55, 56, 57, 68, 153 or 97 in which 1 to 3 bases are deleted, substituted, inserted and/or added can be used.

[0037] When dCpf1 is used as the nuclease-deficient CRISPR effector protein, a base sequence encoding gRNA can be designed as a DNA sequence encoding crRNA with particular RNA attached to the 5'-terminal. Such RNA attached to the 5'-terminal of crRNA and a DNA sequence encoding said RNA can be appropriately selected by those of ordinary skill in the art according to the dCpf1 to be used. For example, when dFnCpf1 is used, a base sequence in which SEQ ID NO:116; AATTTCTACTGTT GTAGAT is attached to the 5'-side of the targeting sequence can be used as a base sequence encoding gRNA (when transcribed to RNA, the sequences of the underlined parts form base pairs to form a stem-loop structure). The sequence to be added to the 5'-terminal may be a sequence generally used for various Cpf1 proteins in which at least 1 to 6 bases are deleted, substituted, inserted and/or added, as long as gRNA can recruit a fusion protein to the expression regulatory region after transcription.

[0038] When dCas9 is used as the CRISPR effector protein, a base sequence encoding gRNA can be designed as a DNA sequence in which a DNA sequence encoding known tracrRNA is linked to the 3'-terminal of a DNA sequence encoding crRNA. Such tracrRNA and a DNA sequence encoding the tracrRNA can be appropriately selected by those of ordinary skill in the art according to the dCas9 to be used. For example, when dSaCas9 is used, the base sequence set forth in SEQ ID NO: 117 is used as the DNA sequence encoding tracrRNA. The DNA sequence encoding tracrRNA may be a base sequence encoding tracrRNA generally used for various Cas9 proteins in which at least 1 to 6 bases are deleted, substituted, inserted and/or added, as long as gRNA can recruit a fusion protein to the expression regulatory region after transcription.

[0039] A polynucleotide comprising a base sequence encoding gRNA designed in this way can be chemically synthesized using a known DNA synthesis method.

[0040] In another embodiment of the present invention, the polynucleotide of the present invention may comprise at least two different base sequences encoding a gRNA. For example, the polynucleotide can comprise at least two different base sequences encoding the guide RNA, wherein the at least two different base sequences are selected from a base sequence comprising a sequence set forth in SEQ ID NO: 54, 55, 56, 57, 68, 153 or 97.

[0041] (5) Promoter sequence

In one embodiment of the present invention, a promoter sequence may be operably linked to the upstream of each of a base sequence encoding fusion protein of nuclease-deficient CRISPR effector protein and transcriptional repressor and/or a base sequence encoding gRNA. The promoter to be possibly linked is not particularly limited as long

as it shows a promoter activity in the target cell. Examples of the promoter sequence possibly linked to the upstream of the base sequence encoding gRNA include, but are not limited to, U6 promoter, SNR6 promoter, SNR52 promoter, SCR1 promoter, RPR1 promoter, U3 promoter, H1 promoter, and tRNA promoter, which are pol III promoters, and the like. In one embodiment of the present invention, U6 promoter can be used as the promoter sequence for the base sequence encoding the guide RNA. In one embodiment of the present invention, when a polynucleotide comprises two or more base sequences respectively encoding a guide RNA, a single promoter sequence may be operably linked to the upstream of the two or more base sequences. In another embodiment, when a polynucleotide comprises two or more base sequences respectively encoding a guide RNA, a promoter sequence may be operably linked to the upstream of each of the two or more base sequences, wherein the promoter sequence operably linked to each base sequence may be the same or different.

[0042] As the aforementioned promoter sequence possibly linked to the upstream of the base sequence encoding fusion protein, a ubiquitous promoter or neuron-specific promoter may be used. Examples of the ubiquitous promoter include, but are not limited to, EF-1a promoter, EFS promoter, CMV (cytomegalovirus) promoter, hTERT promoter, SRa promoter, SV40 promoter, LTR promoter, CAG promoter, RSV (Rous sarcoma virus) promoter, and the like. In one embodiment of the present invention, EFS promoter, CMV promoter or CAG promoter can be used as the ubiquitous promoter. Examples of the neuron-specific promoter include, but are not limited to, neuron-specific enolase (NSE) promoter, human neurofilament light chain (NEFL) promoter. The aforementioned promoter may have any modification and/or alteration as long as it has promoter activity in the target cell.

[0043] In one embodiment of the present invention, U6 is used as a promoter for a base sequence encoding the guide RNA, and CMV promoter can be used as the promoter sequence for the base sequence encoding the fusion protein.

[0044] (6) Other base sequence

Furthermore, the polynucleotide of the present invention may further comprise known sequences such as polyadenylation (polyA) signal, Kozak consensus sequence and the like besides those mentioned above for the purpose of improving the translation efficiency of mRNA produced by transcription of a base sequence encoding a fusion protein of nuclease-deficient CRISPR effector protein and transcription repressor. For example, polyadenylation signal in the present invention may include hGH polyA, bGH polyA, 2x sNRP-1 polyA (see US7557197B2, which is incorporated herein by reference in its entirety), and so on. In addition, the polynucleotide of the present invention may comprise a base sequence encoding a linker sequence, a base sequence encoding NLS and/or a base sequence encoding a tag. Futhermore, the

polynucleotide of the present invention may comprise an intervening sequence. A preferred example of the intervening sequence is a sequence encoding IRES (Internal ribosome entry site), 2A peptide. The 2A peptide is a peptide sequence of around 20 amino acid residues derived from virus, is recognized by a protease present in the cell (2A peptidase), and is cleaved at the position of 1 residue from the C terminal. Multiple genes linked as one unit by 2A peptide are transcribed and translated as one unit, and then cleaved by 2A peptidase. Examples of the 2A peptidase include F2A (derived from foot-and-mouth disease virus), E2A (derived from equine rhinitis A virus), T2A (derived from Thosea asigna virus), and P2A (derived from porcine teschovirus-1).

[0045] (7) Exemplified embodiments of the present invention

In one embodiment of the present invention, a polynucleotide is provided comprising:

a base sequence encoding a fusion protein of a nuclease-deficient CRISPR effector protein and a transcriptional repressor,

a promoter sequence for the base sequence encoding the fusion protein of the nuclease-deficient CRISPR effector protein and the transcriptional repressor,

one or two base sequences respectively encoding a guide RNA, wherein the one or two base sequences are selected from a base sequence comprising a sequence set forth in SEQ ID NO: 54, 55, 56, 57, 68, 153 or 97, or the base sequence comprising a sequence set forth in SEQ ID NO: 54, 55, 56, 57, 68, 153 or 97, in which 1 to 3 bases are deleted, substituted, inserted, and/or added, and

a promoter sequence for the base sequence encoding the gRNA,

wherein the nuclease-deficient CRISPR effector protein is dSaCas9 or dSaCas9[-25],

wherein the transcriptional repressor is selected from the group KRAB, MeCP2, SIN3A, HDT1, MBD2B, NIPP1, and HP1A,

wherein the promoter sequence for the base sequence encoding the fusion protein is selected from the group EFS promoter, CMV promoter and CAG promoter, and

wherein the promoter sequence for the base sequence encoding the gRNA is selected from the group U6 promoter, SNR6 promoter, SNR52 promoter, SCR1 promoter, RPR1 promoter, U3 promoter, and H1 promoter.

[0046] In one embodiment of the present invention, a polynucleotide is provided comprising:

a base sequence encoding a fusion protein of a nuclease-deficient CRISPR effector protein and a transcriptional repressor,

CMV promoter for the base sequence encoding the fusion protein of the nuclease-deficient CRISPR effector protein and the transcriptional repressor,

one or two base sequences respectively encoding a guide RNA, wherein the one or

two base sequences are selected from a base sequence comprising a sequence set forth in SEQ ID NO: 54, 55, 56, 57, 68, 153 or 97, or a base sequence comprising a sequence set forth in SEQ ID NO: 54, 55, 56, 57, 68, 153 or 97 in which 1 to 3 bases are deleted, substituted, inserted, and/or added, and
U6 promoter for the base sequence encoding the guide RNA,
wherein the nuclease-deficient CRISPR effector protein is dSaCas9, and
wherein the transcriptional repressor is KRAB.

[0047] 2. Vector

The present invention provides a vector comprising the polynucleotide of the present invention (hereinafter sometimes referred to as "the vector of the present invention"). The vector of the present invention may be a plasmid vector or a viral vector.

[0048] When the vector of the present invention is a plasmid vector, the plasmid vector to be used is not particularly limited and may be any plasmid vector such as cloning plasmid vector and expression plasmid vector. The plasmid vector is prepared by inserting the polynucleotide of the present invention into a plasmid vector by a known method.

[0049] When the vector of the present invention is a viral vector, the viral vector to be used is not particularly limited and examples thereof include, but are not limited to, adenovirus vector, adeno-associated virus (AAV) vector, lentivirus vector, retrovirus vector, Sendaivirus vector and the like. In the present specification, the "virus vector" or "viral vector" also includes derivatives thereof. Considering the use in gene therapy, AAV vector is preferably used for the reasons such that it can express transgene for a long time, and it is derived from a non-pathogenic virus and has high safety.

[0050] A viral vector comprising the polynucleotide of the present invention can be prepared by a known method. In brief, a plasmid vector for virus expression into which the polynucleotide of the present invention has been inserted is prepared, the vector is transfected into an appropriate host cell to allow for transient production of a viral vector comprising the polynucleotide of the present invention, and the viral vector is collected.

[0051] In one embodiment of the present invention, when AAV vector is used, the serotype of the AAV vector is not particularly limited as long as expression of the human MAPT gene in the target can be activated, and any of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAVrh.10 and the like may be used (for the various serotypes of AAV, see, for example, WO 2005/033321 and EP2341068 (A1), which are incorporated herein by reference in their entireties). Examples of the variants of AAV include, but are not limited to, new serotype with a modified capsid (e.g., WO 2012/057363, which is incorporated herein by reference in its entirety) and the like. For example, in one embodiment of the present invention, a new serotype with a modified capsid improving infectivity for muscle cells can be used, such as AAV_{587}

MTP, AAV_{588}MTP, AAV-B1, AAVM41, AAVS1_P1, and AAVS10_P1, and the like (see Yu et al., Gene Ther. 2009 Aug;16(8):953-62, Choudhury et al., Mol Ther. 2016 Aug;24(7):1247-57, Yang et al., Proc Natl Acad Sci U S A. 2009 Mar 10;106(10):3946-51, and WO2019/207132, which are incorporated herein by reference in their entireties).

[0052] When an AAV vector is prepared, a known method such as (1) a method using a plasmid, (2) a method using a baculovirus, (3) a method using a herpes simplex virus, (4) a method using an adenovirus, or (5) a method using yeast can be used (e.g., Appl Microbiol Biotechnol. 2018; 102(3): 1045-1054, etc., which is incorporated herein by reference in its entirety). For example, when an AAV vector is prepared by a method using a plasmid, first, a vector plasmid comprising inverted terminal repeat (ITR) at both ends of wild-type AAV genomic sequence and the polynucleotide of the present invention inserted in place of the DNA encoding Rep protein and capsid protein is prepared. On the other hand, the DNA encoding Rep protein and capsid protein necessary for forming virus particles are inserted into other plasmids. Furthermore, a plasmid comprising genes (E1A, E1B, E2A, VA and E4orf6) responsible for the helper action of adenovirus necessary for proliferation of AAV is prepared as an adenovirus helper plasmid. The co-transfection of these three kinds of plasmids into the host cell causes the production of recombinant AAV (i.e., AAV vector) in the cell. As the host cell, a cell capable of supplying a part of the gene products (proteins) of the genes responsible for the aforementioned helper action (e.g., 293 cell, etc.) is preferably used. When such cell is used, it is not necessary to carry the gene encoding a protein that can be supplied from the host cell in the aforementioned adenoviral helper plasmid. The produced AAV vector is present in the nucleus. Thus, a desired AAV vector is prepared by destroying the host cell with freeze-thawing, collecting the virus and then subjecting the virus fraction to separation and purification by density gradient ultracentrifugation method using cesium chloride, column method or the like.

[0053] AAV vector has great advantages in terms of safety, gene transduction efficiency and the like, and is used for gene therapy. However, it is known that the size of a polynucleotide that can be packaged in AAV vector is limited. For example, in one embodiment of the present invention, the entire length including the base length of a polynucleotide comprising a base sequence encoding a fusion protein of dSaCas9 and miniVR or microVR, a base sequence encoding gRNA targeting the expression regulatory region of the human MAPT gene, and EFS promoter sequence or CK8 promoter sequence and U6 promoter sequence as the promoter sequences, and ITR parts is about 4.85 kb, and they can be packaged in a single AAV vector.

[0054] 3. Pharmaceutical composition

The present invention also provides a pharmaceutical composition comprising the

polynucleotide of the present invention or the vector of the present invention (hereinafter sometimes referred to as "the pharmaceutical composition of the present invention"). The pharmaceutical composition of the present invention can be used for treating or preventing tauopathy including AD.

[0055] The pharmaceutical composition of the present invention comprises the polynucleotide of the present invention or the vector of the present invention as an active ingredient, and may be prepared as a formulation comprising such active ingredient (i.e., the polynucleotide of the present invention or the vector of the present invention) and, generally, a pharmaceutically acceptable carrier.

[0056] The pharmaceutical composition of the present invention is administered parenterally, and may be administered topically or systemically. The pharmaceutical composition of the present invention can be administered by, but are not limited to, for example, intravenous administration, intraarterial administration, subcutaneous administration, intraperitoneal administration, or intramuscular administration.

[0057] The dose of the pharmaceutical composition of the present invention to a subject is not particularly limited as long as it is an effective amount for the treatment and/or prevention. It may be appropriately optimized according to the active ingredient, dosage form, age and body weight of the subject, administration schedule, administration method and the like.

[0058] In one embodiment of the present invention, the pharmaceutical composition of the present invention can be not only administered to the subject affected with tauopathy including AD but also prophylactically administered to subjects who may develop tauopathy including AD in the future based on the genetic background analysis and the like. The term "treatment" in the present specification also includes remission of disease, in addition to the cure of diseases. In addition, the term "prevention" may also include delaying the onset of disease, in addition to prophylaxis of the onset of disease. The pharmaceutical composition of the present invention can also be referred to as "the agent of the present invention" or the like.

[0059] 4. Method for treatment or prevention of DMD or BMD

The present invention also provides a method for treating or preventing tauopathy including AD, comprising administering the polynucleotide of the present invention or the vector of the present invention to a subject in need thereof (hereinafter sometimes referred to as "the method of the present invention"). In addition, the present invention includes the polynucleotide of the present invention or the vector of the present invention for use in the treatment or prevention of tauopathy including AD. Furthermore, the present invention includes use of the polynucleotide of the present invention or the vector of the present invention in the manufacture of a pharmaceutical composition for the treatment or prevention of tauopathy including AD.

[0060] The method of the present invention can be practiced by administering the aforementioned pharmaceutical composition of the present invention to a subject affected with tauopathy including AD, and the dose, administration route, subject and the like are the same as those mentioned above.

[0061] Measurement of the symptoms may be performed before the start of the treatment using the method of the present invention and at any timing after the treatment to determine the response of the subject to the treatment.

[0062] The method of the present invention can improve the functions of the skeletal muscle and/or cardiac muscle of the subject. Muscles to be improved in the function thereof are not particularly limited, and any muscles and muscle groups are exemplified.

[0063] 5. Ribonucleoprotein

The present invention provides a ribonucleoprotein comprising the following (hereinafter sometimes referred to as "RNP of the present invention"):

(c) a fusion protein of a nuclease-deficient CRISPR effector protein and a transcription repressor, and

(d) a guide RNA targeting a continuous region of 18 to 24 nucleotides in length in a region set forth in SEQ ID NO: 54, 55, 56, 57, 68, 153 or 97 in the expression regulatory region of human MAPT gene.

[0064] As the nuclease-deficient CRISPR effector protein, transcription repressor, and guide RNA comprised in the RNP of the present invention, the nuclease-deficient CRISPR effector protein, transcription repressor, and guide RNA explained in detail in the above-mentioned section of "1. Polynucleotide" can be used. The fusion protein of nuclease-deficient CRISPR effector protein and transcription repressor to be comprised in the RNP of the present invention can be produced by, for example, introducing a polynucleotide encoding the fusion protein into the cell, bacterium, or other organism to allow for the expression, or an in vitro translation system by using the polynucleotide. In addition, guide RNA comprised in the RNP of the present invention can be produced by, for example, chemical synthesis or an in vitro transcription system by using a polynucleotide encoding the guide RNA. The thus-prepared fusion protein and guide RNA are mixed to prepare the RNP of the present invention. Where necessary, other substances such as gold particles may be mixed. To directly deliver the RNP of the present invention to the target cell, tissue and the like, the RNP may be encapsulated in a lipid nanoparticle (LNP) by a known method. The RNP of the present invention can be introduced into the target cell, tissue and the like by a known method. For example, Lee K., et al., Nat Biomed Eng. 2017; 1:889-901, WO 2016/153012, which are incorporated herein by reference in their entireties, and the like can be referred to for encapsulation in LNP and introduction method.

[0065] In one embodiment of the present invention, the guide RNA comprised in RNP of the

present invention targets continuous 18 to 24 nucleotides in length, preferably 20 to 23 nucleotides in length, more preferably 21 to 23 nucleotides in length, in the region of "45,887,381-45,962,898" existing in the GRCh38/hg38 of human chromosome 17 (Chr 17).

[0066] 6. Others

The present invention also provides a composition or kit comprising the following for suppression of the expression of the human MAPT gene:

(e) a fusion protein of a nuclease-deficient CRISPR effector protein and a transcription repressor, or a polynucleotide encoding the fusion protein, and

(f) a guide RNA targeting a continuous region of 18 to 24 nucleotides in length in a region set forth in SEQ ID NO: 54, 55, 56, 57, 68, 153 or 97 in the expression regulatory region of human MAPT gene, or a polynucleotide encoding the guide RNA.

[0067] The present invention also provides a method for treating or preventing tauopathy including AD, comprising administering the following (e) and (f):

(e) a fusion protein of a nuclease-deficient CRISPR effector protein and a transcription repressor, or a polynucleotide encoding the fusion protein, and

(f) a guide RNA targeting a continuous region of 18 to 24 nucleotides in length in a region set forth in SEQ ID NO: 54, 55, 56, 57, 68, 153 or 97 in the expression regulatory region of human MAPT, or a polynucleotide encoding the guide RNA.

[0068] The present invention also provides use of the following (e) and (f):

(e) a fusion protein of a nuclease-deficient CRISPR effector protein and a transcription repressor, or a polynucleotide encoding the fusion protein, and

(f) a guide RNA targeting a continuous region of 18 to 24 nucleotides in length in a region set forth in SEQ ID NO: 54, 55, 56, 57, 68, 153 or 97 in the expression regulatory region of human MAPT gene, or a polynucleotide encoding the guide RNA,

in the manufacture of a pharmaceutical composition for the treatment or prevention of tauopathy including AD.

[0069] As the nuclease-deficient CRISPR effector protein, transcription repressor, guide RNA, as well as polynucleotides encoding them and vectors in which they are carried in these inventions, those explained in detail in the above-mentioned sections of "1. Polynucleotide", "2. Vector" and "5. Ribonucleoprotein" can be used. The dose, administration route, subject, formulation and the like of the above-mentioned (e) and (f) are the same as those explained in the section of "3. Pharmaceutical composition".

[0070] Other features of the invention will become apparent in the course of the following descriptions of exemplary embodiments which are given for illustration of the invention and are not intended to be limiting thereof.

Examples

[0071] The examples describe the use of a fusion protein of dCas9 with a transcriptional repressor to suppress gene expression, in the defined expression regulatory region of human MAPT gene that leads to the selective suppression of human MAPT gene expression. The example also describes the definition of a specific genomic region that confers selective suppression of the human MAPT gene without minimally affecting the expression of other genes. The method of the present invention to suppress human MAPT gene expression represents a novel therapeutic or preventive strategy for the tauopathy including AD as described and illustrated herein.

[0072] Example 1

(1) Experimental Methods

Cell Culture and Transfection

SK-N-AS (American Type Culture Collection) cells were seeded 24 hours prior to transfection in 12-well plates at a density of 100,000 cells per well and cultured in DMEM media supplemented with 10% FBS and 2 mM fresh L-glutamine, 1 mM sodium pyruvate and non-essential amino acids. Cells were transfected with 1000 ng sgRNA containing px601-CMV-dSaCas9-KRAB-P2A-Puro (modified from GenScript) plasmid using 3.0 μl of TransIT-VirusGEN (Mirus Bio), according to manufacturer's instructions.

[0073] For gene expression analysis, the transfected cells were harvested at 72 h after transfection and lysed in RLT buffer (Qiagen) to extract total RNA using RNeasy kit (Qiagen).

[0074] Gene expression analysis

[0075] For Taqman analysis, 1.5 μg of total RNA was used to generate cDNA using TaqMan High-Capacity RNA-to-cDNA Kit (Applied Biosystems) in 20 μl volume. The generated cDNA was diluted 10-fold and 3.33 μl was used per Taqman reaction. The Taqman primers and probes for the MAPT and HPRT gene were obtained from Applied Biosystems. Taqman reaction was run using Taqman gene expression master mix (ThermoFisher) in ThermoFisher QuantStudio 5 Real-Time PCR System and analyzed using QuantStudio 5 analysis software.

Taqman probe product IDs:

MAPT: Hs00902193_m1 (FAM-MGB)

HPRT: Hs99999909_m1 (VIC PL)

Taqman qPCR condition:

Step 1; 50°C 2 min

Step 2; 95°C 2 min

Step 3; 95°C 1 sec

Step 4; 60°C 20 sec

Repeat Steps 3 and 4; 45 times

[0076] Selection of sgRNA Sequence

The location of the guide RNA target sites relative to the MAPT gene is shown in Fig. 1. The selected guide RNA sequences (Table 1) or control sgRNA guide sequences (Table 2) were fused with the tracer RNA sequence to form single-molecule guide RNA (sgRNA) sequences, and were cloned into px601-CMV-dSaCas9-KRAB-P2A-Puro (modified from GenScript). The sgRNA expression is driven by the hU6 promoter, and the vector expresses the puromycin gene under a CMV/P2A promoter to facilitate tracking and selection of the sgRNA expressing cells. Three control sgRNA guides (Table 2) were selected from the Human CRISPR Knockout Pooled Library (Sanjana N. E. et al, Nature Methods, 11(8), p.783, 2014).

[0077] (2) Results

Suppression of MAPT gene expression by the RNP

The suppression of MAPT transcript by the ninety-six sgRNAs are shown (Fig. 2), where MAPT transcript expression was normalized to the mRNA levels of the HPRT gene. Detected MAPT expression levels after transfection with control sgRNA guides were set to 1.0. sgRNA# 54, 55, 56, 57, and 68 showed ≥90% suppression (Fig. 2). The experiments detailed were conducted at least three times, and the mean-fold suppression values and standard deviations are shown.

[0078] Table 1 List of sa sgRNA guides (with NNGRRT PAM sequence) within 'UCSC Genome Browser on Human Dec. 2013 (GRCh38/hg38) Assembly; chromosome 17: 45,887,381- 45,962,898'.

[0079]

[Table 1-1]

SEQ ID No.	Guide RNA (sa sgRNA)	Position	Strand	Sequence	PAM	Specificity Score	Efficiency Score
1	1	45961816	-1	ACTGCACTCCAGCCTGGGTGA	CAGGGT	4.2707957	11.10541033
2	2	45961820	1	GTTTCACCCTGTCTCACCCAGGC	TGGAGT	35.0477918	7.38177467
3	3	45961824	-1	ATTACACCACTGCACTCCAGC	CTGGGT	19.503033	54.5412524
4	4	45961869	-1	CTACTGGGAGGCTGAGGCAG	GAGAAT	13.1602659	14.44961198
5	5	45961906	1	TGCCTCAGCCTCCCAGTAGC	TGGGAT	48.3443421	5.93261184
6	6	45961960	1	TTATTGTATTTTTAGTAGAGA	TGGGGT	7.2384615	1.89303944
7	7	45962005	-1	TGGGTGGATCACCTGAGGTCA	GGGGAT	77.1434169	6.39485671
8	8	45962023	-1	CACTTTGGGAGGCCGAGGTGG	GTGGAT	39.4034708	18.9296388
9	9	45962037	-1	TCAGCACTTTGGGAGGCCGAG	GTGGGT	31.821238	31.2362459
10	10	45962096	1	CCTGGCCGTCACCTGGTGTG	TTGAAT	75.6281371	1.77914941
11	11	45962151	1	AGATGTAAATAACGCTTGGGC	AGGAAT	94.3928814	9.96943822
12	12	45962165	1	CTTGGCCAGGAATAGTGAGCA	CCGGAT	69.6805397	3.89982682
13	13	45962171	1	CAGGAATAGTGAGCACCGGAT	GAGGAT	87.4269238	11.1391751
14	14	45962195	1	GATGGGCGGCCACTGTTAGA	GAGGGT	94.4779443	43.0454744
15	15	45962272	-1	AGGAGTGTTCGAGGGCAGAGT	GGGAGT	46.3644966	5.2111691
16	16	45962278	-1	GTTCTGAGGAGTGTTCGAGGG	CAGAGT	53.512248	15.1839364
17	17	45962293	-1	AAGAGGAGAGTAATAAGTTCTG	AGAGAT	69.2107704	35.7827944

[0080] [Table 1-2]

18	18	45962307	-1	TCACCTGGGAAGAAGAGGA	CAGGAT	39.53770646	5.00525373
19	19	45962331	1	TTCCCCAGGTGACTTTGAAC	CAGGAT	77.6067082	25.3739037
20	20	45962351	1	CCAGAMTGGCTGAGCCAAGCC	AAGAGT	74.313096	8.1697096
21	21	45962387	1	TGGAAGATCACCTGGGAAGT	ACAGGT	92.4905661	3.51725961
22	22	45962440	1	TACANCATGCACCAAGACCAA	GAGGGT	81.5773043	63.9077130
23	23	45962513	1	GGCCCAGATCACTGCAAGCCA	AGGGGT	78.3669171	22.4204299
24	24	45962538	1	GTGGTAGGAACAGTTTGCATC	CAGAAT	80.3998337	7.57883596
25	25	45962547	-1	ATTTAAAATTTCTTTGCAATT	CTGGAT	44.678716	1.35233247
26	26	45962605	1	GTAAAGTAAAGCCTCATTAAT	TTGAGT	55.7696345	2.75396296
27	27	45962634	-1	GTCTGGCATTAATCTCATTGC	TTGAGT	76.3653348	4.70638165
28	28	45962680	-1	TGCTGGAGCTTCCAAAGTGC	TTGGAT	26.7935865	13.2396766
29	29	45962696	1	CACTTTGGAAGGCCCAGCCAG	GAGGAT	34.93137	30.2924901
30	30	45962712	-1	GGTCTCAATTCCTGCCCTCA	AGGGAT	76.867643	1.73623484
31	31	45962713	1	GCAGGAGATCCCTTGAGAYC	AGGAAT	25.7962436	4.81696985
32	32	45962759	-1	ATTTTAAATTATTTAGAGA	CGGTGT	33.8490644	7.01995763
33	33	45962814	1	ATGTCTATAGTCCTAGCTAGT	CAGGAT	23.1152609	13.3784542
34	34	45962803	-1	TGATCCTTCTTGCCTCAGTATC	CTGAGT	70.752644	12.3488214
35	35	45962830	1	CTACTCAGGATGCTGAGCCAG	AAGGAT	26.6698191	9.87147671
36	36	45962847	1	GCAGAAGGATCACTTGAGCCC	AGGAGT	67.4490464	22.7859368
37	37	45893871	-1	AGTGGATGATTTCTATGTAG	GAGGGT	77.3291451	16.9076608
38	38	45893590	-1	ATCAAGTTTAAGCCCAAGTAG	TGGGAT	78.0130617	67.2473985

[0081]

[Table 1-3]

39	39	45693633	-1	GCCCCAGCTTCGGCTTAGC	TTGCAT	84.965362	2.0071445
40	40	45693660	1	GCTTGGGGCCTGGGGGAGCAG	CAGAAT	74.2151504	32.61015535
41	41	45693716	-1	GCTGTAAATAGAGCTTGAGT	CTGAAT	65.5490417	60.3036159
42	42	45693843	-1	GAAAAAAAATCTTAATTAG	ACGAAT	38.227743	4.28508603
43	43	45693885	-1	TTTACTTTAAAAAAAAGAAC	GTGAAT	39.0517239	37.3185904
44	44	45693917	1	TACAGTTCTAATGTATTGTAA	CTGAGT	73.9591349	57.6996631
45	45	45693952	1	TTTAAGCCGATTTCTTAAGCA	AAGCAT	69.1307684	23.6637612
46	46	45693968	-1	CCTTTTTTGTTTACTCACCAG	GTGAAT	72.020046	29.0300062
47	47	45694050	1	GGGGGCGGTTCCGGGACTACG	AAGGGT	98.364443	16.0031071
48	48	45694080	-1	GGCCTTCCAACGTAGCCGGCCC	TCGAGT	81.4721884	4.01239061
49	49	45694175	-1	GCTAGGCCAGTGTTCCGCCC	CTGGGT	86.9498758	21.1902526
50	50	45694206	-1	GTTGTCCTCCTTTTGGCCATG	CGGCAT	86.481861	27.1648396
51	51	45694230	-1	AGTCTTTGTGTAGTTGCGGCA	GTGCGT	89.5691395	20.8766619
52	52	45694234	-1	TTGGAGTCTTTGTTGTCGTTGT	GGGGGT	92.9659815	1.8785473
53	53	45694254	-1	GCTTTCTCCACCTCCTGTAGT	TGGAGT	74.7956696	15.3497161
54	54	45694675	-1	CTGCTGTTGTGTGGGGAGCTC	GTGGGT	76.9773506	1.96971761
55	55	45694745	-1	GAGGCGAGGGGGGGCGGCC	AGGGGT	47.5758005	0.0980397
56	56	45694874	1	GCCTGGAAAGGGACCTGAGCA	AGGCAT	60.1540743	7.22036518
57	57	45694890	1	GAGCCAGGGATGCAGGCACCC	GTGAGT	81.3048047	22.0833632
58	58	45694916	1	TGCGCGGTGTGTGTGTGTGTG	GAGGGT	60.288787	17.5078756
59	59	45694946	-1	CAGCCTCCACCTTGGTGTCTGC	GCCAAT	65.8772768	1.77179237

[0082]

[Table 1-4]

60	60	45894956	-1	CCTCCCCGCACAGCCTCCACC	TGGGCT	63.1187292	12.71127654
61	61	45894967	1	CCCAGTTGGAGCCTGTACCAG	CAGAGT	66.989345	8.752411839
62	62	45895026	-1	TTGCCCCAAAAGGCCTCCAGTC	GAGAGT	74.4118079	4.653267229
63	63	45895080	1	TGTGTGTGTGTGTGTGTGG	AGAGGT	17.6160322	21.170253
64	64	45895115	1	GTCCCATAACGACCCCCGAAA	CCCAAT	97.3700561	22.352532
65	65	45895117	-1	GCGATTTCAGATTCCGTTTC	GGGGGT	90.6758263	1.149615899
66	66	45895138	-1	TGCCCAACAGCCGCAGGACA	GCGCAT	65.9105328	3.879317232
67	67	45895170	1	GCCATCACTCTAAGAAGACAC	CTGCAT	70.4087054	23.42499104
68	68	45895175	1	CAGTCTAAGAAGACGTGCA	TCAGGT	83.2675292	6.555771012
69	69	45897307	-1	GCACAACCATTCTGAGCACAT	CAGAGT	73.7901057	10.17656111
70	70	45897630	-1	ACTCCACTACACCCTCGCACCA	CAGAGT	87.392231	32.14260631
71	71	45897827	-1	GCAGTCGATCACCTCAAGTC	AGCAGT	79.2541241	4.626566735
72	72	45900065	-1	AGCTCAGCAAGCACATCTCTGCA	CTCTAT	58.8196471	45.03200387
73	73	45900134	-1	TCCTCCCCATCAAATCTAAAC	TACAGT	80.0773966	34.8808509
74	74	45900384	-1	AGGTCAGAGCCCTTGTTCCA	AAGAAT	73.1531089	2.277256539
75	75	45900607	1	ATGTTCACAGGAAGAGCAAAG	CCCCGT	48.0066423	66.8501815
76	76	45900828	-1	GACTACTGAACTTAGATTCAA	CTGAAT	86.546618	12.51052683
77	77	45900958	-1	ATGATAAGCTGAGTTTTAGAG	CTGTGAT	66.6749579	65.98034787
78	78	45900235	-1	ACTCCACTTCTACCTGCCCAA	CAGAGT	66.0161798	52.8030303
79	79	45900443	-1	CACTTTCCAGCCAGCAGCTAG	GTGCAT	50.3759205	26.4257077
80	80	45900650	1	CCCAGTGTTGGGCCAACATGA	CTTCCT	79.7054939	6.401723064

[0083]

[Table 1-5]

81	81	45889813	1	GCAACAAGTCCTTCCTATAG	GGGAT	86.8612477	24.8501093
82	82	45889978	1	AGGCTTTTATCATATTGCCA	TAGGT	81.8505267	57.2184178
83	83	45890204	-1	ATACTTTTTATGTGGGGTGG	GGGAT	29.2430136	8.4303134 3
84	84	45890436	-1	GCTTGAGGCAGGTCATCATT	TAGAT	81.4023508	21.7393466
85	85	45890561	-1	CAACTCTTCGACCAGTCTTG	GTGAT	85.2046243	32.6405628
86	86	45890768	1	TCTAAGGTCATACAGATGGC	TAGAT	87.0357152	29.7348071
87	87	45890974	1	ACCTACATTGACTAAATTATC	TGAAT	77.4168613	6.691677391
88	88	45891162	1	AAATGTGAAATTTGAATGTAG	ACGAT	53.0514588	14.4789416
89	89	45891388	-1	GTCCTGTATCCTGATTGATAC	AGGAT	86.586271	12.5791488
90	90	45891637	1	TTTTTGTGATTTTAGTAGAGA	TGAGT	42.507869	2.50646081
91	91	45891855	-1	TTTACTCTTCCTTTCCCCCAC	CAGAAT	89.3524073	44.2056074
92	92	45892062	-1	GGGACATTTCCAGTCTCTAGA	AGAGT	81.0469934	52.032287
93	93	45892213	1	TTTGTAGGCAAAGGAAAACCT	CAGAAT	59.9843427	53.8381039
94	94	45892406	1	CTTTTACATATTTTTGAGCAA	GAGAGT	50.1306495	58.8116789
95	95	45892605	-1	CTGTCTCAGCCTCCCAGCTAC	TGGGAT	69.3861361	5.4157682 4
96	96	45892786	1	CTTCTGAATACTGATCTAGT	ACAGGT	76.9342922	5.4996672 8

[0084] Table 2 List of control sgRNA guide sequences.

[0085] [Table 2]

SEQ ID No.	Control Guide	Sequence
98	1	ACGGAGGCTAAGCGTCGCAA
99	2	CGCTTCCGCGGCCCGTTCAA
100	3	GTAGGCGCGCCGCTCTCTAC

[0086] Example 2

(1) Experimental Methods

Cell Culture and Transfection

SK-N-AS (American Type Culture Collection) cells were seeded 24-72 hours prior to transfection in 12-well plates at a density of 75,000-200,000 cells per well and cultured in DMEM media supplemented with 10% FBS and 2 mM fresh L-glutamine, 1 mM sodium pyruvate and non-essential amino acids. Cells were transfected with 1000 ng sgRNA containing px601-CMV-dSaCas9-KRAB-P2A-Puro (modified from GenScript) plasmid using 3.0 μl of TransIT-VirusGEN (Mirus Bio), according to manufacturer's instructions. 24-36 hours following transfection, transfected cells were enriched by puromycin selection (1.5 μg/ml in DMEM). Cells were harvested at 72 h after transfection and lysed in RLT buffer (Qiagen) to extract total RNA using RNeasy kit (Qiagen).

[0087] Gene expression analysis

For Taqman analysis, max 1.5 μg of total RNA was used to generate cDNA using TaqMan High-Capacity RNA-to-cDNA Kit (Applied Biosystems) in 10 μl volume. The generated cDNA was diluted 10-fold and 3.33 μl was used per Taqman reaction. The Taqman primers and probes for the MAPT and HPRT gene were obtained from Applied Biosystems. Taqman reaction was run using Taqman gene expression master mix (ThermoFisher) in ThermoFisher QuantStudio 5 Real-Time PCR System and analyzed using QuantStudio 5 analysis software.

Taqman probe product IDs:

MAPT: Hs00902193_m1 (FAM-MGB)

HPRT: Hs99999909_m1 (VIC PL)

Taqman qPCR condition:

Step 1; 50°C 2 min

Step 2; 95°C 2 min

Step 3; 95°C 1 sec

Step 4; 60°C 20 sec

Repeat Steps 3 and 4; 45 times

[0088] Selection of sgRNA Sequence

The location of the guide RNA target sites relative to the MAPT gene is shown in Fig. 1. The selected guide RNA sequences (Table 3) were fused with the tracer RNA sequence to form single-molecule guide RNA (sgRNA) sequences, and were cloned into px601-CMV-dSaCas9-KRAB-P2A-Puro (modified from GenScript). The sgRNA expression is driven by the hU6 promoter, and the vector expresses the puromycin gene under a CMV/P2A promoter mechanism to facilitate tracking and selection of the sgRNA expressing cells. Three control sgRNA guides (Table 2) were selected from the Human CRISPR Knockout Pooled Library (Sanjana N. E. et al, Nature Methods, 11(8), p.783, 2014).

[0089] (2) Results

Suppression of MAPT gene expression by the RNP

The suppression of MAPT transcript by the additional thirty-eight sgRNAs are shown (Fig. 3), where MAPT transcript expression was normalized to the mRNA levels of the HPRT gene. Detected MAPT expression levels after transfection with control sgRNA guides were set to 1.0. sgRNA# 123, 127 and 132 showed close to 80% suppression whereas 113 and 106 showed 70% suppression. The experiments detailed were conducted at least three times, and the mean -fold suppression values and standard deviations are shown.

[0090] Table 3 List of sa sgRNA guides (with NNGRRT PAM sequence) within 'UCSC Genome Browser on Human Dec. 2013 (GRCh38/hg38) Assembly; chromosome 17: 45,887,381- 45,962,898'.

[0091]

[Table 3-1]

SEQ ID No.	Guide RNA (sa sgRNA)	Position	Strand	Sequence	PAM	Specificity Score	Efficiency Score
115	97	45895706	-1	AGGTGCAGGGAGGGGCCCTTGCA	GGGAGT	99.3096335	4.733708607
119	98	45895244	-1	CAGGGCAGTGAAGCCCTGT	CGGAT	99.6206557	32.383784
120	99	45895315	-1	CCCAGCCCCCAAATGTCCCCT	ACGGGT	77.3756342	15.4724932
121	100	45895349	1	GGAGAAATCGAGCAGATGGGG	AGGGGT	48.0823599	19.4042152
122	101	45895435	1	CGCCGTGCCTGAGAACAGTGC	GCGGAT	80.2954811	20.1560618
123	102	45895454	-1	TGCCTTTGCGAGCGTGCACAG	TGGGAT	87.1186343	41.36942026
124	103	45895467	1	CACTGTGTACGCTCGCAAAGG	CAGGGT	96.1501184	10.186814
125	104	45895510	-1	GCGAACGCGGACCAGCGCCG	CCGAGT	85.3329528	15.10555389
126	105	45895549	1	CACGCACTAGCCGCCAGCCACGC	ACGGAT	83.9131281	4.1878061
127	106	45895583	1	GGGCTGCAGGTGCATCTCGGG	GCGGAT	78.7301006	6.03387682
128	107	45895684	1	GGCTCGCCCCTCACTGCCGCA	GTGGGT	85.1877935	55.616367
129	108	45895706	-1	TCCTCCCCCTCCTCCCCCAC	CAGGGT	71.2853148	16.62158252
130	109	45895716	1	CCCTGGTGAGCGAGGAAGGG	GAGGAT	41.7697132	2.35704489
131	110	45895751	-1	AAAAAAGGGGGCTGGGGC	GGGAGT	41.1972073	0.55405919
132	111	45896830	1	TCTTTACGGTGCCATGCCAAA	CCGGGT	98.5160082	54.2601482
133	112	45896030	1	CTGGTTTCTGGCTTTTATTCT	GAGAGT	45.2770507	0.69271586
134	113	45896037	-1	GGGAGGTTGACTCAACACCCT	CAGAAT	84.4628587	37.0688894
135	114	45896107	1	TCCTCATTTCCGAGCCCATTG	TTGGAT	86.5360958	11.15351283

[0092]

[Table 3-2]

136	115	45896124	1	ATTGTTGGATCTCGAGGCTTG	CTGGGT	90.2563231	4.75122805
137	116	45896139	1	GGCTTGCTGGGTTCGATGAAC	TCGAGT	96.6052647	1.64593323
138	117	45896149	-1	GCCGGGGGTCGGGGGGTTGAC	TCGAGT	81.9105554	2.00201999
139	118	45896159	-1	TTCCATGCGTGCCGGGGGTCG	GGGGGT	91.9530435	1.44849023
140	119	45896167	-1	CACGCCCGTTCCATGCGTGCC	GGGGGT	96.1743145	0.31699411
141	120	45892794	1	TACTGATCTAACTAGGGGTTG	CAGGGT	89.6918865	9.65934561
142	121	45892881	1	ACCAAGATAGAGGTCTTGAAC	TAGGAT	86.6149258	2.22133977
143	122	45892928	1	CAACAAAAAGTCAATTCCAGG	CTGAGT	59.6496529	60.3179983
144	123	45892932	-1	CATGAGCCACTGCACTCAGCC	TGGAAT	62.8908565	9.07396863
145	124	45892965	-1	CACCTCAGCCTCCCAAAGCGT	TGGGAT	71.7665018	21.2102322
146	125	45892979	1	AACGCTTTGGGAGGCTGAGGT	GGGAGT	50.6652373	9.9538773
147	126	45893095	-1	CAATCTTCCCACCTCAGCCTT	CTGAGT	65.0078225	25.505003
148	127	45893120	1	TGGGAAGATTGCTTGAGCCCC	AGGAGT	81.4308321	1.44369449
149	128	45893172	-1	GAGACAAGGTCTTGCTCAGGC	TGGAGT	42.5862188	20.7088644
150	129	45893212	-1	TGTACACCTTAGAAAAGTCAG	TGGAAT	74.3622004	57.8656558
151	130	45893283	-1	TCTGCCCTAGCCTTCTGACTA	CAGAAT	81.7937919	12.1321814
152	131	45893341	1	GTGGTTCTCACTCCTACTTCT	TTGGGT	71.6156126	3.48872191
153	132	45893341	-1	GGGGCATATACCCAAAGAAGT	AGGAGT	76.8761349	39.4682588
154	133	45893425	1	AAGGTCAGTTCCAGAAACTGT	GTGAAT	67.2726045	17.7360205
155	134	45893485	1	AACATATGTTGATTTTTTTAAA	AAGAAT	22.7028856	17.540826

Industrial Applicability

[0093] According to the present invention, the expression of MAPT gene in human cells can be suppressed. Thus, the present invention is expected to be extremely useful for the treatment and/or prevention of AD.

[0094] Where a numerical limit or range is stated herein, the endpoints are included. Also, all values and subranges within a numerical limit or range are specifically included as if explicitly written out.

[0095] As used herein the words “a” and “an” and the like carry the meaning of “one or more.”

[0096] Obviously, numerous modifications and variations of the present invention are possible in light of the above teachings. It is, therefore, to be understood that, within the scope of the appended claims, the invention may be practiced otherwise than as specifically described herein.

[0097] All patents and other references mentioned above are incorporated in full herein by this reference, the same as if set forth at length.

[0098] This application is based on US provisional patent application No. 63/049,736 (filing date: July 9, 2020), and US provisional patent application No. 63/212,429 (filing date: June 18, 2021), both filed in US, the contents of which are incorporated in full herein.