

COMPOSITIONS AND METHODS FOR MODULATING SPLICING AND PROTEIN EXPRESSION

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 62/888,887, filed August 19, 2019, and U.S. Provisional Application No. 63/049,262, filed July 08, 2020, each of which is incorporated herein by reference in its entirety.

REFERENCE TO SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on August 19, 2020, is named 47991-727_601_SL.txt and is 17,694,293 bytes in size.

BACKGROUND

[0003] Alternative splicing events in genes can lead to non-productive or less productive mRNA transcripts, and therapeutic agents which can target the alternative splicing events in genes can modulate the expression level of functional proteins in patients and/or inhibit aberrant protein expression. Such therapeutic agents can be used to treat a condition or disease caused by protein deficiency.

SUMMARY

[003a] In one aspect, disclosed herein is a composition comprising a non-sense mediated RNA decay alternative exon (NSAE)-modulating agent that interacts with a target motif within a pre-processed mRNA transcript to modulate splicing at an alternative 5' or 3' splice site of a pre-processed mRNA transcript in a cell, thereby promoting inclusion of a canonical exon in a processed mRNA transcript that is processed from the pre-processed mRNA transcript, and promoting exclusion of an alternative exon from the pre-processed mRNA transcript in the cell, wherein the target motif is located: (i) in an intronic region between two canonical exons, (ii) in one of the two canonical exons, or (iii) in a region spanning both an intron and canonical exon; and wherein the alternative exon comprises: (1) only a portion of a canonical exon, or (2) a canonical exon and at least a portion of an intron upstream of the canonical exon and adjacent to the canonical exon.

[003b] In another aspect, disclosed herein is the use of the composition disclosed herein in manufacturing a medicament for treating or preventing a disease or condition in a subject in need thereof, wherein the disease or condition is a disease or condition caused by a deficient amount or activity of the target protein.

[003c] In another aspect, disclosed herein is the use of the composition disclosed herein in manufacturing a medicament for modulating expression of the target protein.

[003d] In yet another aspect, disclosed herein is a pharmaceutical composition comprising the composition disclosed herein, and a pharmaceutically acceptable excipient and/or a delivery vehicle.

[003e] In yet another aspect, disclosed herein is a composition comprising a non-sense mediated RNA decay alternative exon (NSAE)-modulating agent that interacts with a target motif within a pre-processed

mRNA transcript to modulate splicing at an alternative 5' or 3' splice site of a pre-processed mRNA transcript, thereby modulating inclusion of a canonical exon in a processed mRNA transcript that is processed from the pre-processed mRNA transcript, and modulating exclusion of an alternative exon from the pre-processed mRNA transcript, wherein the target motif is located: (i) in an intronic region between two canonical exons, (ii) in one of the two canonical exons, or (iii) in a region spanning both an intron and canonical exon; and wherein the alternative exon comprises: (1) only a portion of a canonical exon, or (2) a canonical exon and at least a portion of an intron adjacent to the canonical exon.

[003f] In yet another aspect, disclosed herein is a composition comprising a modified antisense oligonucleotide sequence or a viral delivery system encoding a polynucleotide comprising an antisense oligonucleotide sequence, wherein the antisense oligonucleotide sequence has at least about 80% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1-165 and 1301-10225.

[003g] In yet another aspect, disclosed herein is the use of the composition disclosed herein in manufacturing a medicament for treating or preventing a disease or condition in a subject in need thereof, wherein the disease or condition is a disease or condition caused by a deficient amount or activity of the target protein.

[003h] In yet another aspect, disclosed herein is the composition disclosed herein for use in therapy.

[0004] In some examples, provided herein is a composition comprising a non-sense mediated RNA decay alternative exon (NSAE)-modulating agent that interacts with a target motif within a pre-processed mRNA transcript to modulate exclusion of an NSAE from a processed mRNA transcript and to modulate inclusion of a canonical exon in the processed mRNA transcript, wherein the target motif is located (i) in an intronic region between two canonical exons, (ii) in one of the two canonical exons, or (iii) in a region spanning both an intron and canonical exon; and wherein the NSAE comprises (i) only a portion of a canonical exon, or (ii) a canonical exon and at least a portion of an intron adjacent to the canonical exon; wherein the NSAE-modulating agent modulates exclusion of an NSAE from the processed mRNA transcript and modulates inclusion of a canonical exon in the processed mRNA transcript.

[0005] In some examples, provided herein is a composition comprising a non-sense mediated RNA decay alternative exon (NSAE)-modulating agent that modulates expression of a target protein in a cell comprising a pre-processed mRNA transcript (pre-mRNA) that encodes the target protein and comprises: an alternative nonsense mediated RNA decay-inducing (NMD) exon comprising an alternative 5' splice site downstream of the 5' splice site of a canonical exon and within the canonical exon, or upstream of the 5' splice site of the canonical exon and within an intron, wherein the NSAE-modulating agent modulates processing of an mRNA transcript from the pre-processed mRNA transcript by modulating splicing of the pre-mRNA at the 5' alternative splice site, wherein the splicing of the pre-mRNA at the 5' alternative splice site modulates the expression of the target protein in a cell.

[0006] In some examples, provided herein is a composition comprising a non-sense mediated RNA decay alternative exon (NSAE)-modulating agent that modulates expression of a target protein in a cell comprising a pre-processed mRNA transcript (pre-mRNA) that encodes the target protein and comprises: an alternative nonsense mediated RNA decay-inducing (NMD) exon comprising an alternative 3' splice site upstream of the 3' splice site of a canonical exon and within the canonical exon, or downstream of the 3' splice site of the canonical exon and within an intron, wherein the NSAE-modulating agent modulates processing of an mRNA transcript from the pre-processed mRNA transcript by modulating splicing of the pre-mRNA at the 3' alternative splice site, and wherein the splicing of the pre-mRNA at the 3' alternative splice site modulates the expression of the target protein in a cell.

[0007] In some examples, provided herein is a composition comprising a non-sense mediated RNA decay alternative 5' or 3' splice site (NSASS)-modulating agent that interacts with a target motif within a pre-processed mRNA transcript to modulate splicing at an alternative 5' or 3' splice site of a pre-processed mRNA transcript and to modulate inclusion of a canonical exon in a processed mRNA transcript that is processed from the pre-processed mRNA transcript, wherein the target motif is located in an intronic region between two canonical exons, in one of the two canonical exons, or in a region spanning both an intron and canonical exon; wherein modulating splicing at the alternative 5' or 3' splice site of the pre-processed mRNA transcript modulates exclusion of an alternative exon from the processed mRNA transcript, wherein the alternative exon comprises only a portion of a canonical exon, or a canonical exon and at least a portion of an intron adjacent to the canonical exon; and wherein the NSASS-modulating agent modulates exclusion of the alternative exon from the processed mRNA transcript and modulates inclusion of a canonical exon in the processed mRNA transcript.

[0008] In some examples, provided herein is a composition comprising a non-sense mediated RNA decay alternative 5' or 3' splice site (NSASS)-modulating agent that modulates expression of a target protein in a cell comprising a pre-processed mRNA transcript (pre-mRNA) that encodes the target protein, wherein the pre-mRNA comprises an alternative exon comprising an alternative 5' splice site downstream of the 5' splice site of a canonical exon and within the canonical exon, or upstream of the 5' splice site of the canonical exon and within an intron; wherein the NSASS-modulating agent modulates processing of an mRNA transcript from the pre-processed mRNA transcript by modulating splicing of the pre-mRNA at the 5' alternative splice site, wherein the splicing of the pre-mRNA at the 5' alternative splice site modulates the expression of the target protein in a cell.

[0009] In some examples, provided herein is a composition comprising a non-sense mediated RNA decay alternative exon (NSASS)-modulating agent that modulates expression of a target protein in a cell comprising a pre-processed mRNA transcript (pre-mRNA) that encodes the target protein, wherein the pre-mRNA comprises an alternative exon comprising an alternative 3' splice site upstream of the 3' splice site of a canonical exon and within the canonical exon, or downstream of the 3' splice site of the canonical exon and within an intron, wherein the NSASS-modulating agent modulates processing of an mRNA transcript from the pre-processed mRNA transcript by modulating splicing of the pre-mRNA at the 3'

alternative splice site, and wherein the splicing of the pre-mRNA at the 3' alternative splice site modulates the expression of the target protein in a cell.

[0010] In some examples, the agent is a small molecule. In some examples the agent is a polypeptide. In some examples, the polypeptide is a nucleic acid binding protein. In some examples, the nucleic acid binding protein contains a TAL-effector or zinc finger binding domain. In some examples, the nucleic acid binding protein is a Cas family protein. In some examples, the polypeptide is accompanied by or complexed with one or more nucleic acid molecules. In some examples, the Agent is an antisense oligomer (ASO) complementary to the targeted region of the pre-mRNA. In some examples, the Agent is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, complementary to the targeted region of the pre-mRNA encoding the target protein. In some examples, the Agent is an antisense oligomer, and wherein the Agent comprises a backbone modification comprising a phosphorothioate linkage or a phosphorodiamidate linkage. In some examples, the Agent is an antisense oligomer, and wherein the Agent comprises a phosphorodiamidate morpholino. In some examples, the Agent is an antisense oligomer, and wherein the Agent comprises a locked nucleic acid. In some examples, the Agent is an antisense oligomer, and wherein the Agent comprises a peptide nucleic acid. In some examples, the Agent is an antisense oligomer, and wherein the Agent comprises a 2'-O-methyl. In some examples, the Agent is an antisense oligomer, and wherein the Agent comprises a 2'-Fluoro, or a 2'-O-methoxyethyl moiety. In some examples, the Agent is an antisense oligomer, and wherein the Agent comprises at least one modified sugar moiety. In some examples, each sugar moiety is a modified sugar moiety. In some examples, the Agent is an antisense oligomer, and wherein the Agent 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. Described herein, in some examples, is a composition of a nucleic acid molecule that encodes for the NSAE-modulating agent. In some examples, the nucleic acid molecule is incorporated into a viral delivery system. In some examples, the viral delivery system is an adenovirus-associated vector.

[0011] In some examples, provided herein is a method of modulating protein expression, comprising: (a) contacting a non-sense mediated RNA decay alternative exon (NSAE)-modulating agent to a target motif within a pre-processed mRNA transcript, wherein the NSAE comprises (i) only a portion of a canonical exon, or (ii) a canonical exon and at least a portion of an intron adjacent to the canonical exon; (b) processing a mRNA transcript from the pre-processed mRNA transcript thereby forming a processed mRNA transcript, wherein the NSAE-modulating agent modulates exclusion of an NSAE from the

processed mRNA transcript and modulates inclusion of the canonical exon in the processed mRNA transcript; and (c) translating the processed mRNA transcript wherein the exclusion of the NSAE and inclusion of the canonical exon modulates protein expression relative to the protein expression of an equivalent mRNA transcript comprising the NSAE instead of the canonical exon. In some examples, the target motif is located in an intronic region between two canonical exons. In some examples, the target motif is located in one of the two canonical exons. In some examples, the target motif is located in a region spanning both an intron and a canonical exon.

[0012] In some examples, provided herein is a method of modulating expression of a target protein by a cell having a pre-processed mRNA transcript (pre-mRNA) that encodes the target protein and comprises: an alternative nonsense mediated RNA decay-inducing (NMD) exon comprising an alternative 3' splice site upstream of the 3' splice site of a canonical exon and within the canonical exon, or downstream of the 3' splice site of the canonical exon and within an intron, the method comprising contacting a non-sense mediated RNA decay alternative exon (NSAE)-modulating agent to the cell, wherein the non-sense mediated RNA decay alternative exon (NSAE)-modulating agent modulates processing of an mRNA transcript from the pre-processed mRNA transcript by modulating splicing of the pre-mRNA at the 3' alternative splice site, and wherein the splicing of the pre-mRNA at the 3' alternative splice site modulates the expression of the target protein.

[0013] In some examples, provided herein is a method of modulating expression of a target protein by a cell having a pre-processed mRNA transcript (pre-mRNA) that encodes the target protein and comprises: an alternative nonsense mediated RNA decay-inducing (NMD) exon comprising an alternative 5' splice site downstream of the 5' splice site of a canonical exon and within the canonical exon, or upstream of the 5' splice site of the canonical exon and within an intron, the method comprising contacting a non-sense mediated RNA decay alternative exon (NSAE)-modulating agent to the cell, wherein the non-sense mediated RNA decay alternative exon (NSAE)-modulating agent modulates processing of an mRNA transcript from the pre-processed mRNA transcript by modulating splicing of the pre-mRNA at the 5' alternative splice site, and wherein the splicing of the pre-mRNA at the 5' alternative splice site modulates the expression of the target protein.

[0014] In some examples, the non-sense mediated RNA decay alternative exon (NSAE)-modulating agent binds to a targeted portion of the pre-processed mRNA transcript. In some examples, the wherein the non-sense mediated RNA decay alternative exon (NSAE)-modulating agent binds to a factor involved in splicing of the NSAE or NMD exon. In some examples, the wherein the non-sense mediated RNA decay alternative exon (NSAE)-modulating agent inhibits activity of a factor involved in splicing of the NMD exon. In some examples, the wherein the non-sense mediated RNA decay alternative exon (NSAE)-modulating agent interferes with binding of a factor involved in splicing of the NMD exon to a region of the targeted portion of the pre-processed mRNA transcript. In some examples, modulation of splicing of the pre-mRNA increases the expression of the target protein. In some examples, the level the target protein in the cell 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 level of processed mRNA encoding the target protein in a control cell. In some examples, modulation of splicing of the pre-mRNA increases production of the processed mRNA encoding the target protein. In some examples, the level of processed mRNA encoding the target protein in the cell contacted with the therapeutic agent is increased about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to the level of processed mRNA encoding the target protein in a control cell. In some examples, the target protein is the canonical isoform of the protein. In some examples, the target protein is SynGAP1. In some examples, the target protein is any one selected from the group consisting of ABCA5, ABCA7, ABCC3, ABCC5, ABCC8, ABCD1, ABR, ACAD9, ACAP1, ACOX2, ACSF3, ACTN4, ADAM17, ADAMTS13, AKR1E2, ALAD, ALG3, ANKRD29, ANKS3, ANO4, AP3B2, AP3M1, AP5Z1, ARNTL, ASAP3, ATRX, BBS2, BBS4, BRD9, CALM3, CARS1, CASP9, CATSPER1, CCDC40, CCDC88B, CD4, CD58, CES2, CHFR, CLCN2, CNOT3, CNTROB, COL5A3, CPSF1, CSPP1, CTF1, CTH, CYP3A5, DAB2IP, DBN1, DDX51, DEAF1, DGKE, DHDDS, DMKN, DNHD1, DOCK8, DOK5, EIF2B5, ELAC2, ELP1, ENTPD4, ESRRA, ESS2, ETAA1, ETHE1, FANCA, FASTK, FASTKD3, FPGS, FREM1, GAS8, GCK, GGA1, GGA3, GGT1, GLMN, GON4L, GRB14, HDAC3, HELQ, HP1BP3, HPS1, HPS4, IDUA, IFT122, IL17RC, IMPDH1, INVS, IRF3, KCTD3, KLKB1, KYAT1, LAMC3, LDAH, LIMS2, MALT1, MAP3K7, MAPK13, MCAT, MCEE, MPI, MSTO1, NBEAL2, NLE1, NLRC5, NOM1, NOP58, NPHP1, NPR1, NUP188, OSGEP, PABPC4, PCOLCE, PHKA2, PIDD1, PKD1, PLA2G6, PLD2, PLEKHG5, PNPO, POLE, PON2, PRMT7, PRODH, PRPF3, PRPF4, PYCR1, RAD52, REXO1, RFX5, RHBDF2, RMND1, ROBO3, RPGRI1, RTTN, RUFY3, SEMA3B, SEMA3F, SH2D3A, SIK3, SIRT3, SKIV2L, SLC2A13, SLC12A7, SLC22A3, SLC25A13, SLC25A37, SLC27A5, SLC30A9, SLC30A10, SMPD1, SMTN, SNRPB, SP140, STAMBP, STXBP2, SULF2, SYNGAP1, SYNJ2, TAZ, TBL2, TDRD7, TECPR2, TFB1M, TM6SF1, TNK2, TOE1, TOR1B, TRAPPC6B, TRIP4, TRMT2A, TRMU, TRPV4, TTC19, TYMP, UMPS, UROD, WDR11, WDR62, WIPI1, WRAP53, XPO1, YY1AP1, and ZC3H14. In some examples, the non-sense mediated RNA decay alternative exon (NSAE)-modulating agent is a composition described herein.

[0015] In some examples, provided herein is a pharmaceutical composition comprising: a therapeutic agent comprising a composition described herein; and a pharmaceutically acceptable excipient and/or a delivery vehicle.

[0016] In some examples, provided herein is a method of treating or preventing a disease or condition in a subject in need thereof, the method comprising: administering to the subject a pharmaceutical composition described herein.

[0017] In some examples, provided herein is a method of treating or preventing a disease or condition in a subject in need thereof, the method comprising: administering to the subject a pharmaceutical composition comprising: (a) a non-sense mediated RNA decay alternative exon (NSAE)-modulating agent that interacts with a target motif within a pre-processed mRNA transcript to modulate exclusion of an NSAE from a processed mRNA transcript and to modulate inclusion of a canonical exon in the processed mRNA transcript, wherein the NSAE comprises (i) only a portion of a canonical exon, or (ii) a canonical exon and at least a portion of an intron adjacent to the canonical exon; and (b) a pharmaceutically acceptable excipient and/or a delivery vehicle, wherein the disease or condition is treated or prevented in the subject by the administration of the NSAE-modulating agent by a modulation in expression of a protein translated from the processed mRNA transcript.

[0018] In some examples, provided herein is a method of treating a disease or condition in a subject in need thereof by modulating expression of a target protein in a cell of the subject, wherein the cell of the subject has a pre-processed mRNA transcript (pre-mRNA) that encodes the target protein and comprises: (a) an exon followed by an intron flanking a 3' splice site of the exon; and (b) an alternative nonsense mediated RNA decay-inducing (NMD) exon comprising an alternative 3' splice site upstream of the 3' splice site of the exon and within the exon, or downstream of the 3' splice site of the exon and within the intron, the method comprising contacting a therapeutic agent to the cell, wherein the therapeutic agent modulates processing of an mRNA transcript from the pre-processed mRNA transcript by modulating splicing of the pre-mRNA at the 3' alternative splice site, and wherein the splicing of the pre-mRNA at the 3' alternative splice site modulates the expression of the target protein in the cell of the subject.

[0019] In some examples, provided herein is a method of treating a disease or condition in a subject in need thereof by modulating expression of a target protein in a cell of the subject, wherein the cell of the subject has a pre-processed mRNA transcript (pre-mRNA) that encodes the target protein and comprises: (a) an exon preceded by an intron flanking a 5' splice site of the exon; and (b) an alternative nonsense mediated RNA decay-inducing (NMD) exon comprising an alternative 5' splice site downstream of the 5' splice site of the exon and within the exon, or upstream of the 5' splice site of the exon and within the intron, the method comprising contacting a therapeutic agent to the cell, wherein the therapeutic agent modulates processing of an mRNA transcript from the pre-processed mRNA transcript by modulating splicing of the pre-mRNA at the 5' alternative splice site, and wherein the splicing of the pre-mRNA at the 5' alternative splice site modulates the expression of the target protein in the cell of the subject.

[0020] In some examples the disease is autosomal dominant mental retardation, epileptic encephalopathy, or autism. In some examples, the disease or the condition is caused by a deficient

amount or activity of the target protein. In some examples, the therapeutic agent increases the level of the processed mRNA encoding the target protein in the cell. In some examples, the therapeutic agent increases the expression of the target protein in the cell. In some examples, the level of processed mRNA encoding the target protein in the cell contacted with the therapeutic agent is increased about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to the level of processed mRNA encoding the target protein in a control cell. In some examples, the level the target protein in the cell 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 level of processed mRNA encoding the target protein in a control cell. In some examples, the method further comprises assessing mRNA levels or expression levels of the target protein. In some examples, the method further comprises assessing the subject's genome for at least one genetic mutation associated with the disease.

[0021] In some examples, the disease or the condition is caused by an excess amount or activity of the target protein. In some examples, the therapeutic agent decreases the level of the processed mRNA encoding the target protein in the cell. In some examples, the therapeutic agent decreases the expression of the target protein in the cell. In some examples, the level of processed mRNA encoding the target protein in the cell contacted with the therapeutic agent is decreased about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to the level of processed mRNA encoding the target protein in a control cell. In some examples, the level the target protein in the cell is decreased about 1.1 to about 10-fold, about 1.5 to about 10-fold, about

2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to the level of processed mRNA encoding the target protein in a control cell.

[0022] In some examples, at least one genetic mutation is within a locus of a gene associated with the disease. In some examples, at least one genetic mutation is within a locus associated with expression of a gene associated with the disease. In some examples, at least one genetic mutation is within the SYNGAP1 gene locus. In some examples, at least one genetic mutation is within a locus associated with SYNGAP1 gene expression. In some examples, at least one genetic mutation is within a target gene locus, wherein the target gene locus is any one selected from the group consisting of ABCA5, ABCA7, ABCC3, ABCC5, ABCC8, ABCD1, ABR, ACAD9, ACAP1, ACOX2, ACSF3, ACTN4, ADAM17, ADAMTS13, AKR1E2, ALAD, ALG3, ANKRD29, ANKS3, ANO4, AP3B2, AP3M1, AP5Z1, ARNTL, ASAP3, ATRX, BBS2, BBS4, BRD9, CALM3, CARS1, CASP9, CATSPER1, CCDC40, CCDC88B, CD4, CD58, CES2, CHFR, CLCN2, CNOT3, CNTROB, COL5A3, CPSF1, CSPP1, CTF1, CTH, CYP3A5, DAB2IP, DBN1, DDX51, DEAF1, DGKE, DHDDS, DMKN, DNHD1, DOCK8, DOK5, EIF2B5, ELAC2, ELP1, ENTPD4, ESRRA, ESS2, ETAA1, ETHE1, FANCA, FASTK, FASTKD3, FPGS, FREM1, GAS8, GCK, GGA1, GGA3, GGT1, GLMN, GON4L, GRB14, HDAC3, HELQ, HP1BP3, HPS1, HPS4, IDUA, IFT122, IL17RC, IMPDH1, INVS, IRF3, KCTD3, KLKB1, KYAT1, LAMC3, LDAH, LIMS2, MALT1, MAP3K7, MAPK13, MCAT, MCEE, MPI, MSTO1, NBEAL2, NLE1, NLRC5, NOM1, NOP58, NPHP1, NPR1, NUP188, OSGEP, PABPC4, PCOLCE, PHKA2, PIDD1, PKD1, PLA2G6, PLD2, PLEKHG5, PNPO, POLE, PON2, PRMT7, PRODH, PRPF3, PRPF4, PYCR1, RAD52, REXO1, RFX5, RHBDF2, RMND1, ROBO3, RPGRIP1, RTTN, RUFY3, SEMA3B, SEMA3F, SH2D3A, SIK3, SIRT3, SKIV2L, SLC2A13, SLC12A7, SLC22A3, SLC25A13, SLC25A37, SLC27A5, SLC30A9, SLC30A10, SMPD1, SMTN, SNRPB, SP140, STAMBP, STXBP2, SULF2, SYNGAP1, SYNJ2, TAZ, TBL2, TDRD7, TECPR2, TFB1M, TM6SF1, TNK2, TOE1, TOR1B, TRAPPC6B, TRIP4, TRMT2A, TRMU, TRPV4, TTC19, TYMP, UMPS, UROD, WDR11, WDR62, WIPI1, WRAP53, XPO1, YY1AP1, and ZC3H14 gene locus. In some examples, at least one genetic mutation is within a locus associated with a target gene expression, wherein the target gene is any one selected from the group consisting of ABCA5, ABCA7, ABCC3, ABCC5, ABCC8, ABCD1, ABR, ACAD9, ACAP1, ACOX2, ACSF3, ACTN4, ADAM17, ADAMTS13, AKR1E2, ALAD, ALG3, ANKRD29, ANKS3, ANO4, AP3B2, AP3M1, AP5Z1, ARNTL, ASAP3, ATRX, BBS2, BBS4, BRD9, CALM3, CARS1, CASP9, CATSPER1, CCDC40, CCDC88B, CD4, CD58, CES2, CHFR, CLCN2, CNOT3, CNTROB, COL5A3, CPSF1, CSPP1, CTF1, CTH, CYP3A5, DAB2IP, DBN1, DDX51, DEAF1, DGKE, DHDDS, DMKN, DNHD1, DOCK8, DOK5, EIF2B5, ELAC2, ELP1, ENTPD4,

ESRRA, ESS2, ETAA1, ETHE1, FANCA, FASTK, FASTKD3, FPGS, FREM1, GAS8, GCK, GGA1, GGA3, GGT1, GLMN, GON4L, GRB14, HDAC3, HELQ, HP1BP3, HPS1, HPS4, IDUA, IFT122, IL17RC, IMPDH1, INVS, IRF3, KCTD3, KLKB1, KYAT1, LAMC3, LDAH, LIMS2, MALT1, MAP3K7, MAPK13, MCAT, MCEE, MPI, MSTO1, NBEAL2, NLE1, NLRC5, NOM1, NOP58, NPHP1, NPR1, NUP188, OSGEP, PABPC4, PCOLCE, PHKA2, PIDD1, PKD1, PLA2G6, PLD2, PLEKHG5, PNPO, POLE, PON2, PRMT7, PRODH, PRPF3, PRPF4, PYCR1, RAD52, REXO1, RFX5, RHBDF2, RMND1, ROBO3, RPGRI1, RTTN, RUFY3, SEMA3B, SEMA3F, SH2D3A, SIK3, SIRT3, SKIV2L, SLC2A13, SLC12A7, SLC22A3, SLC25A13, SLC25A37, SLC27A5, SLC30A9, SLC30A10, SMPD1, SMTN, SNRNP, SP140, STAMBP, STXBP2, SULF2, SYNGAP1, SYNJ2, TAZ, TBL2, TDRD7, TECPR2, TFB1M, TM6SF1, TNK2, TOE1, TOR1B, TRAPPC6B, TRIP4, TRMT2A, TRMU, TRPV4, TTC19, TYMP, UMPS, UROD, WDR11, WDR62, WIPI1, WRAP53, XPO1, YY1AP1, and ZC3H14 genes. 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 cell or the cells is ex vivo, or in a tissue, or organ ex vivo. In some examples, the therapeutic agent is administered to the subject by intracerebroventricular injection, intraperitoneal injection, intramuscular injection, intrathecal injection, subcutaneous injection, oral administration, synovial injection, intravitreal administration, subretinal injection, topical application, implantation, or intravenous injection.

[0023] In some examples, provided herein is a therapeutic agent for use in a method described herein.

[0024] In some examples, provided herein is a pharmaceutical composition comprising a therapeutic agent described herein and a pharmaceutically acceptable excipient.

[0025] In some examples, provided herein is a method of treating a subject in need thereof, comprising administering a pharmaceutical composition described herein by intracerebroventricular injection, intraperitoneal injection, intramuscular injection, intrathecal injection, subcutaneous injection, oral administration, synovial injection, intravitreal administration, subretinal injection, topical application, implantation, or intravenous injection to the subject.

BRIEF DESCRIPTION OF THE DRAWINGS

[0026] Figure 1A depicts an exemplary schematic depicting a wild type cell expressing a pre-mRNA that is alternatively spliced to generate a productive mRNA and a non-productive mRNA. While the non-productive mRNA is degraded by NMD, the productive mRNA is translated into normal protein levels.

[0027] Figure 1B depicts an exemplary schematic depicting a wild type cell treated with a TANGO (Targeted Augmentation of Nuclear Gene Output) ASO targeting the non-productive AS event (e.g. exon inclusion) that prevents the inclusion of the non-productive exon and leads to an increase of the productive mRNA. Increased level of the productive mRNA results in increased protein level beyond 100%.

[0028] Figure 1C depicts an exemplary schematic depicting an example of a haploinsufficient cell containing a wild-type allele (orange) and a mutant allele (red). Both alleles express a pre-mRNA that is alternatively spliced to generate a productive mRNA and a non-productive mRNA. While the non-

CLAIMS

1. A composition comprising a non-sense mediated RNA decay alternative exon (NSAE)-modulating agent that interacts with a target motif within a pre-processed mRNA transcript to modulate splicing at an alternative 5' or 3' splice site of a pre-processed mRNA transcript in a cell, thereby promoting inclusion of a canonical exon in a processed mRNA transcript that is processed from the pre-processed mRNA transcript, and promoting exclusion of an alternative exon from the pre-processed mRNA transcript in the cell,
wherein the target motif is located: (i) in an intronic region between two canonical exons, (ii) in one of the two canonical exons, or (iii) in a region spanning both an intron and canonical exon; and
wherein the alternative exon comprises:
(1) only a portion of a canonical exon, or
(2) a canonical exon and at least a portion of an intron upstream of the canonical exon and adjacent to the canonical exon.
2. The composition of claim 1, wherein the processed mRNA transcript encodes a target protein, and wherein the NSAE-modulating agent increases expression of the target protein in the cell.
3. The composition of claim 1, wherein the target protein is selected from the group consisting of ABCC3, ABCC5, ABCC8, ACOX2, ACSF3, ADAM17, ADAMTS13, ALAD, ANKS3, ANO4, AP3B2, AP5Z1, ARNTL, BBS2, BRD9, CALM3, CARS1, CASP9, CD58, CHFR, CLCN2, CNOT3, CNTROB, CTF1, CYP3A5, DHDDS, DNHD1, ENTPD4, ESRRA, ESS2, ETAA1, FASTKD3, FREM1, GAS8, GGA3, GGT1, GLMN, GRB14, HDAC3, HP1BP3, HPS4, IDUA, IFT122, IMPDH1, KCTD3, KYAT1, MAP3K7, NBEAL2, NOP58, NPHP1, NUP188, PHKA2, PIDD1, PKD1, PLA2G6, PLD2, PLEKHG5, PRMT7, PRODH, PRPF3, PRPF4, RAD52, REXO1, RFX5, RUFY3, SEMA3B, SEMA3F, SH2D3A, SIRT3, SKIV2L, SLC25A13, SLC2A13, SLC30A10, SLC30A9, SMTN, STXBP2, SYNGAP1, TBL2, TECPR2, TNK2, TOE1, TRMT2A, TRPV4, UROD, WDR62, WRAP53 and XPO1.
4. The composition of claim 1, wherein the alternative 3' splice site flanks the alternative exon, is downstream of a 3' splice site flanking the canonical exon, and is within the canonical exon.
5. The composition of claim 1, wherein the alternative 3' splice site flanks the alternative exon, is upstream of a 3' splice site flanking the canonical exon, and is within the intron immediately upstream of the canonical exon.
6. The composition of claim 1, wherein the NSAE-modulating agent is a small molecule or a polypeptide.
7. The composition of claim 1, wherein the NSAE-modulating agent is a nucleic acid binding protein.
8. The composition of claim 7, wherein the nucleic acid binding protein contains a TAL-effector, zinc finger binding domain, a Cas family protein.

9. The composition of claim 1, wherein the NSAE-modulating agent is an antisense oligomer (ASO), or a viral delivery system encoding the antisense oligomer.
10. The composition of claim 9, wherein the antisense oligomer is at least 75% complementary to the target motif of the pre-mRNA encoding the target protein.
11. The composition of claim 9, wherein the NSAE-modulating agent is the antisense oligomer, and wherein the antisense oligomer comprises a backbone modification comprising a phosphorothioate linkage or a phosphorodiamidate linkage.
12. The composition of claim 9, wherein the NSAE-modulating agent is the antisense oligomer, and wherein the antisense oligomer comprises a phosphorodiamidate morpholino, a locked nucleic acid, or a peptide nucleic acid.
13. The composition of claim 9, wherein the NSAE-modulating agent is the antisense oligomer, and wherein the antisense oligomer comprises a 2'-O-methyl moiety, a 2'-Fluoro moiety, or a 2'-O-methoxyethyl moiety.
14. The composition of claim 9, wherein the NSAE-modulating agent is the antisense oligomer, and wherein the antisense oligomer comprises at least one modified sugar moiety.
15. The composition of claim 9, wherein the antisense oligomer consists of from 8 to 50 nucleobases.
16. The composition of claim 1, wherein target protein is SYNGAP1 protein, and wherein the pre-mRNA comprises a sequence selected from the group consisting of premrna_ENST00000293748.9, premrna_ENST00000418600.7, premrna_ENST00000428982.4, premrna_ENST00000449372.7, premrna_ENST00000470232.1, premrna_ENST00000479510.2, premrna_ENST00000628646.2, premrna_ENST00000629380.3, premrna_ENST00000635885.1, premrna_ENST00000636075.1, premrna_ENST00000636116.1, premrna_ENST00000636146.1, premrna_ENST00000636193.1, premrna_ENST00000636436.1, premrna_ENST00000636443.1, premrna_ENST00000636640.1, premrna_ENST00000636731.1, premrna_ENST00000636905.1, premrna_ENST00000637052.1, premrna_ENST00000637194.1, premrna_ENST00000637490.1, premrna_ENST00000637587.1, premrna_ENST00000637671.1, premrna_ENST00000637721.1, premrna_ENST00000637911.1, premrna_ENST00000638127.1, premrna_ENST00000638142.2, premrna_ENST00000644458.1, premrna_ENST00000645250.1, and premrna_ENST00000646630.1.
17. The composition of claim 1, wherein target protein is SYNGAP1 protein, and wherein the target motif of the 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 a sequence selected from the group consisting of SEQ ID NOs: 166-169 and 980-983, and complements thereof.

18. The composition of claim 1, wherein target protein is SYNGAP1 protein, and wherein the target motif of the *SYNGAP1* pre-mRNA comprises a sequence that is complementary to at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 contiguous nucleic acids of a sequence selected from the group consisting of SEQ ID NOs: 1-165 and 4300-4352, and a complement thereof.

19. The composition of claim 1, wherein the NSAE-modulating agent comprises a sequence that comprises has at least about 80% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1-165 and 4300-4352.

20. Use of the composition of any one of claims 1 to 19 in manufacturing a medicament for treating or preventing a disease or condition in a subject in need thereof, wherein the disease or condition is a disease or condition caused by a deficient amount or activity of the target protein.

21. The use according to claim 20, wherein the disease or condition is autosomal dominant mental retardation, epileptic encephalopathy, or autism.

22. The use according to claim 20, wherein the NSAE-modulating agent increases the level of the processed mRNA encoding the target protein in the cell.

23. The use according to claim 22, wherein the level of processed mRNA encoding the target protein in the cell contacted with the NSAE-modulating agent is increased about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to the level of processed mRNA encoding the target protein in a control cell that is not contacted with the NSAE-modulating agent.

24. The use according to claim 20, wherein the NSAE-modulating agent increases the expression of the target protein in the cell.

25. The use according to claim 24, wherein the level of the target protein in the cell contacted with the NSAE-modulating agent is increased about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-

fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to the level of the target protein in a control cell that is not contacted with the NSAE-modulating agent.

26. The use according to claim 20, wherein at least one genetic mutation is within a locus of a gene associated with the disease.

27. The use according to claim 20, wherein at least one genetic mutation is within a locus associated with expression of a gene associated with the disease.

28. The use according to claim 20, wherein at least one genetic mutation is within the SYNGAP1 gene locus.

29. The use according to claim 20, wherein at least one genetic mutation is within a locus associated with SYNGAP1 gene expression.

30. The use according to claim 20, wherein the subject is a human.

31. The use according to claim 20, wherein the composition is administered to the subject by intracerebroventricular injection, intraperitoneal injection, intramuscular injection, intrathecal injection, subcutaneous injection, oral administration, synovial injection, intravitreal administration, subretinal injection, topical application, implantation, or intravenous injection.

32. Use of the composition of any one of claims 1 to 19 in manufacturing a medicament for modulating expression of the target protein.

33. A pharmaceutical composition comprising the composition of any one of claims 1 to 19, and a pharmaceutically acceptable excipient and/or a delivery vehicle.

34. A composition comprising a non-sense mediated RNA decay alternative exon (NSAE)-modulating agent that interacts with a target motif within a pre-processed mRNA transcript to modulate splicing at an alternative 5' or 3' splice site of a pre-processed mRNA transcript, thereby modulating inclusion of a canonical exon in a processed mRNA transcript that is processed from the pre-processed mRNA transcript, and modulating exclusion of an alternative exon from the pre-processed mRNA transcript,

wherein the target motif is located: (i) in an intronic region between two canonical exons, (ii) in one of the two canonical exons, or (iii) in a region spanning both an intron and canonical exon; and

wherein the alternative exon comprises:

(1) only a portion of a canonical exon, or

(2) a canonical exon and at least a portion of an intron adjacent to the canonical exon.

35. A composition comprising a modified antisense oligonucleotide sequence or a viral delivery system encoding a polynucleotide comprising an antisense oligonucleotide sequence, wherein the antisense

oligonucleotide sequence has at least about 80% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1-165 and 1301-10225.

36. Use of the composition of claim 34 or 35 in manufacturing a medicament for treating or preventing a disease or condition in a subject in need thereof, wherein the disease or condition is a disease or condition caused by a deficient amount or activity of the target protein.

37. The use according to claim 36, wherein the disease or condition is autosomal dominant mental retardation, epileptic encephalopathy or autism.

38. The use according to claim 37, wherein the disease or condition is autosomal dominant mental retardation.

39. The composition of any one of claims 1 to 38 for use in therapy.