

COMPOSITIONS AND METHODS FOR TREATING CONDITIONS ASSOCIATED WITH UBE3A OVEREXPRESSION

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of and priority to U.S. Provisional Application No. 63/609,567, filed 13 December 2023, the entire disclosure of which is hereby incorporated herein by reference in its entirety for all purposes.

REFERENCE TO A SEQUENCE LISTING

[0002] This application contains a Sequence Listing which has been submitted electronically in XML format. The Sequence Listing XML is incorporated herein by reference. Said XML file, created 25 October 2024, is named UXN150-01WO_SL, and is 3,671,742 bytes in size.

TECHNICAL FIELD OF THE DISCLOSURE

[0003] This disclosure relates to antisense nucleic acid compounds for inhibiting expression of ubiquitin protein ligase E3A (*UBE3A*) in neurons and compositions comprising the same for use in the treatment of conditions associated with *UBE3A* overexpression such as chromosome 15q11.2-q13.3 duplication syndrome (Dup15q syndrome).

BACKGROUND OF THE INVENTION

[0004] Ubiquitin protein ligase E3A (*UBE3A*) poly-ubiquitinates cytoplasmic and nuclear proteins, targeting them for proteasomal degradation. This protein is expressed in many cell types, including in neurons, where it is highly localized to axon terminals, but it is also found in nuclei and mitochondria. In neurons, *UBE3A* is thought to locally regulate individual synapses through its activity in axon terminals and globally regulate neuronal physiology through its chromatin regulation activity in nuclei. *UBE3A* may be involved in the epigenetic regulation of neuronal imprinted genes by way of its regulation of DNA methylation and chromatin modeling. (See Lopez, Simon Jesse, David J. Segal, and Janine M. LaSalle. "UBE3A: An E3 ubiquitin ligase with genome-wide impact in neurodevelopmental disease." *Frontiers in Molecular Neuroscience* 11 (2019): 476).

[0005] The ubiquitin protein ligase E3A (*UBE3A*) gene is located within the human 15q11.2-q13.3 locus, a unique and highly regulated genetic locus that is parentally imprinted in neuronal cells. Specifically, the maternal allele is selectively methylated whereas the paternal allele is

non-methylated and expressed in neurons. At the same time, the paternal *UBE3A* allele is silenced in neurons by expression of the *UBE3A* anti-sense transcript (*UBE3A-ATS*). This unique regulation gives rise to three different human neurodevelopmental disorders stemming from mutations in the 15q11.2-q13.3 locus: deletion of the maternal allele gives rise to Angelman syndrome (AS) due to loss of *UBE3A* expression caused by silencing of the paternal allele; deletion of the paternal allele gives rise to Prader-Willi syndrome (PWS); and duplication of the maternal allele gives rise to chromosome 15q11.2-q13.3 duplication syndrome (Dup15q syndrome). (Lopez, et al., 2019; see also Copping, Nycole A., et al. "Neuronal overexpression of Ube3a isoform 2 causes behavioral impairments and neuroanatomical pathology relevant to 15q11.2-q13.3 duplication syndrome." *Human Molecular Genetics* 26.20 (2017): 3995-4010).

[0006] Symptoms of Dup15q syndrome present with a high degree of diversity among patients, but frequently include hypotonia, speech/language disorder, developmental delay, behavior challenges, and abnormal EEG. Patients may also present with characteristic facial features, reduced growth, autism, sensory processing disorders, seizures, and hyperpigmentation. Dup15q syndrome patients usually have one of two forms of genetic disruption giving rise to the syndrome: maternal interstitial duplications and maternal isodicentric ("idic(15)") duplications. Idic(15) patients are tetrasomic for the 15q11.2-q13 region (2 extra copies of the region, with 3 maternal copies and 1 paternal) and maternal interstitial duplication patients are triploid for the region (1 extra copy, with 2 maternal copies and 1 paternal). (See Kalsner, Louisa, and Stormy J. Chamberlain. "Prader-Willi, Angelman, and 15q11-q13 duplication syndromes." *Pediatric Clinics* 62.3 (2015): 587-606).

[0007] Estimates vary, but it is believed that Dup15q syndrome affects between 1 in 5,000 to 1 in 20,000 individuals, yet no disease-modifying therapy exists. Others have described antisense oligonucleotides targeting human *UBE3A* (see United States Pat. App. Pub. No. US/2022/0259601A1 and Elamin, Marwa, et al. "The role of *UBE3A* in the autism and epilepsy-related Dup15q syndrome using patient-derived, CRISPR-corrected neurons." *Stem Cell Reports* 18.4 (2023): 884-898); still, an effective ASO for treating Dup15q syndrome has yet to be identified. Thus, there remains a high unmet need for therapeutic approaches to treating Dup15q syndrome and other disorders arising from excess *UBE3A* expression or activity. The present disclosure addresses this need by providing novel nucleic acid molecules that target human *UBE3A* nucleic acids, including *UBE3A* genomic regions and transcripts, and reduce *UBE3A* expression in human neurons.

BRIEF SUMMARY OF INVENTION

[0008] This disclosure provides compositions comprising novel nucleic acid molecules that can be used to inhibit or reduce expression of *UBE3A* in neurons. The disclosure further provides methods of using these compositions for the prevention or treatment of conditions associated with *UBE3A* overexpression in CNS neurons of a subject. More specifically, embodiments of this disclosure provide antisense nucleic acid compounds (i.e., “antisense compounds”) targeting a human *UBE3A* nucleic acid, and methods of their use for the treatment of Dup15q syndrome.

[0009] In one aspect, provided herein are antisense oligonucleotides (ASOs) comprising at least 16 contiguous nucleosides and having a nucleotide sequence selected from SEQ ID NO: 285 through SEQ ID NO: 562. The ASOs may comprise one or more of: a) a gap segment consisting of linked deoxynucleosides; b) a 5' segment consisting of at least 2 linked nucleosides; c) a 3' segment consisting of at least 2 linked nucleosides; d) at least one phosphorothioate internucleoside linkage; e) at least one nucleoside comprising a modified sugar; and f) at least one nucleoside comprising a modified nucleobase.

[0010] The gap segment of the ASOs may be positioned between the 5' segment and the 3' segment. The gap segment may comprise 5 to 15 linked nucleosides.

[0011] The 3' segment of the ASOs may comprise 2-5 linked nucleosides.

[0012] The 5' segment of the ASOs may comprise 2-5 linked nucleosides.

[0013] In some embodiments, at least one nucleoside of the 5' segment of the ASOs and at least one nucleoside of the 3' segment of the ASOs may comprise a modified sugar. In some embodiments, each nucleoside of the 5' segment and each nucleoside of the 3' segment comprises a modified sugar.

[0014] The modified sugar of the ASOs may comprise a bicyclic sugar. The bicyclic sugar may be selected from the group consisting of: 2'-O(CH₂)₂OCH₃ (MOE); 4'-(CH₂)—O-2' (LNA); 4'-(CH₂)₂—O-2' (ENA); and 4'-CH(CH₃)—O-2' (cEt).

[0015] In some embodiments, each internucleoside linkage of the ASOs is a phosphorothioate internucleoside linkage.

[0016] In some embodiments, a 5-methylcytosine nucleobase is substituted in place of a non-5-methyl cytosine residue in the ASOs.

[0017] The ASO may comprise a 3-10-3 LNA-DNA-LNA gapmer, wherein all internucleoside linkages are phosphorothioate internucleoside linkages.

[0018] The ASO may comprise a 3-11-3 LNA-DNA-LNA gapmer, wherein all

internucleoside linkages are phosphorothioate internucleoside linkages.

[0019] The ASO may comprise a 3-12-3 LNA-DNA-LNA gapmer, wherein all internucleoside linkages are phosphorothioate internucleoside linkages.

[0020] The ASO may comprise a 4-11-5 MOE-DNA-MOE gapmer, wherein all internucleoside linkages are phosphorothioate internucleoside linkages.

[0021] The ASO may comprise a nucleotide sequence according to SEQ ID NO: 295, 307, 309, 310, 327, 328, 329, 330, 340, 341, 342, 343, 351, 356, 359, 366, 394, 402, 523, 527, 528, 529, 539, 542, 549, 550, 551, or 553.

[0022] In some embodiments, the ASO is a gapmer according to Compound ID No. 16-63326LNA, 17-29823LNA, 17-29855LNA, 17-29858LNA, 17-63264LNA, 17-63289LNA, 17-63290LNA, 17-63291LNA, 17-63324LNA, 17-63325LNA, 17-63326LNA, 17-63327LNA, 17-63458LNA, 17-67260LNA, 18-435LNA, 18-29854LNA, 18-63323LNA, 18-63458LNA, 17-63278LNA, 17-63286LNA, 17-63287LNA, 17-63288LNA, 17-63437LNA, 17-29853LNA, 18-63282LNA, 18-63285LNA, 18-63286LNA, or 18-63325LNA.

[0023] Also provided herein is an antisense oligonucleotide (ASO) comprising: a 5' segment of 3 linked locked nucleic acid (LNA) nucleosides, a gap segment of linked deoxynucleosides, and a 3' segment of 3 linked LNA nucleosides, wherein all internucleoside linkages are phosphorothioate internucleoside linkages, and wherein ASO comprises the nucleotide sequence of SEQ ID NO: 550.

[0024] Also provided herein is an antisense oligonucleotide (ASO) comprising: a 5' segment of 3 linked locked nucleic acid (LNA) nucleosides, a gap segment of linked deoxynucleosides, and a 3' segment of 3 linked LNA nucleosides, wherein all internucleoside linkages are phosphorothioate internucleoside linkages, and wherein ASO comprises the nucleotide sequence of SEQ ID NO: 542.

[0025] Also provided herein is an antisense oligonucleotide (ASO) comprising: a 5' segment of 3 linked locked nucleic acid (LNA) nucleosides, a gap segment of linked deoxynucleosides, and a 3' segment of 3 linked LNA nucleosides, wherein all internucleoside linkages are phosphorothioate internucleoside linkages, and wherein ASO comprises the nucleotide sequence of SEQ ID NO: 394.

[0026] Also provided herein is an antisense oligonucleotide (ASO) comprising: a 5' segment of 3 linked locked nucleic acid (LNA) nucleosides, a gap segment of linked deoxynucleosides, and a 3' segment of 3 linked LNA nucleosides, wherein all internucleoside linkages are phosphorothioate internucleoside linkages, and wherein ASO comprises the nucleotide sequence of SEQ ID NO: 328.

[0027] Also provided herein is an antisense oligonucleotide (ASO) comprising: a 5' segment of 3 linked locked nucleic acid (LNA) nucleosides, a gap segment of linked deoxynucleosides, and a 3' segment of 3 linked LNA nucleosides, wherein all internucleoside linkages are phosphorothioate internucleoside linkages, and wherein ASO comprises the nucleotide sequence of SEQ ID NO: 342.

[0028] Also provided herein is an antisense oligonucleotide (ASO) comprising: a 5' segment of 3 linked locked nucleic acid (LNA) nucleosides, a gap segment of linked deoxynucleosides, and a 3' segment of 3 linked LNA nucleosides, wherein all internucleoside linkages are phosphorothioate internucleoside linkages, and wherein ASO comprises the nucleotide sequence of SEQ ID NO: 356.

[0029] Also provided herein is an antisense oligonucleotide (ASO) comprising: a 5' segment of 3 linked locked nucleic acid (LNA) nucleosides, a gap segment of linked deoxynucleosides, and a 3' segment of 3 linked LNA nucleosides, wherein all internucleoside linkages are phosphorothioate internucleoside linkages, and wherein ASO comprises the nucleotide sequence of SEQ ID NO: 295.

[0030] Also provided herein is an antisense oligonucleotide (ASO) comprising: a 5' segment of 3 linked locked nucleic acid (LNA) nucleosides, a gap segment of linked deoxynucleosides, and a 3' segment of 3 linked LNA nucleosides, wherein all internucleoside linkages are phosphorothioate internucleoside linkages, and wherein ASO comprises the nucleotide sequence of SEQ ID NO: 351.

[0031] Also provided herein is an antisense oligonucleotide (ASO) comprising: a 5' segment of 3 linked locked nucleic acid (LNA) nucleosides, a gap segment of linked deoxynucleosides, and a 3' segment of 3 linked LNA nucleosides, wherein all internucleoside linkages are phosphorothioate internucleoside linkages, and wherein ASO comprises the nucleotide sequence of SEQ ID NO: 307.

[0032] In some embodiments, all cytosine nucleosides of the ASOs are replaced with 5-methylcytosine nucleosides.

[0033] In some embodiments, the ASOs comprise a 2'OMe-modified nucleoside at position 2 in the DNA gap.

[0034] Also provided herein are compositions comprising ASOs or salt thereof and a pharmaceutically acceptable carrier.

[0035] The composition may be a pharmaceutical formulation.

[0036] Also provided are methods of reducing the level of a *UBE3A* RNA in a cell, comprising contacting the cell with the ASO composition, thereby reducing the level of the

UBE3A RNA in the cell.

[0037] Also provided are methods of inhibiting expression of *UBE3A* protein in a cell, comprising contacting the cell with the ASO or composition, thereby reducing expression of *UBE3A* protein in the cell.

[0038] In some embodiments, the cell is a central nervous system neuron.

[0039] In some embodiments, the cell is triploid or tetraploid for the *UBE3A* gene.

[0040] The cell may comprise a maternal duplication of the *UBE3A* allele.

[0041] The methods described herein may be performed on a cell in vitro. In some embodiments, the cell may be in a subject.

[0042] The subject may be a human and the ASO or composition may be administered to the subject.

[0043] In some embodiments, the ASO is administered to the subject via intravenous, subcutaneous, intramuscular, intraperitoneal, intrathecal, intra cisterna magna, or intraventricular (i.e., intracerebroventricular) injection.

[0044] Also provided are methods of treating, preventing, or ameliorating a disease associated with overexpression of *UBE3A* in central nervous system neurons in a subject, comprising administering to the subject an ASO or composition thereof described herein, thereby treating, preventing, or ameliorating the disease. The disease may be Dup15q syndrome. In some instances, the subject may have an idic(15) genotype.

[0045] In some embodiments, the methods may comprise administering to the subject a second pharmaceutical agent for treating, preventing, or ameliorating one or more symptoms of Dup15q syndrome. In some instances, the subject may have an idic(15) genotype.

[0046] In some embodiments, the ASOs or compositions thereof described herein are for use in a method of treating, preventing, or ameliorating a disease associated with overexpression of *UBE3A* in central nervous system neurons in a subject. The disease may be Dup15q syndrome. In some instances, the subject may have an idic(15) genotype.

[0047] Also provided are uses of an ASO or composition thereof described herein for the treatment of a disease associated with increased levels of *UBE3A* protein in central nervous system neurons in a subject. The disease may be Dup15q syndrome. In some instances, the subject may have an idic(15) genotype.

[0048] Also provided are uses of an ASO or composition thereof described herein for the preparation of a medicament for the treatment of a disease associated with increased levels of *UBE3A* protein in central nervous system neurons of a subject. The disease may be Dup15q syndrome. In some instances, the subject may have an idic(15) genotype.

BRIEF DESCRIPTION OF DRAWINGS

[0049] FIG. 1 shows the dose response curve of ASO 18-63285LNA in normal human H9 neurons treated for 10 days as measured using multiplex QuantiGene assay.

[0050] FIG. 2 shows the dose response curve of ASO 17-29853LNA in normal human H9 neurons treated for 10 days as measured using multiplex QuantiGene assay.

[0051] FIG. 3 shows the dose response curve of ASO 18-63323LNA in normal human H9 neurons treated for 10 days as measured using multiplex QuantiGene assay.

[0052] FIG. 4 shows the dose response curve of ASO 17-67260LNA in normal human H9 neurons treated for 10 days as measured using multiplex QuantiGene assay.

[0053] FIG. 5 shows the dose response curve of ASO 16-63326LNA in normal human H9 neurons treated for 10 days as measured using multiplex QuantiGene assay.

[0054] FIG. 6 shows the dose response curve of ASO 17-63458LNA in normal human H9 neurons treated for 10 days as measured using multiplex QuantiGene assay.

[0055] FIG. 7 shows the dose response curve of ASO 17-29823LNA in normal human H9 neurons treated for 10 days as measured using multiplex QuantiGene assay.

[0056] FIG. 8 shows effects of 3 μ M ASO treatment for 10 days on *UBE3A* mRNA levels in iPSC-derived Dup15q-idic(15) neurons (triplicates) compared to untreated controls.

[0057] FIG. 9 shows effects of 3 μ M ASO treatment for 10 days on UBE3A protein levels in iPSC-derived Dup15q-idic(15) neurons compared to untreated controls.

[0058] FIG. 10 shows dose response curves of select ASOs (17-29853LNA and 17-29823LNA) treated for 10 days.

[0059] FIG. 11 shows dose-dependent UBE3A protein knockdown as confirmed by Western blot for ASO 17-29823LNA.

[0060] FIG. 12 shows in vivo target engagement of select ASOs in mouse brain (ASO1 = 18-63285LNA; ASO2 = 17-29853LNA; ASO3 = 18-63286LNA; ASO4 = 17-63286LNA; and ASO10 = 17-63458LNA).

DETAILED DESCRIPTION OF THE INVENTION

[0061] This disclosure provides antisense nucleic acid compounds including, e.g., antisense oligonucleotides (ASOs), for use in therapeutic applications. In some embodiments, the antisense compounds including ASOs and compositions or formulations thereof of this disclosure can be used for ameliorating, preventing or treating conditions associated with excess *UBE3A* expression, including, e.g., chromosome 15q11.2-13.1 duplication syndrome,

or Dup15q syndrome. Genetic and clinical evidence suggest that overexpression of the maternal *UBE3A* allele is the major contributor to the pathology observed in Dup15q syndrome, a neurodevelopmental disorder caused by maternal duplication of a genetic region on chromosome 15 called Prader-Willi Syndrome/Angelman Syndrome Critical Region (PWS/AS-CR). In CNS neurons, *UBE3A* is expressed only on the maternal chromosome as its expression on the paternal chromosome is epigenetically silenced. Full presentation of Dup15q syndrome occurs when *UBE3A* is overexpressed on the maternal chromosome. It is presently submitted that reduction of *UBE3A* overexpression in the CNS may ameliorate the neurological deficits observed in Dup15q syndrome individuals. Thus, antisense nucleic acid compounds are provided herein which are useful in reducing overexpression of *UBE3A* and/or normalizing *UBE3A* levels in Dup15q syndrome individuals.

[0062] In some embodiments, the antisense compounds of the disclosure reduce levels of a human *UBE3A* nucleic acid in a subject upon administration to the subject. In some embodiments, the antisense compounds of the disclosure reduce levels of *UBE3A* protein in a subject upon administration to the subject. The antisense compounds may reduce expression of *UBE3A* in a subject upon administration to the subject to levels seen under genetically normal conditions, i.e., with one paternal and one maternal copy present. In these manners, the antisense compounds provided herein may slow, ameliorate, or reverse the effects of *UBE3A* overexpression in neurons.

[0063] Thus, this disclosure encompasses synthetic, purified, antisense nucleic acid compounds such as antisense oligonucleotides (“ASOs”) for therapeutic use in reducing *UBE3A* nucleic acid and/or reducing *UBE3A* protein levels upon administration to a subject. The antisense compounds may contain natural and modified nucleotides, natural and modified internucleoside linkages, varying nucleotide lengths, and/or varying nucleoside (RNA and DNA) compositions, as provided herein.

I. Definitions

[0064] Throughout the present specification, the terms “about” and/or “approximately” may be used in conjunction with numerical values and/or ranges. The term “about” is understood to mean those values near to a recited value. Furthermore, the phrases “less than about [a value]” or “greater than about [a value]” should be understood in view of the definition of the term “about” provided herein. The terms “about” and “approximately” may be used interchangeably. Unless otherwise noted, the term “about” when immediately preceding a numerical value means $\pm 10\%$ of the numerical value.

[0065] Throughout the present specification, numerical ranges are provided for certain quantities. It is to be understood that these ranges comprise endpoints and all subranges therein, including each integer in and between a disclosed range. Thus, the range “from 50 to 80” includes all possible ranges therein (e.g., 51-79, 52-78, 53-77, 54-76, 55-75, 60-70, etc.) as well as each individual integer from 50 to 80 (e.g., 50, 51, 52, 53, 54, etc.). Where ranges are provided in the form of fractions, percentages, decimals, and the like, such ranges likewise include all possible subranges therein and each individual fraction, percentage, decimal, etc. in and between the disclosed range. For example, the range “from 0.1 to 1.0” includes all possible ranges therein (e.g., 0.2 to 0.9, etc.) and each individual 1/10th decimal from 0.1 to 1.0 (e.g., 0.1, 0.2, 0.3, 0.4, etc.). Furthermore, all values within a given range may be an endpoint for the range encompassed thereby (e.g., the range 50-80 includes the ranges with endpoints such as 55-80, 50-75, etc.).

[0066] The term “a” or “an” refers to one or more of that entity; for example, “an antisense oligonucleotide targeting *UBE3A*” refers to one or more such antisense oligonucleotides or at least one such antisense oligonucleotide. As such, the terms “a” (or “an”), “one or more” and “at least one” are used interchangeably herein.

[0067] The terms “comprise,” “comprising,” and the like, as used in this specification and in the claims are used in the non-limiting sense to mean that items following the word are included, but items not specifically mentioned are not excluded. The present invention may suitably “comprise”, “consist of”, or “consist essentially of”, the steps, elements, and/or reagents described in the claims.

[0068] “Antisense activity” means any detectable and/or measurable change attributable to the hybridization of an antisense compound to its target nucleic acid. Antisense activity includes knockdown or elimination of expression of a gene targeted by a compound having antisense activity.

[0069] “Antisense compound” means a compound comprising or consisting of an oligonucleotide at least a portion of which is complementary to a target nucleic acid to which it is capable of hybridizing, resulting in at least one antisense activity. An antisense oligonucleotide (ASO) is an “antisense compound”.

[0070] “Antisense oligonucleotide” or “ASO” means short (typically 12 to 25 nucleotides), single-stranded, synthetic DNA or RNA or hybrid DNA/RNA molecules designed to target either a coding or a non-coding nucleic acid such as an mRNA or other gene transcript sequence by complementary base-pairing in order to modulate its expression level.

[0071] “Bicyclic nucleoside” or “BNA” means a nucleoside comprising a bicyclic sugar

moiety.

[0072] “Bicyclic sugar moiety” means a modified sugar moiety comprising a 4 to 7 membered ring (including but not limited to a furanosyl) comprising a bridge connecting two atoms of the 4 to 7 membered ring to form a second ring, resulting in a bicyclic structure. In some embodiments, the 4 to 7 membered ring is a sugar ring. In certain embodiments the 4 to 7 membered ring is a furanosyl. In certain such embodiments, the bridge connects the 2'-carbon and the 4'-carbon of the furanosyl.

[0073] “Ubiquitin protein ligase E3A” or “UBE3A” means any protein encoded by a *UBE3A* nucleic acid. The *UBE3A* gene gives rise to 33 known transcript variants. Each variant transcript and each processed mRNA arising from such variants may be a *UBE3A* nucleic acid, as used herein. In some embodiments, the *UBE3A* nucleic acid has the sequence set forth at RefSeqGene NG_009268.1 (human chromosome 15 (NC_000015.10) nucleotide positions 25,333,728 to 25,439,056 (genomic context)). In some embodiments, the *UBE3A* nucleic acid has the sequence set forth at GRCh38.p14 (hg38) chr15:25371667-25439024 (SEQ ID NO: 1) (referred to herein as the “UBE3A target region”). In some embodiments, the *UBE3A* nucleic acid has a sequence set forth in a variant transcript of the human *UBE3A* gene, including, e.g., RefSeq NM_000462.5, NM_001354505.1, NM_001354506.2, NM_001354507.2, NM_001354508.2, NM_001354509.2, NM_001354511.2, NM_001354512.2, NM_001354513.2, NM_001354523.2, NM_001354526.1, NM_001354538.2, NM_001354539.2, NM_001354540.2, NM_001354541.2, NM_001354542.2, NM_001354543.2, NM_001354544.2, NM_001354545.2, NM_001354546.2, NM_001354547.2, NM_001354548.2, NM_001354549.2, NM_001354550.2, NM_001354551.2, NM_001374461.1, NM_130838.4, or NM_130839.5. In some instances, throughout the specification, the term UBE3A is used as shorthand to refer to the UBE3A gene and/or the protein expressed from a *UBE3A* nucleic acid such as *UBE3A* mRNA.

[0074] “Complementary” in reference to oligomeric compounds (e.g., linked nucleosides, oligonucleotides, or nucleic acids) means the capacity of such oligomeric compounds or regions thereof to hybridize to another oligomeric compound or region thereof through nucleobase complementarity under stringent conditions. Complementary oligomeric compounds need not have nucleobase complementarity at each nucleoside. Rather, some mismatches are tolerated. In some embodiments, complementary oligomeric compounds or regions are complementary at 70% of the nucleobases (70% complementary). In certain embodiments, complementary oligomeric compounds or regions are 80% complementary. In certain embodiments, complementary oligomeric compounds or regions are 90%

complementary. In certain embodiments, complementary oligomeric compounds or regions are 95% complementary. In certain embodiments, complementary oligomeric compounds or regions are 100% complementary.

[0075] “Conjugate” means a compound comprising two molecules that are covalently linked. In some embodiments, a conjugate comprises an antibody or other targeting agent and a modified oligonucleotide.

[0076] “Constrained ethyl nucleoside” or “cEt” means a nucleoside comprising a bicyclic sugar moiety comprising a 4'-CH(CH₃)—O-2' bridge.

[0077] “Expression” means the process by which a gene ultimately results in a protein. Expression includes, but is not limited to, transcription, post-transcriptional modification (e.g., splicing, polyadenylation, addition of 5'-cap), and translation.

[0078] “Hybridization” or “base-pairing” means the pairing of complementary oligomeric compounds (e.g., an antisense compound and its target nucleic acid). While not limited to a particular mechanism, the most common mechanism of pairing involves hydrogen bonding, which may be Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding, between complementary nucleobases.

[0079] “Internucleoside linkage” means a covalent linkage between adjacent nucleosides in an oligonucleotide.

[0080] “Locked nucleic acid nucleoside” or “LNA” means a nucleoside comprising a bicyclic sugar moiety comprising a 4'-CH₂—O-2' bridge.

[0081] “Mismatch” means a nucleobase of a first oligomeric compound that is not capable of pairing with a nucleobase at a corresponding position of a second oligomeric compound, when the first and second oligomeric compound are aligned. Either or both of the first and second oligomeric compounds may be oligonucleotides.

[0082] “Modified internucleoside linkage” means any internucleoside linkage other than a naturally occurring internucleoside linkage.

[0083] “Modified nucleobases” include universal bases, hydrophobic bases, promiscuous bases, size-expanded bases, and fluorinated bases. Particular examples of modified nucleobases include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil; 5-propynylcytosine; 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl (—C≡C—CH₃) uracil and cytosine and other alkynyl derivatives of pyrimidine bases, 6-azo uracil,

cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 2-F-adenine, 2-amino-adenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine, 3-deazaguanine and 3-deazaadenine. Further modified nucleobases include tricyclic pyrimidines such as phenoxazine cytidine([5,4-b][1,4]benzoxazin-2(3H)-one), phenothiazine cytidine (1H-pyrimido[5,4-b][1,4]benzothiazin-2(3H)-one), G-clamps such as a substituted phenoxazine cytidine (e.g. 9-(2-aminoethoxy)-H-pyrimido[5,4-b][1,4]benzoxazin-2(3H)-one), carbazole cytidine (2H-pyrimido[4,5-b]indol-2-one), pyridoindole cytidine (H-pyrido[3',2':4,5]pyrrolo[2,3-d]pyrimidin-2-one). Modified nucleobases may also include those in which the purine or pyrimidine base is replaced with other heterocycles, for example 7-deaza-adenine, 7-deazaguanosine, 2-aminopyridine and 2-pyridone.

[0084] “Modified nucleoside” means a nucleoside comprising at least one chemical modification compared to naturally occurring RNA or DNA nucleosides. Modified nucleosides may comprise a modified sugar moiety and/or a modified nucleobase.

[0085] “Modified oligonucleotide” means an oligonucleotide comprising at least one modified nucleoside and/or at least one modified internucleoside linkage. Examples of modified oligonucleotides include single-stranded and double-stranded compounds, such as, antisense oligonucleotides, siRNAs, shRNAs, ssRNAs, and occupancy-based compounds.

[0086] “Modified sugar” means a substituted sugar moiety or a sugar surrogate present in a modified nucleoside. Substituted sugar moieties include, but are not limited to furanosyls comprising substituents at the 2'-position, the 3'-position, the 5'-position, and/or the 4'-position. Certain substituted sugar moieties are bicyclic sugar moieties. Examples of modified sugars include 2'-substituted sugar moieties (a furanosyl comprising a substituent at the 2'-position other than H or OH). Sugar surrogates are structures that do not comprise a furanosyl but are capable of substituting for the naturally occurring sugar moiety of a nucleoside, such that the resulting nucleoside sub-units are capable of linking together and/or linking to other nucleosides to form an oligomeric compound which is capable of hybridizing to a complementary oligomeric compound. Such structures include rings comprising a different number of atoms than furanosyl (e.g., 4, 6, or 7-membered rings); replacement of the oxygen of a furanosyl with a non-oxygen atom (e.g., carbon, sulfur, or nitrogen); or both a change in the number of atoms and a replacement of the oxygen. Such structures may also comprise substitutions corresponding to those described for substituted sugar moieties (e.g., 6-membered

carbocyclic bicyclic sugar surrogates optionally comprising additional substituents). Sugar surrogates also include more complex sugar replacements (e.g., the non-ring systems of peptide nucleic acid). Sugar surrogates include without limitation morpholinos, cyclohexenyls and cyclohexitols. Bicyclic sugar moieties comprise a 4 to 7 membered ring (including but not limited to a furanosyl) comprising a bridge connecting two atoms of the 4 to 7 membered ring to form a second ring, resulting in a bicyclic structure. In certain embodiments, the 4 to 7 membered ring is a sugar ring. In certain embodiments the 4 to 7 membered ring is a furanosyl. In certain such embodiments, the bridge connects the 2'-carbon and the 4'-carbon of the furanosyl.

[0087] “Modulation” means a change of amount or quality of a molecule, function, or activity following an intervention when compared to the amount or quality of the molecule, function, or activity prior to or without the intervention. For example, modulation includes the change, either an increase (stimulation or induction) or a decrease (inhibition or reduction) in gene expression. As a further example, modulation of expression can include a change in splice site selection of pre-mRNA processing, resulting in a change in the absolute or relative amount of a particular splice-variant compared to the amount in the absence of modulation.

[0088] “MOE” means $\text{—OCH}_2\text{CH}_2\text{OCH}_3$.

[0089] “mRNA” means “messenger RNA”, i.e., an RNA molecule that encodes a protein.

[0090] “Naturally occurring internucleoside linkage” means a 3' to 5' phosphodiester linkage.

[0091] “Naturally occurring sugar moiety” means a ribofuranosyl as found in naturally occurring RNA or a deoxyribofuranosyl as found in naturally occurring DNA.

[0092] “Nucleobase” means a group of atoms that can be linked to a sugar moiety to create a nucleoside that is capable of incorporation into an oligonucleotide, and wherein the group of atoms is capable of bonding with a complementary naturally occurring nucleobase of another oligonucleotide or nucleic acid. Nucleobases may be naturally occurring or may be modified. “Unmodified nucleobase” or “naturally occurring nucleobase” means the naturally occurring heterocyclic nucleobases of RNA or DNA: the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) (including 5-methylcytosine), and uracil (U).

[0093] “Nucleoside” refers to a compound comprising a nucleobase moiety and a sugar moiety. Nucleosides include, but are not limited to, naturally occurring nucleosides (as found in DNA and RNA) and modified nucleosides. Nucleosides may be linked to a phosphate moiety.

[0094] “Nucleotide” means a nucleoside further comprising a phosphate linking group. “Linked nucleosides” may or may not be linked by phosphate linkages and thus include “linked

nucleotides.” “Linked nucleosides” are nucleosides that are connected in a continuous sequence (i.e., no additional nucleosides are present between those that are linked).

[0095] “Oligomeric compound” means a polymeric structure comprising two or more sub-structures. In certain embodiments, an oligomeric compound comprises an oligonucleotide. In certain embodiments, an oligomeric compound consists of an oligonucleotide.

[0096] “Oligonucleotide” means a compound comprising a plurality of linked nucleosides. In certain embodiments, an oligonucleotide comprises one or more unmodified ribonucleosides (RNA) and/or unmodified deoxyribonucleosides (DNA) and/or one or more modified nucleosides.

[0097] “Pharmaceutically acceptable carrier or diluent” means any substance suitable for use in administering to an animal. In certain embodiments, a pharmaceutically acceptable carrier or diluent is sterile saline. In certain embodiments, such sterile saline is pharmaceutical grade saline.

[0098] “Polynucleotide” refers to an “oligomer,” i.e., a molecule comprising at least two monomers and includes oligonucleotides such as DNA oligonucleotides, RNA oligonucleotides, and mixed DNA/RNA oligonucleotides, as well as polynucleotides including DNA polynucleotides, RNA polynucleotides, and mixed DNA/RNA polynucleotides, as well as polynucleotides bearing synthetic or modified monomers such as modified nucleotides described herein.

[0099] “Pre-mRNA” means an RNA transcript that has not been fully processed into mRNA. Pre-RNA includes one or more intron.

[0100] “Subject” refers to a human or any non-human animal (e.g., mouse, rat, rabbit, dog, cat, cattle, swine, sheep, horse or primate). A human includes pre- and post-natal forms. In many embodiments, a subject is a human being. A subject can be a patient, which refers to a human presenting to a medical provider for diagnosis or treatment of a disease. The term “subject” is used herein interchangeably with “individual” or “patient” unless the context requires otherwise. A subject can be afflicted with or is susceptible to a disease or disorder but may or may not display symptoms of the disease or disorder.

[0101] “Substituted sugar moiety” means a furanosyl that is not a naturally occurring sugar moiety. Substituted sugar moieties include, but are not limited to furanosyls comprising substituents at the 2’-position, the 3’-position, the 5’-position and/or the 4’-position. Certain substituted sugar moieties are bicyclic sugar moieties.

[0102] “Sugar moiety” means a naturally occurring sugar moiety or a modified sugar moiety of a nucleoside.

[0103] “Sugar surrogate” means a structure that does not comprise a furanosyl and that is capable of replacing the naturally occurring sugar moiety of a nucleoside, such that the resulting nucleoside sub-units are capable of linking together and/or linking to other nucleosides to form an oligomeric compound which is capable of hybridizing to a complementary oligomeric compound. Such structures include rings comprising a different number of atoms than furanosyl (e.g., 4, 6, or 7-membered rings); replacement of the oxygen of a furanosyl with a non-oxygen atom (e.g., carbon, sulfur, or nitrogen); or both a change in the number of atoms and a replacement of the oxygen. Such structures may also comprise substitutions corresponding to those described for substituted sugar moieties (e.g., 6-membered carbocyclic bicyclic sugar surrogates optionally comprising additional substituents). Sugar surrogates also include more complex sugar replacements (e.g., the non-ring systems of peptide nucleic acid). Sugar surrogates include without limitation morpholinos, cyclohexenyls and cyclohexitols.

[0104] “Targeting” or “targeted to” means the association of an antisense compound to a particular sequence or region of a nucleic acid molecule. An antisense compound targets a target nucleic acid if it is sufficiently complementary to the target nucleic acid to allow hybridization under physiological conditions.

[0105] “2’-(ara)-F” refers to a 2’-F substituted nucleoside, wherein the fluoro group is in the arabino position.

[0106] “2’-deoxynucleoside” means a nucleoside comprising 2’-H furanosyl sugar moiety, as found in naturally occurring deoxyribonucleosides (DNA). In certain embodiments, a 2’-deoxynucleoside may comprise a modified nucleobase or may comprise an RNA nucleobase (e.g., uracil).

[0107] “2’-F nucleoside” refers to a nucleoside comprising a sugar comprising fluorine at the 2’ position. Unless otherwise indicated, the fluorine in a 2’-F nucleoside is in the ribo position (replacing the OH of a natural ribose).

[0108] “2’-substituted nucleoside” means a nucleoside comprising a substituent at the 2’-position other than H or OH. Unless otherwise indicated, a 2’-substituted nucleoside is not a bicyclic nucleoside.

[0109] “2’-substituted sugar moiety” means a furanosyl comprising a substituent at the 2’-position other than H or OH. Unless otherwise indicated, a 2’-substituted sugar moiety is not a bicyclic sugar moiety (i.e., the 2’-substituent of a 2’-substituted sugar moiety does not form a bridge to another atom of the furanosyl ring).

[0110] “3’-endo-furanosyl nucleoside” means an RNA-like nucleoside that comprises a

substituted sugar moiety that has a 3'-endo conformation. 3'-endo-furanosyl nucleosides include, but are not limited to: 2'-MOE ("MOE"), 2'-F, 2'-OMe ("OMe"), locked nucleic acids (LNA), 2'-O,4'-C-ethylene-bridged nucleic acids ("ENA"), and 2'-O-Ethyl (cEt) nucleosides.

[0111] "Acyl," means a radical formed by removal of a hydroxyl group from an organic acid and has the general Formula —C(O)—X where X is typically aliphatic, alicyclic or aromatic. Examples include aliphatic carbonyls, aromatic carbonyls, aliphatic sulfonyls, aromatic sulfinyls, aliphatic sulfinyls, aromatic phosphates, aliphatic phosphates and the like. Acyl groups as used herein may optionally include further substituent groups.

[0112] "Alicyclic" means a cyclic ring system wherein the ring is aliphatic. The ring system can comprise one or more rings wherein at least one ring is aliphatic. Preferred alicyclics include rings having from about 5 to about 9 carbon atoms in the ring. Alicyclic as used herein may optionally include further substituent groups.

[0113] "Aliphatic" means a straight or branched hydrocarbon radical containing up to 24 carbon atoms wherein the saturation between any two carbon atoms is a single, double or triple bond. An aliphatic group preferably contains from 1 to about 24 carbon atoms, more typically from 1 to about 12 carbon atoms with from 1 to about 6 carbon atoms being more preferred. The straight or branched chain of an aliphatic group may be interrupted with one or more heteroatoms that include nitrogen, oxygen, sulfur and phosphorus. Such aliphatic groups interrupted by heteroatoms include without limitation, polyalkoxys, such as polyalkylene glycols, polyamines, and polyimines. Aliphatic groups as used herein may optionally include further substituent groups.

[0114] "Alkenyl," means a straight or branched hydrocarbon chain radical containing up to 24 carbon atoms and having at least one carbon-carbon double bond. Examples of alkenyl groups include without limitation, ethenyl, propenyl, butenyl, 1-methyl-2-buten-1-yl, dienes such as 1,3-butadiene and the like. Alkenyl groups typically include from 2 to about 24 carbon atoms, more typically from 2 to about 12 carbon atoms with from 2 to about 6 carbon atoms being more preferred. Alkenyl groups as used herein may optionally include one or more further substituent groups.

[0115] "Alkoxy" means a radical formed between an alkyl group and an oxygen atom wherein the oxygen atom is used to attach the alkoxy group to a parent molecule. Examples of alkoxy groups include without limitation, methoxy, ethoxy, propoxy, isopropoxy, n-butoxy, sec-butoxy, tert-butoxy, n-pentoxy, neopentoxy, n-hexoxy and the like. Alkoxy groups as used herein may optionally include further substituent groups.

[0116] "Alkyl," means a saturated straight or branched hydrocarbon radical containing up to

24 carbon atoms. Examples of alkyl groups include without limitation, methyl, ethyl, propyl, butyl, isopropyl, n-hexyl, octyl, decyl, dodecyl and the like. Alkyl groups typically include from 1 to about 24 carbon atoms, more typically from 1 to about 12 carbon atoms (C1-C12 alkyl) with from 1 to about 6 carbon atoms being more preferred.

[0117] “Alkynyl,” means a straight or branched hydrocarbon radical containing up to 24 carbon atoms and having at least one carbon-carbon triple bond. Examples of alkynyl groups include, without limitation, ethynyl, 1-propynyl, 1-butyne, and the like. Alkynyl groups typically include from 2 to about 24 carbon atoms, more typically from 2 to about 12 carbon atoms with from 2 to about 6 carbon atoms being more preferred. Alkynyl groups as used herein may optionally include one or more further substituent groups.

[0118] “Aminoalkyl” means an amino substituted C1-C12 alkyl radical. The alkyl portion of the radical forms a covalent bond with a parent molecule. The amino group can be located at any position and the aminoalkyl group can be substituted with a further substituent group at the alkyl and/or amino portions.

[0119] “Aralkyl” and “arylalkyl” mean an aromatic group that is covalently linked to a C1-C12 alkyl radical. The alkyl radical portion of the resulting aralkyl (or arylalkyl) group forms a covalent bond with a parent molecule. Examples include without limitation, benzyl, phenethyl and the like. Aralkyl groups as used herein may optionally include further substituent groups attached to the alkyl, the aryl or both groups that form the radical group.

[0120] “Aryl” and “aromatic” mean a mono- or polycyclic carbocyclic ring system radicals having one or more aromatic rings. Examples of aryl groups include without limitation, phenyl, naphthyl, tetrahydronaphthyl, indanyl, indenyl and the like. Preferred aryl ring systems have from about 5 to about 20 carbon atoms in one or more rings. Aryl groups as used herein may optionally include further substituent groups.

[0121] “Furanosyl” means a structure comprising a 5-membered ring comprising four carbon atoms and one oxygen atom.

[0122] “Halo” and “halogen,” mean an atom selected from fluorine, chlorine, bromine and iodine.

[0123] “Heteroaryl,” and “heteroaromatic,” mean a radical comprising a mono- or polycyclic aromatic ring, ring system or fused ring system wherein at least one of the rings is aromatic and includes one or more heteroatoms. Heteroaryl is also meant to include fused ring systems including systems where one or more of the fused rings contain no heteroatoms. Heteroaryl groups typically include one ring atom selected from sulfur, nitrogen or oxygen. Examples of heteroaryl groups include without limitation, pyridinyl, pyrazinyl, pyrimidinyl,

pyrrolyl, pyrazolyl, imidazolyl, thiazolyl, oxazolyl, isooxazolyl, thiadiazolyl, oxadiazolyl, thiophenyl, furanyl, quinolinyl, isoquinolinyl, benzimidazolyl, benzooxazolyl, quinoxalinyl and the like. Heteroaryl radicals can be attached to a parent molecule directly or through a linking moiety such as an aliphatic group or hetero atom. Heteroaryl groups as used herein may optionally include further substituent groups.

II. Overview

[0124] The antisense compounds targeting human *UBE3A* nucleic acids described herein and compositions or formulations comprising the same may be used to ameliorate, prevent, or treat any symptom or disorder associated with excess *UBE3A* expression in a subject, such as symptoms or disorders arising from a maternal duplication within the 15q11.2-q13.3 region, e.g., Dup15q syndrome. For example, the antisense compounds targeting human *UBE3A* nucleic acids may be used to ameliorate, prevent, or treat any symptom or disease associated with excess UBE3A protein in cells, e.g., neurons, of a subject. In some embodiments, the subject may present with Dup15q syndrome.

[0125] The antisense compounds targeting human *UBE3A* nucleic acids described herein may be any antisense compound useful for inhibiting expression of the target gene, i.e., *UBE3A*. Example sequences for portions of antisense compounds targeting human *UBE3A* nucleic acids are provided in Table 1. For example, antisense compounds include single and double stranded nucleic acid compounds and may suppress target gene expression via RNaseH-mediated mRNA decay, steric hindrance-based mechanisms, RNA-induced silencing complex (RISC), or guide strand-directed nuclease-mediated mRNA decay mechanisms. Thus, antisense compounds targeting human *UBE3A* nucleic acids described herein may comprise antisense oligonucleotides (ASOs), RNAi compounds such as small interfering RNA (siRNA) and short hairpin RNA (shRNA), or nuclease guide RNA (gRNA) compounds for use with nucleases such as zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), Cas9, and similar nucleases. In general, persons skilled in the art are aware of modifications needed to adapt the antisense compounds disclosed herein, such as those in Table 1, to be suitable for use as, e.g., an ASO, an siRNA, an shRNA, or a gRNA. Such modifications include, e.g., incorporation of sense and/or antisense strands, preparation of single or double stranded compositions, use of DNA or RNA nucleosides, and oligonucleotide length, among others.

[0126] In some embodiments, the antisense compounds of the disclosure are antisense oligonucleotides or “ASOs”. The ASOs of the disclosure are designed to target human *UBE3A* nucleic acids, such as *UBE3A* mRNA, and thereby modulate its expression. The ASOs can be

administered to a subject in need (e.g., a patient with Dup15q syndrome) and reduce functionally active *UBE3A* mRNA or UBE3A protein levels in the subject. Thus, the ASOs described herein and compositions or formulations comprising the same can be used for preventing, treating, ameliorating, or reversing any symptoms of *UBE3A* overexpression in a subject. In an exemplary embodiment, the subject is a human patient presenting with a maternal duplication within the 15q11.2-q13.3 region, resulting in excess expression of UBE3A protein in neurons. In some embodiments, a composition or formulation comprising an ASO of the disclosure can be delivered to the central nervous system of the subject.

III. Antisense Compounds Targeting Human *UBE3A* Transcript

[0127] In certain embodiments, the present invention provides antisense nucleic acid compounds targeting human *UBE3A* nucleic acids (including mRNA transcripts of the *UBE3A* gene) to suppress expression of the *UBE3A* gene. The antisense compounds may comprise oligonucleotides. In some embodiments, oligonucleotides of the disclosure comprise one or more chemical modifications such as one or more nucleoside modification (including modifications to the sugar moiety and/or the nucleobase) and/or modifications to one or more internucleoside linkage.

A. Modified Nucleosides

[0128] The antisense compounds of the disclosure may comprise or consist of oligonucleotides comprising at least one modified nucleoside. Such modified nucleosides may comprise a modified sugar moiety, a modified nucleobase, or both a modified sugar moiety and a modified nucleobase.

i. Sugar Modifications

[0129] The antisense compounds described herein may comprise one or more nucleosides comprising a modified sugar to confer desirable properties, such as enhanced nuclease stability or increased binding affinity with a target nucleic acid relative to antisense compounds comprising non-modified sugar moieties. Modified sugars may be substituted sugar moieties, bicyclic or tricyclic sugar moieties, or sugar surrogates.

[0130] Substituted sugar moieties may comprise one or more substituent, including but not limited to substituents at the 2' and/or 5' positions. Examples of sugar substituents suitable for the 2'-position include, but are not limited to: 2'-F, 2'-OCH₃ ("OMe" or "O-methyl"), and 2'-O(CH₂)₂OCH₃ ("MOE"). In certain embodiments, sugar substituents at the 2' position are selected from allyl, amino, azido, thio, O-allyl substitutions. Examples of sugar substituents at

the 5'-position, include, but are not limited to: 5'-methyl (R or S); 5'-vinyl, 5'-vinylphosphonate, and 5'-methoxy.

[0131] Nucleosides comprising 2'-substituted sugar moieties are referred to as 2'-substituted nucleosides. In certain embodiments, a 2'-substituted nucleoside comprises a 2'-substituent group selected from halo, allyl, amino, azido, O—C1-C10 alkoxy; O—C1-C10 substituted alkoxy, SH, CN, OCN, CF₃, OCF₃, O-alkyl, S-alkyl, N(R_m)-alkyl; O-alkenyl, S-alkenyl, or N(R_m)-alkenyl; O-alkynyl, S-alkynyl, N(R_m)-alkynyl; O-alkylenyl-O-alkyl, alkynyl, alkaryl, aralkyl, O-alkaryl, O-aralkyl, O(CH₂)₂SCH₃, O—(CH₂)₂—O—N(R_m)(R_n) or O—CH₂—C(=O)—N(R_m)(R_n), where each R_m and R_n is, independently, H, an amino protecting group or substituted or unsubstituted C1-C10 alkyl.

[0132] In certain embodiments, a 2'-substituted nucleoside comprises a 2'-substituent group selected from F, NH₂, N₃, OCF₃, O—CH₃, O(CH₂)₃NH₂, CH₂—CH=CH₂, O—CH₂—CH=CH₂, OCH₂CH₂OCH₃, O(CH₂)₂SCH₃, O—(CH₂)₂—O—N(R_m)(R_n), O(CH₂)₂O(CH₂)₂N(CH₃)₂, and N-substituted acetamide (O—CH₂—C(=O)—N(R_m)(R_n)) where each R_m and R_n is, independently, H, an amino protecting group or substituted or unsubstituted C1-C10 alkyl.

[0133] Certain modified sugar moieties comprise a bridging sugar substituent that forms a second ring resulting in a bicyclic sugar moiety. In certain such embodiments, the bicyclic sugar moiety comprises a bridge between the 4' and the 2' furanose ring atoms. Examples of such 4' to 2' sugar substituents, include, but are not limited to: —[C(R_a)(R_b)]_n—, —[C(R_a)(R_b)]_n—O—, —C(R_aR_b)—N(R)—O— or, —C(R_aR_b)—O—N(R)—; 4'-CH₂-2', 4'-(CH₂)₂-2', 4'-(CH₂)₃-2', 4'-(CH₂)—O-2' (LNA); 4'-(CH₂)—S-2'; 4'-(CH₂)₂—O-2' (ENA); 4'-CH(CH₃)—O-2' (cEt) and 4'-CH(CH₂OCH₃)—O-2', and analogs thereof (see, e.g., U.S. Pat. No. 7,399,845, issued on Jul. 15, 2008); 4'-C(CH₃)(CH₃)—O-2' and analogs thereof, (see, e.g., WO/2009/006478); 4'-CH₂—N(OCH₃)-2' and analogs thereof (see, e.g., WO/2008/150729); 4'-CH₂—O—N(CH₃)-2' (see, e.g., US/2004/0171570); 4'-CH₂—O—N(R)-2', and 4'-CH₂—N(R)—O-2', wherein each R is, independently, H, a protecting group, or C1-C12 alkyl; 4'-CH₂—N(R)—O-2', wherein R is H, C1-C12 alkyl, or a protecting group (see, U.S. Pat. No. 7,427,672, issued on Sep. 23, 2008); 4'-CH₂—C(H)(CH₃)-2' (see, e.g., Chattopadhyaya, et al., J. Org. Chem., 2009, 74, 118-134); and 4'-CH₂—C(=CH₂)-2' and analogs thereof (see WO/2008/154401).

[0134] Nucleosides comprising bicyclic sugar moieties are referred to as bicyclic nucleosides or BNAs. Bicyclic nucleosides include, but are not limited to, (A) α-L-Methyleneoxy (4'-CH₂—O-2') BNA, (B) β-D-Methyleneoxy (4'-CH₂—O-2') BNA (also referred to as locked

nucleic acid or LNA), (C) Ethyleneoxy (4'-(CH₂)₂—O-2') BNA, (D) Aminoxy (4'-CH₂—O—N(R)-2') BNA, (E) Oxyamino (4'-CH₂—N(R)—O-2') BNA, (F) Methyl(methyleneoxy) (4'-CH(CH₃)—O-2') BNA (also referred to as constrained ethyl or cEt), (G) methylene-thio (4'-CH₂—S-2') BNA, (H) methylene-amino (4'-CH₂-N(R)-2') BNA, (I) methyl carbocyclic (4'-CH₂—CH(CH₃)-2') BNA, (J) propylene carbocyclic (4'-(CH₂)₃-2') BNA, and (M) 4'-CH₂—O—CH₂-2'.

[0135] Additional bicyclic sugar moieties are known in the art, for example: Singh et al., Chem. Commun., 1998, 4, 455-456; Koshkin et al., Tetrahedron, 1998, 54, 3607-3630; Wahlestedt et al., Proc. Natl. Acad. Sci. U.S.A., 2000, 97, 5633-5638; Kumar et al., Bioorg. Med. Chem. Lett., 1998, 8, 2219-2222; Singh et al., J. Org. Chem., 1998, 63, 10035-10039; Srivastava et al., J. Am. Chem. Soc., 129(26) 8362-8379 (Jul. 4, 2007); Elayadi et al., Curr. Opinion Inven. Drugs, 2001, 2, 558-561; Braasch et al., Chem. Biol., 2001, 8, 1-7; Orum et al., Curr. Opinion Mol. Ther., 2001, 3, 239-243; U.S. Pat. Nos. 7,053,207, 6,268,490, 6,770,748, 6,794,499, 7,034,133, 6,525,191, 6,670,461, and 7,399,845; WO/2004/106356, WO/1994/14226, WO/2005/021570, and WO/2007/134181; U.S. Patent Application Publication Nos. US/2004/0171570, US/2007/0287831, and US/2008/0039618; and WO/2008/150729A2, WO/2008/154401A2, and WO/2009/006478A2.

[0136] In certain embodiments, bicyclic sugar moieties and nucleosides incorporating such bicyclic sugar moieties are further defined by isomeric configuration. For example, a nucleoside comprising a 4'-2' methylene-oxy bridge, may be in the α -L configuration or in the β -D configuration. Previously, α -L-methyleneoxy (4'-CH₂—O-2') bicyclic nucleosides have been incorporated into antisense oligonucleotides that showed antisense activity (Frieden et al., Nucleic Acids Research, 2003, 21, 6365-6372).

[0137] In certain embodiments, substituted sugar moieties comprise one or more non-bridging sugar substituent and one or more bridging sugar substituent (e.g., 5'-substituted and 4'-2' bridged sugars). (See WO/2007/134181, wherein LNA is substituted with, for example, a 5'-methyl or a 5'-vinyl group).

[0138] In certain embodiments, modified sugar moieties are sugar surrogates. In certain such embodiments, the oxygen atom of the naturally occurring sugar is substituted, e.g., with a sulfur, carbon or nitrogen atom. In certain such embodiments, such modified sugar moiety also comprises bridging and/or non-bridging substituents as described above. For example, certain sugar surrogates comprise a 4'-sulfur atom and a substitution at the 2'-position (see, e.g., U.S. Patent Application Publication US/2005/0130923) and/or the 5' position.

[0139] In certain embodiments, sugar surrogates comprise rings having other than 5-atoms.

For example, in certain embodiments, a sugar surrogate comprises a six-membered tetrahydropyran. Such tetrahydropyrans may be further modified or substituted. Nucleosides comprising such modified tetrahydropyrans include, but are not limited to, hexitol nucleic acid (HNA), anitol nucleic acid (ANA), mannitol nucleic acid (MNA) (see Leumann, C J. *Bioorg. & Med. Chem.* (2002) 10:841-854), and fluoro HNA (F-HNA).

[0140] Many other bicyclic and tricyclic sugar and sugar surrogate ring systems are known in the art that can be used to modify nucleosides (see, e.g., review article: Leumann, J. C, *Bioorganic & Medicinal Chemistry*, 2002, 10, 841-854).

[0141] In certain embodiments, sugar surrogates comprise rings having more than 5 atoms and more than one heteroatom. For example, nucleosides comprising morpholino sugar moieties and their use in antisense compounds has been reported (see for example: Braasch et al., *Biochemistry*, 2002, 41, 4503-4510; and U.S. Pat. Nos. 5,698,685; 5,166,315; 5,185,444; and 5,034,506).

[0142] In certain embodiments, morpholinos may be modified, for example by adding or altering various substituent groups from the above morpholino structure. Such sugar surrogates are referred to herein as “modified morpholinos.”

[0143] Combinations of modifications are also provided without limitation, such as 2'-F-5'-methyl substituted nucleosides (see WO/2008/101157 for other disclosed 5',2'-bis substituted nucleosides) and replacement of the ribosyl ring oxygen atom with S and further substitution at the 2'-position (see U.S. Patent Application Publication US/20050130923) or alternatively 5'-substitution of a bicyclic nucleic acid (see WO/2007/134181, wherein a 4'-CH₂—O-2' bicyclic nucleoside is further substituted at the 5' position with a 5'-methyl or a 5'-vinyl group). The synthesis and preparation of carbocyclic bicyclic nucleosides along with their oligomerization and biochemical studies have also been described (see, e.g., Srivastava et al., *J. Am. Chem. Soc.* 2007, 129(26), 8362-8379).

ii. Modified Nucleobases

[0144] The antisense compounds described herein may comprise one or more nucleosides comprising modified nucleobases.

[0145] Modified nucleobases may include universal bases, hydrophobic bases, promiscuous bases, size-expanded bases, and fluorinated bases. Particular examples of modified nucleobases include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil; 5-propynylcytosine; 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl

derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl ($\text{—C}\equiv\text{C—CH}_3$) uracil and cytosine and other alkynyl derivatives of pyrimidine bases, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 2-F-adenine, 2-amino-adenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine, 3-deazaguanine and 3-deazaadenine. Further modified nucleobases include tricyclic pyrimidines such as phenoxazine cytidine([5,4-b][1,4]benzoxazin-2(3H)-one), phenothiazine cytidine (1H-pyrimido[5,4-b][1,4]benzothiazin-2(3H)-one), G-clamps such as a substituted phenoxazine cytidine (e.g. 9-(2-aminoethoxy)-H-pyrimido[5,4-b][1,4]benzoxazin-2(3H)-one), carbazole cytidine (2H-pyrimido[4,5-b]indol-2-one), pyridoindole cytidine (H-pyrido[3',2':4,5]pyrrolo[2,3-d]pyrimidin-2-one). Modified nucleobases may also include those in which the purine or pyrimidine base is replaced with other heterocycles, for example 7-deaza-adenine, 7-deazaguanosine, 2-aminopyridine and 2-pyridone.

B. Modified Internucleoside Linkages

[0146] Individual nucleosides of the antisense compounds described herein may be linked together using any internucleoside linkage to form oligonucleotides. Internucleoside linkages include phosphorus containing internucleoside linkages including, but not limited to, phosphodiester (P=O), phosphotriesters, methylphosphonates, phosphoramidates, and phosphorothioates (P=S). Non-phosphorus containing internucleoside linkages include, but are not limited to, methylenemethylimino ($\text{—CH}_2\text{—N(CH}_3\text{)—O—CH}_2\text{—}$), thiodiester (—O—C(O)—S—), thionocarbamate (—O—C(O)(NH)—S—); siloxane ($\text{—O—Si(H)}_2\text{—O—}$); and N,N'-dimethylhydrazine ($\text{—CH}_2\text{—N(CH}_3\text{)—N(CH}_3\text{)—}$). Modified linkages, compared to natural phosphodiester linkages, may improve nuclease resistance of the oligonucleotide. In some embodiments, internucleoside linkages having a chiral atom can be prepared as a racemic mixture, or as separate enantiomers. Representative chiral linkages include, but are not limited to, alkylphosphonates and phosphorothioates. Methods of preparing phosphorous-containing and non-phosphorous-containing internucleoside linkages are known to those skilled in the art.

C. Oligonucleotide Motifs

[0147] Antisense compounds described herein may comprise oligonucleotides having one or

more chemical modification, i.e., one or more modified sugars and/or one or more modified nucleobases and/or one or more modified internucleoside linkages. These chemical modifications (sugar modifications, nucleobase modifications, and/or linkage modifications) may be present in a given oligonucleotide according to a defined pattern or motif. The patterns of chemical modifications of sugar moieties, internucleoside linkages, and nucleobases may be independent of one another. Thus, an oligonucleotide may be described by its sugar modification motif, internucleoside linkage motif and/or nucleobase modification motif (independent of the sequence of nucleobases).

i. Sugar Motifs

[0148] Oligonucleotides described herein may comprise one or more type of modified sugar moieties and/or naturally occurring sugar moieties arranged along an oligonucleotide or region thereof in a defined pattern.

[0149] For example, the oligonucleotides may comprise or consist of a region having a gapmer sugar motif, which comprises two external regions or segments and a central or internal region or gap. The three regions of a gapmer sugar motif (the 5'-segment, the gap segment, and the 3'-segment) form a contiguous sequence of nucleosides wherein at least some of the sugar moieties of the nucleosides of each of the 5' and 3' segments differ from at least some of the sugar moieties of the nucleosides of the gap. Specifically, at least the sugar moieties of the nucleosides of each 5' and 3' segment that are closest to the gap (the 3'-most nucleoside of the 5'-segment and the 5'-most nucleoside of the 3'-segment) differ from the sugar moiety of the neighboring gap nucleosides, thus defining the boundary between the 3' and 5' segments and the gap. In some embodiments, the sugar moieties within the gap are the same as one another. In some embodiments, the gap includes one or more nucleoside having a sugar moiety that differs from the sugar moiety of one or more other nucleosides of the gap. In some embodiments, the sugar motifs of the 5' and the 3' segments are the same as one another (symmetric sugar gapmer). In certain embodiments, the sugar motifs of the 5'-segment differs from the sugar motif of the 3'-segment (asymmetric sugar gapmer).

[0150] Segments of a gapmer may be different lengths or equal lengths. For example, in some instances, a gapmer consists of a 5 nucleoside 5' segment, a 5 nucleoside gap segment, and a 5 nucleoside 3' segment. In other instances, a gapmer consists of, e.g., a 3 nucleoside 5' segment, a 9 nucleoside gap segment, and a 3 nucleoside 3' segment. In still other instances, a gapmer consists of, e.g., a 2 nucleoside 5' segment, a 10 nucleoside gap segment, and a 3 nucleoside 3' segment. Thus, each of the segments may be of differing lengths compared to

the other segments. The lengths of 5' and 3' segments can vary, typically from 2 nucleosides to 5 nucleosides in length. The length of the gap segment can also vary, typically from 5 nucleosides to 15 nucleosides in length.

[0151] Gapmers can be referred to according to their segment lengths in the 5' to 3' direction. For instance, a gapmer consisting of a 3 nucleoside 5' segment, a 10 nucleoside gap, and a 3 nucleoside 3' segment can be referred to as a 3-10-3 gapmer, more specifically a 3-10-3 16mer. For specific gapmers where sugar modifications are defined, the gapmers can be more specifically referred to by defining the sugar modifications of each segment. For example, a gapmer consisting of a 3 nucleoside 5' segment having 4'-CH₂-2', 4'-(CH₂)₂-2', 4'-(CH₂)₃-2', or 4'-(CH₂)—O-2' (LNA) sugar modifications, a gap having 10 linked deoxynucleosides, and a 3 nucleoside 3' segment having 2'-O(CH₂)₂OCH₃ ("MOE") sugar modifications can be referred to as a 3-10-3 LNA-DNA-MOE gapmer, more specifically a 3-10-3 16mer.

[0152] Gapmers may comprise one or more modified sugar at a specific position in one or more segment. That is, gapmers may comprise a sugar modification at a specific nucleoside position within the 5' segment, the gap segment, and/or the 3' segment. In some embodiments, gapmers described herein comprise a 2'OMe modified sugar at a specific position within the DNA gap. For example, gapmers may comprise a 2'OMe modified sugar at the nucleoside of position 2 in the DNA gap.

ii. Nucleobase Modification Motifs

[0153] Oligonucleotides described herein may comprise chemical modifications to nucleobases arranged along the oligonucleotide or region/segment thereof in a defined pattern or nucleobase modification motif. In certain embodiments, each nucleobase is modified.

[0154] Oligonucleotides may comprise a block of modified nucleobases. In certain such embodiments, the block is at the 3'-end of the oligonucleotide. In certain embodiments the block is within 3 nucleotides of the 3'-end of the oligonucleotide. In certain such embodiments, the block is at the 5'-end of the oligonucleotide. In certain embodiments the block is within 3 nucleotides of the 5'-end of the oligonucleotide.

[0155] Nucleobase modifications can depend on the base at a particular position of an oligonucleotide. For example, in certain embodiments each purine or each pyrimidine in an oligonucleotide is modified. In certain embodiments, each adenine is modified. In certain embodiments, each guanine is modified. In certain embodiments, each thymine is modified. In certain embodiments, each cytosine is modified. In certain embodiments, each uracil is modified.

[0156] In some embodiments, oligonucleotides comprise one or more nucleosides comprising a modified nucleobase. In certain embodiments, oligonucleotides having a gapmer sugar motif comprise a nucleoside comprising a modified nucleobase. In certain such embodiments, one nucleoside comprising a modified nucleobase is in the central gap of an oligonucleotide having a gapmer sugar motif. In certain embodiments, the sugar is an unmodified 2' deoxynucleoside. In certain embodiments, the modified nucleobase is selected from: a 2-thio pyrimidine and a 5-propyne pyrimidine.

[0157] In some embodiments, some, all, or none of the cytosine moieties in an oligonucleotide are 5-methylcytosine. 5-methylcytosine is not a “modified nucleobase.” Accordingly, unless otherwise indicated, unmodified nucleobases include both cytosine residues having a 5-methyl and those lacking a 5-methyl. In some embodiments, the methylation state of all or some cytosine nucleobases is specified.

D. Length of Antisense Compounds

[0158] The present invention provides antisense nucleic acid compounds including oligonucleotides of differing nucleotide lengths. For example, the antisense compounds may consist of 8 to 9, 8 to 10, 8 to 11, 8 to 12, 8 to 13, 8 to 14, 8 to 15, 8 to 16, 8 to 17, 8 to 18, 8 to 19, 8 to 20, 8 to 21, 8 to 22, 8 to 23, 8 to 24, 9 to 10, 9 to 11, 9 to 12, 9 to 13, 9 to 14, 9 to 15, 9 to 16, 9 to 17, 9 to 18, 9 to 19, 9 to 20, 9 to 21, 9 to 22, 9 to 23, 9 to 24, 9 to 25, 10 to 11, 10 to 12, 10 to 13, 10 to 14, 10 to 15, 10 to 16, 10 to 17, 10 to 18, 10 to 19, 10 to 20, 10 to 21, 10 to 22, 10 to 23, 10 to 24, 10 to 25, 11 to 12, 11 to 13, 11 to 14, 11 to 15, 11 to 16, 11 to 17, 11 to 18, 11 to 19, 11 to 20, 11 to 21, 11 to 22, 11 to 23, 11 to 24, 11 to 25, 12 to 13, 12 to 14, 12 to 15, 12 to 16, 12 to 17, 12 to 18, 12 to 19, 12 to 20, 12 to 21, 12 to 22, 12 to 23, 12 to 24, 12 to 25, 13 to 14, 13 to 15, 13 to 16, 13 to 17, 13 to 18, 13 to 19, 13 to 20, 13 to 21, 13 to 22, 13 to 23, 13 to 24, 13 to 25, 14 to 15, 14 to 16, 14 to 17, 14 to 18, 14 to 19, 14 to 20, 14 to 21, 14 to 22, 14 to 23, 14 to 24, 14 to 25, 15 to 16, 15 to 17, 15 to 18, 15 to 19, 15 to 20, 15 to 21, 15 to 22, 15 to 23, 15 to 24, 15 to 25, 16 to 17, 16 to 18, 16 to 19, 16 to 20, 16 to 21, 16 to 22, 16 to 23, 16 to 24, 16 to 25, 17 to 18, 17 to 19, 17 to 20, 17 to 21, 17 to 22, 17 to 23, 17 to 24, 17 to 25, 18 to 19, 18 to 20, 18 to 21, 18 to 22, 18 to 23, 18 to 24, 18 to 25, 19 to 20, 19 to 21, 19 to 22, 19 to 23, 19 to 24, 19 to 25, 20 to 21, 20 to 22, 20 to 23, 20 to 24, 20 to 25, 21 to 22, 21 to 23, 21 to 24, 21 to 25, 22 to 23, 22 to 24, 22 to 25, 23 to 24, 23 to 25 or 24 to 25 linked nucleosides. In some embodiments, an antisense oligonucleotide of the disclosure can be from about 12 nucleotides to about 25 nucleotides in length, such as 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleotides in length. Generally, oligonucleotides having a length of

n linked nucleosides are referred to as “nmers” such that an oligonucleotide of 16 linked nucleosides is referred to as a 16mer.

E. Conjugations for Improved Biodistribution

[0159] Antisense compounds described herein may be modified by attachment of one or more conjugate groups. In general, conjugate groups modify one or more properties of the attached antisense compound including but not limited to pharmacodynamics, pharmacokinetics, stability, binding, absorption, cellular distribution, cellular uptake, biodistribution, charge, and clearance. Conjugate groups are used in the chemical arts and are linked directly or via an optional conjugate linking moiety or conjugate linking group to a parent compound such as an antisense compound, such as an oligonucleotide. Conjugate groups include without limitation, intercalators, reporter molecules, polyamines, polyamides, polyethylene glycols, thioethers, polyethers, cholesterol, thiocholesterols, cholic acid moieties, folate, lipids, phospholipids, biotin, phenazine, phenanthridine, anthraquinone, adamantane, acridine, fluoresceins, rhodamines, coumarins and dyes.

[0160] In some embodiments, conjugate groups are attached to oligonucleotides by a conjugate linking group. In certain such embodiments, conjugate linking groups, including, but not limited to, bifunctional linking moieties such as those known in the art are amenable to the compounds provided herein. Conjugate linking groups are useful for attachment of conjugate groups, such as chemical stabilizing groups, functional groups, reporter groups and other groups to selective sites in a parent compound such as for example an antisense compound. In general, a bifunctional linking moiety comprises a hydrocarbyl moiety having two functional groups. One of the functional groups is selected to bind to a parent molecule or compound of interest and the other is selected to bind essentially any selected group such as chemical functional group or a conjugate group. In some embodiments, the conjugate linker comprises a chain structure or an oligomer of repeating units such as ethylene glycol or amino acid units. Examples of functional groups that are routinely used in a bifunctional linking moiety include, but are not limited to, electrophiles for reacting with nucleophilic groups and nucleophiles for reacting with electrophilic groups. In some embodiments, bifunctional linking moieties include amino, hydroxyl, carboxylic acid, thiol, unsaturations (e.g., double or triple bonds), and the like.

[0161] Some nonlimiting examples of conjugate linking moieties include pyrrolidine, 8-amino-3,6-dioxaoctanoic acid (ADO), succinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC) and 6-aminohexanoic acid (AHEx or AHA). Other linking groups

include, but are not limited to, substituted C1-C10 alkyl, substituted or unsubstituted C2-C10 alkenyl or substituted or unsubstituted C2-C10 alkynyl, wherein a nonlimiting list of preferred substituent groups includes hydroxyl, amino, alkoxy, carboxy, benzyl, phenyl, nitro, thiol, thioalkoxy, halogen, alkyl, aryl, alkenyl and alkynyl.

[0162] Conjugate groups may be attached to either or both ends (that is, 5' and/or 3' ends) of an oligonucleotide (terminal conjugate groups) and/or at any internal position. In some embodiments, conjugate groups are at the 5'-end of an antisense compound described herein, or near the 5'-end. In some embodiments, conjugate groups are at the 3'-end of an antisense compound described herein, or near the 3'-end.

[0163] In some embodiments, conjugate groups may interact with the lipid components of the cell membrane, bind to specific cell surface proteins or receptors, and/or penetrate the cell through endogenous transport mechanisms carrying the antisense compound with them.

[0164] The conjugate can be attached at the 5'- and/or the 3'-end of a sense and/or antisense strand of an antisense compound via a covalent attachment such as a nucleic acid or non-nucleic acid linker. The conjugate can be attached through a carbamate group or other linking group (see, e.g., U.S. Patent Application Publication Nos. US/2005/0074771, US/2005/0043219, and US/2005/0158727).

[0165] In some embodiments, the conjugate group may be a molecular entity that facilitates the delivery of the antisense compound into a cell or may be a molecule that comprises a drug or label. Examples of conjugate molecules suitable for attachment to antisense compounds of the present disclosure include, without limitation, lipophilic molecules (e.g., fatty acids), cholesterol, glycols such as polyethylene glycol (PEG), human serum albumin (HSA), carotenoids, terpenes, bile acids, folates (e.g., folic acid, folate analogs and derivatives thereof), sugars (e.g., galactose, galactosamine, N-acetyl galactosamine, glucose, mannose, fructose, fucose, etc.), phospholipids, peptides, ligands for cellular receptors capable of mediating cellular uptake, antibodies, aptamers, and combinations thereof (see, e.g., U.S. Patent Application Publication Nos. US/2003/0130186, US/2004/0110296, and US/2004/0249178; and U.S. Pat. No. 6,753,423).

[0166] The conjugate group used and the extent of conjugation to the antisense compound can be evaluated for improved pharmacokinetic profiles, bioavailability, biodistribution, and/or stability of the antisense compound. As such, one skilled in the art can screen antisense compounds having various conjugates attached thereto to identify conjugates having improved properties using any of a variety of well-known in vitro cell culture techniques or in vivo animal models.

[0167] In some embodiments, an antisense compound disclosed herein may be conjugated to a lipophilic molecule (for example, a long chain fatty acid or LCFA), an antibody (for example, anti-transferrin receptor antibody), an aptamer, a ligand, a peptide, or a polymer.

[0168] In some embodiments, an antisense compound disclosed herein may be conjugated to a ligand or receptor having specific binding activity to a cognate receptor or ligand of a neuron. Thus, the antisense compounds may be specifically targeted to neurons by binding receptors or ligands expressed thereon.

[0169] In some embodiments, an antisense compound disclosed herein may be conjugated to an antibody. In some embodiments, the antibody is a neuron-targeting antibody, e.g., an antibody having specificity for a neuron-specific cell surface marker.

IV. Pharmaceutical Formulations

[0170] In some aspects, this application provides compositions containing antisense nucleic acid compounds described herein and a suitable carrier such as a buffer, solvent, or diluent. The compositions may be liquid, wherein the antisense compound is dissolved in a suitable solvent or diluent, or may be solid or lyophilized, wherein the antisense compound is present in dry form with a suitable carrier or buffer. Such compositions containing antisense compounds can further be formulated for particular uses, such as for achieving a pharmacological effect in a cell based system and/or for pharmaceutical application. Thus, the disclosure provides pharmaceutical formulations containing a polynucleotide such as an ASO of the disclosure and a pharmaceutically acceptable carrier.

[0171] A pharmaceutical formulation can be capable of local or systemic administration. In some aspects, a pharmaceutical formulation can be capable of any mode of administration. In certain aspects, the administration can be by any route, including intravenous, intrathecal, intracerebroventricular, subcutaneous, pulmonary, intramuscular, intraperitoneal, dermal, oral, inhalation or nasal administration.

[0172] The present disclosure provides pharmaceutical formulations comprising one or more antisense compound described herein. Such pharmaceutical formulations may comprise a suitable pharmaceutically acceptable diluent or carrier, such as a pharmaceutical grade diluent or carrier. In some embodiments, a pharmaceutical formulation comprises a sterile saline solution and one or more antisense compound. In certain embodiments, such pharmaceutical formulations may consist of a sterile saline solution and one or more antisense compound. In some embodiments, a pharmaceutical formulation comprises one or more antisense compound and sterile water. In certain embodiments, a pharmaceutical formulation consists of one or more

antisense compound and sterile water. In some embodiments, a pharmaceutical formulation comprises one or more antisense compound and phosphate-buffered saline (PBS).

[0173] The antisense compounds of the disclosure may be admixed with pharmaceutically acceptable active and/or inert substances for the preparation of pharmaceutical formulations or formulations depending on intended route of administration, extent of disease, or dose to be administered.

[0174] Pharmaceutical formulations provided herein comprising antisense compounds encompass any pharmaceutically acceptable salts, esters, or salts of such esters. In certain embodiments, pharmaceutical formulations comprising antisense compounds comprise one or more oligonucleotide which, upon administration to an animal, including a human, is capable of providing (directly or indirectly) the biologically active metabolite or residue thereof. Accordingly, for example, the disclosure is also drawn to pharmaceutically acceptable salts of antisense compounds, prodrugs, pharmaceutically acceptable salts of such prodrugs, and other bioequivalents. Suitable pharmaceutically acceptable salts include, but are not limited to, sodium and potassium salts.

[0175] A prodrug can include the incorporation of additional nucleosides at one or both ends of an antisense compound which are cleaved by endogenous nucleases within the body, to form the active antisense nucleic acid compound.

[0176] In certain embodiments, a pharmaceutical formulation provided herein comprises a delivery system. Examples of delivery systems include, but are not limited to, liposomes and emulsions. Certain delivery systems are useful for preparing certain pharmaceutical formulations including those comprising hydrophobic compounds. In certain embodiments, certain organic solvents such as dimethylsulfoxide are used.

[0177] Lipid moieties have been used in nucleic acid therapies and delivery systems therefor in a variety of compositions and methods. For example, the nucleic acid may be introduced into preformed liposomes or lipoplexes made of mixtures of cationic lipids and neutral lipids. In certain methods, DNA complexes with mono- or poly-cationic lipids are formed without the presence of a neutral lipid. In certain embodiments, a lipid moiety is selected to increase distribution of a pharmaceutical agent to a particular cell or tissue. In certain embodiments, a lipid moiety is selected to increase distribution of a pharmaceutical agent to fat tissue. In certain embodiments, a lipid moiety is selected to increase distribution of a pharmaceutical agent to muscle tissue.

[0178] In some embodiments, an antisense compound may be delivered via liposomes, nanoparticles, lipid nanoparticles (LNPs), polymers, microparticles, microcapsules, micelles,

or extracellular vesicles and formulations comprising the same.

[0179] In some embodiments, an antisense compound may be delivered via a lipid nanoparticle (LNP). Examples of LNPs capable of delivering antisense compounds are described in WO/2015/074085, WO/2016/081029, WO/2017/117530, WO/2018/118102, WO/2018/119163, WO/2018/222926, WO/2019/191780, and WO/2020/154746. In some embodiments, an LNP may be decorated with targeting moiety, e.g., an antibody, a receptor, or a fragment thereof capable of binding to a target ligand.

[0180] In some embodiments, a lipid nanoparticle comprises (a) a nucleic acid (e.g., an antisense compound described herein), (b) a cationic lipid, (c) an aggregation reducing agent (such as a PEG-lipid), (d) optionally a non-cationic lipid (such as a neutral lipid), and (e) optionally a sterol. In one embodiment, the lipid nanoparticle comprises (i) at least one cationic lipid; (ii) a neutral lipid, e.g., DSPC; (iii) a sterol, e.g., cholesterol; and (iv) a PEG-lipid, in a molar ratio of about 20-65% cationic lipid: 5-25% neutral lipid: 25-55% sterol: 0.5-15% PEG-lipid. In some embodiments, the cationic lipid is selected from ATX-002, ATX-081, ATX-095, or ATX-126, as described in WO/2018/222926.

[0181] In some embodiments, an antisense compound is delivered via a nanocarrier comprising a molecule enabling specific receptor-mediated endosomal uptake. In one embodiment, said molecule can enable receptor binding, endosomal uptake, controlled breakdown of the endosomal membrane, and release of the antisense compound into a target cell. Examples of nanocarriers capable of delivering antisense compounds are described in WO/2009/141257. In some embodiments, the nanocarrier is a lipid-based nanocarrier, e.g., a lipid nanoparticle (LNP).

[0182] Pharmaceutical formulations provided herein may comprise one or more modified oligonucleotides and one or more excipients. In certain such embodiments, excipients are selected from water, salt solutions, alcohol, polyethylene glycols, gelatin, lactose, amylase, magnesium stearate, talc, silicic acid, viscous paraffin, hydroxymethylcellulose, and polyvinylpyrrolidone.

[0183] Pharmaceutical formulations provided herein may comprise a co-solvent system comprising, for example, benzyl alcohol, a nonpolar surfactant, a water-miscible organic polymer, and an aqueous phase. In certain embodiments, such co-solvent systems are used for hydrophobic compounds. A non-limiting example of such a co-solvent system is the VPD co-solvent system, which is a solution of absolute ethanol comprising 3% w/v benzyl alcohol, 8% w/v of the nonpolar surfactant Polysorbate 80™ and 65% w/v polyethylene glycol 300. The proportions of such co-solvent systems may be varied considerably without significantly

altering their solubility and toxicity characteristics. Furthermore, the identity of co-solvent components may be varied; for example: other surfactants may be used instead of Polysorbate 80™; the fraction size of polyethylene glycol may be varied; other biocompatible polymers may replace polyethylene glycol, e.g., polyvinyl pyrrolidone; and other sugars or polysaccharides may substitute for dextrose.

[0184] Pharmaceutical formulations as described herein may be formulated for, i.e., prepared for purposes of, oral administration, nasal administration, buccal administration, or administration by injection (e.g., intravenous, intrathecal, intracerebroventricular, subcutaneous, intramuscular, etc.). For example, a pharmaceutical formulation may comprise a carrier and be formulated in aqueous solution, such as water or physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiological saline buffer. In certain embodiments, other ingredients are included (e.g., ingredients that aid in solubility or serve as preservatives). In certain embodiments, injectable suspensions are prepared using appropriate liquid carriers, suspending agents and the like. Certain pharmaceutical formulations for injection are presented in unit dosage form, e.g., in ampoules or in multi-dose containers. Certain pharmaceutical formulations for injection are suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Certain solvents suitable for use in pharmaceutical formulations for injection include, but are not limited to, lipophilic solvents and fatty oils, such as sesame oil, synthetic fatty acid esters, such as ethyl oleate or triglycerides, and liposomes.

V. Methods of Administration

[0185] An effective dose of an antisense compound or pharmaceutical formulation comprising an antisense compound of this disclosure can be an amount that is sufficient to reduce the amount of *UBE3A* mRNA or *UBE3A* protein in a cell or tissue that expresses the *UBE3A* gene under physiological conditions. For example, an effective dose of the antisense compounds provided herein may reduce the level of *UBE3A* mRNA expressed in a neuron of a Dup15q syndrome individual. In some instances, the effective dose reduces the level of *UBE3A* expression from the levels seen in a tetraploid idic(15) individual to the levels seen in a diploid individual. In some instances, the effective dose reduces the level of *UBE3A* expression from the levels seen in a triploid Dup15q syndrome individual to the levels seen in a diploid individual. In these ways, an effective dose may normalize the level of *UBE3A* expression in neurons of a Dup15q individual relative to a subject having no maternal duplication in the *UBE3A* allele. An effective dose of an antisense compound or pharmaceutical

formulation comprising an antisense compound of this disclosure reduces the level of *UBE3A* mRNA or UBE3A protein expressed in a central nervous system neuron by about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more compared to a neuron that expresses the *UBE3A* gene under physiological conditions in the absence of the effective dose of an antisense compound or pharmaceutical formulation thereof described herein. In some embodiments, an effective dose reduces the amount of *UBE3A* mRNA or UBE3A protein by 1/3 or by about 1/2. In some embodiments, the effective dose reduces the amount of *UBE3A* mRNA or UBE3A protein by more than 1/2.

[0186] A therapeutically effective dose can be an amount of an antisense compound or formulation thereof that is sufficient to reduce, slow, halt, or reverse the effects of *UBE3A* overexpression in central nervous system neuron of a subject having Dup15q syndrome. In some embodiments, a therapeutically effective dose reduces the amount of *UBE3A* mRNA or UBE3A protein by about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more compared to neurons of the subject absent the therapeutically effective dose of an antisense compound or pharmaceutical formulation thereof described herein. In some embodiments, a therapeutically effective dose of the antisense compounds or formulations thereof provided herein normalize *UBE3A* expression levels in the central nervous system of a Dup15q syndrome individual to levels seen in an individual who is diploid for the *UBE3A* allele, i.e., does not have a maternal duplication in the allele.

[0187] A therapeutically effective dose can be administered to a subject in one or more separate administrations, and by different routes. As will be appreciated in the art, a therapeutically effective dose or a therapeutically effective amount is largely determined based on the total amount of the therapeutic agent contained in the pharmaceutical formulations of the present disclosure. Generally, a therapeutically effective amount is sufficient to achieve a meaningful benefit to the subject (e.g., treating, modulating, curing, preventing and/or ameliorating a disease or condition associated with *UBE3A* overexpression in central nervous system neurons). For example, a therapeutically effective amount may be an amount sufficient to achieve a desired therapeutic and/or prophylactic effect. Generally, the amount of a therapeutic agent (e.g., an ASO described herein) administered to a subject in need thereof will depend upon the characteristics of the subject. Such characteristics include ploidy of the *UBE3A* allele, disease severity, general health, age, sex and body weight of the subject. One of ordinary skill in the art armed with the present disclosure may be able to determine appropriate dosages depending on these and other related factors such as pharmacodynamic and pharmacokinetic properties of the therapeutic agents described herein. In addition, both

objective and subjective assays may optionally be employed to identify optimal dosage ranges.

[0188] Therapeutically effective doses of the antisense compounds described herein can be administered to subjects by appropriate administration routes. For example, doses may be administered via local or systemic administration. In some aspects, doses of the antisense compounds are administered to a subject via intravenous, intrathecal, intracerebroventricular, subcutaneous, pulmonary, intramuscular, intraperitoneal, dermal, oral, inhalation or nasal administration. In some embodiments, therapeutically effective doses of the antisense compounds are administered directly to the central nervous system of a subject. CNS delivery routes suitable for administration of the antisense compounds and pharmaceutical formulations thereof described herein include, e.g., intrathecal injection, intra cisterna magna injection, and intraventricular (intracerebroventricular) injection. In some embodiments, the compounds and formulations described herein are administered by intrathecal injection into the cerebrospinal fluid (CSF) of a subject in a reclined, e.g., Trendelenburg, position with or without a post-injection fluid flush to disperse the injected fluid and increase CNS distribution in the subject.

[0189] Methods provided herein contemplate single as well as multiple administrations of a therapeutically effective amount of the polynucleotide (e.g., an ASO targeting *UBE3A* nucleic acids) described herein. Pharmaceutical formulations comprising a polynucleotide described herein can be administered at regular intervals, depending on the nature, severity and extent of the subject's condition (e.g., the severity of a subject's disease state and the associated symptoms). In some embodiments, a therapeutically effective amount of the polynucleotide of the present disclosure may be administered periodically at regular intervals, e.g., once every year, once every six months, once every four months, once every three months, once every two months, once a month, once every two weeks, once a week, or more frequently than once a week. For example, a therapeutically effective amount of an ASO of the present disclosure may be administered weekly, once every two weeks, or monthly.

[0190] In some embodiments, the pharmaceutical formulations of the present disclosure are formulated such that they are suitable for extended-release of the polynucleotide contained therein. Such extended-release compositions may be conveniently administered to a subject at extended dosing intervals. Also contemplated herein are pharmaceutical formulations which are formulated for depot administration (e.g., subcutaneously, intramuscularly) to either deliver or release a polynucleotide of the disclosure over extended periods of time. The extended-release means employed may be combined with modifications made to the polynucleotide to enhance stability.

[0191] In some embodiments, administering a therapeutically effective dose of a

composition or formulation comprising a polynucleotide of the disclosure can result in decreased levels of UBE3A protein in CNS neurons of a treated subject. In some embodiments, administering a composition or formulation comprising a polynucleotide of the disclosure results in a 1/3, 1/2, 1/3 to 1/2, or greater than 1/2 decrease in levels of UBE3A protein in neurons of the subject relative to a baseline UBE3A protein level in the subject prior to treatment or relative to a baseline UBE3A protein level in age-matched or disease-state-matched or genotype-matched subjects having Dup15q syndrome. In certain embodiments, administering a therapeutically effective dose of a composition or formulation comprising a polynucleotide of the disclosure will result in a decrease in levels of UBE3A protein relative to baseline UBE3A protein levels in CNS tissues of the subject prior to treatment.

[0192] In some embodiments, a therapeutically effective dose, when administered regularly, results in reduced expression of UBE3A in CNS neurons as compared to baseline levels prior to treatment. In some embodiments, administering a therapeutically effective dose of a compound or formulation comprising an antisense compound of the disclosure results in the expression of UBE3A in CNS neurons of the subject at a level at or below about 1/3 or 1/2, or at or below about 75%, about 70%, about 65%, about 60%, about 55%, about 50%, about 45%, about 40%, about 35%, about 30%, or about 25% compared to expression in neurons of the subject prior to treatment.

[0193] A therapeutically effective dose of an antisense compound described herein or formulation thereof may comprise about 0.01 to about 50 mg of the antisense compound per kg body weight of the subject. For instance, the therapeutically effective dose may be about 0.1 to about 50 mg/kg body weight, about 0.1-25 mg/kg, about 0.1-20 mg/kg, about 0.1-15 mg/kg, about 0.1-10 mg/kg, about 0.1-5 mg/kg, about 0.1-1 mg/kg, about 1 mg/kg, about 2 mg/kg, about 3 mg/kg, about 4 mg/kg, about 5 mg/kg, about 6 mg/kg, about 7 mg/kg, about 8 mg/kg, about 9 mg/kg, about 10 mg/kg, about 11 mg/kg, about 12 mg/kg, about 13 mg/kg, about 14 mg/kg, about 15 mg/kg, about 16 mg/kg, about 17 mg/kg, about 18 mg/kg, about 19 mg/kg, about 20 mg/kg, about 21 mg/kg, about 22 mg/kg, about 23 mg/kg, about 24 mg/kg, about 25 mg/kg, about 1-50 mg/kg, about 5-50 mg/kg, about 10-50 mg/kg, about 15-50 mg/kg, about 20-50 mg/kg, or about 25-50 mg/kg body weight of the subject.

[0194] In some embodiments, a therapeutically effective dose of an antisense compound described herein or formulation thereof may comprise about 1 mg, about 2 mg, about 3 mg, about 4 mg, about 5 mg, about 6 mg, about 7 mg, about 8 mg, about 9 mg, about 10 mg, about 11 mg, about 12 mg, about 13 mg, about 14 mg, about 15 mg, about 16 mg, about 17 mg, about 18 mg, about 19 mg, or about 20 mg of the antisense compound. The therapeutically effective

dose of an antisense compound may be based on the subject's age, sex, disease severity, *UBE3A* copy number, or a combination thereof.

VI. Methods of Treatment

[0195] The present disclosure provides antisense compounds and formulations thereof for use in ameliorating, preventing, delaying onset of, or treating conditions associated with increased expression of *UBE3A* in central nervous system neurons in a subject, such as symptoms or disorders arising from a maternal duplication in the *UBE3A* gene of a subject. Thus, also provided are methods for ameliorating, preventing, delaying onset of, or treating conditions associated with increased UBE3A protein levels in the CNS of a subject, such as symptoms or disorders arising from a maternal duplication in the *UBE3A* gene of a subject. In some embodiments, the subject is diagnosed with Dup15q syndrome. In some embodiments, the subject is diagnosed with isodicentric duplication Dup15q syndrome (idic(15)). In some embodiments, the methods comprise administering to the subject an antisense compound or a pharmaceutical formulation thereof. In some embodiments, the methods may further comprise the administration of one or more additional therapeutics (i.e., a “second pharmaceutical agents”) before, simultaneous with, or after administration of the antisense compound or pharmaceutical formulation thereof.

[0196] Pharmaceutical agents that may be co-administered as a second pharmaceutical agent according to the methods described herein include, e.g., growth hormones, seizure medications (such as lorazepam, diazepam, rufinamide, phenytoin, valproic acid, felbamate, levetiracetam, lamotrigine, clobazam, carbamazepine, topiramate, oxcarbazepine, lacosamide, zonisamide, prednisone/prednisolone, and vigabatrin) medications for abnormal behaviors (such as clonidine, sertraline, risperidone, quetiapine, and olanzapine) and antidepressants.

[0197] Throughout the description, where compositions are described as having, including, or comprising specific components, or where processes and methods are described as having, including, or comprising specific steps, it is contemplated that, additionally, there are compositions of the present disclosure that consist essentially of, or consist of, the recited components, and that there are processes and methods according to the present disclosure that consist essentially of, or consist of, the recited steps.

[0198] In the application, where an element or component is said to be included in and/or selected from a list of recited elements or components, it will be understood that the element

or component can be any one of the recited elements or components, or the element or component can be selected from a group consisting of two or more of the recited elements or components.

[0199] Further, it will be understood that elements and/or features of a composition or a method described herein can be combined in a variety of ways without departing from the spirit and scope of the present disclosure, whether explicit or implicit herein. For example, where reference is made to a particular compound, that compound can be used in various embodiments of compositions of the present disclosure and/or in methods of the present disclosure, unless otherwise understood from the context. In other words, within this application, embodiments have been described and depicted in a way that enables a clear and concise application to be written and drawn, but it is intended and will be appreciated that embodiments may be variously combined or separated without parting from the present teachings and disclosure(s). For example, it will be appreciated that all features described and depicted herein can be applicable to all aspects of the disclosure(s) described and depicted herein. All of the features disclosed in this specification may be combined in any combination. Each feature disclosed in this specification may be replaced by an alternative feature serving the same, equivalent, or similar purpose.

[0200] It will be understood that the expression “at least one of” includes individually each of the recited objects after the expression and the various combinations of two or more of the recited objects unless otherwise understood from the context and use. The expression “and/or” in connection with three or more recited objects will be understood to have the same meaning unless otherwise understood from the context.

[0201] The use of the term “include,” “includes,” “including,” “have,” “has,” “having,” “contain,” “contains,” or “containing,” including grammatical equivalents thereof, will be understood generally as open-ended and non-limiting, for example, not excluding additional unrecited elements or steps, unless otherwise specifically stated or understood from the context.

[0202] The use of any and all examples, or exemplary language herein, for example, “for instance,” “such as,” “for example,” “e.g.,” or “including” is intended merely to illustrate better the present disclosure and does not pose a limitation on the scope of the disclosure unless claimed. No language in the specification will be construed as indicating any non-claimed element as essential to the practice of the subject matter of the present disclosure.

[0203] It is understood that this disclosure is not limited to the particular methodology, protocols, materials, and reagents described, as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and

is not intended to limit the scope of the present disclosure, which will be encompassed by the appended claims.

[0204] While antisense nucleic acid compounds, compositions, formulations, and methods described herein have been described with specificity according to certain embodiments, the following examples serve only to illustrate examples of these compounds, compositions, formulations, and methods, and are not intended to limit the same.

[0205] Although the sequence listing accompanying this filing identifies each sequence as either “RNA” or “DNA” as required, in reality, those sequences may be modified with any combination of chemical modifications. One of skill in the art will readily appreciate that such designation as “RNA” or “DNA” to describe modified oligonucleotides is, in certain instances, arbitrary. For example, an oligonucleotide comprising a nucleoside comprising a 2'-OH sugar moiety and a thymine base could be described as a DNA having a modified sugar (2'-OH for the natural 2'-H of DNA) or as an RNA having a modified base (thymine (methylated uracil) for natural uracil of RNA).

[0206] Accordingly, nucleic acid sequences provided herein, including, but not limited to those in the sequence listing, are intended to encompass nucleic acids containing any combination of natural or modified RNA and/or DNA, including, but not limited to such nucleic acids having modified nucleobases. By way of further example and without limitation, an antisense nucleic acid compound having the nucleobase sequence “ATCGATCG” encompasses any nucleic acid compounds having such nucleobase sequence, whether modified or unmodified, including, but not limited to, such compounds comprising RNA bases, such as those having sequence “AUCGAUCG” and those having some DNA bases and some RNA bases such as “AUCGATCG” and nucleic acid compounds having other modified or naturally occurring bases, such as “ATmeCGAUCG,” wherein meC indicates a cytosine base comprising a methyl group at the 5-position.

[0207] All publications, patents and patent applications, including any drawings, sequences, and appendices therein referred to throughout the present description are incorporated by reference in their entirety for all purposes to the same extent as if each individual publication, patent or patent application, drawing, sequence, or appendix was specifically and individually indicated to be incorporated by reference in its entirety for all purposes.

ENUMERATED EMBODIMENTS

[0208] The scope of this disclosure is indicated by the appended claims and the following enumerated embodiments, and all changes that come within the meaning and range of

equivalency of the claims and enumerated embodiments are intended to be embraced therein. Thus, the disclosure provides:

[0209] Embodiment 1. A nucleic acid compound, such as an antisense oligonucleotide (ASO), comprising at least 16 contiguous nucleosides and having a nucleotide sequence selected from SEQ ID NO: 285 through SEQ ID NO: 562, comprising one or more of:

- [0210]** a) a gap segment consisting of linked deoxynucleosides;
- [0211]** b) a 5' segment consisting of at least 2 linked nucleosides;
- [0212]** c) a 3' segment consisting of at least 2 linked nucleosides;
- [0213]** d) at least one phosphorothioate internucleoside linkage;
- [0214]** e) at least one nucleoside comprising a modified sugar; and
- [0215]** f) at least one nucleoside comprising a modified nucleobase.

[0216] Embodiment 2. The nucleic acid compound of Embodiment 1, wherein the gap segment is positioned between the 5' segment and the 3' segment.

[0217] Embodiment 3. The nucleic acid compound of Embodiment 1 or 2, wherein the gap segment comprises 5 to 15 linked nucleosides.

[0218] Embodiment 4. The nucleic acid compound of any one of Embodiments 1-3, wherein the 3' segment comprises 2-5 linked nucleosides.

[0219] Embodiment 5. The nucleic acid compound of any one of Embodiments 1-4, wherein the 5' segment comprises 2-5 linked nucleosides.

[0220] Embodiment 6. The nucleic acid compound of any one of Embodiments 1-5, wherein at least one nucleoside of the 5' segment and at least one nucleoside of the 3' segment comprises a modified sugar.

[0221] Embodiment 7. The nucleic acid compound of any one of Embodiments 1-6, wherein each nucleoside of the 5' segment and each nucleoside of the 3' segment comprises a modified sugar.

[0222] Embodiment 8. The nucleic acid compound of any one of Embodiments 6 or 7, wherein the modified sugar comprises a bicyclic sugar.

[0223] Embodiment 9. The nucleic acid compound of Embodiment 8, wherein the bicyclic sugar is selected from the group consisting of: 2'-O(CH₂)₂OCH₃ (MOE); 4'-(CH₂)—O-2' (LNA); 4'-(CH₂)₂—O-2' (ENA); and 4'-CH(CH₃)—O-2' (cEt).

[0224] Embodiment 10. The nucleic acid compound of any one of Embodiments 1-9, wherein each internucleoside linkage is a phosphorothioate internucleoside linkage.

[0225] Embodiment 11. The nucleic acid compound of any one of Embodiments 1-10, comprising a 5-methylcytosine nucleobase in place of a non-5-methyl cytosine residue.

[0226] Embodiment 12. The nucleic acid compound of any one of Embodiments 1-11, comprising a 3-10-3 LNA-DNA-LNA gapmer, wherein all internucleoside linkages are phosphorothioate internucleoside linkages.

[0227] Embodiment 13. The nucleic acid compound of any one of Embodiments 1-11, comprising a 3-11-3 LNA-DNA-LNA gapmer, wherein all internucleoside linkages are phosphorothioate internucleoside linkages.

[0228] Embodiment 14. The nucleic acid compound of any one of Embodiments 1-11, comprising a 3-12-3 LNA-DNA-LNA gapmer, wherein all internucleoside linkages are phosphorothioate internucleoside linkages.

[0229] Embodiment 15. The nucleic acid compound of any one of Embodiments 1-11, comprising a 4-11-5 MOE-DNA-MOE gapmer, wherein all internucleoside linkages are phosphorothioate internucleoside linkages.

[0230] Embodiment 16. The nucleic acid compound of any one of Embodiments 1-15, wherein the nucleic acid compound comprises a nucleotide sequence according to SEQ ID NO: 295, 307, 309, 310, 327, 328, 329, 330, 340, 341, 342, 343, 351, 356, 359, 366, 394, 402, 523, 527, 528, 529, 539, 542, 549, 550, 551, or 553.

[0231] Embodiment 17. The nucleic acid compound of Embodiment 1, wherein the nucleic acid compound is a gapmer according to Compound ID No. 16-63326LNA, 17-29823LNA, 17-29855LNA, 17-29858LNA, 17-63264LNA, 17-63289LNA, 17-63290LNA, 17-63291LNA, 17-63324LNA, 17-63325LNA, 17-63326LNA, 17-63327LNA, 17-63458LNA, 17-67260LNA, 18-435LNA, 18-29854LNA, 18-63323LNA, 18-63458LNA, 17-63278LNA, 17-63286LNA, 17-63287LNA, 17-63288LNA, 17-63437LNA, 17-29853LNA, 18-63282LNA, 18-63285LNA, 18-63286LNA, or 18-63325LNA.

[0232] Embodiment 18. An antisense oligonucleotide (ASO) comprising: a 5' segment of 3 linked locked nucleic acid (LNA) nucleosides, a gap segment of linked deoxynucleosides, and a 3' segment of 3 linked LNA nucleosides, wherein all internucleoside linkages are phosphorothioate internucleoside linkages, and wherein ASO comprises the nucleotide sequence of SEQ ID NO: 550.

[0233] Embodiment 19. An antisense oligonucleotide (ASO) comprising: a 5' segment of 3 linked locked nucleic acid (LNA) nucleosides, a gap segment of linked deoxynucleosides, and a 3' segment of 3 linked LNA nucleosides, wherein all internucleoside linkages are phosphorothioate internucleoside linkages, and wherein ASO comprises the nucleotide sequence of SEQ ID NO: 542.

[0234] Embodiment 20. An antisense oligonucleotide (ASO) comprising: a 5' segment of

3 linked locked nucleic acid (LNA) nucleosides, a gap segment of linked deoxynucleosides, and a 3' segment of 3 linked LNA nucleosides, wherein all internucleoside linkages are phosphorothioate internucleoside linkages, and wherein ASO comprises the nucleotide sequence of SEQ ID NO: 394.

[0235] Embodiment 21. An antisense oligonucleotide (ASO) comprising: a 5' segment of 3 linked locked nucleic acid (LNA) nucleosides, a gap segment of linked deoxynucleosides, and a 3' segment of 3 linked LNA nucleosides, wherein all internucleoside linkages are phosphorothioate internucleoside linkages, and wherein ASO comprises the nucleotide sequence of SEQ ID NO: 328.

[0236] Embodiment 22. An antisense oligonucleotide (ASO) comprising: a 5' segment of 3 linked locked nucleic acid (LNA) nucleosides, a gap segment of linked deoxynucleosides, and a 3' segment of 3 linked LNA nucleosides, wherein all internucleoside linkages are phosphorothioate internucleoside linkages, and wherein ASO comprises the nucleotide sequence of SEQ ID NO: 342.

[0237] Embodiment 23. An antisense oligonucleotide (ASO) comprising: a 5' segment of 3 linked locked nucleic acid (LNA) nucleosides, a gap segment of linked deoxynucleosides, and a 3' segment of 3 linked LNA nucleosides, wherein all internucleoside linkages are phosphorothioate internucleoside linkages, and wherein ASO comprises the nucleotide sequence of SEQ ID NO: 356.

[0238] Embodiment 24. An antisense oligonucleotide (ASO) comprising: a 5' segment of 3 linked locked nucleic acid (LNA) nucleosides, a gap segment of linked deoxynucleosides, and a 3' segment of 3 linked LNA nucleosides, wherein all internucleoside linkages are phosphorothioate internucleoside linkages, and wherein ASO comprises the nucleotide sequence of SEQ ID NO: 295.

[0239] Embodiment 25. An antisense oligonucleotide (ASO) comprising: a 5' segment of 3 linked locked nucleic acid (LNA) nucleosides, a gap segment of linked deoxynucleosides, and a 3' segment of 3 linked LNA nucleosides, wherein all internucleoside linkages are phosphorothioate internucleoside linkages, and wherein ASO comprises the nucleotide sequence of SEQ ID NO: 351.

[0240] Embodiment 26. An antisense oligonucleotide (ASO) comprising: a 5' segment of 3 linked locked nucleic acid (LNA) nucleosides, a gap segment of linked deoxynucleosides, and a 3' segment of 3 linked LNA nucleosides, wherein all internucleoside linkages are phosphorothioate internucleoside linkages, and wherein ASO comprises the nucleotide sequence of SEQ ID NO: 307.

- [0241] Embodiment 27. The nucleic acid compound or ASO of any one of Embodiments 1-26, wherein all cytosine nucleosides are replaced with 5-methylcytosine nucleosides.
- [0242] Embodiment 28. The nucleic acid compound or ASO of any one of Embodiments 1-27, comprising a 2'OMe-modified nucleoside at position 2 in the DNA gap.
- [0243] Embodiment 29. A composition comprising the nucleic acid compound or ASO of any one of Embodiments 1-28 or salt thereof and a pharmaceutically acceptable carrier.
- [0244] Embodiment 30. The composition of Embodiment 29, wherein the composition is a pharmaceutical formulation.
- [0245] Embodiment 31. A method of reducing the level of a *UBE3A* RNA in a cell, comprising contacting the cell with the nucleic acid compound or ASO of any one of Embodiments 1-28 or the composition of Embodiment 29 or 30, thereby reducing the level of the *UBE3A* RNA in the cell.
- [0246] Embodiment 32. A method of inhibiting expression of UBE3A protein in a cell, comprising contacting the cell with the nucleic acid compound or ASO of any one of Embodiments 1-28 or the composition of Embodiment 29 or 30, thereby reducing expression of UBE3A protein in the cell.
- [0247] Embodiment 33. The method of Embodiment 31 or 32, wherein the cell is a central nervous system neuron.
- [0248] Embodiment 34. The method of any one of Embodiments 31-33, wherein the cell is triploid or tetraploid for the *UBE3A* gene.
- [0249] Embodiment 35. The method of any one of Embodiments 31-34, wherein the cell comprises a maternal duplication of the *UBE3A* allele.
- [0250] Embodiment 36. The method of any one of Embodiments 31-35, wherein the cell is in vitro.
- [0251] Embodiment 37. The method of any one of Embodiments 31-35, wherein the cell is in a subject.
- [0252] Embodiment 38. The method of Embodiment 37, wherein the subject is a human and the nucleic acid compound, ASO, or composition is administered to the subject.
- [0253] Embodiment 39. The method of Embodiment 38, wherein the nucleic acid compound or ASO is administered to the subject via intravenous, subcutaneous, intramuscular, intraperitoneal, intrathecal, intra cisterna magna, or intraventricular (intracerebroventricular) injection.
- [0254] Embodiment 40. A method of treating, preventing, or ameliorating a disease associated with overexpression of *UBE3A* in central nervous system neurons in a subject,

comprising administering to the subject an nucleic acid compound or ASO of any one of Embodiments 1-28 or the composition of Embodiment 29 or 30, thereby treating, preventing, or ameliorating the disease.

[0255] Embodiment 41. The method of Embodiment 40, wherein the disease is Dup15q syndrome.

[0256] Embodiment 42. The method of Embodiment 40 or 41, wherein the subject has an idic(15) genotype.

[0257] Embodiment 43. The method of any one of Embodiments 37-42, comprising administering to the subject a second pharmaceutical agent for treating, preventing, or ameliorating one or more symptoms of Dup15q syndrome.

[0258] Embodiment 44. An nucleic acid compound or ASO according to any one of Embodiments 1-28 or the composition of Embodiment 29 or 30 for use in a method of treating, preventing, or ameliorating a disease associated with overexpression of *UBE3A* in central nervous system neurons in a subject.

[0259] Embodiment 45. The nucleic acid compound or ASO for use of Embodiment 44, wherein the disease is Dup15q syndrome.

[0260] Embodiment 46. The nucleic acid compound or ASO for use of Embodiment 44 or 45, wherein the subject has an idic(15) genotype.

[0261] Embodiment 47. Use of an nucleic acid compound or ASO of any one of Embodiments 1-28 or the composition of Embodiment 29 or 30 for the treatment of a disease associated with increased levels of UBE3A protein in central nervous system neurons in a subject.

[0262] Embodiment 48. Use of an nucleic acid compound or ASO of any one of Embodiments 1-28 or the composition of Embodiment 29 or 30 for the preparation of a medicament for the treatment of a disease associated with increased levels of UBE3A protein in central nervous system neurons of a subject.

[0263] Embodiment 49. The use of Embodiment 47 or 48, wherein the disease is Dup15q syndrome.

[0264] Embodiment 50. The use of any one of Embodiments 47-49, wherein the subject has an idic(15) genotype.

EXAMPLES

[0265] The disclosure now being generally described will be more readily understood by reference to the following examples, which are included merely for purposes of illustration of

certain aspects and embodiments of the present disclosure, and are not intended to limit the disclosure.

Example 1: ASO Design and Initial Screen

[0266] Antisense oligonucleotides of 16, 17, 18, and 20 nucleotide lengths (i.e., 16-, 17-, 18- and 20-mers, respectively) were designed targeting hg38 chr15:25371667-25439024, a portion of the human *UBE3A* gene comprising at least a portion of the 5' UTR through at least a portion of exon 7. The target region is provided herein as SEQ ID NO: 1. The target region was selected in part to emphasize the 5' region of the *UBE3A* gene to encourage more rapid decay of mRNA transcripts and thus more potent ASOs.

[0267] In designing antisense compounds targeting the human *UBE3A* target region sequence (SEQ ID NO: 1), repeat regions were filtered out, 3' guanine (G) residues were avoided, melting temperature and GC content were evaluated and taken into account, off target interactions within the human genome were avoided, and homology of target sequences among human, non-human primate (*macaca fascicularis*), pig (*sus scrofa*) and rodent (*mus musculus*) model organisms was favored. According to these parameters, an initial set of ASOs was designed, synthesized, and evaluated in an in vitro potency screen (Table 1).

[0268] Each ASO is ascribed a unique Compound ID having the following informational components: [ASO nucleotide length]-[start position][optional SNP alteration][chemical modification]. The [ASO nucleotide length] component of each Compound ID represents the length in linked nucleosides of the ASO (e.g., "16", "17", "18", or "20"). The [start position] component represents the nucleotide position of the first 5' residue targeted by the ASO relative to SEQ ID NO: 1 (e.g., "480" or "63462", etc.). The [optional SNP alteration] component represents certain ASOs having single nucleotide mismatches introduced relative to the target *UBE3A* sequence to elucidate potential impacts of single nucleotide polymorphisms (SNPs) falling within target sequences in individuals, where a single nucleotide within the ASO, either in the middle portion (SNPM) or near the 3' end of the ASO (SNPE), was altered according to the underlined residues provided for SNPM and SNPE ASOs in Table 1. Last, the [chemical modification] component represents whether each ASO is locked nucleic acid modified or 2'-O-methoxy-ethyl modified, as further described below.

[0269] The initial set of ASOs screened included locked nucleic acid (LNA-modified) and 2'-O-methoxy-ethyl (MOE-modified) molecules. LNA-modified ASOs comprised 3 linked 5' LNA-modified nucleosides, followed by 10-12 linked DNA nucleosides, followed by 3 linked 3' LNA-modified nucleosides, with fully phosphorothioate-modified internucleoside linkages,

i.e., 3-10-3 and 3-12-3 LNA-DNA-LNA gapmers. MOE-modified ASOs comprised 4 linked 5' MOE-modified nucleosides followed by 11 linked DNA nucleosides, followed by 5 linked 3' MOE-modified nucleosides, with mixed phosphorothioate-modified and phosphodiester internucleoside linkages, i.e., 4-11-5 MOE-DNA-MOE gapmers. All ASOs synthesized and screened had 5-methylcytosine in place of all cytosine residues.

[0270] Certain ASO sequences were used as positive and negative controls throughout the studies described herein. Scrambled ASOs 17-SCRA1LNA (GATTACAGATTACACAT, SEQ ID NO: 563), 18-SCRA2LNA (CATCATCATCATCATCAT, SEQ ID NO: 564), and 20-SCRA3MOE (ACATCATCATCATCATCATC, SEQ ID NO: 565) were used as negative controls. As positive controls, 20-CTRL1MOE (GGATTCAACTGCTGTCCTTG, SEQ ID NO: 566) and 20-CTRL2MOE (TGAGCTATCACCTATCCTTG, SEQ ID NO: 567) were used. Positive control ASO 20-CTRL1MOE was described in United States Pat. App. Pub. No. US/2022/0259601A1 as ASO-015 (SEQ ID NO: 298 therein) and 20-CTRL2MOE was described in Elamin et al., Stem Cell Reports 18.4 (2023): 884-898 (see Supplemental Information therein).

[0271] Briefly, mixed population human normal H9 neurons were differentiated in house from commercially available neural progenitor H9 line (Millipore-Sigma, cat# SCR055, ENStem Human Neural Progenitor Expansion Kit). A master cell bank of cryopreserved differentiated H9 neurons was generated and served as a source of neurons used in ASO selection experiments. H9 neurons were thawed and plated at 120,000 cells per well on 96 well plates, then cultured for 2 weeks to establish mature neuronal cultures. Neurons were treated with ASOs at 3 μ M final concentration by gymnosol and cultured for 6 or 10 days without medium change, after which medium was removed and cells were lysed. Levels of *UBE3A* mRNA were evaluated using QuantiGene assay (Thermo Fisher Scientific), a hybridization-based assay for direct measurement of RNA transcripts. Both singleplex and multiplex assays were used to identify a panel of selected ASOs with consistently potent knockdown results across experiments (Table 2).

[0272] Table 2 provides *UBE3A* knockdown efficiency of top-performing ASOs identified by the initial singleplex and multiplex potency screens presented as a percentage of *UBE3A* mRNA (“%KD”) measured relative to untreated control neurons (i.e., “-50%” means a 50% reduction relative to untreated controls and “50%” means a 50% increase relative to untreated controls, as an example). Calculated standard deviation (“St Dev”) and coefficient of variation (“%CV”) are also reported for each ASO in Table 2.

Table 1. Human *UBE3A* ASOs – Initial Screen

Compound ID	Sequence	SEQ ID NO	Compound ID	Sequence	SEQ ID NO
16-480LNA	TGGTCGTAG TCGCCCT	285	18-63462LNA	GGTAAGCTGA GCTTGCTC	404
16-482LNA	ACTGGTCGT AGTCGCC	286	18-63463LNA	AGGTAAGCTG AGCTTGCT	405
16-29845LNA	CCAGTCTAG CTGCTAC	287	18-64667LNA	CCCCCGCAGC TGGCTGCA	406
16-29896LNA	ACATCAGGG TGATCAC	288	18-64668LNA	TCCCCCGCAG CTGGCTGC	407
16-29907LNA	GCCATTCGG TGACATC	289	20-431MOE	GTTCTCTTTC GCTGCCGCCA	408
16-29916LNA	CAAGCTGTG GCCATT	290	20-433MOE	TCGTTCTCTTT CGCTGCCGC	409
16-29917LNA	ACAAGCTGT GGCCATT	291	20-434MOE	CTCGTTCTCTT TCGCTGCCG	410
16-31774LNA	GCAAAACAA AAAATAA	292	20-436MOE	GCCTCGTTCT CTTTCGCTGC	411
16-31920LNA	TAAGTACCC CAAGATT	293	20-437MOE	CGCCTCGTTC TCTTTCGCTG	412
16-63309LNA	CCACAGCCC TCAGTTA	294	20-439MOE	ACCGCCTCGT TCTCTTTCGC	413
16-63326LNA	TCGTGCAGG CTTCATT	295	20-440MOE	CACCGCCTCG TTCTCTTTCG	414
17-435LNA	CGTTCTCTTT CGCTGCC	296	20-441MOE	CCACCGCCTC GTTCTCTTTC	415
17-442LNA	ACCGCCTCG TTCTCTTT	297	20-442MOE	GCCACCGCCT CGTTCTCTTT	416
17-479LNA	TGGTCGTAG TCGCCCTC	298	20-480MOE	TCACTGGTCG TAGTCGCCCT	417
17-480LNA	CTGGTCGTA GTCGCCCT	299	20-27066MOE	ATTCTAAAT CAGGCTCTTA	418
17-481LNA	ACTGGTCGT AGTCGCCC	300	20-27073MOE	CTGGTGAATT CTAAATCAG	419
17-482LNA	CACTGGTCG TAGTCGCC	301	20-27084MOE	CTTCTGAGGA GCTGGTGAAT	420
17-27055LNA	CTCTTACAG ATTTTAA	302	20-27085MOE	ACTTCTGAGG AGCTGGTGAA	421
17-27064LNA	AAAATCAGG CTCTTACA	303	20-27086MOE	AACTTCTGAG GAGCTGGTGA	422
17-27069LNA	ATTCTAAAA TCAGGCTC	304	20-27087MOE	AAACTTCTGA GGAGCTGGTG	423
17-27100LNA	ATATTTCGC CAAACCTC	305	20-27088MOE	CAAACCTCTG AGGAGCTGGT	424
17-29817LNA	CGTAGGCTT AATAACTC	306	20-27090MOE	GCCAAACTTC TGAGGAGCTG	425

17-29823LNA	TCTGAGCGT AGGCTTAA	307	20-27091MOE	CGCCAAACTT CTGAGGAGCT	426
17-29839LNA	CTAGCTGCT ACCTTGAT	308	20-27092MOE	TCGCCAAACT TCTGAGGAGC	427
17-29855LNA	AGGTTGTCA CACCAGTC	309	20-27093MOE	TTCGCCAAAC TTCTGAGGAG	428
17-29858LNA	AACAGGTTG TCACACCA	310	20-29830MOE	GCTACCTTGA TCTGAGCGTA	429
17-29859LNA	AAACAGGTT GTCACACC	311	20-29831MOE	TGCTACCTTG ATCTGAGCGT	430
17-29879LNA	AGCTTTGAG TCACTGAT	312	20-29836MOE	CTAGCTGCTA CCTTGATCTG	431
17-29906LNA	GCCATTCGG TGACATCA	313	20-29838MOE	GTCTAGCTGC TACCTTGATC	432
17-29907LNA	GGCCATTCG GTGACATC	314	20-29840MOE	CAGTCTAGCT GCTACCTTGA	433
17-29916LNA	ACAAGCTGT GGCCATTC	315	20-29841MOE	CCAGTCTAGC TGCTACCTTG	434
17-29917LNA	TACAAGCTG TGGCCATT	316	20-29846MOE	TCACACCAGT CTAGCTGCTA	435
17-29918LNA	TTACAAGCT GTGGCCAT	317	20-29847MOE	GTCACACCAG TCTAGCTGCT	436
17-29919LNA	TTTACAAGC TGTGGCCA	318	20-29852MOE	AGGTTGTCAC ACCAGTCTAG	437
17-29920LNA	TTTTACAAG CTGTGGCC	319	20-29853MOE	CAGGTTGTCA CACCAGTCTA	438
17-33529LNA	CGTCAGACT GAGGTTCT	320	20-29854MOE	ACAGGTTGTC ACACCAGTCT	439
17-33531LNA	GTCGTCAGA CTGAGGTT	321	20-29885MOE	GGTGATCACA GCTTTGAGTC	440
17-33541LNA	TAGCTTCAA TGTCGTCA	322	20-29904MOE	GGCCATTCGG TGACATCAGG	441
17-33545LNA	CGGCTAGCT TCAATGTC	323	20-29905MOE	TGGCCATTCG GTGACATCAG	442
17-33548LNA	ATTCGGCTA GCTTCAAT	324	20-29906MOE	GTGGCCATTC GGTGACATCA	443
17-33549LNA	CATTCGGCT AGCTTCAA	325	20-29907MOE	TGTGGCCATT CGGTGACATC	444
17-63262LNA	TTGCAGCTG CTCGCTTC	326	20-29909MOE	GCTGTGGCCA TTCGGTGACA	445
17-63264LNA	CTTTGCAGC TGCTCGCT	327	20-29910MOE	AGCTGTGGCC ATTCGGTGAC	446
17-63289LNA	GGTGGTAGT AGCGTTCT	328	20-29911MOE	AAGCTGTGGC CATTCGGTGA	447
17-63290LNA	TGGTGGTAG TAGCGTTC	329	20-29912MOE	CAAGCTGTGG CCATTCGGTG	448
17-63291LNA	CTGGTGGTA GTAGCGTT	330	20-29915MOE	TTACAAGCTG TGGCCATTCG	449

17-63306LNA	ACAGCCCTC AGTTAACT	331	20-33523MOE	CAGACTGAGG TTCTCCTGAT	450
17-63306SNPELN A (ACAGCCCT CAGTTAACT (SEQ ID NO: 331)): T to A	ACAGCCCTC AGTTAACA	332	20-33524MOE	TCAGACTGAG GTTCTCCTGA	451
17-63306SNPML NA (ACAGCCCT CAGTTAACT (SEQ ID NO: 331)): C to A	ACAGCCCTA AGTTAACT	333	20-33525MOE	GTCAGACTGA GGTTCCTG	452
17-63307LNA	CACAGCCCT CAGTTAAC	334	20-33526MOE	CGTCAGACTG AGGTTCTCCT	453
17-63308LNA	CCACAGCCC TCAGTTAA	335	20-33528MOE	GTCGTCAGAC TGAGGTTCTC	454
17-63309LNA	TCCACAGCC CTCAGTTA	336	20-33529MOE	TGTCGTCAGA CTGAGGTTCT	455
17-63320LNA	CAGGCTTCA TTTCCACA	337	20-33530MOE	ATGTCGTCAG ACTGAGGTTT	456
17-63321LNA	GCAGGCTTC ATTTCAC	338	20-33532MOE	CAATGTCGTC AGACTGAGGT	457
17-63323LNA	GTGCAGGCT TCATTTC	339	20-33536MOE	GCTTCAATGT CGTCAGACTG	458
17-63324LNA	CGTGCAGGC TTCATTTC	340	20-33540MOE	GCTAGCTTCA ATGTCGTCAG	459
17-63325LNA	TCGTGCAGG CTTCATTT	341	20-33541MOE	GGCTAGCTTC AATGTCGTC	460
17-63326LNA	TTCGTGCAG GCTTCATT	342	20-33542MOE	CGGCTAGCTT CAATGTCGTC	461
17-63327LNA	ATTCGTGCA GGCTTCAT	343	20-33543MOE	TCGGCTAGCT TCAATGTCGT	462
17-63340LNA	AAGCACAAA ACTCATTC	344	20-33544MOE	TTCGGCTAGC TTCAATGTCG	463
17-63348LNA	TGGACAGGA AGCACAAA	345	20-63263MOE	TGCTTTCAG CTGCTCGCTT	464
17-63351LNA	AGTTGGACA GGAAGCAC	346	20-63264MOE	ATGCTTTGCA GCTGCTCGCT	465
17-63352LNA	AAGTTGGAC AGGAAGCA	347	20-63265MOE	GATGCTTTGC AGCTGCTCGC	466
17-63364LNA	CCATACGAA GAAAAGTT	348	20-63266MOE	AGATGCTTTG CAGCTGCTCG	467
17-63410LNA	TTAATCTTA TAAAGCTC	349	20-63288MOE	CTGGTGGTAG TAGCGTTCTA	468

17-63457LNA	CTGAGCTTG CTCCTTTC	350	20-63289MOE	ACTGGTGGTA GTAGCGTTCT	469
17-63458LNA	GCTGAGCTT GCTCCTTT	351	20-63290MOE	AACTGGTGGT AGTAGCGTTC	470
17-63463LNA	GGTAAGCTG AGCTTGCT	352	20-63293MOE	GTAACTGGT GGTAGTAGCG	471
17-63467LNA	TCAAGGTAA GCTGAGCT	353	20-63301MOE	AGCCCTCAGT TAACTGGTGG	472
17-67212LNA	GTAAAGTAA GTCACATC	354	20-63302MOE	CAGCCCTCAG TTAACTGGTG	473
17-67242LNA	AATCAAGA ATTCATA	355	20-63303MOE	ACAGCCCTCA GTAACTGGT	474
17-67260LNA	TCCTCTCTT CTCTACA	356	20-63304MOE	CACAGCCCTC AGTTAACTGG	475
17-67307LNA	CAGCACTAG AAAAAACT	357	20-63305MOE	CCACAGCCCT CAGTTAACTG	476
17-67312LNA	TGCCTCAGC ACTAGAAA	358	20-63306MOE	TCCACAGCCC TCAGTTAACT	477
18-435LNA	TCGTTCTCTT TCGCTGCC	359	20-63307MOE	TTCCACAGCC CTCAGTTAAC	478
18-438LNA	GCCTCGTTC TCTTTCGCT	360	20- 63307SNPEMO E (TTCCACAGC CCTCAGTTAA C_(SEQ ID NO: 478)): A to G	TTCCACAGCC CTCAGTTGAC	479
18-480LNA	ACTGGTCGT AGTCGCCCT	361	20- 63307SNPMMO E (TTCCACAGC CCTCAGTTAA C_(SEQ ID NO: 478)): C to G	TTCCACAGCG CTCAGTTAAC	480
18-482LNA	TCACTGGTC GTAGTCGCC	362	20-63310MOE	CATTTCACAC GCCCTCAGTT	481
18-861LNA	CCTGTCTGG GGCCCCGGC	363	20-63311MOE	TCATTTCAC AGCCCTCAGT	482
18-27091LNA	CCAAACTTC TGAGGAGCT	364	20-63317MOE	CAGGCTTCAT TTCCACAGCC	483
18-29838LNA	CTAGCTGCT ACCTTGATC	365	20-63318MOE	GCAGGCTTCA TTTCCACAGC	484
18-29854LNA	AGGTTGTCA CACCAGTCT	366	20-63319MOE	TGCAGGCTTC ATTTCACAG	485
18-29906LNA	GGCCATTCTG GTGACATCA	367	20-63320MOE	GTGCAGGCTT CATTTCACAC	486
18-29907LNA	TGGCCATTC GGTGACATC	368	20-63321MOE	CGTGCAGGCT TCATTTCAC	487

18-29916LNA	TACAAGCTG TGGCCATTC	369	20-63322MOE	TCGTGCAGGC TTCATTTC	488
18-29920LNA	CTTTTACAA GCTGTGGCC	370	20-63323MOE	TTCGTGCAGG CTTCATTTC	489
18-33523LNA	GACTGAGGT TCTCCTGAT	371	20-63327MOE	CTCATTCGTG CAGGCTTCAT	490
18-33526LNA	TCAGACTGA GGTTCTCCT	372	20-63332MOE	CAAAACTCAT TCGTGCAGGC	491
18-33526SNPELN A (TCAGACTG AGGTTCTCC T ₁ (SEQ ID NO: 372)): T ₁ to C	TCAGACTGA GGTTCTCCC	373	20-63344MOE	GGACAGGAA GCACAAAAC T	492
18-33526SNPML NA (TCAGACTG AGGTTCTCC T ₁ (SEQ ID NO: 372)): G ₁ to C	TCAGACTGA CGTTCTCCT	374	20-63411MOE	TGCATTAATC TTATAAAGCT	493
18-33528LNA	CGTCAGACT GAGGTTCTC	375	20-63450MOE	GCTTGCTCCT TTCTTGGAGG	494
18-33530LNA	GTCGTCAGA CTGAGGTTC	376	20-63451MOE	AGCTTGCTCC TTTCTTGGAG	495
18-33531LNA	TGTCGTCAG ACTGAGGTT	377	20-63452MOE	GAGCTTGCTC CTTTCTTGG	496
18-33538LNA	GCTTCAATG TCGTCAGAC	378	20-63453MOE	TGAGCTTGCT CCTTCTTGG	497
18-33542LNA	GCTAGCTTC AATGTCGTC	379	20-63454MOE	CTGAGCTTGC TCCTTCTTG	498
18-33545LNA	TCGGCTAGC TTCAATGTC	380	20-63455MOE	GCTGAGCTTG CTCCTTCTT	499
18-63262LNA	TTTGCAGCT GCTCGCTTC	381	20-63456MOE	AGCTGAGCTT GCTCCTTCT	500
18-63263LNA	CTTTGCAGC TGCTCGCTT	382	20-63457MOE	AAGCTGAGCT TGCTCCTTTC	501
18-63264LNA	GCTTTGCAG CTGCTCGCT	383	20-63459MOE	GTAAGCTGAG CTTGCTCCTT	502
18-63268LNA	AGATGCTTT GCAGCTGCT	384	20-63460MOE	GGTAAGCTGA GCTTGCTCCT	503
18-63290LNA	CTGGTGGTA GTAGCGTTC	385	20-63461MOE	AGGTAAGCTG AGCTTGCTCC	504
18-63291LNA	ACTGGTGGT AGTAGCGTT	386	20-63462MOE	AAGGTAAGCT GAGCTTGCTC	505

18-63306LNA	CACAGCCCT CAGTTAACT	387	20-63463MOE	CAAGGTAAGC TGAGCTTGCT	506
18-63307LNA	CCACAGCCC TCAGTTAAC	388	20-63464MOE	TCAAGGTAAG CTGAGCTTGC	507
18-63308LNA	TCCACAGCC CTCAGTTAA	389	20-63478MOE	CACCTTTCGA GTTCTCAAGG	508
18-63309LNA	TTCCACAGC CCTCAGTTA	390	20-63479MOE	GCACCTTTCG AGTTCTCAAG	509
18-63320LNA	GCAGGCTTC ATTTCCACA	391	20-67202MOE	AAGTCACATC TAGAAAATCA	510
18-63321LNA	TGCAGGCTT CATTTCCAC	392	20-67248MOE	TCTCTACATA ATTCAAGAAT	511
18-63322LNA	GTGCAGGCT TCATTTCCA	393	20-67296MOE	GAAAAAACTC TTCCAATAAC	512
18-63323LNA	CGTGCAGGC TTCATTTCC	394	20-67319MOE	TCTGTACCAA TGCCCTCAGCA	513
18-63324LNA	TCGTGCAGG CTTCATTTT	395	20-67320MOE	CTCTGTACCA ATGCCTCAGC	514
18-63327LNA	CATTCGTGC AGGCTTCAT	396	20-67321MOE	GCTCTGTACC AATGCCTCAG	515
18-63346LNA	GGACAGGA AGCACAAAA C	397	20-67323MOE	AAGCTCTGTA CCAATGCCTC	516
18-63350LNA	AGTTGGACA GGAAGCACA	398	17-SCRA1LNA	GATTACAGAT TACACAT	563
18-63351LNA	AAGTTGGAC AGGAAGCAC	399	18-SCRA2LNA	CATCATCATC ATCATCAT	564
18-63456LNA	CTGAGCTTG CTCCTTTCT	400	20-SCRA3MOE	ACATCATCAT CATCATCATC	565
18-63457LNA	GCTGAGCTT GCTCCTTTC	401	20-CTRL1MOE	GGATTCAACT GCTGTCCTTG	566
18-63458LNA	AGCTGAGCT TGCTCCTTT	402	20-CTRL2MOE	TGAGCTATCA CCTATCCTTG	567
18-63460LNA	TAAGCTGAG CTTGCTCCT	403			

Table 2. Selected ASOs from Initial Screen

Compound ID	Sequence	SEQ ID NO	%KD	St Dev	%CV
17-63290LNA	TGGTGGTAGTAGCGTTC	329	-74%	0.002	7%
16-63326LNA	TCGTGCAGGCTTCATT	295	-67%	0.001	2%
17-63326LNA	TTCGTGCAGGCTTCATT	342	-65%	0.001	4%
17-63291LNA	CTGGTGGTAGTAGCGTT	330	-65%	0.003	8%
17-29823LNA	TCTGAGCGTAGGCTTAA	307	-65%	0.003	9%
18-435LNA	TCGTTCTCTTTCGCTGCC	359	-64%	0.001	4%
17-63289LNA	GGTGGTAGTAGCGTTCT	328	-64%	0.003	8%

17-63458LNA	GCTGAGCTTGCTCCTTT	351	-63%	0.001	2%
17-29855LNA	AGGTTGTCACACCAGTC	309	-63%	0.008	22%
17-29858LNA	AACAGGTTGTCACACCA	310	-62%	0.001	2%
17-63324LNA	CGTGCAGGCTTCATTTC	340	-61%	0.012	29%
18-63323LNA	CGTGCAGGCTTCATTTC	394	-61%	0.001	4%
17-63264LNA	CTTTGCAGCTGCTCGCT	327	-61%	0.005	12%
18-29854LNA	AGGTTGTCACACCAGTCT	366	-61%	0.005	11%
17-67260LNA	TCCTCTCTTTCTCTACA	356	-60%	0.002	5%
17-63325LNA	TCGTGCAGGCTTCATTT	341	-60%	0.007	15%
18-63458LNA	AGCTGAGCTTGCTCCTTT	402	-60%	0.005	12%
17-63327LNA	ATTCGTGCAGGCTTCAT	343	-58%	0.009	19%

[0273] Analysis of the ASOs described in Table 2 revealed that many of top-performing ASOs clustered in regions of the target *UBE3A* locus, suggesting these regions were highly susceptible to knockdown by antisense mechanisms. These “hot spots” were then used as target sequences for the design of additional ASOs, as described in Example 2.

Example 2: Identification of Hot Spots & Secondary ASO Screen

[0274] The discovery of certain regions in the *UBE3A* target sequence justified design and screening of additional ASOs targeting these hot spots, regions where highly potent ASOs clustered within the *UBE3A* target sequence. As the initial screen did not include all designed ASOs but only a selection closely tiled along the *UBE3A* target sequence for hot spots identification, all the remaining ASOs designed for these hot spot regions were subsequently tested. The additional ASOs were screened for knockdown potency in normal human neurons. Neurons were cultured as in Example 1 and were treated with 3 μ M ASO for 10 days. Results of the secondary screen of hot spot ASOs are provided in Table 3, where *UBE3A* knockdown efficiency is presented as a percentage of *UBE3A* mRNA (“%KD”) measured relative to untreated control neurons (i.e., “-50%” means a 50% reduction relative to untreated controls and “50%” means a 50% increase relative to untreated controls, as an example). Expression levels normalized to untreated controls (“Norm to UT”) are also provided.

Table 3. Hot Spot ASOs – Secondary Screen

Compound ID	Sequence	SEQ ID NO	%K D	Norm to UT
18-63285LNA	GGTAGTAGCGTTCTATTA	550	-82%	0.18
17-29853LNA	GTTGTCACACCAGTCTA	542	-81%	0.19
17-29853LNA	GTTGTCACACCAGTCTA	542	-79%	0.21
17-63286LNA	GGTAGTAGCGTTCTATT	527	-74%	0.26
18-63286LNA	TGGTAGTAGCGTTCTATT	551	-71%	0.29
18-63325LNA	TTCGTGCAGGCTTCATTT	553	-70%	0.30
17-63437LNA	GAGGGATGAGGATCACA	539	-68%	0.32
17-63278LNA	CGTTCTATTAGATGCTT	523	-67%	0.33
17-63288LNA	GTGGTAGTAGCGTTCTA	529	-65%	0.35
17-63287LNA	TGGTAGTAGCGTTCTAT	528	-65%	0.35
18-63282LNA	AGTAGCGTTCTATTAGAT	549	-65%	0.35
17-63278LNA	CGTTCTATTAGATGCTT	523	-64%	0.36
20-432MOE	CGTTCTCTTTCGCTGCCGCC	562	-62%	0.38
18-63437LNA	GGAGGGATGAGGATCACA	555	-62%	0.38
18-63287LNA	GTGGTAGTAGCGTTCTAT	552	-62%	0.38
17-29854LNA	GGTTGTCACACCAGTCT	543	-61%	0.39
17-435LNA	CGTTCTCTTTCGCTGCC	296	-59%	0.41
17-29822LNA	CTGAGCGTAGGCTTAAT	521	-58%	0.42
17-63282LNA	GTAGCGTTCTATTAGAT	525	-57%	0.43
20-CTRL2MOE	TGAGCTATCACCTATCCTTG	567	-57%	0.43
17-29817LNA	CGTAGGCTTAATAACTC	306	-55%	0.45
17-63285LNA	GTAGTAGCGTTCTATTA	526	-55%	0.45
18-63439LNA	TTGGAGGGATGAGGATCA	556	-55%	0.45
17-63343LNA	AGGAAGCACAAAACCTCA	533	-54%	0.46
17-63343LNA	AGGAAGCACAAAACCTCA	533	-53%	0.47
20-CTRL1MOE	GGATTCAACTGCTGTCCTTG	566	-51%	0.49
17-63441LNA	CTTGGAGGGATGAGGAT	540	-49%	0.51
18-63326LNA	ATTTCGTGCAGGCTTCATT	554	-48%	0.52
17-63296LNA	GTAACTGGTGGTAGTA	530	-48%	0.52
18-29859LNA	AAAACAGGTTGTCACACC	544	-48%	0.52
17-63299LNA	TCAGTTAACTGGTGGTA	531	-46%	0.54
17-63346LNA	GACAGGAAGCACAAAAC	534	-44%	0.56
18-63440LNA	CTTGGAGGGATGAGGATC	557	-43%	0.57
18-29838LNA	CTAGCTGCTACCTTGATC	365	-43%	0.57

17-63342LNA	GGAAGCACAAAACATCAT	532	-41%	0.59
17-29821LNA	TGAGCGTAGGCTTAATA	520	-40%	0.60
18-63279LNA	AGCGTTCTATTAGATGCT	548	-40%	0.60
17-29847LNA	ACACCAGTCTAGCTGCT	541	-39%	0.61
18-63274LNA	TCTATTAGATGCTTTGCA	546	-36%	0.64
18-63278LNA	GCGTTCTATTAGATGCTT	547	-36%	0.64
16-480LNA	TGGTCGTAGTCGCCCT	285	-36%	0.64
20-424MOE	TTCGCTGCCGCCACCGCCG G	559	-34%	0.66
20-426MOE	CTTTCGCTGCCGCCACCGCC	561	-32%	0.68
17-29820LNA	GAGCGTAGGCTTAATAA	519	-30%	0.70
20-425MOE	TTTCGCTGCCGCCACCGCC G	560	-26%	0.74
17-63279LNA	GCGTTCTATTAGATGCT	524	-25%	0.75
17-63274LNA	CTATTAGATGCTTTGCA	522	-25%	0.75
17-63402LNA	ATAAAGCTCGAGGGCTT	538	-24%	0.76
17-479LNA	TGGTCGTAGTCGCCCTC	298	-24%	0.76
17-480LNA	CTGGTCGTAGTCGCCCT	299	-22%	0.78
17-480LNA	CTGGTCGTAGTCGCCCT	299	-20%	0.80
18-63447LNA	CTCCTTTCTTGGAGGGAT	558	-20%	0.80
17-29818LNA	GCGTAGGCTTAATAACT	517	-20%	0.80
17-63340LNA	AAGCACAAAACATCATC	344	-19%	0.81
17-29819LNA	AGCGTAGGCTTAATAAC	518	-19%	0.81
17-63399LNA	AAGCTCGAGGGCTTTAA	536	-16%	0.84
17-63398LNA	AGCTCGAGGGCTTTAAT	535	-14%	0.86
18-29860LNA	AAAAACAGGTTGTCACAC	545	-14%	0.86
17-63401LNA	TAAAGCTCGAGGGCTTT	537	-11%	0.89
18-SCRA2LNA	CATCATCATCATCATCAT	564	-3%	0.97

Example 3: Replicate Study Confirms Potency of Top Performing ASOs

[0275] Results from initial and secondary ASO screens were evaluated and an augmented panel of the top 30 ASOs was selected for repeat studies to confirm knockdown potency in normal human neurons. Neurons were cultured as in Example 1 and were treated with 3 μ M ASO for 10 days. Normalized *UBE3A* mRNA levels were determined using multiplex QuantiGene assay. Results are provided in Table 4.

Table 4. Replicate Potency Study Results

Compound ID	Sequence	SEQ ID NO	%KD
18-63285LNA	GGTAGTAGCGTTCTATTA	550	-95%
17-29853LNA	GTTGTCACACCAGTCTA	542	-88%
18-63286LNA	TGGTAGTAGCGTTCTATT	551	-82%

18-435LNA	TCGTTCTCTTTTCGCTGCC	359	-81%
17-63286LNA	GGTAGTAGCGTTCTATT	527	-80%
17-63324LNA	CGTGCAGGCTTCATTTC	340	-78%
18-63323LNA	CGTGCAGGCTTCATTTC	394	-77%
17-63325LNA	TCGTGCAGGCTTCATTT	341	-76%
17-63289LNA	GGTGGTAGTAGCGTTCT	328	-76%
17-63326LNA	TTCGTGCAGGCTTCATT	342	-76%
17-63290LNA	TGGTGGTAGTAGCGTTC	329	-75%
17-67260LNA	TCCTCTCTTTCTCTACA	356	-75%
17-63291LNA	CTGGTGGTAGTAGCGTT	330	-74%
16-63326LNA	TCGTGCAGGCTTCATT	295	-74%
17-63327LNA	ATTCGTGCAGGCTTCAT	343	-73%
17-63458LNA	GCTGAGCTTGCTCCTTT	351	-73%
18-63458LNA	AGCTGAGCTTGCTCCTTT	402	-73%
17-29823LNA	TCTGAGCGTAGGCTTAA	307	-73%
17-29855LNA	AGGTTGTCACACCAGTC	309	-72%
18-29854LNA	AGGTTGTCACACCAGTCT	366	-72%
17-29823LNA	TCTGAGCGTAGGCTTAA	307	-70%
17-29855LNA	AGGTTGTCACACCAGTC	309	-70%
18-63325LNA	TTCGTGCAGGCTTCATTT	553	-65%
17-63287LNA	TGGTAGTAGCGTTCTAT	528	-64%
17-63264LNA	CTTTGCAGCTGCTCGCT	327	-61%
17-63278LNA	CGTTCTATTAGATGCTT	523	-59%
17-63288LNA	GTGGTAGTAGCGTTCTA	529	-56%
17-63437LNA	GAGGGATGAGGATCACA	539	-55%
17-29858LNA	AACAGGTTGTCACACCA	310	-53%
18-63282LNA	AGTAGCGTTCTATTAGAT	549	-51%

[0276] Select ASOs from the replicated potency study shown in Table 4 were carried forward for evaluation of dose response and calculation of half-maximal inhibitory concentration (IC₅₀).

Example 4: Dose Response Studies in Normal Human Neurons

[0277] Dose response studies were carried out to determine IC₅₀ values for select ASOs following the confirmatory potency screen of Example 3. Semi-log dilutions ranging from 30 μ M to 1 nM of select ASOs were applied to normal human neurons (H9) for 10 days (Table 5). *UBE3A* mRNA levels were measured using multiplex QuantiGene assay. IC₅₀ values were calculated by generation of four parameters nonlinear regression curves using GraphPad Prism software. IC₅₀, confidence intervals (“Conf. Int.”) and R-squared (“R-sq.”) analysis are provided in Table 5. Dose response curves for each of the select ASOs are shown in FIG. 1

through FIG. 7.

Table 5. IC50 Analysis of Select ASOs in Human Neurons

Compound ID	Sequence	SEQ ID NO	IC50	Conf. Int.	R-sq.	FIG.
18-63285LNA	GGTAGTAGCGTTCTA TTA	550	37 nM	32 to 43	0.987 6	1
17-29853LNA	GTTGTCACACCAGTC TA	542	52 nM	44 to 61	0.985 5	2
18-63323LNA	CGTGCAGGCTTCATT TCC	394	112 nM	84 to 150	0.961 3	3
17-67260LNA	TCCTCTCTTTCTCTAC A	356	130 nM	109 to 155	0.985 7	4
16-63326LNA	TCGTGCAGGCTTCAT T	295	57 nM	49 to 67	0.987 3	5
17-63458LNA	GCTGAGCTTGCTCCTT T	351	151 nM	128 to 179	0.991 4	6
17-29823LNA	TCTGAGCGTAGGCTT AA	307	105 nM	91 to 121	0.972 1	7

[0278] Based on this analysis, potency of the ASOs was determined and candidates were rank-ordered according to potency.

Example 5: Potent Reduction of Over-Expressed *UBE3A* RNA and Protein in Dup15q-idic Patient iPSC-Derived Neurons

[0279] Patient-derived induced pluripotent stem cells (iPSCs) from a Dup15q patient having idic(15) genotype (Dup1-8), as well as, its engineered isogenic control iPSC line (Dup 1-8-corr), in which supernumerary chromosome containing additional 2 copies of *UBE3A* was removed by CRISPR approach (Elamin et al, 2022), were purchased from the University of Connecticut (UConn, Technology Commercialization Services Office of the Vice President for Research). iPSCs were differentiated into neurons by highly efficient SMAD inhibition-mediated neural induction method using STEMdiff SMADi Neural Induction Kit (Stem Cell Technologies). Relative *UBE3A* genomic DNA levels in iPSC lines were evaluated by qPCR and were found to be approximately 2x control (corrected) levels in Dup15q idic line as expected in view of the tetraploid idic(15) genotype compared to wild type diploid cells (data not shown). Relative *UBE3A* mRNA levels in differentiated neurons were evaluated by multiplex QuantiGene assay and were found to be approximately 2x control levels in Dup15q idic neurons. Due to silencing of paternal allele of *UBE3A* by antisense transcript (ATS) in neurons, the expected level of *UBE3A* mRNA in Dup15q idic compared to isogenic control

neurons is 3:1. Nevertheless, the relationship of copy number to expressed levels seems not to be linear, as concluded from studies in postmortem brain tissue from Dup15q patients (Scoles et al., 2011; Molecular Autism, 2(1)).

[0280] To evaluate effects on *UBE3A* mRNA levels, iPSC-derived Dup15q-idic(15) neurons were cultured in the presence of 3 μ M ASOs for 10 days (triplicates). *UBE3A* mRNA was measured and compared to untreated controls. Results are shown in FIG. 8.

[0281] To evaluate effects on UBE3A protein levels, iPSC-derived Dup15q-idic(15) neurons were cultured in the presence of 3 μ M ASOs for 10 days (triplicates). UBE3A protein was measured (from single well, N=1) and compared to untreated controls. Results are shown in FIG. 9.

[0282] In conclusion, in the context of *UBE3A* over-expression in human iPSC-derived Dup15q-idic(15) neurons, ASOs identified in these studies show potent reduction of *UBE3A* mRNA and protein levels.

Example 6: Dose Response Studies in Dup15q-idic(15) Neurons

[0283] Dose response studies were carried out in Dup15q-idic(15) neurons to determine IC50 values for select ASOs (17-29853LNA and 17-29823LNA). Semi-log dilutions ranging from 30 μ M to 1 nM of each ASO were applied to idic(15) neurons in culture for 10 days. Results, including IC50, confidence intervals ("CI") and R-squared analysis are provided in FIG. 10.

[0284] Dose-dependent UBE3A protein knockdown was also confirmed by Western blot for ASO 17-29823LNA, as shown in FIG. 11, where relative UBE3A protein levels following treatment with semi-log dilutions from 30 μ M to 1 nM of 17-29823LNA are shown.

[0285] These data confirm that, despite over expression of UBE3A, dose dependent target reduction follows similar pattern in Dup15q idic neurons compared to normal human neurons (H9).

Example 7. In Vivo Tolerability Study

[0286] 12 candidate ASOs were selected for in vivo tolerability screening in mice. Of 12 ASOs tested, ASOs according to Compound ID 18-63285LNA, 17-63286LNA, and 17-63458LNA were found to have favorable tolerability profiles.

Example 8. In Vivo Target Engagement

[0287] 18-63285LNA (ASO1), 17-29853LNA (ASO2), 18-63286LNA (ASO3), 17-63286LNA (ASO4), and 17-63458LNA (ASO10) are expected based on nucleotide sequence

to have compatibility with the mouse Ube3a gene. To test target engagement of these ASOs in mice, Ube3a mRNA levels were measured in mouse brain cortex and hippocampus following CNS delivery using Quantigene RNA assay (N=2). As shown in FIG. 12, all ASOs tested show up to 30% Ube3a knockdown in mouse brain regions post single bolus dose.

ANTISENSE COMPOUND SEQUENCES DESCRIBED HEREIN

SEQ ID NO	Start position with respect to SEQ ID NO: 1	Sequence	SEQ ID NO	Start position with respect to SEQ ID NO: 1	Sequence
2	480	TGGTCGTAGTCGCCCT	144	27092	TCGCCAAACTTCTGAG GAGC
3	482	ACTGGTCGTAGTCGCC	145	27093	TTCGCCAAACTTCTGA GGAG
4	29845	CCAGTCTAGCTGCTAC	146	29830	GCTACCTTGATCTGAG CGTA
5	29896	ACATCAGGGTGATCAC	147	29831	TGCTACCTTGATCTGA GCGT
6	29907	GCCATTCGGTGACATC	148	29836	CTAGCTGCTACCTTGA TCTG
7	29916	CAAGCTGTGGCCATTC	149	29838	GTCTAGCTGCTACCTT GATC
8	29917	ACAAGCTGTGGCCATT	150	29840	CAGTCTAGCTGCTACC TTGA
9	31774	GCAAAACAAAAAATA A	151	29841	CCAGTCTAGCTGCTAC CTTG
10	31920	TAAGTACCCCAAGATT	152	29846	TCACACCAGTCTAGCT GCTA
11	63309	CCACAGCCCTCAGTTA	153	29847	GTCACACCAGTCTAGC TGCT
12	63326	TCGTGCAGGCTTCATT	154	29852	AGGTTGTCACACCAGT CTAG
13	435	CGTTCTCTTTCGCTGC C	155	29853	CAGGTTGTCACACCAG TCTA
14	442	ACCGCCTCGTTCTCTT T	156	29854	ACAGGTTGTCACACCA GTCT
15	479	TGGTCGTAGTCGCCCT C	157	29885	GGTGATCACAGCTTTG AGTC
16	480	CTGGTCGTAGTCGCCC T	158	29904	GGCCATTCGGTGACAT CAGG
17	481	ACTGGTCGTAGTCGCC C	159	29905	TGGCCATTCGGTGACA TCAG
18	482	CACTGGTCGTAGTCGC C	160	29906	GTGGCCATTCGGTGAC ATCA

19	27055	CTCTTACAGATTTTAA A	161	29907	TGTGGCCATTCGGTGA CATC
20	27064	AAAATCAGGCTCTTAC A	162	29909	GCTGTGGCCATTCGGT GACA
21	27069	ATTCTAAAATCAGGCT C	163	29910	AGCTGTGGCCATTCGG TGAC
22	27100	ATATTTCGCCAAACTT C	164	29911	AAGCTGTGGCCATTCG GTGA
23	29817	CGTAGGCTTAATAACT C	165	29912	CAAGCTGTGGCCATTC GGTG
24	29823	TCTGAGCGTAGGCTTA A	166	29915	TTACAAGCTGTGGCCA TTCG
25	29839	CTAGCTGCTACCTTGA T	167	33523	CAGACTGAGGTTCTCC TGAT
26	29855	AGGTTGTCACACCAGT C	168	33524	TCAGACTGAGGTTCTC CTGA
27	29858	AACAGGTTGTCACACC A	169	33525	GTCAGACTGAGGTTCT CCTG
28	29859	AAACAGGTTGTCACAC C	170	33526	CGTCAGACTGAGGTTC TCCT
29	29879	AGCTTTGAGTCACTGA T	171	33528	GTCGTCAGACTGAGGT TCTC
30	29906	GCCATTCGGTGACATC A	172	33529	TGTCGTCAGACTGAGG TTCT
31	29907	GGCCATTCGGTGACAT C	173	33530	ATGTCGTCAGACTGAG GTTC
32	29916	ACAAGCTGTGGCCATT C	174	33532	CAATGTCGTCAGACTG AGGT
33	29917	TACAAGCTGTGGCCAT T	175	33536	GCTTCAATGTCGTCAG ACTG
34	29918	TTACAAGCTGTGGCCA T	176	33540	GCTAGCTTCAATGTCG TCAG
35	29919	TTTACAAGCTGTGGCC A	177	33541	GGCTAGCTTCAATGTC GTCA
36	29920	TTTTACAAGCTGTGGC C	178	33542	CGGCTAGCTTCAATGT CGTC
37	33529	CGTCAGACTGAGGTTC T	179	33543	TCGGCTAGCTTCAATG TCGT
38	33531	GTCGTCAGACTGAGGT T	180	33544	TTCGGCTAGCTTCAAT GTCG
39	33541	TAGCTTCAATGTCGTC A	181	63263	TGCTTTGCAGCTGCTC GCTT
40	33545	CGGCTAGCTTCAATGT C	182	63264	ATGCTTTGCAGCTGCT CGCT
41	33548	ATTCGGCTAGCTTCAA T	183	63265	GATGCTTTGCAGCTGC TCGC
42	33549	CATTCGGCTAGCTTCA A	184	63266	AGATGCTTTGCAGCTG CTCG

43	63262	TTGCAGCTGCTCGCTT C	185	63288	CTGGTGGTAGTAGCGT TCTA
44	63264	CTTTGCAGCTGCTCGC T	186	63289	ACTGGTGGTAGTAGCG TTCT
45	63289	GGTGGTAGTAGCGTTC T	187	63290	AACTGGTGGTAGTAGC GTTC
46	63290	TGGTGGTAGTAGCGTT C	188	63293	GTAACTGGTGGTAGT AGCG
47	63291	CTGGTGGTAGTAGCGT T	189	63301	AGCCCTCAGTTAACTG GTGG
48	63306	ACAGCCCTCAGTTAAC T	190	63302	CAGCCCTCAGTTAACT GGTG
49	63306S NPE	ACAGCCCTCAGTTAAC A	191	63303	ACAGCCCTCAGTTAAC TGGT
50	63306S NPM	ACAGCCCTAAGTTAAC T	192	63304	CACAGCCCTCAGTTAA CTGG
51	63307	CACAGCCCTCAGTTAA C	193	63305	CCACAGCCCTCAGTTA ACTG
52	63308	CCACAGCCCTCAGTTA A	194	63306	TCCACAGCCCTCAGTT AACT
53	63309	TCCACAGCCCTCAGTT A	195	63307	TTCCACAGCCCTCAGT TAAC
54	63320	CAGGCTTCATTTCAC A	196	63307S NPE	TTCCACAGCCCTCAGT TGAC
55	63321	GCAGGCTTCATTTCAC C	197	63307S NPM	TTCCACAGCGCTCAGT TAAC
56	63323	GTGCAGGCTTCATTTC C	198	63310	CATTTCACAGCCCTC AGTT
57	63324	CGTGCAGGCTTCATT C	199	63311	TCATTTCACAGCCCT CAGT
58	63325	TCGTGCAGGCTTCATT T	200	63317	CAGGCTTCATTTCAC AGCC
59	63326	TTCGTGCAGGCTTCAT T	201	63318	GCAGGCTTCATTTCAC CAGC
60	63327	ATTCGTGCAGGCTTCAT T	202	63319	TGCAGGCTTCATTTC ACAG
61	63340	AAGCACAAAACATCATT C	203	63320	GTGCAGGCTTCATTTC CACA
62	63348	TGGACAGGAAGCACAA AA	204	63321	CGTGCAGGCTTCATT CCAC
63	63351	AGTTGGACAGGAAGC AC	205	63322	TCGTGCAGGCTTCATT TCCA
64	63352	AAGTTGGACAGGAAG CA	206	63323	TTCGTGCAGGCTTCAT TTCC
65	63364	CCATACGAAGAAAAG TT	207	63327	CTCATTCGTGCAGGCT TCAT
66	63410	TTAATCTTATAAAGCT C	208	63332	CAAACTCATTCGTGC AGGC

67	63457	CTGAGCTTGCTCCTTT C	209	63344	GGACAGGAAGCACAA AACTC
68	63458	GCTGAGCTTGCTCCTT T	210	63411	TGCATTAATCTTATAA AGCT
69	63463	GGTAAGCTGAGCTTGC T	211	63450	GCTTGCTCCTTTCTTG GAGG
70	63467	TCAAGGTAAGCTGAG CT	212	63451	AGCTTGCTCCTTTCTT GGAG
71	67212	GTTAAGTAAGTCACAT C	213	63452	GAGCTTGCTCCTTTCT TGGA
72	67242	AATTCAAGAATTTTCAT A	214	63453	TGAGCTTGCTCCTTTC TTGG
73	67260	TCCTCTCTTTCTCTACA	215	63454	CTGAGCTTGCTCCTTT CTTG
74	67307	CAGCACTAGAAAAAA CT	216	63455	GCTGAGCTTGCTCCTT TCTT
75	67312	TGCCTCAGCACTAGAA A	217	63456	AGCTGAGCTTGCTCCT TTCT
76	435	TCGTTCTCTTTCGCTG CC	218	63457	AAGCTGAGCTTGCTCC TTTC
77	438	GCCTCGTTCTCTTTCG CT	219	63459	GTAAGCTGAGCTTGCT CCTT
78	480	ACTGGTCGTAGTCGCC CT	220	63460	GGTAAGCTGAGCTTGC TCCT
79	482	TCACTGGTCGTAGTCG CC	221	63461	AGGTAAGCTGAGCTTG CTCC
80	861	CCTGTCTGGGGCCCCG GC	222	63462	AAGGTAAGCTGAGCTT GCTC
81	27091	CCAAACTTCTGAGGAG CT	223	63463	CAAGGTAAGCTGAGCT TGCT
82	29838	CTAGCTGCTACCTTGA TC	224	63464	TCAAGGTAAGCTGAGC TTGC
83	29854	AGGTTGTCACACCAGT CT	225	63478	CACCTTTCGAGTTCTC AAGG
84	29906	GGCCATTTCGGTGACAT CA	226	63479	GCACCTTTCGAGTTCT CAAG
85	29907	TGGCCATTTCGGTGACA TC	227	67202	AAGTCACATCTAGAAA ATCA
86	29916	TACAAGCTGTGGCCAT TC	228	67248	TCTCTACATAATTCAA GAAT
87	29920	CTTTTACAAGCTGTGG CC	229	67296	GAAAAAACTCTTCCAA TAAC
88	33523	GACTGAGGTTCTCCTG AT	230	67319	TCTGTACCAATGCCTC AGCA
89	33526	TCAGACTGAGGTTCTC CT	231	67320	CTCTGTACCAATGCCT CAGC
90	33526S NPE	TCAGACTGAGGTTCTC CC	232	67321	GCTCTGTACCAATGCC TCAG

91	33526S NPM	TCAGACTGACGTTCTC CT	233	67323	AAGCTCTGTACCAATG CCTC
92	33528	CGTCAGACTGAGGTTC TC	234	29818	GCGTAGGCTTAATAAC T
93	33530	GTCGTCAGACTGAGGT TC	235	29819	AGCGTAGGCTTAATAA C
94	33531	TGTCGTCAGACTGAGG TT	236	29820	GAGCGTAGGCTTAATA A
95	33538	GCTTCAATGTCGTCAG AC	237	29821	TGAGCGTAGGCTTAAT A
96	33542	GCTAGCTTCAATGTCG TC	238	29822	CTGAGCGTAGGCTTAA T
97	33545	TCGGCTAGCTTCAATG TC	239	63274	CTATTAGATGCTTTGC A
98	63262	TTTGCAGCTGCTCGCT TC	240	63278	CGTTCTATTAGATGCT T
99	63263	CTTTGCAGCTGCTCGC TT	241	63279	GCGTTCTATTAGATGC T
100	63264	GCTTTGCAGCTGCTCG CT	242	63282	GTAGCGTTCTATTAGA T
101	63268	AGATGCTTTGCAGCTG CT	243	63285	GTAGTAGCGTTCTATT A
102	63290	CTGGTGGTAGTAGCGT TC	244	63286	GGTAGTAGCGTTCTAT T
103	63291	ACTGGTGGTAGTAGCG TT	245	63287	TGGTAGTAGCGTTCTA T
104	63306	CACAGCCCTCAGTTAA CT	246	63288	GTGGTAGTAGCGTTCT A
105	63307	CCACAGCCCTCAGTTA AC	247	63296	GTAACTGGTGGTAGT A
106	63308	TCCACAGCCCTCAGTT AA	248	63299	TCAGTTAACTGGTGGT A
107	63309	TTCCACAGCCCTCAGT TA	249	63342	GGAAGCACAAAACCTC AT
108	63320	GCAGGCTTCATTTCCA CA	250	63343	AGGAAGCACAAAACCT CA
109	63321	TGCAGGCTTCATTTCC AC	251	63346	GACAGGAAGCACAAA AC
110	63322	GTGCAGGCTTCATTT CA	252	63398	AGCTCGAGGGCTTTAA T
111	63323	CGTGCAGGCTTCATTT CC	253	63399	AAGCTCGAGGGCTTTA A
112	63324	TCGTGCAGGCTTCATT TC	254	63401	TAAAGCTCGAGGGCTT T
113	63327	CATTCGTGCAGGCTTC AT	255	63402	ATAAAGCTCGAGGGCT T
114	63346	GGACAGGAAGCACAA AAC	256	63437	GAGGGATGAGGATCA CA

115	63350	AGTTGGACAGGAAGC ACA	257	63441	CTTGGAGGGATGAGG AT
116	63351	AAGTTGGACAGGAAG CAC	258	29847	ACACCAGTCTAGCTGC T
117	63456	CTGAGCTTGCTCCTTT CT	259	29853	GTTGTCACACCAGTCT A
118	63457	GCTGAGCTTGCTCCTT TC	260	29854	GGTTGTCACACCAGTC T
119	63458	AGCTGAGCTTGCTCCT TT	261	29859	AAAACAGGTTGTCACA CC
120	63460	TAAGCTGAGCTTGCTC CT	262	29860	AAAAACAGGTTGTCAC AC
121	63462	GGTAAGCTGAGCTTGC TC	263	63274	TCTATTAGATGCTTTG CA
122	63463	AGGTAAGCTGAGCTTG CT	264	63278	GCGTTCTATTAGATGC TT
123	64667	CCCCCGCAGCTGGCTG CA	265	63279	AGCGTTCTATTAGATG CT
124	64668	TCCCCCGCAGCTGGCT GC	266	63282	AGTAGCGTTCTATTAG AT
125	431	GTTCTCTTTCGCTGCC GCCA	267	63285	GGTAGTAGCGTTCTAT TA
126	433	TCGTTCTCTTTCGCTG CCGC	268	63286	TGGTAGTAGCGTTCTA TT
127	434	CTCGTTCTCTTTCGCT GCCG	269	63287	GTGGTAGTAGCGTTCT AT
128	436	GCCTCGTTCTCTTTCG CTGC	270	63325	TTCGTGCAGGCTTCAT TT
129	437	CGCCTCGTTCTCTTTC GCTG	271	63326	ATTCGTGCAGGCTTCA TT
130	439	ACCGCCTCGTTCTCTT TCGC	272	63437	GGAGGGATGAGGATC ACA
131	440	CACCGCCTCGTTCTCT TTCG	273	63439	TTGGAGGGATGAGGA TCA
132	441	CCACCGCCTCGTTCTC TTTC	274	63440	CTTGGAGGGATGAGG ATC
133	442	GCCACCGCCTCGTTCT CTTT	275	63447	CTCCTTTCTTGGAGGG AT
134	480	TCACTGGTCGTAGTCG CCCT	276	424	TTCGCTGCCGCCACCG CCGG
135	27066	ATTCTAAAATCAGGCT CTTA	277	425	TTTCGCTGCCGCCACC GCCG
136	27073	CTGGTGAATTCTAAAA TCAG	278	426	CTTTCGCTGCCGCCAC CGCC
137	27084	CTTCTGAGGAGCTGGT GAAT	279	432	CGTTCTCTTTCGCTGC CGCC
138	27085	ACTTCTGAGGAGCTGG TGAA	280	SCRA1	GATTACAGATTACACA T

139	27086	AACTTCTGAGGAGCTG GTGA	281	SCRA2	CATCATCATCATCATC AT
140	27087	AAACTTCTGAGGAGCT GGTG	282	SCRA3	ACATCATCATCATCAT CATC
141	27088	CAAACCTTCTGAGGAGC TGGT	283	CTRL1	GGATTCAACTGCTGTC CTTG
142	27090	GCCAAACTTCTGAGGA GCTG	284	CTRL2	TGAGCTATCACCTATC CTTG
143	27091	CGCCAAACTTCTGAGG AGCT			

CLAIMS

WHAT IS CLAIMED IS:

1. An antisense oligonucleotide (ASO) comprising at least 16 contiguous nucleosides and having a nucleotide sequence selected from SEQ ID NO: 267 (GGTAGTAGCGTTCTATTA), SEQ ID NO: 244 (GGTAGTAGCGTTCTATT), SEQ ID NO: 68 (GCTGAGCTTGCTCCTTT), SEQ ID NO: 111 (CGTGCAGGCTTCATTTC), SEQ ID NO: 268 (TGGTAGTAGCGTTCTATT), SEQ ID NO: 259 (GTTGTCACACCAGTCTA), SEQ ID NO: 58 (TCGTGCAGGCTTCATTT), SEQ ID NO: 57 (CGTGCAGGCTTCATTTC), SEQ ID NO: 12 (TCGTGCAGGCTTCATT), SEQ ID NO: 73 (TCCTCTCTTTCTCTACA), SEQ ID NO: 46 (TGGTGGTAGTAGCGTTC), and SEQ ID NO: 47 (CTGGTGGTAGTAGCGTT), and wherein the ASO comprises one or more of:
 - a) a gap segment consisting of linked deoxynucleosides;
 - b) a 5' segment consisting of at least 2 linked nucleosides;
 - c) a 3' segment consisting of at least 2 linked nucleosides;
 - d) at least one phosphorothioate internucleoside linkage;
 - e) at least one nucleoside comprising a modified sugar; and
 - f) at least one nucleoside comprising a modified nucleobase.
2. The ASO of claim 1, wherein the gap segment is positioned between the 5' segment and the 3' segment.
3. The ASO of claim 1 or 2, wherein the gap segment comprises 5 to 15 linked nucleosides.
4. The ASO of any one of claims 1-3, wherein the 3' segment comprises 2-5 linked nucleosides.
5. The ASO of any one of claims 1-4, wherein the 5' segment comprises 2-5 linked nucleosides.
6. The ASO of any one of claims 1-5, wherein at least one nucleoside of the 5' segment and at least one nucleoside of the 3' segment comprises a modified sugar.

7. The ASO of any one of claims 1-6, wherein each nucleoside of the 5' segment and each nucleoside of the 3' segment comprises a modified sugar.
8. The ASO of any one of claims 6 or 7, wherein the modified sugar comprises a bicyclic sugar.
9. The ASO of claim 8, wherein the bicyclic sugar is selected from the group consisting of: 2'-O(CH₂)₂OCH₃ (MOE); 4'-(CH₂)—O-2' (LNA); 4'-(CH₂)₂—O-2' (ENA); and 4'-CH(CH₃)—O-2' (cEt).
10. The ASO of any one of claims 1-9, wherein each internucleoside linkage is a phosphorothioate internucleoside linkage.
11. The ASO of any one of claims 1-10, comprising a 5-methylcytosine nucleobase in place of a non-5-methyl cytosine residue.
12. The ASO of any one of claims 1-11, comprising a 3-10-3 LNA-DNA-LNA gapmer, wherein all internucleoside linkages are phosphorothioate internucleoside linkages.
13. The ASO of any one of claims 1-11, comprising a 3-11-3 LNA-DNA-LNA gapmer, wherein all internucleoside linkages are phosphorothioate internucleoside linkages.
14. The ASO of any one of claims 1-11, comprising a 3-12-3 LNA-DNA-LNA gapmer, wherein all internucleoside linkages are phosphorothioate internucleoside linkages.
15. The ASO of any one of claims 1-11, comprising a 4-11-5 MOE-DNA-MOE gapmer, wherein all internucleoside linkages are phosphorothioate internucleoside linkages.
16. An antisense oligonucleotide (ASO), wherein the ASO is a gapmer according to Compound ID No. 16-63326LNA, 17-29823LNA, 17-29855LNA, 17-29858LNA, 17-63264LNA, 17-63289LNA, 17-63290LNA, 17-63291LNA, 17-63324LNA, 17-63325LNA, 17-63326LNA, 17-63327LNA, 17-63458LNA, 17-67260LNA, 18-435LNA, 18-29854LNA, 18-63323LNA, 18-63458LNA, 17-63278LNA, 17-63286LNA, 17-63287LNA, 17-63288LNA, 17-63437LNA, 17-29853LNA, 18-63282LNA, 18-63285LNA, 18-63286LNA, or 18-63325LNA.

17. An antisense oligonucleotide (ASO) comprising: a 5' segment of 3 linked locked nucleic acid (LNA) nucleosides, a gap segment of linked deoxynucleosides, and a 3' segment of 3 linked LNA nucleosides, wherein all internucleoside linkages are phosphorothioate internucleoside linkages, and wherein ASO comprises the nucleotide sequence of SEQ ID NO: 550.
18. An antisense oligonucleotide (ASO) comprising: a 5' segment of 3 linked locked nucleic acid (LNA) nucleosides, a gap segment of linked deoxynucleosides, and a 3' segment of 3 linked LNA nucleosides, wherein all internucleoside linkages are phosphorothioate internucleoside linkages, and wherein ASO comprises the nucleotide sequence of SEQ ID NO: 542.
19. An antisense oligonucleotide (ASO) comprising: a 5' segment of 3 linked locked nucleic acid (LNA) nucleosides, a gap segment of linked deoxynucleosides, and a 3' segment of 3 linked LNA nucleosides, wherein all internucleoside linkages are phosphorothioate internucleoside linkages, and wherein ASO comprises the nucleotide sequence of SEQ ID NO: 394.
20. An antisense oligonucleotide (ASO) comprising: a 5' segment of 3 linked locked nucleic acid (LNA) nucleosides, a gap segment of linked deoxynucleosides, and a 3' segment of 3 linked LNA nucleosides, wherein all internucleoside linkages are phosphorothioate internucleoside linkages, and wherein ASO comprises the nucleotide sequence of SEQ ID NO: 328.
21. An antisense oligonucleotide (ASO) comprising: a 5' segment of 3 linked locked nucleic acid (LNA) nucleosides, a gap segment of linked deoxynucleosides, and a 3' segment of 3 linked LNA nucleosides, wherein all internucleoside linkages are phosphorothioate internucleoside linkages, and wherein ASO comprises the nucleotide sequence of SEQ ID NO: 342.
22. An antisense oligonucleotide (ASO) comprising: a 5' segment of 3 linked locked nucleic acid (LNA) nucleosides, a gap segment of linked deoxynucleosides, and a 3' segment of 3 linked LNA nucleosides, wherein all internucleoside linkages are phosphorothioate internucleoside linkages, and wherein ASO comprises the nucleotide sequence of SEQ ID NO: 356.

23. An antisense oligonucleotide (ASO) comprising: a 5' segment of 3 linked locked nucleic acid (LNA) nucleosides, a gap segment of linked deoxynucleosides, and a 3' segment of 3 linked LNA nucleosides, wherein all internucleoside linkages are phosphorothioate internucleoside linkages, and wherein ASO comprises the nucleotide sequence of SEQ ID NO: 295.
24. An antisense oligonucleotide (ASO) comprising: a 5' segment of 3 linked locked nucleic acid (LNA) nucleosides, a gap segment of linked deoxynucleosides, and a 3' segment of 3 linked LNA nucleosides, wherein all internucleoside linkages are phosphorothioate internucleoside linkages, and wherein ASO comprises the nucleotide sequence of SEQ ID NO: 351.
25. An antisense oligonucleotide (ASO) comprising: a 5' segment of 3 linked locked nucleic acid (LNA) nucleosides, a gap segment of linked deoxynucleosides, and a 3' segment of 3 linked LNA nucleosides, wherein all internucleoside linkages are phosphorothioate internucleoside linkages, and wherein ASO comprises the nucleotide sequence of SEQ ID NO: 307.
26. The ASO of any one of claims 1-25, wherein all cytosine nucleosides are replaced with 5-methylcytosine nucleosides.
27. The ASO of any one of claims 1-26, comprising a 2'OMe-modified nucleoside at position 2 in the DNA gap.
28. A composition comprising the ASO of any one of claims 1-27 or salt thereof and a pharmaceutically acceptable carrier.
29. The composition of claim 28, wherein the composition is a pharmaceutical formulation.
30. A method of reducing the level of a *UBE3A* RNA in a cell, comprising contacting the cell with the ASO of any one of claims 1-28 or the composition of claim 28 or 29, thereby reducing the level of the *UBE3A* RNA in the cell.
31. A method of inhibiting expression of UBE3A protein in a cell, comprising contacting the cell with the ASO of any one of claims 1-27 or the composition of claim 28 or 29, thereby reducing expression of UBE3A protein in the cell.

32. The method of claim 30 or 31, wherein the cell is a central nervous system neuron.
33. The method of any one of claims 30-32, wherein the cell is triploid or tetraploid for the *UBE3A* gene.
34. The method of any one of claims 30-33, wherein the cell comprises a maternal duplication of the *UBE3A* allele.
35. The method of any one of claims 30-34, wherein the cell is in vitro.
36. The method of any one of claims 30-34, wherein the cell is in a subject.
37. The method of claim 36, wherein the subject is a human and the ASO or composition is administered to the subject.
38. The method of claim 37, wherein the ASO is administered to the subject via intravenous, subcutaneous, intramuscular, intraperitoneal, intrathecal, intra cisterna magna, or intraventricular (intracerebroventricular) injection.
39. A method of treating, preventing, or ameliorating a disease associated with overexpression of *UBE3A* in central nervous system neurons in a subject, comprising administering to the subject an ASO of any one of claims 1-27 or the composition of claim 28 or 29, thereby treating, preventing, or ameliorating the disease.
40. The method of claim 39, wherein the disease is Dup15q syndrome.
41. The method of claim 39 or 40, wherein the subject has an idic(15) genotype.
42. The method of any one of claims 36-41, comprising administering to the subject a second pharmaceutical agent for treating, preventing, or ameliorating one or more symptoms of Dup15q syndrome.
43. An ASO according to any one of claims 1-27 or the composition of claim 28 or 29 for use in a method of treating, preventing, or ameliorating a disease associated with overexpression of *UBE3A* in central nervous system neurons in a subject.
44. The ASO for use of claim 43, wherein the disease is Dup15q syndrome.
45. The ASO for use of claim 43 or 44, wherein the subject has an idic(15) genotype.

46. Use of an ASO of any one of claims 1-27 or the composition of claim 28 or 29 for the treatment of a disease associated with increased levels of UBE3A protein in central nervous system neurons in a subject.
47. Use of an ASO of any one of claims 1-27 or the composition of claim 28 or 29 for the preparation of a medicament for the treatment of a disease associated with increased levels of UBE3A protein in central nervous system neurons of a subject.
48. The use of claim 46 or 47, wherein the disease is Dup15q syndrome.
49. The use of any one of claims 46-48, wherein the subject has an idic(15) genotype.