COMPOSITIONS AND METHODS FOR SELECTIVE GENE REGULATION

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 62/854,238, filed May 29, 2019; U.S. Provisional Patent Application No. 62/857,727, filed June 5, 2019; and U.S. Provisional Patent Application No. 63/008,569, filed April 10, 2020, each of which is incorporated herein by reference in its entirety.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on May 28, 2020, is named 46482-724_601_SL.txt and is 418,483 bytes in size.

BACKGROUND

[0003] A broad range of human diseases are associated with abnormal expression of genes. In some cases, a genetic mutation in a gene causes it to be dysregulated, downregulated, or not expressed at all, resulting in haploinsufficiency. In some cases, a genetic mutation in a gene causes it to be upregulated, resulting in overexpression of the gene. Many challenges exist in treating genetic disorders or diseases. One approach is gene therapy, which involves therapeutic delivery of a nucleic acid into a patient's cells. However, various challenges associated with gene therapy remain unsolved, such as unwanted immune response elicited by gene therapy, off-target effects, limitations on cloning capacity of gene therapy vehicles (e.g., viruses), sustaining the therapeutic effect over a longer period of time, etc. The central nervous system (CNS) poses many unique challenges for the development of a therapy that addresses the underlying impairment in a gene and/or protein expression. While there are drugs that help to manage symptoms of CNS diseases/disorders, many CNS diseases/disorders, e.g, Dravet syndrome, lack specific treatments or a cure. Thus, there is a need for novel compositions and methods capable of modulating the expression of any endogenous gene to help reverse the effects of a disease or disorder, in particular, a therapy with reduced immunogenicity, reduced off-target effects, increased specificity for a target gene, and/or increased therapeutic efficacy.

SUMMARY

[0003a] In one aspect, provided herein is a polynucleotide comprising a nucleic acid sequence encoding an engineered transcription factor, wherein the engineered transcription factor

comprises an amino acid sequence of SEQ ID NO: 127, wherein the engineered transcription factor increases expression of a SCN1A gene in a cell.

[0003b] In one aspect, provided herein is a polynucleotide comprising a nucleic acid sequence encoding an engineered transcription factor comprising a zinc finger DNA binding domain (DBD) and a transcription activation domain, wherein the zinc finger DBD comprises SEQ ID NOS: 152-157, and wherein the engineered transcription factor does not comprise an HA tag (SEQ ID NO: 171).

[0003c] In one aspect, provided herein is an expression vector comprising the polynucleotide as described herein.

[0003d] In one aspect, provided herein is a composition comprising (i) the polynucleotide as described herein and (ii) one or more pharmaceutically acceptable carrier(s).

[0003e] In one aspect, provided herein is a polynucleotide comprising a nucleic acid sequence encoding an engineered transcription factor comprises a sequence having at least 90% identity to SEQ ID NO: 127, wherein the polynucleotide is operably linked to a nucleic acid sequence comprising a microRNA binding site.

[0003f] In one aspect, provided herein is a cell comprising the polynucleotide as described herein.

[0003g] In one aspect, provided herein is a method of manufacturing an adeno-associated virus (AAV) vector comprising: (a) culturing a cell as described herein under conditions for producing recombinant AAV virions; (b) harvesting the host cell culture; and (c) purifying AAV virions produced by the host cell.

[0003h] In one aspect, provided herein is a polynucleotide comprising a parvalbumin (PV) neuron selective microRNA binding site, wherein the microRNA binding site comprises a sequence having at least 90% sequence identity to SEQ ID NO: 14 or 15, and wherein the microRNA binding site reduces expression of a transgene in excitatory neurons.

[0003i] In one aspect, provided herein is an expression cassette comprising (i) the PV neuron selective microRNA binding site as described herein and (ii) a promoter, an enhancer, or a combination of a promoter and an enhancer.

[0003j] In one aspect, provided herein is an expression cassette comprising a regulatory element operably linked to a transgene and at least one microRNA binding site, wherein the regulatory element drives expression of the transgene at a higher level in parvalbumin (PV) neurons than in other cell types, wherein the microRNA binding site reduces expression of the transgene in excitatory neurons, and wherein the expression cassette does not comprise SEQ ID NO: 67.

[0003k] In one aspect, provided herein is a host cell comprising the viral vector as described

[0004] herein.In one example, the application provides an expression cassette comprising a sequence encoding a non-naturally occurring transcription factor which increases expression of the SCN1A gene in a cell, wherein the non-naturally occurring transcription factor comprises a DNA

binding domain (DBD) operably linked to at least two transcription activating domains (TAD) in the following manner: TAD1-TAD2-DBD, DBD-TAD3-TAD4, or TAD1-TAD2-DBD-TAD3-TAD4. In certain examples, TAD1, TAD2, TAD3 and TAD4 are independently selected from the following: VP16, VP64, Viper, CITED2, CITED4, CREB3 or functional fragments thereof. In certain examples, TAD1 and TAD2 are the same TAD. In certain examples, TAD1 and TAD2 are CITED2, or a functional fragment thereof. In certain examples, TAD1 and TAD2 are CITED4, or a functional fragment thereof. In certain examples, TAD3 and TAD4 are the same TAD. In certain examples, TAD3 and TAD4 are CITED2, or a functional fragment thereof. In certain examples, TAD3 and TAD4 are CITED4, or a functional fragment thereof. In certain examples, TAD1, TAD2, TAD3 and TAD4 are the same TAD. In certain examples, TAD1, TAD2, TAD3 and TAD4 are CITED2, or a functional fragment thereof. In certain examples, TAD1, TAD2, TAD3 and TAD4 are CITED4, or a functional fragment thereof.

[0005] In certain examples, there is no linker between the at least two TAD domains.

[0006] In certain examples, there is a linker between the at least two TAD domains. In certain examples, the linker comprises or consists of GGSGGGSG (SEQ ID NO: 177) or GGSGGGSGGGSGGGSG (SEQ ID NO: 178).

[0007] In certain examples, the DBD binds to a genomic region having 18-27 nucleotides.

[0008] In certain examples, the DBD comprises at least 80% sequence identity to its closest human counterpart. In certain examples, the DBD comprises at least 90% sequence identity to its closest human counterpart. In certain examples, the DBD and the at least two TAD each comprise at least 80% sequence identity to their closest human counterparts. In certain examples, the DBD and the at least two TAD each comprise at least 90% sequence identity to their closest human counterparts.

[0009] In certain examples, the DBD comprises a guide RNA and a nuclease inactivated Cas protein. In certain examples, the nuclease inactivated Cas protein is a nuclease inactivated Cas9.

[0010] In certain examples, the DBD comprises a zinc finger domain. In certain examples, the DBD comprises six to nine zinc finger domains. In certain examples, the DBD comprises six zinc fingers. In certain examples, the DBD binds to a genomic region having 18 nucleotides. In certain examples, the DBD comprises nine zinc fingers. In certain examples, the DBD binds to a genomic region having 27 nucleotides.

[0011] In certain examples, the DBD comprises a sequence having at least 95% sequence identity to any of SEQ ID NOs: 148-151. In certain examples, the DBD comprises a sequence having any one of SEQ ID NOs: 148-151.

[0012] In certain examples, the DBD is derived from human EGR1 or human EGR3.

[0013] In certain examples, the DBD comprises a sequence having at least 90% identity to any one of SEQ ID NOs: 77-98. In certain examples, the DBD comprises SEQ ID NOs: 77-98.

[0014] In certain examples, the DBD comprises a sequence having at least 90% identity to SEQ ID NO: 92. In certain examples, the DBD comprises SEQ ID NO: 92.

[0015] In certain examples, the non-naturally occurring transcription factor comprises a sequence having at least 90% identity to SEQ ID NO: 130 or 131. In certain examples, the non-naturally occurring transcription factor comprises SEQ ID NO: 130 or 131.

[0016] In certain examples, the expression cassette comprises a nucleotide sequence having at least 90% identity to any one of SEQ ID NOs: 72 or 73. In certain examples, the expression cassette comprises a nucleotide sequence of any one of SEQ ID NOs: 72 or 73.

[0017] In certain examples, the expression cassette further comprises a regulatory element that drives expression of the transcription factor at a higher level in PV neurons than in other cell types. In certain examples, the regulatory element comprises any one of SEQ ID NOs: 1-4. In certain examples, the regulatory element comprises SEQ ID NO: 2 or 3.

[0018] In certain examples, the expression cassette further comprises a PV selective microRNA binding site. In certain examples, the PV selective microRNA binding site comprises at least 90% identity to any one of SEQ ID NOs: 7, 14 or 15. In certain examples, the PV selective microRNA binding site comprises any one of SEQ ID NOs: 7, 14, or 15.

[0019] In certain examples, the expression cassette is a part of a viral vector. In certain examples, the viral vector is an AAV virus. In certain examples, the AAV virus is an AAV9 virus or a scAAV9 virus. In certain examples, the viral vector is a Lentivirus.

[0020] In another example, the application provides an expression cassette comprising a sequence encoding a non-naturally occurring transcription factor which increases expression of the SCN1A gene in a cell, wherein the non-naturally occurring transcription factor comprises a DNA binding domain operably linked to a transcription activating domain, wherein the DNA binding domain is a zinc finger protein comprising the sequence LEPGEKP – [YKCPECGKSFS X HQRTH TGEKP]n - YKCPECGKSFS X HQRTH – TGKKTS (SEQ ID NO: 147), and wherein there is no HA tag (SEQ ID NO: 303) between the DNA binding domain and the transcription activating domain. In certain examples, the transcription activating domain comprises a VP16, VPR or VP64 sequence, or a functional fragment thereof. In certain examples, the transcription activating domain comprises VP64.

[0021] In certain examples, the DNA binding domain binds to a genomic region having 18-27 nucleotides. In certain examples, the DNA binding domain is a zinc finger domain comprising SEQ ID NO: 147 wherein n = 6 to 9. In certain examples, the DNA binding domain is a zinc finger domain comprising SEQ ID NO: 147 wherein n = 6. In certain examples, the DNA binding domain binds to a genomic region having 18 nucleotides. In certain examples, the DNA binding domain is a zinc finger domain comprising SEQ ID NO: 147 wherein n = 9. In certain examples, the DNA binding domain binds to a genomic region having 27 nucleotides.

[0022] In certain examples, the DNA binding domain comprises a sequence having at least 95% sequence identity to any of SEQ ID NOs: 148-151. In certain examples, the DNA binding domain comprises a sequence having any one of SEQ ID NOs: 148-151.

[0023] In certain examples, the DNA binding domain comprises a sequence having at least 90% identity to any one of SEQ ID NOs: 77-91. In certain examples, the DNA binding domain comprises any one of SEQ ID NOs: 77-91.

[0024] In certain examples, the expression cassette further comprises a regulatory element that drives expression of the transcription factor at a higher level in PV neurons than in other cell types. In certain examples, the regulatory element comprises any one of SEQ ID NOs: 1-4. In certain examples, the regulatory element comprises SEQ ID NO: 2 or 3.

[0025] In certain examples, the non-naturally occurring transcription factor comprises a sequence having at least 90% identity to SEQ ID NO: 127. In certain examples, the non-naturally occurring transcription factor comprises SEQ ID NO: 127.

[0026] In certain examples, the expression cassette comprises a nucleotide sequence having at least 90% identity to any one of SEQ ID NOs: 93 or 71. In certain examples, the expression cassette comprises a nucleotide sequence of any one of SEQ ID NOs: 93 or 71.

[0027] In certain examples, the expression cassette further comprises a PV selective microRNA binding site. In certain examples, the PV selective microRNA binding site comprises at least 90% identity to any one of SEQ ID NOs: 7, 14 or 15. In certain examples, the PV selective microRNA binding site comprises any one of SEQ ID NOs: 7, 14, or 15.

[0028] In certain examples, the expression cassette is a part of a viral vector. In certain examples, the viral vector is an AAV virus. In certain examples, the AAV virus is an AAV9 virus or a scAAV9 virus. In certain examples, the viral vector is a Lentivirus.

In another example, the application provides a polynucleotide comprising a PV selective microRNA binding site comprising a sequence having at least 80% sequence identity to SEQ ID NO: 14 or 15, wherein the microRNA binding site reduces expression of the transgene in excitatory

neurons. In certain examples, the PV selective microRNA binding site comprises SEQ ID NO: 14. In certain examples, the PV selective microRNA binding site comprises SEQ ID NO: 15. In another example, the application provides an expression cassette comprising the PV selective microRNA binding site and a promoter and/or enhancer. In certain examples, the promoter and/or enhancer is a PV selective regulatory element that drives expression of the transgene at a higher level in parvalbumin (PV) neurons than in other cell types. In certain examples, the PV selective regulatory element is operably linked to a transgene.

[0029] In another example, the application provides an expression cassette comprising a regulatory element operably linked to a transgene and at least one microRNA binding site, wherein the regulatory element drives expression of the transgene at a higher level in parvalbumin (PV) neurons than in other cell types, and wherein the microRNA binding site reduces expression of the transgene in excitatory neurons. In certain examples, the expression cassette does not comprise SEQ ID NO: 67. In certain examples, the microRNA binding site comprises at least one binding site for MIR128 (SEQ ID NO: 9). In certain examples, the microRNA binding site comprises at least one binding site for MIR221 (SEQ ID NO: 11). In certain examples, the microRNA binding site comprises at least one binding site for MIR222 (SEQ ID NO: 13). In certain examples, the microRNA binding site comprises at least one binding site for MIR128 (SEQ ID NO: 9) and at least one binding site for MIR221 (SEQ ID NO: 11). In certain examples, the microRNA binding site comprises at least one binding site for MIR128 (SEQ ID NO: 9), at least one binding site for MIR221 (SEQ ID NO: 11), and at least one binding site for MIR222 (SEQ ID NO: 13). In certain examples, the microRNA binding site comprises a sequence having at least 90% identity to any one of SEQ ID NOs: 7, 14 or 15. In certain examples, the microRNA binding site comprises SEQ ID NO: 7, 14 or 15.

[0030] In certain examples, the transgene encodes a polypeptide comprising a non-naturally occurring transcription factor which increases expression of the SCN1A gene in a cell. In certain examples, the transcription factor binds to a genomic region having 18-27 nucleotides. In certain examples, the transcription factor comprises a DNA binding domain. In certain examples, the transcription factor comprises a DNA binding domain and a transcription activating domain.

[0031] In certain examples, the DNA binding domain comprises at least 80% sequence identity to its closest human counterpart. In certain examples, the DNA binding domain comprises at least 90% sequence identity to its closest human counterpart. In certain examples, the DNA binding domain and the transcription activating domain both comprise at least 80% sequence identity to their closest human counterparts. In certain examples, the DNA binding domain and

the transcription activating domain both comprise at least 90% sequence identity to their closest human counterparts.

[0032] In certain examples, the DNA binding domain comprises a guide RNA and a nuclease inactivated Cas protein. In certain examples, the nuclease inactivated Cas protein is a nuclease inactivated Cas9.

[0033] In certain examples, the DNA binding domain comprises a zinc finger domain. In certain examples, the DNA binding domain comprises six to nine zinc finger domains. In certain examples, the DNA binding domain comprises six zinc fingers. In certain examples, the DNA binding domain binds to a genomic region having 18 nucleotides. In certain examples, the DNA binding domain comprises nine zinc fingers. In certain examples, the DNA binding domain binds to a genomic region having 27 nucleotides.

[0034] In certain examples, the DNA binding domain comprises a sequence having at least 95% sequence identity to any of SEQ ID NOs: 148-151. In certain examples, the DNA binding domain comprises a sequence having any one of SEQ ID NOs: 148-151. In certain examples, the DNA binding domain comprises a sequence having at least 90% identity to any one of SEQ ID NOs: 92-98. In certain examples, the DNA binding domain comprises any one of SEQ ID NOs: 92-98.

[0035] In certain examples, the DNA binding domain is a zinc finger protein comprising the sequence LEPGEKP – [YKCPECGKSFS X HQRTH TGEKP]n - YKCPECGKSFS X HQRTH – TGKKTS (SEQ ID NO: 147).

[0036] In certain examples, the DNA binding domain comprises a sequence having at least 90% identity to any one of SEQ ID NOs: 77-91. In certain examples, the DNA binding domain comprises any one of SEQ ID NOs: 77-91.

[0037] In certain examples, the DNA binding domain is derived from human EGR1 or human EGR3.

[0038] In certain examples, the transcription activating domain comprises a VP16, VPR, VP64, CITED2, CITED4, or CREB3 sequence, or a functional fragment thereof. In certain examples, the transcription activating domain comprises a human CITED2, CITED4, or CREB3 sequence, or a functional fragment thereof.

[0039] In certain examples, the regulatory element comprises a sequence having any one of SEQ ID NOs: 1-4. In certain examples, the regulatory element comprises a sequence having SEQ ID NO: 2 or 3.

[0040] In certain examples, the non-naturally occurring transcription factor comprises a sequence having at least 90% identity to any one of SEQ ID NOs: 105, 106, and 127-129. In certain examples, the non-naturally occurring transcription factor comprises any one of SEQ ID NOs: 105, 106, and 127-129.

[0041] In certain examples, the transgene comprises a nucleotide sequence having at least 90% identity to any one of SEQ ID NOs: 71, 74, 75, 76 or 184. In certain examples, the transgene comprises any one of SEQ ID NOs: 71, 74, 75, 76 or 184.

[0042] In certain examples, the expression cassette is a part of a viral vector. In certain examples, the viral vector is an AAV virus. In certain examples, AAV virus is an AAV9 virus or a scAAV9 virus. In certain examples, the viral vector is a Lentivirus.

[0043] In another example, the application provides a method for selective expression of a transgene in parvalbumin (PV) neurons of a primate comprising administering to a primate a viral vector comprising a transgene and at least one microRNA binding site, wherein the microRNA binding site reduces expression of the transgene in excitatory neurons.

[0044] In certain examples, the viral vector further comprises a regulatory element operably linked to the transgene, wherein the regulatory element drives expression of the transgene at a higher level in parvalbumin (PV) neurons than in other cell types.

[0045] In certain examples, the microRNA binding site comprises at least one binding site for MIR128 (SEQ ID NO: 9). In certain examples, the microRNA binding site comprises at least one binding site for MIR221 (SEQ ID NO: 11). In certain examples, the microRNA binding site comprises at least one binding site for MIR222 (SEQ ID NO: 13). In certain examples, the microRNA binding site comprises at least one binding site for MIR128 (SEQ ID NO: 9) and at least one binding site for MIR221 (SEQ ID NO: 11). In certain examples, the microRNA binding site comprises at least one binding site for MIR128 (SEQ ID NO: 9), at least one binding site for MIR221 (SEQ ID NO: 11), and at least one binding site for MIR222 (SEQ ID NO: 13). In certain examples, the microRNA binding site comprises a sequence having at least 90% identity to any one of SEQ ID NOs: 7, 14 or 15. In certain examples, the microRNA binding site comprises SEQ ID NO: 7, 14 or 15.

[0046] In certain examples, the transgene comprises a sequence encoding a non-naturally occurring transcription factor which increases expression of the SCN1A gene in a cell.

[0047] In certain examples, the transcription factor binds to a genomic region having 18-27 nucleotides.

[0048] In certain examples, the transcription factor comprises a DNA binding domain.

[0049] In certain examples, the transcription factor comprises a DNA binding domain and a transcription activating domain.

[0050] In certain examples, the DNA binding domain comprises at least 80% sequence identity to its closest human counterpart. In certain examples, the DNA binding domain comprises at least 90% sequence identity to its closest human counterpart. In certain examples, the DNA binding domain and the transcription activating domain both comprise at least 80% sequence identity to their closest human counterparts. In certain examples, the DNA binding domain and the transcription activating domain both comprise at least 90% sequence identity to their closest human counterparts.

[0051] In certain examples, the DNA binding domain comprises a guide RNA and a nuclease inactivated Cas protein. In certain examples, the nuclease inactivated Cas protein is a nuclease inactivated Cas9.

[0052] In certain examples, the DNA binding domain comprises a zinc finger domain. In certain examples, the DNA binding domain comprises six to nine zinc finger domains. In certain examples, the DNA binding domain comprises six zinc fingers. In certain examples, the DNA binding domain binds to a genomic region having 18 nucleotides. In certain examples, the DNA binding domain comprises nine zinc fingers. In certain examples, the DNA binding domain binds to a genomic region having 27 nucleotides.

[0053] In certain examples, the DNA binding domain comprises a sequence having at least 95% sequence identity to any of SEQ ID NOs: 148-151. In certain examples, the DNA binding domain comprises a sequence having any one of SEQ ID NOs: 148-151.

[0054] In certain examples, the DNA binding domain is derived from human EGR1 or human EGR3.

[0055] In certain examples, the DNA binding domain comprises a sequence having at least 90% identity to any one of SEQ ID NOs: 92-98. In certain examples, the DNA binding domain comprises any one of SEQ ID NOs: 92-98.

[0056] In certain examples, the DNA binding domain is a zinc finger protein comprising the sequence LEPGEKP – [YKCPECGKSFS X HQRTH TGEKP]n - YKCPECGKSFS X HQRTH – TGKKTS (SEQ ID NO: 147).

[0057] In certain examples, the DNA binding domain comprises a sequence having at least 90% identity to any one of SEQ ID NOs: 77-91. In certain examples, the DNA binding domain comprises any one of SEQ ID NOs: 77-91.

[0058] In certain examples, the transcription activating domain comprises a VP16, VPR, VP64, CITED2, CITED4, or CREB3 sequence, or a functional fragment thereof. In certain examples, the transcription activating domain comprises a human CITED2, CITED4, or CREB3 sequence, or a functional fragment thereof.

[0059] In certain examples, the regulatory element comprises a sequence having any one of SEQ ID NOs: 1-4. In certain examples, the regulatory element comprises a sequence having SEQ ID NO: 2 or 3.

[0060] In certain examples, the non-naturally occurring transcription factor comprises a sequence having at least 90% identity to any one of SEQ ID NOs: 105, 106, and 127-129. In certain examples, the non-naturally occurring transcription factor comprises any one of SEQ ID NOs: 105, 106, and 127-129.

[0061] In certain examples, the transgene comprises a nucleotide sequence having at least 90% identity to any one of SEQ ID NOs: 71, 74, 75, 76 or 184. In certain examples, the transgene comprises any one of SEQ ID NOs: 71, 74, 75, 76 or 184.

[0062] In certain examples, the viral vector is an AAV virus. In certain examples, the AAV virus is an AAV9 virus or a scAAV9 virus. In certain examples, the viral vector is a Lentivirus.

[0063] In certain examples, the primate is a human. In certain examples, the primate is a non-human primate. In certain examples, the non-human primate is an old world monkey, an orangutan, a gorilla, a chimpanzee, a marmoset, a crab-eating macaque, a rhesus macaque or a pig-tailed macaque.

[0064] In another example, the application provides an expression cassette comprising a sequence encoding a non-naturally occurring transcription factor which increases expression of the SCN1A gene in a cell, wherein the non-naturally occurring transcription factor comprises a sequence having at least 90% identity to SEQ ID NO: 128 or 129. In certain examples, the non-naturally occurring transcription factor comprises SEQ ID NO: 128 or 129.

[0065] In another example, the application provides a method of increasing expression of SCN1A in a cell by administering any of the expression cassettes provided herein. In certain examples, the cell is a neuronal cell. In certain examples, the neuronal cell is selected from the group consisting of unipolar, bipolar, multipolar, or pseudounipolar neurons. In certain examples, the cell is GABAergic neuron. In certain examples, the cell is a PV neuron. In certain examples, the cell is a non-neuronal cell. In certain examples, the cell is a glial cell. In certain examples, the glial cell is selected from the group consisting of astrocytes, oligodendrocytes, ependymal cells, Schwann cells, and satellite cells. In certain examples, the cell is within a

subject. In certain examples, the subject is a mammal. In certain examples, the subject is a human. In certain examples, increasing expression of SCN1A treats a disease, disorder or symptom. In certain examples, the disorder is a central nervous system disorder. In certain examples, the disorder is epilepsy associated with SCN1A haploinsufficiency. In certain examples, the haploinsufficiency is the result of the subject being heterozygous for a loss of function mutation of the SCN1A gene. In certain examples, the disorder is epilepsy associated with an insertion, deletion, or substitution in the SCN1A gene. In certain examples, the disorder is epilepsy associated with a point mutation in the SCN1A gene. In certain examples, the disorder is Dravet Syndrome. In certain examples, a symptom of the central nervous system disorder is neuronal hyperactivity. In certain examples, treating the central nervous system disorder comprises reducing neuronal hyperactivity. In certain examples, a symptom of the central nervous system disorder is seizures. In certain examples, treating the central nervous system disorder comprises reducing the frequency of seizures. In certain examples, treating the central nervous system disorder comprises reducing the severity of seizures.

[0066] In another example, the application provides a method of increasing expression of SCN1A in the CNS by administering any one of the expression cassettes provided herein. In certain examples, the expression cassette is administered via unilateral intracerebroventricular (ICV) administration. In certain examples, the expression cassette is administered via bilateral intracerebroventricular (ICV) administration. In certain examples, the increased expression of SCN1A occurs in the brain. In certain examples, the increased expression of SCN1A occurs in the frontal cortex, parietal cortex, temporal cortex, hippocampus, medulla, and/or occipital cortex. In certain examples, the increased expression of SCN1A occurs in the spine. In certain examples, the increased expression of SCN1A occurs in the spinal cord and/or dorsal root ganglion.

INCORPORATION BY REFERENCE

[0067] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

CLAIMS

WHAT IS CLAIMED IS:

1. A polynucleotide comprising a nucleic acid sequence encoding an engineered transcription factor, wherein the engineered transcription factor comprises an amino acid sequence of SEQ ID NO: 127, wherein the engineered transcription factor increases expression of a SCN1A gene in a cell.

2. A polynucleotide comprising a nucleic acid sequence encoding an engineered transcription factor comprising a zinc finger DNA binding domain (DBD) and a transcription activation domain, wherein the zinc finger DBD comprises SEQ ID NOS: 152-157, and wherein the engineered transcription factor does not comprise an HA tag (SEQ ID NO: 171).

3. The polynucleotide of claim 2, wherein the zinc finger DBD comprises a sequence of SEQ ID NO: 148.

4. The polynucleotide of claim 3, wherein the zinc finger DBD comprises a sequence having at least 90% identity to SEQ ID NO: 77.

5. The polynucleotide of claim 3, wherein the zinc finger DBD comprises a sequence having at least 90% identity to SEQ ID NO: 92.

6. The polynucleotide of claim 3, wherein the zinc finger DBD comprises a sequence having at least 90% identity to SEQ ID NO: 96.

7. The polynucleotide of claim 3, wherein the transcription activating domain is (i) derived from VP16, VPR, VP64, VP128, p65, p300, CITED2, CITED4, EGR1, or EGR3, (ii) a variant of any one of (i), or a functional fragment of any one of (i).

8. The polynucleotide of claim 3, wherein transcription activating domain comprises an amino acid sequence having at least 90% identity to a sequence of SEQ ID NO: 132, 133, 134, 135, or 224.

9. The polynucleotide of claim 3, wherein the engineered transcription factor comprises an amino acid sequence having at least 90% identity to a sequence of SEQ ID NO: 100, 102, 105, 106, 107, 108, 109, 110, 111, 112, SEQ ID NO: 113, 127, 128, 129, 130, 131, 205, 207, 209, 213, or 217.

10. The polynucleotide of claim 9, wherein the engineered transcription factor comprises an amino acid sequence of SEQ ID NO:100, 102, or 127.

11. The polynucleotide of claim 1 or 2, further comprising a regulatory element operably linked to the nucleic acid sequence encoding the engineered transcription factor to form an expression cassette.

12. The polynucleotide of claim 11, wherein the regulatory element comprises a GAD2 promoter, a human synapsin promoter, a CBA promoter, a CMV promoter, a minCMV promoter, a TATA box, a super core promoter, an EF1α promoter, or a combination thereof.

13. The polynucleotide of claim 11, wherein the regulatory element is a parvalbumin (PV) neuron selective regulatory element.

14. The polynucleotide of claim 13, wherein the PV neuron selective regulatory element comprises any one of SEQ ID NOS: 1-4.

15. The polynucleotide of claim 14, wherein the regulatory element comprises SEQ ID NO: 2.

16. The polynucleotide of claim 11, wherein the expression cassette further comprises an element that inhibits expression of the engineered transcription factor in excitatory neurons.

17. The polynucleotide of claim 16, wherein the element that inhibits expression of the engineered transcription factor in excitatory neurons is an element that promotes mRNA degradation.

18. The polynucleotide of claim 16, wherein the element that inhibits expression of the engineered transcription factor in excitatory neurons comprises a microRNA binding site.

19. The polynucleotide of claim 18, wherein the microRNA binding site comprises a sequence of SEQ ID NO: 9, 11, or 13.

20. The polynucleotide of claim 18, wherein the microRNA binding site comprises SEQ ID NO:9 and SEQ ID NO:11.

21. The polynucleotide of claim 18, wherein the element that inhibits expression of the engineered transcription factor comprises at least 6 microRNA binding sites.

22. The polynucleotide of claim 18, wherein the element that inhibits expression of the engineered transcription factor comprises a sequence having at least 90% identity to SEQ ID NO: 7, 14, or 15.

23. The polynucleotide of claim 18, wherein the element that inhibits expression of the engineered transcription factor comprises SEQ ID NO: 7.

24. The polynucleotide of claim 11, wherein the expression cassette comprises a nucleotide sequence having at least 90% identity to a sequence of SEQ ID NO:67, SEQ ID NO:68, 69, 70, 71, 74, 75, 76, or 184.

25. An expression vector comprising the polynucleotide of claim 11.

26. The expression vector of claim 25, wherein the expression vector is a viral vector.

27. The expression vector of claim 26, wherein the viral vector is an adeno-associated virus (AAV) virus.

28. The expression vector of claim 27, wherein the AAV virus has a serotype selected from the group consisting of: AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, rh10, avian AAV, bovine AAV, canine AAV, equine AAV, primate AAV, non-primate AAV, ovine AAV, scAAV, scAAV1, scAAV2, scAAV5, scAAV8, scAAV9, and a hybrid of any one thereof.

29. The expression vector of claim 27 or 28, wherein the expression cassette further comprises a 5′ AAV inverted terminal repeat (ITR) sequence and a 3′ AAV ITR sequence.

30. The expression vector of claim 29, wherein the 5′ AAV ITR sequence and the 3′ AAV ITR sequence are each, independently, an ITR sequence derived from AAV1, AAV2, AAV5, AAV8, or AAV9.

31. The expression vector of any one of claims 27-30, wherein the expression cassette comprises a nucleic acid sequence having at least 90% sequence identity to a sequence of SEQ ID NO: 67, 68, 69, 70, 71, 74, 75, 76, or 184.

32. The expression vector of claim 31, wherein the expression cassette comprises the sequence of SEQ ID NO: 67.

33. The expression vector of claim 31, wherein the expression cassette comprises the sequence of SEQ ID NO: 71.

34. The expression vector of claim 31, wherein the expression cassette comprises the sequence of SEQ ID NO: 74.

35. The expression vector of claim 31, wherein the expression cassette comprises the sequence of SEQ ID NO: 75.

36. The expression vector of claim 31, wherein the expression cassette comprises the sequence of SEQ ID NO: 76.

37. The expression vector of claim 31, wherein the expression cassette comprises the sequence of SEQ ID NO: 184.

38. A composition comprising (i) the polynucleotide of any one of claims 1-37 and (ii) one or more pharmaceutically acceptable carrier(s).

39. A polynucleotide comprising a nucleic acid sequence encoding an engineered transcription factor comprises a sequence having at least 90% identity to SEQ ID NO: 127,

wherein the polynucleotide is operably linked to a nucleic acid sequence comprising a microRNA binding site.

40. The polynucleotide of claim 39, wherein the engineered transcription factor increases expression of the SCN1A gene in a cell.

41. The polynucleotide of claim 40, wherein the cell is a human cell.

42. The polynucleotide of any one of claims 39-41, wherein the microRNA binding site binds to a microRNA that promotes PV neuron selective expression of the engineered transcription factor.

43. The polynucleotide of any one of claims 39-42, wherein the microRNA binding site comprises a sequence of SEQ ID NO: 9, 11, or 13.

44. The polynucleotide of any one of claims 39-42, wherein the nucleic acid sequence comprising a microRNA binding site comprises a sequence having at least 90% identity to SEQ ID NO: 7, 14, or 15.

45. The polynucleotide of claim 44, wherein the nucleic acid sequence comprising the microRNA binding site comprises SEQ ID NO: 7.

46. The polynucleotide of any one of claims 39-45, wherein the polynucleotide further comprises a PV neuron selective regulatory element operably linked to the nucleic acid sequence encoding the engineered transcription factor to form an expression cassette.

47. The polynucleotide of claim 46, wherein the PV neuron selective regulatory element comprises SEQ ID NO: 2.

48. The polynucleotide of claim 46 or 47, wherein the expression cassette is incorporated into an expression vector.

49. The polynucleotide of claim 48, wherein the expression vector is a viral vector.

50. The polynucleotide of claim 49, wherein the viral vector is an adeno-associated virus (AAV) vector.

51. A cell comprising the polynucleotide of any one of claims 2-50.

52. The cell of claim 51, wherein the zinc finger DBD comprises a sequence of SEQ ID NO: 148.

53. The cell of claim 51, wherein the zinc finger DBD comprises a sequence of SEQ ID NO: 77, 92, or 96.

54. The cell of any one of claims 51-53, wherein the transcription activation domain comprises a VPR, VP64, VP16, VP128, p300, CITED2, or CITED4 sequence.

55. The cell of any one of claims 51-54, wherein the engineered transcription factor comprises an amino acid sequence having at least 95% identity to a sequence of SEQ ID NO: 100, 102, 105, 106, 107, 108, 109, 110, 111, 112, 113, 127, 128, 129, 130, 131, 205, 207, 209, 213, or 217.

56. The cell of any one of claims 51-55, wherein the polynucleotide further comprises a regulatory element operably linked to the nucleic acid sequence encoding the engineered transcription factor to form an expression cassette.

57. The cell of claim 56, wherein the regulatory element comprises a PV neuron selective regulatory element that promotes PV neuron selective expression of the engineered transcription factor.

58. The cell of claim 57, wherein the PV neuron selective regulatory element comprises SEQ ID NO:2.

59. The cell of any one of claims 56-58, further comprising a microRNA binding site operably linked to the nucleic acid sequence encoding the engineered transcription factor.

60. The cell of claim 59, wherein the microRNA binding site comprises a sequence of SEQ ID NO: 9, 11, or 13.

61. The cell of any one of claims 56-60, wherein the expression cassette comprises a nucleotide sequence having at least 90% identity to SEQ ID NO: 71.

62. The cell of any one of claims 56-61, wherein the expression cassette is flanked by a 5′ AAV ITR and a 3′ AAV ITR.

63. The cell of claim 62, further comprising an AAV rep gene, an AAV cap gene, an adenoviral helper gene, or a combination thereof.

64. The cell of claim 63, wherein the cell is a 293 cell, an A549 cell, or a HeLa cell.

65. A method of manufacturing an adeno-associated virus (AAV) vector comprising: (a) culturing a cell according to any one of claims 51-64 under conditions for producing recombinant AAV virions; (b) harvesting the host cell culture; and (c) purifying AAV virions produced by the host cell.

66. The method of claim 65, wherein the purifying comprises equilibrium centrifugation, flow-through anionic exchange filtration, tangential flow filtration for concentrating the rAAV virion, rAAV capture by apatite chromatography, heat inactivation of a helper virus, rAAV capture by hydrophobic interaction chromatography, buffer exchange by size exclusion chromatography, nanofiltration, and rAAV capture by anionic exchange chromatography, cationic exchange chromatography, or affinity chromatography.

67. A polynucleotide comprising a parvalbumin (PV) neuron selective microRNA binding site, wherein the microRNA binding site comprises a sequence having at least 90% sequence identity to SEQ ID NO: 14 or 15, and wherein the microRNA binding site reduces expression of a transgene in excitatory neurons.

68. The polynucleotide of claim 67, wherein the PV neuron selective microRNA binding site comprises SEQ ID NO: 14.

69. The polynucleotide of claim 67, wherein the PV neuron selective microRNA binding site comprises SEQ ID NO: 15.

70. An expression cassette comprising (i) the PV neuron selective microRNA binding site of any one of claims 67-69 and (ii) a promoter, an enhancer, or a combination of a promoter and an enhancer.

71. The expression cassette of claim 70, wherein the promoter, the enhancer, or the combination of the promoter and the enhancer comprises a PV neuron selective regulatory element that drives expression of the transgene at a higher level in parvalbumin (PV) neurons than in other cell types.

72. The expression cassette of claim 71, wherein the PV neuron selective regulatory element is operably linked to a transgene.

73. An expression cassette comprising a regulatory element operably linked to a transgene and at least one microRNA binding site, wherein the regulatory element drives expression of the transgene at a higher level in parvalbumin (PV) neurons than in other cell types, wherein the microRNA binding site reduces expression of the transgene in excitatory neurons, and wherein the expression cassette does not comprise SEQ ID NO: 67.

74. The expression cassette of claim 73, wherein the microRNA binding site comprises at least one binding site for MIR128 (SEQ ID NO: 9).

75. The expression cassette of claim 73 or 74, wherein the microRNA binding site comprises at least one binding site for MIR221 (SEQ ID NO: 11).

76. The expression cassette of any one of claims 73-76, wherein the microRNA binding site comprises at least one binding site for MIR222 (SEQ ID NO: 13).

77. The expression cassette of claim 73, wherein the microRNA binding site comprises at least one binding site for MIR128 (SEQ ID NO: 9) and at least one binding site for MIR221 (SEQ ID NO: 11).

78. The expression cassette of claim 73, wherein the microRNA binding site comprises at least one binding site for MIR128 (SEQ ID NO: 9), at least one binding site for MIR221 (SEQ ID NO: 11), and at least one binding site for MIR222 (SEQ ID NO: 13).

79. The expression cassette of any one of claims 73-75, wherein the microRNA binding site comprises a sequence having at least 90% identity to SEQ ID NO: 7, 14 or 15.

80. The expression cassette of claim 79, wherein the microRNA binding site comprises SEQ ID NO: 7.

81. The expression cassette of claim 79, wherein the microRNA binding site comprises SEQ ID NO: 14.

82. The expression cassette of claim 79, wherein the microRNA binding site comprises SEQ ID NO: 15.

83. The expression cassette of any one of claims 73-82, wherein the transgene encodes an engineered transcription factor which increases expression of a SCN1A gene in a cell.

84. The expression cassette of claim 83, wherein the engineered transcription factor binds to a genomic region having 18-27 nucleotides.

85. The expression cassette of claim 83 or 84, wherein the engineered transcription factor comprises a DNA binding domain.

86. The expression cassette of any one of claims 83-85, wherein the engineered transcription factor comprises a DNA binding domain and a transcription activating domain.

87. The expression cassette of claim 85 or 86, wherein the DNA binding domain comprises a sequence that is at least 80% sequence identity to its closest human counterpart.

88. The expression cassette of claim 87, wherein the DNA binding domain comprises a sequence that is at least 90% sequence identity to its closest human counterpart.

89. The expression cassette of claim 86, wherein the DNA binding domain and the transcription activating domain both comprise sequences that have at least 80% sequence identity to their closest human counterparts.

90. The expression cassette of claim 86, wherein the DNA binding domain and the transcription activating domain both comprise sequences that have at least 90% sequence identity to their closest human counterparts.

91. The expression cassette of any one of claims 85-90, wherein the DNA binding domain further comprises a guide RNA and a nuclease inactivated Cas protein.

92. The expression cassette of claim 91, wherein the nuclease inactivated Cas protein is a nuclease inactivated Cas9.

93. The expression cassette of any one of claims 85-92, wherein the DNA binding domain comprises a zinc finger domain.

94. The expression cassette of claim 93, wherein the DNA binding domain comprises from six to nine zinc finger domains.

95. The expression cassette of claim 94, wherein the DNA binding domain comprises six zinc finger domains.

96. The expression cassette of claim 95, wherein the DNA binding domain binds to a genomic region having 18 nucleotides.

97. The expression cassette of claim 94, wherein the DNA binding domain comprises nine zinc finger domains.

98. The expression cassette of claim 97, wherein the DNA binding domain binds to a genomic region having 27 nucleotides.

99. The expression cassette of any one of claims 85-90 and 92-98, wherein the DNA binding domain comprises a sequence having at least 95% sequence identity to any of SEQ ID NOS: 148-151.

100. The expression cassette of claim 99, wherein the DNA binding domain comprises a sequence having any one of SEQ ID NOS: 148-151.

101. The expression cassette of any one of claims 85-90 and 92-98, wherein the DNA binding domain comprises a sequence having at least 90% identity to any one of SEQ ID NOS: 92-98.

102. The expression cassette of claim 101, wherein the DNA binding domain comprises any one of SEQ ID NOS: 92-98.

103. The expression cassette of any one of claims 85-90 and 92-98, wherein the DNA binding domain is a zinc finger protein comprising the sequence LEPGEKP – [YKCPECGKSFS X HQRTH TGEKP]n - YKCPECGKSFS X HQRTH – TGKKTS (SEQ ID NO: 147).

104. The expression cassette of claim 102 or 103, wherein the DNA binding domain comprises a sequence having at least 90% identity to any one of SEQ ID NOS: 77-91.

105. The expression cassette of any one of claims 104, wherein the DNA binding domain comprises any one of SEQ ID NOS: 77-91.

106. The expression cassette of any one of claims 85-90 and 92-102, wherein the DNA binding domain is derived from human EGR1 or human EGR3.

107. The expression cassette of any one of claims 86-90, wherein the transcription activating domain comprises (i) a VP16, VPR, VP64, CITED2, CITED4, or CREB3 sequence, or (ii) a functional fragment of any one of (i).

108. The expression cassette of any one of claims 86-90, wherein the transcription activating domain comprises (i) a human CITED2, CITED4, or CREB3 sequence, or (ii) a functional fragment of any one of (i).

109. The expression cassette of any one of claims 72-108, wherein the regulatory element comprises a sequence of any one of SEQ ID NOS: 1-4.

110. The expression cassette of claim 109, wherein the regulatory element comprises a sequence of SEQ ID NO: 2 or 3.

111. The expression cassette of claim 83, wherein the engineered transcription factor comprises a sequence having at least 90% identity to any one of SEQ ID NOS: 105, 106, and 127-129.

112. The expression cassette of claim 111, wherein the engineered transcription factor comprises any one of SEQ ID NOS: 105, 106, and 127-129.

113. The expression cassette of claim 72 or 73, wherein the transgene comprises a nucleotide sequence having at least 90% identity to SEQ ID NO: 71, 74, 75, 76 or 184.

114. The expression cassette of claim 113, wherein the transgene comprises any one of SEQ ID NO: 71, 74, 75, 76 or 184.

115. The expression cassette of any one of claims 72-114, wherein the expression cassette is a part of a viral vector.

116. The expression cassette of claim 115, wherein the viral vector is an AAV virus.

117. The expression cassette of claim 116, wherein the AAV virus is an AAV9 virus or a scAAV9 virus.

118. The expression cassette of claim 115, wherein the viral vector is a Lentivirus.

119. A host cell comprising the viral vector of any one of any one of claims 115-118.

120. A method of manufacturing an adeno-associated virus (AAV) vector comprising: (a) culturing a host cell according to claim 119 under conditions for producing recombinant AAV virions; (b) harvesting the host cell culture; and (c) purifying AAV virions produced by the host cell.

121. The method of claim 120, wherein the purifying comprises equilibrium centrifugation, flow-through anionic exchange filtration, tangential flow filtration for concentrating the rAAV virion, rAAV capture by apatite chromatography, heat inactivation of a helper virus, rAAV capture by hydrophobic interaction chromatography, buffer exchange

by size exclusion chromatography, nanofiltration, and rAAV capture by anionic exchange chromatography, cationic exchange chromatography, or affinity chromatography.