【CLAIMS】

【Claim 1】

Use of a composition capable of editing a PMP22 nucleic acid sequence, ~~,~~ comprising:

a *Streptococcus pyogenes*-derived Cas9 protein or a ~~Cas9~~ DNA encoding the ~~sequence capable of expressing a~~ Cas9 protein; and

a guide ~~nucleic acid~~RNA, comprising a~~n~~ crRNA and a tracrRNA~~sequence of guide domain and an sgRNA backbone sequence~~, or a DNA encoding the guide RNA in the manufacture of a medicament for treatment of Charcot-Marie-Tooth disease type 1A (CMT1A);

wherein the ~~sequence of the guide domain and the sg~~crRNA ~~backbone sequence are linked sequentially from~~consists of 5′ ~~–~~[a guide domain]-[a first complementary domain]~~ to -~~ 3′ end;

wherein the ~~sg~~tracrRNA ~~backbone sequence is at least 80% or more homologous to a sequence selected from a group~~ consist~~sing~~ of

~~a).~~ 5'-[second complementary donain]-[proximal domain]-[tail domain] ~~GUUUUAGUCCCUGAAAAGGGACUAAAAUAAAGAGUUUGCGGGA-CUCUGCGGGGUUACAAUCCCCUAAAACCGCUUUU~~-3' and

wherein the first complementary domain of the crRNA is bound with the second complementary domain of the tracrRNA to form a double strand,

wherein the first complementary domain and the tracrRNA are interact with the Cas9 protein to form a gRNA-Cas complex,~~b). 5'-GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGU-UAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC-3'~~

wherein the sequence of the guide domain comprises a corresponding RNA

sequence of a target sequence of TATA-Box in the nucleic acid sequence of a PMP22 gene, ~~wherein the target sequence is an 18 to 25 nucleotide sequence of a PMP22 gene~~

wherein the target sequence is selected from a group consisting of: SEQ ID NOs: 14 to 17.

【Claim 2】

The use of the composition according to claim 1,

wherein the guide RNA is a chimeric single stranded guide RNA,

wherein the crRNA of the guide RNA and the tracrRNA of the guide RNA are linked via a linker, and the linker is covalently linked between the crRNA and the tracrRNA~~Cas9 protein is originated from *Streptococcus pyogenes*, wherein the sgRNA backbone sequence is at least 80% or more homologous to~~

~~5' GUUUUAGUCCCUGAAAAGGGACUAAAAUAAAGAGUUUGCGGGA CUCUGCGGGGUUACAAUCCCCUAAAACCGCUUUU 3'~~.

【Claim 3】

The use of the composition according to claim 2, wherein a RNA sequence of the linker is 5′-GAAA-3′~~the target sequence is selected from any one of SEQ ID NOs: 14 to 29 in the nucleic acid sequences of the PMP22 gene~~.

【Claim 4】

The use of the composition according to any one of claims 1 to 3, wherein

a sequence of the first complementary domain of the crRNA of the guide RNA consists of 5′-GUUUUAGAGCUA-3′,

wherein a sequence of the tracrRNA of the guide RNA consists of a sequence of which 5′-UAGCAAGUUAAAAU-3′, 5′-AAGGCUAGUCCG-3′, and 5′-UUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC-3′ are linked sequentially from 5′ to 3′ end~~the Cas9 protein is originated from *Campylobacter jejuni*, wherein the sgRNA backbone sequence is at least 80% or more homologous to 5'-GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC-3'~~.

【Claim 5】

The use of the composition according to any one of claims 1 to 4, wherein the ~~target sequence is selected from any one of SEQ ID NOs: 30 to 40 in the nucleic acid sequences of the PMP22.~~composition

~~【Claim 6】~~

~~The use of the composition according to any one of claims 1 to 5, wherein the~~ composition comprises the Cas9 protein and the guide RNA,

Wherein the Cas9 protein is complexed with the guide RNA as a~~for gene manipulation is form of~~ ribonucleoprotein(RNP).

~~【Claim 7】~~【Claim 6】

The use of the composition according to any one of claims 1 to ~~5~~4, wherein the composition comprises the DNA encoding the Cas9 protein and the DNA encoding the guide RNA~~for gene manipulation is form of plasmid vector~~.

~~【Claim 8】~~【Claim 7】

The use of the composition according to ~~any one of~~ claim~~s 1 to 5~~6, wherein the composition is ~~formed in~~ a viral vector comprising the DNA encoding the Cas9 protein and the DNA encoding the guide RNA~~system~~.

~~【Claim 9】~~【Claim 8】

The use of the composition of claim ~~8~~7, wherein the viral vector ~~includes~~ comprises one or more selected from a retrovirus, a lentivirus, an adenovirus, adeno-associated virus (AAV), vaccinia virus, a poxvirus and a herpes simplex virus.

~~【Claim 10】~~【Claim 9】

~~Use of a~~A composition capable of editing a PMP22 nucleic acid sequence, comprising:

a *Streptococcus pyogenes*-derived Cas9 protein or a ~~Cas9~~ DNA ~~sequence capable of expressing~~encoding the Cas9 protein; and

a guide ~~nucleic acid~~RNA, comprising a tracrRNA, and a crRNA, or a DNA encoding the guide RNA, ~~in the manufacture of a medicament for treatment of Charcot-Marie-Tooth disease type 1A (CMT1A),~~

wherein the ~~sequence of the~~ crRNA consists of: 5′-[~~includes an RNA sequence of~~a guide domain]-[~~and~~ a first complementary domain]-3′,

~~wherein the sequence of the guide domain and the first complementary domain are linked sequenctially from 5′ to 3′ end,~~

~~wherein the first complementary domain is at least 80% or more homologous to a sequence selected from a group consisting of 5′ GUUUUAGAGCUA 3′, and 5′ GUUUUAGUCCCUUUUUAAAUUUCUU 3′.,~~

wherein the tracrRNA ~~has a sequence at least 80% or more homologous to a sequence selected from a group~~ consist~~sing~~ of:~~,~~

~~a sequence~~ 5′- second complementary domain]-[proximal domain]-[tail domain]-~~UAGCAAGUUAAAAU 3′., and 5′ AAGGCUAGUCCG 3′ that are linked sequentially from 5' end to~~ 3'-~~end~~,

~~a sequence 5′ UAGCAAGUUAAAAU 3′, 5′ AAGGCUAGUCCG 3′, and 5′ AAGGCUUAGUCCG 3′ that are linked sequentially from 5'-end to 3'-end,~~

~~a sequence 5′-AAGAAAUUUAAAAAGGGACUAAAAU 3′., and 5′ AAAGAGUUUGC 3′ that are linked sequentially from 5' end to 3' end, and~~

~~a sequence 5′ AAGAAAUUUAAAAAGGGACUAAAAU 3′., 5′ AAAGAGUUUGC 3′, and 5′~~

~~UUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC-3′ that are linked sequentially from 5'-end to 3'-end,~~

wherein the first complementary domain of the crRNA is bound with the second complementary domain of the tracrRNA to form a double strand,

wherein the first complementary domain and the tracrRNA can interact with the Cas9 protein to form a gRNA-Cas complex,

wherein the sequence of the guide domain comprises a corresponding RNA ~~sequence of the guide domain comprises a corresponding RNA~~ sequence of a target sequence of TATA Box in the nucleic acid sequence of a PMP22 gene, wherein the target sequence is selected from a group consisting of: SEQ ID NOs: 14 to 17~~an 18 to 25 nucleotide sequence of a PMP22 gene~~.

~~【Claim 11】~~

~~A kit for gene manipulation, comprising:~~

~~(a) a guide nucleic acid capable of forming complementary bonds with respect to the target sequences of SEQ ID NOs: 14 to 40 in the nucleic acid sequences of a PMP22 gene, respectively the guide nucleic acid, comprising an RNA sequence of guide domain and an sgRNA backbone sequence, or a DNA encoding the guide RNA wherein the sequence of the guide domain and the sgRNA backbone sequence are linked sequentially from 5′ to 3′ end;~~

~~wherein the sgRNA backbone sequence is at least 80% or more homologous to a sequence selected from a group consisting of~~

~~i). 5'-GUUUUAGUCCCUGAAAAGGGACUAAAAUAAAGAGUUUGCGGGA CUCUGCGGGGUUACAAUCCCCUAAAACCGCUUUU-3' and~~

~~ii). 5'-GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGU UAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC-3'; and~~

~~(b) an editor protein including one or more proteins selected from the group~~

~~consisting of a Streptococcus pyogenes derived Cas9 protein, a Campylobacter jejuni derived Cas9 protein, a Streptococcus thermophilus derived Cas9 protein, a Streptococcus aureus derived Cas9 protein, a Neisseria meningitidis derived Cas9 protein, and a Cpf1 protein, respectively, or a nucleic acid sequence encoding the same.~~

【Claim 12】

A composition comprising:

a Cas9 protein or a Cas9 DNA sequence capable of expressing a Cas9 protein; and

a guide nucleic acid, comprising an RNA sequence of guide domain and an sgRNA backbone sequence, or a DNA encoding the guide RNA;

wherein the sequence of the guide domain and the sgRNA backbone sequence are linked sequentially from 5′ to 3′ end;

wherein the sgRNA backbone sequence is at least 80% or more homologous to a sequence selected from a group consisting of

a). 5' GUUUUAGUCCCUGAAAAGGGACUAAAAUAAAGAGUUUGCGGGA CUCUGCGGGGUUACAAUCCCCUAAAACCGCUUUU 3' and

b). 5' GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGU UAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC 3'

wherein the sequence of the guide domain comprises a corresponding RNA sequence of a target sequence of TATA Box in the nucleic acid sequence of a PMP22 gene, wherein the target sequence is an 18 to 25 nucleotide sequence of a PMP22 gene.

【Claim 13】【Claim 10】

The composition according to claim 129, wherein the Cas9 protein is originated from *Streptococcus pyogenes*, wherein the sg guide RNA is a chimeric single stranded guide RNA, of which the crRNA of the guide RNA and the tracrRNA of the guide RNA are covalently linked via a linkerbackbone sequence is at least 80% or more homologous to

5' GUUUUAGUCCCUGAAAAGGGACUAAAAUAAAGAGUUUGCGGGA CUCUGCGGGGUUACAAUCCCCUAAAACCGCUUUU 3',and

Wherein the target sequence is selected from any one of SEQ ID NOs: 14 to 29 in the nucleic acid sequences of the PMP22 gene.

~~【Claim 14】~~【Claim 11】 –

The composition according to claim ~~12~~10, wherein the ~~Cas9 protein is originated from Campylobacter jejuni, wherein the sg~~RNA ~~backbone~~ sequence of the linker is ~~at least 80% or more homologous to~~

5'~~-GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGU-UAUCAACUU~~GAAA~~AAGUGGCACCGAGUCGGUGC~~-3'~~,~~

~~wherein the the target sequence is selected from any one of SEQ ID NOs: 30 to 40 in the nucleic acid sequences of the PMP22 gene~~.

~~【Claim 15】~~【Claim 12】

The composition according to any one of claims ~~12–~~9 to ~~14~~11, wherein the sequence of the first complementary domain of the crRNA of the guide RNA consists of 5′-GUUUUAGAGCUA-3′,

wherein a sequence of the tracrRNA of the guide RNA consists of a sequence of which 5′-UAGCAAGUUAAAAU-3′, 5′-AAGGCUAGUCCG-3′, and 5′-UUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC-3′ are linked sequentially from 5′ to 3′ end.

【Claim 13】

The composition according to any one of claims 9 to 12, wherein the composition is a ~~for gene manipulation is form of~~ ribonucleoprotein(RNP) comprising the Cas9 protein and the guide RNA.

~~【Claim 16】~~【Claim 14】

The composition according to any one of claims ~~12–~~9 to ~~14~~12, wherein the composition comprises the DNA encoding the Cas9 protein and the DNA encoding the guide RNA~~for gene manipulation is form of plasmid vector~~.

~~【Claim 17】~~【Claim 15】

The composition according to ~~any one of~~ claim~~s 12 to~~ 14, wherein the composition is ~~formed in~~ a viral vector comprising the DNA encoding the Cas9 protein and the

DNA encoding the guide RNA~~system~~.

~~【Claim 18】~~【Claim 16】

The composition according to claim ~~17~~15, wherein the viral vector ~~includes~~ comprises one or more selected from a retrovirus, a lentivirus, an adenovirus, adeno-associated virus (AAV), vaccinia virus, a poxvirus and a herpes simplex virus.

~~【Claim 19】~~

~~A composition comprising:~~

~~a Cas9 protein or a Cas9 DNA sequence capable of expressing the Cas9 protein; and~~

~~a guide nucleic acid, comprising a tracrRNA, and a crRNA, or a DNA encoding~~

~~the guide RNA,~~

~~wherein the sequence of the crRNA includes an RNA sequence of guide domain and a first complementary domain,~~

~~wherein the sequence of the guide domain and the first complementary domain are linked sequenctially from 5′ to 3′ end,~~

~~wherein the first complementary domain is at least 80% or more homologous to a sequence selected from a group consisting of 5′-GUUUUAGAGCUA-3′, and 5′-GUUUUAGUCCCUUUUUAAAUUUCUU-3′.,~~

~~wherein the tracrRNA has a sequence at least 80% or more homologous to a sequence selected from a group consisting of,~~

~~a sequence 5′-UAGCAAGUUAAAAU-3′., and 5′-AAGGCUAGUCCG-3′ that are linked sequentially from 5'-end to 3'-end,~~

~~a sequence 5′-UAGCAAGUUAAAAU-3′, 5′-AAGGCUAGUCCG-3′, and 5′-AAGGCUUAGUCCG-3′ that are linked sequentially from 5'-end to 3'-end,~~

~~a sequence 5′-AAGAAAUUUAAAAAGGGACUAAAAU-3′., and 5′-AAAGAGUUUGC-3′ that are linked sequentially from 5'-end to 3'-end, and~~

~~a sequence 5′-AAGAAAUUUAAAAAGGGACUAAAAU-3′., 5′-AAAGAGUUUGC-3′, and 5′-UUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC-3′ that are linked sequentially from 5'-end to 3'-end,~~

~~wherein the sequence of the guide domain comprises a corresponding RNA sequence of a target sequence of TATA Box in the nucleic acid sequence of a PMP22 gene, wherein the target sequence is selected from any one of SEQ ID NOs: 14 to 40 in the nucleic acid sequences of the PMP22 gene.~~

~~【Claim 20】~~【Claim 17】

The composition according to any one of claims ~~12~~ 9 to ~~19~~16, for use in treating Charcot-Marie-Tooth disease type 1A (CMT1A).