

ANTISENSE OLIGOMERS FOR TREATMENT OF AUTOSOMAL DOMINANT MENTAL RETARDATION-5 AND DRAVET SYNDROME

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 62/267,251, filed December 14, 2015, which application is incorporated herein by reference.

REFERENCE TO A SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been filed electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on December 12, 2016, is named 47991_708_601_SL.txt and is 1,460,063 bytes in size.

BACKGROUND OF THE INVENTION

[0003] Mental retardation is the most prevalent handicap of children affecting 1 to 3% of the population. Autosomal Dominant Mental Retardation-5 (MRD5) is a prevalent nonsyndromic form of the disorder characterized by the lack of associated morphologic, radiologic, and metabolic features. A case study identified de novo genetic lesions in the *SYNGAP1* gene that result in the production of truncated proteins in approximately 3% of patients with unexplained nonsyndromic mental retardation. SYNGAP1 is a GTPase-activating enzyme that is selectively expressed in the brain and required for normal development (Hamden, *et al.*, 2009, NEJM 360: 599-605).

[0004] Dravet Syndrome (DS), also known as severe myoclonic epilepsy of infancy or SMEI, was first described by Dravet in 1978. It is a childhood epilepsy characterized by an onset of seizures during the first year of life that do not remit. Mutations in the *SCN1A* gene, which is part of the *SCN1A-SCN2A-SCN3A* gene cluster that encodes alpha-pore forming subunits of the neuronal voltage gated sodium channel, are associated with development of the disease (Miller, *et al.*, 1993-2015, GeneReviews, Eds. Pagon RA, *et al.* Seattle (WA): University of Washington, Seattle, Bookshelf ID: NBK1318, and Mulley, *et al.*, 2005, Hum. Mutat. 25: 535-542).

SUMMARY

[0004a] In one aspect, provided herein is a pharmaceutical composition comprising an antisense oligomer (ASO), wherein the ASO is complementary to a targeted portion of a retained-intron-containing pre-mRNA (RIC pre-mRNA) that encodes SCN1A protein, wherein the RIC pre-mRNA comprises a retained intron, an exon flanking 5' splice site of the retained intron, and an exon flanking 3' splice site of the retained intron, and wherein binding of the ASO to the RIC pre-mRNA will cause a retained intron to be constitutively spliced from the RIC pre-mRNA in cells that express the RIC pre-mRNA, thereby increasing the level of functional RNA encoding SCN1A protein in the cells.

[0004b] In another aspect, provided herein is a pharmaceutical composition as described herein for use as a medicament.

[0004c] In another aspect, provided herein is a use of a pharmaceutical composition as described herein in the manufacture of a medicament for treating Dravet Syndrome (DS) in a subject.

[0005] Disclosed herein, in some examples, are methods of treating a Autosomal Dominant Mental Retardation 5 (MRD5) or Dravet Syndrome (DS) in a subject in need thereof, by increasing the expression of a target protein or functional RNA by cells of the subject, wherein the cells have a retained-intron-containing pre-mRNA (RIC pre-mRNA), the RIC pre-mRNA comprising a retained intron, an exon flanking the 5' splice site, an exon flanking the 3' splice site, and wherein the RIC pre-mRNA encodes the target protein or functional RNA, the method comprising contacting the cells of the subject with an antisense oligomer (ASO) complementary to a targeted portion of the RIC pre-mRNA encoding the target protein or functional RNA, whereby the retained intron is constitutively spliced from the RIC pre-mRNA encoding the target protein or functional RNA, thereby increasing the level of mRNA encoding the target

protein or functional RNA, and increasing the expression of the target protein or functional RNA in the cells of the subject.

[0006] Disclosed herein, in some examples, are methods of increasing expression of a target protein, wherein the target protein is SYNGAP1 or SCN1A, by cells having a retained-intron-containing pre-mRNA (RIC pre-mRNA), the RIC pre-mRNA comprising a retained intron, an exon flanking the 5' splice site of the retained intron, an exon flanking the 3' splice site of the retained intron, and wherein the RIC pre-mRNA encodes SYNGAP1 or SCN1A protein, the method comprising contacting the cells with an antisense oligomer (ASO) complementary to a targeted portion of the RIC pre-mRNA encoding SYNGAP1 or SCN1A protein, whereby the retained intron is constitutively spliced from the RIC pre-mRNA encoding SYNGAP1 or SCN1A protein, thereby increasing the level of mRNA encoding SYNGAP1 or SCN1A protein, and increasing the expression of SYNGAP1 or SCN1A protein in the cells.

[0007] In some examples of any of the aforementioned methods, the method is a method of treating MRD5 and the target protein is SYNGAP1, or the method is a method of treating DS and the target protein is SCN1A. In some examples, the target protein or the functional RNA is a compensating protein or a compensating functional RNA that functionally augments or replaces a target protein or functional RNA that is deficient in amount or activity in the subject. In some examples, the cells are in or from a subject having a condition caused by a deficient amount or activity of SYNGAP1 or SCN1A protein. In some examples, the deficient amount of the target protein is caused by haploinsufficiency of the target protein, wherein the subject has a first allele encoding a functional target protein, and a second allele from which the target protein is not produced, or a second allele encoding a nonfunctional target protein, and wherein the antisense oligomer binds to a targeted portion of a RIC pre-mRNA transcribed from the first allele. In some examples, the subject has a condition caused by a disorder resulting from a deficiency in the amount or function of the target protein, wherein the subject has (a) a first mutant allele from which (i) the target protein is produced at a reduced level compared to production from a wild-type allele, (ii) the target protein is produced in a form having reduced function compared to an equivalent wild-type protein, or (iii) the target protein is not produced, and (b) a second mutant allele from which (i) the target protein is produced at a reduced level compared to production from a wild-type allele, (ii) the target protein is produced in a form having reduced function compared to an equivalent wild-type protein, or (iii) the target protein is not produced, and wherein when the subject has a first mutant allele (a)(iii), the second mutant allele is (b)(i) or (b)(ii), and wherein when the subject has a second mutant allele (b)(iii), the first mutant allele is (a)(i) or (a)(ii), and wherein the RIC pre-mRNA is transcribed from either the first mutant allele that is (a)(i) or (a)(ii), and/or the second allele that is (b)(i) or (b)(ii). In some examples, the target protein is produced in a form having reduced function compared to the equivalent wild-type protein. In some examples, the target protein is produced in a form that is fully-functional compared to the equivalent wild-type protein.

[0008] In some examples of any of the aforementioned methods, the targeted portion of the RIC pre-mRNA is in the retained intron within the region +6 relative to the 5' splice site of the retained intron to -16 relative to the 3' splice site of the retained intron. In some examples, the targeted portion of the

RIC pre-mRNA is in the retained intron within: (a) the region +6 to +499 relative to the 5' splice site of the retained intron; or (b) the region -16 to -496 relative to the 3' splice site of the retained intron. In some examples, the targeted portion of the RIC pre-mRNA is within: (a) the region +2e to -4e in the exon flanking the 5' splice site of the retained intron; or (b) the region +2e to -4e in the exon flanking the 3' splice site of the retained intron. In some examples, the targeted portion of the RIC pre-mRNA is within: (a) the region -4e to -1,054e relative to the 5' splice site of the retained intron; (b) the region +6 to +499 relative to the 5' splice site of the retained intron; (c) the region -16 to -496 relative to the 3' splice site of the retained intron; or (d) the region +2e to +1,912e relative to the 3' splice site of the retained intron.

[0009] In some examples of any of the aforementioned methods, the target protein is SCN1A. In some examples, the RIC pre-mRNA comprises a sequence with at least about 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to any one of SEQ ID NOs: 4-7. In some examples, the RIC pre-mRNA is encoded by a genetic sequence with at least about 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to SEQ ID NO: 1. In some examples, the targeted portion of the RIC pre-mRNA comprises a sequence with at least 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to a region comprising at least 8 contiguous nucleic acids of SEQ ID NO: 2593. In some examples, the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complimentary to any one of SEQ ID NOs: 10-1037. In some examples, the targeted portion of the RIC pre-mRNA is within the region -264e to +496 relative to the 5' splice site of the retained intron 21 or within the region -496 to +37e relative to the 3' splice site of the retained intron 21. In some examples, the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complimentary to any one of SEQ ID NOs: 10-266 or 524-1037. In some examples, the targeted portion of the RIC pre-mRNA is within the region -264e to -4e relative to the 5' splice site of the retained intron 21. In some examples, the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complimentary to any one of SEQ ID NOs: 10-62, 524-576, or 781-833. In some examples, the targeted portion of the RIC pre-mRNA is in retained intron 21 within the region +6 to +496 relative to the 5' splice site of the retained intron 21. In some examples, the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complimentary to any one of SEQ ID NOs: 63-162, 577-675, or 834-932. In some examples, the targeted portion of the RIC pre-mRNA is in retained intron 21 within the region -16 to -496 relative to the 3' splice site of the retained intron 21. In some examples, the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complimentary to any one of SEQ ID NOs: 163-258, 676-772, or 933-1029. In some examples, the targeted portion of the RIC pre-mRNA is within the region +2e to +37e relative to the 3' splice site of the retained intron 21. In some examples, the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complimentary to any one of SEQ ID NOs: 259-266, 773-780, or 1030-1037.

[0010] In some examples of any of the aforementioned methods, the target protein is SCN1A. In some examples, the targeted portion of the RIC pre-mRNA is within the region -264e to +496 relative to the 5' splice site of the retained intron 23 or within the region -496 to +37e relative to the 3' splice site

of the retained intron 23. In some examples, the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complimentary to any one of SEQ ID NOs: 267-523. In some examples, the targeted portion of the RIC pre-mRNA is within the region -264e to -4e relative to the 5' splice site of the retained intron 23. In some examples, the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complimentary to any one of SEQ ID NOs: 267-319. In some examples, the targeted portion of the RIC pre-mRNA is in retained intron 23 within the region +6 to +496 relative to the 5' splice site of the retained intron 23. In some examples, the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complimentary to any one of SEQ ID NOs: 320-418. In some examples, the targeted portion of the RIC pre-mRNA is in retained intron 23 within the region -16 to -496 relative to the 3' splice site of the retained intron 23. In some examples, the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complimentary to any one of SEQ ID NOs: 419-515. In some examples, the targeted portion of the RIC pre-mRNA is within the region +2e to +37e relative to the 3' splice site of the retained intron 23. In some examples, the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complimentary to any one of SEQ ID NOs: 516-523.

[0011] In some examples of any of the aforementioned methods, the target protein is SYNGAP1. In some examples, the RIC pre-mRNA comprises a sequence with at least about 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to any one of SEQ ID NOs: 8 or 9. In some examples, the RIC pre-mRNA is encoded by a genetic sequence with at least about 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to SEQ ID NO: 2 or 3. In some examples, the targeted portion of the RIC pre-mRNA comprises a sequence with at least 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to a region comprising at least 8 contiguous nucleic acids of SEQ ID NO: 2592, 2594, or 2595. In some examples, the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complimentary to any one of SEQ ID NOs: 1038-2591. In some examples, the targeted portion of the RIC pre-mRNA is within the region -73e to +499 relative to the 5' splice site of the retained intron 18 or within the region -496 to +1,912e relative to the 3' splice site of the retained intron 19. In some examples, the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complimentary to any one of SEQ ID NOs: 1038-1509 or 1815-2286. In some examples, the targeted portion of the RIC pre-mRNA is within the region -73e to -4e relative to the 5' splice site of the retained intron 18. In some examples, the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complimentary to any one of SEQ ID NOs: 1038-1050 or 1815-1827. In some examples, the targeted portion of the RIC pre-mRNA is in retained intron 18 within the region +6 to +499 relative to the 5' splice site of the retained intron 18. In some examples, the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complimentary to any one of SEQ ID NOs: 1051-1136 or 1828-1913. In some examples, the targeted portion of the RIC pre-mRNA is in retained intron 19 within the region -16 to -496 relative to the 3' splice site of the retained intron 19. In some examples, the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complimentary to any one of SEQ ID NOs: 1137-1228 or 1914-2005. In some examples, the

targeted portion of the RIC pre-mRNA is within the region +17e to +1,912e relative to the 3' splice site of the retained intron 19. In some examples, the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complimentary to any one of SEQ ID NOs: 1229-1509 or 2006-2286.

[0012] In some examples of any of the aforementioned methods, the target protein is SYNGAP1. In some examples, the targeted portion of the RIC pre-mRNA is within the region -1,054e to +251 relative to the 5' splice site of the retained intron 15 or within the region -256 to +157e relative to the 3' splice site of the retained intron 15. In some examples, the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complimentary to any one of SEQ ID NOs: 1510-1814 or 2287-2591. In some examples, the targeted portion of the RIC pre-mRNA is within the region -4e to -1,054e relative to the 5' splice site of the retained intron 15. In some examples, the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complimentary to any one of SEQ ID NOs: 1510-1686 or 2287-2463. In some examples, the targeted portion of the RIC pre-mRNA is in retained intron 15 within the region +6 to +251 relative to the 5' splice site of the retained intron 15. In some examples, the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complimentary to any one of SEQ ID NOs: 1687-1736 or 2464-2513. In some examples, the targeted portion of the RIC pre-mRNA is in retained intron 15 within the region -16 to -256 relative to the 3' splice site of the retained intron 15. In some examples, the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complimentary to any one of SEQ ID NOs: 1737-1785 or 2514-2562. In some examples, the targeted portion of the RIC pre-mRNA is within the region +2e to +157e relative to the 3' splice site of the retained intron 15. In some examples, the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complimentary to any one of SEQ ID NOs: 1786-1814 or 2563-2591.

[0013] In some examples of any of the aforementioned methods, the antisense oligomer does not increase the amount of the target protein or the functional RNA by modulating alternative splicing of pre-mRNA transcribed from a gene encoding the functional RNA or target protein. In some examples, the antisense oligomer does not increase the amount of the target protein or the functional RNA by modulating aberrant splicing resulting from mutation of the gene encoding the target protein or the functional RNA. In some examples, the RIC pre-mRNA was produced by partial splicing of a full-length pre-mRNA or partial splicing of a wild-type pre-mRNA. In some examples, the mRNA encoding the target protein or functional RNA is a full-length mature mRNA, or a wild-type mature mRNA. In some examples, the target protein produced is full-length protein, or wild-type protein. In some examples, the total amount of the mRNA encoding the target protein or functional RNA produced in the cell contacted with the antisense oligomer is increased about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least

about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to the total amount of the mRNA encoding the target protein or functional RNA produced in a control cell. In some examples, the total amount of target protein produced by the cell contacted with the antisense oligomer is increased about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to the total amount of target protein produced by a control cell.

[0014] In some examples of any of the aforementioned methods, the antisense oligomer comprises a backbone modification comprising a phosphorothioate linkage or a phosphorodiamidate linkage. In some examples, the antisense oligomer comprises a phosphorodiamidate morpholino, a locked nucleic acid, a peptide nucleic acid, a 2'-O-methyl, a 2'-Fluoro, or a 2'-O-methoxyethyl moiety. In some examples, the antisense oligomer comprises at least one modified sugar moiety. In some examples, each sugar moiety is a modified sugar moiety. In some examples, the antisense oligomer consists of from 8 to 50 nucleobases, 8 to 40 nucleobases, 8 to 35 nucleobases, 8 to 30 nucleobases, 8 to 25 nucleobases, 8 to 20 nucleobases, 8 to 15 nucleobases, 9 to 50 nucleobases, 9 to 40 nucleobases, 9 to 35 nucleobases, 9 to 30 nucleobases, 9 to 25 nucleobases, 9 to 20 nucleobases, 9 to 15 nucleobases, 10 to 50 nucleobases, 10 to 40 nucleobases, 10 to 35 nucleobases, 10 to 30 nucleobases, 10 to 25 nucleobases, 10 to 20 nucleobases, 10 to 15 nucleobases, 11 to 50 nucleobases, 11 to 40 nucleobases, 11 to 35 nucleobases, 11 to 30 nucleobases, 11 to 25 nucleobases, 11 to 20 nucleobases, 11 to 15 nucleobases, 12 to 50 nucleobases, 12 to 40 nucleobases, 12 to 35 nucleobases, 12 to 30 nucleobases, 12 to 25 nucleobases, 12 to 20 nucleobases, or 12 to 15 nucleobases. In some examples, the antisense oligomer is at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, complementary to the targeted portion of the RIC pre-mRNA encoding the protein.

[0015] In some examples of any of the aforementioned methods, the cell comprises a population of RIC pre-mRNAs transcribed from the gene encoding the target protein or functional RNA, wherein the population of RIC pre-mRNAs comprises two or more retained introns, and wherein the antisense oligomer binds to the most abundant retained intron in the population of RIC pre-mRNAs. In some examples, the binding of the antisense oligomer to the most abundant retained intron induces splicing out of the two or more retained introns from the population of RIC pre-mRNAs to produce mRNA encoding the target protein or functional RNA. In some examples, the cell comprises a population of RIC pre-mRNAs transcribed from the gene encoding the target protein or functional RNA, wherein the population of RIC pre-mRNAs comprises two or more retained introns, and wherein the antisense

oligomer binds to the second most abundant retained intron in the population of RIC pre-mRNAs. In some examples, the binding of the antisense oligomer to the second most abundant retained intron induces splicing out of the two or more retained introns from the population of RIC pre-mRNAs to produce mRNA encoding the target protein or functional RNA. In some examples, the method further comprises assessing SYNGAP1 or SCN1A protein expression.

[0016] In some examples of any of the aforementioned methods, the MRD5 is treated and wherein the antisense oligomer binds to a targeted portion of a *SYNGAP1* RIC pre-mRNA, wherein the targeted portion is within a sequence selected from SEQ ID NOs: 1038-2591, or wherein DS is treated and wherein the antisense oligomer binds to a targeted portion of a *SCN1A* RIC pre-mRNA, wherein the targeted portion is within a sequence selected from SEQ ID NOs: 10-1037. In some examples, the subject is a human. In some examples, the subject is a non-human animal. In some examples, the subject is a fetus, an embryo, or a child. In some examples, the cells are *ex vivo*. In some examples, the antisense oligomer is administered by intrathecal injection, intracerebroventricular injection, intraperitoneal injection, intramuscular injection, subcutaneous injection, or intravenous injection of the subject. In some examples, the 9 nucleotides at -3e to -1e of the exon flanking the 5' splice site and +1 to +6 of the retained intron, are identical to the corresponding wild-type sequence. In some examples, the 16 nucleotides at -15 to -1 of the retained intron and +1e of the exon flanking the 3' splice site are identical to the corresponding wild-type sequence. Disclosed herein, in some examples, are antisense oligomers as described in any of the aforementioned methods.

[0017] Disclosed herein, in some examples, are antisense oligomers comprising a sequence with at least about 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to any one of SEQ ID NOs: 10-2591.

[0018] Also disclosed herein, in some examples, are pharmaceutical compositions comprising the antisense oligomer of claim 90 or 91 and a pharmaceutically acceptable excipient, diluent, or carrier.

[0019] Disclosed herein, in some examples, are methods of treating a subject in need thereof, by administering the pharmaceutical composition of claim 92 by intrathecal injection, intracerebroventricular injection, intraperitoneal injection, intramuscular injection, subcutaneous injection, or intravenous injection.

[0020] Disclosed herein, in some examples, are compositions comprising an antisense oligomer for use in a method of increasing expression of a target protein or a functional RNA by cells to treat MRD5 or DS in a subject in need thereof, associated with a deficient protein or deficient functional RNA, wherein the deficient protein or deficient functional RNA is deficient in amount or activity in the subject, wherein the antisense oligomer enhances constitutive splicing of a retained intron-containing pre-mRNA (RIC pre-mRNA) encoding the target protein or the functional RNA, wherein the target protein is: (a) the deficient protein; or (b) a compensating protein which functionally augments or replaces the deficient protein or in the subject; and wherein the functional RNA is: (c) the deficient RNA; or (d) a compensating functional RNA which functionally augments or replaces the deficient functional RNA in the subject; wherein the RIC pre-mRNA comprises a retained intron, an exon flanking the 5' splice site and an exon flanking the 3' splice site, and wherein the retained intron is spliced from the RIC pre-mRNA encoding the

target protein or the functional RNA, thereby increasing production or activity of the target protein or the functional RNA in the subject.

[0021] Disclosed herein, in some examples, are compositions comprising an antisense oligomer for use in a method of treating a condition associated with SYNGAP1 or SCN1A protein in a subject in need thereof, the method comprising the step of increasing expression of SYNGAP1 or SCN1A protein by cells of the subject, wherein the cells have a retained-intron-containing pre-mRNA (RIC pre-mRNA) comprising a retained intron, an exon flanking the 5' splice site of the retained intron, an exon flanking the 3' splice site of the retained intron, and wherein the RIC pre-mRNA encodes the SYNGAP1 or SCN1A protein, the method comprising contacting the cells with the antisense oligomer, whereby the retained intron is constitutively spliced from the RIC pre-mRNA transcripts encoding SYNGAP1 or SCN1A protein, thereby increasing the level of mRNA encoding SYNGAP1 or SCN1A protein, and increasing the expression of SYNGAP1 or SCN1A protein, in the cells of the subject. In some examples, the condition is a disease or disorder. In some examples, the disease or disorder is AD mental retardation 5 or Dravet syndrome. In some examples, the target protein and RIC pre-mRNA are encoded by the *SYNGAP1* or *SCN1A* gene. In some examples, the antisense oligomer targets a portion of the RIC pre-mRNA that is in the retained intron within the region +6 relative to the 5' splice site of the retained intron to -16 relative to the 3' splice site of the retained intron. In some examples, the antisense oligomer targets a portion of the RIC pre-mRNA that is in the retained intron within: (a) the region +6 to +499 relative to the 5' splice site of the retained intron; or (b) the region -16 to -496 relative to the 3' splice site of the retained intron. In some examples, the antisense oligomer targets a portion of the RIC pre-mRNA that is within the region about 100 nucleotides downstream of the 5' splice site of the at least one retained intron, to about 100 nucleotides upstream of the 3' splice site of the at least one retained intron. In some examples, the targeted portion of the RIC pre-mRNA is within: (a) the region +2e to -4e in the exon flanking the 5' splice site of the retained intron; or (b) the region +2e to -4e in the exon flanking the 3' splice site of the retained intron. In some examples, the targeted portion of the RIC pre-mRNA is within: (a) the region -4e to -1,054e relative to the 5' splice site of the retained intron; (b) the region +6 to +499 relative to the 5' splice site of the retained intron; (c) the region -16 to -496 relative to the 3' splice site of the retained intron; or (d) the region +2e to +1,912e relative to the 3' splice site of the retained intron.

[0022] In some examples of any of the aforementioned compositions, the target protein is SCN1A. In some examples, the RIC pre-mRNA comprises a sequence with at least about 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to any one of SEQ ID NOs: 4-7. In some examples, the RIC pre-mRNA is encoded by a genetic sequence with at least about 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to SEQ ID NO: 1. In some examples, the targeted portion of the RIC pre-mRNA comprises a sequence with at least 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to a region comprising at least 8 contiguous nucleic acids of SEQ ID NO: 2593. In some examples, the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complimentary to any one of SEQ ID NOs: 10-1037. In some examples, the targeted portion of the RIC pre-mRNA is

within the region -264e to +496 relative to the 5' splice site of the retained intron 21 or within the region -496 to +37e relative to the 3' splice site of the retained intron 21. In some examples, the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complimentary to any one of SEQ ID NOs: 10-266 or 524-1037. In some examples, the targeted portion of the RIC pre-mRNA is within the region -264e to -4e relative to the 5' splice site of the retained intron 21. In some examples, the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complimentary to any one of SEQ ID NOs: 10-62, 524-576, or 781-833. In some examples, the targeted portion of the RIC pre-mRNA is in retained intron 21 within the region +6 to +496 relative to the 5' splice site of the retained intron 21. In some examples, the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complimentary to any one of SEQ ID NOs: 63-162, 577-675, or 834-932. In some examples, the targeted portion of the RIC pre-mRNA is in retained intron 21 within the region -16 to -496 relative to the 3' splice site of the retained intron 21. In some examples, the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complimentary to any one of SEQ ID NOs: 163-258, 676-772, or 933-1029. In some examples, the targeted portion of the RIC pre-mRNA is within the region +2e to +37e relative to the 3' splice site of the retained intron 21. In some examples, the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complimentary to any one of SEQ ID NOs: 259-266, 773-780, or 1030-1037.

[0023] In some examples of any of the aforementioned compositions, the target protein is SCN1A. In some examples, the targeted portion of the RIC pre-mRNA is within the region -264e to +496 relative to the 5' splice site of the retained intron 23 or within the region -496 to +37e relative to the 3' splice site of the retained intron 23. In some examples, the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complimentary to any one of SEQ ID NOs: 267-523. In some examples, the targeted portion of the RIC pre-mRNA is within the region -264e to -4e relative to the 5' splice site of the retained intron 23. In some examples, the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complimentary to any one of SEQ ID NOs: 267-319. In some examples, the targeted portion of the RIC pre-mRNA is in retained intron 23 within the region +6 to +496 relative to the 5' splice site of the retained intron 23. In some examples, the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complimentary to any one of SEQ ID NOs: 320-418. In some examples, the targeted portion of the RIC pre-mRNA is in retained intron 23 within the region -16 to -496 relative to the 3' splice site of the retained intron 23. In some examples, the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complimentary to any one of SEQ ID NOs: 419-515. In some examples, the targeted portion of the RIC pre-mRNA is within the region +2e to +37e relative to the 3' splice site of the retained intron 23. In some examples, the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complimentary to any one of SEQ ID NOs: 516-523.

[0024] In some examples of any of the aforementioned compositions, the target protein is SYNGAP1. In some examples, the RIC pre-mRNA comprises a sequence with at least about 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to any one of SEQ ID NOs: 8 or 9. In some

examples, the RIC pre-mRNA is encoded by a genetic sequence with at least about 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to SEQ ID NO: 2 or 3. In some examples, the targeted portion of the RIC pre-mRNA comprises a sequence with at least 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to a region comprising at least 8 contiguous nucleic acids of SEQ ID NO: 2592, 2594, or 2595. In some examples, the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complimentary to any one of SEQ ID NOs: 1038-2591. In some examples, the targeted portion of the RIC pre-mRNA is within the region -73e to +499 relative to the 5' splice site of the retained intron 18 or within the region -496 to +1,912e relative to the 3' splice site of the retained intron 19. In some examples, the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complimentary to any one of SEQ ID NOs: 1038-1509 or 1815-2286. In some examples, the targeted portion of the RIC pre-mRNA is within the region -73e to -4e relative to the 5' splice site of the retained intron 18. In some examples, the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complimentary to any one of SEQ ID NOs: 1038-1050 or 1815-1827. In some examples, the targeted portion of the RIC pre-mRNA is in retained intron 18 within the region +6 to +499 relative to the 5' splice site of the retained intron 18. In some examples, the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complimentary to any one of SEQ ID NOs: 1051-1136 or 1828-1913. In some examples, the targeted portion of the RIC pre-mRNA is in retained intron 19 within the region -16 to -496 relative to the 3' splice site of the retained intron 19. In some examples, the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complimentary to any one of SEQ ID NOs: 1137-1228 or 1914-2005. In some examples, the targeted portion of the RIC pre-mRNA is within the region +17e to +1,912e relative to the 3' splice site of the retained intron 19. In some examples, the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complimentary to any one of SEQ ID NOs: 1229-1509 or 2006-2286.

[0025] In some examples of any of the aforementioned compositions, the target protein is SYNGAP1. In some examples, the targeted portion of the RIC pre-mRNA is within the region -1,054e to +251 relative to the 5' splice site of the retained intron 15 or within the region -256 to +157e relative to the 3' splice site of the retained intron 15. In some examples, the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complimentary to any one of SEQ ID NOs: 1510-1814 or 2287-2591. In some examples, the targeted portion of the RIC pre-mRNA is within the region -4e to -1,054e relative to the 5' splice site of the retained intron 15. In some examples, the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complimentary to any one of SEQ ID NOs: 1510-1686 or 2287-2463. In some examples, the targeted portion of the RIC pre-mRNA is in retained intron 15 within the region +6 to +251 relative to the 5' splice site of the retained intron 15. In some examples, the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complimentary to any one of SEQ ID NOs: 1687-1736 or 2464-2513. In some examples, the targeted portion of the RIC pre-mRNA is in retained intron 15 within the region -16 to -256 relative to the 3' splice site of the retained intron 15. In some examples, the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complimentary to

any one of SEQ ID NOs: 1737-1785 or 2514-2562. In some examples, the targeted portion of the RIC pre-mRNA is within the region +2e to +157e relative to the 3' splice site of the retained intron 15. In some examples, the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complimentary to any one of SEQ ID NOs: 1786-1814 or 2563-2591.

[0026] In some examples of any of the aforementioned compositions, the antisense oligomer does not increase the amount of target protein or functional RNA by modulating alternative splicing of the pre-mRNA transcribed from a gene encoding the target protein or functional RNA. In some examples, the antisense oligomer does not increase the amount of the functional RNA or functional protein by modulating aberrant splicing resulting from mutation of the gene encoding the target protein or functional RNA. In some examples, the RIC pre-mRNA was produced by partial splicing from a full-length pre-mRNA or a wild-type pre-mRNA. In some examples, the mRNA encoding the target protein or functional RNA is a full-length mature mRNA, or a wild-type mature mRNA. In some examples, the target protein produced is full-length protein, or wild-type protein. In some examples, the retained intron is a rate-limiting intron. In some examples, the retained intron is the most abundant retained intron in said RIC pre-mRNA. In some examples, the retained intron is the second most abundant retained intron in said RIC pre-mRNA.

[0027] In some examples of any of the aforementioned compositions, the antisense oligomer comprises a backbone modification comprising a phosphorothioate linkage or a phosphorodiamidate linkage. In some examples, the antisense oligomer is an antisense oligonucleotide. In some examples, the antisense oligomer comprises a phosphorodiamidate morpholino, a locked nucleic acid, a peptide nucleic acid, a 2'-O-methyl, a 2'-Fluoro, or a 2'-O-methoxyethyl moiety. In some examples, the antisense oligomer comprises at least one modified sugar moiety. In some examples, each sugar moiety is a modified sugar moiety. In some examples, the antisense oligomer consists of from 8 to 50 nucleobases, 8 to 40 nucleobases, 8 to 35 nucleobases, 8 to 30 nucleobases, 8 to 25 nucleobases, 8 to 20 nucleobases, 8 to 15 nucleobases, 9 to 50 nucleobases, 9 to 40 nucleobases, 9 to 35 nucleobases, 9 to 30 nucleobases, 9 to 25 nucleobases, 9 to 20 nucleobases, 9 to 15 nucleobases, 10 to 50 nucleobases, 10 to 40 nucleobases, 10 to 35 nucleobases, 10 to 30 nucleobases, 10 to 25 nucleobases, 10 to 20 nucleobases, 10 to 15 nucleobases, 11 to 50 nucleobases, 11 to 40 nucleobases, 11 to 35 nucleobases, 11 to 30 nucleobases, 11 to 25 nucleobases, 11 to 20 nucleobases, 11 to 15 nucleobases, 12 to 50 nucleobases, 12 to 40 nucleobases, 12 to 35 nucleobases, 12 to 30 nucleobases, 12 to 25 nucleobases, 12 to 20 nucleobases, or 12 to 15 nucleobases.

[0028] Disclosed herein, in some examples, are pharmaceutical compositions comprising the antisense oligomer of any of the compositions of claims 94 to 167, and a pharmaceutically acceptable excipient, diluent, or carrier. Also disclosed herein, in some examples, are methods of treating a subject in need thereof, by administering the pharmaceutical composition of claim 168 by intrathecal injection, intracerebroventricular injection, intraperitoneal injection, intramuscular injection, subcutaneous injection, or intravenous injection.

[0029] Disclosed herein, in some examples, are pharmaceutical compositions comprising: an antisense oligomer that hybridizes to a target sequence of a *SCN1A* or *SYNGAP1* mRNA transcript, wherein the *SCN1A* or *SYNGAP1* mRNA transcript comprises a retained intron, wherein the antisense oligomer induces splicing out of the retained intron from the *SCN1A* or *SYNGAP1* mRNA transcript; and a pharmaceutically acceptable excipient, diluent, or carrier. In some examples, the *SCN1A* or *SYNGAP1* mRNA transcript is a *SCN1A* or *SYNGAP1* RIC pre-mRNA transcript. In some examples, the targeted portion of the *SCN1A* or *SYNGAP1* RIC pre-mRNA transcript is in the retained intron within the region +500 relative to the 5' splice site of the retained intron to -500 relative to the 3' spliced site of the retained intron. In some examples, the *SCN1A* or *SYNGAP1* RIC pre-mRNA transcript is encoded by a genetic sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to any one of SEQ ID NOs: 1-3. In some examples, the *SCN1A* or *SYNGAP1* RIC pre-mRNA transcript comprises a sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to any one of SEQ ID NOs: 4-9. In some examples, the antisense oligomer comprises a backbone modification comprising a phosphorothioate linkage or a phosphorodiamidate linkage. In some examples, the antisense oligomer is an antisense oligonucleotide. In some examples, the antisense oligomer comprises a phosphorodiamidate morpholino, a locked nucleic acid, a peptide nucleic acid, a 2'-O-methyl, a 2'-Fluoro, or a 2'-O-methoxyethyl moiety. In some examples, the antisense oligomer comprises at least one modified sugar moiety. In some examples, the antisense oligomer comprises from 8 to 50 nucleobases, 8 to 40 nucleobases, 8 to 35 nucleobases, 8 to 30 nucleobases, 8 to 25 nucleobases, 8 to 20 nucleobases, 8 to 15 nucleobases, 9 to 50 nucleobases, 9 to 40 nucleobases, 9 to 35 nucleobases, 9 to 30 nucleobases, 9 to 25 nucleobases, 9 to 20 nucleobases, 9 to 15 nucleobases, 10 to 50 nucleobases, 10 to 40 nucleobases, 10 to 35 nucleobases, 10 to 30 nucleobases, 10 to 25 nucleobases, 10 to 20 nucleobases, 10 to 15 nucleobases, 11 to 50 nucleobases, 11 to 40 nucleobases, 11 to 35 nucleobases, 11 to 30 nucleobases, 11 to 25 nucleobases, 11 to 20 nucleobases, 11 to 15 nucleobases, 12 to 50 nucleobases, 12 to 40 nucleobases, 12 to 35 nucleobases, 12 to 30 nucleobases, 12 to 25 nucleobases, 12 to 20 nucleobases, or 12 to 15 nucleobases. In some examples, the antisense oligomer is at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or is 100% complementary to a targeted portion of the *SCN1A* or *SYNGAP1* RIC pre-mRNA transcript. In some examples, the targeted portion of the *SCN1A* or *SYNGAP1* RIC pre-mRNA transcript is within a sequence selected from SEQ ID NOs: 2592-2595. In some examples, the antisense oligomer comprises a nucleotide sequence that is at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any one of SEQ ID NOs: 10-2591. In some examples, the antisense oligomer comprises a nucleotide sequence selected from SEQ ID NOs: 10-2591. In some examples, the pharmaceutical composition is formulated for intrathecal injection, intracerebroventricular injection, intraperitoneal injection, intramuscular injection, subcutaneous injection, or intravenous injection.

[0030] Disclosed herein, in some examples, are methods of inducing processing of a deficient *SCN1A* or *SYNGAP1* mRNA transcript to facilitate removal of a retained intron to produce a fully

processed *SCN1A* or *SYNGAP1* mRNA transcript that encodes a functional form of a *SCN1A* or *SYNGAP1* protein, the method comprising: (a) contacting an antisense oligomer to a target cell of a subject; (b) hybridizing the antisense oligomer to the deficient *SCN1A* or *SYNGAP1* mRNA transcript, wherein the deficient *SCN1A* or *SYNGAP1* mRNA transcript is capable of encoding the functional form of a *SCN1A* or *SYNGAP1* protein and comprises at least one retained intron; (c) removing the at least one retained intron from the deficient *SCN1A* or *SYNGAP1* mRNA transcript to produce the fully processed *SCN1A* or *SYNGAP1* mRNA transcript that encodes the functional form of *SCN1A* or *SYNGAP1* protein; and (d) translating the functional form of *SCN1A* or *SYNGAP1* protein from the fully processed *SCN1A* or *SYNGAP1* mRNA transcript. In some examples, the retained intron is an entire retained intron. In some examples, the deficient *SCN1A* or *SYNGAP1* mRNA transcript is a *SCN1A* or *SYNGAP1* pre-mRNA transcript.

[0031] Disclosed herein, in some examples, are methods of treating a subject having a condition caused by a deficient amount or activity of *SCN1A* or *SYNGAP1* protein comprising administering to the subject an antisense oligomer comprising a nucleotide sequence with at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any one of SEQ ID NOs: 10-2591.

INCORPORATION BY REFERENCE

[0032] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0033] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings.

[0034] **FIG. 1** depicts a schematic representation of an exemplary retained-intron-containing (RIC) pre-mRNA transcript. In FIG. 1, the 5' splice site consensus sequence is indicated with underlined letters (letters are nucleotides; upper case: exonic portion; and lower case: intronic portion) from -3e to -1e and +1 to +6 (numbers labeled "e" are exonic and unlabeled numbers are intronic). The 3' splice site consensus sequence is indicated with underlined letters (letters are nucleotides; upper case: exonic portion and lower case: intronic portion) from -15 to -1 and +1e (numbers labeled "e" are exonic and unlabeled numbers are intronic). Intronic target regions for ASO screening comprise nucleotides +6 relative to the 5' splice site of the retained intron (arrow at left) to -16 relative to the 3' splice site of the retained intron (arrow at right). In embodiments, intronic target regions for ASO screening comprise nucleotides +6 to +496 relative to the 5' splice site of the retained intron and -16 to -499 relative to the 3' splice site of the retained intron. Exonic target regions comprise nucleotides +2e to -4e in the exon flanking the 5' splice site of the retained intron and +2e to -4e in the exon flanking the 3' splice site of the retained intron. "n"

or “N” denote any nucleotide, “y” denotes pyrimidine. The sequences shown represent consensus sequences for mammalian splice sites and individual introns and exons need not match the consensus sequences at every position.

[0035] FIGS. 2A-B depict a schematic representation of the Targeted Augmentation of Nuclear Gene Output (TANGO) approach. FIG. 2A shows a cell divided into nuclear and cytoplasmic compartments. In the nucleus, a pre-mRNA transcript of a target gene consisting of exons (rectangles) and introns (connecting lines) undergoes splicing to generate an mRNA, and this mRNA is exported to the cytoplasm and translated into target protein. For this target gene, the splicing of intron 1 is inefficient and a retained intron-containing (RIC) pre-mRNA accumulates primarily in the nucleus, and if exported to the cytoplasm, is degraded, leading to no target protein production. FIG. 2B shows an example of the same cell divided into nuclear and cytoplasmic compartments. Treatment with an antisense oligomer (ASO) promotes the splicing of intron 1 and results in an increase in mRNA, which in turn translates into higher levels of target protein.

[0036] FIG. 3 depicts intron-retention in the *SYNGAP1* gene with intron 15 shown in detail. The identification of intron-retention events in the *SYNGAP1* gene using RNA sequencing (RNAseq) is shown, visualized in the UCSC genome browser. The upper panel shows the read density corresponding to the *SYNGAP1* transcript expressed in HCN (human cortical neurons) and localized in either the cytoplasmic (top) or nuclear fraction (bottom). At the bottom of this panel, a graphic representation of the *SYNGAP1* gene is shown to scale. The read density is shown as peaks. The highest read density corresponds to exons (black boxes), while no reads are observed for the majority of the introns (lines with arrow heads) in either cellular fraction. Higher read density is detected for introns 15 and 18/19 (pointed by the arrows) in the nuclear fraction compared to the cytoplasmic fraction indicating that splicing efficiency of introns 15 and 18/19 is low, resulting in intron retention. The retained-intron containing pre-mRNA transcripts are retained in the nucleus and are not exported out to the cytoplasm. The read density for intron 15 in HCN is shown in detail in the lower panel indicating 25% intron retention as calculated by bioinformatic analysis.

[0037] FIG. 4 depicts an exemplary *SYNGAP1* gene intron 15 (IVS15) ASO walk. A graphic representation of the ASO walk performed for *SYNGAP1* IVS 15 targeting sequences immediately downstream of the 5' splice site or upstream of the 3' splice site using 2'-O-Me ASOs, PS backbone, is shown. ASOs were designed to cover these regions by shifting 5 nucleotides at a time. The *SYNGAP1* exon-intron structure is drawn to scale.

[0038] FIG. 5 depicts intron-retention in the *SYNGAP1* gene with introns 18 and 19 shown in detail. Intron retention in the *SYNGAP1* gene was identified by RNA sequencing (RNAseq), visualized in the UCSC genome browser, as described herein in the Examples. The read density for introns 18 and 19 in HCN is shown in detail in the lower panel. Introns 18 and 19 flank exon 19, an alternatively spliced exon. Even though exon 19 is not annotated, evidence of its existence is shown by the RNAseq data, such that a clear peak is observed in the cytoplasmic fraction; however the read density corresponding to exon 19 in the cytoplasmic fraction is lower than that of the constitutively spliced exons in *SYNGAP1*. The read

density for intron 18/19 in HCN is shown in detail in the lower panel indicating 42% intron retention as calculated by bioinformatic analysis.

[0039] FIG. 6 depicts an exemplary *SYNGAP1* gene intron 18 (IVS18) and intron 19 (IVS19) ASO walk. A graphic representation of the ASO walk performed for *SYNGAP1* IVS 18 and 19 targeting sequences immediately downstream of the 5' splice site of intron 18 or upstream of the 3' splice site of intron 19 using 2'-O-Me ASOs, PS backbone, is shown. The splice site intronic regions flanking alternative exon 19 are not targeted to avoid affecting the inclusion level of exon 19. ASOs were designed to cover these regions by shifting 5 nucleotides at a time. The *SYNGAP1* exon-intron structure is drawn to scale.

[0040] FIG. 7A depicts a schematic of the RefSeq Genes for *SYNGAP1* corresponding to NM_006772.

[0041] FIG. 7B depicts an exemplary graph showing the average (n=3) fold change in expression levels of *SYNGAP1* mRNA without intron 15 in ARPE-19 cells treated for 24 hrs with 80 nM of the indicated ASOs over mock treated cells. Data is normalized to RPL32 expression.

[0042] FIG. 7C depicts a schematic of the RefSeq Genes for *SYNGAP1* corresponding to NM_006772.

[0043] FIG. 7D depicts an exemplary graph showing the average (n=3) fold change in expression levels of *SYNGAP1* mRNA without intron 18 in ARPE-19 cells treated for 24 hrs with 80 nM of the indicated ASOs over mock treated cells. Data is normalized to RPL32 expression.

[0044] FIG. 7E depicts an exemplary graph showing the average (n=3) fold change in expression levels of *SYNGAP1* mRNA without intron 18 in ARPE-19 cells treated for 24 hrs with 80 nM of the indicated ASOs over mock treated cells. Data is normalized to RPL32 expression.

[0045] FIG. 8A depicts a schematic of a portion of the RefSeq Gene for *SCN1A* corresponding to NM_006920. Primers used for the RT-PCR assay in FIG. 8B and FIG. 8C are shown.

[0046] FIG. 8B depicts an agarose gel showing expression levels of *SCN1A* mRNA as measured by RT-PCR in the indicated cells (VM and CX: ReNcell Neuroprogenitor cells; SK-N-AS: Neuroblastoma cells; C: cytoplasmic fraction; N: nuclear fraction).

[0047] FIG. 8C depicts an agarose gel showing intron 21 retention levels in SK-N-AS cells (left) and VM cells (right) as measured by RT-PCR (VM and CX: ReNcell Neuroprogenitor cells; SK-N-AS: Neuroblastoma cells; C: cytoplasmic fraction; N: nuclear fraction).

[0048] FIG. 8D depicts an exemplary graph showing the average (n=2) fold change in expression levels of *SCN1A* mRNA without intron 21 in SK-N-AS cells treated for 24 hrs with 80 nM of the indicated ASOs over mock treated cells. Data is normalized to RPL32 expression.

[0049] FIG. 8E depicts an exemplary graph showing the average (n=2) fold change in expression levels of *SCN1A* mRNA without intron 21 in SK-N-AS cells treated for 24 hrs with 80 nM of the indicated ASOs over mock treated cells. Data is normalized to RPL32 expression.

[0050] FIG. 8F depicts an exemplary graph showing the average (n=2) fold change in expression levels of *SCN1A* mRNA without intron 21 in SK-N-AS cells treated for 24 hrs with 40 nM, 80 nM or 120 nM of the indicated ASOs (spanning the exon 21-exon 22 splice junction) over mock treated cells. Data is normalized to RPL32 expression.

[0051] FIG. 8G depicts an exemplary graph showing the average (n=2) fold change in expression levels of *SCN1A* mRNA without intron 21 in SK-N-AS cells treated for 24 hrs with 80 nM of the indicated ASOs over mock treated cells. Data is normalized to RPL32 expression.

[0052] FIG 8H depicts the sequences of the ASOs used in the experiments shown in FIG. 8D and FIG. 8E.

[0053] FIG 8I depicts the sequences of the ASOs used in the experiments shown in FIG. 8G.

DETAILED DESCRIPTION OF THE INVENTION

[0054] Individual introns in primary transcripts of protein-coding genes having more than one intron are spliced from the primary transcript with different efficiencies. In most cases only the fully spliced mRNA is exported through nuclear pores for subsequent translation in the cytoplasm. Unspliced and partially spliced transcripts are detectable in the nucleus. It is generally thought that nuclear accumulation of transcripts that are not fully spliced is a mechanism to prevent the accumulation of potentially deleterious mRNAs in the cytoplasm that may be translated to protein. For some genes, splicing of the least efficient intron is a rate-limiting post-transcriptional step in gene expression, prior to translation in the cytoplasm.

[0055] Substantial levels of partially-spliced transcripts encoding the *SCN1A* protein, deficient in AD mental retardation 5, and partially-spliced transcripts encoding the *SYNGAP1* protein, deficient in Dravet syndrome, have been discovered in the nucleus of human cells. These *SCN1A* and *SYNGAP1* pre-mRNA species comprise at least one retained intron. The present invention provides compositions and methods for upregulating splicing of one or more retained *SYNGAP1* or *SCN1A* introns that are rate-limiting for the nuclear stages of gene expression to increase steady-state production of fully-spliced, mature mRNA, and thus, translated *SYNGAP1* or *SCN1A* protein levels. These compositions and methods utilize antisense oligomers (ASOs) that promote constitutive splicing at intron splice sites of a retained-intron-containing *SYNGAP1* or *SCN1A* pre-mRNA (RIC pre-mRNA) that accumulates in the nucleus. Thus, in embodiments, *SYNGAP1* or *SCN1A* protein is increased using the methods of the invention to treat a condition caused by *SYNGAP1* or *SCN1A* deficiency, respectively.

[0056] In other embodiments, the methods of the invention are used to increase *SYNGAP1* or *SCN1A* production to treat a condition in a subject in need thereof. In embodiments, the subject has condition in which *SYNGAP1* or *SCN1A* is not necessarily deficient relative to wild-type, but where an increase in *SYNGAP1* or *SCN1A* mitigates the condition nonetheless. In embodiments, the condition is caused by a *SYNGAP1* or *SCN1A* haploinsufficiency.

Autosomal Dominant Mental Retardation 5

[0057] Mental retardation is the most frequently occurring severe handicap of children. The nonsyndromic form of the disorder is the most common, characterized by the absence of dysmorphic features. Monoallelic lesions are sufficient to cause the disorder that is characterized by global delay of development, hypotonia, and moderate to severe mental retardation. The genetic factors involved in nonsyndromic mental retardation remain poorly understood. Linkage and cytogenetic analyses have led to the identification of 29 X-linked and 5 autosomal recessive genes associated with nonsyndromic mental

retardation, which together account for less than 10% of cases (Hamden, *et al.*, 2009, incorporated herein by reference). Moreover, autosomal dominant genes have yet to be identified, which in turn decreases the likelihood of identifying families that are amenable to linkage analysis. However, the fact that de novo chromosomal rearrangements, usually involving a change in copy number of genomic regions, represent the most commonly recognized cause of mental retardation indicates that monoallelic lesions are sufficient to cause this disorder. This raises the possibility that smaller de novo genetic lesions, such as point mutations, also contribute to the pathogenesis of the disorder. A case study identified de novo genetic lesions in the *SYNGAP1* gene that result in the production of truncated proteins in approximately 3% of patients with unexplained nonsyndromic mental retardation. The patients identified in the study were heterozygous for the protein-truncating mutations that comprised two nonsense mutations and one deletion (Hamdan, *et al.*, 2009). The disease is described, *e.g.*, at OMIM #612621 (Online Mendelian Inheritance in Man, Johns Hopkins University, 1966-2015), incorporated by reference herein.

[0058] *SYNGAP1* is a GTPase-activating enzyme that is selectively expressed in the brain. A component of the N-methyl-D-aspartate (NMDA)-receptor complex that mediates glutamate signaling, *SYNGAP1* acts downstream of the receptor, blocking insertion of the α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor at the postsynaptic membrane by inhibiting the RAS-ERK signaling pathway. *SYNGAP1* is required for normal development; mice homozygous for null alleles of the gene die shortly after birth. By contrast, mice heterozygous for the null allele have impaired synaptic plasticity and learning, consistent with the role of *SYNGAP1* as a component of the NMDA-receptor complex (Hamdan, *et al.*, 2009).

[0059] Alternative splicing of the *SYNGAP1* gene results in the expression of three isoforms of *SYNGAP1* (Hamdan, *et al.*, 2009). *SYNGAP1* possesses a RASGAP domain that activates Ras GTPases. A PDZ-binding motif, QTRV, is present in isoform 2 that mediates interaction with other components of the NMDA-receptor complex. The RASGAP and QTRV domains regulate synaptic plasticity and dendritic-spine formation that are necessary for learning and development. Protein-truncating mutations associated with mental retardation lacked either the complete RASGAP domain or QTRV motifs, consistent with their role in the pathogenesis of mental retardation.

Dravet Syndrome

[0060] Dravet syndrome (DS), otherwise known as severe myoclonic epilepsy of infancy (SMEI), is an epileptic encephalopathy presenting in the first year of life. Dravet syndrome is an increasingly recognized epileptic encephalopathy in which the clinical diagnosis is supported by the finding of sodium channel gene mutations in approximately 70–80% of patients. Mutations of ion channel genes play a major role in the pathogenesis of a range of epilepsy syndromes, resulting in some epilepsies being regarded as channelopathies. Voltage-gated sodium channels (VGSCs) play an essential role in neuronal excitability; therefore, it is not surprising that many mutations associated with DS have been identified in the gene encoding a VGSC subunit. The disease is described by, *e.g.*, Mulley, *et al.*, 2005, and the disease description at OMIM #607208 (Online Mendelian Inheritance in Man, Johns Hopkins University, 1966-2015), both incorporated by reference herein.

[0061] Between 70% and 80% of patients carry sodium channel $\alpha 1$ subunit gene (*SCN1A*) abnormalities, and truncating mutations account for about 40%, and have a significant correlation with an earlier age of seizures onset. Sequencing mutations are found in about 70% of cases and comprise truncating (40%) and missense mutations (40%) with the remaining being splice-site changes. Most mutations are de novo, but familial mutations occur in 5–10% of cases and are usually missense in nature. The remaining *SCN1A* mutations comprise splice-site and missense mutations, most of which fall into the pore-forming region of the sodium channel. At present, over 500 mutations have been associated with DS and are randomly distributed along the gene (Mulley, *et al.*, Neurol. 2006, 67, 1094-1095).

[0062] The *SCN1A* gene is located in the cluster of sodium channel genes on human chromosome 2q24 and encodes the α -pore forming subunits known as Nav1.1 of the neuronal voltage gated sodium channel. The *SCN1A* gene spans approximately 100 kb of genomic DNA and comprises 26 exons. The *SCN1A* protein consists of four domains, each with six-transmembrane segments. Two splice variants have been identified that result in a long and short isoform that differ in the presence or absence of 11 amino acids in the cytoplasmic loop between domains 1 and 2, in exon 11 (Miller, *et al.*, 1993-2015, and Mulley, *et al.*, 2005, 25, 535-542, incorporated herein by reference).

[0063] The identification of genes associated with nonsyndromic mental retardation and Dravet syndrome that encode proteins in well-characterized synaptic pathways offers the possibility of developing pharmacologic treatments to target the disorders and reduce their associated symptoms.

Retained Intron Containing Pre-mRNA (RIC Pre-mRNA)

[0064] In embodiments, the methods of the present invention exploit the presence of retained-intron-containing pre-mRNA (RIC pre-mRNA) transcribed from the *SYNGAP1* or *SCN1A* and encoding *SYNGAP1* or *SCN1A* protein, in the cell nucleus. Splicing of the identified *SYNGAP1* or *SCN1A* RIC pre-mRNA species to produce mature, fully-spliced, *SYNGAP1* or *SCN1A* mRNA, is induced using ASOs that stimulate splicing out of the retained introns. The resulting mature *SYNGAP1* or *SCN1A* mRNA can be exported to the cytoplasm and translated, thereby increasing the amount of *SYNGAP1* or *SCN1A* protein in the patient's cells and alleviating symptoms of AD mental retardation 5 or Dravet syndrome. This method, described further below, is known as Targeted Augmentation of Nuclear Gene Output (TANGO).

SYNGAP1 Nuclear Transcripts

[0065] As described herein in the Examples, the *SYNGAP1* gene was analyzed for intron-retention events and retention of intron 15 and 18/19 was observed. RNA sequencing (RNAseq), visualized in the UCSC genome browser, showed *SYNGAP1* transcripts expressed in human cortical neurons (HCN) and localized in either the cytoplasmic or nuclear fraction. In both fractions, reads were not observed for the majority of the introns. However, higher read density was detected for introns 15 and 18/19 in the nuclear fraction compared to the cytoplasmic fraction indicating that splicing efficiency of introns 15 and 18/19 is low, resulting in intron retention. The retained-intron containing pre-mRNA transcripts are retained in the nucleus and are not exported out to the cytoplasm. The read density for introns 18/19 indicated 42% intron retention. The percent intron retention (PIR) value for introns 18/19 was obtained by averaging

four values (54, 50, 35, 29), each determined in HCN cells using one of four different algorithms. The read density for intron 15 indicated 25% intron retention. The percent intron retention (PIR) value for intron 15 was obtained by averaging four values (49, 0, 26 and 23), each determined in HCN cells using one of four different algorithms.

[0066] In some embodiments, the ASOs disclosed herein target a RIC pre-mRNA transcribed from a *SCN1A* genomic sequence. In some embodiments, the ASO targets a RIC pre-mRNA transcript from a *SCN1A* genomic sequence comprising retained intron 21. In some embodiments, the ASO targets a RIC pre-mRNA transcript from a *SCN1A* genomic sequence comprising retained intron 23. In some embodiments, the ASO targets a RIC pre-mRNA transcript of SEQ ID NO: 1. In some embodiments, the ASO targets a RIC pre-mRNA transcript of SEQ ID NO: 1 comprising a retained intron 21. In some embodiments, the ASO targets a RIC pre-mRNA transcript of SEQ ID NO: 1 comprising a retained intron 23. In some embodiments, the ASOs disclosed herein target a *SCN1A* RIC pre-mRNA sequence. In some embodiments, the ASO targets a *SCN1A* RIC pre-mRNA sequence comprising a retained intron 21. In some embodiments, the ASO targets a *SCN1A* RIC pre-mRNA sequence comprising a retained intron 23. In some embodiments, the ASO targets a *SCN1A* RIC pre-mRNA sequence according to any one of SEQ ID NOs: 4-7. In some embodiments, the ASO targets a *SCN1A* RIC pre-mRNA sequence according to any one of SEQ ID NOs: 4, 6, or 7 comprising a retained intron 21. In some embodiments, the ASO targets a *SCN1A* RIC pre-mRNA sequence according to SEQ ID NO: 5 comprising a retained intron 23. In some embodiments, the ASOs disclosed herein target SEQ ID NO: 2593. In some embodiments, the ASO has a sequence according to any one of SEQ ID NOs: 10-1037.

[0067] In some embodiments, the ASO targets exon 21 of a *SCN1A* RIC pre-mRNA comprising a retained intron 21. In some embodiments, the ASO targets an exon 21 sequence upstream (or 5') from the 5' splice site of a *SCN1A* RIC pre-mRNA comprising a retained intron 21. In some embodiments, the ASO targets an exon sequence about 4 to about 264 nucleotides upstream (or 5') from the 5' splice site of a *SCN1A* RIC pre-mRNA comprising a retained intron 21. In some embodiments, the ASO has a sequence according to any one of SEQ ID NOs: 10-62, 524-576, or 781-833.

[0068] In some embodiments, the ASO targets intron 21 in a *SCN1A* RIC pre-mRNA comprising a retained intron 21. In some embodiments, the ASO targets an intron 21 sequence downstream (or 3') from the 5' splice site of a *SCN1A* RIC pre-mRNA comprising a retained intron 21. In some embodiments, the ASO targets an intron 21 sequence about 6 to about 496 nucleotides downstream (or 3') from the 5' splice site of a *SCN1A* RIC pre-mRNA comprising a retained intron 21. In some embodiments, the ASO has a sequence according to any one of SEQ ID NOs: 63-161, 577-675, or 834-932.

[0069] In some embodiments, the ASO targets an intron 21 sequence upstream (or 5') from the 3' splice site of a *SCN1A* RIC pre-mRNA comprising a retained intron 21. In some embodiments, the ASO targets an intron 21 sequence about 16 to about 496 nucleotides upstream (or 5') from the 3' splice site of a *SCN1A* RIC pre-mRNA comprising a retained intron 21. In some embodiments, the ASO has a sequence according to any one of SEQ ID NOs: 162-258, 676-772, or 933-1029.

[0070] In some embodiments, the ASO targets exon 22 in a *SCN1A* RIC pre-mRNA comprising a retained intron 21. In some embodiments, the ASO targets an exon 22 sequence downstream (or 3') from the 3' splice site of a *SCN1A* RIC pre-mRNA comprising a retained intron 21. In some embodiments, the ASO targets an exon 22 sequence about 2 to about 37 nucleotides downstream (or 3') from the 3' splice site of a *SCN1A* RIC pre-mRNA comprising a retained intron 21. In some embodiments, the ASO has a sequence according to any one of SEQ ID NOs: 259-266, 773-780, or 1030-1037.

[0071] In some embodiments, the ASO targets exon 23 of a *SCN1A* RIC pre-mRNA comprising a retained intron 23. In some embodiments, the ASO targets an exon 23 sequence upstream (or 5') from the 5' splice site of a *SCN1A* RIC pre-mRNA comprising a retained intron 23. In some embodiments, the ASO targets an exon sequence about 4 to about 264 nucleotides upstream (or 5') from the 5' splice site of a *SCN1A* RIC pre-mRNA comprising a retained intron 23. In some embodiments, the ASO has a sequence according to any one of SEQ ID NOs: 267-319.

[0072] In some embodiments, the ASO targets intron 23 in a *SCN1A* RIC pre-mRNA comprising a retained intron 23. In some embodiments, the ASO targets an intron 23 sequence downstream (or 3') from the 5' splice site of a *SCN1A* RIC pre-mRNA comprising a retained intron 23. In some embodiments, the ASO targets an intron 23 sequence about 6 to about 496 nucleotides downstream (or 3') from the 5' splice site of a *SCN1A* RIC pre-mRNA comprising a retained intron 23. In some embodiments, the ASO has a sequence according to any one of SEQ ID NOs: 320-419.

[0073] In some embodiments, the ASO targets an intron 23 sequence upstream (or 5') from the 3' splice site of a *SCN1A* RIC pre-mRNA comprising a retained intron 23. In some embodiments, the ASO targets an intron 23 sequence about 16 to about 496 nucleotides upstream (or 5') from the 3' splice site of a *SCN1A* RIC pre-mRNA comprising a retained intron 23. In some embodiments, the ASO has a sequence according to any one of SEQ ID NOs: 420-515.

[0074] In some embodiments, the ASO targets exon 24 in a *SCN1A* RIC pre-mRNA comprising a retained intron 23. In some embodiments, the ASO targets an exon 24 sequence downstream (or 3') from the 3' splice site of a *SCN1A* RIC pre-mRNA comprising a retained intron 23. In some embodiments, the ASO targets an exon 24 sequence about 2 to about 37 nucleotides downstream (or 3') from the 3' splice site of a *SCN1A* RIC pre-mRNA comprising a retained intron 23. In some embodiments, the ASO has a sequence according to any one of SEQ ID NOs: 516-523.

[0075] In some embodiments, the ASOs disclosed herein target a RIC pre-mRNA transcribed from a *SYNGAP1* genomic sequence. In some embodiments, the ASO targets a RIC pre-mRNA transcript from a *SYNGAP1* genomic sequence comprising retained intron 18. In some embodiments, the ASO targets a RIC pre-mRNA transcript from a *SYNGAP1* genomic sequence comprising retained intron 19. In some embodiments, the ASO targets a RIC pre-mRNA transcript from a *SYNGAP1* genomic sequence comprising retained intron 15. In some embodiments, the ASO targets a RIC pre-mRNA transcript of SEQ ID NO: 2. In some embodiments, the ASO targets a RIC pre-mRNA transcript of SEQ ID NO: 3. In some embodiments, the ASO targets a RIC pre-mRNA transcript of SEQ ID NO: 2 comprising a retained intron 18. In some embodiments, the ASO targets a RIC pre-mRNA transcript of SEQ ID NO: 2

comprising a retained intron 19. In some embodiments, the ASO targets a RIC pre-mRNA transcript of SEQ ID NO: 2 comprising a retained intron 15. In some embodiments, the ASO targets a RIC pre-mRNA transcript of SEQ ID NO: 3 comprising a retained intron 18. In some embodiments, the ASO targets a RIC pre-mRNA transcript of SEQ ID NO: 3 comprising a retained intron 19. In some embodiments, the ASO targets a RIC pre-mRNA transcript of SEQ ID NO: 3 comprising a retained intron 15. In some embodiments, the ASOs disclosed herein target a *SYNGAP1* RIC pre-mRNA sequence. In some embodiments, the ASO targets a *SYNGAP1* RIC pre-mRNA sequence comprising a retained intron 18. In some embodiments, the ASO targets a *SYNGAP1* RIC pre-mRNA sequence comprising a retained intron 19. In some embodiments, the ASO targets a *SYNGAP1* RIC pre-mRNA sequence comprising a retained intron 15. In some embodiments, the ASO targets a *SYNGAP1* RIC pre-mRNA sequence according to any one of SEQ ID NOs: 8-9. In some embodiments, the ASO targets a *SYNGAP1* RIC pre-mRNA sequence according to any one of SEQ ID NOs: 8-9 comprising a retained intron 18. In some embodiments, the ASO targets a *SYNGAP1* RIC pre-mRNA sequence according to any one of SEQ ID NOs: 8-9 comprising a retained intron 19. In some embodiments, the ASO targets a *SYNGAP1* RIC pre-mRNA sequence according to any one of SEQ ID NOs: 8-9 comprising a retained intron 15. In some embodiments, the ASOs disclosed herein target any one of SEQ ID NOs: 2592, 2594, or 2595. In some embodiments, the ASO has a sequence according to any one of SEQ ID NOs: 1038-2591.

[0076] In some embodiments, the ASO targets an exon sequence of a *SYNGAP1* RIC pre-mRNA comprising a retained intron 18 or intron 19. In some embodiments, the ASO targets an exon sequence upstream (or 5') from the 5' splice site of a *SYNGAP1* RIC pre-mRNA comprising a retained intron 18 or intron 19. In some embodiments, the ASO targets an exon sequence about 4 to about 73 nucleotides upstream (or 5') from the 5' splice site of a *SYNGAP1* RIC pre-mRNA comprising a retained intron 18 or intron 19. In some embodiments, the ASO has a sequence according to any one of SEQ ID NOs: 1038-1050 or 1815-1827.

[0077] In some embodiments, the ASO targets intron 18 or intron 19 in a *SYNGAP1* RIC pre-mRNA comprising a retained intron 18 or intron 19. In some embodiments, the ASO targets a sequence downstream (or 3') from the 5' splice site of a *SYNGAP1* RIC pre-mRNA comprising a retained intron 18 or intron 19. In some embodiments, the ASO targets a sequence about 6 to about 499 nucleotides downstream (or 3') from the 5' splice site of a *SYNGAP1* RIC pre-mRNA comprising a retained intron 18 or intron 19. In some embodiments, the ASO has a sequence according to any one of SEQ ID NOs: 1051-1136 or 1828-1913.

[0078] In some embodiments, the ASO targets an intron 18 or intron 19 sequence upstream (or 5') from the 3' splice site of a *SYNGAP1* RIC pre-mRNA comprising a retained intron 18 or intron 19. In some embodiments, the ASO targets a sequence about 16 to about 496 nucleotides upstream (or 5') from the 3' splice site of a *SYNGAP1* RIC pre-mRNA comprising a retained intron 18 or intron 19. In some embodiments, the ASO has a sequence according to any one of SEQ ID NOs: 1137-1228 or 1914-2005.

[0079] In some embodiments, the ASO targets an exon sequence in a *SYNGAP1* RIC pre-mRNA comprising a retained intron 18 or intron 19. In some embodiments, the ASO targets an exon sequence

downstream (or 3') from the 3' splice site of a *SYNGAP1* RIC pre-mRNA comprising a retained intron 18 or intron 19. In some embodiments, the ASO targets an exon sequence about 17 to about 1,912 nucleotides downstream (or 3') from the 3' splice site of a *SYNGAP1* RIC pre-mRNA comprising a retained intron 18 or intron 19. In some embodiments, the ASO has a sequence according to any one of SEQ ID NOs: 1229-1509 or 2006-2286.

[0080] In some embodiments, the ASO targets exon 15 of a *SYNGAP1* RIC pre-mRNA comprising a retained intron 15. In some embodiments, the ASO targets an exon 15 sequence upstream (or 5') from the 5' splice site of a *SYNGAP1* RIC pre-mRNA comprising a retained intron 15. In some embodiments, the ASO targets an exon sequence about 4 to about 1,054 nucleotides upstream (or 5') from the 5' splice site of a *SYNGAP1* RIC pre-mRNA comprising a retained intron 15. In some embodiments, the ASO has a sequence according to any one of SEQ ID NOs: 1510-1686 or 2287-2463.

[0081] In some embodiments, the ASO targets intron 15 in a *SYNGAP1* RIC pre-mRNA comprising a retained intron 15. In some embodiments, the ASO targets an intron 15 sequence downstream (or 3') from the 5' splice site of a *SYNGAP1* RIC pre-mRNA comprising a retained intron 15. In some embodiments, the ASO targets an intron 15 sequence about 6 to about 251 nucleotides downstream (or 3') from the 5' splice site of a *SYNGAP1* RIC pre-mRNA comprising a retained intron 15. In some embodiments, the ASO has a sequence according to any one of SEQ ID NOs: 1687-1736 or 2464-2513.

[0082] In some embodiments, the ASO targets an intron 15 sequence upstream (or 5') from the 3' splice site of a *SYNGAP1* RIC pre-mRNA comprising a retained intron 15. In some embodiments, the ASO targets an intron 15 sequence about 16 to about 256 nucleotides upstream (or 5') from the 3' splice site of a *SYNGAP1* RIC pre-mRNA comprising a retained intron 15. In some embodiments, the ASO has a sequence according to any one of SEQ ID NOs: 1737-1785 or 2514-2562.

[0083] In some embodiments, the ASO targets exon 16 in a *SYNGAP1* RIC pre-mRNA comprising a retained intron 15. In some embodiments, the ASO targets an exon 16 sequence downstream (or 3') from the 3' splice site of a *SYNGAP1* RIC pre-mRNA comprising a retained intron 15. In some embodiments, the ASO targets an exon 16 sequence about 2 to about 157 nucleotides downstream (or 3') from the 3' splice site of a *SYNGAP1* RIC pre-mRNA comprising a retained intron 15. In some embodiments, the ASO has a sequence according to any one of SEQ ID NOs: 1786-1814 or 2563-2591.

[0084] In embodiments, the targeted portion of the *SYNGAP1* RIC pre-mRNA is in intron 15, 18, or 19. The *SYNGAP1* intron numbering used herein corresponds to the mRNA sequence at NM_006772.2. In embodiments, hybridization of an ASO to the targeted portion of the RIC pre-mRNA results in enhanced splicing at the splice site (5' splice site or 3' splice site) of at least one of retained introns 15, 18, or 19, and subsequently increases SYNGAP1 protein production. It is understood that the intron numbering may change in reference to a different *SYNGAP1* isoform sequence. One of skill in the art can determine the corresponding intron number in any isoform based on an intron sequence provided herein or using the number provided in reference to the mRNA sequence at NM_006772.2. One of skill in the art also can determine the sequences of flanking exons in any *SYNGAP1* isoform for targeting using the methods of

the invention, based on an intron sequence provided herein or using the intron number provided in reference to the mRNA sequence at NM_006772.2.

[0085] In embodiments, the targeted portion of the *SCN1A* RIC pre-mRNA is in intron 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 (intron numbering corresponding to the mRNA sequence at NM_001202435.1). In embodiments, hybridization of an ASO to the targeted portion of the RIC pre-mRNA results in enhanced splicing at the splice site (5' splice site or 3' splice site) of at least one of retained introns 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25, and subsequently increases SCN1A protein production. In embodiments, the targeted portion of the *SCN1A* RIC pre-mRNA is in intron 21 or 23. One of skill in the art can determine the corresponding intron number in any isoform based on an intron sequence provided herein or using the number provided in reference to the mRNA sequence at NM_006920, NM_001202435, NM_001165964, or NM_001165963. One of skill in the art also can determine the sequences of flanking exons in any *SCN1A* isoform for targeting using the methods of the invention, based on an intron sequence provided herein or using the intron number provided in reference to the mRNA sequence at NM_006920, NM_001202435, NM_001165964, or NM_001165963.

[0086] The degree of intron retention can be expressed as percent intron retention (PIR), the percentage of transcripts in which a given intron is retained. In brief, PIR can be calculated as the percentage of the average number of reads mapping to the exon–intron junctions, over the sum of the average of the exon–intron junction reads plus the exon–exon junction reads.

[0087] PIR values for *SCN1A* have been reported, *e.g.*, by Braunschweig, *et al.*, 2014, (see, *e.g.*, Supplemental Table S9), incorporated by reference herein in its entirety. In embodiments, the methods and compositions of the invention are used to increase the expression of *SCN1A* by inducing constitutive splicing of a retained intron of an *SCN1A* RIC-pre-mRNA. In embodiments, the retained intron is any of introns 1-25. In embodiments, the retained intron is any of introns 2, 4, 6, 13, 14, 15, 16, 17, 18, 20, 21, 22, 23, 24, and 25. In embodiments, the retained intron is any of introns 15, 18, and 19. In embodiments, the retained intron can be any *SCN1A* intron. In embodiments, the retained intron is intron 21. In embodiments, the retained intron is intron 23. The *SCN1A* intron numbering used herein corresponds to the mRNA sequence at NM_001202435.1. It is understood that the intron numbering may change in reference to a different *SCN1A* isoform sequence.

SYNGAP1 or SCN1A Protein Expression

[0088] As described above, SYNGAP1 mutations in AD nonsyndromic mental retardation are spread across the entire protein, and there is a high de novo mutation rate. Linkage and cytogenetic analysis have identified 29 X-linked and 5 autosomal recessive genes associated with AD nonsyndromic mental retardation, which account for less than 10% of cases. This suggests that smaller de novo genetic lesions, such as point mutations, may contribute to disease pathogenesis.

[0089] Also described above, SCN1A mutations in Dravet Syndrome are spread across the entire protein. More than 100 novel mutations have been identified throughout the gene with the more debilitating arising de novo. These comprise of truncations (47%), missense (43%), deletions (3%), and splice site

mutations (7%). The percentage of subjects carrying SCN1A mutations varies between 33 and 100%. The majority of mutations are novel changes (88%).

[0090] In embodiments, the methods described herein are used to increase the production of a functional SYNGAP1 or SCN1A protein. As used herein, the term “functional” refers to the amount of activity or function of a SYNGAP1 or SCN1A protein that is necessary to eliminate any one or more symptoms of a treated condition, *e.g.*, AD mental retardation 5 or Dravet syndrome. In embodiments, the methods are used to increase the production of a partially functional SYNGAP1 or SCN1A protein. As used herein, the term “partially functional” refers to any amount of activity or function of the SYNGAP1 or SCN1A protein that is less than the amount of activity or function that is necessary to eliminate or prevent any one or more symptoms of a disease or condition. In some embodiments, a partially functional protein or RNA will have at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% less activity relative to the fully functional protein or RNA.

[0091] In embodiments, the method is a method of increasing the expression of the SYNGAP1 or SCN1A protein by cells of a subject having a RIC pre-mRNA encoding the SYNGAP1 or SCN1A protein, wherein the subject has AD mental retardation 5 or Dravet syndrome caused by a deficient amount of activity of SYNGAP1 or SCN1A protein, and wherein the deficient amount of the SYNGAP1 or SCN1A protein is caused by haploinsufficiency of the SYNGAP1 or SCN1A protein. In such an embodiment, the subject has a first allele encoding a functional SYNGAP1 or SCN1A protein, and a second allele from which the SYNGAP1 or SCN1A protein is not produced. In another such embodiment, the subject has a first allele encoding a functional SYNGAP1 or SCN1A protein, and a second allele encoding a nonfunctional SYNGAP1 or SCN1A protein. In another such embodiment, the subject has a first allele encoding a functional SYNGAP1 or SCN1A protein, and a second allele encoding a partially functional SYNGAP1 or SCN1A protein. In any of these embodiments, the antisense oligomer binds to a targeted portion of the RIC pre-mRNA transcribed from the first allele (encoding functional SYNGAP1 or SCN1A protein), thereby inducing constitutive splicing of the retained intron from the RIC pre-mRNA, and causing an increase in the level of mature mRNA encoding functional SYNGAP1 or SCN1A protein, and an increase in the expression of the SYNGAP1 or SCN1A protein in the cells of the subject.

[0092] In embodiments, the subject has a first allele encoding a functional SYNGAP1 or SCN1A protein, and a second allele encoding a partially functional SYNGAP1 or SCN1A protein, and the antisense oligomer binds to a targeted portion of the RIC pre-mRNA transcribed from the first allele (encoding functional SYNGAP1 or SCN1A protein) or a targeted portion of the RIC pre-mRNA transcribed from the second allele (encoding partially functional SYNGAP1 or SCN1A protein), thereby inducing constitutive splicing of the retained intron from the RIC pre-mRNA, and causing an increase in the level of mature mRNA encoding the SYNGAP1 or SCN1A protein, and an increase in the expression of functional or partially functional SYNGAP1 or SCN1A protein in the cells of the subject.

[0093] In related embodiments, the method is a method of using an ASO to increase the expression of a protein or functional RNA. In embodiments, an ASO is used to increase the expression of SYNGAP1 or SCN1A protein in cells of a subject having a RIC pre-mRNA encoding SYNGAP1 or SCN1A protein, wherein the subject has a deficiency, *e.g.*, AD mental retardation 5 or Dravet syndrome, in the amount or function of a SYNGAP1 or SCN1A protein.

[0094] In embodiments, the RIC pre-mRNA transcript that encodes the protein that is causative of the disease or condition is targeted by the ASOs described herein. In some embodiments, a RIC pre-mRNA transcript that encodes a protein that is not causative of the disease is targeted by the ASOs. For example, a disease that is the result of a mutation or deficiency of a first protein in a particular pathway may be ameliorated by targeting a RIC pre-mRNA that encodes a second protein, thereby increasing production of the second protein. In some embodiments, the function of the second protein is able to compensate for the mutation or deficiency of the first protein (which is causative of the disease or condition).

[0095] In embodiments, the subject has:

(a) a first mutant allele from which

- (i) the SYNGAP1 or SCN1A protein is produced at a reduced level compared to production from a wild-type allele,
- (ii) the SYNGAP1 or SCN1A protein is produced in a form having reduced function compared to an equivalent wild-type protein, or
- (iii) the SYNGAP1 or SCN1A protein or functional RNA is not produced; and

(b) a second mutant allele from which

- (i) the SYNGAP1 or SCN1A protein is produced at a reduced level compared to production from a wild-type allele,
- (ii) the SYNGAP1 or SCN1A protein is produced in a form having reduced function compared to an equivalent wild-type protein, or
- (iii) the SYNGAP1 or SCN1A protein is not produced, and

wherein the RIC pre-mRNA is transcribed from the first allele and/or the second allele. In these embodiments, the ASO binds to a targeted portion of the RIC pre-mRNA transcribed from the first allele or the second allele, thereby inducing constitutive splicing of the retained intron from the RIC pre-mRNA, and causing an increase in the level of mRNA encoding SYNGAP1 or SCN1A protein and an increase in the expression of the target protein or functional RNA in the cells of the subject. In these embodiments, the target protein or functional RNA having an increase in expression level resulting from the constitutive splicing of the retained intron from the RIC pre-mRNA is either in a form having reduced function compared to the equivalent wild-type protein (partially-functional), or having full function compared to the equivalent wild-type protein (fully-functional).

[0096] In embodiments, the level of mRNA encoding SYNGAP1 or SCN1A protein is increased 1.1 to 10-fold, when compared to the amount of mRNA encoding SYNGAP1 or SCN1A protein that is produced in a control cell, *e.g.*, one that is not treated with the antisense oligomer or one that is treated with an antisense oligomer that does not bind to the targeted portion of the *SYNGAP1* or *SCN1A* RIC pre-mRNA.

[0097] In embodiments, the condition caused by a deficient amount or activity of SYNGAP1 or SCN1A protein is not a condition caused by alternative or aberrant splicing of the retained intron to which the ASO is targeted. In embodiments, the condition caused by a deficient amount or activity of the SYNGAP1 or SCN1A protein is not a condition caused by alternative or aberrant splicing of any retained intron in a RIC pre-mRNA encoding the SYNGAP1 or SCN1A protein. In embodiments, alternative or aberrant splicing may occur in a pre-mRNA transcribed from the *SYNGAP1* or *SCN1A* gene, however the compositions and methods of the invention do not prevent or correct this alternative or aberrant splicing.

[0098] In embodiments, a subject treated using the methods of the invention expresses a partially functional SYNGAP1 or SCN1A protein from one allele, wherein the partially functional SYNGAP1 or SCN1A protein is caused by a frameshift mutation, a nonsense mutation, a missense mutation, or a partial gene deletion. In embodiments, a subject treated using the methods of the invention expresses a nonfunctional SYNGAP1 or SCN1A protein from one allele, wherein the nonfunctional SYNGAP1 or SCN1A protein is caused by a frameshift mutation, a nonsense mutation, a missense mutation, a partial gene deletion, in one allele. In embodiments, a subject treated using the methods of the invention has a *SYNGAP1* or *SCN1A* whole gene deletion, in one allele.

[0099] In embodiments, a subject has a SYNGAP1 or SCN1A missense mutation. A subject can have a SYNGAP1 and a SCN1A mutation. In some cases, a SYNGAP1 mutation can be a truncating mutation. A SYNGAP1 truncating mutation can be a K138X, R579X, or L813RfsX22 mutation. A K138X mutation can result in a truncated SYNGAP1 protein that may lack a RASGAP domain. Said K138X mutation can encode a c.412A→T mutation. In some cases, a R579X mutation may truncate SYNGAP1 in a region following a RASGAP domain. In embodiments, a c.2438delT may also truncate SYNGAP1 in a region following a RASGAP domain. In some cases, a L813RfsX22 mutation may encode a c.2438delT sequence of mutation. Said mutations can occur upstream of the 3' terminus of a SYNGAP1 gene that can be alternatively spliced. In embodiments, mutations in said 3' terminus may alter the encoding of a C-terminal portion of SYNGAP1 protein.

[00100] The present invention also discloses a splicing process that can produce at least three isoforms of said SYNGAP1. Isoforms of the present invention may also be mutated. In some cases, a SYNGAP1 isoform can consist of at least 1343 amino acids. An isoform may comprise functional domains. A first SYNGAP1 isoform can contain functional domains that are mutated. A SYNGAP1 isoform can have a pleckstrin homology domain, a C2 domain, RASGAP, SH3, CC domain, and a CamKII binding domain. A K138X mutation can be upstream of a pleckstrin homology domain. A K138X mutation can be within a pleckstrin homology domain. A K138X mutation can be downstream of a pleckstrin homology domain. A R579X mutation can be in a region of a RASGAP domain. A R579X mutation can also be adjacent to a RASGAP domain. Similarly, a L813RfsX22 mutation can be adjacent to a SH3 SYNGAP1 domain. A L813RfsX22 mutation can also be within a SH3 SYNGAP1 domain.

[00101] In embodiments, a mutation can also be present on a second or third isoform of said SYNGAP1.

[00102] In embodiments, a subject can have a mutation that is not determined. A mutation that is not determined may be a SYNGAP1 mutation or a SCN1A mutation. A mutation that is not determined may

be a SYNGAP1 mutation. In some cases, a SYNGAP1 mutation can be I1115T. Said, I1115T mutation may code a c.3344T→C mutation. In certain embodiments, mutations in SYNGAP1 may be associated with diseases that are not nonsyndromic mental retardation. Mutations in SYNGAP1 can be associated with autism spectrum disorders and schizophrenia. In some cases, mutations that are associated with SYNGAP1 can be a polymorphism.

[00103] In embodiments of the present invention, a subject can have a mutation in *SCN1A*. Mutations in *SCN1A* can be spread throughout said gene. SCN1A protein can consist of four domains. Said SCN1A domains can have transmembrane segments. Mutations in said SCN1A protein may arise throughout said protein. Said SCN1A protein may consist of at least two isoforms. Mutations in SCN1A may comprise of R931C, R946C, M934I, R1648C, or R1648H. In some cases, mutations may be observed in a C-terminus of a SCN1A protein. Mutations in a SCN1A protein may also be found in loops between segments 5 and 6 of the first three domains of said SCN1A protein. In some cases, mutations may be observed in an N-terminus of a SCN1A protein. Mutations within SCN1A can be R222X, R712X, I227S, R1892X, W952X, R1245X, R1407X, W1434R, c.4338+1G>A, S1516X, L1670fsX1678, or K1846fsX1856. Mutations that can be targeted with the present invention may also encode a pore of an ion channel.

[00104] In embodiments, the present invention can be used to treat Dravet syndrome. In other embodiments, the present invention can be used to treat severe myoclonic epilepsy of infancy (SMEI). In other embodiments, the present invention can treat borderline Dravet syndrome. The present invention can also treat borderline SMEI. Additionally, the present invention can treat generalized epilepsy with febrile seizures plus (GEFS+). GEFS+ may be associated with mutations in epilepsy-associated ion channel subunits such as SCN1B or GABRG2. The present invention can also treat sodium channelopathies. Sodium channelopathies may be associated with mutations in SCN1A. Sodium channelopathies may also be associated with subunits of said SCN1A, such as the beta subunit, SCN1B. In some cases, additional diseases associated with SCN1A mutations may also be treated with the present invention. Related SCN1A diseases associated with SCN1A mutations can be atypical myotonia congenita, hyperkalemic periodic paralysis, or paramyotonia congenita.

[00105] In embodiments, a subject having any SYNGAP1 or SCN1A mutation known in the art and described in the literature referenced above (*e.g.*, by Hamdan, *et al.*, 2009, Mulley, *et al.*, 2005) is treated using the methods and compositions of the present invention. In embodiments, the mutation can be within any *SYNGAP1* or *SCN1A* intron.

Use of TANGO for Increasing SYNGAP1 or SCN1A Protein Expression

[00106] As described above, in embodiments, Targeted Augmentation of Nuclear Gene Output (TANGO) is used in the methods of the invention to increase expression of a SYNGAP1 or SCN1A protein. In these embodiments, a retained-intron-containing pre-mRNA (RIC pre-mRNA) encoding SYNGAP1 or SCN1A protein is present in the nucleus of a cell. Cells having a *SYNGAP1* or *SCN1A* RIC pre-mRNA that comprises a retained intron, an exon flanking the 5' splice site, and an exon flanking the 3' splice site, encoding the SYNGAP1 or SCN1A protein, are contacted with antisense oligomers (ASOs) that are complementary to a targeted portion of the RIC pre-mRNA. Hybridization of the ASOs to the

targeted portion of the RIC pre-mRNA results in enhanced splicing at the splice site (5' splice site or 3' splice site) of the retained intron and subsequently increases target protein production.

[00107] The terms “pre-mRNA,” and “pre-mRNA transcript” may be used interchangeably and refer to any pre-mRNA species that contains at least one intron. In embodiments, pre-mRNA or pre-mRNA transcripts comprise a 5'-7-methylguanosine cap and/or a poly-A tail. In embodiments, pre-mRNA or pre-mRNA transcripts comprise both a 5'-7-methylguanosine cap and a poly-A tail. In some embodiments, the pre-mRNA transcript does not comprise a 5'-7-methylguanosine cap and/or a poly-A tail. A pre-mRNA transcript is a non-productive messenger RNA (mRNA) molecule if it is not translated into a protein (or transported into the cytoplasm from the nucleus).

[00108] As used herein, a “retained-intron-containing pre-mRNA” (“RIC pre-mRNA”) is a pre-mRNA transcript that contains at least one retained intron. The RIC pre-mRNA contains a retained intron, an exon flanking the 5' splice site of the retained intron, an exon flanking the 3' splice site of the retained intron, and encodes the target protein. An “RIC pre-mRNA encoding a target protein” is understood to encode the target protein when fully spliced. A “retained intron” is any intron that is present in a pre-mRNA transcript when one or more other introns, such as an adjacent intron, encoded by the same gene have been spliced out of the same pre-mRNA transcript. In some embodiments, the retained intron is the most abundant intron in RIC pre-mRNA encoding the target protein. In embodiments, the retained intron is the most abundant intron in a population of RIC pre-mRNAs transcribed from the gene encoding the target protein in a cell, wherein the population of RIC pre-mRNAs comprises two or more retained introns. In embodiments, an antisense oligomer targeted to the most abundant intron in the population of RIC pre-mRNAs encoding the target protein induces splicing out of two or more retained introns in the population, including the retained intron to which the antisense oligomer is targeted or binds. In embodiments, a mature mRNA encoding the target protein is thereby produced. The terms “mature mRNA,” and “fully-spliced mRNA,” are used interchangeably herein to describe a fully processed mRNA encoding a target protein (*e.g.*, mRNA that is exported from the nucleus into the cytoplasm and translated into target protein) or a fully processed functional RNA. The term “productive mRNA,” also can be used to describe a fully processed mRNA encoding a target protein. In embodiments, the targeted region is in a retained intron that is the most abundant intron in a RIC pre-mRNA encoding the SYNGAP1 or SCN1A protein. In embodiments, the most retained intron in a RIC pre-mRNA encoding the SYNGAP1 protein is intron 18/19. In embodiments, the most retained intron in a RIC pre-mRNA encoding the SYNGAP1 protein is intron 15.

[00109] In embodiments, a retained intron is an intron that is identified as a retained intron based on a determination of at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, or at least about 50%, retention. In embodiments, a retained intron is an intron that is identified as a retained intron based on a determination of about 5% to about 100%, about 5% to about 95%, about 5% to about 90%, about 5% to about 85%, about 5% to about 80%, about 5% to about 75%, about 5% to about 70%, about 5% to about 65%, about 5% to about 60%, about 5% to about 55%, about 5% to about 50%, about 5% to about

45%, about 5% to about 40%, about 5% to about 35%, about 5% to about 30%, about 5% to about 25%, about 5% to about 20%, about 5% to about 15%, about 10% to about 100%, about 10% to about 95%, about 10% to about 90%, about 10% to about 85%, about 10% to about 80%, about 10% to about 75%, about 10% to about 70%, about 10% to about 65%, about 10% to about 60%, about 10% to about 55%, about 10% to about 50%, about 10% to about 45%, about 10% to about 40%, about 10% to about 35%, about 10% to about 30%, about 10% to about 25%, about 10% to about 20%, about 15% to about 100%, about 15% to about 95%, about 15% to about 90%, about 15% to about 85%, about 15% to about 80%, about 15% to about 75%, about 15% to about 70%, about 15% to about 65%, about 15% to about 60%, about 15% to about 55%, about 15% to about 50%, about 15% to about 45%, about 15% to about 40%, about 15% to about 35%, about 15% to about 30%, about 15% to about 25%, about 20% to about 100%, about 20% to about 95%, about 20% to about 90%, about 20% to about 85%, about 20% to about 80%, about 20% to about 75%, about 20% to about 70%, about 20% to about 65%, about 20% to about 60%, about 20% to about 55%, about 20% to about 50%, about 20% to about 45%, about 20% to about 40%, about 20% to about 35%, about 20% to about 30%, about 25% to about 100%, about 25% to about 95%, about 25% to about 90%, about 25% to about 85%, about 25% to about 80%, about 25% to about 75%, about 25% to about 70%, about 25% to about 65%, about 25% to about 60%, about 25% to about 55%, about 25% to about 50%, about 25% to about 45%, about 25% to about 40%, or about 25% to about 35%, retention. ENCODE data (described by, *e.g.*, Tilgner, *et al.*, 2012, “Deep sequencing of subcellular RNA fractions shows splicing to be predominantly co-transcriptional in the human genome but inefficient for lncRNAs,” *Genome Research* 22(9):1616-25) can be used to aid in identifying retained introns.

[00110] As used herein, the term “comprise” or variations thereof such as “comprises” or “comprising” are to be read to indicate the inclusion of any recited feature (*e.g.*, in the case of an antisense oligomer, a defined nucleobase sequence) but not the exclusion of any other features. Thus, as used herein, the term “comprising” is inclusive and does not exclude additional, unrecited features (*e.g.*, in the case of an antisense oligomer, the presence of additional, unrecited nucleobases).

[00111] In embodiments of any of the compositions and methods provided herein, “comprising” may be replaced with “consisting essentially of” or “consisting of.” The phrase “consisting essentially of” is used herein to require the specified feature(s) (*e.g.*, nucleobase sequence) as well as those which do not materially affect the character or function of the claimed invention. As used herein, the term “consisting” is used to indicate the presence of the recited feature (*e.g.*, nucleobase sequence) alone (so that in the case of an antisense oligomer consisting of a specified nucleobase sequence, the presence of additional, unrecited nucleobases is excluded).

[00112] In embodiments, the targeted region is in a retained intron that is the second most abundant intron in a RIC pre-mRNA encoding the SYNGAP1 or SCN1A protein. For example, the second most abundant retained intron may be targeted rather than the most abundant retained intron due to the uniqueness of the nucleotide sequence of the second most abundant retained intron, ease of ASO design to target a particular nucleotide sequence, and/or amount of increase in protein production resulting from targeting the intron with an ASO. In embodiments, the retained intron is the second most abundant intron

in a population of RIC pre-mRNAs transcribed from the gene encoding the target protein in a cell, wherein the population of RIC pre-mRNAs comprises two or more retained introns. In embodiments, an antisense oligomer targeted to the second most abundant intron in the population of RIC pre-mRNAs encoding the target protein induces splicing out of two or more retained introns in the population, including the retained intron to which the antisense oligomer is targeted or binds. In embodiments, fully-spliced (mature) RNA encoding the target protein is thereby produced. In embodiments, the second-most retained intron in a RIC pre-mRNA encoding the SYNGAP1 protein is intron 15. In embodiments, the second-most retained intron in a RIC pre-mRNA encoding the SYNGAP1 protein is intron 18/19.

[00113] In embodiments, an ASO is complementary to a targeted region that is within a non-retained intron in a RIC pre-mRNA. In embodiments, the targeted portion of the RIC pre-mRNA is within: the region +6 to +100 relative to the 5' splice site of the non-retained intron; or the region -16 to -100 relative to the 3' splice site of the non-retained intron. In embodiments, the targeted portion of the RIC pre-mRNA is within the region +100 relative to the 5' splice site of the non-retained intron to -100 relative to the 3' splice site of the non-retained intron. As used to identify the location of a region or sequence, "within" is understood to include the residues at the positions recited. For example, a region +6 to +100 includes the residues at positions +6 and +100. In embodiments, fully-spliced (mature) RNA encoding the target protein is thereby produced.

[00114] In embodiments, the retained intron of the RIC pre-mRNA is an inefficiently spliced intron. As used herein, "inefficiently spliced" may refer to a relatively low frequency of splicing at a splice site adjacent to the retained intron (5' splice site or 3' splice site) as compared to the frequency of splicing at another splice site in the RIC pre-mRNA. The term "inefficiently spliced" may also refer to the relative rate or kinetics of splicing at a splice site, in which an "inefficiently spliced" intron may be spliced or removed at a slower rate as compared to another intron in a RIC pre-mRNA.

[00115] In embodiments, the 9-nucleotide sequence at -3e to -1e of the exon flanking the 5' splice site and +1 to +6 of the retained intron is identical to the corresponding wild-type sequence. In embodiments, the 16 nucleotide sequence at -15 to -1 of the retained intron and +1e of the exon flanking the 3' splice site is identical to the corresponding wild-type sequence. As used herein, the "wild-type sequence" refers to the nucleotide sequence for *SYNGAP1* or *SCN1A* in the published reference genome deposited in the NCBI repository of biological and scientific information (operated by National Center for Biotechnology Information, National Library of Medicine, 8600 Rockville Pike, Bethesda, MD USA 20894). Also used herein, a nucleotide position denoted with an "e" indicates the nucleotide is present in the sequence of an exon (e.g., the exon flanking the 5' splice site or the exon flanking the 3' splice site). As used herein, the "wild-type sequence" refers to the canonical sequence available at NCBI Gene ID 8831 for *SYNGAP*, and NCBI GENE ID 6323 for *SCN1A*.

[00116] The methods involve contacting cells with an ASO that is complementary to a portion of a pre-mRNA encoding SYNGAP1 or SCN1A protein, resulting in increased expression of *SYNGAP1* or *SCN1A*. As used herein, "contacting" or administering to cells refers to any method of providing an ASO in immediate proximity with the cells such that the ASO and the cells interact. A cell that is contacted

with an ASO will take up or transport the ASO into the cell. The method involves contacting a condition or disease-associated or condition or disease-relevant cell with any of the ASOs described herein. In some embodiments, the ASO may be further modified or attached (*e.g.*, covalently attached) to another molecule to target the ASO to a cell type, enhance contact between the ASO and the condition or disease-associated or condition or disease-relevant cell, or enhance uptake of the ASO.

[00117] As used herein, the term “increasing protein production” or “increasing expression of a target protein” means enhancing the amount of protein that is translated from an mRNA in a cell. A “target protein” may be any protein for which increased expression/production is desired.

[00118] In embodiments, contacting a cell that expresses a *SYNGAP1* or *SCN1A* RIC pre-mRNA with an ASO that is complementary to a targeted portion of the *SYNGAP1* or *SCN1A* RIC pre-mRNA transcript results in a measurable increase in the amount of the SYNGAP1 or SCN1A protein (*e.g.*, a target protein) encoded by the pre-mRNA. Methods of measuring or detecting production of a protein will be evident to one of skill in the art and include any known method, for example, Western blotting, flow cytometry, immunofluorescence microscopy, and ELISA.

[00119] In embodiments, contacting cells with an ASO that is complementary to a targeted portion of a *SYNGAP1* or *SCN1A* RIC pre-mRNA transcript results in an increase in the amount of SYNGAP1 or SCN1A protein produced by at least 10, 20, 30, 40, 50, 60, 80, 100, 150, 200, 250, 300, 350, 400, 450, 500, or 1000%, compared to the amount of the protein produced by a cell in the absence of the ASO/absence of treatment. In embodiments, the total amount of SYNGAP1 or SCN1A protein produced by the cell to which the antisense oligomer was contacted is increased about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to the amount of target protein produced by an control compound. A control compound can be, for example, an oligonucleotide that is not complementary to the targeted portion of the RIC pre-mRNA.

[00120] In some embodiments, contacting cells with an ASO that is complementary to a targeted portion of a *SYNGAP1* or *SCN1A* RIC pre-mRNA transcript results in an increase in the amount of mRNA encoding *SYNGAP1* or *SCN1A*, including the mature mRNA encoding the target protein. In some embodiments, the amount of mRNA encoding *SYNGAP1* or *SCN1A* protein, or the mature mRNA encoding the SYNGAP1 or SCN1A protein, is increased by at least 10, 20, 30, 40, 50, 60, 80, 100, 150, 200, 250, 300, 350, 400, 450, 500, or 1000%, compared to the amount of the protein produced by a cell in the absence of the ASO/absence of treatment. In embodiments, the total amount of the mRNA encoding SYNGAP1 or SCN1A protein, or the mature mRNA encoding SYNGAP1 or SCN1A protein produced in the cell to which the antisense oligomer was contacted is increased about 1.1 to about 10-fold, about 1.5 to

about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold compared to the amount of mature RNA produced in an untreated cell, *e.g.*, an untreated cell or a cell treated with a control compound. A control compound can be, for example, an oligonucleotide that is not complementary to the targeted portion of the *SYNGAP1* or *SCN1A* RIC pre-mRNA.

Constitutive Splicing of a Retained Intron from a RIC pre-mRNA

[00121] The methods and antisense oligonucleotide compositions provided herein are useful for increasing the expression of SYNGAP1 or SCN1A protein in cells, for example, in a subject having AD mental retardation 5 or Dravet syndrome caused by a deficiency in the amount or activity of SYNGAP1 or SCN1A protein, by increasing the level of mRNA encoding SYNGAP1 or SCN1A protein, or the mature mRNA encoding SYNGAP1 or SCN1A protein. In particular, the methods and compositions as described herein induce the constitutive splicing of a retained intron from a *SYNGAP1* or *SCN1A* RIC pre-mRNA transcript encoding SYNGAP1 or SCN1A protein, thereby increasing the level of mRNA encoding SYNGAP1 or SCN1A protein, or the mature mRNA encoding SYNGAP1 or SCN1A protein and increasing the expression of SYNGAP1 or SCN1A protein.

[00122] Constitutive splicing of a retained intron from a RIC pre-mRNA correctly removes the retained intron from the RIC pre-mRNA, wherein the retained intron has wild-type splice sequences. Constitutive splicing, as used herein, does not encompass splicing of a retained intron from a RIC pre-mRNA transcribed from a gene or allele having a mutation that causes alternative splicing or aberrant splicing of a pre-mRNA transcribed from the gene or allele. For example, constitutive splicing of a retained intron, as induced using the methods and antisense oligonucleotides provided herein, does not correct aberrant splicing in or influence alternative splicing of a pre-mRNA to result in an increased expression of a target protein or functional RNA.

[00123] In embodiments, constitutive splicing correctly removes a retained intron from a *SYNGAP1* or *SCN1A* RIC pre-mRNA, wherein the *SYNGAP1* or *SCN1A* RIC pre-mRNA is transcribed from a wild-type gene or allele, or a polymorphic gene or allele, that encodes a fully-functional target protein or functional RNA, and wherein the gene or allele does not have a mutation that causes alternative splicing or aberrant splicing of the retained intron.

[00124] In some embodiments, constitutive splicing of a retained intron from a *SYNGAP1* or *SCN1A* RIC pre-mRNA encoding SYNGAP1 or SCN1A protein correctly removes a retained intron from a *SYNGAP1* or *SCN1A* RIC pre-mRNA encoding SYNGAP1 or SCN1A protein, wherein the *SYNGAP1* or *SCN1A* RIC pre-mRNA is transcribed from a gene or allele from which the target gene or functional RNA is produced at a reduced level compared to production from a wild-type allele, and wherein the gene or

allele does not have a mutation that causes alternative splicing or aberrant splicing of the retained intron. In these embodiments, the correct removal of the constitutively spliced retained intron results in production of target protein or functional RNA that is functional when compared to an equivalent wild-type protein or functional RNA.

[00125] In other embodiments, constitutive splicing correctly removes a retained intron from a *SYNGAP1* or *SCN1A* RIC pre-mRNA, wherein the *SYNGAP1* or *SCN1A* RIC pre-mRNA is transcribed from a gene or allele that encodes a target protein or functional RNA produced in a form having reduced function compared to an equivalent wild-type protein or functional RNA, and wherein the gene or allele does not have a mutation that causes alternative splicing or aberrant splicing of the retained intron. In these embodiments, the correct removal of the constitutively spliced retained intron results in production of partially functional target protein, or functional RNA that is partially functional when compared to an equivalent wild-type protein or functional RNA.

[00126] “Correct removal” of the retained intron by constitutive splicing refers to removal of the entire intron, without removal of any part of an exon.

[00127] In embodiments, an antisense oligomer as described herein or used in any method described herein does not increase the amount of mRNA encoding SYNGAP1 or SCN1A protein or the amount of SYNGAP1 or SCN1A protein by modulating alternative splicing or aberrant splicing of a pre-mRNA transcribed from *SCN1A* or *SYNGAP1*. Modulation of alternative splicing or aberrant splicing can be measured using any known method for analyzing the sequence and length of RNA species, *e.g.*, by RT-PCR and using methods described elsewhere herein and in the literature. In embodiments, modulation of alternative or aberrant splicing is determined based on an increase or decrease in the amount of the spliced species of interest of at least 10% or 1.1-fold. In embodiments, modulation is determined based on an increase or decrease at a level that is at least 10% to 100% or 1.1 to 10-fold, as described herein regarding determining an increase in mRNA encoding SYNGAP1 or SCN1A protein in the methods of the invention.

[00128] In embodiments, the method is a method wherein the *SYNGAP1* or *SCN1A* RIC pre-mRNA was produced by partial splicing of a wild-type *SYNGAP1* or *SCN1A* pre-mRNA. In embodiments, the method is a method wherein the *SYNGAP1* or *SCN1A* RIC pre-mRNA was produced by partial splicing of a full-length wild-type *SYNGAP1* or *SCN1A* pre-mRNA. In embodiments, the *SYNGAP1* or *SCN1A* RIC pre-mRNA was produced by partial splicing of a full-length *SYNGAP1* or *SCN1A* pre-mRNA. In these embodiments, a full-length *SYNGAP1* or *SCN1A* pre-mRNA may have a polymorphism in a splice site of the retained intron that does not impair correct splicing of the retained intron as compared to splicing of the retained intron having the wild-type splice site sequence.

[00129] In embodiments, the mRNA encoding SYNGAP1 or SCN1A protein is a full-length mature mRNA, or a wild-type mature mRNA. In these embodiments, a full-length mature mRNA may have a polymorphism that does not affect the activity of the target protein or the functional RNA encoded by the mature mRNA, as compared to the activity of SYNGAP1 or SCN1A protein encoded by the wild-type mature mRNA.

[00130] One aspect of the present disclosure is a composition comprising antisense oligomers that enhances splicing by binding to a targeted portion of a *SYNGAP1* or *SCN1A* RIC pre-mRNA. As used herein, the terms “ASO” and “antisense oligomer” are used interchangeably and refer to an oligomer such as a polynucleotide, comprising nucleobases that hybridizes to a target nucleic acid (*e.g.*, a *SYNGAP1* or *SCN1A* RIC pre-mRNA) sequence by Watson-Crick base pairing or wobble base pairing (G-U). The ASO may have exact sequence complementary to the target sequence or near complementarity (*e.g.*, sufficient complementarity to bind the target sequence and enhancing splicing at a splice site). ASOs are designed so that they bind (hybridize) to a target nucleic acid (*e.g.*, a targeted portion of a pre-mRNA transcript) and remain hybridized under physiological conditions. Typically, if they hybridize to a site other than the intended (targeted) nucleic acid sequence, they hybridize to a limited number of sequences that are not a target nucleic acid (to a few sites other than a target nucleic acid). Design of an ASO can take into consideration the occurrence of the nucleic acid sequence of the targeted portion of the pre-mRNA transcript or a sufficiently similar nucleic acid sequence in other locations in the genome or cellular pre-mRNA or transcriptome, such that the likelihood the ASO will bind other sites and cause “off-target” effects is limited. Any antisense oligomers known in the art, for example in PCT Application No. PCT/US2014/054151, published as WO 2015/035091, titled “Reducing Nonsense-Mediated mRNA Decay,” incorporated by reference herein, can be used to practice the methods described herein.

[00131] In some embodiments, ASOs “specifically hybridize” to or are “specific” to a target nucleic acid or a targeted portion of a RIC pre-mRNA. Typically such hybridization occurs with a T_m substantially greater than 37°C, preferably at least 50°C, and typically between 60°C to approximately 90°C. Such hybridization preferably corresponds to stringent hybridization conditions. At a given ionic strength and pH, the T_m is the temperature at which 50% of a target sequence hybridizes to a complementary oligonucleotide.

[00132] Oligomers, such as oligonucleotides, are “complementary” to one another when hybridization occurs in an antiparallel configuration between two single-stranded polynucleotides. A double-stranded polynucleotide can be “complementary” to another polynucleotide, if hybridization can occur between one of the strands of the first polynucleotide and the second. Complementarity (the degree to which one polynucleotide is complementary with another) is quantifiable in terms of the proportion (*e.g.*, the percentage) of bases in opposing strands that are expected to form hydrogen bonds with each other, according to generally accepted base-pairing rules. The sequence of an antisense oligomer (ASO) need not be 100% complementary to that of its target nucleic acid to hybridize. In certain embodiments, ASOs can comprise at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence complementarity to a target region within the target nucleic acid sequence to which they are targeted. For example, an ASO in which 18 of 20 nucleobases of the oligomeric compound are complementary to a target region, and would therefore specifically hybridize, would represent 90 percent complementarity. In this example, the remaining non-complementary nucleobases may be clustered together or interspersed with complementary nucleobases

and need not be contiguous to each other or to complementary nucleobases. Percent complementarity of an ASO with a region of a target nucleic acid can be determined routinely using BLAST programs (basic local alignment search tools) and PowerBLAST programs known in the art (Altschul, *et al.*, J. Mol. Biol., 1990, 215, 403-410; Zhang and Madden, Genome Res., 1997, 7, 649-656).

[00133] An ASO need not hybridize to all nucleobases in a target sequence and the nucleobases to which it does hybridize may be contiguous or noncontiguous. ASOs may hybridize over one or more segments of a pre-mRNA transcript, such that intervening or adjacent segments are not involved in the hybridization event (*e.g.*, a loop structure or hairpin structure may be formed). In certain embodiments, an ASO hybridizes to noncontiguous nucleobases in a target pre-mRNA transcript. For example, an ASO can hybridize to nucleobases in a pre-mRNA transcript that are separated by one or more nucleobase(s) to which the ASO does not hybridize.

[00134] The ASOs described herein comprise nucleobases that are complementary to nucleobases present in a target portion of a RIC pre-mRNA. The term ASO embodies oligonucleotides and any other oligomeric molecule that comprises nucleobases capable of hybridizing to a complementary nucleobase on a target mRNA but does not comprise a sugar moiety, such as a peptide nucleic acid (PNA). The ASOs may comprise naturally-occurring nucleotides, nucleotide analogs, modified nucleotides, or any combination of two or three of the preceding. The term “naturally occurring nucleotides” includes deoxyribonucleotides and ribonucleotides. The term “modified nucleotides” includes nucleotides with modified or substituted sugar groups and/or having a modified backbone. In some embodiments, all of the nucleotides of the ASO are modified nucleotides. Chemical modifications of ASOs or components of ASOs that are compatible with the methods and compositions described herein will be evident to one of skill in the art and can be found, for example, in U.S. Patent No. 8,258,109 B2, U.S. Patent No. 5,656,612, U.S. Patent Publication No. 2012/0190728, and Dias and Stein, Mol. Cancer Ther. 2002, 347-355, herein incorporated by reference in their entirety.

[00135] The nucleobase of an ASO may be any naturally occurring, unmodified nucleobase such as adenine, guanine, cytosine, thymine and uracil, or any synthetic or modified nucleobase that is sufficiently similar to an unmodified nucleobase such that it is capable of hydrogen bonding with a nucleobase present on a target pre-mRNA. Examples of modified nucleobases include, without limitation, hypoxanthine, xanthine, 7-methylguanine, 5, 6-dihydrouracil, 5-methylcytosine, and 5-hydroxymethoylcytosine.

[00136] The ASOs described herein also comprise a backbone structure that connects the components of an oligomer. The term “backbone structure” and “oligomer linkages” may be used interchangeably and refer to the connection between monomers of the ASO. In naturally occurring oligonucleotides, the backbone comprises a 3'-5' phosphodiester linkage connecting sugar moieties of the oligomer. The backbone structure or oligomer linkages of the ASOs described herein may include (but are not limited to) phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoranilate, phosphoramidate, and the like. See, *e.g.*, LaPlanche, *et al.*, Nucleic Acids Res. 14:9081 (1986); Stec, *et al.*, J. Am. Chem. Soc. 106:6077 (1984), Stein, *et al.*, Nucleic Acids Res. 16:3209 (1988), Zon, *et al.*, Anti Cancer Drug Design 6:539 (1991); Zon, *et al.*, Oligonucleotides and

Analogues: A Practical Approach, pp. 87-108 (F. Eckstein, Ed., Oxford University Press, Oxford England (1991)); Stec, *et al.*, U.S. Pat. No. 5,151,510; Uhlmann and Peyman, Chemical Reviews 90:543 (1990).

In some embodiments, the backbone structure of the ASO does not contain phosphorous but rather contains peptide bonds, for example in a peptide nucleic acid (PNA), or linking groups including carbamate, amides, and linear and cyclic hydrocarbon groups. In some embodiments, the backbone modification is a phosphothioate linkage. In some embodiments, the backbone modification is a phosphoramidate linkage.

[00137] In embodiments, the stereochemistry at each of the phosphorus internucleotide linkages of the ASO backbone is random. In embodiments, the stereochemistry at each of the phosphorus internucleotide linkages of the ASO backbone is controlled and is not random. For example, U.S. Pat. App. Pub. No. 2014/0194610, "Methods for the Synthesis of Functionalized Nucleic Acids," incorporated herein by reference, describes methods for independently selecting the handedness of chirality at each phosphorous atom in a nucleic acid oligomer. In embodiments, an ASO used in the methods of the invention, including, but not limited to, any of the ASOs set forth herein in Table 1, comprises an ASO having phosphorus internucleotide linkages that are not random. In embodiments, a composition used in the methods of the invention comprises a pure diastereomeric ASO. In embodiments, a composition used in the methods of the invention comprises an ASO that has diastereomeric purity of at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, about 100%, about 90% to about 100%, about 91% to about 100%, about 92% to about 100%, about 93% to about 100%, about 94% to about 100%, about 95% to about 100%, about 96% to about 100%, about 97% to about 100%, about 98% to about 100%, or about 99% to about 100%.

[00138] In embodiments, the ASO has a nonrandom mixture of Rp and Sp configurations at its phosphorus internucleotide linkages. For example, it has been suggested that a mix of Rp and Sp is required in antisense oligonucleotides to achieve a balance between good activity and nuclease stability (Wan, *et al.*, 2014, "Synthesis, biophysical properties and biological activity of second generation antisense oligonucleotides containing chiral phosphorothioate linkages," Nucleic Acids Research 42(22): 13456-13468, incorporated herein by reference). In embodiments, an ASO used in the methods of the invention, including, but not limited to, any of the ASOs set forth herein in Table 1, comprises about 5-100% Rp, at least about 5% Rp, at least about 10% Rp, at least about 15% Rp, at least about 20% Rp, at least about 25% Rp, at least about 30% Rp, at least about 35% Rp, at least about 40% Rp, at least about 45% Rp, at least about 50% Rp, at least about 55% Rp, at least about 60% Rp, at least about 65% Rp, at least about 70% Rp, at least about 75% Rp, at least about 80% Rp, at least about 85% Rp, at least about 90% Rp, or at least about 95% Rp, with the remainder Sp, or about 100% Rp. In embodiments, an ASO used in the methods of the invention, including, but not limited to, any of the ASOs set forth herein in Table 1, comprises about 10% to about 100% Rp, about 15% to about 100% Rp, about 20% to about 100% Rp, about 25% to about 100% Rp, about 30% to about 100% Rp, about 35% to about 100% Rp, about 40% to about 100% Rp, about 45% to about 100% Rp, about 50% to about 100% Rp, about 55% to

about 100% Rp, about 60% to about 100% Rp, about 65% to about 100% Rp, about 70% to about 100% Rp, about 75% to about 100% Rp, about 80% to about 100% Rp, about 85% to about 100% Rp, about 90% to about 100% Rp, or about 95% to about 100% Rp, about 20% to about 80% Rp, about 25% to about 75% Rp, about 30% to about 70% Rp, about 40% to about 60% Rp, or about 45% to about 55% Rp, with the remainder Sp.

[00139] In embodiments, an ASO used in the methods of the invention, including, but not limited to, any of the ASOs set forth herein in Table 1, comprises about 5-100% Sp, at least about 5% Sp, at least about 10% Sp, at least about 15% Sp, at least about 20% Sp, at least about 25% Sp, at least about 30% Sp, at least about 35% Sp, at least about 40% Sp, at least about 45% Sp, at least about 50% Sp, at least about 55% Sp, at least about 60% Sp, at least about 65% Sp, at least about 70% Sp, at least about 75% Sp, at least about 80% Sp, at least about 85% Sp, at least about 90% Sp, or at least about 95% Sp, with the remainder Rp, or about 100% Sp. In embodiments, an ASO used in the methods of the invention, including, but not limited to, any of the ASOs set forth herein in Table 1, comprises about 10% to about 100% Sp, about 15% to about 100% Sp, about 20% to about 100% Sp, about 25% to about 100% Sp, about 30% to about 100% Sp, about 35% to about 100% Sp, about 40% to about 100% Sp, about 45% to about 100% Sp, about 50% to about 100% Sp, about 55% to about 100% Sp, about 60% to about 100% Sp, about 65% to about 100% Sp, about 70% to about 100% Sp, about 75% to about 100% Sp, about 80% to about 100% Sp, about 85% to about 100% Sp, about 90% to about 100% Sp, or about 95% to about 100% Sp, about 20% to about 80% Sp, about 25% to about 75% Sp, about 30% to about 70% Sp, about 40% to about 60% Sp, or about 45% to about 55% Sp, with the remainder Rp.

[00140] Any of the ASOs described herein may contain a sugar moiety that comprises ribose or deoxyribose, as present in naturally occurring nucleotides, or a modified sugar moiety or sugar analog, including a morpholine ring. Non-limiting examples of modified sugar moieties include 2' substitutions such as 2'-O-methyl (2'-O-Me), 2'-O-methoxyethyl (2'MOE), 2'-O-aminoethyl, 2'F; N3'->P5' phosphoramidate, 2'dimethylaminooxyethoxy, 2'dimethylaminoethoxyethoxy, 2'-guanidinidinium, 2'-O-guanidininium ethyl, carbamate modified sugars, and bicyclic modified sugars. In some embodiments, the sugar moiety modification is selected from 2'-O-Me, 2'F, and 2'MOE. In some embodiments, the sugar moiety modification is an extra bridge bond, such as in a locked nucleic acid (LNA). In some embodiments the sugar analog contains a morpholine ring, such as phosphorodiamidate morpholino (PMO). In some embodiments, the sugar moiety comprises a ribofuransyl or 2'deoxyribofuransyl modification. In some embodiments, the sugar moiety comprises 2'4'-constrained 2'-O-methoxyethyl (cMOE) modifications. In some embodiments, the sugar moiety comprises cEt 2', 4' constrained 2'-O ethyl BNA modifications. In some embodiments, the sugar moiety comprises tricycloDNA (tcDNA) modifications. In some embodiments, the sugar moiety comprises ethylene nucleic acid (ENA) modifications. In some embodiments, the sugar moiety comprises MCE modifications. Modifications are known in the art and described in the literature, *e.g.*, by Jarver, *et al.*, 2014, "A Chemical View of Oligonucleotides for Exon Skipping and Related Drug Applications," *Nucleic Acid Therapeutics* 24(1): 37-47, incorporated by reference for this purpose herein.

[00141] In some examples, each monomer of the ASO is modified in the same way, for example each linkage of the backbone of the ASO comprises a phosphorothioate linkage or each ribose sugar moiety comprises a 2'-O-methyl modification. Such modifications that are present on each of the monomer components of an ASO are referred to as "uniform modifications." In some examples, a combination of different modifications may be desired, for example, an ASO may comprise a combination of phosphorodiamidate linkages and sugar moieties comprising morpholine rings (morpholinos). Combinations of different modifications to an ASO are referred to as "mixed modifications" or "mixed chemistries."

[00142] In some embodiments, the ASO comprises one or more backbone modification. In some embodiments, the ASO comprises one or more sugar moiety modification. In some embodiments, the ASO comprises one or more backbone modification and one or more sugar moiety modification. In some embodiments, the ASO comprises 2'-MOE modifications and a phosphorothioate backbone. In some embodiments, the ASO comprises a phosphorodiamidate morpholino (PMO). In some embodiments, the ASO comprises a peptide nucleic acid (PNA). Any of the ASOs or any component of an ASO (*e.g.*, a nucleobase, sugar moiety, backbone) described herein may be modified in order to achieve desired properties or activities of the ASO or reduce undesired properties or activities of the ASO. For example, an ASO or one or more component of any ASO may be modified to enhance binding affinity to a target sequence on a pre-mRNA transcript; reduce binding to any non-target sequence; reduce degradation by cellular nucleases (*i.e.*, RNase H); improve uptake of the ASO into a cell and/or into the nucleus of a cell; alter the pharmacokinetics or pharmacodynamics of the ASO; and modulate the half-life of the ASO.

[00143] In some embodiments, the ASOs are comprised of 2'-O-(2-methoxyethyl) (MOE) phosphorothioate-modified nucleotides. ASOs comprised of such nucleotides are especially well-suited to the methods disclosed herein; oligomers having such modifications have been shown to have significantly enhanced resistance to nuclease degradation and increased bioavailability, making them suitable, for example, for oral delivery in some embodiments described herein. See *e.g.*, Geary, *et al.*, J Pharmacol Exp Ther. 2001; 296(3):890-7; Geary, *et al.*, J Pharmacol Exp Ther. 2001; 296(3):898-904.

[00144] Methods of synthesizing ASOs will be known to one of skill in the art. Alternatively or in addition, ASOs may be obtained from a commercial source.

[00145] Unless specified otherwise, the left-hand end of single-stranded nucleic acid (*e.g.*, pre-mRNA transcript, oligonucleotide, ASO, etc.) sequences is the 5' end and the left-hand direction of single or double-stranded nucleic acid sequences is referred to as the 5' direction. Similarly, the right-hand end or direction of a nucleic acid sequence (single or double stranded) is the 3' end or direction. Generally, a region or sequence that is 5' to a reference point in a nucleic acid is referred to as "upstream," and a region or sequence that is 3' to a reference point in a nucleic acid is referred to as "downstream." Generally, the 5' direction or end of an mRNA is where the initiation or start codon is located, while the 3' end or direction is where the termination codon is located. In some aspects, nucleotides that are upstream of a reference point in a nucleic acid may be designated by a negative number, while nucleotides that are downstream of a reference point may be designated by a positive number. For example, a

reference point (*e.g.*, an exon-exon junction in mRNA) may be designated as the “zero” site, and a nucleotide that is directly adjacent and upstream of the reference point is designated “minus one,” *e.g.*, “-1,” while a nucleotide that is directly adjacent and downstream of the reference point is designated “plus one,” *e.g.*, “+1.”

[00146] In some embodiments, the ASOs are complementary to (and bind to) a targeted portion of a *SCN1A* RIC pre-mRNA that is downstream (in the 3' direction) of the 5' splice site of the retained intron in a *SCN1A* RIC pre-mRNA (*e.g.*, the direction designated by positive numbers relative to the 5' splice site) (FIG. 1). In some embodiments, the ASOs are complementary to a targeted portion of the *SCN1A* RIC pre-mRNA that is within the region about +6 to about +500 relative to the 5' splice site of the retained intron. In some embodiments, the ASO is not complementary to nucleotides +1 to +5 relative to the 5' splice site (the first five nucleotides located downstream of the 5' splice site). In some embodiments, the ASOs may be complementary to a targeted portion of a *SCN1A* RIC pre-mRNA that is within the region between nucleotides +6 and +496 relative to the 5' splice site of the retained intron. In some aspects, the ASOs are complementary to a targeted portion that is within the region about +6 to about +500, about +6 to about +490, about +6 to about +480, about +6 to about +470, about +6 to about +460, about +6 to about +450, about +6 to about +440, about +6 to about +430, about +6 to about +420, about +6 to about +410, about +6 to about +400, about +6 to about +390, about +6 to about +380, about +6 to about +370, about +6 to about +360, about +6 to about +350, about +6 to about +340, about +6 to about +330, about +6 to about +320, about +6 to about +310, about +6 to about +300, about +6 to about +290, about +6 to about +280, about +6 to about +270, about +6 to about +260, about +6 to about +250, about +6 to about +240, about +6 to about +230, about +6 to about +220, about +6 to about +210, about +6 to about +200, about +6 to about +190, about +6 to about +180, about +6 to about +170, about +6 to about +160, about +6 to about +150, about +6 to about +140, about +6 to about +130, about +6 to about +120, about +6 to about +110, about +6 to about +100, about +6 to about +90, about +6 to about +80, about +6 to about +70, about +6 to about +60, about +6 to about +50, about +6 to about +40, about +6 to about +30, or about +6 to about +20 relative to 5' splice site of the retained intron.

[00147] In some embodiments, the ASOs are complementary to (and bind to) a targeted portion of a *SCN1A* RIC pre-mRNA that is upstream (in the 5' direction) of the 5' splice site of the retained intron in a *SCN1A* RIC pre-mRNA (*e.g.*, the direction designated by negative numbers relative to the 5' splice site) (FIG. 1). In some embodiments, the ASOs are complementary to a targeted portion of the *SCN1A* RIC pre-mRNA that is within the region about -4e to about -270e relative to the 5' splice site of the retained intron. In some embodiments, the ASO is not complementary to nucleotides -1e to -3e relative to the 5' splice site (the first three nucleotides located upstream of the 5' splice site). In some embodiments, the ASOs may be complementary to a targeted portion of a *SCN1A* RIC pre-mRNA that is within the region between nucleotides -4e and -264e relative to the 5' splice site of the retained intron. In some aspects, the ASOs are complementary to a targeted portion that is within the region about -4e to about -270e, about -4e to about -260e, about -4e to about -250e, about -4e to about -240e, about -4e to about -230e, about -4e to about -220e, about -4e to about -210e, about -4e to about -200e, about -4e to about -190e, about -4e to

about -180e, about -4e to about -170e, about -4e to about -160e, about -4e to about -150e, about -4e to about -140e, about -4e to about -130e, about -4e to about -120e, about -4e to about -110e, about -4e to about -100e, about -4e to about -90e, about -4e to about -80e, about -4e to about -70e, about -4e to about -60e, about -4e to about -50e, about -4e to about -40e, about -4e to about -30e, or about -4e to about -20e relative to 5' splice site of the retained intron.

[00148] In some embodiments, the ASOs are complementary to a targeted region of a *SCN1A* RIC pre-mRNA that is upstream (in the 5' direction) of the 3' splice site of the retained intron in a *SCN1A* RIC pre-mRNA (e.g., in the direction designated by negative numbers) (FIG. 1). In some embodiments, the ASOs are complementary to a targeted portion of the *SCN1A* RIC pre-mRNA that is within the region about -16 to about -500 relative to the 3' splice site of the retained intron. In some embodiments, the ASO is not complementary to nucleotides -1 to -15 relative to the 3' splice site (the first 15 nucleotides located upstream of the 3' splice site). In some embodiments, the ASOs are complementary to a targeted portion of the *SCN1A* RIC pre-mRNA that is within the region -16 to -496 relative to the 3' splice site of the retained intron. In some aspects, the ASOs are complementary to a targeted portion that is within the region about -16 to about -500, about -16 to about -490, about -16 to about -480, about -16 to about -470, about -16 to about -460, about -16 to about -450, about -16 to about -440, about -16 to about -430, about -16 to about -420, about -16 to about -410, about -16 to about -400, about -16 to about -390, about -16 to about -380, about -16 to about -370, about -16 to about -360, about -16 to about -350, about -16 to about -340, about -16 to about -330, about -16 to about -320, about -16 to about -310, about -16 to about -300, about -16 to about -290, about -16 to about -280, about -16 to about -270, about -16 to about -260, about -16 to about -250, about -16 to about -240, about -16 to about -230, about -16 to about -220, about -16 to about -210, about -16 to about -200, about -16 to about -190, about -16 to about -180, about -16 to about -170, about -16 to about -160, about -16 to about -150, about -16 to about -140, about -16 to about -130, about -16 to about -120, about -16 to about -110, about -16 to about -100, about -16 to about -90, about -16 to about -80, about -16 to about -70, about -16 to about -60, about -16 to about -50, about -16 to about -40, or about -16 to about -30 relative to 3' splice site of the retained intron.

[00149] In some embodiments, the ASOs are complementary to a targeted region of a *SCN1A* RIC pre-mRNA that is downstream (in the 3' direction) of the 3' splice site of the retained intron in a *SCN1A* RIC pre-mRNA (e.g., in the direction designated by positive numbers) (FIG. 1). In some embodiments, the ASOs are complementary to a targeted portion of the *SCN1A* RIC pre-mRNA that is within the region of about +2e to about +40e relative to the 3' splice site of the retained intron. In some embodiments, the ASO is not complementary to nucleotides +1e relative to the 3' splice site (the first nucleotide located downstream of the 3' splice site). In some embodiments, the ASOs may be complementary to a targeted portion of a *SCN1A* RIC pre-mRNA that is within the region between nucleotides +2e and +37e relative to the 3' splice site of the retained intron. In some aspects, the ASOs are complementary to a targeted portion that is within the region about +2e to about +40e, about +2e to about +30e, about +2e to about +20e, or about +2e to about +10e relative to 3' splice site of the retained intron.

[00150] In some embodiments, the ASOs are complementary to (and bind to) a targeted portion of a *SYNGAP1* RIC pre-mRNA that is downstream (in the 3' direction) of the 5' splice site of the retained intron in a *SYNGAP1* RIC pre-mRNA (e.g., the direction designated by positive numbers relative to the 5' splice site) (FIG. 1). In some embodiments, the ASOs are complementary to a targeted portion of the *SYNGAP1* RIC pre-mRNA that is within the region about +6 to about +500 relative to the 5' splice site of the retained intron. In some embodiments, the ASO is not complementary to nucleotides +1 to +5 relative to the 5' splice site (the first five nucleotides located downstream of the 5' splice site). In some embodiments, the ASOs may be complementary to a targeted portion of a *SYNGAP1* RIC pre-mRNA that is within the region between nucleotides +6 and +496 relative to the 5' splice site of the retained intron. In some embodiments, the ASOs may be complementary to a targeted portion of a *SYNGAP1* RIC pre-mRNA that is within the region between nucleotides +6 and +251 relative to the 5' splice site of the retained intron. In some aspects, the ASOs are complementary to a targeted portion that is within the region about +6 to about +500, about +6 to about +490, about +6 to about +480, about +6 to about +470, about +6 to about +460, about +6 to about +450, about +6 to about +440, about +6 to about +430, about +6 to about +420, about +6 to about +410, about +6 to about +400, about +6 to about +390, about +6 to about +380, about +6 to about +370, about +6 to about +360, about +6 to about +350, about +6 to about +340, about +6 to about +330, about +6 to about +320, about +6 to about +310, about +6 to about +300, about +6 to about +290, about +6 to about +280, about +6 to about +270, about +6 to about +260, about +6 to about +250, about +6 to about +240, about +6 to about +230, about +6 to about +220, about +6 to about +210, about +6 to about +200, about +6 to about +190, about +6 to about +180, about +6 to about +170, about +6 to about +160, about +6 to about +150, about +6 to about +140, about +6 to about +130, about +6 to about +120, about +6 to about +110, about +6 to about +100, about +6 to about +90, about +6 to about +80, about +6 to about +70, about +6 to about +60, about +6 to about +50, about +6 to about +40, about +6 to about +30, or about +6 to about +20 relative to 5' splice site of the retained intron.

[00151] In some embodiments, the ASOs are complementary to (and bind to) a targeted portion of a *SYNGAP1* RIC pre-mRNA that is upstream (in the 5' direction) of the 5' splice site of the retained intron in a *SYNGAP1* RIC pre-mRNA (e.g., the direction designated by negative numbers relative to the 5' splice site) (FIG. 1). In some embodiments, the ASOs are complementary to a targeted portion of the *SYNGAP1* RIC pre-mRNA that is within the region about -4e to about -1,060e relative to the 5' splice site of the retained intron. In some embodiments, the ASO is not complementary to nucleotides -1e to -3e relative to the 5' splice site (the first three nucleotides located upstream of the 5' splice site). In some embodiments, the ASOs may be complementary to a targeted portion of a *SYNGAP1* RIC pre-mRNA that is within the region between nucleotides -4e and -1054e relative to the 5' splice site of the retained intron. In some embodiments, the ASOs may be complementary to a targeted portion of a *SYNGAP1* RIC pre-mRNA that is within the region between nucleotides -4e and -73e relative to the 5' splice site of the retained intron. In some aspects, the ASOs are complementary to a targeted portion that is within the region about -4e to about -1,060e, about -4e to about -1,050e, about -4e to about -1,040e, about -4e to about -1,030e, about -4e to about -1,020e, about -4e to about -1,010e, about -4e to about -1,000e, about -4e to about -990e,

about -4e to about -980e, about -4e to about -970e, about -4e to about -960e, about -4e to about -950e, about -4e to about -940e, about -4e to about -930e, about -4e to about -920e, about -4e to about -910e, about -4e to about -900e, about -4e to about -890e, about -4e to about -880e, about -4e to about -870e, about -4e to about -860e, about -4e to about -850e, about -4e to about -840e, about -4e to about -830e, about -4e to about -820e, about -4e to about -810e, about -4e to about -800e, about -4e to about -790e, about -4e to about -780e, about -4e to about -770e, about -4e to about -760e, about -4e to about -750e, about -4e to about -740e, about -4e to about -730e, about -4e to about -720e, about -4e to about -710e, about -4e to about -700e, about -4e to about -690e, about -4e to about -680e, about -4e to about -670e, about -4e to about -660e, about -4e to about -650e, about -4e to about -640e, about -4e to about -630e, about -4e to about -620e, about -4e to about -610e, about -4e to about -600e, about -4e to about -590e, about -4e to about -580e, about -4e to about -570e, about -4e to about -560e, about -4e to about -550e, about -4e to about -540e, about -4e to about -530e, about -4e to about -520e, about -4e to about -510e, about -4e to about -500e, about -4e to about -490e, about -4e to about -480e, about -4e to about -470e, about -4e to about -460e, about -4e to about -450e, about -4e to about -440e, about -4e to about -430e, about -4e to about -420e, about -4e to about -410e, about -4e to about -400e, about -4e to about -390e, about -4e to about -380e, about -4e to about -370e, about -4e to about -360e, about -4e to about -350e, about -4e to about -340e, about -4e to about -330e, about -4e to about -320e, about -4e to about -310e, about -4e to about -300e, about -4e to about -290e, about -4e to about -280e, about -4e to about -270e, about -4e to about -260e, about -4e to about -250e, about -4e to about -240e, about -4e to about -230e, about -4e to about -220e, about -4e to about -210e, about -4e to about -200e, about -4e to about -190e, about -4e to about -180e, about -4e to about -170e, about -4e to about -160e, about -4e to about -150e, about -4e to about -140e, about -4e to about -130e, about -4e to about -120e, about -4e to about -110e, about -4e to about -100e, about -4e to about -90e, about -4e to about -80e, about -4e to about -70e, about -4e to about -60e, about -4e to about -50e, about -4e to about -40e, about -4e to about -30e, or about -4e to about -20e relative to 5' splice site of the retained intron.

[00152] In some embodiments, the ASOs are complementary to a targeted region of a *SYNGAP1* RIC pre-mRNA that is upstream (in the 5' direction) of the 3' splice site of the retained intron in a *SYNGAP1* RIC pre-mRNA (e.g., in the direction designated by negative numbers) (FIG. 1). In some embodiments, the ASOs are complementary to a targeted portion of the *SYNGAP1* RIC pre-mRNA that is within the region of about -16 to about -500 relative to the 3' splice site of the retained intron. In some embodiments, the ASO is not complementary to nucleotides -1 to -15 relative to the 3' splice site (the first 15 nucleotides located upstream of the 3' splice site). In some embodiments, the ASOs are complementary to a targeted portion of the *SYNGAP1* RIC pre-mRNA that is within the region -16 to -256 relative to the 3' splice site of the retained intron. In some embodiments, the ASOs are complementary to a targeted portion of the *SYNGAP1* RIC pre-mRNA that is within the region -16 to -496 relative to the 3' splice site of the retained intron. In some aspects, the ASOs are complementary to a targeted portion that is within the region about -16 to about -500, about -16 to about -490, about -16 to about -480, about -16 to about -470, about -16 to about -460, about -16 to about -450, about -16 to about -440, about -16 to

about -430, about -16 to about -420, about -16 to about -410, about -16 to about -400, about -16 to about -390, about -16 to about -380, about -16 to about -370, about -16 to about -360, about -16 to about -350, about -16 to about -340, about -16 to about -330, about -16 to about -320, about -16 to about -310, about -16 to about -300, about -16 to about -290, about -16 to about -280, about -16 to about -270, about -16 to about -260, about -16 to about -250, about -16 to about -240, about -16 to about -230, about -16 to about -220, about -16 to about -210, about -16 to about -200, about -16 to about -190, about -16 to about -180, about -16 to about -170, about -16 to about -160, about -16 to about -150, about -16 to about -140, about -16 to about -130, about -16 to about -120, about -16 to about -110, about -16 to about -100, about -16 to about -90, about -16 to about -80, about -16 to about -70, about -16 to about -60, about -16 to about -50, about -16 to about -40, or about -16 to about -30 relative to 3' splice site of the retained intron.

[00153] In some embodiments, the ASOs are complementary to a targeted region of a *SYNGAP1* RIC pre-mRNA that is downstream (in the 3' direction) of the 3' splice site of the retained intron in a *SYNGAP1* RIC pre-mRNA (*e.g.*, in the direction designated by positive numbers) (FIG. 1). In some embodiments, the ASOs are complementary to a targeted portion of the *SYNGAP1* RIC pre-mRNA that is within the region of about +2e to about +1,920e relative to the 3' splice site of the retained intron. In some embodiments, the ASO is not complementary to nucleotides +1e relative to the 3' splice site (the first nucleotide located downstream of the 3' splice site). In some embodiments, the ASO is not complementary to nucleotides +16e relative to the 3' splice site (the first sixteen nucleotides located downstream of the 3' splice site). In some embodiments, the ASOs may be complementary to a targeted portion of a *SYNGAP1* RIC pre-mRNA that is within the region between nucleotides +2e and +157e relative to the 3' splice site of the retained intron. In some embodiments, the ASOs may be complementary to a targeted portion of a *SYNGAP1* RIC pre-mRNA that is within the region between nucleotides +2e and +1,912e relative to the 3' splice site of the retained intron. In some aspects, the ASOs are complementary to a targeted portion that is within the region about +2e to about +1,920e, about +2e to about +1,910e, about +2e to about +1,900e, about +2e to about +1,890e, about +2e to about +1,880e, about +2e to about +1,870e, about +2e to about +1,860e, about +2e to about +1,850e, about +2e to about +1,840e, about +2e to about +1,830e, about +2e to about +1,820e, about +2e to about +1,810e, about +2e to about +1,800e, about +2e to about +1,790e, about +2e to about +1,780e, about +2e to about +1,770e, about +2e to about +1,760e, about +2e to about +1,750e, about +2e to about +1,740e, about +2e to about +1,730e, about +2e to about +1,720e, about +2e to about +1,710e, about +2e to about +1,700e, about +2e to about +1,690e, about +2e to about +1,680e, about +2e to about +1,670e, about +2e to about +1,660e, about +2e to about +1,650e, about +2e to about +1,640e, about +2e to about +1,630e, about +2e to about +1,620e, about +2e to about +1,610e, about +2e to about +1,600e, about +2e to about +1,590e, about +2e to about +1,580e, about +2e to about +1,570e, about +2e to about +1,560e, about +2e to about +1,550e, about +2e to about +1,540e, about +2e to about +1,530e, about +2e to about +1,520e, about +2e to about +1,510e, about +2e to about +1,500e, about +2e to about +1,490e, about +2e to about +1,480e, about +2e to about +1,470e, about +2e to about +1,460e, about +2e to about +1,450e, about +2e to about +1,440e, about +2e to about +1,430e, about +2e to about +1,420e, about +2e to about +1,410e, about +2e to about

+1,400e, about +2e to about +1,390e, about +2e to about +1,380e, about +2e to about +1,370e, about +2e to about +1,360e, about +2e to about +1,350e, about +2e to about +1,340e, about +2e to about +1,330e, about +2e to about +1,320e, about +2e to about +1,310e, about +2e to about +1,300e, about +2e to about +1,290e, about +2e to about +1,280e, about +2e to about +1,270e, about +2e to about +1,260e, about +2e to about +1,250e, about +2e to about +1,240e, about +2e to about +1,230e, about +2e to about +1,220e, about +2e to about +1,210e, about +2e to about +1,200e, about +2e to about +1,190e, about +2e to about +1,180e, about +2e to about +1,170e, about +2e to about +1,160e, about +2e to about +1,150e, about +2e to about +1,140e, about +2e to about +1,130e, about +2e to about +1,120e, about +2e to about +1,110e, about +2e to about +1,100e, about +2e to about +1,090e, about +2e to about +1,080e, about +2e to about +1,070e, about +2e to about +1,060e, about +2e to about +1,050e, about +2e to about +1,040e, about +2e to about +1,030e, about +2e to about +1,020e, about +2e to about +1,010e, about +2e to about +1,000e, about +2e to about +990e, about +2e to about +980e, about +2e to about +970e, about +2e to about +960e, about +2e to about +950e, about +2e to about +940e, about +2e to about +930e, about +2e to about +920e, about +2e to about +910e, about +2e to about +900e, about +2e to about +890e, about +2e to about +880e, about +2e to about +870e, about +2e to about +860e, about +2e to about +850e, about +2e to about +840e, about +2e to about +830e, about +2e to about +820e, about +2e to about +810e, about +2e to about +800e, about +2e to about +790e, about +2e to about +780e, about +2e to about +770e, about +2e to about +760e, about +2e to about +750e, about +2e to about +740e, about +2e to about +730e, about +2e to about +720e, about +2e to about +710e, about +2e to about +700e, about +2e to about +690e, about +2e to about +680e, about +2e to about +670e, about +2e to about +660e, about +2e to about +650e, about +2e to about +640e, about +2e to about +630e, about +2e to about +620e, about +2e to about +610e, about +2e to about +600e, about +2e to about +590e, about +2e to about +580e, about +2e to about +570e, about +2e to about +560e, about +2e to about +550e, about +2e to about +540e, about +2e to about +530e, about +2e to about +520e, about +2e to about +510e, about +2e to about +500e, about +2e to about +490e, about +2e to about +480e, about +2e to about +470e, about +2e to about +460e, about +2e to about +450e, about +2e to about +440e, about +2e to about +430e, about +2e to about +420e, about +2e to about +410e, about +2e to about +400e, about +2e to about +390e, about +2e to about +380e, about +2e to about +370e, about +2e to about +360e, about +2e to about +350e, about +2e to about +340e, about +2e to about +330e, about +2e to about +320e, about +2e to about +310e, about +2e to about +300e, about +2e to about +290e, about +2e to about +280e, about +2e to about +270e, about +2e to about +260e, about +2e to about +250e, about +2e to about +240e, about +2e to about +230e, about +2e to about +220e, about +2e to about +210e, about +2e to about +200e, about +2e to about +190e, about +2e to about +180e, about +2e to about +170e, about +2e to about +160e, about +2e to about +150e, about +2e to about +140e, about +2e to about +130e, about +2e to about +120e, about +2e to about +110e, about +2e to about +100e, about +2e to about +90e, about +2e to about +80e, about +2e to about +70e, about +2e to about +60e, about +2e to about +50e, about +2e to about +40e, about +2e to about +30e, or about +2e to about +20e relative to 3' splice site of the retained intron.

[00154] In embodiments, the targeted portion of the *SYNGAP1* or *SCN1A* RIC pre-mRNA is within the region +100 relative to the 5' splice site of the retained intron to -100 relative to the 3' splice site of the retained intron.

[00155] The ASOs may be of any length suitable for specific binding and effective enhancement of splicing. In some embodiments, the ASOs consist of 8 to 50 nucleobases. For example, the ASO may be 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 40, 45, or 50 nucleobases in length. In some embodiments, the ASOs consist of more than 50 nucleobases. In some embodiments, the ASO is from 8 to 50 nucleobases, 8 to 40 nucleobases, 8 to 35 nucleobases, 8 to 30 nucleobases, 8 to 25 nucleobases, 8 to 20 nucleobases, 8 to 15 nucleobases, 9 to 50 nucleobases, 9 to 40 nucleobases, 9 to 35 nucleobases, 9 to 30 nucleobases, 9 to 25 nucleobases, 9 to 20 nucleobases, 9 to 15 nucleobases, 10 to 50 nucleobases, 10 to 40 nucleobases, 10 to 35 nucleobases, 10 to 30 nucleobases, 10 to 25 nucleobases, 10 to 20 nucleobases, 10 to 15 nucleobases, 11 to 50 nucleobases, 11 to 40 nucleobases, 11 to 35 nucleobases, 11 to 30 nucleobases, 11 to 25 nucleobases, 11 to 20 nucleobases, 11 to 15 nucleobases, 12 to 50 nucleobases, 12 to 40 nucleobases, 12 to 35 nucleobases, 12 to 30 nucleobases, 12 to 25 nucleobases, 12 to 20 nucleobases, 12 to 15 nucleobases, 13 to 50 nucleobases, 13 to 40 nucleobases, 13 to 35 nucleobases, 13 to 30 nucleobases, 13 to 25 nucleobases, 13 to 20 nucleobases, 14 to 50 nucleobases, 14 to 40 nucleobases, 14 to 35 nucleobases, 14 to 30 nucleobases, 14 to 25 nucleobases, 14 to 20 nucleobases, 15 to 50 nucleobases, 15 to 40 nucleobases, 15 to 35 nucleobases, 15 to 30 nucleobases, 15 to 25 nucleobases, 15 to 20 nucleobases, 20 to 50 nucleobases, 20 to 40 nucleobases, 20 to 35 nucleobases, 20 to 30 nucleobases, 20 to 25 nucleobases, 25 to 50 nucleobases, 25 to 40 nucleobases, 25 to 35 nucleobases, or 25 to 30 nucleobases in length. In some embodiments, the ASOs are 18 nucleotides in length. In some embodiments, the ASOs are 15 nucleotides in length. In some embodiments, the ASOs are 25 nucleotides in length.

[00156] In some embodiments, two or more ASOs with different chemistries but complementary to the same targeted portion of the RIC pre-mRNA are used. In some embodiments, two or more ASOs that are complementary to different targeted portions of the RIC pre-mRNA are used.

[00157] In embodiments, the antisense oligonucleotides of the invention are chemically linked to one or more moieties or conjugates, *e.g.*, a targeting moiety or other conjugate that enhances the activity or cellular uptake of the oligonucleotide. Such moieties include, but are not limited to, a lipid moiety, *e.g.*, as a cholesterol moiety, a cholesteryl moiety, an aliphatic chain, *e.g.*, dodecandiol or undecyl residues, a polyamine or a polyethylene glycol chain, or adamantane acetic acid. Oligonucleotides comprising lipophilic moieties and preparation methods have been described in the published literature. In embodiments, the antisense oligonucleotide is conjugated with a moiety including, but not limited to, an abasic nucleotide, a polyether, a polyamine, a polyamide, a peptides, a carbohydrate, *e.g.*, N-acetylgalactosamine (GalNAc), N-Ac-Glucosamine (GluNAc), or mannose (*e.g.*, mannose-6-phosphate), a lipid, or a polyhydrocarbon compound. Conjugates can be linked to one or more of any nucleotides comprising the antisense oligonucleotide at any of several positions on the sugar, base or phosphate group, as understood in the art and described in the literature, *e.g.*, using a linker. Linkers can include a

bivalent or trivalent branched linker. In embodiments, the conjugate is attached to the 3' end of the antisense oligonucleotide. Methods of preparing oligonucleotide conjugates are described, *e.g.*, in U.S. Pat. No. 8,450,467, "Carbohydrate conjugates as delivery agents for oligonucleotides," incorporated by reference herein.

[00158] In some embodiments, the nucleic acid to be targeted by an ASO is a *SYNGAP1* or *SCN1A* RIC pre-mRNA expressed in a cell, such as a eukaryotic cell. In some embodiments, the term "cell" may refer to a population of cells. In some embodiments, the cell is in a subject. In some embodiments, the cell is isolated from a subject. In some embodiments, the cell is *ex vivo*. In some embodiments, the cell is a condition or disease-relevant cell or a cell line. In some embodiments, the cell is in vitro (*e.g.*, in cell culture).

Pharmaceutical Compositions

[00159] Pharmaceutical compositions or formulations comprising the antisense oligonucleotide of the described compositions and for use in any of the described methods can be prepared according to conventional techniques well known in the pharmaceutical industry and described in the published literature. In embodiments, a pharmaceutical composition or formulation for treating a subject comprises an effective amount of any antisense oligomer as described above, or a pharmaceutically acceptable salt, solvate, hydrate or ester thereof, and a pharmaceutically acceptable diluent. The antisense oligomer of a pharmaceutical formulation may further comprise a pharmaceutically acceptable excipient, diluent or carrier.

[00160] Pharmaceutically acceptable salts are suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response, etc., and are commensurate with a reasonable benefit/risk ratio. (See, *e.g.*, S. M. Berge, et al., J. Pharmaceutical Sciences, 66: 1-19 (1977), incorporated herein by reference for this purpose. The salts can be prepared in situ during the final isolation and purification of the compounds, or separately by reacting the free base function with a suitable organic acid. Examples of pharmaceutically acceptable, nontoxic acid addition salts are salts of an amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid or by using other documented methodologies such as ion exchange. Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p-toluenesulfonate, undecanoate, valerate salts, and the like. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like. Further pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary

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

[00161] In embodiments, the compositions are formulated into any of many possible dosage forms such as, but not limited to, tablets, capsules, gel capsules, liquid syrups, soft gels, suppositories, and enemas. In embodiments, the compositions are formulated as suspensions in aqueous, non-aqueous or mixed media. Aqueous suspensions may further contain substances that increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. The suspension may also contain stabilizers. In embodiments, a pharmaceutical formulation or composition of the present invention includes, but is not limited to, a solution, emulsion, microemulsion, foam or liposome-containing formulation (*e.g.*, cationic or noncationic liposomes).

[00162] The pharmaceutical composition or formulation of the present invention may comprise one or more penetration enhancer, carrier, excipients or other active or inactive ingredients as appropriate and well known to those of skill in the art or described in the published literature. In embodiments, liposomes also include sterically stabilized liposomes, *e.g.*, liposomes comprising one or more specialized lipids. These specialized lipids result in liposomes with enhanced circulation lifetimes. In embodiments, a sterically stabilized liposome comprises one or more glycolipids or is derivatized with one or more hydrophilic polymers, such as a polyethylene glycol (PEG) moiety. In embodiments, a surfactant is included in the pharmaceutical formulation or compositions. The use of surfactants in drug products, formulations and emulsions is well known in the art. In embodiments, the present invention employs a penetration enhancer to effect the efficient delivery of the antisense oligonucleotide, *e.g.*, to aid diffusion across cell membranes and /or enhance the permeability of a lipophilic drug. In embodiments, the penetration enhancers are a surfactant, fatty acid, bile salt, chelating agent, or non-chelating nonsurfactant.

[00163] In embodiments, the pharmaceutical formulation comprises multiple antisense oligonucleotides. In embodiments, the antisense oligonucleotide is administered in combination with another drug or therapeutic agent.

Treatment of Subjects

[00164] Any of the compositions provided herein may be administered to an individual. "Individual" may be used interchangeably with "subject" or "patient." An individual may be a mammal, for example a human or animal such as a non-human primate, a rodent, a rabbit, a rat, a mouse, a horse, a donkey, a goat, a cat, a dog, a cow, a pig, or a sheep. In embodiments, the individual is a human. In embodiments, the individual is a fetus, an embryo, or a child. In other embodiments, the individual may be another eukaryotic organism, such as a plant. In some embodiments, the compositions provided herein are administered to a cell *ex vivo*.

[00165] In some embodiments, the compositions provided herein are administered to an individual as a method of treating a disease or disorder. In some embodiments, the individual has a genetic disease, such as any of the diseases described herein. In some embodiments, the individual is at risk of having the disease, such as any of the diseases described herein. In some embodiments, the individual is at increased risk of having a disease or disorder caused by insufficient amount of a protein or insufficient activity of a

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

[00166] Suitable routes for administration of ASOs of the present invention may vary depending on cell type to which delivery of the ASOs is desired. Multiple tissues and organs are affected by AD mental retardation 5 and Dravet syndrome, with the brain being the most significantly affected tissue. The ASOs of the present invention may be administered to patients parenterally, for example, by intrathecal injection, intracerebroventricular injection, intraperitoneal injection, intramuscular injection, subcutaneous injection, or intravenous injection.

[00167] In embodiments, the antisense oligonucleotide is administered with one or more agents capable of promoting penetration of the subject antisense oligonucleotide across the blood-brain barrier by any method known in the art. For example, delivery of agents by administration of an adenovirus vector to motor neurons in muscle tissue is described in U.S. Pat. No. 6,632,427, “Adenoviral-vector-mediated gene transfer into medullary motor neurons,” incorporated herein by reference. Delivery of vectors directly to the brain, *e.g.*, the striatum, the thalamus, the hippocampus, or the substantia nigra, is described, *e.g.*, in U.S. Pat. No. 6,756,523, “Adenovirus vectors for the transfer of foreign genes into cells of the central nervous system particularly in brain,” incorporated herein by reference.

[00168] In embodiments, the antisense oligonucleotides are linked or conjugated with agents that provide desirable pharmaceutical or pharmacodynamic properties. In embodiments, the antisense oligonucleotide is coupled to a substance, known in the art to promote penetration or transport across the blood-brain barrier, *e.g.*, an antibody to the transferrin receptor. In embodiments, the antisense oligonucleotide is linked with a viral vector, *e.g.*, to render the antisense compound more effective or increase transport across the blood-brain barrier. In embodiments, osmotic blood brain barrier disruption is assisted by infusion of sugars, *e.g.*, meso erythritol, xylitol, D(+) galactose, D(+) lactose, D(+) xylose, dulcitol, myo-inositol, L(-) fructose, D(-) mannitol, D(+) glucose, D(+) arabinose, D(-) arabinose, cellobiose, D(+) maltose, D(+) raffinose, L(+) rhamnose, D(+) melibiose, D(-) ribose, adonitol, D(+) arabitol, L(-) arabitol, D(+) fucose, L(-) fucose, D(-) lyxose, L(+) lyxose, and L(-) lyxose, or amino acids, *e.g.*, glutamine, lysine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glycine, histidine, leucine, methionine, phenylalanine, proline, serine, threonine, tyrosine, valine, and taurine. Methods and materials for enhancing blood brain barrier penetration are described, *e.g.*, in U.S. Pat. No. 9,193,969, “Compositions and methods for selective delivery of oligonucleotide molecules to specific neuron types,” U.S. Pat. No. 4,866,042, “Method for the delivery of genetic material across the blood brain barrier,” U.S. Pat. No. 6,294,520, “Material for passage through the blood-brain barrier,” and U.S. Pat. No. 6,936,589, “Parenteral delivery systems,” each incorporated herein by reference.

[00169] In embodiments, an ASO of the invention is coupled to a dopamine reuptake inhibitor (DRI), a selective serotonin reuptake inhibitor (SSRI), a noradrenaline reuptake inhibitor (NRI), a norepinephrine-dopamine reuptake inhibitor (NDRI), and a serotonin-norepinephrine-dopamine reuptake inhibitor (SNDRI), using methods described in, *e.g.*, U.S. Pat. No. 9,193,969, incorporated herein by reference.

[00170] In embodiments, subjects treated using the methods and compositions are evaluated for improvement in condition using any methods known and described in the art.

Methods of Identifying Additional ASOs that Enhance Splicing

[00171] Also within the scope of the present invention are methods for identifying (determining) additional ASOs that enhance splicing of a *SYNGAP1* or *SCN1A* RIC pre-mRNA, specifically at the target intron. ASOs that specifically hybridize to different nucleotides within the target region of the pre-mRNA may be screened to identify (determine) ASOs that improve the rate and/or extent of splicing of the target intron. In some embodiments, the ASO may block or interfere with the binding site(s) of a splicing repressor(s)/silencer. Any method known in the art may be used to identify (determine) an ASO that when hybridized to the target region of the intron results in the desired effect (*e.g.*, enhanced splicing, protein or functional RNA production). These methods also can be used for identifying ASOs that enhance splicing of the retained intron by binding to a targeted region in an exon flanking the retained intron, or in a non-retained intron. An example of a method that may be used is provided below.

[00172] A round of screening, referred to as an ASO “walk” may be performed using ASOs that have been designed to hybridize to a target region of a pre-mRNA. For example, the ASOs used in the ASO walk can be tiled every 5 nucleotides from approximately 100 nucleotides upstream of the 5' splice site of the retained intron (*e.g.*, a portion of sequence of the exon located upstream of the target/retained intron) to approximately 100 nucleotides downstream of the 5' splice site of the target/retained intron 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.*, a portion of sequence of the exon located downstream of the target/retained intron). For example, a first ASO of 15 nucleotides in length may be designed to specifically hybridize to nucleotides +6 to +20 relative to the 5' splice site of the target/retained intron. A second ASO is designed to specifically hybridize to nucleotides +11 to +25 relative to the 5' splice site of the target/retained intron. ASOs are designed as such spanning the target region of the pre-mRNA. In embodiments, the ASOs can be tiled more closely, *e.g.*, every 1, 2, 3, or 4 nucleotides. Further, the ASOs can be tiled from 100 nucleotides downstream of the 5' splice site, to 100 nucleotides upstream of the 3' splice site. In some embodiments, the ASOs can be tiled from about 1,160 nucleotides upstream of the 5' splice site, to about 500 nucleotides downstream of the 5' splice site. In some embodiments, the ASOs can be tiled from about 500 nucleotides upstream of the 3' splice site, to about 1,920 nucleotides downstream of the 3' splice site.

[00173] One or more ASOs, or a control ASO (an ASO with a scrambled sequence, sequence that is not expected to hybridize to the target region) are delivered, for example by transfection, into a disease-relevant cell line that expresses the target pre-mRNA (*e.g.*, the RIC pre-mRNA described elsewhere herein). The splicing-inducing effects of each of the ASOs may be assessed by any method known in the

art, for example by reverse transcriptase (RT)-PCR using primers that span the splice junction, as described herein (see, *e.g.*, Example 1). A reduction or absence of the RT-PCR product produced using the primers spanning the splice junction in ASO-treated cells as compared to in control ASO-treated cells indicates that splicing of the target intron has been enhanced. In some embodiments, the splicing efficiency, the ratio of spliced to unspliced pre-mRNA, the rate of splicing, or the extent of splicing may be improved using the ASOs described herein. The amount of protein or functional RNA that is encoded by the target pre-mRNA can also be assessed to determine whether each ASO achieved the desired effect (*e.g.*, enhanced protein production). Any method known in the art for assessing and/or quantifying protein production, such as Western blotting, flow cytometry, immunofluorescence microscopy, and ELISA, can be used.

[00174] A second round of screening, referred to as an ASO “micro-walk” may be performed using ASOs that have been designed to hybridize to a target region of a pre-mRNA. The ASOs used in the ASO micro-walk are tiled every 1 nucleotide to further refine the nucleotide acid sequence of the pre-mRNA that when hybridized with an ASO results in enhanced splicing.

[00175] Regions defined by ASOs that promote splicing of the target intron are explored in greater detail by means of an ASO “micro-walk”, involving ASOs spaced in 1-nt steps, as well as longer ASOs, typically 18-25 nt.

[00176] As described for the ASO walk above, the ASO micro-walk is performed by delivering one or more ASOs, or a control ASO (an ASO with a scrambled sequence, sequence that is not expected to hybridize to the target region), for example by transfection, into a disease-relevant cell line that expresses the target pre-mRNA. The splicing-inducing effects of each of the ASOs may be assessed by any method known in the art, for example by reverse transcriptase (RT)-PCR using primers that span the splice junction, as described herein (see, *e.g.*, Example 1). A reduction or absence of the RT-PCR product produced using the primers spanning the splice junction in ASO-treated cells as compared to in control ASO-treated cells indicates that splicing of the target intron has been enhanced. In some embodiments, the splicing efficiency, the ratio of spliced to unspliced pre-mRNA, the rate of splicing, or the extent of splicing may be improved using the ASOs described herein. The amount of protein or functional RNA that is encoded by the target pre-mRNA can also be assessed to determine whether each ASO achieved the desired effect (*e.g.*, enhanced protein production). Any method known in the art for assessing and/or quantifying protein production, such as Western blotting, flow cytometry, immunofluorescence microscopy, and ELISA, can be used.

[00177] ASOs that when hybridized to a region of a pre-mRNA result in enhanced splicing and increased protein production may be tested *in vivo* using animal models, for example transgenic mouse models in which the full-length human gene has been knocked-in or in humanized mouse models of disease. Suitable routes for administration of ASOs may vary depending on the disease and/or the cell types to which delivery of the ASOs is desired. ASOs may be administered, for example, by intrathecal injection, intracerebroventricular injection, intraperitoneal injection, intramuscular injection, subcutaneous injection, or intravenous injection. Following administration, the cells, tissues, and/or organs of the model animals

may be assessed to determine the effect of the ASO treatment by for example evaluating splicing (efficiency, rate, extent) and protein production by methods known in the art and described herein. The animal models may also be any phenotypic or behavioral indication of the disease or disease severity. [00178] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

EXAMPLES

[00179] The present invention will be more specifically illustrated by the following Examples. However, it should be understood that the present invention is not limited by these examples in any manner.

Example 1: Identification of Intron Retention Events in *SYNGAP1* Transcripts by RNAseq using Next Generation Sequencing

[00180] Whole transcriptome shotgun sequencing was carried out using next generation sequencing to reveal a snapshot of transcripts produced by the *SYNGAP1* gene to identify intron-retention events. For this purpose, polyA+ RNA from nuclear and cytoplasmic fractions of HCN (human cortical neurons) was isolated and cDNA libraries constructed using Illumina's TruSeq Stranded mRNA library Prep Kit. The libraries were pair-end sequenced resulting in 100-nucleotide reads that were mapped to the human genome (Feb. 2009, GRCh37/hg19 assembly). The sequencing results for *SYNGAP1* are shown in FIG. 3. Briefly, FIG. 3 shows the mapped reads visualized using the UCSC genome browser (operated by the UCSC Genome Informatics Group (Center for Biomolecular Science & Engineering, University of California, Santa Cruz, 1156 High Street, Santa Cruz, CA 95064) and described by, *e.g.*, Rosenbloom, *et al.*, 2015, "The UCSC Genome Browser database: 2015 update," *Nucleic Acids Research* 43, Database Issue, doi: 10.1093/nar/gku1177) and the coverage and number of reads can be inferred by the peak signals. The height of the peaks indicates the level of expression given by the density of the reads in a particular region. A schematic representation of *SYNGAP1* (drawn to scale) is provided by the UCSC genome browser (below the read signals) so that peaks can be matched to *SYNGAP1* exonic and intronic regions. Based on this display, we identified three introns (15, 18 and 19 indicated by arrows; corresponding to NM_006772 intron 15, NM_006772 intron 18, and NM_006772 intron 19, respectively) that have high read density in the nuclear fraction of HCN, but have very low to no reads in the cytoplasmic fraction of these cells (as shown for intron 15 in the bottom diagram of FIG. 3 and for introns 18 and 19 in the bottom diagram of FIG. 5). This indicates that these introns are retained and that the intron-15, intron-18, and intron-19 containing transcripts remain in the nucleus, and suggests that these retained *SYNGAP1* RIC pre-mRNAs are non-productive, as they are not exported out to the cytoplasm.

Example 2: Design of ASO-Walk Targeting Intron 15 of *SYNGAP1*

[00181] An ASO walk was designed to target intron 15 using the method described herein (FIG. 4; Table 1, SEQ ID NOs: 1510 to 1814 and 2287 to 2591). A region immediately upstream and downstream of the intron 15 5' splice site spanning nucleotides +251 to -1,054e and a region immediately upstream and downstream of intron 15 3' splice site spanning nucleotides -256 to +157e were utilized to design ASOs to target retained intron 15 *SYNGAP1* RIC pre-mRNAs. Table 1 lists exemplary ASOs that were designed and their target sequences. From this design, 2'-O-Me RNA, PS backbone, 18-mer ASOs shifted by 5-nucleotide intervals were produced and utilized to target *SYNGAP1* RIC pre-mRNAs to increase *SYNGAP1* protein production (see FIG. 4 and FIG. 7B).

Table 1. List of ASOs targeting *SYNGAP1*

Gene SEQ ID NO:	Pre-mRNA SEQ ID NO:	ASOs	Retained Intron	Target Sequence SEQ ID NO:
<i>SYNGAP1</i> SEQ ID NO. 2	<i>SYNGAP1</i> :NM_006772 SEQ ID NO. 8	SEQ ID NOs: 1038 - 1509	Intron 18	SEQ ID NO: 2594
		SEQ ID NOs: 1510 - 1814	Intron 15	SEQ ID NO: 2592
<i>SYNGAP1</i> SEQ ID NO. 3	<i>SYNGAP1</i> :NM_006772 SEQ ID NO. 9	SEQ ID NOs: 1815 - 2286	Intron 18	SEQ ID NO: 2595
		SEQ ID NOs: 2287 - 2591	Intron 15	SEQ ID NO: 2592

Example 3: Design of ASO-Walk Targeting Intron 18 and 19 of *SYNGAP1*

[00182] An ASO walk was designed to target introns 18 and 19 using the method described herein (FIG. 6; Table 1, SEQ ID NOs: 1815 to 2286 and 1038 to 1509). A region immediately upstream and downstream of the intron 18 5' splice site spanning nucleotides +499 to -73e to and a region immediately upstream and downstream of the intron 19 3' splice site spanning nucleotides -496 to +1,912e were utilized to design ASOs to target retained intron 18 or 19 *SYNGAP1* RIC pre-mRNAs. Table 1 lists exemplary ASOs that were designed and their target sequences. From this design, 2'-O-Me RNA, PS backbone, 18-mer ASOs shifted by 5-nucleotide intervals were produced and utilized to target *SYNGAP1* RIC pre-mRNAs to increase *SYNGAP1* protein production (see FIG. 6 and FIGS. 7D-E). The splice site intronic regions flanking alternative exon 19 are not targeted to avoid affecting the inclusion level of exon 19.

Example 4: Improved Splicing Efficiency via ASO-Targeting of *SYNGAP1* Introns 15, 18, or 19 Increases Transcript Levels

[00183] To determine an increase in target gene intron splicing efficiency with ASOs we used the method described herein. ARPE-19 cells, a human retinal epithelium cell line (American Type Culture Collection (ATCC), USA) were mock-transfected, or transfected with the targeting ASOs described in Examples 2 and 3. Cells were transfected using Lipofectamine RNAiMax transfection reagent (Thermo Fisher)

according to vendor's specifications. Briefly, ASOs were plated in 96-well tissue culture plates and combined with RNAiMax diluted in Opti-MEM. Cells were detached using trypsin and resuspended in full medium, and approximately 25,000 cells were added the ASO-transfection mixture. Transfection experiments were carried out in triplicate plate replicates. Final ASO concentration was 80 nM. Media was changed 6h post-transfection, and cells harvested at 24h, using the Cells-to-Ct lysis reagent, supplemented with DNase (Thermo Fisher), according to vendor's specifications. cDNA was generated with Cells-to-Ct RT reagents (Thermo Fisher) according to vendor's specifications. To quantify the amount of splicing at the intron of interest, quantitative PCR was carried out using Taqman assays with probes spanning the corresponding exon-exon junction (Thermo Fisher). Taqman assays were carried out according to vendor's specifications, on a QuantStudio 7 Flex Real-Time PCR system (Thermo Fisher). Target gene assay values were normalized to RPL32 (deltaCt) and plate-matched mock transfected samples (delta-delta Ct), generating fold-change over mock quantitation ($2^{-(\text{delta-delta Ct})}$). Average fold-change over mock of the three plate replicates for ASOs targeting retained intron 15 is plotted in FIG. 7B. ASOs targeted to positions -31 and -36 demonstrated increased target gene expression by >1.5-fold, implying an increase in splicing at that target intron. Average fold-change over mock of the three plate replicates for ASOs targeting retained intron 18 is plotted in FIG. 7D. ASOs targeted to positions +36 and +41 demonstrated increased target gene expression by >~2-fold, implying an increase in splicing at that target intron. Average fold-change over mock of the three plate replicates for ASOs targeting retained intron 19 is plotted in FIG. 7E. ASOs targeted to positions -55 and -60 demonstrated increased target gene expression by >2-fold, implying an increase in splicing at that target intron. Together with whole transcriptome data confirming retention of the target intron (Example 1), these results confirm that ASOs can improve the splicing efficiency of a rate limiting intron.

Example 5: Identification of Intron Retention Events in *SCN1A* Transcripts by RNAseq Using Next Generation Sequencing

[00184] *SCN1A* intron 21 retention was confirmed using an RT-PCR assay described in Figure 8C.

Intron 21 is retained in SK-N-AS and RenCell VM cells indicated by the presence of a band in the nuclear fraction.

Example 6: Design of ASO-Walk Targeting Intron 21 of *SCN1A*

[00185] An ASO walk was designed to target intron 21 using the method described herein (Table 2, SEQ ID NOs: 10-266 and 524-1037). A region immediately upstream and downstream of the intron 21 5' splice site spanning nucleotides -264e to +496 and a region immediately upstream and downstream of intron 21 3' splice site spanning nucleotides -496 to +37e were utilized to design ASOs to target retained intron 21 *SCN1A* RIC pre-mRNAs. Table 2 lists exemplary ASOs that were designed and their target sequences. From this design, 2'-O-Me RNA, PS backbone, 18-mer ASOs shifted by 5-nucleotide intervals were produced and utilized to target *SCN1A* RIC pre-mRNAs to increase *SCN1A* protein production (see FIGS. 8D-E, Table 3-4).

Table 2. List of ASOs targeting *SCN1A*

Gene SEQ ID NO:	Pre-mRNA SEQ ID NO:	ASOs	Retained Intron	Target Sequence SEQ ID NO:
<i>SCN1A</i> SEQ ID NO. 1	<i>SCN1A</i> :NM_006920 SEQ ID NO. 4	SEQ ID NOs: 10 - 266	Intron 21	SEQ ID NO: 2593
	<i>SCN1A</i> :NM_00120243 5 SEQ ID NO. 5	SEQ ID NOs: 267 - 523	Intron 23	SEQ ID NO: 2593
	<i>SCN1A</i> :NM_00116596 4 SEQ ID NO. 6	SEQ ID NOs: 524 - 780	Intron 21	SEQ ID NO: 2593
	<i>SCN1A</i> :NM_00116596 3 SEQ ID NO. 7	SEQ ID NOs: 781 - 1037	Intron 21	SEQ ID NO: 2593

Table 3. *SCN1A* Intron 21 ASO Macrowalk

SEQ ID NO:	Name	Sequence (5'-3')	Retained Intron
63	SCN1A-IVS21+6	CAGAGAAAAUAGUGUUCA	21
64	SCN1A-IVS21+11	AUAUUCAGAGAAAAUAGU	21
65	SCN1A-IVS21+16	UAAAAUAUUCAGAGAAA	21
66	SCN1A-IVS21+21	AACAAUAAAAUAUUCAG	21
67	SCN1A-IVS21+26	UUCCAAACAAUAAAAUA	21
68	SCN1A-IVS21+31	UAUUAUCCAAACAAUAA	21
69	SCN1A-IVS21+36	UUUGUUAUUAUCCAAAC	21
70	SCN1A-IVS21+41	AUUAUUUUGUUAUUAUUC	21
71	SCN1A-IVS21+46	AUGUCAUUAUUUUGUUAU	21
72	SCN1A-IVS21+51	GAUGUAUGUCAUUAUUUU	21
73	SCN1A-IVS21+56	UAAUAGAUGUAUGUCAUU	21
74	SCN1A-IVS21+61	CUAAAUAUAGAUGUAUG	21
75	SCN1A-IVS21+66	AGGAACUAAAUAUAGAU	21
76	SCN1A-IVS21+71	UUCUUAGGAACUAAUAA	21
77	SCN1A-IVS21+76	ACUUUUUCUUAGGAACUA	21
78	SCN1A-IVS21+81	UAUAUACUUUUUCUUAGG	21
258	SCN1A-IVS21-16	UGCAUGUUUUACUUUGGA	21
257	SCN1A-IVS21-21	GUUUUACUUUGGAGUAAA	21
256	SCN1A-IVS21-26	ACUUUGGAGUAAAAUAA	21

SEQ ID NO:	Name	Sequence (5'-3')	Retained Intron
255	SCN1A-IVS21-31	GGAGUAAAAAUAAUUUAG	21
254	SCN1A-IVS21-36	AAAAAUAAUUUAGACCUG	21
253	SCN1A-IVS21-41	UAAUUUAGACCUGAUGUU	21
252	SCN1A-IVS21-46	UAGACCUGAUGUUUAAUA	21
251	SCN1A-IVS21-51	CUGAUGUUUAAUAAAUAU	21
250	SCN1A-IVS21-56	GUUUAAUAAAUUUCUUA	21
249	SCN1A-IVS21-61	AUAAAUUUCUUACUGAU	21
248	SCN1A-IVS21-66	UAUUCUUACUGAUUAAU	21
247	SCN1A-IVS21-71	UUACUGAUUAAUUUUC	21
246	SCN1A-IVS21-76	GAUAAUUUUUCAAAGG	21
245	SCN1A-IVS21-81	AAUUUCAAAGGGAAUA	21

Table 4. SCN1A Intron 21 ASO Microwalk

SEQ ID NO:	Name	Sequence (5'-3')	Retained Intron
2596	SCN1A-IVS21-27	CUUUGGAGUAAAAUAAU	21
2597	SCN1A-IVS21-28	UUUGGAGUAAAAUAAUU	21
2598	SCN1A-IVS21-29	UUGGAGUAAAAUAAUUU	21
2599	SCN1A-IVS21-30	UGGAGUAAAAUAAUUUA	21
2600	SCN1A-IVS21-32	GAGUAAAAUAAUUUAGA	21
2601	SCN1A-IVS21-33	AGUAAAAUAAUUUAGAC	21
2602	SCN1A-IVS21-34	GUAAAAUAAUUUAGACC	21
2603	SCN1A-IVS21-35	UAAAAUAAUUUAGACCU	21
2604	SCN1A-IVS21-72	UACUGAUUAAUUUUCAA	21
2605	SCN1A-IVS21-73	ACUGAUUAAUUUUCAAA	21
2606	SCN1A-IVS21-74	CUGAUUAAUUUUCAAAA	21
2607	SCN1A-IVS21-75	UGAUUAAUUUUCAAAAG	21
2608	SCN1A-IVS21-77	AUAUAAUUUUCAAAAGGG	21
2609	SCN1A-IVS21-78	UAUAUAAUUUUCAAAAGGGA	21
2610	SCN1A-IVS21-79	AUAUAAUUUUCAAAAGGGAA	21

2611	SCN1A-IVS21-80	UAAUUUUCAAAAGGGAAU	21
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Example 7: Design of ASO-Walk Targeting Intron 23 of *SCN1A*

[00186] An ASO walk can be designed to target intron 23 using the methods described herein (Table 2, SEQ ID NOs: 267-523). A region immediately upstream and downstream of the intron 23 5' splice site spanning nucleotides -264e to +496 and a region immediately upstream and downstream of intron 23 3' splice site spanning nucleotides -496 to +37e are utilized to design ASOs to target retained intron 23 *SCN1A* RIC pre-mRNAs. Table 2 lists exemplary ASOs that are designed and their target sequences. From this design, 2'-O-Me RNA, PS backbone, 18-mer ASOs shifted by 5-nucleotide intervals can be produced and utilized to target *SCN1A* RIC pre-mRNAs to increase SCN1A protein production.

Example 8: Improved Splicing Efficiency via ASO-Targeting of *SCN1A* Retained Introns Increases Transcript Levels

[00187] To determine whether we can achieve an increase in *SCN1A* expression by improving splicing efficiency of *SCN1A* intron 21 using ASOs, RT-PCR products are evaluated by Taqman RT-qPCR. To this end, cells are mock-transfected, or transfected with ASOs described for intron 21 in Table 3, independently, using RNAiMAX (Invitrogen) delivery reagent. Experiments are performed using 80 nM ASOs for 24 hrs. To quantify the amount of splicing at the intron of interest, quantitative PCR was carried out using Taqman assays with probes spanning the corresponding exon-exon junction (Thermo Fisher). Taqman assays were carried out according to vendor's specifications, on a QuantStudio 7 Flex Real-Time PCR system (Thermo Fisher). Target gene assay values were normalized to RPL32 (deltaCt) and plate-matched mock transfected samples (delta-delta Ct), generating fold-change over mock quantitation ($2^{-(\text{delta-delta Ct})}$). Average fold-change over mock of the three plate replicates is plotted in FIGS. 8D-E. Several ASOs were identified that increase the target gene expression by $>\sim 1.25$ -fold implying an increase in splicing at that target intron.

[00188] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising an antisense oligomer (ASO), wherein the ASO is complementary to a targeted portion of a retained-intron-containing pre-mRNA (RIC pre-mRNA) that encodes SCN1A protein, wherein the RIC pre-mRNA comprises a retained intron, an exon flanking 5' splice site of the retained intron, and an exon flanking 3' splice site of the retained intron, and wherein binding of the ASO to the RIC pre-mRNA will cause a retained intron to be constitutively spliced from the RIC pre-mRNA in cells that express the RIC pre-mRNA, thereby increasing the level of functional RNA encoding SCN1A protein in the cells.
2. A pharmaceutical composition according to claim 1 for use as a medicament.
3. Use of a pharmaceutical composition according to claim 1 in the manufacture of a medicament for treating Dravet Syndrome (DS) in a subject.
4. A pharmaceutical composition according to claim 1 or 2, or a use according to claim 3, wherein the retained intron is intron 21 and the targeted portion of the RIC pre-mRNA is within the region +6 relative to the 5' splice site of the retained intron 21 to -16 relative to the 3' splice site of the retained intron 21.
5. A pharmaceutical composition according to claim 1, claim 2 or claim 4 or a use according to claims 3 or 4, wherein the RIC pre-mRNA comprises a sequence with at least 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to a sequence selected from SEQ ID NOs: 4-7.
6. A pharmaceutical composition according to claim 1, claim 2, claim 4 or claim 5, or a use according to any of claims 3 to 5, wherein the targeted portion of the RIC pre-mRNA comprises a sequence with at least 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to a region comprising at least 8 contiguous nucleic acids of SEQ ID NO: 2593.
7. A pharmaceutical composition according to claim 1, claim 2, or any of claims 4 to 6, or a use according to any of claims 3 to 6, wherein the ASO comprises a sequence with at least 80%, 85%, 90%, 95%, 97%, or 100% identity to a sequence selected from SEQ ID NOs: 246, 255, 10-245, 247-254, 256-266 or 524-1037, preferably SEQ ID NOs: 246 or 255.
8. A pharmaceutical composition according to claim 1, claim 2 or any of claims 4 to 7, or a use according to any of claims 3 to 7, wherein either:

i) the SCN1A protein is produced in a form having reduced function compared to an equivalent wild-type protein; or

ii) the SCN1A protein is produced in a form that is fully-functional compared to an equivalent wild-type protein.

9. A pharmaceutical composition according to claim 1, claim 2 or any of claims 4 to 8, or a use according to any of claims 3 to 8, wherein the ASO comprises a backbone modification comprising a phosphorothioate linkage or a phosphorodiamidate linkage; or a modified sugar moiety or a sugar analog, optionally comprising a phosphorodiamidate morpholino, a locked nucleic acid, a peptide nucleic acid, a 2'-O-methyl moiety, a 2'-Fluoro moiety, or a 2'-O-methoxyethyl moiety.

10. A pharmaceutical composition according to claim 1, claim 2 or any of claims 4 to 9, or a use according to any of claims 3 to 9, wherein the ASO consists of from 8 to 50 nucleobases.

11. A pharmaceutical composition according claim 2, or a use according to any of claims 3 to 10, wherein the SCN1A protein is deficient in amount or activity in the subject to be treated.

12. A pharmaceutical composition according to claim 2, or a use according to any of claims 3 to 11, wherein the SCN1A protein is deficient in amount or activity in the subject to be treated, and the deficient amount of the SCN1A protein is caused by haploinsufficiency of the SCN1A protein, wherein the subject has a first allele encoding a functional SCN1A protein, and a second allele from which the SCN1A protein is not produced, or a second allele encoding a nonfunctional SCN1A protein, and wherein the ASO binds to a targeted portion of a RIC pre-mRNA transcribed from the first allele.

13. A pharmaceutical composition according to claim 2, or a use according to any of claims 3 to 11, wherein the SCN1A protein is deficient in amount or activity in the subject to be treated, and the subject has

a. a first mutant allele from which

- i. the SCN1A protein is produced at a reduced level compared to production from a wild-type allele,
- ii. the SCN1A protein is produced in a form having reduced function compared to an equivalent wild-type SCN1A protein, or
- iii. the SCN1A protein is not produced, and

b. a second mutant allele from which

- i. the SCN1A protein is produced at a reduced level compared to production from a wild-type allele,

- ii. the SCN1A protein is produced in a form having reduced function compared to an equivalent wild-type SCN1A protein, or
- iii. the SCN1A protein is not produced, and

wherein when the subject has first mutant allele a.iii., the second mutant allele is b.i. or b.ii., wherein when the subject has a second mutant allele b.iii., the first mutant allele is a.i. or a.ii., and wherein the RIC pre-mRNA is transcribed from the first mutant allele that is a.i. or a.ii. or the second allele that is b.i. or b.ii.

14. A pharmaceutical composition according to claim 2, or a use according to any of claims 3 to 13, wherein the ASO is to be administered by intrathecal injection, intracerebroventricular injection, intraperitoneal injection, intramuscular injection, subcutaneous injection, or intravenous injection of the subject.

15. A pharmaceutical composition according to claim 1, claim 2 or any of claims 4 to 8, or a use according to any of claims 3 to 14, wherein the ASO does not increase the amount of SCN1A protein or functional RNA encoding SCN1A protein in the cells by modulating alternative splicing of pre-mRNA transcribed from a gene encoding the functional RNA or SCN1A protein.