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ANTISENSE OLIGONUCLEOTIDE TARGETING APOE ϵ 4 AND USES THEREOF

RELATED APPLICATIONS

[0001] This application claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Application, U.S.S.N. 63/228,555, filed August 2, 2021, the entire contents of which are incorporated herein by reference.

FEDERALLY SPONSORED RESEARCH

[0001] This invention was made with government support under grant number DA048787, awarded by the National Institutes of Health. The government has certain rights in the invention.

REFERENCE TO AN ELECTRONIC SEQUENCE LISTING

[0002] The contents of the electronic sequence listing (H082470390WO00-SEQ-LJG.xml; Size: 109,330 bytes; and Date of Creation: August 1, 2022) is herein incorporated by reference in its entirety.

BACKGROUND

[0003] Alzheimer's disease (AD) is the leading cause of dementia and the sixth leading cause of death in the United States. Currently, 5.8 million Americans over 65 years of age live with Alzheimer's disease. By 2050, AD is projected to affect 13.8 million Americans with a cost of \$1.1 trillion to the United States economy. There is currently no cure for AD, nor treatment that slows disease progression.

[0004] Alzheimer's disease is a complex dementia developing as a result of multiple genetic and environmental factors, and genome-wide association studies have identified the ϵ 4 allele of the apolipoprotein E (ApoE) gene as the major genetic risk factor for late-onset AD. In the central nervous system, the ApoE protein is highly expressed in nonneuronal cells, mainly astrocytes, and is the predominant apolipoprotein of high-density lipoproteins (HDL), thus playing a critical role in cholesterol metabolism. ApoE has three relatively common allelic variants (ϵ 2, ϵ 3, ϵ 4) defined by two single-nucleotide polymorphisms (SNPs) at amino acids 112 and 158 in the mature protein. Inheriting one copy of ϵ 4 increases the risk of developing AD three-fold, while inheriting two copies of ϵ 4 increases the risk of developing AD between

8- to 12-fold as compared to inheriting two copies of the neutral $\epsilon 3$ variant. ApoE $\epsilon 4$ not only increases the risk of developing AD but is also associated with an earlier age of AD onset. While the frequency of ApoE alleles varies among racial and ethnic groups, between 26.3-38.8% of Americans carry at least one copy of $\epsilon 4$. This percentage rises to 65% of individuals diagnosed with AD, illustrating the causative role of $\epsilon 4$ in AD.

[0005] Despite being a known risk factor for AD for over 30 years, there are no effective therapeutic agents targeting ApoE $\epsilon 4$. ApoE $\epsilon 4$ has only recently begun to emerge as a promising therapeutic target, with no active clinical trials currently targeting ApoE, and $\epsilon 4$ remains understudied. Limited therapeutic research targeting ApoE to slow AD pathogenesis has used animal models homozygous for $\epsilon 4$, although less than 5% of individuals are homozygous $\epsilon 4/\epsilon 4$. In contrast, between 25-30% of individuals are heterozygous for $\epsilon 4$ (either $\epsilon 2/\epsilon 4$ or $\epsilon 3/\epsilon 4$). Importantly, heterozygous carriers of $\epsilon 4$ account for the majority of individuals diagnosed with AD (56%), while homozygous carriers of $\epsilon 4$ account for merely 11% of those individuals. Thus, a therapeutic specifically targeting $\epsilon 4$ in heterozygous carriers is both massively understudied and holds great therapeutic potential to a large majority of individuals diagnosed with AD. The present disclosure describes a therapeutic for AD through the selective targeting and degradation of ApoE $\epsilon 4$ mRNA mediated by allele specific antisense oligonucleotide (ASO) technology in individuals carrying at least one ApoE $\epsilon 4$ allele. In heterozygous individuals, this therapy shifts the balance of ApoE expression towards the more protective ($\epsilon 2$) or neutral ($\epsilon 3$) allele, thus reducing the risk of developing AD.

SUMMARY

[0006] In some embodiments, the disclosure provides inhibitory nucleic acids (*e.g.*, ASOs, siRNAs, shRNAs, miRNAs, mixmers, aptamers) complementary with ApoE $\epsilon 4$ RNA that are useful for reducing levels of ApoE $\epsilon 4$. ApoE $\epsilon 4$ is associated with a higher risk of developing cognitive and neurological diseases such as stroke, Parkinson's disease, Lou Gehrig's disease, Charcot-Marie-Tooth disease, multiple sclerosis, diabetic neuropathy, sleep apnea, Lewy body disease, ischemia of the central nervous system, Alzheimer's disease, and poor prognosis following head trauma. Particularly, ApoE $\epsilon 4$ is associated with a higher risk of developing Alzheimer's disease (AD), *e.g.*, in a subject having or suspected of having AD. In some embodiments, the inhibitory nucleic acids (*e.g.*, ASOs, siRNAs, shRNAs, miRNAs, mixmers, aptamers) are designed to direct RNase H mediated

degradation of the target ApoE ϵ 4 RNA. In some embodiments, the inhibitory nucleic acids (*e.g.*, ASOs, siRNAs, shRNAs, miRNAs, mixmers, aptamers) are designed to have specific binding affinity properties to ApoE ϵ 4 RNA (*e.g.*, specifically binds to ApoE ϵ 4 RNA as compared to ApoE ϵ 3 or ApoE ϵ 2 RNA) or to a specific region of the ApoE ϵ 4 RNA (for example a region of exon 4 of the ApoE ϵ 4 RNA).

[0007] The present disclosure, at least in part, relates to inhibitory nucleic acids (*e.g.*, antisense oligonucleotides (ASOs), siRNAs, shRNAs, miRNAs, mixmers, aptamers), for inhibiting expression or activity of an ApoE ϵ 4 allele that includes a cytidine at rs429358, wherein the inhibitory nucleic acid comprises a region of complementarity at least 90% complementary to at least 5, at least 6, 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, at least 16, at least 17, at least 18, at least 19, or at least 20 consecutive nucleotides of an ApoE ϵ 4 mRNA. In some embodiments, the inhibitory nucleic acid comprises a region of complementarity at least 90% complementary to a region of exon 4 of an ApoE ϵ 4 mRNA.

[0008] In some embodiments, the ApoE ϵ 4 is human ApoE ϵ 4. In some embodiments, the inhibitory nucleic acids comprise a region of complementarity to at least 5, at least 6, 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, at least 16, at least 17, at least 18, at least 19, or at least 20 consecutive nucleotides of SEQ ID NO: 1. In some embodiments, the inhibitory nucleic acids comprise a region of complementarity to at least 5, at least 6, 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, at least 16, at least 17, at least 18, at least 19, or at least 20 consecutive nucleotides of SEQ ID NO: 2.

[0009] In some embodiments, the inhibitory nucleic acid (*e.g.*, ASOs, siRNAs, shRNAs, miRNAs, mixmers, aptamers) is an RNA, a DNA or a hybrid nucleic acid. In some embodiments, the inhibitory nucleic acid is single-stranded, double-stranded, or comprises both single-stranded and double-stranded regions.

[0010] In some embodiments, the inhibitory nucleic acid is an antisense oligonucleotide. In some embodiments, the antisense oligonucleotide is capable of promoting RNase H mediated RNA degradation of the ApoE ϵ 4 mRNA.

[0011] In some embodiments, the antisense oligonucleotide is a gapmer comprising a 5'-X-Y-Z-3' configuration, wherein X comprises 0 to 7 linked nucleotides; Y comprises 5 to 12 linked 2'-deoxyribonucleotides, and Z comprises 2 to 7 linked nucleotides. In some embodiments, one of the nucleotides in X is a 2'-modified nucleotide. In some embodiments,

more than one nucleotide in X is a 2'-modified nucleotide. In some embodiments, each nucleotide in X is a 2'-modified nucleotide. In some embodiments, at least one of the nucleotides in Z is a 2'-modified nucleotide. In some embodiments, each nucleotide in Z is a 2'-modified nucleotide. In some embodiments, one or more of the internucleotide linkages in the X, Y or Z region is a modified internucleotide linkage. In some embodiments, every internucleotide linkage in X is a modified internucleotide linkage. In some embodiments, every internucleotide linkage in Y is a modified internucleotide linkage. In some embodiments, every internucleotide linkage in Z is a modified internucleotide linkage. In some embodiments, every internucleotide linkage in each of the X, Y and Z regions is a modified internucleotide linkage. In some embodiments, one or more of the internucleotide linkages in the X, Y or Z region is a phosphorothioate linkage. In some embodiments, every internucleotide linkage in X is a phosphorothioate linkage. In some embodiments, every internucleotide linkage in Y is a phosphorothioate linkage. In some embodiments, every internucleotide linkage in Z is a phosphorothioate linkage. In some embodiments, every internucleotide linkage in each of the X, Y and Z regions is a phosphorothioate linkage.

[0012] In some embodiments, the 2'-modified nucleotide is a non-bicyclic 2'-modified nucleotide. In some embodiments, the 2'-modified nucleotide is a 2'-O-methoxyethyl (2'-MOE) modified nucleotide, a 2'-O-methyl (2'-O-Me) modified nucleotide, a 2'-fluoro (2'-F) modified nucleotide, a 2'-O-aminopropyl (2'-O-AP) modified nucleotide, a 2'-O-dimethylaminoethyl (2'-O-DMAOE) modified nucleotide, a 2'-O-dimethylaminopropyl (2'-O-DMAP) modified nucleotide, a 2'-O-dimethylaminoethoxyethyl (2'-O-DMAEOE) modified nucleotide, or a 2'-O-N-methylacetamido (2'-O-NMA) modified nucleotide. In some embodiments, the 2'-modified nucleotide is a 2'-O-methoxyethyl (2'-MOE) modified nucleotide. In some embodiments, the 2'-modified nucleotide is a 2'-4' bicyclic nucleotide. In some embodiments, the 2'-modified nucleotide is a Locked Nucleic Acid (LNA), a constrained ethyl (cEt) nucleotide, or an Ethylene-bridged Nucleic Acid (ENA). In some embodiments, the antisense oligonucleotide comprises a nucleotide sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to any one of SEQ ID NOs: 3-12, 20-29, or 42-45.

[0013] In some embodiments, the antisense oligonucleotide comprises a 5'-X-Y-Z-3' configuration of:

X	Y	Z
EEEEE	(D) ₇	EEEEE

EEEE (D)₉ EEEE

wherein “E” is a 2′-MOE modified ribonucleoside; and “D” is 2′-deoxyribonucleoside.

[0014] In some embodiments, the antisense oligonucleotide comprises one or more phosphorothioate internucleotide linkages, one or more methylphosphonate internucleotide linkages, one or more phosphorodithioate internucleotide linkages, one or more boron phosphonate internucleotide linkages, one or more phosphonocarboxylate internucleotide linkages, and/or one or more phosphonoacetate internucleotide linkages. In some embodiments, each internucleotide linkage in the antisense oligonucleotide is a phosphorothioate internucleotide linkage, a methylphosphonate internucleotide linkage, a phosphorodithioate internucleotide linkage, a boron phosphonate internucleotide linkage, a phosphonocarboxylate internucleotide linkage, and/or a phosphonoacetate internucleotide linkage. In some embodiments, the ends of the antisense oligonucleotide are modified to make them resistant to RNase H degradation. In some embodiments, the ends of the antisense oligonucleotide comprise 2′-MOE modified ribonucleosides.

[0015] In some embodiments, the antisense oligonucleotide is selected from the following sequences:

5′ – oG*oC*oC*oG*oC*dG*dC*dA*dC*dG*dT*dC*oC*oT*oC*oC*oA – 3′ (SEQ ID NO: 20);
 5′ – oG*oG*oC*oC*oG*dC*dG*dC*dA*dC*dG*dT*oC*oC*oT*oC*oC – 3′ (SEQ ID NO: 21);
 5′ – oC*oG*oG*oC*oC*dG*dC*dG*dC*dA*dC*dG*oT*oC*oC*oT*oC – 3′ (SEQ ID NO: 22);
 5′ – oG*oC*oG*oG*oC*dC*dG*dC*dG*dC*dA*dC*oG*oT*oC*oC*oT – 3′ (SEQ ID NO: 23);
 5′ – oG*oG*oC*oG*oG*dC*dC*dG*dC*dG*dC*dA*oC*oG*oT*oC*oC – 3′ (SEQ ID NO: 24);
 5′ – oA*oG*oG*oC*oG*dG*dC*dC*dG*dC*dG*dC*oA*oC*oG*oT*oC – 3′ (SEQ ID NO: 25);
 5′ – oG*oC*oC*oG*oC*dG*dC*dA*dC*dG*dT*dC*dC*dT*oC*oC*oA*oT*oG – 3′ (SEQ ID NO: 26);

5′ – oG*oG*oC*oC*oG*dC*dG*dC*dA*dC*dG*dT*dC*dC*oT*oC*oC*oA*oT – 3′ (SEQ ID NO: 27);

5′ – oC*oG*oG*oC*oC*dG*dC*dG*dC*dA*dC*dG*dT*dC*oC*oT*oC*oC*oA – 3′ (SEQ ID NO: 28);

5′ – oG*oC*oG*oG*oC*dC*dG*dC*dG*dC*dA*dC*dG*dT*oC*oC*oT*oC*oC – 3′ (SEQ ID NO: 29);

5′ – oA* oG* oG* oC* oG* dG* dC* dC* dG* dC* dG* dC* dA* dC* oG* oT* oC* oC* oT* – 3′ (SEQ ID NO: 44); and

5′ – oG* oG* oC* oG* oG* dC* dC* dG* dC* dG* dC* dA* dC* dG* oT* oC* oC* oT* oC* – 3′ (SEQ ID NO: 45),

wherein “oN” is 2'- MOE modified ribonucleoside; “dN” is 2'-deoxyribonucleoside; and “*” indicates phosphorothioate internucleotide linkage.

[0016] In some embodiments, the inhibitory nucleic acid is capable of inhibiting the expression and/or activity of ApoE ε4 by steric hindrance, inhibiting mRNA maturation, destabilizing pre-mRNA or RNase H mediated degradation.

[0017] In some embodiments, the inhibitory nucleic acid is an siRNA. In some embodiments, the siRNA comprises an antisense strand that comprises a region of complementarity of at least 3, 4, 5, 6, 7, 8, 9, or 10 consecutive nucleotides of SEQ ID NO: 1 or SEQ ID NO: 2. In some embodiments, the antisense strand comprises at least 15 consecutive nucleotides of any one of SEQ ID NOs: 46-57, wherein each T is optionally U. In some embodiments, the antisense strand consists of any one of SEQ ID NOs: 46-57, wherein each T is optionally U. In some embodiments, the siRNA further comprises a sense strand that hybridizes to the antisense strand. In some embodiments, each nucleotide of the sense strand hybridizes (i.e. is complementary) to a nucleotide of the antisense strand. In some embodiments, at least 80%, at least 85%, at least 90% or at least 95% of the nucleotides of the sense strand hybridize (i.e. are complementary) to nucleotides of the antisense strand. In some embodiments, the sense strand and the antisense strand are connected by a loop.

[0018] In some embodiments, the inhibitory nucleic acid is an shRNA. In some embodiments, the shRNA comprises an antisense portion that comprises a region of complementarity of at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 consecutive nucleotides of SEQ ID NO: 1 or SEQ ID NO: 2. In some embodiments, the antisense strand comprises at least 15 consecutive nucleotides of any one of SEQ ID NOs: 46-57. In some embodiments, the antisense strand consists of any one of SEQ ID NOs: 46-57.

[0019] In some embodiments, the shRNA further comprises a sense portion that hybridizes to the antisense portion. In some embodiments, the sense portion of the antisense portion of the shRNA is connected by a single-stranded nucleic acid hairpin. In some embodiments, the hairpin is at least 3, at least 4, at least 5, at least 10 or at least 15 nucleotides long.

[0020] In some embodiments, the inhibitory nucleic acid is a miRNA.

[0021] In some embodiments, the inhibitory nucleic acid is a mixmer.

[0022] In some embodiments, the inhibitory nucleic acid is an aptamer.

[0023] In some aspects, the present disclosure also provides a pharmaceutical composition comprising any of the inhibitory nucleic acids described herein, and a pharmaceutically acceptable excipient.

[0024] In some aspects, the present disclosure also provides a method of inhibiting the expression or activity of ApoE ϵ 4 in a cell, the method comprising contacting the cell with the inhibitory nucleic acid as described herein in an amount effective for promoting internalization of the inhibitory nucleic acid into the cell.

[0025] In some aspects, the present disclosure also provides a method of inhibiting the expression or activity of ApoE ϵ 4 in a subject, the method comprising administering to the subject an inhibitory nucleic acid or a pharmaceutical composition as described herein in an amount effective for promoting internalization of the inhibitory nucleic acid into the subject, or into a cell of a subject. In some embodiments, the inhibitory nucleic acid promotes RNase H mediated degradation of ApoE ϵ 4.

[0026] In some aspects, the present disclosure also provides a method of preventing, treating, slowing the progression and/or reversing Alzheimer's disease in a subject in need thereof, the method comprising administering to the subject an effective amount of an inhibitory nucleic acid or a pharmaceutical composition described herein.

[0027] In some aspects, the present disclosure provides inhibitory nucleic acids and pharmaceutical compositions thereof for use in preventing, treating, slowing the progression and/or reversing Alzheimer's disease in a subject.

[0028] In some embodiments, the subject is at risk of developing Alzheimer's disease. In some embodiments, the subject is a heterozygous for ApoE ϵ 4 allele. In some embodiments, the subject is ApoE ϵ 2/ ϵ 4 heterozygous. In some embodiments, the subject is ApoE ϵ 3/ ϵ 4 heterozygous. In some embodiments, the subject is a homozygous for ApoE ϵ 4 allele. In some embodiments the subject has been screened for Alzheimer's disease using a computerized cognitive testing device. In some embodiments, the cognitive testing device is the Cantab Mobile, the Cognigram, the Cognivue, the Cognision and the Automated Neuropsychological Assessment Metrics (ANAM) device. In some embodiments, the subject has been genetically screened for the presence of a gene known to cause autosomal dominant Alzheimer's disease (ADAD) or "familial Alzheimer's". In some embodiments, the subject has been screened for a biomarker that indicates (or detects) amyloid beta plaque formation.

[0029] In some embodiments, the subject is a human. In some embodiments, the subject is a non-human mammal.

[0030] In some embodiments, the administration is systemic administration. In some embodiments, the systemic administration comprises intravenous administration, intramuscular administration, intraperitoneal administration, or subcutaneous administration.

[0031] In some embodiments, the administration is direct administration to the central nervous system (CNS). In some embodiments, the direct administration to the CNS comprises intracerebral injection, intraparenchymal injection, intrathecal injection, or intracerebroventricular administration.

[0032] In some aspects, the present disclosure also provides a kit comprising an antisense oligonucleotide which comprises the inhibitory nucleic acid, or the pharmaceutical composition described herein.

[0033] The details of one or more embodiments of the invention are set forth in the description below. Other features or advantages of the present invention will be apparent from the following drawing and detailed description of certain embodiments and also from the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0034] FIG. 1 shows schematic targeting of antisense oligonucleotides (ASOs) to APOE ϵ 4. This schematic illustrates the binding of ASOs to exon 4 of ApoE ϵ 4 RNA. Single-nucleotide polymorphism (SNP) rs429358 is illustrated by the triangle.

[0035] FIGs. 2A-2B show the ApoE ϵ 3 or ApoE ϵ 4 reporter plasmids and the experimental design to test the efficacy of the ASOs *in vitro*. FIG. 2A shows the map of the plasmids that express either ApoE ϵ 3 or ApoE ϵ 4 cDNA and firefly luciferase fused by a P2A self-cleaving peptide sequence. FIG. 2B shows the experimental steps of testing the ability of ASOs in knocking down ApoE ϵ 3 or ApoE ϵ 4.

[0036] FIGs. 3A-3K show the results of the ASOs in knocking down ApoE ϵ 3 or ApoE ϵ 4 using the reporter plasmids.

[0037] The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate certain embodiments, and together with the written description, serve to provide non-limiting examples of certain aspects of the compositions and methods disclosed herein.

DETAILED DESCRIPTION

[0038] Some aspects of the disclosure provide inhibitory nucleic acids (*e.g.*, ASOs, siRNAs, shRNAs, miRNAs, mixmers, aptamers) designed to target Apolipoprotein E (ApoE) ϵ 4 RNA to modulate the expression or the activity of ApoE ϵ 4. In some embodiments, the inhibitory nucleic acid (*e.g.*, ASOs, siRNAs, shRNAs, miRNAs, mixmers, aptamers) is an

RNA, a DNA or a hybrid nucleic acid. In some embodiments, the inhibitory nucleic acid is single-stranded, double-stranded, or comprises both single-stranded and double-stranded regions.

[0039] In some embodiments, the disclosure provides inhibitory nucleic acids (*e.g.*, ASOs, siRNAs, shRNAs, miRNAs, mixmers, aptamers) complementary with ApoE ϵ 4 RNA that are useful for reducing levels of ApoE ϵ 4. ApoE ϵ 4 is associated with a higher risk of developing cognitive and neurological diseases such as stroke, Parkinson's disease, Lou Gehrig's disease, Charcot–Marie–Tooth disease, multiple sclerosis, diabetic neuropathy, sleep apnea, Lewy body disease, ischemia of the central nervous system, Alzheimer's disease, and poor prognosis following head trauma. Particularly, ApoE ϵ 4 is associated with a higher risk of developing Alzheimer's disease (AD), *e.g.*, in a subject having or suspected of having AD. In some embodiments, the inhibitory nucleic acids (*e.g.*, ASOs, siRNAs, shRNAs, miRNAs, mixmers, aptamers) are designed to direct RNase H mediated degradation of the target ApoE ϵ 4 RNA. In some embodiments, the inhibitory nucleic acids (*e.g.*, ASOs, siRNAs, shRNAs, miRNAs, mixmers, aptamers) are designed to have specific binding affinity properties to ApoE ϵ 4 RNA (*e.g.*, specifically binds to ApoE ϵ 4 RNA as compared to ApoE ϵ 3 or ApoE ϵ 2 RNA) or to a specific region of the ApoE ϵ 4 RNA (for example a region of exon 4 of the ApoE ϵ 4 RNA).

I. Inhibitory Nucleic Acids Targeting ApoE ϵ 4

[0040] A “nucleic acid” sequence refers to a DNA or RNA sequence. An inhibitory nucleic acid, as used herein, refers to nucleic acids capable of inhibiting expression or activity of a target gene (*e.g.*, DNA, RNA, or protein of the target gene), for example ApoE ϵ 4. Non-limiting examples of inhibitory nucleic acids include *e.g.*, dsRNA, siRNA, shRNA, miRNA, amiRNA, antisense oligonucleotides (ASOs), DNA or RNA aptamers, *etc.* An ASO is a small chain of nucleotides, generally 18-30 nucleotides long, that targets messenger RNA (mRNA) and is capable of altering mRNA expression through a variety of mechanisms, including ribonuclease H mediated decay of the pre-mRNA, direct steric blockage, and exon content modulation through splicing site binding on pre-mRNA. A small interfering RNA (siRNA), also known as short interfering RNA or silencing RNA, is a double-stranded non-coding RNA molecules, typically 20-27 base pairs in length, that interferes with the expression of specific genes with complementary nucleotide sequences by degrading mRNA after transcription, preventing translation. A short hairpin RNA or small hairpin RNA (shRNA) is

an artificial RNA molecules with a tight hairpin turn that can be used to silence target gene expression via RNA interference (RNAi). A microRNA (abbreviated miRNA) is a small single-stranded non-coding RNA molecule (containing about 22 nucleotides) that functions in RNA silencing and post-transcriptional regulation of gene expression via base-pairing with complementary sequences within mRNA molecules. An amiRNA is an artificial miRNA. A mixmer is an oligomer consisting of alternating short stretches of LNA and DNA. An LNA, or Locked Nucleic Acid, also known as bridged nucleic acid (BNA), or inaccessible RNA, is a modified RNA nucleotide in which the ribose moiety is modified with an extra bridge connecting the 2' oxygen and 4' carbon.

[0041] Some aspects of the disclosure provide inhibitory nucleic acids (*e.g.*, ASOs, siRNAs, shRNAs, miRNAs, mixmers, aptamers) designed to target Apolipoprotein E (ApoE) ϵ 4 RNA to modulate the expression or the activity of ApoE ϵ 4. In some embodiments, the disclosure provides inhibitory nucleic acids (*e.g.*, ASOs, siRNAs, shRNAs, miRNAs, mixmers, aptamers) complementary to ApoE ϵ 4 RNA that are useful for reducing RNA or protein expression levels of ApoE ϵ 4, which is known to be associated with a higher risk of developing Alzheimer's disease (AD), *e.g.*, in a subject having or suspected of having AD. In some embodiments, the inhibitory nucleic acids (*e.g.*, ASOs, siRNAs, shRNAs, miRNAs, mixmers, aptamers) are designed to promote RNase H mediated degradation of the target ApoE ϵ 4 RNA. In some embodiments, the inhibitory nucleic acids (*e.g.*, ASOs, siRNAs, shRNAs, miRNAs, mixmers, aptamers) are designed to have specific binding affinity properties to ApoE ϵ 4 RNA (*e.g.*, specifically binds to ApoE ϵ 4 RNA as compared to ApoE ϵ 3 or ApoE ϵ 2 RNA) or to a specific region of the ApoE ϵ 4 RNA (for example a region of exon 4 of the ApoE ϵ 4 RNA). In some embodiments, the specific binding affinity is enabled by complementarity between the inhibitory nucleic acid and the target region of ApoE ϵ 4 RNA, wherein the inhibitory nucleic acid has at least 80%, at least 85% or at least 90% complementarity with the target region of ApoE ϵ 4 RNA.

[0042] In some embodiments, such inhibitory nucleic acids are capable of targeting ApoE ϵ 4 gene in a cell of the central nervous system (CNS), *e.g.*, via specifically binding to ApoE ϵ 4 RNA in the neurons following delivery to the CNS. In some embodiments, the inhibitory nucleic acid specifically targets the ApoE ϵ 4 gene in a cell. In some embodiments, the inhibitory nucleic acid comprises a region of complementarity to a ApoE ϵ 4 allele. Exemplary inhibitory nucleic acids targeting the ApoE ϵ 4 RNA are described in further detail

herein, however, it should be appreciated that the exemplary inhibitory nucleic acids provided herein are not meant to be limiting.

[0043] The apolipoprotein E (APOE) gene is involved in the development of AD (*e.g.*, late-onset AD). In humans, APOE gene is located on chromosome 19. ApoE protein has several isoforms, including ApoE ϵ 2, which is protective against AD; ApoE ϵ 3, which is neutral to the development of AD; and ApoE ϵ 4, which is associated with increased risk for developing late-onset AD. Homozygous patients who carry two copies of ApoE ϵ 4 (*e.g.*, subjects that are APOE ϵ 4^{+/+}) are at an even greater risk of developing late-onset AD as compared to heterozygous patients who carry only one copy of ApoE ϵ 4 and one copy of either ApoE ϵ 2 or ApoE ϵ 3. The ApoE isoforms are defined by two single nucleotide polymorphisms (SNPS) at amino acids 112 and 158 in the mature protein (or at amino acid 130 and amino acid 176 when the signal peptide is included). ApoE ϵ 2 contains Cys¹¹²/Cys¹⁵⁸ and has been observed to be associated with type III hyperlipoproteinemia and other diseases but also plays a neuroprotective role. ApoE ϵ 3 contains Cys¹¹²/Arg¹⁵⁸ and is the most common ApoE allele. ApoE ϵ 4 contains Arg¹¹²/Arg¹⁵⁸ and has been observed to be associated with late-onset Alzheimer's disease, atherosclerosis, unfavorable outcomes in traumatic brain injury (TBI), and other diseases. ApoE ϵ 4 differs from both ApoE ϵ 2 and ApoE ϵ 3 by a T to C SNP at rs429358 (in ApoE ϵ 2 and ApoE ϵ 3 mRNA, the T is a U) , resulting in a single amino acid change (Cys¹¹² to Arg¹¹²).

[0044] In some embodiments, human ApoE ϵ 4 mRNA with SNP rs429358 (in bold face and underlined) comprises a nucleotide sequence set forth in SEQ ID NO: 13.

AUGAGCUCAGGGGCCUCUAGAAAGAGCUGGGACCCUGGGAACCCUGGCCUCCAGACUGGCC
AAUCACAGGCAGGAAGAUGAAGGUUCUGUGGGCUGCGUUGCUGGUCACAUUCCUGGCAGGAU
GCCAGGCCAAGGUGGAGCAAGCGGUGGAGACAGAGCCGGAGCCGAGCUGCGCCAGCAGACC
GAGUGGCAGAGCGGCCAGCGCUGGGAAACUGGCACUGGGUCGCUUUUGGGAUUACCUGCGCUG
GGUGCAGACACUGUCUGAGCAGGUGCAGGAGGAGCUGCUCAGCUGCCAGGUCACCCAGGAAC
UGAGGGCGCUGAUGGACGAGACCAUGAAGGAGUUGAAGGCCUACAAAUCGGAACUGGAGGAA
CAACUGACCCCGUGGCGGAGGAGACGCGGGCACGGCUGUCCAAGGAGCUGCAGGCGGCGCA
GGCCCGGCUGGGCGCGGACAUGGAGGACGUGCGCGGCCGCCUGGUGCAGUACCGCGGCGAGG
UGCAGGCCAUGCUCGGCCAGAGCACCGAGGAGCUGCGGGUGCGCCUCGCCUCCACCUGCGC
AAGCUGCGUAAGCGGCUCUCCGCGAUGCCGAUGACCUGCAGAAGCGCCUGGCAGUGUACCA
GGCCGGGGCCCCGCGAGGGCGCCGAGCGCGGCCUCAGCGCCAUCCGCGAGCGCCUGGGGCCCC
UGGUGGAACAGGGCGCGUGCGGGCCGCCACUGUGGGCUGCCUGGCGGCCAGCCGCUACAG
GAGCGGGCCCAGGCCUGGGGCGAGCGGCUGCGCGCGCGGAUGGAGGAGAUGGGCAGCCGGAC
C (SEQ ID NO: 13)

[0045] In some embodiment, human ApoE ϵ 3 mRNA with SNP rs429358 (in bold face and underlined) comprises a nucleotide sequence set forth in SEQ ID NO: 14.

AUGAGCUCAGGGGCCUCUAGAAAGAGCUGGGACCCUGGGAACCCUGGCCUCCAGACUGGCC
 AAUCACAGGCAGGAAGAUGAAGGUUCUGUGGGCUGCGUUGCUGGUCACAUUCCUGGCAGGAU
 GCCAGGCCAAGGUGGAGCAAGCGGUGGAGACAGAGCCGGAGCCGAGCUGCGCCAGCAGACC
 GAGUGGCAGAGCGGCCAGCGCUGGGAACUGGCACUGGGUCGCUUUUGGGAUUACCUGCGCUG
 GGUGCAGACACUGUCUGAGCAGGUGCAGGAGGAGCUGCUCAGCUCACCCAGGUCACCCAGGAAC
 UGAGGGCGCUGAUGGACGAGACCAUGAAGGAGUUGAAGGCCUACAAAUCGGAACUGGAGGAA
 CAACUGACCCCGGUGGCGGAGGAGACGCGGGCACGGCUGUCCAAGGAGCUGCAGGCGGCGCA
 GGCCCGGCUGGGCGCGGACAUGGAGGACGUGGCGGGCCCGCCUGGUGCAGUACCGCGGCGAGG
 UGCAGGCCAUGCUCGGCCAGAGCACCGAGGAGCUGCGGGUGCGCCUCGCCUCCACCUGCGC
 AAGCUGCGUAAGCGGCUCCUCCGCGAUGCCGAUGACCUGCAGAAGCGCCUGGCAGUGUACCA
 GGCCGGGGCCCGCGAGGGCGCCGAGCGCGGCCUCAGCGCCAUCCGCGAGCGCCUGGGGCCCC
 UGGUGGAACAGGGCCGCGUGCGGGCCGCCACUGUGGGCUCUCCUGGCCGGCCAGCCGCUACAG
 GAGCGGGCCCAGGCCUGGGGCGAGCGGCUGCGCGCGCGGAUGGAGGAGAUGGGCAGCCGGAC
 C (SEQ ID NO: 14)

[0046] In some embodiments, an inhibitory nucleic acid specifically targets (e.g., comprises a region that has at least 80%, at least 85% or at least 90% complementarity with) one ApoE isoform RNA (e.g., ApoE ϵ 4 RNA). In some embodiments, an inhibitory nucleic acid specifically targets (e.g., comprises a region that has at least 80%, at least 85% or at least 90% complementarity with) the SNP rs429358 of ApoE ϵ 4 RNA. In some embodiments, an inhibitory nucleic acid specifically targets (e.g., comprises a region of complementarity to) the ApoE ϵ 4 allele that comprises a cytidine at SNP rs429358. In some embodiments, an inhibitory nucleic acid targets (e.g., comprises a region that has at least 80%, at least 85% or at least 90% complementarity with) a nucleotide sequence at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 1 or SEQ ID NO: 2, wherein the target nucleotide sequence is 10 to 200 nucleotides long.

[0047] Exemplary ApoE target RNA sequences are set forth in SEQ ID NOs: 1 or 2:

GGCGCGGACAUGGAGGACGUGCGCGGCCGCCUGGUGCA (SEQ ID NO: 1)

GGCGCGGACAUGGAGGACGUCCGCGGCCGCCUGGUGCA (SEQ ID NO: 2)

[0048] In some embodiments, an inhibitory nucleic acid comprises a region of complementarity to at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18 at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, or more consecutive nucleotides to an ApoE ϵ 4 mRNA. In some embodiments, an inhibitory nucleic acid comprises a region of complementarity to at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18 at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, or more consecutive nucleotides to an ApoE ϵ 4 allele that comprises a cytidine at

SNP rs429358. ApoE ϵ 4 mRNA. In some embodiments, an inhibitory nucleic acid comprises a region of complementarity to at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18 at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, or more consecutive nucleotides to the nucleotide sequence of SEQ ID NO: 1. In some embodiments, an inhibitory nucleic acid comprises a region of complementarity to at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18 at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, or more consecutive nucleotides to the nucleotide sequence of SEQ ID NO: 2. In some embodiments, an inhibitory nucleic acid comprises a region of complementarity to at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18 at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, or more consecutive nucleotides, except for at least 1, at least 2, at least 3, at least 4, or at least 5 mismatch. In some embodiments, an inhibitory nucleic acid comprises a region of complementarity to at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18 at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, or more consecutive nucleotides, except for at least 1, at least 2, at least 3, at least 4, or at least 5 mismatch within the region of complementarity to the nucleotide sequence of SEQ ID NO: 2. As used herein, the term “region of complementarity” refers to a nucleotide sequence, *e.g.*, of an oligonucleotide, that is sufficiently complementary to a cognate nucleotide sequence, *e.g.*, of ApoE ϵ 4 mRNA, such that the two nucleotide sequences are capable of annealing to one another under physiological conditions (*e.g.*, in a cell). In some embodiments, a region of complementarity is fully complementary to a cognate nucleotide sequence of ApoE ϵ 4 mRNA. However, in some embodiments, a region of complementarity is partially complementary to a cognate nucleotide sequence of the ApoE ϵ 4 mRNA (*e.g.*, at least 80%, 90%, 95% or 99% complementarity). In some embodiments, a region of complementarity contains 1, 2, 3, or 4 mismatches compared with a cognate nucleotide sequence of ApoE ϵ 4 mRNA. Complementary, as used herein, refers to the capacity for precise pairing between two nucleotide sequences or two sets of nucleotide sequences. In particular, complementarity is a term that characterizes an extent of hydrogen bond pairing that brings about binding between two nucleotides or two sets of nucleotides. For example, if a base at one position of an oligonucleotide is capable of hydrogen bonding with a base at the corresponding position of ApoE ϵ 4 mRNA, then the bases are considered to be

complementary to each other at that position. Base pairings may include both canonical Watson-Crick base pairing and non-Watson-Crick base pairing (*e.g.*, Wobble base pairing and Hoogsteen base pairing). For example, in some embodiments, for complementary base pairings, adenosine-type bases (A) are complementary to thymidine-type bases (T) or uracil-type bases (U), cytosine-type bases (C) are complementary to guanosine-type bases (G), and universal bases such as 3-nitropyrrole or 5-nitroindole can hybridize to and are considered complementary to any of A, C, G, U, or T. Inosine (I) has also been considered in the art to be a universal base and is considered complementary to any A, C, U, or T.

[0049] In some embodiments, an inhibitory nucleic acid targeting (*e.g.*, comprises a region of complementarity to) ApoE ϵ 4 mRNA is an oligonucleotide. As used herein, the term “oligonucleotide” refers to an oligomeric nucleic acid compound of up to 10, up to 15, up to 20, up to 25, up to 30, up to 35, up to 40, up to 45, up to 50, up to 55, up to 60, up to 65, up to 70, up to 75, up to 80, up to 85, up to 90, up to 95, up to 100, up to 120, up to 140, up to 160, up to 180 or up to 200 nucleotides in length. Examples of oligonucleotides include, but are not limited to, RNAi oligonucleotides (*e.g.*, siRNAs, shRNAs), microRNAs, antisense oligonucleotide (*e.g.*, gapmers, mixmers), phosphorodiamidite morpholinos, peptide nucleic acids, aptamers, *etc.* Oligonucleotides may be single-stranded or double-stranded. In some embodiments, an oligonucleotide may comprise one or more modified nucleotides (*e.g.* 2'-O-methyl sugar modifications, purine or pyrimidine modifications). In some embodiments, an oligonucleotide may comprise one or more modified internucleotide linkage. In some embodiments, an oligonucleotide may comprise one or more phosphorothioate linkages, which may be in the Rp or Sp stereochemical conformation.

i. Antisense Oligonucleotides

[0050] In some embodiments, an inhibitory nucleic acid targeting (*e.g.*, comprises a region of complementarity to) ApoE ϵ 4 mRNA is an antisense oligonucleotide. As used herein, the term “antisense oligonucleotide” refers to an oligomeric compound, at least a portion of which is at least partially complementary to ApoE ϵ 4 mRNA to which it hybridizes, wherein such hybridization results in at least one antisense activity (*e.g.*, inhibition of expression or activity of ApoE ϵ 4).

[0051] In some embodiments, an antisense oligonucleotides targeting ApoE ϵ 4 mRNA are designed to cause RNase H-mediated degradation of ApoE ϵ 4 mRNA. In some

embodiments, an oligonucleotide may have a region of complementarity to ApoE ϵ 4 gene sequences of multiple species, *e.g.*, selected from human, mouse and non-human species.

[0052] In some embodiments, an antisense oligonucleotide targeting ApoE ϵ 4 mRNA comprises a region of complementarity to at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18 at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, or more consecutive nucleotides to an ApoE ϵ 4 allele that comprises a cytidine at SNP rs429358. In some embodiments, an antisense oligonucleotide targeting ApoE ϵ 4 mRNA comprises a region of complementarity to ApoE ϵ 4 mRNA as set forth in SEQ ID NO: 13. In some embodiments, an antisense oligonucleotide targeting ApoE ϵ 4 mRNA comprises a region of complementarity to at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18 at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, or more consecutive nucleotides to the nucleotide sequence of SEQ ID NO: 1. In some embodiments, an antisense oligonucleotide targeting ApoE ϵ 4 mRNA comprises a region of complementarity to at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18 at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, or more consecutive nucleotides to the nucleotide sequence of SEQ ID NO: 2. In some embodiments, an antisense oligonucleotide targeting ApoE ϵ 4 mRNA comprises a region of complementarity to at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18 at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, or more consecutive nucleotides, except for at least 1, at least 2, at least 3, at least 4, or at least 5 mismatches. In some embodiments, an antisense oligonucleotide targeting ApoE ϵ 4 mRNA comprises a region of complementarity to at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18 at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, or more consecutive nucleotides, except for at least 1, at least 2, at least 3, at least 4, or at least 5 mismatches within the region of complementarity to the nucleotide sequence of SEQ ID NO: 2.

[0053] Antisense oligonucleotides may be of a variety of different lengths. In some embodiments, an oligonucleotide is 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 75, or more nucleotides in length. In some embodiments, the oligonucleotide is 8 to 50 nucleotides in length, 8 to 40 nucleotides in

length, 8 to 30 nucleotides in length, 10 to 15 nucleotides in length, 10 to 20 nucleotides in length, 15 to 25 nucleotides in length, or 21 to 23 nucleotides in length.

[0054] In some embodiments, an antisense oligonucleotide for purposes of the present disclosure specifically hybridizes (e.g. has complementarity to) ApoE ϵ 4 mRNA when binding of the antisense oligonucleotide sequence to ApoE ϵ 4 mRNA interferes with the normal function of the ApoE ϵ 4 mRNA to cause a loss of activity (e.g., inhibiting translation) or expression (e.g., degrading ApoE ϵ 4 mRNA), and there is a sufficient degree of complementarity to avoid non-specific binding of the antisense oligonucleotide sequence to non-target sequences 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. Thus, in some embodiments, an antisense oligonucleotide may be at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% complementary to the consecutive nucleotides of ApoE ϵ 4 mRNA. In some embodiments, a complementary nucleotide sequence need not be 100% complementary to that of ApoE ϵ 4 mRNA to be specifically hybridizable or specific for ApoE ϵ 4 mRNA.

[0055] In some embodiments, an antisense oligonucleotide comprises a region of complementarity to ApoE ϵ 4 mRNA that is in the range of 8 to 15, 8 to 30, 8 to 40, or 10 to 50, or 5 to 50, or 5 to 40 nucleotides in length. In some embodiments, a region of complementarity of an oligonucleotide to ApoE ϵ 4 mRNA 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, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100 nucleotides in length. In some embodiments, the region of complementarity is complementary with at least 8 consecutive nucleotides of ApoE ϵ 4 mRNA. In some embodiments, an oligonucleotide may contain 1, 2, or 3 base mismatches compared to the portion of the consecutive nucleotides of ApoE ϵ 4 mRNA. In some embodiments the oligonucleotide may have up to 3 mismatches over 15 bases, or up to 2 mismatches over 10 bases.

[0056] In some embodiments, an antisense oligonucleotide comprises a region of complementarity to nucleotide sequence set forth in any one of SEQ ID NOs: 1, 2, or 13. In some embodiments, an antisense oligonucleotide comprises at least 10, 11, 12, 13, 14, 15, 16,

17, 18, 19, or 20 nucleotides (*e.g.*, consecutive nucleotides) that are complementary to a nucleotide sequence set forth in any one of SEQ ID NOs: 1, 2, or 13. In some embodiments, an antisense oligonucleotide comprises a sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100% complementary with at least 10, at least 12 or at least 15 consecutive nucleotides of any one of SEQ ID NO: 1, 2, and 13. In some embodiments, an antisense oligonucleotide comprises a sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100% complementary with at least 10, at least 12 or at least 15 consecutive nucleotides of the region surrounding a SNP in any one of SEQ ID NO: 1, 2, and 13. In some embodiments, an antisense oligonucleotide comprises a sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100% complementary with at least 10, at least 12 or at least 15 consecutive nucleotides of the region extending over 30 or fewer base pairs on either or both sides of a SNP. In some embodiments, an antisense oligonucleotide comprises a sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100% complementary with at least 10, at least 12 or at least 15 consecutive nucleotides of the region extending over 20 or fewer base pairs on either or both sides of a SNP. In some embodiments, the SNP is at rs429358.

[0057] An antisense oligonucleotide described herein may be modified, *e.g.*, comprise a modified sugar moiety, a modified internucleotide linkage, a modified nucleobase, a modified nucleotide, and/or (*e.g.*, and) combinations thereof. In addition, in some embodiments, an antisense oligonucleotide may exhibit one or more of the following properties: do not mediate alternative splicing; are not immune stimulatory; are nuclease resistant; have improved cell uptake compared to unmodified oligonucleotides; are not toxic to cells or mammals; have improved endosomal exit internally in a cell; minimizes TLR stimulation; or avoid pattern recognition receptors. Any of the modified chemistries or formats of oligonucleotides described herein can be combined with each other. For example, one, two, three, four, five, or more different types of modifications can be included within the same oligonucleotide.

[0058] In some embodiments, certain nucleotide modifications may be used that make an oligonucleotide into which they are incorporated more resistant to nuclease digestion than the native oligodeoxynucleotide or oligoribonucleotide molecules; these modified oligonucleotides survive intact for a longer time than unmodified oligonucleotides. Specific examples of modified oligonucleotides include those comprising modified backbones, for example, modified internucleotide linkages, such as phosphorothioates, methylphosphonates, phosphorodithioates, boron phosphonates, phosphonocarboxylates, phosphonoacetates,

phosphotriesters, methyl phosphonates, short chain alkyl or cycloalkyl intersugar linkages or short chain heteroatomic or heterocyclic intersugar linkages. Accordingly, oligonucleotides of the disclosure can be stabilized against nucleolytic degradation, such as by the incorporation of a modification, *e.g.*, a nucleotide modification.

[0059] In some embodiments, an antisense oligonucleotide may be of up to 50 or up to 100 nucleotides in length in which 2 to 10, 2 to 15, 2 to 16, 2 to 17, 2 to 18, 2 to 19, 2 to 20, 2 to 25, 2 to 30, 2 to 40, 2 to 45, or more nucleotides of the oligonucleotide are modified nucleotides. An antisense oligonucleotide may be of 8 to 30 nucleotides in length in which 2 to 10, 2 to 15, 2 to 16, 2 to 17, 2 to 18, 2 to 19, 2 to 20, 2 to 25, 2 to 30 nucleotides of the oligonucleotide are modified nucleotides. An antisense oligonucleotide may be of 8 to 15 nucleotides in length in which 2 to 4, 2 to 5, 2 to 6, 2 to 7, 2 to 8, 2 to 9, 2 to 10, 2 to 11, 2 to 12, 2 to 13, 2 to 14 nucleotides of the oligonucleotide are modified nucleotides. Optionally, the oligonucleotides may have every nucleotide except 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides modified. Oligonucleotide modifications are described further herein.

[0060] In some embodiments, an antisense oligonucleotide described herein comprises at least one nucleoside modified at the 2' position of the sugar. In some embodiments, an oligonucleotide comprises at least one 2'-modified nucleoside. In some embodiments, all of the nucleosides in the oligonucleotide are 2'-modified nucleosides.

[0061] In some embodiments, an antisense oligonucleotide described herein comprises one or more non-bicyclic 2'-modified nucleosides, *e.g.*, 2'-deoxy, 2'-fluoro (2'-F), 2'-O-methyl (2'-O-Me), 2'-O-methoxyethyl (2'-MOE), 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), 2'-O-dimethylaminoethoxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified nucleoside. In some embodiments, an antisense oligonucleotide comprises one or more 2'-O-methoxyethyl (2'-MOE) modified nucleoside. In some embodiments, each of the nucleosides of the antisense oligonucleotide is a 2'-O-methoxyethyl (2'-MOE) modified nucleoside.

[0062] In some embodiments, an antisense oligonucleotide described herein comprises one or more 2'-4' bicyclic nucleosides in which the ribose ring comprises a bridge moiety connecting two atoms in the ring, *e.g.*, connecting the 2'-O atom to the 4'-C atom via a methylene (LNA) bridge, an ethylene (ENA) bridge, or a (S)-constrained ethyl (cEt) bridge. Examples of LNAs are described in International Patent Application Publication WO 2008/043753, published on April 17, 2008, and entitled "*RNA Antagonist Compounds For The Modulation Of PCSK9*," the contents of which are incorporated herein by reference in its

entirety. Examples of ENAs are provided in International Patent Publication No. WO 2005/042777, published on May 12, 2005, and entitled “*APP/ENA Antisense*”; Morita *et al.*, *Nucleic Acid Res.*, Suppl 1:241-242, 2001; Surono *et al.*, *Hum. Gene Ther.*, 15:749-757, 2004; Koizumi, *Curr. Opin. Mol. Ther.*, 8:144-149, 2006 and Horie *et al.*, *Nucleic Acids Symp. Ser (Oxf)*, 49:171-172, 2005; the disclosures of which are incorporated herein by reference in their entireties. Examples of cEt are provided in US Patents 7,101,993; 7,399,845 and 7,569,686, each of which is herein incorporated by reference in its entirety.

[0063] In some embodiments, an antisense oligonucleotide comprises a modified nucleoside disclosed in one of the following US Patents or Patent Application Publications: US Patent 7,399,845, issued on July 15, 2008, and entitled “*6-Modified Bicyclic Nucleic Acid Analogs*”; US Patent 7,741,457, issued on June 22, 2010, and entitled “*6-Modified Bicyclic Nucleic Acid Analogs*”; US Patent 8,022,193, issued on September 20, 2011, and entitled “*6-Modified Bicyclic Nucleic Acid Analogs*”; US Patent 7,569,686, issued on August 4, 2009, and entitled “*Compounds And Methods For Synthesis Of Bicyclic Nucleic Acid Analogs*”; US Patent 7,335,765, issued on February 26, 2008, and entitled “*Novel Nucleoside And Oligonucleotide Analogues*”; US Patent 7,314,923, issued on January 1, 2008, and entitled “*Novel Nucleoside And Oligonucleotide Analogues*”; US Patent 7,816,333, issued on October 19, 2010, and entitled “*Oligonucleotide Analogues And Methods Utilizing The Same*” and US Publication Number 2011/0009471 now US Patent 8,957,201, issued on February 17, 2015, and entitled “*Oligonucleotide Analogues And Methods Utilizing The Same*,” the entire contents of each of which are incorporated herein by reference.

[0064] In some embodiments, an antisense oligonucleotide comprises at least one modified nucleoside that results in an increase in T_m of the oligonucleotide in a range of 1 °C, 2 °C, 3 °C, 4 °C, or 5 °C compared with an oligonucleotide that does not have the at least one modified nucleoside. The antisense oligonucleotide may have a plurality of modified nucleosides that result in a total increase in T_m of the oligonucleotide in a range of 2 °C, 3 °C, 4 °C, 5 °C, 6 °C, 7 °C, 8 °C, 9 °C, 10 °C, 15 °C, 20 °C, 25 °C, 30 °C, 35 °C, 40 °C, 45 °C or more compared with an oligonucleotide that does not have the modified nucleoside.

[0065] An antisense oligonucleotide may comprise a mix of nucleosides of different kinds. For example, an antisense oligonucleotide may comprise a mix of 2'-deoxyribonucleosides or ribonucleosides and 2'-fluoro modified nucleosides. An antisense oligonucleotide may comprise a mix of deoxyribonucleosides or ribonucleosides and 2'-O-Me modified nucleosides. An antisense oligonucleotide may comprise a mix of 2'-fluoro modified nucleosides and 2'-O-methyl modified nucleosides. An antisense oligonucleotide may

comprise a mix of bridged nucleosides and 2'-fluoro or 2'-O-methyl modified nucleotides. An antisense oligonucleotide may comprise a mix of non-bicyclic 2'-modified nucleosides (*e.g.*, 2'-O-MOE) and 2'-4' bicyclic nucleosides (*e.g.*, LNA, ENA, cEt). An antisense oligonucleotide may comprise a mix of 2'-fluoro modified nucleosides and 2'-O-Me modified nucleosides. An antisense oligonucleotide may comprise a mix of 2'-4' bicyclic nucleosides and 2'-MOE, 2'-fluoro, or 2'-O-Me modified nucleosides. An antisense oligonucleotide may comprise a mix of non-bicyclic 2'-modified nucleosides (*e.g.*, 2'-MOE, 2'-fluoro, or 2'-O-Me) and 2'-4' bicyclic nucleosides (*e.g.*, LNA, ENA, cEt).

[0066] An antisense oligonucleotide may comprise alternating nucleosides of different kinds. For example, an oligonucleotide may comprise alternating 2'-deoxyribonucleosides or ribonucleosides and 2'-fluoro modified nucleosides. An antisense oligonucleotide may comprise alternating deoxyribonucleosides or ribonucleosides and 2'-O-Me modified nucleosides. An antisense oligonucleotide may comprise alternating 2'-fluoro modified nucleosides and 2'-O-Me modified nucleosides. An antisense oligonucleotide may comprise alternating bridged nucleosides and 2'-fluoro or 2'-O-methyl modified nucleotides. An antisense oligonucleotide may comprise alternating non-bicyclic 2'-modified nucleosides (*e.g.*, 2'-O-MOE) and 2'-4' bicyclic nucleosides (*e.g.*, LNA, ENA, cEt). An antisense oligonucleotide may comprise alternating 2'-4' bicyclic nucleosides and 2'-MOE, 2'-fluoro, or 2'-O-Me modified nucleosides. An antisense oligonucleotide may comprise alternating non-bicyclic 2'-modified nucleosides (*e.g.*, 2'-MOE, 2'-fluoro, or 2'-O-Me) and 2'-4' bicyclic nucleosides (*e.g.*, LNA, ENA, cEt).

[0067] In some embodiments, an antisense oligonucleotide described herein comprises a 5'-vinylphosphonate modification, one or more abasic residues, and/or one or more inverted abasic residues.

[0068] In some embodiments, an antisense oligonucleotide may contain a phosphorothioate or other modified internucleotide linkage. In some embodiments, an antisense oligonucleotide comprises phosphorothioate internucleotide linkages. In some embodiments, an antisense oligonucleotide comprises phosphorothioate internucleotide linkages between at least two nucleotides. In some embodiments, an antisense oligonucleotide comprises phosphorothioate internucleotide linkages between all nucleotides. For example, in some embodiments, an antisense oligonucleotide comprises modified internucleotide linkages at the first, second, and/or (*e.g.*, and) third internucleotide linkage at the 5' or 3' end of the nucleotide sequence.

[0069] Phosphorus-containing linkages that may be used include, but are not limited to, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters,

aminoalkylphosphotriesters, methyl and other alkyl phosphonates comprising 3'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates comprising 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs of these, and those having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'; see US Patent Nos. 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,196; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,306; 5,550,111; 5,563,253; 5,571,799; 5,587,361; and 5,625,050.

[0070] In some embodiments, an antisense oligonucleotide may have heteroatom backbones, such as methylene(methylimino) or MMI backbones; amide backbones (see De Mesmaeker *et al.*, *Ace. Chem. Res.* 1995, 28:366-374); morpholino backbones (see Summerton and Weller, U.S. Pat. No. 5,034,506); or peptide nucleic acid (PNA) backbones (wherein the phosphodiester backbone of the oligonucleotide is replaced with a polyamide backbone, the nucleotides being bound directly or indirectly to the aza nitrogen atoms of the polyamide backbone, see Nielsen *et al.*, *Science* 1991, 254, 1497).

[0071] In some embodiments, internucleotidic phosphorus atoms of antisense oligonucleotides are chiral, and the properties of the antisense oligonucleotides by adjusted based on the configuration of the chiral phosphorus atoms. In some embodiments, appropriate methods may be used to synthesize P-chiral oligonucleotide analogs in a stereocontrolled manner (*e.g.*, as described in Oka N, Wada T, Stereocontrolled synthesis of oligonucleotide analogs containing chiral internucleotidic phosphorus atoms. *Chem Soc Rev.* 2011 Dec;40(12):5829-43.) In some embodiments, phosphorothioate containing antisense oligonucleotides comprise nucleoside units that are joined together by either substantially all Sp or substantially all Rp phosphorothioate intersugar linkages are provided. In some embodiments, such phosphorothioate oligonucleotides having substantially chirally pure intersugar linkages are prepared by enzymatic or chemical synthesis, as described, for example, in US Patent 5,587,261, issued on December 12, 1996, the contents of which are incorporated herein by reference in their entirety. In some embodiments, chirally controlled oligonucleotides provide selective cleavage patterns of ApoE ϵ 4 mRNA. For example, in some embodiments, a chirally controlled oligonucleotide provides single site cleavage within a complementary sequence of a nucleic acid, as described, for example, in US Patent

Application Publication 2017-0037399 A1, published on February 2, 2017, entitled “Chiral Design,” the contents of which are incorporated herein by reference in their entirety.

[0072] In some embodiments, an ASO comprises deoxynucleotides. In some embodiments, an ApoE ϵ 4-targeting antisense oligonucleotide comprises at least 15 consecutive nucleosides of (*e.g.*, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, or at least 19) the nucleotide sequence of any one of SEQ ID NOs: 30-41. In some embodiments, an ApoE ϵ 4-targeting antisense oligonucleotide comprises the nucleotide sequence of any one of SEQ ID NOs: 30-41. In some embodiments, any of the ApoE ϵ 4-targeting antisense oligonucleotide as set forth in any one of SEQ ID NOs: 30-41 comprises one or more modified nucleoside as described herein.

Table 1: ASO Sequences

ASO ID	Sequence (5' to 3')
ASO1	GCCGCGCACGTCCTCCA (SEQ ID NO: 30)
ASO2	GGCCGCGCACGTCCTCC (SEQ ID NO: 31)
ASO3	CGGCCGCGCACGTCCTC (SEQ ID NO: 32)
ASO4	GCGGCCGCGCACGTCCT (SEQ ID NO: 33)
ASO5	GGCGGCCGCGCACGTCC (SEQ ID NO: 34)
ASO6	AGGCGGCCGCGCACGTC (SEQ ID NO: 35)
ASO7	GCCGCGCACGTCCTCCATG (SEQ ID NO: 36)
ASO8	GGCCGCGCACGTCCTCCAT (SEQ ID NO: 37)
ASO9	CGGCCGCGCACGTCCTCCA (SEQ ID NO: 38)
ASO10	GCGGCCGCGCACGTCCTCC (SEQ ID NO: 39)
ASO11	AGGCGGCCGCGCACGTCCT (SEQ ID NO: 40)
ASO12	GGCGGCCGCGCACGTCCTC (SEQ ID NO: 41)

[0073] In some embodiments, an antisense oligonucleotide targeting ApoE ϵ 4 mRNA comprises a gapmer motif. “Gapmer” means a chimeric antisense compound in which an internal region having a plurality of nucleotides that support RNase H cleavage is positioned between external regions having one or more nucleotides, wherein the nucleotides comprising the internal region are chemically distinct from the nucleotide or nucleotides comprising the external regions. The internal region can be referred to as a “gap segment” and the external regions can be referred to as “wing segments.” In some embodiments, an antisense oligonucleotide targeting ApoE ϵ 4 mRNA comprises one or more modified nucleotides, and/or (*e.g.*, and) one or more modified internucleotide linkages. In some embodiments, the internucleotide linkage is a phosphorothioate linkage. In some embodiments, an antisense oligonucleotide targeting ApoE ϵ 4 mRNA comprises a full phosphorothioate backbone. In

some embodiments, the ends of the antisense oligonucleotide are modified to make them resistant to RNase H degradation. In some embodiments, the ends of the antisense oligonucleotide comprise 2'-MOE modified ribonucleosides.

[0074] A gapmer oligonucleotide generally has the formula 5'-X-Y-Z-3', with X and Z as flanking regions around a gap region Y. In some embodiments, the flanking region X of formula 5'-X-Y-Z-3' is also referred to as the X region, flanking sequence X, 5' wing region X, or 5' wing segment. In some embodiments, the flanking region Z of formula 5'-X-Y-Z-3' is also referred to as the Z region, flanking sequence Z, 3' wing region Z, or 3' wing segment. In some embodiments, the gap region Y of formula 5'-X-Y-Z-3' is also referred to as the Y region, Y segment, or gap-segment Y. In some embodiments, each nucleoside in the gap region Y is a 2'-deoxyribonucleoside, and neither the 5' wing region X or the 3' wing region Z contains any 2'-deoxyribonucleosides.

[0075] In some embodiments, the Y region is a contiguous stretch of nucleotides, *e.g.*, a region of 6 or more DNA nucleotides. In some embodiments, the gapmer binds to the ApoE ϵ 4 mRNA, at which point an RNase is recruited and then cleaves the ApoE ϵ 4 mRNA. In some embodiments, the Y region is flanked both 5' and 3' by regions X and Z comprising modified nucleosides, *e.g.*, one to six modified nucleosides. Examples of modified nucleosides include, but are not limited to, 2'-modified nucleosides (*e.g.*, 2'-MOE, 2'-O-Me, 2'-F) or 2'-4' bicyclic nucleosides (*e.g.*, LNA, cEt, ENA). In some embodiments, the flanking sequences X and Z may be 1-20 nucleotides, 1-8 nucleotides, or 1-5 nucleotides in length. The flanking sequences X and Z may be of similar length or of dissimilar lengths. In some embodiments, the gap-segment Y may be a nucleotide sequence of 5-20 nucleotides, 5-15 nucleotides, or 6-10 nucleotides in length. In some embodiments, one or more of the internucleotide linkages in the X, Y or Z region is a modified internucleotide linkage. In some embodiments, every internucleotide linkage in X is a modified internucleotide linkage. In some embodiments, every internucleotide linkage in Y is a modified internucleotide linkage. In some embodiments, every internucleotide linkage in Z is a modified internucleotide linkage. In some embodiments, every internucleotide linkage in each of the X, Y and Z regions is a modified internucleotide linkage. In some embodiments, one or more of the internucleotide linkages in the X, Y or Z region is a phosphorothioate linkage. In some embodiments, every internucleotide linkage in X is a phosphorothioate linkage. In some embodiments, every internucleotide linkage in Y is a phosphorothioate linkage. In some embodiments, every internucleotide linkage in Z is a phosphorothioate linkage. In some

embodiments, every internucleotide linkage in each of the X, Y and Z regions is a phosphorothioate linkage.

[0076] In some embodiments, the gap region of the gapmer oligonucleotides may contain modified nucleotides known to be acceptable for efficient RNase H action in addition to DNA nucleotides, such as C4'-substituted nucleotides, acyclic nucleotides, and arabino-configured nucleotides. In some embodiments, the gap region Y comprises one or more unmodified internucleotide linkages. In some embodiments, one or both flanking regions each independently comprise one or more phosphorothioate internucleotide linkages (*e.g.*, phosphorothioate internucleotide linkages or other linkages) between at least two, at least three, at least four, at least five, or more nucleotides. In some embodiments, the gap region and two flanking regions each independently comprise modified internucleotide linkages (*e.g.*, phosphorothioate internucleotide linkages or other linkages) between at least two, at least three, at least four, at least five, or more nucleotides.

[0077] A gapmer may be produced using any methods known in the art. Representative U.S. Patents, U.S. Patent Publications, and PCT publications that teach the preparation of gapmers include, but are not limited to, U.S. Patent Nos. 5,013,830; 5,149,797; 5,220,007; 5,256,775; 5,366,878; 5,403,711; 5,491,133; 5,565,350; 5,623,065; 5,652,355; 5,652,356; 5,700,922; 5,898,031; 7,015,315; 7,101,993; 7,399,845; 7,432,250; 7,569,686; 7,683,036; 7,750,131; 8,580,756; 9,045,754; 9,428,534; 9,695,418; 10,017,764; 10,260,069; 9,428,534; 8,580,756; U.S. Patent Publication Nos. US-2005-0074801, US-2009-0221685; US-2009-0286969, US-2010-0197762, and US-2011-0112170; PCT Publication Nos. WO 2004/069991; WO 2005/023825; WO 2008/049085, and WO 2009/090182; and EP Patent No. EP 2149605, each of which is herein incorporated by reference in its entirety.

[0078] In some embodiments, the gapmer is 10-40 nucleotides in length. For example, the gapmer may be 10-40, 10-35, 10-30, 10-25, 10-20, 10-15, 15-40, 15-35, 15-30, 15-25, 15-20, 20-40, 20-35, 20-30, 20-25, 25-40, 25-35, 25-30, 30-40, 30-35, or 35-40 nucleotides in length. In some embodiments, the gapmer is 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 nucleotides in length.

[0079] In some embodiments, the gap region Y in the gapmer is 5-20 nucleotides in length. For example, the gap region Y may be 5-20, 5-15, 5-10, 10-20, 10-15, or 15-20 nucleosides in length. In some embodiments, the gap region Y is 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides in length. In some embodiments, each nucleotide in the gap

region Y is a 2'-deoxyribonucleoside. In some embodiments, all nucleotides in the gap region Y are 2'-deoxyribonucleotides.

[0080] In some embodiments, the 5' wing region of the gapmer (X in the 5'-X-Y-Z-3' formula) and the 3' wing region of the gapmer (Z in the 5'-X-Y-Z-3' formula) are independently 1-20 nucleotides long. For example, the 5' wing region of the gapmer (X in the 5'-X-Y-Z-3' formula) and the 3' wing region of the gapmer (Z in the 5'-X-Y-Z-3' formula) may be independently 1-20, 1-15, 1-10, 1-7, 1-5, 1-3, 1-2, 2-5, 2-7, 3-5, 3-7, 5-20, 5-15, 5-10, 10-20, 10-15, or 15-20 nucleotides long. In some embodiments, the 5' wing region of the gapmer (X in the 5'-X-Y-Z-3' formula) and the 3' wing region of the gapmer (Z in the 5'-X-Y-Z-3' formula) are independently 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides long. In some embodiments, the 5' wing region of the gapmer (X in the 5'-X-Y-Z-3' formula) and the 3' wing region of the gapmer (Z in the 5'-X-Y-Z-3' formula) are of the same length. In some embodiments, the 5' wing region of the gapmer (X in the 5'-X-Y-Z-3' formula) and the 3' wing region of the gapmer (Z in the 5'-X-Y-Z-3' formula) are of different lengths. In some embodiments, the 5' wing region of the gapmer (X in the 5'-X-Y-Z-3' formula) is longer than the 3' wing region of the gapmer (Z in the 5'-X-Y-Z-3' formula). In some embodiments, the 5' wing region of the gapmer (X in the 5'-X-Y-Z-3' formula) is shorter than the 3' wing region of the gapmer (Z in the 5'-X-Y-Z-3' formula).

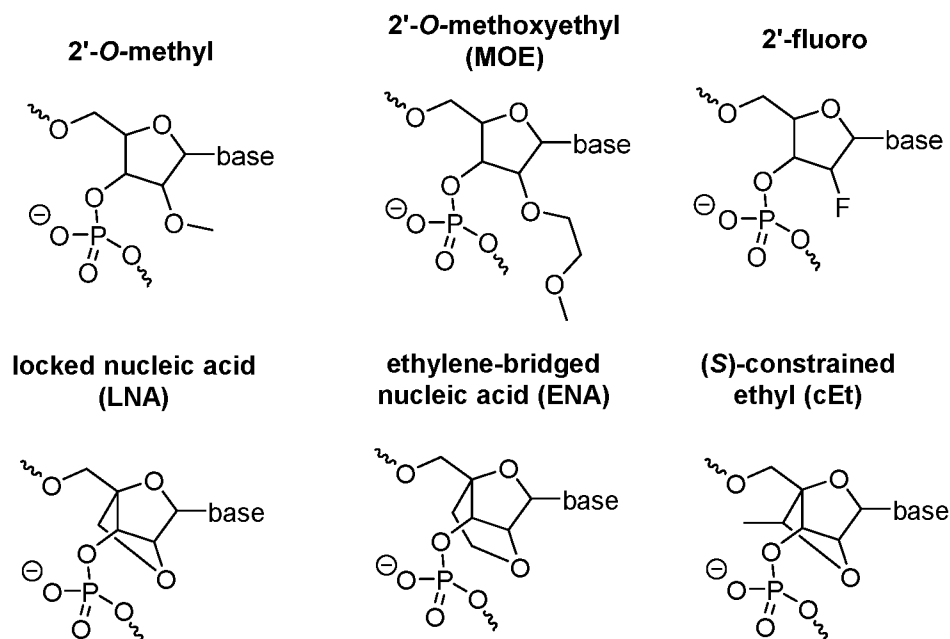
[0081] In some embodiments, the gapmer oligonucleotide has the formula 5'-X-Y-Z-3'. In some embodiments, the number of nucleotides in each of the X, Y, and Z regions, respectively, is indicated by a combination of 3 numbers as follows: 5-7-5, 5-9-5, 5-10-5, 4-12-4, 3-14-3, 2-16-2, 1-18-1, 3-10-3, 2-10-2, 1-10-1, 2-8-2, 4-6-4, 3-6-3, 2-6-2, 4-7-4, 3-7-3, 2-7-2, 4-8-4, 3-8-3, 2-8-2, 1-8-1, 2-9-2, 1-9-1, 2-10-2, 1-10-1, 1-12-1, 1-16-1, 2-15-1, 1-15-2, 1-14-3, 3-14-1, 2-14-2, 1-13-4, 4-13-1, 2-13-3, 3-13-2, 1-12-5, 5-12-1, 2-12-4, 4-12-2, 3-12-3, 1-11-6, 6-11-1, 2-11-5, 5-11-2, 3-11-4, 4-11-3, 1-17-1, 2-16-1, 1-16-2, 1-15-3, 3-15-1, 2-15-2, 1-14-4, 4-14-1, 2-14-3, 3-14-2, 1-13-5, 5-13-1, 2-13-4, 4-13-2, 3-13-3, 1-12-6, 6-12-1, 2-12-5, 5-12-2, 3-12-4, 4-12-3, 1-11-7, 7-11-1, 2-11-6, 6-11-2, 3-11-5, 5-11-3, 4-11-4, 1-18-1, 1-17-2, 2-17-1, 1-16-3, 1-16-3, 2-16-2, 1-15-4, 4-15-1, 2-15-3, 3-15-2, 1-14-5, 5-14-1, 2-14-4, 4-14-2, 3-14-3, 1-13-6, 6-13-1, 2-13-5, 5-13-2, 3-13-4, 4-13-3, 1-12-7, 7-12-1, 2-12-6, 6-12-2, 3-12-5, 5-12-3, 1-11-8, 8-11-1, 2-11-7, 7-11-2, 3-11-6, 6-11-3, 4-11-5, 5-11-4, 1-18-1, 1-17-2, 2-17-1, 1-16-3, 3-16-1, 2-16-2, 1-15-4, 4-15-1, 2-15-3, 3-15-2, 1-14-5, 2-14-4, 4-14-2, 3-14-3, 1-13-6, 6-13-1, 2-13-5, 5-13-2, 3-13-4, 4-13-3, 1-12-7, 7-12-1, 2-12-6, 6-12-2, 3-12-5, 5-12-3, 1-11-8, 8-11-1, 2-11-7, 7-11-2, 3-11-6, 6-11-3, 4-11-5, 5-11-4, 1-19-1, 1-18-2, 2-18-1, 1-17-3, 3-17-1, 2-17-2, 1-16-4, 4-16-1, 2-16-3, 3-16-2, 1-15-5, 2-15-4, 4-15-2, 3-

15-3, 1-14-6, 6-14-1, 2-14-5, 5-14-2, 3-14-4, 4-14-3, 1-13-7, 7-13-1, 2-13-6, 6-13-2, 3-13-5, 5-13-3, 4-13-4, 1-12-8, 8-12-1, 2-12-7, 7-12-2, 3-12-6, 6-12-3, 4-12-5, 5-12-4, 2-11-8, 8-11-2, 3-11-7, 7-11-3, 4-11-6, 6-11-4, 5-11-5, 1-20-1, 1-19-2, 2-19-1, 1-18-3, 3-18-1, 2-18-2, 1-17-4, 4-17-1, 2-17-3, 3-17-2, 1-16-5, 2-16-4, 4-16-2, 3-16-3, 1-15-6, 6-15-1, 2-15-5, 5-15-2, 3-15-4, 4-15-3, 1-14-7, 7-14-1, 2-14-6, 6-14-2, 3-14-5, 5-14-3, 4-14-4, 1-13-8, 8-13-1, 2-13-7, 7-13-2, 3-13-6, 6-13-3, 4-13-5, 5-13-4, 2-12-8, 8-12-2, 3-12-7, 7-12-3, 4-12-6, 6-12-4, 5-12-5, 3-11-8, 8-11-3, 4-11-7, 7-11-4, 5-11-6, 6-11-5, 1-21-1, 1-20-2, 2-20-1, 1-20-3, 3-19-1, 2-19-2, 1-18-4, 4-18-1, 2-18-3, 3-18-2, 1-17-5, 2-17-4, 4-17-2, 3-17-3, 1-16-6, 6-16-1, 2-16-5, 5-16-2, 3-16-4, 4-16-3, 1-15-7, 7-15-1, 2-15-6, 6-15-2, 3-15-5, 5-15-3, 4-15-4, 1-14-8, 8-14-1, 2-14-7, 7-14-2, 3-14-6, 6-14-3, 4-14-5, 5-14-4, 2-13-8, 8-13-2, 3-13-7, 7-13-3, 4-13-6, 6-13-4, 5-13-5, 1-12-10, 10-12-1, 2-12-9, 9-12-2, 3-12-8, 8-12-3, 4-12-7, 7-12-4, 5-12-6, 6-12-5, 4-11-8, 8-11-4, 5-11-7, 7-11-5, 6-11-6, 1-22-1, 1-21-2, 2-21-1, 1-21-3, 3-20-1, 2-20-2, 1-19-4, 4-19-1, 2-19-3, 3-19-2, 1-18-5, 2-18-4, 4-18-2, 3-18-3, 1-17-6, 6-17-1, 2-17-5, 5-17-2, 3-17-4, 4-17-3, 1-16-7, 7-16-1, 2-16-6, 6-16-2, 3-16-5, 5-16-3, 4-16-4, 1-15-8, 8-15-1, 2-15-7, 7-15-2, 3-15-6, 6-15-3, 4-15-5, 5-15-4, 2-14-8, 8-14-2, 3-14-7, 7-14-3, 4-14-6, 6-14-4, 5-14-5, 3-13-8, 8-13-3, 4-13-7, 7-13-4, 5-13-6, 6-13-5, 4-12-8, 8-12-4, 5-12-7, 7-12-5, 6-12-6, 5-11-8, 8-11-5, 6-11-7, or 7-11-6.

[0082] In some embodiments, one or more nucleosides in the 5' wing region of the gapmer (X in the 5'-X-Y-Z-3' formula) or the 3' wing region of the gapmer (Z in the 5'-X-Y-Z-3' formula) are modified nucleosides. In some embodiments, the modified nucleoside is a 2'-modified nucleoside.

[0083] As used herein, the terms "2'-modified nucleoside" and "2'-modified ribonucleoside" are used interchangeably and refer to a nucleoside having a sugar moiety modified at the 2' position. In some embodiments, the 2'-modified nucleoside is a 2'-4' bicyclic nucleoside, where the 2' and 4' positions of the sugar are bridged (*e.g.*, via a methylene, an ethylene, or a (S)-constrained ethyl bridge). In some embodiments, the 2'-modified nucleoside is a non-bicyclic 2'-modified nucleoside, *e.g.*, where the 2' position of the sugar moiety is substituted. Non-limiting examples of 2'-modified nucleosides include: 2'-deoxy, 2'-fluoro (2'-F), 2'-O-methyl (2'-O-Me), 2'-O-methoxyethyl (2'-MOE), 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), 2'-O-dimethylaminoethoxyethyl (2'-O-DMAEOE), 2'-O-N-methylacetamido (2'-O-NMA), locked nucleic acid (LNA, methylene-bridged nucleic acid), ethylene-bridged nucleic acid (ENA), and (S)-constrained ethyl-bridged nucleic acid (cEt). In some embodiments, the 2'-modified nucleosides described herein are modified

nucleotides, such as 2'-modified nucleosides (*e.g.*, 2'-MOE, 2'-O-Me, 2'-F) or 2'-4' bicyclic nucleosides (*e.g.*, LNA, cEt, ENA), and the oligonucleotides comprising the 2'-modified nucleosides have increased affinity to a ApoE ϵ 4 mRNA, relative to an unmodified oligonucleotide. Examples of structures of 2'-modified nucleosides are provided below:



[0084] In some embodiments, the 2'-modified nucleoside is a 2'-4' bicyclic nucleoside or a non-bicyclic 2'-modified nucleoside. In some embodiments, the high-affinity modified nucleoside is a 2'-4' bicyclic nucleoside (*e.g.*, LNA, cEt, or ENA) or a non-bicyclic 2'-modified nucleoside (*e.g.*, 2'-fluoro (2'-F), 2'-O-methyl (2'-O-Me), 2'-O-methoxyethyl (2'-MOE), 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), 2'-O-dimethylaminoethoxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA)). In some embodiments, the modified nucleoside is a 2'-O-methoxyethyl (2'-MOE) modified nucleoside. In some embodiments, the modified nucleoside is a Hachimoji nucleoside.

[0085] In some embodiments, one or more nucleosides in the 5' wing region of the gapmer (X in the 5'-X-Y-Z-3' formula) are modified nucleosides, such as 2'-modified nucleosides (*e.g.*, 2'-MOE, 2'-O-Me, 2'-F) or 2'-4' bicyclic nucleosides (*e.g.*, LNA, cEt, ENA). In some embodiments, each nucleoside in the 5' wing region of the gapmer (X in the 5'-X-Y-Z-3' formula) is a high-affinity modified nucleoside. In some embodiments, one or more nucleosides in the 3' wing region of the gapmer (Z in the 5'-X-Y-Z-3' formula) are modified nucleosides, such as 2'-modified nucleosides (*e.g.*, 2'-MOE, 2'-O-Me, 2'-F) or 2'-4' bicyclic nucleosides (*e.g.*, LNA, cEt, ENA). In some embodiments, each nucleoside in the 3' wing

region of the gapmer (Z in the 5'-X-Y-Z-3' formula) is a modified nucleoside, such as 2'-modified nucleosides (*e.g.*, 2'-MOE, 2'-O-Me, 2'-F) or 2'-4' bicyclic nucleosides (*e.g.*, LNA, cEt, ENA). In some embodiments, one or more nucleosides in the 5' wing region of the gapmer (X in the 5'-X-Y-Z-3' formula) are modified nucleosides, such as 2'-modified nucleosides (*e.g.*, 2'-MOE, 2'-O-Me, 2'-F) or 2'-4' bicyclic nucleosides (*e.g.*, LNA, cEt, ENA), and one or more nucleosides in the 3' wing region of the gapmer (Z in the 5'-X-Y-Z-3' formula) are modified nucleosides, such as 2'-modified nucleosides (*e.g.*, 2'-MOE, 2'-O-Me, 2'-F) or 2'-4' bicyclic nucleosides (*e.g.*, LNA, cEt, ENA). In some embodiments, each nucleoside in the 5' wing region of the gapmer (X in the 5'-X-Y-Z-3' formula) is a modified nucleoside, such as 2'-modified nucleosides (*e.g.*, 2'-MOE, 2'-O-Me, 2'-F) or 2'-4' bicyclic nucleosides (*e.g.*, LNA, cEt, ENA), and each nucleoside in the 3' wing region of the gapmer (Z in the 5'-X-Y-Z-3' formula) is a modified nucleoside, such as 2'-modified nucleosides (*e.g.*, 2'-MOE, 2'-O-Me, 2'-F) or 2'-4' bicyclic nucleosides (*e.g.*, LNA, cEt, ENA).

[0086] In some embodiments, the 5' wing region of the gapmer (X in the 5'-X-Y-Z-3' formula) comprises the same modified nucleosides (*e.g.*, 2'-modified nucleosides (*e.g.*, 2'-MOE, 2'-O-Me, 2'-F) or 2'-4' bicyclic nucleosides (*e.g.*, LNA, cEt, ENA)) as the 3' wing region of the gapmer (Z in the 5'-X-Y-Z-3' formula). For example, the 5' wing region of the gapmer (X in the 5'-X-Y-Z-3' formula) and the 3' wing region of the gapmer (Z in the 5'-X-Y-Z-3' formula) may comprise one or more non-bicyclic 2'-modified nucleosides (*e.g.*, 2'-MOE or 2'-O-Me). In another example, the 5' wing region of the gapmer (X in the 5'-X-Y-Z-3' formula) and the 3' wing region of the gapmer (Z in the 5'-X-Y-Z-3' formula) may comprise one or more 2'-4' bicyclic nucleosides (*e.g.*, LNA or cEt). In some embodiments, each nucleoside in the 5' wing region of the gapmer (X in the 5'-X-Y-Z-3' formula) and the 3' wing region of the gapmer (Z in the 5'-X-Y-Z-3' formula) is a non-bicyclic 2'-modified nucleosides (*e.g.*, 2'-MOE or 2'-O-Me).

[0087] In some embodiments, one or more nucleosides in the 5' wing region of the gapmer (X in the 5'-X-Y-Z-3' formula) are modified nucleosides with reduced toxicity. In some embodiments, each nucleoside in the 5' wing region of the gapmer (X in the 5'-X-Y-Z-3' formula) is a modified nucleoside with reduced toxicity. In some embodiments, one or more nucleosides in the 3' wing region of the gapmer (Z in the 5'-X-Y-Z-3' formula) are modified nucleosides with reduced toxicity. In some embodiments, each nucleoside in the 3' wing region of the gapmer (Z in the 5'-X-Y-Z-3' formula) is a modified nucleoside with reduced toxicity. In some embodiments, one or more nucleosides in the 5' wing region of the gapmer (X in the 5'-X-Y-Z-3' formula) are modified nucleosides with reduced toxicity and one or

more nucleosides in the 3' wing region of the gapmer (Z in the 5'-X-Y-Z-3' formula) are modified nucleosides with reduced toxicity. In some embodiments, each nucleoside in the 5' wing region of the gapmer (X in the 5'-X-Y-Z-3' formula) is a modified nucleoside with reduced toxicity, and each nucleoside in the 3' wing region of the gapmer (Z in the 5'-X-Y-Z-3' formula) is a modified nucleoside with reduced toxicity.

[0088] In some embodiments, the gapmer comprises a 5'-X-Y-Z-3' configuration, wherein X and Z is independently 1-7 (*e.g.*, 1, 2, 3, 4, 5, 6, or 7) nucleosides in length, and Y is 6-10 (*e.g.*, 6, 7, 8, 9, or 10) nucleosides in length, wherein each nucleoside in X and Z is a non-bicyclic 2'-modified nucleosides (*e.g.*, 2'-MOE), and each nucleoside in Y is a 2'-deoxyribonucleoside. In some embodiments, the 5' wing region of the gapmer (X in the 5'-X-Y-Z-3' formula) comprises the same modified nucleosides (*e.g.*, 2'-modified nucleosides (*e.g.*, 2'-MOE, 2'-O-Me, 2'-F) or 2'-4' bicyclic nucleosides (*e.g.*, LNA, cEt, ENA)) as the 3' wing region of the gapmer (Z in the 5'-X-Y-Z-3' formula). For example, the 5' wing region of the gapmer (X in the 5'-X-Y-Z-3' formula) may comprise one or more non-bicyclic 2'-modified nucleosides (*e.g.*, 2'-MOE), and the 3' wing region of the gapmer (Z in the 5'-X-Y-Z-3' formula) may comprise one or more non-bicyclic 2'-modified nucleosides (*e.g.*, 2'-MOE). In some embodiments, the gapmer comprises a 5'-X-Y-Z-3' configuration, wherein X and Z is independently 1-7 (*e.g.*, 1, 2, 3, 4, 5, 6, or 7) nucleosides in length, and Y is 6-10 (*e.g.*, 6, 7, 8, 9, or 10) nucleosides in length, wherein each nucleoside in X is a non-bicyclic 2'-modified nucleosides (*e.g.*, 2'-MOE), each nucleoside in Z is a non-bicyclic 2'-modified nucleosides (*e.g.*, 2'-MOE), and each nucleoside in Y is a 2'-deoxyribonucleoside. In some embodiments, the 5' wing region of the gapmer (X in the 5'-X-Y-Z-3' formula) comprises different modified nucleosides compared to the 3' wing region of the gapmer (Z in the 5'-X-Y-Z-3' formula).

[0089] Non-limiting examples of gapmer configurations comprises non-bicyclic 2'-modified nucleoside (*e.g.*, 2'-MOE or 2'-O-Me) and/or 2'-4' bicyclic nucleosides (*e.g.*, LNA or cEt) in the 5' wing region of the gapmer (X in the 5'-X-Y-Z-3' formula) and/or the 3' wing region of the gapmer (Z in the 5'-X-Y-Z-3' formula) include: EEEEE-(D)₇-EEEE, EEEEE-(D)₉-EEEE, BBB-(D)_n-BBBAA; KKK-(D)_n-KKKAA; LLL-(D)_n-LLLAA; BBB-(D)_n-BBBEE; KKK-(D)_n-KKKEE; LLL-(D)_n-LLLEE; BBB-(D)_n-BBBAA; KKK-(D)_n-KKKAA; LLL-(D)_n-LLLAA; BBB-(D)_n-BBBEE; KKK-(D)_n-KKKEE; LLL-(D)_n-LLLEE; BBB-(D)_n-BBBAAA; KKK-(D)_n-KKKAAA; LLL-(D)_n-LLLAAA; BBB-(D)_n-BBBEEE; KKK-(D)_n-KKKEEE; LLL-(D)_n-LLLEEE; BBB-(D)_n-BBBAAA; KKK-(D)_n-KKKAAA; LLL-(D)_n-LLLAAA; BBB-(D)_n-BBBEEE; KKK-(D)_n-KKKEEE; LLL-(D)_n-LLLEEE; BABA-(D)_n-ABAB;

KAKA-(D)_n-AKAK; LALA-(D)_n-ALAL; BEBE-(D)_n-EBEB; KEKE-(D)_n-EKEK; LELE-(D)_n-ELEL; BABA-(D)_n-ABAB; KAKA-(D)_n-AKAK; LALA-(D)_n-ALAL; BEBE-(D)_n-EBEB; KEKE-(D)_n-EKEK; LELE-(D)_n-ELEL; ABAB-(D)_n-ABAB; AKAK-(D)_n-AKAK; ALAL-(D)_n-ALAL; EBEB-(D)_n-EBEB; EKEK-(D)_n-EKEK; ELEL-(D)_n-ELEL; ABAB-(D)_n-ABAB; AKAK-(D)_n-AKAK; ALAL-(D)_n-ALAL; EBEB-(D)_n-EBEB; EKEK-(D)_n-EKEK; ELEL-(D)_n-ELEL; AABB-(D)_n-BBAA; BBAA-(D)_n-AABB; AAKK-(D)_n-KKAA; AALL-(D)_n-LLAA; EEBB-(D)_n-BBEE; EEKK-(D)_n-KKEE; EELL-(D)_n-LLEE; AABB-(D)_n-BBAA; AAKK-(D)_n-KKAA; AALL-(D)_n-LLAA; EEBB-(D)_n-BBEE; EEKK-(D)_n-KKEE; EELL-(D)_n-LLEE; BBB-(D)_n-BBA; KKK-(D)_n-KKA; LLL-(D)_n-LLA; BBB-(D)_n-BBE; KKK-(D)_n-KKE; LLL-(D)_n-LLE; BBB-(D)_n-BBA; KKK-(D)_n-KKA; LLL-(D)_n-LLA; BBB-(D)_n-BBE; KKK-(D)_n-KKE; LLL-(D)_n-LLE; BBB-(D)_n-BBA; KKK-(D)_n-KKA; LLL-(D)_n-LLA; BBB-(D)_n-BBE; KKK-(D)_n-KKE; LLL-(D)_n-LLE; ABBB-(D)_n-BBBA; AKKK-(D)_n-KKKA; ALLL-(D)_n-LLLA; EBBB-(D)_n-BBBE; EKKK-(D)_n-KKKE; ELLL-(D)_n-LLLE; ABBB-(D)_n-BBBA; AKKK-(D)_n-KKKA; ALLL-(D)_n-LLLA; EBBB-(D)_n-BBBE; EKKK-(D)_n-KKKE; ELLL-(D)_n-LLLE; ABBB-(D)_n-BBBAA; AKKK-(D)_n-KKKAA; ALLL-(D)_n-LLLAA; EBBB-(D)_n-BBBEE; EKKK-(D)_n-KKKEE; ELLL-(D)_n-LLLEE; ABBB-(D)_n-BBBAA; AKKK-(D)_n-KKKAA; ALLL-(D)_n-LLLAA; EBBB-(D)_n-BBBEE; EKKK-(D)_n-KKKEE; ELLL-(D)_n-LLLEE; AABBB-(D)_n-BBB; AAKKK-(D)_n-KKK; AALLL-(D)_n-LLL; EEBBB-(D)_n-BBB; EEKKK-(D)_n-KKK; EELLL-(D)_n-LLL; AABBB-(D)_n-BBB; AAKKK-(D)_n-KKK; AALLL-(D)_n-LLL; EEBBB-(D)_n-BBB; EEKKK-(D)_n-KKK; EELLL-(D)_n-LLL; AABBB-(D)_n-BBBA; AAKKK-(D)_n-KKKA; AALLL-(D)_n-LLLA; EEBBB-(D)_n-BBBE; EEKKK-(D)_n-KKKE; EELLL-(D)_n-LLLE; AABBB-(D)_n-BBBA; AAKKK-(D)_n-KKKA; AALLL-(D)_n-LLLA; EEBBB-(D)_n-BBBE; EEKKK-(D)_n-KKKE; EELLL-(D)_n-LLLE; ABBAABB-(D)_n-BB; AKKAACK-(D)_n-KK; ALLAALLL-(D)_n-LL; EBBEEBB-(D)_n-BB; EKKEEKK-(D)_n-KK; ELLEELL-(D)_n-LL; ABBAABB-(D)_n-BB; AKKAACK-(D)_n-KK; ALLAALLL-(D)_n-LL; EBBEEBB-(D)_n-BB; EKKEEKK-(D)_n-KK; ELLEELL-(D)_n-LL; ABBABB-(D)_n-BBB; AKKAKK-(D)_n-KKK; ALLALLL-(D)_n-LLL; EBBEBB-(D)_n-BBB; EKKEKK-(D)_n-KKK; ELLELL-(D)_n-LLL; ABBABB-(D)_n-BBB; AKKAKK-(D)_n-KKK; ALLALLL-(D)_n-LLL; EBBEBB-(D)_n-BBB; EKKEKK-(D)_n-KKK; ELLELL-(D)_n-LLL; EEK-(D)_n-EEEEEEEEE; EEK-(D)_n-EEEEEEEEE; EK-(D)_n-EEEEEEEEE; EK-(D)_n-EEEKK; K-(D)_n-EEEKEKE; K-(D)_n-EEEKEKEE; K-(D)_n-EEKEK; EK-(D)_n-EEEKEKE; EK-(D)_n-EEEKEK; EEK-(D)_n-EEEKEK; EEK-(D)_n-KEEKE; EK-(D)_n-EEKEK; EK-(D)_n-KEEK; EEK-(D)_n-EEEKEK; EK-(D)_n-KEEKEE; EK-(D)_n-EEKEKE; EK-(D)_n-EEEKEKE; and EK-(D)_n-EEEEKEK; wherein “A” represents a 2'-modified nucleoside; “B” represents a 2'-4' bicyclic

nucleoside; “K” represents a constrained ethyl nucleoside (cEt); “L” represents an LNA nucleoside; and “E” represents a 2'-MOE modified ribonucleoside; “D” represents a 2'-deoxyribonucleoside; “n” represents the length of the gap segment (Y in the 5'-X-Y-Z-3' configuration) and is an integer between 1-20, inclusive.

[0090] In some embodiments, any of the gapmers described herein comprise one or more modified nucleotide linkages (*e.g.*, a phosphorothioate linkage) in each of the X, Y, and Z regions. In some embodiments, each internucleotide linkage in any of the gapmers described herein is a phosphorothioate linkage. In some embodiments, each of the X, Y, and Z regions independently comprises a combination of phosphorothioate linkages and phosphodiester linkages. In some embodiments, each internucleotide linkage in the gap region Y is a phosphorothioate linkage, the 5' wing region X comprises a combination of phosphorothioate linkages and phosphodiester linkages, and the 3' wing region Z comprises a combination of phosphorothioate linkages and phosphodiester linkages.

[0091] Non-limiting examples of gapmers targeting SEQ ID NO: 1 and SEQ ID NO: 2 of ApoE ϵ 4 mRNA are provided in Table 2.

Table 2. ApoE ϵ 4-targeting gapmers

ASO ID	Gapmer Sequence (5' to 3')	Gapmer configuration* 5'-X-Y-Z'-3'	Gapmer Structure** (5' to 3')
ASO1	GCCGCGCACGTCCTC CA (SEQ ID NO: 3)	EEEE-(D) ₇ - EEEE Full PS backbone	oG*oC*oC*oG*oC*dG*dC*dA* dC*dG*dT*dC*oC*oT*oC*oC* oA (SEQ ID NO: 20)
ASO2	GGCCGCGCACGTCCT CC (SEQ ID NO: 4)	EEEE-(D) ₇ - EEEE Full PS backbone	oG*oG*oC*oC*oG*dC*dG*dC* dA*dC*dG*dT*oC*oC*oT*oC* oC (SEQ ID NO: 21)
ASO3	CGGCCGCGCACGTCC TC (SEQ ID NO: 5)	EEEE-(D) ₇ - EEEE Full PS backbone	oC*oG*oG*oC*oC*dG*dC*dG* dC*dA*dC*dG*oT*oC*oC*oT* oC (SEQ ID NO: 22)
ASO4	GCGGCCGCGCACGTC CT (SEQ ID NO: 6)	EEEE-(D) ₇ - EEEE Full PS backbone	oG*oC*oG*oG*oC*dC*dG*dC* dG*dC*dA*dC*oG*oT*oC*oC* oT (SEQ ID NO: 23)
ASO5	GGCGGCCGCGCACGT CC (SEQ ID NO: 7)	EEEE-(D) ₇ - EEEE Full PS backbone	oG*oG*oC*oG*oG*dC*dC*dG* dC*dG*dC*dA*oC*oG*oT*oC* oC (SEQ ID NO: 24)

ASO6	AGGCGGCCGCGCACGTC (SEQ ID NO: 8)	EEEE-(D) ₇ - EEEE Full PS backbone	oA*oG*oG*oC*oG*dG*dC*dC* dG*dC*dG*dC*oA*oC*oG*oT* oC (SEQ ID NO: 25)
ASO7	GCCGCGCACGTCCTCATG (SEQ ID NO: 9)	EEEE-(D) ₉ - EEEE Full PS backbone	oG*oC*oC*oG*oC*dG*dC*dA* dC*dG*dT*dC*dC*dT*oC*oC* oA*oT*oG (SEQ ID NO: 26)
ASO8	GGCCGCGCACGTCCTCCAT (SEQ ID NO: 10)	EEEE-(D) ₉ - EEEE Full PS backbone	oG*oG*oC*oC*oG*dC*dG*dC* dA*dC*dG*dT*dC*dC*oT*oC* oC*oA*oT (SEQ ID NO: 27)
ASO9	CGGCCGCGCACGTCTCCA (SEQ ID NO: 11)	EEEE-(D) ₉ - EEEE Full PS backbone	oC*oG*oG*oC*oC*dG*dC*dG* dC*dA*dC*dG*dT*dC*oC*oT* oC*oC*oA (SEQ ID NO: 28)
ASO10	GCGGCCGCGCACGTCCTCC (SEQ ID NO: 12)	EEEE-(D) ₉ - EEEE Full PS backbone	oG*oC*oG*oG*oC*dC*dG*dC* dG*dC*dA*dC*dG*dT*oC*oC* oT*oC*oC (SEQ ID NO: 29)
ASO11	AGGCGGCCGCGCACGTCCT (SEQ ID NO: 42)	EEEE-(D) ₉ - EEEE Full PS backbone	oA* oG* oG* oC* oG* dG* dC* dC* dG* dC* dG* dC* dA* dC* oG* oT* oC* oC* oT* (SEQ ID NO: 44)
ASO12	GGCGGCCGCGCACGTCCTC (SEQ ID NO: 43)	EEEE-(D) ₉ - EEEE Full PS backbone	oG* oG* oC* oG* oG* dC* dC* dG* dC* dG* dC* dA* dC* dG* oT* oC* oC* oT* oC* (SEQ ID NO: 45)

*“E” is a 2'-MOE modified ribonucleoside; “D” is 2'-deoxyribonucleoside; “10” or “8” is the number of the 2'-deoxyribonucleoside in Y; and “PS” is phosphorothioate internucleotide linkage.

** “dN” is 2'-deoxyribonucleoside; “oN” is 2'-MOE modified ribonucleoside; “oT” is 5-methyl-2'-MOE-thymidine; and “*” indicates phosphorothioate internucleotide linkage.

[0092] In some embodiments, an ApoE ε4-targeting antisense oligonucleotide (e.g., gapmer) described herein is 15-20 nucleosides (e.g., 15, 16, 17, 18, 19, or 20 nucleosides) in length, comprises a region of complementarity to at least 15 consecutive nucleosides (e.g., at least 15, at least 16, at least 17, at least 18, at least 19, or at least 20) of SEQ ID NOs: 12, or 13 and comprises a 5'-X-Y-Z-3' configuration, wherein X comprises 3-5 (e.g., 3, 4, or 5) linked nucleosides, wherein at least one of the nucleosides in X is a 2'-modified nucleoside (e.g., 2'-MOE modified nucleoside); wherein Y comprises 6-10 (e.g., 6, 7, 8, 9, or 10) linked 2'-deoxyribonucleosides; wherein Z comprises 3-5 (e.g., 3, 4, or 5) linked nucleosides; and wherein at least one of the nucleosides in Z is a 2'-modified nucleoside (e.g., 2'-MOE modified nucleoside).

[0093] In some embodiments, an ApoE ϵ 4-targeting antisense oligonucleotide (e.g., gapmer) comprises at least 15 consecutive nucleosides of (e.g., at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, or at least 19) the nucleotide sequence of any one of SEQ ID NOs: 3-12, 20-29, or 42-45 and comprises a 5'-X-Y-Z-3' configuration, wherein X comprises 3-5 (e.g., 3, 4, or 5) linked nucleosides, wherein at least one of the nucleosides in X is a 2'-modified nucleoside (e.g., 2'-MOE modified nucleoside); wherein Y comprises 6-10 (e.g., 6, 7, 8, 9, or 10) linked 2'-deoxyribonucleosides; wherein Z comprises 3-5 (e.g., 3, 4, or 5) linked nucleosides; and wherein at least one of the nucleosides in Z is a 2'-modified nucleoside (e.g., 2'-MOE modified nucleoside).

[0094] In some embodiments, an ApoE ϵ 4-targeting antisense oligonucleotide (e.g., gapmer) comprises the nucleotide sequence of any one of SEQ ID Nos: 3-12, 20-29, or 42-45, and comprises a 5'-X-Y-Z-3' configuration, wherein X comprises 3-5 (e.g., 3, 4, or 5) linked nucleosides, wherein at least one of the nucleosides in X is a 2'-modified nucleoside (e.g., 2'-MOE modified nucleoside); wherein Y comprises 6-10 (e.g., 6, 7, 8, 9, or 10) linked 2'-deoxyribonucleosides; and wherein Z comprises 3-5 (e.g., 3, 4, or 5) linked nucleosides, wherein at least one of the nucleosides in Z is a 2'-modified nucleoside (e.g., 2'-MOE modified nucleoside).

[0095] In some embodiments, each nucleoside in X is a 2'-modified nucleoside, and/or (e.g., and) each nucleoside in Z is a 2'-modified nucleoside. In some embodiments, the 2'-modified nucleoside is a non-bicyclic 2'-modified nucleoside (e.g., 2'-MOE modified nucleoside).

[0096] In some embodiments, the ApoE ϵ 4-targeting antisense oligonucleotide (e.g., gapmer) comprises one or more phosphorothioate internucleotide linkages. In some embodiments, each internucleotide linkage in the ApoE ϵ 4-targeting antisense oligonucleotide is a phosphorothioate internucleotide linkage. In some embodiments, the ApoE ϵ 4-targeting antisense oligonucleotide comprises one or more phosphodiester internucleotide linkages, optionally wherein the phosphodiester internucleotide linkages are in X and or Z. In some embodiments, the ApoE ϵ 4-targeting antisense oligonucleotide comprises one or more phosphorothioate internucleotide linkages and one or more phosphodiester internucleotide linkages. In some embodiments, each internucleotide linkage in the gap region Y is a phosphorothioate internucleotide linkage, X comprises one or more phosphorothioate internucleotide linkages and one or more phosphodiester internucleotide linkages, and Z comprises one or more phosphorothioate internucleotide linkages and one or more phosphodiester internucleotide linkages.

[0097] In some embodiments, the oligonucleotide comprises a 5'-X-Y-Z-3' configuration of:

X	Y	Z
EEEEEE	(D) ₇	EEEEEE
EEE	(D) ₉	EEE

wherein “E” is a 2'-MOE modified ribonucleoside; “D” is 2'-deoxyribonucleoside; and “7” or “9” is the number of the 2'-deoxyribonucleoside in Y, and wherein the antisense oligonucleotide comprises phosphorothioate internucleotide linkages.

[0098] In some embodiments, the ApoE ε4-targeting antisense oligonucleotide is selected from ASOs 1-12 listed in Tables 1 and 2. In some embodiments, any one of the ApoE ε4-targeting antisense oligonucleotides can be in salt form, *e.g.*, sodium, potassium, or magnesium salts.

[0099] In some embodiments, the 5' or 3' nucleoside (*e.g.*, terminal nucleoside) of any one of the oligonucleotides described herein (*e.g.*, the oligonucleotides listed in Table 1) is conjugated to an amine group, optionally via a spacer. In some embodiments, the spacer comprises an aliphatic moiety. In some embodiments, the spacer comprises a polyethylene glycol moiety. In some embodiments, a phosphodiester linkage is present between the spacer and the 5' or 3' nucleoside of the oligonucleotide. In some embodiments, the 5' or 3' nucleoside (*e.g.*, terminal nucleoside) of any of the oligonucleotides described herein (*e.g.*, the oligonucleotides listed in Tables 1 and 2) is conjugated to a spacer that is a substituted or unsubstituted aliphatic, substituted or unsubstituted heteroaliphatic, substituted or unsubstituted carbocyclylene, substituted or unsubstituted heterocyclylene, substituted or unsubstituted arylene, substituted or unsubstituted heteroarylene, -O-, -N(R^A)-, -S-, -C(=O)-, -C(=O)O-, -C(=O)NR^A-, -NR^AC(=O)-, -NR^AC(=O)R^A-, -C(=O)R^A-, -NR^AC(=O)O-, -NR^AC(=O)N(R^A)-, -OC(=O)-, -OC(=O)O-, -OC(=O)N(R^A)-, -S(O)₂NR^A-, -NR^AS(O)₂-, or a combination thereof; each R^A is independently hydrogen or substituted or unsubstituted alkyl. In certain embodiments, the spacer is a substituted or unsubstituted alkylene, substituted or unsubstituted heterocyclylene, substituted or unsubstituted heteroarylene, -O-, -N(R^A)-, or -C(=O)N(R^A)₂, or a combination thereof.

[00100] In some embodiments, the 5' or 3' nucleoside of any one of the oligonucleotides described herein (*e.g.*, the oligonucleotides listed in Tables 1 and 2) is conjugated to a compound of the formula -NH₂-(CH₂)_n-, wherein n is an integer from 1 to 12, inclusive. In some embodiments, n is 6, 7, 8, 9, 10, 11, or 12. In some embodiments, a phosphodiester linkage is present between the moiety of the formula NH₂-(CH₂)_n- and the 5' or 3' nucleoside of the oligonucleotide. In some embodiments, a moiety of the formula NH₂-

(CH₂)₆- is conjugated to the oligonucleotide via a reaction between 6-amino-1-hexanol (NH₂-(CH₂)₆-OH) and the 5' phosphate of the oligonucleotide. In some embodiments, other aminoalcohols can be used to effect the conjugation.

ii. RNA Interference (RNAi)

[00101] It should be appreciated that, in some embodiments, oligonucleotides in one format (*e.g.*, antisense oligonucleotides) may be suitably adapted to another format (*e.g.*, siRNA oligonucleotides) by incorporating functional sequences (*e.g.*, antisense strand sequences) from one format to the other format, as known in the art and described in Atri et al., “Chapter 6 - MicroRNAs in diagnosis and therapeutics” *AGO-Driven Non-Coding RNAs: Codes to Decode the Therapeutics of Diseases*, ed. Bibekanand Mallick, Elsevier 2019, Pages 137-177, <https://doi.org/10.1016/B978-0-12-815669-8.00006-3>.

[00102] In some embodiments, the ApoE ε4-targeting inhibitory nucleic acids provided herein are small interfering RNAs (siRNA), also known as short interfering RNA or silencing RNA. siRNA, is a class of double-stranded RNA molecules, typically about 20-25 base pairs in length that target nucleic acids (*e.g.*, mRNAs) for degradation via the RNA interference (RNAi) pathway in cells. The specificity of siRNA molecules may be determined by the binding of the antisense strand of the molecule to its target RNA. Effective siRNA molecules are generally less than 30 to 35 base pairs in length to prevent the triggering of non-specific RNA interference pathways in the cell via the interferon response, although longer siRNA can also be effective. In some embodiments, the siRNA molecules are 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, or more base pairs in length. In some embodiments, the siRNA molecules are 8 to 30 base pairs in length, 10 to 15 base pairs in length, 10 to 20 base pairs in length, 15 to 25 base pairs in length, 19 to 21 base pairs in length, or 21 to 23 base pairs in length.

[00103] Following selection of an appropriate target RNA sequence, siRNA molecules that comprise a nucleotide sequence complementary to all or a portion of the target sequence, *i.e.*, an antisense sequence, can be designed and prepared using methods known in the art (see, *e.g.*, PCT Publication Number WO 2004/016735; and U.S. Patent Publication Nos. 2004-0077574 and 2008-0081791; each of which is incorporated herein by reference).

[00104] The siRNA molecule can be double-stranded (*i.e.*, a dsRNA molecule comprising an antisense strand and a complementary sense strand) or single-stranded (*i.e.*, a ssRNA molecule comprising just an antisense strand). The siRNA molecules can comprise a duplex (*i.e.* comprising annealed sense and antisense strands with a 3' overhang), asymmetric

duplex (*i.e.* a duplex with 3' and 5' antisense overhangs), hairpin (*i.e.* when two regions of the same strand, usually complementary in nucleotide sequence when read in opposite directions, base-pair to form a double helix that ends in an unpaired loop), or asymmetric hairpin (*i.e.* hairpin with a strand overhang) secondary structure, having self-complementary sense and antisense strands. In some embodiments, the ApoE ϵ 4-targeting inhibitory nucleic acid described herein is an siRNA comprising an antisense strand and a sense strand.

[00105] In some embodiments, the antisense strand of the siRNA molecule is 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, or more nucleotides in length. In some embodiments, the antisense strand is 8 to 50 nucleotides in length, 8 to 40 nucleotides in length, 8 to 30 nucleotides in length, 10 to 15 nucleotides in length, 10 to 20 nucleotides in length, 15 to 25 nucleotides in length, 19 to 21 nucleotides in length, or 21 to 23 nucleotides in lengths.

[00106] In some embodiments, the sense strand of the siRNA molecule is 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, or more nucleotides in length. In some embodiments, the sense strand is 8 to 50 nucleotides in length, 8 to 40 nucleotides in length, 8 to 30 nucleotides in length, 10 to 15 nucleotides in length, 10 to 20 nucleotides in length, 15 to 25 nucleotides in length, 19 to 21 nucleotides in length, or 21 to 23 nucleotides in lengths.

[00107] In some embodiments, siRNA molecules comprise an antisense strand comprising a region of complementarity to a target region in a ApoE ϵ 4 mRNA (*e.g.*, the SNP rs429358). In some embodiments, the region of complementarity is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% complementary to a target region in a ApoE ϵ 4 mRNA (*e.g.*, the SNP rs429358). In some embodiments, the target region is a region of consecutive nucleotides in the ApoE ϵ 4 mRNA (*e.g.*, the SNP rs429358). In some embodiments, the target region extends over 30 or fewer base pairs on either or both sides of a SNP. In some embodiments, the target region extends over 20 or fewer base pairs on either or both sides of a SNP. In some embodiments, the SNP is rs429358. In some embodiments, a complementary nucleotide sequence need not be 100% complementary to that of its target to be specifically hybridizable or specific for ApoE ϵ 4 mRNA.

[00108] In some embodiments, siRNA molecules comprise an antisense strand that comprises a region of complementarity to an ApoE ϵ 4 mRNA (*e.g.*, the SNP rs429358) sequence and the region of complementarity is in the range of 8 to 15, 8 to 30, 8 to 40, or 10

to 50, or 5 to 50, or 5 to 40 nucleotides in length. In some embodiments, the region of complementarity 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 nucleotides in length. In some embodiments, the region of complementarity is complementary to at least 6, 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, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, or more consecutive nucleotides of an ApoE ϵ 4 mRNA (*e.g.*, the SNP rs429358). In some embodiments, the region of complementarity comprises a nucleotide sequence that contains no more than 1, 2, 3, 4, or 5 base mismatches compared to the complementary portion of a ApoE ϵ 4 mRNA (*e.g.*, the SNP rs429358). In some embodiments, the region of complementarity comprises a nucleotide sequence that has up to 3 mismatches over 15 bases, up to 2 mismatches over 10 bases, or up to 1 mismatch over 5 bases.

[00109] In some embodiments, siRNA molecules comprise an antisense strand comprising a nucleotide sequence that is complementary (*e.g.*, at least 85%, at least 90%, at least 95%, or 100%) to a target RNA sequence as set forth in any one of SEQ ID NOs: 1, 2, or 13. In some embodiments, siRNA molecules comprise an antisense strand comprising a nucleotide sequence that is at least 85%, at least 90%, at least 95%, or 100% identical to the sequence as set forth in any one of SEQ ID NOs: 1, 2 or 13. In some embodiments, siRNA molecules comprise an antisense strand comprising at least 6, 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, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, or more consecutive nucleotides of the sequence as set forth in any one of SEQ ID NOs: 1, 2, or 13.

[00110] Double-stranded siRNA may comprise RNA strands that are the same length or different lengths. Double-stranded siRNA molecules can also be assembled from a single oligonucleotide in a stem-loop structure, wherein self-complementary sense and antisense regions of the siRNA molecule are linked by means of a nucleic acid based or non-nucleic acid-based linker(s), as well as circular single-stranded RNA having two or more loop structures and a stem comprising self-complementary sense and antisense strands, wherein the circular RNA can be processed either *in vivo* or *in vitro* to generate an active siRNA molecule capable of mediating RNAi. Small hairpin RNA (shRNA) molecules are also contemplated herein. These molecules comprise a specific antisense sequence in addition to the reverse complement (sense) sequence, typically separated by a spacer or loop sequence.

Cleavage of the spacer or loop provides a single-stranded RNA molecule and its reverse complement, such that they may anneal to form a dsRNA molecule (optionally with additional processing steps that may result in the addition or removal of one, two, three, or more nucleotides from the 3' end and/or (*e.g.*, and) the 5' end of either or both strands). A spacer can be of a sufficient length to permit the antisense and sense sequences to anneal and form a double-stranded structure (or stem) prior to cleavage of the spacer (and, optionally, subsequent processing steps that may result in the addition or removal of one, two, three, four, or more nucleotides from the 3' end and/or (*e.g.*, and) the 5' end of either or both strands). A spacer sequence may be an unrelated nucleotide sequence that is situated between two complementary nucleotide sequence regions which, when annealed into a double-stranded nucleic acid, comprise a shRNA.

[00111] The overall length of the siRNA molecules can vary from about 14 to about 100 nucleotides depending on the type of siRNA molecule being designed. Generally between about 14 and about 50 of these nucleotides are complementary to the RNA target sequence, *i.e.*, constitute the specific antisense sequence of the siRNA molecule. For example, when the siRNA is a double- or single-stranded siRNA, the length can vary from about 14 to about 50 nucleotides, whereas when the siRNA is a shRNA or circular molecule, the length can vary from about 40 nucleotides to about 100 nucleotides.

[00112] An siRNA molecule may comprise a 3' overhang at one end of the molecule, the other end may be blunt-ended or have also an overhang (5' or 3'). When the siRNA molecule comprises an overhang at both ends of the molecule, the length of the overhangs may be the same or different. In one embodiment, the siRNA molecule of the present disclosure comprises 3' overhangs of about 1 to about 3 nucleotides on both ends of the molecule. In some embodiments, the siRNA molecule comprises 3' overhangs of about 1 to about 3 nucleotides on the sense strand. In some embodiments, the siRNA molecule comprises 3' overhangs of about 1 to about 3 nucleotides on the antisense strand. In some embodiments, the siRNA molecule comprises 3' overhangs of about 1 to about 3 nucleotides on both the sense strand and the antisense strand.

[00113] In some embodiments, the siRNA or shRNA molecule comprises one or more modified nucleotides (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more). In some embodiments, the siRNA or shRNA molecule comprises one or more modified nucleotides and/or (*e.g.*, and) one or more modified internucleotide linkages. In some embodiments, the modified nucleotide is a modified sugar moiety (*e.g.* a 2' modified nucleotide). In some embodiments, the siRNA molecule comprises one or more 2' modified nucleotides, *e.g.*, a 2'-deoxy, 2'-

fluoro (2'-F), 2'-O-methyl (2'-O-Me), 2'-O-methoxyethyl (2'-MOE), 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), 2'-O-dimethylaminoethoxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA). In some embodiments, each nucleotide of the siRNA molecule is a modified nucleotide (*e.g.*, a 2'-modified nucleotide). In some embodiments, the siRNA molecule comprises one or more 2'-O-methyl modified nucleotides. In some embodiments, the siRNA molecule comprises one or more 2'-F modified nucleotides. In some embodiments, the siRNA molecule comprises one or more 2'-O-methyl and 2'-F modified nucleotides.

[00114] In some embodiments, the siRNA or shRNA molecule contains a phosphorothioate or other modified internucleotide linkage. In some embodiments, the siRNA molecule comprises phosphorothioate internucleotide linkages. In some embodiments, the siRNA molecule comprises phosphorothioate internucleotide linkages between at least two nucleotides. In some embodiments, the siRNA molecule comprises phosphorothioate internucleotide linkages between all nucleotides. In some embodiments, the siRNA molecule comprises modified internucleotide linkages at the first, second, and/or (*e.g.*, and) third internucleotide linkage at the 5' or 3' end of the siRNA molecule.

[00115] In some embodiments, the modified internucleotide linkages are phosphorus-containing linkages. In some embodiments, phosphorus-containing linkages that may be used include, but are not limited to, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates comprising 3' alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates comprising 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs of these, and those having inverted polarity wherein adjacent pairs of nucleosides are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'; see U.S. Patent Nos. 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,196; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,306; 5,550,111; 5,563,253; 5,571,799; 5,587,361; and 5,625,050; each of which is incorporated herein by reference.

[00116] Any of the modified chemistries or formats of siRNA or shRNA molecules described herein can be combined with each other. For example, one, two, three, four, five, or more different types of modifications can be included within the same siRNA molecule.

[00117] In some embodiments, the antisense strand and/or the sense strand of the siRNA or shRNA molecule comprises one or more modified nucleotides (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more). In some embodiments, the antisense strand and/or the sense strand of the siRNA or shRNA molecule comprises one or more modified nucleotides and/or (*e.g.*, and) one or more modified internucleotide linkages. In some embodiments, the modified nucleotide comprises a modified sugar moiety (*e.g.* a 2' modified nucleotide). In some embodiments, the antisense strand and/or the sense strand of the siRNA or shRNA molecule comprises one or more 2' modified nucleotides, *e.g.*, a 2'-deoxy, 2'-fluoro (2'-F), 2'-O-methyl (2'-O-Me), 2'-O-methoxyethyl (2'-MOE), 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), 2'-O-dimethylaminoethoxyethyl (2'-O-DMAEOE), or 2'-O—N-methylacetamido (2'-O—NMA). In some embodiments, each nucleotide of the antisense strand and/or the sense strand of the siRNA molecule is a modified nucleotide (*e.g.*, a 2'-modified nucleotide). In some embodiments, the antisense strand and/or the sense strand of the siRNA or shRNA molecule comprises one or more phosphorodiamidate morpholinos. In some embodiments, the antisense strand and/or the sense strand of the siRNA molecule is a phosphorodiamidate morpholino oligomer (PMO).

[00118] In some embodiments, the antisense strand and/or the sense strand of the siRNA or shRNA molecule contains a phosphorothioate or other modified internucleotide linkage. In some embodiments, the antisense strand and/or the sense strand of the siRNA or shRNA molecule comprises phosphorothioate internucleotide linkages. In some embodiments, the antisense strand and/or the sense strand of the siRNA or shRNA molecule comprises phosphorothioate internucleotide linkages between at least two nucleotides. In some embodiments, the antisense strand and/or the sense strand of the siRNA or shRNA molecule comprises phosphorothioate internucleotide linkages between all nucleotides. For example, in some embodiments, the antisense strand and/or the sense strand of the siRNA or shRNA molecule comprises modified internucleotide linkages at the first, second, and/or (*e.g.*, and) third internucleotide linkage at the 5' or 3' end of the siRNA or shRNA molecule. In some embodiments, the two internucleotide linkages at the 3' end of the antisense strands are phosphorothioate internucleotide linkages. In some embodiments, the modified internucleotide linkages are phosphorus-containing linkages.

[00119] Any of the modified chemistries or formats of the antisense strand and/or the sense strand of the siRNA molecule described herein can be combined with each other. For

example, one, two, three, four, five, or more different types of modifications can be included within the same antisense strand.

[00120] In some embodiments, the antisense and/or sense strand of the siRNA or shRNA molecule comprises modifications that enhance or reduce RNA-induced silencing complex (RISC) loading. In some embodiments, the antisense strand of the siRNA or shRNA molecule comprises modifications that enhance RISC loading. In some embodiments, the sense strand of the siRNA molecule comprises modifications that reduce RISC loading and reduce off-target effects. In some embodiments, the antisense strand of the siRNA or shRNA molecule comprises a 2'-methoxyethyl (2'-MOE) modification. The addition of the 2'-methoxyethyl (2'-MOE) group at the cleavage site improves both the specificity and silencing activity of siRNAs by facilitating the oriented RNA-induced silencing complex (RISC) loading of the modified strand, as described in Song *et al.*, (2017) *Mol. Ther. Nucleic Acids* 9:242-250, incorporated herein by reference in its entirety. In some embodiments, the antisense strand of the siRNA or shRNA molecule comprises a 2'-O-Me-phosphorodithioate modification, which increases RISC loading as described in Wu *et al.*, (2014) *Nat. Commun.* 5:3459, incorporated herein by reference in its entirety.

[00121] In some embodiments, the sense strand of the siRNA or shRNA molecule comprises a 5'-morpholino, which reduces RISC loading of the sense strand and improves antisense strand selection and RNAi activity, as described in Kumar *et al.*, (2019) *Chem. Commun. (Camb)* 55(35):5139-5142, incorporated herein by reference in its entirety. In some embodiments, the sense strand of the siRNA molecule is modified with a synthetic RNA-like high affinity nucleotide analogue, Locked Nucleic Acid (LNA), which reduces RISC loading of the sense strand and further enhances antisense strand incorporation into RISC, as described in Elman *et al.*, (2005) *Nucleic Acids Res.* 33(1): 439-447, incorporated herein by reference in its entirety. In some embodiments, the sense strand of the siRNA molecule comprises a 5' unlocked nucleic acid (UNA) modification, which reduces RISC loading of the sense strand and improves silencing potency of the antisense strand, as described in Snead *et al.*, (2013) *Mol. Ther. Nucleic Acids* 2(7):e103, incorporated herein by reference in its entirety. In some embodiments, the sense strand of the siRNA molecule comprises a 5-nitroindole modification, which decreased the RNAi potency of the sense strand and reduces off-target effects as described in Zhang *et al.*, (2012) *Chembiochem* 13(13):1940-1945, incorporated herein by reference in its entirety. In some embodiments, the sense strand comprises a 2'-O-methyl (2'-O-Me) modification, which reduces RISC loading and the off-target effects of the sense strand, as described in Zheng *et al.*, *FASEB* (2013) 27(10): 4017-

4026, incorporated herein by reference in its entirety. In some embodiments, the sense strand of the siRNA molecule is fully substituted with morpholino, 2'-MOE or 2'-O-Me residues, and are not recognized by RISC as described in Kole *et al.*, (2012) *Nature Reviews. Drug Discovery* 11(2):125-140, incorporated herein by reference in its entirety. In some embodiments, the antisense strand of the siRNA molecule comprises a MOE modification and the sense strand comprises an 2'-O-Me modification (see *e.g.*, Song *et al.*, (2017) *Mol. Ther. Nucleic Acids* 9:242-250).

[00122] Any of the ApoE ϵ 4 antisense oligonucleotides as set forth in Tables 1 and 2 can be adapted to be the antisense strand of the siRNA or shRNA. Exemplary antisense strand of the RNAi (*e.g.*, siRNA or shRNA) molecules targeting ApoE ϵ 4 are set forth in Table 3.

Table 3. Antisense strand of siRNA or shRNA targeting ApoE ϵ 4:

RNAi ID	Antisense strand sequence (5' to 3')
RNAi1	GCCGCGCACGUCCUCCA (SEQ ID NO: 46)
RNAi2	GGCCGCGCACGUCCUCC (SEQ ID NO: 47)
RNAi3	CGGCCGCGCACGUCCUC (SEQ ID NO: 48)
RNAi4	GCGGCCGCGCACGUCCU (SEQ ID NO: 49)
RNAi5	GGCGGCCGCGCACGUCC (SEQ ID NO: 50)
RNAi6	AGGCGGCCGCGCACGUC (SEQ ID NO: 51)
RNAi7	GCCGCGCACGUCCUCCAUG (SEQ ID NO: 52)
RNAi8	GGCCGCGCACGUCCUCCAU (SEQ ID NO: 53)
RNAi9	CGGCCGCGCACGUCCUCCA (SEQ ID NO: 54)
RNAi10	GCGGCCGCGCACGUCCUCC (SEQ ID NO: 55)
RNAi11	AGGCGGCCGCGCACGUCCU (SEQ ID NO: 56)
RNAi12	GGCGGCCGCGCACGUCCUC (SEQ ID NO: 57)

[00123] In some embodiments, the ApoE ϵ 4-targeting siRNA or shRNA comprises an antisense strand that is 18-25 nucleosides (*e.g.*, 18, 19, 20, 21, 22, 23, 24, or 25 nucleosides) in length and comprises a region of complementarity to the sequence as set forth in any one of SEQ ID NOs: 1, 2, or 13, wherein the region of complementarity is at least 13 nucleotides (*e.g.*, 13, 14, 15, 16, 17, 18, or 19 nucleotides) in length. In some embodiments, the antisense strand is 17 nucleotides in length and comprises a region of complementarity to the sequence as set forth in any one of SEQ ID NOs: 1, 2, or 13, wherein the region of complementarity is 19 nucleotides in length. In some embodiments, the region of complementarity is fully complementarity with all or a portion of its target sequence. In some embodiments, the region of complementarity includes 1, 2, 3, or more mismatches.

[00124] In some embodiments, the ApoE ϵ 4-targeting siRNA or shRNA comprises an antisense strand that comprises at least 15 consecutive nucleosides of (*e.g.*, at least 15, at least 16, at least 17, at least 18, at least 19, or at least 20) the sequence of any one of SEQ ID NOs: 46-57. In some embodiments, the ApoE ϵ 4-targeting siRNA or shRNA further comprises a sense strand that comprises at least 15 consecutive nucleosides (*e.g.*, at least 15, at least 16, at least 17, at least 18, at least 19, or at least 20) complementary to the sequence of any one of SEQ ID NOs: 46-57. In some embodiment, the one or more modified nucleosides are selected from 2'-O-Me and 2'-F modified nucleosides.

[00125] In some embodiments, the 5' or 3' nucleoside (*e.g.*, terminal nucleoside) of the antisense strand of any one of the oligonucleotides described herein (*e.g.*, the oligonucleotides listed in Table 3) is conjugated to an amine group, optionally via a spacer. In some embodiments, the 5' or 3' nucleoside (*e.g.*, terminal nucleoside) of the sense strand of any one of the oligonucleotides described herein (*e.g.*, sense strand of the oligonucleotides listed in Table 3) is conjugated to an amine group, optionally via a spacer.

[00126] In some embodiments, the spacer comprises an aliphatic moiety. In some embodiments, the spacer comprises a polyethylene glycol moiety. In some embodiments, the 5' or 3' nucleoside (*e.g.*, terminal nucleoside) of the antisense strand or the sense strand of any of the oligonucleotides described herein (*e.g.*, the oligonucleotides listed in Tables 3) is conjugated to a spacer that is a substituted or unsubstituted aliphatic, substituted or unsubstituted heteroaliphatic, substituted or unsubstituted carbocyclene, substituted or unsubstituted heterocyclene, substituted or unsubstituted arylene, substituted or unsubstituted heteroarylene, -O-, -N(R^A)-, -S-, -C(=O)-, -C(=O)O-, -C(=O)NR^A-, -NR^AC(=O)-, -NR^AC(=O)R^A-, -C(=O)R^A-, -NR^AC(=O)O-, -NR^AC(=O)N(R^A)-, -OC(=O)-, -OC(=O)O-, -OC(=O)N(R^A)-, -S(O)₂NR^A-, -NR^AS(O)₂-, or a combination thereof; each R^A is independently hydrogen or substituted or unsubstituted alkyl. In certain embodiments, the spacer is a substituted or unsubstituted alkylene, substituted or unsubstituted heterocyclene, substituted or unsubstituted heteroarylene, -O-, -N(R^A)-, or -C(=O)N(R^A)₂, or a combination thereof. In some embodiments, a phosphodiester linkage is present between the spacer and the 5' or 3' nucleoside of the oligonucleotide.

[00127] In some embodiments, the 5' or 3' nucleoside of the sense strand or the antisense strand of any one of the oligonucleotides described herein (*e.g.*, the oligonucleotides listed in Table 3) is conjugated to a moiety of the formula -NH₂-(CH₂)_n-, wherein n is an integer from 1 to 12, inclusive. In some embodiments, n is 6, 7, 8, 9, 10, 11, or 12, inclusive. In some embodiments, a phosphodiester linkage is present between the

moiety of the formula $-\text{NH}_2-(\text{CH}_2)_n-$ and the 5' or 3' nucleoside of the sense strand or the antisense strand of the oligonucleotide.

iii. microRNA (miRNAs)

[00128] In another aspect, an ApoE ϵ 4-targeting inhibitory nucleic acid may be a microRNA (miRNA). MicroRNAs (referred to as “miRNAs”) are small non-coding RNAs, belonging to a class of regulatory molecules that control gene expression by binding to complementary sites on a target transcript, *e.g.*, an ApoE ϵ 4 RNA transcript. Typically, miRNAs are generated from large RNA precursors (termed pri-miRNAs) that are processed in the nucleus into approximately 70 nucleotide pre-miRNAs, which fold into imperfect stem-loop structures.

[00129] These pre-miRNAs typically undergo an additional processing step within the cytoplasm where mature miRNAs of 18-25 nucleotides in length are excised from one side of the pre-miRNA hairpin by an RNase III enzyme, Dicer. Any of the RNAi sequences described herein can be adapted to mature miRNA sequences that can be encoded by pre-miRNA. It is within the skill of one of ordinary skill in the art to design pre-miRNA sequences encoding a mature miRNA.

[00130] As used herein, miRNAs include pri-miRNA, pre-miRNA, mature miRNA, and fragments and variants thereof that retain the biological activity of a mature miRNA. In one embodiment, the size range of the ApoE ϵ 4-targeting miRNA is from 21 nucleotides to 170 nucleotides. In another embodiment, the size range of the ApoE ϵ 4-targeting miRNA is from 70 to 170 nucleotides in length. In another embodiment, mature ApoE ϵ 4-targeting miRNAs 21 to 25 nucleotides in length are used. In one embodiment, the target region of the miRNA sequence includes the SNP rs429358 and up to 25 nucleotides on either or both sides of the SNP rs429358.

iv. Mixmers

[00131] In another aspect, an oligonucleotide described herein may be an ApoE ϵ 4-targeting mixmer or include an ApoE ϵ 4-targeting mixmer sequence pattern. In general, mixmers are oligonucleotides that comprise both naturally and non-naturally occurring nucleosides or comprise two different types of non-naturally occurring nucleosides typically in an alternating pattern. Mixmers generally have higher binding affinity than unmodified oligonucleotides and may be used to specifically bind a target molecule, *e.g.*, to block a

binding site on the target molecule. Generally, mixmers do not recruit an RNase to the target molecule, for instance ApoE ϵ 4 RNA, and thus do not promote cleavage of the ApoE ϵ 4 RNA. Such oligonucleotides that are incapable of recruiting RNase H have been described, for example, see WO 2007/112754 or WO 2007/112753, each of which is incorporated herein by reference.

[00132] In some embodiments, the ApoE ϵ 4-targeting mixmer comprises or consists of a repeating pattern of nucleoside analogues and naturally occurring nucleosides, or one type of nucleoside analogue and a second type of nucleoside analogue. However, a mixmer need not comprise a repeating pattern and may instead comprise any arrangement of modified nucleosides, as described herein, and naturally occurring nucleosides or any arrangement of one type of modified nucleoside and a second type of modified nucleoside. The repeating pattern may, for instance, be every second or every third nucleoside is a modified nucleoside, such as an LNA, and the remaining nucleosides are naturally occurring nucleosides, such as DNA nucleosides (e.g., A, T, G, C), or are a 2' substituted nucleoside analogues, such as 2'-MOE or 2' fluoro analogues, or any other modified nucleosides described herein. It is recognized that the repeating pattern of modified nucleosides, such as LNAs, may be combined with modified nucleosides at fixed positions—*e.g.* at the 5' or 3' termini.

[00133] In some embodiments, an ApoE ϵ 4-targeting mixmer does not comprise a region of more than 5, more than 4, more than 3, or more than 2 consecutive naturally occurring nucleosides, such as DNA nucleosides (e.g., A, T, G, C). In some embodiments, the mixmer comprises at least a region consisting of at least two consecutive modified nucleoside, such as at least two consecutive LNAs. In some embodiments, the ApoE ϵ 4-targeting mixmer comprises a region consisting of at least three consecutive modified nucleoside units, such as at least three consecutive LNAs.

[00134] In some embodiments, the ApoE ϵ 4-targeting mixmer does not comprise a region of more than 7, more than 6, more than 5, more than 4, more than 3, or more than 2 consecutive nucleoside analogues, such as LNAs. In some embodiments, LNA units may be replaced with other nucleoside analogues, such as those referred to herein.

[00135] ApoE ϵ 4-targeting mixmers may be designed to include a mixture of affinity enhancing modified nucleosides, such as the non-limiting examples LNA nucleosides and 2'-O-Me nucleosides. In some embodiments, an ApoE ϵ 4-targeting mixmer comprises modified internucleotide linkages (*e.g.*, phosphorothioate internucleotide linkages or other linkages as

described herein) between at least two, at least three, at least four, at least five or more nucleosides.

[00136] An ApoE ϵ 4-targeting mixmer may be produced using any suitable method. Representative U.S. patents, U.S. patent publications, and PCT publications that teach the preparation of mixmers include U.S. Patent Publication Nos. US-2006-0128646, US-2009-0209748, US-2009-0298916, US-2011-0077288, and US-2012-0322851, and U.S. Patent No. 7,687,617; each of which is incorporated herein by reference.

[00137] In some embodiments, an ApoE ϵ 4-targeting mixmer comprises one or more morpholino nucleosides. For example, in some embodiments, the ApoE ϵ 4-targeting mixmer may comprise morpholino nucleosides mixed (*e.g.*, in an alternating manner) with one or more other nucleosides (*e.g.*, deoxyribonucleosides or ribonucleosides) or modified nucleosides (*e.g.*, 2'-MOE, LNA, 2'-O-Me nucleosides).

v. Aptamers

[00138] In yet another aspect, inhibitory nucleic acids provided herein may be in the form of ApoE ϵ 4-targeting aptamers. Generally, an aptamer is any nucleic acid that binds specifically to a target, such as a small molecule, protein, or nucleic acid. In some embodiments, the aptamer is a DNA aptamer or an RNA aptamer. In some embodiments, an ApoE ϵ 4-targeting nucleic acid aptamer is a single-stranded DNA or RNA (ssDNA or ssRNA). It is to be understood that a single-stranded nucleic acid aptamer may form helices and/or (*e.g.*, and) loop structures. The nucleic acid that forms the nucleic acid aptamer may comprise naturally occurring nucleotides, modified nucleotides, naturally occurring nucleotides with any type of modified linker as described herein inserted between one or more nucleotides, modified nucleotides with any type of modified linker as described herein inserted between one or more nucleotides, or a combination of thereof. Exemplary publications and patents describing aptamers and method of producing aptamers include, *e.g.*, Jon R. Lorsch and, Jack W. Szostak, Chance and Necessity in the Selection of Nucleic Acid Catalysts, *Accounts of Chemical Research* 1996, 29 (2):103-110; ; S.D. Jayasena, Aptamers: an emerging class of molecules that rival antibodies in diagnostics, *Clinical Chemistry* 1999, 45(9):1628-1650; U.S. Patent Nos. 5,270,163; 5,567,588; 5,650,275; 5,670,637; 5,683,867; 5,696,249; 5,789,157; 5,843,653; 5,864,026; 5,989,823; 6,569,630; and 8,318,438; and published PCT application WO 99/31275, each of which is incorporated herein by reference.

[00139] In some embodiments, the oligonucleotides in Table 1 comprises deoxynucleotide. In some embodiments, one or more of the thymine bases (T's) in any one of the oligonucleotides provided in Tables 2 and 3 may optionally be uracil bases (U's), and/or one or more of the U's may optionally be T's.

II. Pharmaceutical Composition

[00140] Inhibitory nucleic acids (*e.g.*, ASOs, siRNAs, shRNAs, miRNAs, mixmers, aptamers) provided herein may be formulated in any suitable manner. Generally, inhibitory nucleic acids (*e.g.*, ASOs, siRNAs, shRNAs, miRNAs, mixmers, aptamers) provided herein are formulated in a manner suitable for pharmaceutical use as described herein. For example, inhibitory nucleic acids (*e.g.*, ASOs, siRNAs, shRNAs, miRNAs, mixmers, aptamers) can be delivered to a subject using a formulation that minimizes degradation, facilitates delivery and/or (*e.g.*, and) uptake, and/or (*e.g.*, and) provides another beneficial property to the inhibitory nucleic acids (*e.g.*, ASOs, siRNAs, shRNAs, miRNAs, mixmers, aptamers) in the formulation. In some embodiments, provided herein are compositions comprising inhibitory nucleic acids (*e.g.*, ASOs, siRNAs, shRNAs, miRNAs, mixmers, aptamers) and pharmaceutically acceptable excipients. Such compositions can be suitably formulated such that when administered to a subject, either into the immediate environment of a target cell (*e.g.*, cell of the central nervous system, neuron, glial cell, astrocyte, oligodendrocyte, microglial cell and ependymal cell), or systemically, a sufficient amount of the inhibitory nucleic acids (*e.g.*, ASOs, siRNAs, shRNAs, miRNAs, mixmers, aptamers) enter target cells (*e.g.*, cells of the CNS, neurons, glial cells, astrocytes, oligodendrocytes, microglia and ependymal cells). In some embodiments, inhibitory nucleic acids (*e.g.*, ASOs, siRNAs, shRNAs, miRNAs, mixmers, aptamers) are formulated in buffered solutions, such as phosphate-buffered saline solutions, liposomes, micellar structures, and capsids, for example as described in US Patent No. 9,950,068, US Patent No. 9,050,373, US Patent No. 9,314,529, US Patent No. 8,877,237, US Patent Application Publication No. 20200085974 and US Patent Application Publication No. 20110274745, all of which are herein incorporated by reference.

[00141] It should be appreciated that, in some embodiments, compositions may include one or more types of inhibitory nucleic acids provided herein (*e.g.*, ASOs, siRNAs, shRNAs, miRNAs, mixmers, aptamers).

[00142] In some embodiments, inhibitory nucleic acids (*e.g.*, ASOs, siRNAs, shRNAs, miRNAs, mixmers, aptamers) are formulated in water or in an aqueous solution (*e.g.*, water

with pH adjustments). In some embodiments, inhibitory nucleic acids (*e.g.*, ASOs, siRNAs, shRNAs, miRNAs, mixmers, aptamers) are formulated in a basic buffered aqueous solution (*e.g.*, PBS) with a pH of 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9.0, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9, 10.0, 10.1, 10.2, 10.3, 10.4, 10.5, 10.6, 10.7, 10.8, 10.9, 11.0, 11.1, 11.2, 11.3, 11.4, 11.5, 11.6, 11.7, 11.8, 11.9, 12.0, 12.1, 12.2, 12.3, 12.4, 12.5, 12.6, 12.7, 12.8, 12.9, 13.0, 13.1, 13.2, 13.3, 13.4, 13.5, 13.6, 13.7, 13.8, or 13.9. In some embodiments, formulations as disclosed herein comprise an excipient. In some embodiments, an excipient confers to a composition improved stability, improved absorption, improved solubility, and/or (*e.g.*, and) therapeutic enhancement of the inhibitory nucleic acid. In some embodiments, an excipient is a buffering agent (*e.g.*, sodium citrate, sodium phosphate, a tris base, or sodium hydroxide), a sugar (*e.g.*, lactose, sucrose, mannitol or sorbitol), a vehicle (*e.g.*, a buffered solution, petrolatum, dimethyl sulfoxide, alcohol, or mineral oil, micelle, liposome, capsid, lipid nanoparticle), a salt, a cellulose preparation, a polymer, a lipid, an antioxidant, a preservative, a calcium phosphate, a binder (*e.g.*, starch, gelatin, methyl cellulose, hydroxypropyl methylcellulose, sodium carboxymethylcellulose, and/or polyvinyl pyrrolidone), or any combination thereof.

[00143] In some embodiments, an inhibitory nucleic acid (*e.g.*, ASO, siRNA, shRNA, miRNA, mixmer, aptamer) is lyophilized for extending its shelf-life and then made into a solution before use (*e.g.*, before administration to a subject). Accordingly, an excipient in a composition comprising an inhibitory nucleic acid (*e.g.*, ASO, siRNA, shRNA, miRNA, mixmer, aptamer) described herein may be a lyoprotectant (*e.g.*, mannitol, lactose, polyethylene glycol, or polyvinyl pyrrolidone) or a collapse temperature modifier (*e.g.*, dextran, ficoll, or gelatin).

[00144] In some embodiments, a pharmaceutical composition is formulated to be compatible with its intended route of administration. As used herein, the terms “administering” or “administration” means to provide an inhibitory nucleic acids (*e.g.*, ASOs, siRNAs, shRNAs, miRNAs, mixmers, aptamers) to a subject in a manner that is physiologically and/or (*e.g.*, and) pharmacologically useful (*e.g.*, to treat a condition in the subject). Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, administration, or direct administration to the CNS (*e.g.*, intracerebral injection, intraventricular injection, intracisternal injection, intraparenchymal injection, intrathecal injection, and any combination of the foregoing).

[00145] Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the instant

preparation of sterile injectable solutions or dispersions. The excipient can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol, and the like), and suitable mixtures thereof. In some embodiments, formulations include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, and sodium chloride, in the composition. Sterile injectable solutions can be prepared by incorporating the inhibitory nucleic acids (*e.g.*, ASOs, siRNAs, shRNAs, miRNAs, mixmers, aptamers) in a required amount in a selected solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization.

[00146] In some embodiments, a composition may contain at least about 0.1%, at least about 0.5%, at least about 1%, at least about 2%, at least about 3%, at least about 4%, at least about 5%, at least about 10%, at least about 12%, at least about 15%, at least about 18%, or at least about 20% of an inhibitory nucleic acid (*e.g.*, ASOs, siRNAs, shRNAs, miRNAs, mixmers, aptamers) described herein, or more, although the percentage of the inhibitory nucleic acid may be between about 1% and about 80% or more of the weight or volume of the total composition. Factors such as solubility, bioavailability, biological half-life, route of administration, product shelf life, as well as other pharmacological considerations will be contemplated by one skilled in the art of preparing such pharmaceutical formulations, and as such, a variety of dosages and treatment regimens may be desirable.

III. Therapeutic Applications

[00147] Inhibitory nucleic acids (*e.g.*, ASOs, siRNAs, shRNAs, miRNAs, mixmers, aptamers) described herein are effective in treating, preventing, slowing the progression and/or reversing Alzheimer's Disease (AD) in a subject having, suspected of having AD, or susceptible of developing AD (*e.g.*, carrying the ApoE ϵ 4 allele). Inhibitory nucleic acids (*e.g.*, ASOs, siRNAs, shRNAs, miRNAs, mixmers, aptamers) described herein can also be used in treating, preventing, slowing the progression and/or reversing other cognitive and neurological diseases, including but not limited to stroke, Parkinson's disease, Lou Gehrig's disease, Charcot-Marie-Tooth disease, multiple sclerosis, diabetic neuropathy, sleep apnea, Lewy body disease, and ischemia of the central nervous system. As used herein, the term "subject" refers to a mammal. In some embodiments, the subject is a non-human primate or rodent. In some embodiments, the subject is a human. In some embodiments, the subject is a patient, *e.g.*, a human patient that has or is suspected of having a disease. In some embodiments, the subject is a human patient who has or is suspected of having a disease

resulting from carrying a disease-associated-allele, *e.g.*, an ApoE ϵ 4 allele. As used herein, the term “treating” or “treatment” refers to the application or administration of a composition including one or more active agents (*e.g.*, inhibitory nucleic acids described herein) to a subject, who has a disease or disorder (*e.g.*, AD), a symptom of the disease or disorder (*e.g.*, AD), or a predisposition toward the disease or disorder (*e.g.*, AD), with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve, or affect the disease or disorder, a symptom of the disease or disorder, or the predisposition toward the disease or disorder. Alleviating a target disease/disorder includes delaying or preventing the development or progression of the disease or disorder, or reducing the severity of the disease or disorder.

[00148] In some embodiments, inhibitory nucleic acids (*e.g.*, ASOs, siRNAs, shRNAs, miRNAs, mixmers, aptamers) are effective in treating AD. In some embodiments, inhibitory nucleic acids (*e.g.*, ASOs, siRNAs, shRNAs, miRNAs, mixmers, aptamers) are effective in preventing, slowing the progression and/or reversing AD. In some embodiments, AD is associated with the presence of one or more ApoE ϵ 4 alleles.

[00149] In some embodiments, a subject may be a human subject, a non-human primate subject, a rodent subject, or any other suitable mammalian subject. In some embodiments, a subject may have AD. In some embodiments, a subject has one or more ApoE ϵ 4 alleles, which may increase the risk of the subject developing AD. In some embodiments, a subject is suffering from one or more symptoms of AD, *e.g.*, memory loss, poor judgment leading to bad decisions, loss of spontaneity and sense of initiative, taking longer to complete normal daily tasks, repeating questions, trouble handling money and paying bills, wandering and getting lost, losing things or misplacing them in odd places, mood and personality changes, increased anxiety and/or aggression, *etc.* In some embodiments, a subject is not yet suffering from any symptoms of AD.

[00150] An aspect of the disclosure includes a method involving administering to a subject an effective amount of an inhibitory nucleic acid (*e.g.*, ASOs, siRNAs, shRNAs, miRNAs, mixmers, aptamers) as described herein. In some embodiments, an effective amount of a pharmaceutical composition that comprises an inhibitory nucleic acid (*e.g.*, ASO, siRNA, shRNA, miRNA, mixmer, aptamer) can be administered to a subject in need of treatment. In some embodiments, a pharmaceutical composition comprising an inhibitory nucleic acid (*e.g.*, ASO, siRNA, shRNA, miRNA, mixmer, aptamer) as described herein may be administered by a suitable route, which may include intravenous administration, *e.g.*, as a bolus or by continuous infusion over a period of time, or by direct injection into the CNS. In

some embodiments, a pharmaceutical composition may be in solid form, aqueous form, or a liquid form. In some embodiments, an aqueous or liquid form may be lyophilized. In some embodiments, a lyophilized form may be reconstituted with an aqueous or liquid solution.

[00151] Compositions for intravenous administration may contain various carriers, such as vegetable oils, dimethylactamide, dimethylformamide, ethyl lactate, ethyl carbonate, isopropyl myristate, ethanol, and polyols (glycerol, propylene glycol, liquid polyethylene glycol, and the like). For intravenous injection, water soluble inhibitory nucleic acids can be administered by the drip method, whereby a pharmaceutical formulation containing the inhibitory nucleic acids and physiologically acceptable excipients is infused. Physiologically acceptable excipients may include, for example, 5% dextrose, 0.9% saline, Ringer's solution or other suitable excipients. Intramuscular preparations, can be dissolved and administered in a pharmaceutical excipient such as Water-for-Injection, 0.9% saline, or 5% glucose solution.

[00152] In some embodiments, a pharmaceutical composition that comprises an inhibitory nucleic acid (*e.g.*, ASOs, siRNAs, shRNAs, miRNAs, mixmers, aptamers) is administered via site-specific or local delivery techniques, *e.g.*, direct injection into the CNS. Examples of these techniques include intracerebral injection, intraventricular injection, intracisternal injection, intraparenchymal injection, intrathecal injection, and any combination of the foregoing.

[00153] In some embodiments, a pharmaceutical composition that comprises an inhibitory nucleic acids (*e.g.*, ASOs, siRNAs, shRNAs, miRNAs, mixmers, aptamers) is administered at an effective concentration that confers a therapeutic effect on the subject. Effective amounts vary, as recognized by those skilled in the art, depending on the severity of the disease, unique characteristics of the subject being treated, *e.g.* age, physical conditions, health, or weight, the duration of the treatment, the nature of any concurrent therapies, the route of administration and related factors. These related factors are known to those in the art and may be addressed with no more than routine experimentation. In some embodiments, an effective concentration is the maximum dose that is considered to be safe for the patient. In some embodiments, an effective concentration will be the lowest possible concentration that provides maximum efficacy.

[00154] Empirical considerations, *e.g.* the half-life of the inhibitory nucleic acid in a subject, generally will contribute to determination of the concentration of pharmaceutical composition that is used for treatment. The frequency of administration may be empirically determined and adjusted to maximize the efficacy of the treatment.

[00155] The efficacy of treatment may be assessed using any suitable methods. In some embodiments, the efficacy of treatment may be assessed by evaluation of symptoms associated with AD, through measures of a subject's self-reported outcomes, *e.g.* mobility, self-care, usual activities, pain/discomfort, and anxiety/depression, or other wellness and quality-of-life indicators.

[00156] In some embodiments, a pharmaceutical composition that comprises an inhibitory nucleic acids (*e.g.*, ASOs, siRNAs, shRNAs, miRNAs, mixmers, aptamers) described herein is administered to a subject at an effective concentration sufficient to inhibit the activity or expression of a target gene (*e.g.*, ApoE ϵ 4) by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95% relative to a control, *e.g.* baseline level of gene expression prior to treatment, or compared to a control population.

[00157] In some embodiments, a single dose or administration of a pharmaceutical composition that comprises an inhibitory nucleic acid (*e.g.*, ASO, siRNA, shRNA, miRNA, mixmer, aptamer) described herein to a subject is sufficient to inhibit activity or expression of ApoE ϵ 4 for at least 1-5, 1-10, 5-15, 10-20, 15-30, 20-40, 25-50, or more days. In some embodiments, a single dose or administration of a pharmaceutical composition that comprises an inhibitory nucleic acid (*e.g.*, ASO, siRNA, shRNA, miRNA, mixmer, aptamer) described herein to a subject is sufficient to inhibit the activity or expression of ApoE ϵ 4 for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 15, 20, or 24 weeks. In some embodiments, a single dose or administration of a pharmaceutical composition that comprises an inhibitory nucleic acids (*e.g.*, ASOs, siRNAs, shRNAs, miRNAs, mixmers, aptamers) described herein to a subject is sufficient to inhibit activity or expression of a target gene for at least 1-5, 1-10, 2-5, 2-10, 4-8, 4-12, 5-10, 5-12, 5-15, 8-12, 8-15, 10-12, 10-15, 10-20, 12-15, 12-20, 15-20, or 15-25 weeks. In some embodiments, a single dose or administration of a pharmaceutical composition that comprises an inhibitory nucleic acid (*e.g.*, ASO, siRNA, shRNA, miRNA, mixmer, aptamer) described herein to a subject is sufficient to inhibit the activity or expression of a ApoE ϵ 4 for at least 1, 2, 3, 4, 5, or 6 months.

[00158] In some embodiments, a pharmaceutical composition may comprise more than one inhibitory nucleic acid (*e.g.*, ASOs, siRNAs, shRNAs, miRNAs, mixmers, aptamers). In some embodiments, a pharmaceutical composition may further comprise any other suitable therapeutic agent for treatment of a subject, *e.g.*, a human subject having AD. In some embodiments, the other therapeutic agents may enhance or supplement the effectiveness of

the inhibitory nucleic acids (*e.g.*, ASOs, siRNAs, shRNAs, miRNAs, mixmers, aptamers) described herein. In some embodiments, the other therapeutic agents may function to treat a different symptom or disease than the inhibitory nucleic acids (*e.g.*, ASOs, siRNAs, shRNAs, miRNAs, mixmers, aptamers) described herein. In some embodiments, the pharmaceutical composition is for use in treating, preventing, slowing the progression and/or reversing cognitive and neurological diseases in a subject, including but not limited to Alzheimer's disease (AD), stroke, Parkinson's disease, Lou Gehrig's disease, Charcot–Marie–Tooth disease, multiple sclerosis, diabetic neuropathy, sleep apnea, Lewy body disease, and ischemia of the central nervous system. In some embodiments, the subject is a non-human primate or rodent. In some embodiments, the subject is a human. In some embodiments, the subject is a patient, *e.g.*, a human patient that has or is suspected of having or is at risk of developing a disease. In some embodiments, the subject is a patient, *e.g.*, a human patient that has or is suspected of having or is at risk of developing Alzheimer's disease (AD). In some embodiments, the subject is a human patient who has or is suspected of having a disease resulting from carrying a disease-associated-allele, *e.g.*, an ApoE ϵ 4 allele.

IV. Kits and Related Composition

[00159] The agents described herein may, in some embodiments, be assembled into pharmaceutical or research kits to facilitate their use in therapeutic or research applications. A kit may include one or more containers housing the inhibitory nucleic acids of the disclosure and instructions for use. Specifically, such kits may include one or more inhibitory nucleic acids described herein, along with instructions describing the intended application and the proper use of these inhibitory nucleic acids. 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 inhibitory nucleic acids. Kits for research purposes may contain the components in appropriate concentrations or quantities for performing various experiments.

[00160] In some embodiments, the instant disclosure relates to a kit for administering an inhibitory nucleic acid (*e.g.*, ASO, siRNA, shRNA, miRNA, mixmer, aptamer) as described herein. In some embodiments, the kit comprising a container housing the inhibitory nucleic acid (*e.g.*, ASO, siRNA, shRNA, miRNA, mixmer, aptamer), and devices (*e.g.*, syringe) for extracting the inhibitory nucleic acid (*e.g.*, ASO, siRNA, shRNA, miRNA, mixmer, aptamer.) from the container. In some embodiments, the device for extracting the

inhibitory nucleic acids (*e.g.*, ASOs, siRNAs, shRNAs, miRNAs, mixmers, aptamers) from the container is also used for administration (*e.g.*, injection).

[00161] In some embodiments, the instant disclosure relates to a kit for producing an inhibitory nucleic acid (*e.g.*, ASOs, siRNAs, shRNAs, miRNAs, mixmers, aptamers).

[00162] In some embodiments, the instant disclosure relates to a kit for treating, preventing, slowing the progression and/or reversing a cognitive or neurological disease in a subject, including but not limited to Alzheimer's disease (AD), stroke, Parkinson's disease, Lou Gehrig's disease, Charcot–Marie–Tooth disease, multiple sclerosis, diabetic neuropathy, sleep apnea, Lewy body disease, and ischemia of the central nervous system. In some embodiments, the kit is for inhibiting the expression or activity of an ApoE ϵ 4 allele in a target cell (*e.g.*, cells of the CNS).

[00163] The kit may be designed to facilitate practicing the methods described herein by researchers, clinicians or healthcare professionals and can take many different 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 reconstitutable or otherwise processable (*e.g.*, to an active form), for example, by the addition of a suitable solvent or other medium (for example, water or a cell culture medium), which may or may not be provided in the kit. As used herein, “instructions” can include a component of instruction and/or promotion, and typically involve written instructions on or associated with the packaging. 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, CD-ROM, website links for downloadable file, *etc.*), Internet, and/or web-based communications, *etc.* 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 human or animal administration.

[00164] 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 administering it to a subject. The kit may include a container housing an inhibitory nucleic acid (*e.g.*, ASO, siRNA, shRNA, miRNA, mixmer, aptamer) described herein. The inhibitory nucleic acid (*e.g.*, ASO, siRNA, shRNA, miRNA, mixmer, aptamer) may be in the form of a liquid, gel, or solid (*e.g.*, powder). The inhibitory nucleic acid (*e.g.*, ASO, siRNA,

shRNA, miRNA, mixmer, aptamer) may be prepared sterilely, packaged in a syringe, and shipped refrigerated. Alternatively, the inhibitory nucleic acid (*e.g.*, ASO, siRNA, shRNA, miRNA, mixmer, aptamer) may be housed in a vial or other container for storage. A second container may have other pharmaceutical agents or excipients prepared sterilely.

Alternatively, the kit may include the inhibitory nucleic acid (*e.g.*, ASO, siRNA, shRNA, miRNA, mixmer, aptamer) premixed and shipped in a syringe, vial, tube, or other container.

V. General techniques

[00165] The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry, and immunology, which are within the skill of the art. *Molecular Cloning: A Laboratory Manual*, second edition (Sambrook, *et al.*, 1989) Cold Spring Harbor Press; *Oligonucleotide Synthesis* (M. J. Gait, ed., 1984); *Methods in Molecular Biology*, Humana Press; *Cell Biology: A Laboratory Notebook* (J. E. Cellis, ed., 1998) Academic Press; *Animal Cell Culture* (R. I. Freshney, ed., 1987); *Introduction to Cell and Tissue Culture* (J. P. Mather and P. E. Roberts, 1998) Plenum Press; *Cell and Tissue Culture: Laboratory Procedures* (A. Doyle, J. B. Griffiths, and D. G. Newell, eds., 1993-8) J. Wiley and Sons; *Methods in Enzymology* (Academic Press, Inc.); *Handbook of Experimental Immunology* (D. M. Weir and C. C. Blackwell, eds.); *Gene Transfer Vectors for Mammalian Cells* (J. M. Miller and M. P. Calos, eds., 1987); *Current Protocols in Molecular Biology* (F. M. Ausubel, *et al.*, eds., 1987); *PCR: The Polymerase Chain Reaction* (Mullis, *et al.*, eds., 1994); *Current Protocols in Immunology* (J. E. Coligan *et al.*, eds., 1991); *Short Protocols in Molecular Biology* (Wiley and Sons, 1999); *Immunobiology* (C. A. Janeway and P. Travers, 1997); *Antibodies* (P. Finch, 1997); *Antibodies: a practical approach* (D. Catty, ed., IRL Press, 1988-1989); *Monoclonal antibodies: a practical approach* (P. Shepherd and C. Dean, eds., Oxford University Press, 2000); *Using antibodies: a laboratory manual* (E. Harlow and D. Lane (Cold Spring Harbor Laboratory Press, 1999); *The Antibodies* (M. Zanetti and J. D. Capra, eds., Harwood Academic Publishers, 1995)

[00166] Without further elaboration, it is believed that one skilled in the art can, based on the present disclosure, utilize the present invention to its fullest extent. The following specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever. All publications

cited herein are incorporated by reference for the purposes or subject matter referenced herein.

[00167] Exemplary embodiments of the invention will be 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.

EXAMPLES

[00168] Example 1: Antisense Oligonucleotides targeting ApoE ϵ 4

[00169] ASOs are short, single-stranded oligonucleotides that bind to complementary target RNA by Watson-Crick base pairing in a sequence-specific manner. ASOs can modulate RNA in a variety of ways, for example, by activating RNase H. RNase H is a nuclear enzyme that recognizes, binds to, and cleaves the RNA strand of RNA-DNA heteroduplexes. The activity of RNase H is extremely sequence specific; a single nucleotide mismatch in the RNA-DNA heteroduplex results in a 3-5-fold decrease in RNA cleavage. The present disclosure aims to design ASOs that allow for allelic specific targeting of ϵ 4 in ApoE ϵ 4 carriers, *e.g.*, ApoE ϵ 4 heterozygous. As mentioned previously, the three common allelic variants of ApoE are defined by two SNPs (rs 7412 and rs429358) at amino acid positions 112 and 158 respectively: ϵ 2 (Cys₁₁₂; Cys₁₅₈), ϵ 3 (Cys₁₁₂; Arg₁₅₈), and ϵ 4 (Arg₁₁₂; Arg₁₅₈). ϵ 4 differs from both ϵ 2 and ϵ 3 by a T $\rightarrow$ C SNP at rs429358 in the fourth exon of the ApoE locus, resulting in a single amino acid change (Cys₁₁₂ $\rightarrow$ Arg₁₁₂). A set of allele specific ASOs targeting the SNP at rs429358 was designed to selectively target and degrade ϵ 4 at the RNA level (FIG. 1, Tables 1-3).

[00170] To test the efficacy of the ASOs *in vitro*, plasmids that express either ApoE 3 or ApoE 4 cDNA and firefly luciferase fused by a P2A self-cleaving peptide sequence were generated (FIG. 2A). As P2A acts at translation, ApoE and Luciferase RNA is a single transcript. Thus, ASOs targeting ApoE RNA will also degrade Luciferase RNA. HEK 293T cells were transfected with either ApoE 3 or ApoE 4 Luciferase plasmid and a Renilla plasmid for internal normalization of transfection efficiency. One hour after transfection, both ASOs were applied to wells. 24 hours after transfection 293Ts were harvested and Luciferase and Renilla fluorescence were measured according to standard dual-reporter protocol (FIG. 2B). Luciferase expression was normalized to Renilla expression to control for differences in transfection efficiency across wells. Luciferase: Renilla ratios from wells that received ASOs

were normalized to Luciferase: Renilla ratios of wells that did not receive ASO to generate dose response curves for individual ASOs (FIGs. 3A-3K).

[00171] To test the efficacy of the ASOs *in vivo*, mice that are homozygous for a humanized knock in of heterozygous ApoE (hApoE 3/4) and also carry mutations that lead to the development of AD, such as the widely used APP-PS1/21 and 5XFAD mouse models of AD, were used. ApoE genotype has been previously shown to affect AD severity and progression in these models and their well characterized pathological and behavioral tests are well suited as a readout of AD progression and therapeutic efficacy.

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OTHER EMBODIMENTS

[00192] All of the features disclosed in this specification may be combined in any combination. Each feature disclosed in this specification may be replaced by an alternative feature serving the same, equivalent, or similar purpose. Thus, unless expressly stated otherwise, each feature disclosed is only an example of a generic series of equivalent or similar features.

[00193] From the above description, one skilled in the art can easily ascertain the essential characteristics of the present invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions. Thus, other embodiments are also within the claims.

EQUIVALENTS

[00194] While several inventive embodiments have been described and illustrated herein, those of ordinary skill in the art will readily envision a variety of other means and/or structures for performing the function and/or obtaining the results and/or one or more of the advantages described herein, and each of such variations and/or modifications is deemed to be within the scope of the inventive embodiments described herein. More generally, those skilled in the art will readily appreciate that all parameters, dimensions, materials, and configurations described herein are meant to be exemplary and that the actual parameters, dimensions, materials, and/or configurations will depend upon the specific application or applications for which the inventive teachings is/are used. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific inventive embodiments described herein. It is, therefore, to be understood that the foregoing embodiments are presented by way of example only and that, within the scope of the appended claims and equivalents thereto, inventive embodiments may be practiced otherwise than as specifically described and claimed. Inventive embodiments of the present disclosure are directed to each individual feature, system, article, material, kit, and/or method described herein. In addition, any combination of two or more such features, systems, articles, materials, kits, and/or methods, if such features, systems, articles, materials, kits, and/or methods are not mutually inconsistent, is included within the inventive scope of the present disclosure.

[00195] All definitions, as defined and used herein, should be understood to control over dictionary definitions, definitions in documents incorporated by reference, and/or ordinary meanings of the defined terms.

[00196] All references, patents and patent applications disclosed herein are incorporated by reference with respect to the subject matter for which each is cited, which in some cases may encompass the entirety of the document.

[00197] The indefinite articles “a” and “an,” as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to mean “at least one.”

[00198] As used herein, the term “approximately” or “about,” as applied to one or more values of interest, refers to a value that is similar to a stated reference value. In certain embodiments, the term “approximately” or “about” refers to a range of values that fall within 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of the stated reference value unless otherwise stated or

otherwise evident from the context (except where such number would exceed 100% of a possible value).

[00199] 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, *i.e.*, “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.*

[00200] 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 (*i.e.* “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.” “Consisting essentially of,” when used in the claims, shall have its ordinary meaning as used in the field of patent law.

[00201] 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 any one 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 non-limiting 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.*

[00202] It should also be understood that, unless clearly indicated to the contrary, in any methods claimed 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.

CLAIMS

What is claimed is:

1. An inhibitory nucleic acid for inhibiting expression or activity of an ApoE ϵ 4 allele that comprises a cytidine at rs429358, wherein the inhibitory nucleic acid comprises a region of complementarity at least 90% complementary to at least 12 consecutive nucleotides of an ApoE ϵ 4 mRNA.
2. The inhibitory nucleic acid of claim 1, wherein the ApoE ϵ 4 is human ApoE ϵ 4.
3. The inhibitory nucleic acid of claim 2, wherein the inhibitory nucleic acid comprises a region of complementarity to at least 12 consecutive nucleotides to SEQ ID NO: 1.
4. The inhibitory nucleic acid of claim 2, wherein the inhibitory nucleic acid comprises a region of complementarity to at least 10 consecutive nucleotides to SEQ ID NO: 2.
5. The inhibitory nucleic acid of claim 2, wherein the inhibitory nucleic acid comprises a region of complementarity to a target region comprised within 30 base pairs on either or both sides of rs429358.
6. The inhibitory nucleic acid of any one of claims 1-5, wherein the inhibitory nucleic acid is an RNA, a DNA or a hybrid nucleic acid, and wherein the inhibitory nucleic acid is single-stranded, double-stranded, or comprises both single-stranded and double-stranded regions.
7. The inhibitory nucleic acid of any one of claims 1-6, wherein the inhibitory nucleic acid is an antisense oligonucleotide.
8. The inhibitory nucleic acid of claim 7, wherein the antisense oligonucleotide is capable of promoting RNase H mediated RNA degradation of the ApoE ϵ 4 mRNA.

9. The inhibitory nucleic acid of claim 8, wherein the antisense oligonucleotide is a gapmer comprising a 5'-X-Y-Z-3' configuration, wherein
- X comprises 0 to 7 linked ribonucleosides;
 - Y comprises 5 to 12 linked 2'-deoxyribonucleosides, and
 - Z comprises 2 to 7 linked ribonucleosides.
10. The inhibitory nucleic acid of claim 9, wherein at least one of the nucleosides in X is a 2'-modified nucleoside.
11. The inhibitory nucleic acid of claims 9 or 10, wherein each nucleoside in X is a 2'-modified nucleoside.
12. The inhibitory nucleic acid of any one of claims 9-11, wherein at least one of the nucleosides in Z is a 2'-modified nucleoside.
13. The inhibitory nucleic acid of any one of claims 9-12, wherein each nucleoside in Z is a 2'-modified nucleoside.
14. The inhibitory nucleic acid of any one of claims 10-13, wherein the 2'-modified nucleoside is a non-bicyclic 2'-modified nucleoside.
15. The inhibitory nucleic acid of any one of claims 10-14, wherein the 2'-modified nucleoside is a 2'-O-methoxyethyl (2'-MOE) modified nucleoside, a 2'-O-methyl (2'-O-Me) modified nucleoside, a 2'-fluoro (2'-F) modified nucleoside, a 2'-O-aminopropyl (2'-O-AP) modified nucleoside, a 2'-O-dimethylaminoethyl (2'-O-DMAOE) modified nucleoside, a 2'-O-dimethylaminopropyl (2'-O-DMAP) modified nucleoside, a 2'-O-dimethylaminoethoxyethyl (2'-O-DMAEOE) modified nucleoside, or a 2'-O-N-methylacetamido (2'-O-NMA) modified nucleoside.
16. The inhibitory nucleic acid of claim 15, wherein the 2'-modified nucleoside is a 2'-O-methoxyethyl (2'-MOE) modified nucleoside.
17. The inhibitory nucleic acid of any one of claims 10-13, wherein the 2'-modified nucleoside is a 2'-4' bicyclic nucleoside.

18. The inhibitory nucleic acid of claim 17, wherein the 2'-modified nucleoside is a LNA modified nucleoside, a cEt modified nucleoside, or a ENA modified nucleoside.

19. The inhibitory nucleic acid of any one of claims 7-18, wherein the antisense oligonucleotide comprises a nucleotide sequence at least 90% identical to any one of SEQ ID NOs: 3-12, 20-29, or 42-45.

20. The inhibitory nucleic acid of any one of claims 7-19, wherein the antisense oligonucleotide comprises a 5'-X-Y-Z-3' configuration of:

X	Y	Z
EEEEEE	(D) ₇	EEEEEE
EEEEEE	(D) ₉	EEEEEE

wherein "E" is a 2'-MOE modified ribonucleoside; and "D" is 2'-deoxyribonucleoside.

21. The inhibitory nucleic acid of any one of claims 7-20, wherein the antisense oligonucleotide comprises one or more phosphorothioate internucleotide linkages, one or more methylphosphonate internucleotide linkages, one or more phosphorodithioate internucleotide linkages, one or more boron phosphonate internucleotide linkages, one or more phosphonocarboxylate internucleotide linkages, and/or one or more phosphonoacetate internucleotide linkages.

22. The inhibitory nucleic acid of any one of claims 7-21, wherein each internucleotide linkage in the antisense oligonucleotide is a phosphorothioate internucleotide linkage, a phosphorothioate internucleotide linkage, a methylphosphonate internucleotide linkage, a phosphorodithioate internucleotide linkage, a boron phosphonate internucleotide linkage, a phosphonocarboxylate internucleotide linkage, and/or a phosphonoacetate internucleotide linkage.

23. The inhibitory nucleic acid of any one of claims 7-22, wherein the antisense oligonucleotide is selected from:

5' – oG*oC*oC*oG*oC*dG*dC*dA*dC*dG*dT*dC*oC*oT*oC*oC*oA – 3' (SEQ ID NO: 20);

5' – oG*oG*oC*oC*oG*dC*dG*dC*dA*dC*dG*dT*oC*oC*oT*oC*oC – 3' (SEQ ID NO: 21);

5' – oC*oG*oG*oC*oC*dG*dC*dG*dC*dA*dC*dG*oT*oC*oC*oT*oC – 3' (SEQ ID NO: 22);

5' – oG*oC*oG*oG*oC*dC*dG*dC*dG*dC*dA*dC*oG*oT*oC*oC*oT – 3' (SEQ ID NO: 23);

5' – oG*oG*oC*oG*oG*dC*dC*dG*dC*dG*dC*dA*oC*oG*oT*oC*oC – 3' (SEQ ID NO: 24);

5' – oA*oG*oG*oC*oG*dG*dC*dC*dG*dC*dG*dC*oA*oC*oG*oT*oC – 3' (SEQ ID NO: 25);

5' – oG*oC*oC*oG*oC*dG*dC*dA*dC*dG*dT*dC*dC*dT*oC*oC*oA*oT*oG – 3' (SEQ ID NO: 26);

5' – oG*oG*oC*oC*oG*dC*dG*dC*dA*dC*dG*dT*dC*dC*oT*oC*oC*oA*oT – 3' (SEQ ID NO: 27);

5' – oC*oG*oG*oC*oC*dG*dC*dG*dC*dA*dC*dG*dT*dC*oC*oT*oC*oC*oA – 3' (SEQ ID NO: 28);

5' – oG*oC*oG*oG*oC*dC*dG*dC*dG*dC*dA*dC*dG*dT*oC*oC*oT*oC*oC – 3' (SEQ ID NO: 29);

5' – oA* oG* oG* oC* oG* dG* dC* dC* dG* dC* dG* dC* dA* dC* oG* oT* oC* oC* oT* – 3' (SEQ ID NO: 44); and

5' – oG* oG* oC* oG* oG* dC* dC* dG* dC* dG* dC* dA* dC* dG* oT* oC* oC* oT* oC* – 3' (SEQ ID NO: 45),

wherein “oN” is 2'- MOE modified ribonucleoside; “dN” is 2'-deoxyribonucleoside; and “*” indicates a phosphorothioate internucleotide linkage.

24. The inhibitory nucleic acid of claim 7, wherein the inhibitory nucleic acid is capable of inhibiting expression and/or activity of ApoE ε4 by steric hindrance, inhibiting mRNA maturation, destabilizing pre-mRNA or RNase H mediated degradation.

25. The inhibitory nucleic acid of any one of claims 1-6, wherein the inhibitory nucleic acid is an siRNA.

26. The inhibitory nucleic acid of claim 25, wherein the siRNA comprises an antisense strand that comprises a region of complementarity of at least 10 consecutive nucleotides of SEQ ID NO: 1 or SEQ ID NO: 2.

27. The inhibitory nucleic acid of claims 25 or 26, wherein the antisense strand comprises at least 15 consecutive nucleotides of any one of SEQ ID NOs: 3-12, 20-29, or 30-57, wherein each T is optionally U.
28. The inhibitory nucleic acid of any one of claims 25-27, wherein the antisense strand consists of any one of SEQ ID NOs: 3-12, 20-29, or 30-57, wherein each T is optionally U.
29. The inhibitory nucleic acid of any one of claims 25-28, wherein the siRNA further comprises a sense strand that hybridizes to the antisense strand.
30. The inhibitory nucleic acid of any one of claims 1-6, wherein the inhibitory nucleic acid is an shRNA.
31. The inhibitory nucleic acid of claim 30, wherein the shRNA comprises an antisense portion that comprises a region of complementarity of at least 15 consecutive nucleotides of SEQ ID NO: 1 or SEQ ID NO: 2.
32. The inhibitory nucleic acid of claims 30 or 31, wherein the antisense strand comprises at least 15 consecutive nucleotides of any one of SEQ ID NOs: 46-57, wherein each T is optionally U.
33. The inhibitory nucleic acid of any one of claims 30-32, wherein the antisense strand consists of any one of SEQ ID NOs: 46-57, wherein each T is optionally U.
34. The inhibitory nucleic acid of any one of claims 31-33, wherein the shRNA further comprises a sense portion that hybridizes to the antisense portion.
35. The inhibitory nucleic acid of claim 34, wherein the sense portion of the antisense portion of the shRNA is connected by a single-stranded nucleic acid hairpin.
36. The inhibitory nucleic acid of any one of claims 1-6, wherein the inhibitory nucleic acid is a miRNA.

37. The inhibitory nucleic acid of any one of claims 1-6, wherein the inhibitory nucleic acid is a mixmer.
38. The inhibitory nucleic acid of any one of claims 1-6, wherein the inhibitory nucleic acid is an aptamer.
39. A pharmaceutical composition comprising the inhibitory nucleic acid of any one of claims 1-38, and a pharmaceutically acceptable excipient.
40. A method of inhibiting the expression or activity of ApoE ϵ 4 in a cell, the method comprising contacting the cell with the inhibitory nucleic acid of any one of claims 1-38 in an amount effective for promoting internalization of the inhibitory nucleic acid into the cell.
41. A method of inhibiting the expression or activity of ApoE ϵ 4 in a subject, the method comprising administering to the subject the inhibitory nucleic acid of any one of claims 1-38, or the pharmaceutical composition of claim 39 in an amount effective for promoting internalization of the inhibitory nucleic acid into the subject, or into a cell of a subject.
42. The method of claims 40 or 41, wherein the inhibitory nucleic acid promotes RNase H mediated degradation of ApoE ϵ 4.
43. A method of preventing, treating, slowing the progression and/or reversing Alzheimer's disease in a subject in need thereof, the method comprising administering to the subject an effective amount of the inhibitory nucleic acid of any one of claims 1-38, or the pharmaceutical composition of claim 39.
44. The method of claim 43, wherein the subject is a heterozygous for ApoE ϵ 4 allele.
45. The method of claim 44, wherein the subject is ApoE ϵ 2/ ϵ 4 heterozygous.
46. The method of claim 44, wherein the subject is ApoE ϵ 3/ ϵ 4 heterozygous.
47. The method of claim 43, wherein subject is a homozygous for ApoE ϵ 4 allele.

48. The method of any one of claims 43-47, wherein the subject is a human.
49. The method of any one of claims 43-47, wherein the subject is a non-human mammal.
50. The method of any one of claims 43-49, wherein the administration is systemic administration.
51. The method of claim 50, wherein the systemic administration comprises intravenous administration, intramuscular administration, intraperitoneal administration, or subcutaneous administration.
52. The method of any one of claims 43-49, wherein administration is direct administration into the central nervous system (CNS).
53. The method of claim 52, wherein the direct administration into the CNS comprises intracerebral injection, intraparenchymal injection, intrathecal injection, or intracerebroventricular administration.
54. The inhibitory nucleic acid of any one of claims 1-38, or the pharmaceutical composition of claim 39, for use in preventing or treating Alzheimer's disease in a subject.
55. The use of claim 54, wherein the subject is a heterozygous for ApoE ϵ 4 allele.
56. The use of claim 55, wherein the subject is ApoE ϵ 2/ ϵ 4 heterozygous.
57. The use of claim 55, wherein the subject is ApoE ϵ 3/ ϵ 4 heterozygous.
58. The use of claim 54, wherein subject is a homozygous for ApoE ϵ 4 allele.
59. The use of any one of claims 54-58, wherein the subject is a human.
60. The use of any one of claims 54-58, wherein the subject is a non-human mammal.

61. The use of any one of claims 54-60, wherein the subject is administered by systemic administration.
62. The use of claim 61, wherein the systemic administration comprises intravenous administration, intramuscular administration, intraperitoneal administration, or subcutaneous administration.
63. The use of any one of claims 54-60, wherein the subject is administered by direct administration to the central nervous system (CNS).
64. The method of claim 63, wherein the direct administration to the CNS comprises intracerebral injection, intraparenchymal injection, intrathecal injection, or intracerebroventricular administration.
65. A kit comprising an antisense oligonucleotide which comprises the inhibitory nucleic acid of any one of claims 1-38, or the pharmaceutical composition of claim 39.

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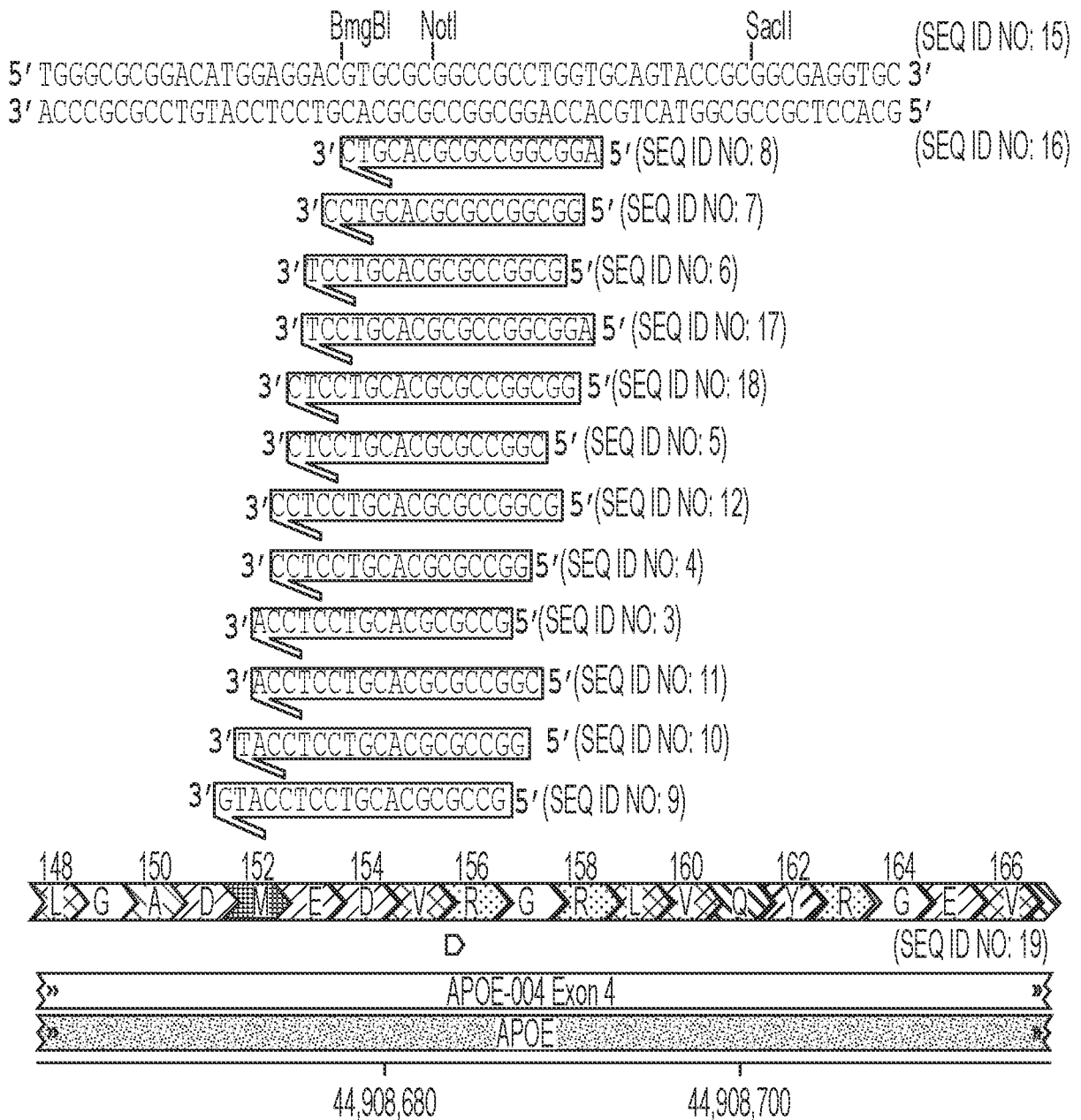


FIG. 1

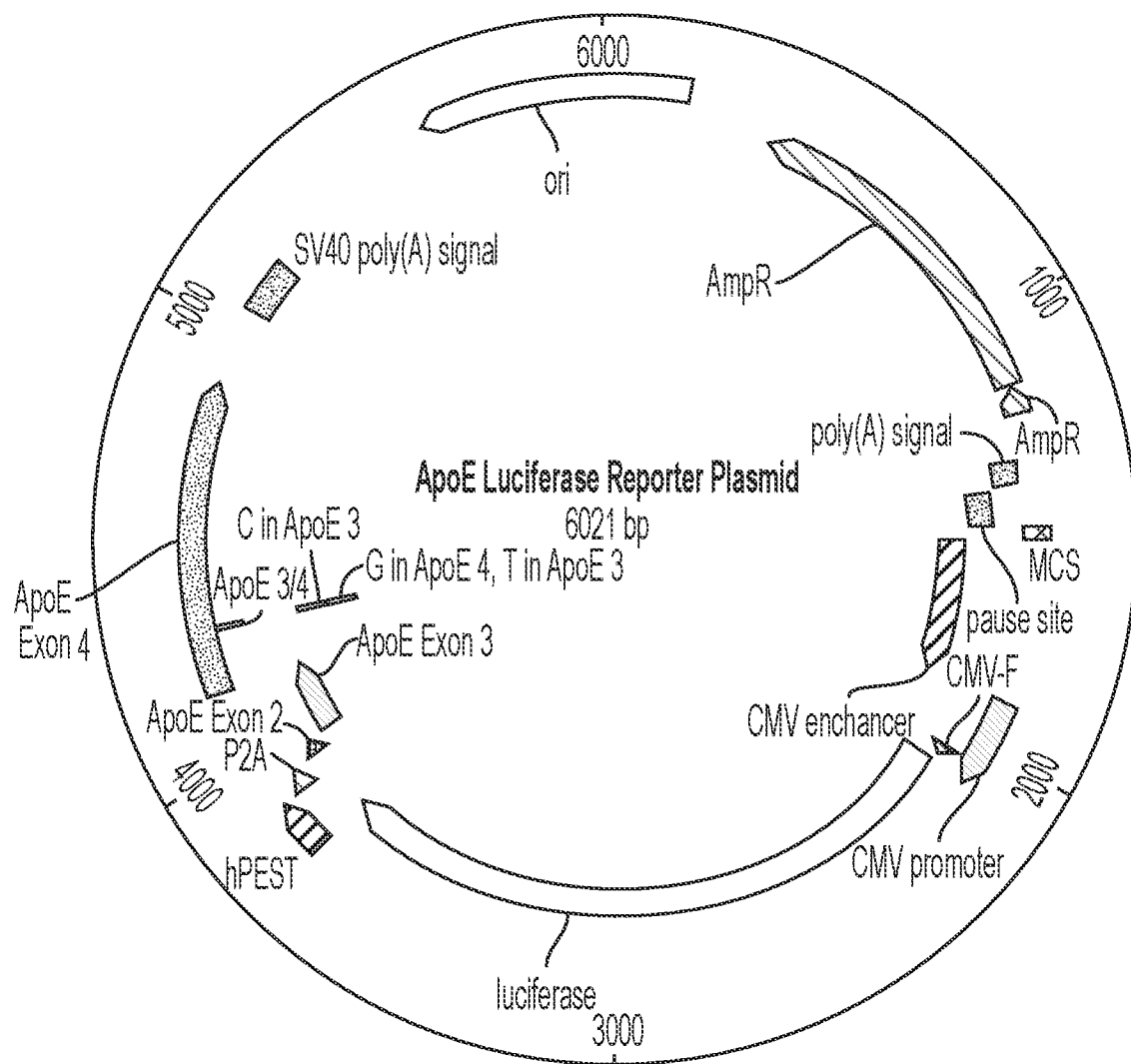


FIG. 2A

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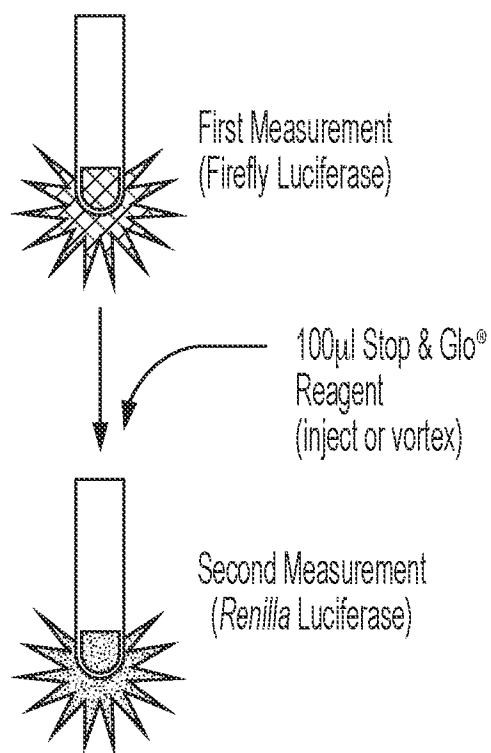
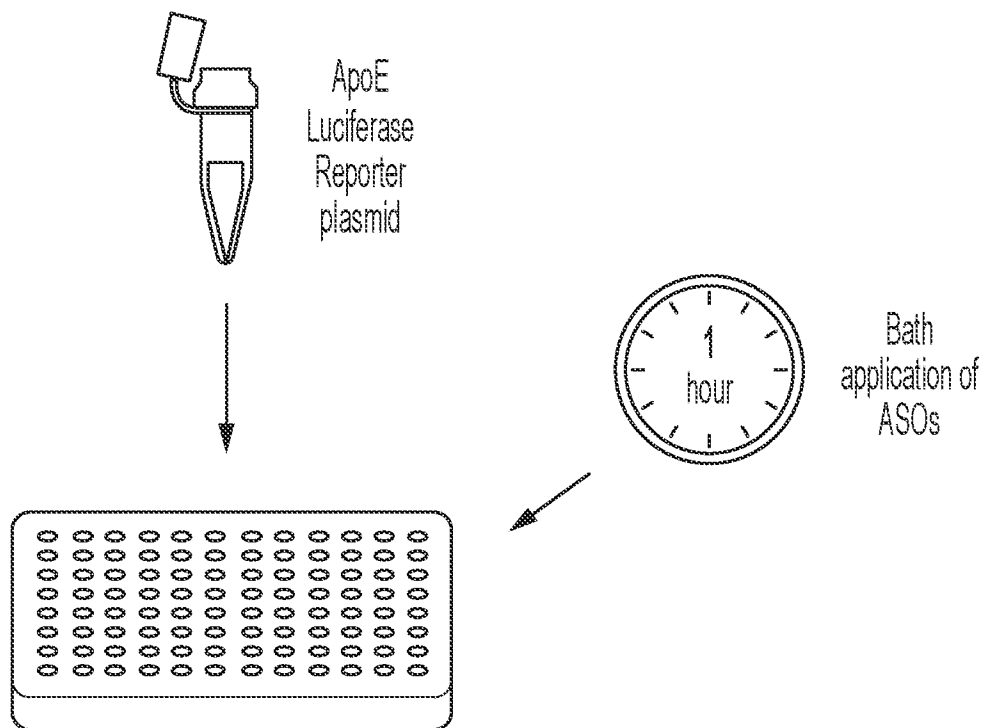


FIG. 2B

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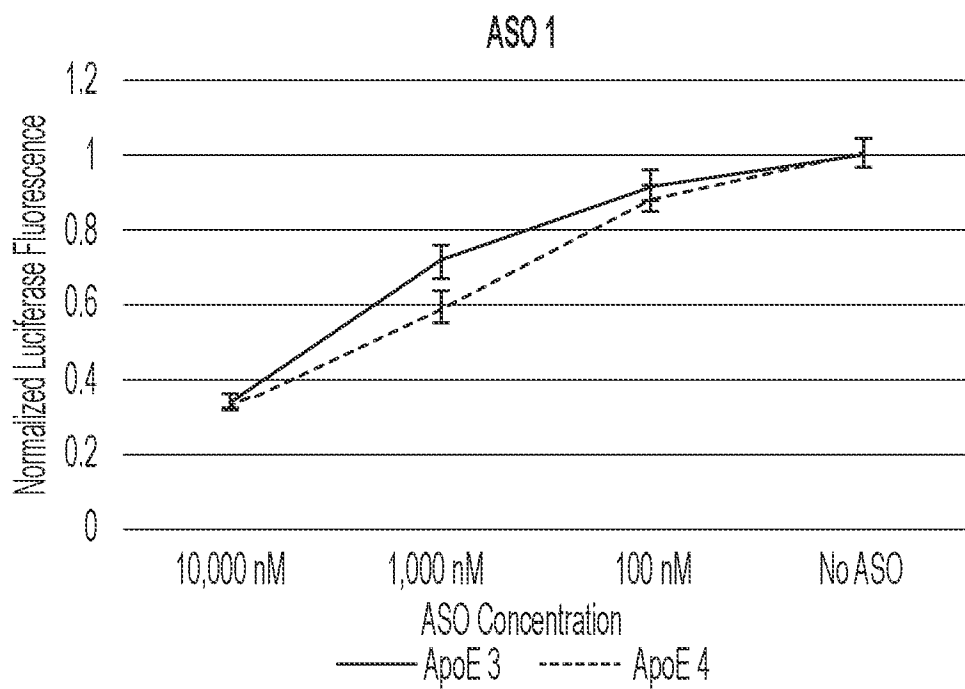


FIG. 3A

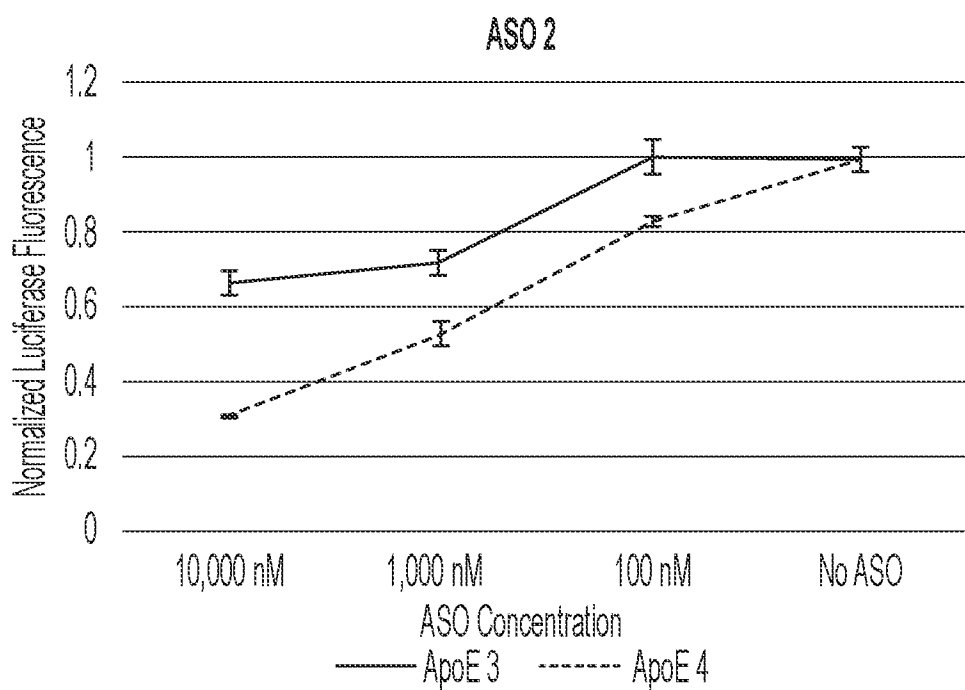


FIG. 3B

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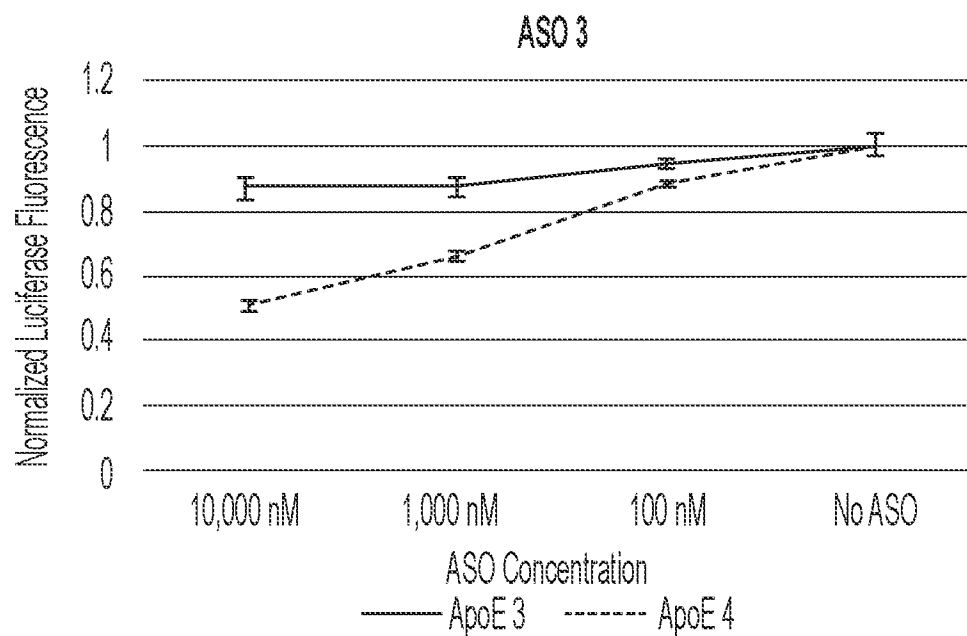


FIG. 3C

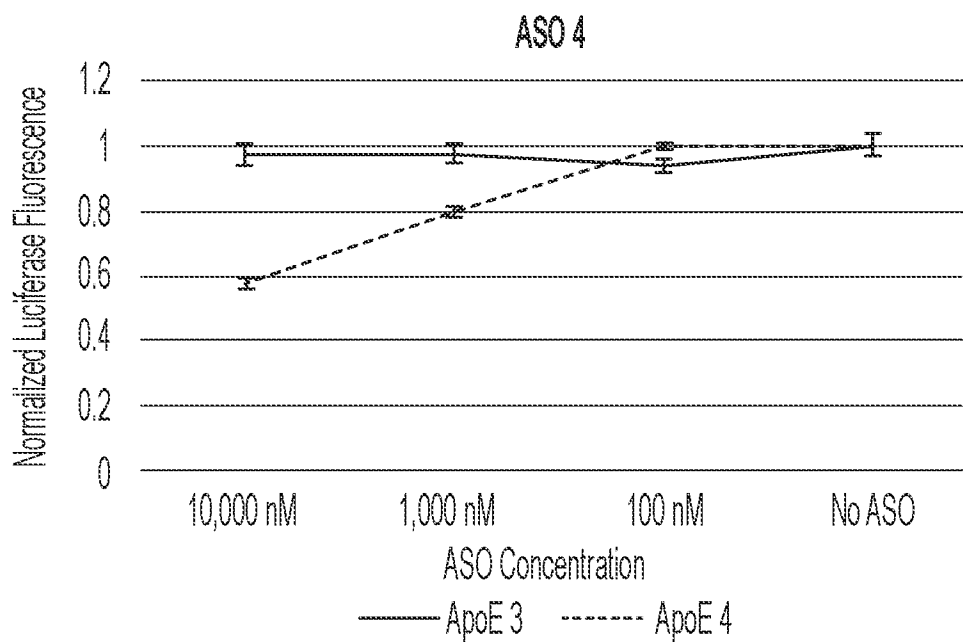


FIG. 3D

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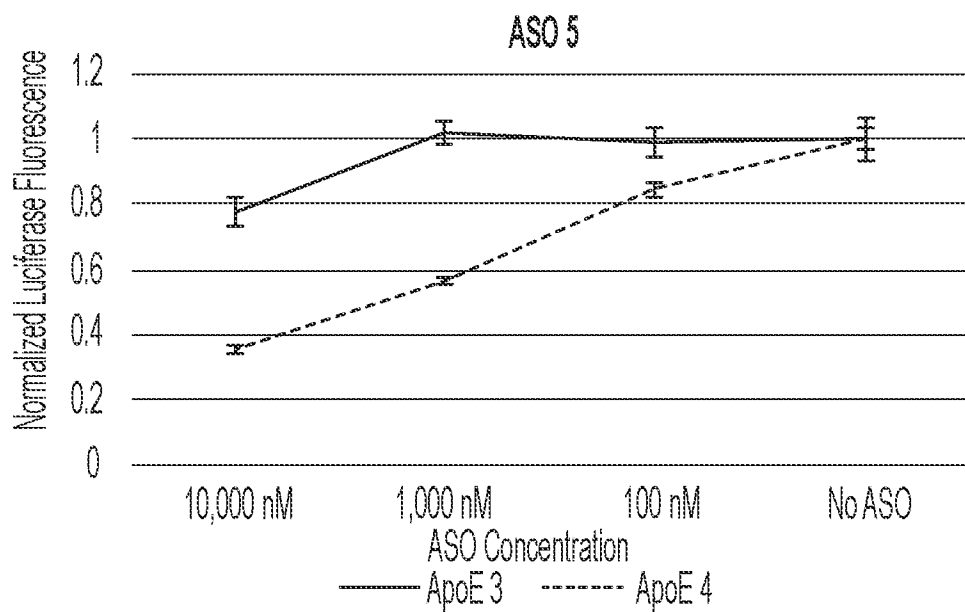


FIG. 3E

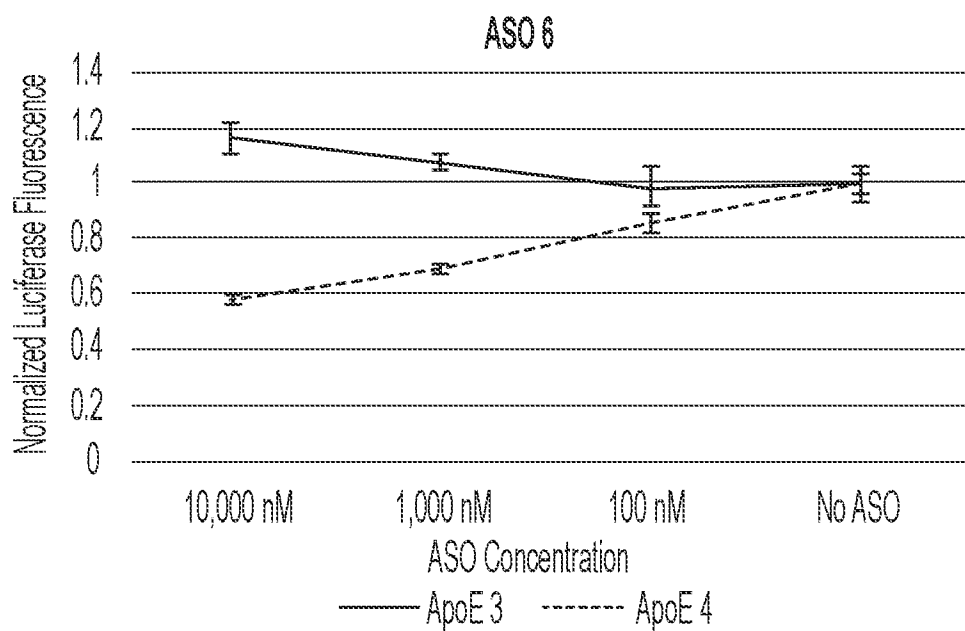


FIG. 3F

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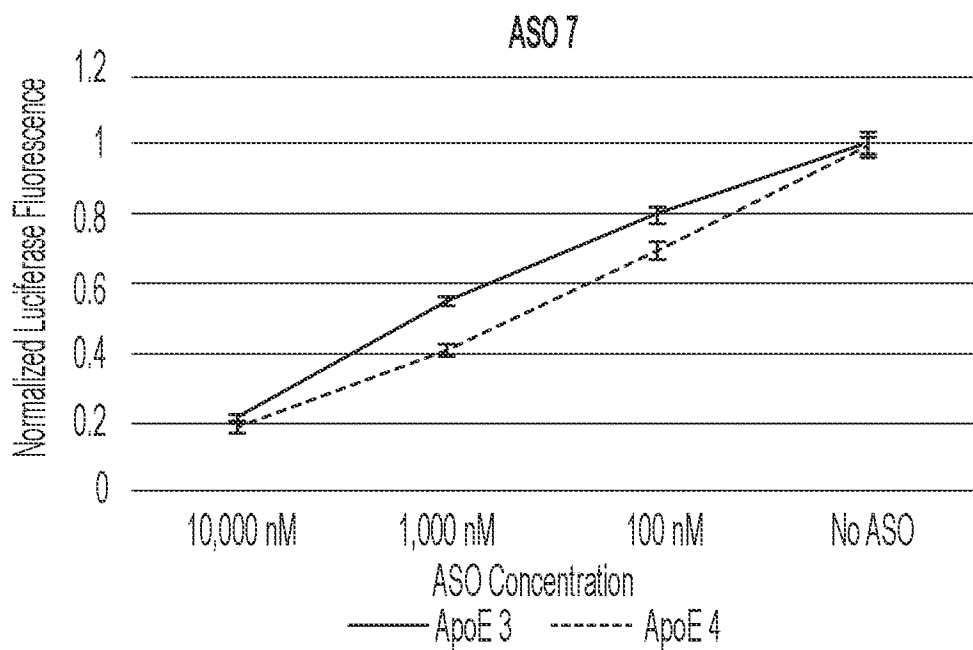


FIG. 3G

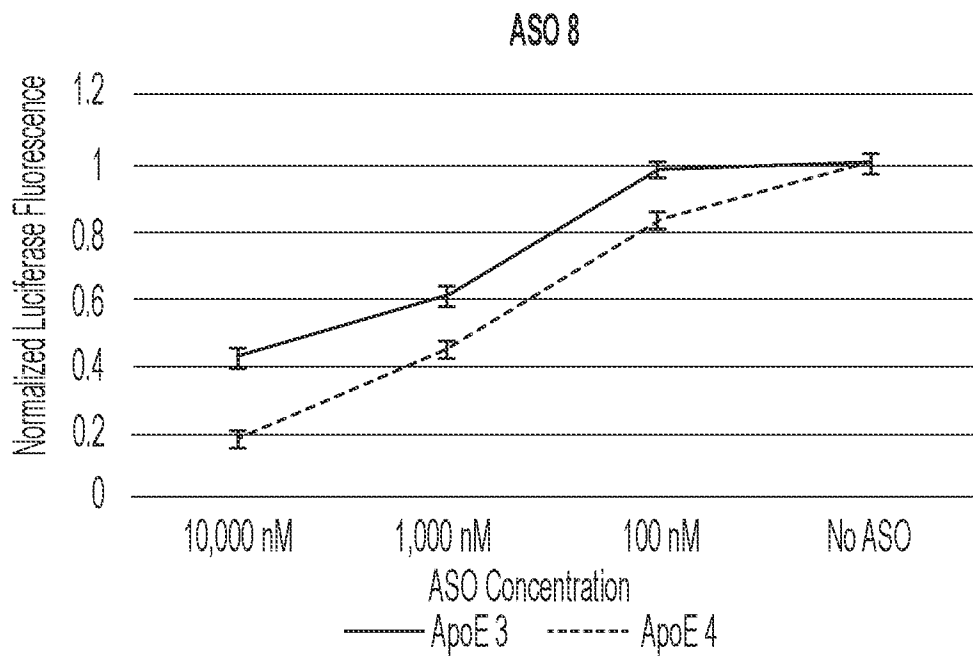


FIG. 3H

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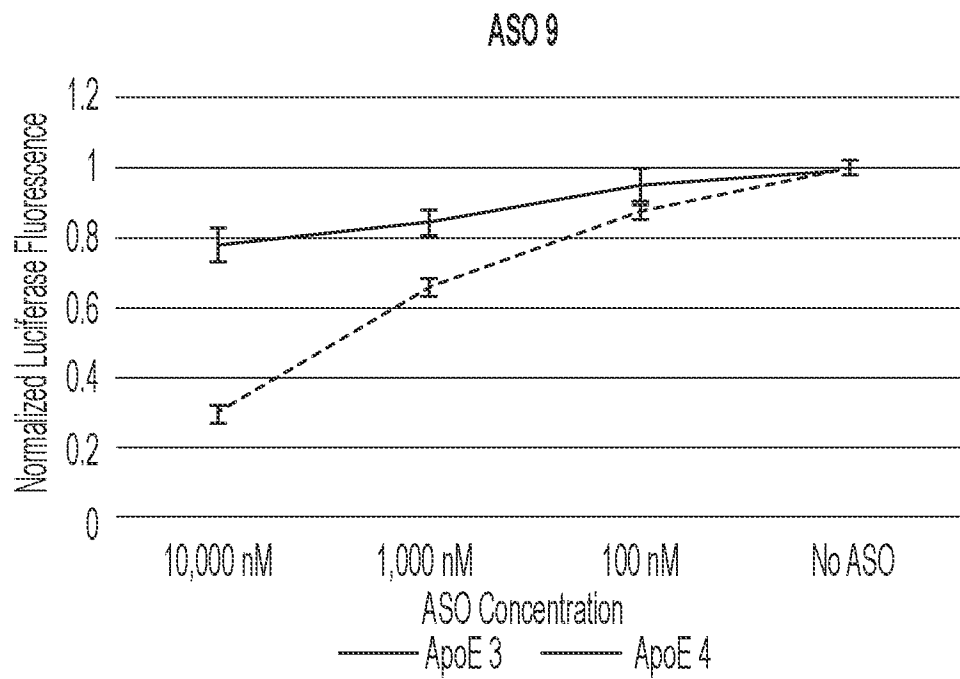


FIG. 3I

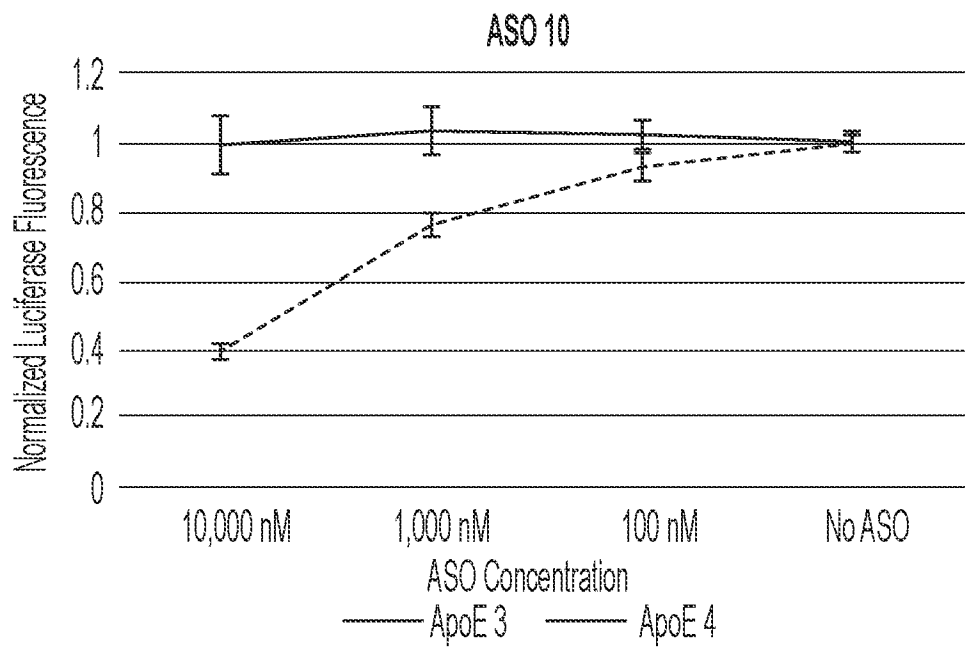


FIG. 3J

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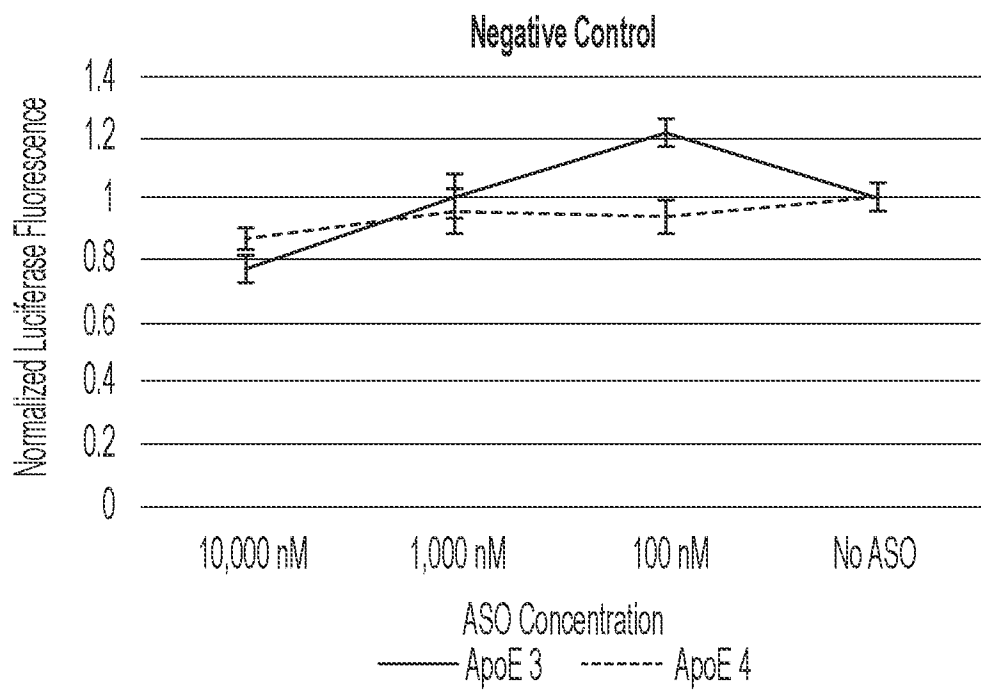


FIG. 3K