

[006] Several RNA-based therapeutics are known that can inhibit or reduce *APOC3* expression. For example, Intl. Patent Application Publication Nos. WO 2010/083615, WO 2012/177947, WO 2016/011123, WO 2016/081444, and WO 2019/051402, as well as CN Patent Application Publication No. 108239644, describe double-stranded (ds) RNAi constructs for inhibiting or reducing *APOC3* expression, as well as methods of using the same for treating or preventing lipid metabolism conditions, diseases and/or disorders such as obesity and cardiometabolic disorders. Also, Intl. Patent Application Publication No. WO 2014/205451 describes antisense oligonucleotides for inhibiting or reducing *APOC3* expression.

[007] Despite the existence of some therapeutics directed toward *APOC3*, there is a need for additional therapeutics for inhibiting or reducing *APOC3* expression for treating liver disease.

SUMMARY

[008] To address this need, the disclosure describes compositions and methods for treating a disease, disorder, and/or condition related to *APOC3* expression. The disclosure is based, in part, on discovering and developing ds oligonucleotides (*e.g.*, RNAi oligonucleotides) for selectively inhibiting and/or reducing *APOC3* expression in, for example, the liver. Accordingly, target sequences within *APOC3* have been identified, and RNAi oligonucleotides that bind to these target sequences and inhibit *APOC3* mRNA expression have been generated. As shown herein, the RNAi oligonucleotides inhibit human and non-human primate (NHP) *APOC3* expression in the liver. Without being bound by theory, the RNAi oligonucleotides herein are useful for treating a disease, disorder or condition associated with *APOC3* expression (*e.g.*, hypertriglyceridemia or another dyslipidemia). In general, the RNAi oligonucleotides herein are useful for treating a disease, disorder, or condition associated with aberrant *APOC3* expression (*e.g.*, *APOC3* gain-of-function polymorphisms). In particular, the RNAi oligonucleotides herein are useful for treating a disease, disorder, or condition associated with mutant *APOC3* expression.

[009] Accordingly, the disclosure describes RNAi oligonucleotides for reducing or inhibiting *APOC3* expression that include a sense strand and/or an antisense strand, where the sense strand has a sequence as set forth in Table 2, and where the antisense strand has a sequence as set forth in Table 2.

[0010] In some examples, the sense strand has a sequence as set forth in Table 2 (*e.g.*, any one of the odd numbers of SEQ ID NOs:9 to 170), especially any one of SEQ ID NOs:37, 43, 45, 87, 89, 99, 101, and 105.

[0011] In some examples, the antisense strand having a sequence as set forth in Table 2 (*e.g.*, any one of the even numbers of SEQ ID NOs:9 to 170), especially any one of SEQ ID NOs:38, 44, 46, 88, 90, 100, 102, and 106.

[0012] Alternatively, the disclosure describes RNAi oligonucleotides for reducing or inhibiting *APOC3* expression that include a sense strand and/or an antisense strand, where the sense strand has a sequence as set forth in Table 3, and where the antisense strand has a sequence as set forth in Table 3.

[0013] In some examples, the sense strand has a sequence as set forth in Table 3 (*e.g.*, any one of the odd numbers of SEQ ID NOs:171 to 332), especially any one of SEQ ID NOs:199, 205, 207, 249, 251, 261, 263, and 267.

[0014] In some examples, the antisense strand has a sequence as set forth in Table 3 (*e.g.*, any one of the even numbers of SEQ ID NOs:171 to 332), especially any one of SEQ ID NOs:200, 206, 208, 250, 252, 262, 264, and 268.

[0015] Alternatively, RNAi oligonucleotides are described for reducing or inhibiting *APOC3* expression that include a sense strand and an antisense strand, where the sense and antisense strands form a duplex region, and where the antisense strand has a region of complementarity to a *APOC3* mRNA target sequence of any one of SEQ ID NOs:334 to 341.

[0016] In any of the examples above, the sense strand is from about 15 nucleotides to about 50 nucleotides in length. In some examples, the sense strand is from about 20 nucleotides to about 40 nucleotides in length. In some examples, the sense strand is 36 nucleotides in length.

[0017] In any of the examples above, the antisense strand is from about 15 nucleotides to about 30 nucleotides in length. In some examples, the antisense strand is from about 20 nucleotides to about 25 nucleotides. In some examples, the antisense strand is 22 nucleotides in length.

[0018] In any of the examples above, the duplex region is from about 19 nucleotides in length to about 21 nucleotides in length. In certain embodiment, the duplex region is 20 nucleotides in length.

[0019] In any of the examples above, the region of complementarity is at least 15 contiguous nucleotides in length. In some examples, the region of complementarity is from

about 19 contiguous nucleotides in length to about 21 contiguous nucleotides in length. In other examples, the region of complementarity is 19 contiguous nucleotides in length, 20 contiguous nucleotides in length, or 21 contiguous nucleotides in length.

[0020] In any of the examples above, the RNAi oligonucleotides include on the sense strand a 3' end a stem-loop set forth as: S1-L-S2, where S1 is complementary to S2, and where L forms a loop between S1 and S2 of about 3 to about 5 nucleotides in length.

[0021] In any of the examples above, the antisense strand, the sense strand, or both have an overhang sequence. In some examples, the antisense strand includes a 3'-overhang of 1 or more nucleotides in length. In other examples, the 3'-overhang sequence is 2 nucleotides in length such as, for example, GG.

[0022] Oligonucleotides also are described that include an antisense strand and a sense strand, where the antisense strand can be from about 21 nucleotides to about 27 nucleotides in length and has a region of complementarity to *APOC3*, wherein the sense strand includes a stem-loop at its 3' end set forth as: S1-L-S2, wherein S1 is complementary to S2, wherein L forms a loop between S1 and S2 from about 3 nucleotides to about 5 nucleotides in length, and wherein the antisense strand and the sense strand form a duplex structure of at least about 19 nucleotides in length but are not covalently linked.

[0023] In some examples, the loop L is a triloop (triL) or a tetraloop (L). In some examples, L is a tetraloop of 4 nucleotides in length. In other examples, L includes a sequence 5'-GAAA-3'.

[0024] In some examples, S1 and S2 are 1-10 nucleotides in length and have the same length. In other examples, S1 and S2 are 1 nucleotide, 2 nucleotides, 3 nucleotides, 4 nucleotides, 5 nucleotides, 6 nucleotides, 7 nucleotides, 8 nucleotides, 9 nucleotides, or 10 nucleotides in length. In other examples, S1 and S2 are 6 nucleotides in length. In certain examples, the stem-loop comprises the sequence 5'-GCAGCCGAAAGGCUGC-3' (SEQ ID NO:333).

[0025] In some examples, the sense strand is 25 nucleotides in length and the antisense strand is 27 nucleotides in length. In other examples, the sense strand is 36 nucleotides in length and the antisense strand is 22 nucleotides in length.

[0026] In the examples above, the duplex region includes a 3'-overhang sequence on the antisense strand. In some examples, the 3'-overhang sequence on the antisense strand is 2 nucleotides in length.

[0027] In any of the examples above, at least one nucleotide in an oligonucleotide is a modified nucleotide. In some examples, all nucleotides in the oligonucleotide are modified except for nucleotides in the stem-loop (*i.e.*, S1-L-S2). In other examples, all nucleotides in the oligonucleotide are modified except for nucleotides in the loop (*i.e.*, L).

[0028] In some examples, the modified nucleotide includes a 2'-modification such as, for example, 2'-aminoethyl (EA), 2'-fluoro (2'-F), 2'-O-methyl (2'-OMe), 2'-O-methoxyethyl (2'-MOE) and 2'-deoxy-2'-fluoro- β -arabinonucleic acid (2'-FANA). In certain examples, all nucleotides in an oligonucleotide include a 2'-modification such as, for example, 2'-F or 2'-OMe.

[0029] In any of the examples above, at least one nucleotide in an oligonucleotide includes a modified internucleotide linkage. In some examples, the modified internucleotide linkage is a phosphorothioate linkage.

[0030] In any of the examples above, a 4'-carbon of a sugar of a 5'-nucleotide of the antisense strand includes a phosphate analog such as, for example, an oxymethylphosphonate, vinylphosphonate or malonyl phosphonate. Alternatively, or optionally, the phosphate analog is a 4'-phosphate analog including 5'-methoxyphosphonate-4'-oxy.

[0031] In any of the examples above, at least one nucleotide of an oligonucleotide can be conjugated to one or more targeting ligands such as, for example, an amino sugar, carbohydrate, cholesterol, lipid, or polypeptide. In some examples, the targeting ligand is a N-acetylgalactosamine (GalNAc) moiety. In other examples, the GalNAc moiety is a monovalent GalNAc moiety, a bivalent GalNAc moiety, a trivalent GalNAc moiety, or a tetravalent GalNAc moiety.

[0032] In some examples, the targeting ligands are conjugated to one or more nucleotides of L of the stem loop. In certain examples, up to 4 nucleotides of L of the stem-loop are each conjugated to a monovalent GalNAc moiety.

[0033] In certain examples, one or more nucleotides at positions 8, 9, 10, or 11 of the sense strand are modified with a 2'-F. In other examples, the sugar moiety at each nucleotide at positions 1 to 7, 12 to 27 and 31 to 36 in the sense strand is modified with a 2'-OMe. In certain examples, nucleotides at positions 8 to 11 of the sense strand are modified with a 2'-F, and positions 1 to 7, 12 to 27 and 31 to 36 are modified with a 2'-OMe.

[0034] In certain other examples, the sense strand includes a 2'-F modified nucleotide at positions 8 to 11, a 2'-OMe modified nucleotide at positions 1 to 7, 12 to 27 and 31 to 36, a

GalNAc-conjugated nucleotide at position 28, 29 and 30, and a phosphorothioate linkage between positions 1 and 2.

[0035] In certain other examples, one or more nucleotides at positions 2 to 5, 7, 10 and 14 of the antisense strand are modified with 2'-F, and one or more nucleotides at positions 1, 6, 8-9, 11-13 and 15-22 modified with a 2'-OMe. In other examples, the antisense strand includes a 2'-F-modified nucleotide at positions 2 to 5, 7, 10 and 14, and a 2'-OMe-modified nucleotide at positions 1, 6, 8 to 9, 11 to 13 and 15 to 22.

[0036] In certain examples, the antisense strand includes a 2'-F modified nucleotide at positions 2 to 5, 7, 10 and 14, a 2'-OMe at positions 1, 6, 8 to 9, 11 to 13 and 15 to 22, and a phosphorothioate linkage between positions 1 and 2, positions 2 and 3, positions 3 and 4, positions 20 and 21, and positions 21 and 22.

[0037] In certain examples, the oligonucleotides have a modification pattern as shown in FIG. 1A, 1B or 1C.

[0038] FIG. 1A depicts modification pattern 1, wherein the modification pattern of each strand is illustrated below:

Sense Strand: 5' mX-S-mX-fX-mX-mX-mX-mX-fX-fX-fX-mX-fX-fX-mX-mX-mX-fX-mX-mX-mX-mX-mX-mX-mX-mX-[X-GalNAc]-[X-GalNAc]-[X-GalNAc]-[X-GalNAc]-mX-mX-mX-mX-mX-mX 3'

Hybridized to:

Antisense Strand: 5' [MePhosphonate-4O-mX]-S-fX-S-fX-S-mX-fX-mX-fX-fX-mX-fX-mX-fX-mX-fX-mX-fX-mX-mX-fX-mX-S-mX-S-mX 3';

or represented as follows:

Sense Strand: 5' [mXs][mX][fX][mX][mX][mX][mX][fX][fX][fX][mX][fX][fX][mX][mX][mX][fX][mX][mX][mX][mX][mX][mX][mX][mX][X-GalNAc][X-GalNAc][X-GalNAc][X-GalNAc][mX][mX][mX][mX][mX][mX] 3'

Hybridized to:

Antisense Strand: 5' [MePhosphonate-4O-mX]-S-fX-S-fX-S-mX-fX-mX-fX-mX-mX-fX-mX-fX-mX-fX-mX-fX-mX-fX-mX-S-mX-S-mX 3';

or represented as follows:

Sense Strand: 5' [mXs][mX][fX][mX][fX][mX][mX][fX][fX][fX][fX][mX][fX][mX][mX][mX][fX][mX][mX][mX][mX][mX][mX][mX][mX][mX][X-GalNAc][X-GalNAc][X-GalNAc][X-GalNAc][mX][mX][mX][mX][mX][mX] 3'

Hybridized to:

Antisense Strand: 5' [MePhosphonate-4O-mXs][fXs][fXs][mX][fX][mX][fX][mX][mX][fX][mX][fX][mX][fX][mX][fX][mX][fX][mX][fX][mXs][mXs][mX] 3'.

Modification key for the modification patterns 1-3 above:

Symbol	Modification/linkage
Key 1	
mX	2'-O-methyl modified nucleotide
fX	2'- fluoro modified nucleotide
-S-	phosphorothioate linkage
-	phosphodiester linkage
[MePhosphonate-4O-mX]	5'-methoxyphosphonate-4'-oxy modified nucleotide
X-GalNAc	ademX-GalNAc or prgX-peg-GalNAc
Key 2	
[mXs]	2'-O-methyl modified nucleotide with a phosphorothioate linkage to the neighboring nucleotide
[fXs]	2'- fluoro modified nucleotide with a phosphorothioate linkage to the neighboring nucleotide
[mX]	2'-O-methyl modified nucleotide with phosphodiester linkages to neighboring nucleotides
[fX]	2'- fluoro modified nucleotide with phosphodiester linkages to neighboring nucleotides

[0041] In any of the examples above, the oligonucleotide is a RNAi oligonucleotide. In some examples, the RNAi oligonucleotide includes a sense strand having a nucleotide sequence as set forth in Table 2, especially any one of SEQ ID NOs:37, 43, 45, 87, 89, 99, 101, and 105. In certain examples, the RNAi oligonucleotide includes a sense strand having a nucleotide sequence as set forth in Table 3, especially any one of SEQ ID NOs:199, 205, 207,

249, 251, 261, 263, and 267. In some examples, the RNAi oligonucleotide includes an antisense strand having a nucleotide sequence as set for the in Table 2, especially any one of SEQ ID NOs:38, 44, 46, 88, 90, 100, 102, and 106. In certain examples, the RNAi oligonucleotide includes an antisense strand having a nucleotide sequence as set forth the in Table 3, especially any one of SEQ ID NOs:200, 206, 208, 250, 252, 262, 264, and 268.

[0042] In certain examples, the RNAi oligonucleotide includes a sense strand having a nucleotide sequence of any one of SEQ ID NOs:37, 43, 45, 87, 89, 99, 101 and 105, and an antisense strand having a nucleotide sequence of any one of SEQ ID NOs:38, 44, 46, 88, 90, 100, 102 and 106.

[0043] In certain examples, the RNAi oligonucleotide includes a sense strand having a nucleotide sequence of any one of SEQ ID NOs:37, 89 and 101, and an antisense strand having a nucleotide sequence of any one of SEQ ID NOs:38, 90 and 102.

[0044] In certain other examples, the sense strand, and the antisense strand of the RNAi oligonucleotide, respectively, are selected from:

- (a) SEQ ID NOs:37 and 38,
- (b) SEQ ID NOs:43 and 44,
- (c) SEQ ID NOs:45 and 46,
- (d) SEQ ID NOs:87 and 88,
- (e) SEQ ID NOs:89 and 90,
- (f) SEQ ID NOs:99 and 100,
- (g) SEQ ID NOs:101 and 102, and
- (h) SEQ ID NOs:105 and 106.

[0045] In certain examples, the RNAi oligonucleotide includes a sense strand having a nucleotide sequence of any one of SEQ ID NOs:199, 205, 207, 249, 251, 261, 263, and 267, and an antisense strand having a nucleotide sequence of any one of SEQ ID NOs:200, 206, 208, 250, 252, 262, 264, and 268.

[0046] In certain examples, the RNAi oligonucleotide includes a sense strand having a nucleotide sequence of any one of SEQ ID NOs:199, 251 and 263, and an antisense strand having a nucleotide sequence of any one of SEQ ID NOs:200, 252 and 264.

[0047] In certain other examples, the sense strand, and the antisense strand of the RNAi oligonucleotide, respectively, are selected from:

- (a') SEQ ID NOs:199 and 200,
- (b') SEQ ID NOs:205 and 206,

- (c') SEQ ID NOs:207 and 208,
- (d') SEQ ID NOs:249 and 250,
- (e') SEQ ID NOs:251 and 252,
- (f') SEQ ID NOs:261 and 262,
- (g') SEQ ID NOs:263 and 264, and
- (h') SEQ ID NOs:267 and 268.

[0048] Oligonucleotides also are described for inhibiting or reducing *APOC3* expression that include a sense strand and an antisense strand, where the sense strand and the antisense strand form a duplex region, where all nucleotides of the sense strand and the antisense strand include a modification of a base, a sugar and/or an internucleotide linkage, where the antisense strand includes a region of complementarity to a *APOC3* mRNA target sequence of one of SEQ ID NOs:334 to 341, and where the region of complementarity is at least about 15 contiguous nucleotides in length.

[0049] In other examples, pharmaceutical compositions are described that include at least one oligonucleotide herein, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier, delivery agent or excipient. In some examples, the pharmaceutical compositions include an additional therapeutic agent such as, for example, a lipid-lowering agent, an antidiabetic agent, or anti-obesity agent.

[0050] In other examples, methods are described for reducing *APOC3* expression in a cell, a population of cells, a tissue, an organ, or an individual that include at least a step of administering/contacting the cell, the population of cells, the tissue, the organ, or the individual with an oligonucleotide herein or a pharmaceutical composition herein. In some examples, reducing *APOC3* expression includes reducing an amount or level of *APOC3* mRNA, an amount or level of *APOC3* protein, or both in the cell, the population of cells, the tissue, the organ, or the individual. In some examples, the cell, the cell population, the tissue, the organ, or the individual has a disease, disorder, or condition associated with *APOC3* expression. In certain examples, the disease, disorder, or condition associated with *APOC3* expression is hypertriglyceridemia, high non-HDL cholesterol, liver steatosis, insulin resistance or even atherosclerotic cardiovascular disease (ASCVD).

[0051] In other examples, methods are described for treating an individual having or suspected of having a disease, disorder or condition associated with *APOC3* expression. The methods include at least a step of administering to an individual in need thereof an effective amount of an oligonucleotide herein or a pharmaceutical composition herein. In some examples, the

disease, disorder, or condition associated with *APOC3* expression is hypertriglyceridemia, high non-HDL cholesterol, liver steatosis, insulin resistance or even ASCVD. In some examples, the oligonucleotide or pharmaceutical composition is administered daily, weekly, monthly, quarterly, yearly *via* subcutaneous (SQ) administration, especially monthly or quarterly.

[0052] In some examples, the individual has NAFLD, liver steatosis, non-alcoholic steatohepatitis (NASH), alcoholic hepatitis (AH), alcoholic liver disease (ALD), cholangiocarcinoma (CCA), cirrhosis, , hepatic fibrosis, hepatic inflammation, hepatocellular carcinoma (HCC), primary sclerosing cholangitis (PSC), hyperlipidemia, diabetes, and /or obesity.

[0053] In any of the examples above, the methods may comprise additional steps such as measuring or obtaining genotype information, *APOC3* expression, APOC3 protein levels, the individual's weight and/or blood glucose and/or cholesterol and/or LPL and/or TG and then comparing such obtained values to one or more baseline values or previously obtained values to assess the effectiveness of contacting or administering. In some examples, the additional step comprises confirming that the individual has an *APOC3* gain-of-function polymorphism. In some examples, the additional step comprises confirming that the individual has a single-nucleotide polymorphism (SNP) of rs5128, rs2854116, rs2854117, rs2070666, or mutation of 1100C>T, 2845T>G or Gln38Lys.

[0054] In any of the examples above, the methods can include administering the RNAi oligonucleotide or pharmaceutical composition simultaneously, separately, or sequentially with a second composition or a second therapeutic agent. In some examples, the second composition or a second therapeutic agent is a APOC3 antibody or fragment thereof, a lipid-lowering agent, an antidiabetic agent or anti-obesity agent. In some examples, the second composition or second therapeutic agent is administered with a frequency same as the RNAi oligonucleotide (*i.e.*, every other day, twice a week, or even weekly). In other examples, the second composition or second therapeutic agent is administered with a frequency distinct from the RNAi oligonucleotide. Likewise, in other examples, the second composition or second therapeutic agent is administered *via* the same route as the RNAi oligonucleotide (*e.g.*, SQ). In still other examples, the second composition or second therapeutic agent is administered *via* a route that differs from the RNAi oligonucleotide).

[0055] In other examples, uses are described for the RNAi oligonucleotides herein for treating a disease, disorder or condition associated with *APOC3* expression, which optionally are

administered simultaneously, separately, or sequentially (*i.e.*, in combination) with a second composition or second therapeutic agent.

[0056] In other examples, uses are described for the RNAi oligonucleotides herein in manufacturing a medicament for treating a disease, disorder, or condition associated with *APOC3* expression, where the medicament optionally further includes a second composition or second therapeutic agent.

[0057] In other examples, kits are described that include at least one oligonucleotide herein, an optional pharmaceutically acceptable carrier, and a package insert having instructions for administering the same to an individual having a disease, disorder, or condition associated with *APOC3* expression.

[0058] An advantage of the oligonucleotides and compositions herein is that suppressed *APOC3* expression exerts a beneficial effect on the entire spectrum of dyslipidemia.

[0058a] In one aspect, the disclosure refers to a RNAi oligonucleotide for reducing apolipoprotein C-III (*APOC3*) expression, the oligonucleotide comprising a sense strand and an antisense strand, wherein the sense strand and the antisense strand form a duplex region, wherein the antisense strand comprises a region of complementarity to an *APOC3* mRNA target sequence of any one of SEQ ID NOS: 338, 334 to 337, and 339 to 341, and wherein the region of complementarity is at least 15 contiguous nucleotides in length.

[0058b] In another aspect, the disclosure refers to a RNAi oligonucleotide for reducing apolipoprotein C-III (*APOC3*) expression, the oligonucleotide comprising a sense strand of 18 to 36 nucleotides in length, optionally a sense strand of 36 nucleotides in length and an antisense strand of 22 nucleotides in length, wherein the sense strand and the antisense strand form a duplex region, wherein the 3' end of the sense strand comprises a stem-loop set forth as S1-L-S2, wherein S1 is complementary to S2, wherein L forms a loop between S1 and S2 of 3 to 5 nucleotides in length, wherein the antisense strand comprises a region of complementarity to an *APOC3* mRNA target sequence of any one of SEQ ID NOS: 338, 334 to 337, and 339 to 341, and wherein the region of complementarity is at least 19 contiguous nucleotides in length, optionally 20 nucleotides in length.

[0058c] In yet another aspect, the disclosure refers to a RNAi oligonucleotide for reducing apolipoprotein C-III (*APOC3*) expression, the oligonucleotide comprising a sense strand of 36 nucleotides in length and an antisense strand of 22 nucleotides in length, wherein the sense strand and the antisense strand form a duplex region of at least 19 nucleotides in length, optionally 20 nucleotides in length, wherein the 3' end of the sense strand comprises a

stem-loop set forth as S1-L-S2, wherein S1 is complementary to S2, wherein L forms a loop between S1 and S2 of 3 to 5 nucleotides in length, wherein the antisense strand comprises a region of complementarity to an APOC3 mRNA target sequence of any one of SEQ ID NOS: 338, 334 to 337, and 339 to 341, and wherein the region of complementarity is at least 19 contiguous nucleotides in length, optionally 20 nucleotides in length.

[0058d] In a further aspect, the disclosure refers to a RNAi oligonucleotide for reducing apolipoprotein C-III (*APOC3*) expression, the oligonucleotide comprising a sense strand and an antisense strand, wherein the sense strand and the antisense strand form a duplex region, wherein all nucleotides comprising the sense strand and antisense strand are modified, optionally wherein the 4'-carbon of the sugar of the 5'-nucleotide of the antisense strand comprises a phosphate analog, wherein the antisense strand comprises a region of complementarity to an APOC3 mRNA target sequence of any one of SEQ ID NOS: 338, 334 to 337, and 339 to 341, and wherein the region of complementarity is at least 15 contiguous nucleotides in length.

[0058e] In another aspect, the disclosure refers to a RNAi oligonucleotide for reducing apolipoprotein C-III (*APOC3*) expression, the oligonucleotide comprising a sense strand and an antisense strand, wherein the sense strand and the antisense strand form a duplex region, wherein all nucleotides comprising the sense strand and the antisense strand are modified, wherein the antisense strand and the sense strand comprise 1 or more 2'-fluoro and 2'-O-methyl modified nucleotides and at least one phosphorothioate linkage, wherein the 4'-carbon of the sugar of the 5'-nucleotide of the antisense strand comprises a phosphate analog, wherein the antisense strand comprises a region of complementarity to an APOC3 mRNA target sequence of any one of SEQ ID NOS: 338, 334 to 337, and 339 to 341, and wherein the region of complementarity is at least 15 contiguous nucleotides in length.

[0058f] In yet another aspect, the disclosure refers to a RNAi oligonucleotide for reducing apolipoprotein C-III (*APOC3*) expression, the oligonucleotide comprising a sense strand and an antisense strand selected from a row set forth in Table 2 or Table 3, or a pharmaceutically acceptable salt thereof.

[0058g] In yet another aspect, the disclosure refers to a pharmaceutical composition comprising: the RNAi oligonucleotide as described herein, or a pharmaceutically acceptable salt thereof; and a pharmaceutically acceptable carrier, delivery agent or excipient.

[0058h] In a further aspect, the disclosure refers to use of an RNAi oligonucleotide as described herein, or a pharmaceutically acceptable salt thereof, or the pharmaceutical composition as

described herein, in the manufacture of a medicament for treating an individual having a disease, disorder or condition associated with apolipoprotein C-III (*APOC3*) expression, optionally wherein the disease, disorder or condition associated with *APOC3* expression is acute coronary diseases (ACD), atherosclerotic cardiovascular disease (ASCVD), alcoholic hepatitis (AH), alcoholic liver disease (ALD), cardiometabolic disease, cholangiocarcinoma (CCA), cirrhosis, coronary heart diseases (CHD), diabetes, hepatic fibrosis, hepatic inflammation, hepatocellular carcinoma (HCC), hyperlipidemia, hypertriglyceridemia, high non-HDL cholesterol, insulin resistance, liver steatosis, metabolic syndromes (MetS), non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), obesity, or primary sclerosing cholangitis (PSC).

[0058i] In another aspect, the disclosure refers to use of an *in vitro* method of reducing apolipoprotein C-III (*APOC3*) expression in a cell, a population of cells, the method comprising the step of: contacting the cell or the population of cells with the RNAi oligonucleotide as described herein, or a pharmaceutically acceptable salt thereof, or the pharmaceutical composition as described herein; optionally wherein reducing *APOC3* expression comprises reducing an amount or level of *APOC3* mRNA, an amount or level of *APOC3* protein, or both.

[0058j] In yet another aspect, the disclosure refers to use of an RNAi oligonucleotide as described herein, or a pharmaceutically acceptable salt thereof, or the pharmaceutical composition as described herein, in the manufacture of a medicament for reducing apolipoprotein C-III (*APOC3*) expression in an individual; optionally wherein reducing *APOC3* expression comprises reducing an amount or level of *APOC3* mRNA, an amount or level of *APOC3* protein, or both.

[0058k] In a further aspect, the disclosure refers to use of the RNAi oligonucleotide as described herein, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for use in combination therapy for treating a disease or condition associated with apolipoprotein C-III (*APOC3*) expression, wherein said medicament is to be administered in combination with a second composition or therapeutic agent.

[0058l] In another aspect, the disclosure refers to the RNAi oligonucleotide as described herein, or a pharmaceutically acceptable salt thereof, or the pharmaceutical composition as described herein, for use as a medicament.

[0058m] In yet another aspect, the disclosure refers to a kit comprising the RNAi oligonucleotide as described herein, or a pharmaceutically acceptable salt thereof, an optional pharmaceutically acceptable carrier, and a package insert comprising instructions for

administration to an individual having a disease, disorder or condition associated with apolipoprotein C-III (*APOC3*) expression; optionally wherein the disease, disorder or condition associated with apolipoprotein C-III (*APOC3*) expression is acute coronary diseases (ACD), atherosclerotic cardiovascular disease (ASCVD), alcoholic hepatitis (AH), alcoholic liver disease (ALD), cardiometabolic disease, cholangiocarcinoma (CCA), cirrhosis, coronary heart diseases (CHD), diabetes, hepatic fibrosis, hepatic inflammation, hepatocellular carcinoma (HCC), hyperlipidemia, hypertriglyceridemia, high non-HDL cholesterol, insulin resistance, liver steatosis, metabolic syndromes (MetS), non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), obesity, or primary sclerosing cholangitis (PSC).

BRIEF DESCRIPTION OF THE DRAWINGS

[0059] The advantages, effects, features, and objects other than those set forth above will become more readily apparent when consideration is given to the detailed description below. Such detailed description refers to the following drawing(s), where:

[0060] FIGS. 1A-1C disclose schematics depicting the structure and chemical modification pattern for 3 generic GalNAc-conjugated *APOC3* oligonucleotides (M1, M2 and M3, respectively).

DETAILED DESCRIPTION

Overview

[0061] Dyslipidemia refers to unhealthy levels of one or more kinds of lipid (fat) in blood. There are three main types of lipids: high-density lipoprotein (HDL), low-density lipoprotein (LDL), and TGs. Dyslipidemia is divided up into primary and secondary types. Primary dyslipidemia is inherited, whereas secondary dyslipidemia is an acquired condition (*i.e.*, develops from other causes such as, for example, obesity or diabetes).

[0062] RNA interference (RNAi) is a process of introducing exogenous RNA into a cell leading to specific degradation of the mRNA encoding the targeted protein with a resultant decrease in target gene expression.

[0063] In humans, *APOC3* is 99 amino acids in length (residues 1-20, however, are a signal peptide that is subsequently cleaved) with a predicted molecular weight of 8.8 kDa. Exemplary

CLAIMS

1. A RNAi oligonucleotide for reducing apolipoprotein C-III (*APOC3*) expression, the oligonucleotide comprising a sense strand and an antisense strand, wherein the sense strand and the antisense strand form a duplex region, wherein the antisense strand comprises a region of complementarity to an *APOC3* mRNA target sequence of any one of SEQ ID NOS: 338, 334 to 337, and 339 to 341, and wherein the region of complementarity is at least 15 contiguous nucleotides in length.
2. The RNAi oligonucleotide of Claim 1, wherein the sense strand is 15 to 50 nucleotides in length, optionally wherein the sense strand is 18 to 36 nucleotides in length; and/or wherein the antisense strand is 15 to 30 nucleotides in length, optionally wherein the antisense strand is 22 nucleotides in length, and where in the antisense strand and the sense strand form a duplex region of at least 19 nucleotides in length, optionally at least 20 nucleotides in length; and/or wherein the region of complementarity is at least 19 contiguous nucleotides in length, optionally at least 20 nucleotides in length.
3. The RNAi oligonucleotide of Claim 1 or 2, wherein the 3' end of the sense strand comprises a stem-loop set forth as S1-L-S2, wherein S1 is complementary to S2, and wherein L forms a loop between S1 and S2 of 3 to 5 nucleotides in length.
4. A RNAi oligonucleotide for reducing apolipoprotein C-III (*APOC3*) expression, the oligonucleotide comprising a sense strand of 18 to 36 nucleotides in length, optionally a sense strand of 36 nucleotides in length and an antisense strand of 22 nucleotides in length, wherein the sense strand and the antisense strand form a duplex region, wherein the 3' end of the sense strand comprises a stem-loop set forth as S1-L-S2, wherein S1 is complementary to S2, wherein L forms a loop between S1 and S2 of 3 to 5 nucleotides in length, wherein the antisense strand comprises a region of complementarity to an *APOC3* mRNA target sequence of any one of SEQ ID NOS: 338, 334 to 337, and 339 to 341, and wherein the region of complementarity is at least 19 contiguous nucleotides in length, optionally 20 nucleotides in length.
5. A RNAi oligonucleotide for reducing apolipoprotein C-III (*APOC3*) expression, the oligonucleotide comprising a sense strand of 36 nucleotides in length and an antisense strand of 22 nucleotides in length, wherein the sense strand and the antisense strand form a duplex

region of at least 19 nucleotides in length, optionally 20 nucleotides in length, wherein the 3' end of the sense strand comprises a stem-loop set forth as S1-L-S2, wherein S1 is complementary to S2, wherein L forms a loop between S1 and S2 of 3 to 5 nucleotides in length, wherein the antisense strand comprises a region of complementarity to an APOC3 mRNA target sequence of any one of SEQ ID NOS: 338, 334 to 337, and 339 to 341, and wherein the region of complementarity is at least 19 contiguous nucleotides in length, optionally 20 nucleotides in length.

6. The RNAi oligonucleotide of any one of Claims 3 to 5, wherein L is a triloop or a tetraloop; optionally wherein the tetraloop comprises a sequence of 5'-GAAA-3'.

7. The RNAi oligonucleotide of Claim 6, wherein S1 and S2 are 1-10 nucleotides in length and have the same length; optionally wherein S1 and S2 are 6 nucleotides in length.

8. The RNAi oligonucleotide of Claim 6 or 7, wherein the stem-loop comprises the sequence 5'-GCAGCCGAAAGGCUGC-3' (SEQ ID NO:333).

9. The RNAi oligonucleotide of any one of Claims 1 to 8, wherein the antisense strand comprises a 3' overhang sequence of one or more nucleotides in length; optionally wherein the 3' overhang sequence is 2 nucleotides in length, and optionally wherein the 3' overhang sequence is GG.

10. The RNAi oligonucleotide of any one of the preceding claims comprising at least one modified nucleotide; optionally wherein the modified nucleotide comprises a 2'-modification, and/or wherein the 2'-modification is a modification selected from the group consisting of 2'-aminoethyl, 2'-fluoro, 2'-O-methyl, 2'-O-methoxyethyl, and 2'-deoxy-2'-fluoro- β -D-arabinonucleic acid.

11. The RNAi oligonucleotide of Claim 10, wherein all nucleotides are modified, and optionally wherein the modification is a 2'-modification selected from the group consisting of 2'-fluoro and 2'-O-methyl.

12. The RNAi oligonucleotide of Claim 11, wherein one or more nucleotides at positions 8, 9, 10, or 11 of the sense strand are modified with 2'-fluoro, and/or wherein one or more

nucleotides at positions 2, 3, 4, 5, 7, 10, or 14 of the antisense strand are modified with 2'-fluoro, and/or wherein one or more nucleotides at positions 1, 2, 3, 4, 5, 6, 7, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 31, 32, 33, 34, 35, or 36 of the sense strand are modified with 2'-O-methyl, and/or wherein one or more nucleotides at positions 1, 6, 8, 9, 11, 12, 13, 15, 16, 17, 18, 19, 20, 21, or 22 of the antisense strand are modified with 2'-O-methyl.

13. The RNAi oligonucleotide of any one of the preceding claims comprising at least one modified internucleotide linkage; optionally wherein the at least one modified internucleotide linkage is a phosphorothioate linkage.

14. The RNAi oligonucleotide of Claim 13, wherein the internucleotide linkage of nucleotides at positions 1 and 2 of the sense strand are modified with the phosphorothioate linkage, and/or wherein the internucleotide linkage of one or more nucleotides at positions 1 and 2, 2 and 3, 3 and 4, 20 and 21, or 21 and 22 of the antisense strand are modified with the phosphorothioate linkage.

15. The RNAi oligonucleotide of any one of Claims 1 to 14, wherein the RNAi oligonucleotide has a modification pattern as shown in FIG. 1A, FIG. 1B, or FIG. 1C, and/or wherein the RNAi oligonucleotide has the modification pattern shown in FIG. 1B.

16. The RNAi oligonucleotide of any one of the preceding claims, wherein the 4'-carbon of the sugar of the 5'-nucleotide of the antisense strand comprises a phosphate analog; optionally wherein the phosphate analog is oxymethylphosphonate, vinylphosphonate or malonyl phosphonate, and optionally wherein the phosphate analog is a 4'-phosphate analog comprising 5'-methoxyphosphonate-4'-oxy.

17. The RNAi oligonucleotide of any one of the preceding claims, wherein at least one nucleotide of the oligonucleotide is conjugated to one or more targeting ligands; optionally wherein each targeting ligand comprises a carbohydrate, amino sugar, cholesterol, polypeptide, or lipid; optionally wherein each targeting ligand comprises a N-acetyl galactosamine (GalNAc) moiety; optionally wherein the GalNAc moiety is a monovalent GalNAc moiety, a bivalent GalNAc moiety, a trivalent GalNAc moiety, or a tetravalent GalNAc moiety.

18. The RNAi oligonucleotide of any one of Claims 3 to 17, wherein up to 4 nucleotides of L of the stem-loop are each conjugated to a monovalent GalNAc moiety.

19. The RNAi oligonucleotide of any one of the preceding claims, wherein the sense strand comprises a nucleotide sequence of any one of SEQ ID NOs: 89, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, and 169, and/or wherein the antisense strand comprises a nucleotide sequence of any one of SEQ ID NOs: 90, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, and 170.

20. The RNAi oligonucleotide of any one of the preceding claims, wherein the sense strand and the antisense strands comprise nucleotide sequences selected from the group consisting of:

- (a) SEQ ID NOs:89 and 90,
- (b) SEQ ID NOs:37 and 38,
- (c) SEQ ID NOs:43 and 44,
- (d) SEQ ID NOs:45 and 46,
- (e) SEQ ID NOs:87 and 88,
- (f) SEQ ID NOs:99 and 100,
- (g) SEQ ID NOs:101 and 102, and
- (h) SEQ ID NOs:105 and 106.

21. The RNAi oligonucleotide of any one of the preceding claims, wherein the sense strand and the antisense strands comprise nucleotide sequences selected from the group consisting of:

- (a') SEQ ID NOs:251 and 252,
- (b') SEQ ID NOs:199 and 200,
- (c') SEQ ID NOs:205 and 206,
- (d') SEQ ID NOs:207 and 208,
- (e') SEQ ID NOs:249 and 250,
- (f') SEQ ID NOs:261 and 262,

(g') SEQ ID NOs:263 and 264, and

(h') SEQ ID NOs:267 and 268.

22. A RNAi oligonucleotide for reducing apolipoprotein C-III (*APOC3*) expression, the oligonucleotide comprising a sense strand and an antisense strand, wherein the sense strand and the antisense strand form a duplex region, wherein all nucleotides comprising the sense strand and antisense strand are modified, optionally wherein the 4'-carbon of the sugar of the 5'-nucleotide of the antisense strand comprises a phosphate analog, wherein the antisense strand comprises a region of complementarity to an *APOC3* mRNA target sequence of any one of SEQ ID NOs: 338, 334 to 337, and 339 to 341, and wherein the region of complementarity is at least 15 contiguous nucleotides in length.

23. A RNAi oligonucleotide for reducing apolipoprotein C-III (*APOC3*) expression, the oligonucleotide comprising a sense strand and an antisense strand, wherein the sense strand and the antisense strand form a duplex region, wherein all nucleotides comprising the sense strand and the antisense strand are modified, wherein the antisense strand and the sense strand comprise 1 or more 2'-fluoro and 2'-O-methyl modified nucleotides and at least one phosphorothioate linkage, wherein the 4'-carbon of the sugar of the 5'-nucleotide of the antisense strand comprises a phosphate analog, wherein the antisense strand comprises a region of complementarity to an *APOC3* mRNA target sequence of any one of SEQ ID NOs: 338, 334 to 337, and 339 to 341, and wherein the region of complementarity is at least 15 contiguous nucleotides in length.

24. The RNAi oligonucleotide of Claim 22 or 23, wherein the sense strand comprises of any one of SEQ ID NOs: 89, 37, 43, 45, 87, 99, 101, and 105; or wherein the sense strand comprises of any one of SEQ ID NOs: 251, 199, 205, 207, 249, 261, 263, and 267, and/or wherein the antisense strand comprises of any one of SEQ ID NOs: 90, 38, 44, 46, 88, 100, 102, and 106; or wherein the antisense strand comprises of any one of SEQ ID NOs: 252, 200, 206, 208, 250, 262, 264, and 268.

25. A RNAi oligonucleotide for reducing apolipoprotein C-III (*APOC3*) expression, the oligonucleotide comprising a sense strand and an antisense strand selected from a row set forth in Table 2 or Table 3, or a pharmaceutically acceptable salt thereof.

26. A pharmaceutical composition comprising:
the RNAi oligonucleotide of any one of Claims 1 to 25, or a pharmaceutically acceptable salt thereof; and
a pharmaceutically acceptable carrier, delivery agent or excipient.
27. Use of an RNAi oligonucleotide of any one of claims 1 to 25, or a pharmaceutically acceptable salt thereof, or the pharmaceutical composition of claim 26, in the manufacture of a medicament for treating an individual having a disease, disorder or condition associated with apolipoprotein C-III (*APOC3*) expression,
optionally wherein the disease, disorder or condition associated with *APOC3* expression is acute coronary diseases (ACD), atherosclerotic cardiovascular disease (ASCVD), alcoholic hepatitis (AH), alcoholic liver disease (ALD), cardiometabolic disease, cholangiocarcinoma (CCA), cirrhosis, coronary heart diseases (CHD), diabetes, hepatic fibrosis, hepatic inflammation, hepatocellular carcinoma (HCC), hyperlipidemia, hypertriglyceridemia, high non-HDL cholesterol, insulin resistance, liver steatosis, metabolic syndromes (MetS), non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), obesity, or primary sclerosing cholangitis (PSC).
28. An *in vitro* method of reducing apolipoprotein C-III (*APOC3*) expression in a cell, a population of cells, the method comprising the step of:
contacting the cell or the population of cells with the RNAi oligonucleotide of any one of Claims 1 to 25, or a pharmaceutically acceptable salt thereof, or the pharmaceutical composition of Claim 26;
optionally wherein reducing *APOC3* expression comprises reducing an amount or level of *APOC3* mRNA, an amount or level of *APOC3* protein, or both.
29. Use of an RNAi oligonucleotide of any one of claims 1 to 25, or a pharmaceutically acceptable salt thereof, or the pharmaceutical composition of claim 26, in the manufacture of a medicament for reducing apolipoprotein C-III (*APOC3*) expression in an individual; optionally wherein reducing *APOC3* expression comprises reducing an amount or level of *APOC3* mRNA, an amount or level of *APOC3* protein, or both.
30. The use of Claim 29, wherein the individual has a disease, disorder or condition associated with *APOC3* expression; optionally wherein the disease, disorder or condition

associated with *APOC3* expression is acute coronary diseases (ACD), atherosclerotic cardiovascular disease (ASCVD), alcoholic hepatitis (AH), alcoholic liver disease (ALD), cardiometabolic disease, cholangiocarcinoma (CCA), cirrhosis, coronary heart diseases (CHD), diabetes, hepatic fibrosis, hepatic inflammation, hepatocellular carcinoma (HCC), hyperlipidemia, hypertriglyceridemia, high non-HDL cholesterol, insulin resistance, liver steatosis, metabolic syndromes (MetS), non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), obesity, or primary sclerosing cholangitis (PSC).

31. Use of the RNAi oligonucleotide of any one of Claims 27 or 29 to 30, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for use in combination therapy for treating a disease or condition associated with apolipoprotein C-III (*APOC3*) expression, wherein said medicament is to be administered in combination with a second composition or therapeutic agent.

32. The RNAi oligonucleotide of any one of Claims 1 to 25, or a pharmaceutically acceptable salt thereof, or the pharmaceutical composition of Claim 26, for use as a medicament.

33. A kit comprising the RNAi oligonucleotide of any one of Claims 1 to 25, or a pharmaceutically acceptable salt thereof, an optional pharmaceutically acceptable carrier, and a package insert comprising instructions for administration to an individual having a disease, disorder or condition associated with apolipoprotein C-III (*APOC3*) expression; optionally wherein the disease, disorder or condition associated with apolipoprotein C-III (*APOC3*) expression is acute coronary diseases (ACD), atherosclerotic cardiovascular disease (ASCVD), alcoholic hepatitis (AH), alcoholic liver disease (ALD), cardiometabolic disease, cholangiocarcinoma (CCA), cirrhosis, coronary heart diseases (CHD), diabetes, hepatic fibrosis, hepatic inflammation, hepatocellular carcinoma (HCC), hyperlipidemia, hypertriglyceridemia, high non-HDL cholesterol, insulin resistance, liver steatosis, metabolic syndromes (MetS), non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), obesity, or primary sclerosing cholangitis (PSC).