

ANTISENSE OLIGOMERS FOR TREATMENT OF CONDITIONS AND DISEASES**CROSS-REFERENCE**

[0001] This application claims the benefit of U.S. Provisional Application No. 62/811,511, filed on February 27, 2019 which is incorporated herein by reference in its entirety.

BACKGROUND

[0002] Nervous system disorders are often associated with channelopathy, characterized by the disturbed function of ion channels that mediate neuronal excitability, neuronal interactions, and brain functions at large. Mutations in the SCN1A gene, which is part of the SCN1A-SCN2A-SCN3A gene cluster that encodes alpha-pore forming subunits of the neuronal voltage gated sodium channel, are associated with development of disease number of diseases and conditions, such as Dravet Syndrome (DS) (Miller, et al., 1993-2015, GeneReviews, Eds. Pagon RA, et al. Seattle (WA): University of Washington, Seattle, Bookshelf ID: NBK1318, and Mulley, et al., 2005, Hum. Mutat. 25: 535-542).

SUMMARY

[0003] Disclosed herein, in certain embodiments, is a method of modulating expression of SCN1A protein in a cell having an mRNA that contains a non-sense mediated RNA decay-inducing exon (NMD exon mRNA) and encodes SCN1A protein, the method comprising contacting a therapeutic agent to the cell, whereby the therapeutic agent modulates splicing of the NMD exon from the NMD exon mRNA encoding SCN1A protein, thereby modulating the level of processed mRNA encoding SCN1A protein, and modulating expression of SCN1A protein in the cell, wherein the therapeutic agent binds to a targeted portion of the NMD exon mRNA encoding SCN1A, and wherein the targeted portion is: from about 1000 nucleotides upstream from the 5' end of an NMD-inducing exon (NIE) to about 100 nucleotides upstream from the 5' end of the NIE; or from about 100 nucleotides downstream of the 3' end of the NIE to about 1000 nucleotides downstream of the 3' end of the NIE. In some embodiments, the therapeutic agent interferes with binding of a factor involved in splicing of the NMD exon from a region of the targeted portion. In some embodiments, the targeted portion is at most about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides upstream of 5' end of the NIE. In some embodiments, the targeted portion is at least about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides,

about 60 nucleotides, about 50 nucleotides, about 40 nucleotides, about 30 nucleotides, about 20 nucleotides, about 10 nucleotides, about 5 nucleotides, about 4 nucleotides, about 2 nucleotides, about 1 nucleotides upstream of 5' end of the NIE. In some embodiments, the targeted portion is at most about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides downstream of 3' end of the NIE. In some embodiments, the targeted portion is at least about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides, about 40 nucleotides, about 30 nucleotides, about 20 nucleotides, about 10 nucleotides, about 5 nucleotides, about 4 nucleotides, about 2 nucleotides, about 1 nucleotides downstream of 3' end of the NIE. In some embodiments, the therapeutic agent is an antisense oligomer (ASO). In some embodiments, the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% identity to any one of SEQ ID NOs: 12-731. In some embodiments, the therapeutic agent promotes exclusion of the NMD exon from the processed mRNA encoding SCN1A protein. In some embodiments, exclusion of the NMD exon from the processed mRNA encoding SCN1A protein in the cell contacted with the therapeutic agent is increased about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to exclusion of the NMD exon from the processed mRNA encoding SCN1A protein in a control cell. In some embodiments, the therapeutic agent increases level of the processed mRNA encoding SCN1A protein in the cell. In some embodiments, an amount of the processed mRNA encoding SCN1A protein in the cell contacted with the therapeutic agent is increased about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least

about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to an total amount of the processed mRNA encoding SCN1A protein in a control cell.

[0004] Disclosed herein, in certain embodiments, is a method of treating a disease or condition in a subject in need thereof by modulating expression of SCN1A protein in a cell of the subject, comprising: contacting the cell of the subject with a therapeutic agent that modulates splicing of a non-sense mediated mRNA decay-inducing exon (NMD exon) from an mRNA in the cell that contains the NMD exon and encodes SCN1A, thereby modulating the level of processed mRNA encoding the SCN1A protein, and modulating expression of SCN1A protein in the cell of the subject; wherein the therapeutic agent binds to a targeted portion of the NMD exon mRNA encoding SCN1A, and wherein the targeted portion is: from about 1000 nucleotides upstream from the 5' end of an NMD-inducing exon (NIE) to about 100 nucleotides upstream from the 5' end of the NIE; or from about 100 nucleotides downstream of the 3' end of the NIE to about 1000 nucleotides downstream of the 3' end of the NIE. In some embodiments, the therapeutic agent interferes with binding of a factor involved in splicing of the NMD exon from a region of the targeted portion. In some embodiments, the targeted portion is at most about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides upstream of 5' end of the NIE. In some embodiments, the targeted portion is at least about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides, about 40 nucleotides, about 30 nucleotides, about 20 nucleotides, about 10 nucleotides, about 5 nucleotides, about 4 nucleotides, about 2 nucleotides, about 1 nucleotides upstream of 5' end of the NIE. In some embodiments, the targeted portion is at most about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides downstream of 3' end of the NIE. In some embodiments, the targeted portion is at least about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides, about 40 nucleotides, about 30 nucleotides, about 20 nucleotides, about 10 nucleotides, about 5 nucleotides, about 4 nucleotides, about 2 nucleotides, about 1 nucleotides downstream of 3' end of the NIE. In some embodiments, the therapeutic agent is an antisense oligomer (ASO). In some embodiments, the ASO comprises a sequence that is at least about

80%, 85%, 90%, 95%, 97%, or 100% identity to any one of SEQ ID NOs: 12-731. In some embodiments, the therapeutic agent promotes exclusion of the NMD exon from the processed mRNA encoding SCN1A protein. In some embodiments, exclusion of the NMD exon from the processed mRNA encoding SCN1A protein in the cell contacted with the therapeutic agent is increased about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to exclusion of the NMD exon from the processed mRNA encoding SCN1A protein in a control cell. In some embodiments, the therapeutic agent increases level of the processed mRNA encoding SCN1A protein in the cell. In some embodiments, an amount of the processed mRNA encoding SCN1A protein in the cell contacted with the therapeutic agent is increased about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to an total amount of the processed mRNA encoding SCN1A protein in a control cell. In some embodiments, the disease or condition is induced by a loss-of-function mutation in *Na_v1.1*. In some embodiments, the disease or condition is associated with haploinsufficiency of the *SCN1A* gene, and wherein the subject has a first allele encoding a functional SCN1A, and a second allele from which SCN1A is not produced or produced at a reduced level, or a second allele encoding a nonfunctional SCN1A or a partially functional SCN1A. In some embodiments, the disease or condition is encephalopathy. In some embodiments, the encephalopathy is epileptic encephalopathy. In some embodiments, the disease or condition is Dravet Syndrome (DS); severe myoclonic epilepsy of infancy (SMEI)-borderland (SMEB); Febrile seizure (FS); epilepsy, generalized, with febrile seizures plus (GEFS+); epileptic encephalopathy, early infantile, 13; cryptogenic generalized epilepsy; cryptogenic focal epilepsy; myoclonic-astatic epilepsy; Lennox-Gastaut syndrome; West syndrome; idiopathic spasms; early myoclonic encephalopathy; progressive

myoclonic epilepsy; alternating hemiplegia of childhood; unclassified epileptic encephalopathy; sudden unexpected death in epilepsy (SUDEP); sick sinus syndrome 1; autism; or malignant migrating partial seizures of infancy. In some embodiments, GEFS+ is epilepsy, generalized, with febrile seizures plus, type 2. In some embodiments, the Febrile seizure is Febrile seizures, familial, 3A. In some embodiments, SMEB is SMEB without generalized spike wave (SMEB-SW), SMEB without myoclonic seizures (SMEB-M), SMEB lacking more than one feature of SMEI (SMEB-O), or intractable childhood epilepsy with generalized tonic-clonic seizures (ICEGTC).

[0005] Disclosed herein, in certain embodiments, is a method of modulating expression of SCN1A protein in a cell having an mRNA that contains a non-sense mediated RNA decay-inducing exon (NMD exon mRNA) and encodes SCN1A protein, the method comprising contacting a therapeutic agent to the cell, whereby the therapeutic agent modulates splicing of the NMD exon from the NMD exon mRNA encoding SCN1A protein, thereby modulating the level of processed mRNA encoding SCN1A protein, and modulating expression of SCN1A protein in the cell, wherein the therapeutic agent binds to a targeted portion of the NMD exon mRNA encoding SCN1A, and wherein the targeted portion is from about 1000 nucleotides upstream from the 5' end of an NMD-inducing exon (NIE) to about 1000 nucleotides downstream of the 3' end of the NIE.

[0006] Disclosed herein, in certain embodiments, is a method of treating a disease or condition in a subject in need thereof by modulating expression of SCN1A protein in a cell of the subject, comprising: contacting the cell of the subject with a therapeutic agent that modulates splicing of a non-sense mediated mRNA decay-inducing exon (NMD exon) from an mRNA in the cell that contains the NMD exon and encodes SCN1A, thereby modulating the level of processed mRNA encoding the SCN1A protein, and modulating expression of SCN1A protein in the cell of the subject; wherein the therapeutic agent binds to a targeted portion of the NMD exon mRNA encoding SCN1A, and wherein the targeted portion is from about 1000 nucleotides upstream from the 5' end of an NMD-inducing exon (NIE) to about 1000 nucleotides downstream of the 3' end of the NIE.

[0007] Disclosed herein, in certain embodiments, is an antisense oligomer (ASO) comprising a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% identity to any one of SEQ ID NOs: 12-731.

[0008] Disclosed herein, in certain embodiments, is an antisense oligomer (ASO) consisting of a sequence selected from SEQ ID NOs: 12-731.

[0009] Disclosed herein, in certain embodiments, is a method of treating a disease or condition in a subject in need thereof by modulating expression of SCN1A protein in a cell of the subject, comprising: contacting the cell of the subject with an ASO comprising a sequence that is at least about 80%, 85%,

90%, 95%, 97%, or 100% identity to any one of SEQ ID NOs: 12-731; or an ASO consisting of a sequence selected from SEQ ID NOs: 12-731.

[0010] Disclosed herein, in certain embodiments, is a kit comprising an ASO comprising a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% identity to any one of SEQ ID NOs: 12-731; or an ASO consisting of a sequence selected from SEQ ID NOs: 12-731.

INCORPORATION BY REFERENCE

[0011] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0013] **FIG. 1** depicts a schematic representation of a target mRNA that contains a non-sense mediated RNA decay-inducing exon (NMD exon mRNA) and therapeutic agent-mediated exclusion of the nonsense-mediated mRNA decay-inducing exon to increase expression of the full-length target protein or functional RNA. **FIG. 1A** shows a cell divided into nuclear and cytoplasmic compartments. In the nucleus, a pre-mRNA transcript of a target gene undergoes splicing to generate mRNA, and this mRNA is exported to the cytoplasm and translated into target protein. For this target gene, some fraction of the mRNA contains a nonsense-mediated mRNA decay-inducing exon (NMD exon mRNA) that is degraded in the cytoplasm, thus leading to no target protein production. **FIG. 1B** shows an example of the same cell divided into nuclear and cytoplasmic compartments. Treatment with a therapeutic agent, such as an antisense oligomer (ASO), promotes the exclusion of the nonsense-mediated mRNA decay-inducing exon and results in an increase in mRNA, which is in turn translated into higher levels of target protein. **FIG. 1C** is a schematic representation of therapeutic ASO-mediated exclusion of a nonsense-mediated mRNA decay-inducing exon, which decreases non-productive mRNA and increases productive mRNA and increases expression of the full-length target protein from the productive mRNA.

[0014] **FIG. 2** depicts identification of an exemplary nonsense-mediated mRNA decay (NMD)-inducing exon in the *SCN1A* gene. The identification of the NMD-inducing exon in the *SCN1A* gene using comparative genomics is shown, visualized in the UCSC genome browser. The upper panel

shows a graphic representation of the *SCN1A* gene to scale. The conservation level across 100 vertebrate species is shown as peaks. The highest peaks correspond to exons (black boxes), while no peaks are observed for the majority of the introns (lines with arrow heads). Peaks of conservation were identified in intron 20 (NM_006920), shown in the middle panel. Inspection of the conserved sequences identified an exon-like sequence of 64 bp (bottom panel, sequence highlighted in grey) flanked by 3' and 5' splice sites (underlined sequence), which we refer to as exon 20x. Inclusion of this exon leads to a frameshift and the introduction of a premature termination codon in exon 21 rendering the transcript a target of NMD.

[0015] FIG. 3A depicts confirmation of NMD-inducing exon via cycloheximide treatment. RT-PCR analysis using cytoplasmic RNA from DMSO-treated (CHX-) or cycloheximide-treated (CHX+) Neuro 2A (mouse neural progenitor cells) and primers in exon 21 and a downstream exon confirmed the presence of a band corresponding to the NMD-inducing exon (21x). The identity of the product was confirmed by sequencing. Densitometry analysis of the bands was performed to calculate percent exon 21x inclusion of total *SCN1A* transcript. Treatment of Neuro 2A with cycloheximide (CHX+) to inhibit NMD led to a 2-fold increase of the product corresponding to the NMD-inducing exon 21x in the cytoplasmic fraction (cf. light grey bar, CHX-, to dark grey bar, CHX+).

[0016] FIG. 3B depicts confirmation of NMD-inducing exon via cycloheximide treatment. RT-PCR analysis using cytoplasmic RNA from DMSO-treated (CHX-) or cycloheximide-treated (CHX+) RenCell VM (human neural progenitor cells) and primers in exon 20 and exon 23 confirmed the presence of a band corresponding to the NMD-inducing exon (20x). The identity of the product was confirmed by sequencing. Densitometry analysis of the bands was performed to calculate percent exon 20x inclusion of total *SCN1A* transcript. Treatment of RenCell VM with cycloheximide (CHX+) to inhibit NMD led to a 2-fold increase of the product corresponding to the NMD-inducing exon 20x in the cytoplasmic fraction (cf. light grey bar, CHX-, to dark grey bar, CHX+).

[0017] FIG. 4. depicts an exemplary graphic representation of an ASO walk performed for *SCN1A* exon 20x region targeting two indicated regions (region 1 and region 2) upstream of the 3' splice site of exon 20x and two indicated regions (region 3 and region 4) downstream of the 5' splice site of exon 20x. ASOs were designed to cover these regions by shifting 5 nucleotides at a time.

[0018] FIG. 5A depicts *SCN1A* exon 20x region ASOs selected from an extended ASO walk evaluated by RT-PCR. A representative PAGE shows SYBR-safe-stained RT-PCR products of *SCN1A* mock-treated, control ASO treated (NT), *SCN1A* exon 20x region ASOs from an extended walk in RenCells via nucleofection at 1uM for 24 hrs. Mock = No ASO; control NT = non-targeting control; Posctrl = positive control.

[0019] FIG. 5B depicts a graph plotting the percent exon 20x inclusion from the data in FIG. 5A.

[0020] FIG. 5C depicts a graph of qPCR results of an extended ASO walk using the samples of FIG. 5A normalized to RPL32 internal control and the fold-change of SCN1A mRNA relative to mock is plotted.

DETAILED DESCRIPTION OF THE INVENTION

Splicing and Nonsense-mediated mRNA Decay

[0021] Intervening sequences or introns are removed by a large and highly dynamic RNA-protein complex termed the spliceosome, which orchestrates complex interactions between primary transcripts, small nuclear RNAs (snRNAs) and a large number of proteins. Spliceosomes assemble ad hoc on each intron in an ordered manner, starting with recognition of the 5' splice site (5'ss) by U1 snRNA or the 3' splice site (3'ss) by the U2 pathway, which involves binding of the U2 auxiliary factor (U2AF) to the 3'ss region to facilitate U2 binding to the branch point sequence (BPS). U2AF is a stable heterodimer composed of a U2AF2-encoded 65-kD subunit (U2AF65), which binds the polypyrimidine tract (PPT), and a U2AF1-encoded 35-kD subunit (U2AF35), which interacts with highly conserved AG dinucleotides at 3'ss and stabilizes U2AF65 binding. In addition to the BPS/PPT unit and 3'ss/5'ss, accurate splicing requires auxiliary sequences or structures that activate or repress splice site recognition, known as intronic or exonic splicing enhancers or silencers. These elements allow genuine splice sites to be recognized among a vast excess of cryptic or pseudo-sites in the genome of higher eukaryotes, which have the same sequences but outnumber authentic sites by an order of magnitude. Although they often have a regulatory function, the exact mechanisms of their activation or repression are poorly understood.

[0022] The decision of whether to splice or not to splice can be typically modeled as a stochastic rather than deterministic process, such that even the most defined splicing signals can sometimes splice incorrectly. However, under normal conditions, pre-mRNA splicing proceeds at surprisingly high fidelity. This is attributed in part to the activity of adjacent cis-acting auxiliary exonic and intronic splicing regulatory elements (ESRs or ISRs). Typically, these functional elements are classified as either exonic or intronic splicing enhancers (ESEs or ISEs) or silencers (ESSs or ISSs) based on their ability to stimulate or inhibit splicing, respectively. Although there is now evidence that some auxiliary cis-acting elements may act by influencing the kinetics of spliceosome assembly, such as the arrangement of the complex between U1 snRNP and the 5'ss, it seems very likely that many elements function in concert with trans-acting RNA-binding proteins (RBPs). For example, the serine- and arginine-rich family of RBPs (SR proteins) is a conserved family of proteins that have a key role in defining exons. SR proteins promote exon recognition by recruiting components of the pre-spliceosome to adjacent splice sites or by antagonizing the effects of ESSs in the vicinity. The

repressive effects of ESSs can be mediated by members of the heterogeneous nuclear ribonucleoprotein (hnRNP) family and can alter recruitment of core splicing factors to adjacent splice sites. In addition to their roles in splicing regulation, silencer elements are suggested to have a role in repression of pseudo-exons, sets of decoy intronic splice sites with the typical spacing of an exon but without a functional open reading frame. ESEs and ESSs, in cooperation with their cognate trans-acting RBPs, represent important components in a set of splicing controls that specify how, where and when mRNAs are assembled from their precursors.

[0023] The sequences marking the exon-intron boundaries are degenerate signals of varying strengths that can occur at high frequency within human genes. In multi-exon genes, different pairs of splice sites can be linked together in many different combinations, creating a diverse array of transcripts from a single gene. This is commonly referred to as alternative pre-mRNA splicing. Although most mRNA isoforms produced by alternative splicing can be exported from the nucleus and translated into functional polypeptides, different mRNA isoforms from a single gene can vary greatly in their translation efficiency. Those mRNA isoforms with premature termination codons (PTCs) at least 50 bp upstream of an exon junction complex are likely to be targeted for degradation by the nonsense-mediated mRNA decay (NMD) pathway. Mutations in traditional (BPS/PPT/3'ss/5'ss) and auxiliary splicing motifs can cause aberrant splicing, such as exon skipping or cryptic (or pseudo-) exon inclusion or splice-site activation, and contribute significantly to human morbidity and mortality. Both aberrant and alternative splicing patterns can be influenced by natural DNA variants in exons and introns.

[0024] Given that exon-intron boundaries can occur at any of the three positions of a codon, it is clear that only a subset of alternative splicing events can maintain the canonical open reading frame. For example, only exons that are evenly divisible by 3 can be skipped or included in the mRNA without any alteration of reading frame. Splicing events that do not have compatible phases will induce a frame-shift. Unless reversed by downstream events, frame-shifts can certainly lead to one or more PTCs, probably resulting in subsequent degradation by NMD. NMD is a translation-coupled mechanism that eliminates mRNAs containing PTCs. NMD can function as a surveillance pathway that exists in all eukaryotes. NMD can reduce errors in gene expression by eliminating mRNA transcripts that contain premature stop codons. Translation of these aberrant mRNAs could, in some cases, lead to deleterious gain-of-function or dominant-negative activity of the resulting proteins. NMD targets not only transcripts with PTCs but also a broad array of mRNA isoforms expressed from many endogenous genes, suggesting that NMD is a master regulator that drives both fine and coarse adjustments in steady-state RNA levels in the cell.

[0025] A NMD-inducing exon (NIE) is an exon or a pseudo-exon that is a region within an intron and can activate the NMD pathway if included in a mature RNA transcript. In the constitutive splicing events, the intron containing an NIE is usually spliced out, but the intron or a portion thereof (e.g. NIE) can be retained during alternative or aberrant splicing events. Mature mRNA transcripts containing such an NIE can be non-productive due to frame shift which induce NMD pathway. Inclusion of a NIE in mature RNA transcripts can downregulate gene expression. mRNA transcripts containing an NIE can be referred as “NIE containing mRNA” or “NMD exon mRNA” in the current disclosure.

[0026] Cryptic (or pseudo- splice sites) have the same splicing recognition sequences as genuine splice sites but are not used in the splicing reactions. They outnumber genuine splice sites in the human genome by an order of a magnitude and are normally repressed by thus far poorly understood molecular mechanisms. Cryptic 5' splice sites have the consensus NNN/GUNNNN or NNN/GCNNNN where N is any nucleotide and / is the exon-intron boundary. Cryptic 3' splice sites have the consensus NAG/N. Their activation is positively influenced by surrounding nucleotides that make them more similar to the optimal consensus of authentic splice sites, namely MAG/GURAGU and YAG/G, respectively, where M is C or A, R is G or A, and Y is C or U.

[0027] Splice sites and their regulatory sequences can be readily identified by a skilled person using suitable algorithms publicly available, listed for example in Kralovicova, J. and Vorechovsky, I. (2007) Global control of aberrant splice site activation by auxiliary splicing sequences: evidence for a gradient in exon and intron definition. *Nucleic Acids Res.*, 35, 6399-6413, (<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2095810/pdf/gkm680.pdf>)

[0028] The cryptic splice sites or splicing regulatory sequences may compete for RNA-binding proteins such as U2AF with a splice site of the NIE. In one embodiment, an agent may bind to the cryptic splice site or splicing regulatory sequences to prevent the binding of RNA-binding proteins and thereby favoring utilization of the NIE splice sites.

[0029] In one embodiment, the cryptic splice site may not comprise the 5' or 3' splice site of the NIE. The cryptic splice site may be at least 10 nucleotides upstream of the NIE 5' splice site. The cryptic splice site may be at least 20 nucleotides upstream of the NIE 5' splice site. The cryptic splice site may be at least 50 nucleotides upstream of the NIE 5' splice site. The cryptic splice site may be at least 100 nucleotides upstream of the NIE 5' splice site. The cryptic splice site may be at least 200 nucleotides upstream of the NIE 5' splice site.

[0030] The cryptic splice site may be at least 10 nucleotides downstream of the NIE 3' splice site. The cryptic splice site may be at least 20 nucleotides downstream of the NIE 3' splice site. The cryptic splice site may be at least 50 nucleotides downstream of the NIE 3' splice site. The cryptic splice site

may be at least 100 nucleotides downstream of the NIE 3' splice site. The cryptic splice site may be at least 200 nucleotides downstream of the NIE 3' splice site.

Target Transcripts

[0031] In some embodiments, the methods of the present disclosure exploit the presence of NIE in the pre-mRNA transcribed from the *SCN1A* gene. Splicing of the identified *SCN1A* NIE pre-mRNA species to produce functional mature *SCN1A* mRNA can be induced using a therapeutic agent such as an ASO that stimulates exon skipping of an NIE. Induction of exon skipping can result in inhibition of an NMD pathway. The resulting mature *SCN1A* mRNA can be translated normally without activating NMD pathway, thereby increasing the amount of SCN1A protein in the patient's cells and alleviating symptoms of a condition associated with SCN1A deficiency, such as Dravet Syndrome (DS); Epilepsy, generalized, with febrile seizures plus, type 2; Febrile seizures, familial, 3A; Autism; Epileptic encephalopathy, early infantile, 13; Sick sinus syndrome 1; Alzheimer's disease; or SUDEP.

[0032] In various embodiments, the present disclosure provides a therapeutic agent which can target *SCN1A* mRNA transcripts to modulate, e.g., enhance or inhibit, splicing or protein expression level. The therapeutic agent can be a small molecule, polynucleotide, or polypeptide. In some embodiments, the therapeutic agent is an ASO. Various regions or sequences on the *SCN1A* pre-mRNA can be targeted by a therapeutic agent, such as an ASO. In some embodiments, the ASO targets a *SCN1A* pre-mRNA transcript containing an NIE. In some embodiments, the ASO targets a sequence within an NIE of a *SCN1A* pre-mRNA transcript. In some embodiments, the ASO targets a sequence upstream (or 5') from the 5' end of an NIE (3'ss) of a *SCN1A* pre-mRNA transcript. In some embodiments, the ASO targets a sequence downstream (or 3') from the 3' end of an NIE (5'ss) of a *SCN1A* pre-mRNA transcript. In some embodiments, the ASO targets a sequence that is within an intron flanking on the 5' end of the NIE of a *SCN1A* pre-mRNA transcript. In some embodiments, the ASO targets a sequence that is within an intron flanking the 3' end of the NIE of a *SCN1A* pre-mRNA transcript. In some embodiments, the ASO targets a sequence comprising an NIE-intron boundary of a *SCN1A* pre-mRNA transcript. An NIE-intron boundary can refer to the junction of an intron sequence and an NIE region. The intron sequence can flank the 5' end of the NIE, or the 3' end of the NIE. In some embodiments, the ASO targets a sequence within an exon of a *SCN1A* pre-mRNA transcript. In some embodiments, the ASO targets a sequence within an intron of a *SCN1A* pre-mRNA transcript. In some embodiments, the ASO targets a sequence comprising both a portion of an intron and a portion of an exon.

[0033] In some embodiments, a therapeutic agent described herein modulates binding of a factor involved in splicing of the NMD exon mRNA.

[0034] In some embodiments, a therapeutic agent described herein interferes with binding of a factor involved in splicing of the NMD exon mRNA.

[0035] In some embodiments, a therapeutic agent described herein prevents binding of a factor involved in splicing of the NMD exon mRNA.

[0036] In some embodiments, a therapeutic agent targets a targeted portion located in an intronic region between two canonical exonic regions of the NMD exon mRNA encoding SCN1A, and wherein the intronic region contains the NMD exon.

[0037] In some embodiments, a therapeutic agent targets a targeted portion at least partially overlaps with the NMD exon.

[0038] In some embodiments, a therapeutic agent targets a targeted portion that is at least partially overlaps with an intron upstream of the NMD exon.

[0039] In some embodiments, a therapeutic agent targets a targeted portion within the NMD exon.

[0040] In some embodiments, a therapeutic agent targets a targeted portion comprising at least about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more consecutive nucleotides of the NMD exon. In some embodiments, a therapeutic agent targets a targeted portion comprising at most about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more consecutive nucleotides of the NMD exon. In some embodiments, a therapeutic agent targets a targeted portion comprising about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more consecutive nucleotides of the NMD exon.

[0041] In some embodiments, a therapeutic agent targets a targeted portion proximal to the NMD exon.

[0042] In some embodiments, the ASO targets a sequence from about 1 to about 5000 nucleotides downstream from the 5' end of the intron comprising the NIE. In some embodiments, the ASO targets a sequence from about 1 to about 20 nucleotides, about 20 to about 50 nucleotides, about 50 to about 100 nucleotides, about 100 to about 150 nucleotides, about 150 to about 200 nucleotides, about 200 to about 250 nucleotides, about 250 to about 300, about 250 to about 300 nucleotides, about 350 to about 400 nucleotides, about 450 to about 500 nucleotides, about 550 to about 600 nucleotides, about 650 to about 700 nucleotides, about 750 to about 800 nucleotides, about 850 to about 900 nucleotides, about 950 to about 1000 nucleotides, about 1050 to about 1100 nucleotides, about 1150 to about 1200 nucleotides, about 1250 to about 1300 nucleotides, about 1350 to about 1400 nucleotides, about 1450 to about 1500 nucleotides, about 1550 to about 1600 nucleotides, about 1650 to about 1700 nucleotides, about 1750 to about 1800 nucleotides, about 1850 to about 1900 nucleotides, about 1950 to about 2000 nucleotides, about 2000 to about 3000 nucleotides, about 3000 to about 4000 nucleotides, or about 4000 to about 5000 nucleotides, downstream from the 5' end of the intron comprising the NIE. In some embodiments, the ASO targets a sequence from about 1 to about 20

nucleotides, about 20 to about 50 nucleotides, about 50 to about 100 nucleotides, about 100 to about 150 nucleotides, about 150 to about 200 nucleotides, about 200 to about 250 nucleotides, about 250 to about 300, about 250 to about 300 nucleotides, about 350 to about 400 nucleotides, about 450 to about 500 nucleotides, about 550 to about 600 nucleotides, about 650 to about 700 nucleotides, about 750 to about 800 nucleotides, about 850 to about 900 nucleotides, or about 950 to about 1000 nucleotides downstream from the 5' end of the intron comprising the NIE.

[0043] In some embodiments, the ASO targets a sequence at least about 1 nucleotide, at least about 10 nucleotides, at least about 20 nucleotides, at least about 50 nucleotides, at least about 80 nucleotides, at least about 85 nucleotides, at least about 90 nucleotides, at least about 95 nucleotides, at least about 96 nucleotides, at least about 97 nucleotides, at least about 98 nucleotides, at least about 99 nucleotides, at least about 100 nucleotides, at least about 101 nucleotides, at least about 102 nucleotides, at least about 103 nucleotides, at least about 104 nucleotides, at least about 105 nucleotides, at least about 110 nucleotides, at least about 120 nucleotides, at least about 150 nucleotides, at least about 200 nucleotides, at least about 300 nucleotides, at least about 400 nucleotides, at least about 500 nucleotides, at least about 600 nucleotides, at least about 700 nucleotides, at least about 800 nucleotides, at least about 900 nucleotides, at least about 1000 nucleotides, at least about 1200 nucleotides, at least about 1400 nucleotides, at least about 1500 nucleotides, at least about 1600 nucleotides, at least about 1800 nucleotides, at least about 2000 nucleotides, at least about 3000 nucleotides, at least about 4000 nucleotides, or at least about 5000 nucleotides downstream from the 5' end of the intron comprising the NIE.

[0044] In some embodiments, the ASO targets a sequence from about 1 to about 2000 nucleotides upstream (or 5') from the 5' end of the NIE. In some embodiments, the ASO targets a sequence from about 1 to about 20 nucleotides, about 20 to about 50 nucleotides, about 50 to about 100 nucleotides, about 100 to about 150 nucleotides, about 150 to about 200 nucleotides, about 200 to about 250 nucleotides, about 250 to about 300, about 250 to about 300 nucleotides, about 350 to about 400 nucleotides, about 450 to about 500 nucleotides, about 550 to about 600 nucleotides, about 650 to about 700 nucleotides, about 750 to about 800 nucleotides, about 850 to about 900 nucleotides, about 950 to about 1000 nucleotides, about 1050 to about 1100 nucleotides, about 1150 to about 1200 nucleotides, about 1250 to about 1300 nucleotides, about 1350 to about 1400 nucleotides, about 1450 to about 1500 nucleotides, about 1550 to about 1600 nucleotides, about 1650 to about 1700 nucleotides, about 1750 to about 1800 nucleotides, about 1850 to about 1900 nucleotides, or about 1950 to about 2000 nucleotides upstream (or 5') from the 5' end of the NIE. In some embodiments, the ASO targets a sequence from about 1 to about 20 nucleotides, about 20 to about 50 nucleotides, about 50 to about 100 nucleotides, about 100 to about 150 nucleotides, about 150 to about 200

nucleotides, about 200 to about 250 nucleotides, about 250 to about 300, about 250 to about 300 nucleotides, about 350 to about 400 nucleotides, about 450 to about 500 nucleotides, about 550 to about 600 nucleotides, about 650 to about 700 nucleotides, about 750 to about 800 nucleotides, about 850 to about 900 nucleotides, or about 950 to about 1000 nucleotides upstream (or 5') from the 5' end of the NIE.

[0045] In some embodiments, the ASO targets a sequence at least about 1 nucleotide, at least about 10 nucleotides, at least about 20 nucleotides, at least about 50 nucleotides, at least about 80 nucleotides, at least about 85 nucleotides, at least about 90 nucleotides, at least about 95 nucleotides, at least about 96 nucleotides, at least about 97 nucleotides, at least about 98 nucleotides, at least about 99 nucleotides, at least about 100 nucleotides, at least about 101 nucleotides, at least about 102 nucleotides, at least about 103 nucleotides, at least about 104 nucleotides, at least about 105 nucleotides, at least about 110 nucleotides, at least about 120 nucleotides, at least about 150 nucleotides, at least about 200 nucleotides, at least about 300 nucleotides, at least about 400 nucleotides, at least about 500 nucleotides, at least about 600 nucleotides, at least about 700 nucleotides, at least about 800 nucleotides, at least about 900 nucleotides, at least about 1000 nucleotides, at least about 1200 nucleotides, at least about 1400 nucleotides, at least about 1500 nucleotides, at least about 1600 nucleotides, at least about 1800 nucleotides, or at least about 2000 nucleotides upstream (or 5') from the 5' end of the NIE.

[0046] In some embodiments, the ASO targets a sequence from about 1 to about 500 nucleotides downstream from the 5' end of the NIE. In some embodiments, the ASO targets a sequence at least about 1 nucleotide, at least about 10 nucleotides, at least about 20 nucleotides, at least about 50 nucleotides, at least about 80 nucleotides, at least about 85 nucleotides, at least about 90 nucleotides, at least about 95 nucleotides, at least about 96 nucleotides, at least about 97 nucleotides, at least about 98 nucleotides, at least about 99 nucleotides, at least about 100 nucleotides, at least about 101 nucleotides, at least about 102 nucleotides, at least about 103 nucleotides, at least about 104 nucleotides, at least about 105 nucleotides, at least about 110 nucleotides, at least about 120 nucleotides, at least about 150 nucleotides, at least about 200 nucleotides, at least about 300 nucleotides, at least about 400 nucleotides, or at least about 500 nucleotides downstream from the 5' end of the NIE region.

[0047] In some embodiments, the ASO targets a sequence from about 1 to about 500 nucleotides upstream from the 3' end of the NIE. In some embodiments, the ASO targets a sequence at least about 1 nucleotide, at least about 10 nucleotides, at least about 20 nucleotides, at least about 50 nucleotides, at least about 80 nucleotides, at least about 85 nucleotides, at least about 90 nucleotides, at least about 95 nucleotides, at least about 96 nucleotides, at least about 97 nucleotides, at least about 98 nucleotides,

at least about 99 nucleotides, at least about 100 nucleotides, at least about 101 nucleotides, at least about 102 nucleotides, at least about 103 nucleotides, at least about 104 nucleotides, at least about 105 nucleotides, at least about 110 nucleotides, at least about 120 nucleotides, at least about 150 nucleotides, at least about 200 nucleotides, at least about 300 nucleotides, at least about 400 nucleotides, or at least about 500 nucleotides upstream from the 3' end of the NIE region.

[0048] In some embodiments, the ASO targets a sequence from about 1 to about 2000 nucleotides downstream (or 3') from the 3' end of the NIE. In some embodiments, the ASO targets a sequence from about 1 to about 20 nucleotides, about 20 to about 50 nucleotides, about 50 to about 100 nucleotides, about 100 to about 150 nucleotides, about 150 to about 200 nucleotides, about 200 to about 250 nucleotides, about 250 to about 300, about 250 to about 300 nucleotides, about 350 to about 400 nucleotides, about 450 to about 500 nucleotides, about 550 to about 600 nucleotides, about 650 to about 700 nucleotides, about 750 to about 800 nucleotides, about 850 to about 900 nucleotides, about 950 to about 1000 nucleotides, about 1050 to about 1100 nucleotides, about 1150 to about 1200 nucleotides, about 1250 to about 1300 nucleotides, about 1350 to about 1400 nucleotides, about 1450 to about 1500 nucleotides, about 1550 to about 1600 nucleotides, about 1650 to about 1700 nucleotides, about 1750 to about 1800 nucleotides, about 1850 to about 1900 nucleotides, or about 1950 to about 2000 nucleotides downstream (or 3') from the 3' end of the NIE. In some embodiments, the ASO targets a sequence from about 1 to about 20 nucleotides, about 20 to about 50 nucleotides, about 50 to about 100 nucleotides, about 100 to about 150 nucleotides, about 150 to about 200 nucleotides, about 200 to about 250 nucleotides, about 250 to about 300, about 250 to about 300 nucleotides, about 350 to about 400 nucleotides, about 450 to about 500 nucleotides, about 550 to about 600 nucleotides, about 650 to about 700 nucleotides, about 750 to about 800 nucleotides, about 850 to about 900 nucleotides, or about 950 to about 1000 nucleotides downstream (or 3') from the 3' end of the NIE.

[0049] In some embodiments, the ASO targets a sequence at least about 1 nucleotide, at least about 10 nucleotides, at least about 20 nucleotides, at least about 50 nucleotides, at least about 80 nucleotides, at least about 85 nucleotides, at least about 90 nucleotides, at least about 95 nucleotides, at least about 96 nucleotides, at least about 97 nucleotides, at least about 98 nucleotides, at least about 99 nucleotides, at least about 100 nucleotides, at least about 101 nucleotides, at least about 102 nucleotides, at least about 103 nucleotides, at least about 104 nucleotides, at least about 105 nucleotides, at least about 110 nucleotides, at least about 120 nucleotides, at least about 150 nucleotides, at least about 200 nucleotides, at least about 300 nucleotides, at least about 400 nucleotides, at least about 500 nucleotides, at least about 600 nucleotides, at least about 700 nucleotides, at least about 800 nucleotides, at least about 900 nucleotides, at least about 1000 nucleotides, at least about 1200

nucleotides, at least about 1400 nucleotides, at least about 1500 nucleotides, at least about 1600 nucleotides, at least about 1800 nucleotides, or at least about 2000 nucleotides downstream (or 3') from the 3' end of the NIE.

[0050] In some embodiments, the ASO targets a sequence from about 1 to about 5000 nucleotides upstream from the 3' end of the intron comprising the NIE. In some embodiments, the ASO targets a sequence from about 1 to about 20 nucleotides, about 20 to about 50 nucleotides, about 50 to about 100 nucleotides, about 100 to about 150 nucleotides, about 150 to about 200 nucleotides, about 200 to about 250 nucleotides, about 250 to about 300, about 250 to about 300 nucleotides, about 350 to about 400 nucleotides, about 450 to about 500 nucleotides, about 550 to about 600 nucleotides, about 650 to about 700 nucleotides, about 750 to about 800 nucleotides, about 850 to about 900 nucleotides, about 950 to about 1000 nucleotides, about 1050 to about 1100 nucleotides, about 1150 to about 1200 nucleotides, about 1250 to about 1300 nucleotides, about 1350 to about 1400 nucleotides, about 1450 to about 1500 nucleotides, about 1550 to about 1600 nucleotides, about 1650 to about 1700 nucleotides, about 1750 to about 1800 nucleotides, about 1850 to about 1900 nucleotides, about 1950 to about 2000 nucleotides, about 2000 to about 3000 nucleotides, about 3000 to about 4000 nucleotides, or about 4000 to about 5000 nucleotides, upstream from the 3' end of the intron comprising the NIE. In some embodiments, the ASO targets a sequence from about 1 to about 20 nucleotides, about 20 to about 50 nucleotides, about 50 to about 100 nucleotides, about 100 to about 150 nucleotides, about 150 to about 200 nucleotides, about 200 to about 250 nucleotides, about 250 to about 300, about 250 to about 300 nucleotides, about 350 to about 400 nucleotides, about 450 to about 500 nucleotides, about 550 to about 600 nucleotides, about 650 to about 700 nucleotides, about 750 to about 800 nucleotides, about 850 to about 900 nucleotides, or about 950 to about 1000 nucleotides upstream from the 3' end of the intron comprising the NIE.

[0051] In some embodiments, the ASO targets a sequence at least about 1 nucleotide, at least about 10 nucleotides, at least about 20 nucleotides, at least about 50 nucleotides, at least about 80 nucleotides, at least about 85 nucleotides, at least about 90 nucleotides, at least about 95 nucleotides, at least about 96 nucleotides, at least about 97 nucleotides, at least about 98 nucleotides, at least about 99 nucleotides, at least about 100 nucleotides, at least about 101 nucleotides, at least about 102 nucleotides, at least about 103 nucleotides, at least about 104 nucleotides, at least about 105 nucleotides, at least about 110 nucleotides, at least about 120 nucleotides, at least about 150 nucleotides, at least about 200 nucleotides, at least about 300 nucleotides, at least about 400 nucleotides, at least about 500 nucleotides, at least about 600 nucleotides, at least about 700 nucleotides, at least about 800 nucleotides, at least about 900 nucleotides, at least about 1000 nucleotides, at least about 1200 nucleotides, at least about 1400 nucleotides, at least about 1500 nucleotides, at least about 1600

nucleotides, at least about 1800 nucleotides, at least about 2000 nucleotides, at least about 3000 nucleotides, at least about 4000 nucleotides, or at least about 5000 nucleotides upstream from the 3' end of the intron comprising the NIE.

[0052] In some embodiments, the ASO targets a sequence from about 4 to about 300 nucleotides upstream (or 5') from the 5' end of the NIE. In some embodiments, the ASO targets a sequence from about 1 to about 20 nucleotides, about 20 to about 50 nucleotides, about 50 to about 100 nucleotides, about 100 to about 150 nucleotides, about 150 to about 200 nucleotides, about 200 to about 250 nucleotides, about 250 to about 300, about 250 to about 300 nucleotides, about 350 to about 400 nucleotides, about 450 to about 500 nucleotides, about 550 to about 600 nucleotides, about 650 to about 700 nucleotides, about 750 to about 800 nucleotides, about 850 to about 900 nucleotides, about 950 to about 1000 nucleotides, about 1050 to about 1100 nucleotides, about 1150 to about 1200 nucleotides, about 1250 to about 1300 nucleotides, about 1350 to about 1400 nucleotides, or about 1450 to about 1500 nucleotides upstream (or 5') from the 5' end of the NIE region. In some embodiments, the ASO may target a sequence more than 300 nucleotides upstream from the 5' end of the NIE. In some embodiments, the ASO targets a sequence from about 4 to about 300 nucleotides downstream (or 3') from the 3' end of the NIE. In some embodiments, the ASO targets a sequence about 1 to about 20 nucleotides, about 20 to about 50 nucleotides, about 50 to about 100 nucleotides, about 100 to about 150 nucleotides, about 150 to about 200 nucleotides, about 200 to about 250 nucleotides, about 250 to about 300 nucleotides, about 350 to about 400 nucleotides, about 450 to about 500 nucleotides, about 550 to about 600 nucleotides, about 650 to about 700 nucleotides, about 750 to about 800 nucleotides, about 850 to about 900 nucleotides, about 950 to about 1000 nucleotides, about 1050 to about 1100 nucleotides, about 1150 to about 1200 nucleotides, about 1250 to about 1300 nucleotides, about 1350 to about 1400 nucleotides, or about 1450 to about 1500 nucleotides downstream from the 3' end of the NIE. In some embodiments, the ASO targets a sequence more than 300 nucleotides downstream from the 3' end of the NIE.

[0053] In some embodiments, the ASO targets a sequence from about 4 to about 300 nucleotides upstream (or 5') from the 5' end of the NIE. In some embodiments, the ASO targets a sequence at least about 1 nucleotide, at least about 10 nucleotides, at least about 20 nucleotides, at least about 50 nucleotides, at least about 80 nucleotides, at least about 85 nucleotides, at least about 90 nucleotides, at least about 95 nucleotides, at least about 96 nucleotides, at least about 97 nucleotides, at least about 98 nucleotides, at least about 99 nucleotides, at least about 100 nucleotides, at least about 101 nucleotides, at least about 102 nucleotides, at least about 103 nucleotides, at least about 104 nucleotides, at least about 105 nucleotides, at least about 110 nucleotides, at least about 120 nucleotides, at least about 150 nucleotides, at least about 200 nucleotides, at least about 300

nucleotides, at least about 400 nucleotides, at least about 500 nucleotides, at least about 600 nucleotides, at least about 700 nucleotides, at least about 800 nucleotides, at least about 900 nucleotides, or at least about 1000 nucleotides upstream (or 5') from the 5' end of the NIE region. In some embodiments, the ASO targets a sequence about 4 to about 300 nucleotides downstream (or 3') from the 3' end of the NIE. In some embodiments, the ASO targets a sequence at least about 1 nucleotide, at least about 10 nucleotides, at least about 20 nucleotides, at least about 50 nucleotides, at least about 80 nucleotides, at least about 85 nucleotides, at least about 90 nucleotides, at least about 95 nucleotides, at least about 96 nucleotides, at least about 97 nucleotides, at least about 98 nucleotides, at least about 99 nucleotides, at least about 100 nucleotides, at least about 101 nucleotides, at least about 102 nucleotides, at least about 103 nucleotides, at least about 104 nucleotides, at least about 105 nucleotides, at least about 110 nucleotides, at least about 120 nucleotides, at least about 150 nucleotides, at least about 200 nucleotides, at least about 300 nucleotides, at least about 400 nucleotides, at least about 500 nucleotides, at least about 600 nucleotides, at least about 700 nucleotides, at least about 800 nucleotides, at least about 900 nucleotides, or at least about 1000 nucleotides downstream from the 3' end of the NIE. In some embodiments, the ASO targets a sequence more than 300 nucleotides downstream from the 3' end of the NIE.

[0054] In some embodiments, the ASO targets a sequence from about 4 to about 300 nucleotides upstream (or 5') from the 5' end of the NIE. In some embodiments, the ASO targets a sequence at most about 10 nucleotides, at most about 20 nucleotides, at most about 50 nucleotides, at most about 80 nucleotides, at most about 85 nucleotides, at most about 90 nucleotides, at most about 95 nucleotides, at most about 96 nucleotides, at most about 97 nucleotides, at most about 98 nucleotides, at most about 99 nucleotides, at most about 100 nucleotides, at most about 101 nucleotides, at most about 102 nucleotides, at most about 103 nucleotides, at most about 104 nucleotides, at most about 105 nucleotides, at most about 110 nucleotides, at most about 120 nucleotides, at most about 150 nucleotides, at most about 200 nucleotides, at most about 300 nucleotides, at most about 400 nucleotides, at most about 500 nucleotides, at most about 600 nucleotides, at most about 700 nucleotides, at most about 800 nucleotides, at most about 900 nucleotides, at most about 1000 nucleotides, at most about 1100 nucleotides, at most about 1200 nucleotides, at most about 1300 nucleotides, at most about 1400 nucleotides, or at most about 1500 nucleotides upstream (or 5') from the 5' end of the NIE region. In some embodiments, the ASO targets a sequence about 4 to about 300 nucleotides downstream (or 3') from the 3' end of the NIE. In some embodiments, the ASO targets a sequence at most about 10 nucleotides, at most about 20 nucleotides, at most about 50 nucleotides, at most about 80 nucleotides, at most about 85 nucleotides, at most about 90 nucleotides, at most about 95 nucleotides, at most about 96 nucleotides, at most about 97 nucleotides, at most about 98

nucleotides, at most about 99 nucleotides, at most about 100 nucleotides, at most about 101 nucleotides, at most about 102 nucleotides, at most about 103 nucleotides, at most about 104 nucleotides, at most about 105 nucleotides, at most about 110 nucleotides, at most about 120 nucleotides, at most about 150 nucleotides, at most about 200 nucleotides, at most about 300 nucleotides, at most about 400 nucleotides, at most about 500 nucleotides, at most about 600 nucleotides, at most about 700 nucleotides, at most about 800 nucleotides, at most about 900 nucleotides, or at most about 1000 nucleotides, at most about 1100 nucleotides, at most about 1200 nucleotides, at most about 1300 nucleotides, at most about 1400 nucleotides, or at most about 1500 nucleotides downstream from the 3' end of the NIE. In some embodiments, the ASO targets a sequence more than 300 nucleotides downstream from the 3' end of the NIE.

[0055] In some embodiments, the NIE (exon 23) as described herein is located between GRCh38/hg38: chr2: 166007230 and chr2: 166007293. In some embodiments, the 5' end of the NIE is located at GRCh38/hg38: chr2: 166007230. In some embodiments, the 3' end of the NIE is located at GRCh38/hg38: chr2: 166007293.

[0056] In some embodiments, the ASO targets a sequence from about 1 to about 2000 nucleotides upstream (or 5') from genomic site GRCh38/hg38: chr2: 166007230. In some embodiments, the ASO targets a sequence from about 1 to about 20 nucleotides, about 20 to about 50 nucleotides, about 50 to about 100 nucleotides, about 100 to about 150 nucleotides, about 150 to about 200 nucleotides, about 200 to about 250 nucleotides, about 250 to about 300, about 250 to about 300 nucleotides, about 350 to about 400 nucleotides, about 450 to about 500 nucleotides, about 550 to about 600 nucleotides, about 650 to about 700 nucleotides, about 750 to about 800 nucleotides, about 850 to about 900 nucleotides, about 950 to about 1000 nucleotides, about 1050 to about 1100 nucleotides, about 1150 to about 1200 nucleotides, about 1250 to about 1300 nucleotides, about 1350 to about 1400 nucleotides, about 1450 to about 1500 nucleotides, about 1550 to about 1600 nucleotides, about 1650 to about 1700 nucleotides, about 1750 to about 1800 nucleotides, about 1850 to about 1900 nucleotides, or about 1950 to about 2000 nucleotides upstream (or 5') from genomic site GRCh38/hg38: chr2: 166007230. In some embodiments, the ASO targets a sequence from about 1 to about 20 nucleotides, about 20 to about 50 nucleotides, about 50 to about 100 nucleotides, about 100 to about 150 nucleotides, about 150 to about 200 nucleotides, about 200 to about 250 nucleotides, about 250 to about 300, about 250 to about 300 nucleotides, about 350 to about 400 nucleotides, about 450 to about 500 nucleotides, about 550 to about 600 nucleotides, about 650 to about 700 nucleotides, about 750 to about 800 nucleotides, about 850 to about 900 nucleotides, or about 950 to about 1000 nucleotides upstream (or 5') from genomic site GRCh38/hg38: chr2: 166007230.

[0057] In some embodiments, the ASO targets a sequence at least about 1 nucleotide, at least about 10 nucleotides, at least about 20 nucleotides, at least about 50 nucleotides, at least about 80 nucleotides, at least about 85 nucleotides, at least about 90 nucleotides, at least about 95 nucleotides, at least about 96 nucleotides, at least about 97 nucleotides, at least about 98 nucleotides, at least about 99 nucleotides, at least about 100 nucleotides, at least about 101 nucleotides, at least about 102 nucleotides, at least about 103 nucleotides, at least about 104 nucleotides, at least about 105 nucleotides, at least about 110 nucleotides, at least about 120 nucleotides, at least about 150 nucleotides, at least about 200 nucleotides, at least about 300 nucleotides, at least about 400 nucleotides, at least about 500 nucleotides, at least about 600 nucleotides, at least about 700 nucleotides, at least about 800 nucleotides, at least about 900 nucleotides, at least about 1000 nucleotides, at least about 1200 nucleotides, at least about 1400 nucleotides, at least about 1500 nucleotides, at least about 1600 nucleotides, at least about 1800 nucleotides, or at least about 2000 nucleotides upstream (or 5') from genomic site GRCh38/hg38: chr2: 166007230.

[0058] In some embodiments, the ASO targets a sequence from about 1 to about 500 nucleotides downstream from genomic site GRCh38/hg38: chr2: 166007230. In some embodiments, the ASO targets a sequence at least about 1 nucleotide, at least about 10 nucleotides, at least about 20 nucleotides, at least about 50 nucleotides, at least about 80 nucleotides, at least about 85 nucleotides, at least about 90 nucleotides, at least about 95 nucleotides, at least about 96 nucleotides, at least about 97 nucleotides, at least about 98 nucleotides, at least about 99 nucleotides, at least about 100 nucleotides, at least about 101 nucleotides, at least about 102 nucleotides, at least about 103 nucleotides, at least about 104 nucleotides, at least about 105 nucleotides, at least about 110 nucleotides, at least about 120 nucleotides, at least about 150 nucleotides, at least about 200 nucleotides, at least about 300 nucleotides, at least about 400 nucleotides, or at least about 500 nucleotides downstream from genomic site GRCh38/hg38: chr2: 166007230.

[0059] In some embodiments, the ASO targets a sequence from about 1 to about 500 nucleotides upstream from genomic site GRCh38/hg38: chr2: 166007293. In some embodiments, the ASO targets a sequence at least about 1 nucleotide, at least about 10 nucleotides, at least about 20 nucleotides, at least about 50 nucleotides, at least about 80 nucleotides, at least about 85 nucleotides, at least about 90 nucleotides, at least about 95 nucleotides, at least about 96 nucleotides, at least about 97 nucleotides, at least about 98 nucleotides, at least about 99 nucleotides, at least about 100 nucleotides, at least about 101 nucleotides, at least about 102 nucleotides, at least about 103 nucleotides, at least about 104 nucleotides, at least about 105 nucleotides, at least about 110 nucleotides, at least about 120 nucleotides, at least about 150 nucleotides, at least about 200 nucleotides, at least about 300

nucleotides, at least about 400 nucleotides, or at least about 500 nucleotides upstream from genomic site GRCh38/hg38: chr2: 166007293.

[0060] In some embodiments, the ASO targets a sequence from about 1 to about 2000 nucleotides downstream (or 3') from genomic site GRCh38/hg38: chr2: 166007293. In some embodiments, the ASO targets a sequence from about 1 to about 20 nucleotides, about 20 to about 50 nucleotides, about 50 to about 100 nucleotides, about 100 to about 150 nucleotides, about 150 to about 200 nucleotides, about 200 to about 250 nucleotides, about 250 to about 300, about 250 to about 300 nucleotides, about 350 to about 400 nucleotides, about 450 to about 500 nucleotides, about 550 to about 600 nucleotides, about 650 to about 700 nucleotides, about 750 to about 800 nucleotides, about 850 to about 900 nucleotides, about 950 to about 1000 nucleotides, about 1050 to about 1100 nucleotides, about 1150 to about 1200 nucleotides, about 1250 to about 1300 nucleotides, about 1350 to about 1400 nucleotides, about 1450 to about 1500 nucleotides, about 1550 to about 1600 nucleotides, about 1650 to about 1700 nucleotides, about 1750 to about 1800 nucleotides, about 1850 to about 1900 nucleotides, or about 1950 to about 2000 nucleotides downstream (or 3') from genomic site GRCh38/hg38: chr2: 166007293. In some embodiments, the ASO targets a sequence from about 1 to about 20 nucleotides, about 20 to about 50 nucleotides, about 50 to about 100 nucleotides, about 100 to about 150 nucleotides, about 150 to about 200 nucleotides, about 200 to about 250 nucleotides, about 250 to about 300, about 250 to about 300 nucleotides, about 350 to about 400 nucleotides, about 450 to about 500 nucleotides, about 550 to about 600 nucleotides, about 650 to about 700 nucleotides, about 750 to about 800 nucleotides, about 850 to about 900 nucleotides, or about 950 to about 1000 nucleotides downstream (or 3') from genomic site GRCh38/hg38: chr2: 166007293.

[0061] In some embodiments, the ASO targets a sequence at least about 1 nucleotide, at least about 10 nucleotides, at least about 20 nucleotides, at least about 50 nucleotides, at least about 80 nucleotides, at least about 85 nucleotides, at least about 90 nucleotides, at least about 95 nucleotides, at least about 96 nucleotides, at least about 97 nucleotides, at least about 98 nucleotides, at least about 99 nucleotides, at least about 100 nucleotides, at least about 101 nucleotides, at least about 102 nucleotides, at least about 103 nucleotides, at least about 104 nucleotides, at least about 105 nucleotides, at least about 110 nucleotides, at least about 120 nucleotides, at least about 150 nucleotides, at least about 200 nucleotides, at least about 300 nucleotides, at least about 400 nucleotides, at least about 500 nucleotides, at least about 600 nucleotides, at least about 700 nucleotides, at least about 800 nucleotides, at least about 900 nucleotides, at least about 1000 nucleotides, at least about 1200 nucleotides, at least about 1400 nucleotides, at least about 1500 nucleotides, at least about 1600 nucleotides, at least about 1800 nucleotides, or at least about 2000 nucleotides downstream (or 3') from genomic site GRCh38/hg38: chr2: 166007293.

[0062] In some embodiments, the intron comprising the NIE is located between GRCh38/hg38: chr2: 166002754 and chr2: 166009718. In some embodiments, the 5' end of the intron comprising the NIE is located at GRCh38/hg38: chr2: 166002754. In some embodiments, the 3' end of the intron comprising the NIE is located at GRCh38/hg38: chr2: 166009718.

[0063] In some embodiments, the ASO targets a sequence from about 1 to about 5000 nucleotides downstream from genomic site GRCh38/hg38: chr2: 166002754. In some embodiments, the ASO targets a sequence from about 1 to about 20 nucleotides, about 20 to about 50 nucleotides, about 50 to about 100 nucleotides, about 100 to about 150 nucleotides, about 150 to about 200 nucleotides, about 200 to about 250 nucleotides, about 250 to about 300, about 250 to about 300 nucleotides, about 350 to about 400 nucleotides, about 450 to about 500 nucleotides, about 550 to about 600 nucleotides, about 650 to about 700 nucleotides, about 750 to about 800 nucleotides, about 850 to about 900 nucleotides, about 950 to about 1000 nucleotides, about 1050 to about 1100 nucleotides, about 1150 to about 1200 nucleotides, about 1250 to about 1300 nucleotides, about 1350 to about 1400 nucleotides, about 1450 to about 1500 nucleotides, about 1550 to about 1600 nucleotides, about 1650 to about 1700 nucleotides, about 1750 to about 1800 nucleotides, about 1850 to about 1900 nucleotides, about 1950 to about 2000 nucleotides, about 2000 to about 3000 nucleotides, about 3000 to about 4000 nucleotides, or about 4000 to about 5000 nucleotides, downstream from genomic site GRCh38/hg38: chr2: 166002754. In some embodiments, the ASO targets a sequence from about 1 to about 20 nucleotides, about 20 to about 50 nucleotides, about 50 to about 100 nucleotides, about 100 to about 150 nucleotides, about 150 to about 200 nucleotides, about 200 to about 250 nucleotides, about 250 to about 300, about 250 to about 300 nucleotides, about 350 to about 400 nucleotides, about 450 to about 500 nucleotides, about 550 to about 600 nucleotides, about 650 to about 700 nucleotides, about 750 to about 800 nucleotides, about 850 to about 900 nucleotides, or about 950 to about 1000 nucleotides downstream from genomic site GRCh38/hg38: chr2: 166002754.

[0064] In some embodiments, the ASO targets a sequence at least about 1 nucleotide, at least about 10 nucleotides, at least about 20 nucleotides, at least about 50 nucleotides, at least about 80 nucleotides, at least about 85 nucleotides, at least about 90 nucleotides, at least about 95 nucleotides, at least about 96 nucleotides, at least about 97 nucleotides, at least about 98 nucleotides, at least about 99 nucleotides, at least about 100 nucleotides, at least about 101 nucleotides, at least about 102 nucleotides, at least about 103 nucleotides, at least about 104 nucleotides, at least about 105 nucleotides, at least about 110 nucleotides, at least about 120 nucleotides, at least about 150 nucleotides, at least about 200 nucleotides, at least about 300 nucleotides, at least about 400 nucleotides, at least about 500 nucleotides, at least about 600 nucleotides, at least about 700 nucleotides, at least about 800 nucleotides, at least about 900 nucleotides, at least about 1000 nucleotides, at least about 1200

nucleotides, at least about 1400 nucleotides, at least about 1500 nucleotides, at least about 1600 nucleotides, at least about 1800 nucleotides, at least about 2000 nucleotides, at least about 3000 nucleotides, at least about 4000 nucleotides, or at least about 5000 nucleotides downstream from genomic site GRCh38/hg38: chr2: 166002754.

[0065] In some embodiments, the ASO targets a sequence from about 1 to about 5000 nucleotides upstream from genomic site GRCh38/hg38: chr2: 166007229. In some embodiments, the ASO targets a sequence from about 1 to about 20 nucleotides, about 20 to about 50 nucleotides, about 50 to about 100 nucleotides, about 100 to about 150 nucleotides, about 150 to about 200 nucleotides, about 200 to about 250 nucleotides, about 250 to about 300, about 250 to about 300 nucleotides, about 350 to about 400 nucleotides, about 450 to about 500 nucleotides, about 550 to about 600 nucleotides, about 650 to about 700 nucleotides, about 750 to about 800 nucleotides, about 850 to about 900 nucleotides, about 950 to about 1000 nucleotides, about 1050 to about 1100 nucleotides, about 1150 to about 1200 nucleotides, about 1250 to about 1300 nucleotides, about 1350 to about 1400 nucleotides, about 1450 to about 1500 nucleotides, about 1550 to about 1600 nucleotides, about 1650 to about 1700 nucleotides, about 1750 to about 1800 nucleotides, about 1850 to about 1900 nucleotides, about 1950 to about 2000 nucleotides, about 2000 to about 3000 nucleotides, about 3000 to about 4000 nucleotides, or about 4000 to about 5000 nucleotides, upstream from genomic site GRCh38/hg38: chr2: 166007229. In some embodiments, the ASO targets a sequence from about 1 to about 20 nucleotides, about 20 to about 50 nucleotides, about 50 to about 100 nucleotides, about 100 to about 150 nucleotides, about 150 to about 200 nucleotides, about 200 to about 250 nucleotides, about 250 to about 300, about 250 to about 300 nucleotides, about 350 to about 400 nucleotides, about 450 to about 500 nucleotides, about 550 to about 600 nucleotides, about 650 to about 700 nucleotides, about 750 to about 800 nucleotides, about 850 to about 900 nucleotides, or about 950 to about 1000 nucleotides upstream from genomic site GRCh38/hg38: chr2: 166007229.

[0066] In some embodiments, the ASO targets a sequence at least about 1 nucleotide, at least about 10 nucleotides, at least about 20 nucleotides, at least about 50 nucleotides, at least about 80 nucleotides, at least about 85 nucleotides, at least about 90 nucleotides, at least about 95 nucleotides, at least about 96 nucleotides, at least about 97 nucleotides, at least about 98 nucleotides, at least about 99 nucleotides, at least about 100 nucleotides, at least about 101 nucleotides, at least about 102 nucleotides, at least about 103 nucleotides, at least about 104 nucleotides, at least about 105 nucleotides, at least about 110 nucleotides, at least about 120 nucleotides, at least about 150 nucleotides, at least about 200 nucleotides, at least about 300 nucleotides, at least about 400 nucleotides, at least about 500 nucleotides, at least about 600 nucleotides, at least about 700 nucleotides, at least about 800 nucleotides, at least about 900 nucleotides, at least about 1000 nucleotides, at least about 1200

nucleotides, at least about 1400 nucleotides, at least about 1500 nucleotides, at least about 1600 nucleotides, at least about 1800 nucleotides, at least about 2000 nucleotides, at least about 3000 nucleotides, at least about 4000 nucleotides, or at least about 5000 nucleotides upstream from genomic site GRCh38/hg38: chr2: 166007229.

[0067] In some embodiments, the ASO targets a sequence from about 1 to about 5000 nucleotides downstream from genomic site GRCh38/hg38: chr2: 166007294. In some embodiments, the ASO targets a sequence from about 1 to about 20 nucleotides, about 20 to about 50 nucleotides, about 50 to about 100 nucleotides, about 100 to about 150 nucleotides, about 150 to about 200 nucleotides, about 200 to about 250 nucleotides, about 250 to about 300, about 250 to about 300 nucleotides, about 350 to about 400 nucleotides, about 450 to about 500 nucleotides, about 550 to about 600 nucleotides, about 650 to about 700 nucleotides, about 750 to about 800 nucleotides, about 850 to about 900 nucleotides, about 950 to about 1000 nucleotides, about 1050 to about 1100 nucleotides, about 1150 to about 1200 nucleotides, about 1250 to about 1300 nucleotides, about 1350 to about 1400 nucleotides, about 1450 to about 1500 nucleotides, about 1550 to about 1600 nucleotides, about 1650 to about 1700 nucleotides, about 1750 to about 1800 nucleotides, about 1850 to about 1900 nucleotides, about 1950 to about 2000 nucleotides, about 2000 to about 3000 nucleotides, about 3000 to about 4000 nucleotides, or about 4000 to about 5000 nucleotides, downstream from genomic site GRCh38/hg38: chr2: 166007294. In some embodiments, the ASO targets a sequence from about 1 to about 20 nucleotides, about 20 to about 50 nucleotides, about 50 to about 100 nucleotides, about 100 to about 150 nucleotides, about 150 to about 200 nucleotides, about 200 to about 250 nucleotides, about 250 to about 300, about 250 to about 300 nucleotides, about 350 to about 400 nucleotides, about 450 to about 500 nucleotides, about 550 to about 600 nucleotides, about 650 to about 700 nucleotides, about 750 to about 800 nucleotides, about 850 to about 900 nucleotides, or about 950 to about 1000 nucleotides downstream from genomic site GRCh38/hg38: chr2: 166007294.

[0068] In some embodiments, the ASO targets a sequence at least about 1 nucleotide, at least about 10 nucleotides, at least about 20 nucleotides, at least about 50 nucleotides, at least about 80 nucleotides, at least about 85 nucleotides, at least about 90 nucleotides, at least about 95 nucleotides, at least about 96 nucleotides, at least about 97 nucleotides, at least about 98 nucleotides, at least about 99 nucleotides, at least about 100 nucleotides, at least about 101 nucleotides, at least about 102 nucleotides, at least about 103 nucleotides, at least about 104 nucleotides, at least about 105 nucleotides, at least about 110 nucleotides, at least about 120 nucleotides, at least about 150 nucleotides, at least about 200 nucleotides, at least about 300 nucleotides, at least about 400 nucleotides, at least about 500 nucleotides, at least about 600 nucleotides, at least about 700 nucleotides, at least about 800 nucleotides, at least about 900 nucleotides, at least about 1000 nucleotides, at least about 1200

nucleotides, at least about 1400 nucleotides, at least about 1500 nucleotides, at least about 1600 nucleotides, at least about 1800 nucleotides, at least about 2000 nucleotides, at least about 3000 nucleotides, at least about 4000 nucleotides, or at least about 5000 nucleotides downstream from genomic site GRCh38/hg38: chr2: 166007294.

[0069] In some embodiments, the ASO targets a sequence from about 1 to about 5000 nucleotides upstream from genomic site GRCh38/hg38: chr2: 166009718. In some embodiments, the ASO targets a sequence from about 1 to about 20 nucleotides, about 20 to about 50 nucleotides, about 50 to about 100 nucleotides, about 100 to about 150 nucleotides, about 150 to about 200 nucleotides, about 200 to about 250 nucleotides, about 250 to about 300, about 250 to about 300 nucleotides, about 350 to about 400 nucleotides, about 450 to about 500 nucleotides, about 550 to about 600 nucleotides, about 650 to about 700 nucleotides, about 750 to about 800 nucleotides, about 850 to about 900 nucleotides, about 950 to about 1000 nucleotides, about 1050 to about 1100 nucleotides, about 1150 to about 1200 nucleotides, about 1250 to about 1300 nucleotides, about 1350 to about 1400 nucleotides, about 1450 to about 1500 nucleotides, about 1550 to about 1600 nucleotides, about 1650 to about 1700 nucleotides, about 1750 to about 1800 nucleotides, about 1850 to about 1900 nucleotides, about 1950 to about 2000 nucleotides, about 2000 to about 3000 nucleotides, about 3000 to about 4000 nucleotides, or about 4000 to about 5000 nucleotides, upstream from genomic site GRCh38/hg38: chr2: 166009718. In some embodiments, the ASO targets a sequence from about 1 to about 20 nucleotides, about 20 to about 50 nucleotides, about 50 to about 100 nucleotides, about 100 to about 150 nucleotides, about 150 to about 200 nucleotides, about 200 to about 250 nucleotides, about 250 to about 300, about 250 to about 300 nucleotides, about 350 to about 400 nucleotides, about 450 to about 500 nucleotides, about 550 to about 600 nucleotides, about 650 to about 700 nucleotides, about 750 to about 800 nucleotides, about 850 to about 900 nucleotides, or about 950 to about 1000 nucleotides upstream from genomic site GRCh38/hg38: chr2: 166009718.

[0070] In some embodiments, the ASO targets a sequence at least about 1 nucleotide, at least about 10 nucleotides, at least about 20 nucleotides, at least about 50 nucleotides, at least about 80 nucleotides, at least about 85 nucleotides, at least about 90 nucleotides, at least about 95 nucleotides, at least about 96 nucleotides, at least about 97 nucleotides, at least about 98 nucleotides, at least about 99 nucleotides, at least about 100 nucleotides, at least about 101 nucleotides, at least about 102 nucleotides, at least about 103 nucleotides, at least about 104 nucleotides, at least about 105 nucleotides, at least about 110 nucleotides, at least about 120 nucleotides, at least about 150 nucleotides, at least about 200 nucleotides, at least about 300 nucleotides, at least about 400 nucleotides, at least about 500 nucleotides, at least about 600 nucleotides, at least about 700 nucleotides, at least about 800 nucleotides, at least about 900 nucleotides, at least about 1000 nucleotides, at least about 1200

nucleotides, at least about 1400 nucleotides, at least about 1500 nucleotides, at least about 1600 nucleotides, at least about 1800 nucleotides, at least about 2000 nucleotides, at least about 3000 nucleotides, at least about 4000 nucleotides, or at least about 5000 nucleotides upstream from genomic site GRCh38/hg38: chr2: 166009718.

[0071] In some embodiments, the NIE as described herein is located between GRCh37/hg19: chr2:166,863,740 and GRCh37/hg19: chr2:166,863,803, as depicted in **FIG. 2**. In some embodiments, the 5' end of the NIE is located at GRCh37/hg19: chr2:166,863,803. In some embodiments, the 3' end of the NIE is located at GRCh37/hg19: chr2:166,863,740.

[0072] In some embodiments, In some embodiments, the ASO targets a sequence from about 4 to about 300 nucleotides upstream (or 5') from genomic site GRCh37/hg19: chr2:166,863,803. In some embodiments, the ASO targets a sequence about 1 to about 20 nucleotides, about 20 to about 50 nucleotides, about 50 to about 100 nucleotides, about 100 to about 150 nucleotides, about 150 to about 200 nucleotides, about 200 to about 250 nucleotides, about 250 to about 300, about 250 to about 300 nucleotides, about 350 to about 400 nucleotides, about 450 to about 500 nucleotides, about 550 to about 600 nucleotides, about 650 to about 700 nucleotides, about 750 to about 800 nucleotides, about 850 to about 900 nucleotides, about 950 to about 1000 nucleotides, about 1050 to about 1100 nucleotides, about 1150 to about 1200 nucleotides, about 1250 to about 1300 nucleotides, about 1350 to about 1400 nucleotides, or about 1450 to about 1500 nucleotides upstream (or 5') from genomic site GRCh37/hg19: chr2:166,863,803. In some embodiments, the ASO may target a sequence more than 300 nucleotides upstream from genomic site GRCh37/hg19: chr2:166,863,803. In some embodiments, the ASO targets a sequence from about 4 to about 300 nucleotides downstream (or 3') from GRCh37/hg19: chr2:166,863,740. In some embodiments, the ASO targets a sequence about 1 to about 20 nucleotides, about 20 to about 50 nucleotides, about 50 to about 100 nucleotides, about 100 to about 150 nucleotides, about 150 to about 200 nucleotides, about 200 to about 250 nucleotides, about 250 to about 300 nucleotides, about 350 to about 400 nucleotides, about 450 to about 500 nucleotides, about 550 to about 600 nucleotides, about 650 to about 700 nucleotides, about 750 to about 800 nucleotides, about 850 to about 900 nucleotides, about 950 to about 1000 nucleotides, about 1050 to about 1100 nucleotides, about 1150 to about 1200 nucleotides, about 1250 to about 1300 nucleotides, about 1350 to about 1400 nucleotides, or about 1450 to about 1500 nucleotides downstream from GRCh37/hg19: chr2:166,863,740. In some embodiments, the ASO targets a sequence more than 300 nucleotides downstream from GRCh37/hg19: chr2:166,863,740.

[0073] In some embodiments, the ASO targets a sequence from about 4 to about 300 nucleotides upstream (or 5') from genomic site GRCh37/hg19: chr2:166,863,803. In some embodiments, the ASO targets a sequence at least about 1 nucleotide, at least about 10 nucleotides, at least about 20

nucleotides, at least about 50 nucleotides, at least about 80 nucleotides, at least about 85 nucleotides, at least about 90 nucleotides, at least about 95 nucleotides, at least about 96 nucleotides, at least about 97 nucleotides, at least about 98 nucleotides, at least about 99 nucleotides, at least about 100 nucleotides, at least about 101 nucleotides, at least about 102 nucleotides, at least about 103 nucleotides, at least about 104 nucleotides, at least about 105 nucleotides, at least about 110 nucleotides, at least about 120 nucleotides, at least about 150 nucleotides, at least about 200 nucleotides, at least about 300 nucleotides, at least about 400 nucleotides, at least about 500 nucleotides, at least about 600 nucleotides, at least about 700 nucleotides, at least about 800 nucleotides, at least about 900 nucleotides, or at least about 1000 nucleotides upstream (or 5') from genomic site GRCh37/hg19: chr2:166,863,803. In some embodiments, the ASO targets a sequence from about 4 to about 300 nucleotides downstream (or 3') from GRCh37/hg19: chr2:166,863,740. In some embodiments, the ASO targets a sequence at least about 1 nucleotide, at least about 10 nucleotides, at least about 20 nucleotides, at least about 50 nucleotides, at least about 80 nucleotides, at least about 85 nucleotides, at least about 90 nucleotides, at least about 95 nucleotides, at least about 96 nucleotides, at least about 97 nucleotides, at least about 98 nucleotides, at least about 99 nucleotides, at least about 100 nucleotides, at least about 101 nucleotides, at least about 102 nucleotides, at least about 103 nucleotides, at least about 104 nucleotides, at least about 105 nucleotides, at least about 110 nucleotides, at least about 120 nucleotides, at least about 150 nucleotides, at least about 200 nucleotides, at least about 300 nucleotides, at least about 400 nucleotides, at least about 500 nucleotides, at least about 600 nucleotides, at least about 700 nucleotides, at least about 800 nucleotides, at least about 900 nucleotides, or at least about 1000 nucleotides downstream from GRCh37/hg19: chr2:166,863,740. In some embodiments, the ASO targets a sequence more than 300 nucleotides downstream from GRCh37/hg19: chr2:166,863,740.

[0074] In some embodiments, the ASO targets a sequence from about 4 to about 300 nucleotides upstream (or 5') from genomic site GRCh37/hg19: chr2:166,863,803. In some embodiments, the ASO targets a sequence at most about 10 nucleotides, at most about 20 nucleotides, at most about 50 nucleotides, at most about 80 nucleotides, at most about 85 nucleotides, at most about 90 nucleotides, at most about 95 nucleotides, at most about 96 nucleotides, at most about 97 nucleotides, at most about 98 nucleotides, at most about 99 nucleotides, at most about 100 nucleotides, at most about 101 nucleotides, at most about 102 nucleotides, at most about 103 nucleotides, at most about 104 nucleotides, at most about 105 nucleotides, at most about 110 nucleotides, at most about 120 nucleotides, at most about 150 nucleotides, at most about 200 nucleotides, at most about 300 nucleotides, at most about 400 nucleotides, at most about 500 nucleotides, at most about 600 nucleotides, at most about 700 nucleotides, at most about 800 nucleotides, at most about 900

nucleotides, at most about 1000 nucleotides, at most about 1100 nucleotides, at most about 1200 nucleotides, at most about 1300 nucleotides, at most about 1400 nucleotides, or at most about 1500 nucleotides upstream (or 5') from genomic site GRCh37/hg19: chr2:166,863,803. In some embodiments, the ASO targets a sequence from about 4 to about 300 nucleotides downstream (or 3') from GRCh37/hg19: chr2:166,863,740. In some embodiments, the ASO targets a sequence at most about 10 nucleotides, at most about 20 nucleotides, at most about 50 nucleotides, at most about 80 nucleotides, at most about 85 nucleotides, at most about 90 nucleotides, at most about 95 nucleotides, at most about 96 nucleotides, at most about 97 nucleotides, at most about 98 nucleotides, at most about 99 nucleotides, at most about 100 nucleotides, at most about 101 nucleotides, at most about 102 nucleotides, at most about 103 nucleotides, at most about 104 nucleotides, at most about 105 nucleotides, at most about 110 nucleotides, at most about 120 nucleotides, at most about 150 nucleotides, at most about 200 nucleotides, at most about 300 nucleotides, at most about 400 nucleotides, at most about 500 nucleotides, at most about 600 nucleotides, at most about 700 nucleotides, at most about 800 nucleotides, at most about 900 nucleotides, or at most about 1000 nucleotides, at most about 1100 nucleotides, at most about 1200 nucleotides, at most about 1300 nucleotides, at most about 1400 nucleotides, or at most about 1500 nucleotides downstream from GRCh37/hg19: chr2:166,863,740. In some embodiments, the ASO targets a sequence more than 300 nucleotides downstream from GRCh37/hg19: chr2:166,863,740.

[0075] As described herein in the Examples, the *SCN1A* gene (**SEQ ID NO. 1**) was analyzed for NIE and inclusion of a portion of intron 20 (see **SEQ ID NO. 6** which encodes the Intron 20 pre-mRNA) (this portion is referred as Exon 23 or Exon 20x throughout the present disclosure) was observed. In some embodiments, the ASOs disclosed herein target a NIE containing pre-mRNA (**SEQ ID NO. 2**) transcribed from a *SCN1A* genomic sequence. In some embodiments, the ASO targets a NIE containing pre-mRNA transcript from a *SCN1A* genomic sequence comprising a portion of intron 20. In some embodiments, the ASO targets a NIE containing pre-mRNA transcript from a *SCN1A* genomic sequence comprising exon 23 (or exon 20x) (**SEQ ID NO. 4**). In some embodiments, the ASO targets a NIE containing pre-mRNA transcript of **SEQ ID NO. 2 or 9**. In some embodiments, the ASO targets a NIE containing pre-mRNA transcript of **SEQ ID NO. 2 or 9** comprising an NIE. In some embodiments, the ASO targets a NIE containing pre-mRNA transcript of **SEQ ID NO. 2** comprising exon 23 (or exon 20x) (**SEQ ID NO. 7**). In some embodiments, the ASOs disclosed herein target a *SCN1A* pre-mRNA sequence (**SEQ ID NO. 2 or 9**). In some embodiments, the ASO targets a *SCN1A* pre-mRNA sequence comprising an NIE (**SEQ ID NO. 7 or 11**). In some embodiments, the ASO targets a *SCN1A* pre-mRNA sequence according to any one of **SEQ ID NOs: 6, 7, 10, or 11**. In some embodiments, the ASO has a sequence according to any one of **SEQ ID NOs: 12-731**. In some

embodiments, the ASO has a sequence according to any one of **SEQ ID NOs: 12-371**. In some embodiments, the ASO has a sequence according to any one of **SEQ ID NOs: 372-731**.

[0076] In some embodiments, the *SCN1A* NIE containing pre-mRNA transcript is encoded by a genetic sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to **SEQ ID NO.: 1 or 8**. In some embodiments, the *SCN1A* NIE pre-mRNA transcript comprises a sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to any one of **SEQ ID NOs.: 2-7 and 9-11**.

[0077] In some embodiments, the *SCN1A* NIE containing pre-mRNA transcript (or NMD exon mRNA) comprises a sequence with at least about 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to any one of **SEQ ID NOs: 2, 6, 7, 9, 10, and 12**. In some embodiments, *SCN1A* NIE containing pre-mRNA transcript (or NMD exon mRNA) is encoded by a sequence with at least about 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to **SEQ ID NOs: 1 and 8**. In some embodiments, the targeted portion of the NMD exon mRNA comprises a sequence with at least 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to a region comprising at least 8 contiguous nucleic acids of **SEQ ID NOs: 2, 6, 7, 9, 10, and 12**.

[0078] In some embodiments, the ASO targets a sequence upstream from the 5' end of an NIE. For example, ASOs targeting a sequence upstream from the 5' end of an NIE (e.g. exon 23 (or exon 20x) in human *SCN1A*, or exon 21x in mouse *SCN1A*) can comprise a sequence with at least 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to any one of **SEQ ID NOs: 12-191 or 372-551**. For another example, ASOs targeting a sequence upstream from the 5' end of an NIE (e.g. exon 23 (or exon 20x) in human *SCN1A*, or exon 21x in mouse *SCN1A*) can comprise a sequence with at least 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to any one of **SEQ ID NOs: 12-191**. For an additional example, ASOs targeting a sequence upstream from the 5' end of an NIE (e.g. exon 23 (or exon 20x) in human *SCN1A*, or exon 21x in mouse *SCN1A*) can comprise a sequence with at least 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to any one of **SEQ ID NOs: 372-551**.

[0079] In some embodiments, the ASO targets exon 23 (or exon 20x) in a *SCN1A* NIE containing pre-mRNA comprising exon 23. In some embodiments, the ASO targets an exon 23 sequence downstream (or 3') from the 5' end of the exon 23 of a *SCN1A* pre-mRNA. In some embodiments, the ASO targets an exon 23 sequence upstream (or 5') from the 3' end of the exon 20x of a *SCN1A* pre-mRNA.

[0080] In some embodiments, the ASO targets a sequence downstream from the 3' end of an NIE. For example, ASOs targeting a sequence downstream from the 3' end of an NIE (e.g. exon 23 (or exon 20x) in human *SCN1A*, or exon 21x in mouse *SCN1A*) can comprise a sequence with at least 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to any one of **SEQ ID NOs: 192-371 or 552-731**. For another example, ASOs targeting a sequence downstream from the 3' end of an NIE (e.g. exon 23

(or exon 20x) in human *SCN1A*, or exon 21x in mouse *SCN1A*) can comprise a sequence with at least 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to any one of **SEQ ID NOs: 192-371**. For an additional example, ASOs targeting a sequence downstream from the 3' end of an NIE (e.g. exon 23 (or exon 20x) in human *SCN1A*, or exon 21x in mouse *SCN1A*) can comprise a sequence with at least 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to any one of **SEQ ID NOs: 552-731**.

[0081] In some embodiments, the targeted portion of the *SCN1A* NIE containing pre-mRNA is in intron 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 (intron numbering corresponding to the mRNA sequence at NM_006920). In some embodiments, hybridization of an ASO to the targeted portion of the NIE pre-mRNA results in exon skipping of at least one of NIE within intron 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25, and subsequently increases SCN1A protein production. In some embodiments, hybridization of an ASO to the targeted portion of the NIE pre-mRNA inhibits or blocks exon skipping of at least one of NIE within intron 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25, and subsequently decreases SCN1A protein production. In some embodiments, the targeted portion of the *SCN1A* NIE containing pre-mRNA is in intron 20. One of skill in the art can determine the corresponding intron number in any isoform based on an intron sequence provided herein or using the number provided in reference to the mRNA sequence at NM_006920, NM_001202435, NM_001165964, or NM_001165963. One of skill in the art also can determine the sequences of flanking exons in any *SCN1A* isoform for targeting using the methods of the invention, based on an intron sequence provided herein or using the intron number provided in reference to the mRNA sequence at NM_006920, NM_001202435, NM_001165964, or NM_001165963.

[0082] In some embodiments, the methods and compositions of the present disclosure are used to modulate, e.g., increase or decrease, the expression of SCN1A by inducing or inhibiting exon skipping of a pseudo-exon of an *SCN1A* NIE containing pre-mRNA. In some embodiments, the pseudo-exon is a sequence within any of introns 1-25. In some embodiments, the pseudo-exon is a sequence within any of introns 2, 4, 6, 13, 14, 15, 16, 17, 18, 20, 21, 22, 23, 24, and 25. In some embodiments, the pseudo-exon is a sequence within any of introns 15, 18, and 19. In some embodiments, the pseudo-exon can be any *SCN1A* intron or a portion thereof. In some embodiments, the pseudo-exon is within intron 20. The *SCN1A* intron numbering used herein corresponds to the mRNA sequence at NM_006920. It is understood that the intron numbering may change in reference to a different *SCN1A* isoform sequence.

SCN1A Protein

[0083] The *SCN1A* gene can encode SCN1A (sodium channel, voltage-gated, type I, alpha subunit) protein, which can also be referred to as alpha-subunit of voltage-gated sodium channel Na_v1.1. Also

described above, SCN1A mutations in DS are spread across the entire protein. More than 100 novel mutations have been identified throughout the gene with the more debilitating arising de novo. These comprise of truncations (47%), missense (43%), deletions (3%), and splice site mutations (7%). The percentage of subjects carrying SCN1A mutations varies between 33 and 100%. The majority of mutations are novel changes (88%).

[0084] In some embodiments, the methods described herein are used to modulate, e.g., increase or decrease, the production of a functional SCN1A protein. As used herein, the term “functional” refers to the amount of activity or function of a SCN1A protein that is necessary to eliminate any one or more symptoms of a treated condition, e.g., Dravet syndrome; Epilepsy, generalized, with febrile seizures plus, type 2; Febrile seizures, familial, 3A; Autism; Epileptic encephalopathy, early infantile, 13; Sick sinus syndrome 1; Alzheimer’s disease; or SUDEP. In some embodiments, the methods are used to increase the production of a partially functional SCN1A protein. As used herein, the term “partially functional” refers to any amount of activity or function of the SCN1A protein that is less than the amount of activity or function that is necessary to eliminate or prevent any one or more symptoms of a disease or condition. In some embodiments, a partially functional protein or RNA will have at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% less activity relative to the fully functional protein or RNA.

[0085] In some embodiments, the method is a method of increasing the expression of the SCN1A protein by cells of a subject having a NIE containing pre-mRNA encoding the SCN1A protein, wherein the subject has Dravet syndrome caused by a deficient amount of activity of SCN1A protein, and wherein the deficient amount of the SCN1A protein is caused by haploinsufficiency of the SCN1A protein. In such an embodiment, the subject has a first allele encoding a functional SCN1A protein, and a second allele from which the SCN1A protein is not produced. In another such embodiment, the subject has a first allele encoding a functional SCN1A protein, and a second allele encoding a nonfunctional SCN1A protein. In another such embodiment, the subject has a first allele encoding a functional SCN1A protein, and a second allele encoding a partially functional SCN1A protein. In any of these embodiments, the antisense oligomer binds to a targeted portion of the NIE containing pre-mRNA transcribed from the second allele, thereby inducing exon skipping of the pseudo-exon from the pre-mRNA, and causing an increase in the level of mature mRNA encoding functional SCN1A protein, and an increase in the expression of the SCN1A protein in the cells of the subject.

[0086] In related embodiments, the method is a method of using an ASO to increase the expression of a protein or functional RNA. In some embodiments, an ASO is used to increase the expression of SCN1A protein in cells of a subject having a NIE containing pre-mRNA encoding SCN1A protein,

wherein the subject has a deficiency, e.g., Dravet Syndrome (DS) (also known as SMEI); severe myoclonic epilepsy of infancy (SMEI)-borderland (SMEB); Febrile seizure (FS); epilepsy, generalized, with febrile seizures plus (GEFS+); epileptic encephalopathy, early infantile, 13; cryptogenic generalized epilepsy; cryptogenic focal epilepsy; myoclonic-astatic epilepsy; Lennox-Gastaut syndrome; West syndrome; idiopathic spasms; early myoclonic encephalopathy; progressive myoclonic epilepsy; alternating hemiplegia of childhood; unclassified epileptic encephalopathy; sudden unexpected death in epilepsy (SUDEP); sick sinus syndrome 1; early infantile SCN1A encephalopathy; early infantile epileptic encephalopathy (EIEE); or autism, in the amount or function of a SCN1A protein. In some embodiments, an ASO is used to increase the expression of SCN1A protein in cells of a subject, wherein the subject has a deficiency, e.g., Epileptic encephalopathy, early infantile, 13; in the amount or function of a SCN8A protein. In some embodiments, an ASO is used to increase the expression of SCN1A protein in cells of a subject, wherein the subject has a deficiency, e.g., Sick sinus syndrome 1; in the amount or function of a SCN5A protein.

[0087] In some embodiments, the NIE containing pre-mRNA transcript that encodes the protein that is causative of the disease or condition is targeted by the ASOs described herein. In some embodiments, a NIE containing pre-mRNA transcript that encodes a protein that is not causative of the disease is targeted by the ASOs. For example, a disease that is the result of a mutation or deficiency of a first protein in a particular pathway may be ameliorated by targeting a NIE containing pre-mRNA that encodes a second protein, thereby increasing production of the second protein. In some embodiments, the function of the second protein is able to compensate for the mutation or deficiency of the first protein (which is causative of the disease or condition).

[0088] In some embodiments, the subject has:

- (a) a first mutant allele from which
 - (i) the SCN1A protein is produced at a reduced level compared to production from a wild-type allele,
 - (ii) the SCN1A protein is produced in a form having reduced function compared to an equivalent wild-type protein, or
 - (iii) the SCN1A protein or functional RNA is not produced; and
- (b) a second mutant allele from which
 - (i) the SCN1A protein is produced at a reduced level compared to production from a wild-type allele,
 - (ii) the SCN1A protein is produced in a form having reduced function compared to an equivalent wild-type protein, or
 - (iii) the SCN1A protein is not produced, and

wherein the NIE containing pre-mRNA is transcribed from the first allele and/or the second allele. In these embodiments, the ASO binds to a targeted portion of the NIE containing pre-mRNA transcribed from the first allele or the second allele, thereby inducing exon skipping of the pseudo-exon from the NIE containing pre-mRNA, and causing an increase in the level of mRNA encoding SCN1A protein and an increase in the expression of the target protein or functional RNA in the cells of the subject. In these embodiments, the target protein or functional RNA having an increase in expression level resulting from the exon skipping of the pseudo-exon from the NIE containing pre-mRNA is either in a form having reduced function compared to the equivalent wild-type protein (partially-functional), or having full function compared to the equivalent wild-type protein (fully-functional).

[0089] In some embodiments, the level of mRNA encoding SCN1A protein is increased 1.1 to 10-fold, when compared to the amount of mRNA encoding SCN1A protein that is produced in a control cell, *e.g.*, one that is not treated with the antisense oligomer or one that is treated with an antisense oligomer that does not bind to the targeted portion of the *SCN1A* NIE containing pre-mRNA.

[0090] In some embodiments, a subject treated using the methods of the present disclosure expresses a partially functional SCN1A protein from one allele, wherein the partially functional SCN1A protein is caused by a frameshift mutation, a nonsense mutation, a missense mutation, or a partial gene deletion. In some embodiments, a subject treated using the methods of the invention expresses a nonfunctional SCN1A protein from one allele, wherein the nonfunctional SCN1A protein is caused by a frameshift mutation, a nonsense mutation, a missense mutation, a partial gene deletion, in one allele. In some embodiments, a subject treated using the methods of the invention has a *SCN1A* whole gene deletion, in one allele.

[0091] In some embodiments, the method is a method of decreasing the expression of the SCN1A protein by cells of a subject having a NIE containing pre-mRNA encoding the SCN1A protein, and wherein the subject has a gain-of-function mutation in Nav1.1. In such an embodiment, the subject has an allele from which the SCN1A protein is produced in an elevated amount or an allele encoding a mutant SCN1A that induces increased activity of Nav1.1 in the cell. In some embodiments, the increased activity of Nav1.1 is characterized by a prolonged or near persistent sodium current mediated by the mutant Nav1.1 channel, a slowing of fast inactivation, a positive shift in steady-state inactivation, higher channel availability during repetitive stimulation, increased non-inactivated depolarization-induced persistent sodium currents, delayed entry into inactivation, accelerated recovery from fast inactivation, and/or rescue of folding defects by incubation at lower temperature or co-expression of interacting proteins. In any of these embodiments, the antisense oligomer binds to a targeted portion of the NIE containing pre-mRNA transcribed from the second allele, thereby inhibiting or blocking exon skipping of the pseudo-exon from the pre-mRNA, and causing a decrease

in the level of mature mRNA encoding functional SCN1A protein, and a decrease in the expression of the SCN1A protein in the cells of the subject.

[0092] In related embodiments, the method is a method of using an ASO to decrease the expression of a protein or functional RNA. In some embodiments, an ASO is used to decrease the expression of SCN1A protein in cells of a subject having a NIE containing pre-mRNA encoding SCN1A protein. In some embodiments, the subject has a gain-of-function mutation in $Na_v1.1$, e.g., migraine. In some embodiments, an ASO is used to decrease the expression of SCN1A protein in cells of a subject, the subject has a gain-of-function mutation in $Na_v1.1$, e.g., migraine, familial hemiplegic, 3.

[0093] In some embodiments, the level of mRNA encoding SCN1A protein is decreased 1.1 to 10-fold, when compared to the amount of mRNA encoding SCN1A protein that is produced in a control cell, e.g., one that is not treated with the antisense oligomer or one that is treated with an antisense oligomer that does not bind to the targeted portion of the *SCN1A* NIE containing pre-mRNA.

[0094] In some embodiments, a subject treated using the methods of the present disclosure expresses a mutant SCN1A protein from one allele, wherein the mutant SCN1A protein is caused by a frameshift mutation, a nonsense mutation, a missense mutation, or a partial gene deletion, and wherein the mutant SCN1A protein causes an elevated activity level of $Na_v1.1$. In some embodiments, a subject treated using the methods of the present disclosure expresses an elevated amount of SCN1A protein from one allele due to a frameshift mutation, a nonsense mutation, a missense mutation, or a partial gene deletion.

[0095] In embodiments of the present invention, a subject can have a mutation in *SCN1A*. Mutations in *SCN1A* can be spread throughout said gene. SCN1A protein can consist of four domains. Said SCN1A domains can have transmembrane segments. Mutations in said SCN1A protein may arise throughout said protein. Said SCN1A protein may consist of at least two isoforms. Mutations in SCN1A may comprise of R931C, R946C, M934I, R1648C, or R1648H. In some cases, mutations may be observed in a C-terminus of a SCN1A protein. Mutations in a SCN1A protein may also be found in loops between segments 5 and 6 of the first three domains of said SCN1A protein. In some cases, mutations may be observed in an N-terminus of a SCN1A protein. Exemplary mutations within SCN1A include, but are not limited to, R222X, R712X, I227S, R1892X, W952X, R1245X, R1407X, W1434R, c.4338+1G>A, S1516X, L1670fsX1678, or K1846fsX1856. Mutations that can be targeted with the present invention may also encode a pore of an ion channel.

[0096] In some embodiments, the methods and compositions described herein can be used to treat DS. In other embodiments, the methods and compositions described herein can be used to treat severe myoclonic epilepsy of infancy (SMEI). In other embodiments, the methods and compositions described herein can be used to treat borderline Dravet syndrome; Epilepsy, generalized, with febrile seizures

plus, type 2; Febrile seizures, familial, 3A; Migraine, familial hemiplegic, 3; Autism; Epileptic encephalopathy, early infantile, 13; Sick sinus syndrome 1; Alzheimer's disease or SUDEP. The methods and compositions described herein can also be used to treat borderline SMEI. Additionally, the methods and compositions described herein can be used to treat generalized epilepsy with febrile seizures plus (GEFS+). GEFS+ may be associated with mutations in epilepsy-associated ion channel subunits such as SCN1B or GABRG2. The methods and compositions described herein can also be used to treat sodium channelopathies. Sodium channelopathies may be associated with mutations in SCN1A. Sodium channelopathies may also be associated with subunits of SCN1A, such as the beta subunit, SCN1B. In some cases, additional diseases associated with SCN1A mutations may also be treated with the present disclosure. Related SCN1A diseases associated with SCN1A mutations include, but are not limited to, atypical myotonia congenita, hyperkalemic periodic paralysis, and paramyotonia congenita.

[0097] In some embodiments, a subject having any SCN1A mutation known in the art and described in the literature referenced above (*e.g.*, by Hamdan, *et al.*, 2009, Mulley, *et al.*, 2005) can be treated using the methods and compositions described herein. In some embodiments, the mutation is within any *SCN1A* intron or exon.

Exon Inclusion

[0098] As used herein, a "NIE containing pre-mRNA" is a pre-mRNA transcript that contains at least one pseudo-exon. Alternative or aberrant splicing can result in inclusion of the at least one pseudo-exon in the mature mRNA transcripts. The terms "mature mRNA," and "fully-spliced mRNA," are used interchangeably herein to describe a fully processed mRNA. Inclusion of the at least one pseudo-exon can be non-productive mRNA and lead to NMD of the mature mRNA. NIE containing mature mRNA may sometimes lead to aberrant protein expression.

[0099] In some embodiments, the included pseudo-exon is the most abundant pseudo-exon in a population of NIE containing pre-mRNAs transcribed from the gene encoding the target protein in a cell. In some embodiments, the included pseudo-exon is the most abundant pseudo-exon in a population of NIE containing pre-mRNAs transcribed from the gene encoding the target protein in a cell, wherein the population of NIE containing pre-mRNAs comprises two or more included pseudo-exons. In some embodiments, an antisense oligomer targeted to the most abundant pseudo-exon in the population of NIE containing pre-mRNAs encoding the target protein induces exon skipping of one or two or more pseudo-exons in the population, including the pseudo-exon to which the antisense oligomer is targeted or binds. In embodiments, the targeted region is in a pseudo-exon that is the most abundant pseudo-exon in a NIE containing pre-mRNA encoding the SCN1A protein.

[00100] The degree of exon inclusion can be expressed as percent exon inclusion, e.g., the percentage of transcripts in which a given pseudo-exon is included. In brief, percent exon inclusion can be calculated as the percentage of the amount of RNA transcripts with the exon inclusion, over the sum of the average of the amount of RNA transcripts with exon inclusion plus the average of the amount of RNA transcripts with exon exclusion.

[00101] In some embodiments, an included pseudo-exon is an exon that is identified as an included pseudo-exon based on a determination of at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, or at least about 50%, inclusion. In embodiments, a included pseudo-exon is an exon that is identified as a included pseudo-exon based on a determination of about 5% to about 100%, about 5% to about 95%, about 5% to about 90%, about 5% to about 85%, about 5% to about 80%, about 5% to about 75%, about 5% to about 70%, about 5% to about 65%, about 5% to about 60%, about 5% to about 55%, about 5% to about 50%, about 5% to about 45%, about 5% to about 40%, about 5% to about 35%, about 5% to about 30%, about 5% to about 25%, about 5% to about 20%, about 5% to about 15%, about 10% to about 100%, about 10% to about 95%, about 10% to about 90%, about 10% to about 85%, about 10% to about 80%, about 10% to about 75%, about 10% to about 70%, about 10% to about 65%, about 10% to about 60%, about 10% to about 55%, about 10% to about 50%, about 10% to about 45%, about 10% to about 40%, about 10% to about 35%, about 10% to about 30%, about 10% to about 25%, about 10% to about 20%, about 15% to about 100%, about 15% to about 95%, about 15% to about 90%, about 15% to about 85%, about 15% to about 80%, about 15% to about 75%, about 15% to about 70%, about 15% to about 65%, about 15% to about 60%, about 15% to about 55%, about 15% to about 50%, about 15% to about 45%, about 15% to about 40%, about 15% to about 35%, about 15% to about 30%, about 15% to about 25%, about 20% to about 100%, about 20% to about 95%, about 20% to about 90%, about 20% to about 85%, about 20% to about 80%, about 20% to about 75%, about 20% to about 70%, about 20% to about 65%, about 20% to about 60%, about 20% to about 55%, about 20% to about 50%, about 20% to about 45%, about 20% to about 40%, about 20% to about 35%, about 20% to about 30%, about 25% to about 100%, about 25% to about 95%, about 25% to about 90%, about 25% to about 85%, about 25% to about 80%, about 25% to about 75%, about 25% to about 70%, about 25% to about 65%, about 25% to about 60%, about 25% to about 55%, about 25% to about 50%, about 25% to about 45%, about 25% to about 40%, or about 25% to about 35%, inclusion. ENCODE data (described by, e.g., Tilgner, *et al.*, 2012, “Deep sequencing of subcellular RNA fractions shows splicing to be predominantly co-transcriptional in the human genome but inefficient for lncRNAs,” *Genome Research* 22(9):1616-25) can be used to aid in identifying exon inclusion.

[00102] In some embodiments, contacting cells with an ASO that is complementary to a targeted portion of a *SCN1A* pre-mRNA transcript results in an increase in the amount of SCN1A protein produced by at least 10, 20, 30, 40, 50, 60, 80, 100, 150, 200, 250, 300, 350, 400, 450, 500, or 1000%, compared to the amount of the protein produced by a cell in the absence of the ASO/absence of treatment. In some embodiments, the total amount of SCN1A protein produced by the cell to which the antisense oligomer is contacted is increased about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to the amount of target protein produced by a control compound. A control compound can be, for example, an oligonucleotide that is not complementary to a targeted portion of the pre-mRNA.

[00103] In some embodiments, contacting cells with an ASO that is complementary to a targeted portion of a *SCN1A* pre-mRNA transcript results in a decrease in the amount of SCN1A protein produced by at least 10, 20, 30, 40, 50, 60, 80, 100, 150, 200, 250, 300, 350, 400, 450, 500, or 1000%, compared to the amount of the protein produced by a cell in the absence of the ASO/absence of treatment. In some embodiments, the total amount of SCN1A protein produced by the cell to which the antisense oligomer is contacted is decreased about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to the amount of target protein produced by a control compound. A control compound can be, for example, an oligonucleotide that is not complementary to a targeted portion of the pre-mRNA.

[00104] In some embodiments, contacting cells with an ASO that is complementary to a targeted portion of a *SCN1A* pre-mRNA transcript results in an increase in the amount of mRNA encoding SCN1A, including the mature mRNA encoding the target protein. In some embodiments, the amount of mRNA encoding SCN1A protein, or the mature mRNA encoding the SCN1A protein, is increased

by at least 10, 20, 30, 40, 50, 60, 80, 100, 150, 200, 250, 300, 350, 400, 450, 500, or 1000%, compared to the amount of the protein produced by a cell in the absence of the ASO/absence of treatment. In some embodiments, the total amount of the mRNA encoding SCN1A protein, or the mature mRNA encoding SCN1A protein produced in the cell to which the antisense oligomer is contacted is increased about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold compared to the amount of mature RNA produced in an untreated cell, *e.g.*, an untreated cell or a cell treated with a control compound. A control compound can be, for example, an oligonucleotide that is not complementary to a targeted portion of the SCN1A NIE containing pre-mRNA.

[00105] In some embodiments, contacting cells with an ASO that is complementary to a targeted portion of a *SCN1A* pre-mRNA transcript results in a decrease in the amount of mRNA encoding SCN1A, including the mature mRNA encoding the target protein. In some embodiments, the amount of mRNA encoding SCN1A protein, or the mature mRNA encoding the SCN1A protein, is decreased by at least 10, 20, 30, 40, 50, 60, 80, 100, 150, 200, 250, 300, 350, 400, 450, 500, or 1000%, compared to the amount of the protein produced by a cell in the absence of the ASO/absence of treatment. In some embodiments, the total amount of the mRNA encoding SCN1A protein, or the mature mRNA encoding SCN1A protein produced in the cell to which the antisense oligomer is contacted is decreased about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold compared to the amount of mature RNA produced in an untreated cell, *e.g.*, an untreated cell or a cell treated with a control compound. A control compound can be, for example, an oligonucleotide that is not complementary to a targeted portion of the SCN1A NIE containing pre-mRNA.

[00106] The NIE can be in any length. In some embodiments, the NIE comprises a full sequence of an intron, in which case, it can be referred to as intron retention. In some embodiments, the NIE can be a portion of the intron. In some embodiments, the NIE can be a 5' end portion of an intron including a 5'ss sequence. In some embodiments, the NIE can be a 3' end portion of an intron including a 3'ss sequence. In some embodiments, the NIE can be a portion within an intron without inclusion of a 5'ss sequence. In some embodiments, the NIE can be a portion within an intron without inclusion of a 3'ss sequence. In some embodiments, the NIE can be a portion within an intron without inclusion of either a 5'ss or a 3'ss sequence. In some embodiments, the NIE can be from 5 nucleotides to 10 nucleotides in length, from 10 nucleotides to 15 nucleotides in length, from 15 nucleotides to 20 nucleotides in length, from 20 nucleotides to 25 nucleotides in length, from 25 nucleotides to 30 nucleotides in length, from 30 nucleotides to 35 nucleotides in length, from 35 nucleotides to 40 nucleotides in length, from 40 nucleotides to 45 nucleotides in length, from 45 nucleotides to 50 nucleotides in length, from 50 nucleotides to 55 nucleotides in length, from 55 nucleotides to 60 nucleotides in length, from 60 nucleotides to 65 nucleotides in length, from 65 nucleotides to 70 nucleotides in length, from 70 nucleotides to 75 nucleotides in length, from 75 nucleotides to 80 nucleotides in length, from 80 nucleotides to 85 nucleotides in length, from 85 nucleotides to 90 nucleotides in length, from 90 nucleotides to 95 nucleotides in length, or from 95 nucleotides to 100 nucleotides in length. In some embodiments, the NIE can be at least 10 nucleotides, at least 20 nucleotides, at least 30 nucleotides, at least 40 nucleotides, at least 50 nucleotides, at least 60 nucleotides, at least 70 nucleotides, at least 80 nucleotides in length, at least 90 nucleotides, or at least 100 nucleotides in length. In some embodiments, the NIE can be from 100 to 200 nucleotides in length, from 200 to 300 nucleotides in length, from 300 to 400 nucleotides in length, from 400 to 500 nucleotides in length, from 500 to 600 nucleotides in length, from 600 to 700 nucleotides in length, from 700 to 800 nucleotides in length, from 800 to 900 nucleotides in length, from 900 to 1,000 nucleotides in length. In some embodiments, the NIE may be longer than 1,000 nucleotides in length.

[00107] Inclusion of a pseudo-exon can lead to a frameshift and the introduction of a premature termination codon (PIC) in the mature mRNA transcript rendering the transcript a target of NMD. Mature mRNA transcript containing NIE can be non-productive mRNA transcript which does not lead to protein expression. The PIC can be present in any position downstream of an NIE. In some embodiments, the PIC can be present in any exon downstream of an NIE. In some embodiments, the PIC can be present within the NIE. For example, inclusion of exon 20x in an mRNA transcript encoded by the *SCN1A* gene can induce a PIC in the mRNA transcript, e.g., a PIC in exon 21 of the mRNA transcript.

Therapeutic Agents

[00108] In various embodiments of the present disclosure, compositions and methods comprising a therapeutic agent are provided to modulate protein expression level of SCN1A. In some embodiments, provided herein are compositions and methods to modulate alternative splicing of *SCN1A* pre-mRNA. In some embodiments, provided herein are compositions and methods to induce exon skipping in the splicing of SCN1A pre-mRNA, e.g., to induce skipping of a pseudo-exon during splicing of SCN1A pre-mRNA. In other embodiments, therapeutic agents may be used to induce the inclusion of an exon in order to decrease the protein expression level.

[00109] In some embodiment, a therapeutic agent disclosed herein is a small molecule, a polypeptide, or a polynucleic acid polymer. In some instances, the therapeutic agent is a small molecule. In some instances, the therapeutic agent is a polypeptide. In some instances, the therapeutic agent is a polynucleic acid polymer. In some cases, the therapeutic agent is a repressor agent. In additional cases, the therapeutic agent is an enhancer agent.

[00110] A therapeutic agent disclosed herein can be a NIE repressor agent. A therapeutic agent may comprise a polynucleic acid polymer.

[00111] According to one aspect of the present disclosure, provided herein is a method of treatment or prevention of a condition associated with a functional-SCN1A protein deficiency, comprising administering a NIE repressor agent to a subject to increase levels of functional SCN1A protein, wherein the agent binds to a region of the pre-mRNA transcript to decrease inclusion of the NIE in the mature transcript. For example, provided herein is a method of treatment or prevention of a condition associated with a functional-SCN1A protein deficiency, comprising administering a NIE repressor agent to a subject to increase levels of functional SCN1A protein, wherein the agent binds to a region of an intron containing an NIE (e.g., intron 20 in human *SCN1A* gene) of the pre-mRNA transcript or to a NIE-activating regulatory sequence in the same intron.

[00112] Where reference is made to reducing NIE inclusion in the mature mRNA, the reduction may be complete, e.g., 100%, or may be partial. The reduction may be clinically significant. The reduction/correction may be relative to the level of NIE inclusion in the subject without treatment, or relative to the amount of NIE inclusion in a population of similar subjects. The reduction/correction may be at least 10% less NIE inclusion relative to the average subject, or the subject prior to treatment. The reduction may be at least 20% less NIE inclusion relative to an average subject, or the subject prior to treatment. The reduction may be at least 40% less NIE inclusion relative to an average subject, or the subject prior to treatment. The reduction may be at least 50% less NIE inclusion relative to an average subject, or the subject prior to treatment. The reduction may be at least 60% less NIE inclusion relative to an average subject, or the subject prior to treatment. The reduction may be at least 80% less

NIE inclusion relative to an average subject, or the subject prior to treatment. The reduction may be at least 90% less NIE inclusion relative to an average subject, or the subject prior to treatment.

[00113] Where reference is made to increasing active-SCN1A protein levels, the increase may be clinically significant. The increase may be relative to the level of active-SCN1A protein in the subject without treatment, or relative to the amount of active-SCN1A protein in a population of similar subjects. The increase may be at least 10% more active-SCN1A protein relative to the average subject, or the subject prior to treatment. The increase may be at least 20% more active-SCN1A protein relative to the average subject, or the subject prior to treatment. The increase may be at least 40% more active-SCN1A protein relative to the average subject, or the subject prior to treatment. The increase may be at least 50% more active-SCN1A protein relative to the average subject, or the subject prior to treatment. The increase may be at least 80% more active-SCN1A protein relative to the average subject, or the subject prior to treatment. The increase may be at least 100% more active-SCN1A protein relative to the average subject, or the subject prior to treatment. The increase may be at least 200% more active-SCN1A protein relative to the average subject, or the subject prior to treatment. The increase may be at least 500% more active-SCN1A protein relative to the average subject, or the subject prior to treatment.

[00114] In embodiments wherein the NIE repressor agent comprises a polynucleic acid polymer, the polynucleic acid polymer may be about 50 nucleotides in length. The polynucleic acid polymer may be about 45 nucleotides in length. The polynucleic acid polymer may be about 40 nucleotides in length. The polynucleic acid polymer may be about 35 nucleotides in length. The polynucleic acid polymer may be about 30 nucleotides in length. The polynucleic acid polymer may be about 24 nucleotides in length. The polynucleic acid polymer may be about 25 nucleotides in length. The polynucleic acid polymer may be about 20 nucleotides in length. The polynucleic acid polymer may be about 19 nucleotides in length. The polynucleic acid polymer may be about 18 nucleotides in length. The polynucleic acid polymer may be about 17 nucleotides in length. The polynucleic acid polymer may be about 16 nucleotides in length. The polynucleic acid polymer may be about 15 nucleotides in length. The polynucleic acid polymer may be about 14 nucleotides in length. The polynucleic acid polymer may be about 13 nucleotides in length. The polynucleic acid polymer may be about 12 nucleotides in length. The polynucleic acid polymer may be about 11 nucleotides in length. The polynucleic acid polymer may be about 10 nucleotides in length. The polynucleic acid polymer may be between about 10 and about 50 nucleotides in length. The polynucleic acid polymer may be between about 10 and about 45 nucleotides in length. The polynucleic acid polymer may be between about 10 and about 40 nucleotides in length. The polynucleic acid polymer may be between about 10 and about 35 nucleotides in length. The polynucleic acid polymer may be between about 10 and about 30 nucleotides in length.

The polynucleic acid polymer may be between about 10 and about 25 nucleotides in length. The polynucleic acid polymer may be between about 10 and about 20 nucleotides in length. The polynucleic acid polymer may be between about 15 and about 25 nucleotides in length. The polynucleic acid polymer may be between about 15 and about 30 nucleotides in length. The polynucleic acid polymer may be between about 12 and about 30 nucleotides in length.

[00115] The sequence of the polynucleic acid polymer may be at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.5% complementary to a target sequence of an mRNA transcript, e.g., a partially processed mRNA transcript. The sequence of the polynucleic acid polymer may be 100% complementary to a target sequence of a pre-mRNA transcript.

[00116] The sequence of the polynucleic acid polymer may have 4 or fewer mismatches to a target sequence of the pre-mRNA transcript. The sequence of the polynucleic acid polymer may have 3 or fewer mismatches to a target sequence of the pre-mRNA transcript. The sequence of the polynucleic acid polymer may have 2 or fewer mismatches to a target sequence of the pre-mRNA transcript. The sequence of the polynucleic acid polymer may have 1 or fewer mismatches to a target sequence of the pre-mRNA transcript. The sequence of the polynucleic acid polymer may have no mismatches to a target sequence of the pre-mRNA transcript.

[00117] The polynucleic acid polymer may specifically hybridize to a target sequence of the pre-mRNA transcript. For example, the polynucleic acid polymer may have 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or 100% sequence complementarity to a target sequence of the pre-mRNA transcript. The hybridization may be under high stringent hybridization conditions.

[00118] The polynucleic acid polymer may have a sequence with at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.5% sequence identity to a sequence selected from the group consisting of **SEQ ID NOs: 12-731**. The polynucleic acid polymer may have a sequence with 100% sequence identity to a sequence selected from the group consisting of **SEQ ID NOs: 12-731**. In some instances, the polynucleic acid polymer may have a sequence with at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.5% sequence identity to a sequence selected from the group consisting of **SEQ ID NOs: 12-371**. In some cases, the polynucleic acid polymer may have a sequence with 100% sequence identity to a sequence selected from the group consisting of **SEQ ID NOs: 12-371**. In some instances, the polynucleic acid polymer may have a sequence with at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.5% sequence identity to a sequence selected from the group consisting of **SEQ ID NOs: 372-731**. In some cases, the

polynucleic acid polymer may have a sequence with 100% sequence identity to a sequence selected from the group consisting of **SEQ ID NOs: 372-731**.

[00119] Where reference is made to a polynucleic acid polymer sequence, the skilled person will understand that one or more substitutions may be tolerated, optionally two substitutions may be tolerated in the sequence, such that it maintains the ability to hybridize to the target sequence; or where the substitution is in a target sequence, the ability to be recognized as the target sequence. References to sequence identity may be determined by BLAST sequence alignment using standard/default parameters. For example, the sequence may have 99% identity and still function according to the present disclosure. In other embodiments, the sequence may have 98% identity and still function according to the present disclosure. In another embodiment, the sequence may have 95% identity and still function according to the present disclosure. In another embodiment, the sequence may have 90% identity and still function according to the present disclosure.

Antisense Oligomers

[00120] Provided herein is a composition comprising an antisense oligomer that induces exon skipping by binding to a targeted portion of a *SCN1A* NIE containing pre-mRNA. As used herein, the terms “ASO” and “antisense oligomer” are used interchangeably and refer to an oligomer such as a polynucleotide, comprising nucleobases that hybridizes to a target nucleic acid (*e.g.*, a *SCN1A* NIE containing pre-mRNA) sequence by Watson-Crick base pairing or wobble base pairing (G-U). The ASO may have exact sequence complementary to the target sequence or near complementarity (*e.g.*, sufficient complementarity to bind the target sequence and enhancing splicing at a splice site). ASOs are designed so that they bind (hybridize) to a target nucleic acid (*e.g.*, a targeted portion of a pre-mRNA transcript) and remain hybridized under physiological conditions. Typically, if they hybridize to a site other than the intended (targeted) nucleic acid sequence, they hybridize to a limited number of sequences that are not a target nucleic acid (to a few sites other than a target nucleic acid). Design of an ASO can take into consideration the occurrence of the nucleic acid sequence of the targeted portion of the pre-mRNA transcript or a sufficiently similar nucleic acid sequence in other locations in the genome or cellular pre-mRNA or transcriptome, such that the likelihood the ASO will bind other sites and cause “off-target” effects is limited. Any antisense oligomers known in the art, for example in PCT Application No. PCT/US2014/054151, published as WO 2015/035091, titled “Reducing Nonsense-Mediated mRNA Decay,” incorporated by reference herein, can be used to practice the methods described herein.

[00121] In some embodiments, ASOs “specifically hybridize” to or are “specific” to a target nucleic acid or a targeted portion of a NIE containing pre-mRNA. Typically such hybridization occurs with a T_m substantially greater than 37 °C, preferably at least 50 °C, and typically between 60 °C to

approximately 90 °C. Such hybridization preferably corresponds to stringent hybridization conditions. At a given ionic strength and pH, the T_m is the temperature at which 50% of a target sequence hybridizes to a complementary oligonucleotide.

[00122] Oligomers, such as oligonucleotides, are “complementary” to one another when hybridization occurs in an antiparallel configuration between two single-stranded polynucleotides. A double-stranded polynucleotide can be “complementary” to another polynucleotide, if hybridization can occur between one of the strands of the first polynucleotide and the second. Complementarity (the degree to which one polynucleotide is complementary with another) is quantifiable in terms of the proportion (*e.g.*, the percentage) of bases in opposing strands that are expected to form hydrogen bonds with each other, according to generally accepted base-pairing rules. The sequence of an antisense oligomer (ASO) need not be 100% complementary to that of its target nucleic acid to hybridize. In certain embodiments, ASOs can comprise at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence complementarity to a target region within the target nucleic acid sequence to which they are targeted. For example, an ASO in which 18 of 20 nucleobases of the oligomeric compound are complementary to a target region, and would therefore specifically hybridize, would represent 90 percent complementarity. In this example, the remaining non-complementary nucleobases may be clustered together or interspersed with complementary nucleobases and need not be contiguous to each other or to complementary nucleobases. Percent complementarity of an ASO with a region of a target nucleic acid can be determined routinely using BLAST programs (basic local alignment search tools) and PowerBLAST programs known in the art (Altschul, *et al.*, J. Mol. Biol., 1990, 215, 403-410; Zhang and Madden, Genome Res., 1997, 7, 649-656).

[00123] An ASO need not hybridize to all nucleobases in a target sequence and the nucleobases to which it does hybridize may be contiguous or noncontiguous. ASOs may hybridize over one or more segments of a pre-mRNA transcript, such that intervening or adjacent segments are not involved in the hybridization event (*e.g.*, a loop structure or hairpin structure may be formed). In certain embodiments, an ASO hybridizes to noncontiguous nucleobases in a target pre-mRNA transcript. For example, an ASO can hybridize to nucleobases in a pre-mRNA transcript that are separated by one or more nucleobase(s) to which the ASO does not hybridize.

[00124] The ASOs described herein comprise nucleobases that are complementary to nucleobases present in a targeted portion of a NIE containing pre-mRNA. The term ASO embodies oligonucleotides and any other oligomeric molecule that comprises nucleobases capable of hybridizing to a complementary nucleobase on a target mRNA but does not comprise a sugar moiety, such as a peptide nucleic acid (PNA). The ASOs may comprise naturally-occurring nucleotides, nucleotide analogs,

modified nucleotides, or any combination of two or three of the preceding. The term “naturally occurring nucleotides” includes deoxyribonucleotides and ribonucleotides. The term “modified nucleotides” includes nucleotides with modified or substituted sugar groups and/or having a modified backbone. In some embodiments, all of the nucleotides of the ASO are modified nucleotides. Chemical modifications of ASOs or components of ASOs that are compatible with the methods and compositions described herein will be evident to one of skill in the art and can be found, for example, in U.S. Patent No. 8,258,109 B2, U.S. Patent No. 5,656,612, U.S. Patent Publication No. 2012/0190728, and Dias and Stein, *Mol. Cancer Ther.* 2002, 347-355, herein incorporated by reference in their entirety.

[00125] One or more nucleobases of an ASO may be any naturally occurring, unmodified nucleobase such as adenine, guanine, cytosine, thymine and uracil, or any synthetic or modified nucleobase that is sufficiently similar to an unmodified nucleobase such that it is capable of hydrogen bonding with a nucleobase present on a target pre-mRNA. Examples of modified nucleobases include, without limitation, hypoxanthine, xanthine, 7-methylguanine, 5, 6-dihydrouracil, 5-methylcytosine, and 5-hydroxymethylcytosine.

[00126] The ASOs described herein also comprise a backbone structure that connects the components of an oligomer. The term “backbone structure” and “oligomer linkages” may be used interchangeably and refer to the connection between monomers of the ASO. In naturally occurring oligonucleotides, the backbone comprises a 3'-5' phosphodiester linkage connecting sugar moieties of the oligomer. The backbone structure or oligomer linkages of the ASOs described herein may include (but are not limited to) phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoraniladate, phosphoramidate, and the like. See, *e.g.*, LaPlanche, *et al.*, *Nucleic Acids Res.* 14:9081 (1986); Stec, *et al.*, *J. Am. Chem. Soc.* 106:6077 (1984), Stein, *et al.*, *Nucleic Acids Res.* 16:3209 (1988), Zon, *et al.*, *Anti-Cancer Drug Design* 6:539 (1991); Zon, *et al.*, *Oligonucleotides and Analogues: A Practical Approach*, pp. 87-108 (F. Eckstein, Ed., Oxford University Press, Oxford England (1991)); Stec, *et al.*, U.S. Pat. No. 5,151,510; Uhlmann and Peyman, *Chemical Reviews* 90:543 (1990). In some embodiments, the backbone structure of the ASO does not contain phosphorous but rather contains peptide bonds, for example in a peptide nucleic acid (PNA), or linking groups including carbamate, amides, and linear and cyclic hydrocarbon groups. In some embodiments, the backbone modification is a phosphothioate linkage. In some embodiments, the backbone modification is a phosphoramidate linkage.

[00127] In embodiments, the stereochemistry at each of the phosphorus internucleotide linkages of the ASO backbone is random. In embodiments, the stereochemistry at each of the phosphorus internucleotide linkages of the ASO backbone is controlled and is not random. For example, U.S. Pat. App. Pub. No. 2014/0194610, “Methods for the Synthesis of Functionalized Nucleic Acids,”

incorporated herein by reference, describes methods for independently selecting the handedness of chirality at each phosphorous atom in a nucleic acid oligomer. In embodiments, an ASO used in the methods of the invention, including, but not limited to, any of the ASOs set forth herein in Tables 5 and 6, comprises an ASO having phosphorus internucleotide linkages that are not random. In embodiments, a composition used in the methods of the invention comprises a pure diastereomeric ASO. In embodiments, a composition used in the methods of the invention comprises an ASO that has diastereomeric purity of at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, about 100%, about 90% to about 100%, about 91% to about 100%, about 92% to about 100%, about 93% to about 100%, about 94% to about 100%, about 95% to about 100%, about 96% to about 100%, about 97% to about 100%, about 98% to about 100%, or about 99% to about 100%.

[00128] In embodiments, the ASO has a nonrandom mixture of Rp and Sp configurations at its phosphorus internucleotide linkages. For example, it has been suggested that a mix of Rp and Sp is required in antisense oligonucleotides to achieve a balance between good activity and nuclease stability (Wan, *et al.*, 2014, "Synthesis, biophysical properties and biological activity of second generation antisense oligonucleotides containing chiral phosphorothioate linkages," *Nucleic Acids Research* 42(22): 13456-13468, incorporated herein by reference). In embodiments, an ASO used in the methods of the invention, including, but not limited to, any of the ASOs set forth herein in **SEQ ID NOs: 12-731**, comprises about 5-100% Rp, at least about 5% Rp, at least about 10% Rp, at least about 15% Rp, at least about 20% Rp, at least about 25% Rp, at least about 30% Rp, at least about 35% Rp, at least about 40% Rp, at least about 45% Rp, at least about 50% Rp, at least about 55% Rp, at least about 60% Rp, at least about 65% Rp, at least about 70% Rp, at least about 75% Rp, at least about 80% Rp, at least about 85% Rp, at least about 90% Rp, or at least about 95% Rp, with the remainder Sp, or about 100% Rp. In embodiments, an ASO used in the methods of the invention, including, but not limited to, any of the ASOs set forth herein in **SEQ ID NOs: 12-731**, comprises about 10% to about 100% Rp, about 15% to about 100% Rp, about 20% to about 100% Rp, about 25% to about 100% Rp, about 30% to about 100% Rp, about 35% to about 100% Rp, about 40% to about 100% Rp, about 45% to about 100% Rp, about 50% to about 100% Rp, about 55% to about 100% Rp, about 60% to about 100% Rp, about 65% to about 100% Rp, about 70% to about 100% Rp, about 75% to about 100% Rp, about 80% to about 100% Rp, about 85% to about 100% Rp, about 90% to about 100% Rp, or about 95% to about 100% Rp, about 20% to about 80% Rp, about 25% to about 75% Rp, about 30% to about 70% Rp, about 40% to about 60% Rp, or about 45% to about 55% Rp, with the remainder Sp.

[00129] In embodiments, an ASO used in the methods of the invention, including, but not limited to, any of the ASOs set forth herein in **SEQ ID NOs: 12-731**, comprises about 5-100% Sp, at least about 5% Sp, at least about 10% Sp, at least about 15% Sp, at least about 20% Sp, at least about 25% Sp, at least about 30% Sp, at least about 35% Sp, at least about 40% Sp, at least about 45% Sp, at least about 50% Sp, at least about 55% Sp, at least about 60% Sp, at least about 65% Sp, at least about 70% Sp, at least about 75% Sp, at least about 80% Sp, at least about 85% Sp, at least about 90% Sp, or at least about 95% Sp, with the remainder Rp, or about 100% Sp. In embodiments, an ASO used in the methods of the invention, including, but not limited to, any of the ASOs set forth herein in **SEQ ID NOs: 12-731**, comprises about 10% to about 100% Sp, about 15% to about 100% Sp, about 20% to about 100% Sp, about 25% to about 100% Sp, about 30% to about 100% Sp, about 35% to about 100% Sp, about 40% to about 100% Sp, about 45% to about 100% Sp, about 50% to about 100% Sp, about 55% to about 100% Sp, about 60% to about 100% Sp, about 65% to about 100% Sp, about 70% to about 100% Sp, about 75% to about 100% Sp, about 80% to about 100% Sp, about 85% to about 100% Sp, about 90% to about 100% Sp, or about 95% to about 100% Sp, about 20% to about 80% Sp, about 25% to about 75% Sp, about 30% to about 70% Sp, about 40% to about 60% Sp, or about 45% to about 55% Sp, with the remainder Rp.

[00130] Any of the ASOs described herein may contain a sugar moiety that comprises ribose or deoxyribose, as present in naturally occurring nucleotides, or a modified sugar moiety or sugar analog, including a morpholine ring. Non-limiting examples of modified sugar moieties include 2' substitutions such as 2'-O-methyl (2'-O-Me), 2'-O-methoxyethyl (2'MOE), 2'-O-aminoethyl, 2'F; N3'->P5' phosphoramidate, 2'dimethylaminooxyethoxy, 2'dimethylaminoethoxyethoxy, 2'-guanidinidium, 2'-O-guanidinium ethyl, carbamate modified sugars, and bicyclic modified sugars. In some embodiments, the sugar moiety modification is selected from 2'-O-Me, 2'F, and 2'MOE. In some embodiments, the sugar moiety modification is an extra bridge bond, such as in a locked nucleic acid (LNA). In some embodiments the sugar analog contains a morpholine ring, such as phosphorodiamidate morpholino (PMO). In some embodiments, the sugar moiety comprises a ribofuransyl or 2'deoxyribofuransyl modification. In some embodiments, the sugar moiety comprises 2'4'-constrained 2'O-methoxyethyl (cMOE) modifications. In some embodiments, the sugar moiety comprises cEt 2', 4' constrained 2'-O ethyl BNA modifications. In some embodiments, the sugar moiety comprises tricycloDNA (tcDNA) modifications. In some embodiments, the sugar moiety comprises ethylene nucleic acid (ENA) modifications. In some embodiments, the sugar moiety comprises MCE modifications. Modifications are known in the art and described in the literature, *e.g.*, by Jarver, *et al.*, 2014, "A Chemical View of Oligonucleotides for Exon Skipping and Related Drug

Applications,” *Nucleic Acid Therapeutics* 24(1): 37-47, incorporated by reference for this purpose herein.

[00131] In some embodiments, each monomer of the ASO is modified in the same way, for example each linkage of the backbone of the ASO comprises a phosphorothioate linkage or each ribose sugar moiety comprises a 2'-O-methyl modification. Such modifications that are present on each of the monomer components of an ASO are referred to as “uniform modifications.” In some examples, a combination of different modifications may be desired, for example, an ASO may comprise a combination of phosphorodiamidate linkages and sugar moieties comprising morpholine rings (morpholinos). Combinations of different modifications to an ASO are referred to as “mixed modifications” or “mixed chemistries.”

[00132] In some embodiments, the ASO comprises one or more backbone modifications. In some embodiments, the ASO comprises one or more sugar moiety modification. In some embodiments, the ASO comprises one or more backbone modifications and one or more sugar moiety modifications. In some embodiments, the ASO comprises a 2'MOE modification and a phosphorothioate backbone. In some embodiments, the ASO comprises a phosphorodiamidate morpholino (PMO). In some embodiments, the ASO comprises a peptide nucleic acid (PNA). Any of the ASOs or any component of an ASO (*e.g.*, a nucleobase, sugar moiety, backbone) described herein may be modified in order to achieve desired properties or activities of the ASO or reduce undesired properties or activities of the ASO. For example, an ASO or one or more components of any ASO may be modified to enhance binding affinity to a target sequence on a pre-mRNA transcript; reduce binding to any non-target sequence; reduce degradation by cellular nucleases (*i.e.*, RNase H); improve uptake of the ASO into a cell and/or into the nucleus of a cell; alter the pharmacokinetics or pharmacodynamics of the ASO; and/or modulate the half-life of the ASO.

[00133] In some embodiments, the ASOs are comprised of 2'-O-(2-methoxyethyl) (MOE) phosphorothioate-modified nucleotides. ASOs comprised of such nucleotides are especially well-suited to the methods disclosed herein; oligomers having such modifications have been shown to have significantly enhanced resistance to nuclease degradation and increased bioavailability, making them suitable, for example, for oral delivery in some embodiments described herein. See *e.g.*, Geary, *et al.*, *J Pharmacol Exp Ther.* 2001; 296(3):890-7; Geary, *et al.*, *J Pharmacol Exp Ther.* 2001; 296(3):898-904.

[00134] Methods of synthesizing ASOs will be known to one of skill in the art. Alternatively or in addition, ASOs may be obtained from a commercial source.

[00135] Unless specified otherwise, the left-hand end of single-stranded nucleic acid (*e.g.*, pre-mRNA transcript, oligonucleotide, ASO, etc.) sequences is the 5' end and the left-hand direction of single or

double-stranded nucleic acid sequences is referred to as the 5' direction. Similarly, the right-hand end or direction of a nucleic acid sequence (single or double stranded) is the 3' end or direction. Generally, a region or sequence that is 5' to a reference point in a nucleic acid is referred to as "upstream," and a region or sequence that is 3' to a reference point in a nucleic acid is referred to as "downstream." Generally, the 5' direction or end of an mRNA is where the initiation or start codon is located, while the 3' end or direction is where the termination codon is located. In some aspects, nucleotides that are upstream of a reference point in a nucleic acid may be designated by a negative number, while nucleotides that are downstream of a reference point may be designated by a positive number. For example, a reference point (*e.g.*, an exon-exon junction in mRNA) may be designated as the "zero" site, and a nucleotide that is directly adjacent and upstream of the reference point is designated "minus one," *e.g.*, "-1," while a nucleotide that is directly adjacent and downstream of the reference point is designated "plus one," *e.g.*, "+1."

[00136] In some embodiments, the ASOs are complementary to (and bind to) a targeted portion of a *SCN1A* NIE containing pre-mRNA that is downstream (in the 3' direction) of the 5' splice site (or 3' end of the NIE) of the included exon in a *SCN1A* NIE containing pre-mRNA (*e.g.*, the direction designated by positive numbers relative to the 5' splice site). In some embodiments, the ASOs are complementary to a targeted portion of the *SCN1A* NIE containing pre-mRNA that is within the region about +1 to about +500 relative to the 5' splice site (or 3' end) of the included exon. In some embodiments, the ASOs may be complementary to a targeted portion of a *SCN1A* NIE containing pre-mRNA that is within the region between nucleotides +6 and +496 relative to the 5' splice site (or 3' end) of the included exon. In some aspects, the ASOs are complementary to a targeted portion that is within the region about +1 to about +500, about +1 to about +490, about +1 to about +480, about +1 to about +470, about +1 to about +460, about +1 to about +450, about +1 to about +440, about +1 to about +430, about +1 to about +420, about +1 to about +410, about +1 to about +400, about +1 to about +390, about +1 to about +380, about +1 to about +370, about +1 to about +360, about +1 to about +350, about +1 to about +340, about +1 to about +330, about +1 to about +320, about +1 to about +310, about +1 to about +300, about +1 to about +290, about +1 to about +280, about +1 to about +270, about +1 to about +260, about +1 to about +250, about +1 to about +240, about +1 to about +230, about +1 to about +220, about +1 to about +210, about +1 to about +200, about +1 to about +190, about +1 to about +180, about +1 to about +170, about +1 to about +160, about +1 to about +150, about +1 to about +140, about +1 to about +130, about +1 to about +120, about +1 to about +110, about +1 to about +100, about +1 to about +90, about +1 to about +80, about +1 to about +70, about +1 to about +60, about +1 to about +50, about +1 to about +40, about +1 to about +30, or about +1 to about +20 relative to 5' splice site (or 3' end) of the included exon. In some aspects, the

ASOs are complementary to a targeted portion that is within the region from about +1 to about +100, from about +100 to about +200, from about +200 to about +300, from about +300 to about +400, or from about +400 to about +500 relative to 5' splice site (or 3' end) of the included exon.

[00137] In some embodiments, the ASOs are complementary to (and bind to) a targeted portion of a *SCN1A* NIE containing pre-mRNA that is upstream (in the 5' direction) of the 5' splice site (or 3' end) of the included exon in a *SCN1A* NIE containing pre-mRNA (e.g., the direction designated by negative numbers relative to the 5' splice site). In some embodiments, the ASOs are complementary to a targeted portion of the *SCN1A* NIE containing pre-mRNA that is within the region about -4 to about -270 relative to the 5' splice site (or 3' end) of the included exon. In some embodiments, the ASOs may be complementary to a targeted portion of a *SCN1A* NIE containing pre-mRNA that is within the region between nucleotides -1 and -264 relative to the 5' splice site (or 3' end) of the included exon. In some aspects, the ASOs are complementary to a targeted portion that is within the region about -1 to about -270, about -1 to about -260, about -1 to about -250, about -1 to about -240, about -1 to about -230, about -1 to about -220, about -1 to about -210, about -1 to about -200, about -1 to about -190, about -1 to about -180, about -1 to about -170, about -1 to about -160, about -1 to about -150, about -1 to about -140, about -1 to about -130, about -1 to about -120, about -1 to about -110, about -1 to about -100, about -1 to about -90, about -1 to about -80, about -1 to about -70, about -1 to about -60, about -1 to about -50, about -1 to about -40, about -1 to about -30, or about -1 to about -20 relative to 5' splice site (or 3' end) of the included exon. In some aspects, the ASOs are complementary to a targeted portion that is within the region from about -1 to about -50, from about -50 to about -100, from about -100 to about -150, from about -150 to about -200, or from about -200 to about -250 relative to 5' splice site (or 3' end) of the included exon.

[00138] In some embodiments, the ASOs are complementary to a targeted region of a *SCN1A* NIE containing pre-mRNA that is upstream (in the 5' direction) of the 3' splice site (or 5' end) of the included exon in a *SCN1A* NIE containing pre-mRNA (e.g., in the direction designated by negative numbers). In some embodiments, the ASOs are complementary to a targeted portion of the *SCN1A* NIE containing pre-mRNA that is within the region about -1 to about -500 relative to the 3' splice site (or 5' end) of the included exon. In some embodiments, the ASOs are complementary to a targeted portion of the *SCN1A* NIE containing pre-mRNA that is within the region -1 to -496 relative to the 3' splice site of the included exon. In some aspects, the ASOs are complementary to a targeted portion that is within the region about -1 to about -500, about -1 to about -490, about -1 to about -480, about -1 to about -470, about -1 to about -460, about -1 to about -450, about -1 to about -440, about -1 to about -430, about -1 to about -420, about -1 to about -410, about -1 to about -400, about -1 to about -390, about -1 to about -380, about -1 to about -370, about -1 to about -360, about -1 to about -350,

about -1 to about -340, about -1 to about -330, about -1 to about -320, about -1 to about -310, about -1 to about -300, about -1 to about -290, about -1 to about -280, about -1 to about -270, about -1 to about -260, about -1 to about -250, about -1 to about -240, about -1 to about -230, about -1 to about -220, about -1 to about -210, about -1 to about -200, about -1 to about -190, about -1 to about -180, about -1 to about -170, about -1 to about -160, about -1 to about -150, about -1 to about -140, about -1 to about -130, about -1 to about -120, about -1 to about -110, about -1 to about -100, about -1 to about -90, about -1 to about -80, about -1 to about -70, about -1 to about -60, about -1 to about -50, about -1 to about -40, or about -1 to about -30 relative to 3' splice site of the included exon. In some aspects, the ASOs are complementary to a targeted portion that is within the region from about -1 to about -100, from about -100 to about -200, from about -200 to about -300, from about -300 to about -400, or from about -400 to about -500 relative to 3' splice site of the included exon.

[00139] In some embodiments, the ASOs are complementary to a targeted region of a *SCN1A NIE containing* pre-mRNA that is downstream (in the 3' direction) of the 3' splice site (5' end) of the included exon in a *SCN1A NIE containing* pre-mRNA (e.g., in the direction designated by positive numbers). In some embodiments, the ASOs are complementary to a targeted portion of the *SCN1A NIE containing* pre-mRNA that is within the region of about +1 to about +100 relative to the 3' splice site of the included exon. In some aspects, the ASOs are complementary to a targeted portion that is within the region about +1 to about +90, about +1 to about +80, about +1 to about +70, about +1 to about +60, about +1 to about +50, about +1 to about +40, about +1 to about +30, about +1 to about +20, or about +1 to about +10 relative to 3' splice site of the included exon.

[00140] In some embodiments, the targeted portion of the *SCN1A NIE containing* pre-mRNA is within the region +100 relative to the 5' splice site (3' end) of the included exon to -100 relative to the 3' splice site (5' end) of the included exon. In some embodiments, the targeted portion of the *SCN1A NIE containing* pre-mRNA is within the NIE. In some embodiments, the targeted portion of the *SCN1A NIE containing* pre-mRNA comprises a pseudo-exon and intron boundary.

[00141] The ASOs may be of any length suitable for specific binding and effective enhancement of splicing. In some embodiments, the ASOs consist of 8 to 50 nucleobases. For example, the ASO may be 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 40, 45, or 50 nucleobases in length. In some embodiments, the ASOs consist of more than 50 nucleobases. In some embodiments, the ASO is from 8 to 50 nucleobases, 8 to 40 nucleobases, 8 to 35 nucleobases, 8 to 30 nucleobases, 8 to 25 nucleobases, 8 to 20 nucleobases, 8 to 15 nucleobases, 9 to 50 nucleobases, 9 to 40 nucleobases, 9 to 35 nucleobases, 9 to 30 nucleobases, 9 to 25 nucleobases, 9 to 20 nucleobases, 9 to 15 nucleobases, 10 to 50 nucleobases, 10 to 40 nucleobases, 10 to 35 nucleobases, 10 to 30 nucleobases, 10 to 25 nucleobases, 10 to 20 nucleobases, 10 to 15 nucleobases,

11 to 50 nucleobases, 11 to 40 nucleobases, 11 to 35 nucleobases, 11 to 30 nucleobases, 11 to 25 nucleobases, 11 to 20 nucleobases, 11 to 15 nucleobases, 12 to 50 nucleobases, 12 to 40 nucleobases, 12 to 35 nucleobases, 12 to 30 nucleobases, 12 to 25 nucleobases, 12 to 20 nucleobases, 12 to 15 nucleobases, 13 to 50 nucleobases, 13 to 40 nucleobases, 13 to 35 nucleobases, 13 to 30 nucleobases, 13 to 25 nucleobases, 13 to 20 nucleobases, 14 to 50 nucleobases, 14 to 40 nucleobases, 14 to 35 nucleobases, 14 to 30 nucleobases, 14 to 25 nucleobases, 14 to 20 nucleobases, 15 to 50 nucleobases, 15 to 40 nucleobases, 15 to 35 nucleobases, 15 to 30 nucleobases, 15 to 25 nucleobases, 15 to 20 nucleobases, 20 to 50 nucleobases, 20 to 40 nucleobases, 20 to 35 nucleobases, 20 to 30 nucleobases, 20 to 25 nucleobases, 25 to 50 nucleobases, 25 to 40 nucleobases, 25 to 35 nucleobases, or 25 to 30 nucleobases in length. In some embodiments, the ASOs are 18 nucleotides in length. In some embodiments, the ASOs are 15 nucleotides in length. In some embodiments, the ASOs are 25 nucleotides in length.

[00142] In some embodiments, two or more ASOs with different chemistries but complementary to the same targeted portion of the NIE containing pre-mRNA are used. In some embodiments, two or more ASOs that are complementary to different targeted portions of the NIE containing pre-mRNA are used.

[00143] In embodiments, the antisense oligonucleotides of the invention are chemically linked to one or more moieties or conjugates, *e.g.*, a targeting moiety or other conjugate that enhances the activity or cellular uptake of the oligonucleotide. Such moieties include, but are not limited to, a lipid moiety, *e.g.*, as a cholesterol moiety, a cholesteryl moiety, an aliphatic chain, *e.g.*, dodecandiol or undecyl residues, a polyamine or a polyethylene glycol chain, or adamantane acetic acid. Oligonucleotides comprising lipophilic moieties and preparation methods have been described in the published literature. In embodiments, the antisense oligonucleotide is conjugated with a moiety including, but not limited to, an abasic nucleotide, a polyether, a polyamine, a polyamide, a peptides, a carbohydrate, *e.g.*, N-acetylgalactosamine (GalNAc), N-Ac-Glucosamine (GluNAc), or mannose (*e.g.*, mannose-6-phosphate), a lipid, or a polyhydrocarbon compound. Conjugates can be linked to one or more of any nucleotides comprising the antisense oligonucleotide at any of several positions on the sugar, base or phosphate group, as understood in the art and described in the literature, *e.g.*, using a linker. Linkers can include a bivalent or trivalent branched linker. In embodiments, the conjugate is attached to the 3' end of the antisense oligonucleotide. Methods of preparing oligonucleotide conjugates are described, *e.g.*, in U.S. Pat. No. 8,450,467, "Carbohydrate conjugates as delivery agents for oligonucleotides," incorporated by reference herein.

[00144] In some embodiments, the nucleic acid to be targeted by an ASO is a *SCN1A* NIE containing pre-mRNA expressed in a cell, such as a eukaryotic cell. In some embodiments, the term "cell" may

refer to a population of cells. In some embodiments, the cell is in a subject. In some embodiments, the cell is isolated from a subject. In some embodiments, the cell is *ex vivo*. In some embodiments, the cell is a condition or disease-relevant cell or a cell line. In some embodiments, the cell is *in vitro* (e.g., in cell culture).

Pharmaceutical Compositions

[00145] Pharmaceutical compositions or formulations comprising the agent, e.g., antisense oligonucleotide, of the described compositions and for use in any of the described methods can be prepared according to conventional techniques well known in the pharmaceutical industry and described in the published literature. In embodiments, a pharmaceutical composition or formulation for treating a subject comprises an effective amount of any antisense oligomer as described herein, or a pharmaceutically acceptable salt, solvate, hydrate or ester thereof. The pharmaceutical formulation comprising an antisense oligomer may further comprise a pharmaceutically acceptable excipient, diluent or carrier.

[00146] Pharmaceutically acceptable salts are suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response, etc., and are commensurate with a reasonable benefit/risk ratio. (See, e.g., S. M. Berge, et al., J. Pharmaceutical Sciences, 66: 1-19 (1977), incorporated herein by reference for this purpose. The salts can be prepared in situ during the final isolation and purification of the compounds, or separately by reacting the free base function with a suitable organic acid. Examples of pharmaceutically acceptable, nontoxic acid addition salts are salts of an amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid or by using other documented methodologies such as ion exchange. Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p-toluenesulfonate, undecanoate, valerate salts, and the like. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like. Further pharmaceutically acceptable salts include, when appropriate,

nontoxic ammonium, quaternary ammonium, and amine cations formed using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, loweralkyl sulfonate and aryl sulfonate.

[00147] In embodiments, the compositions are formulated into any of many possible dosage forms such as, but not limited to, tablets, capsules, gel capsules, liquid syrups, soft gels, suppositories, and enemas. In embodiments, the compositions are formulated as suspensions in aqueous, non-aqueous or mixed media. Aqueous suspensions may further contain substances that increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. The suspension may also contain stabilizers. In embodiments, a pharmaceutical formulation or composition of the present invention includes, but is not limited to, a solution, emulsion, microemulsion, foam or liposome-containing formulation (*e.g.*, cationic or noncationic liposomes).

[00148] The pharmaceutical composition or formulation described herein may comprise one or more penetration enhancers, carriers, excipients or other active or inactive ingredients as appropriate and well known to those of skill in the art or described in the published literature. In embodiments, liposomes also include sterically stabilized liposomes, *e.g.*, liposomes comprising one or more specialized lipids. These specialized lipids result in liposomes with enhanced circulation lifetimes. In embodiments, a sterically stabilized liposome comprises one or more glycolipids or is derivatized with one or more hydrophilic polymers, such as a polyethylene glycol (PEG) moiety. In embodiments, a surfactant is included in the pharmaceutical formulation or compositions. The use of surfactants in drug products, formulations and emulsions is well known in the art. In embodiments, the present invention employs a penetration enhancer to effect the efficient delivery of the antisense oligonucleotide, *e.g.*, to aid diffusion across cell membranes and /or enhance the permeability of a lipophilic drug. In embodiments, the penetration enhancers are a surfactant, fatty acid, bile salt, chelating agent, or non-chelating nonsurfactant.

[00149] In embodiments, the pharmaceutical formulation comprises multiple antisense oligonucleotides. In embodiments, the antisense oligonucleotide is administered in combination with another drug or therapeutic agent.

Combination Therapies

[00150] In some embodiments, the ASOs disclosed in the present disclosure can be used in combination with one or more additional therapeutic agents. In some embodiments, the one or more additional therapeutic agents can comprise a small molecule. For example, the one or more additional therapeutic agents can comprise a small molecule described in WO2016128343A1, WO2017053982A1, WO2016196386A1, WO201428459A1, WO201524876A2, WO2013119916A2, and WO2014209841A2, which are incorporated by reference herein in their entirety. In some embodiments, the one or more additional therapeutic agents comprise an ASO that can be used to

correct intron retention. In some embodiments, the one or more other agents are selected from the ASOs listed in **Table 4**.

Treatment of Subjects

[00151] Any of the compositions provided herein may be administered to an individual. “Individual” may be used interchangeably with “subject” or “patient.” An individual may be a mammal, for example a human or animal such as a non-human primate, a rodent, a rabbit, a rat, a mouse, a horse, a donkey, a goat, a cat, a dog, a cow, a pig, or a sheep. In embodiments, the individual is a human. In embodiments, the individual is a fetus, an embryo, or a child. In other embodiments, the individual may be another eukaryotic organism, such as a plant. In some embodiments, the compositions provided herein are administered to a cell *ex vivo*.

[00152] In some embodiments, the compositions provided herein are administered to an individual as a method of treating a disease or disorder. In some embodiments, the individual has a genetic disease, such as any of the diseases described herein. In some embodiments, the individual is at risk of having a disease, such as any of the diseases described herein. In some embodiments, the individual is at increased risk of having a disease or disorder caused by insufficient amount of a protein or insufficient activity of a protein. If an individual is “at an increased risk” of having a disease or disorder caused by insufficient amount of a protein or insufficient activity of a protein, the method involves preventative or prophylactic treatment. For example, an individual may be at an increased risk of having such a disease or disorder because of family history of the disease. Typically, individuals at an increased risk of having such a disease or disorder benefit from prophylactic treatment (*e.g.*, by preventing or delaying the onset or progression of the disease or disorder). In embodiments, a fetus is treated in utero, *e.g.*, by administering the ASO composition to the fetus directly or indirectly (*e.g.*, via the mother).

[00153] Suitable routes for administration of ASOs of the present invention may vary depending on cell type to which delivery of the ASOs is desired. Multiple tissues and organs are affected by Dravet syndrome; Epilepsy, generalized, with febrile seizures plus, type 2; Febrile seizures, familial, 3A; Migraine, familial hemiplegic, 3; Autism; Epileptic encephalopathy, early infantile, 13; Sick sinus syndrome 1; Alzheimer’s disease or SUDEP, with the brain being the most significantly affected tissue. The ASOs of the present invention may be administered to patients parenterally, for example, by intrathecal injection, intracerebroventricular injection, intraperitoneal injection, intramuscular injection, subcutaneous injection, intravitreal injection, or intravenous injection.

[00154] In some embodiments, the disease or condition is induced by a mutation in $Na_v1.1$ (a protein encoded by the *SCN1A* gene). In some instances, the mutation is a loss-of-function mutation in $Na_v1.1$. In some cases, the loss-of-function mutation in $Na_v1.1$ comprises one or more mutations that decreases

or impairs the function of Na_v1.1 (e.g., by 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more) relative to the function of a wild-type Na_v1.1. In some cases, the loss-of-function mutation in Na_v1.1 comprises one or more mutations that result in a disease phenotype. Exemplary loss-of-function mutations include, but are not limited to, R859C, T875M, V1353L, I1656M, R1657C, A1685V, M1841T, and R1916G.

[00155] In other instances, the mutation is a gain-of-function mutation in Na_v1.1. In such cases, the gain-of-function mutation comprises one or more mutations that prolongs activation of Na_v1.1 (e.g., by 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more) relative to the function of a wild-type Na_v1.1. In such cases, the gain-of-function mutation in Na_v1.1 comprises one or more mutations that result in a disease phenotype. Exemplary gain-of-function mutations include, but are not limited to, D188V, W1204R, R1648H, and D1866Y.

[00156] In some embodiments, the disease or condition is an encephalopathy. In some cases, the encephalopathy is induced by a loss-of-function mutation in Na_v1.1.

[00157] In some embodiments, the encephalopathy is epileptic encephalopathy. Exemplary epileptic encephalopathies include, but are not limited to, Dravet Syndrome (DS) (also known as severe myoclonic epilepsy of infancy or SMEI); severe myoclonic epilepsy of infancy (SMEI)-borderland (SMEB); Febrile seizure (FS); epilepsy, generalized, with febrile seizures plus (GEFS+); epileptic encephalopathy, early infantile, 13; cryptogenic generalized epilepsy; cryptogenic focal epilepsy; myoclonic-astatic epilepsy; Lennox-Gastaut syndrome; West syndrome; idiopathic spasms; early myoclonic encephalopathy; progressive myoclonic epilepsy; alternating hemiplegia of childhood; unclassified epileptic encephalopathy; sudden unexpected death in epilepsy (SUDEP); early infantile SCN1A encephalopathy; early infantile epileptic encephalopathy (EIEE); or sick sinus syndrome 1. In some embodiments, the disease or condition is epileptic encephalopathy, optionally selected from Dravet Syndrome (DS) (also known as severe myoclonic epilepsy of infancy or SMEI); severe myoclonic epilepsy of infancy (SMEI)-borderland (SMEB); Febrile seizure (FS); epilepsy, generalized, with febrile seizures plus (GEFS+); epileptic encephalopathy, early infantile, 13; cryptogenic generalized epilepsy; cryptogenic focal epilepsy; myoclonic-astatic epilepsy; Lennox-Gastaut syndrome; West syndrome; idiopathic spasms; early myoclonic encephalopathy; progressive myoclonic epilepsy; alternating hemiplegia of childhood; unclassified epileptic encephalopathy; sudden unexpected death in epilepsy (SUDEP); and sick sinus syndrome 1.

[00158] In some instances, GEFS+ is epilepsy, generalized, with febrile seizures plus, type 2.

[00159] In some instances, the Febrile seizure is Febrile seizures, familial, 3A.

[00160] In some instances, SMEB is SMEB without generalized spike wave (SMEB-SW), SMEB without myoclonic seizures (SMEB-M), SMEB lacking more than one feature of SMEI (SMEB-O), or intractable childhood epilepsy with generalized tonic-clonic seizures (ICEGTC).

[00161] In some embodiments, the diseases or conditions induced by a loss-of-function mutation in $Na_v1.1$ include, but are not limited to, Dravet Syndrome (DS) (also known as SMEI); severe myoclonic epilepsy of infancy (SMEI)-borderland (SMEB); Febrile seizure (FS); epilepsy, generalized, with febrile seizures plus (GEFS+); epileptic encephalopathy, early infantile, 13; cryptogenic generalized epilepsy; cryptogenic focal epilepsy; myoclonic-astatic epilepsy; Lennox-Gastaut syndrome; West syndrome; idiopathic spasms; early myoclonic encephalopathy; progressive myoclonic epilepsy; alternating hemiplegia of childhood; unclassified epileptic encephalopathy; sudden unexpected death in epilepsy (SUDEP); sick sinus syndrome 1; early infantile *SCN1A* encephalopathy; early infantile epileptic encephalopathy (EIEE); autism; or malignant migrating partial seizures of infancy.

[00162] In some embodiments, the disease or condition is induced by a gain-of-function mutation in $Na_v1.1$. Exemplary diseases or conditions associated with a gain-of-function mutation in $Na_v1.1$ include, but are not limited to, migraine. In some instances, the disease or condition induced by a gain-of-function mutation in $Na_v1.1$ is migraine.

[00163] In some instances, the migraine is migraine, familial hemiplegic, 3.

[00164] In some embodiments, the disease or condition is a $Na_v1.1$ genetic epilepsy. The $Na_v1.1$ genetic epilepsy can include a loss-of-function mutation in $Na_v1.1$ or a gain-of-function mutation in $Na_v1.1$. In some cases, the $Na_v1.1$ genetic epilepsy includes one or more hereditary mutations. In other cases, the $Na_v1.1$ genetic epilepsy includes one or more de novo mutations. In some cases, the $Na_v1.1$ genetic epilepsy includes Dravet Syndrome (DS) (also known as severe myoclonic epilepsy of infancy or SMEI); severe myoclonic epilepsy of infancy (SMEI)-borderland (SMEB); Febrile seizure (FS); epilepsy, generalized, with febrile seizures plus (GEFS+); epileptic encephalopathy, early infantile, 13; cryptogenic generalized epilepsy; cryptogenic focal epilepsy; myoclonic-astatic epilepsy; Lennox-Gastaut syndrome; West syndrome; idiopathic spasms; early myoclonic encephalopathy; progressive myoclonic epilepsy; alternating hemiplegia of childhood; unclassified epileptic encephalopathy; early infantile *SCN1A* encephalopathy; early infantile epileptic encephalopathy (EIEE); sudden unexpected death in epilepsy (SUDEP); or malignant migrating partial seizures of infancy. In some cases, the $Na_v1.1$ genetic epilepsy associated with a loss-of-function mutation in $Na_v1.1$ includes Dravet Syndrome (DS) (also known as severe myoclonic epilepsy of infancy or SMEI); severe myoclonic epilepsy of infancy (SMEI)-borderland (SMEB); Febrile seizure (FS); epilepsy, generalized, with febrile seizures plus (GEFS+); epileptic encephalopathy, early infantile, 13; cryptogenic generalized epilepsy; cryptogenic focal epilepsy; myoclonic-astatic epilepsy; Lennox-Gastaut syndrome; West

syndrome; idiopathic spasms; early myoclonic encephalopathy; progressive myoclonic epilepsy; alternating hemiplegia of childhood; unclassified epileptic encephalopathy; early infantile *SCN1A* encephalopathy; early infantile epileptic encephalopathy (EIEE); sudden unexpected death in epilepsy (SUDEP); malignant migrating partial seizures of infancy.

[00165] In some embodiments, the disease or condition is associated with a haploinsufficiency of the *SCN1A* gene. Exemplary diseases or conditions associated with a haploinsufficiency of the *SCN1A* gene include, but are not limited to, Dravet Syndrome (DS) (also known as SMEI); severe myoclonic epilepsy of infancy (SMEI)-borderland (SMEB); Febrile seizure (FS); epilepsy, generalized, with febrile seizures plus (GEFS+); epileptic encephalopathy, early infantile, 13; cryptogenic generalized epilepsy; cryptogenic focal epilepsy; myoclonic-astatic epilepsy; Lennox-Gastaut syndrome; West syndrome; idiopathic spasms; early myoclonic encephalopathy; progressive myoclonic epilepsy; alternating hemiplegia of childhood; unclassified epileptic encephalopathy; sudden unexpected death in epilepsy (SUDEP); sick sinus syndrome 1; early infantile *SCN1A* encephalopathy; early infantile epileptic encephalopathy (EIEE); or malignant migrating partial seizures of infancy. In some cases, the disease or condition is Dravet Syndrome (DS) (also known as SMEI); severe myoclonic epilepsy of infancy (SMEI)-borderland (SMEB); Febrile seizure (FS); epilepsy, generalized, with febrile seizures plus (GEFS+); epileptic encephalopathy, early infantile, 13; cryptogenic generalized epilepsy; cryptogenic focal epilepsy; myoclonic-astatic epilepsy; Lennox-Gastaut syndrome; West syndrome; idiopathic spasms; early myoclonic encephalopathy; progressive myoclonic epilepsy; alternating hemiplegia of childhood; unclassified epileptic encephalopathy; sudden unexpected death in epilepsy (SUDEP); sick sinus syndrome 1; early infantile *SCN1A* encephalopathy; early infantile epileptic encephalopathy (EIEE); or malignant migrating partial seizures of infancy.

[00166] In some cases, the disease or condition is Dravet Syndrome (DS).

[00167] Dravet syndrome (DS), otherwise known as severe myoclonic epilepsy of infancy (SMEI), is an epileptic encephalopathy presenting in the first year of life. Dravet syndrome is an increasingly recognized epileptic encephalopathy in which the clinical diagnosis is supported by the finding of sodium channel gene mutations in approximately 70–80% of patients. Mutations of ion channel genes play a major role in the pathogenesis of a range of epilepsy syndromes, resulting in some epilepsies being regarded as channelopathies. Voltage-gated sodium channels (VGSCs) play an essential role in neuronal excitability; therefore, it is not surprising that many mutations associated with DS have been identified in the gene encoding a VGSC subunit. The disease is described by, e.g., Mulley, et al., 2005, and the disease description at OMIM #607208 (Online Mendelian Inheritance in Man, Johns Hopkins University, 1966-2015), both incorporated by reference herein.

[00168] Between 70% and 80% of patients carry sodium channel $\alpha 1$ subunit gene (*SCN1A*) abnormalities, and truncating mutations account for about 40%, and have a significant correlation with an earlier age of seizures onset. Sequencing mutations are found in about 70% of cases and comprise truncating (40%) and missense mutations (40%) with the remaining being splice-site changes. Most mutations are de novo, but familial mutations occur in 5–10% of cases and are usually missense in nature. The remaining *SCN1A* mutations comprise splice-site and missense mutations, most of which fall into the pore-forming region of the sodium channel. At present, over 500 mutations have been associated with DS and are randomly distributed along the gene (Mulley, et al., *Neurol.* 2006, 67, 1094-1095).

[00169] The *SCN1A* gene is located in the cluster of sodium channel genes on human chromosome 2q24 and encodes the α -pore forming subunits known as Nav1.1 of the neuronal voltage gated sodium channel. The *SCN1A* gene spans approximately 100 kb of genomic DNA and comprises 26 exons. The SCN1A protein consists of four domains, each with six-transmembrane segments. Two splice variants have been identified that result in a long and short isoform that differ in the presence or absence of 11 amino acids in the cytoplasmic loop between domains 1 and 2, in exon 11 (Miller, et al., 1993-2015, and Mulley, et al., 2005, 25, 535-542, incorporated herein by reference).

[00170] Alternative splicing events in *SCN1A* gene can lead to non-productive mRNA transcripts which in turn can lead to aberrant protein expression, and therapeutic agents which can target the alternative splicing events in *SCN1A* gene can modulate the expression level of functional proteins in DS patients and/or inhibit aberrant protein expression. Such therapeutic agents can be used to treat a condition caused by SCN1A protein deficiency.

[00171] One of the alternative splicing events that can lead to non-productive mRNA transcripts is the inclusion of an extra exon in the mRNA transcript that can induce non-sense mediated mRNA decay. The present disclosure provides compositions and methods for modulating alternative splicing of *SCN1A* to increase the production of protein-coding mature mRNA, and thus, translated functional SCN1A protein. These compositions and methods include antisense oligomers (ASOs) that can cause exon skipping and promote constitutive splicing of *SCN1A* pre-mRNA. In various embodiments, functional SCN1A protein can be increased using the methods of the disclosure to treat a condition caused by SCN1A protein deficiency.

[00172] In some cases, the disease or condition is SMEB.

[00173] In some cases, the disease or condition is GEFS+.

[00174] In some cases, the disease or condition is a Febrile seizure (e.g., Febrile seizures, familial, 3A).

[00175] In some cases, the disease or condition is autism (also known as autism spectrum disorder or ASD).

[00176] In some cases, the disease or condition is migraine (e.g., migraine, familial hemiplegic, 3).

[00177] In some cases, the disease or condition is Alzheimer's disease.

[00178] In some embodiments, the disease or condition is *SCN2A* encephalopathy.

[00179] In some embodiments, the disease or condition is *SCN8A* encephalopathy.

[00180] In some embodiments, the disease or condition is *SCN5A* arrhythmia.

[00181] In embodiments, the antisense oligonucleotide is administered with one or more agents capable of promoting penetration of the subject antisense oligonucleotide across the blood-brain barrier by any method known in the art. For example, delivery of agents by administration of an adenovirus vector to motor neurons in muscle tissue is described in U.S. Pat. No. 6,632,427, "Adenoviral-vector-mediated gene transfer into medullary motor neurons," incorporated herein by reference. Delivery of vectors directly to the brain, e.g., the striatum, the thalamus, the hippocampus, or the substantia nigra, is described, e.g., in U.S. Pat. No. 6,756,523, "Adenovirus vectors for the transfer of foreign genes into cells of the central nervous system particularly in brain," incorporated herein by reference.

[00182] In embodiments, the antisense oligonucleotides are linked or conjugated with agents that provide desirable pharmaceutical or pharmacodynamic properties. In embodiments, the antisense oligonucleotide is coupled to a substance, known in the art to promote penetration or transport across the blood-brain barrier, e.g., an antibody to the transferrin receptor. In embodiments, the antisense oligonucleotide is linked with a viral vector, e.g., to render the antisense compound more effective or increase transport across the blood-brain barrier. In embodiments, osmotic blood brain barrier disruption is assisted by infusion of sugars, e.g., meso erythritol, xylitol, D(+) galactose, D(+) lactose, D(+) xylose, dulcitol, myo-inositol, L(-) fructose, D(-) mannitol, D(+) glucose, D(+) arabinose, D(-) arabinose, cellobiose, D(+) maltose, D(+) raffinose, L(+) rhamnose, D(+) melibiose, D(-) ribose, adonitol, D(+) arabitol, L(-) arabitol, D(+) fucose, L(-) fucose, D(-) lyxose, L(+) lyxose, and L(-) lyxose, or amino acids, e.g., glutamine, lysine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glycine, histidine, leucine, methionine, phenylalanine, proline, serine, threonine, tyrosine, valine, and taurine. Methods and materials for enhancing blood brain barrier penetration are described, e.g., in U.S. Pat. No. 9,193,969, "Compositions and methods for selective delivery of oligonucleotide molecules to specific neuron types," U.S. Pat. No. 4,866,042, "Method for the delivery of genetic material across the blood brain barrier," U.S. Pat. No. 6,294,520, "Material for passage through the blood-brain barrier," and U.S. Pat. No. 6,936,589, "Parenteral delivery systems," each incorporated herein by reference.

[00183] In embodiments, an ASO of the invention is coupled to a dopamine reuptake inhibitor (DRI), a selective serotonin reuptake inhibitor (SSRI), a noradrenaline reuptake inhibitor (NRI), a norepinephrine-dopamine reuptake inhibitor (NDRI), and a serotonin-norepinephrine-dopamine reuptake inhibitor (SNDRI), using methods described in, *e.g.*, U.S. Pat. No. 9,193,969, incorporated herein by reference.

[00184] In embodiments, subjects treated using the methods and compositions are evaluated for improvement in condition using any methods known and described in the art.

Methods of Identifying Additional ASOs that Induce Exon Skipping

[00185] Also within the scope of the present disclosure are methods for identifying or determining ASOs that induce exon skipping of a *SCN1A* NIE containing pre-mRNA. For example, a method can comprise identifying or determining ASOs that induce pseudo-exon skipping of a *SCN1A* NIE containing pre-mRNA. ASOs that specifically hybridize to different nucleotides within the target region of the pre-mRNA may be screened to identify or determine ASOs that improve the rate and/or extent of splicing of the target intron. In some embodiments, the ASO may block or interfere with the binding site(s) of a splicing repressor(s)/silencer. Any method known in the art may be used to identify (determine) an ASO that when hybridized to the target region of the exon results in the desired effect (*e.g.*, pseudo-exon skipping, protein or functional RNA production). These methods also can be used for identifying ASOs that induce exon skipping of the included exon by binding to a targeted region in an intron flanking the included exon, or in a non-included exon. An example of a method that may be used is provided below.

[00186] A round of screening, referred to as an ASO “walk” may be performed using ASOs that have been designed to hybridize to a target region of a pre-mRNA. For example, the ASOs used in the ASO walk can be tiled every 5 nucleotides from approximately 100 nucleotides upstream of the 3’ splice site of the included exon (*e.g.*, a portion of sequence of the exon located upstream of the target/included exon) to approximately 100 nucleotides downstream of the 3’ splice site of the target/included exon and/or from approximately 100 nucleotides upstream of the 5’ splice site of the included exon to approximately 100 nucleotides downstream of the 5’ splice site of the target/included exon (*e.g.*, a portion of sequence of the exon located downstream of the target/included exon). For example, a first ASO of 15 nucleotides in length may be designed to specifically hybridize to nucleotides +6 to +20 relative to the 3’ splice site of the target/included exon. A second ASO may be designed to specifically hybridize to nucleotides +11 to +25 relative to the 3’ splice site of the target/included exon. ASOs are designed as such spanning the target region of the pre-mRNA. In embodiments, the ASOs can be tiled more closely, *e.g.*, every 1, 2, 3, or 4 nucleotides. Further, the ASOs can be tiled from 100 nucleotides downstream of the 5’ splice site, to 100 nucleotides upstream of the 3’ splice site. In some

embodiments, the ASOs can be tiled from about 1,160 nucleotides upstream of the 3' splice site, to about 500 nucleotides downstream of the 5' splice site. In some embodiments, the ASOs can be tiled from about 500 nucleotides upstream of the 3' splice site, to about 1,920 nucleotides downstream of the 3' splice site.

[00187] One or more ASOs, or a control ASO (an ASO with a scrambled sequence, sequence that is not expected to hybridize to the target region) are delivered, for example by transfection, into a disease-relevant cell line that expresses the target pre-mRNA (*e.g.*, a NIE containing pre-mRNA described herein). The exon skipping effects of each of the ASOs may be assessed by any method known in the art, for example by reverse transcriptase (RT)-PCR using primers that span the splice junction, as described in **Example 4**. A reduction or absence of a longer RT-PCR product produced using the primers spanning the region containing the included exon (*e.g.* including the flanking exons of the NIE) in ASO-treated cells as compared to in control ASO-treated cells indicates that splicing of the target NIE has been enhanced. In some embodiments, the exon skipping efficiency (or the splicing efficiency to splice the intron containing the NIE), the ratio of spliced to unspliced pre-mRNA, the rate of splicing, or the extent of splicing may be improved using the ASOs described herein. The amount of protein or functional RNA that is encoded by the target pre-mRNA can also be assessed to determine whether each ASO achieved the desired effect (*e.g.*, enhanced functional protein production). Any method known in the art for assessing and/or quantifying protein production, such as Western blotting, flow cytometry, immunofluorescence microscopy, and ELISA, can be used.

[00188] A second round of screening, referred to as an ASO “micro-walk” may be performed using ASOs that have been designed to hybridize to a target region of a pre-mRNA. The ASOs used in the ASO micro-walk are tiled every 1 nucleotide to further refine the nucleotide acid sequence of the pre-mRNA that when hybridized with an ASO results in exon skipping (or enhanced splicing of NIE).

[00189] Regions defined by ASOs that promote splicing of the target intron are explored in greater detail by means of an ASO “micro-walk”, involving ASOs spaced in 1-nt steps, as well as longer ASOs, typically 18-25 nt.

[00190] As described for the ASO walk above, the ASO micro-walk is performed by delivering one or more ASOs, or a control ASO (an ASO with a scrambled sequence, sequence that is not expected to hybridize to the target region), for example by transfection, into a disease-relevant cell line that expresses the target pre-mRNA. The splicing-inducing effects of each of the ASOs may be assessed by any method known in the art, for example by reverse transcriptase (RT)-PCR using primers that span the NIE, as described herein (see, *e.g.*, **Example 4**). A reduction or absence of a longer RT-PCR product produced using the primers spanning the NIE in ASO-treated cells as compared to in control ASO-treated cells indicates that exon skipping (or splicing of the target intron containing an NIE) has

been enhanced. In some embodiments, the exon skipping efficiency (or the splicing efficiency to splice the intron containing the NIE), the ratio of spliced to unspliced pre-mRNA, the rate of splicing, or the extent of splicing may be improved using the ASOs described herein. The amount of protein or functional RNA that is encoded by the target pre-mRNA can also be assessed to determine whether each ASO achieved the desired effect (*e.g.*, enhanced functional protein production). Any method known in the art for assessing and/or quantifying protein production, such as Western blotting, flow cytometry, immunofluorescence microscopy, and ELISA, can be used.

[00191] ASOs that when hybridized to a region of a pre-mRNA result in exon skipping (or enhanced splicing of the intron containing a NIE) and increased protein production may be tested *in vivo* using animal models, for example transgenic mouse models in which the full-length human gene has been knocked-in or in humanized mouse models of disease. Suitable routes for administration of ASOs may vary depending on the disease and/or the cell types to which delivery of the ASOs is desired. ASOs may be administered, for example, by intrathecal injection, intracerebroventricular injection, intraperitoneal injection, intramuscular injection, subcutaneous injection, intravitreal injection, or intravenous injection. Following administration, the cells, tissues, and/or organs of the model animals may be assessed to determine the effect of the ASO treatment by for example evaluating splicing (efficiency, rate, extent) and protein production by methods known in the art and described herein. The animal models may also be any phenotypic or behavioral indication of the disease or disease severity.

[00192] As described herein in various examples, exon 20x in human *SCN1A* gene is equivalent to exon 21x in mouse *SCN1A* gene.

[00193] Also within the scope of the present disclosure is a method to identify or validate an NMD-inducing exon in the presence of an NMD inhibitor, for example, cycloheximide. An exemplary method is provided in **FIG. 3** and **Example 2**.

SPECIFIC EMBODIMENTS

[00194] Embodiment 1. A method of modulating expression of SCN1A protein in a cell having an mRNA that contains a non-sense mediated RNA decay-inducing exon (NMD exon mRNA) and encodes SCN1A protein, the method comprising contacting a therapeutic agent to the cell, whereby the therapeutic agent modulates splicing of the NMD exon from the NMD exon mRNA encoding SCN1A protein, thereby modulating the level of processed mRNA encoding SCN1A protein, and modulating expression of SCN1A protein in the cell, wherein the therapeutic agent binds to a targeted portion of the NMD exon mRNA encoding SCN1A, and wherein the targeted portion is: from about 1000 nucleotides upstream from the 5' end of an NMD-inducing exon (NIE) to about 100 nucleotides upstream from the 5' end of the NIE; or from about 100 nucleotides downstream of the 3' end of the NIE to about 1000 nucleotides downstream of the 3' end of the NIE.

[00195] Embodiment 2. A method of treating a disease or condition in a subject in need thereof by modulating expression of SCN1A protein in a cell of the subject, comprising: contacting the cell of the subject with a therapeutic agent that modulates splicing of a non-sense mediated mRNA decay-inducing exon (NMD exon) from an mRNA in the cell that contains the NMD exon and encodes SCN1A, thereby modulating the level of processed mRNA encoding the SCN1A protein, and modulating expression of SCN1A protein in the cell of the subject; wherein the therapeutic agent binds to a targeted portion of the NMD exon mRNA encoding SCN1A, and wherein the targeted portion is: from about 1000 nucleotides upstream from the 5' end of an NMD-inducing exon (NIE) to about 100 nucleotides upstream from the 5' end of the NIE; or from about 100 nucleotides downstream of the 3' end of the NIE to about 1000 nucleotides downstream of the 3' end of the NIE.

[00196] Embodiment 3. The method of embodiment 1 or 2, wherein the therapeutic agent interferes with binding of a factor involved in splicing of the NMD exon from a region of the targeted portion.

[00197] Embodiment 4. The method of embodiment 1 or 2, wherein the targeted portion is at most about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides upstream of 5' end of the NIE.

[00198] Embodiment 5. The method of embodiment 1 or 2, wherein the targeted portion is at least about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides, about 40 nucleotides, about 30 nucleotides, about 20 nucleotides, about 10 nucleotides, about 5 nucleotides, about 4 nucleotides, about 2 nucleotides, about 1 nucleotides upstream of 5' end of the NIE.

[00199] Embodiment 6. The method of embodiment 1 or 2, wherein the targeted portion is at most about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides downstream of 3' end of the NIE.

[00200] Embodiment 7. The method of embodiment 1 or 2, wherein the targeted portion is at least about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides, about 40 nucleotides, about 30 nucleotides, about 20 nucleotides, about 10 nucleotides, about 5 nucleotides, about 4 nucleotides, about 2 nucleotides, about 1 nucleotides downstream of 3' end of the NIE.

[00201] Embodiment 8. The method of any one of the embodiments 1-7, wherein the therapeutic agent is an antisense oligomer (ASO).

[00202] Embodiment 9. The method of embodiment 8, wherein the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% identity to any one of SEQ ID NOs: 12-731.

[00203] Embodiment 10. The method of any one of the embodiments 1-9, wherein the therapeutic agent promotes exclusion of the NMD exon from the processed mRNA encoding SCN1A protein.

[00204] Embodiment 11. The method of embodiment 10, wherein exclusion of the NMD exon from the processed mRNA encoding SCN1A protein in the cell contacted with the therapeutic agent is increased about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to exclusion of the NMD exon from the processed mRNA encoding SCN1A protein in a control cell.

[00205] Embodiment 12. The method of embodiment 10, wherein the therapeutic agent increases level of the processed mRNA encoding SCN1A protein in the cell.

[00206] Embodiment 13. The method of embodiment 10, wherein an amount of the processed mRNA encoding SCN1A protein in the cell contacted with the therapeutic agent is increased about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to an total amount of the processed mRNA encoding SCN1A protein in a control cell.

[00207] Embodiment 14. The method of embodiment 2, wherein the disease or condition is induced by a loss-of-function mutation in Nav1.1.

[00208] Embodiment 15. The method of embodiment 14, wherein the disease or condition is associated with haploinsufficiency of the SCN1A gene, and wherein the subject has a first allele

encoding a functional SCN1A, and a second allele from which SCN1A is not produced or produced at a reduced level, or a second allele encoding a nonfunctional SCN1A or a partially functional SCN1A.

[00209] Embodiment 16. The method of embodiment 14, wherein the disease or condition is encephalopathy.

[00210] Embodiment 17. The method of embodiment 16, wherein the encephalopathy is epileptic encephalopathy.

[00211] Embodiment 18. The method of embodiment 14, wherein the disease or condition is Dravet Syndrome (DS); severe myoclonic epilepsy of infancy (SMEI)-borderland (SMEB); Febrile seizure (FS); epilepsy, generalized, with febrile seizures plus (GEFS+); epileptic encephalopathy, early infantile, 13; cryptogenic generalized epilepsy; cryptogenic focal epilepsy; myoclonic-astatic epilepsy; Lennox-Gastaut syndrome; West syndrome; idiopathic spasms; early myoclonic encephalopathy; progressive myoclonic epilepsy; alternating hemiplegia of childhood; unclassified epileptic encephalopathy; sudden unexpected death in epilepsy (SUDEP); sick sinus syndrome 1; autism; or malignant migrating partial seizures of infancy.

[00212] Embodiment 19. The method of embodiment 18, wherein GEFS+ is epilepsy, generalized, with febrile seizures plus, type 2.

[00213] Embodiment 20. The method of embodiment 18, wherein the Febrile seizure is Febrile seizures, familial, 3A.

[00214] Embodiment 21. The method of embodiment 18, wherein SMEB is SMEB without generalized spike wave (SMEB-SW), SMEB without myoclonic seizures (SMEB-M), SMEB lacking more than one feature of SMEI (SMEB-O), or intractable childhood epilepsy with generalized tonic-clonic seizures (ICEGTC).

[00215] Embodiment 22. The method of embodiments 1 or 2, wherein the ASO consists of a sequence selected from SEQ ID NOs: 72 or 432.

[00216] Embodiment 23. The method of embodiments 1 or 2, wherein the ASO consists of a sequence selected from SEQ ID NOs: 73 or 433.

[00217] Embodiment 24. The method of embodiments 1 or 2, wherein the ASO consists of a sequence selected from SEQ ID NOs: 76 or 436.

[00218] Embodiment 25. The method of embodiments 1 or 2, wherein the ASO consists of a sequence selected from SEQ ID NOs: 181 or 541.

[00219] Embodiment 26. The method of embodiments 1 or 2, wherein the ASO consists of a sequence selected from SEQ ID NOs: 220 or 580.

[00220] Embodiment 27. A method of modulating expression of SCN1A protein in a cell having an mRNA that contains a non-sense mediated RNA decay-inducing exon (NMD exon mRNA) and

encodes SCN1A protein, the method comprising contacting a therapeutic agent to the cell, whereby the therapeutic agent modulates splicing of the NMD exon from the NMD exon mRNA encoding SCN1A protein, thereby modulating the level of processed mRNA encoding SCN1A protein, and modulating expression of SCN1A protein in the cell, wherein the therapeutic agent binds to a targeted portion of the NMD exon mRNA encoding SCN1A, and wherein the targeted portion is from about 1000 nucleotides upstream from the 5' end of an NMD-inducing exon (NIE) to about 1000 nucleotides downstream of the 3' end of the NIE.

[00221] Embodiment 28. A method of treating a disease or condition in a subject in need thereof by modulating expression of SCN1A protein in a cell of the subject, comprising: contacting the cell of the subject with a therapeutic agent that modulates splicing of a non-sense mediated mRNA decay-inducing exon (NMD exon) from an mRNA in the cell that contains the NMD exon and encodes SCN1A, thereby modulating the level of processed mRNA encoding the SCN1A protein, and modulating expression of SCN1A protein in the cell of the subject; wherein the therapeutic agent binds to a targeted portion of the NMD exon mRNA encoding SCN1A, and wherein the targeted portion is from about 1000 nucleotides upstream from the 5' end of an NMD-inducing exon (NIE) to about 1000 nucleotides downstream of the 3' end of the NIE.

[00222] Embodiment 29. An antisense oligomer (ASO) comprising a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% identity to any one of SEQ ID NOs: 12-731.

[00223] Embodiment 30. An antisense oligomer (ASO) consisting of a sequence selected from SEQ ID NOs: 12-731.

[00224] Embodiment 31. A method of treating a disease or condition in a subject in need thereof by modulating expression of SCN1A protein in a cell of the subject, comprising: contacting the cell of the subject with an ASO of embodiment 29 or embodiment 30.

[00225] Embodiment 32. A kit comprising an ASO of embodiment 29 or embodiment 30.

[00226] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

EXAMPLES

[00227] The present invention will be more specifically illustrated by the following Examples. However, it should be understood that the present invention is not limited by these examples in any manner.

Example 1: Identification of NMD-inducing Exon Inclusion Events in *SCN1A* Transcripts by RNAseq using Next Generation Sequencing

[00228] Whole transcriptome shotgun sequencing was carried out using next generation sequencing to reveal a snapshot of transcripts produced by the *SCN1A* gene to identify NIE inclusion events. For this purpose, polyA⁺ RNA from nuclear and cytoplasmic fractions of HCN (human cortical neurons) was isolated and cDNA libraries constructed using Illumina's TruSeq Stranded mRNA library Prep Kit. The libraries were pair-end sequenced resulting in 100-nucleotide reads that were mapped to the human genome (Feb. 2009, GRCh37/hg19 assembly). The sequencing results for *SCN1A* are shown in **Fig. 2**. Briefly, **Fig. 2** shows the mapped reads visualized using the UCSC genome browser (operated by the UCSC Genome Informatics Group (Center for Biomolecular Science & Engineering, University of California, Santa Cruz, 1156 High Street, Santa Cruz, CA 95064) and described by, *e.g.*, Rosenbloom, *et al.*, 2015, "The UCSC Genome Browser database: 2015 update," *Nucleic Acids Research* 43, Database Issue, doi: 10.1093/nar/gku1177) and the coverage and number of reads can be inferred by the peak signals. The height of the peaks indicates the level of expression given by the density of the reads in a particular region. The upper panel shows a graphic representation of the *SCN1A* gene to scale. The conservation level across 100 vertebrate species is shown as peaks. The highest peaks correspond to exons (black boxes), while no peaks are observed for the majority of the introns (lines with arrow heads). Peaks of conservation were identified in intron 20 (NM_006920), shown in the middle panel. Inspection of the conserved sequences identified an exon-like sequence of 64 bp (bottom panel, sequence highlighted in grey) flanked by 3' and 5' splice sites (underlined sequence). Inclusion of this exon leads to a frameshift and the introduction of a premature termination codon in exon 21 rendering the transcript a target of NMD.

[00229] Exemplary *SCN1A* gene, pre-mRNA, exon, and intron sequences are summarized in **Table 1**. The sequence for each exon or intron is summarized in **Table 2**.

Table 1. List of target *SCN1A* gene and pre-mRNA sequences.

Species	SEQ ID NO.	Sequence Type
Human	SEQ ID NO. 1	<i>SCN1A</i> gene (NC_000002.12)
	SEQ ID NO. 2	<i>SCN1A</i> pre-mRNA (encoding <i>e.g.</i> , <i>SCN1A</i> mRNA NM_006920.5)
	SEQ ID NO. 3	Intron 22 gene (GRCh38/hg38 assembly) (coordinate: chr2 166002754 166007229)

	SEQ ID NO. 4	Exon 23 (Exon 20x) gene (GRCh38/hg38 assembly) (coordinate: chr2 166007230 166007293)
	SEQ ID NO. 5	Intron 23 gene (GRCh38/hg38 assembly) (coordinate: chr2 166007294 166009718)
	SEQ ID NO. 6	IVS 22+IVS 23 pre-mRNA (pre-mRNA sequence of introns 22 and 23)
	SEQ ID NO. 7	Exon 23 (Exon 20x) pre-mRNA
Mouse	SEQ ID NO. 8	SCN1A gene (NC_000068.7)
	SEQ ID NO. 9	SCN1A pre-mRNA (encoding e.g., SCN1A mRNA NM_001313997.1)
	SEQ ID NO. 10	Intron 21 pre-mRNA
	SEQ ID NO. 11	Exon 21x pre-mRNA

Table 2. Sequences of target exon or intron in SCN1A pre-mRNA transcripts.

SEQ ID NO.	Sequence Type	Sequence
3	Intron 22 gene	gtaagaaaaatgaagAACCTgaagtattgtatatagccaaaataaactaaatttagaaaaagga aaatctatgcatgcaaaaggaaatggcaaatcttgcaaaattgctactttattgtttatctgtgcatatttactt ctagggtgatgcaagagaaataggcctctctgaaatgataataatcatttatctgctgtctatttaaagta actttatttctaatccatcttgggagtttctacaaatctatatacaaaaaaagctgatcattattaaagta ctatgtgtaagtataatggaatcctaaagtaaatctatatcagggtacttattcttggatgatatactgtact taacgagtttctgaaaaataatgtgaatcacacatgtgcctaagtatgagtgttaagaaaaaatgaagg agttgttaaaactttgtctgtataatgccaaagtgtgattattgaatatattcaagattagatggttagatatt aagtgtgactgaatttataaaactagtaataactaacttaagattacatacaaatccacatcattttataaca ataaagtaaaacacttataatgaacagaaaaataatgttgaactcattactataggttaattatataacactt aacttgcatcttattgtgcagagtcacacaaaatgtattttatcctttcaagatgcaataatcattttccatc atgcataacagattagaatgttgcattattgacttatttccatgcctttttacggcatgaagcattagtta tagatataataataaaaaatagttctgtcttttttaaaaaaatattatcaaaacaaacactgaattgtgt gattccaatagaaaaacactgctctttcacctcctaagggtgtagttactttatggaaactaagctgtattgta gacttccatttgactttgtagattgtttatagccttatgttcttctcaagcttattataaatgtcactttgtaa gaacgtaggacttgccttcgatttccctaacatataatgaactttgtcctcattatcgacaactcagaacaat ataatacaagtagtcccttttatttctcacagagagcctcaattttcaccaaaatgttaacagaaattatctc tgggtgtataagaatgaatgtgttttcaatgaatgtcactttgtttgttcagactggcagtttcagttctg gagaaaaaaatgtcatttgtgtacattctactgaaaacatgttgctgaatcaaaataatattttatatgg cttggaatctgaacaatgctaaacattgaaaatattataaacctttacatttgaccatttgaaagttatta aattcattgggtcaagtgtcagatatttcatacattacattcatttctataaaaaagctgatcttatcgggata cttttaattttctcagaaataaccataatctataattattaatcaataatgccttttataataaagagggttagttttg aaacttggagtttttagacataaaatcctataatgtctgatagtgatataactaatagtttaaatgtgcagattt atgaatatggctctatttctcataatgacaacatacacacagcactaaatgactaatcttcaatacgtgtt tggcattgtagatgcaaaataacgttataatgattctatttttatacttctagtgtttggatattttttgtaa aatataatcatgaatgatgtgaggttgatataagaatgatattgattgggaagtgaattgaacat gtcagaaactctcatttaattctttgcccctagcagcataaaatcacaatagctgcgtcaaagcgttaactca ggcactcattttattttgtgttctgtatttttcaaagcatgtgctttatgcaacattactgaataaagcatgtt gtacagtgcttgataagaagttagaagtaacaataaattatcatcagttgcactttgtgtttgtcatgtttt atgcacatttctggctgacagcttttaaacatttattgtatttcaaatttccagtcacaaattttcaactgttaaaa ttaaactgagtgaattgatgtcgtgaatatctagggtaaaataaaattgtgttaattttgatttttaatttct aacctaggaatcttaataccttcttttcaaaagaactcaagcttaattggatagggaacagacgggaga gcatcatgaacaaaaagtaacaccaaattgtctgtcatatcagatttctaactaataacaaactatatttct attttgtatag

4	Exon 23 (Exon 20x) gene	GATAATCTTGCTCCAACCTTGGATGGGGTGGAGCGCTGGTTCCTCCCCTGAGCCCTTTATTATGG
5	Intron 23 gene	gtactgtattaccccttttctacctttaatccttgactgtgacttatgtgtagtgagggtgaggaggaggattg ggaagggtactattatgaccacagtagggaaaatacatttattacatcctaatccctcttttaattgtctt aaatttcatttgaaaaaaaaaacctttatgaattaccctctgtggattttaaccccaatgggtgatatctt attaagttcattgaatatgatttagttatgtgtatatggagttatccatctttgggggagattactggattggtga gggcggggggacccctgggtgtagaatgattatgtgaaaaacaatttaactgttaagctcatgatactgttg aggcatacagccctgctgttttagtacattgggtcctgaaattaccagtttagataccatcagttgat tattgatgtatgagcagatactagggtgcaatattcagggttcataagactggattgattgtgaccactc tcattttttatgtgtaagttcatatgggttattttcaaatgttaacaaggcaaaaatatataagaataagt gaataagcacatgtgaattgtgtgtaacaaaaagttagaataaaaaatccacttattgaaattgcaga atagaatacacatcagaaataaaacacacgtcttatcatgagtattagaataaaatttaaggcataaact cacttcttagaataagtaactcccaactaacttctaggattttaaaacataacacagtgaaacatacataa acataactctacattttatttcttaagtttaagtgattatatacaagaagaaggtttattatcagagagaca gaaaaagtcagaattttgtttggatcaccaatatacatagcttcaaaaaaacgtcttataataaaaccac aacataatttttttagatttttaagaagattctatttcttcttatacttaaaaaatggatgattcctacttggcc acttttttttattcacatagattttcttatttctattagagaagcactagaattcatgaatagttgatttgaa gttcaagtaattaattcagataaaaagacatttctcatgtatgaaaaattctaatgtgaattgcataat tatcaatcctcatttagttagacttattttaaaaatgcaggtaatgaaccagaaatagaattggttgctga gagtagagaaacttattgatgtgtttgaaaaaaagcttctgagaagaacaacctctagtacagtat taattcattagatagctccttctcagacatttcttcatgtagcctgaaagtcaatttgaattgttctttcc aatttattcagactaattctgcctacttcttcccccaagaaccaattactgcagcttattgagactgaaa aaagttaatacacctccttcttctgaaccaagggaatggcttgggaactctgggaaaagacaatctttcta tgatcttcttcttaatttaatacatcataaataatgactatagcttgtataataaaactccccaaactgtgcc agatgttttctaagataaagttatttattgttcacaaaaaaataaaacttttctctggccaaatgtatgcaa cttggcaaatcatatcctgaagtgactgtcagagtagacatgcttgcgtcataaattccatagagttcgtt aactctaatacaatccccagtttcaagtaaacctctcaaacataattactaagcacaactctcctgtgc tcagttcctaattattctcatcccatattcagaaataacatttaaaattatgctttagatcaataactaatct aaacttgccttcaataaccattcattttgtcaaccattatttattctatattcaaaactctctgtatgttctta tattcaagacactcaaggccctggagattcacgaacatatgttgcactttaaatttttagaaaactttacaa tctgtcaggattacactgaactctagtagacagtaatatgggtaccagataagtgaggagcaactctccac gtagactggaaacagcactaaatgtctattataggtacttctgaacttaactgttttaacctcattttctca tatgccaaatgagaacgcaactgaattatctgtacagttctgtcagtagaattctgattctgaattca aagggggaaacattctcttttttggaggctaaactgggggacaaagttaggctccatgaagaagtgc ctattgaaactaagcctttaagaggggagagattttcagaagaggagctattagacaagggaatttcaatgt aaatggcatctcaatcacctggcaattatattagcacacgggtattatattaattgaagtgcatgaagtata gatgaccagggaagttaaaactggaaatagattgtggagtgtatgaataccaaggtaagaaaaatat ttgttagttaccagagagccaataaataactttcaagtgaggacttggggaagtaattacattcatagat taaatgaaggagaaggttaggagacagatgacagtgcaagtagaataacagagggcagttctaggt ggtgactgtgagaatggaaaagaggtggcaagctgagaaacgtttcaagaaaaaatgtgagacagg taatgtgaaaaagaaaatcgagaaataggtatagataatcagtggtctgctactctaaattgggtgttgaa ggcaaaatacgtattttaattagtactctgtgtatatacactagaacacagcattgtaattcggatagtgaca aaataatcagaaaagaagggaatagtaactgatttcaatttccaaatctctaattcgaagaatctaatc tattcatccatttaaaataaattatataacgagaatttgaagtccattgtattaatgcagacagtcagatga gataaggcaaaagtgcaggtgcagcttggttagttgcatcgccacatcattggttctgctgataactc aaccaaaataattttcatactcatccctccacttggcattactggtattcttatttcttggccacttatca cactgtttatgttcccagaaggcctagagttctttacaggctttaaacagggatcagaagtataagaatt ggctcatgtatttttttcagacaggcaggttaaaaaaattgttctaaaaatacactggcatcaaatggcaa atagaagatgtttgacgactacttccattggatcagactgacaagaataatacaagcacataggtggaatt aaacttagcttaattgtccaagtttgaggcagctgcccctataagcattttagggtctgttttagcttccct cttagccactcctgtgcagctccagtgagggtatggaggaaaaagcaagggaagccatccctatgttgt tccaaacatgaacactcaagatttttaactagtgttcagaaagtaagagggggaaaaacatccttctatag aaaaaaaaaaaagtagataataattgaacacagaacttcatgtatcacatcagatttgagaactatgtatg gcatccctcttttcttatttcttaagaatgatttctattatgtttcatttgaataaagtttttgaaataactcag taaatgaacaactgacatgactggagcttgaataaacgatgtgatctaatgaataacataatgcaa

[illegible]

[illegible]

[illegible]

[illegible]

SEQ ID NO.	SEQ ID NO.	SEQ ID NO.	
SEQ ID NO. 1	SEQ ID NO. 2	SEQ ID NOs: 12-731	Exon 23 (Exon 20x)

Table 4. Sequences of ASOs targeting human *SCN1A*

SEQUENCE NAME	Chr2 Start	Chr2 End	SEQ ID NO:	ASO sequence	SEQ ID NO:	ASO sequence
SCN1A-IVS22-986	166864788	166864806	12	ATGAATTTAATA AACTTT	372	AUGAAUUUAAUA AACUUU
SCN1A-IVS22-981	166864783	166864801	13	GACCAATGAATT TAATAA	373	GACCAAUGAAUU UAAUAA
SCN1A-IVS22-976	166864778	166864796	14	CACTTGACCAAT GAATTT	374	CACUUGACCAAU GAAUUU
SCN1A-IVS22-971	166864773	166864791	15	CTGAGCACTTGA CCAATG	375	CUGAGCACUUGA CCAUG
SCN1A-IVS22-966	166864768	166864786	16	AATATCTGAGCA CTTGAC	376	AAUAUCUGAGCA CUUGAC
SCN1A-IVS22-961	166864763	166864781	17	ATGGAAATATCT GAGCAC	377	AUGGAAUAUCU GAGCAC
SCN1A-IVS22-956	166864758	166864776	18	AATGTATGGAAA TATCTG	378	AAUGUAUGGAAA UAUCUG
SCN1A-IVS22-951	166864753	166864771	19	AGTGTAAATGTAT GGAAAT	379	AGUGUAAUGUAU GGAAAU
SCN1A-IVS22-946	166864748	166864766	20	AATGAAGTGTA TGTATG	380	AAUGAAGUGUAA UGUAUG
SCN1A-IVS22-941	166864743	166864761	21	ATAGAAATGAAG TGTAAT	381	AUAGAAAUGAAG UGUAAU
SCN1A-IVS22-936	166864738	166864756	22	TTTTATAGAAAT GAAGT	382	UUUUUAUAGAAA UGAAGU
SCN1A-IVS22-931	166864733	166864751	23	CAGCTTTTTTATA GAAAT	383	CAGCUUUUUUAU AGAAAU
SCN1A-IVS22-926	166864728	166864746	24	AAGATCAGCTTT TTTATA	384	AAGAUCAGCUUU UUUAUA
SCN1A-IVS22-921	166864723	166864741	25	CCGATAAGATCA GCTTTT	385	CCGAUAAGAUA GCUUUU
SCN1A-IVS22-916	166864718	166864736	26	GTATACCGATAA GATCAG	386	GUUAACCGAUAA GAUCAG
SCN1A-IVS22-911	166864713	166864731	27	TAAAAGTATACC GATAAG	387	UAAAAGUAUACC GAUAAG
SCN1A-IVS22-906	166864708	166864726	28	AAAATTAAGT ATACCG	388	AAAAUUAAGU AUACCG
SCN1A-IVS22-901	166864703	166864721	29	CTGAGAAAATTA AAAGTA	389	CUGAGAAAAUUA AAAGUA
SCN1A-IVS22-896	166864698	166864716	30	TATTTCTGAGAA AATTAA	390	UAUUUCUGAGAA AAUUA
SCN1A-IVS22-891	166864693	166864711	31	ATGGTTATTTCTG AGAAA	391	AUGGUUAUUUCU GAGAAA
SCN1A-IVS22-886	166864688	166864706	32	TAGATATGGTTA TTTCTG	392	UAGAUUGGUUA UUUCUG
SCN1A-IVS22-881	166864683	166864701	33	AATTATAGATAT GGTTAT	393	AAUUUAUGAUU GGUUAU

SCN1A- IVS22-876	166864 678	166864 696	34	TTAATAATTATA GATATG	394	UAAUAAUUAUA GAUUG
SCN1A- IVS22-871	166864 673	166864 691	35	ATTGATTAATAA TTATAG	395	AUUGAUUAAUAA UUAUAG
SCN1A- IVS22-866	166864 668	166864 686	36	GCATTATTGATTA ATAAT	396	GCAUUAUUGAUU AAUAAU
SCN1A- IVS22-861	166864 663	166864 681	37	AAAAGGCATTAT TGATTA	397	AAAAGGCAUUUA UGAUUA
SCN1A- IVS22-856	166864 658	166864 676	38	AATATAAAAGGC ATTATT	398	AAUAUAAAAGGC AUUAUU
SCN1A- IVS22-851	166864 653	166864 671	39	CTTTTAATATAAA AGGCA	399	CUUUUAAUUAUA AAGGCA
SCN1A- IVS22-846	166864 648	166864 666	40	AACCTCTTTTAAT ATAAA	400	AACCUCUUUUAA UAUAAA
SCN1A- IVS22-841	166864 643	166864 661	41	AAACTAACCTCT TTTAAT	401	AAACUAACCUCU UUUAAU
SCN1A- IVS22-836	166864 638	166864 656	42	TTCAAAAACATA CCTCTT	402	UUCAAAAACUAA CCUCUU
SCN1A- IVS22-831	166864 633	166864 651	43	CAAGTTTCAAAA ACTAAC	403	CAAGUUUCAAAA ACUAAAC
SCN1A- IVS22-826	166864 628	166864 646	44	AACTCCAAGTTT CAAAAA	404	AACUCCAAGUUU CAAAAA
SCN1A- IVS22-821	166864 623	166864 641	45	TCTAAAACCTCA AGTTTC	405	UCUAAAACUCCA AGUUUC
SCN1A- IVS22-816	166864 618	166864 636	46	TTATGTCTAAAA CTCCAA	406	UUAUGUCUAAAA CUCCAA
SCN1A- IVS22-811	166864 613	166864 631	47	GGATTTTATGTCT AAAAC	407	GGAUUUUAUGUC UAAAAAC
SCN1A- IVS22-806	166864 608	166864 626	48	TATAAGGATTTT ATGTCT	408	UAUAAGGAUUUU AUGUCU
SCN1A- IVS22-801	166864 603	166864 621	49	GCATTTATAAGG ATTTTA	409	GCAUUUAUAAGG AUUUUA
SCN1A- IVS22-796	166864 598	166864 616	50	TATCAGCATTTAT AAGGA	410	UAUCAGCAUUUA UAAGGA
SCN1A- IVS22-791	166864 593	166864 611	51	ATCACTATCAGC ATTTAT	411	AUCACUAUCAGC AUUUUAU
SCN1A- IVS22-786	166864 588	166864 606	52	GTTATATCACTAT CAGCA	412	GUUAUAUCACUA UCAGCA
SCN1A- IVS22-781	166864 583	166864 601	53	TATTAGTTATATC ACTAT	413	UAUUAGUUAUUA CACUAU
SCN1A- IVS22-776	166864 578	166864 596	54	TAAACTATTAGTT ATATC	414	UAAACUAUUAGU UAUAUC
SCN1A- IVS22-771	166864 573	166864 591	55	CCATTTAAACTAT TAGTT	415	CCAUUUAAACUA UUAGUU
SCN1A- IVS22-766	166864 568	166864 586	56	TCTGACCATTTAA ACTAT	416	UCUGACCAUUUA AACUAU
SCN1A- IVS22-761	166864 563	166864 581	57	ATAAATCTGACC ATTTAA	417	AUAAAUCUGACC AUUUAA
SCN1A- IVS22-756	166864 558	166864 576	58	TATTCATAAATCT GACCA	418	UAUUCAUAAAUC UGACCA
SCN1A- IVS22-751	166864 553	166864 571	59	AGCATATTCAT AAATCT	419	AGCCAUAUUCAU AAAUCU
SCN1A- IVS22-746	166864 548	166864 566	60	AATAGAGCCATA TTCATA	420	AAUAGAGCCAUA UUCAUA

SCN1A- IVS22-741	166864 543	166864 561	61	TGAGGAATAGAG CCATAT	421	UGAGGAAUAGAG CCAUAU
SCN1A- IVS22-736	166864 538	166864 556	62	CATTATGAGGAA TAGAGC	422	CAUUAUGAGGAA UAGAGC
SCN1A- IVS22-731	166864 533	166864 551	63	GTTGTCATTATGA GGAAT	423	GUUGUCAUUAUG AGGAAU
SCN1A- IVS22-726	166864 528	166864 546	64	TGTATGTTGTCAT TATGA	424	UGUAUGUUGUCA UUAUGA
SCN1A- IVS22-721	166864 523	166864 541	65	CTGTGTGTATGTT GTCAT	425	CUGUGUGUAUGU UGUCAU
SCN1A- IVS22-716	166864 518	166864 536	66	TAGTGCTGTGTGT ATGTT	426	UAGUGCUGUGUG UAUGUU
SCN1A- IVS22-711	166864 513	166864 531	67	CATTTTAGTGCTG TGTGT	427	CAUUUUAGUGCU GUGUGU
SCN1A- IVS22-706	166864 508	166864 526	68	TTAGTCATTTTAG TGCTG	428	UUAGUCAUUUUA GUGCUG
SCN1A- IVS22-701	166864 503	166864 521	69	AGAGATTAGTCA TTTTAG	429	AGAGAUUAGUCA UUUUAG
SCN1A- IVS22-696	166864 498	166864 516	70	ATTGAAGAGATT AGTCAT	430	AUUGAAGAGAUU AGUCAU
SCN1A- IVS22-691	166864 493	166864 511	71	CACGTATTGAAG AGATTA	431	CACGUUUUGAAG AGAUUA
SCN1A- IVS22-686	166864 488	166864 506	72	CCAAACACGTAT TGAAGA	432	CCAAACACGUUU UGAAGA
SCN1A- IVS22-681	166864 483	166864 501	73	CAATGCCAAACA CGTATT	433	CAAUGCCAAACA CGUAUU
SCN1A- IVS22-676	166864 478	166864 496	74	CTCTACAATGCC AAACAC	434	CUCUACAAUGCC AAACAC
SCN1A- IVS22-671	166864 473	166864 491	75	TTTGACTCTACAA TGCCA	435	UUUGACUCUACA AUGCCA
SCN1A- IVS22-666	166864 468	166864 486	76	GTTATTTTGACTC TACAA	436	GUUAUUUUGACU CUACAA
SCN1A- IVS22-661	166864 463	166864 481	77	ATAACGTTATTTT GACTC	437	AUAACGUUAUUU UGACUC
SCN1A- IVS22-656	166864 458	166864 476	78	CAATTATAACGT TATTTT	438	CAAUUAUAACGU UAUUUU
SCN1A- IVS22-651	166864 453	166864 471	79	AGAATCAATTAT AACGTT	439	AGAAUCAUUUAU AACGUU
SCN1A- IVS22-646	166864 448	166864 466	80	AAAATAGAATCA ATTATA	440	AAAAUAGAAUCA AUUAUA
SCN1A- IVS22-641	166864 443	166864 461	81	TATAAAAAATAG AATCAA	441	UAUAAAAAAUAG AAUCAA
SCN1A- IVS22-636	166864 438	166864 456	82	AGAAGTATAAAA AATAGA	442	AGAAGUAUAAAA AAUAGA
SCN1A- IVS22-631	166864 433	166864 451	83	ACACTAGAAGTA TAAAAA	443	ACACUAGAAGUA UAAAAA
SCN1A- IVS22-626	166864 428	166864 446	84	TCCAAACACTAG AAGTAT	444	UCCAAACACUAG AAGUAU
SCN1A- IVS22-621	166864 423	166864 441	85	AAATATCCAAAC ACTAGA	445	AAAUUCCAAAC ACUAGA
SCN1A- IVS22-616	166864 418	166864 436	86	AAATAAAATATC CAAACA	446	AAAUAAAAUAUC CAAACA
SCN1A- IVS22-611	166864 413	166864 431	87	TTACAAAATAAA ATATCC	447	UUACAAAAUAAA AUAUCC

SCN1A- IVS22-606	166864 408	166864 426	88	TATTTTACAAAA TAAAA	448	UAUUUUUACAAA AUAAAA
SCN1A- IVS22-601	166864 403	166864 421	89	GATTATATTTTA CAAAA	449	GAUUUAUUUUU ACAAAA
SCN1A- IVS22-596	166864 398	166864 416	90	TTCATGATTATAT TTTTA	450	UUCAUGAUUAUA UUUUUA
SCN1A- IVS22-591	166864 393	166864 411	91	CATCATTCATGAT TATAT	451	CAUCAUUCUGA UUAUAU
SCN1A- IVS22-586	166864 388	166864 406	92	CTCACCATCATTC ATGAT	452	CUCACCAUCAUU CAUGAU
SCN1A- IVS22-581	166864 383	166864 401	93	CCAACCTCACCA TCATTC	453	CCAACCUCACCA UCAUUC
SCN1A- IVS22-576	166864 378	166864 396	94	TATATCCAACCTC ACCAT	454	UAUAUCCAACCU CACCAU
SCN1A- IVS22-571	166864 373	166864 391	95	ATTCTTATATCCA ACCTC	455	AUUCUUAUAUCC AACCUC
SCN1A- IVS22-566	166864 368	166864 386	96	TCATCATTCCTTAT ATCCA	456	UCAUCAUUCUUA UAUCCA
SCN1A- IVS22-561	166864 363	166864 381	97	CATAATCATCATT CTTAT	457	CAUAAUCAUCAU UCUUAU
SCN1A- IVS22-556	166864 358	166864 376	98	CCAATCATAATC ATCATT	458	CCAUAUCAAAUC AUCAUU
SCN1A- IVS22-551	166864 353	166864 371	99	ACTTCCCAATCAT AATCA	459	ACUUCCCAAUCA UAAUCA
SCN1A- IVS22-546	166864 348	166864 366	100	ATCTCACTTCCCA ATCAT	460	AUCUCACUUCCC AAUCAU
SCN1A- IVS22-541	166864 343	166864 361	101	TTCAAATCTCACT TCCCA	461	UUCAAAUCUCAC UUCCCA
SCN1A- IVS22-536	166864 338	166864 356	102	GCATGTTCAAAT CTCACT	462	GCAUGUUCAAAU CUCACU
SCN1A- IVS22-531	166864 333	166864 351	103	TCTGAGCATGTTC AAATC	463	UCUGAGCAUGUU CAAUUC
SCN1A- IVS22-526	166864 328	166864 346	104	GAGTTTCTGAGC ATGTTC	464	GAGUUUCUGAGC AUGUUC
SCN1A- IVS22-521	166864 323	166864 341	105	AATGAGAGTTTC TGAGCA	465	AAUGAGAGUUUC UGAGCA
SCN1A- IVS22-516	166864 318	166864 336	106	AATTAAATGAGA GTTTCT	466	AAUUAAAUGAGA GUUUCU
SCN1A- IVS22-511	166864 313	166864 331	107	CAAAGAATTAAA TGAGAG	467	CAAAGAAUUAAA UGAGAG
SCN1A- IVS22-506	166864 308	166864 326	108	TAGGGCAAAGAA TTAAAT	468	UAGGGCAAAGAA UUAAAU
SCN1A- IVS22-501	166864 303	166864 321	109	GCTGCTAGGGCA AAGAAT	469	GCUGCUAGGGCA AAGAAU
SCN1A- IVS22-496	166864 298	166864 316	110	TTTATGCTGCTAG GGCAA	470	UUUAUGCUGCUA GGGCAA
SCN1A- IVS22-491	166864 293	166864 311	111	GTGATTTTATGCT GCTAG	471	GUGAUUUUAUGC UGCUAG
SCN1A- IVS22-486	166864 288	166864 306	112	CTATTGTGATTTT ATGCT	472	CUAUUGUGAUUU UAUGCU
SCN1A- IVS22-481	166864 283	166864 301	113	CGCAGCTATTGT GATTTT	473	CGCAGCUAUUGU GAUUUU
SCN1A- IVS22-476	166864 278	166864 296	114	TTTGACGCAGCT ATTGTG	474	UUUGACGCAGCU AUUGUG

SCN1A- IVS22-471	166864 273	166864 291	115	TACGCTTTGACG CAGCTA	475	UACGCUUUGACG CAGCUA
SCN1A- IVS22-466	166864 268	166864 286	116	TGAGTTACGCTTT GACGC	476	UGAGUUACGCUU UGACGC
SCN1A- IVS22-461	166864 263	166864 281	117	GTGCCTGAGTTA CGCTTT	477	GUGCCUGAGUUA CGCUUU
SCN1A- IVS22-456	166864 258	166864 276	118	AATGAGTGCCTG AGTTAC	478	AAUGAGUGCCUG AGUUAC
SCN1A- IVS22-451	166864 253	166864 271	119	AATAAAATGAGT GCCTGA	479	AAUAAAAUGAGU GCCUGA
SCN1A- IVS22-446	166864 248	166864 266	120	ACAAAAATAAAA TGAGTG	480	ACAAAAAUAAAA UGAGUG
SCN1A- IVS22-441	166864 243	166864 261	121	GAACAACAAAAA TAAAT	481	GAACAACAAAAA UAAAAU
SCN1A- IVS22-436	166864 238	166864 256	122	TAACAGAACAAC AAAAAT	482	UACAGAACAAC AAAAAU
SCN1A- IVS22-431	166864 233	166864 251	123	AAAAATAACAGA ACAACA	483	AAAAAUACAGA ACAACA
SCN1A- IVS22-426	166864 228	166864 246	124	TTTGAAAAAATA ACAGAA	484	UUUGAAAAAAUA ACAGAA
SCN1A- IVS22-421	166864 223	166864 241	125	CATGCTTTGAAA AAATAA	485	CAUGC UUUGAAA AAAUAA
SCN1A- IVS22-416	166864 218	166864 236	126	AAGCACATGCTT TGAAAA	486	AAGCACAU GCUU UGAAAA
SCN1A- IVS22-411	166864 213	166864 231	127	CATAAAAGCACA TGCTTT	487	CAUAAAAGCACA UGCUUU
SCN1A- IVS22-406	166864 208	166864 226	128	TGTTGCATAAAA GCACAT	488	UGUUGCAUAAAA GCACAU
SCN1A- IVS22-401	166864 203	166864 221	129	AGTAATGTTGCA TAAAAG	489	AGUAAUGUUGCA UAAAAG
SCN1A- IVS22-396	166864 198	166864 216	130	TATTCAGTAATGT TGCAT	490	UAUUCAGUAAUG UUGCAU
SCN1A- IVS22-391	166864 193	166864 211	131	TGCTTTATTCAGT AATGT	491	UGC UUUAUUCAG UAAUGU
SCN1A- IVS22-386	166864 188	166864 206	132	CAACATGCTTTAT TCAGT	492	CAACAUGCUUUA UUCAGU
SCN1A- IVS22-381	166864 183	166864 201	133	CTGTACAACATG CTTTAT	493	CUGUACAACAUG CUUUUAU
SCN1A- IVS22-376	166864 178	166864 196	134	AAGCACTGTACA ACATGC	494	AAGCACUGUACA ACAUGC
SCN1A- IVS22-371	166864 173	166864 191	135	TTATCAAGCACT GTACAA	495	UUAUCAAGCACU GUACAA
SCN1A- IVS22-366	166864 168	166864 186	136	ACTTCTTATCAAG CACTG	496	ACUUCUUAUCAA GCACUG
SCN1A- IVS22-361	166864 163	166864 181	137	TTCTAACTTCTTA TCAAG	497	UUCUAA CUUCU AUCAAG
SCN1A- IVS22-356	166864 158	166864 176	138	TTACTTTCTAACT TCTTA	498	UUACUUUCU AAC UUCUUA
SCN1A- IVS22-351	166864 153	166864 171	139	ATTTGTTACTTTC TAACT	499	AUUUGUUACUUU CUAACU
SCN1A- IVS22-346	166864 148	166864 166	140	AATTTATTTGTTA CTTTC	500	AAUUUAUUUGUU ACUUUC
SCN1A- IVS22-341	166864 143	166864 161	141	ATGATAATTTATT TGTTA	501	AUGAUAAUUUAU UUGUUA

SCN1A- IVS22-336	166864 138	166864 156	142	ACGTGATGATAA TTTATT	502	ACGUGAUGAUAA UUUAUU
SCN1A- IVS22-331	166864 133	166864 151	143	GTGCAACGTGAT GATAAT	503	GUGCAACGUGAU GAUAAU
SCN1A- IVS22-326	166864 128	166864 146	144	ACAAAGTGCAAC GTGATG	504	ACAAAGUGCAAC GUGAUG
SCN1A- IVS22-321	166864 123	166864 141	145	AAAACACAAAGT GCAACG	505	AAAACACAAAGU GCAACG
SCN1A- IVS22-316	166864 118	166864 136	146	CATGCAAAACAC AAAGTG	506	CAUGCAAAACAC AAAGUG
SCN1A- IVS22-311	166864 113	166864 131	147	TAAACATGCAA AACACA	507	UAAAAAUGCAA AACACA
SCN1A- IVS22-306	166864 108	166864 126	148	GTGCATAAAACA TGCAAA	508	GUGCAUAAACA UGCAAA
SCN1A- IVS22-301	166864 103	166864 121	149	GAAATGTGCATA AAACAT	509	GAAAUGUGCAUA AAACAU
SCN1A- IVS22-296	166864 098	166864 116	150	AGCCAGAAATGT GCATAA	510	AGCCAGAAUGU GCAUAA
SCN1A- IVS22-291	166864 093	166864 111	151	CTGTCAGCCAGA AATGTG	511	CUGUCAGCCAGA AAUGUG
SCN1A- IVS22-286	166864 088	166864 106	152	AAAAGCTGTCAG CCAGAA	512	AAAAGCUGUCAG CCAGAA
SCN1A- IVS22-281	166864 083	166864 101	153	TGTTTAAAAGCT GTCAGC	513	UGUUUAAAAGCU GUCAGC
SCN1A- IVS22-276	166864 078	166864 096	154	ATAAATGTTTAA AAGCTG	514	AUAAAUGUUUAA AAGCUG
SCN1A- IVS22-271	166864 073	166864 091	155	ATACAATAAATG TTTAAA	515	AUACAAUAAAUG UUUAAA
SCN1A- IVS22-266	166864 068	166864 086	156	TTGAAATACAAT AAATGT	516	UUGAAAUACAAU AAAUGU
SCN1A- IVS22-261	166864 063	166864 081	157	GAAATTTGAAAT ACAATA	517	GAAAUUUGAAAU ACAAUA
SCN1A- IVS22-256	166864 058	166864 076	158	GACTGGAAATTT GAAATA	518	GACUGGAAAUUU GAAUAU
SCN1A- IVS22-251	166864 053	166864 071	159	ATTGGAAGTGA AATTTG	519	AUUUGGACUGGA AAUUUG
SCN1A- IVS22-246	166864 048	166864 066	160	GAAAAATTTGGA CTGGAA	520	GAAAAAUUUGGA CUGGAA
SCN1A- IVS22-241	166864 043	166864 061	161	AAGTTGAAAAAT TTGGAC	521	AAGUUGAAAAAU UUGGAC
SCN1A- IVS22-236	166864 038	166864 056	162	TTTACAAGTTGA AAAATT	522	UUUACAAGUUGA AAAAUU
SCN1A- IVS22-231	166864 033	166864 051	163	TTAATTTTACAAG TTGAA	523	UUAAUUUUACAA GUUGAA
SCN1A- IVS22-226	166864 028	166864 046	164	TCAGTTTAATTTT ACAAG	524	UCAGUUUAAUUU UACAAG
SCN1A- IVS22-221	166864 023	166864 041	165	TTCACTCAGTTTA ATTTT	525	UUCACUCAGUUU AAUUUU
SCN1A- IVS22-216	166864 018	166864 036	166	ATCAATTCCTC AGTTTA	526	AUCAAUUCACUC AGUUUA
SCN1A- IVS22-211	166864 013	166864 031	167	ACGACATCAATT CACTCA	527	ACGACAUCAAUU CACUCA
SCN1A- IVS22-206	166864 008	166864 026	168	TATTCACGACAT CAATTC	528	UAUUCACGACAU CAAUUC

SCN1A- IVS22-201	166864 003	166864 021	169	CTAGATATTCAC GACATC	529	CUAGAUAUUCAC GACAUC
SCN1A- IVS22-196	166863 998	166864 016	170	TTACCCTAGATAT TCACG	530	UUACCCUAGAU UUCACG
SCN1A- IVS22-191	166863 993	166864 011	171	TTATTTTACCCTA GATAT	531	UUAUUUUACCCU AGAUAU
SCN1A- IVS22-186	166863 988	166864 006	172	AAATTTTATTTTA CCCTA	532	AAAUUUUAUUUU ACCCUA
SCN1A- IVS22-181	166863 983	166864 001	173	AACACAAATTTT ATTTTA	533	AACACAAAUUUU AUUUUA
SCN1A- IVS22-176	166863 978	166863 996	174	ATTTAAACACAA ATTTTA	534	AUUUAAACACAA AUUUUA
SCN1A- IVS22-171	166863 973	166863 991	175	TACAAATTTAAA CACAAA	535	UACAAAUUUAAA CACAAA
SCN1A- IVS22-166	166863 968	166863 986	176	AAAAATACAAAT TTAAAC	536	AAAAUACAAAU UAAAC
SCN1A- IVS22-161	166863 963	166863 981	177	AAATTAATAATA CAAATT	537	AAAUUAAAAAUA CAAAUU
SCN1A- IVS22-156	166863 958	166863 976	178	TTAGGAAATTAA AAATAC	538	UUAGGAAAUUAA AAAUAC
SCN1A- IVS22-151	166863 953	166863 971	179	CTAGGTTAGGAA ATTAAA	539	CUAGGUUAGGAA AUUAAA
SCN1A- IVS22-146	166863 948	166863 966	180	ATTTCTAGGTTA GGAAA	540	AUUUCCUAGGUU AGGAAA
SCN1A- IVS22-141	166863 943	166863 961	181	TTAAGATTTCTTA GGTTA	541	UUAAGAUUCCU AGGUUA
SCN1A- IVS22-136	166863 938	166863 956	182	GGTATTTAAGAT TTCCTA	542	GGUAUUUAAGAU UCCCUA
SCN1A- IVS22-131	166863 933	166863 951	183	AAGAAGGTATTT AAGATT	543	AAGAAGGUUUU AAGAUU
SCN1A- IVS22-126	166863 928	166863 946	184	TGAAAAAGAAGG TATTTA	544	UGAAAAAGAAGG UAUUUA
SCN1A- IVS22-121	166863 923	166863 941	185	TCTTTTGAAAAA GAAGGT	545	UCUUUUGAAAAA GAAGGU
SCN1A- IVS22-116	166863 918	166863 936	186	TGAGTCTTTTGA AAAAG	546	UGAGUUCUUUUG AAAAAG
SCN1A- IVS22-111	166863 913	166863 931	187	AGACTTGAGTTC TTTTGA	547	AGACUUGAGUUC UUUUGA
SCN1A- IVS22-106	166863 908	166863 926	188	CATTAAGACTTG AGTTCT	548	CAUUAAGACUUG AGUUCU
SCN1A- IVS22-101	166863 903	166863 921	189	CTATCCATTAAG ACTTGA	549	CUAUCCAUAAG ACUUGA
SCN1A- IVS22-096	166863 898	166863 916	190	TTCCCTATCCAT TAAGA	550	UUUCCCUAUCCA UUAAGA
SCN1A- IVS22-091	166863 893	166863 911	191	GTCTGTTTCCCTA TCCAT	551	GUCUGUUUCCCU AUCCA
SCN1A- IVS23+091	166863 631	166863 649	192	TTCCCTACTGTG GTGCA	552	UUUCCCUACUGU GGUGCA
SCN1A- IVS23+096	166863 626	166863 644	193	TGTATTTTCCCTA CTGTG	553	UGUAUUUCCCU ACUGUG
SCN1A- IVS23+101	166863 621	166863 639	194	AATAATGTATTT CCCTA	554	AAUAAUGUAUUU UCCCUA
SCN1A- IVS23+106	166863 616	166863 634	195	ATGTAAATAATG TATTTT	555	AUGUAAUAAUG UAUUUU

SCN1A- IVS23+111	166863 611	166863 629	196	TTAGGATGTAAA TAATGT	556	UUAGGAUGUAAA UAAUGU
SCN1A- IVS23+116	166863 606	166863 624	197	AGGGATTAGGAT GTAAAT	557	AGGGAUUAGGAU GUAAAU
SCN1A- IVS23+121	166863 601	166863 619	198	AAAGAGGGAATT AGGATG	558	AAAGAGGGGAAUU AGGAUG
SCN1A- IVS23+126	166863 596	166863 614	199	ATTGAAAAGAAG GGATTA	559	AUUGAAAAGAAG GGAUUA
SCN1A- IVS23+131	166863 591	166863 609	200	AGACAATTGAAA AGAGGG	560	AGACAAUUGAAA AGAGGG
SCN1A- IVS23+136	166863 586	166863 604	201	ATTTAAGACAAT TGAAAA	561	AUUUAAGACAAU UGAAAA
SCN1A- IVS23+141	166863 581	166863 599	202	ATGAAATTTAAG ACAATT	562	AUGAAAUUUAAG ACAAUU
SCN1A- IVS23+146	166863 576	166863 594	203	TTCAAATGAAAT TTAAGA	563	UUCAAAUGAAAU UUAAGA
SCN1A- IVS23+151	166863 571	166863 589	204	TTTTTTTCAAATG AAATT	564	UUUUUUUCAAUU GAAAUU
SCN1A- IVS23+156	166863 566	166863 584	205	TTTTTTTTTTTTTC AAATG	565	UUUUUUUUUUUU CAAUG
SCN1A- IVS23+161	166863 561	166863 579	206	AAGGTTTTTTTTTT TTTTTC	566	AAGGUUUUUUUU UUUUUC
SCN1A- IVS23+166	166863 556	166863 574	207	TCATAAAGGTTTT TTTTT	567	UCAUAAAGGUUU UUUUUU
SCN1A- IVS23+171	166863 551	166863 569	208	TAAATTCATAAA GGTTTT	568	UAAAUUCAUAAA GGUUUU
SCN1A- IVS23+176	166863 546	166863 564	209	GAGGGTAAATTC ATAAAG	569	GAGGGUAAAUUC AUAAAG
SCN1A- IVS23+181	166863 541	166863 559	210	CCACAGAGGGTA AATTCA	570	CCACAGAGGGUA AAUUCA
SCN1A- IVS23+186	166863 536	166863 554	211	AAAATCCACAGA GGGTAA	571	AAAAUCCACAGA GGGUAA
SCN1A- IVS23+191	166863 531	166863 549	212	GGTTAAAATCC ACAGAG	572	GGGUAAAAAUCC ACAGAG
SCN1A- IVS23+196	166863 526	166863 544	213	CATTGGGATTAA AATCCA	573	CAUUGGGAUUAA AAUCCA
SCN1A- IVS23+201	166863 521	166863 539	214	TCAACCATTAGG GTAAA	574	UCAACCAUUAGG GUUAAA
SCN1A- IVS23+206	166863 516	166863 534	215	AGATATCAACCA TTGGGA	575	AGAUUAUCAACCA UUGGGA
SCN1A- IVS23+211	166863 511	166863 529	216	AATAAAGATATC AACCAT	576	AAUAAAGAUUUC AACCAU
SCN1A- IVS23+216	166863 506	166863 524	217	AACTTAATAAAG ATATCA	577	AACUUAUAAAG AUAUCA
SCN1A- IVS23+221	166863 501	166863 519	218	AATGAACTTAA TAAAGA	578	AAUGAAACUUAU UAAAGA
SCN1A- IVS23+226	166863 496	166863 514	219	TATTCAATGAAA CTTAAT	579	UAUUCAAUGAAA CUUAAU
SCN1A- IVS23+231	166863 491	166863 509	220	AATCATATTCAA TGAAAC	580	AAUCAUAUUCAA UGAAAC
SCN1A- IVS23+236	166863 486	166863 504	221	AACATAATCATA TTCAAT	581	AACAAAUCAUA UUCAAU
SCN1A- IVS23+241	166863 481	166863 499	222	CACATAACTAAA TCATAT	582	CACAUACUAAA UCAUAU

SCN1A- IVS23+246	166863 476	166863 494	223	ATATACACATAA CTAAAT	583	AUAUACACAUAA CUAAAAU
SCN1A- IVS23+251	166863 471	166863 489	224	ACTCCATATACA CATAAC	584	ACUCCAUUAACA CAUAAC
SCN1A- IVS23+256	166863 466	166863 484	225	GGATAACTCCAT ATACAC	585	GGAUAAACUCCAU AUACAC
SCN1A- IVS23+261	166863 461	166863 479	226	AAGATGGATAAC TCCATA	586	AAGAUGGAUAAC UCCAU
SCN1A- IVS23+266	166863 456	166863 474	227	CCCCAAAGATGG ATAACT	587	CCCCAAAGAUGG AUAACU
SCN1A- IVS23+271	166863 451	166863 469	228	AATCTCCCCAAA GATGGA	588	AAUCUCCCCAAA GAUGGA
SCN1A- IVS23+276	166863 446	166863 464	229	CCAGTAATCTCC CCAAAG	589	CCAGUAAUCUCC CCAAAG
SCN1A- IVS23+281	166863 441	166863 459	230	CCAATCCAGTAA TCTCCC	590	CCAAUCCAGUAA UCUCCC
SCN1A- IVS23+286	166863 436	166863 454	231	CCTCACCAATCC AGTAAT	591	CCUCACCAAUCC AGUAAU
SCN1A- IVS23+291	166863 431	166863 449	232	CCCGCCCTCACC AATCCA	592	CCCGCCCUCACCA AUCCA
SCN1A- IVS23+296	166863 426	166863 444	233	GGTCCCCCGCCC TCACCA	593	GGUCCCCCGCCC UCACCA
SCN1A- IVS23+301	166863 421	166863 439	234	ACCAGGGTCCCC CGCCCT	594	ACCAGGGUCCCC CGCCCU
SCN1A- IVS23+306	166863 416	166863 434	235	TCTACACCAGGG TCCCCC	595	UCUACACCAGGG UCCCCC
SCN1A- IVS23+311	166863 411	166863 429	236	ATCATTCTACACC AGGGT	596	AUCAUUCUACAC CAGGGU
SCN1A- IVS23+316	166863 406	166863 424	237	ACATAATCATTCT ACACC	597	ACAUAUAUAUUC UACACC
SCN1A- IVS23+321	166863 401	166863 419	238	TTTTACATAATC ATTCT	598	UUUUCACAUAAU CAUUCU
SCN1A- IVS23+326	166863 396	166863 414	239	TTGTTTTTTCACA TAATC	599	UUGUUUUUUCAC AUAUC
SCN1A- IVS23+331	166863 391	166863 409	240	TTAAATTGTTTTT TCACA	600	UUAAAUUGUUUU UUCACA
SCN1A- IVS23+336	166863 386	166863 404	241	ACAAGTTAAATT GTTTTT	601	ACAAGUAAAUAU GUUUUU
SCN1A- IVS23+341	166863 381	166863 399	242	GCTTAACAAGTT AAATTG	602	GCUUAACAAGUU AAAUUG
SCN1A- IVS23+346	166863 376	166863 394	243	CATGAGCTTAAC AAGTTA	603	CAUGAGCUAAC AAGUUA
SCN1A- IVS23+351	166863 371	166863 389	244	AGTATCATGAGC TTAACA	604	AGUAUCAUGAGC UUAACA
SCN1A- IVS23+356	166863 366	166863 384	245	CAAACAGTATCA TGAGCT	605	CAAACAGUAUCA UGAGCU
SCN1A- IVS23+361	166863 361	166863 379	246	TGCCTCAAACAG TATCAT	606	UGCCUCAACAG UAUCAU
SCN1A- IVS23+366	166863 356	166863 374	247	CTGTATGCCTCA AACAGT	607	CUGUAUGCCUCA AACAGU
SCN1A- IVS23+371	166863 351	166863 369	248	AGGGACTGTATG CCTCAA	608	AGGGACUGUAUG CCUCAA
SCN1A- IVS23+376	166863 346	166863 364	249	ACAGCAGGGACT GTATGC	609	ACAGCAGGGACU GUAUGC

SCN1A- IVS23+381	166863 341	166863 359	250	ACTAAACAGCAA GGGCTG	610	ACUAAACAGCAA GGGCUG
SCN1A- IVS23+386	166863 336	166863 354	251	AATGTACTAAAC AGCAGG	611	AAUGUACUAAAC AGCAGG
SCN1A- IVS23+391	166863 331	166863 349	252	AGACCAATGTAC TAAACA	612	AGACCAAUGUAC UAAACA
SCN1A- IVS23+396	166863 326	166863 344	253	GACCCAGACCAA TGTACT	613	GACCCAGACCAA UGUACU
SCN1A- IVS23+401	166863 321	166863 339	254	TTCAGGACCCAG ACCAAT	614	UUCAGGACCCAG ACCAAU
SCN1A- IVS23+406	166863 316	166863 334	255	TAATTTTTCAGGA CCCAGA	615	UAAUUUUCAGGA CCCAGA
SCN1A- IVS23+411	166863 311	166863 329	256	ACTGGTAATTTTC AGGAC	616	ACUGGUAUUUU CAGGAC
SCN1A- IVS23+416	166863 306	166863 324	257	ATCTAACTGGTA ATTTTC	617	AUCUAAUCUGUA AUUUUC
SCN1A- IVS23+421	166863 301	166863 319	258	ATGGTATCTAAC TGGTAA	618	AUGGUAUCUAA UGGUAA
SCN1A- IVS23+426	166863 296	166863 314	259	AACTGATGGTAT CTAACT	619	AACUGAUGGUAA CUAACU
SCN1A- IVS23+431	166863 291	166863 309	260	TAATCAACTGAT GGTATC	620	UAAUCAACUGAU GGUAUC
SCN1A- IVS23+436	166863 286	166863 304	261	ATCAATAATCAA CTGATG	621	AUCAUAAUCAA CUGAUG
SCN1A- IVS23+441	166863 281	166863 299	262	TACATATCAATA ATCAAC	622	UACAUUAUCAA AUCAAC
SCN1A- IVS23+446	166863 276	166863 294	263	GCTCATACATAT CAATAA	623	GCUCAUACAUU CAAUAA
SCN1A- IVS23+451	166863 271	166863 289	264	TATCTGCTCATAC ATATC	624	UAUCUGCUCAUA CAUAUC
SCN1A- IVS23+456	166863 266	166863 284	265	CCTAGTATCTGCT CATAC	625	CCUAGUAUCUGC UCAUAC
SCN1A- IVS23+461	166863 261	166863 279	266	TGCACCCTAGTA TCTGCT	626	UGCACCCUAGUA UCUGCU
SCN1A- IVS23+466	166863 256	166863 274	267	AATATTGCACCC TAGTAT	627	AAUAUUGCACCC UAGUAU
SCN1A- IVS23+471	166863 251	166863 269	268	CCTGAAATATTG CACCT	628	CCUGAAAUAUUG CACCCU
SCN1A- IVS23+476	166863 246	166863 264	269	TGAAACCTGAAA TATTGC	629	UGAAACCUGAAA UAUUGC
SCN1A- IVS23+481	166863 241	166863 259	270	TCTTATGAAACCT GAAAT	630	UCUUAUGAAACC UGAAAU
SCN1A- IVS23+486	166863 236	166863 254	271	ACCAGTCTTATG AAACCT	631	ACCAGUCUUAUG AAACCU
SCN1A- IVS23+491	166863 231	166863 249	272	TCAATACCAGTC TTATGA	632	UCAAUACCAGUC UUAUGA
SCN1A- IVS23+496	166863 226	166863 244	273	CACAATCAATAC CAGTCT	633	CACAAUCAAUAC CAGUCU
SCN1A- IVS23+501	166863 221	166863 239	274	GTGGTCACAATC AATACC	634	GUGGUCACAAUC AAUACC
SCN1A- IVS23+506	166863 216	166863 234	275	TGAGAGTGGTCA CAATCA	635	UGAGAGUGGUCA CAAUCA
SCN1A- IVS23+511	166863 211	166863 229	276	AAAAATGAGAGT GGTCAC	636	AAAAAUGAGAGU GGUCAC

SCN1A- IVS23+516	166863 206	166863 224	277	CAATAAAAAATG AGAGTG	637	CAAUAAAAAUG AGAGUG
SCN1A- IVS23+521	166863 201	166863 219	278	TTACACAATAAA AAATGA	638	UUACACAAUAAA AAAUGA
SCN1A- IVS23+526	166863 196	166863 214	279	TGAACTTACACA ATAAAA	639	UGAACUUACACA AUAAAA
SCN1A- IVS23+531	166863 191	166863 209	280	CCATATGAACTT ACACAA	640	CCAUAUGAACUU ACACAA
SCN1A- IVS23+536	166863 186	166863 204	281	TAACCCCATATG AACTTA	641	UAACCCCAUAUG AACUUA
SCN1A- IVS23+541	166863 181	166863 199	282	GAAAATAACCCC ATATGA	642	GAAAAUAACCCC AUAUGA
SCN1A- IVS23+546	166863 176	166863 194	283	ATTTTGAAAATA ACCCCA	643	AUUUUGAAAAUA ACCCCA
SCN1A- IVS23+551	166863 171	166863 189	284	TTAACATTTTGAA ATAAA	644	UUAACAUUUUGA AAAUA
SCN1A- IVS23+556	166863 166	166863 184	285	CCTGTTTAACATT TTGAA	645	CCUUGUUAACAU UUUGAA
SCN1A- IVS23+561	166863 161	166863 179	286	TTTTGCCTTGTTA ACATT	646	UUUUGCCUUGUU AACAUU
SCN1A- IVS23+566	166863 156	166863 174	287	TATATTTTTGCCT TGTTA	647	UAUAUUUUUGCC UUGUUA
SCN1A- IVS23+571	166863 151	166863 169	288	CTTAATATATTTT TGCCT	648	CUUAAUAUAUUU UUGCCU
SCN1A- IVS23+576	166863 146	166863 164	289	TATTTCTTAATAT ATTTT	649	UAUUUCUAAUA UAUUUU
SCN1A- IVS23+581	166863 141	166863 159	290	TCAACTATTTCTT AATAT	650	UCAACUAUUUCU UAAUAU
SCN1A- IVS23+586	166863 136	166863 154	291	CTTATTCAACTAT TTCTT	651	CUUAUUC AACUA UUUCUU
SCN1A- IVS23+591	166863 131	166863 149	292	ATGTGCTTATTCA ACTAT	652	AUGUGCUUAUUC AACUAU
SCN1A- IVS23+596	166863 126	166863 144	293	TTCAATGTGCTT ATCA	653	UUCACAUGUGCU UAUUA
SCN1A- IVS23+601	166863 121	166863 139	294	CACAATTCACAT GTGCTT	654	CACAAUUCACAU GUGCUU
SCN1A- IVS23+606	166863 116	166863 134	295	TACAACACAATT CACATG	655	UACAACACAAUU CACAUG
SCN1A- IVS23+611	166863 111	166863 129	296	TTGTTTACAACAC AATTC	656	UUGUUUACAACA CAAUUC
SCN1A- IVS23+616	166863 106	166863 124	297	ACTTTTTGTTTAC AACAC	657	ACUUUUUGUUUA CAACAC
SCN1A- IVS23+621	166863 101	166863 119	298	TTCTAACTTTTTG TTTAC	658	UUCUAACUUUUU GUUUAC
SCN1A- IVS23+626	166863 096	166863 114	299	TTTTATTCTAACT TTTTG	659	UUUUUAUCUAAC UUUUUG
SCN1A- IVS23+631	166863 091	166863 109	300	GATTTTTTTTATTC TAACT	660	GAUUUUUUUAUU CUAACU
SCN1A- IVS23+636	166863 086	166863 104	301	AAGTGGATTTTTT TATTC	661	AAGUGGAUUUUU UUAUUC
SCN1A- IVS23+641	166863 081	166863 099	302	CAAATAAGTGGA TTTTTT	662	CAAAUAAGUGGA UUUUUU
SCN1A- IVS23+646	166863 076	166863 094	303	TAATTCAAATAA GTGGAT	663	UAAUUCAAAUA GUGGAU

SCN1A- IVS23+651	166863 071	166863 089	304	CTGCATAATTCA AATAAG	664	CUGCAUAAUUCA AAUAAAG
SCN1A- IVS23+656	166863 066	166863 084	305	CTATTCTGCATAA TTCAA	665	CUAUUCUGCAUA AUUCA
SCN1A- IVS23+661	166863 061	166863 079	306	GTATTCTATTCTG CATAA	666	GUAUUCUAUUCU GCAUAA
SCN1A- IVS23+666	166863 056	166863 074	307	GGTATGTATTCTA TTCTG	667	GGUAUGUAUUCU AUUCUG
SCN1A- IVS23+671	166863 051	166863 069	308	TTCTAGGTATGTA TTCTA	668	UUCUAGGUAUGU AUUCUA
SCN1A- IVS23+676	166863 046	166863 064	309	TTTATTTCTAGGT ATGTA	669	UUUAUUUCUAGG UAUGUA
SCN1A- IVS23+681	166863 041	166863 059	310	TTTGTTTTATTTC TAGGT	670	UUUGUUUUUUUU CUAGGU
SCN1A- IVS23+686	166863 036	166863 054	311	ACGTTTTTGT TTT ATTTC	671	ACGUUUUUUGUUU UAUUUC
SCN1A- IVS23+691	166863 031	166863 049	312	ATAAGACGTTTTT GTTTT	672	AUAAGACGUUUU UGUUUU
SCN1A- IVS23+696	166863 026	166863 044	313	TCATGATAAGAC GTTTTT	673	UCAUGAUAAAGAC GUUUUU
SCN1A- IVS23+701	166863 021	166863 039	314	AATACTCATGAT AAGACG	674	AAUACUCAUGAU AAGACG
SCN1A- IVS23+706	166863 016	166863 034	315	ATCTTAATACTCA TGATA	675	AUCUUAUACUC AUGAUA
SCN1A- IVS23+711	166863 011	166863 029	316	ATTTTATCTTAAT ACTCA	676	AUUUUAUCUUA UACUCA
SCN1A- IVS23+716	166863 006	166863 024	317	CTTAAATTTTATC TTAAT	677	CUUAAUUUUUAU CUUAAU
SCN1A- IVS23+721	166863 001	166863 019	318	TATGCCTTAAATT TTATC	678	UAUGCCUUAUU UUUAUC
SCN1A- IVS23+726	166862 996	166863 014	319	GAGTTTATGCCTT AAATT	679	GAGUUUAUGCCU UAAAUU
SCN1A- IVS23+731	166862 991	166863 009	320	GAAGTGAGTTTA TGCCTT	680	GAAGUGAGUUUA UGCCUU
SCN1A- IVS23+736	166862 986	166863 004	321	TCTAAGAAGTGA GTTTAT	681	UCUAAGAAGUGA GUUUUAU
SCN1A- IVS23+741	166862 981	166862 999	322	CTTATTCTAAGA AGTGAG	682	CUAAUUCUAAGA AGUGAG
SCN1A- IVS23+746	166862 976	166862 994	323	AGTTACTTATTCT AAGAA	683	AGUUACUUAUUC UAAGAA
SCN1A- IVS23+751	166862 971	166862 989	324	TTGGGAGTTACTT ATTCT	684	UUGGGAGUUACU UAUUCU
SCN1A- IVS23+756	166862 966	166862 984	325	GTTAGTTGGGAG TTACTT	685	GUUAGUUGGGAG UUACUU
SCN1A- IVS23+761	166862 961	166862 979	326	AGAAAGTTAGTT GGGAGT	686	AGAAAGUUAGUU GGGAGU
SCN1A- IVS23+766	166862 956	166862 974	327	ATCCTAGAAAGT TAGTTG	687	AUCCUAGAAAGU UAGUUG
SCN1A- IVS23+771	166862 951	166862 969	328	TTAAAATCCTAG AAAGTT	688	UUAAAAUCCUAG AAAGUU
SCN1A- IVS23+776	166862 946	166862 964	329	ATGTTTTTAAAATC CTAGA	689	AUGUUUUAAAAU CCUAGA
SCN1A- IVS23+781	166862 941	166862 959	330	GTGTTATGTTTTA AAATC	690	GUGUUAUGUUUU AAAAUC

SCN1A- IVS23+786	166862 936	166862 954	331	TCACTGTGTTATG TTTTA	691	UCACUGUGUUUAU GUUUUA
SCN1A- IVS23+791	166862 931	166862 949	332	TGTTTTCACTGTG TTATG	692	UGUUUUCACUGU GUUAUG
SCN1A- IVS23+796	166862 926	166862 944	333	ATGTATGTTTTCA CTGTG	693	AUGUAUGUUUUC ACUGUG
SCN1A- IVS23+801	166862 921	166862 939	334	TGTTTATGTATGT TTTCA	694	UGUUUAUGUAUG UUUUA
SCN1A- IVS23+806	166862 916	166862 934	335	AGTTATGTTTATG TATGT	695	AGUUAUGUUUAU GUAUGU
SCN1A- IVS23+811	166862 911	166862 929	336	TGTAGAGTTATG TTTATG	696	UGUAGAGUUAUG UUUAUG
SCN1A- IVS23+816	166862 906	166862 924	337	TAAATGTAGAG TTATGT	697	UAAAAUGUAGAG UUAUGU
SCN1A- IVS23+821	166862 901	166862 919	338	ATAAATAAAATG TAGAGT	698	AUAAAAUAAAUG UAGAGU
SCN1A- IVS23+826	166862 896	166862 914	339	TAAGAATAAATA AAATGT	699	UAAGAAUAAAUA AAAUGU
SCN1A- IVS23+831	166862 891	166862 909	340	AACTTTAAAGAAT AAATAA	700	AACUUUAAGAAU AAAUAA
SCN1A- IVS23+836	166862 886	166862 904	341	ACTTAAACTTTA AGAATA	701	ACUAAAACUUUA AGAAUA
SCN1A- IVS23+841	166862 881	166862 899	342	AATACACTTAAA CTTTAA	702	AAUACACUAAAA CUUUAA
SCN1A- IVS23+846	166862 876	166862 894	343	TGTATAATACAC TTAAAC	703	UGUAUAAUACAC UAAAC
SCN1A- IVS23+851	166862 871	166862 889	344	CTTCTTGATAAT ACACT	704	CUUCUUGUAUAA UACACU
SCN1A- IVS23+856	166862 866	166862 884	345	CTCTTCTTCTGT ATAAT	705	CUCUUCUUCUUG UAUAAU
SCN1A- IVS23+861	166862 861	166862 879	346	ATAAACTCTTCTT CTTGT	706	AUAAACUCUUCU UCUUGU
SCN1A- IVS23+866	166862 856	166862 874	347	CGAATATAAACT CTTCTT	707	CGAAUAUAAACU CUUCUU
SCN1A- IVS23+871	166862 851	166862 869	348	TCTCTCGAATATA AACTC	708	UCUCUCGAAUAU AAACUC
SCN1A- IVS23+876	166862 846	166862 864	349	TTCTGTCTCTCGA ATATA	709	UUCUGUCUCUCG AAUAUA
SCN1A- IVS23+881	166862 841	166862 859	350	ACTTTTTCTGTCT CTCGA	710	ACUUUUUCUGUC UCUCGA
SCN1A- IVS23+886	166862 836	166862 854	351	TTCTGACTTTTTC TGTCT	711	UUCUGACUUUUU CUGUCU
SCN1A- IVS23+891	166862 831	166862 849	352	AAAAATTCTGAC TTTTTC	712	AAAAAUUCUGAC UUUUUC
SCN1A- IVS23+896	166862 826	166862 844	353	CAAACAAAAATT CTGACT	713	CAAACAAAAAUU CUGACU
SCN1A- IVS23+901	166862 821	166862 839	354	TGATCCAAACAA AAATTC	714	UGAUCCAAACAA AAAUUC
SCN1A- IVS23+906	166862 816	166862 834	355	ATTGGTGATCCA AACAAA	715	AUUGGUGAUCCA AACAAA
SCN1A- IVS23+911	166862 811	166862 829	356	GATATATTGGTG ATCCAA	716	GAUAUAUUGGUG AUCCAA
SCN1A- IVS23+916	166862 806	166862 824	357	GCTATGATATATT GGTGA	717	GCUAUGAUUAU UGGUGA

SCN1A- IVS23+921	166862 801	166862 819	358	TGTAAGCTATGA TATATT	718	UGUAAGCUAUGA UAUAUU
SCN1A- IVS23+926	166862 796	166862 814	359	TTTTTTGTAAGCT ATGAT	719	UUUUUUGUAAGC UAUGAU
SCN1A- IVS23+931	166862 791	166862 809	360	ACAGTTTTTTTGT AAGCT	720	ACAGUUUUUUUG UAAGCU
SCN1A- IVS23+936	166862 786	166862 804	361	TTAAGACAGTTTT TTTGT	721	UUAAGACAGUUU UUUUGU
SCN1A- IVS23+941	166862 781	166862 799	362	TTTAATTAAGAC AGTTTT	722	UUUAAUUAAGAC AGUUUU
SCN1A- IVS23+946	166862 776	166862 794	363	TGGGTTTTAATTA AGACA	723	UGGGUUUUAAUU AAGACA
SCN1A- IVS23+951	166862 771	166862 789	364	TGTTGTGGGTTTT AATTA	724	UGUUGUGGGUUU UAAUUA
SCN1A- IVS23+956	166862 766	166862 784	365	AATTATGTTGTG GGTTTT	725	AAUUAUGUUGUG GGUUUU
SCN1A- IVS23+961	166862 761	166862 779	366	AAAAAAATTATG TTGTGG	726	AAAAAAUUAUG UUGUGG
SCN1A- IVS23+966	166862 756	166862 774	367	AATCTAAAAAAA TTATGT	727	AAUCUAAAAAAA UUAUGU
SCN1A- IVS23+971	166862 751	166862 769	368	TTAAAAATCTAA AAAAAT	728	UUAAAAAUCUAA AAAAAU
SCN1A- IVS23+976	166862 746	166862 764	369	CTTCTTAAAAAT CTAAA	729	CUUUCUAAAAAA UCUAAA
SCN1A- IVS23+981	166862 741	166862 759	370	AGAATCTTTCTTA AAAAT	730	AGAAUCUUUCUU AAAAAU
SCN1A- IVS23+986	166862 736	166862 754	371	ATAATAGAATCT TTCTTA	731	AUAAUAGAAUCU UUCUUA

Example 4: SCN1A Exon 23 (Exon 20x) Region Extended ASO Walk Evaluated by RT-PCR

[00232] ASO walk sequences can be evaluated by for example RT-PCR. In FIG. 5A, a representative PAGE shows SYBR-safe-stained RT-PCR products of SCN1A mock-treated, control ASO treated, targeting the exon 20x region as described herein in the Example 3 and in the description of FIG. 3, at 1 μ M concentration in RenCells by nucleofection. Two products, one comprising the exon 20x and one excluding exon 20x were quantified and the percent exon 20x inclusion is plotted in the bar graph (FIG. 5B). Taqman q PCR products were also normalized to RPL32 internal control and fold-change relative to mock is plotted in the bar graph (FIG. 5C).

[00233] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

CLAIMS

What is claimed is:

1. A method of modulating expression of SCN1A protein in a cell having an mRNA that contains a non-sense mediated RNA decay-inducing exon (NMD exon mRNA) and encodes SCN1A protein, the method comprising contacting a therapeutic agent to the cell, whereby the therapeutic agent modulates splicing of the NMD exon from the NMD exon mRNA encoding SCN1A protein, thereby modulating the level of processed mRNA encoding SCN1A protein, and modulating expression of SCN1A protein in the cell, wherein the therapeutic agent binds to a targeted portion of the NMD exon mRNA encoding SCN1A, and wherein the targeted portion is:
 - from about 1000 nucleotides upstream from the 5' end of an NMD-inducing exon (NIE) to about 100 nucleotides upstream from the 5' end of the NIE; or
 - from about 100 nucleotides downstream of the 3' end of the NIE to about 1000 nucleotides downstream of the 3' end of the NIE.
2. A method of treating a disease or condition in a subject in need thereof by modulating expression of SCN1A protein in a cell of the subject, comprising:
 - contacting the cell of the subject with a therapeutic agent that modulates splicing of a non-sense mediated mRNA decay-inducing exon (NMD exon) from an mRNA in the cell that contains the NMD exon and encodes SCN1A, thereby modulating the level of processed mRNA encoding the SCN1A protein, and modulating expression of SCN1A protein in the cell of the subject; wherein the therapeutic agent binds to a targeted portion of the NMD exon mRNA encoding SCN1A, and wherein the targeted portion is:
 - from about 1000 nucleotides upstream from the 5' end of an NMD-inducing exon (NIE) to about 100 nucleotides upstream from the 5' end of the NIE; or
 - from about 100 nucleotides downstream of the 3' end of the NIE to about 1000 nucleotides downstream of the 3' end of the NIE.
3. The method of claim 1 or 2, wherein the therapeutic agent interferes with binding of a factor involved in splicing of the NMD exon from a region of the targeted portion.
4. The method of claim 1 or 2, wherein the targeted portion is at most about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides upstream of 5' end of the NIE.

5. The method of claim 1 or 2, wherein the targeted portion is at least about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides, about 40 nucleotides, about 30 nucleotides, about 20 nucleotides, about 10 nucleotides, about 5 nucleotides, about 4 nucleotides, about 2 nucleotides, about 1 nucleotides upstream of 5' end of the NIE.
6. The method of claim 1 or 2, wherein the targeted portion is at most about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides downstream of 3' end of the NIE.
7. The method of claim 1 or 2, wherein the targeted portion is at least about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides, about 40 nucleotides, about 30 nucleotides, about 20 nucleotides, about 10 nucleotides, about 5 nucleotides, about 4 nucleotides, about 2 nucleotides, about 1 nucleotides downstream of 3' end of the NIE.
8. The method of any one of the claims 1-7, wherein the therapeutic agent is an antisense oligomer (ASO).
9. The method of claim 8, wherein the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% identity to any one of SEQ ID NOs: 12-731.
10. The method of any one of the claims 1-9, wherein the therapeutic agent promotes exclusion of the NMD exon from the processed mRNA encoding SCN1A protein.
11. The method of claim 10, wherein exclusion of the NMD exon from the processed mRNA encoding SCN1A protein in the cell contacted with the therapeutic agent is increased about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-

fold, compared to exclusion of the NMD exon from the processed mRNA encoding SCN1A protein in a control cell.

12. The method of claim 10, wherein the therapeutic agent increases level of the processed mRNA encoding SCN1A protein in the cell.
13. The method of claim 10, wherein an amount of the processed mRNA encoding SCN1A protein in the cell contacted with the therapeutic agent is increased about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to an total amount of the processed mRNA encoding SCN1A protein in a control cell.
14. The method of claim 2, wherein the disease or condition is induced by a loss-of-function mutation in *Nav1.1*.
15. The method of claim 14, wherein the disease or condition is associated with haploinsufficiency of the *SCN1A* gene, and wherein the subject has a first allele encoding a functional SCN1A, and a second allele from which SCN1A is not produced or produced at a reduced level, or a second allele encoding a nonfunctional SCN1A or a partially functional SCN1A.
16. The method of claim 14, wherein the disease or condition is encephalopathy.
17. The method of claim 16, wherein the encephalopathy is epileptic encephalopathy.
18. The method of claim 14, wherein the disease or condition is Dravet Syndrome (DS); severe myoclonic epilepsy of infancy (SMEI)-borderland (SMEB); Febrile seizure (FS); epilepsy, generalized, with febrile seizures plus (GEFS+); epileptic encephalopathy, early infantile, 13; cryptogenic generalized epilepsy; cryptogenic focal epilepsy; myoclonic-astatic epilepsy; Lennox-Gastaut syndrome; West syndrome; idiopathic spasms; early myoclonic encephalopathy; progressive myoclonic epilepsy; alternating hemiplegia of childhood; unclassified epileptic encephalopathy; sudden unexpected death in epilepsy (SUDEP); sick sinus syndrome 1; autism; or malignant migrating partial seizures of infancy.
19. The method of claim 18, wherein GEFS+ is epilepsy, generalized, with febrile seizures plus, type 2.

20. The method of claim 18, wherein the Febrile seizure is Febrile seizures, familial, 3A.
21. The method of claim 18, wherein SMEB is SMEB without generalized spike wave (SMEB-SW), SMEB without myoclonic seizures (SMEB-M), SMEB lacking more than one feature of SMEI (SMEB-O), or intractable childhood epilepsy with generalized tonic-clonic seizures (ICEGTC).
22. The method of claims 1 or 2, wherein the ASO consists of a sequence selected from SEQ ID NOs: 72 or 432.
23. The method of claims 1 or 2, wherein the ASO consists of a sequence selected from SEQ ID NOs: 73 or 433.
24. The method of claims 1 or 2, wherein the ASO consists of a sequence selected from SEQ ID NOs: 76 or 436.
25. The method of claims 1 or 2, wherein the ASO consists of a sequence selected from SEQ ID NOs: 181 or 541.
26. The method of claims 1 or 2, wherein the ASO consists of a sequence selected from SEQ ID NOs: 220 or 580.
27. A method of modulating expression of SCN1A protein in a cell having an mRNA that contains a non-sense mediated RNA decay-inducing exon (NMD exon mRNA) and encodes SCN1A protein, the method comprising contacting a therapeutic agent to the cell, whereby the therapeutic agent modulates splicing of the NMD exon from the NMD exon mRNA encoding SCN1A protein, thereby modulating the level of processed mRNA encoding SCN1A protein, and modulating expression of SCN1A protein in the cell, wherein the therapeutic agent binds to a targeted portion of the NMD exon mRNA encoding SCN1A, and wherein the targeted portion is from about 1000 nucleotides upstream from the 5' end of an NMD-inducing exon (NIE) to about 1000 nucleotides downstream of the 3' end of the NIE.
28. A method of treating a disease or condition in a subject in need thereof by modulating expression of SCN1A protein in a cell of the subject, comprising:
 - contacting the cell of the subject with a therapeutic agent that modulates splicing of a non-sense mediated mRNA decay-inducing exon (NMD exon) from an mRNA in the cell that contains the NMD exon and encodes SCN1A, thereby modulating the level of processed mRNA encoding the SCN1A protein, and modulating expression of SCN1A protein in the cell of the subject; wherein the therapeutic agent binds to a targeted portion of the NMD exon mRNA encoding SCN1A, and wherein the targeted portion is from about 1000 nucleotides upstream from the 5' end of an NMD-inducing exon (NIE) to about 1000 nucleotides downstream of the 3' end of the NIE.

29. An antisense oligomer (ASO) comprising a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% identity to any one of SEQ ID NOs: 12-731.
30. An antisense oligomer (ASO) consisting of a sequence selected from SEQ ID NOs: 12-731.
31. A method of treating a disease or condition in a subject in need thereof by modulating expression of SCN1A protein in a cell of the subject, comprising:
contacting the cell of the subject with an ASO of claim 29 or claim 30.
32. A kit comprising an ASO of claim 29 or claim 30.