DESCRIPTION

Title of Invention: OLIGODENDROCYTE-SPECIFIC PROMOTER, MIRNA SPECIFIC TO PLP1 GENE, VECTOR INCLUDING SAID PROMOTER AND/OR MIRNA, AND PHARMACEUTICAL COMPOSITION INCLUDING SAID VECTOR

Technical Field

[0001] The present invention relates to an oligodendrocyte-specific promoter, in particular, to an oligodendrocyte-specific promoter comprising a nucleic acid having a sequence identity of at least 90% to a nucleotide sequence set forth in SEQ ID NO: 1. The present invention also relates to an miRNA specific to a PLP1 gene. Further, the present invention relates to a vector comprising the promoter and/or the miRNA, and a pharmaceutical composition comprising the vector.

Background Art

[0002] Hypomyelinating leukodystrophies is a collective term for brain diseases in children caused by poor development of the brain white matter, and currently 11 diseases have been known. A representative disease among them is Pelizaeus-Merzbacher disease (PMD). PMD is a rare intractable disease that is characterized in that myelination of the central nervous system is disordered and causes developmental failure of motor functions and neurological symptoms immediately after birth. The estimated incidence in Japan is 1.45 individuals per 100000 newborn male infants. Currently, there is no fundamental cure.

[0003] Myelin is generated by oligodendrocytes (oligodendroglia), one of glial cells, in the brain, and has a multilayer structure of insulating phospholipid present around the axon of a neuron. A primary protein constituting myelin is proteolipid protein (PLP), Myelin basic protein (MBP), human 2',3'-cyclic nucleotide phosphodiesterase (CNP), and so on are also known as other constituents. PMD is caused by abnormalities of a gene for proteolipid protein (PLP1 gene). The most frequent mutation among such abnormalities is PLP1 duplications (about 60%). PLP1 duplication leads to overexpression of the PLP1 gene, thereby normalization of its expression level is expected to provide therapeutic effect.

[0004] Non Patent Literature 1 disclosed expression of adeno-associated virus (AAV)-mediated green fluorescent protein (GFP) driven by myelin basic protein (MBP) promoter or glial fibrillary acidic protein (GFAP) promoter in the brain of mice under development, and reported that the expression of GFAP promoter-driven GFP was highly specific to astrocytes after injection of a vector into the brain of neonatal mice and adult mice. Non Patent Literature 1 also reported the selectivity of the MBP promoter for oligodendrocytes was poor after AAV delivery to right after birth, but was superior after injection of a vector 10 days after birth. This document suggested that direct injection of AAV driven by a cell type-specific promoter into the developed brain after birth generates targeted long-term transgene expression in glial cells.

[0005] Non Patent Literature 2 disclosed development of target gene therapy for oligodendrocytes in Pelizaeus-Merzbacher-like disease, and

reported that a GJC2/Cx47 gene was inserted into the downstream of a myelin basic protein promoter, and administered to 10-day-old mice via single intracerebral injection into the internal capsule with an adeno-associated virus (AAV.MBP.Cx47myc) vector.

[0006] Each of Non Patent Literatures 1 and 2 discloses gene therapy and a promoter therefor with use of an AAV vector, but did not describe at all gene therapy for abnormality of the PLP1 gene and a promoter or miRNA therefor.

Citation List

Non Patent Literature

[0007] Non Patent Literature 1: von Jonquieres G et al., Glial promoter selectivity following AAV-delivery to the immature brain. PLoS One. 2013 Jun 14; 8(6) : e65646.

Non Patent Literature 2: Georgiou E et al., Gene therapy targeting oligodendrocytes provides therapeutic benefit in a leukodystrophy model. Brain. 2017 Mar 1; 140(3): 599-616.

Summary of Invention

Technical Problem

[0008] An object of the present invention is to provide a vector capable of oligodendrocyte-specifically suppressing expression of the PLP1 gene for treating PMD caused by abnormality of the PLP1 gene, and a promoter and miRNA therefor, and a pharmaceutical composition comprising the vector.

Solution to Problem

[0009] The present inventors found that a human 2',3'-cyclic nucleotide phosphodiesterase (CNP) promoter having a sequence set forth in SEQ ID NO: 1 can drive gene expression in an oligodendrocyte-specific and highly efficient manner, and that expression of the PLP1 gene is successfully suppressed with an AAV vector comprising the promoter and a PLP1 gene-specific miRNA operably linked to the downstream of the promoter, thus completing the present invention.

[0010] Specifically, the present invention relates to the followings.

[1] An oligodendrocyte-specific promoter comprising a nucleic acid having a sequence identity of at least 90% to a nucleotide sequence set forth in SEQ ID NO: 1.

[2] The promoter according to [1], comprising a nucleic acid having the nucleotide sequence set forth in SEQ ID NO: 1.

[3] An oligodendrocyte-specific vector comprising the promoter according to [1] or [2].

[4] The vector according to [3], further comprising an miRNA sequence specific to a human PLP1 gene operably linked to the promoter.

[5] An miRNA comprising a pair of nucleotide sequences consisting of an antisense sequence and a sense sequence and being specific to a PLP1 gene, wherein the pair of nucleotide sequences is selected from the group consisting of pairs of an antisense sequence set forth in a left column of Table 1 and a sense sequence set forth in the just right of the antisense sequence in a right column of the table.

[6] The miRNA according to [5], comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 2 to 7 and 24 to 51.

[7] The miRNA according to [5], comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 2 to 5, 7, 24, 26, 29 to 32, 35, 36, 42, and 51.

[8] The miRNA according to [5], comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 2, 29, 31, and 32.

[9] A vector comprising a sequence of the miRNA according to any one of [5] to [8].

[10] The vector according to [9], wherein the sequence of the miRNA is operably linked to an oligodendrocyte-specific promoter.

[11] The vector according to [10], wherein the oligodendrocyte-specific promoter is the promoter according to [1] or [2].

[12] The vector according to any one of [3], [4], and [9] to [11], wherein the vector is an adeno-associated virus (AAV) vector.

[13] A pharmaceutical composition comprising the vector according to any one of [3], [4], and [9] to [12].

[14] The pharmaceutical composition according to [13], wherein the pharmaceutical composition is a pharmaceutical composition for treating a disease associated with abnormality in a PLP1 gene.

[15] The pharmaceutical composition according to [14], wherein the disease is Pelizaeus-Merzbacher disease, spastic paraplegia type 2, or multiple sclerosis.

[16] The pharmaceutical composition according to [14], wherein the disease is Pelizaeus-Merzbacher disease.

[17] The pharmaceutical composition according to [13], wherein the pharmaceutical composition is a PLP1 gene expression suppressor which suppresses expression of the PLP1 gene.

[18] A method for treating a disease associated with abnormality of a PLP1 gene, the method comprising administering an effective amount of the vector according to any one of [3], [4], and [9] to [12] to a patient in need of treatment.

[19] The method according to [18], wherein the disease is Pelizaeus-Merzbacher disease, spastic paraplegia type 2, or multiple sclerosis.

[20] The method according to [18], wherein the disease is Pelizaeus-Merzbacher disease.

[21] A method for suppressing expression of a PLP1 gene, the method comprising administering an effective amount of the vector according to any one of [3], [4], and [9] to [12] to a patient in need of treatment.

[22] The vector according to any one of [3], [4], and [9] to [12] for use in treatment of a disease associated with abnormality of a PLP1 gene.

[23] The vector according to [22], wherein the disease is Pelizaeus-Merzbacher disease, spastic paraplegia type 2, or multiple sclerosis.

[24] The vector according to [22], wherein the disease is Pelizaeus-Merzbacher disease.

Advantageous Effects of Invention

[0011] The AAV vector comprising the promoter of the present invention is capable of expressing a gene in an oligodendrocyte-specific and highly efficient manner, and moreover successfully suppresses expression of the PLP1 gene through inclusion of an miRNA sequence specific to the human PLP1 gene operably linked to the promoter, and

thus can serve as a therapeutic drug for PMD caused by PLP1 duplication, etc.

Brief Description of Drawings

[0012] [Figure 1] Figure 1 shows a schematic diagram of an AAV vector of Example 1.

[Figure 2] Figure 2 shows photographs representing results of immunofluorescence staining on specificity of AAV-expressing cells in Example 2.

[Figure 3] Figure 3 shows a graph representing results of site-by-site measurement of suppression of expression (knockdown) in terms of PLP1 gene expression levels on the basis of fluorescence intensity generated by PLP1 immunofluorescence staining in Example 3.

[Figure 4] Figure 4 shows graphs representing results of measurement of suppression of expression (knockdown) in terms of PLP1 gene expression levels through quantitative PCR in Example 4. (A) and (B) show relative expression levels of the PLP1 gene and relative expression levels of the Olig2 gene, respectively.

[Figure 5] Figure 5 shows photographs representing results of histological analysis through immunostaining after administration to PLP1-Tg mice in Example 5.

[Figure 6] Figure 6 shows photographs representing results of micromorphological analysis with an electron microscope after administration to PLP1-Tg mice in Example 5.

[Figure 7] Figure 7 shows a graph representing life-prolonging effect provided by administration to PLP1-Tg mice in Example 5.

[Figure 8] Figure 8 shows a graph representing body weight gain effect provided by administration to PLP1-Tg mice in Example 5.

[Figure 9] Figure 9 shows a graph representing results of measurement of suppression of expression (knockdown) in terms of PLP1 gene expression levels through quantitative PCR in Example 6.

[Figure 10] Figure 10 shows a graph representing results of measurement of suppression of expression (knockdown) in terms of PLP1 gene expression levels through quantitative PCR in Example 7.

[Figure 11] Figure 11 shows a graph representing results of measurement of suppression of expression (knockdown) in terms of PLP1 gene expression levels through quantitative PCR in Example 8.

[Figure 12] Figure 12 shows a graph representing results of measurement of suppression of expression (knockdown) in terms of PLP1 gene expression levels through quantitative PCR in Example 9.

[Figure 13] Figure 13 shows a graph representing results of site-by-site measurement of suppression of expression (knockdown) in terms of PLP1 gene expression levels on the basis of fluorescence intensity generated by PLP1 immunofluorescence staining in Example 10.

Description of Embodiments

[0013] [Promoter]

The promoter of the present embodiment is an oligodendrocyte-specific promoter comprising a nucleic acid having a sequence identity of at least 90%, preferably having a sequence identity of at least 95%, more preferably having a sequence identity of at least 98%, to a nucleotide sequence set forth in SEQ ID NO: 1, particularly

preferably comprising a nucleic acid having the nucleotide sequence set forth in SEQ ID NO: 1. In addition to the nucleic acid having a sequence identity of at least 90% to the nucleotide sequence set forth in SEQ ID NO: 1, the promoter may include in an end of the promoter nucleotide sequences of sites for cloning into vectors such as restriction enzyme sites and an att sequence for Gateway cloning.

[0014] The nucleic acid having the nucleotide sequence set forth in SEQ ID NO: 1 is a human CNP promoter, and derived from chr17:40,118,309-40,120,101 of the human genome reference sequence (GRCh37/hg19). The human CNP gene is a gene expressed specifically in oligodendrocytes in the brain, and the specificity is inferred to be determined by the promoter sequence. The present inventors found that the human CNP promoter sequence set forth in SEQ ID NO: 1 is capable of oligodendrocyte-specificcally expressing genes other than the CNP gene that are disposed in the downstream of the promoter.

[0015] The promoter of the present embodiment is a promoter that specifically operates in oligodendrocytes, whereby genes operably linked to the downstream of the promoter are expressed specifically in oligodendrocytes. The promoter of the present embodiment can be obtained through a gene engineering approach. For example, a gene for the human CNP promoter is obtained from a human genome, and some nucleotides are deleted, inserted, or substituted to obtain a promoter having a desired nucleotide sequence. Alternatively, a promoter having a desired nucleotide sequence can be obtained through partial or complete synthesis.

[0016] [miRNA]

An miRNA typically includes an antisense sequence and a sense sequence, and can form a hairpin structure, thereby processed into a mature miRNA. The antisense strand in the mature miRNA bonds to a target mRNA to decompose the mRNA or cause translational repression, thereby suppressing expression of the target gene. The miRNA of the present embodiment is an miRNA specific to the PLP1 gene, in particular, to the human PLP1 gene, includes an antisense sequence and a sense sequence, and is capable of suppressing expression of the PLP1 gene.

[0017] It is preferable that a 5' flanking sequence, an antisense sequence, a stem-loop sequence, a sense sequence, and a 3' flanking sequence be disposed in the order from the 5' side in the miRNA of the present embodiment. The configuration is required to be such that the 5' flanking sequence has a length of 25 to 100 nucleotides, the stem-loop sequence has a length of 4 to 30 nucleotides, and the 3' flanking sequence has a length of 25 to 100 nucleotides.

[0018] Each flanking sequence needs to be a flanking sequence known to be generally expressed in oligodendrocytes, and, for example, a 5' flanking sequence and 3' flanking sequence that are known to be sequences derived from miR155 can be used. In addition to the 5' flanking sequence and 3' flanking sequence derived from miR155, a 5' flanking sequence and 3' flanking sequence of any of miR-138, miR-219, miR-9, miR-23a, miR-388, and miR-297c, which are known to be expressed in oligodendrocytes, can be used.

[0019] The miRNA sequence specific to the human PLP1 gene can be

designed, for example, by using software such as BLOCK-iT (TM) RNAi Designer (Thermo Fisher Scientific, USA), and, for example, the following human PLP1-miRNA candidate nucleotide sequences can be used.

Human PLP1-miRNA candidate nucleotide sequence 1 (SEQ ID NO: 2):

5'-
AAAGGAAGAAGAAAGAGGCAGGTTTTGGCCACTGACTGACC
TGCCTCTCTTCTTCCTTT -3'

Human PLP1-miRNA candidate nucleotide sequence 2 (SEQ ID NO: 3):

5'-
AACACCAGGAGCCACACAACGGTTTTGGCCACTGACTGACCG
TTGTGTCTCCTGGTGTT -3'

Human PLP1-miRNA candidate nucleotide sequence 3 (SEQ ID NO: 4):

5'-
TTCCATGGGAGAACACCATACGTTTTGGCCACTGACTGACGTA
TGGTGCTCCCATGGAA -3'

Human PLP1-miRNA candidate nucleotide sequence 4 (SEQ ID NO: 5):

5'-
TGAGCAGGGAAACCAGTGTAGGTTTTGGCCACTGACTGACCT
ACACTGTTCCCTGCTCA-3'

Human PLP1-miRNA candidate nucleotide sequence 5 (SEQ ID NO: 6):

5'-
AGGGCTTTCTGATTGACAGCCGTTTTGGCCACTGACTGACGG
CTGTCACAGAAAGCCCT -3'

Human PLP1-miRNA candidate nucleotide sequence 6 (SEQ ID NO: 7):

5'-
ACCCCAAAGAAACACAATCCAGTTTTGGCCACTGACTGACtgg
attgtttctttggggt -3'

Human PLP1-miRNA candidate nucleotide sequence 7 (SEQ ID NO: 24):

5'-
ACAAATGCAGCAATAAACAGGGTTTTGGCCACTGACTGACCC
TGTTTAGCTGCATTTGT -3'

Human PLP1-miRNA candidate nucleotide sequence 8 (SEQ ID NO: 25):

5'-
AATAGACTGGCAGGTGGTCCAGTTTTGGCCACTGACTGACTG
GACCACGCCAGTCTATT -3'

Human PLP1-miRNA candidate nucleotide sequence 9 (SEQ ID NO: 26):

5'-
AAAGAATGAGCTTGATGTTGGGTTTTGGCCACTGACTGACCC
AACATCGCTCATTCTTT -3'

Human PLP1-miRNA candidate nucleotide sequence 10 (SEQ ID NO: 27):

5'-

AGATACTCATAGTCTTGGTAGGTTTTGGCCACTGACTGACCTA
CCAAGTATGAGTATCT -3'

Human PLP1-miRNA candidate nucleotide sequence 11 (SEQ ID NO: 28):

5'-
AAGCCCATGTCTTTGGGACTCGTTTTGGCCACTGACTGACGA
GTCCCAGACATGGGCTT -3'

[0020] Alternatively, the miRNA sequence specific to the human PLP1 gene can be designed in such a manner that artificial siRNAs are designed as described in Examples, and an optimum sequence is selected from the siRNAs to design the miRNA.

[0021] The miRNA of the present embodiment comprises a pair of nucleotide sequences consisting of an antisense sequence and a sense sequence, and the pair of nucleotide sequences may be selected from the group consisting of pairs of an antisense sequence set forth in a left column of Table 1 and a sense sequence set forth in the just right of the antisense sequence in a right column of the table.

[Table 1]

Table 1. Antisense and sense sequences for miRNA designed for human PLP1 gene

Antisense sequence	Sense sequence
AAAGGAAGAAGAAAGAGGCAG (SEQ ID NO: 52)	CTGCCTCTCTTCTTCCTTT (SEQ ID NO: 53)
AACACCAGGAGCCACACAACG (SEQ ID NO: 54)	CGTTGTGTCTCCTGGTGTT (SEQ ID NO: 55)
TTCCATGGGAGAACACCATAC (SEQ ID NO: 56)	GTATGGTGCTCCCATGGAA (SEQ ID NO: 57)
TGAGCAGGGAAACCAGTGTAG (SEQ ID NO: 58)	CTACACTGTTCCCTGCTCA (SEQ ID NO: 59)
AGGGCTTTCTGATTGACAGCC (SEQ ID NO: 60)	GGCTGTCACAGAAAGCCCT (SEQ ID NO: 61)
ACCCCAAAGAAACACAATCCA (SEQ ID NO: 62)	TGGATTGTTTCTTTGGGGT (SEQ ID NO: 63)
ACAAATGCAGCAATAAACAGG (SEQ ID NO: 64)	CCTGTTTAGCTGCATTTGT (SEQ ID NO: 65)
AATAGACTGGCAGGTGGTCCA (SEQ ID NO: 66)	TGGACCACGCCAGTCTATT (SEQ ID NO: 67)
AAAGAATGAGCTTGATGTTGG (SEQ ID NO: 68)	CCAACATCGCTCATTCTTT (SEQ ID NO: 69)
AGATACTCATAGTCTTGGTAG (SEQ ID NO: 70)	CTACCAAGTATGAGTATCT (SEQ ID NO: 71)
AGAAACACAATCCAGTGGCCA (SEQ ID NO: 72)	TGGCCACTATTGTGTTTCT (SEQ ID NO: 73)
AAATAGGTCTCAATTAGCTTT (SEQ ID NO: 74)	AAAGCTAAGAGACCTATTT (SEQ ID NO: 75)
AAGAAACACAATCCAGTGGCC (SEQ ID NO: 76)	GGCCACTGTTGTGTTTCTT (SEQ ID NO: 77)
TAAACAGGTGGAAGGTCATTT (SEQ ID NO: 78)	AAATGACCCCACCTGTTTA (SEQ ID NO: 79)
TTGTAGTCGCCAAAGATCTGC (SEQ ID NO: 80)	GCAGATCTGGCGACTACAA (SEQ ID NO: 81)
AATTAGAGCCTCCATTCCTTT (SEQ ID NO: 82)	AAAGGAATAGGCTCTAATT (SEQ ID NO: 83)
TTAAGGACGGCAAAGTTGTAA (SEQ ID NO: 84)	TTACAACTGCCGTCCTTAA (SEQ ID NO: 85)
TTTAAGGACGGCAAAGTTGTA (SEQ ID NO: 86)	TACAACTTCCGTCCTTAAA (SEQ ID NO: 87)
ATGTCTTTGGGACTCTGACTC (SEQ ID NO: 88)	GAGTCAGACCCAAAGACAT (SEQ ID NO: 89)
TATCTATCCTGTGTCTACCAG (SEQ ID NO: 90)	CTGGTAGACAGGATAGATA (SEQ ID NO: 91)
AAATTACTTTCTGATCCTCAG (SEQ ID NO: 92)	CTGAGGATGAAAGTAATTT (SEQ ID NO: 93)
TCTAACAAGCCCATGTCTTTG (SEQ ID NO: 94)	CAAAGACAGGCTTGTTAGA (SEQ ID NO: 95)
AATTACTTTCTGATCCTCAGG (SEQ ID NO: 96)	CCTGAGGAAGAAAGTAATT (SEQ ID NO: 97)
AGTAAATGTACACAGGCACAG (SEQ ID NO: 98)	CTGTGCCTGTACATTTACT (SEQ ID NO: 99)
TAAGTAAGGTTGGCTGAGTTA (SEQ ID NO: 100)	TAACTCAGAACCTTACTTA (SEQ ID NO: 101)
TTCTGTGGGTGAAAGATCCTT (SEQ ID NO: 102)	AAGGATCTCACCCACAGAA (SEQ ID NO: 103)
AGAAGATGCTGACAACACCCT (SEQ ID NO: 104)	AGGGTGTTCAGCATCTTCT (SEQ ID NO: 105)
AATTGTAGCCGGCTGGCTAGT (SEQ ID NO: 106)	ACTAGCCACGGCTACAATT (SEQ ID NO: 107)
AGATTTGGGCAAACGCTCTTA (SEQ ID NO: 108)	TAAGAGCGTGCCCAAATCT (SEQ ID NO: 109)
ATTCTACGCTCCCTTATGCTG (SEQ ID NO: 110)	CAGCATAAGAGCGTAGAAT (SEQ ID NO: 111)
TGGTAATAGAGAGACCAGAAT (SEQ ID NO: 112)	ATTCTGGTCTCTATTACCA (SEQ ID NO: 113)
TATAGATGGCAAGAGGACCAA (SEQ ID NO: 114)	TTGGTCCTTGCCATCTATA (SEQ ID NO: 115)
AAACCAGTGTAGCTGCAGCCC (SEQ ID NO: 116)	GGGCTGCATACACTGGTTT (SEQ ID NO: 117)

[0022] The miRNA of the present embodiment preferably includes any of the following pairs of nucleotide sequences.

- A pair of nucleotide sequences consisting of SEQ ID NO: 52 as an antisense sequence and SEQ ID NO: 53 as a sense sequence

- A pair of nucleotide sequences consisting of SEQ ID NO: 54 as an

antisense sequence and SEQ ID NO: 55 as a sense sequence

- A pair of nucleotide sequences consisting of SEQ ID NO: 56 as an antisense sequence and SEQ ID NO: 57 as a sense sequence

- A pair of nucleotide sequences consisting of SEQ ID NO: 58 as an antisense sequence and SEQ ID NO: 59 as a sense sequence

- A pair of nucleotide sequences consisting of SEQ ID NO: 62 as an antisense sequence and SEQ ID NO: 63 as a sense sequence

- A pair of nucleotide sequences consisting of SEQ ID NO: 64 as an antisense sequence and SEQ ID NO: 65 as a sense sequence

- A pair of nucleotide sequences consisting of SEQ ID NO: 68 as an antisense sequence and SEQ ID NO: 69 as a sense sequence

- A pair of nucleotide sequences consisting of SEQ ID NO: 72 as an antisense sequence and SEQ ID NO: 73 as a sense sequence

- A pair of nucleotide sequences consisting of SEQ ID NO: 76 as an antisense sequence and SEQ ID NO: 77 as a sense sequence

- A pair of nucleotide sequences consisting of SEQ ID NO: 78 as an antisense sequence and SEQ ID NO: 79 as a sense sequence

- A pair of nucleotide sequences consisting of SEQ ID NO: 84 as an antisense sequence and SEQ ID NO: 85 as a sense sequence

- A pair of nucleotide sequences consisting of SEQ ID NO: 86 as an antisense sequence and SEQ ID NO: 87 as a sense sequence

- A pair of nucleotide sequences consisting of SEQ ID NO: 98 as an antisense sequence and SEQ ID NO: 99 as a sense sequence

- A pair of nucleotide sequences consisting of SEQ ID NO: 116 as an antisense sequence and SEQ ID NO: 117 as a sense sequence

[0023] The miRNA of the present embodiment more preferably

includes any of the following pairs of nucleotide sequences.

- A pair of nucleotide sequences consisting of SEQ ID NO: 52 as an antisense sequence and SEQ ID NO: 53 as a sense sequence
- A pair of nucleotide sequences consisting of SEQ ID NO: 72 as an antisense sequence and SEQ ID NO: 73 as a sense sequence
- A pair of nucleotide sequences consisting of SEQ ID NO: 76 as an antisense sequence and SEQ ID NO: 77 as a sense sequence
- A pair of nucleotide sequences consisting of SEQ ID NO: 78 as an antisense sequence and SEQ ID NO: 79 as a sense sequence

[0024] Moreover, the miRNA of the present embodiment sequence may have a nucleotide sequence selected from the group consisting of SEQ ID NOs: 29 to 51 set forth in Table 2.

[Table 2]

Table 2. miRNA Sequences designed for human PLP1 gene

SEQ ID NO:	pre-miRNA Sequence
29	AGAAACACAATCCAGTGGCCAGTTTTGGCCACTGACTGACTGGCCACTATTGTGTTTCT
30	AAATAGGTCTCAATTAGCTTTGTTTTGGCCACTGACTGACAAAGCTAAGAGACCTATTT
31	AAGAAACACAATCCAGTGGCCGTTTTGGCCACTGACTGACGGCCACTGTTGTGTTTCTT
32	TAAACAGGTGGAAGGTCATTTGTTTTGGCCACTGACTGACAAATGACCCCACCTGTTTA
33	TTGTAGTCGCCAAAGATCTGCGTTTTGGCCACTGACTGACGCAGATCTGGCGACTACAA
34	AATTAGAGCCTCCATTCCTTTGTTTTGGCCACTGACTGACAAAGGAATAGGCTCTAATT
35	TTAAGGACGGCAAAGTTGTAAGTTTTGGCCACTGACTGACTTACAACTGCCGTCCTTAA
36	TTTAAGGACGGCAAAGTTGTAGTTTTGGCCACTGACTGACTACAACTTCCGTCCTTAAA
37	ATGTCTTTGGGACTCTGACTCGTTTTGGCCACTGACTGACGAGTCAGACCCAAAGACAT
38	TATCTATCCTGTGTCTACCAGGTTTTGGCCACTGACTGACCTGGTAGACAGGATAGATA
39	AAATTACTTTCTGATCCTCAGGTTTTGGCCACTGACTGACCTGAGGATGAAAGTAATTT
40	TCTAACAAGCCCATGTCTTTGGTTTTGGCCACTGACTGACCAAAGACAGGCTTGTTAGA
41	AATTACTTTCTGATCCTCAGGGTTTTGGCCACTGACTGACCCTGAGGAAGAAAGTAATT
42	AGTAAATGTACACAGGCACAGGTTTTGGCCACTGACTGACCTGTGCCTGTACATTTACT
43	TAAGTAAGGTTGGCTGAGTTAGTTTTGGCCACTGACTGACTAACTCAGAACCTTACTTA
44	TTCTGTGGGTGAAAGATCCTTGTTTTGGCCACTGACTGACAAGGATCTCACCCACAGAA
45	AGAAGATGCTGACAACACCCTGTTTTGGCCACTGACTGACAGGGTGTTCAGCATCTTCT
46	AATTGTAGCCGGCTGGCTAGTGTTTTGGCCACTGACTGACACTAGCCACGGCTACAATT
47	AGATTTGGGCAAACGCTCTTAGTTTTGGCCACTGACTGACTAAGAGCGTGCCCAAATCT
48	ATTCTACGCTCCCTTATGCTGGTTTTGGCCACTGACTGACCAGCATAAGAGCGTAGAAT
49	TGGTAATAGAGAGACCAGAATGTTTTGGCCACTGACTGACATTCTGGTCTCTATTACCA
50	TATAGATGGCAAGAGGACCAAGTTTTGGCCACTGACTGACTTGGTCCTTGCCATCTATA
51	AAACCAGTGTAGCTGCAGCCCGTTTTGGCCACTGACTGACGGGCTGCATACACTGGTTT

[0025] The miRNA of the present embodiment may have a nucleotide sequence selected from the group consisting of SEQ ID NOs: 2 to 7 and 24 to 51, preferably has a nucleotide sequence selected from the group consisting of SEQ ID NOs: 2 to 5, 7, 24, 26, 29 to 32, 35, 36, 42, and 51, and more preferably has a nucleotide sequence selected from the group consisting of SEQ ID NOs: 2, 29, 31, and 32.

[0026] [Vector]

The oligodendrocyte-specific vector of the present embodiment comprises an oligodendrocyte-specific promoter and/or an miRNA specific to the PLP1 gene. The vector of the present embodiment is a vector for use in treatment of a disease associated with abnormality in

the PLP1 gene, or for the purpose of suppression of expression of the PLP1 gene.

[0027] The vector needs to be a plasmid or vector capable of infecting human cells and expressing a gene, and examples of the vector include adeno-associated virus (AAV), lentivirus, retrovirus, adenovirus, Sendai virus, and plasmids (including complexes with a liposome or a polymer), and the vector is preferably AAV. AAV is a small, helper-dependent envelope-free virus that is classified into the genus Dependovirus in the family Parvoviridae and infects animals including humans, primates, and rodents. The induction of immunoreaction by AAV is very weak, and no pathogenicity has been found for AAV. AAV is capable of infecting both dividing cells and non-dividing cells, and once infecting host cells, AAV can survive out of the nuclear chromosomes and express a gene with a low frequency of insertion of the gene into the chromosomal genome of the host.

[0028] For the AAV, for example, any of the serotypes AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, and AAV9 is preferably used, and a hybrid mosaic AAV vector and a chimeric AAV vector are also preferably used. For the hybrid mosaic AAV vector, for example, any combinations of AAV1 to AAV9 including an AAV1/2 hybrid are available. Examples of the chimeric AAV vector include Olig001 (Powell SK et al., Gene Ther. 2016 Nov; 23 (11): 807-814), which has high tropism for oligodendrocytes.

[0029] The oligodendrocyte-specific promoter in the vector of the present embodiment is not limited to a particular promoter and may be any promoter that operates specifically in oligodendrocytes, and is

preferably the promoter of the present embodiment, that is, the oligodendrocyte-specific promoter is, for example, an oligodendrocyte-specific promoter comprising a nucleic acid preferably having a sequence identity of at least 90% to the nucleotide sequence set forth in SEQ ID NO: 1. The miRNA specific to the PLP1 gene in the vector of the present embodiment is not limited to a particular miRNA and may be any miRNA capable of suppressing expression of the PLP1 gene, and preferably is the miRNA of the present embodiment, that is, the miRNA is an miRNA comprising a pair of nucleotide sequences consisting of an antisense sequence and a sense sequence, wherein the pair of nucleotide sequences is selected from the group consisting of pairs of an antisense sequence set forth in a left column of Table 1 and a sense sequence set forth in the just right of the antisense sequence in a right column of the table.

[0030] The vector of the present embodiment may comprise the oligodendrocyte-specific promoter of the present embodiment and a transgene operably linked to the promoter. The transgene is not limited to a particular transgene, and, for example, an miRNA sequence specific to the PLP1 gene, in particular, an miRNA sequence specific to the human PLP1 gene is preferably used. The miRNA sequence specific to the human PLP1 gene can be designed, for example, by using software such as BLOCK-iT (TM) RNAi Designer (Thermo Fisher Scientific, USA). Alternatively, the miRNA sequence specific to the human PLP1 gene can be designed in such a manner that artificial siRNAs are designed as described in Examples, and an optimum sequence is selected from the siRNAs to design the miRNA. For such

an miRNA specific to the human PLP1 gene, for example, the above-described miRNA of the present embodiment is preferably used.

[0031] The vector of the present embodiment may comprise an oligodendrocyte-specific promoter and the miRNA of the present embodiment that is operably linked to the promoter. The oligodendrocyte-specific promoter is not limited to a particular promoter, and examples of the oligodendrocyte-specific promoter include a myelin basic protein gene promoter and a PLP1 gene promoter. For the oligodendrocyte-specific promoter, the above-described oligodendrocyte-specific promoter of the present embodiment is preferably used. Preferably, the vector of the present embodiment may comprise the oligodendrocyte-specific promoter of the present embodiment and one or more miRNAs of the present embodiment that are operably linked to the promoter.

[0032] The vector of the present embodiment may comprise a reporter gene to confirm gene expression. Examples of the reporter gene include, but are not limited to, GFP, Venus, and tdTomato.

[0033] The vector of the present embodiment can be constructed with ease through gene engineering. For example, an AAV1/2 hybrid vector including the hCNP promoter set forth in SEQ ID NO: 1 and a reporter gene can be produced with a method as described in the following. The hCNP promoter, a coding region for a reporter gene such as Venus, a 3' untranslated region, and an SV40 poly A signal are disposed in the order presented between two NotI sites of a self-complementary AAV vector, and the nucleotide sequence of an miRNA expression cassette is then inserted into the 3' untranslated region. The vector can be

cryopreserved at -20°C until use, and, when the vector is used, AAV293 cells (Takara Bio Inc., Japan) are transfected with the vector together with a serotype-specific helper plasmid and the adenovirus helper plasmid pHelper, and then cultured for 72 hours to achieve amplification in cells or in the culture solution. Depending on the scale, purification can be performed by means of column purification, ultrafiltration, ultracentrifugation, or the like. The vector genome titer of the AAV vector obtained can be determined through quantitative PCR using an AAVpro Titration Kit (Takara Bio Inc., Japan).

[0034] [Pharmaceutical composition]

The pharmaceutical composition of the present embodiment comprises the above-described vector of the present embodiment. The pharmaceutical composition of the present embodiment is preferably a pharmaceutical composition for treating a disease associated with abnormality in the PLP1 gene. Examples of the disease associated with abnormality involving the PLP1 gene include Pelizaeus-Merzbacher disease (PMD) and spastic paraplegia type 2, each of which is caused by duplication of the PLP1 gene, point mutation in the PLP1 gene, or deletion or insertion mutation involving the PLP1 gene, or multiple sclerosis. In addition, the pharmaceutical composition of the present embodiment may be a PLP1 gene expression suppressor capable of suppressing expression of the PLP1 gene.

[0035] The pharmaceutical composition of the present embodiment is preferably an aqueous solution containing an AAV vector, and may have a titer of 5×10^{10} to 5×10^{14} vg/mL, preferably of 5×10^{11} to 5×10^{13} vg/mL.

[0036] The pharmaceutical composition of the present embodiment may contain a pharmaceutically acceptable additive component such as phosphate-buffered saline (PBS), and may further contain an additional therapeutic agent. The pharmaceutical composition of the present embodiment can be produced by dissolving in an aqueous solution such as water and adding other components, as necessary.

[0037] The pharmaceutical composition of the present embodiment may be directly administered to the brain in treating Pelizaeus-Merzbacher disease, and examples of such administration include administration to one site or multiple sites of the brain parenchyma (e.g., the white matter in the cerebral hemisphere, the internal capsule, the cerebellum) or the spinal cord, and intracerebroventricular/intrathecal or intravascular/intraperitoneal administration is also acceptable. Administration can be performed at any time after birth. The amount of injection can vary among administration sites and ages, and an amount of 0.1 to 2 mL/site is appropriate in the case of administration to the brain parenchyma, and it is preferred to administer about 0.5 mL/site. In the case of intracerebroventricular/intrathecal administration, an amount of 0.5 to 2 mL/kg body weight is appropriate, and it is preferred to administer up to about 1 mL/kg body weight. In the case of intravascular or intraperitoneal administration, an amount of 1 to 10 mL/kg body weight is appropriate, and it is preferred to administer up to 5 mL/kg body weight.

[0038] [Therapeutic method]

The therapeutic method of the present embodiment is a method

for treating a disease associated with abnormality of the PLP1 gene, the method comprising administering an effective amount of the above-described vector of the present embodiment to a patient in need of treatment. Alternatively, the therapeutic method of the present embodiment is a method for suppressing expression of the PLP1 gene, the method comprising administering an effective amount of the above-described vector of the present embodiment to a patient in need of treatment.

Examples

[0039] Hereinafter, the present invention will be more specifically described with reference to Examples. However, the present invention is not limited to Examples in the following.

[0040] <Example 1 Construction of Oligodendrocyte-Specific AAV Expression Cassette and AAV Vector Including the Same>

A pre-miRNA sequence (PLP1 miRNA, SEQ ID NO: 9) targeting the mRNA of a mouse PLP1 gene (NM_011123.3, SEQ ID NO: 8) and a pre-miRNA sequence as a negative control (miR-neg, SEQ ID NO: 10) were designed by using BLOCK-iT (TM) RNAi Designer (Thermo Fisher Scientific, USA). The miR-neg is an miRNA sequence that can form a hairpin structure, thereby processed into a mature miRNA, but is predicted not to target known vertebrate genes.

PLP1-miRNA (SEQ ID NO: 9):

5'-

ACTCCAAAGAAACACAATCCAGTTTTGGCCACTGACTGACTG

GATTGTTTCTTTGGAGT -3'

miR-neg (SEQ ID NO: 10):

5'-

GTATGCATCGAATGAGATTCCGTTTTGGCCACTGACTGACGGA

ATCTCTCGATGCATAC -3'

[0041] Each of The PLP1 miRNA sequence and the miR-neg sequence was synthesized as designed, and cloned into a cloning site of a pcDNA (TM) 6.2-GW/miR vector (Thermo Fisher Scientific, USA) included in a BLOCK-iT (TM) Pol II miR RNAi expression vector kit. A 5' flanking sequence (SEQ ID NO: 11) and 3' flanking sequence (SEQ ID NO: 12) derived from mouse miR-155 were respectively disposed at one side and the other side of the PLP1 miRNA sequence or miR-neg sequence. The 5' flanking sequence and 3' flanking sequence derived from mouse miR-155 are respectively the 5' region and 3' region of mouse miR-155 with a portion of hairpin structure excluded therefrom, and the pre-miRNA sequence or miR-neg sequence was disposed between the 5' flanking sequence and the 3' flanking sequence so that these miRNA sequences could be expressed.

5' flanking sequence (SEQ ID NO: 11):

5'- CTGGAGGCTTGCTGAAGGCTGTATGCT -3'

3' flanking sequence (SEQ ID NO: 12):

5'-

CAGGACACAAGGCCTGTTACTAGCACTCACATGGAACAAATG

GCC -3'

[0042] From the resulting pcDNA (TM) 6.2-GW/miR vector, the portion of 5' flanking sequence-PLP1 miRNA sequence (or miR-neg

sequence)-3' flanking sequence was PCR-amplified, the amplified product was cleaved with XhoI/BamHI, and the resultant was inserted into a DNA fragment obtained by cleaving a pW-CAG-Venus-WPRE vector (SEQ ID NO: 13) with XhoI/BamHI and cleaved at both ends of WPRE positioned in the 3' side of a venus (SEQ ID NO: 14) sequence to remove the WPRE sequence. Subsequently, the resulting vector was cleaved with SpeI/EcoRI to remove the CAG promoter, and a DNA fragment was inserted into this site in the 5' side of the Venus sequence, the DNA fragment being obtained by PCR-amplifying a human CNP promoter (SEQ ID NO: 1) of 1.8 kb and cleaved at the both ends with SpeI/EcoRI, to enable oligodendrocyte-specific gene expression. The resulting vector was further cleaved with NotI to remove the backbone of single-stranded AAV derived from pW-CAG-Venus-WPRE, and bonded to the backbone of self-complementary adeno-associated virus (scAAV) obtained by cleaving pscW-PABPN1 (SEQ ID NO: 15) with NotI to afford the final constructs Pscw-hCNP-Venus-PLP1 miRNA (SEQ ID NO: 16) and Pscw-hCNP-Venus-miR-neg (SEQ ID NO: 17) (Figure 1).

[0043] Preparation of an AAV1/2 hybrid vector (scAAV-AAV1/2 vector) was performed in accordance with a method disclosed in Non Patent Literature 1. Specifically, AAV293 cells were co-transfected using PEI with the Pscw-hCNP-Venus-PLP1 miRNA or Pscw-hCNP-Venus-miR-neg, which was an scAAV construct obtained above, the serotype-specific AAV helper plasmids p5E18RXC1 (Xiao W et al., J Virol (1999) 73: 3994-4003) and pAAV-RC (Agilent Technologies) encoding the rep and cap genes of AAV1 and AAV2,

respectively, and the adenovirus helper plasmid pHelper (Agilent Technologies). The cells were collected 72 hours after the transfection, and each scAAV-AAV1/2 vector was purified by using an AAVpro Purification Kit (Takara Bio Inc., Japan). Vector genome titers were determined through quantitative PCR using an AAVpro Titration Kit (Takara Bio Inc., Japan).

[0044] The scAAV-AAV1/2 vectors obtained were designated as the AAV-hCNP-Venus-PLP1 miRNA vector (or PLP1 miRNA vector) and the AAV-hCNP-Venus-miR-neg vector (or miR-neg vector).

[0045] <Example 2 Administration of AAV Vectors to Wild-Type Mice and Expression Thereof>

One microliter of a solution of the PLP1 miRNA vector or miR-neg vector obtained in Example 1 (titer: 1.2×10^{12} vg/mL) was injected into one striatum and the internal capsule of each 10-day-old, wild-type mouse Jcl:B6C3F1 (n = 5 per group). Before the injection, the mice were anesthetized with pentobarbital solution. The injection of the vector was performed with a 33G syringe at a rate of 150 nL/min. After the injection each mouse was left untouched there for 1 minute, and the needle was slowly pulled out of the brain. Thereafter, the mice were returned as a group to their mother, and grown until day 17 after birth.

[0046] Gene expression from each vector was evaluated by detecting the green fluorescent protein Venus on day 17 after birth. Types of Venus-expressing cells were identified through double immunostaining with cell markers including Gst-π (for mature oligodendrocytes), NeuN (for neurons), Iba1 (for microglia), and GFAP (astrocytes).

[0047] For immunostaining, each Jcl:B6C3F1 mouse was anesthetized with Forane inhalation solution, and subsequently subjected to transcardial perfusion with PBS and then with fresh 4% paraformaldehyde. The brain was removed and further fixed at 4°C overnight, and then subjected to substitution with 30% sucrose/PBS and cryopreserved in the embedding medium OCT Compound (TissueTech, Inc.). A coronal section (20 μm) was obtained, and immunostained to check specificity of infected cells expressing Venus. The section was incubated together with blocking solution (PBS containing 0.05% Triton (TM) X-100 and 5% goat serum) at room temperature for 3 hours, and incubated at 4°C overnight with Gst-π (Cell Signaling Technology, Inc., 1:300), GFAP (Millipore Corporation, 1:400), NeuN (Chemicon, 1:400), or Iba1 (Biocare Medical, LLC., 1:500), as primary antibodies. The section was then washed, and incubated together with an appropriate secondary antibody (Thermo Fisher Scientific, USA) at room temperature for 1 hour. Cell nuclei were visualized with 40,60-diamidino-2-phenylindole (DAPI, Sigma-Aldrich Co. LLC). The slide was mounted with ProLong (R) Diamond Mountant (Thermo Fisher Scientific, USA), and photographed with a KEYENCE fluorescence microscope (KEYENCE CORPORATION, Japan).

[0048] Figure 2 shows the results. It is understood from Figure 2 that Venus-positive cells corresponded to Gst-π-positive oligodendrocytes but did not correspond to NeuN-positive cell neurons, GFAP-positive astrocytes, or Iba1-positive microglia. Thus, Figure 2 demonstrates that the miR-neg vector was expressed in an oligodendrocyte-specific and highly efficient manner.

[0049] <Example 3 Evaluation of Suppression Efficiency of Expression (Knockdown) in Terms of PLP1 Gene Expression Levels - Part 1>

One microliter of a solution of each of the two scAAV-AAV1/2 vectors (titer: 1.2×10^{12} vg/mL) obtained in Example 1 was injected into one striatum, the internal capsule, and the cerebellum of each 10-day-old, wild-type mouse Jcl:B6C3F1 (n = 3 per group). The injection method was the same as in Example 2. Thereafter, expression of PLP1 protein in Venus-positive cells was examined through immunostaining to evaluate PLP1 miRNA knockdown efficiencies. Immunostaining was performed with the same method as in Example 2 except that an anti-PLP1 rabbit polyclonal antibody (Numata Y et al., J Biol Chem. 2013 Mar 15; 288 (11): 7451-66) was used as a primary antibody, and an anti-rabbit fluorescent antibody (Thermo Fisher Scientific, USA) was used as a secondary antibody. Each site of the callosum, striatum, and internal capsule were photographed with a KEYENCE fluorescence microscope (KEYENCE CORPORATION, Japan).

[0050] Mean fluorescence intensities of immunostained PLP1 were quantified by using Image J 1.45s (Wayne Rasband, National Institutes of Health, USA), and Figure 3 shows quantitative analysis of fluorescence intensity through PLP1 immunofluorescence staining. It can be seen from Figure 3 that there was almost no difference in expression of PLP1 protein between the side without injection of the miR-neg vector (non-infected side) and the side with vector injection (infected side), whereas for the PLP1 miRNA vector there was 40% of expression-suppressing effect on PLP1 protein in the infected side as

compared with the non-infected side. The result that there was no difference in the number of astrocytes and that of microglia between the non-infected side and the infected side indicates the absence of reactive inflammatory response due to AAV administration.

[0051] <Example 4 Evaluation of Suppression Efficiency of Expression (Knockdown) in Terms of PLP1 Gene Expression Levels - Part 2>

One microliter of a solution of each of the two scAAV-AAV1/2 vectors (titer: 1.2×10^{12} vg/mL) obtained in Example 1 was injected into both striata and the internal capsule of each 10-day-old, wild-type mouse Jcl:B6C3F1 (n = 4 per group). The injection method was the same as in Example 2. Thereafter, expression of the PLP1 mRNA in Venus-positive cells was examined through quantitative PCR to evaluate PLP1 miRNA knockdown efficiency.

[0052] To sort Venus-positive cells, each Jcl:B6C3F1 mouse was anesthetized with Forane inhalation solution, and subjected to transcardial perfusion with 20 mL of PBS. The brain was removed, and roughly fragmented with micro dice scissors. To each brain, 1 mL of Accutase (Millipore Corporation) was added, and the resultant was incubated at 37°C for 30 minutes. To each sample, 2 mL of Hanks' balanced salt solution (HBSS) containing 10% FBS was added, and the tissue was homogenized with a micropipette. Each sample was filtered with a 100-μm cell strainer, and the collected cell suspension was centrifuged. To purify cells from myelin debris, cells were resuspended in HBSS containing 40% Percoll (Amersham plc), and centrifuged at 700 ×g at room temperature for 25 minutes. The upper myelin layer was sucked, and monocytes were resuspended in 1 mL of a

DMEM/F12 medium. These cells were separated by an FACS Canto flow cytometer (BD Biosciences), and the result was analyzed by using the software FlowJo (Tree Star Inc., OR, RRID: NIF-0000 to 30575). The result is plotted as percent to all cells.

[0053] For quantitative PCR, the total RNA was isolated from the selected Venus-positive cells using an RNeasy Mini Kit (Qiagen), and a cDNA was synthesized by using a SuperScript (R) Reverse Transcriptase Kit (Thermo Fisher Scientific, USA). Gene expression was analyzed through quantitative RT-PCR using LightCycler (R) 480 SYBR Green I Master and a LightCycler (R) 480 Instrument with specific primers below (F. Hoffmann-La Roche Ltd., Switzerland). The results were normalized to the house keeping gene β-actin, and relative expression levels of PLP1 and Olig2 were calculated. CP values (crossing point values) obtained for each specimen were converted into log values to plot relative expression levels ± SD, and the plotted results are shown in Figure 4.

PLP1 forward primer (SEQ ID NO: 18): 5'-GTTCCAGAGGCCAACATCAAGCTC -3'

PLP1 reverse primer (SEQ ID NO: 19): 5'-AGCCATACAACAGTCAGGGCATAG -3'

Olig2 forward primer (SEQ ID NO: 20): 5'-GGGAGGTCATGCCTTACGC -3'

Olig2 reverse primer (SEQ ID NO: 21): 5'-CTCCAGCGAGTTGGTGAGC -3'

β-actin forward primer (SEQ ID NO: 22): 5'-CACAGCTTCTTTGCAGCTCCTT -3'

β-actin reverse primer (SEQ ID NO: 23): 5'-GACGACCAGCGCAGCGATA -3'

[0054] It can be seen from Figure 4 that for the expression level of the mRNA of Olig2, an oligodendrocyte marker, there was no significant difference between the miR-neg vector and the PLP1 miRNA vector (B), whereas for the expression level of the PLP1 mRNA, injection of the PLP1 miRNA vector provided approximately 60% of expression-suppressing effect on the PLP1 gene (A).

[0055] <Example 5 Effect of Administration to PLP1 Transgenic (PLP1-Tg) Mice>

To both striata and the internal capsule of each of a litter of 10-day-old (body weight: 5 to 6 g) PLP1-Tg/B6C3 mice overexpressing mouse PLP1, the PLP1 miRNA vector (group with treatment, n = 16) or the miR-neg vector (group with mock treatment, n = 16) was injected to conduct treatment test. PLP1-Tg/B6C3 was prepared by backcrossing PLP1-Tg/BDF1, which was gifted from Dr. Tetsushi Kagawa in National Institute for Physiological Sciences, with a B6C3 strain (Kagawa T et al., Neuron. 1994 Aug; 13 (2): 427-42). For PLP1-Tg-homozygous individuals, 1 μL of a solution of each of the two scAAV-AAV1/2 vectors (titer: 1.2×10^{12} vg/mL) obtained in Example 1 was injected into both striata and the internal capsule of each 10-day-old mouse in the same manner as in Example 2. For a positive control, wild-type litter mate mice injected with the miR-neg vector (n = 16) were used. Some of those mice were removed of brain tissue on day 25 after birth (15 days after the injection) and histological analysis (immunostaining, morphometric analysis) was conducted for the brain

tissue, and the residual mice were observed for body weights and survival rates.

[0056] Histological analysis by immunostaining was carried out as follows. For the group of wild-type mice, and the group of PLP1-Tg mice with treatment and that with mock treatment, where n = 5 per group, each of the callosum, striatum, and internal capsule were immunostained in the same manner as in Example 3 on day 25 after birth (15 days after the injection). For the wild-type mice as a positive control group, PLP1 provided fibrous, strongly stained images corresponding to myelin sheaths in the callosum, striatum, and internal capsule. In analysis of the PLP1-Tg mice, almost no stained image corresponding to myelin sheaths was found for the mice with mock treatment, whereas a stained image indicating abnormal accumulation of PLP1 in the cytoplasm of oligodendrocytes were obtained. For the mice with treatment, by contrast, almost no stained image indicating the accumulation of PLP1 in cytoplasm was found, and a fibrous stained image inferred to correspond to myelin sheaths were found, which revealed that administration of the PLP1 miRNA vector provides improved stained images of PLP1 as compared with abnormal ones. Figure 5 shows micrographs of callosum.

[0057] Micromorphological analysis with an electron microscope was performed in the following manner. For the wild-type mice and the group of PLP1-Tg mice with treatment and that with mock treatment, where n = 2 per group, fixing by reflux was performed with 0.1 M phosphate buffer containing 2% glutaraldehyde and 2% paraformaldehyde on day 25 after birth (15 days after the injection), and

then the brain was removed and further fixed by soaking in the same buffer. Each of the callosum, striatum, and internal capsule was checked for AAV-infected sites under a stereoscopic fluorescence microscope, a tissue section of 600-μm square was cut out, and this was post-fixed with 0.1 M phosphate buffer containing 1% osmium. After dehydration, the tissue section was embedded in epoxy resin, a 70-nm ultrathin section was prepared, and this was observed through an electron microscope (Tecnai Spirit, Thermo Fisher Scientific, USA) at a magnification of 7300×.

[0058] Figure 6 shows electron micrographs. It was clear that for the wild-type mice almost all axons were myelinated in each of the callosum, striatum, and internal capsule. In analysis of the PLP1-Tg mice, the fraction of myelinated axons was clearly low in the mice with mock treatment, and moreover the axons were narrowed. In the mice with treatment, on the other hand, the fraction of myelinated axons was higher, and moreover diameters of axons were generally larger than those in the mice with mock treatment. From these results, it was revealed from the viewpoint of ultrafine morphology that administration of the PLP1 miRNA vector provides therapeutic effect owing to enrichment of myelinated fibers.

[0059] The group of mice with treatment and group of mice with mock treatment, where n = 9 per group, were observed even after 25 days in age until their death to evaluate body weights and survival rates for the groups. Administration to the PLP1-Tg mice was found to provide significant survival rate-improving (life-prolonging) effect (Figure 7) and body weight gain effect (Figure 8), and thus the efficacy of the

present therapy was confirmed.

[0060] The thus-described results revealed that the oligodendrocyte-specific promoter and the AAV vector including the promoter can be applicable as a fundamental technique to develop therapy for PMD, and that the gene expression AAV vector with a PLP1-specific miRNA using the promoter can be an effective approach for treatment of PMD.

[0061] <Example 6 Evaluation of Suppression Efficiency of Expression (Knockdown) in Terms of PLP1 Gene Expression Levels - Part 3>

To evaluate the knockdown efficiency of the human PLP1-miRNA designed with BLOCK-iT (TM) RNAi Designer (Thermo Fisher Scientific, USA), pcDNA (TM) 6.2-hPLP1, which is a plasmid capable of overexpressing a sequence encoding the human PLP1 gene, and pscw.CAG.Venus.PLP1-miRNA, which is a plasmid obtained by cloning a sequence into a 3' untranslated region in the downstream of a cDNA encoding Venus, a fluorescent protein that is expressed under the control of a CAG promoter, were concomitantly introduced into HeLa cells through a transfection method. For the transfection, TransIt-LT1 (Takara Bio Inc.) was used. After 24 hours cells were collected, and the total RNA was extracted by using an RNeasy Mini Kit (Qiagen). For quantitative PCR, a cDNA was synthesized by using a SuperScript (R) Reverse Transcriptase Kit (Thermo Fisher Scientific, USA). Gene expression was analyzed through quantitative PCR using LightCycler (R) 480 SYBR Green I Master and a LightCycler (R) 480 Instrument with specific primers below (F. Hoffmann-La Roche Ltd., Switzerland). The primers used in the quantitative PCR for PLP1 gene expression are

listed in the following.

Human PLP1 forward primer (SEQ ID NO: 280): 5'-GCTCCAACCTTCTGTCCATCT -3'

Human PLP1 reverse primer (SEQ ID NO: 281): 5'-ACGGCAAAGTTGTAAGTGGC -3'

Human β-actin forward primer (SEQ ID NO: 282): 5'-GACAGGATGCAGAAGGAGATTACT -3'

Human β-actin reverse primer (SEQ ID NO: 283): 5'-TGATCCACATCTGCTGGAAGGT -3'

[0062] Figure 9 shows results of the knockdown. Knockdown efficiencies were calculated assuming the PLP1 expression level of cells with miR-neg expression as 1. The results were normalized to the house keeping gene β-actin, and calculated as relative expression levels ± SD. Seven sequences that lower PLP1 gene expression levels were identified (SEQ ID NOs: 2, 3, 4, 5, 7, 24, and 26).

[0063] <Example 7 Evaluation of Suppression Efficiency of Expression (Knockdown) in Terms of PLP1 Gene Expression Levels - Part 4>

A pair of siRNAs consisting of a sense strand and the corresponding antisense strand shown in Table 3 and transfection solution (RNAi MAX, Invitrogen) were mixed together, and the solution prepared was added to U-251MG cells, and the cells were cultured for 48 hours.

[Table 3]

Table 3. siRNA Sequences designed for human PLP1 gene

No.	SEQ ID NO:	Sense/Antisense strand	siRNA Sequence
1	118	hPLP1-001	UGACCUUCCACCUGUUUAUdTdT
	119	hPLP1-001anti	AUAAACAGGUGGAAGGUCAdTdT
2	120	hPLP1-002	GUGCCUGUGUACAUUUACUdTdT
	121	hPLP1-002anti	AGUAAAUGUACACAGGCACdTdT
3	122	hPLP1-003	UUGCCCAAAUCUGCCUAUUdTdT
	123	hPLP1-003anti	AAUAGGCAGAUUUGGGCAAdTdT
4	124	hPLP1-004	AGGAAUGGAGGCUCUAAUUdTdT
	125	hPLP1-004anti	AAUUAGAGCCUCCAUUCCUdTdT
5	126	hPLP1-005	UGGCCACUGGAUUGUGUUUdTdT
	127	hPLP1-005anti	AAACACAAUCCAGUGGCCAdTdT
6	128	hPLP1-006	GCGGGUGUGUCAUUGUUUGdTdT
	129	hPLP1-006anti	CAAACAAUGACACACCCGCdTdT
7	130	hPLP1-007	GACCUUCCACCUGUUUAUUdTdT
	131	hPLP1-007anti	AAUAAACAGGUGGAAGGUCdTdT
8	132	hPLP1-008	ACUCCCUUCUCCUUGAUAAdTdT
	133	hPLP1-008anti	UUAUCAAGGAGAAGGGAGUdTdT
9	134	hPLP1-009	CUGCAAGUCACAAAGGAAUdTdT
	135	hPLP1-009anti	AUUCCUUUGUGACUUGCAGdTdT
10	136	hPLP1-010	GGUCCUCUUGCCAUCUAUAdTdT
	137	hPLP1-010anti	UAUAGAUGGCAAGAGGACCdTdT
11	138	hPLP1-011	CCUCGUUAGGGAAGAGAAAdTdT
	139	hPLP1-011anti	UUUCUCUUCCCUAACGAGGdTdT
12	140	hPLP1-012	GGAGAAGAGGACAAAGAUAdTdT
	141	hPLP1-012anti	UAUCUUUGUCCUCUUCUCCdTdT
13	142	hPLP1-013	GGCCAACAUCAAGCUCAUUdTdT
	143	hPLP1-013anti	AAUGAGCUUGAUGUUGGCCdTdT
14	144	hPLP1-014	AGGCCAACAUCAAGCUCAUdTdT
	145	hPLP1-014anti	AUGAGCUUGAUGUUGGCCUdTdT
15	146	hPLP1-015	CUCCCUUCUCCUUGAUAACdTdT
	147	hPLP1-015anti	GUUAUCAAGGAGAAGGGAGdTdT
16	148	hPLP1-016	GCCUCUUUCUUCUUCCUUUdTdT
	149	hPLP1-016anti	AAAGGAAGAAGAAAGAGGCdTdT
17	150	hPLP1-017	CUGUGCCUGUGUACAUUUAdTdT
	151	hPLP1-017anti	UAAAUGUACACAGGCACAGdTdT
18	152	hPLP1-018	AAGGAAUGGAGGCUCUAAUdTdT
	153	hPLP1-018anti	AUUAGAGCCUCCAUUCCUUdTdT
19	154	hPLP1-019	AGGGUGUUGUCAGCAUCUUdTdT
	155	hPLP1-019anti	AAGAUGCUGACAACACCCUdTdT
20	156	hPLP1-020	ACAGCUGAGUUCCAAAUGAdTdT
	157	hPLP1-020anti	UCAUUUGGAACUCAGCUGUdTdT

No.	SEQ ID NO:	Sense/Antisense strand	siRNA Sequence
21	158	hPLP1-021	GCCACUGGAUUGUGUUUCUdTdT
	159	hPLP1-021anti	AGAAACACAAUCCAGUGGCdTdT
22	160	hPLP1-022	ACAACUUUGCCGUCCUUAAdTdT
	161	hPLP1-022anti	UUAAGGACGGCAAAGUUGUdTdT
23	162	hPLP1-023	GCUGAUGCCAGAAUGUAUGdTdT
	163	hPLP1-023anti	CAUACAUUCUGGCAUCAGCdTdT
24	164	hPLP1-024	UUGCCGUCCUUAAACUCAUdTdT
	165	hPLP1-024anti	AUGAGUUUAAGGACGGCAAdTdT
25	166	hPLP1-025	CUGCCUCUUUCUUCUUCCUdTdT
	167	hPLP1-025anti	AGGAAGAAGAAAGAGGCAGdTdT
26	168	hPLP1-026	CCACUGGAUUGUGUUUCUUdTdT
	169	hPLP1-026anti	AAGAAACACAAUCCAGUGGdTdT
27	170	hPLP1-027	GGAUCAGAAAGUAAUUUCUdTdT
	171	hPLP1-027anti	AGAAAUUACUUUCUGAUCCdTdT
28	172	hPLP1-028	UAGCCAGCCGGCUACAAUUdTdT
	173	hPLP1-028anti	AAUUGUAGCCGGCUGGCUAdTdT
29	174	hPLP1-029	AGCGUAGAAUCUGUGUAGAdTdT
	175	hPLP1-029anti	UCUACACAGAUUCUACGCUdTdT
30	176	hPLP1-030	UCCCUUCUCCUUGAUAACAdTdT
	177	hPLP1-030anti	UGUUAUCAAGGAGAAGGGAdTdT
31	178	hPLP1-031	UUCCAAGGAGCUGAGAAUAdTdT
	179	hPLP1-031anti	UAUUCUCAGCUCCUUGGAAdTdT
32	180	hPLP1-032	UGCCAGAAUGUAUGGUGUUdTdT
	181	hPLP1-032anti	AACACCAUACAUUCUGGCAdTdT
33	182	hPLP1-033	AUGCCUUCCAGUAUGUCAUdTdT
	183	hPLP1-033anti	AUGACAUACUGGAAGGCAUdTdT
34	184	hPLP1-034	CAACUUUGCCGUCCUUAAAdTdT
	185	hPLP1-034anti	UUUAAGGACGGCAAAGUUGdTdT
35	186	hPLP1-035	CCAACAUCAAGCUCAUUCUdTdT
	187	hPLP1-035anti	AGAAUGAGCUUGAUGUUGGdTdT
36	188	hPLP1-036	UGGCCUUACACCUCGUUAGdTdT
	189	hPLP1-036anti	CUAACGAGGUGUAAGGCCAdTdT
37	190	hPLP1-037	AGGACAUCCCGACAAGUUUdTdT
	191	hPLP1-037anti	AAACUUGUCGGGAUGUCCUdTdT
38	192	hPLP1-038	GUCAGAGUCCCAAAGACAUdTdT
	193	hPLP1-038anti	AUGUCUUUGGGACUCUGACdTdT
39	194	hPLP1-039	UUGCAGGAGAAGAGGACAAdTdT
	195	hPLP1-039anti	UUGUCCUCUUCUCCUGCAAdTdT
40	196	hPLP1-040	GGUAGACACAGGAUAGAUAdTdT
	197	hPLP1-040anti	UAUCUAUCCUGUGUCUACCdTdT

No.	SEQ ID NO:	Sense/Antisense strand	siRNA Sequence
41	198	hPLP1-041	UCAGCCAACCUUACUUACAdTdT
	199	hPLP1-041anti	UGUAAGUAAGGUUGGCUGAdTdT
42	200	hPLP1-042	AUGGAACUGCCUCUUUCUUdTdT
	201	hPLP1-042anti	AAGAAAGAGGCAGUUCCAUdTdT
43	202	hPLP1-043	ACUCAGCCAACCUUACUUAdTdT
	203	hPLP1-043anti	UAAGUAAGGUUGGCUGAGUdTdT
44	204	hPLP1-044	CUUCCACUGAUGGAAACAAdTdT
	205	hPLP1-044anti	UUGUUUCCAUCAGUGGAAGdTdT
45	206	hPLP1-045	AUGACCUUCCACCUGUUUAdTdT
	207	hPLP1-045anti	UAAACAGGUGGAAGGUCAUdTdT
46	208	hPLP1-046	UCUCCUGAGGAUCAGAAAGdTdT
	209	hPLP1-046anti	CUUUCUGAUCCUCAGGAGAdTdT
47	210	hPLP1-047	GAGGAUCAGAAAGUAAUUUdTdT
	211	hPLP1-047anti	AAAUUACUUUCUGAUCCUCdTdT
48	212	hPLP1-048	GGAUCUUUCACCCACAGAAdTdT
	213	hPLP1-048anti	UUCUGUGGGUGAAAGAUCCdTdT
49	214	hPLP1-049	CUGAGGAUCAGAAAGUAAUdTdT
	215	hPLP1-049anti	AUUACUUUCUGAUCCUCAGdTdT
50	216	hPLP1-050	CUGGUAGACACAGGAUAGAdTdT
	217	hPLP1-050anti	UCUAUCCUGUGUCUACCAGdTdT
51	218	hPLP1-051	GCAGUUGCUGGUGGCUAAUdTdT
	219	hPLP1-051anti	AUUAGCCACCAGCAACUGCdTdT
52	220	hPLP1-052	AAGACAUGGGCUUGUUAGAdTdT
	221	hPLP1-052anti	UCUAACAAGCCCAUGUCUUdTdT
53	222	hPLP1-053	UUGCUGCCACUUACAACUUdTdT
	223	hPLP1-053anti	AAGUUGUAAGUGGCAGCAAdTdT
54	224	hPLP1-054	UCGUUAGGGAAGAGAAACAdTdT
	225	hPLP1-054anti	UGUUUCUCUUCCCUAACGAdTdT
55	226	hPLP1-055	UCCACUGAUGGAAACAAAGdTdT
	227	hPLP1-055anti	CUUUGUUUCCAUCAGUGGAdTdT
56	228	hPLP1-056	AGAGCGUUUGCCCAAAUCUdTdT
	229	hPLP1-056anti	AGAUUUGGGCAAACGCUCUdTdT
57	230	hPLP1-057	UGAUUGCUGCCACUUACAAdTdT
	231	hPLP1-057anti	UUGUAAGUGGCAGCAAUCAdTdT
58	232	hPLP1-058	GGAGCGUAGAAUCUGUGUAdTdT
	233	hPLP1-058anti	UACACAGAUUCUACGCUCCdTdT
59	234	hPLP1-059	UGAGGAUCAGAAAGUAAUUdTdT
	235	hPLP1-059anti	AAUUACUUUCUGAUCCUCAdTdT
60	236	hPLP1-060	GGUGUUGUCAGCAUCUUCUdTdT
	237	hPLP1-060anti	AGAAGAUGCUGACAACACCdTdT

No.	SEQ ID NO:	Sense/Antisense strand	siRNA Sequence
61	238	hPLP1-061	GUCAAAGCAAGGAUCUUUCdTdT
	239	hPLP1-061anti	GAAAGAUCCUUGCUUUGACdTdT
62	240	hPLP1-062	AGAUCUUUGGCGACUACAAdTdT
	241	hPLP1-062anti	UUGUAGUCGCCAAAGAUCUdTdT
63	242	hPLP1-063	UGCAGGAGAAGAGGACAAAdTdT
	243	hPLP1-063anti	UUUGUCCUCUUCUCCUGCAdTdT
64	244	hPLP1-064	UGGUGGCUAAUGGUGUAACdTdT
	245	hPLP1-064anti	GUUACACCAUUAGCCACCAdTdT
65	246	hPLP1-065	AGCUAAUUGAGACCUAUUUdTdT
	247	hPLP1-065anti	AAAUAGGUCUCAAUUAGCUdTdT
66	248	hPLP1-066	GUGCUGAUGCCAGAAUGUAdTdT
	249	hPLP1-066anti	UACAUUCUGGCAUCAGCACdTdT
67	250	hPLP1-067	UUUCCAAGGAGCUGAGAAUdTdT
	251	hPLP1-067anti	AUUCUCAGCUCCUUGGAAAdTdT
68	252	hPLP1-068	UGUGCCUGUGUACAUUUACdTdT
	253	hPLP1-068anti	GUAAAUGUACACAGGCACAdTdT
69	254	hPLP1-069	CAGGUACAGAGAAGGAAUGdTdT
	255	hPLP1-069anti	CAUUCCUUCUCUGUACCUGdTdT
70	256	hPLP1-070	GCAUAAGGGAGCGUAGAAUdTdT
	257	hPLP1-070anti	AUUCUACGCUCCCUUAUGCdTdT
71	258	hPLP1-071	UCAGGUACAGAGAAGGAAUdTdT
	259	hPLP1-071anti	AUUCCUUCUCUGUACCUGAdTdT
72	260	hPLP1-072	GCUGCAGCUACACUGGUUUdTdT
	261	hPLP1-072anti	AAACCAGUGUAGCUGCAGCdTdT
73	262	hPLP1-073	UCUCCCAUGGAAUGCUUUCdTdT
	263	hPLP1-073anti	GAAAGCAUUCCAUGGGAGAdTdT
74	264	hPLP1-074	UGCCUUCCAGUAUGUCAUCdTdT
	265	hPLP1-074anti	GAUGACAUACUGGAAGGCAdTdT
75	266	hPLP1-075	GCUGCCACUUACAACUUUGdTdT
	267	hPLP1-075anti	CAAAGUUGUAAGUGGCAGCdTdT
76	268	hPLP1-076	ACUUUGCCGUCCUUAAACUdTdT
	269	hPLP1-076anti	AGUUUAAGGACGGCAAAGUdTdT
77	270	hPLP1-077	ACCUUACUUACAGCAUAAGdTdT
	271	hPLP1-077anti	CUUAUGCUGUAAGUAAGGUdTdT
78	272	hPLP1-078	UGGACUACUGAAGCCCUAAdTdT
	273	hPLP1-078anti	UUAGGGCUUCAGUAGUCCAdTdT
79	274	hPLP1-079	CUUCCAGUAUGUCAUCUAUdTdT
	275	hPLP1-079anti	AUAGAUGACAUACUGGAAGdTdT
80	276	hPLP1-080	UCUGGUCUCUCUAUUACCAdTdT
	277	hPLP1-080anti	UGGUAAUAGAGAGACCAGAdTdT
81	278	hPLP1-081	GUCCCAAAGACAUGGGCUUdTdT
	279	hPLP1-081anti	AAGCCCAUGUCUUUGGGACdTdT

[0064] Thereafter, an RNA was extracted from the cells, and PLP1 gene expression was evaluated through a quantitative PCR method (n = 3). A SuperPrep Cell Lysis RT Kit for qPCR (TOYOBO CO., LTD.) was used for RNA preparation and cDNA synthesis. For the quantitative PCR method, a Taqman probe for the PLP1 gene (Hs00166914_m1, Applied Biosystems) and that for the S18 gene (Hs99999901_s1, Applied Biosystems) were used. Results of the gene expression were normalized to the house keeping gene S18.

[0065] Figure 10 shows the results. The vertical axis in Figure 10 represents knockdown efficiencies with reference to U-251MG cells with addition only of transfection solution. Knockdown efficiencies for the PLP1 gene were calculated as relative expression levels assuming the expression level in U-251MG cells with addition only of transfection solution as 1. Selected were 24 pairs of siRNA sequences that lower PLP1 gene expression levels to 50% or less as a mean value both in the first and second trials (Nos. 2, 4, 10, 16, 21, 22, 26, 28, 34, 38, 40, 43, 45, 47, 48, 52, 56, 59, 60, 62, 65, 70, 72, and 80).

[0066] <Example 8 Evaluation of Suppression Efficiency of Expression (Knockdown) in Terms of PLP1 Gene Expression Levels - Part 5>

On the basis of the 24 pairs of siRNA sequences obtained in Example 7, miRNA sequences were designed (Table 2). Among them, miRNA sequences targeting the sequence encoding the human PLP1 gene were selected (SEQ ID NOs: 2, 29, 30, 31, 32, 33, 35, 36, 42, and 51). Among them, SEQ ID NOs: 2, 29, 31, 32, and 33 are each a sequence directed to a sequence common to mice and primates such as common marmosets.

[0067] To evaluate the 10 miRNA sequences, each plasmid obtained by cloning any of the sequences into the downstream of a CAG promoter was introduced through a transfection method into HeLa cells overexpressing the coding sequences of the human PLP1 gene. The experimental details of primers used in quantitative PCR for PLP1 gene expression are the same as in Example 6. Figure 11 shows results of the knockdown. The vertical axis in Figure 11 represents the PLP1 expression levels ± SD relative to the expression when a vector including the miR-neg was added, which was set as 1. Figure 11 indicates that miRNAs having any of SEQ ID NOs: 2, 29, 30, 31, 32, 35, 36, 42, and 51 knocked down the human PLP1 gene.

[0068] <Example 9 Evaluation of Suppression Efficiency of Expression (Knockdown) in Terms of PLP1 Gene Expression Levels - Part 6>

Each of the vectors used in Examples 6 and 8, including an miRNA having a nucleotide sequence set forth in any of SEQ ID NOs: 2, 29, 31, 32, and 35, was transfected into U-251MG cells endogenously expressing the human PLP1 gene to evaluate knockdown efficiencies for PLP1 gene expression. A plasmid obtained by cloning into the downstream of a CAG promoter was introduced into cells through a transfection method, and GFP-expressing cells were then exclusively selected by a FACS method. Thereafter, PLP1 gene expression was analyzed though a quantitative PCR method. For the primers, primers having nucleotide sequences set forth in SEQ ID NOs: 280 to 283 were used.

[0069] Figure 12 shows the results. The vertical axis in Figure 12 represents relative expression levels assuming the PLP1 expression

level when a vector including the miR-neg was added as 1. From Figure 12, vectors including an miRNA having any of SEQ ID NOs: 2, 29, 31, 32, and 35 were found to also have suppressing effect on endogenous PLP1 gene expression.

[0070] <Example 10 Evaluation of Suppression Efficiency of Expression (Knockdown) in Terms of PLP1 Gene Expression Levels - Part 7>

One microliter of a solution of an scAAV-AAV1/2 vector including the miRNA having SEQ ID NO: 2 from Example 9 (titer: 1.2×10^{12} vg/mL) was injected into one striatum, the internal capsule, and the cerebellum of each 10-day-old, wild-type mouse Jcl:B6C3F1 (n = 3 per group). The injection method was the same as in Example 2. Thereafter, expression of PLP1 protein in Venus-positive cells was examined through immunostaining to evaluate PLP1 miRNA knockdown efficiencies. Immunostaining was performed with the same method as in Example 2 except that an anti-PLP1 rabbit polyclonal antibody (Numata Y et al., J Biol Chem. 2013 Mar 15; 288 (11): 7451-66) was used as a primary antibody, and an anti-rabbit fluorescent antibody (Thermo Fisher Scientific, USA) was used as a secondary antibody. Each site of the callosum, striatum, and internal capsule were photographed with a KEYENCE fluorescence microscope (KEYENCE CORPORATION, Japan).

[0071] Mean fluorescence intensities of immunostained PLP1 were quantified by using Image J 1.45s (Wayne Rasband, National Institutes of Health, USA), and Figure 13 shows quantitative analysis of fluorescence intensity through PLP1 immunofluorescence staining.

The vertical axis in Figure 13 represents relative PLP1 expression levels in the infected side with reference to the non-infected side. It can be seen from Figure 13 that there was almost no difference in expression of PLP1 protein between the side without injection of the miR-neg vector (non-infected side) and the side with vector injection (infected side), whereas for the PLP1 miRNA vector there was 40% of expression-suppressing effect on PLP1 protein in the infected side as compared with the non-infected side.

Industrial Applicability

[0072] The promoter of the present invention and the AAV vector of the present invention can serve as a therapeutic drug for PMD caused by PLP1 duplication. In addition, they can serve as a therapeutic drug for PMD caused by PLP1 point mutation. Furthermore, since PLP1 is one of target antigens for multiple sclerosis, it is expected that they can serve as a therapeutic drug for multiple sclerosis.

CLAIMS

[Claim 1]

An oligodendrocyte-specific promoter comprising a nucleic acid having a sequence identity of at least 90% to a nucleotide sequence set forth in SEQ ID NO: 1.

[Claim 2]

The promoter according to claim 1, comprising a nucleic acid having the nucleotide sequence set forth in SEQ ID NO: 1.

[Claim 3]

An oligodendrocyte-specific vector comprising the promoter according to claim 1 or 2.

[Claim 4]

The vector according to claim 3, further comprising an miRNA sequence specific to a human PLP1 gene operably linked to the promoter.

[Claim 5]

An miRNA specific to a PLP1 gene, comprising a pair of nucleotide sequences consisting of an antisense sequence and a sense sequence, wherein the pair of nucleotide sequences is selected from the group consisting of pairs of an antisense sequence set forth in a left column of a table below and a sense sequence set forth in the just right of the antisense sequence in a right column of the table.

[Table 1]

Table 1. Antisense and sense sequences for miRNA designed for human PLP1 gene

Antisense sequence	Sense sequence
AAAGGAAGAAGAAAGAGGCAG (SEQ ID NO: 52)	CTGCCTCTCTTCTTCCTTT (SEQ ID NO: 53)
AACACCAGGAGCCACACAACG (SEQ ID NO: 54)	CGTTGTGTCTCCTGGTGTT (SEQ ID NO: 55)
TTCCATGGGAGAACACCATAC (SEQ ID NO: 56)	GTATGGTGCTCCCATGGAA (SEQ ID NO: 57)
TGAGCAGGGAAACCAGTGTAG (SEQ ID NO: 58)	CTACACTGTTCCCTGCTCA (SEQ ID NO: 59)
AGGGCTTTCTGATTGACAGCC (SEQ ID NO: 60)	GGCTGTCACAGAAAGCCCT (SEQ ID NO: 61)
ACCCCAAAGAAACACAATCCA (SEQ ID NO: 62)	TGGATTGTTTCTTTGGGGT (SEQ ID NO: 63)
ACAAATGCAGCAATAAACAGG (SEQ ID NO: 64)	CCTGTTTAGCTGCATTTGT (SEQ ID NO: 65)
AATAGACTGGCAGGTGGTCCA (SEQ ID NO: 66)	TGGACCACGCCAGTCTATT (SEQ ID NO: 67)
AAAGAATGAGCTTGATGTTGG (SEQ ID NO: 68)	CCAACATCGCTCATTCTTT (SEQ ID NO: 69)
AGATACTCATAGTCTTGGTAG (SEQ ID NO: 70)	CTACCAAGTATGAGTATCT (SEQ ID NO: 71)
AGAAACACAATCCAGTGGCCA (SEQ ID NO: 72)	TGGCCACTATTGTGTTTCT (SEQ ID NO: 73)
AAATAGGTCTCAATTAGCTTT (SEQ ID NO: 74)	AAAGCTAAGAGACCTATTT (SEQ ID NO: 75)
AAGAAACACAATCCAGTGGCC (SEQ ID NO: 76)	GGCCACTGTTGTGTTTCTT (SEQ ID NO: 77)
TAAACAGGTGGAAGGTCATTT (SEQ ID NO: 78)	AAATGACCCCACCTGTTTA (SEQ ID NO: 79)
TTGTAGTCGCCAAAGATCTGC (SEQ ID NO: 80)	GCAGATCTGGCGACTACAA (SEQ ID NO: 81)
AATTAGAGCCTCCATTCCTTT (SEQ ID NO: 82)	AAAGGAATAGGCTCTAATT (SEQ ID NO: 83)
TTAAGGACGGCAAAGTTGTAA (SEQ ID NO: 84)	TTACAACTGCCGTCCTTAA (SEQ ID NO: 85)
TTTAAGGACGGCAAAGTTGTA (SEQ ID NO: 86)	TACAACTTCCGTCCTTAAA (SEQ ID NO: 87)
ATGTCTTTGGGACTCTGACTC (SEQ ID NO: 88)	GAGTCAGACCCAAAGACAT (SEQ ID NO: 89)
TATCTATCCTGTGTCTACCAG (SEQ ID NO: 90)	CTGGTAGACAGGATAGATA (SEQ ID NO: 91)
AAATTACTTTCTGATCCTCAG (SEQ ID NO: 92)	CTGAGGATGAAAGTAATTT (SEQ ID NO: 93)
TCTAACAAGCCCATGTCTTTG (SEQ ID NO: 94)	CAAAGACAGGCTTGTTAGA (SEQ ID NO: 95)
AATTACTTTCTGATCCTCAGG (SEQ ID NO: 96)	CCTGAGGAAGAAAGTAATT (SEQ ID NO: 97)
AGTAAATGTACACAGGCACAG (SEQ ID NO: 98)	CTGTGCCTGTACATTTACT (SEQ ID NO: 99)
TAAGTAAGGTTGGCTGAGTTA (SEQ ID NO: 100)	TAACTCAGAACCTTACTTA (SEQ ID NO: 101)
TTCTGTGGGTGAAAGATCCTT (SEQ ID NO: 102)	AAGGATCTCACCCACAGAA (SEQ ID NO: 103)
AGAAGATGCTGACAACACCCT (SEQ ID NO: 104)	AGGGTGTTCAGCATCTTCT (SEQ ID NO: 105)
AATTGTAGCCGGCTGGCTAGT (SEQ ID NO: 106)	ACTAGCCACGGCTACAATT (SEQ ID NO: 107)
AGATTTGGGCAAACGCTCTTA (SEQ ID NO: 108)	TAAGAGCGTGCCCAAATCT (SEQ ID NO: 109)
ATTCTACGCTCCCTTATGCTG (SEQ ID NO: 110)	CAGCATAAGAGCGTAGAAT (SEQ ID NO: 111)
TGGTAATAGAGAGACCAGAAT (SEQ ID NO: 112)	ATTCTGGTCTCTATTACCA (SEQ ID NO: 113)
TATAGATGGCAAGAGGACCAA (SEQ ID NO: 114)	TTGGTCCTTGCCATCTATA (SEQ ID NO: 115)
AAACCAGTGTAGCTGCAGCCC (SEQ ID NO: 116)	GGGCTGCATACACTGGTTT (SEQ ID NO: 117)

[Claim 6]

The miRNA according to claim 5, comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 2 to 7 and 24 to 51.

[Claim 7]

The miRNA according to claim 5, comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 2 to 5, 7, 24, 26, 29 to 32, 35, 36, 42, and 51.

[Claim 8]

The miRNA according to claim 5, comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 2, 29, 31, and 32.

[Claim 9]

A vector comprising a sequence of the miRNA according to any one of claims 5 to 8.

[Claim 10]

The vector according to claim 9, wherein the sequence of the miRNA is operably linked to an oligodendrocyte-specific promoter.

[Claim 11]

The vector according to claim 10, wherein the oligodendrocyte-specific promoter is the promoter according to claim 1 or 2.

[Claim 12]

The vector according to any one of claims 3, 4, and 9 to 11, wherein the vector is an adeno-associated virus (AAV) vector.

[Claim 13]

A pharmaceutical composition comprising the vector according to any one of claims 3, 4, and 9 to 12.

[Claim 14]

The pharmaceutical composition according to claim 13, wherein the pharmaceutical composition is a pharmaceutical composition for

treating a disease associated with abnormality in a PLP1 gene.

[Claim 15]

The pharmaceutical composition according to claim 14, wherein the disease is Pelizaeus-Merzbacher disease, spastic paraplegia type 2, or multiple sclerosis.

[Claim 16]

The pharmaceutical composition according to claim 14, wherein the disease is Pelizaeus-Merzbacher disease.

[Claim 17]

The pharmaceutical composition according to claim 13, wherein the pharmaceutical composition is a PLP1 gene expression suppressor which suppresses expression of the PLP1 gene.

[Claim 18]

A method for treating a disease associated with abnormality of a PLP1 gene, the method comprising administering an effective amount of the vector according to any one of claims 3, 4, and 9 to 12 to a patient in need of treatment.

[Claim 19]

The method according to claim 18, wherein the disease is Pelizaeus-Merzbacher disease, spastic paraplegia type 2, or multiple sclerosis.

[Claim 20]

The method according to claim 18, wherein the disease is Pelizaeus-Merzbacher disease.

[Claim 21]

A method for suppressing expression of a PLP1 gene, the

method comprising administering an effective amount of the vector according to any one of claims 3, 4, and 9 to 12 to a patient in need of treatment.

[Claim 22]

The vector according to any one of claims 3, 4, and 9 to 12 for use in treatment of a disease associated with abnormality of a PLP1 gene.

[Claim 23]

The vector according to claim 22, wherein the disease is Pelizaeus-Merzbacher disease, spastic paraplegia type 2, or multiple sclerosis.

[Claim 24]

The vector according to claim 22, wherein the disease is Pelizaeus-Merzbacher disease.