neuronal loss of UBE3A precipitates AS. Glia are less likely to play a role, as unlike neurons, they express UBE3A biallelically and at very low levels.

[0006] There are currently no specific treatments available for AS. Individuals affected by AS are managed by supportive symptomatic care.

SUMMARY

[0007] The present invention is based, in part, on the development of optimized UBE3A genes, expression cassettes, and vectors capable of providing therapeutic levels of UBE3A expression for treating disorders associated with UBE3A misexpression such as Angelman Syndrome. Examples of the present invention overcome shortcomings in the art by providing codon-optimized UBE3A genes, expression cassettes, and vectors capable of providing therapeutic levels of UBE3A expression for treating disorders associated with UBE3A expression such as AS.

[0008] Thus, one aspect, the present disclosure refers to a polynucleotide comprising a human UBE3A open reading frame, wherein the human UBE3A open reading frame encodes human UBE3A short isoform and long isoform, optionally wherein the human UBE3A long isoform is human UBE3A long isoform 2 or human UBE3A long isoform 3.

[0009] In another aspect, the present disclosure refers to an expression cassette comprising the polynucleotide as disclosed herein. A further example describes an expression cassette comprising a polynucleotide comprising a human UBE3A open reading frame and vectors, transformed cells, and transgenic animals comprising the polynucleotide of the disclosure. In yet another aspect, the present disclosure refers to a vector comprising the polynucleotide as disclosed herein or the expression cassette as disclosed herein, optionally wherein the vector is a neuro-tropic vector, optionally wherein the vector is a viral vector, optionally an AAV vector such as AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12 AAV13, or any combination or derivative thereof. In a further aspect, the present disclosure refers to a transformed cell comprising the polynucleotide as disclosed herein, the expression cassette as disclosed herein, and/or the vector as disclosed herein, optionally wherein the polynucleotide, expression cassette, and/or vector is stably incorporated into the cell genome.

[0010] in another aspect, the present disclosure refers to a pharmaceutical formulation comprising the polynucleotide, expression cassette, vector, and/or transformed cell as disclosed herein in a pharmaceutically acceptable carrier.

[0011] In another aspect, the present disclosure refers to use of the polynucleotide as disclosed herein, expression cassette as disclosed herein, vector as disclosed herein, transformed cell as disclosed herein, and/or pharmaceutical composition as disclosed herein, in the manufacture of a medicament for treating a disorder associated with aberrant expression of a UBE3A gene or aberrant activity of a UBE3A gene product in a subject in need thereof, comprising the subject to be administered the medicament, such that the UBE3A open reading frame is expressed in the subject. In another aspect, the present disclosure refers to the polynucleotide, expression cassette, vector, transformed cell, and/or pharmaceutical composition as disclosed herein, for use in therapy. In one example, there is described a method of expressing a UBE3A open reading frame in a cell, comprising contacting the cell with the polynucleotide, expression cassette, and/or vector of the invention, thereby expressing the UBE3A open reading frame in the cell.

[0012] A further example describes to a method of expressing a UBE3A open reading frame in a subject, comprising delivering to the subject the polynucleotide, expression cassette, vector, and/or transformed cell of the invention, thereby expressing the UBE3A open reading frame in the subject.

[0013] An additional example describes a method of treating a disorder associated with aberrant expression of a UBE3A gene or aberrant activity of a UBE3A gene product in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the polynucleotide, expression cassette, vector, and/or transformed cell of the invention, such that the UBE3A open reading frame is expressed in the subject.

[0014] Further described herein is a method of treating Angelman Syndrome (AS) in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the polynucleotide, expression cassette, vector, and/or transformed cell of the invention, such that the UBE3A open reading frame is expressed in the subject.

[0015] Described herein is also a polynucleotide, expression cassette, vector, and/or transformed cell of the invention for use in a method of treating a disorder associated with aberrant expression of a UBE3A gene or aberrant activity of a UBE3A gene product in a subject in need thereof.

[0016] These and other aspects of the invention are set forth in more detail in the description of the invention below.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] **FIG. 1** shows a diagram of neuronal regulation of UBE3A, wherein neurons have paternal silencing of UBE3A.

[0018] **FIGS. 2A-2B** show the experimental design for flurothyl kindling and rechallenge (**FIG. 2A**) and generalized seizure thresholds (GST, **FIG. 2B**) for wildtype mice (WT), mice lacking long UBE3A (ISO2-KO), and mice lacking short UBE3A (ISO3-KO).

[0019] **FIG. 3A** shows the N-terminal amino acid sequences of WT human and mouse UBE3A isoforms (SEQ ID NOS:15-19). Mouse isoform 1 (not shown) is truncated and does not express a stable protein.

[0020] **FIG. 3B** shows the 5' DNA sequence of the codon-optimized hUBE3A1>>3 open reading frame (SEQ ID NO:20) (top), in register with corresponding amino acid residues of resultant hUBE3A protein (SEQ ID NO:21) (bottom). Both long and short hUBE3A are encoded in the same reading frame. Kozak sequences of different strengths were engineered to bias translation initiation from the second start codon (CAGG**ATG**A) beginning the short isoform of hUBE3A.

[0021] **FIG. 3C** shows a schematic depicting the full AS gene therapy construct for expressing both long (isoform 3) and short (isoform 1) isoforms of hUBE3A (SEQ ID NOS:22-24).

What is claimed is:

1. A polynucleotide comprising a human UBE3A open reading frame, wherein the human UBE3A open reading frame encodes human UBE3A short isoform and long isoform, optionally wherein the human UBE3A long isoform is human UBE3A long isoform 2 or human UBE3A long isoform 3.

2. The polynucleotide of claim 1, wherein the human UBE3A open reading frame comprises one or more nucleotide modifications to reduce expression of the long isoform as compared to expression of native ORF which lacks the one or more nucleotide modifications to reduce expression and/or wherein the human UBE3A open reading frame comprises one or more nucleotide modifications to enhance expression of the short isoform, optionally wherein the expression of the long isoform is reduced by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% as compared to native ORF not comprising nucleotide modifications to reduce expression, and/or optionally wherein the expression of the short isoform is enhanced by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% as compared to native ORF not comprising nucleotide modifications to enhance expression.

3. The polynucleotide of claim 1 or 2, wherein the human UBE3A open reading frame comprises one or more nucleotide modifications to encode expression of the short isoform at a ratio of about 1:1 to about 15:1, as compared to expression of the long isoform.

4. The polynucleotide of any one of claims 1-3, which comprises a first Kozak sequence comprising SEQ ID NO:6 operably linked to coding sequences for the long isoform.

5. The polynucleotide of any one of claims 1-4, which comprises a second Kozak sequence comprising SEQ ID NO:8 operably linked to coding sequence for the short isoform.

6. The polynucleotide of any one of claims 1-5, wherein the human UBE3A open reading frame is codon-optimized for expression in a human cell, optionally wherein the human UBE3A open reading frame is codon-optimized for intracellular or non-secretory expression in a human cell.

7. The polynucleotide of any one of claims 1-6, wherein the human UBE3A open reading frame comprises the nucleotide sequence of SEQ ID NO:9, SEQ ID NO:26, or SEQ ID NO:27, or a nucleotide sequence having at least about 70% identity thereto.

8. An expression cassette comprising the polynucleotide of any one of claims 1-7.

9. The expression cassette of claim 8, wherein the human UBE3A open reading frame is operably linked to a promoter and/or a polyadenylation signal, optionally, wherein the promoter is a neuron-specific promoter, optionally a human synapsin promoter, and/or optionally wherein the polyadenylation signal is a bovine growth hormone (bGH) polyadenylation signal (bGHpA).

10. The expression cassette of claim 8 or 9, further comprising at least one adeno-associated virus (AAV) inverted terminal repeat (ITR), optionally two AAV ITRs, optionally wherein the AAV ITRs are selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12 and AAV13 ITRs.

11. The expression cassette of any one of claims 8-10, wherein the expression cassette comprises an AAV2 ITR; a human synapsin promoter; the human UBE3A open reading frame comprising human UBE3A short isoform and long isoform 3 further comprising one or more Kozak sequences comprising, consisting essentially of, or consisting of SEQ ID NO:6 and/or SEQ ID NO:8; a bGHpA; and an AAV2 ITR, optionally comprising the nucleotide sequence of SEQ ID NO:14 or a sequence at least about 90% identical thereto.

12. A vector comprising the polynucleotide of any one of claims 1-7 or the expression cassette of any one of claims 8-11, optionally wherein the vector is a neuro-tropic vector, optionally wherein the vector is a viral vector, optionally an AAV vector such as AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12 AAV13, or any combination or derivative thereof.

13. A transformed cell comprising the polynucleotide of any one of claims 1-7, the expression

cassette of any one of claims 8-11, and/or the vector of claim 12, optionally wherein the polynucleotide, expression cassette, and/or vector is stably incorporated into the cell genome.

14. A pharmaceutical composition comprising the polynucleotide of any one of claims 1-7, the expression cassette of any one of claims 8-11, and/or the vector of claim 12, and/or the transformed cell of claim 13 in a pharmaceutically acceptable carrier.

15. The polynucleotide of any one of claims 1-7, expression cassette of any one of claims 8-11, vector of claim 12, transformed cell of claim 13, and/or pharmaceutical composition of claim 14, for use in therapy.

16. Use of the polynucleotide of any one of claims 1-7, expression cassette of any one of claims 8-11, vector of claim 12, transformed cell of claim 13, and/or pharmaceutical composition of claim 14, in the manufacture of a medicament for treating a disorder associated with aberrant expression of a UBE3A gene or aberrant activity of a UBE3A gene product in a subject in need thereof, comprising the subject to be administered the medicament, such that the UBE3A open reading frame is expressed in the subject.

17. Use of the polynucleotide of any one of claims 1-7, expression cassette of any one of claims 8-11, vector of claim 12, transformed cell of claim 13, and/or pharmaceutical composition of claim 14, in the manufacture of a medicament for treating Angelman Syndrome.

18. The polynucleotide, expression cassette, vector, transformed cell, and/or pharmaceutical composition of claim 15, or the use according to any one of claims 16 to 17, wherein the human UBE3A short isoform is expressed at a ratio of about 1:1 to about 15:1 as compared to expression of the long isoform, optionally wherein the subject exhibits symptoms of the disease prior to delivery of the polynucleotide, expression cassette, vector, transformed cell, pharmaceutical composition, and/or the medicament, optionally wherein the polynucleotide, expression cassette, vector, transformed cell, pharmaceutical composition, and/or the medicament is to be delivered *in utero*, optionally wherein the subject is a human, optionally wherein the polynucleotide, expression cassette, vector, transformed cell, pharmaceutical composition, and/or the medicament is to be

delivered to the nervous system of the subject optionally at a dose of at least about 10^2, 10^3, 10^4, 10^5, 10^6, 10^7, 10^8, 10^9, 10^{10}, 10^{11}, 10^{12}, 10^{13}, 10^{14}, 10^{15} or 10^{16} viral genomes/kilogram, optionally from 10^8-10^{14} viral genomes/kilogram, optionally delivered by intravenous, intrathecal, intracerebral, intra-cisterna magna, intraparenchymal, intracerebroventricular, intranasal, intra-aural, intra-ocular, or peri-ocular delivery, or any combination thereof.