UBE3A Genes and Expression Cassettes and Their Use

STATEMENT OF PRIORITY

[0001] This application claims the benefit, under 35 U.S.C. § 119(e), of U.S. Provisional Application No. 62/851,411, filed on May 22, 2019, the entire contents of which are incorporated by reference herein.

STATEMENT REGARDING ELECTRONIC FILING OF A SEQUENCE LISTING

[0002] A Sequence Listing in ASCII text format, submitted under 37 C.F.R. § 1.821, entitled 5470-867WO_ST25.txt, 49,256 bytes in size, generated on May 22, 2020 and filed via EFS-Web, is provided in lieu of a paper copy. This Sequence Listing is hereby incorporated herein by reference into the specification for its disclosures.

FIELD OF THE INVENTION

[0003] This invention relates to polynucleotides comprising UBE3A open reading frame (ORF) sequences, vectors comprising the same, and methods of using the same for delivery of the ORF to a cell or a subject and to treat disorders associated with aberrant expression of a UBE3A gene or aberrant activity of a UBE3A gene product in the subject, such as Angelman Syndrome.

BACKGROUND OF THE INVENTION

[0004] Angelman Syndrome (AS) is a neurodevelopmental disorder affecting approximately 1:15,000 individuals. Individuals with AS have seizures, movement disorders, intellectual disability, and lack speech throughout life. Individuals usually present with ataxia during the first year of life. Loss of a functional UBE3A gene in neurons causes AS.

[0005] UBE3A is located on chromosome 15, and is the only gene within the 15q11-q13 region that exhibits biased expression from the maternal allele. The paternal allele is silenced in neurons due to paternal imprinting. Accordingly, both deletions of maternal 15q11-13 and loss-of-function mutations of maternal UBE3A cause AS (*see* **FIG. 1**). Point mutations that impair the E3 ubiquitin ligase function of UBE3A protein (also known as E6AP ubiquitin-protein ligase (E6AP)) are sufficient to produce the full spectrum of AS phenotypes, highlighting the importance of UBE3A to the pathogenesis of AS. The consensus is that

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 OF THE INVENTION

[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. Aspects 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 of the invention relates 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.

[0009] A further aspect of the invention relates to 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 invention.

[0010] Another aspect of the invention relates to a pharmaceutical formulation comprising the polynucleotide, expression cassette, vector, and/or transformed cell of the invention in a pharmaceutically acceptable carrier.

[0011] An additional aspect of the invention relates to 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 aspect of the invention relates 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 aspect of the invention relates to 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] A further aspect of the invention relates to 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] Another aspect of the invention relates to 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).

[0022] **FIG. 4** shows UBE3A-stained sagittal sections harvested from WT and AS model mice (*Ube3a*$^{m-/p+}$), AS mice exhibit a near complete loss of neuronal UBE3A expression (Jiang *et al*, Neuron, 1998:21(4):799-811).

[0023] **FIG. 5** shows the results of droplet digital PCR experiments to detect either endogenous mouse *Ube3a* transcripts (*mUbe3a*, left panel) or codon-optimized human *UBE3A* transcripts (*hUBE3A-opt*, right panel) reverse transcribed from mouse hippocampal mRNAs. Hippocampi were harvested from adult WT and AS mice following ICV injection of vehicle or AS gene therapy virus (PHP.B/hUBE3A) at P1. Note the selective detection of *hUBE3A-opt* in the gene therapy-treated AS + AAV group.

[0024] **FIG. 6** shows western blotting of P9 mouse whole-brain lysates using a UBE3A antibody that binds both mouse and human UBE3A equally, irrespective of isoform. In a WT sample (far left), a major band representing the short UBE3A isoform is juxtaposed with a minor band immediately upfield that corresponds to long UBE3A (long to short ratio, ~5:1). In a sample from maternal ISO3-KO mice (far right), which lack neuronal expression of short UBE3A, the long:short UBE3A ratio is drastically reduced, enabling very clear resolution of two discrete UBE3A isoform bands. Samples from homozygous ISO3-KO mice (second from left) yield only the long isoform band. Samples from PHP.B/hUBE3A-treated AS mice (second from right) express both short and long hUBE3A isoforms in a ratio of approximately 3:1, thus demonstrating the feasibility of the dual isoform approach.

[0025] **FIGS. 7A-7B** show immunohistochemistry of WT and AS model mice. **FIG. 7A** shows lateral to medial series of UBE3A-stained sagittal sections harvested from mice at varying postnatal ages (P10, P15, P25), following ICV injection of AS gene therapy virus (PHP.B/hUBE3A) at P1. **FIG. 7B** shows the efficiency of hUBE3A gene transfer within hippocampal area CA3 in high-magnification confocal micrographs. Arrows indicate rare neurons (NeuN+) not re-expressing UBE3A. UBE3A protein shows pronounced nuclear localization, consistent with dominant expression of the short hUBE3A isoform.

[0026] **FIG. 8** shows a cytoplasmic-to-nuclear shift in subcellular localization subsequent to hUBE3A gene transfer. **Panels A-C** show UBE3A-stained layer 2/3 neocortex from AS model mice at P10 (**Panel A**), P15 (**Panel B**), and P20 (**Panel C**) following ICV injection of PHP.B/hUBE3A virus at P1. A gradual accumulation of virally transduced hUBE3A in the nucleus is seen over development, faithfully recapitulating spatiotemporal patterns of endogenous UBE3A expression, as shown in **Panels D-F** from Judson *et al.*, J Comparative Neurology, 2014:522(8):1874-1896.

[0027] **FIG. 9** shows the monthly gross weight gain of male WT and AS model mice injected ICV at postnatal days 0-2 with either vehicle or AS gene therapy virus (PHP.B/hUBE3A). Body weights were statistically similar across the experimental groups at one month of age—WT + Vehicle, WT + AAV, AS + Vehicle, AS + AAV—demonstrating good tolerance of hUBE3A gene transfer and a lack of overt toxicity. By two months, and persisting into adulthood, AS + vehicle mice exhibited a statistically significant increase in weight gain. This phenotype was completely rescued in AS + AAV mice.

[0028] **FIG. 10** shows the results of behavioral testing in adult WT and AS model mice ICV injected with either vehicle (WT + Vehicle, AS + Vehicle) or PHP.B/hUBE3A AS gene therapy virus (WT + AAV, AS + AAV) at postnatal days 0-2. **Panel A** shows a schematic of the experimental timeline. **Panel B** summarizes open field experiments testing locomotor activity. Data are displayed both in 5-minute bins (left panel) and as the sum total of activity in a 1-hour recording session (right panel). There was no statistically significant decrease between AS + Vehicle and WT + Vehicle mice as previously reported, though a trend was evident, which did not appear to be rescued in AS + AAV mice. Notably, WT + AAV mice exhibit modest hyperactivity, a potential side effect of UBE3A overexpression. **Panel C** depicts results from the rotarod motor performance assay, which was conducted on two separate days: day 1, consisting of three acquisition trials; day 2, occurring 48 hours later, consisting of 2 retest trials. All trials were of 5 minutes duration. Unlike AS + Vehicle mice, AS + AAV mice significantly overcame their motor deficiencies during acquisition, approaching WT + Vehicle control group levels of performance, which were sustained during retest. **Panel D** shows results for marble burying, an assay that measures spontaneous digging and burrowing behavior. AS + Vehicle mice typically buried very few marbles during a 30-minute session, indicating they have a severe deficit in this ethologically relevant behavior. This phenotype was partially, if not fully, rescued in most AS + AAV mice. **Panel E** demonstrates that PHP.B/hUBE3A treatment significantly improved nest building—another innate behavior severely lacking in AS mice—in the AS + AAV group. PHP.B/hUBE3A-treated AS mice incorporated more of the available nesting material into their nests (left panel) and made nests of higher quality than their vehicle-treated counterparts.

[0029] **FIG. 11** shows the results of flurothyl kindling and rechallenge testing in adult WT and AS model mice ICV injected with either vehicle (WT + Vehicle, AS + Vehicle) or PHP.B/hUBE3A AS gene therapy virus (WT + AAV, AS + AAV) at postnatal days 0-2. **Panel A** shows a schematic of the experimental timeline. **Panel B** summarizes mean group latencies to myoclonic (left panel) and generalized seizure (right panel) over the 8-day

flurothyl kindling period and subsequent flurothyl rechallenge on day 36. AS + Vehicle mice trended strongly toward having decreased myoclonic seizure thresholds (MST) and generalized seizure thresholds (GST) upon rechallenge. Seizure thresholds were normalized in the AS + AAV group, demonstrating the potential for AS gene therapy to protect against exaggerated epileptogenesis.

[0030] **FIG. 12** shows anatomical correlates of enhanced seizure susceptibility following flurothyl seizure kindling. **Panel A** shows WFA staining of perineuronal nets (magenta) in sagittal sections of the dorsal hippocampus. Quantification of mean WFA fluorescence in the dentate gyrus confirmed that enhanced perineuronal net deposition in this structure was unique to AS + Vehicle mice. **Panel B** shows GFAP staining of astrocytes in sagittal sections spanning the hippocampus and cortex. Intensely stained astrocytes in the hippocampus are indicative of reactive astrogliosis—a hallmark of temporal lobe epilepsy—and were only observed in the AS + Vehicle group, as demonstrated by quantification of mean hippocampal GFAP fluorescence.

[0031] **FIG. 13** shows WT (native) coding sequences for UBE3A ORF that encodes both human UBE3A Isoform 3 and Isoform 1, with WT Kozak sequences (SEQ ID NO: 26). The Kozak sequences regulating the respective expressed isoforms are shown in bold, the translation start sites are underlined.

[0032] **FIG. 14** shows WT (native) coding sequences for UBE3A ORF that encodes both human UBE3A Isoform 3 and Isoform 1, with modified Kozak sequences (SEQ ID NO: 27). The Kozak sequences regulating the respective expressed isoforms are shown in bold, the translation start sites are underlined.

DETAILED DESCRIPTION OF THE INVENTION

[0033] The present invention is explained in greater detail below. This description is not intended to be a detailed catalog of all the different ways in which the invention may be implemented, or all the features that may be added to the instant invention. For example, features illustrated with respect to one embodiment may be incorporated into other embodiments, and features illustrated with respect to a particular embodiment may be deleted from that embodiment. In addition, numerous variations and additions to the various embodiments suggested herein will be apparent to those skilled in the art in light of the instant disclosure which do not depart from the instant invention. Hence, the following specification is intended to illustrate some particular embodiments of the invention, and not to exhaustively specify all permutations, combinations and variations thereof.

[0034] Unless the context indicates otherwise, it is specifically intended that the various features of the invention described herein can be used in any combination. Moreover, the present invention also contemplates that in some embodiments of the invention, any feature or combination of features set forth herein can be excluded or omitted. To illustrate, if the specification states that a complex comprises components A, B and C, it is specifically intended that any of A, B or C, or a combination thereof, can be omitted and disclaimed singularly or in any combination.

[0035] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. The terminology used in the description of the invention herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention.

[0036] Nucleotide sequences are presented herein by single strand only, in the 5' to 3' direction, from left to right, unless specifically indicated otherwise. Nucleotides and amino acids are represented herein in the manner recommended by the IUPAC-IUB Biochemical Nomenclature Commission, or (for amino acids) by either the one-letter code, or the three letter code, both in accordance with 37 C.F.R. §1.822 and established usage.

[0037] Except as otherwise indicated, standard methods known to those skilled in the art may be used for production of recombinant and synthetic polypeptides, antibodies or antigen-binding fragments thereof, manipulation of nucleic acid sequences, production of transformed cells, the construction of rAAV constructs, modified capsid proteins, packaging vectors expressing the AAV rep and/or cap sequences, and transiently and stably transfected packaging cells. Such techniques are known to those skilled in the art. *See, e.g.,* SAMBROOK *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL 2nd Ed. (Cold Spring Harbor, NY, 1989); F. M. AUSUBEL *et al.* CURRENT PROTOCOLS IN MOLECULAR BIOLOGY (Green Publishing Associates, Inc. and John Wiley & Sons, Inc., New York).

[0038] All publications, patent applications, patents, nucleotide sequences, amino acid sequences and other references mentioned herein are incorporated by reference in their entirety.

Definitions

[0039] As used in the description of the invention and the appended claims, the singular forms "a," "an" and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise.

[0040] As used herein, "and/or" refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative ("or").

[0041] Moreover, the present invention also contemplates that in some embodiments of the invention, any feature or combination of features set forth herein can be excluded or omitted.

[0042] Furthermore, the term "about," as used herein when referring to a measurable value such as an amount of a compound or agent of this invention, dose, time, temperature, and the like, is meant to encompass variations of ± 10%, ± 5%, ± 1%, ± 0.5%, or even ± 0.1% of the specified amount.

[0043] As used herein, the transitional phrase "consisting essentially of" is to be interpreted as encompassing the recited materials or steps and those that do not materially affect the basic and novel characteristic(s) of the claimed invention. Thus, the term "consisting essentially of" as used herein should not be interpreted as equivalent to "comprising."

[0044] The term "consists essentially of" (and grammatical variants), as applied to a polynucleotide or polypeptide sequence of this invention, means a polynucleotide or polypeptide that consists of both the recited sequence (*e.g.,* SEQ ID NO) and a total of ten or less (*e.g.,* 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) additional nucleotides or amino acids on the 5' and/or 3' or N-terminal and/or C-terminal ends of the recited sequence or between the two ends (*e.g.,* between domains) such that the function of the polynucleotide or polypeptide is not materially altered. The total of ten or less additional nucleotides or amino acids includes the total number of additional nucleotides or amino acids added together. The term "materially altered," as applied to polynucleotides of the invention, refers to an increase or decrease in ability to express the encoded polypeptide of at least about 50% or more as compared to the expression level of a polynucleotide consisting of the recited sequence. The term "materially altered," as applied to polypeptides of the invention, refers to an increase or decrease in biological activity of at least about 50% or more as compared to the activity of a polypeptide consisting of the recited sequence.

[0045] The term "parvovirus" as used herein encompasses the family *Parvoviridae*, including autonomously-replicating parvoviruses and dependoviruses. The autonomous

parvoviruses include members of the genera *Parvovirus*, *Erythrovirus*, *Densovirus*, *Iteravirus*, and *Contravirus*. Exemplary autonomous parvoviruses include, but are not limited to, minute virus of mouse, bovine parvovirus, canine parvovirus, chicken parvovirus, feline panleukopenia virus, feline parvovirus, goose parvovirus, H1 parvovirus, muscovy duck parvovirus, snake parvovirus, and B19 virus. Other autonomous parvoviruses are known to those skilled in the art. *See, e.g.,* FIELDS *et al.*, VIROLOGY, volume 2, chapter 69 (4th ed., Lippincott-Raven Publishers).

[0046] The genus *Dependovirus* contains the adeno-associated viruses (AAV), including but not limited to, AAV type 1, AAV type 2, AAV type 3 (including types 3A and 3B), AAV type 4, AAV type 5, AAV type 6, AAV type 7, AAV type 8, AAV type 9, AAV type 10, AAV type 11, AAV type 12, AAV type 13, avian AAV, bovine AAV, canine AAV, goat AAV, snake AAV, equine AAV, and ovine AAV. *See, e.g.,* FIELDS *et al.*, VIROLOGY, volume 2, chapter 69 (4th ed., Lippincott-Raven Publishers); and **Table 1**.

[0047] The term "adeno-associated virus" (AAV) in the context of the present invention includes without limitation AAV type 1, AAV type 2, AAV type 3 (including types 3A and 3B), AAV type 4, AAV type 5, AAV type 6, AAV type 7, AAV type 8, AAV type 9, AAV type 10, AAV type 11, avian AAV, bovine AAV, canine AAV, equine AAV, and ovine AAV and any other AAV now known or later discovered. *See, e.g.,* BERNARD N. FIELDS *et al.*, VIROLOGY, volume 2, chapter 69 (4th ed., Lippincott-Raven Publishers). A number of additional AAV serotypes and clades have been identified (*see, e.g.,* Gao *et al.*, (2004) *J. Virol.* 78:6381-6388 and **Table 1**), which are also encompassed by the term "AAV.

[0048] The parvovirus particles and genomes of the present invention can be from, but are not limited to, AAV. The genomic sequences of various serotypes of AAV and the autonomous parvoviruses, as well as the sequences of the native ITRs, Rep proteins, and capsid subunits are known in the art. Such sequences may be found in the literature or in public databases such as GenBank. *See, e.g.,* GenBank Accession Numbers NC_002077, NC_001401, NC_001729, NC_001863, NC_001829, NC_001862, NC_000883, NC_001701, NC_001510, NC_006152, NC_006261, AF063497, U89790, AF043303, AF028705, AF028704, J02275, J01901, J02275, X01457, AF288061, AH009962, AY028226, AY028223, AY631966, AX753250, EU285562, NC_001358, NC_001540, AF513851, AF513852 and AY530579; the disclosures of which are incorporated by reference herein for teaching parvovirus and AAV nucleic acid and amino acid sequences. *See also, e.g.,* Bantel-Schaal *et al.*, (1999) *J. Virol.* 73: 939; Chiorini *et al.*, (1997) *J. Virol.* 71:6823; Chiorini *et al.*, (1999) *J. Virol.* 73:1309; Gao *et al.*, (2002) *Proc. Nat. Acad. Sci. USA* 99:11854; Moris *et al.*,

(2004) *Virol.* 33-:375-383; Mori *et al.*, (2004) *Virol.* 330:375; Muramatsu *et al.*, (1996) *Virol.* 221:208; Ruffing *et al.*, (1994) *J. Gen. Virol.* 75:3385; Rutledge *et al.*, (1998) *J. Virol.* 72:309; Schmidt *et al.*, (2008) *J. Virol.* 82:8911; Shade *et al.*, (1986) *J. Virol.* 58:921; Srivastava *et al.*, (1983) *J. Virol.* 45:555; Xiao *et al.*, (1999) *J. Virol.* 73:3994; international patent publications WO 00/28061, WO 99/61601, WO 98/11244; and U.S. Patent No. 6,156,303; the disclosures of which are incorporated by reference herein for teaching parvovirus and AAV nucleic acid and amino acid sequences. *See also* **Table 1**. An early description of the AAV1, AAV2 and AAV3 ITR sequences is provided by Xiao, X., (1996), "Characterization of Adeno-associated virus (AAV) DNA replication and integration," Ph.D. Dissertation, University of Pittsburgh, Pittsburgh, PA (incorporated herein it its entirety).

[0049] A "chimeric" AAV nucleic acid capsid coding sequence or AAV capsid protein is one that combines portions of two or more capsid sequences. A "chimeric" AAV virion or particle comprises a chimeric AAV capsid protein.

[0050] The term "tropism" as used herein refers to preferential but not necessarily exclusive entry of the vector (*e.g.,* virus vector) into certain cell or tissue type(s) and/or preferential but not necessarily exclusive interaction with the cell surface that facilitates entry into certain cell or tissue types, optionally and preferably followed by expression (*e.g.,* transcription and, optionally, translation) of sequences carried by the vector contents (*e.g.,* viral genome) in the cell, *e.g.,* for a recombinant virus, expression of the heterologous nucleotide sequence(s). Those skilled in the art will appreciate that transcription of a heterologous nucleic acid sequence from the viral genome may not be initiated in the absence of trans-acting factors, *e.g.,* for an inducible promoter or otherwise regulated nucleic acid sequence. In the case of a rAAV genome, gene expression from the viral genome may be from a stably integrated provirus and/or from a non-integrated episome, as well as any other form which the virus nucleic acid may take within the cell.

[0051] The term "tropism profile" refers to the pattern of transduction of one or more target cells, tissues and/or organs. Representative examples of chimeric AAV capsids have a tropism profile characterized by efficient transduction of cells of the central nervous system (CNS) with only low transduction of peripheral organs (*see* e.g. US Patent No. 9,636,370 McCown *et al.*, and US patent publication 2017/0360960 Gray *et al.*). Vectors (*e.g.,* virus vectors, *e.g.,* AAV capsids) expressing specific tropism profiles may be referred to as "tropic" for their tropism profile, *e.g.,* neuro-tropic, liver-tropic, *etc.*

Table 1

AAV Serotypes/Isolates	GenBank Accession Number		AAV Serotypes/Isolates	GenBank Accession Number		AAV Serotypes/Isolates	GenBank Accession Number
Clonal Isolates			Hu S17	AY695376		Cy3	AY243019
Avian AAV ATCC VR-865	AY186198, AY629583, NC_004828		Hu T88	AY695375		Cy5	AY243017
Avian AAV strain DA-1	NC_006263, AY629583		Hu T71	AY695374		Rh13	AY243013
Bovine AAV	NC_005889, AY388617		Hu T70	AY695373			
AAV4	NC_001829		Hu T40	AY695372		**Clade E**	
AAV5	AY18065, AF085716		Hu T32	AY695371		Rh38	AY530558
Rh34	AY243001		Hu T17	AY695370		Hu66	AY530626
Rh33	AY243002		Hu LG15	AY695377		Hu42	AY530605
Rh32	AY243003					Hu67	AY530627
AAV10	AY631965		**Clade C**			Hu40	AY530603
AAV11	AY631966		**AAV 3**	NC_001729		Hu41	AY530604
AAV12	DQ813647		**AAV 3B**	NC_001863		Hu37	AY530600
AAV13	EU285562		Hu9	AY530629		Rh40	AY530559
			Hu10	AY530576		Rh2	AY243007
Clade A			Hu11	AY530577		Bb1	AY243023
AAV1	NC_002077, AF063497		Hu53	AY530615		Bb2	AY243022
AAV6	NC_001862		Hu55	AY530617		**Rh10**	AY243015
Hu.48	AY530611		Hu54	AY530616		Hu17	AY530582
Hu 43	AY530606		Hu7	AY530628		Hu6	AY530621
Hu 44	AY530607		Hu18	AY530583		Rh25	AY530557
Hu 46	AY530609		Hu15	AY530580		Pi2	AY530554
			Hu16	AY530581		Pi1	AY530553
Clade B			Hu25	AY530591		Pi3	AY530555
Hu19	AY530584		Hu60	AY530622		Rh57	AY530569
Hu20	AY530586		Ch5	AY243021		Rh50	AY530563
Hu23	AY530589		Hu3	AY530595		Rh49	AY530562
Hu22	AY530588		Hu1	AY530575		Hu39	AY530601
Hu24	AY530590		Hu4	AY530602		Rh58	AY530570
Hu21	AY530587		Hu2	AY530585		Rh61	AY530572
Hu27	AY530592		Hu61	AY530623		Rh52	AY530565
Hu28	AY530593					Rh53	AY530566
Hu29	AY530594		**Clade D**			Rh51	AY530564
Hu63	AY530624		Rh62	AY530573		Rh64	AY530574
Hu64	AY530625		Rh48	AY530561		Rh43	AY530560
Hu13	AY530578		Rh54	AY530567		**AAV8**	AF513852
Hu56	AY530618		Rh55	AY530568		Rh8	AY242997
Hu57	AY530619		Cy2	AY243020		Rh1	AY530556
Hu49	AY530612		**AAV7**	AF513851			
Hu58	AY530620		Rh35	AY243000		**Clade F**	
Hu34	AY530598		Rh37	AY242998		**AAV9** (Hu14)	AY530579
Hu35	AY530599		Rh36	AY242999		Hu31	AY530596
AAV2	NC_001401		Cy6	AY243016		Hu32	AY530597
Hu45	AY530608		Cy4	AY243018			
Hu47	AY530610						
Hu51	AY530613						
Hu52	AY530614						
Hu T41	AY695378						

[0052] The term "disorder associated with aberrant expression of a UBE3A gene" as used herein refers to a disease, disorder, syndrome, or condition that is caused by or a symptom of decreased or altered expression of the UBE3A gene in a subject relative to the expression level in a normal subject or in a population.

[0053] The term "disorder associated with aberrant activity of a UBE3A gene product" as used herein refers to a disease, disorder, syndrome, or condition that is caused by or a symptom of decreased or altered activity of the UBE3A gene product in a subject relative to the activity in a normal subject or in a population. In some embodiments, a disorder associated with aberrant activity of a UBE3A gene product may be Angelman Syndrome.

[0054] Angelman Syndrome (AS) is a monogenic disorder caused by deletions or mutations in the maternal UBE3A allele. The intact paternal allele is silenced in neurons of healthy and AS patients by the antisense DNA transcript *UBE3A-ATS*, as diagramed in **FIG. 1**. There are three human UBE3A protein isoforms (**FIG. 3A**). The shortest (*i.e.,* human isoform 1) has the unique capacity to localize to the nucleus and is expressed in excess of the two long isoforms—human isoforms 2 and 3—which are distinguished by amino acid extensions that align with the N-terminus of long mouse isoform 2 (Miao *et al*, J Neuroscience, 2013;33(1):327-333). Mice deficient for the short isoform (isoform 3 in mice; ISO3-KO) have been shown to broadly phenocopy electrophysiological and behavioral deficits in AS model mice (Avagliano *et al*., Nat Neurosci, 2019:22(8):1235-1247). More recently, the inventors demonstrated that ISO3-KO mice, similar to AS mice (Gu *et al*, J Clin Invest, 2019;129(1):163-168), exhibit enhanced sensitivity to seizure rechallenge following flurothyl kindling. In contrast, a complementary mouse model harboring a selective deletion of the long UBE3A isoform (isoform 2 in mice; ISO2-KO) has thus far failed to express any AS mouse phenotypes (**FIG. 2B**; Avagliano *et al*., Nat Neurosci, 2019:22(8):1235-1247). Recent clinical data similarly supports the critical importance of short UBE3A, but suggests that long UBE3A also contributes to healthy brain development and function, despite being expressed at lower levels (Sadhwani *et al*, Am J Med Genet A, 2018;176(7):1641-1647). While not wishing to be bound to theory, endogenous expression ratios of short and long UBE3A isoforms from an exogenously added expression cassette may be approximated by modulating the relative strength or weakness of the Kozak sequences governing their translation.

[0055] As used herein, a Kozak sequence refers to a nucleotide sequence comprising nucleotides upstream of, downstream of, and encompassing a start/initiation codon (*i.e.,* translation start site). The Kozak sequence is recognized by the ribosome as the translational

start site. Sequence variations (e.g., by natural and/or human-made modifications) from a known Kozak consensus sequence (e.g., of gccRccAUGG (SEQ ID NO:24)) can alter in strength of initiation (*see* **FIG. 3C** Kozak consensus sequence), wherein the underlined nucleotides indicate a start/initiation codon, uppercase letters indicate highly conserved bases, R indicates a conserved purine (most commonly adenine), and lowercase letters indicate common, yet variable, bases. The known consensus Kozak sequence is a consensus representative of the most common nucleotide at each position based on a multitude of human mRNAs; the native/wildtype Kozak sequence(s) of an individual gene and/or open reading frame may vary. As known in the art, a thymidine (T) nucleotide in a DNA sequence corresponds to (*e.g.,* is transcribed as) a uridine (U) nucleotide in a corresponding mRNA (*e.g.,* a DNA ATG start codon corresponds to an mRNA AUG, etc.). Any sequence disclosed herein is considered a disclosure as both a T and a U for DNA and RNA, respectively.

[0056] As used herein, "transduction" of a cell by a virus vector (*e.g.,* an AAV vector) means entry of the vector into the cell and transfer of genetic material into the cell by the incorporation of nucleic acid into the virus vector and subsequent transfer into the cell via the virus vector.

[0057] Unless indicated otherwise, "efficient transduction" or "efficient tropism," or similar terms, can be determined by reference to a suitable positive or negative control (*e.g.,* at least about 50%, 60%, 70%, 80%, 85%, 90%, 95% or more of the transduction or tropism, respectively, of a positive control or at least about 110%, 120%, 150%, 200%, 300%, 500%, 1000% or more of the transduction or tropism, respectively, of a negative control).

[0058] Similarly, it can be determined if a virus "does not efficiently transduce" or "does not have efficient tropism" for a target tissue, or similar terms, by reference to a suitable control. In particular embodiments, the virus vector does not efficiently transduce (*i.e.,* does not have efficient tropism for) tissues outside the CNS, *e.g.,* liver, kidney, gonads and/or germ cells. In particular embodiments, undesirable transduction of tissue(s) (*e.g.,* liver) is 20% or less, 10% or less, 5% or less, 1% or less, 0.1% or less of the level of transduction of the desired target tissue(s) (*e.g.,* CNS cells).

[0059] The terms "5' portion" and "3' portion" are relative terms to define a spatial relationship between two or more elements. Thus, for example, a "3' portion" of a polynucleotide indicates a segment of the polynucleotide that is downstream of another segment. The term "3' portion" is not intended to indicate that the segment is necessarily at the 3' end of the polynucleotide, or even that it is necessarily in the 3' half of the polynucleotide, although it may be. Likewise, a "5' portion" of a polynucleotide indicates a

segment of the polynucleotide that is upstream of another segment. The term "5' portion" is not intended to indicate that the segment is necessarily at the 5' end of the polynucleotide, or even that it is necessarily in the 5' half of the polynucleotide, although it may be.

[0060] As used herein, the term "polypeptide" encompasses both peptides and proteins, unless indicated otherwise.

[0061] A "polynucleotide," "nucleic acid," or "nucleotide sequence" may be of RNA, DNA or DNA-RNA hybrid sequences (including both naturally occurring and non-naturally occurring nucleotides), but is preferably either a single or double stranded DNA sequence.

[0062] The term "regulatory element" refers to a genetic element which controls some aspect of the expression of nucleic acid sequences. For example, a promoter is a regulatory element which facilitates the initiation of transcription of an operably linked coding region. Other regulatory elements are splicing signals, polyadenylation signals, termination signals, etc. The region in a nucleic acid sequence or polynucleotide in which one or more regulatory elements are found may be referred to as a "regulatory region."

[0063] As used herein with respect to nucleic acids, the term "operably linked" refers to a functional linkage between two or more nucleic acids. For example, a promoter sequence may be described as being "operably linked" to a heterologous nucleic acid sequence because the promoter sequence initiates and/or mediates transcription of the heterologous nucleic acid sequence. In some embodiments, the operably linked nucleic acid sequences are contiguous and/or are in the same reading frame.

[0064] The term "open reading frame (ORF)," as used herein, refers to the portion of a polynucleotide (*e.g.,* a gene) that encodes a polypeptide, and is inclusive of the initiation start site (*i.e.,* Kozak sequence) that initiates transcription of the polypeptide. The term "coding region" may be used interchangeably with open reading frame.

[0065] The term "codon-optimized," as used herein, refers to a gene coding sequence that has been optimized to increase expression by substituting one or more codons normally present in a coding sequence (for example, in a wildtype sequence, including, *e.g.,* a coding sequence for UBE3A) with a codon for the same amino acid. This is referred to as a synonymous codon. In this manner, the protein encoded by the gene is identical, but the underlying nucleobase sequence of the gene or corresponding mRNA is different. In some embodiments, the optimization substitutes one or more rare codons (that is, codons for tRNA that occur relatively infrequently in cells from a particular species) with synonymous codons that occur more frequently to improve the efficiency of translation. For example, in human codon-optimization one or more codons in a coding sequence are replaced by codons that

occur more frequently in human cells for the same amino acid. Codon optimization can also increase gene expression through other mechanisms that can improve efficiency of transcription and/or translation. Strategies include, without limitation, increasing total GC content (that is, the percent of guanines and cytosines in the entire coding sequence), decreasing CpG content (that is, the number of CG or GC dinucleotides in the coding sequence), removing cryptic splice donor or acceptor sites, and/or adding or removing ribosomal entry and/or initiation sites, such as Kozak sequences. Desirably, a codon-optimized gene exhibits improved protein expression, for example, the protein encoded thereby is expressed at a detectably greater level in a cell compared with the level of expression of the protein provided by the wildtype gene in an otherwise similar cell. Codon-optimization also provides the ability to distinguish a codon-optimized gene and/or corresponding mRNA from an endogenous gene and/or corresponding mRNA *in vitro* or *in vivo*.

[0066] The term "sequence identity," as used herein, has the standard meaning in the art. As is known in the art, a number of different programs can be used to identify whether a polynucleotide or polypeptide has sequence identity or similarity to a known sequence. Sequence identity or similarity may be determined using standard techniques known in the art, including, but not limited to, the local sequence identity algorithm of Smith & Waterman, *Adv. Appl. Math. 2*:482 (1981), by the sequence identity alignment algorithm of Needleman & Wunsch, *J. Mol. Biol. 48*:443 (1970), by the search for similarity method of Pearson & Lipman, *Proc. Natl. Acad. Sci. USA 85*:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Drive, Madison, WI), the Best Fit sequence program described by Devereux *et al.*, *Nucl. Acid Res. 12*:387 (1984), preferably using the default settings, or by inspection.

[0067] An example of a useful algorithm is PILEUP. PILEUP creates a multiple sequence alignment from a group of related sequences using progressive, pairwise alignments. It can also plot a tree showing the clustering relationships used to create the alignment. PILEUP uses a simplification of the progressive alignment method of Feng & Doolittle, *J. Mol. Evol. 35*:351 (1987); the method is similar to that described by Higgins & Sharp, *CABIOS 5*:151 (1989).

[0068] Another example of a useful algorithm is the BLAST algorithm, described in Altschul *et al.*, *J. Mol. Biol. 215*:403 (1990) and Karlin *et al., Proc. Natl. Acad. Sci. USA 90*:5873 (1993). A particularly useful BLAST program is the WU-BLAST-2 program which

was obtained from Altschul *et al.*, *Meth. Enzymol.*, *266*:460 (1996); blast.wustl/edu/blast/README.html. WU-BLAST-2 uses several search parameters, which are preferably set to the default values. The parameters are dynamic values and are established by the program itself depending upon the composition of the particular sequence and composition of the particular database against which the sequence of interest is being searched; however, the values may be adjusted to increase sensitivity.

[0069] An additional useful algorithm is gapped BLAST as reported by Altschul *et al., Nucleic Acids Res. 25*:3389 (1997).

[0070] A percentage amino acid sequence identity value is determined by the number of matching identical residues divided by the total number of residues of the "longer" sequence in the aligned region. The "longer" sequence is the one having the most actual residues in the aligned region (gaps introduced by WU-Blast-2 to maximize the alignment score are ignored).

[0071] In a similar manner, percent nucleic acid sequence identity is defined as the percentage of nucleotide residues in the candidate sequence that are identical with the nucleotides in the polynucleotide specifically disclosed herein.

[0072] The alignment may include the introduction of gaps in the sequences to be aligned. In addition, for sequences which contain either more or fewer nucleotides than the polynucleotides specifically disclosed herein, it is understood that in one embodiment, the percentage of sequence identity will be determined based on the number of identical nucleotides in relation to the total number of nucleotides. Thus, for example, sequence identity of sequences shorter than a sequence specifically disclosed herein, will be determined using the number of nucleotides in the shorter sequence, in one embodiment. In percent identity calculations relative weight is not assigned to various manifestations of sequence variation, such as insertions, deletions, substitutions, *etc.*

[0073] In one embodiment, only identities are scored positively (+1) and all forms of sequence variation including gaps are assigned a value of "0," which obviates the need for a weighted scale or parameters as described below for sequence similarity calculations. Percent sequence identity can be calculated, for example, by dividing the number of matching identical residues by the total number of residues of the "shorter" sequence in the aligned region and multiplying by 100. The "longer" sequence is the one having the most actual residues in the aligned region.

[0074] As used herein, an "isolated" nucleic acid or nucleotide sequence (*e.g.*, an "isolated DNA" or an "isolated RNA") means a nucleic acid or nucleotide sequence separated or

substantially free from at least some of the other components of the naturally occurring organism or virus, for example, the cell or viral structural components or other polypeptides or nucleic acids commonly found associated with the nucleic acid or nucleotide sequence.

[0075] Likewise, an "isolated" polypeptide means a polypeptide that is separated or substantially free from at least some of the other components of the naturally occurring organism or virus, for example, the cell or viral structural components or other polypeptides or nucleic acids commonly found associated with the polypeptide.

[0076] As used herein, the term "modified," as applied to a polynucleotide or polypeptide sequence, refers to a sequence that differs from a wildtype sequence due to one or more deletions, additions, substitutions, or any combination thereof.

[0077] As used herein, by "isolate" (or grammatical equivalents) a virus vector, it is meant that the virus vector is at least partially separated from at least some of the other components in the starting material.

[0078] By the term "treat," "treating," or "treatment of" (or grammatically equivalent terms) is meant to reduce or to at least partially improve or ameliorate the severity of the subject's condition and/or to alleviate, mitigate or decrease in at least one clinical symptom and/or to delay the progression of the condition.

[0079] As used herein, the term "prevent," "prevents," or "prevention" (and grammatical equivalents thereof) means to delay or inhibit the onset of a disease. The terms are not meant to require complete abolition of disease, and encompass any type of prophylactic treatment to reduce the incidence of the condition or delays the onset of the condition.

[0080] A "therapeutically effective" amount as used herein is an amount that is sufficient to provide some improvement or benefit to the subject. Alternatively stated, a "therapeutically effective" amount is an amount that will provide some alleviation, mitigation, decrease or stabilization in at least one clinical symptom in the subject. Those skilled in the art will appreciate that the therapeutic effects need not be complete or curative, as long as some benefit is provided to the subject.

[0081] A "prevention effective" amount as used herein is an amount that is sufficient to prevent and/or delay the onset of a disease, disorder and/or clinical symptoms in a subject and/or to reduce and/or delay the severity of the onset of a disease, disorder and/or clinical symptoms in a subject relative to what would occur in the absence of the methods of the invention. Those skilled in the art will appreciate that the level of prevention need not be complete, as long as some benefit is provided to the subject.

[0082] The term "heterologous" as used to describe a nucleic acid sequence or protein means that the nucleic acid or protein was derived from a different organism, or a different species of the same organism, than the host cell or subject in which it is expressed. The term "heterologous" when used with reference to a protein or a nucleic acid in a plasmid, expression cassette, or vector, indicates that the protein or the nucleic acid is present with another sequence or subsequence with which the protein or nucleic acid in question is not found in relationship to in nature. Typically the heterologous nucleic acid is a nucleic acid of interest that comprises an open reading frame that encodes a polypeptide, and/or comprises a nontranslated RNA, each of which may be referred to herein as a "transgene". The nucleic acid of interest may encode a therapeutic polypeptide or therapeutic RNA, or a diagnostic polypeptide or diagnostic RNA. The nucleic acid of interest/heterologous nucleic acid is often in the context of an expression cassette.

[0083] As the term is used herein, an "expression cassette" comprises the nucleic acid of interest operably linked/associated with appropriate regulatory element sequences (regulatory elements), for example, transcription/translation control signals, origins of replication, polyadenylation signals, internal ribosome entry sites (IRES), promoters, and/or enhancers, and the like. In some embodiments of the invention, the expression cassette comprises a promoter (e.g., eukaryotic) operably linked to the nucleic acid of interest, and optionally a (eukaryotic) transcription termination sequence. Typically the expression cassette is in the context of a vector (e.g., flanked by vector sequences). When in the context of a viral vector, it is typically flanked by viral packaging signals for viral vectors. For example, for an AAV viral vector, the packaging signals are at least one inverted terminal repeat which is located adjacent the expression cassette, optionally the 5' inverted terminal repeat (ITR) and the 3' ITR flank the expression cassette.

[0084] A "vector" refers to a compound used as a vehicle to carry foreign genetic material into another cell, where it can be replicated and/or expressed. A cloning vector containing foreign nucleic acid is termed a recombinant vector. Examples of nucleic acid vectors are plasmids, viral vectors, cosmids, and artificial chromosomes. Recombinant vectors typically contain an origin of replication, a multicloning site, and a selectable marker. The nucleic acid sequence typically consists of an insert (recombinant nucleic acid or transgene) and a larger sequence that serves as the "backbone" of the vector. The purpose of a vector which transfers genetic information to another cell is typically to isolate, multiply, or express the insert in the target cell. Expression vectors (expression constructs) are for the expression of the exogenous gene in the target cell, and generally have a promoter sequence that drives expression of the

exogenous gene/ORF. Insertion of a vector into the target cell is referred to transformation or transfection for bacterial and eukaryotic cells, although insertion of a viral vector is often called transduction. The term "vector" may also be used in general to describe items to that serve to carry foreign genetic material into another cell, such as, but not limited to, a transformed cell or a nanoparticle.

[0085] As used herein, the term "vector," "virus vector," "delivery vector" (and similar terms) in a specific embodiment generally refers to a virus particle that functions as a nucleic acid delivery vehicle, and which comprises the viral nucleic acid (*i.e.,* the vector genome) packaged within the virion. Virus vectors according to the present invention comprise a chimeric AAV capsid according to the invention and can package an AAV or rAAV genome or any other nucleic acid including viral nucleic acids. Alternatively, in some contexts, the term "vector," "virus vector," "delivery vector" (and similar terms) may be used to refer to the vector genome (*e.g.,* vDNA) in the absence of the virion and/or to a viral capsid that acts as a transporter to deliver molecules tethered to the capsid or packaged within the capsid.

[0086] The virus vectors of the invention can further be duplexed parvovirus particles as described in international patent publication WO 01/92551 (the disclosure of which is incorporated herein by reference in its entirety). Thus, in some embodiments, double stranded (duplex) genomes can be packaged.

[0087] A "recombinant AAV vector genome" or "rAAV genome" is an AAV genome (*i.e.,* vDNA) that comprises at least one inverted terminal repeat (*e.g.,* one, two or three inverted terminal repeats) and one or more heterologous nucleotide sequences. rAAV vectors generally retain the 145 base terminal repeat(s) (TR(s)) in *cis* to generate virus; however, modified AAV TRs and non-AAV TRs including partially or completely synthetic sequences can also serve this purpose. All other viral sequences are dispensable and may be supplied in *trans* (Muzyczka, (1992) *Curr. Topics Microbiol. Immunol.* 158:97). The rAAV vector optionally comprises two TRs (*e.g.,* AAV TRs), which generally will be at the 5' and 3' ends of the heterologous nucleotide sequence(s), but need not be contiguous thereto. The TRs can be the same or different from each other. The vector genome can also contain a single ITR at its 3' or 5' end.

[0088] The term "terminal repeat" or "TR" includes any viral terminal repeat or synthetic sequence that forms a hairpin structure and functions as an inverted terminal repeat (ITR) (*i.e.,* mediates the desired functions such as replication, virus packaging, integration and/or provirus rescue, and the like). The TR can be an AAV TR (referred to herein as an AAV ITR) or a non-AAV TR. For example, a non-AAV TR (referred to herein as a non-AAV ITR)

sequence such as those of other parvoviruses (*e.g.,* canine parvovirus (CPV), mouse parvovirus (MVM), human parvovirus B-19) or the SV40 hairpin that serves as the origin of SV40 replication can be used as a TR, which can further be modified by truncation, substitution, deletion, insertion and/or addition. Further, the TR (ITR)can be partially or completely synthetic, such as the "double-D sequence" as described in United States Patent No. 5,478,745 to Samulski *et al.*

[0089] Parvovirus genomes have palindromic sequences at both their 5' and 3' ends. The palindromic nature of the sequences leads to the formation of a hairpin structure that is stabilized by the formation of hydrogen bonds between the complementary base pairs. This hairpin structure is believed to adopt a "Y" or a "T" shape. *See*, *e.g.,* FIELDS *et al.*, VIROLOGY, volume 2, chapters 69 & 70 (4th ed., Lippincott-Raven Publishers).

[0090] An "AAV inverted terminal repeat" or "AAV ITR" may be from any AAV serotype or isolate, including but not limited to serotypes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10,11, 12, 13 or any other AAV now known or later discovered (*see, e.g.,* **Table 1**). An AAV ITR need not have the native ITR (*e.g.,* a native AAV ITR sequence may be altered by insertion, deletion, truncation and/or missense mutations), as long as the ITR mediates the desired functions, *e.g.,* replication, virus packaging, integration, and/or provirus rescue, and the like.

[0091] The terms "rAAV particle" and "rAAV virion" are used interchangeably here. A "rAAV particle" or "rAAV virion" comprises a rAAV vector genome packaged within an AAV capsid.

[0092] The virus vectors of the invention can further be "targeted" virus vectors (*e.g.,* having a directed tropism) and/or a "hybrid" parvovirus (*i.e.,* in which the viral ITRs and viral capsid are from different parvoviruses) as described in international patent publication WO 00/28004 and Chao *et al.*, (2000) *Mol. Therapy* 2:619.

[0093] Further, the viral capsid or genomic elements can contain other modifications, including insertions, deletions and/or substitutions.

[0094] As used herein, the term "amino acid" encompasses any naturally occurring amino acids, modified forms thereof, and synthetic amino acids, including non-naturally occurring amino acids..

[0095] Naturally occurring, levorotatory (L-) amino acids are shown in **Table 2**.

Table 2

Amino Acid Residue	Abbreviation	
	Three-Letter Code	One-Letter Code
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid (Aspartate)	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid (Glutamate)	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

[0096] Alternatively, the amino acid can be a modified amino acid residue (nonlimiting examples are shown in **Table 3**) or can be an amino acid that is modified by post-translation modification (*e.g.*, acetylation, amidation, formylation, hydroxylation, methylation, phosphorylation or sulfatation).

Table 3: Amino Acid Residue Derivatives

Modified Amino Acid Residue	Abbreviation
2-Aminoadipic acid	Aad
3-Aminoadipic acid	bAad
beta-Alanine, beta-Aminoproprionic acid	bAla
2-Aminobutyric acid	Abu
4-Aminobutyric acid, Piperidinic acid	4Abu
6-Aminocaproic acid	Acp
2-Aminoheptanoic acid	Ahe
2-Aminoisobutyric acid	Aib
3-Aminoisobutyric acid	bAib
2-Aminopimelic acid	Apm
t-butylalanine	t-BuA
Citrulline	Cit
Cyclohexylalanine	Cha
2,4-Diaminobutyric acid	Dbu
Desmosine	Des
2,2'-Diaminopimelic acid	Dpm
2,3-Diaminoproprionic acid	Dpr
N-Ethylglycine	EtGly

N-Ethylasparagine	EtAsn
Homoarginine	hArg
Homocysteine	hCys
Homoserine	hSer
Hydroxylysine	Hyl
Allo-Hydroxylysine	aHyl
3-Hydroxyproline	3Hyp
4-Hydroxyproline	4Hyp
Isodesmosine	Ide
allo-Isoleucine	aIle
Methionine sulfoxide	MSO
N-Methylglycine, sarcosine	MeGly
N-Methylisoleucine	MeIle
6-N-Methyllysine	MeLys
N-Methylvaline	MeVal
2-Naphthylalanine	2-Nal
Norvaline	Nva
Norleucine	Nle
Ornithine	Orn
4-Chlorophenylalanine	Phe(4-Cl)
2-Fluorophenylalanine	Phe(2-F)
3-Fluorophenylalanine	Phe(3-F)
4-Fluorophenylalanine	Phe(4-F)
Phenylglycine	Phg
Beta-2-thienylalanine	Thi

[0097] Further, the non-naturally occurring amino acid can be an "unnatural" amino acid as described by Wang *et al.*, (2006) *Annu. Rev. Biophys. Biomol. Struct.* 35:225-49. These unnatural amino acids can advantageously be used to chemically link molecules of interest to the AAV capsid protein.

[0098] The term "template" or "substrate" is used herein to refer to a polynucleotide sequence that may be replicated to produce the parvovirus viral DNA. For the purpose of vector production, the template will typically be embedded within a larger nucleotide sequence or construct, including but not limited to a plasmid, naked DNA vector, bacterial artificial chromosome (BAC), yeast artificial chromosome (YAC) or a viral vector (*e.g.*, adenovirus, herpesvirus, Epstein-Barr Virus, AAV, baculoviral, retroviral vectors, and the like). Alternatively, the template may be stably incorporated into the chromosome of a packaging cell.

[0099] As used herein, parvovirus or AAV "Rep coding sequences" indicate the nucleic acid sequences that encode the parvoviral or AAV non-structural proteins that mediate viral replication and the production of new virus particles. The parvovirus and AAV replication genes and proteins have been described in, *e.g.*, FIELDS *et al.*, VIROLOGY, volume 2, chapters 69 & 70 (4th ed., Lippincott-Raven Publishers).

[0100] The "Rep coding sequences" need not encode all of the parvoviral or AAV Rep proteins. For example, with respect to AAV, the Rep coding sequences do not need to encode all four AAV Rep proteins (Rep78, Rep 68, Rep52 and Rep40), in fact, it is believed that AAV5 only expresses the spliced Rep68 and Rep40 proteins. In representative embodiments, the Rep coding sequences encode at least those replication proteins that are necessary for viral genome replication and packaging into new virions. The Rep coding sequences will generally encode at least one large Rep protein (*i.e.,* Rep78/68) and one small Rep protein (*i.e.,* Rep52/40). In particular embodiments, the Rep coding sequences encode the AAV Rep78 protein and the AAV Rep52 and/or Rep40 proteins. In other embodiments, the Rep coding sequences encode the Rep68 and the Rep52 and/or Rep40 proteins. In a still further embodiment, the Rep coding sequences encode the Rep68 and Rep52 proteins, Rep68 and Rep40 proteins, Rep78 and Rep52 proteins, or Rep78 and Rep40 proteins.

[0101] As used herein, the term "large Rep protein" refers to Rep68 and/or Rep78. Large Rep proteins of the claimed invention may be either wildtype or synthetic. A wildtype large Rep protein may be from any parvovirus or AAV, including but not limited to serotypes 1, 2, 3a, 3b, 4, 5, 6, 7, 8, 9, 10, 11, or 13, or any other AAV now known or later discovered (*see, e.g.,* **Table 1**). A synthetic large Rep protein may be altered by insertion, deletion, truncation and/or missense mutations.

[0102] Those skilled in the art will further appreciate that it is not necessary that the replication proteins be encoded by the same polynucleotide. For example, for MVM, the NS-1 and NS-2 proteins (which are splice variants) may be expressed independently of one another. Likewise, for AAV, the p19 promoter may be inactivated and the large Rep protein(s) expressed from one polynucleotide and the small Rep protein(s) expressed from a different polynucleotide. Typically, however, it will be more convenient to express the replication proteins from a single construct. In some systems, the viral promoters (*e.g.,* AAV p19 promoter) may not be recognized by the cell, and it is therefore necessary to express the large and small Rep proteins from separate expression cassettes. In other instances, it may be desirable to express the large Rep and small Rep proteins separately, *i.e.,* under the control of separate transcriptional and/or translational control elements. For example, it may be desirable to control expression of the large Rep proteins, so as to decrease the ratio of large to small Rep proteins. In the case of insect cells, it may be advantageous to down-regulate expression of the large Rep proteins (*e.g.,* Rep78/68) to avoid toxicity to the cells (*see, e.g.,* Urabe *et al.*, (2002) *Human Gene Therapy* 13:1935).

[0103] As used herein, the parvovirus or AAV "cap coding sequences" encode the structural proteins that form a functional parvovirus or AAV capsid (*i.e.,* can package DNA and infect target cells). Typically, the cap coding sequences will encode all of the parvovirus or AAV capsid subunits, but less than all of the capsid subunits may be encoded as long as a functional capsid is produced. Typically, but not necessarily, the cap coding sequences will be present on a single nucleic acid molecule.

[0104] The capsid structure of autonomous parvoviruses and AAV are described in more detail in BERNARD N. FIELDS *et al.*, VIROLOGY, volume 2, chapters 69 & 70 (4th ed., Lippincott-Raven Publishers).

[0105] By "substantially retain" a property, it is meant that at least about 75%, 85%, 90%, 95%, 97%, 98%, 99% or 100% of the property (*e.g.,* activity or other measurable characteristic) is retained.

UBE3A Expression Cassettes and Vectors

[0106] The present invention relates to the design of a UBE3A expression cassette to provide therapeutic levels of intracellular (*e.g.,* non-secretory) expression of UBE3A protein (also known as E6AP ubiquitin-protein ligase (E6AP)), the enzyme encoded by the UBE3A gene, and the use of the expression cassette to achieve therapeutic levels of UBE3A/E6AP in a subject.

[0107] Thus, one aspect of the present invention relates to a polynucleotide comprising a mammalian UBE3A open reading frame. The open reading frame is the portion of the UBE3A gene that encodes UBE3A/E6AP. In some embodiments, the mammalian UBE3A open reading frame may be a human UBE3A open reading frame. The human UBE3A open reading frame may encode one or more isoforms of human UBE3A in the same reading frame. For example, in some embodiments, the mammalian UBE3A open reading frame may be a human UBE3A short isoform (*i.e.,* human UBE3A isoform 1), and/or a human UBE3A long isoform (*e.g.,* human UBE3A isoform 2 and/or 3) open reading frame. In some embodiments, the mammalian UBE3A open reading frame may be a human UBE3A isoform 1 and isoform 3 open reading frame. In other embodiments, the mammalian UBE3A open reading frame may be a human UBE3A isoform 1 and isoform 2 open reading frame. As used herein, a mammalian UBE3A open reading frame refers to a nucleotide sequence that encodes mammalian UBE3A/E6AP, *e.g.,* a human UBE3A open reading frame refers to a nucleotide sequence that encodes a human UBE3A/E6AP. Where appropriate for function, as the term UBE3A open reading frame is used herein to refer to an open reading frame that

encodes both a human UBE3A short isoform and a human UBE3A long isoform, the open reading frame includes 5' upstream nucleic acid sequences that encompass a Kozak sequence that influences expression of the long isoform. Such an open reading frame is shown in **FIG. 13** and **FIG. 14**.

SEQ ID NO:1. Wildtype human UBE3A short isoform (isoform 1; GenBank accession # NM_130838.3)

GCTGCCTGCCGGGATACTCGGCCCGCCCAGCCAGTCCTCCCGTCTTGCGCCGCGGCCGCGAGATCCGTGT
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ACCCGACACACCAGGCTTCGCTCGCTCGCGCACCCCTCCGCCGCGTAGCCATCCGCCAGCGCGGGCGCCC
GCCATCCGCCGCCTACTTACGCTTCACCTCTGCCGACCCGGCGCGCTCGGCTGCGGGCGGCGGCGCCTCC
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SEQ ID NO:2. Wildtype human UBE3A long isoform (isoform 2; GenBank accession # NM_000462.5)

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SEQ ID NO:3. Wildtype human UBE3A long isoform (isoform 3; GenBank accession # NM_130839.4)

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ATTAAAGTGAGGAGAAATTAAATACTCTGAGGGCGTTTTATATAATAAATTCGTGAAGAAATGTGTGCTC
TTCA

[0108] While not wishing to be bound to theory, analysis of UBE3A isoform expression in human brain tissue indicated that short UBE3A isoform 1 is expressed at a ratio of at least 4:1, and up to 8:1, as compared to long UBE3A isoforms, depending on the specific brain region assayed (Mele *et al*, Science, 2015; 348(6235):660-665. Thus, in some embodiments, the mammalian UBE3A open reading frame may comprise one or more Kozak sequences that is modified from a WT or control, *e.g.,* comprises, consists essentially of, or consists of a polynucleotide sequence in which 1, 2, 3, 4, 5, or more nucleotides have been substituted, added, and/or deleted compared to a WT or control, *e.g.,* the consensus Kozak sequence and/or a wildtype UBE3A Kozak sequence(s). In some embodiments, the mammalian UBE3A open reading frame may comprise one or more Kozak sequences comprising nucleotide modifications (*e.g.,* substitutions) that reduce and/or enhance initiation activity of one or more start codons (*i.e.,* one or more initiation codons, also known as translation start sites) in the open reading frame. In some embodiments, the mammalian UBE3A open reading frame may comprise one or more Kozak sequences comprising nucleotide modifications (*e.g.,* substitutions) that reduce initiation activity of one or more start codons (*e.g.,* reduce expression of an isoform) by 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or more as compared to a control, *e.g.,* the consensus Kozak sequence, or *e.g.,* a wildtype (*e.g.,* unmodified) UBE3A Kozak sequence. In some embodiments, the mammalian UBE3A open reading frame may comprise one or more Kozak sequences comprising nucleotide modifications (*e.g.,* substitutions) that enhance initiation activity of one or more start codons (*e.g.,* enhance expression of an isoform) by 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, 100%, 150%, 200% or more as compared to a control, *e.g.,* the consensus Kozak sequence, or *e.g.,* a wildtype (*e.g.,* unmodified) UBE3A Kozak sequence. In some embodiments, the mammalian UBE3A open reading frame may comprise one or more nucleotide modifications to provide expression of a short isoform at a ratio of about 1:1 to about 15:1 as compared to expression of a long isoform, *e.g.,* a ratio of about 1:1, 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, 11:1, 12:1, 13:1, 14:1, or 15:1 or any value or range therein. For example, in some embodiments, the mammalian (*e.g.,* human) UBE3A open reading frame may comprise one or more nucleotide modifications to provide expression of a short isoform at a ratio of about 2:1 to about 4.5:1,

about 1:1 to about 10:1, about 3:1 to about 8.5:1, or about 3:1 to about 4:1 as compared to expression of a long isoform. For example, in some embodiments, the mammalian (*e.g.*, human) UBE3A open reading frame may comprise one or more nucleotide modifications to provide expression of a short isoform at a ratio of about 2.5:1, about 3:1, about 4:1, about 6.5:1, about 8:1, or about 10:1 as compared to expression of a long isoform.

[0109] In some embodiments, the mammalian UBE3A open reading frame comprises a first Kozak sequence comprising, consisting essentially of, or consisting of the nucleotide sequence of SEQ ID NO:6 or a sequence at least about 50% identical thereto, *e.g.,* at least about 50, 55, 60, 65, 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical thereto. In some embodiments, the first Kozak sequence (e.g., SEQ ID NO: 6) is operably linked to isoform 3 or isoform 2, such as by replacing or modifying the WT isoform 3 or WT isoform 2 Kozak sequence (SEQ ID NO: 5 or 4 respectively).

SEQ ID NO:4. Wildtype human UBE3A long isoform 2 Kozak sequence (start codon in underline).
UGCAGGAUGG

SEQ ID NO:5. Wildtype human UBE3A long isoform 3 Kozak sequence (start codon in underline).
CACCGAAUGG

SEQ ID NO:6. Modified human UBE3A first Kozak sequence (start codon in underline) with reduced initiation activity.
UUUUUUAUGG

[0110] In some embodiments, the mammalian UBE3A open reading frame comprises a second Kozak sequence comprising, consisting essentially of, or consisting of the nucleotide sequence of SEQ ID NO:8 or a sequence at least about 50% identical thereto, *e.g.,* at least about 50, 55, 60, 65, 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical thereto. In some embodiments, the second Kozak sequence (e.g., SEQ ID NO: 8) is operably linked to short isoform 1 such as by replacing or modifying the WT human UBE3A isoform 1 Kozak sequences (SEQ ID NO: 7).

SEQ ID NO:7. Wildtype human UBE3A short isoform (isoform 1) Kozak sequence (start codon in underline).
AGCCGAAUGA

SEQ ID NO:8. Modified human UBE3A second Kozak sequence (start codon in underline) with enhanced initiation activity.
AGCAGGAUGA

[0111] In some embodiments, the only codon optimized nucleotide sequences of the mammalian UBE3A open reading frame is to one or both Kozak sequences, such as is shown in **FIG. 14** for human UBE3A. In some embodiments, additional (in addition to the Kozak sequences) codon optimized nucleotide sequences are within the mammalian UBE3A open reading frame, to influence expression in mammalian cells. The term "mammal" as used herein includes, but is not limited to, humans, primates, non-human primates (*e.g.,* monkeys and baboons), cattle, sheep, goats, pigs, horses, cats, dogs, rabbits, rodents (*e.g.,* rats, mice, hamsters, and the like), *etc.* Codon optimization is a technique well known in the art and optimal codons for expression in different species are known. The use of a codon-optimized UBE3A sequence allows one to distinguish expression of the transduced sequence from expression of the endogenous UBE3A sequence in a subject. In some embodiments, a polynucleotide comprising a human UBE3A open reading frame of the present invention may be codon-optimized for expression in human cells.

[0112] In some embodiments, a codon-optimized UBE3A open reading frame comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO:9, SEQ ID NO: 26 or SEQ ID NO: 27, or a sequence at least about 70% identical thereto, *e.g.,* at least about 70, 75, 76, 77, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical thereto. In some embodiments, one or both of the Kozak sequences in SEQ ID NO:27 (**FIG. 14**) are not further modified, while the other nucleotide sequences are further codon optimized to generate a UBE3A open reading frame that is at least about 70% identical to the respective sequences in SEQ ID NO: 27 (**FIG. 14**), *e.g.,* at least about 70, 75, 76, 77, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical, when omitting the Kozak sequences from the comparison, thereby preserving the Kozak sequence (SEQ ID NO: 8) operably linked to short isoform 1 and/or the Kozak sequence (SEQ ID NO: 6) is operably linked to isoform 3 or isoform 2.

SEQ ID NO:9. Human codon-optimized UBE3A isoform 1 and isoform 3 open reading frame (hUBE3A1>>3)

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ATGGCCACAGCTTGTAAAAGATCAGGAGAACCTCAGTCTGACGACATTGAAGCTAGCAGGATGAAGAGAGCCGCC
GCAAAGCACCTGATCGAACGCTACTACCATCAGCTCACCGAGGGTTGCGGCAACGAAGCGTGTACCAACGAGTTC
TGTGCCTCCTGCCCCACGTTCCTGCGGATGGATAACAATGCCGCCGCAATCAAGGCGCTTGAACTGTATAAGATC
AACGCCAAGCTGTGCGATCCCCACCCTTCCAAGAAGGGAGCCAGCTCAGCCTACCTGGAAAACTCCAAGGGCGCC
CCTAACAACTCATGCTCCGAGATCAAGATGAATAAGAAGGGCGCCCGGATTGACTTCAAGGATGTGACCTACCTG
ACCGAGGAGAAGGTGTACGAGATCCTGGAACTCTGCCGAGAACGGGAGGACTACTCCCCTCTGATCCGCGTGATC
GGAAGAGTGTTCAGCTCCGCTGAAGCGCTCGTGCAGTCGTTCAGAAAGGTCAAGCAGCACACTAAGGAAGAACTC
AAGTCCCTGCAGGCCAAAGATGAGGATAAGGACGAAGATGAAAAAGAAAAGGCCGCCTGCTCAGCTGCCGCGATG
GAAGAAGATTCAGAGGCCTCCAGCAGCAGGATTGGCGACAGCTCCCAGGGGGACAACAACCTCCAGAAGCTGGGT
CCAGACGATGTGTCGGTGGACATCGACGCCATTCGGAGAGTGTACACCCGACTGCTCTCGAACGAAAAGATCGAG
ACTGCATTCCTGAACGCCCTTGTCTACCTGAGCCCGAACGTGGAATGCGACCTTACTTACCATAACGTCTACTCC
CGGGACCCAAACTACCTGAACCTGTTCATCATCGTGATGGAAAACCGGAACCTCCATTCCCCCGAGTACCTGGAA
ATGGCCCTGCCCCTGTTCTGCAAGGCCATGTCAAAGCTTCCGCTGGCCGCACAAGGAAAGCTGATCCGCTTGTGG
TCCAAGTACAACGCGGACCAGATCAGACGCATGATGGAAACGTTCCAGCAACTGATTACTTACAAGGTCATCTCC
AATGAGTTCAACTCCCGGAATCTTGTGAACGACGATGATGCCATTGTGGCCGCCTCCAAATGCCTGAAGATGGTC
TACTATGCGAACGTCGTGGGCGGGGAAGTCGATACCAACCACAACGAGGAGGACGACGAGGAACCTATCCCTGAG
TCATCAGAACTGACTCTGCAAGAACTGCTGGGCGAAGAACGCCGGAACAAGAAGGGCCCAAGAGTCGACCCGTTG
GAAACCGAACTGGGAGTCAAAACCCTGGACTGCAGAAAGCCTCTGATCCCGTTCGAAGAGTTCATCAACGAACCC
CTGAACGAGGTGCTGGAAATGGACAAGGACTACACCTTCTTCAAGGTCGAAACCGAGAACAAGTTCTCTTTCATG
ACTTGCCCGTTCATTCTGAACGCAGTGACCAAGAACCTGGGCCTCTACTACGACAACCGCATCCGCATGTACAGC
GAACGCCGCATCACCGTGCTGTACTCCCTGGTGCAAGGCCAACAGCTGAACCCCTACCTTCGCCTGAAAGTCCGC
CGCGACCATATCATTGATGACGCTCTCGTGCGCCTTGAGATGATCGCGATGGAGAACCCCGCAGATCTGAAGAAG
CAGCTCTACGTCGAGTTCGAAGGAGAACAGGGGGTGGACGAAGGAGGAGTGTCCAAGGAGTTCTTTCAGCTCGTG
GTGGAGGAAATCTTTAACCCTGACATCGGAATGTTCACCTATGACGAATCCACAAAGCTGTTTTGGTTTAACCCG
TCCTCGTTCGAAACTGAGGGTCAATTCACCCTCATCGGCATTGTGCTGGGACTCGCCATCTACAACAATTGCATC
CTCGACGTGCACTTCCCGATGGTGGTGTACCGCAAACTGATGGGCAAAAAGGGAACCTTCAGAGATCTGGGAGAC
TCACACCCGGTGCTGTACCAGTCGCTCAAGGACTTGTTGGAATACGAAGGGAACGTGGAAGATGATATGATGATT
ACCTTCCAAATCTCGCAGACTGACTTGTTTGGAAACCCTATGATGTACGACCTGAAGGAGAATGGAGACAAGATC
CCGATCACGAACGAAAACCGCAAGGAGTTCGTCAACCTGTACTCCGACTATATTCTGAACAAGAGCGTGGAGAAG
CAGTTTAAGGCTTTCCGCCGGGGATTCCACATGGTCACCAACGAAAGCCCGCTCAAGTACCTCTTTCGGCCCGAA
GAGATTGAACTGCTGATCTGCGGGTCGAGGAATCTGGACTTCCAGGCCCTTGAAGAGACTACTGAGTACGACGGA
GGCTATACCCGGGACTCCGTGCTGATAAGAGAGTTCTGGGAGATCGTGCACTCCTTCACCGATGAGCAGAAGCGG
CTGTTCCTCCAATTCACCACCGGCACTGACAGAGCGCCAGTCGGAGGACTGGGGAAGCTGAAGATGATCATTGCG
AAGAACGGTCCCGACACTGAGAGGTTGCCCACTTCCCACACTTGTTTCAACGTGCTGCTGCTCCCGGAGTACTCC
TCCAAGGAGAAGCTGAAGGAACGGCTCCTGAAGGCCATTACATACGCCAAAGGTTTCGGCATGCTTTAATGA
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[0113] Another aspect of the invention relates to an expression cassette comprising a polynucleotide comprising a mammalian (*e.g.,* human) UBE3A open reading frame of the present invention. In certain embodiments, the polynucleotide is a human codon-optimized sequence, *e.g.,* a polynucleotide comprising the nucleotide sequence of SEQ ID NO:9, or a sequence at least about 70% identical thereto, *e.g.,* at least about 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical thereto. In various embodiments of the invention described herein, the UBE3A nucleotide sequence of SEQ ID NO: 9 further comprises additional 5' base pair sequences (e.g., 6 thymines to result in the Modified Kozak sequence of SEQ ID NO: 6) to include a first Kozak sequence.

[0114] The UBE3A open reading frame in the expression cassette may be operably linked to one or more expression elements that may modify (i.e., enhance, or reduce, or limit)

expression of UBE3A and/or E6AP, such as promoters, enhancers, polyadenylation signals, localization signals, and/or other expression elements known in the art.

[0115] In some embodiments, the polynucleotide is operably linked to a promoter, *e.g.,* a neuron-specific promoter, *e.g.,* human synapsin (hSYN), neuron-specific enolase (NSE), tubulin alpha 1 (Ta1), MeCP2 (MeP).

[0116] In some embodiments, the polynucleotide is operably linked to a human synapsin promoter, *e.g.,* a promoter comprising, consisting essentially of, or consisting of the nucleotide sequence of SEQ ID NO:10 or a sequence at least about 70% identical thereto, *e.g.,* at least about 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical thereto.

SEQ ID NO:10. Human synapsin (hSYN) promoter

GGTACCAGGGCCCTGCGTATGAGTGCAAGTGGGTTTTAGGACCAGGATGAGGCG
GGGTGGGGGTGCCTACCTGACGACCGACCCCGACCCACTGGACAAGCACCCAAC
CCCCATTCCCCAAATTGCGCATCCCCTATCAGAGAGGGGGAGGGGAAACAGGAT
GCGGCGAGGCGCGTGCGCACTGCCAGCTTCAGCACCGCGGACAGTGCCTTCGCC
CCCGCCTGGCGGCGCGCCACCGCCGCCTCAGCACTGAAGGCGCGCTGACGTC
ACTCGCCGGTCCCCCGCAAACTCCCCTTCCCGGCCACCTTGGTCGCGTCCGCGCC
GCCGCCGGCCCAGCCGGACCGCACCACGCGAGGCGCGAGATAGGGGGGCACGG
GCGCGACCATCTGCGCTGCGGCGCCGGCGACTCAGCGCTGCCTCAGTCTGCGGTG
GGCAGCGGAGGAGTCGTGTCGTGCCTGAGAGCGCAGTC

[0117] In some embodiments, the UBE3A open reading frame is operably linked to a polyadenylation signal, *e.g.,* a bovine growth hormone (bGH) polyadenylation signal (bGHpA), *e.g.,* a polyadenylation signal comprising, consisting essentially of, or consisting of the nucleotide sequence of SEQ ID NO:11 or a sequence at least about 70% identical thereto, *e.g.,* at least about 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical thereto.

SEQ ID NO:11. Bovine growth hormone polyadenylation signal (bGHpA)

CTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTT
CCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAAT
TGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAG
GACAGCAAGGGGGAGGATTGGGAAGACAACAGCAGGCATGCTGGGGATGCGGT
GGGCTCTATGGCTTCTGAGGCGGAAAGAACCAGCT

[0118] In some embodiments, the UBE3A open reading frame is operably linked to a polyadenylation signal, *e.g.,* a synthetic polyA (SpA), *e.g.,* a polyadenylation signal comprising, consisting essentially of, or consisting of the nucleotide sequence of SEQ ID

NO:12 or a sequence at least about 70% identical thereto, *e.g.,* at least about 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical thereto.

SEQ ID NO:12. Synthetic polyadenylation signal (SpA)
AATAAAGAGCTCAGATGCATCGATCAGAGTGTGTTGGTTTTTTGTGTG

[0119] In some embodiments, the UBE3A open reading frame is operably linked to a polyadenylation signal, *e.g.,* a simian virus 40 (SV40) polyadenylation signal (SV40pA), *e.g.,* a polyadenylation signal comprising, consisting essentially of, or consisting of the nucleotide sequence of SEQ ID NO:13 or a sequence at least about 70% identical thereto, *e.g.,* at least about 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical thereto.

SEQ ID NO:13. SV40 polyadenylation signal (SV40pA)
AGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTG
AAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTA
TAAGCTGCAATAAACAAGTTAACAACAACAATT

[0120] Those skilled in the art will further appreciate that a variety of promoter/enhancer elements may be used depending on the level and tissue-specific expression desired. The promoter/enhancer may be constitutive or inducible, depending on the pattern of expression desired. The promoter/enhancer may be native or foreign and can be a natural or a synthetic sequence. By foreign, it is intended that the transcriptional initiation region is not found in the wildtype host into which the transcriptional initiation region is introduced.

[0121] Promoter/enhancer elements can be native to the target cell or subject to be treated and/or native to the heterologous nucleic acid sequence. The promoter/enhancer element is generally chosen so that it will function in the target cell(s) of interest. In representative embodiments, the promoter/enhancer element is a mammalian promoter/enhancer element. The promoter/enhance element may be constitutive or inducible.

[0122] Inducible expression control elements are generally used in those applications in which it is desirable to provide regulation over expression of the heterologous nucleic acid sequence(s). Inducible promoters/enhancer elements for gene delivery can be tissue-specific or tissue-preferred promoter/enhancer elements, and include muscle specific or preferred (including cardiac, skeletal and/or smooth muscle), neural tissue specific or preferred (including brain-specific), eye (including retina-specific and cornea-specific), liver specific or

preferred, bone marrow specific or preferred, pancreatic specific or preferred, spleen specific or preferred, and lung specific or preferred promoter/enhancer elements. Other inducible promoter/enhancer elements include hormone-inducible and metal-inducible elements. Exemplary inducible promoters/enhancer elements include, but are not limited to, a Tet on/off element, a RU486-inducible promoter, an ecdysone-inducible promoter, a rapamycin-inducible promoter, and a metallothionein promoter.

[0123] In embodiments wherein the UBE3A open reading frame is transcribed and then translated in the target cells, specific initiation signals are generally employed for efficient translation of inserted protein coding sequences. These exogenous translational control sequences, which may include the ATG initiation codon (*i.e.,* translation start site) and adjacent sequences, can be of a variety of origins, both natural and synthetic. Non-limiting examples of these additional exogenous translational control sequences include Kozak sequences (*e.g.,* additional Kozak consensus sequences).

[0124] In certain embodiments, the expression cassette further comprises at least one adeno-associated virus (AAV) inverted terminal repeat (ITR), *e.g.,* two AAV ITRs. The two ITRs may have the same nucleotide sequence or different nucleotide sequences. The AAV ITRs may be from any AAV serotype or isolate (e.g., AAV type 1, AAV type 2, AAV type 3 (including types 3A and 3B), AAV type 4, AAV type 5, AAV type 6, AAV type 7, AAV type 8, AAV type 9, AAV type 10, AAV type 11, AAV type 12, AAV type 13, or any shown in Table 1). In some embodiments, the ITR(s) is/are from AAV2.. Each ITR independently may be the wildtype sequence or a modified sequence. In some embodiments, a modified ITR may have a D-element deletion (WO 01/92551). A D-element deletion is defined as the removal of that portion of the ITR known as the D-element. The D-element can be alternatively referred to or known as a D region, or D sequence, and/or the nucleotides of the ITR that do not form palindromic hairpin structures. In some embodiments, the expression cassette is an AAV single-stranded vector. In some embodiments, the expression cassette is an AAV genome, *e.g.,* a self-complementary AAV genome.

[0125] In certain embodiments, the expression cassette comprises a promoter, a mammalian UBE3A open reading frame, and a polyadenylation site, optionally in the recited order. In certain embodiments, the expression cassette comprises a promoter, a human UBE3A open reading frame, and a polyadenylation site, optionally in the recited order. In certain embodiments, the expression cassette comprises an AAV ITR, a promoter, a human UBE3A open reading frame, a polyadenylation site, and an AAV ITR, optionally in the recited order. In certain embodiments, the expression cassette comprises a human synapsin promoter, a

human UBE3A open reading frame, and a bGH polyadenylation site, optionally in the recited order. In certain embodiments, the expression cassette comprises an AAV ITR, a human synapsin promoter, a human UBE3A open reading frame, a bGH polyadenylation site, and an AAV ITR, optionally in the recited order. In certain embodiments, the expression cassette comprises an AAV2 ITR, a promoter, a human UBE3A open reading frame, a bGH polyadenylation site, and an AA2V ITR, optionally in the recited order. In certain embodiments, the expression cassette comprises an AAV2 ITR, a human synapsin promoter, a human UBE3A open reading frame comprising human UBE3A short isoform and long isoform, a bGH polyadenylation site, and an AA2V ITR, optionally in the recited order. In certain embodiments, the expression cassette comprises an AAV2 ITR, a human synapsin promoter, a human UBE3A open reading frame comprising human UBE3A short isoform (*i.e.,* isoform 1) and long isoform 3, a bGH polyadenylation site, and an AA2V ITR, optionally in the recited order. In certain embodiments, the expression cassette comprises an AAV2 ITR, a human synapsin promoter, a human UBE3A open reading frame comprising human UBE3A short isoform and long isoform 2, a bGH polyadenylation site, and an AAV2 ITR, optionally in the recited order. The aforementioned components are in operable linkage. In certain embodiments, the expression cassette comprises an AAV2 ITR, a human synapsin promoter, a human UBE3A open reading frame comprising human UBE3A short isoform and a human long isoform (i.e., either isoform 2 or 3) and further comprising one or more Kozak sequences comprising, consisting essentially of, or consisting of SEQ ID NO:6 and/or SEQ ID NO:8 or a sequence at least about 50% identical thereto, *e.g.,* at least about 50, 55, 60, 65, 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical thereto, a bGH polyadenylation site, and an AA2V ITR, optionally in the recited order. The aforementioned components are in operable linkage.

[0126] In some embodiments, the expression cassette comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO:14 or a sequence at least about 70% identical thereto, *e.g.,* at least about 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical thereto.

SEQ ID NO:14. Human UBE3A expression cassette excluding ITRs

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GAATTCGGTACCAGGGCCCTGCGTATGAGTGCAAGTGGGTTTTAGGACCAGGATGAGGCGGGGTGGGGGTGCCTA
CCTGACGACCGACCCCGACCCACTGGACAAGCACCCAACCCCCATTCCCCAAATTGCGCATCCCCTATCAGAGAG
GGGGAGGGGAAACAGGATGCGGCGAGGCGCGTGCGCACTGCCAGCTTCAGCACCGCGGACAGTGCCTTCGCCCCC
GCCTGGCGGCGCGCGCCACCGCCGCCTCAGCACTGAAGGCGCGCTGACGTCACTCGCCGGTCCCCCGCAAACTCC
CCTTCCCGGCCACCTTGGTCGCGTCCGCGCCGCCGCCGGCCCAGCCGGACCGCACCACGCGAGGCGCGAGATAGG
GGGGCACGGGCGCGACCATCTGCGCTGCGGCGCCGGCGACTCAGCGCTGCCTCAGTCTGCGGTGGGCAGCGGAGG
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AGTCGTGTCGTGCCTGAGAGCGCAGTCTCTCCGATTTTTTATGGCCACAGCTTGTAAAAGATCAGGAGAACCTCA
GTCTGACGACATTGAAGCTAGCAGGATGAAGAGAGCCGCCGCAAAGCACCTGATCGAACGCTACTACCATCAGCT
CACCGAGGGTTGCGGCAACGAAGCGTGTACCAACGAGTTCTGTGCCTCCTGCCCCACGTTCCTGCGGATGGATAA
CAATGCCGCCGCAATCAAGGCGCTTGAACTGTATAAGATCAACGCCAAGCTGTGCGATCCCCACCCTTCCAAGAA
GGGAGCCAGCTCAGCCTACCTGGAAAACTCCAAGGGCGCCCCTAACAACTCATGCTCCGAGATCAAGATGAATAA
GAAGGGCGCCCGGATTGACTTCAAGGATGTGACCTACCTGACCGAGGAGAAGGTGTACGAGATCCTGGAACTCTG
CCGAGAACGGGAGGACTACTCCCCTCTGATCCGCGTGATCGGAAGAGTGTTCAGCTCCGCTGAAGCGCTCGTGCA
GTCGTTCAGAAAGGTCAAGCAGCACACTAAGGAAGAACTCAAGTCCCTGCAGGCCAAAGATGAGGATAAGGACGA
AGATGAAAAAGAAAAGGCCGCCTGCTCAGCTGCCGCGATGGAAGAAGATTCAGAGGCCTCCAGCAGCAGGATTGG
CGACAGCTCCCAGGGGGACAACAACCTCCAGAAGCTGGGTCCAGACGATGTGTCGGTGGACATCGACGCCATTCG
GAGAGTGTACACCCGACTGCTCTCGAACGAAAAGATCGAGACTGCATTCCTGAACGCCCTTGTCTACCTGAGCCC
GAACGTGGAATGCGACCTTACTTACCATAACGTCTACTCCCGGGACCCAAACTACCTGAACCTGTTCATCATCGT
GATGGAAAACCGGAACCTCCATTCCCCCGAGTACCTGGAAATGGCCCTGCCCCTGTTCTGCAAGGCCATGTCAAA
GCTTCCGCTGGCCGCACAAGGAAAGCTGATCCGCTTGTGGTCCAAGTACAACGCGGACCAGATCAGACGCATGAT
GGAAACGTTCCAGCAACTGATTACTTACAAGGTCATCTCCAATGAGTTCAACTCCCGGAATCTTGTGAACGACGA
TGATGCCATTGTGGCCGCCTCCAAATGCCTGAAGATGGTCTACTATGCGAACGTCGTGGGCGGGGAAGTCGATAC
CAACCACAACGAGGAGGACGACGAGGAACCTATCCCTGAGTCATCAGAACTGACTCTGCAAGAACTGCTGGGCGA
AGAACGCCGGAACAAGAAGGGCCCAAGAGTCGACCCGTTGGAAACCGAACTGGGAGTCAAAACCCTGGACTGCAG
AAAGCCTCTGATCCCGTTCGAAGAGTTCATCAACGAACCCCTGAACGAGGTGCTGGAAATGGACAAGGACTACAC
CTTCTTCAAGGTCGAAACCGAGAACAAGTTCTCTTTCATGACTTGCCCGTTCATTCTGAACGCAGTGACCAAGAA
CCTGGGCCTCTACTACGACAACCGCATCCGCATGTACAGCGAACGCCGCATCACCGTGCTGTACTCCCTGGTGCA
AGGCCAACAGCTGAACCCCTACCTTCGCCTGAAAGTCCGCCGCGACCATATCATTGATGACGCTCTCGTGCGCCT
TGAGATGATCGCGATGGAGAACCCCGCAGATCTGAAGAAGCAGCTCTACGTCGAGTTCGAAGGAGAACAGGGGGT
GGACGAAGGAGGAGTGTCCAAGGAGTTCTTTCAGCTCGTGGTGGAGGAAATCTTTAACCCTGACATCGGAATGTT
CACCTATGACGAATCCACAAAGCTGTTTTGGTTTAACCCGTCCTCGTTCGAAACTGAGGGTCAATTCACCCTCAT
CGGCATTGTGCTGGGACTCGCCATCTACAACAATTGCATCCTCGACGTGCACTTCCCGATGGTGGTGTACCGCAA
ACTGATGGGCAAAAAGGGAACCTTCAGAGATCTGGGAGACTCACACCCGGTGCTGTACCAGTCGCTCAAGGACTT
GTTGGAATACGAAGGGAACGTGGAAGATGATATGATGATTACCTTCCAAATCTCGCAGACTGACTTGTTTGGAAA
CCCTATGATGTACGACCTGAAGGAGAATGGAGACAAGATCCCGATCACGAACGAAAACCGCAAGGAGTTCGTCAA
CCTGTACTCCGACTATATTCTGAACAAGAGCGTGGAGAAGCAGTTTAAGGCTTTCCGCCGGGGATTCCACATGGT
CACCAACGAAAGCCCGCTCAAGTACCTCTTTCGGCCCGAAGAGATTGAACTGCTGATCTGCGGGTCGAGGAATCT
GGACTTCCAGGCCCTTGAAGAGACTACTGAGTACGACGGAGGCTATACCCGGGACTCCGTGCTGATAAGAGAGTT
CTGGGAGATCGTGCACTCCTTCACCGATGAGCAGAAGCGGCTGTTCCTCCAATTCACCACCGGCACTGACAGAGC
GCCAGTCGGAGGACTGGGGAAGCTGAAGATGATCATTGCGAAGAACGGTCCCGACACTGAGAGGTTGCCCACTTC
CCACACTTGTTTCAACGTGCTGCTGCTCCCGGAGTACTCCTCCAAGGAGAAGCTGAAGGAACGGCTCCTGAAGGC
CATTACATACGCCAAAGGTTTCGGCATGCTTTAATGACTCGAGCGGCCGCGGGATCCCTCGAGACTGTGCCTTCT
AGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTT
TCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAG
GACAGCAAGGGGGAGGATTGGGAAGACAACAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGCTTCTGAGGCG
GAAAGAACCAGCTGACCGCGT

[0127] A further aspect of the invention relates to a vector comprising the polynucleotide or the expression cassette described herein.. Suitable vectors include, but are not limited to, a plasmid, phage, viral vector (*e.g.,* an AAV vector, a lentiviral vector, an adenovirus vector, a herpesvirus vector, an alphavirus vector, or a baculovirus vector), bacterial artificial chromosome (BAC), or yeast artificial chromosome (YAC). For example, the nucleic acid can comprise, consist of, or consist essentially of an AAV vector comprising a 5' and/or 3' terminal repeat (*e.g.,* 5' and/or 3' AAV terminal repeat). In some embodiments, the vector is a delivery vehicle such as a particle (*e.g.,* a microparticle or nanoparticle) or a liposome to which the expression cassette is attached or in which the expression cassette is embedded. The vector may be any delivery vehicle suitable to carry the expression cassette into a cell. In some embodiments, the vector may be a neuro-tropic vector.

[0128] In some embodiments, the vector is a viral vector, *e.g.,* a lentiviral vector and/or an AAV vector. In some embodiments the vector is an AAV vector. The AAV vector may be any AAV serotype or isolate (e.g., AAV type 1, AAV type 2, AAV type 3 (including types 3A and 3B), AAV type 4, AAV type 5, AAV type 6, AAV type 7, AAV type 8, AAV type 9, AAV type 10, AAV type 11, AAV type 12, AAV type 13, or any shown in Table 1). In some embodiments the AAV vector is an AAV9 vector. In some embodiments, the AAV vector may be AAV9 and/or an AAV9-derived variant, such as, but not limited to PHP.B, PHP.eB, or AAV9.HR. In some embodiments, the AAV vector may be a synthetic AAV vector, *e.g.,* Anc80. In some embodiments, the AAV vector may comprise wildtype capsid proteins. In other embodiments, the AAV vector may comprise a modified capsid protein with altered tropism compared to a wildtype capsid protein, *e.g.,* a modified capsid protein is liver-detargeted or has enhanced tropism for particular cells.

[0129] In some embodiments, the vector is a single-stranded AAV (ssAAV) vector. In some embodiments, the vector is a self-complementary or duplexed AAV (scAAV) vector. scAAV vectors are described in international patent publication WO 01/92551 (the disclosure of which is incorporated herein by reference in its entirety). Use of scAAV to express the UBE3A ORF may provide an increase in the number of cells transduced, the copy number per transduced cell, or both.

[0130] An additional aspect of the invention relates to a transformed cell comprising the polynucleotide, expression cassette, and/or vector of the invention. In some embodiments, the polynucleotide, expression cassette, and/or vector is stably incorporated into the cell genome. The cell may be an *in vitro*, *ex vivo*, or *in vivo* cell.

[0131] Another aspect of the invention relates to a transgenic animal comprising the polynucleotide, expression cassette, vector, and/or the transformed cell of the invention. In some embodiments, the animal is a laboratory animal, *e.g.,* a mouse, rat, rabbit, dog, monkey, or non-human primate.

[0132] A further aspect of the invention relates to a pharmaceutical formulation comprising the polynucleotide, expression cassette, vector, and/or transformed cell of the invention in a pharmaceutically acceptable carrier.

[0133] In a specific embodiment, the polynucleotide, expression cassette, vector, and/or transformed cell of the invention is isolated.

[0134] In another specific embodiment, the polynucleotide, expression cassette, vector, and/or transformed cell of the invention is purified.

Methods of Producing Virus Vectors

[0135] The present invention further provides methods of producing virus vectors. In one particular embodiment, the present invention provides a method of producing a recombinant AAV particle, comprising providing to a cell permissive for AAV replication: (a) a recombinant AAV template comprising (i) the polynucleotide or expression cassette of the invention, and (ii) an ITR; (b) a polynucleotide comprising Rep coding sequences and Cap coding sequences; under conditions sufficient for the replication and packaging of the recombinant AAV template; whereby recombinant AAV particles are produced in the cell. Conditions sufficient for the replication and packaging of the recombinant AAV template can be, *e.g.,* the presence of AAV sequences sufficient for replication of the AAV template and encapsidation into AAV capsids (*e.g.,* AAV *rep* sequences and AAV *cap* sequences) and helper sequences from adenovirus and/or herpesvirus. In particular embodiments, the AAV template comprises two AAV ITR sequences, which are located 5' and 3' to the polynucleotide of the invention, although they need not be directly contiguous thereto.

[0136] In some embodiments, the recombinant AAV template comprises an ITR that is not resolved by Rep to make duplexed AAV vectors as described in international patent publication WO 01/92551.

[0137] The AAV template and AAV *rep* and *cap* sequences are provided under conditions such that virus vector comprising the AAV template packaged within the AAV capsid is produced in the cell. The method can further comprise the step of collecting the virus vector from the cell. The virus vector can be collected from the medium and/or by lysing the cells.

[0138] The cell can be a cell that is permissive for AAV viral replication. Any suitable cell known in the art may be employed. In particular embodiments, the cell is a mammalian cell (*e.g.,* a primate or human cell). As another option, the cell can be a trans-complementing packaging cell line that provides functions deleted from a replication-defective helper virus, *e.g.,* 293 cells or other E1a trans-complementing cells.

[0139] The AAV replication and capsid sequences may be provided by any method known in the art. Current protocols typically express the AAV *rep/cap* genes on a single plasmid. The AAV replication and packaging sequences need not be provided together, although it may be convenient to do so. The AAV *rep* and/or *cap* sequences may be provided by any viral or non-viral vector. For example, the *rep/cap* sequences may be provided by a hybrid adenovirus or herpesvirus vector (*e.g.,* inserted into the E1a or E3 regions of a deleted adenovirus vector). EBV vectors may also be employed to express the AAV *cap* and *rep* genes. One advantage of this method is that EBV vectors are episomal, yet will maintain a

high copy number throughout successive cell divisions (*i.e.*, are stably integrated into the cell as extra-chromosomal elements, designated as an "EBV based nuclear episome," *see* Margolski, (1992) *Curr. Top. Microbiol. Immun.* 158:67).

[0140] As a further alternative, the *rep/cap* sequences may be stably incorporated into a cell.

[0141] Typically the AAV *rep/cap* sequences will not be flanked by the TRs, to prevent rescue and/or packaging of these sequences.

[0142] The AAV template can be provided to the cell using any method known in the art. For example, the template can be supplied by a non-viral (*e.g.*, plasmid) or viral vector. In particular embodiments, the AAV template is supplied by a herpesvirus or adenovirus vector (*e.g.*, inserted into the E1a or E3 regions of a deleted adenovirus). As another illustration, Palombo *et al.*, (1998) *J. Virology* 72:5025, describes a baculovirus vector carrying a reporter gene flanked by the AAV TRs. EBV vectors may also be employed to deliver the template, as described above with respect to the *rep/cap* genes.

[0143] In another representative embodiment, the AAV template is provided by a replicating rAAV virus. In still other embodiments, an AAV provirus comprising the AAV template is stably integrated into the chromosome of the cell.

[0144] To enhance virus titers, helper virus functions (*e.g.*, adenovirus or herpesvirus) that promote a productive AAV infection can be provided to the cell. Helper virus sequences necessary for AAV replication are known in the art. Typically, these sequences will be provided by a helper adenovirus or herpesvirus vector. Alternatively, the adenovirus or herpesvirus sequences can be provided by another non-viral or viral vector, *e.g.*, as a non-infectious adenovirus miniplasmid that carries all of the helper genes that promote efficient AAV production as described by Ferrari *et al.*, (1997) *Nature Med.* 3:1295, and U.S. Patent Nos. 6,040,183 and 6,093,570.

[0145] Further, the helper virus functions may be provided by a packaging cell with the helper sequences embedded in the chromosome or maintained as a stable extrachromosomal element. Generally, the helper virus sequences cannot be packaged into AAV virions, *e.g.*, are not flanked by ITRs.

[0146] Those skilled in the art will appreciate that it may be advantageous to provide the AAV replication and capsid sequences and the helper virus sequences (*e.g.*, adenovirus sequences) on a single helper construct. This helper construct may be a non-viral or viral construct. As one nonlimiting illustration, the helper construct can be a hybrid adenovirus or hybrid herpesvirus comprising the AAV *rep/cap* genes.

[0147] In one particular embodiment, the AAV *rep/cap* sequences and the adenovirus helper sequences are supplied by a single adenovirus helper vector. This vector can further comprise the AAV template. The AAV *rep/cap* sequences and/or the AAV template can be inserted into a deleted region (*e.g.,* the E1a or E3 regions) of the adenovirus.

[0148] In a further embodiment, the AAV *rep/cap* sequences and the adenovirus helper sequences are supplied by a single adenovirus helper vector. According to this embodiment, the AAV template can be provided as a plasmid template.

[0149] In another illustrative embodiment, the AAV *rep/cap* sequences and adenovirus helper sequences are provided by a single adenovirus helper vector, and the AAV template is integrated into the cell as a provirus. Alternatively, the AAV template is provided by an EBV vector that is maintained within the cell as an extrachromosomal element (*e.g.,* as an EBV based nuclear episome).

[0150] In a further exemplary embodiment, the AAV *rep/cap* sequences and adenovirus helper sequences are provided by a single adenovirus helper. The AAV template can be provided as a separate replicating viral vector. For example, the AAV template can be provided by an AAV particle or a second recombinant adenovirus particle.

[0151] According to the foregoing methods, the hybrid adenovirus vector typically comprises the adenovirus 5' and 3' *cis* sequences sufficient for adenovirus replication and packaging (*i.e.,* the adenovirus terminal repeats and PAC sequence). The AAV *rep/cap* sequences and, if present, the AAV template are embedded in the adenovirus backbone and are flanked by the 5' and 3' *cis* sequences, so that these sequences may be packaged into adenovirus capsids. As described above, the adenovirus helper sequences and the AAV *rep/cap* sequences are generally not flanked by ITRs so that these sequences are not packaged into the AAV virions.

[0152] Zhang *et al.*, ((2001) *Gene Ther.* 18:704-12) describe a chimeric helper comprising both adenovirus and the AAV *rep* and *cap* genes.

[0153] Herpesvirus may also be used as a helper virus in AAV packaging methods. Hybrid herpesviruses encoding the AAV Rep protein(s) may advantageously facilitate scalable AAV vector production schemes. A hybrid herpes simplex virus type I (HSV-1) vector expressing the AAV-2 *rep* and *cap* genes has been described (Conway *et al.*, (1999) *Gene Ther.* 6:986 and WO 00/17377).

[0154] As a further alternative, the virus vectors of the invention can be produced in insect cells using baculovirus vectors to deliver the *rep/cap* genes and AAV template as described, for example, by Urabe *et al.*, (2002) *Human Gene Ther.* 13:1935-43.

[0155] AAV vector stocks free of contaminating helper virus may be obtained by any method known in the art. For example, AAV and helper virus may be readily differentiated based on size. AAV may also be separated away from helper virus based on affinity for a heparin substrate (Zolotukhin *et al.* (1999) *Gene Therapy* 6:973). Deleted replication-defective helper viruses can be used so that any contaminating helper virus is not replication competent. As a further alternative, an adenovirus helper lacking late gene expression may be employed, as only adenovirus early gene expression is required to mediate packaging of AAV. Adenovirus mutants defective for late gene expression are known in the art (*e.g.,* ts100K and ts149 adenovirus mutants).

Methods of Using UBE3A Vectors

[0156] The present invention also relates to methods for delivering a UBE3A open reading frame to a cell or a subject to increase production of UBE3A and/or E6AP, *e.g.,* for therapeutic or research purposes *in vitro*, *ex vivo*, and/or *in vivo*. Thus, one aspect of the invention relates to a method of expressing a UBE3A open reading frame in a cell, comprising contacting the cell with the polynucleotide, expression cassette, and/or the vector of the invention, thereby expressing the UBE3A open reading frame in the cell. In some embodiments, the cell is an *in vitro* cell, an *ex vivo* cell, and/or an *in vivo* cell. Expression of the present invention *in vitro* may be beneficial for research purposes, *e.g.,* to express a recombinant protein, to study the effect of the protein on the cell, to evaluate efficacy and/or safety prior to expression *in vivo*, and/or the development of research and healthcare diagnostic and/or screening methods.

[0157] Another aspect of the invention relates 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. In some embodiments, the subject is an animal model of a disorder associated with aberrant UBE3A gene expression.

[0158] A further aspect of the invention relates to a method of treating a disorder associated with aberrant expression of a UBE3A gene or aberrant activity of a UBE3A gene product (*e.g.,* E6AP) in a subject in need thereof, comprising delivering to the subject a therapeutically effective amount of the polynucleotide, expression cassette, vector, and/or transformed cell of the invention, thereby treating the disorder associated with aberrant expression of the UBE3A gene or aberrant activity of a UBE3A gene product in the subject. The invention provides a method of treating a disorder associated with aberrant expression of

a UBE3A gene or aberrant activity of a UBE3A gene product (*e.g.,* E6AP) in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the polynucleotide, the expression cassette, vector, and/or transformed cell of the invention, such that the UBE3A open reading frame is expressed in the subject. In some embodiments, the disorder associated with aberrant expression of the UBE3A gene or gene product may be Angelman Syndrome.

[0159] The invention further provides a method of treating Angelman Syndrome in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the polynucleotide, the expression cassette, vector, and/or transformed cell of the invention, such that the UBE3A open reading frame is expressed in the subject.

[0160] In certain embodiments, the polynucleotide, expression cassette, vector, and/or transformed cell is delivered to the subject, *e.g.,* systemically (*e.g.,* intravenously) or directly to the central nervous system (*e.g.,* to the cerebrospinal fluid by intrathecal or intraventricular or intra-cisterna magna injection) of the subject. In some embodiments, the polynucleotide, expression cassette, vector, and/or transformed cell is delivered intravenously. In some embodiments, the polynucleotide, expression cassette, vector, and/or transformed cell is delivered intracerebroventricularly.

[0161] Recombinant virus vectors according to the present invention find use in both veterinary and medical applications. Suitable subjects include avians, reptiles, amphibians, fish, and mammals. The term "mammal" as used herein includes, but is not limited to, humans, primates, non-human primates (*e.g.,* monkeys and baboons), cattle, sheep, goats, pigs, horses, cats, dogs, rabbits, rodents (*e.g.,* rats, mice, hamsters, and the like), *etc.* Human subjects include embryos, fetuses, neonates, infants, juveniles, and adults. Optionally, the subject is "in need of" the methods of the present invention, *e.g.,* because the subject has or is believed at risk for a disorder including those described herein or that would benefit from the delivery of a polynucleotide including those described herein. As a further option, the subject can be a laboratory animal and/or an animal model of disease. Preferably, the subject is a human.

[0162] In certain embodiments, the polynucleotide of the invention is administered to a subject in need thereof as early as possible in the life of the subject, *e.g.,* as soon as the subject is diagnosed with aberrant UBE3A and/or E6AP expression or activity or any of the above-mentioned diseases or disorders. In some embodiments, the polynucleotide is administered to a newborn subject, *e.g.,* after newborn screening has identified aberrant UBE3A and/or E6AP expression or activity. In some embodiments, the polynucleotide is

administered to a subject prior to the age of 10 years, *e.g.,* prior to 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 years of age. In some embodiments, the polynucleotide is administered to juvenile or adult subjects after the age of 10 years. In some embodiments, the polynucleotide is administered to a fetus *in utero*, *e.g.,* after prenatal screening has identified aberrant UBE3A and/or E6AP expression or activity or the presence of one of the above-mentioned diseases or disorders. In some embodiments, the polynucleotide is administered to a subject as soon as the subject develops symptoms associated with aberrant UBE3A and/or E6AP expression or activity or is suspected or diagnosed as having aberrant UBE3A and/or E6AP expression or activity or one of the above-mentioned diseases or disorders. In some embodiments, the polynucleotide is administered to a subject before the subject develops symptoms associated with aberrant UBE3A and/or E6AP expression or activity or disease/disorder, *e.g.,* a subject that is suspected or diagnosed as having aberrant UBE3A and/or E6AP expression or activity or one of the above-mentioned diseases or disorders but has not started to exhibit symptoms.

[0163] In particular embodiments, the present invention provides a pharmaceutical composition comprising a polynucleotide, expression cassette, vector, and/or transformed cell of the invention in a pharmaceutically acceptable carrier and, optionally, other medicinal agents, pharmaceutical agents, stabilizing agents, buffers, carriers, adjuvants, diluents, *etc.* For injection, the carrier will typically be a liquid. For other methods of administration, the carrier may be either solid or liquid. For inhalation administration, the carrier will be respirable, and will preferably be in solid or liquid particulate form. In some embodiments, a pharmaceutical carrier may comprise D-sorbitol (*e.g.,* PBS 5% w/v D-sorbitol).

[0164] By "pharmaceutically acceptable" it is meant a material that is not toxic or otherwise undesirable, *i.e.,* the material may be administered to a subject without causing any undesirable biological effects.

[0165] One aspect of the present invention is a method of transferring a UBE3A open reading frame to a cell *in vitro*. The polynucleotide, expression cassette, and/or vector of the invention may be introduced to the cells in the appropriate amount. The virus vector may be introduced to the cells at the appropriate multiplicity of infection according to standard transduction methods appropriate for the particular target cells. Titers of the virus vector or capsid to administer can vary, depending upon the target cell type and number, and the particular virus vector or capsid, and can be determined by those of skill in the art without undue experimentation. In particular embodiments, at least about 10^3 infectious units, more preferably at least about 10^5 infectious units are introduced to the cell.

[0166] The cell(s) into which the polynucleotide, expression cassette, and/or vector of the invention, *e.g.,* virus vector, can be introduced may be of any type, including but not limited to neural cells (including cells of the peripheral and central nervous systems, in particular, brain cells such as neurons, oligodendrocytes, glial cells, astrocytes), lung cells, cells of the eye (including retinal cells, retinal pigment epithelium, and corneal cells), epithelial cells (*e.g.,* gut and respiratory epithelial cells), skeletal muscle cells (including myoblasts, myotubes and myofibers), diaphragm muscle cells, dendritic cells, pancreatic cells (including islet cells), hepatic cells, a cell of the gastrointestinal tract (including smooth muscle cells, epithelial cells), heart cells (including cardiomyocytes), bone cells (*e.g.,* bone marrow stem cells), hematopoietic stem cells, spleen cells, keratinocytes, fibroblasts, endothelial cells, prostate cells, joint cells (including, *e.g.,* cartilage, meniscus, synovium and bone marrow), germ cells, and the like. Alternatively, the cell may be any progenitor cell. As a further alternative, the cell can be a stem cell (*e.g.,* neural stem cell, liver stem cell). As still a further alternative, the cell may be a cancer or tumor cell. Moreover, the cells can be from any species of origin, as indicated above.

[0167] The polynucleotide, expression cassette, and/or vector of the invention, *e.g.,* virus vector, may be introduced to cells *in vitro* for the purpose of administering the modified cell to a subject. In particular embodiments, the cells have been removed from a subject, the polynucleotide, expression cassette, and/or vector of the invention, *e.g.,* virus vector, is introduced therein, and the cells are then replaced back into the subject. Methods of removing cells from subject for treatment *ex vivo*, followed by introduction back into the subject are known in the art (*see, e.g.,* U.S. patent No. 5,399,346). Alternatively, the polynucleotide, expression cassette, and/or vector of the invention, *e.g.,* virus vector, is introduced into cells from another subject, into cultured cells, or into cells from any other suitable source, and the cells are administered to a subject in need thereof.

[0168] Suitable cells for *ex vivo* gene therapy are as described above. Dosages of the cells to administer to a subject will vary upon the age, condition and species of the subject, the type of cell, the nucleic acid being expressed by the cell, the mode of administration, and the like. Typically, at least about 10^2 to about 10^8 or about 10^3 to about 10^6 cells will be administered per dose in a pharmaceutically acceptable carrier. In particular embodiments, the cells transduced with the virus vector *ex vivo* are administered to the subject in an effective amount in combination with a pharmaceutical carrier.

[0169] A further aspect of the invention is a method of administering the polynucleotide, expression cassette, and/or vector of the invention, *e.g.,* virus vector, to a subject. In

particular embodiments, the method comprises a method of delivering a UBE3A open reading frame to an animal subject, the method comprising: administering an effective amount of a virus vector according to the invention to an animal subject. Administration of the virus vectors of the present invention to a human subject or an animal in need thereof can be by any means known in the art. Optionally, the virus vector is delivered in an effective dose in a pharmaceutically acceptable carrier.

[0170] Dosages of the virus vectors to be administered to a subject will depend upon the mode of administration, the disease or condition to be treated, the individual subject's condition, the particular virus vector, and the nucleic acid to be delivered, and can be determined in a routine manner. Exemplary doses for achieving therapeutic effects are virus titers 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}, 10^{16} transducing units (TU) or more, *e.g.,* about 10^7, 10^8, 10^9, 10^{10}, 10^{11}, 10^{12}, 10^{13}, 10^{14}, or 10^{15} TU, yet more preferably, from about 10^8 – 10^{14} TU is used for the dose, or about 10^{10}, 10^{11}, 10^{12}, 10^{13}, 10^{14}, or 10^{15} TU. Doses and virus titer transducing units may be calculated as vector or viral genomes (vg), and/or vg/kg of the subject. In some embodiments, the dose is 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}, 10^{16} vg/kg of the subject, *e.g.,* about 10^7, 10^8, 10^9, 10^{10}, 10^{11}, 10^{12}, 10^{13}, 10^{14}, or 10^{15} vg/kg of the subject, or from about 10^8 – 10^{14} vg/kg subject, in some embodiments about 10^{10}, 10^{11}, 10^{12}, 10^{13}, 10^{14}, or 10^{15} vg/kg of the subject.

[0171] In some embodiments, a method of the present invention may comprise expressing and/or administering a UBE3A open reading frame at a dose (*e.g.,* viral titer) such that a UBE3A short isoform is expressed at a ratio of about 1:1 to about 15:1 as compared to expression of a long isoform, *e.g.,* a ratio of about 1:1, 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, 11:1, 12:1, 13:1, 14:1, or 15:1 or any value or range therein. For example, in some embodiments, the mammalian (*e.g.,* human) UBE3A open reading frame may be administered such that a short isoform is expressed at a ratio of about 2:1 to about 4.5:1, about 1:1 to about 10:1, about 3:1 to about 8.5:1, or about 3:1 to about 4:1 as compared to expression of a long isoform. In some embodiments, the mammalian (*e.g.,* human) UBE3A open reading frame may be administered such that a short isoform is expressed at a ratio of about 2.5:1, about 3:1, about 4:1, about 6.5:1, about 8:1, or about 10:1 as compared to expression of a long isoform.

[0172] In particular embodiments, more than one administration (*e.g.,* two, three, four or more administrations) may be employed to achieve the desired level of gene expression over a period of various intervals, *e.g.,* daily, weekly, monthly, yearly, *etc.*

[0173] Exemplary modes of administration include oral, rectal, transmucosal, topical, intranasal, inhalation (*e.g.,* via an aerosol), buccal (*e.g.,* sublingual), vaginal, intrathecal, intraocular, transdermal, *in utero* (or *in ovo*), parenteral (*e.g.,* intravenous, subcutaneous, intradermal, intramuscular [including administration to skeletal, diaphragm and/or cardiac muscle], intradermal, intrapleural, intracerebral, and intraarticular), topical (*e.g.,* to both skin and mucosal surfaces, including airway surfaces, and transdermal administration), intro-lymphatic, and the like, as well as direct tissue or organ injection (*e.g.,* to liver, skeletal muscle, cardiac muscle, diaphragm muscle or brain). Administration can also be to a tumor (*e.g.,* in or a near a tumor or a lymph node). The most suitable route in any given case will depend on the nature and severity of the condition being treated and on the nature of the particular vector that is being used. In some embodiments, more than one mode and/or route of administration may be utilized, for example, *e.g.,* intraparenchymal administration and intracerebroventricular administration.

[0174] In some embodiments, the vector comprising the expression cassette (e.g., viral vector, AAV vector) is administered to the CNS, the peripheral nervous system, or both. In some embodiments, the viral vector is administered directly to the CNS, *e.g.,* the brain or the spinal cord. Direct administration can result in high specificity of transduction of CNS cells, *e.g.,* wherein at least 80%, 85%, 90%, 95% or more of the transduced cells are CNS cells. Any method known in the art to administer vectors directly to the CNS can be used. The vector may be introduced into the spinal cord, brainstem (medulla oblongata, pons), midbrain (hypothalamus, thalamus, epithalamus, pituitary gland, substantia nigra, pineal gland), cerebellum, telencephalon (corpus striatum, cerebrum including the occipital, temporal, parietal and frontal lobes, cortex, basal ganglia, hippocampus and amygdala), limbic system, neocortex, corpus striatum, cerebrum, and inferior colliculus. The vector may also be administered to different regions of the eye such as the retina, cornea or optic nerve. The vector may be delivered into the cerebrospinal fluid (*e.g.,* by lumbar puncture) for more disperse administration of the vector.

[0175] The vector comprising the expression cassette (e.g., viral vector, AAV vector) may be administered to the desired region(s) of the CNS by any route known in the art, including but not limited to, intrathecal, intracerebral, intraventricular, intra-cisterna magna, intraparenchymal, intranasal, intra-aural, intra-ocular (*e.g.,* intra-vitreous, sub-retinal, anterior chamber) and peri-ocular (*e.g.,* sub-Tenon's region) delivery or any combination thereof.

[0176] The vector may be administered in a manner that produces a more widespread, diffuse transduction of tissues, including the CNS, the peripheral nervous system, and/or other tissues.

[0177] Typically, the vector comprising the expression cassette (e.g., viral vector, AAV vector) will be administered in a liquid formulation by direct injection (*e.g.,* stereotactic injection) to the desired region or compartment in the CNS and/or other tissues. In some embodiments, the vector can be delivered via a reservoir and/or pump. In other embodiments, the vector may be provided by topical application to the desired region or by intra-nasal administration of an aerosol formulation. Administration to the eye or into the ear, may be by topical application of liquid droplets. As a further alternative, the vector may be administered as a solid, slow-release formulation. Controlled release of parvovirus and AAV vectors is described by international patent publication WO 01/91803.

[0178] Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. Alternatively, one may administer the virus vector in a local rather than systemic manner, for example, in a depot or sustained-release formulation. Further, the virus vector can be delivered dried to a surgically implantable matrix such as a bone graft substitute, a suture, a stent, and the like (*e.g.,* as described in U.S. Patent 7,201,898).

[0179] Pharmaceutical compositions suitable for oral administration can be presented in discrete units, such as capsules, cachets, lozenges, or tablets, each containing a predetermined amount of the composition of this invention; as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water or water-in-oil emulsion. Oral delivery can be performed by complexing a virus vector of the present invention to a carrier capable of withstanding degradation by digestive enzymes in the gut of an animal. Examples of such carriers include plastic capsules or tablets, as known in the art. Such formulations are prepared by any suitable method of pharmacy, which includes the step of bringing into association the composition and a suitable carrier (which may contain one or more accessory ingredients as noted above). In general, the pharmaceutical composition according to embodiments of the present invention are prepared by uniformly and intimately admixing the composition with a liquid or finely divided solid carrier, or both, and then, if necessary, shaping the resulting mixture. For example, a tablet can be prepared by compressing or molding a powder or granules containing the composition, optionally with one or more accessory ingredients. Compressed tablets are prepared by compressing, in a suitable machine, the composition in a free-flowing form, such as a powder or granules

optionally mixed with a binder, lubricant, inert diluent, and/or surface active/dispersing agent(s). Molded tablets are made by molding, in a suitable machine, the powdered compound moistened with an inert liquid binder.

[0180] Pharmaceutical compositions suitable for buccal (sub-lingual) administration include lozenges comprising the composition of this invention in a flavored base, usually sucrose and acacia or tragacanth; and pastilles comprising the composition in an inert base such as gelatin and glycerin or sucrose and acacia.

[0181] Pharmaceutical compositions suitable for parenteral administration can comprise sterile aqueous and non-aqueous injection solutions of the composition of this invention, which preparations are optionally isotonic with the blood of the intended recipient. These preparations can contain anti-oxidants, buffers, bacteriostats and solutes, which render the composition isotonic with the blood of the intended recipient. Aqueous and non-aqueous sterile suspensions, solutions and emulsions can include suspending agents and thickening agents. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like.

[0182] The compositions can be presented in unit/dose or multi-dose containers, for example, in sealed ampoules and vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example, saline or water-for-injection immediately prior to use.

[0183] Extemporaneous injection solutions and suspensions can be prepared from sterile powders, granules and tablets of the kind previously described. For example, an injectable, stable, sterile composition of this invention in a unit dosage form in a sealed container can be provided. The composition can be provided in the form of a lyophilizate, which can be reconstituted with a suitable pharmaceutically acceptable carrier to form a liquid composition suitable for injection into a subject. The unit dosage form can be from about 1 μg to about 10 grams of the composition of this invention. When the composition is substantially water-insoluble, a sufficient amount of emulsifying agent, which is physiologically acceptable, can

be included in sufficient quantity to emulsify the composition in an aqueous carrier. One such useful emulsifying agent is phosphatidyl choline.

[0184] Pharmaceutical compositions suitable for rectal administration can be presented as unit dose suppositories. These can be prepared by admixing the composition with one or more conventional solid carriers, such as for example, cocoa butter and then shaping the resulting mixture.

[0185] Pharmaceutical compositions of this invention suitable for topical application to the skin can take the form of an ointment, cream, lotion, paste, gel, spray, aerosol, or oil. Carriers that can be used include, but are not limited to, petroleum jelly, lanoline, polyethylene glycols, alcohols, transdermal enhancers, and combinations of two or more thereof. In some embodiments, for example, topical delivery can be performed by mixing a pharmaceutical composition of the present invention with a lipophilic reagent (*e.g.,* DMSO) that is capable of passing into the skin.

[0186] Pharmaceutical compositions suitable for transdermal administration can be in the form of discrete patches adapted to remain in intimate contact with the epidermis of the subject for a prolonged period of time. Compositions suitable for transdermal administration can also be delivered by iontophoresis *(see,* for example, *Pharm. Res.* 3:318 (1986)) and typically take the form of an optionally buffered aqueous solution of the composition of this invention. Suitable formulations can comprise citrate or bis\tris buffer (pH 6) or ethanol/water and can contain from 0.1 to 0.2M active ingredient.

[0187] The virus vectors disclosed herein may be administered to the lungs of a subject by any suitable means, for example, by administering an aerosol suspension of respirable particles comprised of the virus vectors, which the subject inhales. The respirable particles may be liquid or solid. Aerosols of liquid particles comprising the virus vectors may be produced by any suitable means, such as with a pressure-driven aerosol nebulizer or an ultrasonic nebulizer, as is known to those of skill in the art. *See, e.g.,* U.S. Patent No. 4,501,729. Aerosols of solid particles comprising the virus vectors may likewise be produced with any solid particulate medicament aerosol generator, by techniques known in the pharmaceutical art.

[0188] The present invention may be as defined in any one of the following numbered paragraphs.

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.

2. The polynucleotide of paragraph 1, wherein the human UBE3A open reading frame encodes human UBE3A short isoform (*i.e.,* isoform 1) and long isoform 2.

3. The polypeptide of paragraph 1, wherein the human UBE3A open reading frame encodes human UBE3A short isoform (*i.e.,* isoform 1) and long isoform 3.

4. The polynucleotide of any one of paragraphs 1-3, which comprises 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.

5. The polynucleotide of paragraph 4, 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.

6. The polynucleotide of any one of paragraphs 1-5, wherein the human UBE3A open reading frame comprises one or more nucleotide modifications to enhance expression of the short isoform (*e.g.,* enhance expression by 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or more) as compared to native ORF not comprising nucleotide modifications to enhance expression.

7. The polynucleotide of paragraph 6, 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.

8. The polynucleotide of any one of paragraphs 1-7, 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.

9. The polynucleotide of any one of paragraphs 1-8, which comprises a first Kozak sequence comprising SEQ ID NO:6 operably linked to coding sequences for the long isoform.

10. The polynucleotide of any one of paragraphs 1-9, which comprises a second Kozak sequence comprising SEQ ID NO:8 operably linked to coding sequence for the short isoform.

11. The polynucleotide of any one of paragraphs 1-10, wherein the human UBE3A open reading frame is codon-optimized for expression in a human cell.

12. The polynucleotide of paragraph 12, wherein the human UBE3A open reading frame is codon-optimized for intracellular or non-secretory expression in a human cell.

13. The polynucleotide of any one of paragraphs 1-12, wherein the human UBE3A open reading frame comprises the nucleotide sequence of SEQ ID NO:9, SEQ ID NO: 25, or SEQ ID NO: 26, or a nucleotide sequence having at least about 70%, 75%, 76%, 77%, 80%, 85%, or 90% identity thereto.

14. An expression cassette comprising the polynucleotide of any one of paragraphs 1-13.

15. The expression cassette of paragraph 14, wherein the human UBE3A open reading frame is operably linked to a promoter.

16. The expression cassette of paragraph 15, wherein the promoter is a neuron-specific promoter.

17. The expression cassette of paragraph 15 or 16, wherein the promoter is a human synapsin promoter.

18. The expression cassette of any one of paragraphs 14-17, wherein the human UBE3A open reading frame is operably linked to a polyadenylation signal.

19. The expression cassette of paragraph 18, wherein the polyadenylation signal is a bovine growth hormone (bGH) polyadenylation signal (bGHpA).

20. The expression cassette of any one of paragraphs 14-19, further comprising at least one adeno-associated virus (AAV) inverted terminal repeat (ITR).

21. The expression cassette of paragraph 20, wherein the expression cassette comprises two AAV ITRs.

22. The expression cassette of paragraph 21, 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.

23. The expression cassette of paragraph 22, wherein the AAV ITRs are AAV2 ITRs.

24. The expression cassette of any one of paragraphs 14-23, wherein the expression cassette is a single stranded AAV genome.

25. The expression cassette of any one of paragraphs 14-24, wherein the expression cassette comprises a promoter, the human UBE3A open reading frame, and a polyadenylation site.

26. The expression cassette of any one of paragraphs 14-25, wherein the expression cassette comprises an AAV ITR, a promoter, the human UBE3A open reading frame, a polyadenylation site, and an AAV ITR.

27. The expression cassette of any one of paragraphs 14-26, 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 2, a bGHpA, and an AAV2 ITR.

28. The expression cassette of any one of paragraphs 14-26, 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, a bGHpA, and an AAV2 ITR.

29. The expression cassette of any one of paragraphs 14-28, 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 2 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.

30. The expression cassette of any one of paragraphs 14-28, 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.

31. The expression cassette of paragraph 30, comprising the nucleotide sequence of SEQ ID NO:14 or a sequence at least about 90% identical thereto.

32. A vector comprising the polynucleotide of any one of paragraphs 1-13 or the expression cassette of any one of paragraphs 14-31.

33. The vector of paragraph 32, wherein the vector is a neuro-tropic vector.

34. The vector of paragraph 32 or 33, wherein the vector is a viral vector.

35. The vector of paragraph 34, wherein the vector is an AAV vector.

36. The vector of paragraph 34, wherein the AAV vector is selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12 AAV13, and combinations thereof.

37. The vector of paragraph 35, wherein the AAV vector is an AAV9 or AAV9-derived vector.

38. The vector of paragraph 37, wherein the AAV9-derived vector is PHP.B, PHP.eB, or AAV9.HR).

39. A transformed cell comprising the polynucleotide of any one of paragraphs 1-13, the expression cassette of any one of paragraphs 14-31, and/or the vector of any one of paragraphs 32-38.

40. The transformed cell of paragraph 39, wherein the polynucleotide, expression cassette, and/or vector is stably incorporated into the cell genome.

41. A transgenic animal comprising the polynucleotide of any one of paragraphs 1-13, the expression cassette of any one of paragraphs 14-31, the vector of any one of paragraphs 32-38, and/or the transformed cell of paragraph 39 or 40.

42. A pharmaceutical composition comprising the polynucleotide of any one of paragraphs 1-13, the expression cassette of any one of paragraphs 14-31, the vector of any one of paragraphs 32-38, and/or the transformed cell of paragraph 39 or 40 in a pharmaceutically acceptable carrier.

43. A method of expressing a UBE3A open reading frame in a cell, comprising contacting the cell with the polynucleotide of any one of paragraphs 1-13, the expression cassette of any one of paragraphs 14-31, and/or the vector of any one of paragraphs 32-38, thereby expressing the UBE3A open reading frame in the cell.

44. A method of expressing a UBE3A open reading frame in a subject, comprising delivering to the subject the polynucleotide of any one of paragraphs 1-13, the expression cassette of any one of paragraphs 14-31, the vector of any one of paragraphs 32-38, and/or the transformed cell of paragraph 39 or 40, thereby expressing the UBE3A open reading frame in the subject.

45. 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 of any one of paragraphs 1-13, the expression cassette of any one of paragraphs 14-31, the vector of any one of paragraphs 32-38, and/or the transformed cell of paragraph 39 or 40, such that the UBE3A open reading frame is expressed in the subject.

46. The method of paragraph 45, wherein the disorder associated with expression of the UBE3A gene is Angelman Syndrome.

47. A method of treating Angelman Syndrome in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the polynucleotide of any one of paragraphs 1-13, the expression cassette of any one of paragraphs 14-31, the vector of any one of paragraphs 32-38, and/or the transformed cell of paragraph 39 or 40, such that the UBE3A open reading frame is expressed in the subject.

48. The method of any one of paragraphs 43-47, 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.

49. The method of any one of paragraphs 44-48, wherein the subject exhibits symptoms of the disease prior to delivery of the polynucleotide, expression cassette, vector, and/or transformed cell.

50. The method of any one of paragraphs 44-49, wherein the polynucleotide, expression cassette, vector, and/or transformed cell is delivered *in utero*.

51. The method of any one of paragraphs 44-50, wherein the subject is a human.

52. The method of any one of paragraphs 44-51, wherein the polynucleotide, expression cassette, vector, and/or transformed cell is delivered to the nervous system of the subject.

53. The method of any one of paragraphs 44-52, wherein the AAV vector is administered to the subject 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.

54. The method of paragraph 52, wherein the polynucleotide, expression cassette, vector, and/or transformed cell is delivered intravenously.

55. The method of paragraph 52, wherein the polynucleotide, expression cassette, vector, and/or transformed cell is delivered by intrathecal, intracerebral, intra-cisterna magna, intraparenchymal, intracerebroventricular, intranasal, intra-aural, intra-ocular, or peri-ocular delivery, or any combination thereof.

56. The method of paragraph 55, wherein the polynucleotide, expression cassette, vector, and/or transformed cell is delivered intrathecally.

57. The method of paragraph 55, wherein the polynucleotide, expression cassette, vector, and/or transformed cell is delivered intracerebroventricularly.

[0189] Having described the present invention, the same will be explained in greater detail in the following examples, which are included herein for illustration purposes only, and which are not intended to be limiting to the invention.

EXAMPLES

Example 1: Generation of human UBE3A codon-optimized construct for intracellular expression.

[0190] A novel Angelman Syndrome therapy was designed that enables co-delivery of both short and long UBE3A isoforms (*e.g.,* human isoforms 1 (also known as "short") and 3 (also known as "long")) at the appropriate ratio of expression, referred to herein as PHP.B/SYN-UBE3A incorporating the open reading frame (ORF) hUBE3A_Isoform1>>3. This is a significant technical advantage, as viral vector packaging limits normally allow for the expression of only one isoform. This construct overcomes the two main challenges: (1) delivery of UBE3A widely throughout the brain; and (2) avoidance of UBE3A overexpression, which may be linked to a syndromic form of autism (Dup15q syndrome). AAV9-derived capsids, when delivered during early life, can widely transduce neurons throughout the brain. While there are other AAV capsids that might enable even more widespread gene transfer in the brains of non-human primates and humans, AAV9-derived capsids were selected to be mindful of limiting UBE3A overexpression.

[0191] In addition, this construct was generated to express intracellular (*e.g.,* cytoplasmic and/or nuclear) UBE3A and differs from some work performed in other labs in that it does not comprise an exogenously added secretory signal, thereby preventing extracellular expression and generation of a secreted form of UBE3A. While not wishing to be bound to

theory, it is believed that such engineering of the UBE3A to result in secreted UBE3A would remove the ability to regulate appropriate levels of expression of UBE3A, which may lead to hazardous overexpression. Overexpression of UBE3A may be linked to Dup15q syndrome, a syndromic form of autism. Secreted UBE3A may also lose regulation of cell-type specificity or biodistribution of UBE3A expression, as well as have altered post-translational modifications and/or altered and/or expanded substrate interaction for its ligase activity. In this respect, the UBE3A product generated from the constructs described herein is thought to generate superior results than have been previously reported.

[0192] **FIG. 3B** shows a schematic of the 5' vector DNA sequence of Kozak-optimized (SEQ ID NO:20) (top) and protein (SEQ ID NO:21) (bottom) codon-optimized hUBE3A1>>3 open reading frame for the vector design to achieve biased expression of the short human UBE3A (hUBE3A) isoform. 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 (AGCAGGATGA; SEQ ID NO:25) beginning the short isoform of hUBE3A (**FIG. 3B** and **FIG. 3C**).

[0193] Expression properties of the construct were examined in an AS mouse model (*Ube3a*$^{m-/p+}$), wherein mice show a complete loss of all UBE3A isoforms in neurons (**FIG. 4;** Jiang *et al*, Neuron, 1998:21(4):799-811). Neonatal mice were intracerebroventricularly (ICV) injected with about 1 x 10^{11} viral genomes (vg) of AAV9-derived variant virus vector PHP.B/SYN-hUBE3A on postnatal day P1 and harvested at various time points for complementary analyses of mRNA and protein expression. **FIG. 5** shows that *hUBE3A* transcripts expressed from the construct can be selectively detected among endogenous mRNAs in a treated subject, due to the presence unique nucleotide sequences introduced through codon-optimization. This aspect of the construct design can be leveraged to faithfully trace PHP.B/SYN-hUBE3A biodistribution, and thereby facilitate the optimization of biodelivery strategies in mice, nonhuman primates, and eventually humans. *hUBE3A* transcripts encoded by the design proved capable of expressing both short and long hUBE3A protein isoforms as shown by the western blotting experiment in **FIG. 6.** Importantly, the ratio of short to long hUBE3A was 3:1, approximating the 4:1 ratio previously observed for endogenous mouse UBE3A isoforms (Avagliano *et al.*, Nat Neurosci, 2019:22(8):1235-1247), and demonstrative of the feasibility of the construct design to achieve desired hUBE3A isoform ratios through the manipulation of Kozak sequence strengths.

[0194] **FIG. 7A** demonstrates successful delivery of the hUBE3A_Isoform1>>3 products to neonatal AS model mice, as evidenced by UBE3A immunohistochemistry (IHC) performed

on postnatal days P10, P15, and P25. Examining the brain structures more closely, costaining of UBE3A and the neuronal marker NeuN showed near-complete co-localization, indicating highly efficient transduction of neurons with PHP.B/SYN-UBE3A (**FIG. 7B**). UBE3A protein showed pronounced nuclear localization, consistent with dominant expression of the short hUBE3A isoform. Arrows indicate rare hippocampal neurons in which UBE3A was not re-expressed, indicating that most neurons in the cortex and hippocampus express hUBE3A_Isoform1>>3 products.

[0195] Qualitative assessments showed effective re-introduction of UBE3A isoforms to the developing brain (**FIG. 8 panels A-F**), at the appropriate levels and in accordance with endogenous patterns of subcellular localization, wherein hUBE3A exhibits a cytoplasmic-to-nuclear shift in subcellular localization subsequent to gene transfer. **FIG. 8 Panels A-C** show UBE3A-stained layer 2/3 neocortex from AS model mice at P10 (**Panel A**), P15 (**Panel B**), and P25 (**Panel C**) following ICV injection of PHP.B/hUBE3A virus at P1. A gradual accumulation of virally transduced hUBE3A in the nucleus was seen over development, a faithful recapitulation of spatiotemporal patterns of endogenous UBE3A expression, as shown in **Panels D-F** from Judson *et al.*, J Comparative Neurology, 2014:522(8):1874-1896.

Example 2: Human UBE3A codon-optimized construct treatment for recovery of anatomical and behavioral phenotypes in AS model mice.

[0196] **FIG. 9** shows that PHP.B/hUBE3A was generally well-tolerated in both WT and AS model mice, as demonstrated by body weights that were equivalent in the gene therapy-treated groups (WT + AAV and AS + AAV) and vehicle-treated WT controls (WT + Vehicle) at one month of age. Vehicle-treated AS mice gained weight excessively into adulthood, consistent both with previous reports (Judson *et al.*, J Neurosci, 2017:37(31):7347-7361), and with clinical observations of adult-onset obesity in AS individuals (Bindels-de Heus *et al.*, Am J Med Genet A, 2020:182(1):53-63). This phenotype was completely rescued by PHP.B/hUBE3A treatment in the AS + AAV group.

[0197] Further testing of the capacity for neonatal ICV PHP.B/hUBE3A treatment to rescue highly penetrant and reproducible AS mouse model deficits was pursued (**FIG. 10, panel A;** Rotaru *et al.*, Neuroscience, 2020:Epub ahead of print). **FIG. 10, panel B** shows a trend toward hypolocomotion in AS + Vehicle mice that was not affected by gene therapy treatment. AS + Vehicle and AS +AAV exhibited similarly poor performance in the first trial of the rotarod task, suggesting that a certain level of motor dysfunction in AS may be impervious to neonatal ICV PHP.B/hUBE3A treatment. However, repeated rotarod testing

revealed a restored capacity for motor learning and plasticity in AS + AAV mice that raised motor performance over successive acquisition trials and sustained it near control levels over time (**FIG. 10, panel C**).

[0198] Innate behaviors including digging and nest building are also known to be severely deficient in AS mice (Rotaru *et al.*, Neuroscience, 2020:Epub ahead of print). **FIG. 10, panels D** and **E** show that these phenotypes are recapitulated in AS + Vehicle mice, as evidenced by their poor performance relative to WT + Vehicle controls on well-established marble burying and nest building assays. The penetrance of these phenotypes was greatly reduced in the AS + AAV group, with many individual subjects performing the tasks at control levels. Nevertheless, there was a small subset of poor AS + AAV responders, which were especially noticeable in the spread of the marble burying data. These results warrant careful *post hoc* analysis of hUBE3A biodistribution to ensure that the true efficacy of the treatment is not underestimated due to technical errors—poor injection targeting or spread—that confound measurements of behavioral rescue.

[0199] **FIG. 11** shows evidence of severe epileptogenesis in AS + Vehicle mice that was revealed through flurothyl seizure kindling and rechallenge. This result was consistent with the findings of a previous study (Gu et al., J Clin Invest, 2018:129(1):163-168), in which AS mice exhibited a similarly heightened sensitivity to flurothyl re-exposure a month after kindling. Moreover, exaggerated epileptogenesis in AS mice was amenable to rescue by tamoxifen-inducible reinstatement of UBE3A during postnatal development, and accordingly, neonatal ICV PHP.B/hUBE3A treatment rendered AS + AAV mice resilient to flurothyl rechallenge (**FIG. 11, panel B**).

[0200] Anatomical and physiological changes to brain circuits define epileptogenesis. Flurothyl-kindled AS mice show an enhanced deposition of perineuronal nets (PNNs) in the hippocampus, specifically in the molecular layer of the dentate gyrus (Gu et al., J Clin Invest, 2018:129(1):163-168). **FIG. 12, panel A** shows that this aberrant PNN phenotype was also penetrant in AS + Vehicle mice, but fully rescued in AS + AAV mice. Reactive astrogliosis is another hallmark of epileptogenic foci. **FIG. 12, panel B** shows that there was a dramatic increase in intensely GFAP-stained astrocytes in the hippocampi of AS + Vehicle mice, which proved to be completely normalized in the AS + AAV group. Collectively, these studies provide corroborating evidence that neonatal ICV PHP.B/hUBE3A treatment could be highly effective in improving seizure outcomes in AS.

[0201] WT + AAV mice served as a *de facto* test of the tolerance of UBE3A overexpression in these studies. In most cases WT + AAV and WT + Vehicle group

performance was similar, suggesting that UBE3A overexpression resulting from PHP.B/hUBE3A treatment is relatively well-tolerated. However, WT + AAV mice did prove to be moderately hyperlocomotive (**FIG. 10, panel B**), and they somewhat underperformed on the marble burying task (**FIG. 10, panel D**). Thus, certain behaviors and underlying neural circuits may potentially be more vulnerable than others to UBE3A overexpression.

Example 3: Construct treatment in human-derived cells *in vitro* and *in vivo*.

[0202] The construct described in Example 1 will be delivered *in vitro* to patient-derived iPSCs which have been differentiated into neurons and which exhibit AS cellular phenotypes. The recipient cells will then be observed for reversal of characteristic AS cellular phenotypes, as compared to an appropriate control cell which has not received the construct.

[0203] AS iPSC lines can be generated from patient peripheral skin or blood cells to which pluripotency is conferred by retroviral or lentiviral vectors expressing key transcription factors OCT4, SOX2, KLF4, MYC, and LIN28 (Takahashi et al., Cell, 2007: 131: 861-872; Sommer et al., Stem Cells, 2008: 27: 543-549). In some preparations, CRISPR/Cas9 editing can be used to introduce AS-causing UBE3A deletions to iPSCs derived from neurotypical patients, thus yielding novel AS iPSC lines with accompanying isogenic controls. AS iPSCs can be differentiated into neurons with neural induction media containing supplements and neural growth factors according to established protocols (Chamberlain et al., Proc. Natl. Acad. Sci., 2010: 107: 17668-17673).

[0204] Patient-derived AS neurons have been shown to exhibit characteristically delayed maturation of electrophysiological properties including hyperpolarization of the resting membrane potential and action potential firing, which is amenable to rescue by paternal UBE3A unsilencing by pharmacological agents (Fink et al., Nat. Comm., 2017: Apr 24;8:15038). Similarly, the construct will be delivered to patient-derived AS neurons (in vitro and in vivo) in order to restore expression of hUBE3A and correct delays in electrophysiological maturation. For proof-of-concept treatment in non-human primates and humans, the construct will be delivered at early intervention stages (e.g., less than 2 years of age) in 1 to 3 patients, with dose escalation as needed based on safety and efficacy studies. Clinical benefit and safety will be assessed (e.g., as compared to appropriate controls).

[0205] The foregoing examples are illustrative of the present invention, and are not to be construed as limiting thereof. Although the invention has been described in detail with reference to preferred embodiments, variations and modifications exist within the scope and spirit of the invention as described and defined in the following claims.

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.

2. The polynucleotide of claim 1, wherein the human UBE3A open reading frame encodes human UBE3A short isoform (*i.e.,* isoform 1) and long isoform 2.

3. The polynucleotide of claim 1, wherein the human UBE3A open reading frame encodes human UBE3A short isoform (*i.e.,* isoform 1) and long isoform 3.

4. The polynucleotide of any one of claims 1-3, 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.

5. The polynucleotide of claim 4, which comprises 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.

6. The polynucleotide of any one of claims 1-5, wherein the human UBE3A open reading frame comprises one or more nucleotide modifications to enhance expression of the short isoform (*e.g.,* enhance expression by 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or more) as compared to native ORF not comprising nucleotide modifications to enhance expression.

7. The polynucleotide of claim 6, 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.

8. The polynucleotide of any one of claims 1-7, 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.

9. The polynucleotide of any one of claims 1-8, which comprises a first Kozak sequence comprising SEQ ID NO:6 operably linked to coding sequences for the long isoform.

10. The polynucleotide of any one of claims 1-9, which comprises a second Kozak sequence comprising SEQ ID NO:8 operably linked to coding sequence for the short isoform.

11. The polynucleotide of any one of claims 1-10, wherein the human UBE3A open reading frame is codon-optimized for expression in a human cell.

12. The polynucleotide of claim 12, wherein the human UBE3A open reading frame is codon-optimized for intracellular or non-secretory expression in a human cell.

13. The polynucleotide of any one of claims 1-12, 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%, 75%, 76%, 77%, 80%, 85%, or 90% identity thereto.

14. An expression cassette comprising the polynucleotide of any one of claims 1-13.

15. The expression cassette of claim 14, wherein the human UBE3A open reading frame is operably linked to a promoter.

16. The expression cassette of claim 15, wherein the promoter is a neuron-specific promoter.

17. The expression cassette of claim 15 or 16, wherein the promoter is a human synapsin promoter.

18. The expression cassette of any one of claims 14-17, wherein the human UBE3A open reading frame is operably linked to a polyadenylation signal.

19. The expression cassette of claim 18, wherein the polyadenylation signal is a bovine growth hormone (bGH) polyadenylation signal (bGHpA).

20. The expression cassette of any one of claims 14-19, further comprising at least one adeno-associated virus (AAV) inverted terminal repeat (ITR).

21. The expression cassette of claim 20, wherein the expression cassette comprises two AAV ITRs.

22. The expression cassette of claim 21, 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.

23. The expression cassette of claim 22, wherein the AAV ITRs are AAV2 ITRs.

24. The expression cassette of any one of claims 14-23, wherein the expression cassette is a single stranded AAV genome.

25. The expression cassette of any one of claims 14-24, wherein the expression cassette comprises a promoter, the human UBE3A open reading frame, and a polyadenylation site.

26. The expression cassette of any one of claims 14-25, wherein the expression cassette comprises an AAV ITR, a promoter, the human UBE3A open reading frame, a polyadenylation site, and an AAV ITR.

27. The expression cassette of any one of claims 14-26, 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 2, a bGHpA, and an AAV2 ITR.

28. The expression cassette of any one of claims 14-26, 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, a bGHpA, and an AAV2 ITR.

29. The expression cassette of any one of claims 14-28, 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 2 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.

30. The expression cassette of any one of claims 14-28, 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.

31. The expression cassette of claim 30, comprising the nucleotide sequence of SEQ ID NO:14 or a sequence at least about 90% identical thereto.

32. A vector comprising the polynucleotide of any one of claims 1-13 or the expression cassette of any one of claims 14-31.

33. The vector of claim 32, wherein the vector is a neuro-tropic vector.

34. The vector of claim 32 or 33, wherein the vector is a viral vector.

35. The vector of claim 34, wherein the vector is an AAV vector.

36. The vector of claim 35, wherein the AAV vector is selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12 AAV13, and combinations thereof.

37. The vector of claim 35, wherein the AAV vector is an AAV9 or AAV9-derived vector.

38. The vector of claim 37, wherein the AAV9-derived vector is PHP.B, PHP.eB, or AAV9.HR).

39. A transformed cell comprising the polynucleotide of any one of claims 1-13, the expression cassette of any one of claims 14-31, and/or the vector of any one of claims 32-38.

40. The transformed cell of claim 39, wherein the polynucleotide, expression cassette, and/or vector is stably incorporated into the cell genome.

41. A transgenic animal comprising the polynucleotide of any one of claims 1-13, the expression cassette of any one of claims 14-31, the vector of any one of claims 32-38, and/or the transformed cell of claim 39 or 40.

42. A pharmaceutical composition comprising the polynucleotide of any one of claims 1-13, the expression cassette of any one of claims 14-31, the vector of any one of claims 32-38, and/or the transformed cell of claim 39 or 40 in a pharmaceutically acceptable carrier.

43. A method of expressing a UBE3A open reading frame in a cell, comprising contacting the cell with the polynucleotide of any one of claims 1-13, the expression cassette of any one of claims 14-31, and/or the vector of any one of claims 32-38, thereby expressing the UBE3A open reading frame in the cell.

44. A method of expressing a UBE3A open reading frame in a subject, comprising delivering to the subject the polynucleotide of any one of claims 1-13, the expression cassette of any one of claims 14-31, the vector of any one of claims 32-38, and/or the transformed cell of claim 39 or 40, thereby expressing the UBE3A open reading frame in the subject.

45. 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 of any one of claims 1-13, the expression cassette of any one of claims 14-31, the vector of any one of claims 32-38, and/or the transformed cell of claim 39 or 40, such that the UBE3A open reading frame is expressed in the subject.

46. The method of claim 45, wherein the disorder associated with expression of the UBE3A gene is Angelman Syndrome.

47. A method of treating Angelman Syndrome in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the polynucleotide of any one of claims 1-13, the expression cassette of any one of claims 14-31, the vector of any one of claims 32-38, and/or the transformed cell of claim 39 or 40, such that the UBE3A open reading frame is expressed in the subject.

48. The method of any one of claims 43-47, 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.

49. The method of any one of claims 44-48, wherein the subject exhibits symptoms of the disease prior to delivery of the polynucleotide, expression cassette, vector, and/or transformed cell.

50. The method of any one of claims 44-49, wherein the polynucleotide, expression cassette, vector, and/or transformed cell is delivered *in utero*.

51. The method of any one of claims 44-50, wherein the subject is a human.

52. The method of any one of claims 44-51, wherein the polynucleotide, expression cassette, vector, and/or transformed cell is delivered to the nervous system of the subject.

53. The method of any one of claims 44-52, wherein the AAV vector is administered to the subject 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.

54. The method of claim 52, wherein the polynucleotide, expression cassette, vector, and/or transformed cell is delivered intravenously.

55. The method of claim 52, wherein the polynucleotide, expression cassette, vector, and/or transformed cell is delivered by intrathecal, intracerebral, intra-cisterna magna, intraparenchymal, intracerebroventricular, intranasal, intra-aural, intra-ocular, or peri-ocular delivery, or any combination thereof.

56. The method of claim 55, wherein the polynucleotide, expression cassette, vector, and/or transformed cell is delivered intrathecally.

57. The method of claim 55, wherein the polynucleotide, expression cassette, vector, and/or transformed cell is delivered intracerebroventricularly.