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(54) Title: COMPOSITIONS AND METHODS FOR TREATING DISORDERS ASSOCIATED WITH LOSS-OF-FUNCTION MUTATIONS IN SCN2A

(57) Abstract: Provided herein are methods for increasing levels of SCN2A protein in a cell, comprising contacting the cell with an antisense oligonucleotide that enhances splicing at a splice site of a retained intron in an intron-retaining SCN2A mRNA or pre-mRNA, wherein the retained intron is selected from among intron 1, 2, 3, 4, 5, 11, 13, 17 and 24 and wherein the antisense oligonucleotide comprises a sequence of nucleobases that is complementary to a target region in the SCN2A mRNA or pre-mRNA. Also provided are antisense oligonucleotides for use in such methods. Also provided are methods for treating disorders associated with a heterozygous loss-of-function mutation in SCN2A, comprising administering to the subject such antisense oligonucleotides.



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COMPOSITIONS AND METHODS FOR TREATING DISORDERS ASSOCIATED WITH LOSS-OF-FUNCTION MUTATIONS IN SCN2A

Field of the Disclosure

[001] The present disclosure relates generally to compositions and methods suitable for treating a disorder associated with loss-of-function mutations in *SCN2A*. More specifically, the disclosure relates to antisense oligonucleotides specific for *SCN2A* and their use for treating a disorder associated with heterozygous loss-of-function mutations of *SCN2A*.

Background to the Disclosure

[002] The *SCN2A* protein (also referred to as sodium voltage-gated channel alpha subunit 2, brain Nav1.2 voltage-gated sodium channel, SCN2A1, SCN2A2, Nav1.2, HBA, NAC2, BFIC3, BFIS3, BFNIS, HBSCI, EIEE11 or HBSCII,) is encoded by the *SCN2A* gene on human chromosome 2 at 2q24.3 (HGNC:10588; NCBI gene:6326; NCBI Reference Sequence: NG_008143.1). *SCN2A* plays a key role in the initiation and propagation of action potentials and the modulation of neuronal and brain excitability. *SCN2A* expression is detected in brain and kidney, however its expression is predominantly in the brain.

[003] In the brain *SCN2A* is heterogeneously expressed, and mutations, dysfunction, and/or dysregulation of the protein or levels of functional protein are associated with various neurodevelopmental disorders. With the large sequencing studies performed in the recent years, *SCN2A* has emerged as the major single gene cause of neurogenetic disorders. *SCN2A* mutations are the second most common cause of severe genetic epilepsy (including developmental and epileptic encephalopathies (DEE)) in the human population (Nakamura et al, 2013; Howell et al, 2015), and are also frequently associated with intellectual disability (Rauch et al, 2012; Li et al, 2016), autism spectrum disorders (Iossifov et al, 2014; Sanders et al, 2015; Wang et al, 2016) and schizophrenia (Carroll et al, 2016).

[004] The *SCN2A* primary transcript is alternatively spliced, resulting in multiple mRNA transcript variants, some of which are known to be developmentally regulated. Some forms of epileptic encephalopathies have been associated with variants in *SCN2A*. Loss-of-function mutations or alterations (e.g. nonsense mutations, large deletion and frameshift mutations) in *SCN2A* may result in a decreased level of functional protein, or the formation of a truncated transcript, leading to the haploinsufficiency. The *SCN2A* gene may have a missense or nonsense mutation in one or both alleles. In some instances, expression of *SCN2A* may be dysregulated by

a primary disorder or a mutation in the gene or a regulatory region, thus resulting in decreased levels of protein or functional protein. In other instances, SCN2A levels are not altered, but increasing levels of functional SCN2A protein provides a therapeutic effect for a neurological disorder or psychiatric disorder.

[005] The only therapies currently available to patients with *SCN2A* or any other disorder associated with a heterozygous loss-of-function mutation in *SCN2A* are those that treat a symptom of the disorder, such as agents to treat epileptic seizures or interventions (e.g. speech therapy, physiotherapy, occupational therapy, etc.) to treat the behavioural or developmental symptoms or intellectual disability. Consequently, there remains a need for agents, compositions and methods for the treatment of MRD5 or any other disorder associated with a heterozygous loss-of-function mutation in *SCN2A*.

Summary of the Disclosure

[006] The present disclosure is predicated, at least in part, on the determination that a number of introns are retained in mature *SCN2A* mRNA in brain tissue, including introns 1, 2, 3, 4, 5, 11, 13, 17 and 24. Introns 2 and 17 in particular have relatively high retention rates.

[007] Intron retention is a form of gene regulation that serves to direct intron-harboured transcripts to nonsense-mediated decay, thereby reducing gene expression (Kurosaki & Maquat, 2016, *J Cell Sci.* 129 (3): 461-467). Intron-retaining transcripts have also been shown to serve as a reservoir of RNAs that undergo splicing and translation whenever their expression is required (Jacob & Smith, 2017, *Hum Genet.* 136 (9): 1043-1057). The process of transcription, which occurs at a rate of 1-4 kb/min, is especially rate-limiting for neuronal activation following neuronal stimuli (Darzacq et al, 2007, *Nat Struct. Mol. Biol.* 14, 796-806). In contrast, splicing of retained introns is a much faster process, taking just seconds to a few minutes (Bayer and Osheim, 1988, *Genes Dev.* 2, 754-765; Singh and Padgett, 2009, *Nat. Struct. Mol. Biol.* 16, 1128-1133). Consequently, neurons can achieve a faster mode of gene regulation using intron retention and subsequent splicing and translation as compared to *de novo* transcription and translation. Intron retention has been demonstrated to occur in a pool of polyadenylated transcripts that are retained in the nucleus. Following neuronal stimulation, they undergo intron excision and are transported to the cytoplasm for further processing, thereby aiding in faster gene regulation.

[008] As demonstrated herein, a retained intron in *SCN2A* mRNA or pre-RNA (such as polyadenylated *SCN2A* mRNA or pre-mRNA transcripts in the nucleus of a cell) can be targeted with antisense oligonucleotides so as to enhance splicing at the

splice site of the retained intron, resulting in an increase in the amount of fully-spliced SCN2A mRNA. Consequently, the antisense oligonucleotides provided herein are useful for increasing the amount of SCN2A produced by a cell. The antisense oligonucleotides provided herein are therefore also useful as therapeutic agents for the treatment of diseases or disorders associated with heterozygous loss-of-function mutations in *SCN2A*, such as severe genetic epilepsy (including developmental and epileptic encephalopathies (DEE)) in the human population, and it is also frequently associated with intellectual disability, autism spectrum disorders and schizophrenia, wherein increasing the levels of SCN2A protein can provide a therapeutic effect.

[0009] Accordingly, in one aspect, provided is a method for increasing levels of SCN2A protein in a cell, comprising contacting the cell with an antisense oligonucleotide that enhances splicing at a splice site of a retained intron in an intron-retaining SCN2A mRNA or pre-mRNA, wherein the retained intron is selected from among introns 1, 2, 3, 4, 5, 11, 13, 17 and 24 and wherein the antisense oligonucleotide comprises a sequence of nucleobases that is complementary to a target region in the SCN2A mRNA or pre-mRNA.

[0010] In an embodiment, the retained intron is intron 2, comprising the nucleotide sequence set forth in SEQ ID NO: 12.

[0011] In another aspect, provided is a method for increasing levels of SCN2A protein in a subject, comprising administering to the subject an antisense oligonucleotide that enhances splicing at a splice site of a retained intron in an intron-retaining SCN2A mRNA or pre-mRNA, wherein the retained intron is selected from among intron 1, 2, 3, 4, 5, 11, 13, 17 and 24 and wherein the antisense oligonucleotide comprises a sequence of nucleobases that is complementary to a target region in the SCN2A mRNA or pre-mRNA. In some embodiments, the subject has a heterozygous loss-of-function mutation in *SCN2A*.

[0012] In some embodiments, the subject has a disorder associated with a heterozygous loss-of-function mutation in *SCN2A*, such as genetic epilepsy (including developmental and epileptic encephalopathies (DEE)), intellectual disability, autism spectrum disorders and schizophrenia.

[0013] Also provided is a method for treating a disorder associated with a heterozygous loss-of-function mutation in *SCN2A*, comprising administering to the subject an antisense oligonucleotide that enhances splicing at a splice site of a retained intron in an intron-retaining SCN2A mRNA or pre-mRNA, wherein the retained intron is selected from among introns 1, 2, 3, 4, 5, 11, 13, 17 and 24, and wherein the

antisense oligonucleotide comprises a sequence of nucleobases that is complementary to a target region in the SCN2A mRNA or pre-mRNA. In particular examples, genetic epilepsy (including developmental and epileptic encephalopathies (DEE)), intellectual disability, autism spectrum disorders and schizophrenia.

[0014] In some embodiments of the methods of the disclosure, the antisense oligonucleotide binds to, or adjacent to, an intron splicing silencer (ISS); binds to nucleotides within a G-quadruplex; or binds to nucleotides with an RNA secondary structure. The ISS may be recognised by a heterogeneous nuclear ribonucleoprotein (hnRNP), such as hnRNPA1 or hnRNP I.

[0015] In one example, the retained intron is intron 2 and the ISS is at positions +8—+12, +33—+36 or +60—+65, relative to the 5' splice site of intron 2. Optionally, the ISS is at positions +8—+12, +33—+36 or +60—+65 relative to the first nucleotide of SEQ ID NO:12.

[0016] In particular embodiments, the retained intron is intron 2 and the target region spans positions -6—+12, -5—+13, -4—+14, -3—+15, -2—+16, -1—+19, 0—+18, +1—+19, +2—+20, +3—+21, +4—+22, +5—+23, +6—+24, +7—+25, +8—+26, +9—+27, +10—+28, +11—+29, +12—+30, +13—+31, +14—+32, +15—+33, +16—+34, +17—+35, +18—+36, +19—+37, +20—+38, +21—+39, +22—+40, +23—+41, +24—+42, +25—+43, +26—+44, +27—+45, +28—+46, +29—+47, +30—+48, +31—+49, +32—+50, +33—+51, +34—+52, +35—+53, +36—+54, +37—+55, +38—+56, +39—+57, +40—+58, +41—+59, +42—+60, +43—+61, +44—+62, +45—+63, +46—+64, +47—+65, +48—+66, +49—+67, +50—+68, +51—+69, +52—+70, +53—+71, +54—+72, +55—+73, +56—+74, +57—+75, +58—+76, +59—+77, +60—+78, +61—+79, +62—+80, +63—+81, +64—+82, +65—+83, +66—+84, +67—+85, +68—+86, +69—+87, +70—+88, +71—+89, +72—+90, +73—+91, +74—+92, +75—+93, +76—+94, +77—+95, +78—+96, +79—+97, +80—+98, +81—+99, relative to the 5' splice site of intron 2, optionally relative to the first nucleotide of SEQ ID NO:12. In some embodiments, the antisense oligonucleotide comprises a sequence having at least or about 70%, 80%, or 90% sequence identity to a sequence set forth in any one of SEQ ID NOs:115-142, or a sequence having at least 8, 9, 10, 11, 12, 13, 14 or 15 contiguous nucleotides from a sequence set forth in any one of SEQ ID NOs: 115-142. In one embodiment, the antisense oligonucleotide comprises the sequence set forth in SEQ ID NO:126 or SEQ ID NO:138, or a sequence comprising at least 8, 9, 10, 11, 12, 13, 14 or 15 contiguous nucleotides from a sequence set forth in SEQ ID NO:126 or SEQ ID NO:138.

[0017] In further embodiments, the retained intron is intron 2 and the target region spans positions -19--1, -20--2, -21--3, -22--4, -23--5, -24--6, -25--7, -26--8, -27--9, -28--10, -29--11, -30--12, -31--13, -32--14, -33--15, -38--20, -39--21, -48--30, -49--31, -51--33, -52--34, -53--35, -54--36, -62--44, -64--46, -65--47, -66--48, -67--49, -76--58, -77--59, -78--60, -79--61, -82--64, -83--65, -84--66, -85--67, -86--68, -87--69, -90--72, -91--73, -92--74, -95--77, -97--79, -98--80, -99--81, -101--83, -102--84, -103--85, -104--86, -105--87, -106--88, -107--89, -108--90, -109--91, -110--92, -111--93, -112--94, -113--95, -114--96, -115--97, -116--98, -117--99, relative to the 3' splice site of intron 2, optionally relative to the last nucleotide of SEQ ID NO:12. In some embodiments, the antisense oligonucleotide comprises a sequence having at least or about 70%, 80%, or 90% sequence identity to a sequence set forth in any one of SEQ ID NOs:143-205, or a sequence having at least 8, 9, 10, 11, 12, 13, 14 or 15 contiguous nucleotides from a sequence set forth in any one of SEQ ID NOs: 143-205. In one embodiment, the antisense oligonucleotide comprises the sequence set forth in SEQ ID NO:155, or a sequence comprising at least 8, 9, 10, 11, 12, 13, 14 or 15 contiguous nucleotides from a sequence set forth in SEQ ID NO:155.

[0018] In the methods of the present disclosure, the antisense oligonucleotide may consist of, for example, from 8 to 50, 8 to 40, 8 to 35, 8 to 30, 8 to 25, 8 to 20, 8 to 15, 9 to 50, 9 to 40, 9 to 35, 9 to 30, 9 to 25, 9 to 20, 9 to 15, 10 to 50, 10 to 40, 10 to 35, 10 to 30, 10 to 25, 10 to 20, 10 to 15, 11 to 50, 11 to 40, 11 to 35, 11 to 30, 11 to 25, 11 to 20, 11 to 15, 12 to 50, 12 to 40, 12 to 35, 12 to 30, 12 to 25, 12 to 20, or 12 to 15 nucleobases. In some embodiments, the antisense oligonucleotide is at least 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% complementary to the target region. In particular embodiments, the antisense oligonucleotide comprises least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 contiguous nucleobases that are 100% complementary to the target region.

[0019] The antisense oligonucleotide utilised in the methods of the present disclosure may comprise at least one modification, *e.g.* a nucleobase modification, a modification of the oligonucleotide backbone or a modification of a ribose sugar. In one embodiment, the antisense oligonucleotide comprises a modified sugar selected from among a 2'-O-methyl (2OMe), 2'-O-methoxy-ethyl (MOE), locked nucleic acids (LNA), 2'-fluoro or S-constrained-ethyl (cEt). In a further embodiment, the antisense oligonucleotide comprises backbone that comprises phosphorothioates.

[0020] In the methods of the disclosure that include administering to the subject an antisense oligonucleotide, the subject may first be determined to have a loss-of-

function mutation in *SCN2A*. In particular embodiments, the subject has been genotyped to identify a heterozygous loss-of-function mutation in *SCN2A*. The antisense oligonucleotide may be administered to the subject by parenteral administration (e.g. subcutaneous administration, intravenous administration, intramuscular administration, intraarterial administration, intraperitoneal administration, or intracranial administration) or intranasal administration (e.g. intrathecal or intracerebroventricular administration). In some embodiments, the antisense oligonucleotide or composition is administered to the subject about every 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or more months.

[0021] In a further aspect, provided herein is an antisense oligonucleotide comprising a sequence of nucleobases that is complementary to a target region in an intron-retaining *SCN2A* mRNA or pre-mRNA, wherein the target region is in a retained intron and wherein the retained intron is selected from among intron 1, 2, 3, 4, 5, 11, 13, 17 and 24.

[0022] In some embodiments, the antisense oligonucleotide binds to, or adjacent to, an intron splicing silencer (ISS); binds to nucleotides within a G-quadruplex; or binds to nucleotides with an RNA secondary structure. In a particular embodiment, the ISS is recognised by a heterogeneous nuclear ribonucleoproteins (hnRNP), e.g. hnRNPA1 or hnRNP I.

[0023] In one embodiment, the retained intron in which the target region is present is intron 2 and the ISS is at positions +8—+12, +33—+36 or +60—+65 relative to the 5' splice site of intron 2. Optionally, the ISS is at positions +8—+12, +33—+36 or +60—+65 relative to the first nucleotide of SEQ ID NO:12.

[0024] In particular embodiments, the retained intron is intron 2 and the target region spans positions -4—+14, -3—+15, -2—+16, +6—+24, +7—+25, +8—26, +9—+27, +10—+28, +14—+32, +19—+37, +20—+38, +21—+39, +22—+40, +23—+41, +24—+42, +25—+43, +26—+44, +27—+45, +28—+46, +29—+47, +30—+48, +31—+49, +32—+50, +33—+51, +34—+52, +35—+53, +43—+61, +45—+63, +46—+64, +49—+67, +52—+70, +59—+77, +64—+82, +65—+83, +68—+86, +69—+87, +70—+88, +71—+89, +72—+90 and +73—+91, relative to the 5' splice site of intron 2, optionally relative to the first nucleotide of SEQ ID NO:12. In some embodiments, the antisense oligonucleotide comprises a sequence having at least or about 70%, 80%, or 90% sequence identity to a sequence set forth in any one of SEQ ID NOs:115-142, or a sequence having at least 8, 9, 10, 11, 12, 13, 14 or 15 contiguous nucleotides from a sequence set forth in any one of SEQ ID NOs: 115-142. In one embodiment, the antisense oligonucleotide comprises the sequence set forth in

SEQ ID NO:126 or SEQ ID NO:138, or a sequence comprising at least 8, 9, 10, 11, 12, 13, 14 or 15 contiguous nucleotides from a sequence set forth in SEQ ID NO:126 or SEQ ID NO:138.

[0025] In further embodiments, the retained intron is intron 2 and the target region spans positions -19--1, -21--3, -30--12, -31--13, -32--14, -62--44, -64--46, -67--49, -76--58, -77--59, -78--60, -79--61 -82--64, -83--65, -84--66, -85--67, -86--68, -87--69, -95--77, -97--79, -100--82, -101--83, -102--84, -103--85, -107--89, -109--91, -111--93, -113--95, and -114--96, relative to the 3' splice site of intron 2, optionally relative to the last nucleotide of SEQ ID NO:12. In some embodiments, the antisense oligonucleotide comprises a sequence having at least or about 70%, 80%, or 90% sequence identity to a sequence set forth in any one of SEQ ID NOs:143-205, or a sequence having at least 8, 9, 10, 11, 12, 13, 14 or 15 contiguous nucleotides from a sequence set forth in any one of SEQ ID NOs: 143-205. In one embodiment, the antisense oligonucleotide comprises the sequence set forth in SEQ ID NO:155, or a sequence comprising at least 8, 9, 10, 11, 12, 13, 14 or 15 contiguous nucleotides from a sequence set forth in SEQ ID NO:155.

[0026] In some embodiments, the antisense oligonucleotide may consist of, for example, from 8 to 50, 8 to 40, 8 to 35, 8 to 30, 8 to 25, 8 to 20, 8 to 15, 9 to 50, 9 to 40, 9 to 35, 9 to 30, 9 to 25, 9 to 20, 9 to 15, 10 to 50, 10 to 40, 10 to 35, 10 to 30, 10 to 25, 10 to 20, 10 to 15, 11 to 50, 11 to 40, 11 to 35, 11 to 30, 11 to 25, 11 to 20, 11 to 15, 12 to 50, 12 to 40, 12 to 35, 12 to 30, 12 to 25, 12 to 20, or 12 to 15 nucleobases. In some embodiments, the antisense oligonucleotide is at least 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% complementary to the target region. In particular embodiments, the antisense oligonucleotide comprises least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 contiguous nucleobases that are 100% complementary to the target region.

[0027] The antisense oligonucleotide may comprise at least one modification, *e.g.* a nucleobase modification, a modification of the oligonucleotide backbone or a modification of a ribose sugar. In one embodiment, the antisense oligonucleotide comprises a modified sugar selected from among a 2'-O-methyl (2OMe), 2'-O-methoxy-ethyl (MOE), locked nucleic acids (LNA), 2'-fluoro or S-constrained-ethyl (cEt). In a further embodiment, the antisense oligonucleotide comprises backbone that comprises phosphorothioates.

[0028] Also provided are compositions comprising an antisense oligonucleotide of the present disclosure, such as pharmaceutical compositions.

[0029] In a further aspect, provided is a use of an antisense oligonucleotide for the treatment of a disorder associated with a heterozygous loss-of-function mutation in *SCN2A*, wherein the antisense oligonucleotide enhances splicing at a splice site of a retained intron in an intron-retaining *SCN2A* mRNA or pre-mRNA, wherein the retained intron is selected from among introns 1, 2, 3, 4, 5, 11, 13, 17 and 24 and wherein the antisense oligonucleotide comprises a sequence of nucleobases that is complementary to a target region in the *SCN2A* mRNA or pre-mRNA.

[0030] In yet another aspect, provided is a use of an antisense oligonucleotide for the treatment of a disorder associated with a heterozygous loss-of-function mutation in *SCN2A*, wherein the antisense oligonucleotide enhances splicing at a splice site of a retained intron in an intron-retaining *SCN2A* mRNA or pre-mRNA, wherein the retained intron is selected from among introns 2 and 17, wherein the antisense oligonucleotide comprises a sequence of nucleobases that is complementary to a target region in the *SCN2A* mRNA or pre-mRNA.

Brief Description of the Drawings

[0031] **Figure 1** is a schematic representation of intron retention in *SCN2A*, as analysed from information obtained from IRBase. The top panel shows a genomic map of *SCN2A* gene from UCSC browser. Thin lines represent the introns and the thick lines/blocks correspond to the exons. The bottom panel shows intron-retention events corresponding to introns in the genomic map. The height of the bars is indicative of the number of recorded events.

[0032] **Figure 2** is a schematic showing the design of primers for each exon-intron pair across the *SCN2A* sequence. **A.** Primers that are specific for intron-retaining transcripts: The forward primer was designed from the sequence of the preceding exon and the reverse primer from the sequence of the intron downstream to the exon. **B.** Primers specific to spliced transcripts: One of the primers was designed such that it spanned the junction of two nearby exons, while the other was designed from the sequence of the preceding or the succeeding exon accordingly.

[0033] **Figure 3** shows relative expression of introns in whole brain *SCN2A* mRNA obtained from two commercial sources. The expression of individual introns across the entire transcript was compared with the averaged exon expression. The results are a representation of three experiments, with the standard error of the mean indicated. **A.** mRNA from source 1 – Ambion. **B.** mRNA from source 2 – Takara.

[0034] **Figure 4** shows relative expression of introns in *SCN2A* mRNA from cell lines. The expression of individual introns across the entire transcript was compared

with the averaged exon expression. The results are a representation of three experiments, with the standard error of the mean indicated. **A.** mRNA from SH-SY5Y cells. **B.** mRNA from SK-N-AS cells.

[0035] **Figure 5** is a schematic of the secondary structure prediction of SCN2A intron 2.

[0036] **Figure 6** is a graphical and schematic representation of modulation of SCN2A transcript expression in the presence of antisense oligonucleotides. Following transfection and incubation, the expression of SCN2A was analysed by qPCR. Mock transfected cells were used as a negative control. The housekeeping gene HPRT1 was used for normalization. The number of biological replicates ranges from 3 to 6. The regions highlighted in red and green are the predicted binding sites for HnRNPA1 and HnRNPI, respectively. **A.** Effect of antisense oligonucleotides targeting the 5' end of SCN2A intron 2 on its expression. **B.** Effect of antisense oligonucleotides targeting the 3' end of SCN2A intron 2 on its expression.

[0037] **Figure 7** shows the effect of antisense oligonucleotides INT2 +6 (SEQ ID NO:126; targeting a region 6 base pairs downstream of 5' of intron 2) on the expression of the SCN2A in is shown, using two sets of primers that cross the exon to exon boundaries of Exons 2 and 3 (**A**), and Exons 18 and 19 (**B**). In comparison, antisense oligonucleotide INT2 +35 (SEQ ID NO:142; targeting a region 35 base pairs downstream of 5' of intron 2) does not result in increase in SCN2A transcripts. Mock transfected cells were used as a negative control.

Detailed Description

[0038] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which the disclosure belongs. All patents, patent applications, published applications and publications, databases, websites and other published materials referred to throughout the entire disclosure, unless noted otherwise, are incorporated by reference in their entirety. In the event that there is a plurality of definitions for terms, those in this section prevail. Where reference is made to a URL or other such identifier or address, it understood that such identifiers can change and particular information on the internet can come and go, but equivalent information can be found by searching the internet. Reference to the identifier evidences the availability and public dissemination of such information.

[0039] As used herein, the singular forms "a", "an" and "the" also include plural aspects (*i.e.* at least one or more than one) unless the context clearly dictates

otherwise. Thus, for example, reference to "a polypeptide" includes a single polypeptide, as well as two or more polypeptides.

[0040] In the context of this specification, the term "about," is understood to refer to a range of numbers that a person of skill in the art would consider equivalent to the recited value in the context of achieving the same function or result.

[0041] Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

[0042] An "antisense oligonucleotide" refers to a single-stranded oligonucleotide having a sequence that permits hybridization to a corresponding region or segment of a target nucleic acid. Reference to an antisense oligonucleotide includes reference to both unmodified and modified antisense oligonucleotides, wherein a modified antisense oligonucleotide contains at least one modified nucleoside and/or modified internucleoside linkage.

[0043] "Complementary," as used herein, refers to the capacity for precise pairing between two nucleobases, such as between a nucleobase in an antisense oligonucleotide and a nucleobase in a SCN2A mRNA or pre-mRNA. The antisense oligonucleotide and the mRNA or pre-mRNA are complementary to each other when a sufficient number of corresponding positions in each molecule are occupied by nucleobases which can hydrogen bond with each other. Thus, "complementary" is used to indicate a sufficient degree of precise pairing over a sufficient number of nucleotides such that stable and specific binding occurs between the antisense oligonucleotide and the mRNA or pre-mRNA. It is understood that the antisense oligonucleotide need not be 100% complementary to the target region in the SCN2A mRNA or pre-mRNA to hybridize thereto. Moreover, an oligonucleotide may be complementary to, and hybridize, over one or more segments such that intervening or adjacent segments are not involved in the hybridization event. "Complementary" as used herein therefore includes reference to less than 100% complementary, such as at least or about 70%, 75%, 80%, 85%, 90% or 95% sequence complementarity.

[0044] As used herein, a "disorder associated with a loss-of-function mutation in SCN2A" refers to a disorder that is associated with, is partially or completely caused by, or has one or more symptoms that are partially or completely caused by, a

mutation in *SCN2A* that results in a loss-of-function phenotype, *i.e.* an decrease in the level (or amount) or activity of *SCN2A*.

[0045] As used herein, "expression of *SCN2A*" refers to the transcription of mRNA from *SCN2A* or the translation of protein from the *SCN2A* mRNA. *SCN2A* expression can be assessed using any method known in the art, including, but not limited to, Northern blot, Western blot and qRT-PCR.

[0046] As used herein, a "loss-of-function mutation" is a mutation in *SCN2A* that results in a decrease in expression and/or activity of the encoded *SCN2A* protein. Expression of the encoded *SCN2A* protein can be assessed using standard assays, such as Western blot. Typically, a loss-of-function mutation results in a decrease of at least or about 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or more of the expression and/or activity of the encoded *SCN2A* protein. In some examples, the loss-of-function mutation results in a complete (*i.e.* 100%) loss of expression or activity of the encoded *SCN2A* protein, such as when the mutation is a mutation (*e.g.* nonsense mutation, large deletion or frameshift mutation) that results in the formation of a truncated transcript that is either not translated or is translated to a non-functional protein. A "heterozygous loss-of-function mutation" in *SCN2A* is one that is present in only one copy of *SCN2A* in the cell (*i.e.* one allele is a wild-type allele) and can lead to haploinsufficiency.

[0047] A "gapmer" as referred to herein is a chimeric antisense oligonucleotide in which an internal region having a plurality of nucleotides that support RNase H cleavage is positioned between external regions having one or more nucleotides, wherein the nucleotides comprising the internal region are chemically distinct from the nucleoside or nucleotides comprising the external regions.

[0048] As used herein, "hybridization" or "binding" or grammatical variations thereof means the pairing of substantially complementary strands of nucleic acids, such as between an antisense oligonucleotide of the disclosure and a *SCN2A* mRNA or pre-mRNA. One mechanism of pairing involves hydrogen bonding, which may be Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding, between complementary nucleobases of the strands of nucleic acids. For example, adenine and thymine or uracil are complementary nucleotides which pair through the formation of hydrogen bonds. Hybridization can occur under varying circumstances. Reference to "hybridizes" or "binds" as used herein means that the antisense oligonucleotide hybridizes or binds to a target region in *SCN2A* mRNA or pre-mRNA by virtue of the complementarity in sequence between the antisense oligonucleotide and the target region, and does not significantly bind to a non-target region.

[0049] The terms "linked" and "attached" are used interchangeably and relate to any type of interaction that join two entities, such as an antisense oligonucleotide and a moiety (*e.g.* a cell penetrating peptide), and include covalent bonds or non-covalent bonds, such as, for example, hydrophobic/hydrophilic interactions, van der Waals forces, ionic bonds or hydrogen bonds.

[0050] The term "exon" refers to a portion of a gene that is present in the mature form of mRNA. Exons include the ORF (open reading frame), *i.e.*, the sequence which encodes protein, as well as the 5' and 3' UTRs (untranslated regions). The UTRs are important for translation of the protein. Algorithms and computer programs are available for predicting exons in DNA sequences (*e.g.* Grail, Grail 2 and Genscan and US 20040219522 for determining an exon-intron junctions).

[0051] The term "intron" refers to a portion of a gene that is not translated into a wild-type protein and while present in genomic DNA and pre-mRNA, it is generally removed in the formation of mature mRNA by splicing.

[0052] The term "messenger RNA" or "mRNA" refers to RNA that is transcribed from genomic DNA and that carries the coding sequence for protein synthesis. The terms "precursor mRNA" or "pre-mRNA" refer to an immature single strand of messenger ribonucleic acid (mRNA) that contains one or more introns and that is directly transcribed from the DNA; for the purposes of the present disclosure, it is considered pre-mRNA until the poly(A) is added and 5' and 3' modifications take place. Pre-mRNA is transcribed by an RNA polymerase from a DNA template in the cell nucleus and is comprised of alternating sequences of introns and exons. In eukaryotes, pre-mRNA is processed into mRNA, which includes removal of the introns, *i.e.*, "splicing", and modifications to the 5' and 3' end (*e.g.*, polyadenylation). mRNA typically comprises from 5' to 3'; a 5' cap (modified guanine nucleotide), 5' UTR (untranslated region), the coding sequence (beginning with a start codon and ending with a stop codon), the 3' UTR, and the poly(A) tail. Eukaryotic pre-mRNAs exist only transiently before being processed into mRNA. As described herein, polyadenylated transcripts in the nucleus of a cell can have one or more retained introns even after initial splicing of the primary transcript and addition of the poly(A) tail. For the purposes of the present disclosure, these transcripts are considered mRNA with retained introns. When a pre-mRNA has been properly processed to an mRNA, it is exported out of the nucleus and translated into a protein by ribosomes in the cytoplasm. The term "fully-spliced mRNA" as used herein means that the mRNA does not contain any introns, or does not contain the intron being targeted by the antisense oligonucleotides and methods according to the present disclosure.

[0053] As used herein, "nucleobase" means a heterocyclic moiety capable of pairing with a base of another nucleic acid, and includes, for example, adenine (A), guanine (G), cytosine (C), thymine (T) and uracil (U). Reference herein to nucleobase also includes reference to a modified nucleobase.

[0054] A "nucleoside" as used herein refers to a nucleobase linked to a sugar. Reference herein to a nucleoside also includes reference to a modified nucleoside, which has a modified sugar moiety or modified nucleobase. A "nucleoside mimetic" includes those structures used to replace the sugar or the sugar and the base and not necessarily the linkage at one or more positions of an oligomeric compound such as for example nucleoside mimetics having morpholino, cyclohexenyl, cyclohexyl, tetrahydropyranyl, bicyclo or tricyclo sugar mimetics *e.g.* non furanose sugar units.

[0055] As used herein, "nucleotide" refers to a nucleoside having a phosphate group covalently linked to the sugar portion of the nucleoside. Reference herein to a nucleotide also includes reference to a modified nucleotide, which has a modified sugar moiety, modified internucleoside linkage, or modified nucleobase. A "nucleotide mimetic" includes those structures used to replace the nucleoside and the linkage at one or more positions of an oligomeric compound such as for example peptide nucleic acids or morpholinos (morpholinos linked by $-N(H)-C(=O)-O-$ or other non-phosphodiester linkage).

[0056] The term "splicing" refers to the modification of a pre-mRNA following transcription, in which introns are removed and exons are joined. Pre-mRNA splicing involves two sequential biochemical reactions. Both reactions involve the spliceosomal transesterification between RNA nucleotides. In a first reaction, the 2'-OH of a specific branch-point nucleotide within an intron, which is defined during spliceosome assembly, performs a nucleophilic attack on the first nucleotide of the intron at the 5' splice site forming a lariat intermediate. In a second reaction, the 3'-OH of the released 5' exon performs a nucleophilic attack at the last nucleotide of the intron at the 3' splice site thus joining the exons and releasing the intron lariat.

[0057] As used herein, the term "sequence identity" or "% identical" or grammatical variations means that in a comparison of two sequences over a specified region the two sequences have the specified number or percentage of identical residues in the same position. Sequences can be aligned by any method known to those of skill in the art. Such methods typically maximize matches, and include methods such as using manual alignments and by using the numerous alignment programs available.

[0058] The term "splice site" refers to the junction between an exon and an intron in a pre-mRNA molecule (also known as a "splice junction"). The "splice site sequence" is the sequence surround the splice site that is capable of being recognised by the splicing machinery of the cell. A 5' splice site (also referred to as a splice donor site) is the splice site at the 5' end of the intron that marks the start of the intron and its boundary with the preceding exon sequence. A 3' splice site (also referred to as a splice acceptor site) is the splice site at the 3' end of the intron that marks the end of the intron and its boundary with the following exon sequence. Numbering used herein in reference to a 5' splice site of an intron is therefore also in reference to the first nucleotide of the intron. Thus, for example, reference to position +1 relative to the 5' splice site of an intron is reference to the first nucleotide in the intron sequence, *e.g.* reference to position +1 relative to the 5' splice site of intron 2 is reference to nucleotide position 1 of the intron 2 sequence, *e.g.* position 1 of SEQ ID NO:12. In another example, reference to positions +18—+27 relative to the 5' splice site of an intron is reference to the 18th through to the 27th nucleotide position of the intron sequence, *e.g.* position 18 through to position 27 of the intron 2 set forth in SEQ ID NO:12. Reference to position -1 relative to the 5' splice site of an intron is reference to the first nucleotide upstream to the intron sequence, *e.g.* reference to position -1 relative to the 5' splice site of intron 2 is reference to nucleotide position 1 upstream of the intron 2 sequence, that is the last nucleotide of the adjacent exon. In another example, reference to positions -1 relative to the 3' splice site of the intron 2 sequence, *is* reference to the final nucleotide of intron 2, *e.g.* final nucleotide of SEQ ID NO:12. In another example, reference to positions -10—-1 relative to the 3' splice site of an intron is reference to the 10th through to the 1st nucleotide position upstream of the 3' splice site, or the final nucleotide ten nucleotides of the intron.

[0059] As used herein the terms "treating" or "treatment" refer to any and all uses which remedy a condition or symptoms, prevent the establishment of a condition or disease, or otherwise prevent, hinder, retard, or reverse the progression of a condition or disease or other undesirable symptoms in any way whatsoever. Thus the terms "treating" and the like are to be considered in their broadest context. For example, treatment does not necessarily imply that a patient is treated until total recovery. In conditions which display or a characterized by multiple symptoms, the treatment or prevention need not necessarily remedy, prevent, hinder, retard, or reverse all of said symptoms, but may prevent, hinder, retard, or reverse one or more of said symptoms. In the context of the present disclosure, symptoms that may be ameliorated, reversed, prevented, retarded or the linked include but are not limited to seizures and spasms.

[0060] The term "subject" as used herein refers to an animal, in particular a mammal and more particularly a primate including a lower primate and even more particularly, a human who can benefit from the protocol of the present disclosure. A subject regardless of whether a human or non-human animal or embryo may be referred to as an individual, subject, animal, patient, host or recipient.

Antisense oligonucleotides for SCN2A

[0061] As demonstrated herein, introns 1, 2, 3, 4, 5, 11, 13, 17 and 24 are retained in mature SCN2A mRNA in brain tissue. Introns 2 and 17 in particular have relatively high retention rates. As demonstrated herein, a retained intron in SCN2A mRNA or pre-RNA can be targeted with antisense oligonucleotides so as to enhance splicing at the splice site of the retained intron, resulting in an increase in the amount of fully-spliced SCN2A mRNA (*i.e.* SCN2A mRNA that does not contain any intron). Such antisense oligonucleotides are therefore useful for increasing the amount of SCN2A produced by a cell, and thus useful as therapeutic agents for the treatment of disorders associated with heterozygous loss-of-function mutations in *SCN2A*, such as genetic epilepsy (including developmental and epileptic encephalopathies (DEE)), intellectual disability, autism spectrum disorders and schizophrenia, where increasing the levels of SCN2A protein can provide a therapeutic effect.

[0062] Thus, provided herein are antisense oligonucleotides that enhance splicing at a splice site of a retained intron in an intron-retaining SCN2A mRNA or pre-mRNA, such as intron 1, 2, 3, 4, 5, 11, 13, 17 and 24. In particular examples, the antisense oligonucleotides enhances splicing at a splice site of intron 2 or intron 17 in an intron-retaining SCN2A mRNA or pre-mRNA.

[0063] The antisense oligonucleotide can function to enhance splicing in one of many ways. In one example, the antisense oligonucleotide binds to, or adjacent to, an intronic splicing silencer (ISS) (also referred to as an ISS site or ISS motif). ISS are *cis*-acting elements (*i.e.* sequences) in the RNA that play a role in silencing or inhibiting splicing at a splice site. The ISS is bound by a RNA-binding protein (RBP) that acts as a silencing repressor. Exemplary RBPs that act as repressors include heterogeneous nuclear ribonucleoproteins (hnRNPs), such as hnRNP A1, A2/B1, C1/C2, E1/E2/E3/E4, F, G, H, I, K, L, M, P, Q1/Q2/Q3 and U and hnRNP A2. The motifs recognised and bound by hnRNPs are not necessarily strict consensus sequences in the classical sense, but can be repeat elements (such as in the case of hnRNP L1, which recognises a CA repeat-rich element) or short and degenerate sequences. Moreover, hnRNPs may recognize specific structures rather than linear sequence motifs, such as in the case of hnRNP F (for review, see *e.g.* Geunes *et al.*, 2016, Hum Genet. 135:851-867; Dvinge,

2018, FEBS Letters, 592:2987-3006). Notwithstanding this, algorithms are available to predict hnRNP binding motifs in RNA molecules (see *e.g.* Piva *et al.*, 2009, Bioinformatics; Piva *et al.*, 2012, Hum Mutat. 2012 Jan;33(1):81-85). Binding of an antisense oligonucleotide to, or adjacent to, an ISS can prevent or inhibit binding of the RBP suppressor (*e.g.* an hnRNP, such as hnRNP A1), thereby enhancing splicing at a splice site of a retained intron, such as intron 1, 2, 3, 4, 5, 11, 13, 17, or 24. In other examples, the antisense oligonucleotide binds to a site in SCN2A mRNA or pre-mRNA that has a propensity to form an RNA secondary structure (*e.g.* stem, hairpin loop, pseudoknot, bulge, internal loop or multiloop), thereby reducing formation of the structure and facilitating efficient recruitment of splicing factors so as to enhance splicing of the retained-intron. In further examples, the antisense oligonucleotide binds to a sequence involved in the formation of G-quadruplexes, which can stabilise the G-quadruplex and enhance splicing (see *e.g.* Rouleau *et al.*, 2015, Nucleic Acids Res. 43(1): 595–606; Ribeiro *et al.* 2015, Hum Genet. 134(1):37-44). Those skilled in the art will appreciate that the composition and distribution of putative Quadruplex forming G-Rich Sequences (QGRS) of SCN2A can be predicted using the computational tool QGRS Mapper, wherein typically sequences with G scores above 19 are considered to have higher propensity for stable G4 structures.

[0064] In some examples, the antisense oligonucleotide of the present disclosure binds to nucleotides (or a target region) within the targeted intron, *i.e.* the intron for which enhanced splicing is to be effected, *e.g.* intron 1, 2, 3, 4, 5, 11, 13, 17 and 24. In other examples, the antisense oligonucleotide of the present disclosure binds to nucleotides (or a target region) in an adjacent exon, while still enhancing splicing at a splice site of the targeted intron.

[0065] In one example, the antisense oligonucleotide binds to a target region within intron 2 in the intron-retaining SCN2A mRNA or pre-mRNA. Accordingly, in some examples, an antisense oligonucleotide of the present disclosure has a sequence of nucleobases that is complementary to a sequence of nucleotides within intron 2 of SCN2A pre-mRNA, such as within the intron 2 set forth as follows:

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GTGAGTTCCTTAGTCAAGTTGCCCTTCACTGCCTAATTTACTAATTGGTTCTGGGCTAGTCCCAGGGATGAT
GGTGAAGAAGGCTGGCCTCCTTCCCCTGTCTAAAGTATCACTAAGATGCTGGATGGGCCTGACCGTGTA
ATGGACCAATGATCCTAGAAAGTCTTTTGAAGCACTCATTGAACTGCATTTGTGAGACAGGCAGAGAA
CTGGTGAGGCATCCTCCAGCGCGGGAATTAAGGAAGGACAAAAGCCTATTCACCTTCTTGAATACAAATT
ATATGCTTAAACAGTGTAATTTGACCCTGATTCCTTAATAATGTTGAGAAGCAAAAAGCTGAACTAGG
AGTCTATTTAAATTTTATTTTTTATTTTGCAGGAGTAGTATCTAAATTCCTCTTTATAGTCTCTAGCTC
TCCATAAGTCACCTTTGATCTTCAGTGGGTTTAATTATTCCTTTATACCACTACTTCTCCTTTCTATFGCT
CTCCACAGAAGGAATAATAGCAGGTGACTTGTAGGTGCCAAATAAGATTCTGAGCAAAGAACACACCTGG
AAAACCTTGAAGTTCTCATGAGAAAAATTTCTAACCAAAAAAAAAAATCAAAGCCTCAATTTTGTGCTTT
ATGTGAATTATAAATGCGGTTTTAAAATACTTACATTTAAACTTGATAAAGTTGCTAAGAATTCCTATGG
CATTGATCACAAATTTTCTTAATAATCCTCATGTCAATTTATCAAATTTAGGAAAGTTTATAGTGCTCAGA
AAAAAAAAAGCATCTATCTTCATGCATATGATGGTAATTATTATGTTATACACTATTTTACAGGGCAATA
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TTTATAAATAATGGTTTTACTTTTTCTTAAATATTCTTAATATATATTCTAAGTTTTATTTTATGTGT
 TGTGTTTTCTTTTCAG
 (SEQ ID NO:12)

[0066] In some examples, the antisense oligonucleotide binds to (*i.e.* comprises a sequence that is complementary to) a target region in intron 2 in an intron-retaining SCN2A mRNA or pre-mRNA, wherein the target region spans positions -6—+12, -5—+13, -4—+14, -3—+15, -2—+16, -1—+19, 0—+18, +1—+19, +2—+20, +3—21, +4—22, +5—+23, +6—+24, +7—+25, +8—26, +9—+27, +10—+28, +11—+29, +12—+30, +13—+31, +14—+32, +15—+33, +16—+34, +17—+35, +18—+36, +19—+37, +20—+38, +21—+39, +22—+40, +23—+41, +24—+42, +25—+43, +26—+44, +27—+45, +28—+46, +29—+47, +30—+48, +31—+49, +32—+50, +33—+51, +34—+52, +35—+53, +36—+54, +37—+55, +38—+56, +39—+57, +40—+58, +41—+59, +42—+60, +43—+61, +44—+62, +45—+63, +46—+64, +47—+65, +48—+66, +49—+67, +50—+68, +51—+69, +52—+70, +53—+71, +54—+72, +55—+73, +56—+74, +57—+75, +58—+76, +59—+77, +60—+78, +61—+79, +62—+80, +63—+81,+64—+82, +65—+83, +66—+84, +67—+85, +68—+86, +69—+87, +70—+88, +71—+89, +72—+90, +73—+91, +74—+92, +75—+93, +76—+94, +77—+95, +78—+96, +79—+97, +80—+98, +81—+99, relative to the 5' splice site of intron 2. In particular embodiments, the antisense oligonucleotide binds to, or adjacent to, an ISS in intron 2. As determined herein, putative ISS recognised by hnRNPA1 are at positions +8—+12 (CTTAG), +33—+36 (ATTTA) or +60—+65(CAGGGA) relative to the 5' splice site of intron 2 (bolded in SEQ ID NO:12, above). Thus, in some embodiments, the antisense oligonucleotide binds to, or adjacent to, nucleotides at positions +8—+12, +33—+36 or +60—+65relative to the 5' splice site of intron 2. For example, the antisense oligonucleotide may bind to one or more of the nucleotides at position +4, +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, +31, +32, +33,+34, +35, +36, +37, +38, +39, or +40 relative to the 5' splice site of intron 2, or may bind to one or more of the nucleotides at position +50, +51, +52, +53, +54, +55, +56, +57, +58, +59, +60, +61, +62, +63, +64, +65, +66, +67, +68, +69, or +70 relative to the 5' splice site of intron 2.

[0067] In one example, the antisense oligonucleotide binds to a target region that spans or is within positions -6—+12, -5—+13, -4—+14, -3—+15, -2—+16, -1—+19, 0—+18, +1—+19, +2—+20, +3—21, +4—22, +5—+23, +6—+24, +7—+25, +8—26, +9—+27, +10—+28, +11—+29, +12—+30, +13—+31, +14—+32, +15—+33, +16—+34, +17—+35, +18—+36, +19—+37, +20—+38, +21—+39, +22—+40, +23—+41, +24—+42, +25—+43, +26—+44, +27—+45, +28—+46, +29—+47, +30—+48, +31—+49, +32—+50, +33—+51, +34—+52, +35—+53, +36—+54, +37—+55,

+38—+56, +39—+57, +40—+58, +41—+59, +42—+60, +43—+61, +44—+62, +45—+63, +46—+64, +47—+65, +48—+66, +49—+67, +50—+68, +51—+69, +52—+70, +53—+71, +54—+72, +55—+73, +56—+74, +57—+75, +58—+76, +59—+77, +60—+78, +61—+79, +62—+80, +63—+81,+64—+82, +65—+83, +66—+84, +67—+85, +68—+86, +69—+87, +70—+88, +71—+89, +72—+90, +73—+91, +74—+92, +75—+93, +76—+94, +77—+95, +78—+96, +79—+97, +80—+98, +81—+99, relative to the 5' splice site of intron 2 (e.g. the intron 2 set forth in SEQ ID NO:12), i.e. the antisense oligonucleotide has a sequence that is complementary to at least one the aforementioned regions.

[0068] In another example, the antisense binds to a target region that spans or is within positions -19--1, -20--2, -21--3, -22--4, -23--5, -24--6, -25--7, -26--8, -27--9, -28--10, -29--11, -30--12, -31--13, -32--14, -33--15, -38--20, -39--21, -48--30, -49--31, -51--33, -52--34, -53--35, -54--36, -62--44, -64--46, -65--47, -66--48, -67--49, -76--58, -77--59, -78--60, -79--61, -82--64, -83--65, -84--66, -85--67, -86--68, -87--69, -90--72, -91--73, -92--74, -95--77, -97--79, -98--80, -99--81, -101--83, -102--84, -103--85, -104--86, -105--87, -106--88, -107--89, -108--90, -109--91, -110--92, -111--93, -112--94,-113--95, -114--96,-115--97, -116--98, -117--99, relative to the 3' splice site of intron 2 (e.g. the intron 2 set forth in SEQ ID NO:12), i.e. the antisense oligonucleotide has a sequence that is complementary to at least one of the aforementioned regions.

[0069] In some examples, the antisense oligonucleotide binds to, and thus comprises a sequence that is complementary to, positions -4—+14, -3—+15, -2—+16, +6—+24, +7—+25, +8—26, +9—+27, +10—+28, +14—+32, +19—+37, +20—+38, +21—+39, +22—+40, +23—+41, +24—+42, +25—+43, +26—+44, +27—+45, +28—+46, +29—+47, +30—+48, +31—+49, +32—+50, +33—+51, +34—+52, +35—+53, +43—+61, +45—+63, +46—+64, +49—+67, +52—+70, +59—+77, +64—+82, +65—+83, +68—+86, +69—+87, +70—+88, +71—+89, +72—+90 and +73—+91, relative to the 5' splice site of intron 2. In some embodiments, the antisense oligonucleotide comprises a sequence having at least or about 70%, 80%, or 90% sequence identity to a sequence set forth in any one of SEQ ID NOs: 115-142, or a sequence having at least 8, 9, 10, 11, 12, 13, 14 or 15 contiguous nucleotides from a sequence set forth in any one of SEQ ID NOs: 115-142. In a particular embodiment, the sequence has at least or about 70%, 80%, or 90% sequence identity to a sequence set forth in any one of SEQ ID NO:126 or SEQ ID NO:138, or a sequence having at least 8, 9, 10, 11, 12, 13, 14 or 15 contiguous nucleotides from a sequence set forth in SEQ ID NO:126 or SEQ ID NO:138.

In a further example, the antisense oligonucleotide binds to, and thus comprises a sequence that is complementary to, positions -19--1, -21--3, -30--12, -31--13, -32--14, -62--44, -64--46, -67--49, -76--58, -77--59, -78--60, -79--61 -82--64, -83--65, -84--66, -85--67, -86--68, -87--69, -95--77, -97--79, -100--82, -101--83, -102--84, -103--85, -107--89, -109--91, -111--93, -113--95, and -114--96, relative to the 3' splice site of intron 2. the antisense oligonucleotide comprises the sequence set forth in any one of SEQ ID NOs:143-205, or a sequence comprising at least 8, 9, 10, 11, 12, 13, 14 or 15 contiguous nucleotides from a sequence set forth in any one of SEQ ID NOs:143-205.

[0070] In one embodiment, the antisense oligonucleotide binds to nucleotides within intron 17 in the intron-retaining SCN2A mRNA or pre-mRNA. Accordingly, in some examples, an antisense oligonucleotide of the present disclosure has a sequence of nucleobases that is complementary to a sequence of nucleotides within intron 17 of SCN2A (nucleotides 120271-130741 of NCBI Reference Sequence: NG_008143.1).

[0071] In one embodiment, the antisense oligonucleotide binds to nucleotides within intron 1 in the intron-retaining SCN2A mRNA or pre-mRNA. Accordingly, in some examples, an antisense oligonucleotide of the present disclosure has a sequence of nucleobases that is complementary to a sequence of nucleotides within intron 1 of SCN2A (nucleotides 5240-61371 of NCBI Reference Sequence: NG_008143.1).

[0072] In another embodiment, the antisense oligonucleotide binds to nucleotides within intron 3 in the intron-retaining SCN2A mRNA or pre-mRNA. Accordingly, in some examples, an antisense oligonucleotide of the present disclosure has a sequence of nucleobases that is complementary to a sequence of nucleotides within intron 3 of SCN2A (nucleotides 62735-73446 of NCBI Reference Sequence: NG_008143.1).

[0073] In yet another embodiment, the antisense oligonucleotide binds to nucleotides within intron 4 in the intron-retaining SCN2A mRNA or pre-mRNA. Accordingly, in some examples, an antisense oligonucleotide of the present disclosure has a sequence of nucleobases that is complementary to a sequence of nucleotides within intron 4 of SCN2A (nucleotides 73537-74264 of NCBI Reference Sequence: NG_008143.1).

[0074] In an embodiment, the antisense oligonucleotide binds to nucleotides within intron 5 in the intron-retaining SCN2A mRNA or pre-mRNA. Accordingly, in some examples, an antisense oligonucleotide of the present disclosure has a sequence of nucleobases that is complementary to a sequence of nucleotides within intron 5 of SCN2A (nucleotides 74394-74950 of NCBI Reference Sequence: NG_008143.1).

[0075] In an embodiment, the antisense oligonucleotide binds to nucleotides within intron 11 in the intron-retaining SCN2A mRNA or pre-mRNA. Accordingly, in some examples, an antisense oligonucleotide of the present disclosure has a sequence of nucleobases that is complementary to a sequence of nucleotides within intron 11 of SCN2A (nucleotides 81358-88754 of NCBI Reference Sequence: NG_008143.1).

[0076] In an embodiment, the antisense oligonucleotide binds to nucleotides within intron 13 in the intron-retaining SCN2A mRNA or pre-mRNA. Accordingly, in some examples, an antisense oligonucleotide of the present disclosure has a sequence of nucleobases that is complementary to a sequence of nucleotides within intron 13 of SCN2A (nucleotides 92584-96928 of NCBI Reference Sequence: NG_008143.1).

[0077] In yet another embodiment, the antisense oligonucleotide binds to nucleotides within intron 24 in the intron-retaining SCN2A mRNA or pre-mRNA. Accordingly, in some examples, an antisense oligonucleotide of the present disclosure has a sequence of nucleobases that is complementary to a sequence of nucleotides within intron 24 of SCN2A (nucleotides 146329-146691 of NCBI Reference Sequence: NG_008143.1).

[0078] The antisense oligonucleotides of the present disclosure can enhance splicing such that the amount or level of the fully-spliced SCN2A mRNA or the amount or level of SCN2A protein in the cell or population of cells that is contacted with the antisense oligonucleotide is increased by at least or about 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 200%, 250%, 300%, 350%, 400%, 450%, 500%, 600%, 700% or more compared to the amount or level of a fully-spliced SCN2A mRNA or of a SCN2A protein in a cell or population of cells that has not been contacted with an antisense oligonucleotide of the present disclosure. Thus, in some instances, the amount or level of a fully-spliced SCN2A mRNA or of a SCN2A protein in the cell or population of cells following exposure to an antisense oligonucleotide of the present disclosure is 1.2x, 1.3x, 1.4x, 1.5x, 1.6x, 1.7x, 1.8x, 1.9x, 2x, 2.1x, 2.2x, 2.3x, 2.4x, 2.5x, 3x, 3.5x, 4x, 4.5x, 5x, 6x, 7x or more compared to the amount or level of a fully-spliced SCN2A mRNA or of a SCN2A protein in a cell or population of cells that has not been exposed to an antisense oligonucleotide of the present disclosure. In some examples, the fully-spliced SCN2A mRNA is that described as NCBI Reference Sequence: NM_001040142.2 (SEQ ID NO:1), and/or derived from nucleotide sequence of NCBI Reference Sequence: NG_008143.1, (SEQ ID NO:2) and/or the SCN2A protein is that described as NCBI Reference Sequence: NP_001035232.1 (SEQ ID NO:3). In other examples, the fully-spliced SCN2A mRNA is that described as NCBI Reference Sequence: NM_001040143.2 (SEQ ID NO:4), and/or the SCN2A protein is that described as NCBI Reference Sequence:

NP_001035233.1 (SEQ ID NO:5). In other examples, the SCN2A mRNA is that described as NCBI Reference Sequence: NM_001371246.1 (SEQ ID NO:6), and/or the SCN2A protein is that described as NCBI Reference Sequence: NP_001358175.1 (SEQ ID NO:7). In other examples, the SCN2A mRNA is that described as NCBI Reference Sequence: NM_001371247.1 (SEQ ID NO:8), and/or the SCN2A protein is that described as NCBI Reference Sequence: NP_001358176.1 (SEQ ID NO:9). In yet another example, the SCN2A mRNA is that described as NCBI Reference Sequence: NM_021007.3 (SEQ ID NO:10), and/or the SCN2A protein is that described as NCBI Reference Sequence: NP_066287.2 (SEQ ID NO:11).

[0079] The antisense oligonucleotides of the present disclosure are typically 8 to 50, nucleobases in length, such as 8 to 50, 8 to 40, 8 to 35, 8 to 30, 8 to 25, 8 to 20, 8 to 15, 9 to 50, 9 to 40, 9 to 35, 9 to 30, 9 to 25, 9 to 20, 9 to 15, 10 to 50, 10 to 40, 10 to 35, 10 to 30, 10 to 25, 10 to 20, 10 to 15, 11 to 50, 11 to 40, 11 to 35, 11 to 30, 11 to 25, 11 to 20, 11 to 15, 12 to 50, 12 to 40, 12 to 35, 12 to 30, 12 to 25, 12 to 20, or 12 to 15 nucleobases in length. Thus, in particular examples, the antisense oligonucleotides are 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, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, or 80 nucleobases in length.

[0080] The antisense oligonucleotides may be 100% complementary across their entire length to a target region of an intron-retaining SCN2A mRNA or pre-mRNA or may be less than 100% complementary. Typically, the antisense oligonucleotides are at least 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% complementary to a target region of an intron-retaining SCN2A mRNA or pre-mRNA, such as a region identified above in intron 5, 8, 9, 12, 13, or 14. The antisense oligonucleotides may contain, for example, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, or at least 20 contiguous nucleobases that are complementary to a target region in an intron-retaining SCN2A mRNA or pre-mRNA. In instances where the antisense oligonucleotides are not 100% complementary, the mismatched or non-complementary nucleobase(s) can be clustered or interspersed with complementary nucleobases and need not be contiguous to each other. The non-complementary nucleobase(s) may be located at the 5' end and/or 3' end of the antisense compound. Alternatively, the non-complementary nucleobase(s) can be at an internal position of the antisense oligonucleotide. When two or more non-complementary nucleobases are present, they can be either contiguous or non-contiguous.

[0081] In particular embodiments, antisense oligonucleotides of the present disclosure are up to 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 nucleobases in length and comprise no more than 6, 5, 4, 3, 2, or 1 non-complementary nucleobase(s) relative to a target region in an intron-retaining SCN2A mRNA or pre-mRNA.

[0082] The antisense oligonucleotides of the present disclosure can be produced using any method known in the art. Typically, the antisense oligonucleotides are produced using chemical synthesis methods. While the antisense oligonucleotides can be unmodified, more typically the antisense oligonucleotides of the present disclosure contain one or more modifications. These modifications can function to, for example, increase stability of the antisense oligonucleotide (e.g. increase resistance of the antisense oligonucleotide to degradation by nucleases), increase affinity of the antisense oligonucleotide to the target mRNA or pre-mRNA, increase steric hindrance by the antisense oligonucleotide, increase RNase H activity, and/or improve intracellular uptake. Exemplary modifications that are well known to those skilled in the art include, but are not limited to, modification of the nucleobase, modification of the backbone phosphate linkages (e.g. phosphodiester, phosphoramidate, or phosphorothioate (PS) modification), modifications of the ribose sugar (e.g. 2'-O-methyl (2OMe), 2'-O-methoxy-ethyl (MOE), locked nucleic acids (LNA), 2'-fluoro and S-constrained-ethyl (cEt) modifications) and other modifications such as replacement of the entire sugar phosphate backbone with polyamide linkages to produce peptide nucleic acids (PNA) and the use of a morpholine ring instead of the ribose ring and phosphoroamidate intersubunit linkages to produce phosphorodiamidate morpholino oligomers (PMO) (broadly reviewed in, for example, Sardone *et al.* (2017) *Molecules* 22(4): 563 Evers *et al.* (2015) *Adv Drug Del Rev* 87:90-103; Kole *et al.* (2012) *Nat Rev Drug Discov.* 11(2): 125-140).

[0083] In particular embodiments, the antisense oligonucleotides of the present disclosure contain one or more modified nucleobases. These can function to, for example, increase stability or binding affinity of the antisense oligonucleotide. Exemplary modified nucleobases include, but are not limited to, N⁶-methyladenine, N²-methylguanine, hypoxanthine, 7-methylguanine, 5-methylcytosine, 5-hydroxymethylcytosine, pseudouracil, 4-thiouracil, 2,6-diaminopurine, orotic acid, agmatidine, lysidine, 2-thiopyrimidine (e.g. 2-thiouracil, 2-thiothymine), G-clamp and its derivatives, 5-substituted pyrimidine (e.g. 5-halouracil, 5-propynyluracil, 5-propynylcytosine, 5-aminomethyluracil, 5-hydroxymethyluracil, 5-aminomethylcytosine, 5-hydroxymethylcytosine, Super T), 7-deazaguanine, 7-deazaadenine, 7-aza-2,6-diaminopurine, 8-aza-7-deazaguanine, 8-aza-7-

deazaadenine, 8-aza-7-deaza-2,6-diaminopurine, Super G, Super A, and N⁴-ethylcytosine, or derivatives thereof; N²-cyclopentylguanine (cPent-G), N²-cyclopentyl-2-aminopurine (cPent-AP), and N²-propyl-2-aminopurine (Pr-AP), pseudouracil or derivatives thereof; and degenerate or universal bases, like 2,6-difluorotoluene or absent bases like abasic sites (*e.g.* 1-deoxyribose, 1,2-dideoxyribose, 1-deoxy-2-O-methylribose; or pyrrolidine derivatives in which the ring oxygen has been replaced with nitrogen (azaribose)). In particular embodiments, the antisense oligonucleotides contain one or more modified nucleobases that increase the binding affinity of the antisense oligonucleotide to the SCN2A mRNA or pre-mRNA, such as 5-methylcytosine (5-me-C), 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine.

[0084] The antisense oligonucleotides of the present disclosure may comprise modified sugar moieties. Exemplary sugar moiety modifications include 2'-O-methyl (2OMe), 2'-O-methoxy-ethyl (MOE), locked nucleic acids (LNA), 2'-fluoro and S-constrained-ethyl (cEt) modifications.

[0085] In particular embodiments, the backbones of the antisense oligonucleotides of the present disclosure comprise phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl or other alkyl phosphonates comprising 3'-alkylene phosphonates or chiral phosphonates, phosphinates, phosphoramidates comprising 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, or boranophosphates. In other embodiments, the backbone has no phosphorus atom. Exemplary oligonucleotide backbones that do not include a phosphorus atom include those that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatom and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These comprise those having morpholino linkages (formed in part from the sugar portion of a nucleoside; see *e.g.* U.S. Patent Nos. 5,698,685, 5,217,866, 5,142,047, 5,034,506, 5,166,315, 5,185,444, 5,521,063, 5,506,337, 8,076,476, 8,299,206 and 7,943,762); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH₂ component parts.

[0086] In one example, the antisense oligonucleotides of the present disclosure are a peptide nucleic acid (PNA). In PNA compounds, the sugar-backbone of an oligonucleotide is replaced with an amide containing backbone, in particular an aminoethylglycine backbone. The nucleobases are retained and are bound directly or indirectly to aza nitrogen atoms of the amide portion of the backbone (see *e.g.* US Patent Nos. 5,539,082; 5,714,331; and 5,719,262).

[0087] In particular embodiments, the antisense oligonucleotides of the present disclosure are partially or completely resistant to RNase H. Such antisense oligonucleotides can include 2'-O-methyl derivatives, and/or phosphorothioate backbones, both of which are resistant to nuclease degradation. In further examples, the antisense oligonucleotides do not activate RNase H, typically by virtue of the presence of one or more structural modifications that sterically hinders or prevent binding of RNase H to a duplex molecule containing the antisense oligonucleotide and the *SCN2A* mRNA or pre-mRNA. For example, such antisense oligonucleotides include those where at least one, or all, of the inter-nucleotide bridging phosphate residues are modified phosphates, such as methyl phosphonates, methyl phosphorothioates, phosphoromorpholidates, phosphoropiperazidates and phosphoramidates. For example, every other one of the internucleotide bridging phosphate residues may be modified as described. In another non-limiting example, such antisense molecules are molecules wherein at least one, or all, of the nucleotides contain a 2' lower alkyl moiety (*e.g.*, C₁-C₄, linear or branched, saturated or unsaturated alkyl, such as methyl, ethyl, ethenyl, propyl, 1-propenyl, 2-propenyl, and isopropyl).

[0088] In other examples, the antisense oligonucleotides of the present disclosure activate RNase H when they form a DNA-RNA duplex with the *SCN2A* mRNA or pre-mRNA. Exemplary of such antisense oligonucleotides are gapmers, which are chimeric molecules containing at least one region modified so as to confer increased resistance to nuclease degradation, increased cellular uptake, increased binding affinity for the target nucleic acid, and a second region that serves as a substrate for RNase H. Gapmers have an internal region having a plurality of nucleotides that support RNase H cleavage. This internal region is positioned between external regions having a plurality of nucleotides that are chemically distinct from the nucleosides of the internal region, and which serve to, for example, increase stability of the antisense oligonucleotide and protect it from nuclease degradation. In certain embodiments, the external regions of the gapmer contain β -D-ribonucleosides, β -D-deoxyribonucleosides, 2'-modified nucleosides (*e.g.* 2'-MOE, and 2'-O-CH₃, among others), bridged nucleic acids (BNAs), or locked nucleic acids (LNAs).

[0089] The antisense oligonucleotides of the present disclosure may also be linked to one or more one or more moieties that enhance the activity, cellular distribution or cellular uptake of the oligonucleotide. Such moieties include but are not limited to lipid moieties such as a cholesterol moiety, cholic acid, a thioether, *e.g.*, hexyl-5-tritylthiol, a thiocholesterol, an aliphatic chain, *e.g.*, dodecandiol or undecyl residues, a phospholipid, *e.g.*, di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate, a polyamine or a polyethylene glycol chain, or 25damantine acetic acid, a palmityl moiety, or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety, carbohydrates, phospholipids, biotin, phenazine, folate, phenanthridine, anthraquinone, acridine, fluoresceins, rhodamines, coumarins, and various dyes.

[0090] In particular embodiments, the antisense oligonucleotides are linked to a cell-penetrating peptide (CPP) that is effective to enhance transport of the compound into cells. The transport moiety can be attached to either terminus of the antisense oligonucleotide, resulting in increased penetration of the antisense oligonucleotides into cells and macromolecular translocation within multiple tissues *in vivo* upon systemic administration. In one embodiment, the cell-penetrating peptide is an arginine-rich peptide transporter. Antisense oligonucleotides linked with arginine-rich CPPs were able to cross the blood-brain barrier and were widely distributed throughout the brain of wild-type mice following systemic delivery (Du *et al.* Hum. Mol. Genet., 20 (2011), pp. 3151–3160). In another embodiment, the cell-penetrating peptide may be Penetratin or the Tat peptide. These peptides are well known in the art and are disclosed, for example, in US Publication No. 20100016215. The transport moieties described above have been shown to greatly enhance cell entry of attached oligomers, relative to uptake of the oligomer in the absence of the attached transport moiety. For example, antisense oligonucleotides linked with arginine-rich CPPs were able to cross the blood-brain barrier and were widely distributed throughout the brain of wild-type mice following systemic delivery (Du *et al.* Hum. Mol. Genet., 20 (2011), pp. 3151–3160). Uptake may be enhanced at least ten-fold, or at least twenty-fold, relative to the unconjugated compound. In other examples, the antisense oligonucleotide 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), or a serotonin-norepinephrine-dopamine reuptake inhibitor (SNDRI), as described in, *e.g.*, US Patent No. 9193969. In further examples, the antisense oligonucleotides are conjugated to peptides collectively known as “angiopeps” which are capable of crossing the blood-brain barrier by receptor-mediated transcytosis using the low-density lipoprotein receptor-related protein-1

(LRP-1), and which allow the delivery of systemically administered antisense-peptide conjugates to the brain (see *e.g.* WO200979790).

[0091] The antisense oligonucleotides can also be modified to have one or more stabilizing groups that are generally attached to one or both termini to enhance properties such as, for example, nuclease stability. Included in stabilizing groups are cap structures. These terminal modifications protect the antisense compound having terminal nucleic acid from exonuclease degradation, and can help in delivery and/or localization within a cell. The cap can be present at the 5'-terminus (5'-cap), or at the 3'-terminus (3'-cap), or can be present on both termini. Cap structures are well known in the art and include, for example, inverted deoxy abasic caps.

Assessment of the antisense oligonucleotides

[0092] Antisense oligonucleotides of the present disclosure can be designed rationally, so as to target a specific region or site in an intron (*e.g.* an ISS, a G-quadruplex or a region with a propensity for secondary structure) and/or by methods such as antisense microwalk or tiling that cover the whole intron or just a region of an intron. For example, the antisense oligonucleotides used in the antisense walk can be tiled every 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 nucleotides from approximately 100 nucleotides upstream of the 5' splice site of the retained intron (*e.g.* in the preceding exon) to approximately 100 nucleotides downstream of the 5' splice site and/or from approximately 100 nucleotides upstream of the 3' splice site of the retained intron to approximately 100 nucleotides downstream of the 3' splice site of the target/retained intron (*e.g.* in the following exon). The activity of these antisense oligonucleotides can then be assessed and confirmed using various techniques known in the art. For example, the ability of the antisense oligonucleotides to enhance splicing, and thereby increase production of fully-spliced SCN2A mRNA and/or SCN2A protein can be assessed using *in vitro* assays to confirm that the antisense oligonucleotides are suitable for use in the methods of the present disclosure. Mouse models can be used to not only assess the ability of the antisense oligonucleotides to increase the level or amount of fully-spliced SCN2A mRNA and/or SCN2A protein *in vivo*, but to also ameliorate symptoms associated with heterozygous loss-of-function SCN2A mutations.

[0093] In one example, cells such as mammalian neuronal cells (*e.g.* SH-SY5Y or SK-N-AS cells) are transfected with an antisense oligonucleotide of the present disclosure. The levels of fully-spliced SCN2A mRNA and intron-retaining SCN2A mRNA can be assessed using qRT-PCR or Northern blot as is well known in the art. The level SCN2A protein can also be assessed, such as by Western blot on total cell lysates or fractions.

[0094] The levels of fully-spliced *SCN2A* mRNA, intron-retaining *SCN2A* mRNA and/or *SCN2A* protein observed when cells are exposed to an antisense oligonucleotide of the present disclosure are compared to the respective levels observed when cells are exposed with a negative control antisense oligonucleotide, so as to determine the change resulting from the antisense oligonucleotide of the present disclosure. Typically, the level of fully-spliced *SCN2A* mRNA and/or *SCN2A* protein is increased by at least or about 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 100%, 110%, 120%, 125%, 30%, 135%, 140%, 145%, 150%, 155%, 160%, 165%, 170%, 175%, 180%, 185%, 190%, 200%, 250%, 300%, 350%, 400% or more. In such instances, the antisense oligonucleotides of the present disclosure can be used for treating a disease or condition associated with a heterozygous loss-of-function mutation in *SCN2A*.

[0095] Mouse models can also be used to assess and confirm the activity of the antisense oligonucleotides of the present disclosure. For example, an antisense oligonucleotide can be administered to a heterozygous *SCN2A* knockout mouse, which displays physical and behavioural traits similar to those observed in patients with *SCN2A*-related intellectual disability (see e.g. Nakajima *et al.* 2019, *Neuropsychopharmacol Rep.* 39(3):223-237; Guo *et al.*, 2009, *Neuropsychopharmacol.* 2009 34(7):1659-72). The ability of the antisense oligonucleotides of the present disclosure to enhance splicing, increase the levels of fully-spliced *SCN2A* mRNA and/or *SCN2A* protein, and/or ameliorate any symptoms associated with the *SCN2A* mutation can then be assessed. In a particular example, *SCN2A* mRNA and/or protein levels in the brain, and in particular the neurons, are assessed. The levels of fully-spliced *SCN2A* mRNA, intron-retaining *SCN2A* mRNA and/or *SCN2A* protein following administration of an antisense oligonucleotide of the present disclosure are compared to the respective levels observed when a negative control antisense oligonucleotide is administered to the mice, so as to determine the change resulting from the antisense oligonucleotide of the present disclosure. Typically, the level of fully-spliced *SCN2A* mRNA and/or *SCN2A* protein is increased by at least or about 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 100%, 110%, 120%, 125%, 30%, 135%, 140%, 145%, 150%, 155%, 160%, 165%, 170%, 175%, 180%, 185%, 190%, 200%, 250%, 300%, 350%, 400% or more. In another example, the effect of administration of an antisense oligonucleotide of the present disclosure on the physical and/or behavioural traits of the mice is assessed. For example, behavioural and electrophysiological measures of memory and seizure in the mice can be assessed as described by Creson *et al.* (eLife 2019;8:e46752).

Compositions

[0096] The present disclosure provides compositions comprising the antisense oligonucleotides described above and herein. In particular examples, provided are pharmaceutical compositions comprising the antisense oligonucleotides and a pharmaceutically acceptable carrier. The compositions can also comprise additional ingredients such as carriers, diluents, stabilizers and excipients. The compositions can include one or more than one antisense oligonucleotide (*e.g.* two or more antisense oligonucleotides targeting the same or different introns), and further may comprise one or more other therapeutic agents.

[0097] The carriers, diluents, stabilizers and excipients can include buffers such as phosphate, citrate, or other organic acids; antioxidants such as ascorbic acid; low molecular weight polypeptides (*e.g.*, less than about 10 residues); proteins such as serum albumin, gelatin or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as Tween™, Pluronic™ or polyethylene glycol (PEG). In some embodiments, the physiologically acceptable carrier is an aqueous pH buffered solution.

[0098] The antisense oligonucleotides may also be formulated in compositions with liposomes, nanoparticles, microparticles, microspheres, lipid particles, vesicles, and the like, for the introduction of the antisense oligonucleotides of the present disclosure into cells. In embodiments, a penetration enhancer is included 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 some embodiments, the penetration enhancer is a surfactant, fatty acid, bile salt, chelating agent, or non-chelating nonsurfactant.

[0099] In embodiments, the antisense oligonucleotide is formulated in the context of a viral vector (*e.g.* adeno-associated viral (AAV) vector) where the vector comprises a genome that encodes an antisense oligonucleotide of the present disclosure.

[00100] Compositions comprising the antisense oligonucleotides encompass compositions comprising any pharmaceutically acceptable salts, esters, or salts of such esters, or any other oligonucleotide which, upon administration to an animal, including a human, is capable of providing (directly or indirectly) the biologically active metabolite or residue thereof. Accordingly, for example, the disclosure also provides

pharmaceutically acceptable salts of the antisense oligonucleotides described herein and other bio equivalents. Suitable pharmaceutically acceptable salts include, but are not limited to, sodium and potassium salts.

Methods

[00101] The antisense oligonucleotides described above and herein can be used to increase levels of fully-spliced SCN2A mRNA and/or SCN2A protein in a cell (*e.g.* a neuronal cell) or in a subject. Consequently, the antisense oligonucleotides described above and herein can be used to treat a disorder associated with a heterozygous loss-of-function mutation in *SCN2A*, *e.g.* epileptic encephalopathy or autism or intellectual disability associated with a heterozygous loss-of-function mutation in *SCN2A*. The methods of the present disclosure therefore include a step of contacting a cell to an antisense oligonucleotide of the present disclosure, and/or administering an antisense oligonucleotide of the present disclosure to a subject. As would be appreciated, the phrase “administering an antisense oligonucleotide” and grammatical variations thereof encompasses embodiments where a composition comprising the antisense oligonucleotide is administered to subject, and embodiments where a composition comprising an agent that encodes the antisense oligonucleotide (*e.g.* a viral vector) is administered to subject. In the latter embodiment, it is understood that the antisense oligonucleotide is expressed *in vivo*, thereby effecting administration of the antisense oligonucleotide to the subject.

[00102] In some examples, the subject presenting with a disease or condition that may be associated with a heterozygous loss-of-function mutation in *SCN2A* is genotyped to confirm the presence of a known heterozygous loss-of-function mutation in *SCN2A* prior to administration of the antisense oligonucleotides and compositions thereof. For example, whole exome sequencing can be performed on the subject. Known heterozygous loss-of-function mutations in *SCN2A* may include, but are not limited to, those described in Vlaskamp *et al.* (Neurology, 2019, 92(2):e96-e97). In other examples, the subject is first genotyped to identify the presence of a mutation in *SCN2A* and this mutation is then confirmed to be a loss-of-function mutation, *e.g.* by assessing the levels of SCN2A mRNA or protein.

[00103] The precise amount or dose of the antisense oligonucleotide administered to the subject depends on, for example, the efficacy of the antisense oligonucleotide, the presence of other moieties (*e.g.* CCPs), the route of administration, the number of dosages administered, and other considerations, such as the weight, age and general state of the subject. Particular dosages and administration protocols can be empirically determined or extrapolated from, for example, studies in animal models or previous

studies in humans, or may be otherwise determined by those skilled in the art using standard procedures.

[00104] The antisense oligonucleotides can be administered by any method and route understood to be suitable by a skilled artisan. Typically, the antisense oligonucleotides are administered parenterally, such as by subcutaneous administration, intravenous administration, intramuscular administration, intraarterial administration, intraperitoneal administration, or intracranial administration, *e.g.*, intrathecal or intracerebroventricular administration. In other embodiments, the antisense oligonucleotides are delivered intranasally. Administration of the antisense oligonucleotides in the methods described herein preferably results in delivery of the antisense oligonucleotides to the central nervous system. In particular embodiments, the antisense oligonucleotides are administered intrathecally or by intracerebroventricular administration. The methods of the present disclosure can involve any combination of any two or more routes.

[00105] The antisense oligonucleotides can be administered to a subject one time or more than one time, including 2, 3, 4, 5 or more times. Where the antisense oligonucleotides are administered more than one time, the time between dosage administration can be, for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or more weeks, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or more months. Selecting an optimal protocol is well within the level of skill of the skilled artisan and may depend on, for example, the half-life of the antisense oligonucleotide and the severity of the condition. In a particular embodiment, the antisense oligonucleotides are administered about every 3 months.

[00106] The antisense oligonucleotides, if desired, can be presented in a package, in a kit or dispenser device, such as a syringe with a needle, or a vial and a syringe with a needle, which can contain one or more unit dosage forms. The kit or dispenser device can be accompanied by instructions for administration.

[00107] In order that the disclosure may be readily understood and put into practical effect, particular exemplary embodiments will now be described by way of the following non-limiting examples.

[00108] The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

EXAMPLES

Example 1 - Materials and Methods

Cell culture

[00109] The cell lines SH-SY5Y and SK-N-AS were obtained from ECACC (European Collection of Authenticated Cell Cultures). The human brain RNA was purchased from Ambion, USA and from Takara-Bio, USA. The cells were grown in Dulbeccos modified Eagle's medium (DMEM) supplemented with 10% FBS. The cells were maintained at 37°C and 5% CO₂. Frozen stocks of the cells were made within passage 6 and stored in liquid nitrogen. Cells were used for experiments below a passage number of 20.

Cell transfection

[00110] Cells were plated in a 96 well plate at a density of 5,000-10,000 cells per well. Transfections were carried out following 18-24 hours of incubation. The instructions provided with the Lipofectamine 3000 transfection reagent from Thermofisher Scientific were followed. Briefly, the transfection reagent was mixed with OptiMEM in one tube, and the ASO was mixed with the same in another tube. The contents of both the tubes were gently mixed together, and following an incubation, the transfection complex was layered onto the cells containing fresh medium. The cells were then incubated for the required time.

RNA isolation

[00111] Total RNA was extracted from cells treated with ASO for the required time periods using the Qiagen Rneasy minikit. Briefly, the cells were pelleted, and RNA was isolated according to the manufacturer's instructions. The genomic DNA elution column was used to free the RNA of any contaminating genomic DNA. RNA quantity and integrity were determined by nanodrop.

Reverse transcription

[00112] A total of 500ug RNA was reverse transcribed to cDNA using the Promega M-MLV reverse transcriptase enzyme as per the kit instructions. The first strand synthesis of cDNA was performed using OligodT primers, which ensured that only mature polyadenylated RNA transcripts were reverse transcribed.

Intron retention analysis

[00113] cDNA from the various tissue/cell samples were analysed using the GoTaq® green kit provided by Promega. The cDNA was added to the reaction mastermix and pipetted into the qPCR plate. The primers for each exon-exon and exon-intron pair were then pipetted into the wells. Each reaction had a technical duplicate

and a water control. Absence of genomic DNA contamination was ensured with the RT minus reactions.

Antisense oligonucleotide (ASO) screening using the Taqman® Fast Advanced Cells to Ct kit

[00114] Cells were seeded in 96 well plates at the required density. Within the following 18-24 hours, transfections were carried out and the cells were incubated up to the time of screening. The further experimental procedures were carried out according to the manufacturer's instructions. Briefly, the medium was aspirated, and the cells were washed with cold PBS. Dnase-containing lysis solution was added to the cells and incubated for 5 min at room temperature. Stop solution was then added to the cells and the cells were incubated for 2 min at room temperature in order to halt the lysis. A total of 20% of lysate was used in the conversion to cDNA using the kit components for reverse transcription. The cDNA was then added to the master mix made using the components for qPCR provided in the kit at a concentration of 25%. Taqman® primers were used for the assay. Each reaction was duplexed with a housekeeping gene for in-well normalization of expression. The ASO treated cells were normalized to mock transfected cells.

Relative gene expression assay

[00115] Cell lysates were collected 24 hours after transfection, and used in taqman based gene expression assays. SCN2A expression levels were measured using the Cells to Ct kit (Invitrogen) real-time RT-PCR kit. Taqman probes targeting the SCN2A exon 2-3 boundary were used, with housekeeping HPRT (Hypoxanthine Guanine Phosphoribosyltransferase) gene used as a control.

Example 2 - Intron retention in human SCN2A

[00116] IRBase (Middleton *et al.*, 2017, Genome Biol. 18: 51) is an RNA sequencing resource of over 2000 human samples, in which a specific intron retention event of a gene in a particular tissue can be assessed. Using this database, the events of intron retention of SCN2A in brain tissue was analysed. As shown in Figure 1, several introns of SCN2A exhibit retention, with intron 11 showing the highest number of events (thin lines represent the introns and the thick lines/blocks correspond to the exons; the height of the bars is indicative of the number of recorded events of intron retention).

[00117] *In vitro* validation of the intron retention was carried out by analysing the levels of sequences corresponding to introns by quantitative PCR. To achieve this, primers were designed that would specifically detect the presence of retained introns relative to the exons flanking them. The use of cDNA that had been reverse transcribed

from DNase-treated polyadenylated RNA ensured that pre-mRNA transcripts, and not genomic DNA, was detected by the primers.

[00118] Intron retention events in the mature SCN2A mRNA were analysed by real-time PCR. Two sets of primers were designed for each Exon-Intron pair across the SCN2A sequence (NG_008143.1; SEQ ID NO:2), consisting of 27 exons and 26 introns:

A: primers that are specific for intron-retaining transcripts: The forward primer was designed from the sequence of the preceding exon and the reverse primer from the sequence of the intron downstream to the exon (Figure 2A).

B: primers specific to spliced transcripts: One of the primers was designed such that it spanned the junction of two nearby exons, while the other was designed from the sequence of the preceding or the succeeding exon accordingly (Figure 2B).

[00119] The primers are set forth below in Table 1.

Table 1: Primers for intron retention analysis

Primer name	Primer sequence	SEQ ID NO.
SCN2A E1-E2F	CTTTATTTTCAGCACTTTCTTATGCAAG	13
SCN2A E1-E2R	TCTTCTGCAATGCGTTGTTC	14
SCN2A E1-I1F	TTTTCCCTCCCTGTTTCTG	15
SCN2A E1-I1R	TCTTCTGCAATGCGTTGTTC	16
SCN2A E2-E3F	GACCCCTACTATATCAATAAGAAAACG	17
SCN2A E2-E3R	AGGGTTGAAGGGAGTTAAAATG	18
SCN2A E2-I2F	AGAAAGTGAGTTCTTAGTCAAGTTGC	19
SCN2A E2-I2R	TGCAGGTTCAAATGAGTGCT	20
SCN2A E3,4,5F	GGTACATTCTTTATTCAATATGCTCATTA	21
SCN2A E3,4,5R	TCTAAACAAAAGCCCCTTGC	22
SCN2A E3-I3F	GGTACATTCATATCCTTTTTCAAATC	23
SCN2A E3-I3R	CACTTAGAAGACGGGGGAAA	24
SCN2A I4-E4F	ATATCGTAGGGGGACCAACC	25
SCN2A I4-E4R	TCTAAACAAAAGCCCCTTGC	26
SCN2A E5-E6F	CACAGTCATTACTTTTGCAATGTG	27
SCN2A E5-E6R	TGTCTGAAATTGTTTTCAATGCT	28
SCN2A I5-E6F	AAGGTGGTACGCCCTTATATC	29
SCN2A I5-E6R	TTGTTTTCAATGCTCGGAGA	30
SCN2A E6-E7F	ATTCCAGACCTGAAGACCATT	31
SCN2A E6-E7R	GAGGCCATTGGAAACATTTA	32
SCN2A E6-I6F	TTCTCCGAGCATTGAAAACA	33
SCN2A E6-I6R	GCAAAGAATCAAATCCATCCA	34
SCN2A E7-E8F	CCTGAAGACCATTGTGGGGG	35
SCN2A E7-E8R	AAAGTGACTTTTATCCTC	36

SCN2A E7-I7F	TGTTTGCAATGGCCTCCAGA	37
SCN2A E7-I7R	AGTCTAAGTCACTTGATTCACATCT	38
SCN2A E8-E9F	AGATGCACGCCAGTGTCTCT	39
SCN2A E8-E9R	CAGTTGATAAAGGTTTTCCAGAGA	40
SCN2A E8-I8F	TAGAGGGGGCAAATGATGCT	41
SCN2A E8-I8R	TGGAGGGTTTTGAGTGAGGA	42
SCN2A E9-E10F	CTTTATCAACTGACACTACGTGCT	43
SCN2A E9-E10R	CCTTCTGTTTCAGCCTCTTCC	44
SCN2A E9-I9F	ACCCCAACTATGGCTACACG	45
SCN2A E9-I9R	GCAATTCTCTTGAGTTCGGTGT	46
SCN2A E10-E11F	CAAGAAGAAGCTCAGGCGG	47
SCN2A E10-E11R	CGATTTTCGGACTCTGTCATT	48
SCN2A E10-I10F	AGGTTTCCGTTTTTCCTTGG	49
SCN2A E10-I10R	AAGGCGGTTTTCTCTCCATT	50
SCN2A E11-E12F	CTCCACACCAGTCTTACTGAGC	51
SCN2A E11-E12R	TTGTCCTCAAAGGTGCTGTG	52
SCN2A E11-I11F	AGGTTTCCGTTTTTCCTTGG	53
SCN2A E11-I11R	AAGGCGGTTTTCTCTCCATT	54
SCN2A E12-E13F	CTACCAGAGGGCACAACACTG	55
SCN2A E12-E13R	CATTGCTCTTTCCTTGTGATG	56
SCN2A E12-I12F	GACTGCAATGGTGTGGTCTC	57
SCN2A E12-I12R	TGGGCAACAACTCAACAAA	58
SCN2A E13-E14F	CATGGAAGAACTGAAGAATCCA	59
SCN2A E13-E14R	TGGGATAGTGCTCCATAGCC	60
SCN2A E13-I13F	TTTTGACCAACACCATGGAA	61
SCN2A E13-I13R	GCAGCTTCTGGAGGCTAAA	62
SCN2A E14-E15F	TGGAAACCTGGTCTTCACAG	63
SCN2A E14-E15R	CGGAATGATCGGAGAAGTCTG	64
SCN2A E14-I14F	CCATCACCATCTGCATTGTC	65
SCN2A E14-I14R	GGGATGCTAAACATTTGATGTC	66
SCN2A E15-E16F	GGCTGCTCCGAGTTTTCAA	67
SCN2A E15-E16R	ACACGATCAGGAAGGAGTGG	68
SCN2A E15-I15F	GGCAAATGTGGAAGGATTGT	69
SCN2A E15-I15R	TGGTGGTATGGCAATCGAAT	70
SCN2A E16-E17F	GGAAATCTAGTGCTTCTGAACCTC	71
SCN2A E16-E17R	GCTTTCTGCTTCTTAAACAAAGG	72
SCN2A E16-I16F	GCCAAACCATGTGCCTTACT	73
SCN2A E16-I16R	GACAATAGGAAGTGGCCTTGA	74
SCN2A E17-E18F	GCAAAGAGAAGCTAAATGCAAC	75
SCN2A E17-E18R	GCCTTCAGGTTCAAGGGATT	76
SCN2A E17-I17F	CCAAATGCTGTTGGAGAATC	77
SCN2A E17-I17R	ACAGGAAGGAAACACGCAAT	78
SCN2A E18-E19F	CTGTTTTACAGAAGACTGTGTACGG	79
SCN2A E18-E19R	GCTCAGCAGAATGATGAAGACA	80
SCN2A E18-I18F	TGAGGAATCCCTTGAACCTG	81

SCN2A E18-I18R	TTCAAACAGCAAATGCAAAAA	82
SCN2A E19-E20F	GGGCTCTCGCCTTTGAAGATA	83
SCN2A E19-E20R	AGGAAGTCTAGCCAGCACCA	84
SCN2A E19-I19F	CAAAGGGAAACTCTGGTGGA	85
SCN2A E19-I19R	TTGAAGGGAAAGGGAAAAGA	86
SCN2A E20-21F	CTGATTGTTGATGTCTCACTGG	87
SCN2A E20-21R	ATTCCTTCAAACCGGGACA	88
SCN2A E20-I20F	ATGCTGCTAAAGTGGGTTGC	89
SCN2A E20-I20R	TGTCAGGCACCATTGCTAGA	90
SCN2A E21-E22F	GAATGAGGGTTGTTGTAAATGC	91
SCN2A E21-E22R	ACCTGGCAGTTTGATTGCTC	92
SCN2A E21-I21F	AGGCCACTGAGAGCTTTGTC	93
SCN2A E21-I21R	GACTCCAGCAGTGCCAAGTA	94
SCN2A E22,23,24F	GTAGCCACGTTTAAGGGATG	95
SCN2A E22,23,24R	GACACCAATGAAAAGATTCAAGG	96
SCN2A E22-I22F	GCCAGGTGGAAAAATGTGAA	97
SCN2A E22-I22R	TTACATCCCTGCCAACAATG	98
SCN2A E23-E24F	CACGAAATGTAGAATTACAACCC	99
SCN2A E23-E24R	GACACCAATGAAAAGATTCAAGG	100
SCN2A E23-I23F	CACGTTTAAGGGATGGATGG	101
SCN2A E23-I23R	CAGAGTGATTAGAGAAAATCATGCTATG	102
SCN2A E24-E25F	GGTGTTCATCATTTTGGAGGTC	103
SCN2A E24-E25R	CGAGGTATGGGTTTTTGTGG	104
SCN2A E24-I24F	CAACCCAAGTATGAAGACAACC	105
SCN2A E24-I24R	TTTGGTGATTGGATTTTGGGA	106
SCN2A E25-E26F	CGACCTGCTAACAAATCCAAG	107
SCN2A E25-E26R	TTCAGCACACATTCTCCAGTG	108
SCN2A E25-I25F	AACCCATACCTCGACCTGCT	109
SCN2A E25-I25R	AGGCTAGGTAGTCCCATTGTT	110
SCN2A E26-E27F	CATTGTAGGAATGTTTCTGGC	111
SCN2A E26-E27R	ACATCCCAAAGATGGCGTAG	112
SCN2A E26-I26F	TGTGGTGGTCATTCTCTCCA	113
SCN2A E26-I26R	CTCATCATTTCAAGCACAGCA	114

[00120] Human brain RNA was used to check for intron retention in SCN2A mRNA. In order to include a wider spectrum of RNA samples, RNA from two commercial suppliers (Ambion and Takara) was obtained. As the primers span the intron, any genomic DNA isolated along with the RNA would be detected and could lead to an overestimation of intron retention. The RNA was therefore treated with DNase before reverse transcription. In addition, reactions without the reverse transcriptase enzyme were included to ensure the absence of genomic DNA. During reverse transcription of the RNA to cDNA, oligodT primers were used in order to favour the selection of transcripts that were mature (polyadenylated) and possessed retained introns. This

prevented the reverse transcription of immature RNA transcripts whose introns were in the process of splicing.

[00121] Using the strategy described above, a number of introns showing varying levels of retention in the first sample of human whole brain RNA (from Ambion) were detected, with the levels ranging from less than 10% to up to 40% of the expression of the exons. The introns showing the highest retention included introns 2 and 17 (Figure 3A). A similar profile of intron retention was observed in the second source of human brain RNA (from Takara), where intron 2 and 17 also showed the highest retention with respect to the exons (Figure 3B).

[00122] A number of introns are retained to varying extents in SCN2A mature mRNA transcripts. On comparison of the retention between two different sources of RNA as a representation of different experimental cohorts, there are common introns that shows retention in both the cohorts in spite of the difference in their intron retention profile.

[00123] Intron retention in two neuronal-like cell lines, SH-SY5Y and SK-N-AS, was assessed as described above. SH-SY5Y and SK-N-AS are transformed neuronal-like cell lines that were derived from metastatic bone tumours and are widely used to study neuronal function.

[00124] Similar to the intron retention profile observed in brain tissue, intron 2 was the highest retained intron at a level of 35% of the exon expression levels when SH-SY5Y cells were assessed (Figure 4A). SK-N-AS cells showed high retention of introns 2 and 17, and although there was a high variation among biological replicates, the retention levels of these introns were 75% of the exon expression. The other introns in this cell line that showed retention were introns 1, 3, 4, 5, 13 and 24 (Figure 4B).

[00125] In summary, among the introns retained in SCN2A mRNA, intron 2 (SEQ ID NO:12) and intron 17 show the highest retention levels among the different cell lines and sources tested.

Example 3 - Identification of antisense oligonucleotides to reduce retention of intron 2

[00126] Targeting of an ASO to a particular sequence can sterically block the access of proteins, such as the spliceosome, to the nucleic acid molecule. Similarly, ASOs can be used to block sites such as splicing enhancer or silencer sequences, thereby altering the splicing propensity of a sequence. Blocking intronic splicing silencer (ISS) sites in the retained introns would in effect induce their splicing. Various tools and studies were therefore performed to identify ASOs that target the pool of SCN2A transcripts that

bear retained introns and block ISS sites, so as to induce splicing and thereby increase the levels of spliced transcripts transported to the cytoplasm for translation into protein.

[00127] Although ISS sequences serve as ideal antisense targets, they are often inconspicuous in long introns. To identify the part(s) of the intron sequence that would potentially increase splicing when targeted, available prediction tools (Human Splicing Finder, SpliceAid2, RBP Map, PESXs and RegRNA 2.0) were utilised for the *in silico* analysis of the intron sequences.

[00128] These online tools predict splicing sites in a sequence, including exonic splicing enhancer (ESE), exonic splicing silencer (ESS), intronic splicing enhancer (ISE) and the intronic splicing silencer (ISS), based on consensus sequences. The available tools used include Human Splicing Finder, SpliceAid, RBP Map, PESXs, RegRNA 2.0.

[00129] Poor splicing factor recruitment results in weakened splicing, and this has been reported to be affected by the secondary structure of the pre-mRNA (Buratti *et al.*, 2004, Mol. Cell Biol. 24(24), 10505-10514). *In-silico* prediction of pre-mRNA secondary structure was performed using prediction tools such as Mfold and RNA fold. Figure 5 shows the secondary structure prediction of SCN2A intron 2. ASOs can be designed to target regions with high propensity for secondary structure.

[00130] Based on published reports, the importance of the role of the splice repressors hnRNPA1 and hnRNP I on the splicing mechanism was inferred (Yimin Hua *et al.*, 2008, Am. J. Hum. Genet. 82, 834-848). The prediction tool SpliceAid2 (<http://www.introni.it/splicing.html>) was used to narrow down the regions of the intronic sequences near the 5' and 3' splice sites that might be bound by these splicing silencers. HnRNPA1 sites were predicted to be at positions +8—+12 (CTTAG), +33—+36 (ATTTA) and +59—+65(CAGGGA) relative to the 5' splice site of intron 2, and at positions -84 — -88 (ATTTA) and -82 — -87 (TTTATA), relative to the 3' splice site of intron 2. HnRNPA1 sites were also predicted for intron 17, and found at positions +21—+26 (AGATAT) relative to the 5' splice site of intron 17, and at positions -53 — -58 (TTTATA), -49 — -53 (ATTTA) and -18 — -24(TTTTTT), relative to the 3' splice site of intron 17.

[00131] The ASOs were designed based on the predictions of the binding sites for the splicing repressors hnRNPA1 and hnRNP I, such that the ASOs would target those sites. The ASOs were 18 nucleotide-long, fully modified oligonucleotides with phosphorothioate (PS) backbone (to increase their stability) and 2'-O-methoxyethylribose (2'-MOE) sugar modifications (to increase binding affinity and

reduce toxicity). A microwalk strategy was used to design the ASOs, starting from the 5' end of intron 2 (see Figure 6B). ASOs with off-target binding sites were excluded.

[00132] Table 2 sets forth the ASOs at the 5' end of intron 2.

Table 2

ASO Name	Sequence	SEQ ID NO.
SCN2A-EX2-6	CTAAGAACTCACTTTCTT	115
SCN2A-EX2-5	ACTAAGAACTCACTTTCT	116
SCN2A-EX2-4	GACTAAGAACTCACTTTC	117
SCN2A-EX2-3	TGACTAAGAACTCACTTT	118
SCN2A-EX2-2	TTGACTAAGAACTCACTT	119
SCN2A-EX2-1	CTTGACTAAGAACTCACT	120
SCN2A-INT2+1	ACTTGACTAAGAACTCAC	121
SCN2A-INT2+2	AACTTGACTAAGAACTCA	122
SCN2A-INT2+3	CAACTTGACTAAGAACTC	123
SCN2A-INT2+4	GCAACTTGACTAAGAACT	124
SCN2A-INT2+5	GGCAACTTGACTAAGAAC	125
SCN2A-INT2+6	AGGCAACTTGACTAAGAA	126
SCN2A-INT2+8	GAAGGCAACTTGACTAAG	127
SCN2A-INT2+9	TGAAGGCAACTTGACTAA	128
SCN2A-INT2+10	GTGAAGGCAACTTGACTA	129
SCN2A-INT2+11	AGTGAAGGCAACTTGACT	130
SCN2A-INT2+12	CAGTGAAGGCAACTTGAC	131
SCN2A-INT2+13	GCAGTGAAGGCAACTTGA	132
SCN2A-INT2+14	GGCAGTGAAGGCAACTTG	133
SCN2A-INT2+15	AGGCAGTGAAGGCAACTT	134
SCN2A-INT2+16	TAGGCAGTGAAGGCAACT	135
SCN2A-INT2+17	ATAGGCAGTGAAGGCAAC	136
SCN2A-INT2+18	AATAGGCAGTGAAGGCAA	137
SCN2A-INT2+19	AAATAGGCAGTGAAGGCA	138
SCN2A-INT2+20	TAAATAGGCAGTGAAGGC	139
SCN2A-INT2+21	GTAAATAGGCAGTGAAGG	140
SCN2A-INT2+22	AGTAAATAGGCAGTGAAG	141
SCN2A-INT2+35	CCCAGAACCAATTAGTAA	142

[00133] Table 3 sets forth the ASOs at the 3' end of intron 2.

Table 3

ASO name	Sequence (3' end)	SEQ. ID NO.
SCN2A-INT2-1	CTGAAAAAGAAAACACAA	143
SCN2A-INT2-2	TGAAAAAGAAAACACAAC	144
SCN2A-INT2-3	GAAAAAGAAAACACAACA	145
SCN2A-INT2-4	AAAAAGAAAACACAACAC	146
SCN2A-INT2-5	AAAAGAAAACACAACACA	147
SCN2A-INT2-6	AAAGAAAACACAACACAT	148
SCN2A-INT2-7	AAGAAAACACAACACATA	149
SCN2A-INT2-8	AGAAAACACAACACATAA	150
SCN2A-INT2-9	GAAAACACAACACATAAA	151
SCN2A-INT2-10	AAAACACAACACATAAAA	152
SCN2A-INT2-11	AAACACAACACATAAAAT	153
SCN2A-INT2-12	AACACAACACATAAAATA	154
SCN2A-INT2-13	ACACAACACATAAAATAA	155
SCN2A-INT2-14	CACAACACATAAAATAAA	156
SCN2A-INT2-15	ACAACACATAAAATAAAA	157
SCN2A-INT2-20	ACATAAAATAAAACTTAG	158
SCN2A-INT2-21	CATAAAATAAAACTTAGA	159
SCN2A-INT2-30	AACTTAGAATATATATT	160
SCN2A-INT2-31	AACTTAGAATATATATTA	161
SCN2A-INT2-33	CTTAGAATATATATTAAG	162
SCN2A-INT2-34	TTAGAATATATATTAAGA	163
SCN2A-INT2-35	TAGAATATATATTAAGAA	164
SCN2A-INT2-36	AGAATATATATTAAGAAT	165
SCN2A-INT2-44	TATTAAGAATATTTAAG	166
SCN2A-INT2-46	TTAAGAATATTTAAGAG	167
SCN2A-INT2-47	TAAGAATATTTAAGAGA	168

SCN2A-INT2-48	AAGAATATTTTAAGAGAA	169
SCN2A-INT2-49	AGAATATTTTAAGAGAAA	170
SCN2A-INT2-58	TAAGAGAAAAGTAAAACC	171
SCN2A-INT2-59	AAGAGAAAAGTAAAACCA	172
SCN2A-INT2-60	AGAGAAAAGTAAAACCAT	173
SCN2A-INT2-61	GAGAAAAGTAAAACCATT	174
SCN2A-INT2-64	AAAAGTAAAACCATTATT	175
SCN2A-INT2-65	AAAGTAAAACCATTATTT	176
SCN2A-INT2-66	AAGTAAAACCATTATTTA	177
SCN2A-INT2-67	AGTAAAACCATTATTTAT	178
SCN2A-INT2-68	GTAAAACCATTATTTATA	179
SCN2A-INT2-69	TAAAACCATTATTTATAA	180
SCN2A-INT2-72	AACCATTATTTATAAATA	181
SCN2A-INT2-73	ACCATTATTTATAAATAT	182
SCN2A-INT2-74	CCATTATTTATAAATATT	183
SCN2A-INT2-77	TTATTTATAAATATTGCC	184
SCN2A-INT2-79	ATTTATAAATATTGCCCT	185
SCN2A-INT2-80	TTTATAAATATTGCCCTG	186
SCN2A-INT2-81	TTATAAATATTGCCCTGT	187
SCN2A-INT2-82	TATAAATATTGCCCTGTA	188
SCN2A-INT2-83	ATAAATATTGCCCTGTAA	189
SCN2A-INT2-84	TAAATATTGCCCTGTAAA	190
SCN2A-INT2-85	AAATATTGCCCTGTAAAA	191
SCN2A-INT2-86	AATATTGCCCTGTAAAAT	192
SCN2A-INT2-87	ATATTGCCCTGTAAAATA	193
SCN2A-INT2-88	TATTGCCCTGTAAAATAG	194
SCN2A-INT2-89	ATTGCCCTGTAAAATAGT	195
SCN2A-INT2-90	TTGCCCTGTAAAATAGTG	196

SCN2A-INT2-91	TGCCCTGTAAAATAGTGT	197
SCN2A-INT2-92	GCCCTGTAAAATAGTGTA	198
SCN2A-INT2-93	CCCTGTAAAATAGTGAT	199
SCN2A-INT2-94	CCTGTAAAATAGTGTATA	200
SCN2A-INT2-95	CTGTAAAATAGTGTATAA	201
SCN2A-INT2-96	TGTAAAATAGTGTATAAC	202
SCN2A-INT2-97	GTAAAATAGTGTATAACA	203
SCN2A-INT2-98	TAAAATAGTGTATAACAT	204
SCN2A-INT2-99	AAAATAGTGTATAACATA	205

[00134] The ASOs were transfected into SH-SY5Y cells with lipofectamine 3000 at a concentration of 200nM, and cells were lysed 24 hours later. Reverse transcription and qPCR analysis was performed as described in Example 1 using the Taqman® Fast Advance Cells to Ct kit (Invitrogen, ThermoFisher Scientific). Taqman primers that were duplexed with a housekeeping gene in order to normalize against variations induced between replicates. The expression of SCN2A in cells following ASO treatment was compared and normalised with cells transfected with mock-transfected cells.

[00135] As shown in Figure 6, among the intron 2 ASOs tested, ASOs SCN2A-INT2+6 consistently resulted in an upregulation of SCN2A mRNA of 1.37 fold, when compared to mock-transfected cells. The other ASOs that gave an upregulation above 1-fold include SCN2A-INT2 -4, -3, -2, +6, +8, +9, +10, +14, +19, +20, +21, +22, +23, +24, +25, +26, +28, +29, +30, +33, +34, +43, +45, +46, +49, +52, +59, +64, +65, +68, +69, +70, +71, +72, +73 at the 5-prime end and SCN2A-INT2 -1, -3, -12, -13, -14, -44, -46, -49, -58, -59, -60, -61, -64, -65, -66, -67, -68, -69, -77, -79, -82, -83, -84, -85, -89, -91, -93, -95, -96 at the 3-prime end. The variation between the biological replicates might be an indication of the varied efficiencies of transfection.

[00136] In order to elucidate the mechanism of action of the intron 2 ASOs on the upregulation of the SCN2A transcript, primers that would amplify only the transcripts without intron 2 were used. These primers bound the intron 2-flanking exons, *i.e.* exons 2 and 3, while the probe spanned the junction between them. They are shown below in Table 4.

Table 4

Primer name	Sequence	SEQ ID NO:
APDJZ44 SCN2A E2-E3 Probe (FAM)	AGTGCCACCCCTGC	206
APDJZ44 SCN2A-E2-E3F	TCTGGACCCCTACTATATCAATAAGAAAA	207
APDJZ44 SCN2A-E2-E3R	GGGTTGAAGGGGAGTTAAAATGTAAAG	208

[00137] As shown in Figure 7, cells transfected with the SCN2A-INT2+6 that caused the highest upregulation of SCN2A mRNA had increased amounts of SCN2A transcripts, as compared to the mock and to the ASO that did not cause any upregulation (SCN2A-INT2+35). This suggested that ASOs that cause upregulation of SCN2A mRNA exert their effect through the splicing out of the retained intron 2.

Other sequences referred to in the disclosure

SEQ ID NO:1; SCN2A transcript 2 (NM_001040142.2):

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SEQ ID NO:2; SCN2A RefSeqGene nucleotide sequence (NG_008143.1):

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SEQ ID NO:3; SCN2A isoform 1 protein (NP_001035232.1):

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SEQ ID NO:4; SCN2A transcript variant 3 (NM_001040143.2):

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SEQ ID NO:5; SCN2A protein isoform 2 (NP_001035233.1):

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SEQ ID NO:6; SCN2A transcript variant 4 (NM_001371246.1)

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SEQ ID NO:7; SCN2A protein isoform 2 (NP_001358175.1):

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SEQ ID NO:8; SCN2A transcript variant 5 (NM_001371247.1):

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SEQ ID NO:9; SCN2A protein isoform 1 (NP_001358176.1):

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SEQ ID NO:10; SCN2A transcript variant1 (NM_021007.3):

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GAAAAAGACAAATCAGAAAAGGAAGCAAAGGGAAAGATATCAGGGAAAGTAAAAAGTAAAAAGAAACCA
AGAATTTTCCATTTGTGATCAATTTGTACAGCCCGTGATGGTGTATGTTTGTGTCAACAGGACTCCC
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TGTGAAACAAATCTCAAACCTGAGTTCAATGTTTATTTGCTTTCAATAGTAATGCCTTATCATTGAAAAGAG
GCTTAAAGAAAAAATAAATCAGCTGATACTCTTGGCATTGCTTGAATCCAATGTTTCCACCTAGTCTTTT
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SEQ ID NO:11; SCN2A protein isoform 1 (NP_066287.2):

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TNCVFM TMSNPPDWTKNVEYTFGTIYTFESLIKILARGFCLEDFTF LRDPWNWLDFTVITFAYVTEFVDL
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VCKISNDCELP RWHMHDFHFLIVFRVLCGEWIETMWDCEVAGQTMCLTVFMMVMVIGNLVVNLFLA
LLLSSFSNDLAATDDDNEMNNLQIAVGRMQKIDFVKRKIREFIQKAFVRKQKALDEIKPLEDLNNKDD
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GKLWNNLRKTCYKIVEHNWFETFIVFMILLSSGALAFEDIYIEQRKTIKTMLEYADKVFTYIFILEMLLK
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VIIDNFNQKKKFGGQDIFMTEEQKYYNAMKKLGSKKPKP IPRANKFQGMVDFVTKQVFDISIMIL
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NFAYVKREVGIDDMFNFTFGNSMICLFQITTSAGWDGLLAPILNSGPPDCDPDKDHPGSSVKGDCGNPS
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VSYEPITTTLKRKQEEVSAIIIQRAYRRYLLKQKVKVSS IYKKDKGKECDGTP IKEDTLIDKLNENSTP
EKTDMPSTTSPPSYDSVTKPEKEKFEKDKSEKEDKGD IRESKK

CLAIMS

1. A method for increasing levels of SCN2A protein in a cell, comprising contacting the cell with an antisense oligonucleotide that enhances splicing at a splice site of a retained intron in an intron-retaining SCN2A mRNA or pre-mRNA, wherein the retained intron is selected from among intron 1, 2, 3, 4, 5, 11, 13, 17 and 24 and wherein the antisense oligonucleotide comprises a sequence of nucleobases that is complementary to a target region in the SCN2A mRNA or pre-mRNA.
2. A method for increasing levels of SCN2A protein in a subject, comprising administering to the subject an antisense oligonucleotide that enhances splicing at a splice site of a retained intron in an intron-retaining SCN2A mRNA or pre-mRNA, wherein the retained intron is selected from among intron 1, 2, 3, 4, 5, 11, 13, 17 and 24 and wherein the antisense oligonucleotide comprises a sequence of nucleobases that is complementary to a target region in the SCN2A mRNA or pre-mRNA.
3. The method of claim 2, wherein the subject has a heterozygous loss-of-function mutation in *SCN2A*.
4. The method of claim 2 or 3, wherein the subject has a disorder associated with a heterozygous loss-of-function mutation in *SCN2A*.
5. The method of claim 4, wherein the disorder associated with a heterozygous loss-of-function mutation in *SCN2A* is genetic epilepsy, developmental and epileptic encephalopathy, intellectual disability, autism spectrum disorders or schizophrenia.
6. A method for treating a disorder associated with a heterozygous loss-of-function mutation in *SCN2A*, comprising administering to the subject an antisense oligonucleotide that enhances splicing at a splice site of a retained intron in an intron-retaining SCN2A mRNA or pre-mRNA, wherein the retained intron is selected from among intron 1, 2, 3, 4, 5, 11, 13, 17 and 24, and wherein the antisense oligonucleotide comprises a sequence of nucleobases that is complementary to a target region in the SCN2A mRNA or pre-mRNA.
7. The method of claim 6, wherein the disorder associated with a heterozygous loss-of-function mutation in *SCN2A* is genetic epilepsy, developmental and epileptic encephalopathy, intellectual disability, autism spectrum disorders or schizophrenia.
8. The method of any one of claims 1 to 7, wherein the antisense oligonucleotide binds to, or adjacent to, an intron splicing silencer (ISS), binds to nucleotides within a G-quadruplex, or binds to nucleotides with an RNA secondary structure.

9. The method of claim 8, wherein the ISS is recognised by a heterogeneous nuclear ribonucleoproteins (hnRNP).
10. The method of claim 9, wherein the hnRNP is hnRNPA1 or hnRNP I.
11. The method of any one of claims 1 to 10, wherein the retained intron is intron 2.
12. The method of claim 11, wherein the ISS is at positions +8—+12, +32—+36, +33—+36, or +60—+65 relative to the 5' splice site of intron 2 and positions -84 — -88 and -82 — -87, relative to the 3' splice site of intron 2.
13. The method of any one of claims 1 to 12, wherein the retained intron is intron 2 and the target region spans positions -4—+14, -3—+15, -2—+16, +6—+24, +7—+25, +8—26, +9—+27, +10—+28, +14—+32, +19—+37, +20—+38, +21—+39, +22—+40, +23—+41, +24—+42, +25—+43, +26—+44, +27—+45, +28—+46, +29—+47, +30—+48, +31—+49, +32—+50, +33—+51, +34—+52, +35—+53, +43—+61, +45—+63, +46—+64, +49—+67, +52—+70, +59—+77, +64—+82, +65—+83, +68—+86, +69—+87, +70—+88, +71—+89, +72—+90 and +73—+91, relative to the 5' splice site of intron 2.
14. The method of any one of claims 1 to 13, wherein the antisense oligonucleotide comprises a sequence having at least or about 70%, 80%, or 90% sequence identity to a sequence set forth in any one of SEQ ID NOs:115-142, or a sequence having at least 8, 9, 10, 11, 12, 13, 14 or 15 contiguous nucleotides from a sequence set forth in any one of SEQ ID NOs: 115-142.
15. The method of any one of claims 1 to 14, wherein the antisense oligonucleotide comprises the sequence set forth in SEQ ID NO:126, or a sequence comprising at least 8, 9, 10, 11, 12, 13, 14 or 15 contiguous nucleotides from a sequence set forth in SEQ ID NO:126.
16. The method of any one of claims 1 to 10, wherein the retained intron is intron 2 and the target region spans positions -19—-1, -21—-3, -30—-12, -31—-13, -32—-14, -62—-44, -64—-46, -67—-49, -76—-58, -77—-59, -78—-60, -79—-61 -82—-64, -83—65, -84—-66, -85—-67, -86—-68, -87—-69, -95—-77, -97—-79, -100—-82, -101—-83, -102—-84, -103—-85, -107—-89, -109—-91, -111—-93, -113—-95, and -114—-96, relative to the 3' splice site of intron 2.
17. The method of any claim 14, wherein the antisense oligonucleotide comprises a sequence having at least or about 70%, 80%, or 90% sequence identity to a

sequence set forth in any one of SEQ ID NOs:143-205, or a sequence having at least 8, 9, 10, 11, 12, 13, 14 or 15 contiguous nucleotides from a sequence set forth in any one of SEQ ID NOs: 143-205.

18. The method of any one of claims 1 to 17, wherein the antisense oligonucleotide consists of from 8 to 50, 8 to 40, 8 to 35, 8 to 30, 8 to 25, 8 to 20, 8 to 15, 9 to 50, 9 to 40, 9 to 35, 9 to 30, 9 to 25, 9 to 20, 9 to 15, 10 to 50, 10 to 40, 10 to 35, 10 to 30, 10 to 25, 10 to 20, 10 to 15, 11 to 50, 11 to 40, 11 to 35, 11 to 30, 11 to 25, 11 to 20, 11 to 15, 12 to 50, 12 to 40, 12 to 35, 12 to 30, 12 to 25, 12 to 20, or 12 to 15 nucleobases.

19. The method of any one of claims 1 to 18, wherein the antisense oligonucleotide is at least 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% complementary to the target region.

20. The method of any one of claims 1 to 19, wherein the antisense oligonucleotide comprises least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 contiguous nucleobases that are 100% complementary to the target region.

21. The method of any one of claims 1 to 20, wherein the antisense oligonucleotide comprises at least one modification.

22. The method of claim 21, wherein the modification is a nucleobase modification, a modification of the oligonucleotide backbone or a modification of a ribose sugar.

23. The method of any one of claims 1 to 22, wherein the antisense oligonucleotide comprises a modified sugar selected from among a 2'-O-methyl (2OMe), 2'-O-methoxy-ethyl (MOE), locked nucleic acids (LNA), 2'-fluoro or S-constrained-ethyl (cEt).

24. The method of any one of claims 1 to 23, wherein antisense oligonucleotide comprises backbone that comprises phosphorothioates.

25. The method of any one of claims 1 to 24, wherein the antisense oligonucleotide activates RNase H.

26. The method of any one of claims 2 to 25, wherein the subject is determined to have a heterozygous loss-of-function mutation in *SCN2A*.

27. The method of any one of claims 2 to 26, wherein the antisense oligonucleotide is administered to the subject by parenteral administration or intranasal administration.
28. The method of claim 27, wherein the parenteral administration is selected from among subcutaneous administration, intravenous administration, intramuscular administration, intraarterial administration, intraperitoneal administration, or intracranial administration.
29. The method of claim 28, wherein intracranial administration is intrathecal or intracerebroventricular administration.
30. The method of any one of claims 2 to 29, wherein the antisense oligonucleotide or composition is administered to the subject about every 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or more months.
31. The method of any one of claims 2 to 30, wherein the antisense oligonucleotide or composition is administered to the subject about every 3 months.
32. An antisense oligonucleotide comprising a sequence of nucleobases that is complementary to a target region in an intron-retaining SCN2A mRNA or pre-mRNA, wherein the target region is in a retained intron and wherein the retained intron is selected from among intron 1, 2, 3, 4, 5, 11, 13, 17 and 24.
33. The antisense oligonucleotide of claim 32, wherein the antisense oligonucleotide binds to, or adjacent to, an intron splicing silencer (ISS), binds to nucleotides within a G-quadruplex, or binds to nucleotides with an RNA secondary structure.
34. The antisense oligonucleotide of claim 33, wherein the ISS is recognised by a heterogeneous nuclear ribonucleoproteins (hnRNP).
35. The antisense oligonucleotide of claim 34, wherein the hnRNP is hnRNPA1 or hnRNP I.
36. The antisense oligonucleotide of claim 34 or 35, wherein the retained intron is intron 2 and the ISS is at positions +8—+12, +33—+36 or +60—+65 relative to the 5' splice site of intron 2 and positions -84 — -88 and -82 — -87, relative to the 3' splice site of intron 2.
37. The antisense oligonucleotide of any one of claims 32 to 36, wherein the retained intron is intron 2 and the target region spans positions -4—+14, -3—+15, -

2—+16, +6—+24, +7—+25, +8—26, +9—+27, +10—+28, +14—+32, +19—+37, +20—+38, +21—+39, +22—+40, +23—+41, +24—+42, +25—+43, +26—+44, +27—+45, +28—+46, +29—+47, +30—+48, +31—+49, +32—+50, +33—+51, +34—+52, +35—+53, +43—+61, +45—+63, +46—+64, +49—+67, +52—+70, +59—+77, +64—+82, +65—+83, +68—+86, +69—+87, +70—+88, +71—+89, +72—+90 and +73—+91, relative to the 5' splice site of intron 2.

38. The antisense oligonucleotide of any one of claims 32 to 37, wherein the antisense oligonucleotide comprises a sequence having at least or about 70%, 80%, or 90% sequence identity to a sequence set forth in any one of SEQ ID NOs:115-205, or a sequence having at least 8, 9, 10, 11, 12, 13, 14 or 15 contiguous nucleotides from a sequence set forth in any one of SEQ ID NOs: 115-205.

39. The antisense oligonucleotide of any one of claims 32 to 38, wherein the antisense oligonucleotide comprises the sequence set forth in SEQ ID NO:126, SEQ ID NO:138 or SEQ ID NO:155, or a sequence comprising at least 8, 9, 10, 11, 12, 13, 14 or 15 contiguous nucleotides from a sequence set forth in SEQ ID NO:126, SEQ ID NO:138 or SEQ ID NO:155.

40. The antisense oligonucleotide of any one of claims 32 to 35, wherein the retained intron is intron 2 and the target region spans positions -19—-1, -21—-3, -30—-12, -31—-13, -32—-14, -62—-44, -64—-46, -67—-49, -76—-58, -77—-59, -78—-60, -79—-61 -82—-64, -83—65, -84—-66, -85—-67, -86—-68, -87—-69, -95—-77, -97—-79, -100—-82, -101—-83, -102—-84, -103—-85, -107—-89, -109—-91, -111—-93, -113—-95, and -114—-96, relative to the 3' splice site of intron 2.

41. The antisense oligonucleotide of claim 40, wherein the antisense oligonucleotide comprises the sequence set forth in any one of SEQ ID NOs:143-205, or a sequence comprising at least 8, 9, 10, 11, 12, 13, 14 or 15 contiguous nucleotides from a sequence set forth in any one of SEQ ID NOs: 143-205.

42. The antisense oligonucleotide of any one of claims 32 to 41, wherein the antisense oligonucleotide consists of from 8 to 50, 8 to 40, 8 to 35, 8 to 30, 8 to 25, 8 to 20, 8 to 15, 9 to 50, 9 to 40, 9 to 35, 9 to 30, 9 to 25, 9 to 20, 9 to 15, 10 to 50, 10 to 40, 10 to 35, 10 to 30, 10 to 25, 10 to 20, 10 to 15, 11 to 50, 11 to 40, 11 to 35, 11 to 30, 11 to 25, 11 to 20, 11 to 15, 12 to 50, 12 to 40, 12 to 35, 12 to 30, 12 to 25, 12 to 20, or 12 to 15 nucleobases.

43. The antisense oligonucleotide of any one of claims 32 to 42, wherein the antisense oligonucleotide is at least 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%,

91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% complementary to the target region.

44. The antisense oligonucleotide of any one of claims 36 to 43, wherein the antisense oligonucleotide comprises least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 contiguous nucleobases that are 100% complementary to the target region.

45. The antisense oligonucleotide of any one of claims 36 to 44, wherein the antisense oligonucleotide comprises at least one modification.

46. The antisense oligonucleotide of claim 45, wherein the modification is a nucleobase modification, a modification of the oligonucleotide backbone or a modification of a ribose sugar.

47. The antisense oligonucleotide of any one of claims 32 to 46, wherein the antisense oligonucleotide comprises a modified sugar selected from among a 2'-O-methyl (2OMe), 2'-O-methoxy-ethyl (MOE), locked nucleic acids (LNA), 2'-fluoro or S-constrained-ethyl (cEt).

48. The antisense oligonucleotide of any one of claims 32 to 47, wherein the antisense oligonucleotide comprises a backbone that comprises phosphorothioates.

49. The antisense oligonucleotide of any one of claims 32 to 48, wherein the antisense oligonucleotide activates RNase H.

50. A composition comprising the antisense oligonucleotide of any one of claims 32 to 49.

51. Use of an antisense oligonucleotide for the treatment of a disorder associated with a heterozygous loss-of-function mutation in *SCN2A*, wherein the antisense oligonucleotide enhances splicing at a splice site of a retained intron in an intron-retaining *SCN2A* mRNA or pre-mRNA, wherein the retained intron is selected from among intron 1, 2, 3, 4, 5, 11, 13, 17 and 24 and wherein the antisense oligonucleotide comprises a sequence of nucleobases that is complementary to a target region in the *SCN2A* mRNA or pre-mRNA.

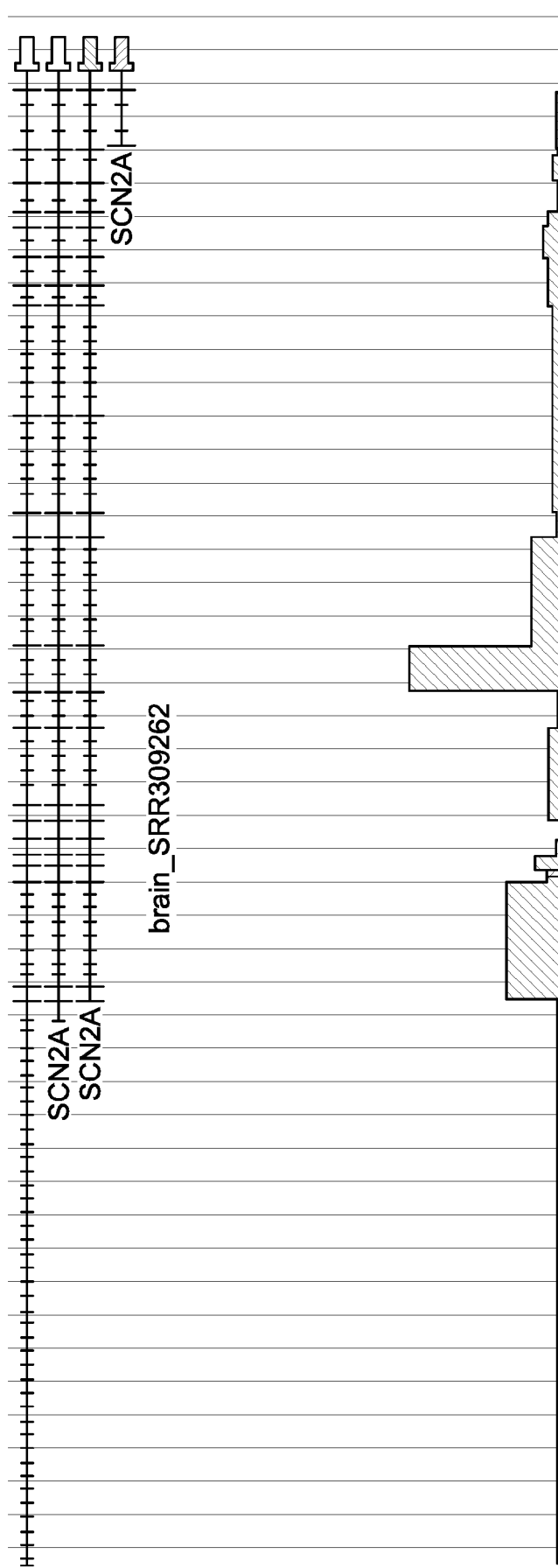
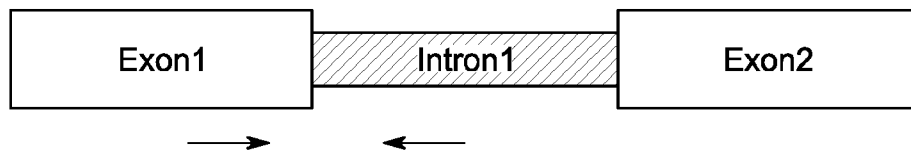


FIGURE 1

A



B

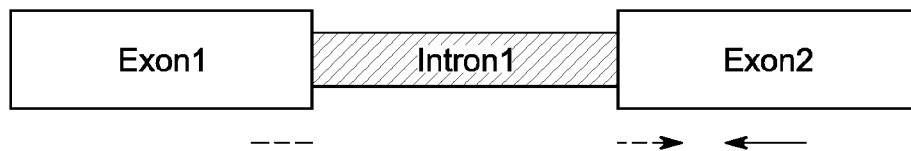
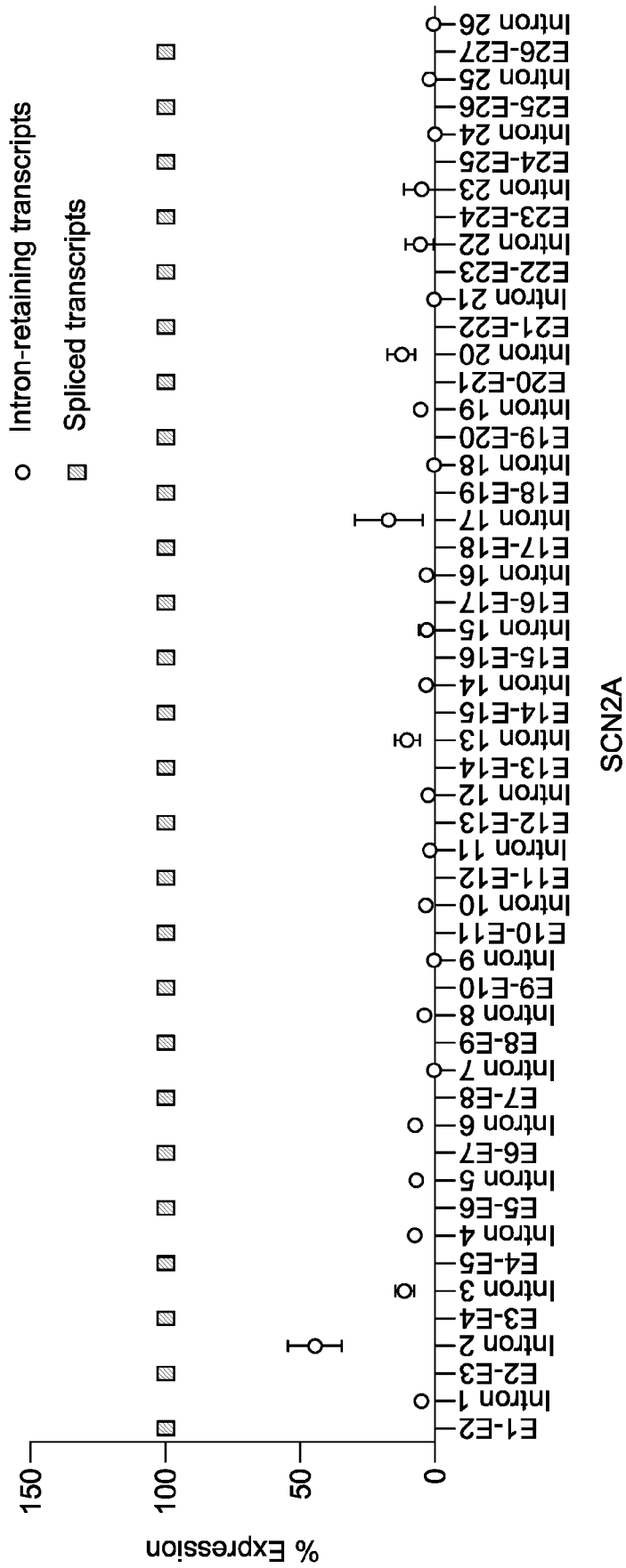


FIGURE 2

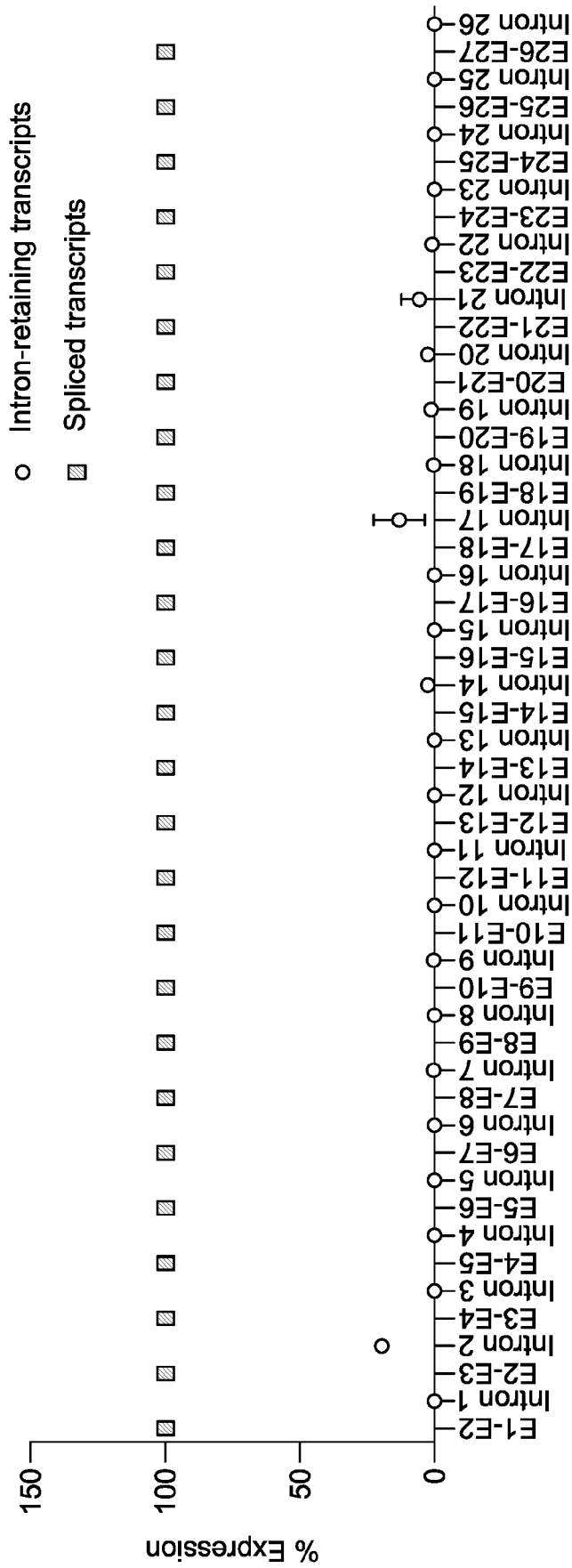
SCN2A Intron retention - Whole brain (Ambion)



SCN2A

FIGURE 3A

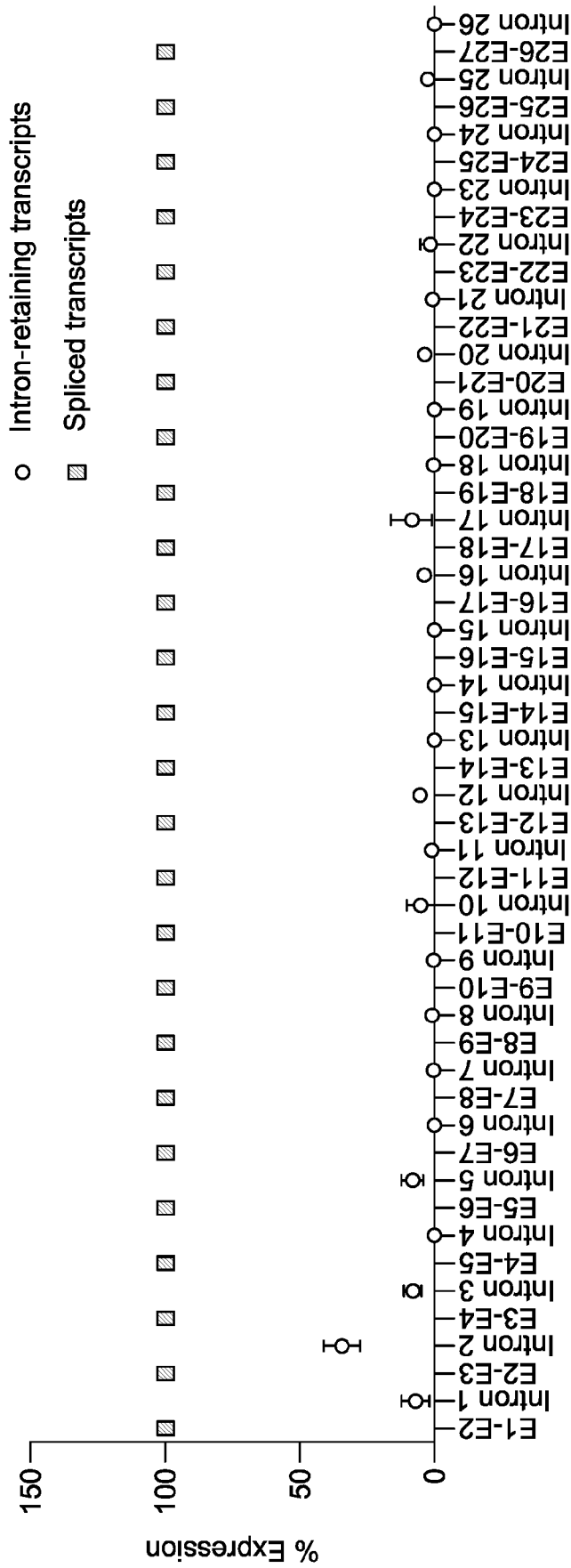
SCN2A Intron retention - Whole brain (Takara)



SCN2A

FIGURE 3B

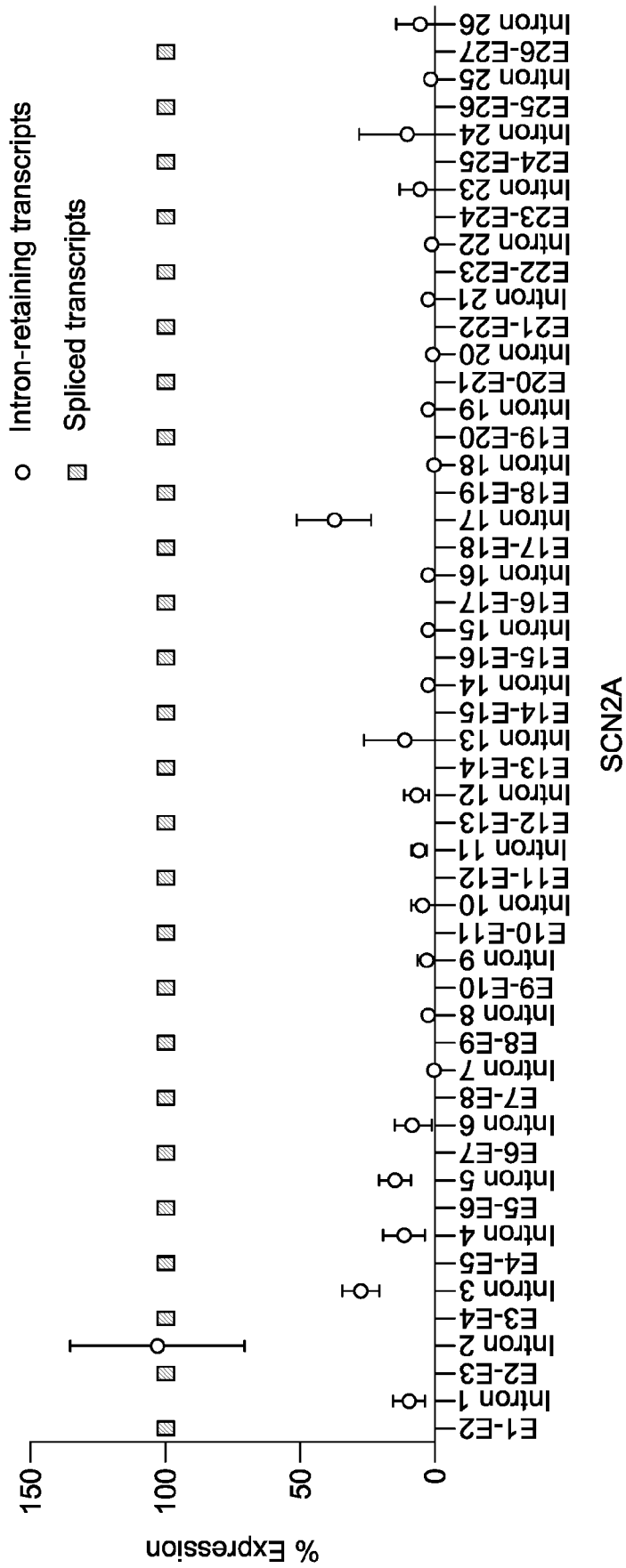
SCN2A Intron retention - SH-SY5Y



SCN2A

FIGURE 4A

SCN2A Intron retention - SK-N-AS



SCN2A

FIGURE 4B

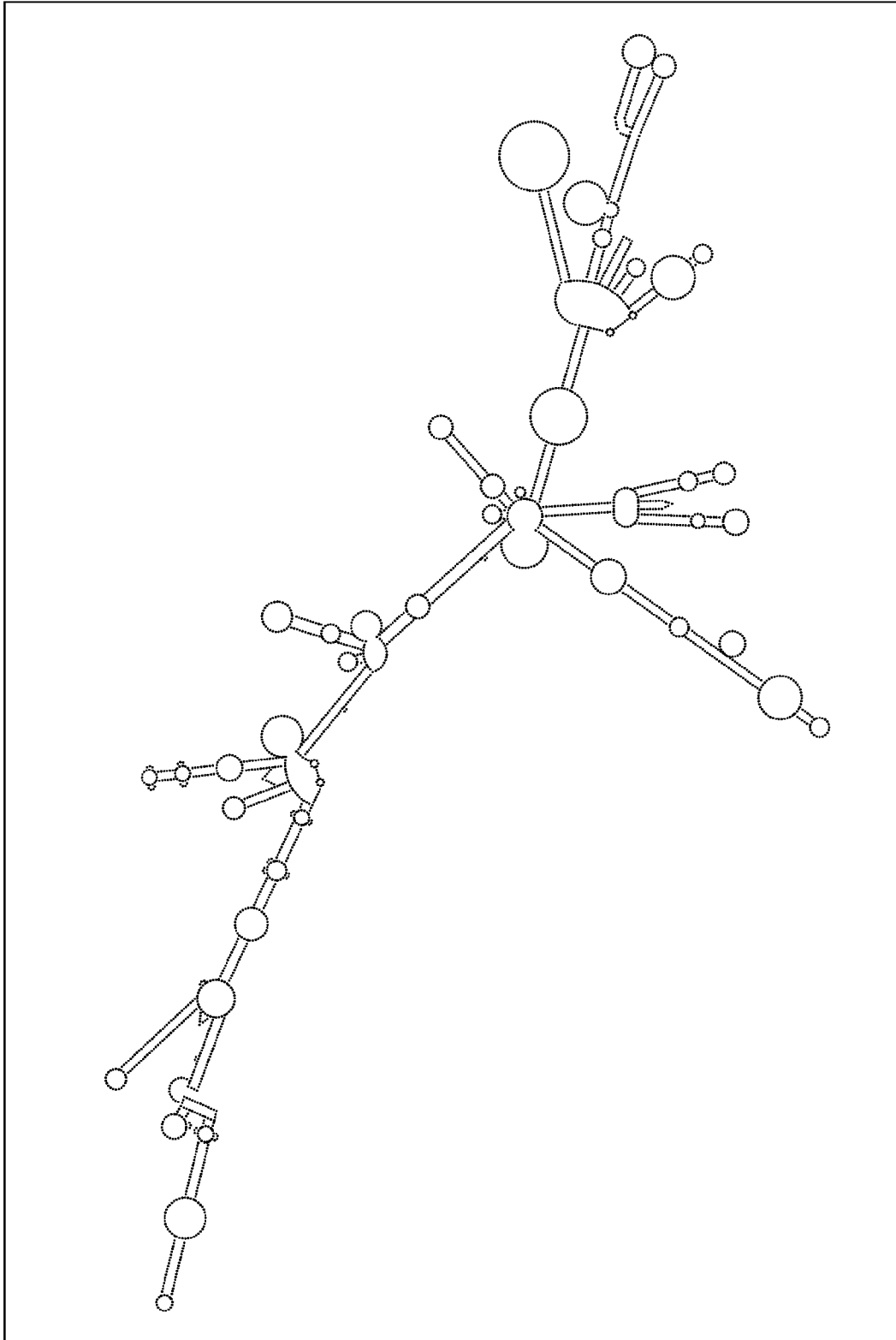


FIGURE 5

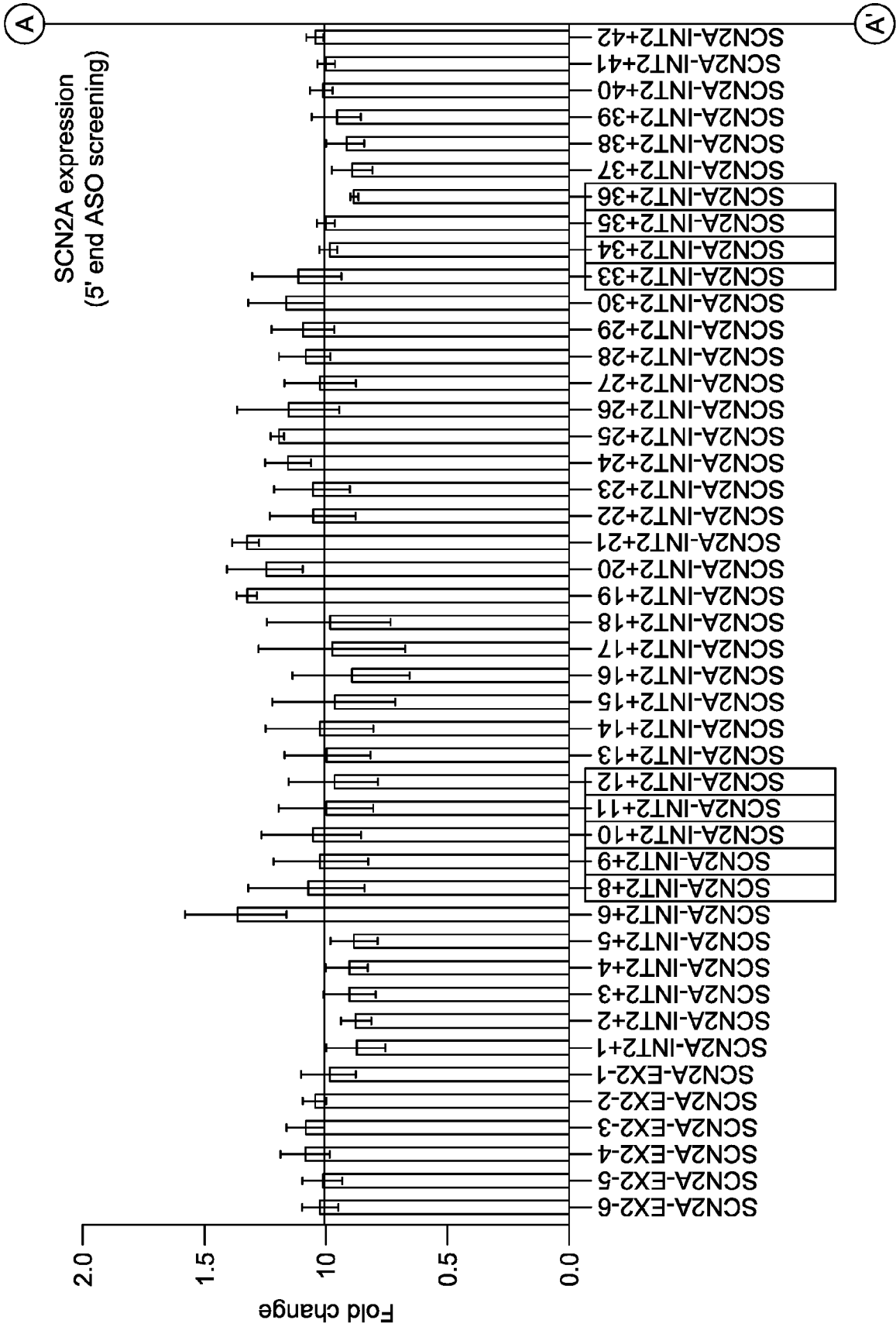


FIGURE 6A

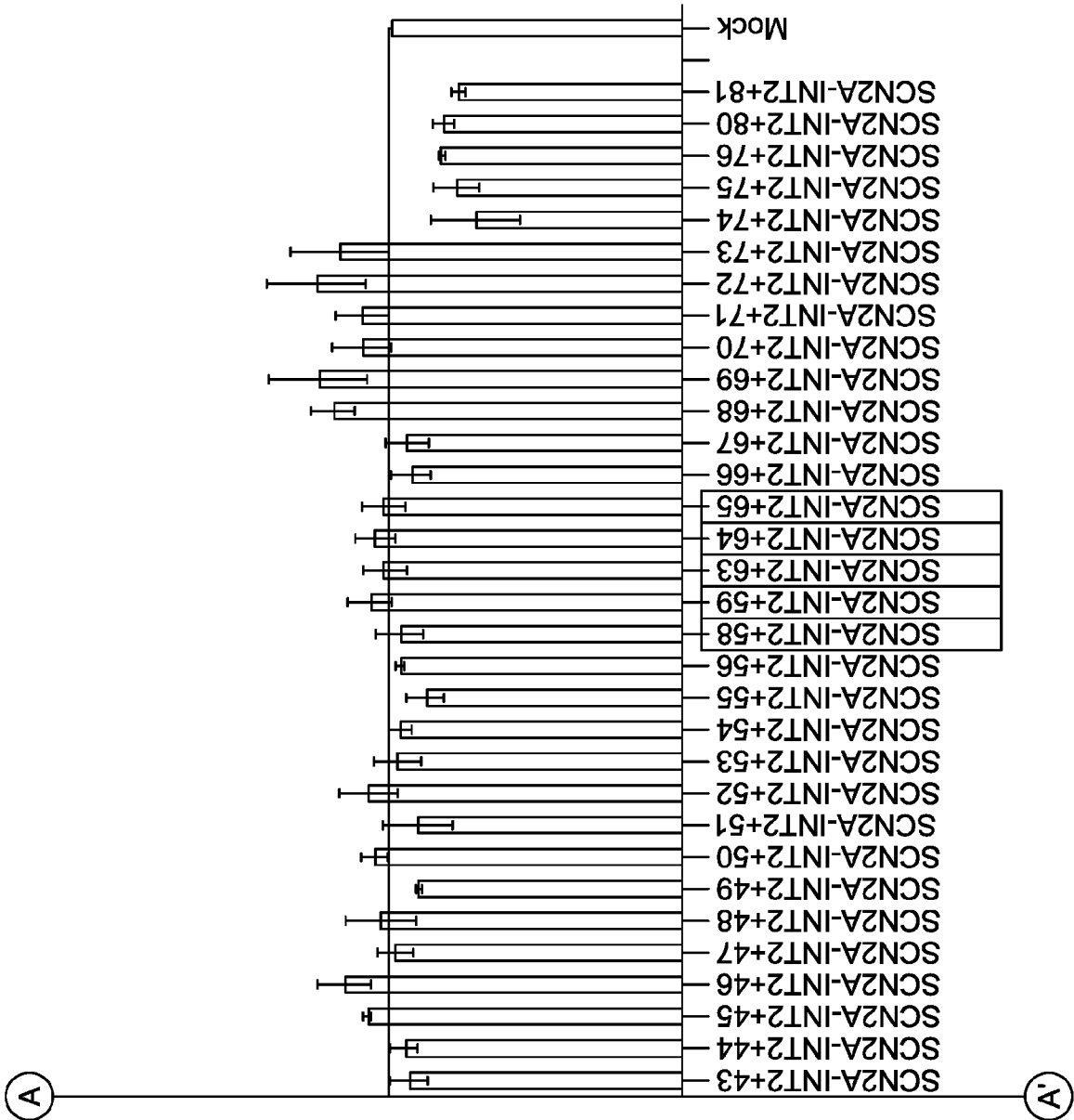


FIGURE 6A(Continued)

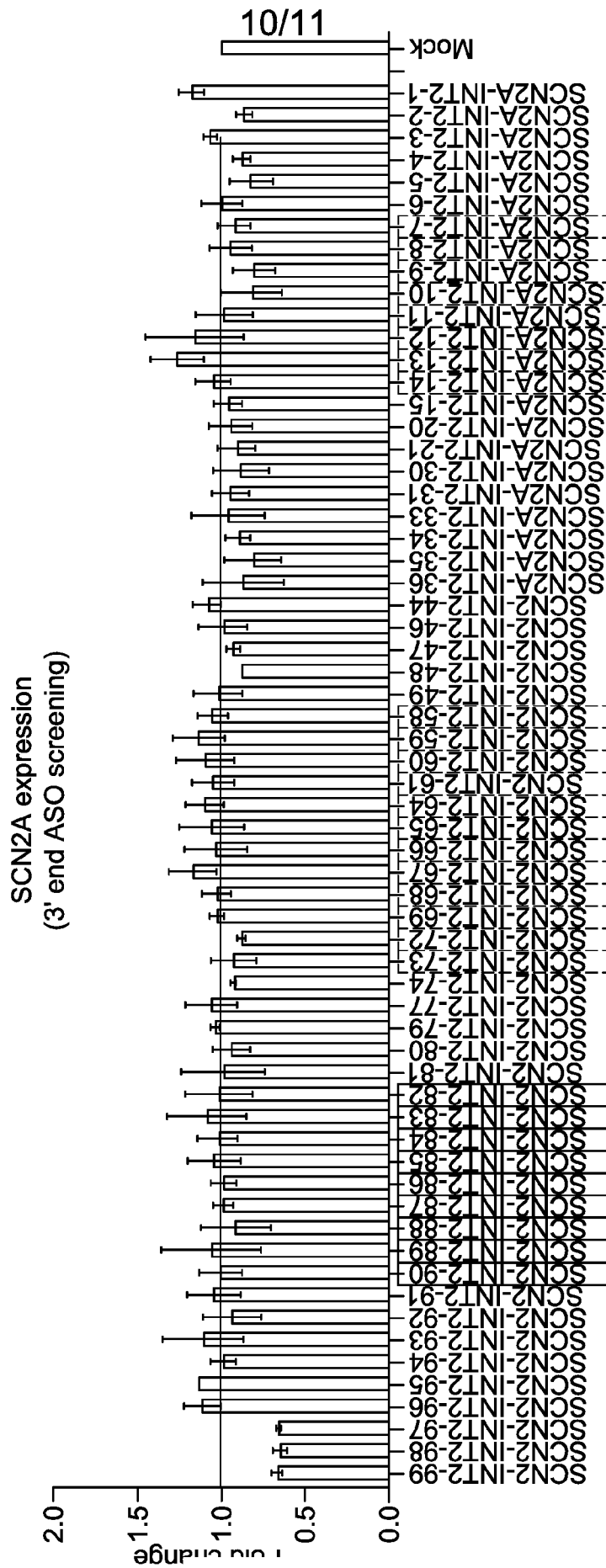


FIGURE 6B

11/11

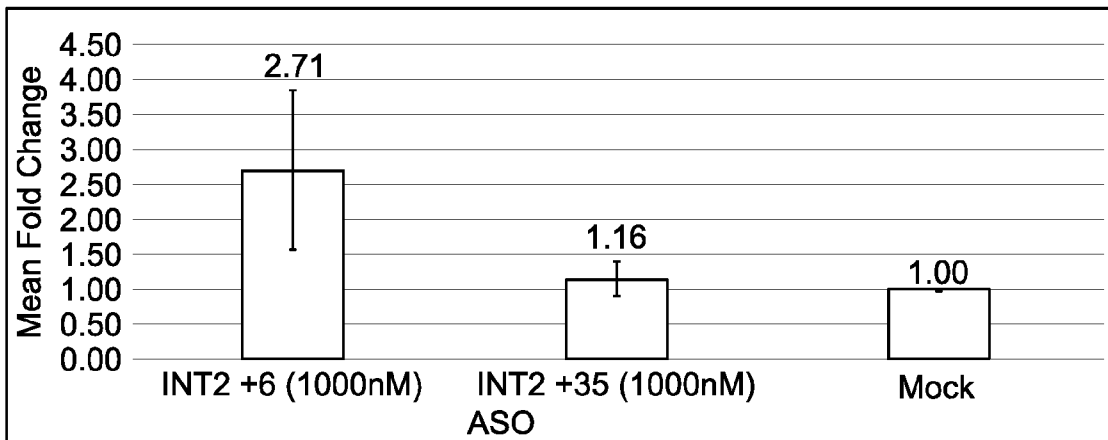
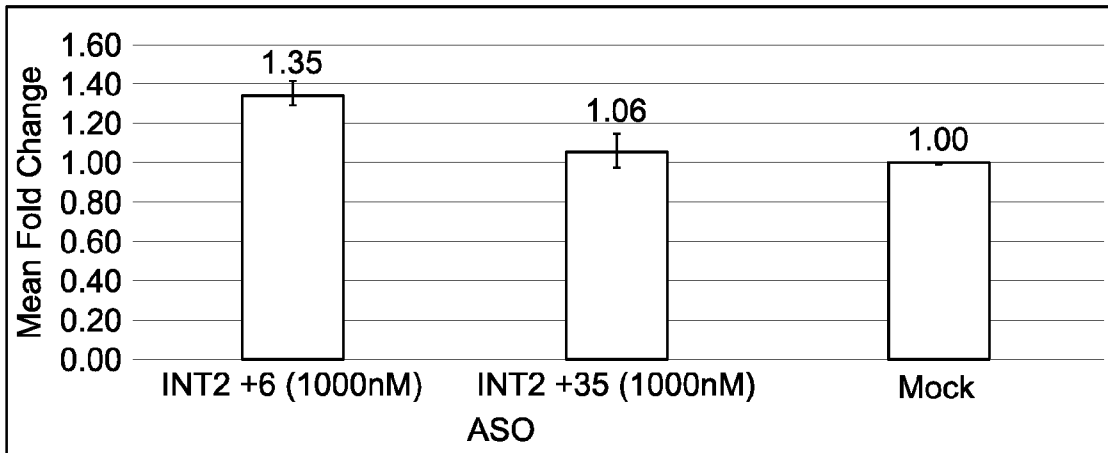


FIGURE 7

INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU2021/050788

A. CLASSIFICATION OF SUBJECT MATTER		
C12N 15/113 (2010.01) A61P 25/18 (2006.01) A61P 25/08 (2006.01) A61K 31/7125 (2006.01) A61K 31/712 (2006.01)		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols)		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)		
PATENW, MEDLINE, BIOSIS, EMBASE, CAPLUS: SCN2A, Nav 1.2, sodium voltage gated channel alpha subunit 2, splicing, intron retention, ASO and like terms. IPC/CPC; C12N15/1138, C12N2320/33		
GenomeQuest: SEQ ID NOs: 12, 115-204 and introns 1, 3, 4, 5, 11, 13, 17 and 24 corresponding to NCBI reference sequence: NG_008143.1 published 12 July 2020		
Google Scholar, Espacenet and IP Australia's internal databases: Applicant and Inventor Search		
Google: SCN2A NCBI mRNA reference		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C		
<input checked="" type="checkbox"/> See patent family annex		
* Special categories of cited documents:		
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention	
"D" document cited by the applicant in the international application	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	
"E" earlier application or patent but published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family	
"O" document referring to an oral disclosure, use, exhibition or other means		
"P" document published prior to the international filing date but later than the priority date claimed		
Date of the actual completion of the international search	Date of mailing of the international search report	
1 October 2021	01 October 2021	
Name and mailing address of the ISA/AU	Authorised officer	
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustralia.gov.au	Stephanie Sun AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. +61262832235	

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C (Continuation).	DOCUMENTS CONSIDERED TO BE RELEVANT	PCT/AU2021/050788
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	STOKE Therapeutics, Inc. Annual Report for the fiscal year ended December 31, 2019, [retrieved from the internet 21 September 2021] <URL: https://www.annualreports.com/HostedData/AnnualReportArchive/s/NASDAQ_STOK_2019.pdf > Published March 23 2020 page 7 'Treatment of autosomal dominant haploinsufficiency diseases with TANGO,' Table on page 11	1-14, 16-51
X	WO 2001077384 A2 (EPIGENOMICS AG) 18 October 2001 page 7, lines 13-19, SEQ ID NO: 98194, 267600, 54977, 163319	32-48, 50
X	US 7655460 B2 (ROULEAU ET AL.) 02 February 2010 Figure 4, Example 4, SEQ ID NO: 200	32-38, 42-48, 50
X	WO 2002006294 A2 (GENAISSANCE PHARMACEUTICALS, INC.) 30 March 2010 page 15, line 17, Claim 18, SEQ ID NO: 89	32-36, 38, 40-48, 50
X	WO 2018007980 A1 (CRISPR THERAPEUTICS AG) 11 January 2018 Claim 30 and 33, SEQ ID NO: 5327, 7114, 7118, 10371, 12381, 49132, 111948, paragraph [00021]-[00022]	32-33, 42-48, 50
X	WO 2013173637 A1 (RANA THERAPEUTICS INC. ET AL.) 21 November 2013 page 5, lines 2-8, page 6, lines 25-30, page 7, lines 13-15, SEQ ID NO: 2024728	32-35, 42-48, 50
X	WO 2010151671 A2 (CURNA, INC.) 29 December 2010 paragraph [0007], [0013], [0018]-[0019], [0125], [00144], SEQ ID NO: 4	32-33, 42-50
X	US 9364495 B2 (HARDEE ET AL.) 14 June 2016 Abstract, Claim 1, col. 8, line 28-29, SEQ ID NO: 15, col. 27, lines 5-6, col. 9, line 66, col. 10, lines 36-38	32-33, 42-50
A	NIH Genome Viewer of NCBI RefSeq genes, curated subset (NM_*, NR_*, NP_* or YP_*) - NM_001040142.2 [retrieved from the internet 23 September 2021] <URL: https://www.ncbi.nlm.nih.gov/genome/gdv/browser/nucleotide/?id=NM_001040142.2 >	
L	NCBI Reference Sequence: NM_001040142.2 [retrieved from the internet on 23 September 2021] < https://www.ncbi.nlm.nih.gov/nucore/1519242486?sat=47&satkey=81167774 > Sequence version published 3 July 2019. Establishes priority of sequencing reads in D9	
P,X	WO 2020154462 A1 (ROGCON U. R., INC.) 30 July 2020 Abstract, Figure 3B, page 62, lines 20-25, Example 2, Claims 1-31	1-14, 16-51

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
- a. forming part of the international application as filed:
 in the form of an Annex C/ST.25 text file.
 on paper or in the form of an image file.
- b. furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
- c. furnished subsequent to the international filing date for the purposes of international search only:
 in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).
 on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).
2. In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:
- SEQ ID NOs: 115-205 searched at 100% identity over 8-50 nucleotides.
- Intron 2 searched as SEQ ID NO: 12 at 100% identity over 8-100 nucleotides.
- All other introns, Intron 1, 3, 4, 5, 11, 13, 17 and 24 searched using the NCBI Reference Sequence: NG_008143.1 published 12 July 2020 as per paragraphs [0070]-[0077] of the specification. Introns were searched at 100% identity over 8-100 nucleotides or 10-50 nucleotides.

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