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 comprising a regulatory element operably linked to a nucleic acid sequence encoding an engineered transcription factor to form an expression cassette, 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 an expression vector comprising the polynucleotide as described herein.

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

[0004] 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

CLAIMS

WHAT IS CLAIMED IS:

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

3. The polynucleotide of claim 1, wherein the regulatory element is a parvalbumin (PV) neuron selective regulatory element.

4. The polynucleotide of claim 3, wherein the PV neuron selective regulatory element comprises any one of SEQ ID NOS: 1-4.

5. The polynucleotide of claim 4, wherein the regulatory element comprises SEQ ID NO: 2.

6. The polynucleotide of claim 1, wherein the expression cassette further comprises an element that inhibits expression of the engineered transcription factor in excitatory neurons.

7. The polynucleotide of claim 6, wherein the element that inhibits expression of the engineered transcription factor in excitatory neurons is an element that promotes mRNA degradation.

8. The polynucleotide of claim 6, wherein the element that inhibits expression of the engineered transcription factor in excitatory neurons comprises a microRNA binding site.

9. The polynucleotide of claim 8, wherein the microRNA binding site comprises a sequence of SEQ ID NO: 9, 11, or 13.

10. The polynucleotide of claim 8, wherein the microRNA binding site comprises SEQ ID NO:9 and SEQ ID NO:11.

11. The polynucleotide of claim 8, wherein the element that inhibits expression of the engineered transcription factor comprises at least 6 microRNA binding sites.

12. The polynucleotide of claim 8, 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.

13. The polynucleotide of claim 8, wherein the element that inhibits expression of the engineered transcription factor comprises SEQ ID NO: 7.

14. The polynucleotide of claim 1, wherein the expression cassette comprises a nucleotide sequence having at least 90% identity to a sequence of SEQ ID NO: 71.

15. An expression vector comprising the polynucleotide of claim 1.

16. The expression vector of claim 15, wherein the expression vector is a viral vector.

17. The expression vector of claim 16, wherein the viral vector is an adeno-associated virus (AAV) virus.

18. The expression vector of claim 17, 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.

19. The expression vector of claim 17 or 18, wherein the expression cassette further comprises a 5′ AAV inverted terminal repeat (ITR) sequence and a 3′ AAV ITR sequence.

20. The expression vector of claim 19, 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.

21. The expression vector of any one of claims 17-20, wherein the expression cassette comprises a nucleic acid sequence having at least 90% sequence identity to a sequence of SEQ ID NO: 71.

22. The expression vector of claim 21, wherein the expression cassette comprises the sequence of SEQ ID NO: 71.

23. A composition comprising (i) the polynucleotide of any one of claims 1-22 and (ii) one or more pharmaceutically acceptable carrier(s).