

## ALLELE-SPECIFIC KNOCKDOWN OF GENE EXPRESSION

## CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This applications claims priority to, and the benefit of, United States (U.S.) Provisional Application Serial No. 63/618,571, filed 8 January 2024 and titled ALLELE-SPECIFIC KNOCKDOWN OF GENE EXPRESSION, which is incorporated by reference herein in its entirety for all purposes.

## INCORPORATION BY REFERENCE

[0002] This application contains a Sequence Listing which has been submitted electronically in XML format and is hereby incorporated by reference in its entirety. In compliance with 37 C.F.R. 1.52(e), the sequence information contained in electronic file named "UM30009PCT Sequence Listing.xml", which was created on 7 January 2025 and is 152,076 bytes in size, is incorporated herein by reference in its entirety.

[0003] U.S. Provisional Patent Application Serial No. 63/396,545, filed on 9 August 2022, and International Patent Application Serial No. PCT/US2023/071895, filed on 9 August 2023, are incorporated by reference herein in their entirety for all purposes.

## STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0004] This invention was made with government support under awards R21NS139270 awarded by the National Institutes of Health. The government has certain rights in this invention.

## BACKGROUND

[0005] For dominantly inherited diseases, there are three main pathogenic mechanisms of gene mutations: (1) Toxic Gain of Function (GoF) wherein the gene mutation confers a new deleterious attribute onto the resultant protein or ribonucleic acid (RNA) such as protein aggregation, mislocalization, or RNA foci, (2) Dominant Negative (DN) where the mutant protein acts to inactivate the remaining wild-type allele, which is often observed for proteins that homodimerize, and (3) Loss of Function (LoF)/Haploinsufficiency, where mutations result in half the level of total active protein which is insufficient for its cellular function.

[0006] For the successful development of gene-based therapeutics, it is essential to establish the mechanism by which gene mutations contribute to pathogenesis. For example, in the case of haploinsufficiency, therapeutics will focus on increasing gene expression, possibly through viral gene delivery. The development of gene-based therapies for dominant disorders based on gain of function or dominant negative mechanisms is more complex. For gain of function mutations, researchers have mainly focused on using RNA interference (RNAi), such as small interfering RNA (siRNA), antisense oligonucleotides (ASOs), and the like, to knockdown gene expression. Currently, nearly all studies utilize RNAi that targets both the mutant and wild-type allele. However, in many cases, reduction of the single wild-type allele may have deleterious consequences. For dominant negative diseases, knockdown of both mutant and wild-type alleles will unlikely be successful as the ratio of mutant:wild-type protein will not change.

[0007] One method to overcome these limitations is to develop RNAi that are specific to the actual mutation. However, the development of RNAi with single base pair discrimination is technically very challenging. This is in part due to the ability for an oligonucleotide to “bubble” over a single base pair mismatch. Further, this would require a unique mutant-specific RNAi for every causative mutation observed within gene. For instance, over 69 different mutations have been identified in the TAR DNA Binding Protein (TARDBP) gene and a unique RNAi would need to be developed for each of the 69 different mutations based on current methodology. Additionally, direct targeting of repeat expansions would not be applicable due to their extensive secondary structure and complex sequence.

[0008] As such, there is need to develop mutant-specific RNAi for the therapeutic treatment of diseases resulting from dominant mutations with a gain of function or dominant negative pathogenic mechanism.

## SUMMARY

[0009] An aspect of the present disclosure provides a method of inhibiting an allele that contains a mutation in a cell, the method comprising: delivering to the cell an antisense oligonucleotide (ASO) (such as an allele-specific antisense oligonucleotide (asASO)) that targets a target variant allele (e.g., an allele of a variant or common variant, and/or an indel, such as an insertion or deletion of one or more bases) that is in cis with the mutation (e.g., the mutation is a gain of function mutation or a dominant negative mutation), wherein the cell (e.g., a subject) is heterozygous for the target variant allele, wherein the antisense oligonucleotide is effective at inhibiting or knocking down the allele (e.g., reducing the expression of the allele).

- [0010] In any aspect or embodiment described herein, the cell is *in vitro*.
- [0011] In any aspect or embodiment described herein, the cell is in a subject.
- [0012] In any aspect or embodiment described herein, the cell is a human cell (e.g., a human cell of the central nervous system).
- [0013] In any aspect or embodiment described herein, the mutation is in superoxide dismutase 1 (SOD1) or chromosome 9 open reading frame 72 (C9Orf72), and the cell is from (i) a subject having one or more symptoms of at least one of amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), or a combination thereof, or (ii) a subject having or suspected of at least one of having amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), or a combination thereof.
- [0014] In any aspect or embodiment described herein, the mutation is in superoxide dismutase 1 (SOD1) or chromosome 9 open reading frame 72 (C9Orf72), and the method is a method of inhibiting expression of a superoxide dismutase 1 (SOD1) gene mutation or a chromosome 9 open reading frame 72 (C9Orf72) gene mutation.
- [0015] In any aspect or embodiment described herein, the mutation is in superoxide dismutase 1 (SOD1), and the method is a method of inhibiting expression of a superoxide dismutase 1 (SOD1) gene mutation.
- [0016] In any aspect or embodiment described herein, the mutation is in chromosome 9 open reading frame 72 (C9Orf72), and the method is a method of inhibiting expression of a chromosome 9 open reading frame 72 (C9Orf72) gene mutation.
- [0017] In any aspect or embodiment described herein, the mutation is in Kinesin family member 5A (KIF5A), and the cell is from (i) a subject having one or more symptoms of at least one of amyotrophic lateral sclerosis, neonatal intractable myoclonus (NEIMY), hereditary spastic paraplegia 10 (SPG10), or a combination thereof, or (ii) a subject having or suspected of having at least one of amyotrophic lateral sclerosis, neonatal intractable myoclonus (NEIMY), hereditary spastic paraplegia 10 (SPG10), or a combination thereof.
- [0018] In any aspect or embodiment described herein, the mutation is in Kinesin family member 5A (KIF5A), and the method is a method of inhibiting the expression of a Kinesin family member 5A (KIF5A) gene mutation.
- [0019] In any aspect or embodiment described herein, the mutation is in Titan protein (TTN), and the cell is from (i) a subject having one or more symptoms of at least one of dilated cardiomyopathy-1G (CMD1G), familial hypertrophic cardiomyopathy-9 (CMH9), myofibrillar myopathy-9 with early respiratory failure (MFM9), tibial muscular dystrophy (TMD), or a combination thereof, or (ii) a subject having or suspected of having at least one of

dilated cardiomyopathy-1G (CMD1G), familial hypertrophic cardiomyopathy-9 (CMH9), myofibrillar myopathy-9 with early respiratory failure (MFM9), tibial muscular dystrophy (TMD), or a combination thereof.

**[0020]** In any aspect or embodiment described herein, the mutation is in Titan protein (TTN), and the method is a method of inhibiting the expression of a Titan protein (TTN) gene mutation.

**[0021]** In any aspect or embodiment described herein, the mutation is in Mitofusin-2 (MFN2), and the cell is from (i) a subject having one or more symptoms of at least one of Charcot-Marie-Tooth (CMT) disease type 2A2A (CMT2A2A), Charcot-Marie-Tooth disease type 6A (CMT6A), or a combination thereof, or (ii) a subject having or suspected of having at least one of Charcot-Marie-Tooth (CMT) disease type 2A2A (CMT2A2A), Charcot-Marie-Tooth disease type 6A (CMT6A), or a combination thereof.

**[0022]** In any aspect or embodiment described herein, the mutation is in Mitofusin-2 (MFN2), and the method is a method of inhibiting the expression of a Mitofusin-2 (MFN2) gene mutation.

**[0023]** In any aspect or embodiment described herein, the mutation is in  $\beta$ -myosin heavy chain (MYH7), and the cell is from (i) a subject having one or more symptoms of at least one of dilated cardiomyopathy-1S (CMD1S), hypertrophic cardiomyopathy-1 (CMH1), autosomal dominant myosin storage congenital myopathy-7A (CMYP7A), Laing distal myopathy, dilated cardiomyopathy-1S (CMD1S), or a combination thereof, or (ii) a subject having or suspected of having at least one of dilated cardiomyopathy-1S (CMD1S), hypertrophic cardiomyopathy-1 (CMH1), autosomal dominant myosin storage congenital myopathy-7A (CMYP7A), Laing distal myopathy, dilated cardiomyopathy-1S (CMD1S), or a combination thereof.

**[0024]** In any aspect or embodiment described herein, the mutation is in  $\beta$ -myosin heavy chain (MYH7), and the method is a method of inhibiting the expression of a  $\beta$ -myosin heavy chain (MYH7) gene mutation.

**[0025]** In any aspect or embodiment described herein, the mutation is in lamin A/C (LMNA), and the cell is from (i) a subject having one or more symptoms of at least one of dilated cardiomyopathy-1A (CMD1A), autosomal dominant Emery-Dreifuss muscular dystrophy 2 (EDMD2), Slovenian type heart-hand syndrome, Hutchinson-Gilford progeria syndrome (HGPS), familial partial lipodystrophy type 2 (FPLD2), Malouf syndrome, congenital muscular dystrophy (MDC), or a combination thereof, or (ii) a subject having or suspected of having at least one of dilated cardiomyopathy-1A (CMD1A), autosomal dominant Emery-Dreifuss muscular dystrophy 2 (EDMD2), Slovenian type heart-hand syndrome,



Hutchinson-Gilford progeria syndrome (HGPS), familial partial lipodystrophy type 2 (FPLD2), Malouf syndrome, congenital muscular dystrophy (MDC), or a combination thereof.

**[0026]** In any aspect or embodiment described herein, the mutation is in lamin A/C (LMNA), and the method is a method of inhibiting the expression of a lamin A/C (LMNA) gene mutation.

**[0027]** In any aspect or embodiment described herein, the mutation is in transactive responsive deoxyribonucleic acid (DNA)-binding protein 43 (TARDBP), and the cell is from (i) a subject having one or more symptoms of at least one of amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), Alzheimer's disease (AD), limbic predominant age-related TDP-43 encephalopathy (LATE), or a combination thereof, or (ii) a subject having or suspected of having at least one of amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), Alzheimer's disease (AD), limbic predominant age-related TDP-43 encephalopathy (LATE), or a combination thereof.

**[0028]** In any aspect or embodiment described herein, the mutation is in transactive responsive deoxyribonucleic acid (DNA)-binding protein 43 (TARDBP), and the method is a method of inhibiting the expression of transactive responsive deoxyribonucleic acid (DNA)-binding protein 43 (TARDBP) gene mutation.

**[0029]** In any aspect or embodiment described herein, (i) the antisense oligonucleotide targets an intronic region of the gene; (ii) the antisense oligonucleotide sequence and/or a target variant allele sequence has a guanine-cytosine (GC) content of 20% to 80% (e.g., 35% to 65% or 40% to 60%); (iii) the antisense oligonucleotide is an oligonucleotide of 10 to 25 nucleotides in length that is complementary with at least 8 contiguous nucleotides of any one of SEQ ID NOs: 1, 2, 64-76, 79, 84, 85, 88, 89, 92, 93, 96, 97, 100, 101, 123, 124, or 125, rs11307260 (SEQ ID NO:79), rs142843265 (SEQ ID NO:64), rs3835416 (SEQ ID NO:125), rs34620383 (SEQ ID NO:65), rs2453565 (SEQ ID NO:66), rs700828 (SEQ ID NO:67), rs774356 (SEQ ID NO:68), rs774357 (SEQ ID NO:69), rs774359 (SEQ ID NO:70), rs2453554 (SEQ ID NO:71), rs2484319 (SEQ ID NO:72), rs2453555 (SEQ ID NO:73), rs2492816 (SEQ ID NO:74), rs3849945 (SEQ ID NO:75), or rs10757668 (SEQ ID NO:76); (iv) the antisense oligonucleotide is an oligonucleotide having the sequence of SEQ ID NO:80-83, 86, 87, 90, 91, 94, 95, 98, 99, 102, 103-122, or 126-152, or the antisense oligonucleotide comprises, consists essentially of, or consists of any antisense oligonucleotide described herein; (v) the mutation is in an A5V mutation in superoxide dismutase 1 (SOD1); (vi) the mutation is in a E134 deletion mutation in superoxide dismutase 1 (SOD1); (vii) the mutation is a repeat expansion within intron 1 of chromosome 9 open reading frame 72 (C9orf72); (viii) the

mutation is a Q331 mutations (e.g., Q331K) in transactive responsive deoxyribonucleic acid (DNA)-binding protein 43 (TARDBP); (ix) the mutation is a M337 mutations (e.g., M337V) in transactive responsive deoxyribonucleic acid (DNA)-binding protein 43 (TARDBP); or (x) a combination thereof.

**[0030]** In any aspect or embodiment described herein, a subject has the target variant allele and the mutation, and the method comprises administering the antisense oligonucleotide to the subject.

**[0031]** Another aspect of the present disclosure provides a method of treating a subject with an allele that contains a mutation, the method comprising: administering to the subject an antisense oligonucleotide (ASO) (such as an allele-specific antisense oligonucleotide (asASO)) that targets a target variant allele (e.g., an allele of a variant or a common variant, and/or an indel, such as an insertion or deletion of one or more bases) that is in cis with the mutation (e.g., is a gain of function mutation or a dominant negative mutation), wherein: (i) the subject is heterozygous for the target variant allele; and (ii) the antisense oligonucleotide is effective at preventing, treating, or ameliorating at least one symptom associated with the mutation.

**[0032]** In any aspect or embodiment described herein, the mutation is in superoxide dismutase 1 (SOD1) or chromosome 9 open reading frame 72 (C9orf72), and the subject (i) has one or more symptoms of amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), or both amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), or (ii) has or is suspected of having amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), or both amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD).

**[0033]** In any aspect or embodiment described herein, the mutation is in Kinesin family member 5A (KIF5A), and the subject (i) has one or more symptoms of at least one of amyotrophic lateral sclerosis, neonatal intractable myoclonus (NEIMY), hereditary spastic paraplegia 10 (SPG10), or a combination thereof, or (ii) has or is suspected of having at least one of amyotrophic lateral sclerosis, neonatal intractable myoclonus (NEIMY), hereditary spastic paraplegia 10 (SPG10), or a combination thereof.

**[0034]** In any aspect or embodiment described herein, the mutation is in Titan protein (TTN), and the subject (i) has one or more symptoms of at least one of dilated cardiomyopathy-1G (CMD1G), familial hypertrophic cardiomyopathy-9 (CMH9), myofibrillar myopathy-9 with early respiratory failure (MFM9), tibial muscular dystrophy (TMD), or a combination thereof, or (ii) has or is suspected of having at least one of dilated cardiomyopathy-1G (CMD1G), familial hypertrophic cardiomyopathy-9 (CMH9), myofibrillar myopathy-9 with early respiratory failure (MFM9), tibial muscular dystrophy (TMD), or a combination thereof.

**[0035]** In any aspect or embodiment described herein, the mutation is in Mitofusin-2 (MFN2), and the subject (i) has one or more symptoms of at least one of Charcot-Marie-Tooth (CMT) disease type 2A2A (CMT2A2A), Charcot-Marie-Tooth disease type 6A (CMT6A), or a combination thereof, or (ii) has or is suspected of having Charcot-Marie-Tooth (CMT) disease type 2A2A (CMT2A2A), Charcot-Marie-Tooth disease type 6A (CMT6A), or a combination thereof.

**[0036]** In any aspect or embodiment described herein, the mutation is in  $\beta$ -myosin heavy chain (MYH7), and the subject (i) has one or more symptoms of at least one of dilated cardiomyopathy-1S (CMD1S), hypertrophic cardiomyopathy-1 (CMH1), autosomal dominant myosin storage congenital myopathy-7A (CMYP7A), Laing distal myopathy, dilated cardiomyopathy-1S (CMD1S), or a combination thereof, or (ii) has or is suspected of having at least one of dilated cardiomyopathy-1S (CMD1S), hypertrophic cardiomyopathy-1 (CMH1), autosomal dominant myosin storage congenital myopathy-7A (CMYP7A), Laing distal myopathy, dilated cardiomyopathy-1S (CMD1S), or a combination thereof.

**[0037]** In any aspect or embodiment described herein, the mutation is in lamin A/C (LMNA), and the subject (i) has one or more symptoms of at least one of dilated cardiomyopathy-1A (CMD1A), autosomal dominant Emery-Dreifuss muscular dystrophy 2 (EDMD2), Slovenian type heart-hand syndrome, Hutchinson-Gilford progeria syndrome (HGPS), familial partial lipodystrophy type 2 (FPLD2), Malouf syndrome, congenital muscular dystrophy (MDC), or a combination thereof, or (ii) has or is suspected of having dilated cardiomyopathy-1A (CMD1A), autosomal dominant Emery-Dreifuss muscular dystrophy 2 (EDMD2), Slovenian type heart-hand syndrome, Hutchinson-Gilford progeria syndrome (HGPS), familial partial lipodystrophy type 2 (FPLD2), Malouf syndrome, congenital muscular dystrophy (MDC), or a combination thereof.

**[0038]** In any aspect or embodiment described herein, the mutation is in transactive responsive deoxyribonucleic acid (DNA)-binding protein 43 (TARDBP), and the subject (i) has one or more symptoms of at least one of amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), Alzheimer's disease (AD), limbic predominant age-related TDP-43 encephalopathy (LATE), or a combination thereof, or (ii) has or is suspected of having at least one of amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), Alzheimer's disease (AD), limbic predominant age-related TDP-43 encephalopathy (LATE), or a combination thereof.

**[0039]** In any aspect or embodiment described herein, the method is a method of treating (i) a subject having one or more symptoms of amyotrophic lateral sclerosis (ALS),

frontotemporal dementia (FTD), or both amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), or (ii) a subject that has or is suspected of having amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), or both amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD).

**[0040]** In any aspect or embodiment described herein, the method is a method of treating (i) a subject having one or more symptoms of at least one of amyotrophic lateral sclerosis, neonatal intractable myoclonus (NEIMY), hereditary spastic paraplegia 10 (SPG10), or a combination thereof, or (ii) a subject that has or is suspected of having at least one of amyotrophic lateral sclerosis, neonatal intractable myoclonus (NEIMY), hereditary spastic paraplegia 10 (SPG10), or a combination thereof.

**[0041]** In any aspect or embodiment described herein, the method is a method of treating (i) a subject having one or more symptoms of at least one of dilated cardiomyopathy-1G (CMD1G), familial hypertrophic cardiomyopathy-9 (CMH9), myofibrillar myopathy-9 with early respiratory failure (MFM9), tibial muscular dystrophy (TMD), or a combination thereof, or (ii) a subject that has or is suspected of having at least one of dilated cardiomyopathy-1G (CMD1G), familial hypertrophic cardiomyopathy-9 (CMH9), myofibrillar myopathy-9 with early respiratory failure (MFM9), tibial muscular dystrophy (TMD), or a combination thereof.

**[0042]** In any aspect or embodiment described herein, the method is a method of treating (i) a subject having one or more symptoms of at least one of Charcot-Marie-Tooth (CMT) disease type 2A2A (CMT2A2A), Charcot-Marie-Tooth disease type 6A (CMT6A), or a combination thereof, or (ii) a subject that has or is suspected of having at least one of Charcot-Marie-Tooth (CMT) disease type 2A2A (CMT2A2A), Charcot-Marie-Tooth disease type 6A (CMT6A), or a combination thereof.

**[0043]** In any aspect or embodiment described herein, the method is a method of treating (i) a subject having one or more symptoms of at least one of dilated cardiomyopathy-1S (CMD1S), hypertrophic cardiomyopathy-1 (CMH1), autosomal dominant myosin storage congenital myopathy-7A (CMYP7A), Laing distal myopathy, dilated cardiomyopathy-1S (CMD1S), or a combination thereof, or (ii) a subject that has or is suspected of having at least one of dilated cardiomyopathy-1S (CMD1S), hypertrophic cardiomyopathy-1 (CMH1), autosomal dominant myosin storage congenital myopathy-7A (CMYP7A), Laing distal myopathy, dilated cardiomyopathy-1S (CMD1S), or a combination thereof.

**[0044]** In any aspect or embodiment described herein, the method is a method of treating (i) a subject having one or more symptoms of at least one of dilated cardiomyopathy-1A (CMD1A), autosomal dominant Emery-Dreifuss muscular dystrophy 2 (EDMD2), Slovenian

type heart-hand syndrome, Hutchinson-Gilford progeria syndrome (HGPS), familial partial lipodystrophy type 2 (FPLD2), Malouf syndrome, congenital muscular dystrophy (MDC), or a combination thereof, or (ii) a subject that has or is suspected of having at least one of dilated cardiomyopathy-1A (CMD1A), autosomal dominant Emery-Dreifuss muscular dystrophy 2 (EDMD2), Slovenian type heart-hand syndrome, Hutchinson-Gilford progeria syndrome (HGPS), familial partial lipodystrophy type 2 (FPLD2), Malouf syndrome, congenital muscular dystrophy (MDC), or a combination thereof.

**[0045]** In any aspect or embodiment described herein, (i) the antisense oligonucleotide targets an intronic region of the gene; (ii) the antisense oligonucleotide sequence and/or a target variant allele sequence has a guanine-cytosine (GC) content of 20% to 80% (e.g., 35% to 65% or 40% to 60%); (iii) the antisense oligonucleotide is an oligonucleotide of 10 to 25 nucleotides in length that is complementary with at least 8 contiguous nucleotides of any one of SEQ ID NOs: 1, 2, 64-76, 79, 84, 85, 88, 89, 92, 93, 96, 97, 100, 101, 123, 124, or 125, rs11307260 (SEQ ID NO:79), rs142843265 (SEQ ID NO:64), rs3835416 (SEQ ID NO:125), rs34620383 (SEQ ID NO:65), rs2453565 (SEQ ID NO:66), rs700828 (SEQ ID NO:67), rs774356 (SEQ ID NO:68), rs774357 (SEQ ID NO:69), rs774359 (SEQ ID NO:70), rs2453554 (SEQ ID NO:71), rs2484319 (SEQ ID NO:72), rs2453555 (SEQ ID NO:73), rs2492816 (SEQ ID NO:74), rs3849945 (SEQ ID NO:75), or rs10757668 (SEQ ID NO:76); (iv) the antisense oligonucleotide is an oligonucleotide having the sequence of SEQ ID NO: 80-83, 86, 87, 90, 91, 94, 95, 98, 99, 102, 103-122, or 126-152, or the antisense oligonucleotide comprises, consists essentially of, or consists of any antisense oligonucleotide described herein; (v) the mutation is in an A5V mutation in superoxide dismutase 1 (SOD1); (vi) the mutation is in a E134 deletion mutation in superoxide dismutase 1 (SOD1); (vii) the mutation is a repeat expansion within intron 1 of chromosome 9 open reading frame 72 (C9Orf72); (viii) the mutation is a Q331 mutations (e.g., Q331K) in transactive responsive deoxyribonucleic acid (DNA)-binding protein 43 (TARDBP); (ix) the mutation is a M337 mutations (e.g., M337V) in transactive responsive deoxyribonucleic acid (DNA)-binding protein 43 (TARDBP); or (x) a combination thereof.

**[0046]** In any aspect or embodiment described herein, (i) the cell or subject is heterozygous for the mutation; (ii) the target variant allele is a common variant; (iii) the target variant allele is an indel (e.g., an insertion or deletion); (iv) the mutation includes a repeat expansion, a single base pair point mutation, a structural variant, a copy number variant, an indel, or a combination thereof; or (v) a combination thereof.

[0047] In any aspect or embodiment described herein, the method further comprises (i) determining or identifying the genotype of the target variant allele of the gene (e.g., by way of sequence and/or the method of haplotyping described herein); (ii) determining or identifying whether the target variant allele is in cis with the mutation (e.g., by way of sequence and/or the method of haplotyping described herein); or (iii) a combination thereof.

[0048] A further aspect of the present disclosure provides a method of determining the haplotype of two variants (e.g., a first or target variant and a second variant or mutation) of a gene in a cell, the method comprising: providing one or more (e.g., at least one, at least two, 1, 2, 3, 4, 5, 6, 7, 8, or more) antisense oligonucleotide (ASO), wherein each of the one or more antisense oligonucleotide targets a different allele of a first or target variant allele (e.g., a common variant; an indel; and/or if an indel, a first antisense oligonucleotide targets the insertion of a first or target variant allele and second antisense oligonucleotide targets the deletion of a first or target variant allele) of the gene; administering each of the one or more antisense oligonucleotide (ASO) to the cell individually; and measuring the expression level of one or more (e.g., at least one, one, at least two, or two) allele (e.g., allele-specific expression) for a second variant allele (e.g., a mutant allele) in each cell an antisense oligonucleotide (ASO) of the one or more antisense oligonucleotides (ASO) was administered.

[0049] In any aspect or embodiment described herein, reduced expression for an allele of the second variant allele indicates that the cell has that second variant allele (e.g., a mutant allele) and the first variant allele that the antisense oligonucleotide targets.

[0050] In any aspect or embodiment described herein, (a) reduced expression indicates that the second variant allele (e.g., a mutation or mutant allele) is in cis with the first variant allele that the antisense oligonucleotide targets; (b) reduced expression for only one allele of the second variant allele indicates that the cell is not heterozygous for the allele of the second variant allele with reduced expression; (c) reduced expression for two alleles of the second variant allele indicates that the cell is heterozygous for the two alleles of the second variant allele with reduced expression and homozygous for the first variant allele that the antisense oligonucleotide targets; or (d) a combination thereof.

[0051] In any aspect or embodiment described herein, (a) reduced expression indicates that the allele of the second variant allele (e.g., a mutation or mutant allele) is in cis with the first variant allele that the antisense oligonucleotide targets; (b) reduced expression for two alleles of the second variant allele indicates that the cell is heterozygous for the two alleles of the second variant allele with reduced expression and homozygous for the first variant allele that the antisense oligonucleotide targets; or (c) a combination thereof.

[0052] In any aspect or embodiment described herein, (i) the first variant allele is a common variant; (ii) the first variant allele is an indel (e.g., an insertion or deletion); (iii) the second variant allele is a mutation that includes a repeat expansion, a single base pair point mutation, a structural variant, a copy number variant, an indel, or a combination thereof; or (iv) a combination thereof.

[0053] In any aspect or embodiment described herein, (i) the antisense oligonucleotide targets an intronic region of the gene; (ii) the antisense oligonucleotide sequence and/or a target variant allele sequence has a guanine-cytosine (GC) content of 20% to 80% (e.g., 35% to 65% or 40% to 60%); (iii) the antisense oligonucleotide is an oligonucleotide of 10 to 25 nucleotides in length; (iv) the antisense oligonucleotide comprises at least one modified nucleotide; (v) the antisense oligonucleotide comprises a phosphorothioate modified linkage; (vi) the antisense oligonucleotide is a morpholino; (vii) the antisense oligonucleotide comprises locked nucleic acids (LNAs) and ribonucleic acids (RNAs) (e.g., alternating locked nucleic acid (LNA) and ribonucleic acid (RNA) nucleotides); (viii) the antisense oligonucleotide comprises locked nucleic acids (LNAs) and deoxyribonucleic acids (DNAs) (e.g., alternating locked nucleic acid (LNA) and deoxyribonucleic acid (DNA) nucleotides); (ix) the antisense oligonucleotide comprises ribonucleic acids (RNAs) and deoxyribonucleic acids (DNAs) (e.g., alternating ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) nucleotides); (x) the antisense oligonucleotide is a gapmer; or (xi) a combination thereof.

[0054] In any aspect or embodiment described herein, the at least one modified nucleotide is a 2'-modified nucleotide. In any aspect or embodiment described herein, each 2'-modified nucleotide includes or is independently the 2'-modified nucleotide is a 2'-deoxy modified nucleotide, 2'-fluoro modified nucleotide, 2'-O-methyl modified nucleotide, 2'-O-methoxyethyl modified nucleotide, 2'-amino modified nucleotide, or 2'-aminoalkoxy modified nucleotide.

[0055] In any aspect or embodiment described herein, the variant is present with at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, or at least 75% of the one or more gene mutations.

[0056] An additional aspect of the present disclosure provides an antisense oligonucleotide (ASO) comprising a nucleic acid sequence that is 10 to 25 nucleotides in length and comprise a region of complementarity that is complementary with at least 8 contiguous nucleotides of a sequence of any one of SEQ ID NOs: 1, 2, 64-76, 79, 84, 85, 88, 89, 92, 93, 96, 97, 100, 101, 123, 124, or 125, rs11307260 (SEQ ID NO:79), rs142843265 (SEQ ID NO:64), rs3835416

(SEQ ID NO:125), rs34620383 (SEQ ID NO:65), rs2453565 (SEQ ID NO:66), rs700828 (SEQ ID NO:67), rs774356 (SEQ ID NO:68), rs774357 (SEQ ID NO:69), rs774359 (SEQ ID NO:70), rs2453554 (SEQ ID NO:71), rs2484319 (SEQ ID NO:72), rs2453555 (SEQ ID NO:73), rs2492816 (SEQ ID NO:74), rs3849945 (SEQ ID NO:75), or rs10757668 (SEQ ID NO:76).

**[0057]** In any aspect or embodiment described herein, the antisense oligonucleotide comprises the nucleic acid sequence of any one of SEQ ID NOs: 80-83, 86, 87, 90, 91, 94, 95, 98, 99, 102, 103-122, or 126-152. In any aspect or embodiment described herein, the antisense oligonucleotide comprises, consists essentially of, or consists of any antisense oligonucleotide described herein.

**[0058]** In any aspect or embodiment described herein, (i) the antisense oligonucleotide comprises at least one modified nucleotide; (ii) the antisense oligonucleotide comprises at least one modified nucleotide that is a 2'-modified nucleotide (e.g., each 2'-modified nucleotide includes or is independently a 2'-deoxy modified nucleotide, 2'-fluoro modified nucleotide, 2'-O-methyl modified nucleotide, 2'-O-methoxyethyl modified nucleotide, 2'-amino modified nucleotide, or 2'-aminoalkoxy modified nucleotide); (iii) the antisense oligonucleotide comprises a phosphorothioate modified linkage; (iv) the antisense oligonucleotide is a morpholino; (v) the antisense oligonucleotide comprises locked nucleic acids (LNAs) and ribonucleic acids (RNAs) (e.g., alternating locked nucleic acid (LNA) and ribonucleic acid (RNA) nucleotides); (vi) the antisense oligonucleotide comprises locked nucleic acids (LNAs) and deoxyribonucleic acids (DNAs) (e.g., alternating locked nucleic acid (LNA) and deoxyribonucleic acid (DNA) nucleotides); (v) the antisense oligonucleotide comprises ribonucleic acids (RNAs) and deoxyribonucleic acids (DNAs) (e.g., alternating ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) nucleotides); (vi) the antisense oligonucleotide is a gapmer; or (vii) a combination thereof.

**[0059]** A further aspect of the present disclosure provides a pharmaceutical composition comprising the antisense oligonucleotide of the present disclosure, and a pharmaceutically-acceptable carrier.

#### BRIEF DESCRIPTION OF DRAWINGS

**[0060]** The accompanying drawings, which are incorporated into and form a part of the specification, illustrate several embodiments of the present disclosure and, together with the description, serve to explain the principles of the present disclosure. The drawings are only for



the purpose of illustrating an embodiment of the present disclosure and are not to be construed as limiting the present disclosure.

**[0061]** FIG. 1 provides a schematic showing the sequence of the two-base pair deletion (C9-deletion, C9-del) that is in linkage disequilibrium with the *C9orf72* repeat expansion relative to the wild-type sequence (see, SEQ ID NO:1 (WT or normal C9) and SEQ ID NO:2 (C9-deletion or C9-mutant)). Provided below the sequences are representative ASOs designed by 1-base pair tiling over the C9-deletion site.

**[0062]** FIG. 2 provides a graph showing the ability of methoxyethyl (MOE) gapmer ASOs that target the two-base pair deletion site to inhibit expression of *C9orf72* in normal fibroblasts that are homozygous for an allele hosting the target two-base pair deletion (C9-del/C9-del; Homozygous Mutant Genotype, which is also referred to herein as “susceptible”) compared to cells that are homozygous for the target wild-type allele (WT/WT; Homozygous Normal Genotype, which is also referred to herein as “resistant”).

**[0063]** FIG. 3A provides a schematic for a dual luciferase assay used to test the effectiveness of potential ASOs.

**[0064]** FIG. 3B provides a schematic for the vector used in a dual luciferase assay. Each variant of the target SNP region is separately cloned into the vector to test the effectiveness of potential ASOs.

**[0065]** FIG. 4A, FIG. 4B, and FIG. 4C provide graphs showing the ability of MOE gapmer ASOs to inhibit expression of an exogenously expressed *C9orf72* target region in HEK cells. ASOs were tested on cells expressing just the luciferase assay vector (no target region expressed), the luciferase vector carrying the wild-type sequence of the indel resistant to the ASOs (Normal (WT) *C9orf72* Target Sequence, SEQ ID NO:1), and the luciferase vector carrying the C9-deletion sequence susceptible to the ASOs (Mutant (C9-del) *C9orf72* Target Sequence, SEQ ID NO:2). Desired ASOs should repress expression of the C9-deletion target sequence and have little to no effect on the WT target sequence or vector alone. This is in comparison to a non-discriminating ASO (Non-Discr; Lagier-Tourenne, C et al. “Targeted degradation of sense and antisense *C9orf72* RNA foci as therapy for ALS and frontotemporal degeneration”. PNAS 2013 Nov 19;110(47):E4530-9), which reduces total levels of both targets but not the vector only construct and scrambled control ASOs (Scram 1 and 2), which do not target either construct. The graph in FIG. 4A shows the screen of the tiled MOE gapmer ASOs, while the data in Fig. 4B confirms the screen data for specific ASOs, and Fig. 4C shows a dose curve of a discriminating ASO #8 versus a scrambled (non-targeting) control ASO.

[0066] FIG. 5 provides a graph showing the ability of a MOE gapmer ASO to inhibit expression of *C9Orf72* in an allele-specific manner in fibroblasts. Two wild-type control fibroblast lines that were homozygous either for the C9-deletion target (C9-del/C9-del; Homozygous Mutant Genotype, which is also referred to herein as “susceptible”) or the wild-type variant (WT/WT; Homozygous Normal Genotype, which is also referred to herein as “resistant”) were used. Treatment with the *C9Orf72* MOE gapmer #8 reduced *C9Orf72* expression in the homozygous mutant genotype cell line, but not in the homozygous normal genotype cell line.

[0067] FIG. 6A, FIG. 6B, and FIG. 6C provide graphs showing the ability of LNA gapmer ASOs to inhibit expression of a *C9Orf72* target region exogenously expressed in HEK cells. ASOs were tested on cells expressing just the luciferase assay vector (no target region expressed), the luciferase vector carrying the wild-type sequence of the indel resistant to the ASOs (Normal (WT) *C9orf72* Target Sequence, SEQ ID NO:1), or the luciferase vector carrying the C9-deletion sequence susceptible to the ASOs (Mutant (C9-del) *C9orf72* Target Sequence, SEQ ID NO:2). Desired ASOs should repress expression of the C9-deletion target sequence and have little to no effect on the WT target sequence or vector alone. This is in comparison to a non-discriminating ASO (Non-Discr; Lagier-Tourenne, C et al. “Targeted degradation of sense and antisense *C9orf72* RNA foci as therapy for ALS and frontotemporal degeneration”. PNAS 2013 Nov 19;110(47):E4530-9) which reduces total levels of both targets or scrambled control ASOs (LNA Scram 1 and 2; Scrambled), which do not target either construct. The graph in FIG. 6A shows the screen of the tiled LNA ASOs, while the data in Fig. 6B confirms the screen data for specific ASOs, and Fig. 6C shows dose curves for discriminating ASOs #9 and #10.

[0068] FIG. 7 provides a graph showing the ability of an LNA gapmer ASO to inhibit expression of *C9Orf72* in an allele-specific manner in fibroblasts. Two control fibroblast lines that were homozygous either for the C9-deletion target (C9-del/C9-del; Homozygous Mutant Genotype, which is also referred to herein as “susceptible”) or the wild-type variant (WT/WT; Homozygous Normal Genotype, which is also referred to herein as “resistant”) were used. Treatment with the *C9Orf72* LNA gapmer #9 reduced *C9Orf72* expression in the homozygous mutant genotype cell line, but not in the homozygous normal genotype cell line. This is in comparison to a non-discriminating ASO (Non-Discr; Lagier-Tourenne, C et al. “Targeted degradation of sense and antisense *C9orf72* RNA foci as therapy for ALS and frontotemporal degeneration”. PNAS 2013 Nov 19;110(47):E4530-9) which reduces total levels of both

targets and a non-targeting control ASO (Scrambled) which does not affect levels of either target.

[0069] FIG. 8 shows the location of the C9 Deletion Variant (target) and a second variant located within the *C9Orf72* transcribed region that can be used to determine the specific transcript frequency in a sample (ASP SNP).

[0070] FIG. 9A, FIG. 9B, and FIG. 9C provide graphs showing partial rescue (i.e., reduction) of *C9Orf72* RNA foci formation in heterozygous patient *C9Orf72* fibroblasts treated with the allele specific LNA gapmer ASO #9. The graph in FIG. 9A shows that treatment with ASO#9 reduces the number of cells that have RNA foci in the total population, while the data in FIG. 9B shows the downward shift in the distribution of cells with certain number of foci per cell. The latter point is summarized in FIG. 9C which shows the average number of RNA foci per cell being reduced with ASO #9 treatment.

[0071] FIG. 10A and FIG. 10B are schematics of the allele-specific knockdown strategy of the present disclosure using antisense oligonucleotide(s) targeting variants, such as common variants and/or indels. Expression levels from each allele resulting from an insertion (“long”) targeting antisense oligonucleotide or a deletion (“short”) targeting antisense oligonucleotide treatment are assessed by RNA sequencing (RNAseq) and/or allele specific droplet digital polymerase chain reaction (ddPCR). Based on the schematic in Fig. 10A, treatment with a long targeting ASO will decrease expression from the WT allele allowing more expression from the mutant allele, while treatment with a short targeting ASO will have the reverse effect.

[0072] FIG. 11A provides a graph that shows the percent of mutant *SOD1* expression as determined by digital droplet PCR in the heterozygous A5V fibroblast cell line 120151 (a patient derived cell line) after a 72-hour treatment with 200 nM of a tiling series of Short *SOD1* MOE gapmers (SOD1 Short-1 through SOD1 Short-11) and a tiling series of Long *SOD1* MOE gapmers (SOD1 Long-1 through SOD1 Long-10), one experiment. This experiment determined that the A5V mutation was in cis with the deletion form of the ASO targeted indel and that SOD1 Short-5 was the most effective at knocking down expression from this allele. SOD1 Long-5, showing the highest expression of *SOD1* from the mutant containing allele, was demonstrated to be the most effective ASO for knocking down expression from the allele containing the insertion form of the ASO targeted indel. SOD1 Short-5 and SOD1 Long-5 being the most effective were subsequently examined in Figures 11C, 12C, 13B, 14B, and 15A and 15B, as Short *SOD1* MOE gapmer and Long *SOD1* MOE gapmer. For comparison, LNA versions of the top *SOD1* MOE gapmer ASOs were made and tested as well, and the data provided herein.

[0073] FIG. 11B and FIG. 11C provide graphs that show the total relative *SOD1* levels observed, as determined by Taqman assay, in the heterozygous A5V fibroblast cell line 220022 (a patient derived cell line) after a 72-hour treatment with 100 nM of Short *SOD1* LNA and Long *SOD1* LNA (two experiments) in Figure 11B and 200 nM of Short *SOD1* MOE gapmer and Long *SOD1* MOE gapmer (one experiment) in Figure 11C. The examined antisense oligonucleotides were all effective at knocking down total *SOD1* expression.

[0074] FIG. 12A, FIG. 12B, and FIG. 12C provide graphs that show the percent of wild-type *SOD1* expression present after allele-specific targeting by the antisense oligonucleotides in the heterozygous A5V fibroblast cell line 220022 (patient derived). The percent of WT *SOD1* allele expression observed after a 72-hour treatment with 100 nM of Short *SOD1* LNA and Long *SOD1* LNA (two experiments) is determined by droplet digital polymerase chain reaction (ddPCR) in Figure 12A, and by bulk RNA sequencing in Figure 12B. The RNA sequencing (RNAseq) allele-specific knockdown counts match the droplet digital polymerase chain reaction (ddPCR) data for the first and second experiments. The percent of WT *SOD1* allele expression observed after a 72-hour treatment with 200 nM of Short *SOD1* MOE gapmer and Long *SOD1* MOE gapmer (one experiment) is determined by droplet digital polymerase chain reaction (ddPCR) in Figure 12C. The examined antisense oligonucleotides demonstrated allele-specific effects, wherein the short antisense oligonucleotides are responsible for the knockdown of mutant allele in the examined cell line.

[0075] FIG. 13A and FIG. 13B provide graphs that show the total relative *SOD1* levels observed, as determined by Taqman assay, in the heterozygous A5V fibroblast cell line 210151 (a patient derived cell line) after a 72-hour treatment with 100 nM of Short *SOD1* LNA and Long *SOD1* LNA (two experiments) in Figure 13A and 200 nM of Short *SOD1* MOE gapmer and Long *SOD1* MOE gapmer (one experiment) in Figure 13B. Thus, *SOD1* knockdown with the examined antisense oligonucleotides was confirmed in multiple *SOD1* patient fibroblast cell lines.

[0076] FIG. 14A and FIG. 14B provide graphs that show the percent of wild-type *SOD1* expression, determined by droplet digital polymerase chain reaction (ddPCR), observed in the heterozygous A5V fibroblast cell line 210151 (patient derived) after a 72-hour treatment with 100 nM of Short *SOD1* LNA and Long *SOD1* LNA (two experiments) in Figure 14A and 200 nM of Short *SOD1* MOE gapmer and Long *SOD1* MOE gapmer (one experiment) in Figure 14B. The examined antisense oligonucleotides confirm allele-specific effects observed in the A5V fibroblast cell line 220022, wherein the short antisense oligonucleotides are responsible for the knockdown of mutant allele in the examined cell line.

[0077] FIG. 15A and FIG. 15B provide a graph that shows that treatment with the same SOD1 MOE gapmer and LNA antisense oligonucleotides (two experiments) that successfully reduce SOD1 levels in two different heterozygous A5V patient fibroblasts, can also target other mutations. Similar to the knockdown studies in the 220022 and 210151 heterozygous A5V patient fibroblast lines described, 200nM of Short and Long SOD1 MOE gapmer and 100nM of Short and Long SOD1 LNA can reduce the total SOD1 levels in a heterozygous Glu134del patient fibroblast line as determined by Taqman assays in Figure 15A and allele specific droplet digital polymerase chain reaction (ddPCR) in Figure 15B. In this cell line, the patient mutation is in cis with the insertion form of the ASO targeted indel, supported by increased wild-type allele expression upon treatment with the Long forms of the ASOs.

[0078] FIG. 16A, FIG. 16B, and FIG. 16C provide graphs showing the results of treating a heterozygous *C9Orf72* mutant fibroblast line derived from a C9ORF72 repeat expansion patient (C9-36) with the Short *C9Orf72* LNA gapmer (LNA #9) the Short *C9Orf72* MOE gapmer (MOE #8), a non-discriminating *C9Orf72* ASO (PNAS 2013; Lagier-Tourenne, C et al. "Targeted degradation of sense and antisense C9orf72 RNA foci as therapy for ALS and frontotemporal degeneration". PNAS 2013 Nov 19;110(47):E4530-9)), and a previously reported allele-specific *C9Orf72* targeting ASO (NATMED 22; Tran, *et al.*, Suppression of mutant C9Orf72 expression by a potent mixed backbone antisense oligonucleotide. Nature Medicine Nat Med. 2022 Jan; 28(1) 117-124)). Total *C9Orf72* levels are reduced after 72hr treatment with 100nM of LNA #9 below those levels observed by similar treatment with NATMED 22 ASO as shown by Taqman assay in Figure 16A. The percent of WT allele expression is increased after 72hr treatment with 100nM of LNA #9 above those levels observed by similar treatment with NATMED 22 ASO as shown by RNA sequencing (RNAseq) analysis in Figure 16B. Total *C9Orf72* levels are reduced after 72hr treatment with 100nM of MOE gapmer #8 similar to treatment with LNA #9 as determined by Taqman assay in Figure 16C. Both MOE gapmer #8 and LNA #9 treatment shows a larger reduction in C9 levels compared to similar treatment with the NATMED 22 ASO.

[0079] FIG. 17 provides a graph that shows *C9Orf72* levels observed, as determined by Taqman assay, in the heterozygous C9-41 fibroblast cell line (a patient derived cell line) after a 72 hr treatment with 100 nM of Short *C9Orf72* LNA LNA #9) the Short *C9Orf72* MOE gapmer (MOE #8), a non-discriminating *C9Orf72* ASO (PNAS 2013; Lagier-Tourenne, C et al. "Targeted degradation of sense and antisense C9orf72 RNA foci as therapy for ALS and frontotemporal degeneration". PNAS 2013 Nov 19;110(47):E4530-9)), and the NATMED 22 *C9Orf72* allele specific ASO (Tran, *et al.*, "Suppression of mutant C9Orf72 expression by a

potent mixed backbone antisense oligonucleotide.” *Nature Medicine* Nat Med. 2022 Jan; 28(1) 117-124). These results are in agreement with those from the C9-36 line and show that a single ASO can target multiple lines.

[0080] FIG. 18 provides a graph of the total C9 levels determined by Taqman assay present after DIV7 gymnotic treatment of C9Orf72 patient IPSC derived cortical neurons with 5uM of MOE gapmer #8 (MOE8) and LNA gapmer #9 (LNA9). This is in comparison to a non-treated control (NT), a non-discriminating ASO (PNAS 2013; Lagier-Tourenne, C *et al.* “Targeted degradation of sense and antisense *C9Orf72* RNA foci as therapy for ALS and frontotemporal degeneration”. PNAS 2013 Nov 19;110(47):E4530-9) which reduces total levels of both targets and a non-targeting control ASO (MSC) which does not affect levels of either target.

[0081] FIG. 19 provides a graph showing the ability of LNA gapmer ASOs to inhibit expression of target regions from different genes utilizing the dual luciferase assay method described herein. ASOs were tested on cells expressing just the luciferase assay vector (no target region expressed), the luciferase vector carrying the wild-type sequence of the indel resistant to the ASOs (see below for each gene), or the luciferase vector carrying the mutant variant of the indel susceptible to the ASOs (see below for each gene). Allele-specific knockdown was achieved for each of Kinesin family member 5A (KIF5A), Titan protein (TTN), Mitofusin-2 (MFN2),  $\beta$ -myosin heavy chain (MYH7), and Lamin A/C (LMNA), thereby further confirming its ability to perform robust allele-specific knockdown in multiple disease/ gene situations. These results were achieved with minimal optimization of a single pair of antisense oligonucleotides for each target examined.

[0082] FIG. 20 provides a graph that shows the percent of mutant *TARDBP* expression as determined by digital droplet PCR in the heterozygous Q331K induced cortical neuron line after DIV7 gymnotic treatment with 5uM of a tiling series of Short TARDBP MOE gapmers (TARDBP Short-1 through TARDBP Short-11) and a tiling series of Long TDP43 MOE gapmers (TARDBP Long-1 through TARDBP Long-10), one experiment. This experiment determined that the Q331K mutation was in cis with the insertion form of the ASO targeted indel and that TARDBP Long-7 and Long-8 were the most effective at knocking down expression from this allele. TARDBP Short-7 and Short-8, showing the highest expression of *TARDBP* from the mutant containing allele, were demonstrated to be the most effective ASO for knocking down expression from the allele containing the deletion form of the ASO targeted indel.

[0083] FIG. 21 provides a graph that shows the percent of mutant *TARDBP* expression as determined by digital droplet PCR in the heterozygous M337V induced cortical neuron line after DIV7 gymnotic treatment with 5uM of the top five performing *TARDBP* MOE gapmers screened in Figure 20 (*TARDBP* Short-5 through *TARDBP* Short-9 and *TARDBP* Long-5 through *TARDBP* Long-9), one experiment. This experiment determined that the M337V mutation was in cis with the deletion form of the ASO targeted indel and confirmed that *TARDBP* ASO 7 and 8 (in this line, *TARDBP* Short-7 and *TARDBP* Short-8) were the most effective at knocking down expression from this allele. Correspondingly, *TARDBP* Long-7 and Long-8, showing the highest expression of TDP43 from the mutant containing allele, were demonstrated to be the most effective ASO for knocking down expression from the allele containing the insertion form of the ASO targeted indel.

[0084] FIG. 22 provides a graph that shows the effect of altering the ASO backbone of the top *TARDBP* MOE #7 on allele specific knockdown of mutant *TARDBP* levels as determined by digital droplet PCR in the heterozygous Q331K and M337V iPSC derived cortical neuron lines after DIV7 gymnotic treatment with 5uM ASO. These treatments were also compared to non-treated mutant *TARDBP* samples (TDP43 UT) and a non-treated WT parent line (KOLF UT). FIG. 22 compares the efficacy of knockdown of *TARDBP* MOE #7 (M) to the same ASOs with altered backbone bonds in two (2M), three (3M), or four (4M) positions of the ASO wings. This experiment determined that altering the ASO backbone in two or three positions of the wings increased the allele specific knockdown of the mutant allele in the M337V and the Q331K lines, respectively.

#### DETAILED DESCRIPTION

[0085] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. The terminology used in the description is for describing particular embodiments only and is not intended to be limiting of the disclosure.

[0086] Where a range of values is provided, it is understood that each intervening value in the range, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise (for example, in the case of a group containing a number of carbon atoms in which case each carbon atom number falling within the range is provided), between the upper and lower limit of that range and any other stated or intervening value in that stated range is encompassed within the disclosure. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges and are also encompassed within the disclosure, subject to

any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either/or both of those included limits are also included in the disclosure.

**[0087]** It should also be understood that, in certain methods or processes described herein that include more than one step or act, the order of the steps or acts of the method is not necessarily limited to the order in which the steps or acts of the method are recited unless the context indicates otherwise.

**[0088]** The following terms are used to describe the present disclosure. In instances where a term is not specifically defined herein, that term is given an art-recognized meaning by those of ordinary skill applying that term in context to its use in describing the present disclosure.

**[0089]** The articles "a" and "an" as used herein and in the appended claims are used herein to refer to one or to more than one (that is, to at least one or one or more of) of the grammatical object of the article unless the context clearly indicates otherwise. By way of example, "an element" means one element or more than one element, unless otherwise indicated.

**[0090]** The phrase "and/or", as used herein in the specification and in the claims, should be understood to mean "either or both" of the elements so conjoined, i.e., elements that are conjunctively present in some cases and disjunctively present in other cases. Multiple elements listed with "and/or" should be construed in the same fashion, that is, "one or more" of the elements so conjoined. Other elements may optionally be present other than the elements specifically identified by the "and/or" clause, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, a reference to "A and/or B", when used in conjunction with open-ended language such as "comprising" can refer, in one embodiment, to A only (optionally including elements other than B); in another embodiment, to B only (optionally including elements other than A); in yet another embodiment, to both A and B (optionally including other elements); etc.

**[0091]** As used herein in the specification and in the claims, "or" should be understood to have the same meaning as "and/or" as defined above. For example, when separating items in a list, "or" or "and/or" shall be interpreted as being inclusive, i.e., the inclusion of at least one, but also including more than one, of a number or list of elements, and, optionally, additional unlisted items. Only terms clearly indicated to the contrary, such as "only one of" or "exactly one of," or, when used in the claims, "consisting of," will refer to the inclusion of exactly one element of a number or list of elements. In general, the term "or" as used herein shall only be interpreted as indicating exclusive alternatives (that is, "one or the other but not both") when preceded by terms of exclusivity, such as "either," "one of," "only one of," or "exactly one of."



**[0092]** As used herein in the specification and in the claims, the phrase “at least one,” in reference to a list of one or more elements, should be understood to mean at least one element selected from anyone or more of the elements in the list of elements, but not necessarily including at least one of each and every element specifically listed within the list of elements and not excluding any combinations of elements in the list of elements. This definition also allows that elements may optionally be present other than the elements specifically identified within the list of elements to which the phrase “at least one” refers, whether related or unrelated to those elements specifically identified. Thus, as a nonlimiting example, “at least one of A and B” (or, equivalently, “at least one of A or B,” or, equivalently “at least one of A and/or B”) can refer, in one embodiment, to at least one, optionally including more than one, A, with no B present (and optionally including elements other than B); in another embodiment, to at least one, optionally including more than one, B, with no A present (and optionally including elements other than A); in yet another embodiment, to at least one, optionally including more than one, A, and at least one, optionally including more than one, B (and optionally including other elements); etc.

**[0093]** In the claims, as well as in the specification above, all transitional phrases such as “comprising,” “including,” “carrying,” “having,” “containing,” “involving,” “holding,” “composed of,” and the like are to be understood to be open-ended (that is, to mean including but not limited to). It is expressly contemplated that all embodiments, and claims reciting one of the open-ended transitional phrases can be written with any other transitional phrase, which may be more limiting, unless clearly precluded by the context or art. Only the transitional phrases “consisting of” and “consisting essentially of” shall be closed or semi-closed transitional phrases, respectively, as set forth in the United States Patent Office Manual of Patent Examining Procedures, Section 2111.03.

**[0094]** The term “administration of” and “administering”, as used herein, refers to giving, providing, applying, or dispensing the compound, for example, of the present disclosure, by any suitable route. In this disclosure suitable routes of administration include oral, intravenous, intramuscular, subcutaneous, inhaled, buccal, transmucosal, and intranasal administration. In the context of administering compounds in the form of a combination, such as a combination of a compound of the disclosure and another active agent compound, the amount of each compound, when administered in combination may be different from that compound administered alone.

**[0095]** The term “patient” or “subject” is used throughout the specification to describe an animal, preferably a human or a domesticated animal, to whom treatment, including

prophylactic treatment, with the compositions according to the present disclosure is provided. For treatment of those diseases, conditions or symptoms that are specific for a specific animal, such as a human patient, the term “patient” refers to that specific animal, including a domesticated animal such as a dog or cat, or a farm animal such as a horse, cow, sheep, etc. In general, in the present disclosure, the terms “patient” and “subject” refer to a human patient unless otherwise stated or implied from the context of the use of the term.

[0096] The terms “effective,” “therapeutically effective,” “effective amount/dose,” “pharmaceutically effective amount/dose,” “pharmaceutically effective amount/dose,” or and “therapeutically effective amount/dose” are used to describe an amount/dose of a compound or composition which, when used within the context of its intended use, and either in a single dose or, more preferably after multiple doses within the context of a treatment regimen, effects an intended result, such as an improvement in or prevention of a disease, disorder, or condition, or amelioration (for example, alleviate to some extent, preferably all) or reduction in one or more symptoms associated with a disease, disorder, or condition. The terms “effective” and “therapeutically effective” subsume all other “effective amount” or “effective concentration” terms, which are otherwise described or used in the present application. The effective amount depends on the type of disease, the composition used, the route of administration, the type of mammal being treated, the physical characteristics of the specific mammal under consideration, concurrent medication, and other factors which those skilled in the medical arts will recognize.

[0097] The term “independently” is used herein to indicate that the variable, which is independently applied, varies independently from application to application.

[0098] The term “optional” or “optionally”, as used herein, means that the subsequently described event or circumstance may or may not occur, and that the description includes instances where said event or circumstance occurs and instances where it does not.

[0099] Aspects of the disclosure relate to methods allele-specific inhibition of gene or protein expression in a cell or subject, particularly gene or protein expression of alleles comprising a gain of function mutation or a dominant negative mutation. In any aspect or embodiment described herein, mutant-specific knockdown/inhibition techniques that are designed to preferentially reduce expression (e.g., gene and/or protein expression) a gain of function mutation or a dominant negative mutation relative to a wild-type allele (e.g., in a cell or subject that is heterozygous for the mutation, such as a cell or subject that has both the mutant allele and the wild-type allele). The techniques of the disclosure described herein directly target the mutant allele comprising the genetic mutation instead of targeting both the mutant and the wild-type alleles, which is important because it means that the techniques

described herein do not significantly impact expression of wild-type alleles in a cell or subject that has a gain of function mutation or a dominant negative mutation (e.g., a cell or subject that is heterozygous for the mutation). It is shown that decreased expression of wild-type alleles can be deleterious to cells and subjects. As an example of the approaches in the art, others have targeted cis mutant variants indirectly (e.g., instead of directly targeting the genetic mutation), which is sometimes successful in targeting repeat expansions.

**[0100]** The present disclosure further provides antisense oligonucleotides, compositions, and methods for inhibiting gene or protein expression, including, for example, mutant superoxide dismutase 1 (SOD1), mutant chromosome 9 open reading frame 72 (C9Orf72), mutant Kinesin family member 5A (KIF5A), mutant Titan protein (TTN), mutant Mitofusin-2 (MFN2), mutant  $\beta$ -myosin heavy chain (MYH7), mutant lamin A/C (LMNA), and/or mutant transactive responsive deoxyribonucleic acid (DNA)-binding protein 43 (TARDBP). In any aspect or embodiment described herein, dysregulated superoxide dismutase 1 (SOD1) or chromosome 9 open reading frame 72 expression is associated with amyotrophic lateral sclerosis (ALS) and/or frontotemporal dementia (FTD). In any aspect or embodiment described herein, dysregulated Kinesin family member 5A (KIF5A) expression is associated with amyotrophic lateral sclerosis, neonatal intractable myoclonus (NEIMY), and/or hereditary spastic paraplegia 10 (SPG10). In any aspect or embodiment described herein, dysregulated Titan protein (TTN) expression is associated with dilated cardiomyopathy-1G (CMD1G), familial hypertrophic cardiomyopathy-9 (CMH9), myofibrillar myopathy-9 with early respiratory failure (MFM9), and/or tibial muscular dystrophy (TMD). In any aspect or embodiment described herein, dysregulated Mitofusin-2 (MFN2) expression is associated with Charcot-Marie-Tooth (CMT) disease type 2A2A (CMT2A2A), and/or Charcot-Marie-Tooth disease type 6A (CMT6A). In any aspect or embodiment described herein, dysregulated  $\beta$ -myosin heavy chain (MYH7) expression is associated with dilated cardiomyopathy-1S (CMD1S), hypertrophic cardiomyopathy-1 (CMH1), autosomal dominant myosin storage congenital myopathy-7A (CMYP7A), Laing distal myopathy, and/or dilated cardiomyopathy-1S (CMD1S). In any aspect or embodiment described herein, dysregulated lamin A/C (LMNA) expression is associated with dilated cardiomyopathy-1A (CMD1A), autosomal dominant Emery-Dreifuss muscular dystrophy 2 (EDMD2), Slovenian type heart-hand syndrome, Hutchinson-Gilford progeria syndrome (HGPS), familial partial lipodystrophy type 2 (FPLD2), Malouf syndrome, and/or congenital muscular dystrophy (MDC). In any aspect or embodiment described herein, dysregulated transactive responsive deoxyribonucleic acid (DNA)-binding protein 43 (TARDBP) expression is associated with amyotrophic lateral

sclerosis (ALS), frontotemporal dementia (FTD), Alzheimer's disease (AD), and/or limbic predominant age-related TDP-43 encephalopathy (LATE).

**[0101]** Aspects of the disclosure relate to improved gene therapy compositions and related methods for treating diseases associated with gain of function mutations or dominant negative mutations (such as those that are associated with ALS or FTD) using the allele-specific antisense oligonucleotides of the present disclosure (e.g., allele-specific antisense oligonucleotides of the present disclosure that target a superoxide dismutase 1 (SOD1) mutant allele, a *C9Orf72* mutant allele, a Kinesin family member 5A (KIF5A) mutant allele, a Titan protein (TTN) mutant allele, a Mitofusin-2 (MFN2) mutant allele, a  $\beta$ -myosin heavy chain (MYH7) mutant allele, a lamin A/C (LMNA) mutant allele, or a transactive responsive deoxyribonucleic acid (DNA)-binding protein 43 (TARDBP) mutant allele).

**[0102]** The inventors of the present disclosure have discovered that directly targeting variants, such as common variants (including indels (genetic insertions or deletions)), can be used to achieve allele discrimination, which eliminates the need for directly targeting repeat expansions or single point mutations/variants and used to target gain of function mutation or dominant negative mutations, thereby not knocking down the other allele (e.g., the normal or wild-type allele).

**[0103]** As an example, the C9-deletion polymorphism indel is located within the transcribed region of the *C9Orf72* gene. In some embodiments, an allele from an indel variant is often located on the same haplotype of the repeat expansion of *C9Orf72* (e.g., in linkage disequilibrium with G<sub>4</sub>C<sub>2</sub> repeats). Further, the variant sequences of each indel are often present in a heterozygous state within *C9Orf72* patients. As such, one allele of the indel variant represents a target for allele-specific ASOs or similar knockdown technology. Further, indels are advantageous in developing allele-specific ASO due to the increased difference between the targeted allele and the non-targeted allele.

**[0104]** In addition to the two base pair C9-deletion, the inventors have identified 10 single nucleotide polymorphisms (SNPs) within the *C9ORF72* gene region which contain an allele that is commonly found on the same haplotype as the repeat expansion. These SNPs are located at rs positions as follows: rs2453565 (SEQ ID NO:66), rs700828 (SEQ ID NO:67), rs774356 (SEQ ID NO:68), rs774357 (SEQ ID NO:69), rs774359 (SEQ ID NO:70), rs2453554 (SEQ ID NO:71), rs2484319 (SEQ ID NO:72), rs2453555 (SEQ ID NO:73), rs2492816 (SEQ ID NO:74), and rs3849945 (SEQ ID NO:75). An example of a targeted two base pair C9-deletion, such as described here, is named rs142843265 (SEQ ID NO:64). The RS position naming convention is as described in Smith, B.N. *et al.*, The *C9ORF72* expansion mutation is a

common cause of ALS +/- FTD in Europe and has a single founder. European Journal of Human Genetics (2013) 21, 102–108.

*Methods of Use, Inhibiting, Treating, and Haplotyping*

**[0105]** An aspect of the present disclosure provides a method of inhibiting a mutant allele in a cell, the method comprising: delivering to the cell an antisense oligonucleotide (ASO) (such as an allele-specific antisense oligonucleotide (asASO)) that targets an allele of a target variant allele that is in cis with the mutation (e.g., the mutation is a gain of function mutation or a dominant negative mutation), wherein the cell (e.g., a subject) is heterozygous for the target variant allele, wherein the antisense oligonucleotide is effective at inhibiting or knocking down the mutant allele (e.g., reducing the expression of the mutant allele). In any aspect or embodiment described herein, the target variant allele is a common variant, an indel (e.g., a genetic insertion or deletion), or a combination thereof. In any aspect or embodiment described herein, the method reduces the expression of the mutant allele in the cell. Thus, a further aspect of the present disclosure provides a method of inhibiting a mutant allele in a cell, the method comprising: delivering to the cell an antisense oligonucleotide (ASO) (such as an allele-specific antisense oligonucleotide (asASO)) that targets an allele of a target variant allele that is cis with the mutation (e.g., the mutation is a gain of function mutation or a dominant negative mutation), wherein the cell (e.g., a subject) is heterozygous for the target variant allele, wherein the antisense oligonucleotide is effective at inhibiting or knocking down the mutant allele (e.g., reducing the expression of the mutant allele). In any aspect or embodiment described herein, the target variant allele is a common variant, an indel (e.g., a genetic insertion or deletion), or a combination thereof. In any aspect or embodiment described herein, the method reduces the expression of the allele or allele that contains the mutation in the cell.

**[0106]** In any aspect or embodiment described herein, the cell is *in vitro*. In any aspect or embodiment described herein, the cell is a human cell (e.g., a human cell of the central nervous system).

**[0107]** In any aspect or embodiment described herein, the cell is in a subject. In any aspect or embodiment described herein, a subject harbors a mutation, and the method comprises administering the antisense oligonucleotide to the subject. For example, an aspect of the present disclosure provides a method of inhibiting the mutant allele in a subject, the method comprising: delivering to the subject an antisense oligonucleotide (ASO) (such as an allele-specific antisense oligonucleotide (asASO)) that targets an allele of a target variant allele that is in cis with the mutation (e.g., the mutation is a gain of function mutation or a dominant

negative mutation), wherein the subject is heterozygous for the target variant allele, wherein the antisense oligonucleotide is effective at inhibiting or knocking down expression of the mutant allele (i.e. reducing the expression of the mutant allele). In any aspect or embodiment described herein, the method reduces the expression of the mutant allele in the subject. Thus, a further aspect of the present disclosure provides a method of inhibiting a mutant allele in a subject, the method comprising: delivering to the subject an antisense oligonucleotide (ASO) (such as an allele-specific antisense oligonucleotide (asASO)) that targets an allele of a target variant allele that is cis with the mutation (e.g., the mutation is a gain of function mutation or a dominant negative mutation), wherein the subject is heterozygous for the target variant allele, wherein the antisense oligonucleotide is effective at inhibiting or knocking down the mutant allele (e.g., reducing the expression of the mutant allele). In any aspect or embodiment described herein, the method reduces the expression of the mutant allele in the subject.

**[0108]** In any aspect or embodiment described herein, the mutation is in superoxide dismutase 1 (SOD1), chromosome 9 open reading frame 72 (C9Orf72), Kinesin family member 5A (KIF5A), Titan protein (TTN), Mitofusin-2 (MFN2),  $\beta$ -myosin heavy chain (MYH7), lamin A/C (LMNA), or transactive responsive deoxyribonucleic acid (DNA)-binding protein 43 (TARDBP). In any aspect or embodiment described herein, the mutation is in superoxide dismutase 1 (SOD1) or chromosome 9 open reading frame 72 (C9Orf72). For example, in any aspect or embodiment described herein, the mutation is in superoxide dismutase 1 (SOD1) or chromosome 9 open reading frame 72 (C9Orf72), and the cell is from (i) a subject having one or more symptoms of amyotrophic lateral sclerosis (ALS) and/or frontotemporal dementia (FTD), or (ii) a subject having or suspected of having amyotrophic lateral sclerosis (ALS) and/or frontotemporal dementia (FTD). Furthermore, in any aspect or embodiment described herein, the mutation is in superoxide dismutase 1 (SOD1) or chromosome 9 open reading frame 72 (C9Orf72), and the method is a method of inhibiting of the superoxide dismutase 1 (SOD1) mutant allele or the chromosome 9 open reading frame 72 (C9Orf72) mutant allele. Additionally, in any aspect or embodiment described herein, the mutation is in superoxide dismutase 1 (SOD1), chromosome 9 open reading frame 72 (C9Orf72), Kinesin family member 5A (KIF5A), Titan protein (TTN), Mitofusin-2 (MFN2),  $\beta$ -myosin heavy chain (MYH7), lamin A/C (LMNA), or transactive responsive deoxyribonucleic acid (DNA)-binding protein 43 (TARDBP), and the method is a method of inhibiting of the superoxide dismutase 1 (SOD1) mutant allele, the chromosome 9 open reading frame 72 (C9Orf72) mutant allele, the Kinesin family member 5A (KIF5A) mutant allele, Titan protein (TTN) mutant allele, Mitofusin-2 (MFN2) mutant allele,  $\beta$ -myosin heavy chain (MYH7) mutant allele, lamin A/C (LMNA)

mutant allele, or transactive responsive deoxyribonucleic acid (DNA)-binding protein 43 (TARDBP) mutant allele.

**[0109]** For example, in any aspect or embodiment described herein, the mutation is in Kinesin family member 5A (KIF5A), and the cell is from (i) a subject having one or more symptoms of amyotrophic lateral sclerosis, neonatal intractable myoclonus (NEIMY), and/or hereditary spastic paraplegia 10 (SPG10), or (ii) a subject having or suspected of having amyotrophic lateral sclerosis, neonatal intractable myoclonus (NEIMY), and/or hereditary spastic paraplegia 10 (SPG10).

**[0110]** For example, in any aspect or embodiment described herein, the mutation is in Titan protein (TTN), and the cell is from (i) a subject having one or more symptoms of dilated cardiomyopathy-1G (CMD1G), familial hypertrophic cardiomyopathy-9 (CMH9), myofibrillar myopathy-9 with early respiratory failure (MFM9), and/or tibial muscular dystrophy (TMD), or (ii) a subject having or suspected of having dilated cardiomyopathy-1G (CMD1G), familial hypertrophic cardiomyopathy-9 (CMH9), myofibrillar myopathy-9 with early respiratory failure (MFM9), and/or tibial muscular dystrophy (TMD).

**[0111]** For example, in any aspect or embodiment described herein, the mutation is in Mitofusin-2 (MFN2), and the cell is from (i) a subject having one or more symptoms of Charcot-Marie-Tooth (CMT) disease type 2A2A (CMT2A2A), and/or Charcot-Marie-Tooth disease type 6A (CMT6A), or (ii) a subject having or suspected of having Charcot-Marie-Tooth (CMT) disease type 2A2A (CMT2A2A), and/or Charcot-Marie-Tooth disease type 6A (CMT6A).

**[0112]** For example, in any aspect or embodiment described herein, the mutation is in  $\beta$ -myosin heavy chain (MYH7), and the cell is from (i) a subject having one or more symptoms of dilated cardiomyopathy-1S (CMD1S), hypertrophic cardiomyopathy-1 (CMH1), autosomal dominant myosin storage congenital myopathy-7A (CMYP7A), and/or Laing distal myopathy, dilated cardiomyopathy-1S (CMD1S), or (ii) a subject having or suspected of having dilated cardiomyopathy-1S (CMD1S), hypertrophic cardiomyopathy-1 (CMH1); autosomal dominant myosin storage congenital myopathy-7A (CMYP7A), and/or Laing distal myopathy; dilated cardiomyopathy-1S (CMD1S).

**[0113]** For example, in any aspect or embodiment described herein, the mutation is in lamin A/C (LMNA), and the cell is from (i) a subject having one or more symptoms of dilated cardiomyopathy-1A (CMD1A), autosomal dominant Emery-Dreifuss muscular dystrophy 2 (EDMD2), Slovenian type heart-hand syndrome, Hutchinson-Gilford progeria syndrome (HGPS), familial partial lipodystrophy type 2 (FPLD2), Malouf syndrome, and/or congenital

muscular dystrophy (MDC), or (ii) a subject having or suspected of having dilated cardiomyopathy-1A (CMD1A), autosomal dominant Emery-Dreifuss muscular dystrophy 2 (EDMD2); Slovenian type heart-hand syndrome, Hutchinson-Gilford progeria syndrome (HGPS); familial partial lipodystrophy type 2 (FPLD2), Malouf syndrome, and/or congenital muscular dystrophy (MDC).

**[0114]** For example, in any aspect or embodiment described herein, the mutation is in transactive responsive deoxyribonucleic acid (DNA)-binding protein 43 (TARDBP), and the cell is from (i) a subject having one or more symptoms of amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), Alzheimer's disease (AD), and/or limbic predominant age-related TDP-43 encephalopathy (LATE), or (ii) a subject having or suspected of having amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), Alzheimer's disease (AD), and/or limbic predominant age-related TDP-43 encephalopathy (LATE).

**[0115]** In any aspect or embodiment described herein, the antisense oligonucleotide targets an intronic region of the target variant allele. In any aspect or embodiment described herein, the antisense oligonucleotide sequence and/or a target variant allele sequence has a guanine-cytosine (GC) content of 35% to 65%. In any aspect or embodiment described herein, the antisense oligonucleotide is an oligonucleotide of 10 to 25 nucleotides in length that is complementary with at least 8 contiguous nucleotides of any one of SEQ ID NOs: 1, 2, 64-76, 79, 84, 85, 88, 89, 92, 93, 96, 97, 100, 101, 123, 124, or 125, rs11307260 (SEQ ID NO:79), rs142843265 (SEQ ID NO:64), rs3835416 (SEQ ID NO:125), rs34620383 (SEQ ID NO:65), rs2453565 (SEQ ID NO:66), rs700828 (SEQ ID NO:67), rs774356 (SEQ ID NO:68), rs774357 (SEQ ID NO:69), rs774359 (SEQ ID NO:70), rs2453554 (SEQ ID NO:71), rs2484319 (SEQ ID NO:72), rs2453555 (SEQ ID NO:73), rs2492816 (SEQ ID NO:74), rs3849945 (SEQ ID NO:75), or rs10757668 (SEQ ID NO:76). In any aspect or embodiment described herein, the antisense oligonucleotide is an oligonucleotide having the sequence of SEQ ID NO:80-83, 86, 87, 90, 91, 94, 95, 98, 99, 102, 103-122, or 126-152. In any aspect or embodiment described herein, or the antisense oligonucleotide comprises, consists essentially of, or consists of any antisense oligonucleotide described herein. In any aspect or embodiment described herein, the mutation is superoxide dismutase 1 (SOD1) mutation (e.g., rs11307260 (SEQ ID NO:79)). In any aspect or embodiment described herein, the mutation is an A5V mutation in superoxide dismutase 1 (SOD1). In any aspect or embodiment described herein, the mutation is a single base pair point mutation in the superoxide dismutase 1 (SOD1) gene. In any aspect or embodiment described herein, the mutation is in a E134 deletion mutation in superoxide dismutase 1 (SOD1). In any aspect or embodiment described herein, the mutation is a single



base pair deletion in the superoxide dismutase 1 (SOD1) gene. In any aspect or embodiment described herein, the mutation is a hexanucleotide repeat expansion in intron 1 of chromosome 9 open reading frame 72 (C9Orf72) gene. In any aspect of embodiment described herein, the mutation is a Q331 mutations (e.g., Q331K) in transactive responsive deoxyribonucleic acid (DNA)-binding protein 43 (TARDBP). In any aspect or embodiment described herein, the mutation is a M337 mutations (e.g., M337V) in transactive responsive deoxyribonucleic acid (DNA)-binding protein 43 (TARDBP). In any aspect or embodiment described herein, a subject has the target variant allele and the mutation, and the method comprises administering the antisense oligonucleotide to the subject.

**[0116]** Another aspect of the present disclosure provides a method of treating a subject with a mutation, the method comprising: administering to the subject an antisense oligonucleotide (ASO) (such as an allele-specific antisense oligonucleotide (asASO)) that targets a target variant allele that is in cis with the mutation (e.g., a gain of function mutation or a dominant negative mutation), wherein: (i) the subject is heterozygous for the target variant allele; and (ii) the antisense oligonucleotide is effective at preventing, treating, or ameliorating at least one symptom associated with the mutation. In any aspect or embodiment described herein, the target variant allele is a common variant, an indel (e.g., a genetic insertion or deletion), or a combination thereof. Thus, further aspect of the present disclosure provides a method of treating a subject with a mutant allele, the method comprising: administering to the subject an antisense oligonucleotide (ASO) (such as an allele-specific antisense oligonucleotide (asASO)) that targets a target variant allele that is cis with the mutation (e.g., a gain of function mutation or a dominant negative mutation), wherein: (i) the subject is heterozygous for the target variant allele; and (ii) the antisense oligonucleotide is effective at preventing, treating, or ameliorating at least one symptom associated with the mutation. In any aspect or embodiment described herein, the target variant allele is a common variant, an indel (e.g., a genetic insertion or deletion), or a combination thereof.

**[0117]** In any aspect or embodiment described herein, the mutation is in superoxide dismutase 1 (SOD1) or chromosome 9 open reading frame 72 (C9Orf72). For example, in any aspect or embodiment described herein, the mutation is in superoxide dismutase 1 (SOD1) or chromosome 9 open reading frame 72 (C9Orf72), and the subject (i) has one or more symptoms of amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), or both amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), or (ii) has or is suspected of having amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), or both amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). By way of further example, in any

aspect or embodiment described herein, the method is a method of treating (i) a subject having one or more symptoms of amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), or both amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), or (ii) a subject that has or suspected of having amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), or both amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD).

**[0118]** For example, in any aspect or embodiment described herein, the mutation is in Kinesin family member 5A (KIF5A), and the subject (i) has one or more symptoms of amyotrophic lateral sclerosis, neonatal intractable myoclonus (NEIMY), and/or hereditary spastic paraplegia 10 (SPG10), or (ii) has or is suspected of having amyotrophic lateral sclerosis, neonatal intractable myoclonus (NEIMY), and/or hereditary spastic paraplegia 10 (SPG10).

**[0119]** For example, in any aspect or embodiment described herein, the mutation is in Titan protein (TTN), and the subject (i) has one or more symptoms of dilated cardiomyopathy-1G (CMD1G), familial hypertrophic cardiomyopathy-9 (CMH9), myofibrillar myopathy-9 with early respiratory failure (MFM9), and/or tibial muscular dystrophy (TMD), or (ii) has or is suspected of having dilated cardiomyopathy-1G (CMD1G), familial hypertrophic cardiomyopathy-9 (CMH9), myofibrillar myopathy-9 with early respiratory failure (MFM9), and/or tibial muscular dystrophy (TMD).

**[0120]** For example, in any aspect or embodiment described herein, the mutation is in Mitofusin-2 (MFN2), and the subject (i) has one or more symptoms of Charcot-Marie-Tooth (CMT) disease type 2A2A (CMT2A2A), and/or Charcot-Marie-Tooth disease type 6A (CMT6A), or (ii) has or is suspected of having Charcot-Marie-Tooth (CMT) disease type 2A2A (CMT2A2A), and/or Charcot-Marie-Tooth disease type 6A (CMT6A).

**[0121]** For example, in any aspect or embodiment described herein, the mutation is in  $\beta$ -myosin heavy chain (MYH7), and the subject (i) has one or more symptoms of dilated cardiomyopathy-1S (CMD1S), hypertrophic cardiomyopathy-1 (CMH1), autosomal dominant myosin storage congenital myopathy-7A (CMYP7A), and/or Laing distal myopathy, dilated cardiomyopathy-1S (CMD1S), or (ii) has or is suspected of having dilated cardiomyopathy-1S (CMD1S), hypertrophic cardiomyopathy-1 (CMH1), autosomal dominant myosin storage congenital myopathy-7A (CMYP7A), and/or Laing distal myopathy, dilated cardiomyopathy-1S (CMD1S).

**[0122]** For example, in any aspect or embodiment described herein, the mutation is in lamin A/C (LMNA), and the subject (i) has one or more symptoms of dilated cardiomyopathy-1A

(CMD1A), autosomal dominant Emery-Dreifuss muscular dystrophy 2 (EDMD2), Slovenian type heart-hand syndrome, Hutchinson-Gilford progeria syndrome (HGPS), familial partial lipodystrophy type 2 (FPLD2), Malouf syndrome, and/or congenital muscular dystrophy (MDC), or (ii) has or is suspected of having dilated cardiomyopathy-1A (CMD1A), autosomal dominant Emery-Dreifuss muscular dystrophy 2 (EDMD2), Slovenian type heart-hand syndrome, Hutchinson-Gilford progeria syndrome (HGPS), familial partial lipodystrophy type 2 (FPLD2), and/or Malouf syndrome, congenital muscular dystrophy (MDC).

**[0123]** For example, in any aspect or embodiment described herein, the mutation is in transactive responsive deoxyribonucleic acid (DNA)-binding protein 43 (TARDBP), and the subject (i) has one or more symptoms of amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), Alzheimer's disease (AD), and/or limbic predominant age-related TDP-43 encephalopathy (LATE), or (ii) has or is suspected of having at least one of amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), Alzheimer's disease (AD), and/or limbic predominant age-related TDP-43 encephalopathy (LATE).

**[0124]** In any aspect or embodiment described herein, the antisense oligonucleotide sequence and/or a target variant allele sequence has a guanine-cytosine (GC) content of about 20% to about 80% (e.g., about 35% to about 65% or about 40% to about 60% or about 45% to about 55%). For example, in any aspect or embodiment described herein, the antisense oligonucleotide sequence and/or a target variant allele sequence has a guanine-cytosine (GC) content of 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 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%, about 25% to about 35%, about 30% to about 80%, about 30% to about 75%, about 30% to about 70%, about 30% to about 65%, about 30% to about 60%, about 30% to about 55%, about 30% to about 50%, about 30% to about 45%, about 30% to about 40%, about 35% to about 80%, about 35% to about 75%, about 35% to about 70%, about 35% to about 65%, about 35% to about 60%, about 35% to about 55%, about 35% to about 50%, about 35% to about 45%, about 40% to about 80%, about 40% to about 75%, about 40% to about 70%, about 40% to about 65%, about 40% to about 60%, about 40% to about 55%, about 40% to about 50%, about 45% to about 80%, about 45% to about 75%, about 45% to about 70%, about 45% to about 65%, about 45% to about 60%, about 45% to about 55%, about 50% to about 80%, about 50% to about 75%, about 50% to

about 70%, about 50% to about 65%, about 50% to about 60%, about 50% to about 80%, about 50% to about 75%, about 50% to about 70%, about 50% to about 65%, about 50% to about 60%, about 55% to about 80%, about 55% to about 75%, about 55% to about 70%, about 55% to about 65%, about 60% to about 80%, about 60% to about 75%, about 60% to about 70%, about 65% to about 80%, about 65% to about 75%, or about 70% to about 80%.

**[0125]** In any aspect or embodiment described herein, the antisense oligonucleotide targets an intronic region of the gene. In any aspect or embodiment described herein, the antisense oligonucleotide is an oligonucleotide of 10 to 25 nucleotides in length that is complementary with at least 8 contiguous nucleotides of any one of SEQ ID NOs: 1, 2, 64-76, 79, 84, 85, 88, 89, 92, 93, 96, 97, 100, 101, 123, 124, or 125, rs11307260 (SEQ ID NO:79), rs142843265 (SEQ ID NO:64), rs3835416 (SEQ ID NO:125), rs34620383 (SEQ ID NO:65), rs2453565 (SEQ ID NO:66), rs700828 (SEQ ID NO:67), rs774356 (SEQ ID NO:68), rs774357 (SEQ ID NO:69), rs774359 (SEQ ID NO:70), rs2453554 (SEQ ID NO:71), rs2484319 (SEQ ID NO:72), rs2453555 (SEQ ID NO:73), rs2492816 (SEQ ID NO:74), rs3849945 (SEQ ID NO:75), or rs10757668 (SEQ ID NO:76). In any aspect or embodiment described herein, the antisense oligonucleotide is an oligonucleotide having the sequence of SEQ ID NO: 80-83, 86, 87, 90, 91, 94, 95, 98, 99, 102, 103-122, or 126-152. In any aspect or embodiment described herein, the antisense oligonucleotide comprises, consists essentially of, or consists of any antisense oligonucleotide described herein. In any aspect or embodiment described herein, the mutation is in an A5V mutation in superoxide dismutase 1 (SOD1). In any aspect or embodiment described herein, the mutation is in a E134 deletion mutation in superoxide dismutase 1 (SOD1). In any aspect or embodiment described herein, the mutation is a hexanucleotide repeat expansion in intron 1 of the chromosome 9 open reading frame 72 (C9orf72) gene. In any aspect or embodiment described herein, the mutation is a Q331 mutations (e.g., Q331K) in transactive responsive deoxyribonucleic acid (DNA)-binding protein 43 (TARDBP). In any aspect or embodiment described herein, the mutation is a M337 mutations (e.g., M337V) in transactive responsive deoxyribonucleic acid (DNA)-binding protein 43 (TARDBP).

**[0126]** In any aspect or embodiment described herein, the cell is heterozygous for the mutation. In any aspect or embodiment described herein, the subject is heterozygous for the mutation. In any aspect or embodiment described herein, the target variant allele is a common variant. In any aspect or embodiment described herein, the target variant allele is an indel (e.g., a genetic insertion or deletion). In any aspect or embodiment described herein, the mutation includes a repeat expansion, a single base pair point mutation, a structural variant, a copy number variant, an indel, or a combination thereof.

[0127] In any aspect or embodiment described herein, the method further comprises determining or identifying the genotype of the target variant within the gene (e.g., by way of sequence and/or the method of haplotyping described herein). For example, in any aspect or embodiment described herein, the method further comprises determining or identifying the genotype of the target variant within the gene by sequencing, the method of haplotyping described herein, or a combination of.

[0128] In any aspect or embodiment described herein, the method further comprises determining or identifying whether the target variant allele is in cis with the mutation (e.g., by way of sequence and/or the method of haplotyping described herein). For example, in any aspect or embodiment described herein, the method further comprises determining or identifying whether the target variant allele is in cis with the mutation by sequencing, the method of haplotyping described herein, or a combination thereof. For example, a target variant allele and the mutation are in cis when an antisense oligonucleotide knockdown or reduces the expression of both the target variant allele and the mutation or second variant allele. Stated another way, a target variant allele and the mutation are in cis when an antisense oligonucleotide that targets a target variant allele knocks down or reduces the expression of the mutation or second variant allele (i.e., in addition to the variant target of the antisense oligonucleotide).

[0129] A further aspect of the present disclosure provides a method of determining the haplotype of two variants (e.g., a first target variant allele and a second variant allele or mutant allele) of a gene in a cell, the method comprising: providing one or more (e.g., at least one, at least two, 1, 2, 3, 4, 5, 6, 7, 8, or more) antisense oligonucleotides (ASO), wherein each of the one or more antisense oligonucleotide targets a different allele of a first target variant allele (e.g., a common variant; an indel; and/or if an indel, a first antisense oligonucleotide targets the insertion of a first target variant allele and a second antisense oligonucleotide targets the deletion of a first target variant allele) of the gene; administering each of the one or more antisense oligonucleotide (ASO) to the cell individually; and measuring the expression level of one or more (e.g., at least one, at least two, 1, 2, 3, 4, 5, 6, 7, 8, or more) allele (e.g., allele-specific expression) for a second variant allele (e.g., a mutation or mutant allele) in each cell an antisense oligonucleotide (ASO) of the one or more antisense oligonucleotide (ASO) was administered. Thus, the method of determining a haplotype of two variants (or method of haplotyping) a gene in a cell comprises administering each of the one or more antisense oligonucleotide (ASO), wherein each of the one or more antisense oligonucleotide targets a different allele of a first or target variant allele (e.g., a common variant; an indel; and/or if an

indel, a first antisense oligonucleotide targets the insertion of a first or target variant allele and second antisense oligonucleotide targets the deletion of a first variant) to the same cell separately (i.e., individually), thereby permitting the expression levels of the one or more allele for the second variant allele to be examined for each antisense oligonucleotide (ASO) independently (i.e., without another antisense oligonucleotide being administered to the same cell). For example, in any aspect or embodiment described herein, two antisense oligonucleotides (ASO) are individually administered for haplotyping. In any aspect or embodiment described herein, the second variant allele is a mutation.

**[0130]** By way of further example, in any aspect or embodiment described herein, one antisense oligonucleotide (ASO) is individually administered for haplotyping. Thus, another further aspect of the present disclosure provides a method of determining the haplotype of two variants (e.g., a first target variant allele and a second variant allele or mutant allele) of a gene in a cell, the method comprising: providing an antisense oligonucleotides (ASO), wherein the antisense oligonucleotide targets an allele of a first target variant allele (e.g., a common variant; an indel; and/or if an indel, a first antisense oligonucleotide targets the insertion of a first or target variant allele and a second antisense oligonucleotide targets the deletion of a first target variant allele) of the gene; administering the antisense oligonucleotide (ASO) to the cell; and measuring the expression level of an allele (e.g., allele-specific expression) for a second variant allele (e.g., a mutation or mutant allele) in the cell the antisense oligonucleotide (ASO) was administered.

**[0131]** Yet another aspect of the present disclosure provides a method of determining the haplotype of two variants (e.g., a first target variant allele and a second variant allele or mutant allele) of a gene in a cell, the method comprising: providing a plurality (e.g., at least two, 2, 3, 4, 5, 6, 7, 8, or more) of antisense oligonucleotides (ASO), wherein each antisense oligonucleotide targets a different allele of a first target variant allele (e.g., a common variant; an indel; and/or if an indel, a first antisense oligonucleotide targets the insertion of a first or target variant allele and a second antisense oligonucleotide targets the deletion of a first target variant allele) of the gene; administering each of the plurality of antisense oligonucleotides (ASO) to the cell individually; and measuring the expression level of a plurality (e.g., at least two or two) of alleles (e.g., allele-specific expression) for a second variant allele (e.g., a mutation or mutant allele) in each cell an antisense oligonucleotide (ASO) of the plurality of antisense oligonucleotides (ASO) was administered. Thus, the method of determining a haplotype of two variants (or method of haplotyping) a gene in a cell comprises administering each of the plurality of antisense oligonucleotides (ASO), wherein each antisense

oligonucleotide targets a different allele of a first or target variant allele (e.g., a common variant; an indel; and/or if an indel, a first antisense oligonucleotide targets the insertion of a first or target variant allele and second antisense oligonucleotide targets the deletion of a first variant) to the same cell separately (i.e., individually), thereby permitting the expression levels of the plurality of alleles for the second variant allele to be examined for each antisense oligonucleotide (ASO) independently (i.e., without another antisense oligonucleotide being administered to the same cell). For example, in any aspect or embodiment described herein, two antisense oligonucleotides (ASO) are individually administered for haplotyping. In any aspect or embodiment described herein, the second variant allele is a mutation.

**[0132]** In any aspect or embodiment described herein, reduced expression for the second variant allele indicates that the cell has that the allele of the second variant allele and the first or target variant allele that the antisense oligonucleotide targets are in cis or on the same haplotype.

**[0133]** In any aspect or embodiment described herein, reduced expression of the second variant allele indicates that the second variant allele (e.g., a mutation or mutant allele) is in cis with the first or target variant allele that the antisense oligonucleotide targets. In any aspect or embodiment described herein, reduced expression for only one allele of the first or target variant allele indicates that the cell is homozygous for the allele of the second variant allele with reduced expression. In any aspect or embodiment described herein, reduced expression for two alleles of the second variant allele (e.g., a mutation or mutant allele) indicates that the cell is heterozygous for the two alleles of the second variant with reduced expression and homozygous for the first or target variant allele that the antisense oligonucleotide targets.

**[0134]** In any aspect or embodiment described herein, (a) reduced expression indicates that the second variant allele (e.g., mutation) is in cis with the first or target variant allele that the antisense oligonucleotide targets;

**[0135]** In any aspect or embodiment described herein, the first or target variant allele is a common variant. In any aspect or embodiment described herein, the first or target variant allele is an indel (e.g., a genetic insertion or deletion). In any aspect or embodiment described herein, the second variant allele is a mutation that includes a repeat expansion, a single base pair point mutation, a structural variant, a copy number variant, an indel, or a combination thereof.

**[0136]** In any aspect or embodiment described herein, the antisense oligonucleotide sequence and/or a target variant allele sequence has a guanine-cytosine (GC) content of about 20% to about 80% (e.g., 35% to 65% or about 40% to about 60% or about 45% to about 55%). For example, in any aspect or embodiment described herein, the antisense oligonucleotide

sequence and/or a target variant allele sequence has a guanine-cytosine (GC) content of 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 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%, about 25% to about 35%, about 30% to about 80%, about 30% to about 75%, about 30% to about 70%, about 30% to about 65%, about 30% to about 60%, about 30% to about 55%, about 30% to about 50%, about 30% to about 45%, about 30% to about 40%, about 35% to about 80%, about 35% to about 75%, about 35% to about 70%, about 35% to about 65%, about 35% to about 60%, about 35% to about 55%, about 35% to about 50%, about 35% to about 45%, about 40% to about 80%, about 40% to about 75%, about 40% to about 70%, about 40% to about 65%, about 40% to about 60%, about 40% to about 55%, about 40% to about 50%, about 45% to about 80%, about 45% to about 75%, about 45% to about 70%, about 45% to about 65%, about 45% to about 60%, about 45% to about 55%, about 50% to about 80%, about 50% to about 75%, about 50% to about 70%, about 50% to about 65%, about 50% to about 60%, about 50% to about 80%, about 50% to about 75%, about 50% to about 70%, about 50% to about 65%, about 50% to about 60%, about 55% to about 80%, about 55% to about 75%, about 55% to about 70%, about 55% to about 65%, about 60% to about 80%, about 60% to about 75%, about 60% to about 70%, about 65% to about 80%, about 65% to about 75%, or about 70% to about 80%.

**[0137]** In any aspect or embodiment described herein, the antisense oligonucleotide targets an intronic region of the gene. In any aspect or embodiment described herein, the antisense oligonucleotide is an oligonucleotide of 10 to 25 nucleotides in length. In any aspect or embodiment described herein, the antisense oligonucleotide comprises at least one modified nucleotide. In any aspect or embodiment described herein, the antisense oligonucleotide comprises a phosphorothioate modified linkage. In any aspect or embodiment described herein, the antisense oligonucleotide is a morpholino. In any aspect or embodiment described herein, the antisense oligonucleotide comprises locked nucleic acids (LNAs) and ribonucleic acids (RNAs) (e.g., alternating locked nucleic acid (LNA) and ribonucleic acid (RNA) nucleotides). In any aspect or embodiment described herein, the antisense oligonucleotide comprises locked nucleic acids (LNAs) and deoxyribonucleic acids (DNAs) (e.g., alternating locked nucleic acid (LNA) and deoxyribonucleic acid (DNA) nucleotides). In any aspect or embodiment described herein, the antisense oligonucleotide comprises ribonucleic acids



(RNAs) and deoxyribonucleic acids (DNAs) (e.g., alternating ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) nucleotides). In any aspect or embodiment described herein, the antisense oligonucleotide is a gapmer.

**[0138]** In any aspect or embodiment described herein, the at least one modified nucleotide is a 2'-modified nucleotide. In any aspect or embodiment described herein, each 2'-modified nucleotide includes or is independently the 2'-modified nucleotide is a 2'-deoxy modified nucleotide, 2'-fluoro modified nucleotide, 2'-O-methyl modified nucleotide, 2'-O-methoxyethyl modified nucleotide, 2'-amino modified nucleotide, or 2'-aminoalkoxy modified nucleotide.

**[0139]** In any aspect or embodiment described herein, the variant is present with at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, or at least 75% of the one or more gene mutations.

**[0140]** As used herein, the terms “treat”, “treatment”, “treating”, and “therapy” refer to therapeutic treatment and prophylactic or preventative manipulations. The terms further include ameliorating existing symptoms, preventing additional symptoms, ameliorating or preventing the underlying causes of symptoms, preventing or reversing causes of symptoms (for example, symptoms associated with ALS and/or FTD). Thus, the terms denote that a beneficial result has been conferred on a subject having a disease, disorder, condition, symptom, or with the potential to develop such a disease, disorder, condition, symptom. Furthermore, treatment may include the application or administration of an inhibitory nucleic acid (e.g., a therapeutic ASO or a pharmaceutical composition comprising an ASO) to a subject, or an isolated tissue or cell line from a subject, who may have a disease, a disorder, a condition, a symptom of disease, disorder, or condition, or a predisposition toward a disease, disorder, or condition, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve or affect the disease, disorder, condition, symptoms of disease, disorder or condition, or the predisposition toward disease, disorder, or condition.

**[0141]** As disclosed herein inhibitory nucleic acids (e.g., antisense oligonucleotides (ASOs)) may be administered by any suitable route. For use in therapy, an effective amount of the antisense nucleic acid and/or other therapeutic agent can be administered to a subject by any mode that delivers the agent to the desired tissue, e.g., neuronal tissue. In any aspect or embodiment described herein, inhibitory nucleic acids (e.g., antisense oligonucleotides (ASOs)) are administered intravenously. Other suitable routes of administration include but are not limited to oral, parenteral, intravenous, intraperitoneal, intranasal, sublingual,

intratracheal, inhalation, subcutaneous, ocular, vaginal, and rectal. Systemic routes include oral and parenteral. Several types of devices are regularly used for administration by inhalation. These types of devices include metered dose inhalers (MDI), breath-actuated MDI, dry powder inhaler (DPI), spacer/holding chambers in combination with MDI, and nebulizers.

**[0142]** For oral administration, the inhibitory nucleic acids (e.g., antisense oligonucleotides (ASOs)) can be formulated readily by combining the active compound(s) with pharmaceutically acceptable carriers well known in the art. Such carriers enable the agents of the disclosure to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a subject to be treated. Pharmaceutical preparations for oral use can be obtained as solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate. Optionally oral formulations may also be formulated in saline or buffers for neutralizing internal acid conditions or may be administered without any carriers.

**[0143]** Delivery of inhibitory nucleic acids (e.g., antisense oligonucleotides (ASOs)) to a subject may be, for example, by administration into the bloodstream of the subject. Administration into the bloodstream may be by injection into a vein, an artery, or any other vascular conduit. Moreover, in certain instances, it may be desirable to deliver inhibitory nucleic acids (e.g., antisense oligonucleotides (ASOs)) to brain tissue, meninges, neuronal cells, glial cells, astrocytes, oligodendrocytes, cerebrospinal fluid (CSF), interstitial spaces and the like. In any aspect or embodiment described herein, inhibitory nucleic acids (e.g., antisense oligonucleotides (ASOs)) may be delivered directly to the spinal cord or brain (e.g., prefrontal cortex) by injection into the ventricular region, as well as to the striatum (e.g., the caudate nucleus or putamen of the striatum), and neuromuscular junction, or cerebellar lobule, with a needle, catheter or related device, using neurosurgical techniques known in the art, such as by stereotactic injection (see, e.g., Stein *et al.*, J Virol 73:3424-3429, 1999; Davidson *et al.*, PNAS 97:3428-3432, 2000; Davidson *et al.*, Nat. Genet. 3:219-223, 1993; and Alisky and Davidson, Hum. Gene Ther. 11:2315-2329, 2000).

[0144] Pharmaceutical preparations that can be used orally include push fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active agents may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. Microspheres formulated for oral administration may also be used. Such microspheres have been well defined in the art. Formulations for oral administration are typically in dosages suitable for such administration.

[0145] For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

[0146] For administration by inhalation, inhibitory nucleic acids (e.g., antisense oligonucleotides (ASOs)) for use according to the present disclosure may be conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of gelatin, for example, for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

[0147] The inhibitory nucleic acids (e.g., antisense oligonucleotides (ASOs)), when it is desirable to deliver them systemically, may be formulated for parenteral administration by injection, for example, by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, for example, in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

[0148] Pharmaceutical formulations for parenteral administration include aqueous solutions of inhibitory nucleic acids (e.g., antisense oligonucleotides (ASOs)) in water-soluble form. Additionally, suspensions of agents may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances that increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also

contain suitable stabilizers or agents that increase the solubility of the agents to allow for the preparation of highly concentrated solutions. Alternatively, agents (e.g., ASOs) may be in powder form for constitution with a suitable vehicle, for example, sterile pyrogen-free water, before use. Inhibitory nucleic acids (e.g., antisense oligonucleotides (ASOs)) may also be formulated in rectal or vaginal compositions such as suppositories or retention enemas, for example, containing conventional suppository bases such as cocoa butter or other glycerides.

[0149] Other delivery systems can include time-release, delayed release or sustained release delivery systems. Such systems can avoid repeated administrations of the agents (e.g., ASOs), increasing convenience to the subject and the physician. Many types of release delivery systems are available. They include polymer base systems such as poly(lactide glycolide), copolyoxalates, polycaprolactones, polyesteramides, polyorthoesters, polyhydroxybutyric acid, and polyanhydrides. Delivery systems also include non-polymer systems that are: lipids including sterols such as cholesterol, cholesterol esters and fatty acids or neutral fats such as mono, di, and tri glycerides; hydrogel release systems; silastic systems; peptide-based systems; wax coatings; compressed tablets using conventional binders and excipients; partially fused implants; and others disclosed herein.

#### *Inhibitory Nucleic Acids*

[0150] In any aspect or embodiment described herein, an inhibitory nucleic acid is an antisense oligonucleotide (ASO). As used herein, the term “antisense oligonucleotide (ASO)” or “antisense nucleic acid,” refers to a nucleic acid that has sequence complementarity to a target sequence and is specifically hybridizable (e.g., under stringent conditions) with a nucleic acid having the target sequence. An antisense oligonucleotide (ASO) is specifically hybridizable when binding of the antisense oligonucleotide (ASO) to the target nucleic acid is sufficient to produce complementary based pairing between the antisense oligonucleotide (ASO) and the target nucleic acid, and there is a sufficient degree of complementarity to avoid non-specific binding of the antisense oligonucleotide (ASO) to non-target nucleic acid under conditions in which specific binding is desired, e.g., under physiological conditions in the case of *in vivo* assays or therapeutic treatment, and in the case of *in vitro* assays, under conditions in which the assays are performed. In any aspect or embodiment described herein, an antisense oligonucleotide (ASO) is a single-stranded oligonucleotide.

[0151] An aspect of the present disclosure provides an inhibitory nucleic acid (e.g., antisense oligonucleotides (ASOs)) are provided that comprise a region of complementarity that is complementary with at least 8, at least 9, at least 10, at least 11, at least 12, at least 13,

at least 14, at least 15, or more contiguous nucleotides (e.g., 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 contiguous nucleotides) of a sequence complementary to the nucleic acid sequence of any one of SEQ ID NOs: 1, 2, 64-67, 79, 84, 85, 88, 89, 92, 93, 96, 97, 100, 101, 123, 124, or 125, rs11307260 (SEQ ID NO:79), rs142843265 (SEQ ID NO:64), rs3835416 (SEQ ID NO:125), rs34620383 (SEQ ID NO:65), rs2453565 (SEQ ID NO:66), rs700828 (SEQ ID NO:67), rs774356 (SEQ ID NO:68), rs774357 (SEQ ID NO:69), rs774359 (SEQ ID NO:70), rs2453554 (SEQ ID NO:71), rs2484319 (SEQ ID NO:72), rs2453555 (SEQ ID NO:73), rs2492816 (SEQ ID NO:74), rs3849945 (SEQ ID NO:75), or rs10757668 (SEQ ID NO:76).

**[0152]** An additional aspect of the present disclosure provides an inhibitory nucleic acid (e.g., antisense oligonucleotides (ASOs)) comprising a nucleic acid sequence that is 10 to 25 nucleotides in length and comprises a region of complementarity that is complementary with at least 8 (e.g., at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25) contiguous nucleotides of a sequence of any one of SEQ ID NOs: 1, 2, 64-76, 79, 84, 85, 88, 89, 92, 93, 96, 97, 100, 101, 123, 124, or 125, rs11307260 (SEQ ID NO:79), rs142843265 (SEQ ID NO:64), rs3835416 (SEQ ID NO:125), rs34620383 (SEQ ID NO:65), rs2453565 (SEQ ID NO:66), rs700828 (SEQ ID NO:67), rs774356 (SEQ ID NO:68), rs774357 (SEQ ID NO:69), rs774359 (SEQ ID NO:70), rs2453554 (SEQ ID NO:71), rs2484319 (SEQ ID NO:72), rs2453555 (SEQ ID NO:73), rs2492816 (SEQ ID NO:74), rs3849945 (SEQ ID NO:75), or rs10757668 (SEQ ID NO:76). In any aspect or embodiment described herein, the antisense oligonucleotide targets an intronic region of the gene.

**[0153]** In any aspect or embodiment described herein, the antisense oligonucleotide sequence and/or a target variant allele sequence has a guanine-cytosine (GC) content of about 20% to about 80% (e.g., about 35% to about 65% or about 40% to about 60% or about 45% to about 55%). For example, in any aspect or embodiment described herein, the antisense oligonucleotide sequence and/or a target variant allele sequence has a guanine-cytosine (GC) content of 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 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%, about 25% to about 35%, about 30% to about 80%, about 30% to about 75%, about 30% to about 70%, about 30% to about

65%, about 30% to about 60%, about 30% to about 55%, about 30% to about 50%, about 30% to about 45%, about 30% to about 40%, about 35% to about 80%, about 35% to about 75%, about 35% to about 70%, about 35% to about 65%, about 35% to about 60%, about 35% to about 55%, about 35% to about 50%, about 35% to about 45%, about 40% to about 80%, about 40% to about 75%, about 40% to about 70%, about 40% to about 65%, about 40% to about 60%, about 40% to about 55%, about 40% to about 50%, about 45% to about 80%, about 45% to about 75%, about 45% to about 70%, about 45% to about 65%, about 45% to about 60%, about 45% to about 55%, about 50% to about 80%, about 50% to about 75%, about 50% to about 70%, about 50% to about 65%, about 50% to about 60%, about 50% to about 80%, about 50% to about 75%, about 50% to about 70%, about 50% to about 65%, about 50% to about 60%, about 55% to about 80%, about 55% to about 75%, about 55% to about 70%, about 55% to about 65%, about 60% to about 80%, about 60% to about 75%, about 60% to about 70%, about 65% to about 80%, about 65% to about 75%, or about 70% to about 80%.

[0154] In any aspect or embodiment described herein, the antisense oligonucleotide comprises the nucleic acid sequence of any one of SEQ ID NOs: 80-83, 86, 87, 90, 91, 94, 95, 98, 99, 102, 103-122, or 125-145. In any aspect or embodiment described herein, the antisense oligonucleotide comprises, consists essentially of, or consists of any antisense oligonucleotide described herein

[0155] In any aspect or embodiment described herein, (i) the antisense oligonucleotide comprises at least one modified nucleotide; (ii) the antisense oligonucleotide comprises at least one modified nucleotide that is a 2'-modified nucleotide (e.g., each 2'-modified nucleotide includes or is independently a 2'-deoxy modified nucleotide, 2'-fluoro modified nucleotide, 2'-O-methyl modified nucleotide, 2'-O-methoxyethyl modified nucleotide, 2'-amino modified nucleotide, or 2'-aminoalkoxy modified nucleotide); (iii) the antisense oligonucleotide comprises a phosphorothioate modified linkage; (iv) the antisense oligonucleotide is a morpholino; (v) the antisense oligonucleotide comprises locked nucleic acids (LNAs) and ribonucleic acids (RNAs) (e.g., alternating locked nucleic acid (LNA) and ribonucleic acid (RNA) nucleotides); (vi) the antisense oligonucleotide comprises locked nucleic acids (LNAs) and deoxyribonucleic acids (DNAs) (e.g., alternating locked nucleic acid (LNA) and deoxyribonucleic acid (DNA) nucleotides); (v) the antisense oligonucleotide comprises ribonucleic acids (RNAs) and deoxyribonucleic acids (DNAs) (e.g., alternating ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) nucleotides); (vi) the antisense oligonucleotide is a gapmer; or (vii) a combination thereof.

**[0156]** In any aspect or embodiment described herein, the disclosure provides inhibitory nucleic acids (e.g., ASOs) that inhibit expression of a superoxide dismutase 1 (SOD1) gene comprising a mutated superoxide dismutase 1 (*SOD1*) allele in cis with an allele of a one-base pair indel (e.g., a genetic insertion or deletion and/or rs11307260 (SEQ ID NO:79)). In any aspect or embodiment described herein, a mutated superoxide dismutase 1 (*SOD1*) allele comprises a mutated superoxide dismutase 1 (*SOD1*) sequence in cis with an allele of a one-base pair indel (e.g., a genetic insertion or deletion, E134 deletion, and/or rs11307260 (SEQ ID NO:79)). In any aspect or embodiment described herein, a mutated superoxide dismutase 1 (*SOD1*) allele comprises a mutated superoxide dismutase 1 (*SOD1*) sequence in cis with an allele of a one-base pair indel (e.g., a genetic insertion or deletion, E134 deletion, and/or rs11307260 (SEQ ID NO:79)). The inhibitory nucleic acids, such as antisense oligonucleotides (ASOs) may, in any aspect or embodiment described herein, preferentially inhibit (e.g., reduce expression of) a superoxide dismutase 1 (*SOD1*) allele comprising an allele of a one-base pair indel (e.g., E134 deletion, rs11307260, or SEQ ID NO:79). In any aspect or embodiment described herein, the superoxide dismutase 1 (*SOD1*) allele comprises an A5V mutation. In any aspect or embodiment described herein, an allele one-base pair SOD1-indel is in cis with the A5V mutation.

**[0157]** In any aspect or embodiment described herein, the disclosure provides inhibitory nucleic acids (e.g., antisense oligonucleotides (ASOs)) that inhibit expression of a chromosome 9 open reading frame 72 (*C9Orf72*) allele harboring a repeat expansion comprising an allele of a two-base pair indel (e.g., a genetic insertion or deletion). In any aspect or embodiment described herein, the mutant chromosome 9 open reading frame 72 (*C9Orf72*) allele comprising an allele of two-base pair deletion comprises SEQ ID NO: 2. The inhibitory nucleic acids, such as antisense oligonucleotides (ASOs) may, in any aspect or embodiment described herein, preferentially inhibit (e.g., reduce expression of) a chromosome 9 open reading frame 72 (*C9Orf72*) mutant allele comprising a two-base pair C9-deletion. In any aspect or embodiment described herein, the chromosome 9 open reading frame 72 (*C9Orf72*) allele comprises a repeat expansion within intron 1. In any aspect or embodiment described herein, the two-base pair C9-deletion (i) is on the same haplotype as, or (ii) is cis with, the repeat expansion within intron 1 of the chromosome 9 open reading frame 72 (*C9Orf72*) gene.

**[0158]** In any aspect or embodiment described herein, the disclosure provides inhibitory nucleic acids (e.g., antisense oligonucleotides (ASOs)) that inhibit expression of a transactive responsive deoxyribonucleic acid (DNA)-binding protein 43 (TARDBP) allele harboring a mutation (e.g., a point mutation). In any aspect or embodiment described herein, the mutant

transactive responsive deoxyribonucleic acid (DNA)-binding protein 43 (TARDBP) allele comprising a point mutation at Q331 (e.g., Q331K mutation). The inhibitory nucleic acids, such as antisense oligonucleotides (ASOs) may, in any aspect or embodiment described herein, preferentially inhibit (e.g., reduce expression of) a transactive responsive deoxyribonucleic acid (DNA)-binding protein 43 (TARDBP) mutant allele comprising a mutation (e.g., a point mutation or Q331K mutation).

**[0159]** In any aspect or embodiment described herein, the inhibitory nucleic acid has a stronger binding affinity for one allele of a variant (e.g., a common variant and/or indel) relative to other alleles of the variant. For example, in any aspect or embodiment described herein, the inhibitory nucleic acid has a binding affinity for a nucleic acid sequence comprising a variant allele (e.g., a common variant and/or indel) that is at least two-fold, three-fold, four-fold, or five-fold stronger than its binding affinity for alternative alleles of the variant. In any aspect or embodiment described herein, the inhibitory nucleic acid has a binding affinity for a nucleic acid sequence comprising a variant allele (e.g., a common variant and/or indel) that is at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% stronger than its binding affinity for alternative alleles of the variant. A binding affinity may be measured using fluorescence-based methods, single-molecule kinetic methods, or any other binding assay known to a person of skill in the art.

**[0160]** In any aspect or embodiment described, the inhibitory nucleic acid inhibits or reduces expression levels (e.g., protein expression, gene expression, RNA expression, and the like) of a mutated allele comprising a mutation and target variant allele (e.g., a common variant and/or an indel) in a cell (e.g., a cell of the central nervous system) or a subject, wherein the mutation is a gain of function mutation or a dominant negative mutation. In any aspect or embodiment described herein, the inhibitory nucleic acid inhibits or reduces expression levels (e.g., protein expression, gene expression, RNA expression, etc.) of a mutated allele comprising a mutation and a target variant allele (e.g., a common variant and/or an indel) by at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100%, relative to a control (e.g., a baseline measurement or a control subject that is not administered the inhibitory nucleic acid), wherein the mutation is a gain of function mutation or a dominant negative mutation. In any aspect or embodiment described herein, the inhibitory nucleic acid inhibits or reduces expression levels of a mutated allele comprising a mutation and a targeted variant allele (e.g., a common variant and/or an indel) but does not substantially inhibit or reduce expression levels of the alternative alleles, wherein the mutation is a gain of function mutation or a dominant negative mutation. In any aspect or embodiment described herein, the inhibitory nucleic acid



inhibits or reduces expression levels of a mutated allele comprising a mutation and a target variant allele (e.g., a common variant and/or an indel) by at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100%, relative to a control (e.g., a baseline measurement or a control subject that is not administered the inhibitory nucleic acid), wherein the mutation is a gain of function mutation or a dominant negative mutation. In any aspect or embodiment described herein, the inhibitory nucleic acid inhibits or reduces expression levels of a mutated allele comprising a mutation and a target variant allele (e.g., a common variant and/or an indel) by at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100%, relative to wild-type allele, wherein the mutation is a gain of function mutation or a dominant negative mutation.

**[0161]** The inhibitory nucleic acid may also be 10-50, or 5-50 bases length. In any aspect or embodiment described herein, the inhibitory nucleic acid is 5 to 30 bases in length (e.g., 10-30, 10-25, 15-25, or 19-22). For example, in any aspect or embodiment described herein, the inhibitory nucleic acid is 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 bases in length, or any combination thereof. In any aspect or embodiment described herein, the inhibitory nucleic acid comprises or consists of a sequence of bases that are at least 80% or 90% complementary to, e.g., at least 5, 10, 15, 20, 25 or 30 bases of, or up to 30 or 40 bases of, the target nucleic acid. In any aspect or embodiment described herein, the inhibitory nucleic acid comprises or consists of a sequence of bases that comprises a sequence of bases with up to 3 mismatches (e.g., 0, 1, 2, or 3 mismatches; up to 1 mismatch; or up to 2 mismatches) over 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 bases of the target nucleic acid.

**[0162]** In any aspect or embodiment described herein, any one or more thymidine (T) nucleotides or uridine (U) nucleotides in a sequence provided herein may be replaced with any other nucleotide suitable for base pairing (e.g., via a Watson-Crick base pair) with an adenosine nucleotide. For example, T may be replaced with U, and U may be replaced with T. In any aspect or embodiment described herein, inhibitory nucleic acids are provided that inhibit expression of genes in a cell of the central nervous system. In any aspect or embodiment described herein, the cell is a neuron, astrocyte, or oligodendrocyte.

**[0163]** In any aspect or embodiment described herein, the cell expresses a mutated allele comprising a target variant allele (e.g., a common variant and/or an indel) and a mutation that is a gain of function mutation or a dominant negative mutation. In any aspect or embodiment described herein, the subject expresses a mutated allele comprising a target variant allele (e.g.,

a common variant and/or an indel) and a mutation that is a gain of function mutation or a dominant negative mutation.

**[0164]** In any aspect or embodiment described herein, an antisense oligonucleotide (ASO) has a region of complementarity that is perfectly complementary to a portion of a target nucleic acid (e.g., target RNA). However, it should be appreciated that, in any aspect and embodiment described herein, an antisense oligonucleotide (ASO) may be used that has less than 100% sequence complementarity with a target nucleic acid. In any aspect or embodiment described herein, the region of complementarity of the antisense oligonucleotide (ASO) is complementary with at least 6 (e.g., at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, or more) consecutive nucleotides of a target nucleic acid. In addition, to minimize the likelihood of off-target effects, in any aspect or embodiment described herein, an antisense oligonucleotide (ASO) is designed to ensure that it does not have a sequence (e.g., of 5 or more consecutive nucleotides) that has high complementarity with an off-target nucleic acid (e.g., a wild-type allele, a different mutant allele, or a combination thereof).

**[0165]** Complementary refers to the capacity for precise pairing between two nucleotides. For example, if a nucleotide at a certain position of an antisense nucleic acid is capable of hydrogen bonding with a nucleotide at the corresponding position of a target nucleic acid (e.g., target RNA), then the antisense nucleic acid and target nucleic acid are considered to be complementary to each other at that position. The antisense nucleic acid and target nucleic acid are complementary to each other when a sufficient number of corresponding positions in each molecule are occupied by nucleotides that can hydrogen bond with each other through their bases. Thus, “complementary” is a term that is used to indicate a sufficient degree of complementarity or precise pairing such that stable and specific binding occurs between the inhibitory nucleic acid (e.g., antisense nucleic acid) and the target nucleic acid. However, it should be appreciated that 100% complementarity is not required. For example, in any aspect or embodiment described herein, an inhibitory nucleic acid (e.g., antisense nucleic acid, an oligonucleotide, or a combination thereof) may be at least 80% (e.g., at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%) complementary to the consecutive nucleotides of a target nucleic acid.

**[0166]** Thus, it is understood in the art that a complementary nucleotide sequence need not be 100% complementary to that of its target to be specifically hybridizable. In any aspect or embodiment described herein, a complementary nucleic acid sequence for purposes of the present disclosure is specifically hybridizable when binding of the sequence to the target

nucleic acid produces the desired inhibition or reduction in expression to occur and there is a sufficient degree of complementarity to avoid non-specific binding to non-target nucleic acids under conditions in which avoidance of non-specific binding is desired, e.g., under physiological conditions in the case of *in vivo* assays or therapeutic treatment, and in the case of *in vitro* assays, under conditions in which the assays are performed under suitable conditions of stringency.

**[0167]** Sequence identity, including determination of sequence complementarity for nucleic acid sequences, may be determined by sequence comparison and alignment algorithms known in the art. To determine the percent identity of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in the first sequence or second sequence for optimal alignment). The nucleotides at corresponding nucleotide positions are then compared. When a position in the first sequence is occupied by the same residue as the corresponding position in the second sequence, then the molecules are identical at that position. In any aspect or embodiment described herein, the percent identity between the two sequences is a function of the number of identical positions shared by the sequences (e.g., % homology = number of identical positions / total number of positions × 100), optionally penalizing the score for the number of gaps introduced and/or length of gaps introduced.

**[0168]** In any aspect or embodiment described herein, an inhibitory nucleic acid (e.g., antisense oligonucleotide (ASO)) of the disclosure have a length in a range of 5 to 40 nucleotides, 5 to 30 nucleotides, 10 to 30 nucleotides, 10 to 25 nucleotides, or 15 to 25 nucleotides. In any aspect or embodiment described herein, an inhibitory nucleic acid (e.g., antisense oligonucleotide (ASO)) has a length of 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, or more.

**[0169]** In any aspect or embodiment described herein, inhibitory nucleic acids (e.g., antisense oligonucleotides (ASOs)) are provided in a homogeneous preparation, e.g., in which at least 85%, at least 90%, at least 95%, or at least 99% of the oligonucleotides are identical. For example, in any aspect or embodiment described herein, homogeneous preparations of oligonucleotides are provided in which at least 85%, at least 90%, at least 95%, or at least 99% of the oligonucleotides in the preparation are 10 to 25 nucleotides in length and comprise a region of complementarity that is complementary with at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleotides of the target sequence comprising the variant (e.g., first variant, common variant, indel, or a combination thereof; such as any of those described herein).

[0170] Antisense nucleic acids of the disclosure may be modified to achieve one or more desired properties (for example, improved cellular uptake, improved stability, reduced immunogenicity, improved potency, improved target hybridization, susceptibility to RNase cleavage, etc.). Antisense nucleic acids can be modified at a base moiety, sugar moiety and/or phosphate backbone. Accordingly, antisense nucleic acids may have one or more modified nucleotides (e.g., a nucleotide analog) and/or one or more backbone modifications (e.g., a modified internucleotide linkage). Antisense nucleic acids may have a combination of modified and unmodified nucleotides. Antisense nucleic acids may also have a combination of modified and unmodified internucleotide linkages.

[0171] In any aspect or embodiment described herein, inhibitory nucleic acid (e.g., antisense oligonucleotide (ASO)) of the present disclosure may include ribonucleotides, deoxyribonucleotides, and combinations thereof. Examples of modified nucleotides which can be used in antisense nucleic acids of the present disclosure include, for example, 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl)uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, and 2,6-diaminopurine.

[0172] In any aspect or embodiment described herein, the inhibitory nucleic acid (e.g., antisense oligonucleotide (ASO)) is a gapmer oligonucleotide. As used herein, a gapmer oligonucleotide has the formula 5'-A-B-C-3', wherein B is a central segment of nucleotides flanked by 'A' and 'C' segments. In any aspect or embodiment described herein, the central 'B' segment comprises contiguous deoxynucleotides. The central segment may comprise 6 to 15 contiguous deoxynucleotides (e.g., 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 contiguous deoxynucleotides). In any aspect or embodiment described herein, the 'A' and/or 'C' flanking segments comprise contiguous ribonucleotides. The flanking segments may comprise 3-10 contiguous ribonucleotides (e.g., 3, 4, 5, 6, 7, 8, 9, or 10 contiguous ribonucleotides). In any aspect or embodiment described herein, the central segment is capable of recruiting an RNase

(e.g., RNase H). In any aspect or embodiment described herein, the gapmer binds to the target nucleic acid to recruit an RNase. In any aspect or embodiment described herein, the flanking segments comprise modified nucleotides. In any aspect or embodiment described herein, each of the nucleotides in a flanking segment are modified nucleotides (e.g., modified ribonucleotides). In any aspect or embodiment described herein, a modified nucleotide is a 2' methoxyethyl modified nucleotide, a 2' o-methyl modified nucleotide, or a locked nucleic acid. In any aspect or embodiment described herein, the two flanking segments comprise the same number of nucleotides. In any aspect or embodiment described herein, the two flanking segments comprise different numbers of nucleotides. In some embodiments, a gapmer is a 5-10-5 gapmer (e.g., comprising a central segment of ten 2' deoxynucleotides flanked by five ribonucleotides, e.g., five 2' methoxyethyl (MOE) ribonucleotides). In any aspect or embodiment described herein, a gapmer is a 4-8-4 gapmer (e.g., comprising a central segment of eight 2' deoxynucleotides flanked by four nucleotides, e.g., four locked nucleic acid (LNA) nucleotides).

**[0173]** In any aspect or embodiment described herein, a modified nucleotide is a 2'-modified nucleotide. For example, in any aspect or embodiment described herein, each of the 2'-modified nucleotide is a 2'-deoxy modified nucleotide, 2'-fluoro modified nucleotide, 2'-O-methyl modified nucleotide, 2'-O-methoxyethyl modified nucleotide, 2'-amino modified nucleotide, or 2'-aminoalkoxy modified nucleotide. In any aspect or embodiment described herein, the 2'-modified nucleotide may comprise a 2'-O-4'-C methylene bridge, such as a locked nucleic acid (LNA) nucleotide. In any aspect or embodiment described herein, the 2'-modified nucleotide may have the 2'-hydroxyl group linked to the 3' or 4' carbon atom of the sugar ring thereby forming a bicyclic sugar moiety. In such aspects and embodiments, the linkage may be a methylene ( $\text{---CH}_2\text{---}$ )<sub>n</sub> group bridging the 2' oxygen atom and the 3' or 4' carbon atom, wherein n is 1 or 2.

**[0174]** In any aspect or embodiment described herein, the inhibitory nucleic acid (e.g., antisense oligonucleotide (ASO)) include combinations of LNA nucleotides and unmodified nucleotides. In any aspect or embodiment described herein, the inhibitory nucleic acid (e.g., antisense oligonucleotide (ASO)) includes combinations of LNA and RNA nucleotides. In any aspect or embodiment described herein, the inhibitory nucleic acid (e.g., antisense oligonucleotide (ASO)) includes combinations of LNA and DNA nucleotides. In any aspect or embodiment described herein, the inhibitory nucleic acid (e.g., antisense oligonucleotide (ASO)) the oligonucleotide modification includes Locked Nucleic Acids (LNAs) in which the

2'-hydroxyl group is linked to the 3' or 4' carbon atom of the sugar ring thereby forming a bicyclic sugar moiety.

**[0175]** In any aspect or embodiment described herein, the inhibitory nucleic acid (e.g., antisense oligonucleotide (ASO)) include nucleobase-modified nucleotides (e.g., nucleotides containing a non-naturally occurring nucleobase instead of a naturally occurring nucleobase). For example, in any aspect or embodiment described herein, bases may be modified to block the activity of adenosine deaminase. In any aspect or embodiment described herein, the modified nucleobases include, but are not limited to, uridine and/or cytidine modified at the 5-position (e.g., 5-(2-amino)propyl uridine, 5-bromo uridine); adenosine and/or guanosines modified at the 8 position (e.g., 8-bromo guanosine); deaza nucleotides (e.g., 7-deaza-adenosine); O- and N-alkylated nucleotides (e.g., N6-methyl adenosine). It should be noted that the above modifications may be combined.

**[0176]** Within the inhibitory nucleic acid (e.g., antisense oligonucleotide (ASO)) of the disclosures, as few as one and as many as all nucleotides can be modified. For example, an oligonucleotide (e.g., an oligonucleotide of 20 nucleotides in length) may contain 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 modified nucleotides. In any aspect or embodiment described herein, a modified oligonucleotide will contain as few modified nucleotides as are necessary to achieve a desired level of *in vitro* or *in vivo* stability and/or bioaccessibility or other desired property.

**[0177]** In any aspect or embodiment described herein, the inhibitory nucleic acid (e.g., antisense oligonucleotide (ASO)) may include nonionic DNA analogs, such as alkyl-phosphates and/or aryl-phosphates (in which the charged phosphonate oxygen is replaced by an alkyl or aryl group), phosphodiester and/or alkylphosphotriesters (in which the charged oxygen moiety is alkylated), or a combination thereof. Nucleic acids which contain a diol, such as tetraethyleneglycol or hexaethyleneglycol, at either or both termini have also been shown to be substantially resistant to nuclease degradation and may be used herein. In any aspect or embodiment described herein, antisense nucleic acids may include at least one lipophilic substituted nucleotide analog and/or a pyrimidine-purine dinucleotide.

**[0178]** In any aspect or embodiment described herein, antisense nucleic acids (e.g., oligonucleotides) may have one or two accessible 5' ends. In any aspect or embodiment described herein, the modified oligonucleotide has two such 5' ends (for example, by attaching two oligonucleotides through a 3'-3' linkage to generate an oligonucleotide having one or two accessible 5' ends). In any aspect or embodiment described herein, the 3'-3'-linkage may be a phosphodiester, phosphorothioate, or any other modified internucleoside bridge. Additionally,

in any aspect or embodiment described herein, 3'3'-linked oligonucleotides where the linkage between the 3' terminal nucleosides is not a phosphodiester, phosphorothioate or other modified bridge, can be prepared using an additional spacer, such as tri-ethylenglycol phosphate moiety or tetra-ethylenglycol phosphate moiety.

**[0179]** In any aspect or embodiment described herein, a phosphodiester internucleotide linkage of an antisense nucleic acid can be replaced with a modified linkage. In any aspect or embodiment described herein, the modified linkage may be selected from, for example, phosphorothioate, phosphorodithioate, NR1R2-phosphoramidate, boranophosphate,  $\alpha$ -hydroxybenzyl phosphonate, phosphate-(C1-C21)—O-alkyl ester, phosphate-[(C6-C12)aryl-(C1-C21)—O-alkyl]ester, (C1-C8)alkylphosphonate and/or (C6-C12)arylphosphonate bridges, and (C7-C12)- $\alpha$ -hydroxymethyl-aryl.

**[0180]** In any aspect or embodiment described herein, phosphate backbone of the inhibitory nucleic acid (e.g., antisense oligonucleotide (ASO)) is modified to generate peptide nucleic acid molecules. As used herein, the terms “peptide nucleic acids” or “PNAs” refer to nucleic acid mimics, e.g., DNA mimics, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols, for example.

**[0181]** In any aspect or embodiment described herein, the inhibitory nucleic acid (e.g., antisense oligonucleotide (ASO)) is formulated as morpholino oligonucleotides. In such embodiments, the riboside moiety of each subunit of an oligonucleotide of the oligonucleotide reagent is converted to a morpholine moiety. In any aspect or embodiment described herein, the morpholinos can be modified, for example, as a peptide conjugated morpholino, a phosphorodiamidate morpholino, and the like.

**[0182]** In any aspect or embodiment described herein, the inhibitory nucleic acid (e.g., antisense oligonucleotide (ASO)) is linked to functional groups such as peptides (e.g., for targeting host cell receptors *in vitro* or *in vivo*), or agents facilitating transport across the cell membrane or the blood-brain barrier. In any aspect or embodiment described herein, the oligonucleotide reagents of the disclosure are modified with chemical moieties (e.g., cholesterol) that improve the *in vivo* pharmacological properties of the oligonucleotide reagents.

#### *Pharmaceutical Compositions*

**[0183]** According to some aspects of the disclosure, compositions are provided that comprise an agent (e.g., inhibitory nucleic acid (e.g., antisense oligonucleotide (ASO) or vector comprising the same) and a carrier. As used herein, the term, “carrier” refers to an organic or inorganic ingredient, natural or synthetic, with which the active ingredient is combined to facilitate an intended use. For example, pharmaceutical compositions are provided that comprise an antisense nucleic acid and a pharmaceutically-acceptable carrier. As used herein the term “pharmaceutically acceptable carrier” refers to a carrier that is suitable for pharmaceutical administration. The term pharmaceutically-acceptable carrier includes compatible solid or liquid fillers, diluents or encapsulating substances that are suitable for administration to a human or other vertebrate animal.

**[0184]** Components of pharmaceutical compositions also are capable of being commingled with the agents of the present disclosure, and with each other, in a manner such that there is no interaction that would substantially impair the desired pharmaceutical efficiency. Pharmaceutical compositions may include solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and other suitable components compatible with pharmaceutical administration. Supplementary active agents can also be incorporated into the compositions. Active ingredients (e.g., inhibitory nucleic acid or antisense oligonucleotide (ASO)) may be admixed or compounded with any conventional, pharmaceutically acceptable carrier or excipient. Pharmaceutical compositions are generally sterile and prepared using aseptic technique. A sterile phosphate-buffered saline is one example of a pharmaceutically acceptable carrier. Other suitable carriers may be used. Pharmaceutical compositions also may comprise suitable solid or gel phase carriers or excipients. Examples of such carriers or excipients include but are not limited to calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols.

**[0185]** Inhibitory nucleic acids may be administered per se (neat) or in the form of a pharmaceutically acceptable salt. When used in medicine the salts are generally pharmaceutically acceptable, but non-pharmaceutically acceptable salts may conveniently be used to prepare pharmaceutically acceptable salts thereof. Such salts include, but are not limited to, those prepared from the following acids: hydrochloric, hydrobromic, sulphuric, nitric, phosphoric, maleic, acetic, salicylic, p-toluene sulphonc, tartaric, citric, methane sulphonc, formic, malonic, succinic, naphthalene-2-sulphonc, and benzene sulphonc. Also, such salts can be prepared as alkaline metal or alkaline earth salts, such as sodium, potassium or calcium salts of the carboxylic acid group.



[0186] Exemplary buffering agents include acetic acid and a salt (1-2% w/v); citric acid and a salt (1-3% w/v); boric acid and a salt (0.5-2.5% w/v); and phosphoric acid and a salt (0.8-2% w/v). Suitable preservatives include benzalkonium chloride (0.003-0.03% w/v); chlorobutanol (0.3-0.9% w/v); parabens (0.01-0.25% w/v) and thimerosal (0.004-0.02% w/v).

[0187] The compositions may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing the agents into association with a carrier that constitutes one or more accessory ingredients. In general, the compositions are prepared by uniformly and intimately bringing the agents into association with a liquid carrier, a finely divided solid carrier, or both, and then, if necessary, shaping the product. Liquid dose units are vials or ampoules. Solid dose units are tablets, capsules and suppositories.

[0188] An effective amount, also referred to as a therapeutically effective amount, of an inhibitory nucleic acid (e.g., antisense oligonucleotide (ASO)) capable of inhibiting a mutated allele comprising a target variant allele and a mutation, wherein the mutation is a gain of function mutation or a dominant negative mutation, is an amount sufficient to ameliorate at least one adverse effect associated with expression, or reduced expression, of the gene in a cell or in an individual in need of such modulation. The therapeutically effective amount to be included in pharmaceutical compositions may be selected based upon several factors, for example, the type, size and condition of the patient to be treated, the intended mode of administration, the capacity of the patient to incorporate the intended dosage form, and the like.

[0189] In any aspect or embodiment described herein, inhibitory nucleic acids may be prepared in a colloidal dispersion system. Colloidal dispersion systems include lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. An example of a colloidal system that may be used in methods provided herein is a liposome. Liposomes are artificial membrane vessels that are useful for delivering antisense nucleic acids *in vivo* or *in vitro*. It has been shown that large unilamellar vesicles can encapsulate large macromolecules. Nucleic acids and other components (e.g., viral vectors) can be encapsulated within the aqueous interior and be delivered to cells in a biologically active form. Liposomes may be targeted to a particular tissue by coupling the liposome to a specific ligand such as a monoclonal antibody, sugar, glycolipid, or protein. Ligands which may be useful for targeting a liposome to, for example, a smooth muscle cell or skeletal muscle cell include, but are not limited to intact or fragments of molecules that interact with muscle cell specific receptors and molecules, such as antibodies, which interact with the cell surface markers. Lipid formulations for transfection are commercially available from QIAGEN, for example, as EFFECTENE™ (a

non-liposomal lipid with a special DNA condensing enhancer) and SUPERFECT™ (a dendrimeric technology). Liposomes are commercially available from Invitrogen, Life Technologies, for example, as LIPOFECTIN™, which is formed of cationic lipids such as N-[1-(2, 3 dioleoyloxy)-propyl]-N, N, N-trimethylammonium chloride (DOTMA) and dioleoyl phosphatidylethanolamine (DOPE), as well as other lipid-based reagents including Lipofectamine and Oligofectamine. Certain cationic lipids, including in particular N-[1-(2, 3 dioleoyloxy)-propyl]-N,N,N-trimethylammonium methyl-sulfate (DOTAP), may be advantageous when combined with the ASO analogs of the disclosure.

**[0190]** In any aspect or embodiment described herein, inhibitory nucleic acids (e.g., antisense oligonucleotides (ASOs)) it formulated with a biocompatible microparticle or implant that is suitable for implantation or administration to a recipient. Bioerodible implants may include a biodegradable polymeric matrix, for example, for containing an exogenous expression construct engineered to express an antisense nucleic acid under the control of an appropriate promoter. The polymeric matrix can be used to achieve sustained release of the therapeutic agent in the subject. A polymeric matrix may be in the form of a microparticle such as a microsphere, in which an antisense nucleic acid and/or other therapeutic agent is dispersed throughout a solid polymeric matrix, or a microcapsule, in which antisense nucleic acid and/or other therapeutic agent is stored in the core of a polymeric shell. Other forms of the polymeric matrix for containing a therapeutic agent include films, coatings, gels, implants, and stents. The matrix composition also can be selected not to degrade, but rather, to release by diffusion over an extended period of time. In some embodiments, inhibitory nucleic acids are administered to the subject via an implant while the other therapeutic agent is administered.

**[0191]** Both non-biodegradable and biodegradable polymeric matrices can be used to deliver ASOs and/or the other therapeutic agent to a subject. Biodegradable matrices are preferred. Such polymers may be natural or synthetic polymers. The polymer is selected based on the period of time over which release is desired, generally in the order of a few hours to a year or longer. Typically, release over a period ranging from between a few hours and three to twelve months may be used. A polymer may be in the form of a hydrogel, for example, a hydrogel that can absorb up to about 90% of its weight in water and which is optionally cross-linked with multi-valent ions or other components, for example, polymers.

**[0192]** Other exemplary compositions that can be used to facilitate uptake of a nucleic acid include calcium phosphate and other chemical mediators of intracellular transport, microinjection compositions, electroporation and homologous recombination compositions

(e.g., for integrating a nucleic acid into a preselected location within the target cell chromosome).

**[0193]** In addition to the formulations described previously, the agents may also be formulated as a depot preparation. Such long-acting formulations may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

**[0194]** Other suitable liquid or solid pharmaceutical preparation forms are, for example, aqueous or saline solutions for inhalation, microencapsulated, encochleated, coated onto microscopic gold particles, contained in liposomes, nebulized, aerosols, pellets for implantation into the skin, or dried onto a sharp object to be scratched into the skin. The pharmaceutical compositions also include granules, powders, tablets, coated tablets, (micro)capsules, suppositories, syrups, emulsions, suspensions, creams, drops or preparations with protracted release of active compounds, in whose preparation excipients and additives and/or auxiliaries such as disintegrants, binders, coating agents, swelling agents, lubricants, flavorings, sweeteners or solubilizers are customarily used as described above. Suitable carriers may be readily selected by one of skill in the art in view of the indication for which the inhibitory nucleic acid (e.g., ASO) is directed. For example, one suitable carrier includes saline, which may be formulated with a variety of buffering solutions (e.g., phosphate buffered saline). Other exemplary carriers include sterile saline, lactose, sucrose, calcium phosphate, gelatin, dextran, agar, pectin, peanut oil, sesame oil, and water. Still others will be apparent to the skilled artisan.

**[0195]** Optionally, the compositions of the invention may contain, in addition to the ASO and carrier(s), other conventional pharmaceutical ingredients, such as preservatives, or chemical stabilizers. Suitable exemplary preservatives include chlorobutanol, potassium sorbate, sorbic acid, sulfur dioxide, propyl gallate, the parabens, ethyl vanillin, glycerin, phenol, and parachlorophenol. Suitable chemical stabilizers include gelatin and albumin.

**[0196]** The pharmaceutical compositions are suitable for use in a variety of drug delivery systems. Delivery vehicles such as liposomes, nanocapsules, microparticles, microspheres, lipid particles, vesicles, and the like, may be used for the introduction of the compositions of the present invention into suitable host cells.

**[0197]** The formation and use of liposomes is generally known to those of skill in the art. Recently, liposomes were developed with improved serum stability and circulation half-times (U.S. Pat. No. 5,741,516). Further, various methods of liposome and liposome like

preparations as potential drug carriers have been described (U.S. Pat. Nos. 5,567,434; 5,552,157; 5,565,213; 5,738,868 and 5,795,587).

[0198] Liposomes have been used successfully with a number of cell types that are normally resistant to transfection by other procedures. In addition, liposomes are free of the DNA length constraints that are typical of viral-based delivery systems. Liposomes have been used effectively to introduce genes, drugs, radiotherapeutic agents, viruses, transcription factors and allosteric effectors into a variety of cultured cell lines and animals. In addition, several successful clinical trials examining the effectiveness of liposome-mediated drug delivery have been completed.

[0199] Liposomes are formed from phospholipids that are dispersed in an aqueous medium and spontaneously form multilamellar concentric bilayer vesicles (also termed multilamellar vesicles (MLVs)). MLVs generally have diameters of from 25 nm to 4  $\mu$ m. Sonication of MLVs results in the formation of small unilamellar vesicles (SUVs) with diameters in the range of 200 to 500 angstroms, containing an aqueous solution in the core.

[0200] Alternatively, nanocapsule formulations of the inhibitory nucleic acid (e.g., ASO) may be used. Nanocapsules can generally entrap substances in a stable and reproducible way. To avoid side effects due to intracellular polymeric overloading, such ultrafine particles (sized around 0.1  $\mu$ m) should be designed using polymers able to be degraded *in vivo*. Biodegradable polyalkyl-cyanoacrylate nanoparticles that meet these requirements are contemplated for use.

[0201] In addition to the methods of delivery described above, the following techniques are also contemplated as alternative methods of delivering the inhibitory nucleic acid (e.g., ASO) compositions to a host. Sonophoresis (e.g., ultrasound) has been used and described in U.S. Pat. No. 5,656,016 as a device for enhancing the rate and efficacy of drug permeation into and through the circulatory system. Other drug delivery alternatives contemplated are intraosseous injection (U.S. Pat. No. 5,779,708), microchip devices (U.S. Pat. No. 5,797,898), ophthalmic formulations, transdermal matrices (U.S. Pat. Nos. 5,770,219 and 5,783,208) and feedback-controlled delivery (U.S. Pat. No. 5,697,899).

#### *Kits and Related Compositions*

[0202] The inhibitory nucleic acids (such as antisense oligonucleotides) and compositions described herein may, in an aspect, be assembled into pharmaceutical or diagnostic or research kits to facilitate their use in therapeutic, diagnostic or research applications. A kit may include one or more containers housing the components of the invention and instructions for use. Specifically, such kits may include one or more agents described herein, along with instructions

describing the intended application and the proper use of these agents. In certain embodiments agents in a kit may be in a pharmaceutical formulation and dosage suitable for a particular application and for a method of administration of the agents. Kits for research purposes may contain the components in appropriate concentrations or quantities for running various experiments.

**[0203]** The kit may be designed to facilitate use of the methods described herein by researchers and can take many forms. Each of the compositions of the kit, where applicable, may be provided in liquid form (e.g., in solution), or in solid form, (e.g., a dry powder). In certain cases, some of the compositions may be constitutable or otherwise processable (e.g., to an active form), for example, by the addition of a suitable solvent or other species (for example, water or a cell culture medium), which may or may not be provided with the kit. As used herein, “instructions” can define a component of instruction and/or promotion, and typically involve written instructions on or associated with packaging of the invention. Instructions also can include any oral or electronic instructions provided in any manner such that a user will clearly recognize that the instructions are to be associated with the kit, for example, audiovisual (e.g., videotape, DVD, and the like.), Internet, and/or web-based communications, and the like. The written instructions may be in a form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which instructions can also reflect approval by the agency of manufacture, use or sale for animal administration.

**[0204]** The kit may contain any one or more of the components described herein in one or more containers. As an example, in one embodiment, the kit may include instructions for mixing one or more components of the kit and/or isolating and mixing a sample and applying to a subject. The kit may include a container housing agents described herein. The agents may be in the form of a liquid, gel or solid (powder). The agents may be prepared sterilely, packaged in syringe and shipped refrigerated. Alternatively, it may be housed in a vial or other container for storage. A second container may have other agents prepared sterilely. Alternatively, the kit may include the active agents premixed and shipped in a syringe, vial, tube, or other container. The kit may have one or more or all of the components required to administer the agents to a subject, such as a syringe, topical application devices, or IV needle tubing and bag.

**[0205]** Exemplary embodiments of the invention are described in more detail by the following examples. These embodiments are exemplary of the invention, which one skilled in the art will recognize is not limited to the exemplary embodiments.

Example 1. Identification of *C9Orf72* indels and SNPs

[0206] Variants of *C9Orf72* that could be used as targets for allele specific antisense technologies were identified using whole genome sequencing (WGS) data from 204 *C9Orf72* patients that were reported to have a repeat expansion. Two insertion/deletion (indel) variants and ten single nucleotide polymorphisms (SNPs) showing a high rate of heterozygosity in the *C9Orf72* patients (around 80%) were identified and evaluated.

[0207] The RS number for the two indels were rs142843265 (SEQ ID NO:64) and rs34620383 (SEQ ID NO:65). Variant alleles in linkage disequilibrium (LD) were consistently present on the same chromosome (travelled together). A two base pair deletion in *C9orf72* (C9-deletion or C9-del; rs142843265 (SEQ ID NO:64)), as shown in FIG. 1, was identified as being in linkage disequilibrium with G<sub>4</sub>C<sub>2</sub> repeat expansions. The heterozygosity frequency of the C9-deletion indel was 79% of *C9Orf72* patients. The inventors identified that a two-base pair deletion (see, e.g., FIG. 1; termed “C9-deletion”) is in linkage disequilibrium with the *C9Orf72* G<sub>4</sub>C<sub>2</sub> repeat expansion. The *C9Orf72* sequence variation comprises a two-base pair C9-deletion comprises SEQ ID NO: 2. Accordingly, targeting this single indel allele could treat approximately 80% of *C9Orf72* patients (e.g., patients having a *C9Orf72* repeat and having or suspected of having ALS or FTD).

[0208] The RS number for the ten SNPs were: rs2453565 (SEQ ID NO:66), rs700828 (SEQ ID NO:67), rs774356 (SEQ ID NO:68), rs774357 (SEQ ID NO:69), rs774359 (SEQ ID NO:70), rs2453554 (SEQ ID NO:71), rs2484319 (SEQ ID NO:72), rs2453555 (SEQ ID NO:73), rs2492816 (SEQ ID NO:74), and rs3849945 (SEQ ID NO:75).

#### Example 2. ASOs Target Indels Display Allele-Specific Knockdown

[0209] Antisense oligonucleotides (ASOs) for targeting mutated *C9Orf72* genes comprising the C9-deletion were designed using 1-base pair tiling over the indel site (see, bottom of FIG. 1). ASOs were synthesized using two different chemistries – (1) 5-10-5 MOE gapmer technology (central segment of ten 2' deoxynucleotides flanked by five 2' methoxyethyl (MOE) ribonucleotides); and (2) 4-8-4 LNA gapmer technology (central segment of eight or nine 2' deoxynucleotides flanked by four locked nucleic acid (LNA) nucleotides). Provided in Table 1 are the specific antisense oligonucleotides that were synthesized and tested.

[0210] In distinct experiments, either cells that were homozygous for the minor allele of rs142843265 (C9-del/C9-del; described in FIG. 1) and cells homozygous for the major allele of rs142843265 (WT/WT; described in FIG. 1) were contacted with the synthesized ASOs (100 nM concentration). As shown in FIG. 2, the ASOs preferentially targeted the cells that were

homozygous for the minor allele of rs142843265 (C9-del/C9-del) for inhibition of *C9orf72* expression. It was found that positioning the segment of the ASO that was complementary to the C9-deletion indel in the center of the ASO often resulted in the best discrimination of C9-deletion inhibition relative to wild-type inhibition. See also FIG. 4A and 6A. For example, an ASO comprising a sequence that was complementary to SEQ ID NO: 12 or 13 resulted in approximately 80% inhibition of *C9orf72* expression in C9-del/C9-del cells, and approximately 5-10% inhibition of *C9orf72* expression in wild-type cells.

**[0211]** A dual luciferase assay was also performed to evaluate the allele-specific ASOs of this Example. In brief, as shown in FIG. 3A, the dual luciferase assay utilized two luciferase genes, wherein the first gene comprised a portion of the *C9orf72* gene at its 3' end. Binding of an ASO to the *C9orf72* gene (SEQ ID NO:1 or SEQ ID NO:2) at the 3' end of the first luciferase gene resulted in lowered expression from the first luciferase gene. The second luciferase gene functioned as an internal control for normalization purposes. Candidate target regions were cloned into luciferase expression vectors (pmirGLO vector, as shown in FIG. 3B). These expression vectors were subsequently transfected into HEK293 cells in a 96-well plate format. Cells were exposed to an antisense oligonucleotide (ASOs) and luciferase expression was measured. Non-treated cells, cells treated with a scrambled ASO (*i.e.*, not specific for *C9orf72* target region, WT or C9-del), and a non-discriminatory ASO (targets both allele target sequences) were used as controls. The average percent inhibition data obtained using this luciferase screening assay is provided in Table 1 and FIGs. 4A (MOE gapmers) and 6A (LNA gapmers).

Table 1. Antisense Oligonucleotides of Example 2

| Name  | Sequence             | SEQ ID NO: | ASO Chemistry | Average Percent Inhibition of C9-del <i>C9orf72</i> Target Region, SEQ ID NO:2 (luciferase screening assay) | Average Percent Inhibition of WT <i>C9orf72</i> Target Region, SEQ ID NO:1 (luciferase screening assay) |
|-------|----------------------|------------|---------------|-------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------|
| ASO 1 | CGAGAAGTAGTACCTTAAAA | 23         | 5-10-5 MOE    | 90%                                                                                                         | 90%                                                                                                     |
| ASO 2 | GAGAAGTAGTACCTTAAAC  | 24         | 5-10-5 MOE    | 81%                                                                                                         | 78%                                                                                                     |
| ASO 3 | AGAAGTAGTACCTTAAACA  | 25         | 5-10-5 MOE    | 3%                                                                                                          | 39%                                                                                                     |

|         |                      |    |               |     |     |
|---------|----------------------|----|---------------|-----|-----|
| ASO 4   | GAAGTAGTACCTTAAAACAT | 26 | 5-10-5<br>MOE | 63% | 79% |
| ASO 5   | AAGTAGTACCTTAAAACATA | 27 | 5-10-5<br>MOE | 10% | 5%  |
| ASO 6   | AGTAGTACCTTAAAACATAT | 28 | 5-10-5<br>MOE | 47% | 76% |
| ASO 7   | GTAGTACCTTAAAACATATC | 29 | 5-10-5<br>MOE | 12% | 77% |
| ASO 8   | TAGTACCTTAAAACATATCA | 30 | 5-10-5<br>MOE | 13% | 79% |
| ASO 9   | AGTACCTTAAAACATATCAG | 31 | 5-10-5<br>MOE | 64% | 90% |
| ASO 10  | GTACCTTAAAACATATCAGA | 32 | 5-10-5<br>MOE | 35% | 83% |
| ASO 11  | TACCTTAAAACATATCAGAG | 33 | 5-10-5<br>MOE | 33% | 77% |
| ASO 12  | ACCTTAAAACATATCAGAGA | 34 | 5-10-5<br>MOE | 47% | 82% |
| ASO 13  | CCTTAAAACATATCAGAGAC | 35 | 5-10-5<br>MOE | 62% | 90% |
| ASO 14  | CTTAAAACATATCAGAGACA | 36 | 5-10-5<br>MOE | 63% | 96% |
| ASO 15  | TTAAAACATATCAGAGACAA | 37 | 5-10-5<br>MOE | 69% | 90% |
| ASO 16  | TAAAACATATCAGAGACAAT | 38 | 5-10-5<br>MOE | 40% | 64% |
| ASO 17  | AAAACATATCAGAGACAATT | 39 | 5-10-5<br>MOE | 89% | 92% |
| ASO 18  | AAACATATCAGAGACAATTT | 40 | 5-10-5<br>MOE | 88% | 92% |
| LNA 1   | AAGTAGTACCTTAAAA     | 41 | 4-8-4 LNA     | 96% | 97% |
| LNA 1b  | AAGTAGTACCTTAAAAC    | 42 | 4-9-4 LNA     | 97% | 97% |
| LNA 2   | AGTAGTACCTTAAAAC     | 43 | 4-8-4 LNA     | 70% | 77% |
| LNA 3   | GTAGTACCTTAAAACA     | 44 | 4-8-4 LNA     | 37% | 70% |
| LNA 4   | TAGTACCTTAAAACAT     | 45 | 4-8-4 LNA     | 23% | 77% |
| LNA 5   | AGTACCTTAAAACATA     | 46 | 4-8-4 LNA     | 46% | 86% |
| LNA 6   | GTACCTTAAAACATAT     | 47 | 4-8-4 LNA     | 14% | 78% |
| LNA 7   | TACCTTAAAACATATC     | 48 | 4-8-4 LNA     | 11% | 83% |
| LNA 7b  | GTACCTTAAAACATATC    | 49 | 4-9-4 LNA     | 23% | 88% |
| LNA 8   | ACCTTAAAACATATCA     | 50 | 4-8-4 LNA     | 0%  | 85% |
| LNA 9   | CCTTAAAACATATCAG     | 51 | 4-8-4 LNA     | 0%  | 78% |
| LNA 10  | CTTAAAACATATCAGA     | 52 | 4-8-4 LNA     | 0%  | 88% |
| LNA 10b | CCTTAAAACATATCAGA    | 53 | 4-9-4 LNA     | 18% | 86% |
| LNA 11  | TTAAAACATATCAGAG     | 54 | 4-8-4 LNA     | 18% | 86% |
| LNA 12  | TAAAACATATCAGAGA     | 55 | 4-8-4 LNA     | 83% | 95% |
| LNA 13  | AAAACATATCAGAGAC     | 56 | 4-8-4 LNA     | 90% | 90% |
| LNA 14  | AAACATATCAGAGACA     | 57 | 4-8-4 LNA     | 96% | 96% |

[0212] Several tested MOE gapmer ASOs (FIG. 4A) as provided in Table 1 were shown to be effective in reducing gene expression in this luciferase assay in an allele-specific manner (i.e., greater reduction in expression of the mutant C9-del allele relative to the normal allele). In particular, it was found that the MOE gapmer of SEQ ID NO: 30 (ASO 8) demonstrated potent allele-specific reduction in gene expression (FIG. 4B). It was further shown that the



MOE gapmer of SEQ ID NO: 30 (ASO 8) was capable of reducing gene expression in a dose-dependent manner (FIG. 4C).

**[0213]** Several tested LNA gapmer ASOs (FIG. 6A) as provided in Table 1 were shown to be effective in reducing gene expression in this luciferase assay in an allele-specific manner (i.e., greater reduction in expression of the mutant C9-del allele relative to the normal allele). In particular, it was found that the LNA gapmers of SEQ ID NO: 51 (LNA 9) and SEQ ID NO: 52 (LNA 10) demonstrated potent allele-specific reduction in gene expression (FIG. 6B). It was further shown that the LNA gapmer of SEQ ID NO: 52 (LNA 10) was capable of reducing gene expression in a dose-dependent manner, while LNA gapmer of SEQ ID NO: 51 (LNA9) had a robust knockdown ability even at the lowest concentrations tested (FIG. 6C).

Example 3. Additional cellular experiments showing efficacy of the ASOs

**[0214]** The efficacy of ASOs synthesized in Example 2 were further evaluated in additional cellular experiments. A qRT-PCR assay was also used to directly measure total *C9orf72* knockdown. Fibroblasts were plated in 6 well plates, grown overnight, then treated with 100nM ASOs for 72hrs. RNA was isolated from the cells, converted to complementary DNA (cDNA), and used as input material for qRT-PCR. 100 ng of each sample was used to probe HPRT (Ctrl) and *C9orf72*. Each sample was run in duplicate and C9 levels were normalized to HPRT and nontreated controls as shown in FIG. 2, FIG. 5, FIG. 7, FIG. 16A, FIG. 16C, and FIG. 17. Allele specific knockdown of the mutant C9 expansion containing allele and relative levels of WT allele were determined by bulk RNAseq analysis.

**[0215]** It was found that the MOE gapmer of SEQ ID NO: 30 (C9\_GM\_9; ASO 8) and the LNA gapmer of SEQ ID NO: 51 (C9\_LNA\_9; ASO 9) were capable of allele-specific reduction in gene expression in fibroblasts. See FIG. 5 (MOE gapmer) and FIG. 7 (LNA gapmer). Control experiments did not demonstrate an allele-specific reduction in gene expression. Notably, a control ASO from Lagier-Tourenne, C et al. "Targeted degradation of sense and antisense C9orf72 RNA foci as therapy for ALS and frontotemporal degeneration". PNAS 2013 Nov 19;110(47):E4530-9 was unable to provide allele-specific reduction in gene expression (FIG. 7)

Example 4: Location of C9 deletion variant and a second variant within the C9orf72 gene

**[0216]** In FIG. 8, the position of the C9 deletion variant (rs142843265), that is the target of the C9orf72 ASOs ) and a second variant within the C9orf72 transcribed region (rs10757668)

which can be used to assess allele-specific transcript knockdown in a sample that is heterozygous for both variants.

Example 5: Experiment confirming reduction of individual allelic expression in cells

**[0217]** The reduction of expression from a specific allele was evaluated in fibroblasts using bulk RNA sequencing. Fibroblasts were plated in 6 well plates, grown overnight, then treated with 100nM ASOs for 72 hours. RNA was isolated from the cells and sent to Yale Genome center for library construction and sequencing. 100M reads were performed per sample and the percentage of WT allele present was calculated based on the presence of the level of rs9103 found in each sample. From this analysis and as shown in Table 2, it was observed that treatment with the LNA gapmer of SEQ ID NO: 51 (C9\_LNA\_9; ASO 9) was capable of mutant allele-specific reduction of gene expression in fibroblasts resulting in a higher portion of gene expression resulting from the WT allele. Notably, NATMED 22 ASO (previously described allele specific *C9Orf72* ASO; Tran, *et al.*, Suppression of mutant *C9Orf72* expression by a potent mixed backbone antisense oligonucleotide. Nature Medicine Nat Med. 2022 Jan; 28(1) 117-124) was able to reduce overall *C9Orf72* expression, but did not raise the portion of the expression of the WT allele as much as LNA #9 (Table below).

Table 2: Reduction of individual allelic expression in fibroblasts

| Treatment        | WT SNP Allele Percent of Total |
|------------------|--------------------------------|
| Non-treated      | 26%%                           |
| NATMED 22 C9 ASO | 52%                            |
| LNA #9           | 68%                            |

Example 6: Experiment showing allele specific ASO treatment of patient cells partially rescue a patient phenotype

**[0218]** The ability of ASOs synthesized in Example 2 to alter known disease phenotypes was evaluated in heterozygous patient *C9Orf72* fibroblasts carrying the C9-deletion variant and ASP SNP. See Figure 8. Fibroblasts were plated in 24 well plates in duplicate – one set on coverslips and one directly on the plate, then treated with 100nM ASOs for 48hrs. A

reduction in C9orf72 expression was confirmed in ASO treated cells using the qRT-PCR assay mentioned in example 3. The duplicate cells on coverslips were processed for RNA foci staining as described in Almeida A, et al “Modeling key pathological features of frontotemporal dementia with C9ORF72 repeat expansion in iPSC-derived human neurons.” *Acta Neuropathol* (2013) 126:385–399, using the probes described in the same paper. As shown in FIG. 9A, FIG. 9B, and FIG. 9C, cells treated with the LNA gapmer of SEQ ID NO: 51 (C9\_LNA\_9; ASO 9) was capable of reducing both the number of foci present in cells and the overall number of cells that had RNA foci present.

Example 7. Development of Allele-specific Antisense Oligonucleotides for Superoxide Dismutase 1 (SOD1)

**[0219]** Bioinformatic analysis of the Superoxide Dismutase 1 (SOD1) gene revealed a common 1 base pair indel in intron 4 (SEQ ID NO:77 (Insertion allele) and SEQ ID NO:78 (Deletion allele)). As determined by the gnomAD database, this indel, rs11307260 SEQ ID NO:79 displays a heterozygosity frequency of 49.8% in the population and is not located in a repetitive region. As such, this indel was selected for the development of allele-specific antisense oligonucleotide. A set of two locked nucleic acid (LNA) gapmers and a set of two methoxyethyl (MOE) antisense oligonucleotide were synthesized for the insertion/deletion alleles with the variant position located near the center. The antisense oligonucleotide that targets the variant that has more bases is referred to herein as the long or insertion antisense oligonucleotide or allele. Conversely, antisense oligonucleotide that targets the variant that has less bases is referred to herein as the short or deletion antisense oligonucleotide or allele.

**[0220]** The sequence of the short (deletion) allele antisense oligonucleotide sequence (Short SOD1 LNA) was:

+C\*+T\*+T\*A\*A\*T\*A\*C\*T\*T\*T\*A\*G\*C\*A\*A\*C\*+A\*+G\*+T, wherein + denotes a locked nucleic acid and \* denotes a nucleic acid with a phosphorothioate backbone/bond (SEQ ID NO:80).

**[0221]** The sequence of the long (insertion) allele antisense oligonucleotide (Long SOD1 LNA) was:

+C\*+T\*+T\*A\*A\*T\*A\*C\*T\*T\*T\*A\*A\*G\*C\*A\*A\*+C\*+A\*+G, wherein + denotes a locked nucleic acid and \* denotes a nucleic acid with a phosphorothioate backbone/bond (SEQ ID NO:81).

[0222] A tiling was performing for short (deletion) methoxyethyl (MOE) antisense oligonucleotides, which are referred to herein as SOD1 Short-1 through SOD1 Short-11. The nucleotide sequence of SOD1 Short-1 through SOD1 Short-11 were:

SOD1 Short-1:

/52MOErA/\*i2MOErA/\*i2MOErA/\*i2MOErT/\*i2MOErC/\*T\*T\*A\*A\*T\*A\*  
C\*T\*T\*T/\*i2MOErA/\*i2MOErG/\*i2MOErC/\*i2MOErA/\*32MOErA/ (SEQ  
ID NO:104), wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and  
\* denotes a nucleic acid with a phosphorothioate backbone/bond.

SOD1 Short-2:

/52MOErA/\*i2MOErA/\*i2MOErT/\*i2MOErC/\*i2MOErT/\*T\*A\*A\*T\*A\*C\*  
T\*T\*T\*A/\*i2MOErG/\*i2MOErC/\*i2MOErA/\*i2MOErA/\*32MOErC/ (SEQ  
ID NO:105), wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and  
\* denotes a nucleic acid with a phosphorothioate backbone/bond.

SOD1 Short-3:

/52MOErA/\*i2MOErT/\*i2MOErC/\*i2MOErT/\*i2MOErT/\*A\*A\*T\*A\*C\*T\*  
T\*T\*A\*G/\*i2MOErC/\*i2MOErA/\*i2MOErA/\*i2MOErC/\*32MOErA/ (SEQ  
ID NO:106), wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and  
\* denotes a nucleic acid with a phosphorothioate backbone/bond.

SOD1 Short-4:

/52MOErT/\*i2MOErC/\*i2MOErT/\*i2MOErT/\*i2MOErA/\*A\*T\*A\*C\*T\*T\*  
T\*A\*G\*C/\*i2MOErA/\*i2MOErA/\*i2MOErC/\*i2MOErA/\*32MOErG/ (SEQ  
ID NO:107), wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and  
\* denotes a nucleic acid with a phosphorothioate backbone/bond.

SOD1 Short-5 (also referred to herein as Short SOD1 MOE):

/52MOErC/\*i2MOErT/\*i2MOErT/\*i2MOErA/\*i2MOErA/\*T\*A\*C\*T\*T\*T\*  
A\*G\*C\*A/\*i2MOErA/\*i2MOErC/\*i2MOErA/\*i2MOErG/\*32MOErT/ (SEQ  
ID NO:82), wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and  
\* denotes a nucleic acid with a phosphorothioate backbone/bond.

SOD1 Short-6:

/52MOErT/\*i2MOErT/\*i2MOErA/\*i2MOErA/\*i2MOErT/\*A\*C\*T\*T\*T\*A\*  
G\*C\*A\*A/\*i2MOErC/\*i2MOErA/\*i2MOErG/\*i2MOErT/\*32MOErA/ (SEQ  
ID NO:108), wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and  
\* denotes a nucleic acid with a phosphorothioate backbone/bond.

SOD1 Short-7:

/52MOErT/\*i2MOErA/\*i2MOErA/\*i2MOErT/\*i2MOErA/\*C\*T\*T\*T\*A\*G\*  
C\*A\*A\*C/\*i2MOErA/\*i2MOErG/\*i2MOErT/\*i2MOErA/\*32MOErT/ (SEQ  
ID NO:109), wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and  
\* denotes a nucleic acid with a phosphorothioate backbone/bond.

SOD1 Short-8:

52MOErA/\*i2MOErA/\*i2MOErT/\*i2MOErA/\*i2MOErC/\*T\*T\*T\*A\*G\*C\*  
A\*A\*C\*A/\*i2MOErG/\*i2MOErT/\*i2MOErA/\*i2MOErT/\*32MOErT/ (SEQ  
ID NO:110), wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and  
\* denotes a nucleic acid with a phosphorothioate backbone/bond.

SOD1 Short-9:

/52MOErA/\*i2MOErT/\*i2MOErA/\*i2MOErC/\*i2MOErT/\*T\*T\*A\*G\*C\*A\*  
A\*C\*A\*G/\*i2MOErT/\*i2MOErA/\*i2MOErT/\*i2MOErT/\*32MOErT/ (SEQ  
ID NO:111), wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and  
\* denotes a nucleic acid with a phosphorothioate backbone/bond.

SOD1 Short-10:

/52MOErT/\*i2MOErA/\*i2MOErC/\*i2MOErT/\*i2MOErT/\*T\*A\*G\*C\*A\*A\*  
C\*A\*G\*T/\*i2MOErA/\*i2MOErT/\*i2MOErT/\*i2MOErT/\*32MOErT/ (SEQ  
ID NO:112), wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and  
\* denotes a nucleic acid with a phosphorothioate backbone/bond.

SOD1 Short-11:

/52MOErA/\*i2MOErC/\*i2MOErT/\*i2MOErT/\*i2MOErT/\*A\*G\*C\*A\*A\*C\*  
A\*G\*T\*A/\*i2MOErT/\*i2MOErT/\*i2MOErT/\*i2MOErT/\*32MOErG/ (SEQ  
ID NO:113), wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and  
\* denotes a nucleic acid with a phosphorothioate backbone/bond.

**[0223]** A tiling was performing for long (insertion) methoxyethyl (MOE) antisense oligonucleotides, which are referred to herein as SOD1 Long-1 through SOD1 Long-10. The nucleotide sequence of SOD1 Long-1 through SOD1 Long-10 were:

SOD1 Long-1:

/52MOErA/\*i2MOErA/\*i2MOErT/\*i2MOErC/\*i2MOErT/\*T\*A\*A\*T\*A\*C\*  
T\*T\*T\*A/\*i2MOErA/\*i2MOErG/\*i2MOErC/\*i2MOErA/\*32MOErA/ (SEQ  
ID NO:114), wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and  
\* denotes a nucleic acid with a phosphorothioate backbone/bond.

SOD1 Long-2:

/52MOErA/\*i2MOErT/\*i2MOErC/\*i2MOErT/\*i2MOErT/\*A\*A\*T\*A\*C\*T\*  
T\*T\*A\*A\*i2MOErG/\*i2MOErC/\*i2MOErA/\*i2MOErA/\*32MOErC/ (SEQ  
ID NO:115), wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and  
\* denotes a nucleic acid with a phosphorothioate backbone/bond.

SOD1 Long-3:

/52MOErT/\*i2MOErC/\*i2MOErT/\*i2MOErT/\*i2MOErA/\*A\*T\*A\*C\*T\*T\*  
T\*A\*A\*G\*i2MOErC/\*i2MOErA/\*i2MOErA/\*i2MOErC/\*32MOErA/ (SEQ  
ID NO:116), wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and  
\* denotes a nucleic acid with a phosphorothioate backbone/bond.

SOD1 Long-4:

/52MOErC/\*i2MOErT/\*i2MOErT/\*i2MOErA/\*i2MOErA/\*T\*A\*C\*T\*T\*T\*  
A\*A\*G\*C\*i2MOErA/\*i2MOErA/\*i2MOErC/\*i2MOErA/\*32MOErG/ (SEQ  
ID NO:117), wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and  
\* denotes a nucleic acid with a phosphorothioate backbone/bond.

SOD1 Long-5 (also referred to herein as Long SOD1 MOE):

/52MOErC/\*i2MOErT/\*i2MOErT/\*i2MOErA/\*i2MOErA/\*T\*A\*C\*T\*T\*T\*  
A\*A\*G\*C\*i2MOErA/\*i2MOErA/\*i2MOErC/\*i2MOErA/\*32MOErG/ (SEQ  
ID NO:83), wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and \*  
denotes a nucleic acid with a phosphorothioate backbone/bond.

SOD1 Long-6:

/52MOErT/\*i2MOErA/\*i2MOErA/\*i2MOErT/\*i2MOErA/\*C\*T\*T\*T\*A\*A\*  
G\*C\*A\*A\*i2MOErC/\*i2MOErA/\*i2MOErG/\*i2MOErT/\*32MOErA/ (SEQ  
ID NO:118), wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and  
\* denotes a nucleic acid with a phosphorothioate backbone/bond.

SOD1 Long-7:

/52MOErA/\*i2MOErA/\*i2MOErT/\*i2MOErA/\*i2MOErC/\*T\*T\*T\*A\*A\*G\*  
C\*A\*A\*C\*i2MOErA/\*i2MOErG/\*i2MOErT/\*i2MOErA/\*32MOErT/ (SEQ  
ID NO:119), wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and  
\* denotes a nucleic acid with a phosphorothioate backbone/bond.

SOD1 Long-8:

/52MOErA/\*i2MOErT/\*i2MOErA/\*i2MOErC/\*i2MOErT/\*T\*T\*A\*A\*G\*C\*  
A\*A\*C\*A\*i2MOErG/\*i2MOErT/\*i2MOErA/\*i2MOErT/\*32MOErT/ (SEQ  
ID NO:120), wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and  
\* denotes a nucleic acid with a phosphorothioate backbone/bond.

SOD1 Long-9:

/52MOErT/\*i2MOErA/\*i2MOErC/\*i2MOErT/\*i2MOErT/\*T\*A\*A\*G\*C\*A\*  
A\*C\*A\*G/\*i2MOErT/\*i2MOErA/\*i2MOErT/\*i2MOErT/\*32MOErT/ (SEQ  
ID NO:121), wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and  
\* denotes a nucleic acid with a phosphorothioate backbone/bond.

SOD1 Long-10:

/52MOErA/\*i2MOErC/\*i2MOErT/\*i2MOErT/\*i2MOErT/\*A\*A\*G\*C\*A\*A\*  
C\*A\*G\*T/\*i2MOErA/\*i2MOErT/\*i2MOErT/\*i2MOErT/\*32MOErT/ (SEQ  
ID NO:122), wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and  
\* denotes a nucleic acid with a phosphorothioate backbone/bond.

[0224] These antisense oligonucleotides were evaluated within a patient derived fibroblast line harboring an A5V mutation (heterozygous A5V fibroblast cell line 120151) using TransIT Oligo transfection with each antisense oligonucleotide (200nM) for 72 hours followed by digital droplet PCT. The assay the percent of mutant *SOD1* expression for each ASO. Thus, Figure 11A provides a graph that show a tiling series of Short SOD1 MOE gapmers (SOD1 Short-1 through SOD1 Short-11) and a tiling series of Long SOD1 MOE gapmers (SOD1 Long-1 through SOD1 Long-10) from one experiment. Of note, Short SOD1 MOE gapmers target the mutant allele in the A5V fibroblast cell line 120151 and the Long SOD1 MOE gapmers target the wildtype allele in the A5V fibroblast cell line 120151. This experiment determined that the A5V mutation was in cis with the deletion form of the ASO targeted indel and that SOD1 Short-5 was the most effective at knocking down expression from this allele. SOD1 Long-5, showing the highest expression of *SOD1* from the mutant containing allele, was demonstrated to be the most effective ASO for knocking down expression from the allele containing the insertion form of the ASO targeted indel. SOD1 Short-5 and SOD1 Long-5 being the most effective were subsequently examined in Figures 11C, 12C, 13B, 14B, and 15A and 15B, as and Long SOD1 MOE gapmer. For comparison, LNA versions of the top SOD1 MOE gapmer ASOs were made and tested as well, and the data provided herein.

[0225] Antisense oligonucleotides Short SOD1 MOE gapmer (also referred to herein as SOD1 Short-5) and SOD1 Long-5 (also referred to herein as Long SOD1 MOE gapmer) were evaluated within a fibroblast line harboring an A5V mutation using TransIT Oligo transfection with each antisense oligonucleotide (100nM LNA or 200nM MOE) for 72 hours followed by Taqman assay, RNA-Seq, and ddPCR. The former assay to determine how total SOD1 levels were affected by ASO treatment and the latter assays to assess allele specific knockdown of each ASO. Figures 11B and 11C show the total relative SOD1 levels observed, as determined

by Taqman qPCR), in A5V fibroblast cell line 220022 after a 72-hour treatment with 100 nM of Short SOD1 LNA and Long SOD1 LNA (two experiments) in Figure 11B and 200 nM of Short SOD1 MOE and Long SOD1 MOE (one experiment) in Figure 11C. The Short SOD1 LNA, Long SOD1 LNA, Short SOD1 MOE, and Long SOD1 MOE were all effective at knocking down total SOD1 expression, with the Short SOD1 LNA and Long SOD1 LNA showing a more robust ability to knock down total SOD1 expression levels.

**[0226]** Figures 12A, 12B, and 12C show the percent of wild-type SOD1 expression, determined by droplet digital polymerase chain reaction (ddPCR) in Figures 12A and 12C or by bulk RNA sequencing in Figure 12B, observed in A5V fibroblast cell line 220022 after a 72-hour treatment with 100 nM of Short SOD1 LNA and Long SOD1 LNA (two experiments) in Figures 12A and 12B and 200 nM of Short SOD1 MOE and Long SOD1 MOE (one experiment) in Figure 12C. The examined antisense oligonucleotides demonstrated allele-specific effects, wherein the short antisense oligonucleotides are responsible for the knockdown of mutant allele in the examined cell line. These results illustrate that with minimal optimization and antisense oligonucleotide design, allele-specific knockdown of a gene (such as SOD1) can be achieved.

**[0227]** Whole cell RNA sequencing (RNAseq) was utilized to examine SOD1 expression levels of the A5V fibroblast cell line 220022 after a 72-hour treatment with 100 nM of Short SOD1 LNA and Long SOD1 LNA (two experiments). For each treatment of each experiment, ribonucleic acid (RNA) was extracted from the treated cells and sequenced at 50M reads. For experiment 1 and experiment 2, the reads were counted and partitioned into wild-type (C) or mutant (T) reads. This data is shown in Table 3 for experiment 1 and Table 4 for experiment 2. The data is further presented as the percent wild-type allele expression in Figure 12B. The RNA sequencing (RNAseq) allele specific knockdown counts match the droplet digital polymerase chain reaction (ddPCR) data for the first and second experiments performed in the A5V fibroblast cell line 220022.

Table 3. Experiment 1 RNA sequencing (RNAseq) data for Short SOD1 LNA and Long SOD1 LNA

|                       | Wild-type | Mutant |
|-----------------------|-----------|--------|
| <b>No Treatment</b>   | 48%       | 52%    |
| <b>Short SOD1 LNA</b> | 80%       | 20%    |
| <b>Long SOD1 LNA</b>  | 33%       | 67%    |



Table 4. Experiment 2 RNA sequencing (RNAseq) data for Short SOD1 LNA and Long SOD1 LNA

|                       | Wild-type | Mutant |
|-----------------------|-----------|--------|
| <b>No Treatment</b>   | 49%       | 51%    |
| <b>Short SOD1 LNA</b> | 79%       | 21%    |
| <b>Long SOD1 LNA</b>  | 32%       | 68%    |

**[0228]** Next, the experiments were repeated as described in A5V fibroblast cell line 210151. Figures 13A and 13B show the total relative SOD1 levels observed, as determined by Taqman qPCR assays in A5V fibroblast cell line 210151 after a 72-hour treatment with 100 nM of Short SOD1 LNA and Long SOD1 LNA (two experiments) in Figure 13A and 200 nM of Short SOD1 MOE and Long SOD1 MOE (one experiment) in Figure 13B. SOD1 knockdown with the examined antisense oligonucleotides was confirmed in A5V fibroblast cell line 210151, wherein a robust knockdown being observed for Short SOD1 LNA and Long SOD1 LNA.

**[0229]** Figures 14A and 14B show the percent of wild-type SOD1 expression, determined by droplet digital polymerase chain reaction (ddPCR), observed in A5V fibroblast cell line 210151 after a 72-hour treatment with 100 nM of Short SOD1 LNA and Long SOD1 LNA (two experiments) in Figure 14A and 200 nM of Short SOD1 MOE and Long SOD1 MOE (one experiment) in Figure 14B. The examined antisense oligonucleotides confirm allele-specific effects observed in the A5V fibroblast cell line 220022, wherein the short antisense oligonucleotides are responsible for the knockdown of mutant allele in the examined cell line.

**[0230]** Figures 15A and 15B show that treatment with the same SOD1 MOE gapmer and LNA antisense oligonucleotides (two experiments) that successfully reduce *SOD1* levels in two different heterozygous A5V patient fibroblasts (Figures 11A, 11B, 12A, 12B, 12C, 13A, 13B, 14A, and 14B) can also target other *SOD1* mutations. Similar to the knockdown studies in the 220022 and 210151 heterozygous A5V patient fibroblast lines described herein, the described SOD1 ASOs (SEQ ID NOs:81-83) reduced the total relative *SOD1* levels observed in a heterozygous Glu134Del patient fibroblast line. In this cell line, the patient mutation is in cis with the insertion form of the ASO targeted indel, supported by increased wild-type allele expression upon treatment with the Long forms of the ASOs. Total *SOD1* levels were determined by Taqman qPCR assays (Figure 15A) or allele specific droplet digital polymerase

chain reaction (ddPCR) (Figure 15B) after a 72-hour treatment with 100 nM of Short SOD1 LNA, 100nM of Long SOD1 LNA, 200 nM of Short SOD1 MOE, or 200 nM of Long SOD1 MOE (two experiments for each) as described previously for C9Orf72 studies in Example 9, except that instead of using the C9Orf72 Taqman probe, a SOD1 probe was used with the HRPT1 control probe.

[0231] As discussed in greater detail above, of the four antisense oligonucleotides examined for *SOD1*, the Short SOD1 LNA and Long SOD1 LNA provided the best knockdown of mutant *SOD1* in three biologically distinct cell lines harboring two distinct ALS related mutations. Droplet digital polymerase chain reaction (ddPCR) of the two A5V cell lines examined, demonstrates that each allele in the patient cell lines is equally expressed. Allele-specific knockdown of the targeted allele was observed for the short antisense oligonucleotides and the long antisense oligonucleotides. The same trend was observed in each of the two distinct A5V cell lines examined, in each experiment.

#### Example 8. Development of Allele-specific Antisense Oligonucleotides for C9ORF72

[0232] From our analysis of WGS data from C9ORF72 patients and controls, a 2-base pair deletion (rs142843265, AAGGTTTCGAGAAGTAGTACCTTAA[AG/-]AACATATCAGAGACAATTTTTTTTA; SEQ ID NO: 64) within intron 3 was identified that is in complete linkage disequilibrium with the repeat expansion allele observed in C9ORF72 patients thus representing an optimal target for allele-specific antisense oligonucleotide. This analysis is corroborated by showing that all European C9ORF72 repeat expansion carriers were derived from a single founder mutation (The C9ORF72 expansion mutation is a common cause of ALS+/-FTD in Europe and has a single founder; Eur J Hum Genet. 2013 Jan;21(1):102-8). Based on the gnomAD/1000 Genomes databases and evaluation of our cohort, it was estimated that 79% of C9ORF72 patients could potentially be targeted with a single allele-specific antisense oligonucleotide targeting the short (deletion) allele. Towards this goal, antisense oligonucleotides were designed using 1-base pair tiling for the short (deletion) allele (termed C9-del). A Short C9ORF72 MOE gapmer and Short C9ORF72 LNA gapmer were synthesized for evaluation and the following sequences.

Short C9ORF72 MOE gapmer:

/52MOErT/\*i2MOErA/\*i2MOErG/\*i2MOErT/\*i2MOErA/\*C\*C\*T\*T\*A\*A\*A\*A  
\*C\*A/\*i2MOErT/\*i2MOErA/\*i2MOErT/\*i2MOErC/\*32MOErA/ (SEQ ID

NO:11), wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic acid with a phosphorothioate backbone/bond

Short C9ORF72 LNA gapmer:

+C\*+C\*+T\*+T\*A\*A\*A\*A\*C\*A\*T\*A\*+T\*+C\*+A\*+G (SEQ ID NO:51), wherein + denotes a locked nucleic acid and \* denotes a nucleic acid with a phosphorothioate backbone/bond

**[0233]** Initial evaluation was performed on two control fibroblast lines that were homozygous either for the short (deletion) allele (C9-del/C9-del) or the long allele (WT/WT). The antisense oligonucleotides (100 nM) were transfected with TransIT Oligo following the manufacturer's instructions, for 72 hours. RNA was collected using a Qiagen RNeasy kit as per the manufacturer's instructions. The RNA was converted to cDNA using a Biorad iSCRIPT RT Kit per the manufacturer's instructions and gene expression was determined by Taqman assays set up per the manufacturer's instructions using probes targeting C9ORF72 and HPRT1 (control). Here, both antisense oligonucleotides (Short C9ORF72 MOE gapmer and Short C9ORF72 LNA gapmer) reduced C9ORF72 expression in the C9-del/C9-del cell line, but not in the WT/WT cell line (see Figures 5 and 7). The Short C9ORF72 LNA gapmer (LNA #9) was further evaluated by treating fibroblast derived from a C9ORF72 repeat expansion patient (C9-36). Taqman assays revealed a reduction of gene expression to as low as 43.2% compared to non-treated cells (Figures 16A, 16C). Further, RNAseq analysis of the same samples showed (Fig 16B) that relative expression of the WT allele was increased when these cells were treated with LNA #9, even to a greater extent than the previously reported allele-specific C9Orf72 targeting ASO, NATMED 22 (Tran, *et al.*, Suppression of mutant C9Orf72 expression by a potent mixed backbone antisense oligonucleotide. *Nat Med.* 2022 Jan;28(1):117-124.

**[0234]** The ability of the investigated allele specific C9Orf72 ASOs, MOE #8 and LNA#9 to reduce total C9 levels was confirmed utilizing the same methodology in a separate, biologically distinct heterozygous C9Orf72 patient fibroblast line (C9-41). The data in Figure 17 confirms the ability of the ASOs to treat patients harboring the C9-del SNP.

**[0235]** FIG. 18 provides a graph of the total C9 levels determined by Taqman assay present after DIV7 gymnotic treatment of the C9Orf72 patient iPSC derived cortical neurons 5  $\mu$ M of MOE gapmer #8 (MOE8) and LNA gapmer #9 (LNA9). This is in comparison to a non-treated control (NT) and 100 nM of a non-discriminating ASO (PNAS 2013; Lagier-

Tourenne, C *et al.* “Targeted degradation of sense and antisense *C9orf72* RNA foci as therapy for ALS and frontotemporal degeneration”. PNAS 2013 Nov 19;110(47):E4530-9), which reduces total levels of both targets and a non-targeting control ASO (MSC) which does not affect levels of either target. Table 1 shows the allele specific knockdown effect of the ASOs, MOE8 and LNA9 in relation to a non-treated control, on expression from the C9 expansion mutation containing allele in iPSC derived cortical neurons as determined by RNA-seq. Allele specific expression levels were determined by assessing counts of each version of the ASP SNP mentioned in Figure 8.

Table 1. Allele specific reduction of mutant expression in mutant iCN cells

|                     | Allele Specific Knockdown |                             |
|---------------------|---------------------------|-----------------------------|
|                     | Mutant Allele Expression  | Wild-Type Allele Expression |
| <b>No Treatment</b> | 40.7%                     | 59.3%                       |
| <b>LNA9</b>         | 8.1%                      | 91.9%                       |
| <b>MOE8</b>         | 25.7%                     | 74.3%                       |

Example 9. Development of Allele-Specific Antisense Oligonucleotides for Additional Target Genes

**[0236]** Antisense nucleotide sets were synthesized for Kinesin family member 5A (KIF5A), Titan protein (TTN), Mitofusin-2 (MFN2),  $\beta$ -myosin heavy chain (MYH7), and lamin A/C (LMNA). Initial assessment of the efficacy of the ASOs was determined using the dual luciferase assays as described herein. The target sequences used in the luciferase constructs were as follows:

Kinesin family member 5A (KIF5A) Short Target Sequence (SEQ ID NO:84)

AATACTTGGAAAAATGCCCAAATGTGAGGAGGGGGGATATGGAGT  
 AGGGGAAGAAAAAGCCTTGATAAGGCTCTGCACAGTGTTGGCATTT  
 CAGTTCTTGGATCACTTTGCAGTGTGGGTCTGGAATCAGATTGCTTG  
 AATTTGAAACT

Kinesin family member 5A (KIF5A) Long Target Sequence (SEQ ID NO:85)

AATACTTGGAAAAATGCCCAAATGTGAGGAGGGGGGATATGGAGT  
 AGGGGAAGAAAAAGCCTTGATAAGGCTCTGACACAGTGTTGGCAT  
 TTCAGTTCTTGGATCACTTTGCAGTGTGGGTCTGGAATCAGATTGCT  
 TGAATTTGAAACT

Titan protein (TTN) Short Target Sequence (SEQ ID NO:88)

TTCTAATACATATATATAAAGGGCAGCCCAACAGGTTTTGATTTTA  
GCGTTGACTTTACACCTTGTTGTATTACTCTCAGCGAGTCCTTTAAC  
CAACCTGAGCCTGAAGTTATTTTCCTGTAAATTGGGGATGCTAATA  
TCAGTCTTAAT

Titan protein (TTN) Long Target Sequence (SEQ ID NO:89)

TTCTAATACATATATATAAAGGGCAGCCCAACAGGTTTTGATTTTA  
GCGTTGACTTTACACCTTGTTGTATTACTCTCAGCGAGTCCTTTAA  
CCAACCTGAGCCTGAAGTTATTTTCCTGTAAATTGGGGATGCTAAT  
ATCAGTCTTAAT

Mitofusin-2 (MFN2) Short Target Sequence (SEQ ID NO:92)

TTGACCTGGATTTGTTCTAAGCAGTGATATTTGTGAAATGACAGAT  
GCTAGTATTTTTCTCTAATACACATTAAAGTAAGTATGTA ACTATTA  
AAAATAGACGACTTTAAAAATAACCACCTACATACGACTATTTTAA  
AGTTCAGATTT

Mitofusin-2 (MFN2) Long Target Sequence (SEQ ID NO:93)

TTGACCTGGATTTGTTCTAAGCAGTGATATTTGTGAAATGACAGAT  
GCTAGTATTTTTCTCTAATACACATTAAAGTAAGTATGTA ACTATT  
AAAAATAGACGACTTTAAAAATAACCACCTACATACGACTATTTTA  
AAGTTCAGATTT

 $\beta$ -myosin Heavy Chain (MYH7) Short Target Sequence (SEQ ID NO:96)

GGAGGAGGTAAAGGTAAGGGCTAGACTCGGGCCACCTGGCCCAAG  
CAAGGAGCACACTGACTAGCCTTGCATCAAATCACTTCCCTTCCC  
GCAATAAGGCTGGCTGTGGACCAACAGTTCTCCAAGAATTCTAAAA  
AGAAATCACAGCC

 $\beta$ -myosin Heavy Chain (MYH7) Long Target Sequence (SEQ ID NO:97)

GGAGGAGGTAAAGGTAAGGGCTAGACTCGGGCCACCTGGCCCAAG  
CAAGGAGCACACTGACTAGCCTTGCATCAACATCACTTCCCTTCCC  
AGCAATAAGGCTGGCTGTGGACCAACAGTTCTCCAAGAATTCTAAA  
AAGAAATCACAGCC

Lamin A/C (LMNA) Short Target Sequence (SEQ ID NO:100)

CTAAGCTTGTCCAGTCAGAAAAGCAGAGGCTGAGGGGTGGCCTTTT  
CTTGAGAACTACATTCAAGTTGCAGCAAGGACAGTGGTCTGAATTT

GACGGGGACAAATGGAAGGGAGATAGGACACATGAGTTCCTTTAG  
GTCTGGCTCAGGG

Lamin A/C (LMNA) Long Target Sequence (SEQ ID NO:101)

CTAAGCTTGTCCAGTCAGAAAAGCAGAGGCTGAGGGGTGGCCTTTT  
CTTGAGAACTACATTCAAGTTGCAGCAAGAAGGACAGTGGTCTGA  
ATTTGACGGGGACAAATGGAAGGGAGATAGGACACATGAGTTCCT  
TTAGGTCTGGCTCAGGG

- [0237] The sequence of allele 1 antisense oligonucleotide sequence for KIF5A was:  
+A\*+A\*+C\*+A\*C\*T\*G\*T\*G\*C\*A\*G\*+A\*+G\*+C\*+C, wherein + denotes a locked nucleic acid and \* denotes a nucleic acid with a phosphorothioate backbone/bond (SEQ ID NO:86).
- [0238] The sequence of allele 2 antisense oligonucleotide sequence for KIF5A was:  
+A\*+A\*+C\*+A\*C\*T\*G\*T\*G\*T\*C\*A\*+G\*+A\*+G\*+C, wherein + denotes a locked nucleic acid and \* denotes a nucleic acid with a phosphorothioate backbone/bond (SEQ ID NO:87).
- [0239] The sequence of allele 1 antisense oligonucleotide sequence for TTN was:  
+T\*+C\*+G\*+C\*T\*G\*A\*G\*G\*A\*G\*T\*+A\*+A\*+T\*+A, wherein + denotes a locked nucleic acid and \* denotes a nucleic acid with a phosphorothioate backbone/bond (SEQ ID NO:90).
- [0240] The sequence of allele 2 antisense oligonucleotide sequence for TTN was:  
+T\*+C\*+G\*+C\*T\*G\*A\*G\*A\*G\*T\*A\*+A\*+T\*+A\*+C, wherein + denotes a locked nucleic acid and \* denotes a nucleic acid with a phosphorothioate backbone/bond (SEQ ID NO:91).
- [0241] The sequence of allele 1 antisense oligonucleotide sequence for MFN2 was:  
+T\*+A\*+C\*+T\*T\*A\*C\*T\*T\*T\*A\*A\*+T\*+G\*+T\*+G, wherein + denotes a locked nucleic acid and \* denotes a nucleic acid with a phosphorothioate backbone/bond (SEQ ID NO:94).
- [0242] The sequence of allele 2 antisense oligonucleotide sequence for MFN2 was:  
+A\*+C\*+T\*+T\*A\*C\*T\*T\*T\*T\*A\*A\*+T\*+G\*+T\*+G, wherein + denotes a locked nucleic acid and \* denotes a nucleic acid with a phosphorothioate backbone/bond (SEQ ID NO:95).
- [0243] The sequence of allele 1 antisense oligonucleotide sequence for MYH7 was:  
+G\*+T\*+G\*+A\*T\*T\*T\*G\*A\*T\*G\*C\*+A\*+A\*+G\*+G, wherein + denotes a locked nucleic acid and \* denotes a nucleic acid with a phosphorothioate backbone/bond (SEQ ID NO:98).
- [0244] The sequence of allele 2 antisense oligonucleotide sequence for MYH7 was:  
+G\*+A\*+A\*+G\*T\*G\*A\*T\*G\*T\*T\*G\*+A\*+T\*+G\*+C, wherein + denotes a locked nucleic acid and \* denotes a nucleic acid with a phosphorothioate backbone/bond (SEQ ID NO:99).
- [0245] The sequence of allele 1 antisense oligonucleotide sequence for LMNA was:  
+C\*+A\*+C\*+T\*G\*T\*C\*C\*T\*T\*G\*C\*+T\*+G\*+C\*+A, wherein + denotes a locked nucleic acid and \* denotes a nucleic acid with a phosphorothioate backbone/bond (SEQ ID NO:102).

[0246] The sequence of allele 2 antisense oligonucleotide sequence for LMNA was:

+A\*+C\*+T\*+G\*T\*C\*C\*T\*T\*C\*T\*T\*+G\*+C\*+T\*+G, wherein + denotes a locked nucleic acid and \* denotes a nucleic acid with a phosphorothioate backbone/bond (SEQ ID NO:103).

[0247] Figure 19 demonstrates that allele-specific knockdown was achieved for each of Kinesin family member 5A (KIF5A), Titan protein (TTN), Mitofusin-2 (MFN2),  $\beta$ -myosin heavy chain (MYH7), and lamin A/C (LMNA). This further confirms the ability to perform, as well as the power of the, allele-specific knockdown with the methods described herein.

Example 10. Development of Allele-Specific Antisense Oligonucleotides for Transactive Responsive Deoxyribonucleic Acid (DNA)-binding Protein 43 (*TARDBP*)

[0248] A tiling was performing for long (insertion) methoxyethyl (MOE) antisense oligonucleotides, which are referred to herein as TARDBP Short-1 through TARDBP Short-11. The nucleotide sequence of TARDBP Short-1 through TARDBP Short-11 were:

TARDBP Short-1:

/52MOErG/\*i2MOErA/\*i2MOErA/\*i2MOErC/\*i2MOErA/\*T\*C\*A\*T\*T\*T\*  
C\*A\*T\*T\*/i2MOErT/\*i2MOErA/\*i2MOErA/\*i2MOErA/\*32MOErC/ (SEQ  
ID NO:126), wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and \*  
denotes a nucleic acid with a phosphorothioate backbone/bond.

TARDBP Short-2:

/52MOErT/\*i2MOErG/\*i2MOErA/\*i2MOErA/\*i2MOErC/\*A\*T\*C\*A\*T\*T\*  
T\*C\*A\*T\*/i2MOErT/\*i2MOErT/\*i2MOErA/\*i2MOErA/\*32MOErA/ (SEQ  
ID NO:127), wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and \*  
denotes a nucleic acid with a phosphorothioate backbone/bond.

TARDBP Short-3:

/52MOErA/\*i2MOErT/\*i2MOErG/\*i2MOErA/\*i2MOErA/\*C\*A\*T\*C\*A\*T\*  
T\*T\*C\*A\*/i2MOErT/\*i2MOErT/\*i2MOErT/\*i2MOErA/\*32MOErA/ (SEQ  
ID NO:128), wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and \*  
denotes a nucleic acid with a phosphorothioate backbone/bond.

TARDBP Short-4:

/52MOErA/\*i2MOErA/\*i2MOErT/\*i2MOErG/\*i2MOErA/\*A\*C\*A\*T\*C\*A\*  
T\*T\*T\*C\*/i2MOErA/\*i2MOErT/\*i2MOErT/\*i2MOErT/\*32MOErA/ (SEQ ID  
NO:129), wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and \*  
denotes a nucleic acid with a phosphorothioate backbone/bond.

TARDBP Short-5:

/52MOErC/\*i2MOErA/\*i2MOErA/\*i2MOErT/\*i2MOErG/\*A\*A\*C\*A\*T\*C\*  
A\*T\*T\*T/\*i2MOErC/\*i2MOErA/\*i2MOErT/\*i2MOErT/\*32MOErT/ (SEQ ID  
NO:130), wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and \*  
denotes a nucleic acid with a phosphorothioate backbone/bond.

TARDBP Short-6:

/52MOErG/\*i2MOErC/\*i2MOErA/\*i2MOErA/\*i2MOErT/\*G\*A\*A\*C\*A\*T\*  
C\*A\*T\*T/\*i2MOErT/\*i2MOErC/\*i2MOErA/\*i2MOErT/\*32MOErT/ (SEQ  
ID NO:131), wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and \*  
denotes a nucleic acid with a phosphorothioate backbone/bond.

TARDBP Short-7:

/52MOErA/\*i2MOErG/\*i2MOErC/\*i2MOErA/\*i2MOErA/\*T\*G\*A\*A\*C\*A\*  
T\*C\*A\*T/\*i2MOErT/\*i2MOErT/\*i2MOErC/\*i2MOErA/\*32MOErT/ (SEQ  
ID NO:132), wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and \*  
denotes a nucleic acid with a phosphorothioate backbone/bond.

TARDBP Short-8:

/52MOErA/\*i2MOErA/\*i2MOErG/\*i2MOErC/\*i2MOErA/\*A\*T\*G\*A\*A\*C\*  
A\*T\*C\*A/\*i2MOErT/\*i2MOErT/\*i2MOErT/\*i2MOErC/\*32MOErA/ (SEQ  
ID NO:133), wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and \*  
denotes a nucleic acid with a phosphorothioate backbone/bond.

TARDBP Short-9:

/52MOErT/\*i2MOErA/\*i2MOErA/\*i2MOErG/\*i2MOErC/\*A\*A\*T\*G\*A\*A\*  
C\*A\*T\*C/\*i2MOErA/\*i2MOErT/\*i2MOErT/\*i2MOErT/\*32MOErC/ (SEQ  
ID NO:134), wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and \*  
denotes a nucleic acid with a phosphorothioate backbone/bond.

TARDBP Short-10:

/52MOErA/\*i2MOErT/\*i2MOErA/\*i2MOErA/\*i2MOErG/\*C\*A\*A\*T\*G\*A\*  
A\*C\*A\*T/\*i2MOErC/\*i2MOErA/\*i2MOErT/\*i2MOErT/\*32MOErT/ (SEQ  
ID NO:135), wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and \*  
denotes a nucleic acid with a phosphorothioate backbone/bond.

TARDBP Short-11:

/52MOErA/\*i2MOErA/\*i2MOErT/\*i2MOErA/\*i2MOErA/\*G\*C\*A\*A\*T\*G\*  
A\*A\*C\*A/\*i2MOErT/\*i2MOErC/\*i2MOErA/\*i2MOErT/\*32MOErT/ (SEQ  
ID NO:136), wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and \*  
denotes a nucleic acid with a phosphorothioate backbone/bond.



[0249] A tiling was performing for long (insertion) methoxyethyl (MOE) antisense oligonucleotides, which are referred to herein as TARDBP Long-1 through TARDBP Long-10. The nucleotide sequence of TARDBP Long-1 through TARDBP Long-10 were:

TARDBP Long-1:

/52MOErG/\*i2MOErA/\*i2MOErA/\*i2MOErC/\*i2MOErA/\*C\*T\*C\*A\*T\*T\*  
T\*C\*A\*T/\*i2MOErT/\*i2MOErT/\*i2MOErA/\*i2MOErA/\*32MOErA/ (SEQ  
ID NO:137), wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and \*  
denotes a nucleic acid with a phosphorothioate backbone/bond.

TARDBP Long-2:

/52MOErT/\*i2MOErG/\*i2MOErA/\*i2MOErA/\*i2MOErC/\*A\*C\*T\*C\*A\*T\*  
T\*T\*C\*A/\*i2MOErT/\*i2MOErT/\*i2MOErT/\*i2MOErA/\*32MOErA/ (SEQ  
ID NO:138), wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and \*  
denotes a nucleic acid with a phosphorothioate backbone/bond.

TARDBP Long-3:

/52MOErA/\*i2MOErT/\*i2MOErG/\*i2MOErA/\*i2MOErA/\*C\*A\*C\*T\*C\*A\*  
T\*T\*T\*C/\*i2MOErA/\*i2MOErT/\*i2MOErT/\*i2MOErT/\*32MOErA/ (SEQ ID  
NO:139), wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and \*  
denotes a nucleic acid with a phosphorothioate backbone/bond.

TARDBP Long-4:

/52MOErA/\*i2MOErA/\*i2MOErT/\*i2MOErG/\*i2MOErA/\*A\*C\*A\*C\*T\*C\*  
A\*T\*T\*T/\*i2MOErC/\*i2MOErA/\*i2MOErT/\*i2MOErT/\*32MOErT/ (SEQ ID  
NO:140), wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and \*  
denotes a nucleic acid with a phosphorothioate backbone/bond.

TARDBP Long-5:

/52MOErC/\*i2MOErA/\*i2MOErA/\*i2MOErT/\*i2MOErG/\*A\*A\*C\*A\*C\*T\*  
C\*A\*T\*T/\*i2MOErT/\*i2MOErC/\*i2MOErA/\*i2MOErT/\*32MOErT/ (SEQ  
ID NO:141), wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and \*  
denotes a nucleic acid with a phosphorothioate backbone/bond.

TARDBP Long-6:

/52MOErG/\*i2MOErC/\*i2MOErA/\*i2MOErA/\*i2MOErT/\*G\*A\*A\*C\*A\*C\*  
T\*C\*A\*T/\*i2MOErT/\*i2MOErT/\*i2MOErC/\*i2MOErA/\*32MOErT/ (SEQ  
ID NO:142), wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and \*  
denotes a nucleic acid with a phosphorothioate backbone/bond.

TARDBP Long-7:

/52MOErA/\*i2MOErG/\*i2MOErC/\*i2MOErA/\*i2MOErA/\*T\*G\*A\*A\*C\*A\*  
C\*T\*C\*A/\*i2MOErT/\*i2MOErT/\*i2MOErT/\*i2MOErC/\*32MOErA/ (SEQ  
ID NO:143), wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and \*  
denotes a nucleic acid with a phosphorothioate backbone/bond.

TARDBP Long-8:

/52MOErA/\*i2MOErA/\*i2MOErG/\*i2MOErC/\*i2MOErA/\*A\*T\*G\*A\*A\*C\*  
A\*C\*T\*C/\*i2MOErA/\*i2MOErT/\*i2MOErT/\*i2MOErT/\*32MOErC/ (SEQ  
ID NO:144), wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and \*  
denotes a nucleic acid with a phosphorothioate backbone/bond.

TARDBP Long-9:

/52MOErT/\*i2MOErA/\*i2MOErA/\*i2MOErG/\*i2MOErC/\*A\*A\*T\*G\*A\*A\*  
C\*A\*C\*T/\*i2MOErC/\*i2MOErA/\*i2MOErT/\*i2MOErT/\*32MOErT/ (SEQ  
ID NO:145), wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and \*  
denotes a nucleic acid with a phosphorothioate backbone/bond.

TARDBP Long-10:

/52MOErA/\*i2MOErT/\*i2MOErA/\*i2MOErA/\*i2MOErG/\*C\*A\*A\*T\*G\*A\*  
A\*C\*A\*C/\*i2MOErT/\*i2MOErC/\*i2MOErA/\*i2MOErT/\*32MOErT/ (SEQ  
ID NO:146), wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and \*  
denotes a nucleic acid with a phosphorothioate backbone/bond.

[0250] FIG. 20 provides a graph that shows the percent of mutant *TARDBP* expression, as determined by digital droplet PCT in the heterozygous Q331K induced cortical neuron line after DIV7 gymnotic treatment with 5  $\mu$ M of a tiling series of Short TARDBP MOE gapmers (TARDBP Short-1 through TARDBP Short-11) and a tiling series of Long TARDBP MOE gapmers (TARDBP Long-1 through TARDBP Long-10), one experiment. This experiment determined that the Q331K mutation was in cis with the insertion form of the ASO targeted indel and that TARDBP Long-7 and Long-8 were the most effective at knocking down expression from this allele. TARDBP Short-7 and Short-8, showing the highest expression of *TARDBP* from the mutant containing allele, were demonstrated to be the most effective ASO for knocking down expression from the allele containing the deletion form of the ASO targeted indel.

[0251] FIG. 21 provides a graph that shows the percent of mutant *TARDBP* expression as determined by digital droplet PCR in the heterozygous M337V induced cortical neuron line after DIV7 gymnotic treatment with 5  $\mu$ M of the top five performing *TARDBP* MOE gapmers screened in Figure 19 (TDP43 Short-5 through TDP43 Short-9 and TDP43 Long-5 through

TDP43 Long-9), one experiment. This experiment determined that the M337V mutation was in cis with the deletion form of the ASO targeted indel and confirmed that TDP43 ASO 7 and 8 (in this line, TARDBP Short-7 and TDP43 Short-8) were the most effective at knocking down expression from this allele. Correspondingly, TARDBP Long-7 and Long-8, showing the highest expression of TARDBP from the mutant containing allele, were demonstrated to be the most effective ASO for knocking down expression from the allele containing the insertion form of the ASO targeted indel.

[0252] FIG. 22 provides a graph that shows the effect of altering the ASO backbone of the top *TARDBP* MOE #7 on allele specific knockdown of mutant *TARDBP* levels as determined by digital droplet PCR in the heterozygous Q331K and M337V iPSC derived cortical neuron lines after DIV7 gymnotic treatment with 5uM ASO. These treatments were also compared to non-treated mutant TARDBP samples (TDP43 UT) and a non-treated WT parent line (KOLF UT).

[0253] The sequence of TARDBP Short 7-2M for *TARDBP* was:

/52MOErA/\*i2MOErG//i2MOErC/\*i2MOErA//i2MOErA/\*T\*G\*A\*A\*C\*A\*T\*C\*  
A\*T\*/i2MOErT//i2MOErT/\*i2MOErC//i2MOErA/\*32MOErT/ (SEQ ID NO:147),  
wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic acid with a phosphorothioate backbone/bond.

[0254] The sequence of TARDBP Short 7-3M for *TARDBP* was:

/52MOErA/\*i2MOErG//i2MOErC//i2MOErA//i2MOErA/\*T\*G\*A\*A\*C\*A\*T\*C\*A  
\*T\*/i2MOErT//i2MOErT//i2MOErC//i2MOErA/\*32MOErT/ (SEQ ID NO:148),  
wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic acid with a phosphorothioate backbone/bond.

[0255] The sequence of TARDBP Short 7-4M for *TARDBP* was:

/52MOErA/\*i2MOErG//i2MOErC//i2MOErA//i2MOErA/T\*G\*A\*A\*C\*A\*T\*C\*A\*  
T/i2MOErT//i2MOErT//i2MOErC//i2MOErA/\*32MOErT/ (SEQ ID NO:149),  
wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic acid with a phosphorothioate backbone/bond.

[0256] The sequence of TARDBP Long 7-2M for *TARDBP* was:

/52MOErA/\*i2MOErG//i2MOErC/\*i2MOErA//i2MOErA/\*T\*G\*A\*A\*C\*A\*C\*T\*  
C\*A\*/i2MOErT/\*i2MOErT//i2MOErT/\*i2MOErC//32MOErA/ (SEQ ID NO:150),  
wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic acid with a phosphorothioate backbone/bond.

[0257] The sequence of TARDBP Long 7-3M for *TARDBP* was:

/52MOErA/\*i2MOErG//i2MOErC//i2MOErA//i2MOErA/\*T\*G\*A\*A\*C\*A\*C\*T\*C  
 \*A\*i2MOErT//i2MOErT//i2MOErT//i2MOErC/\*32MOErA/ (SEQ ID NO:151),  
 wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic  
 acid with a phosphorothioate backbone/bond.

[0258] The sequence of TARDBP Long 7-4M for *TARDBP* was:

/52MOErA/\*i2MOErG//i2MOErC//i2MOErA//i2MOErA/T\*G\*A\*A\*C\*A\*C\*T\*C\*  
 A/i2MOErT//i2MOErT//i2MOErT//i2MOErC/\*32MOErA/ (SEQ ID NO:152),  
 wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic  
 acid with a phosphorothioate backbone/bond.

[0259] FIG. 22 compares the efficacy of knockdown of *TARDBP* MOE #7 (M) to the same ASOs with altered backbone bonds in two (2M), three (3M), or four (4M) positions of the ASO wings. This experiment determined that altering the ASO backbone in two or three positions of the wings increased the allele specific knockdown of the mutant allele in the M337V and the Q331K lines, respectively.

[0260] The contents of all references, patents, pending patent applications and published patents, cited throughout this application are hereby expressly incorporated by reference for all purposes..

[0261] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the disclosure described herein. Such equivalents are intended to be encompassed by the following claims. It is understood that the detailed examples and embodiments described herein are given by way of example for illustrative purposes only, and are in no way considered to be limiting to the disclosure. Various modifications or changes in light thereof will be suggested to persons skilled in the art and are included within the spirit and purview of this application and are considered within the scope of the appended claims. For example, the relative quantities of the ingredients may be varied to optimize the desired effects, additional ingredients may be added, and/or similar ingredients may be substituted for one or more of the ingredients described. Additional advantageous features and functionalities associated with the systems, methods, and processes of the present disclosure will be apparent from the appended claims. Moreover, those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the disclosure described herein. Such equivalents are intended to be encompassed by the following claims.

#### Selected Sequences

**Wild-type C9Orf72 allele (SEQ ID NO: 1)**

AATTGCATGAGTCTTAACGATACAACATAAGACTTAGAAGAAATATTGTGTGGA  
CCTGGGCCTACACCCAGACAGATACCTCAGGGGTACATATGCTCTCCTTCTGTT  
ACAGCTACTTCTAGGGAAAGGTTTCGAGAAGTAGTACCTTAAAGAACATATCAGA  
GACAATTTTTTTTATTTTTACTATGAACAAGTTATCCAAAATTTATTCTGGGCAAA  
CAGAAAAAAAAAGGGAGCAAATATTAATTTGTAGATGCAATTACTATTTTCCTTT  
GTTTACTGATTTAACTCTTTGGGTTTAAG

**C9Orf72 allele comprising a two-base pair deletion (C9-deletion) (SEQ ID NO: 2)**

AATTGCATGAGTCTTAACGATACAACATAAGACTTAGAAGAAATATTGTGTGGA  
CCTGGGCCTACACCCAGACAGATACCTCAGGGGTACATATGCTCTCCTTCTGTT  
ACAGCTACTTCTAGGGAAAGGTTTCGAGAAGTAGTACCTTAAAACATATCAGAGA  
CAATTTTTTTTATTTTTACTATGAACAAGTTATCCAAAATTTATTCTGGGCAAAACA  
GAAAAAAAAAGGGAGCAAATATTAATTTGTAGATGCAATTACTATTTTCCTTTGT  
TTACTGATTTAACTCTTTGGGTTTAAG

**Antisense oligonucleotide C9Orf72 target (Indel +10) (SEQ ID NO: 3)**

TCGAGAAGTAGTACCTTAAA

**Antisense oligonucleotide C9Orf72 target (Indel +9) (SEQ ID NO: 4)**

CGAGAAGTAGTACCTTAAAA

**Antisense oligonucleotide C9Orf72 target (Indel +8) (SEQ ID NO: 5)**

GAGAAGTAGTACCTTAAAAC

**Antisense oligonucleotide C9Orf72 target (Indel +7) (SEQ ID NO: 6)**

AGAAGTAGTACCTTAAAACA

**Antisense oligonucleotide C9Orf72 target (Indel +6) (SEQ ID NO: 7)**

GAAGTAGTACCTTAAAACAT

**Antisense oligonucleotide C9Orf72 target (Indel +5) (SEQ ID NO: 8)**

AAGTAGTACCTTAAAACATA

**Antisense oligonucleotide C9Orf72 target (Indel +4) (SEQ ID NO: 9)**

AGTAGTACCTTAAAACATAT

**Antisense oligonucleotide C9Orf72 target (Indel +3) (SEQ ID NO: 10)**

GTAGTACCTTAAAACATATC

**Antisense oligonucleotide C9Orf72 target (Indel +2) (SEQ ID NO: 11)**

TAGTACCTTAAAACATATCA

**Antisense oligonucleotide C9Orf72 target (Indel +1) (SEQ ID NO: 12)**

AGTACCTTAAAACATATCAG

**Antisense oligonucleotide C9Orf72 target (Indel +0) (SEQ ID NO: 13)**

GTACCTTAAAACATATCAGA

**Antisense oligonucleotide C9Orf72 target (Indel -1) (SEQ ID NO: 14)**

TACCTTAAAACATATCAGAG

**Antisense oligonucleotide C9Orf72 target (Indel -2) (SEQ ID NO: 15)**

ACCTTAAAACATATCAGAGA

**Antisense oligonucleotide C9Orf72 target (Indel -3) (SEQ ID NO: 16)**

CCTTAAAACATATCAGAGAC

**Antisense oligonucleotide C9Orf72 target (Indel -4) (SEQ ID NO: 17)**

CTTAAAACATATCAGAGACA

**Antisense oligonucleotide C9Orf72 target (Indel -5) (SEQ ID NO: 18)**

TTAAAACATATCAGAGACAA

**Antisense oligonucleotide C9Orf72 target (Indel -6) (SEQ ID NO: 19)**

TAAAACATATCAGAGACAAT

**Antisense oligonucleotide C9Orf72 target (Indel -7) (SEQ ID NO: 20)**

AAAACATATCAGAGACAATT

Antisense oligonucleotide *C9Orf72* target (Indel -8) (SEQ ID NO: 21)

AAACATATCAGAGACAATTT

Antisense oligonucleotide *C9Orf72* target (Indel -9) (SEQ ID NO: 22)

AACATATCAGAGACAATTTT

ASO 1 (SEQ ID NO:23)

CGAGAAGTAGTACCTTAAAA

ASO 2 (SEQ ID NO:24)

GAGAAGTAGTACCTTAAAAC

ASO 3 (SEQ ID NO:25)

AGAAGTAGTACCTTAAAACA

ASO 4 (SEQ ID NO:26)

GAAGTAGTACCTTAAAACAT

ASO 5 (SEQ ID NO:27)

AAGTAGTACCTTAAAACATA

ASO 6 (SEQ ID NO:28)

AGTAGTACCTTAAAACATAT

ASO 7 (SEQ ID NO:29)

GTAGTACCTTAAAACATATC

ASO 8 (SEQ ID NO:30)

TAGTACCTTAAAACATATCA

ASO 9 (SEQ ID NO:31)

AGTACCTTAAAACATATCAG

**ASO 10 (SEQ ID NO:32)**

GTACCTTAAAACATATCAGA

**ASO 11 (SEQ ID NO:33)**

TACCTTAAAACATATCAGAG

**ASO 12 (SEQ ID NO:34)**

ACCTTAAAACATATCAGAGA

**ASO 13 (SEQ ID NO:35)**

CCTTAAAACATATCAGAGAC

**ASO 14 (SEQ ID NO:36)**

CTTAAAACATATCAGAGACA

**ASO 15 (SEQ ID NO:37)**

TTAAAACATATCAGAGACAA

**ASO 16 (SEQ ID NO:38)**

TAAAACATATCAGAGACAAT

**ASO 17 (SEQ ID NO:39)**

AAAACATATCAGAGACAATT

**ASO 18 (SEQ ID NO:40)**

AAACATATCAGAGACAATTT

**LNA 1 (SEQ ID NO:41)**

AAGTAGTACCTTAAAA

**LNA 1b (SEQ ID NO:42)**

AAGTAGTACCTTAAAAC



**LNA 2 (SEQ ID NO:43)**

AGTAGTACCTTAAAAC

**LNA 3 (SEQ ID NO:44)**

GTAGTACCTTAAAACA

**LNA 4 (SEQ ID NO:45)**

TAGTACCTTAAAACAT

**LNA 5 (SEQ ID NO:46)**

AGTACCTTAAAACATA

**LNA 6 (SEQ ID NO:47)**

GTACCTTAAAACATAT

**LNA 7 (SEQ ID NO:48)**

TACCTTAAAACATATC

**LNA 7b (SEQ ID NO:49)**

GTACCTTAAAACATATC

**LNA 8 (SEQ ID NO:50)**

ACCTTAAAACATATCA

**LNA 9 (SEQ ID NO:51)**

CCTTAAAACATATCAG

**LNA 10 (SEQ ID NO:52)**

CTTAAAACATATCAGA

**LNA 10b (SEQ ID NO:53)**

CCTTAAAACATATCAGA

**LNA 11 (SEQ ID NO:54)**

TTAAAACATATCAGAG

**LNA 12 (SEQ ID NO:55)**

TAAAACATATCAGAGA

**LNA 13 (SEQ ID NO:56)**

AAAACATATCAGAGAC

**LNA 14 (SEQ ID NO:57)**

AAACATATCAGAGACA

**Forward primer (SEQ ID NO:58)**

ACACTCTTTC CCTACACGAC GCTCTTCCGA TCTACACTCT TTCCCTACAC  
GACGCTCTTCCGATCTGATA TCTCCGGAGC ATT

**Reverse Primer (SEQ ID NO: 59)**

GTGACTGGAG TTCAGACGTG TGCTCTTCCG ATCTNNNNNN NNNN

**C9 ASP Target Primer Forward (SEQ ID NO: 60)**

ACACTCTTTC CCTACACGAC GCTCTTCCGA TCTGATATCT CCGGAGCATT

**C9 ASP Target Primer Reverse (SEQ ID NO: 61)**

GTGACTGGAG TTCAGACGTG TGCTCTTCCG ATCTNNNNNN NNNNTGAAAC  
AATAATCACTCCC

**HPRT Target Primer Forward (SEQ ID NO: 62)**

ACACTCTTTC CCTACACGAC GCTCTTCCGA TCTCATTGTA GCCCTCTGTG TGC

**HPRT Target Primer Reverse (SEQ ID NO: 63)**

GTGACTGGAG TTCAGACGTG TGCTCTTCCG ATCTNNNNNN NNNNCAAGGG  
CATATCCTACAACAAAC

**rs142843265 of Chromosome 9 Open Reading Frame 72 (C9orf72) (SEQ ID NO:64)**

AAGGTTGAGAAAGTAGTACCTTAA[AG/-]AACATATCAGAGACAATTTTTTTTA

**rs34620383 of Chromosome 9 Open Reading Frame 72 (C9orf72) (SEQ ID NO:65)**

TGTTTAGGATTGTTTGTGATTTTCTTTTT[T/-]  
JGAGACAGGGTCTCACTCCGTTGCCAGGCT

**rs2453565 of Chromosome 9 Open Reading Frame 72 (C9orf72) (SEQ ID NO:66)**

TCATCTTTTAAAAAAGTAGAGACC[C/T]TTAGGCAATTAATAAAATAAACTAC

**rs700828 of Chromosome 9 Open Reading Frame 72 (C9orf72) (SEQ ID NO:67)**

TATTAAATATTTTTCATTAGATCA[T/C]AGTTTGAATTTCTCTAGGCATC

**rs774356 of Chromosome 9 Open Reading Frame 72 (C9orf72) (SEQ ID NO:68)**

AATGTAAGTGTTCATTAATCCTTAA[C/T]TAACTCAAGTAGCACTGAAGGAAAG

**rs774357 of Chromosome 9 Open Reading Frame 72 (C9orf72) (SEQ ID NO:69)**

GAAGGGACAGATTTAGAAGGAAAGC[A/G]AAGACAATGAAATCAAGATGAATAA

**rs774359 of Chromosome 9 Open Reading Frame 72 (C9orf72) (SEQ ID NO:70)**

TCTTACTCAATGCTTATAACAACCC[C/T]ACACATTAGGTACTATTACTATTAT

**rs2453554 of Chromosome 9 Open Reading Frame 72 (C9orf72) (SEQ ID NO:71)**

GACTCCCCAAAAACAATCTCTGTG[C/T]ACTACCATAAACTTCAGAAGAACAA

**rs2484319 of Chromosome 9 Open Reading Frame 72 (C9orf72) (SEQ ID NO:72)**

TGCTGAAAGTTTATTTAAAAAAA[A/C]CCATCCTTTAGTAACCTCCACAAC

**rs2453555 of Chromosome 9 Open Reading Frame 72 (C9orf72) (SEQ ID NO:73)**

ACACATTCAATCTAACAAGAATAAT[A/G]ACTAGATCCGTGTAAATTTCTTC

**rs2492816 of Chromosome 9 Open Reading Frame 72 (C9orf72) (SEQ ID NO:74)**

TCTTAACGATACAACATAAGACTTA[A/G]AAGAAATATTGTGTGGACCTGGGCC

**rs3849945 of Chromosome 9 Open Reading Frame 72 (C9orf72) (SEQ ID NO:75)**

TCACTATACTATGCCTTTTATTGTTATTTT[A/G]GAGTGCACCTCCTTCTACTTTTTTT  
TTTTTT

**rs10757668 of Chromosome 9 Open Reading Frame 72 (C9orf72) (SEQ ID NO:76)**

ACTGCATTCCAACGTGTCACATTATC[C/T]AAATGCTCCGGAGATATCTAAACAA

**Superoxide Dismutase 1 (SOD1) Insertion (Long) Target Sequence (SEQ ID NO:77)**

TGAACAAAATTCAAAATACTGTTGCTTAAAGTATTAAGATTTTTTTAGGATT

**Superoxide Dismutase 1 (SOD1) Deletion (Short) Target Sequence (SEQ ID NO:78)**

TGAACAAAATTCAAAATACTGTTGCTTAAAGTATTAAGATTTTTTTAGGATT

**rs11307260 of Superoxide Dismutase 1 (SOD1) (SEQ ID NO:79)**

TGAACAAAATTCAAAATACTGTTGC[T/-]TAAAGTATTAAGATTTTTTTAGGATT

**Short Superoxide Dismutase 1 (SOD1) LNA (SEQ ID NO:80)**

CTTAATACTTTAGCAACAGT

(e.g., +C\*+T\*+T\*A\*A\*T\*A\*C\*T\*T\*T\*A\*G\*C\*A\*A\*C\*+A\*+G\*+T, wherein + denotes a locked nucleic acid and \* denotes a nucleic acid with a phosphorothioate backbone/bond)

**Long Superoxide Dismutase 1 (SOD1) LNA (SEQ ID NO:81)**

CTTAATACTTTAAGCAACAG

(e.g., +C\*+T\*+T\*A\*A\*T\*A\*C\*T\*T\*T\*A\*A\*G\*C\*A\*A\*+C\*+A\*+G, wherein + denotes a locked nucleic acid and \* denotes a nucleic acid with a phosphorothioate backbone/bond)

**Short Superoxide Dismutase 1 (SOD1) MOE (SEQ ID NO:82)**

CTTAATACTTTAGCAACAGT

(e.g.,

/52MOErC/\*i2MOErT/\*i2MOErT/\*i2MOErA/\*i2MOErA/\*T\*A\*C\*T\*T\*T\*A\*G\*C\*A\*/\*i2MOErA/\*i2MOErC/\*i2MOErA/\*i2MOErG/\*32MOErT/, wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic acid with a phosphorothioate backbone/bond)

**Long Superoxide Dismutase 1 (SOD1) MOE (SEQ ID NO:83)**

CTTAATACTTTAAGCAACAG

(e.g.,

/52MOErC/\*i2MOErT/\*i2MOErT/\*i2MOErA/\*i2MOErA/\*T\*A\*C\*T\*T\*A\*A\*G\*C\*/  
i2MOErA/\*i2MOErA/\*i2MOErC/\*i2MOErA/\*32MOErG/, wherein i2MOEr denotes a  
2'-O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic acid with a phosphorothioate  
backbone/bond)

**Kinesin family member 5A (KIF5A) Short Target Sequence (SEQ ID NO:84)**

AATACTTGGA AAAAATGCCCAAATGTGAGGAGGGGGGATATGGAGTAGGGGAAG  
AAAAAGCCTTGATAAGGCTCTGCACAGTGTTGGCATTTCAGTTCTTGGATCACTT  
TGCAGTGTGGGTCTGGAATCAGATTGCTTGAATTTGAAACT

**Kinesin family member 5A (KIF5A) Long Target Sequence (SEQ ID NO:85)**

AATACTTGGA AAAAATGCCCAAATGTGAGGAGGGGGGATATGGAGTAGGGGAAG  
AAAAAGCCTTGATAAGGCTCTGACACAGTGTTGGCATTTCAGTTCTTGGATCACT  
TTGCAGTGTGGGTCTGGAATCAGATTGCTTGAATTTGAAACT

**Short ASO for Kinesin family member 5A (KIF5A) (SEQ ID NO:86)**

AACACTGTGCAGAGCC

(e.g., +A\*+A\*+C\*+A\*C\*T\*G\*T\*G\*C\*A\*G\*+A\*+G\*+C\*+C, wherein + denotes a locked  
nucleic acid and \* denotes a nucleic acid with a phosphorothioate backbone/bond)

**Long ASO for Kinesin family member 5A (KIF5A) (SEQ ID NO:87)**

AACACTGTGTCAGAGC

(e.g., +A\*+A\*+C\*+A\*C\*T\*G\*T\*G\*T\*C\*A\*G\*+A\*+G\*+C, wherein + denotes a locked  
nucleic acid and \* denotes a nucleic acid with a phosphorothioate backbone/bond)

**Titan protein (TTN) Short Target Sequence (SEQ ID NO:88)**

TTCTAATACATATATATAAAGGGCAGCCCAACAGGTTTTGATTTTAGCGTTGACT  
TTACACCTTGTTGTATTACTCTCAGCGAGTCCTTTAACCAACCTGAGCCTGAAGTT  
ATTTTCCTGTAAATTGGGGATGCTAATATCAGTCCTTAAT

**Titan protein (TTN) Long Target Sequence (SEQ ID NO:89)**

TTCTAATACATATATATAAAGGGCAGCCCAACAGGTTTTGATTTTAGCGTTGACT  
 TTACACCTTGTTGTATTACTCCTCAGCGAGTCCTTTAACCAACCTGAGCCTGAAGT  
 TATTTTCCTGTAAATTGGGGATGCTAATATCAGTCTTAAT

**Short ASO for Titan Protein (TTN) (SEQ ID NO:90)**

TCGCTGAGGAGTAATA

(e.g., +T\*+C\*+G\*+C\*T\*G\*A\*G\*G\*A\*G\*T\*+A\*+A\*+T\*+A, wherein + denotes a locked nucleic acid and \* denotes a nucleic acid with a phosphorothioate backbone/bond)

**Long ASO for Titan Protein (TTN) (SEQ ID NO:91)**

TCGCTGAGAGTAATAC

(e.g., +T\*+C\*+G\*+C\*T\*G\*A\*G\*A\*G\*T\*A\*+A\*+T\*+A\*+C, wherein + denotes a locked nucleic acid and \* denotes a nucleic acid with a phosphorothioate backbone/bond)

**Mitofusin-2 (MFN2) Short Target Sequence (SEQ ID NO:92)**

TTGACCTGGATTGTCTAAGCAGTGATATTTGTGAAATGACAGATGCTAGTATT  
 TTTCTCTAATACACATTAAAGTAAGTATGTAAGTATTAATAAATAGACGACTTTAA  
 AAATAACACCTACATACGACTATTTTAAAGTTCAGATTT

**Mitofusin-2 (MFN2) Long Target Sequence (SEQ ID NO:93)**

TTGACCTGGATTGTCTAAGCAGTGATATTTGTGAAATGACAGATGCTAGTATT  
 TTTCTCTAATACACATTAAAGTAAGTATGTAAGTATTAATAAATAGACGACTTTA  
 AAAATAACACCTACATACGACTATTTTAAAGTTCAGATTT

**Short ASO for Mitofusin-2 (MFN2) (SEQ ID NO:94)**

TACTTACTTTAATGTG

(e.g., +T\*+A\*+C\*+T\*T\*A\*C\*T\*T\*T\*A\*A\*+T\*+G\*+T\*+G, wherein + denotes a locked nucleic acid and \* denotes a nucleic acid with a phosphorothioate backbone/bond)

**Long ASO for Mitofusin-2 (MFN2) (SEQ ID NO:95)**

ACTTACTTTTAATGTG

(e.g., +A\*+C\*+T\*+T\*A\*C\*T\*T\*T\*A\*A\*+T\*+G\*+T\*+G, wherein + denotes a locked nucleic acid and \* denotes a nucleic acid with a phosphorothioate backbone/bond)

**β-myosin Heavy Chain (MYH7) Short Target Sequence (SEQ ID NO:96)**

GGAGGAGGTTAAGGTAAGGGCTAGACTCGGGCCACCTGGCCCAAGCAAGGAGC  
 AACTGACTAGCCTTGCATCAAATCACTTCCCTTCCCAGCAATAAGGCTGGCTGT  
 GGACCAACAGTTCTCCAAGAATTCTAAAAAGAAATCACAGCC

**β-myosin Heavy Chain (MYH7) Long Target Sequence (SEQ ID NO:97)**

GGAGGAGGTTAAGGTAAGGGCTAGACTCGGGCCACCTGGCCCAAGCAAGGAGC  
 AACTGACTAGCCTTGCATCAACATCACTTCCCTTCCCAGCAATAAGGCTGGCTG  
 TGGACCAACAGTTCTCCAAGAATTCTAAAAAGAAATCACAGCC

**Short ASO for β-myosin Heavy Chain (MYH7) (SEQ ID NO:98)**

GTGATTTGATGCAAGG

(e.g., +G\*+T\*+G\*+A\*T\*T\*T\*G\*A\*T\*G\*C\*+A\*+A\*+G\*+G, wherein + denotes a locked nucleic acid and \* denotes a nucleic acid with a phosphorothioate backbone/bond)

**Long ASO for β-myosin Heavy Chain (MYH7) (SEQ ID NO:99)**

GAAGTGATGTTGATGC

(e.g., +G\*+A\*+A\*+G\*T\*G\*A\*T\*G\*T\*T\*G\*+A\*+T\*+G\*+C, wherein + denotes a locked nucleic acid and \* denotes a nucleic acid with a phosphorothioate backbone/bond)

**Lamin A/C (LMNA) Short Target Sequence (SEQ ID NO:100)**

CTAAGCTTGTCCAGTCAGAAAAGCAGAGGCTGAGGGGTGGCCTTTTCTTGAGAA  
 CTACATTCAAGTTGCAGCAAGGACAGTGGTCTGAATTTGACGGGGACAAATGGA  
 AGGGAGATAGGACACATGAGTTCCTTTAGGTCTGGCTCAGGG

**Lamin A/C (LMNA) Long Target Sequence (SEQ ID NO:101)**

CTAAGCTTGTCCAGTCAGAAAAGCAGAGGCTGAGGGGTGGCCTTTTCTTGAGAA  
 CTACATTCAAGTTGCAGCAAGAAGGACAGTGGTCTGAATTTGACGGGGACAAAT  
 GGAAGGGAGATAGGACACATGAGTTCCTTTAGGTCTGGCTCAGGG

**Short ASO for Lamin A/C (LMNA) (SEQ ID NO:102)**

CACTGTCCTTGCTGCA

(e.g., +C\*+A\*+C\*+T\*G\*T\*C\*C\*T\*T\*G\*C\*+T\*+G\*+C\*+A, wherein + denotes a locked nucleic acid and \* denotes a nucleic acid with a phosphorothioate backbone/bond)

**Long ASO for Lamin A/C (LMNA) (SEQ ID NO:103)**

ACTGTCCTTCTTGCTG

(e.g., +A\*+C\*+T\*+G\*T\*C\*C\*T\*T\*C\*T\*T\*+G\*+C\*+T\*+G, wherein + denotes a locked nucleic acid and \* denotes a nucleic acid with a phosphorothioate backbone/bond)

**SOD1 Short-1 (SEQ ID NO:104)**

AAATCTTAATACTTTAGCAA

(e.g.,

/52MOErA/\*i2MOErA/\*i2MOErA/\*i2MOErT/\*i2MOErC/\*T\*T\*A\*A\*T\*A\*C\*T\*T\*T\*/

i2MOErA/\*i2MOErG/\*i2MOErC/\*i2MOErA/\*32MOErA/, wherein i2MOEr denotes a

2'-O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic acid with a phosphorothioate

backbone/bond)

**SOD1 Short-2 (SEQ ID NO:105)**

AATCTTAATACTTTAGCAAC

(e.g.,

/52MOErA/\*i2MOErA/\*i2MOErT/\*i2MOErC/\*i2MOErT/\*T\*A\*A\*T\*A\*C\*T\*T\*T\*A\*/

i2MOErG/\*i2MOErC/\*i2MOErA/\*i2MOErA/\*32MOErC/, wherein i2MOEr denotes a 2'-

O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic acid with a phosphorothioate

backbone/bond)

**SOD1 Short-3 (SEQ ID NO:106)**

ATCTTAATACTTTAGCAACA

(e.g.,

/52MOErA/\*i2MOErT/\*i2MOErC/\*i2MOErT/\*i2MOErT/\*A\*A\*T\*A\*C\*T\*T\*T\*A\*G\*/

i2MOErC/\*i2MOErA/\*i2MOErA/\*i2MOErC/\*32MOErA/, wherein i2MOEr denotes a 2'-

O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic acid with a phosphorothioate

backbone/bond)

**SOD1 Short-4 (SEQ ID NO:107)**

TCTTAATACTTTAGCAACAG

(e.g.,

/52MOErT/\*i2MOErC/\*i2MOErT/\*i2MOErT/\*i2MOErA/\*A\*T\*A\*C\*T\*T\*T\*A\*G\*C\*/i



2MOErA/\*i2MOErA/\*i2MOErC/\*i2MOErA/\*32MOErG/, wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic acid with a phosphorothioate backbone/bond)

**SOD1 Short-5 (SEQ ID NO:82)**

CTTAATACTTTAGCAACAGT

(e.g.,

/52MOErC/\*i2MOErT/\*i2MOErT/\*i2MOErA/\*i2MOErA/\*T\*A\*C\*T\*T\*A\*G\*C\*A\*/i2MOErA/\*i2MOErC/\*i2MOErA/\*i2MOErG/\*32MOErT/, wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic acid with a phosphorothioate backbone/bond)

**SOD1 Short-6 (SEQ ID NO:108)**

TTAATACTTTAGCAACAGTA

(e.g.,

/52MOErT/\*i2MOErT/\*i2MOErA/\*i2MOErA/\*i2MOErT/\*A\*C\*T\*T\*T\*A\*G\*C\*A\*A\*/i2MOErC/\*i2MOErA/\*i2MOErG/\*i2MOErT/\*32MOErA/, wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic acid with a phosphorothioate backbone/bond)

**SOD1 Short-7 (SEQ ID NO:109)**

TAATACTTTAGCAACAGTAT

(e.g.,

/52MOErT/\*i2MOErA/\*i2MOErA/\*i2MOErT/\*i2MOErA/\*C\*T\*T\*T\*A\*G\*C\*A\*A\*C\*/i2MOErA/\*i2MOErG/\*i2MOErT/\*i2MOErA/\*32MOErT/, wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic acid with a phosphorothioate backbone/bond)

**SOD1 Short-8 (SEQ ID NO:110)**

AATACTTTAGCAACAGTATT

(e.g.,

/52MOErA/\*i2MOErA/\*i2MOErT/\*i2MOErA/\*i2MOErC/\*T\*T\*T\*A\*G\*C\*A\*A\*C\*A\*/i2MOErG/\*i2MOErT/\*i2MOErA/\*i2MOErT/\*32MOErT/, wherein i2MOEr denotes a

2'-O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic acid with a phosphorothioate backbone/bond)

**SOD1 Short-9 (SEQ ID NO:111)**

ATACTTTAGCAACAGTATTT

(e.g.,

/52MOErA/\*i2MOErT/\*i2MOErA/\*i2MOErC/\*i2MOErT/\*T\*A\*G\*C\*A\*A\*C\*A\*G\*  
/i2MOErT/\*i2MOErA/\*i2MOErT/\*i2MOErT/\*32MOErT/)

**SOD1 Short-10 (SEQ ID NO:112)**

TACTTTAGCAACAGTATTTT

(e.g.,

/52MOErT/\*i2MOErA/\*i2MOErC/\*i2MOErT/\*i2MOErT/\*T\*A\*G\*C\*A\*A\*C\*A\*G\*T\*/  
i2MOErA/\*i2MOErT/\*i2MOErT/\*i2MOErT/\*32MOErT/, wherein i2MOEr denotes a 2'-  
O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic acid with a phosphorothioate  
backbone/bond)

**SOD1 Short-11 (SEQ ID NO:113)**

ACTTTAGCAACAGTATTTTG

(e.g.,

/52MOErA/\*i2MOErC/\*i2MOErT/\*i2MOErT/\*i2MOErT/\*A\*G\*C\*A\*A\*C\*A\*G\*T\*A\*  
/i2MOErT/\*i2MOErT/\*i2MOErT/\*i2MOErT/\*32MOErG/, wherein i2MOEr denotes a 2'-  
O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic acid with a phosphorothioate  
backbone/bond)

**SOD1 Long-1 (SEQ ID NO:114)**

AATCTTAATACTTTAAGCAA

(e.g.,

/52MOErA/\*i2MOErA/\*i2MOErT/\*i2MOErC/\*i2MOErT/\*T\*A\*A\*T\*A\*C\*T\*T\*T\*A\*/  
i2MOErA/\*i2MOErG/\*i2MOErC/\*i2MOErA/\*32MOErA/, wherein i2MOEr denotes a  
2'-O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic acid with a phosphorothioate  
backbone/bond)

**SOD1 Long-2 (SEQ ID NO:115)**

ATCTTAATACTTTAAGCAAC

(e.g.,

/52MOErA/\*i2MOErT/\*i2MOErC/\*i2MOErT/\*i2MOErT/\*A\*A\*T\*A\*C\*T\*T\*T\*A\*A\*/  
i2MOErG/\*i2MOErC/\*i2MOErA/\*i2MOErA/\*i2MOErA/\*32MOErC/, wherein i2MOEr denotes a 2'-  
O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic acid with a phosphorothioate  
backbone/bond)

**SOD1 Long-3 (SEQ ID NO:116)**

TCTTAATACTTTAAGCAACA

(e.g.,

/52MOErT/\*i2MOErC/\*i2MOErT/\*i2MOErT/\*i2MOErA/\*A\*T\*A\*C\*T\*T\*T\*A\*A\*G\*/  
i2MOErC/\*i2MOErA/\*i2MOErA/\*i2MOErC/\*i2MOErA/\*32MOErA/, wherein i2MOEr denotes a 2'-  
O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic acid with a phosphorothioate  
backbone/bond)

**SOD1 Long-4 (SEQ ID NO:117)**

CTTAATACTTTAAGCAACAG

(e.g.,

/52MOErC/\*i2MOErT/\*i2MOErT/\*i2MOErA/\*i2MOErA/\*T\*A\*C\*T\*T\*T\*A\*A\*G\*C\*/  
i2MOErA/\*i2MOErA/\*i2MOErC/\*i2MOErA/\*i2MOErA/\*32MOErG/, wherein i2MOEr denotes a  
2'-O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic acid with a phosphorothioate  
backbone/bond)

**SOD1 Long-5 (SEQ ID NO: 83)**

TTAATACTTTAAGCAACAGT

(e.g.,

/52MOErT/\*i2MOErT/\*i2MOErA/\*i2MOErA/\*i2MOErT/\*A\*C\*T\*T\*T\*A\*A\*G\*C\*A\*/  
i2MOErA/\*i2MOErC/\*i2MOErA/\*i2MOErG/\*i2MOErT/\*32MOErT/)

**SOD1 Long-6 (SEQ ID NO:118)**

TAATACTTTAAGCAACAGTA

(e.g.,

/52MOErT/\*i2MOErA/\*i2MOErA/\*i2MOErT/\*i2MOErA/\*C\*T\*T\*T\*A\*A\*G\*C\*A\*A\*/  
i2MOErC/\*i2MOErA/\*i2MOErG/\*i2MOErT/\*i2MOErA/\*32MOErA/, wherein i2MOEr denotes a 2'-

O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic acid with a phosphorothioate backbone/bond)

**SOD1 Long-7 (SEQ ID NO:119)**

AATACTTTAAGCAACAGTAT

(e.g.,

/52MOErA/\*i2MOErA/\*i2MOErT/\*i2MOErA/\*i2MOErC/\*T\*T\*T\*A\*A\*G\*C\*A\*A\*C\*  
/i2MOErA/\*i2MOErG/\*i2MOErT/\*i2MOErA/\*32MOErT/, wherein i2MOEr denotes a  
2'-O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic acid with a phosphorothioate  
backbone/bond)

**SOD1 Long-8 (SEQ ID NO:120)**

ATACTTTAAGCAACAGTATT

(e.g.,

/52MOErA/\*i2MOErT/\*i2MOErA/\*i2MOErC/\*i2MOErT/\*T\*T\*A\*A\*G\*C\*A\*A\*C\*A\*  
/i2MOErG/\*i2MOErT/\*i2MOErA/\*i2MOErT/\*32MOErT/, wherein i2MOEr denotes a  
2'-O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic acid with a phosphorothioate  
backbone/bond)

**SOD1 Long-9 (SEQ ID NO:121)**

TACTTTAAGCAACAGTATTT

(e.g.,

/52MOErT/\*i2MOErA/\*i2MOErC/\*i2MOErT/\*i2MOErT/\*T\*A\*A\*G\*C\*A\*A\*C\*A\*G\*  
/i2MOErT/\*i2MOErA/\*i2MOErT/\*i2MOErT/\*32MOErT/, wherein i2MOEr denotes a 2'-  
O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic acid with a phosphorothioate  
backbone/bond)

**SOD1 Long-10 (SEQ ID NO:122)**

ACTTTAAGCAACAGTATTTT

(e.g.,

/52MOErA/\*i2MOErC/\*i2MOErT/\*i2MOErT/\*i2MOErT/\*A\*A\*G\*C\*A\*A\*C\*A\*G\*T\*  
/i2MOErA/\*i2MOErT/\*i2MOErT/\*i2MOErT/\*32MOErT/, wherein i2MOEr denotes a 2'-  
O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic acid with a phosphorothioate  
backbone/bond)

**Transactive Responsive Deoxyribonucleic Acid (DNA)-Binding Protein 43 (TARDBP)****Short Target Sequence (SEQ ID NO:123)**

GGGGTCTCCCTGTGTTGTCTAGGCTGCCAGGCTAGTCTTGAACCTATTGGCCTCAC  
 ACAGTCCTCCCACCTTGGCCTCCCAAAGCGCTAGGATTACAGGCATGAGCCACCA  
 TGCCCAGCCTATGTCTTTTGAAAATCGACTGAAATATCACTGCTGCTGTTAATAA  
 AACTAAAAGCTGTATTGGGGGTTTAAATGAAATGATGTTTCATTGCTTATTTTCCT  
 CTGGCTTTAGATAAAATTAATGCTTGTAATCTAAGTTTTGTTGCTACTTTAAATATA  
 TGAATCAGTGGTTTAATCTTCTTTGTTTACATCCCTTATTTCTTATAGATTGCGCA  
 GTCTCTTTGTGGAGAGGACTTGATCATTAAAGGAATCAGCGTTCATATATCCAAT  
 GCCGAACCTAA

**Transactive Responsive Deoxyribonucleic Acid (DNA)-Binding Protein 43 (TARDBP)****Long Target Sequence (SEQ ID NO:124)**

GGGGTCTCCCTGTGTTGTCTAGGCTGCCAGGCTAGTCTTGAACCTATTGGCCTCAC  
 ACAGTCCTCCCACCTTGGCCTCCCAAAGCGCTAGGATTACAGGCATGAGCCACCA  
 TGCCCAGCCTATGTCTTTTGAAAATCGACTGAAATATCACTGCTGCTGTTAATAA  
 AACTAAAAGCTGTATTGGGGGTTTAAATGAAATGAGTGTTTCATTGCTTATTTTC  
 CTCTGGCTTTAGATAAAATTAATGCTTGTAATCTAAGTTTTGTTGCTACTTTAAATA  
 TATGAATCAGTGGTTTAATCTTCTTTGTTTACATCCCTTATTTCTTATAGATTGCGC  
 AGTCTCTTTGTGGAGAGGACTTGATCATTAAAGGAATCAGCGTTCATATATCCAA  
 TGCCGAACCTAA

**rs3835416 of Transactive Responsive Deoxyribonucleic Acid (DNA)-Binding Protein 43 (TARDBP) (SEQ ID NO:125)**

GGGGTCTCCCTGTGTTGTCTAGGCTGCCAGGCTAGTCTTGAACCTATTGGCCTCAC  
 ACAGTCCTCCCACCTTGGCCTCCCAAAGCGCTAGGATTACAGGCATGAGCCACCA  
 TGCCCAGCCTATGTCTTTTGAAAATCGACTGAAATATCACTGCTGCTGTTAATAA  
 AACTAAAAGCTGTATTGGGGGTTTAAATGAAATGA[G/-  
 JTGTTCATTGCTTATTTTTCCTCTGGCTTTAGATAAAATTAATGCTTGTAATCTAAGT  
 TTTGTTGCTACTTTAAATATATGAATCAGTGGTTTAATCTTCTTTGTTTACATCCCT  
 TATTTCTTATAGATTGCGCAGTCTCTTTGTGGAGAGGACTTGATCATTAAAGGAA  
 TCAGCGTTCATATATCCAATGCCGAACCTAA

**TARDBP Short-1 (SEQ ID NO:126)**

GAACATCATTTTCATTTAAAC

(e.g.,

/52MOErG/\*i2MOErA/\*i2MOErA/\*i2MOErC/\*i2MOErA/\*T\*C\*A\*T\*T\*T\*C\*A\*T\*T\*/  
i2MOErT/\*i2MOErA/\*i2MOErA/\*i2MOErA/\*32MOErC/, wherein i2MOEr denotes a 2'-  
O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic acid with a phosphorothioate  
backbone/bond)

**TARDBP Short-2 (SEQ ID NO:127)**

TGAACATCATTTTCATTTAAA

(e.g.,

/52MOErT/\*i2MOErG/\*i2MOErA/\*i2MOErA/\*i2MOErC/\*A\*T\*C\*A\*T\*T\*T\*C\*A\*T\*/  
i2MOErT/\*i2MOErT/\*i2MOErA/\*i2MOErA/\*32MOErA/, wherein i2MOEr denotes a 2'-  
O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic acid with a phosphorothioate  
backbone/bond)

**TARDBP Short-3 (SEQ ID NO:128)**

ATGAACATCATTTTCATTTAA

(e.g.,

/52MOErA/\*i2MOErT/\*i2MOErG/\*i2MOErA/\*i2MOErA/\*C\*A\*T\*C\*A\*T\*T\*T\*C\*A\*/  
i2MOErT/\*i2MOErT/\*i2MOErT/\*i2MOErA/\*32MOErA/, wherein i2MOEr denotes a 2'-  
O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic acid with a phosphorothioate  
backbone/bond)

**TARDBP Short-4 (SEQ ID NO:129)**

AATGAACATCATTTTCATTTA

(e.g.,

/52MOErA/\*i2MOErA/\*i2MOErT/\*i2MOErG/\*i2MOErA/\*A\*C\*A\*T\*C\*A\*T\*T\*T\*C\*/  
i2MOErA/\*i2MOErT/\*i2MOErT/\*i2MOErT/\*32MOErA/, wherein i2MOEr denotes a 2'-  
O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic acid with a phosphorothioate  
backbone/bond)

**TDP43 Short-5 (SEQ ID NO:130)**

CAATGAACATCATTTTCATT

(e.g.,

/52MOErC/\*i2MOErA/\*i2MOErA/\*i2MOErT/\*i2MOErG/\*A\*A\*C\*A\*T\*C\*A\*T\*T\*T\*/  
i2MOErC/\*i2MOErA/\*i2MOErT/\*i2MOErT/\*32MOErT/, wherein i2MOEr denotes a 2'-  
O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic acid with a phosphorothioate  
backbone/bond)

**TARDBP Short-6 (SEQ ID NO:131)**

GCAATGAACATCATTTCATT

(e.g.,

/52MOErG/\*i2MOErC/\*i2MOErA/\*i2MOErA/\*i2MOErT/\*G\*A\*A\*C\*A\*T\*C\*A\*T\*T\*T\*/  
i2MOErT/\*i2MOErC/\*i2MOErA/\*i2MOErT/\*32MOErT/, wherein i2MOEr denotes a 2'-  
O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic acid with a phosphorothioate  
backbone/bond)

**TARDBP Short-7 (SEQ ID NO:132)**

AGCAATGAACATCATTTCAT

(e.g.,

/52MOErA/\*i2MOErG/\*i2MOErC/\*i2MOErA/\*i2MOErA/\*T\*G\*A\*A\*C\*A\*T\*C\*A\*T\*T\*  
i2MOErT/\*i2MOErT/\*i2MOErC/\*i2MOErA/\*32MOErT/, wherein i2MOEr denotes a 2'-  
O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic acid with a phosphorothioate  
backbone/bond)

**TARDBP Short-8 (SEQ ID NO:133)**

AAGCAATGAACATCATTCA

(e.g.,

/52MOErA/\*i2MOErA/\*i2MOErG/\*i2MOErC/\*i2MOErA/\*A\*T\*G\*A\*A\*C\*A\*T\*C\*A\*  
i2MOErT/\*i2MOErT/\*i2MOErT/\*i2MOErC/\*32MOErA/, wherein i2MOEr denotes a 2'-  
O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic acid with a phosphorothioate  
backbone/bond)

**TARDBP Short-9 (SEQ ID NO:134)**

TAAGCAATGAACATCATTC

(e.g.,

/52MOErT/\*i2MOErA/\*i2MOErA/\*i2MOErG/\*i2MOErC/\*A\*A\*T\*G\*A\*A\*C\*A\*T\*C\*

/i2MOErA/\*i2MOErT/\*i2MOErT/\*i2MOErT/\*32MOErC/, wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic acid with a phosphorothioate backbone/bond)

**TARDBP Short-10 (SEQ ID NO:135)**

ATAAGCAATGAACATCATT

(e.g.,

/52MOErA/\*i2MOErT/\*i2MOErA/\*i2MOErA/\*i2MOErG/\*C\*A\*A\*T\*G\*A\*A\*C\*A\*T\*  
/i2MOErC/\*i2MOErA/\*i2MOErT/\*i2MOErT/\*32MOErT/, wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic acid with a phosphorothioate backbone/bond)

**TARDBP Short-11 (SEQ ID NO:136)**

AATAAGCAATGAACATCATT

(e.g.,

/52MOErA/\*i2MOErA/\*i2MOErT/\*i2MOErA/\*i2MOErA/\*G\*C\*A\*A\*T\*G\*A\*A\*C\*A\*  
\*/i2MOErT/\*i2MOErC/\*i2MOErA/\*i2MOErT/\*32MOErT/, wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic acid with a phosphorothioate backbone/bond)

**TARDBP Long-1 (SEQ ID NO:137)**

GAACACTCATTTTCATTTAAA

(e.g.,

/52MOErG/\*i2MOErA/\*i2MOErA/\*i2MOErC/\*i2MOErA/\*C\*T\*C\*A\*T\*T\*T\*C\*A\*T\*/  
i2MOErT/\*i2MOErT/\*i2MOErA/\*i2MOErA/\*32MOErA/, wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic acid with a phosphorothioate backbone/bond)

**TARDBP Long-2 (SEQ ID NO:138)**

TGAACACTCATTTTCATTTAA

(e.g.,

/52MOErT/\*i2MOErG/\*i2MOErA/\*i2MOErA/\*i2MOErC/\*A\*C\*T\*C\*A\*T\*T\*T\*C\*A\*/  
i2MOErT/\*i2MOErT/\*i2MOErT/\*i2MOErA/\*32MOErA/, wherein i2MOEr denotes a 2'-



O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic acid with a phosphorothioate backbone/bond)

**TARDBP Long-3 (SEQ ID NO:139)**

ATGAACACTCATTTTCATTTA

(e.g.,

/52MOErA/\*i2MOErT/\*i2MOErG/\*i2MOErA/\*i2MOErA/\*C\*A\*C\*T\*C\*A\*T\*T\*T\*C\*/  
i2MOErA/\*i2MOErT/\*i2MOErT/\*i2MOErT/\*32MOErA/, wherein i2MOEr denotes a 2'-  
O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic acid with a phosphorothioate  
backbone/bond)

**TARDBP Long-4 (SEQ ID NO:140)**

AATGAACACTCATTTTCATTT

(e.g.,

/52MOErA/\*i2MOErA/\*i2MOErT/\*i2MOErG/\*i2MOErA/\*A\*C\*A\*C\*T\*C\*A\*T\*T\*T\*/  
i2MOErC/\*i2MOErA/\*i2MOErT/\*i2MOErT/\*32MOErT/, wherein i2MOEr denotes a 2'-  
O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic acid with a phosphorothioate  
backbone/bond)

**TARDBP Long-5 (SEQ ID NO:141)**

CAATGAACACTCATTTTCATT

(e.g.,

/52MOErC/\*i2MOErA/\*i2MOErA/\*i2MOErT/\*i2MOErG/\*A\*A\*C\*A\*C\*T\*C\*A\*T\*T\*/  
i2MOErT/\*i2MOErC/\*i2MOErA/\*i2MOErT/\*32MOErT/, wherein i2MOEr denotes a 2'-  
O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic acid with a phosphorothioate  
backbone/bond)

**TARDBP Long-6 (SEQ ID NO:142)**

GCAATGAACACTCATTTTCAT

(e.g.,

/52MOErG/\*i2MOErC/\*i2MOErA/\*i2MOErA/\*i2MOErT/\*G\*A\*A\*C\*A\*C\*T\*C\*A\*T\*/  
i2MOErT/\*i2MOErT/\*i2MOErC/\*i2MOErA/\*32MOErT/, wherein i2MOEr denotes a 2'-  
O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic acid with a phosphorothioate  
backbone/bond)

**TARDBP Long-7 (SEQ ID NO:143)**

AGCAATGAACACTCATTTCA

(e.g.,

/52MOErA/\*i2MOErG/\*i2MOErC/\*i2MOErA/\*i2MOErA/\*T\*G\*A\*A\*C\*A\*C\*T\*C\*A\*  
 /i2MOErT/\*i2MOErT/\*i2MOErT/\*i2MOErC/\*32MOErA/, wherein i2MOEr denotes a 2'-  
 O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic acid with a phosphorothioate  
 backbone/bond)

**TARDBP Long-8 (SEQ ID NO:144)**

AAGCAATGAACACTCATTTTC

(e.g.,

/52MOErA/\*i2MOErA/\*i2MOErG/\*i2MOErC/\*i2MOErA/\*A\*T\*G\*A\*A\*C\*A\*C\*T\*C\*  
 /i2MOErA/\*i2MOErT/\*i2MOErT/\*i2MOErT/\*32MOErC/, wherein i2MOEr denotes a 2'-  
 O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic acid with a phosphorothioate  
 backbone/bond)

**TARDBP Long-9 (SEQ ID NO:145)**

TAAGCAATGAACACTCATTT

(e.g.,

/52MOErT/\*i2MOErA/\*i2MOErA/\*i2MOErG/\*i2MOErC/\*A\*A\*T\*G\*A\*A\*C\*A\*C\*T\*  
 /i2MOErC/\*i2MOErA/\*i2MOErT/\*i2MOErT/\*32MOErT/, wherein i2MOEr denotes a 2'-  
 O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic acid with a phosphorothioate  
 backbone/bond)

**TARDBP Long-10 (SEQ ID NO:146)**

ATAAGCAATGAACACTCATT

(e.g.,

/52MOErA/\*i2MOErT/\*i2MOErA/\*i2MOErA/\*i2MOErG/\*C\*A\*A\*T\*G\*A\*A\*C\*A\*C\*  
 /i2MOErT/\*i2MOErC/\*i2MOErA/\*i2MOErT/\*32MOErT/, wherein i2MOEr denotes a 2'-  
 O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic acid with a phosphorothioate  
 backbone/bond)

**TARDBP SHORT 7-2M (SEQ ID NO:147)**

AGCAATGAACATCATTTTCAT

(e.g.,

/52MOErA/\*i2MOErG/i2MOErC/\*i2MOErA/i2MOErA/\*T\*G\*A\*A\*C\*A\*T\*C\*A\*T\*/i2MOErT/i2MOErT/\*i2MOErC/i2MOErA/\*32MOErT/, wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic acid with a phosphorothioate backbone/bond)

**TARDBP SHORT 7-3M (SEQ ID NO:148)**

AGCAATGAACATCATTTTCAT

(e.g.,

/52MOErA/\*i2MOErG/i2MOErC/i2MOErA/i2MOErA/\*T\*G\*A\*A\*C\*A\*T\*C\*A\*T\*/i2MOErT/i2MOErT//i2MOErC/i2MOErA/\*32MOErT/, wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic acid with a phosphorothioate backbone/bond)

**TARDBP SHORT 7-4M (SEQ ID NO:149)**

AGCAATGAACATCATTTTCAT

(e.g.,

/52MOErA/\*i2MOErG/i2MOErC/i2MOErA/i2MOErA/T\*G\*A\*A\*C\*A\*T\*C\*A\*T/i2MOErT//i2MOErT//i2MOErC/i2MOErA/\*32MOErT/, wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic acid with a phosphorothioate backbone/bond)

**TARDBP LONG 7-2M (SEQ ID NO:150)**

AGCAATGAACACTCATTTTCA

(e.g.,

/52MOErA/\*i2MOErG/i2MOErC/\*i2MOErA/i2MOErA/\*T\*G\*A\*A\*C\*A\*C\*T\*C\*A\*/i2MOErT/\*i2MOErT//i2MOErT/\*i2MOErC//32MOErA/, wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic acid with a phosphorothioate backbone/bond)

**TARDBP LONG 7-3M (SEQ ID NO:151)**

AGCAATGAACACTCATTTTCA

(e.g.,

/52MOErA/\*i2MOErG//i2MOErC//i2MOErA//i2MOErA/\*T\*G\*A\*A\*C\*A\*C\*T\*C\*A\*/i2MOErT//i2MOErT//i2MOErT//i2MOErC/\*i32MOErA/, wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic acid with a phosphorothioate backbone/bond)

**TARDBP LONG 7-4M (SEQ ID NO:152)**

AGCAATGAACACTCATTTCA

(e.g.,

/52MOErA/\*i2MOErG//i2MOErC//i2MOErA//i2MOErA/T\*G\*A\*A\*C\*A\*C\*T\*C\*A/i2MOErT//i2MOErT//i2MOErT//i2MOErC/\*i32MOErA/, wherein i2MOEr denotes a 2'-O-methoxy-ethyl base (2'-MOE) and \* denotes a nucleic acid with a phosphorothioate backbone/bond)

## CLAIMS

What Is Claimed Is:

1. A method of inhibiting an allele that contains a mutation in a cell, the method comprising:  
delivering to the cell an antisense oligonucleotide (ASO), such as an allele-specific antisense oligonucleotide (asASO), that targets a target variant allele (e.g., an allele of a variant or common variant, and/or an indel, such as an insertion or deletion of one or more bases) that is in cis with the mutation (e.g., the mutation is a gain of function mutation or a dominant negative mutation),  
wherein the cell (e.g., a subject) is heterozygous for the target variant allele.
2. The method of claim 1, wherein the cell is *in vitro*.
3. The method of claim 1 or 2, wherein the cell is a human cell (e.g., a human cell of the central nervous system).
4. The method of any one of claims 1-3, wherein  
the mutation is in superoxide dismutase 1 (SOD1) or chromosome 9 open reading frame 72 (C9orf72), and the cell is from (i) a subject having one or more symptoms of at least one of amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), or a combination thereof, or (ii) a subject having or suspected of having at least one of amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), or a combination thereof;  
the mutation is in Kinesin family member 5A (KIF5A), and the cell is from (i) a subject having one or more symptoms of at least one of amyotrophic lateral sclerosis, neonatal intractable myoclonus (NEIMY), hereditary spastic paraplegia 10 (SPG10), or a combination thereof, or (ii) a subject having or suspected of having at least one of amyotrophic lateral sclerosis, neonatal intractable myoclonus (NEIMY), hereditary spastic paraplegia 10 (SPG10), or a combination thereof;  
the mutation is in Titan protein (TTN), and the cell is from (i) a subject having one or more symptoms of at least one of dilated cardiomyopathy-1G (CMD1G), familial hypertrophic cardiomyopathy-9 (CMH9), myofibrillar myopathy-9 with early respiratory failure (MFM9), tibial muscular dystrophy (TMD), or a combination thereof, or (ii) a subject having or suspected of having at least one of dilated cardiomyopathy-1G (CMD1G), familial

hypertrophic cardiomyopathy-9 (CMH9), myofibrillar myopathy-9 with early respiratory failure (MFM9), tibial muscular dystrophy (TMD), or a combination thereof;

the mutation is in Mitofusin-2 (MFN2), and the cell is from (i) a subject having one or more symptoms of at least one of Charcot-Marie-Tooth (CMT) disease type 2A2A (CMT2A2A), Charcot-Marie-Tooth disease type 6A (CMT6A), or a combination thereof, or (ii) a subject having or suspected of having at least one of Charcot-Marie-Tooth (CMT) disease type 2A2A (CMT2A2A), Charcot-Marie-Tooth disease type 6A (CMT6A), or a combination thereof;

the mutation is in  $\beta$ -myosin heavy chain (MYH7), and the cell is from (i) a subject having one or more symptoms of at least one of dilated cardiomyopathy-1S (CMD1S), hypertrophic cardiomyopathy-1 (CMH1), autosomal dominant myosin storage congenital myopathy-7A (CMYP7A), Laing distal myopathy, dilated cardiomyopathy-1S (CMD1S), or a combination thereof, or (ii) a subject having or suspected of having at least one of dilated cardiomyopathy-1S (CMD1S), hypertrophic cardiomyopathy-1 (CMH1), autosomal dominant myosin storage congenital myopathy-7A (CMYP7A), Laing distal myopathy, dilated cardiomyopathy-1S (CMD1S), or a combination thereof;

the mutation is in lamin A/C (LMNA), and the cell is from (i) a subject having one or more symptoms of at least one of dilated cardiomyopathy-1A (CMD1A), autosomal dominant Emery-Dreifuss muscular dystrophy 2 (EDMD2), Slovenian type heart-hand syndrome, Hutchinson-Gilford progeria syndrome (HGPS), familial partial lipodystrophy type 2 (FPLD2), Malouf syndrome, congenital muscular dystrophy (MDC), or a combination thereof, or (ii) a subject having or suspected of having at least one of dilated cardiomyopathy-1A (CMD1A), autosomal dominant Emery-Dreifuss muscular dystrophy 2 (EDMD2), Slovenian type heart-hand syndrome, Hutchinson-Gilford progeria syndrome (HGPS), familial partial lipodystrophy type 2 (FPLD2), Malouf syndrome, congenital muscular dystrophy (MDC), or a combination thereof; or

the mutation is in transactive responsive deoxyribonucleic acid (DNA)-binding protein 43 (TARDBP), and the cell is from (i) a subject having one or more symptoms of at least one of amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), Alzheimer's disease (AD), limbic predominant age-related TDP-43 encephalopathy (LATE), or a combination thereof, or (ii) a subject having or suspected of having at least one of amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), Alzheimer's disease (AD), limbic predominant age-related TDP-43 encephalopathy (LATE), or a combination thereof.

5. The method of any one of claims 1-4, wherein:

the mutation is in superoxide dismutase 1 (SOD1), and the method is a method of inhibiting expression of a superoxide dismutase 1 (SOD1) gene mutation;

the mutation is in chromosome 9 open reading frame 72 (C9Orf72), and the method is a method of inhibiting expression of a chromosome 9 open reading frame 72 (C9Orf72) gene mutation;

the mutation is in Kinesin family member 5A (KIF5A), and the method is a method of inhibiting the expression of a Kinesin family member 5A (KIF5A) gene mutation;

the mutation is in Titan protein (TTN), and the method is a method of inhibiting the expression of a Titan protein (TTN) gene mutation;

the mutation is in Mitofusin-2 (MFN2), and the method is a method of inhibiting the expression of a Mitofusin-2 (MFN2) gene mutation;

the mutation is in  $\beta$ -myosin heavy chain (MYH7), and the method is a method of inhibiting the expression of a  $\beta$ -myosin heavy chain (MYH7) gene mutation;

the mutation is in lamin A/C (LMNA), and the method is a method of inhibiting the expression of a lamin A/C (LMNA) gene mutation; or

the mutation is in transactive responsive deoxyribonucleic acid (DNA)-binding protein 43 (TARDBP), and the method is a method of inhibiting the expression of transactive responsive deoxyribonucleic acid (DNA)-binding protein 43 (TARDBP) gene mutation.

6. The method of any one of claims 1-5, wherein at least one of:

the antisense oligonucleotide targets an intronic region of the gene;

the antisense oligonucleotide sequence and/or a target variant allele sequence has a guanine-cytosine (GC) content of about 20% to about 80%;

the antisense oligonucleotide is an oligonucleotide of 10 to 25 nucleotides in length that is complementary with at least 8 contiguous nucleotides of any one of SEQ ID NOs: 1, 2, 64-76, 79, 84, 85, 88, 89, 92, 93, 96, 97, 100, 101, 123, 124, or 125, rs11307260, rs142843265, rs3835416, rs34620383, rs2453565, rs700828, rs774356, rs774357, rs774359, rs2453554, rs2484319, rs2453555, rs2492816, rs3849945, or rs10757668;

the antisense oligonucleotide is an oligonucleotide having the sequence of SEQ ID NO: 80-83, 86, 87, 90, 91, 94, 95, 98, 99, 102, 103-122, or 126-152, or the antisense oligonucleotide comprises, consists essentially of, or consists of any antisense oligonucleotide described herein;

the mutation is in an A5V mutation in superoxide dismutase 1 (SOD1);

the mutation is in a E134 deletion mutation in superoxide dismutase 1 (SOD1);  
the mutation is a repeat expansion within intron 1 of the chromosome 9 open reading frame 72 (C9orf72);  
the mutation is a Q331 mutations (e.g., Q331K) in transactive responsive deoxyribonucleic acid (DNA)-binding protein 43 (TARDBP);  
the mutation is a M337 mutations (e.g., M337V) in transactive responsive deoxyribonucleic acid (DNA)-binding protein 43 (TARDBP); or  
a combination thereof.

7. The method of any one of claims 1-6, wherein a subject has the target variant allele and the mutation, and the method comprises administering the antisense oligonucleotide to the subject.

8. A method of treating a subject with an allele that contains a mutation, the method comprising:

administering to the subject an antisense oligonucleotide (ASO, such as an allele-specific antisense oligonucleotide (asASO)) that targets a target variant allele (e.g., an allele of a variant or a common variant, and/or an indel, such as an insertion or deletion of one or more bases) that is in cis with the mutation (e.g., the mutation is a gain of function mutation or a dominant negative mutation),

wherein:

the subject is heterozygous for the target variant allele; and  
the antisense oligonucleotide is effective at preventing, treating, or ameliorating at least one symptom associated with the mutation.

9. The method of claim 8, wherein:

the mutation is in superoxide dismutase 1 (SOD1) or chromosome 9 open reading frame 72 (C9orf72), and the subject (i) has one or more symptoms of amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), or both amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), or (ii) has or is suspected of having amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), or both amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD);

the mutation is in Kinesin family member 5A (KIF5A), and the subject (i) has one or more symptoms of at least one of amyotrophic lateral sclerosis, neonatal intractable



myoclonus (NEIMY), hereditary spastic paraplegia 10 (SPG10), or a combination thereof, or (ii) has or is suspected of having at least one of amyotrophic lateral sclerosis, neonatal intractable myoclonus (NEIMY), hereditary spastic paraplegia 10 (SPG10), or a combination thereof;

the mutation is in Titan protein (TTN), and the subject (i) has one or more symptoms of at least one of dilated cardiomyopathy-1G (CMD1G), familial hypertrophic cardiomyopathy-9 (CMH9), myofibrillar myopathy-9 with early respiratory failure (MFM9), tibial muscular dystrophy (TMD), or a combination thereof, or (ii) has or is suspected of having at least one of dilated cardiomyopathy-1G (CMD1G), familial hypertrophic cardiomyopathy-9 (CMH9), myofibrillar myopathy-9 with early respiratory failure (MFM9), tibial muscular dystrophy (TMD), or a combination thereof;

the mutation is in Mitofusin-2 (MFN2), and the subject (i) has one or more symptoms of at least one of Charcot-Marie-Tooth (CMT) disease type 2A2A (CMT2A2A), Charcot-Marie-Tooth disease type 6A (CMT6A), or a combination thereof, or (ii) has or is suspected of having at least one of Charcot-Marie-Tooth (CMT) disease type 2A2A (CMT2A2A), Charcot-Marie-Tooth disease type 6A (CMT6A), or a combination thereof;

the mutation is in  $\beta$ -myosin heavy chain (MYH7), and the subject (i) has one or more symptoms of at least one of dilated cardiomyopathy-1S (CMD1S), hypertrophic cardiomyopathy-1 (CMH1), autosomal dominant myosin storage congenital myopathy-7A (CMYP7A), Laing distal myopathy, dilated cardiomyopathy-1S (CMD1S), or a combination thereof, or (ii) has or is suspected of having at least one of dilated cardiomyopathy-1S (CMD1S), hypertrophic cardiomyopathy-1 (CMH1), autosomal dominant myosin storage congenital myopathy-7A (CMYP7A), Laing distal myopathy, dilated cardiomyopathy-1S (CMD1S), or a combination thereof;

the mutation is in lamin A/C (LMNA), and the subject (i) has one or more symptoms of at least one of dilated cardiomyopathy-1A (CMD1A), autosomal dominant Emery-Dreifuss muscular dystrophy 2 (EDMD2), Slovenian type heart-hand syndrome, Hutchinson-Gilford progeria syndrome (HGPS), familial partial lipodystrophy type 2 (FPLD2), Malouf syndrome, congenital muscular dystrophy (MDC), or a combination thereof, or (ii) has or is suspected of having at least one of dilated cardiomyopathy-1A (CMD1A), autosomal dominant Emery-Dreifuss muscular dystrophy 2 (EDMD2), Slovenian type heart-hand syndrome, Hutchinson-Gilford progeria syndrome (HGPS), familial partial lipodystrophy type 2 (FPLD2), Malouf syndrome, congenital muscular dystrophy (MDC), or a combination thereof; or

the mutation is in transactive responsive deoxyribonucleic acid (DNA)-binding protein 43 (TARDBP), and the subject (i) has one or more symptoms of at least one of amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), Alzheimer's disease (AD), limbic predominant age-related TDP-43 encephalopathy (LATE), or a combination thereof, or (ii) has or is suspected of having at least one of amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), Alzheimer's disease (AD), limbic predominant age-related TDP-43 encephalopathy (LATE), or a combination thereof.

10. The method of claim 9, wherein:

the method is a method of treating (i) a subject having one or more symptoms of amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), or both amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), or (ii) a subject that has or is suspected of having amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), or both amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD);

the method is a method of treating (i) a subject having one or more symptoms of at least one of amyotrophic lateral sclerosis, neonatal intractable myoclonus (NEIMY), hereditary spastic paraplegia 10 (SPG10), or a combination thereof, or (ii) a subject that has or is suspected of having at least one of amyotrophic lateral sclerosis, neonatal intractable myoclonus (NEIMY), hereditary spastic paraplegia 10 (SPG10), or a combination thereof;

the method is a method of treating (i) a subject having one or more symptoms of at least one of dilated cardiomyopathy-1G (CMD1G), familial hypertrophic cardiomyopathy-9 (CMH9), myofibrillar myopathy-9 with early respiratory failure (MFM9), tibial muscular dystrophy (TMD), or a combination thereof, or (ii) a subject that has or is suspected of having at least one of dilated cardiomyopathy-1G (CMD1G), familial hypertrophic cardiomyopathy-9 (CMH9), myofibrillar myopathy-9 with early respiratory failure (MFM9), tibial muscular dystrophy (TMD), or a combination thereof;

the method is a method of treating (i) a subject having one or more symptoms of at least one of Charcot-Marie-Tooth (CMT) disease type 2A2A (CMT2A2A), Charcot-Marie-Tooth disease type 6A (CMT6A), or a combination thereof, or (ii) a subject that has or is suspected of having at least one of Charcot-Marie-Tooth (CMT) disease type 2A2A (CMT2A2A), Charcot-Marie-Tooth disease type 6A (CMT6A), or a combination thereof;

the method is a method of treating (i) a subject having one or more symptoms of at least one of dilated cardiomyopathy-1S (CMD1S), hypertrophic cardiomyopathy-1 (CMH1), autosomal dominant myosin storage congenital myopathy-7A (CMYP7A), Laing distal

myopathy, dilated cardiomyopathy-1S (CMD1S), or a combination thereof, or (ii) a subject that has or is suspected of having at least one of dilated cardiomyopathy-1S (CMD1S), hypertrophic cardiomyopathy-1 (CMH1), autosomal dominant myosin storage congenital myopathy-7A (CMYP7A), Laing distal myopathy, dilated cardiomyopathy-1S (CMD1S), or a combination thereof;

the method is a method of treating (i) a subject having one or more symptoms of at least one of dilated cardiomyopathy-1A (CMD1A), autosomal dominant Emery-Dreifuss muscular dystrophy 2 (EDMD2), Slovenian type heart-hand syndrome, Hutchinson-Gilford progeria syndrome (HGPS), familial partial lipodystrophy type 2 (FPLD2), Malouf syndrome, congenital muscular dystrophy (MDC), or a combination thereof, or (ii) a subject that has or is suspected of having at least one of dilated cardiomyopathy-1A (CMD1A), autosomal dominant Emery-Dreifuss muscular dystrophy 2 (EDMD2), Slovenian type heart-hand syndrome, Hutchinson-Gilford progeria syndrome (HGPS), familial partial lipodystrophy type 2 (FPLD2), Malouf syndrome, congenital muscular dystrophy (MDC), or a combination thereof; or

the method is a method of treating (i) a subject having one or more symptoms of at least one of amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), Alzheimer's disease (AD), limbic predominant age-related TDP-43 encephalopathy (LATE), or a combination thereof, or (ii) a subject that has or suspected of having at least one of amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), Alzheimer's disease (AD), limbic predominant age-related TDP-43 encephalopathy (LATE), or a combination thereof.

11. The method of any one of claims 8-10, wherein at least one of:

the antisense oligonucleotide targets an intronic region of the gene;

the antisense oligonucleotide sequence and/or a target variant allele sequence has a guanine-cytosine (GC) content of about 20% to about 80%

the antisense oligonucleotide is an oligonucleotide of 10 to 25 nucleotides in length that is complementary with at least 8 contiguous nucleotides of any one of SEQ ID NOs: 1, 2, 64-76, 84, 79, 85, 88, 89, 92, 93, 96, 97, 100, 101, 123, 124, or 125, rs11307260, rs142843265, rs3835416, rs34620383, rs2453565, rs700828, rs774356, rs774357, rs774359, rs2453554, rs2484319, rs2453555, rs2492816, rs3849945, or rs10757668;

the antisense oligonucleotide is an oligonucleotide having the sequence of SEQ ID NO: 80-83, 86, 87, 90, 91, 94, 95, 98, 99, 102, 103-122, or 126-152, or the antisense

oligonucleotide comprises, consists essentially of, or consists of any antisense oligonucleotide described herein;

the mutation is in an A5V mutation in superoxide dismutase 1 (SOD1);

the mutation is in a E134 deletion mutation in superoxide dismutase 1 (SOD1);

the mutation is a repeat expansion within intron 1 of chromosome 9 open reading frame 72 (C9orf72);

the mutation is a Q331 mutations (e.g., Q331K) in transactive responsive deoxyribonucleic acid (DNA)-binding protein 43 (TARDBP);

the mutation is a M337 mutations (e.g., M337V) in transactive responsive deoxyribonucleic acid (DNA)-binding protein 43 (TARDBP); or

a combination thereof.

12. The method of any one of claims 1-11, wherein at least one of:

the cell or subject is heterozygous for the mutation;

the target variant allele is a common variant;

the target variant allele is an indel (e.g., an insertion or deletion);

the mutation includes a repeat expansion, a single base pair point mutation, a structural variant, a copy number variant, an indel or a combination thereof; or

a combination thereof.

13. The method of any one of claims 1-12, further comprising at least one of:

determining or identifying the genotype of the target variant allele of the gene (e.g., by way of sequence and/or the method of haplotyping described herein);

determining or identifying whether the target variant allele is in cis with the mutation (e.g., by way of sequence and/or the method of haplotyping described herein); or

a combination thereof.

14. A method of determining the haplotype of two variants (e.g., a first or target variant and a second variant or a mutation) of a gene in a cell, the method comprising:

providing one or more (e.g., at least one, one, at least two, or two) antisense oligonucleotide (ASO), wherein each of the one or more (e.g., one) antisense oligonucleotide targets a different allele of a first or target variant allele (e.g., a common variant; an indel; and/or if an indel, a first antisense oligonucleotide targets the insertion of a first or target

variant allele and second antisense oligonucleotide targets the deletion of a first or target variant allele) of the gene;

administering each of the one or more antisense oligonucleotide (ASO) to the cell individually; and

measuring the expression level of one or more (e.g., at least one, one, at least two, or two) allele (e.g., allele-specific expression) for a second variant allele (e.g., a mutant allele) in each cell an antisense oligonucleotide (ASO) of the one or more antisense oligonucleotides (ASO) was administered.

15. The method of claim 14, wherein reduced expression for an allele of the second variant allele indicates that the cell has that second variant allele and the first or target variant allele that the antisense oligonucleotide targets.

16. The method of claim 14 or 15, wherein at least one of:  
reduced expression indicates that the second variant allele (e.g., a mutation or mutant allele) is in cis with the first or target variant allele that the antisense oligonucleotide targets;  
reduced expression for two alleles of the second variant allele indicates that the cell is heterozygous for the two alleles of the second variant with reduced expression and homozygous for the first or target variant allele that the antisense oligonucleotide targets; or  
a combination thereof.

17. The method of any one of claims 14-16, wherein at least one of:  
the first or target variant allele is a common variant;  
the first or target variant allele is an indel (e.g., an insertion or deletion);  
the second variant allele is a mutant allele that includes a repeat expansion, a single base pair point mutation, a structural variant, a copy number variant, an indel, or a combination thereof; or  
a combination thereof.

18. The method of any one of claims 1-17, wherein at least one of:  
the antisense oligonucleotide targets an intronic region of the gene;  
the antisense oligonucleotide sequence and/or a target variant allele sequence has a guanine-cytosine (GC) content of about 20% to about 80%;  
the antisense oligonucleotide is an oligonucleotide of 10 to 25 nucleotides in length;

the antisense oligonucleotide comprises at least one modified nucleotide;  
the antisense oligonucleotide comprises a phosphorothioate modified linkage;  
the antisense oligonucleotide is a morpholino;  
the antisense oligonucleotide comprises locked nucleic acids (LNAs) and ribonucleic acids (RNAs) (e.g., alternating locked nucleic acid (LNA) and ribonucleic acid (RNA) nucleotides);  
the antisense oligonucleotide comprises locked nucleic acids (LNAs) and deoxyribonucleic acids (DNAs) (e.g., alternating locked nucleic acid (LNA) and deoxyribonucleic acid (DNA) nucleotides);  
the antisense oligonucleotide comprises ribonucleic acids (RNAs) and deoxyribonucleic acids (DNAs) (e.g., alternating ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) nucleotides);  
the antisense oligonucleotide is a gapmer; or  
a combination thereof.

19. The method of claim 18, wherein the at least one modified nucleotide is a 2'-modified nucleotide (e.g., each 2'-modified nucleotide includes or is independently a 2'-deoxy modified nucleotide, 2'-fluoro modified nucleotide, 2'-O-methyl modified nucleotide, 2'-O-methoxyethyl modified nucleotide, 2'-amino modified nucleotide, or 2'-aminoalkoxy modified nucleotide).

20. The method of any one of claims 1-19, wherein the variant is present with at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, or at least 75% of the one or more gene mutations.

21. An antisense oligonucleotide (ASO) comprising a nucleic acid sequence that is 10 to 25 nucleotides in length and comprises a region of complementarity that is complementary with at least 8 contiguous nucleotides of a sequence of any one of SEQ ID NOs:1, 2, 64-76, 79, 84, 85, 88, 89, 92, 93, 96, 97, 100, 101, 123, 124, or 125, rs11307260, rs142843265, rs3835416, rs34620383, rs2453565, rs700828, rs774356, rs774357, rs774359, rs2453554, rs2484319, rs2453555, rs2492816, rs3849945, or rs10757668.

22. The antisense oligonucleotide of claim 21, wherein

the antisense oligonucleotide comprises, consists essentially of, or consists of, the nucleic acid sequence of any one of SEQ ID NOs: 80-83, 86, 87, 90, 91, 94, 95, 98, 99, 102, 103-122, or 126-152; or

the antisense oligonucleotide comprises, consists essentially of, or consists of any antisense oligonucleotide described herein.

23. The antisense oligonucleotide of claim 21 or 22, wherein at least one of:

the antisense oligonucleotide comprises at least one modified nucleotide;

the antisense oligonucleotide comprises at least one modified nucleotide that is a 2'-modified nucleotide (e.g., each 2'-modified nucleotide includes or is independently a 2'-deoxy modified nucleotide, 2'-fluoro modified nucleotide, 2'-O-methyl modified nucleotide, 2'-O-methoxyethyl modified nucleotide, 2'-amino modified nucleotide, or 2'-aminoalkoxy modified nucleotide);

the antisense oligonucleotide comprises a phosphorothioate modified linkage;

the antisense oligonucleotide is a morpholino;

the antisense oligonucleotide comprises locked nucleic acids (LNAs) and ribonucleic acids (RNAs) (e.g., alternating locked nucleic acid (LNA) and ribonucleic acid (RNA) nucleotides);

the antisense oligonucleotide comprises locked nucleic acids (LNAs) and deoxyribonucleic acids (DNAs) (e.g., alternating locked nucleic acid (LNA) and deoxyribonucleic acid (DNA) nucleotides);

the antisense oligonucleotide comprises ribonucleic acids (RNAs) and deoxyribonucleic acids (DNAs) (e.g., alternating ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) nucleotides);

the antisense oligonucleotide is a gapmer; or

a combination thereof.

24. A pharmaceutical composition comprising the antisense oligonucleotide of any of one of claims 21-23, and a pharmaceutically-acceptable carrier.