activation and proflieration of hepatic stellate cells and excessive generation and deposition of extracellular matrix (Chen *et al*. (2015) *World J. Gastroenterol*., 21(3):794-802).

Currently, treatments for NAFLD are directed towards weight loss and treatment of any secondary conditions, such as insulin resistance or dyslipidemia. To date, no pharmacologic treatments for NAFLD have been approved. Therefore, there is a need for therapies for subjects suffering from NAFLD.

Summary

The present disclosure provides iRNA compositions which affect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of a gene encoding Patatin-Like Phospholipase Domain Containing 3 (PNPLA3). The Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) may be within a cell, *e.g.,* a cell within a subject, such as a human subject.

In one aspect, the present disclosure refers to a double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) in a cell, wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of nucleotides 1214-1234 of SEQ ID NO:1, and the antisense strand comprises at least 15 contiguous nucleotides from the corresponding nucleotide sequence of SEQ ID NO:2.

In another aspect, the present disclosure refers to a double stranded ribonucleic acid (dsRNA) for inhibiting expression of Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) in a cell, wherein said dsRNA comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises a region of complementarity to an mRNA encoding PNPLA3, and wherein the region of complementarity comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the antisense nucleotide sequences in any one of Tables 2-11, 21, 24, 27, 30, 32, 33, 36, 37, 49 or 50.

In another aspect, the present disclosure refers to a double stranded ribonucleic acid (dsRNA) for inhibiting expression of Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) in a cell, wherein said dsRNA comprises a sense strand and an antisense strand forming a double stranded region, wherein a) the sense strand comprises at least 15 contiguous nucleotides differing by no more than three nucleotides from any one of the nucleotide sequence of nucleotides 677-721; 683-721; 773-817; 1185-1295; 1185-1241; 1202-1295; 1202-1241; 1255-1295; 1738-1792; 1901-1945; 1920-1945; 2108-2208; 2108-2166; 2108-2136, 2121-2166; 2121-2208; 2169-2208; 2176-2208; or 2239-2265 of SEQ ID NO: 1, and the antisense strand comprises at least 15 contiguous nucleotides from the corresponding nucleotide sequence of SEQ ID NO:2; or b) the sense strand comprises at least 15 contiguous nucleotides differing by no more than three nucleotides from any one of the nucleotide sequence of nucleotides 574-596; 677-699; 683-705; 699-721; 773-795; 795-817; 1185-1207; 1192-1214; 1202-1224; 1208-1230; 1209-1231; 1210-1232; 1211-1233; 1212-1234; 1213-1233; 1214-1234; 1214-1236; 1215-1237; 1216-1238; 1219-1237; 1219-1241; 1255-1275; 1256-1276; 1257-

1275; 1257-1277; 1258-1278; 1259-1279; 1260-1278; 1260-1280; 1261-1281; 1262-1282; 1263-1283; 1264-1282; 1264-1284; 1265-1285; 1267-1285; 1266-1286; 1266-1288; 1267-1285; 1267-1287; 1268-1290; 1269-1289; 1270-1290; 1271-1291; 1272-1292; 1273-1293; 1274-1294; 1275-1295; 1631-1653; 1738-1760; 1739-1761; 1740-1760; 1740-1762; 1741-1763; 1744-1766; 1746-1766; 1750-1772; 1751-1773; 1752-1774; 1753-1775; 1754-1776; 1755-1777; 1756-1778; 1757-1779; 1758-1780; 1759-1781; 1760-1782; 1761-1783; 1762-1782; 1762-1784; 1763-1785; 1764-1786; 1765-1787; 1766-1786; 1766-1788; 1767-1787; 1768-1788; 1767-1789; 1769-1789; 1770-1788; 1770-1790; 1771-1791; 1772-1792; 1815-1837; 1901-1923, 1920-1942, 1923-1945; 2112-2130; 2169-2191; 2171-2191; 2176-2198, 2177-2199, 2178-2200; 2179-2201, 2180-2202; 2181-2203; 2183-2205; 2184-2206; 2186-2208; 2239-2261; 2241-2263; 2242-2264; or 2243-2265 of SEQ ID NO: 1, and the antisense strand comprises at least 15 contiguous nucleotides from the corresponding nucleotide sequence of SEQ ID NO:2.

In another aspect, the present disclosure refers to a cell containing the dsRNA agent as disclosed herein.

In another aspect, the present disclosure refers to a pharmaceutical composition for inhibiting expression of a gene encoding Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) comprising the dsRNA agent as disclosed herein.

In another aspect, the present disclosure refers to the use of the dsRNA agent as disclosed herein, or the pharmaceutical composition as disclosed herein in the manufacture of a medicament for inhibiting expression of a Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) gene in a cell.

In another aspect, the present disclosure refers to the use of the dsRNA agent as disclosed herein, or the pharmaceutical composition as disclosed herein in the manufacture of a medicament for treating a subject having a disorder that would benefit from reduction in Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) expression.

In another aspect, the present disclosure refers to a kit comprising the dsRNA agent as disclosed herein or the pharmaceutical composition as disclosed herein.

In another aspect, the present disclosure refers to a vial comprising the dsRNA agent as disclosed herein or the pharmaceutical composition as disclosed herein.

In another aspect, the present disclosure refers to a syringe comprising the dsRNA agent as disclosed herein or the pharmaceutical composition as disclosed herein.

In an example, the disclosure provides a double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) in a cell, wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15, *e.g.,* 15, 16, 17, 18, 19, or 20, contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from the nucleotide sequence of SEQ ID NO:1 and the antisense strand comprises at least 15, *e.g.,* 15, 16, 17, 18, 19, or 20, contiguous nucleotides differing by no more than 1, 2, or 3 nucleotides from the nucleotide sequence of SEQ ID NO:2. In one example, the dsRNA agent comprises at least one thermally destabilizing nucleotide modification, *e.g.,* an abasic modification; a mismatch

with the opposing nucleotide in the duplex; and destabilizing sugar modification, a 2'-deoxy modification, an acyclic nucleotide, an unlocked nucleic acids (UNA), or a glycerol nucleic acid (GNA), *e.g.,* the antisense strand comprises at least one thermally destabilizing nucleotide modification.

In another example, the present disclosure provides a double stranded ribonucleic acid (dsRNA) for inhibiting expression of Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) in a cell, wherein said dsRNA comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises a region of complementarity to an mRNA encoding Patatin-Like Phospholipase Domain Containing 3 (PNPLA3), and wherein the region of complementarity comprises at least 15, *e.g.,* 15, 16, 17, 18, 19, or 20, contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from any one of the antisense nucleotide sequences in any one of Tables 2-11, 21, 24, 27, 30, 32, 33, 36, 37, 49 or 50.

In one example, the present disclosure provides a double stranded ribonucleic acid (dsRNA) for inhibiting expression of Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) in a cell, wherein said dsRNA comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15, *e.g.,* 15, 16, 17, 18, 19, or 20, contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from any one of the nucleotide sequence of nucleotides 677-721; 683-721; 773-817; 1185-1295; 1185-1241; 1202-1295; 1202-1241; 1255-1295; 1738-1792; 1901-1945; 1920-1945; 2108-2208; 2108-2166; 2108-2136, 2121-2166; 2121-2208; 2169-2208; 2176-2208; or 2239-2265 of the nucleotide sequence of SEQ ID NO:1, and

the antisense strand comprises at least 19 contiguous nucleotides from the corresponding nucleotide sequence of SEQ ID NO:2.

In one example, the present disclosure provides a double stranded ribonucleic acid (dsRNA) for inhibiting expression of Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) in a cell, wherein said dsRNA comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15, *e.g.,* 15, 16, 17, 18, 19, or 20, contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from any one of the nucleotide sequence of nucleotides 574-596; 677-699; 683-705; 699-721; 773-795; 795-817; 1185-1207; 1192-1214; 1202-1224; 1208-1230; 1209-1231; 1210-1232; 1211-1233; 1212-1234; 1213-1233; 1214-1234; 1214-1236; 1215-1237; 1216-1238; 1219-1237; 1219-1241; 1255-1275; 1256-1276; 1257-1275; 1257-1277; 1258-1278; 1259-1279; 1260-1278; 1260-1280; 1261-1281; 1262-1282; 1263-1283; 1264-1282; 1264-1284; 1265-1285; 1267-1285; 1266-1286; 1266-1288; 1267-1285; 1267-1287; 1268-1290; 1269-1289; 1270-1290; 1271-1291; 1272-1292; 1273-1293; 1274-1294; 1275-1295; 1631-1653; 1738-1760; 1739-1761; 1740-1760; 1740-1762; 1741-1763; 1744-1766; 1746-1766; 1750-1772; 1751-1773; 1752-1774; 1753-1775; 1754-1776; 1755-1777; 1756-1778; 1757-1779; 1758-1780; 1759-1781; 1760-1782; 1761-1783; 1762-1782; 1762-1784; 1763-1785; 1764-1786; 1765-1787; 1766-1786; 1766-1788; 1767-1787; 1768-1788; 1767-1789; 1769-1789; 1770-1788; 1770-1790; 1771-1791; 1772-1792; 1815-1837; 1901-1923, 1920-1942, 1923-1945; 2112-2130; 2169-2191; 2171-2191; 2176-2198, 2177-2199, 2178-2200; 2179-2201, 2180-2202; 2181-2203; 2183-2205; 2184-2206; 2186-2208; 2239-2261; 2241-2263; 2242-2264; or 2243-2265 of the nucleotide sequence of SEQ ID NO:1, and the antisense strand comprises at least 19 contiguous nucleotides from the corresponding nucleotide sequence of SEQ ID NO:2.

In one example, the antisense strand comprises at least 15, *e.g.,* 15, 16, 17, 18, 19, or 20, contiguous nucleotides differing by nor more than 0, 1, 2, or 3 nucleotides from any one of the antisense strand nucleotide sequences of a duplex selected from the group consisting of AD-517197.2; AD-517258.2; AD-516748.2; AD-516851.2; AD-519351.2; AD-519754.2; AD-519828.2; AD-520018.2; AD-520035.2; AD-520062.2; AD-520064.2; AD-520065.2; AD-520067.2; AD-75289.2; AD-520069.2; AD-520099.2; AD-67575.7; AD-520101.2; AD-67605.7; AD-1193323.1; AD-1193344.1; AD-1193350.1; AD-1193365.1; AD-1193379.1; AD-1193407.1; AD-1193421.1; AD-1193422.1; AD-1193429.1; AD-1193437.1; AD-1193443.1; AD-1193471.1; and AD-1193481.1.

In one example, the antisense strand comprises at least 15, *e.g.,* 15, 16, 17, 18, 19, or 20, contiguous nucleotides differing by nor more than 0, 1, 2, or 3 nucleotides from any one of the antisense strand nucleotide sequences of a duplex selected from the group consisting of AD-519345.1, AD-519346.1, AD-519347.1, AD-67554.7, AD-519752.3, AD-1010731.1, AD-1010732.1, AD-519343.1, AD-519344.1, AD-519349.1, AD-519350.1, AD-519753.2, AD-519932.1, AD-519935.2, AD-520018.6, AD-517837.2, AD-805635.2, AD-519329.2, AD-520063.2, AD-519757.2, AD-805631.2, AD-516917.2, AD-516828.2, AD-518983.2, AD-805636.2, AD-519754.7, AD-520062.2, AD-67575.9, AD-518923.3, AD-520053.4, AD-519667.2, AD-519773.2, AD-519354.2, AD-520060.4, AD-520061.4, AD-1010733.2, AD-1010735.2; AD-1193323.1; AD-1193344.1; AD-

1193350.1; AD-1193365.1; AD-1193379.1; AD-1193407.1; AD-1193421.1; AD-1193422.1; AD-1193429.1; AD-1193437.1; AD-1193443.1; AD-1193471.1; and AD-1193481.1.

In one example, the present disclosure provides a double stranded ribonucleic acid (dsRNA) for inhibiting expression of Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) in a cell, wherein the dsRNA comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than three nucleotides from any one of the nucleotide sequence of nucleotides 1200-1250, 1205-1250, 11210-1250, 1200-1245, 1200-1240, 1200-1235, 1200-1237, 1205-1237, 1210-1232, 1212-1237, 1212-1234, 1250-1300, 1255-1300, 1260-1300, 1250-1295, 1250-1390, 1250-1285, 1250-1282, 1255-1282, 1260-1282, 1262-1300, 1262-1295, 1262-1390, and 1262-1282 of SEQ ID NO: 1, and the antisense strand comprises at least 15 contiguous nucleotides from the corresponding nucleotide sequence of SEQ ID NO:2.

In one example, the antisense strand comprises at least 15, *e.g.,* 15, 16, 17, 18, 19, or 20, contiguous nucleotides differing by nor more than 0, 1, 2, or 3 nucleotides from any one of the antisense strand nucleotide sequences of a duplex selected from the group consisting of AD-519345, AD-1193350, AD-1193365, AD-1193437 and AD-519347.

In one example, the antisense strand comprises at least 15, *e.g.,* 15, 16, 17, 18, 19, or 20, contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from the antisense strand nucleotide sequence of duplex AD-519351.

In one example, the antisense strand comprises at least 15, *e.g.,* 15, 16, 17, 18, 19, or 20, contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from the antisense strand nucleotide sequence of duplex AD-1193350.

In one example, the antisense strand comprises at least 15, *e.g.,* 15, 16, 17, 18, 19, or 20, contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from the antisense strand nucleotide sequence of duplex AD-1193365.

In one example, the antisense strand comprises at least 15, *e.g.,* 15, 16, 17, 18, 19, or 20, contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from the antisense strand nucleotide sequence of duplex AD-1193437.

In one example, the antisense strand comprises at least 15, *e.g.,* 15, 16, 17, 18, 19, or 20, contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from the antisense strand nucleotide sequence of duplex AD-519347.

In one example, the dsRNA agent comprises at least one modified nucleotide.

In one example, substantially all of the nucleotides of the sense strand; substantially all of the nucleotides of the antisense strand comprise a modification; or substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand comprise a modification.

In one example, all of the nucleotides of the sense strand comprise a modification; all of the nucleotides of the antisense strand comprise a modification; or all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand comprise a modification.

In one example, at least one of the modified nucleotides is selected from the group consisting of a deoxy-nucleotide, a 3'-terminal deoxy-thymine (dT) nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an unlocked nucleotide, a conformationally restricted nucleotide, a constrained ethyl nucleotide, an abasic nucleotide, a 2'-amino-modified nucleotide, a 2'-O-allyl-modified nucleotide, 2'-C-alkyl-modified nucleotide, 2'-hydroxly-modified nucleotide, a 2'-methoxyethyl modified nucleotide, a 2'-O-alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, a non-natural base comprising nucleotide, a tetrahydropyran modified nucleotide, a 1,5-anhydrohexitol modified nucleotide, a cyclohexenyl modified nucleotide, a nucleotide comprising a phosphorothioate group, a nucleotide comprising a methylphosphonate group, a nucleotide comprising a 5'-phosphate, a nucleotide comprising a 5'-phosphate mimic, a thermally destabilizing nucleotide, a glycol modified nucleotide (GNA), and a 2-O-(N-methylacetamide) modified nucleotide; and combinations thereof.

In one example, the modifications on the nucleotides are selected from the group consisting of LNA, HNA, CeNA, 2′-methoxyethyl, 2′-O-alkyl, 2′-O-allyl, 2′-C- allyl, 2′-fluoro, 2′-deoxy, 2'-hydroxyl, and glycol; and combinations thereof.

In one example, at least one of the modified nucleotides is selected from the group consisting of a deoxy-nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a glycol modified nucleotide (GNA), *e.g.,* Ggn, Cgn, Tgn, or Agn, and, a vinyl-phosphonate nucleotide; and combinations thereof.

In another example, at least one of the modifications on the nucleotides is a thermally destabilizing nucleotide modification.

In one example, the thermally destabilizing nucleotide modification is selected from the group consisting of an abasic modification; a mismatch with the opposing nucleotide in the duplex; and destabilizing sugar modification, a 2'-deoxy modification, an acyclic nucleotide, an unlocked nucleic acids (UNA), and a glycerol nucleic acid (GNA).

In some examples, the modified nucleotide comprises a short sequence of 3'-terminal deoxy-thymine nucleotides (dT).

In some examples, the modifications on the nucleotides are 2'-O-methyl, GNA and 2'fluoro modifications.

In some examples, the dsRNA agent further comprises at least one phosphorothioate internucleotide linkage. In some examples, the dsRNA agent comprises 6-8 phosphorothioate internucleotide linkages. In one example, the phosphorothioate or methylphosphonate internucleotide linkage is at the 3'-terminus of one strand. Optionally, the strand is the antisense strand. In another example, the strand is the sense strand. In a related example, the phosphorothioate or methylphosphonate internucleotide linkage is at the 5'-terminus of one strand. Optionally, the strand is the antisense strand. In another example, the strand is the sense strand. In another example, the phosphorothioate or methylphosphonate internucleotide linkage is at the both the 5'- and 3'-terminus of one strand. Optionally, the strand is the antisense strand. In another example, the strand is the sense strand.

The double stranded region may be 19-30 nucleotide pairs in length;19-25 nucleotide pairs in length;19-23 nucleotide pairs in length; 23-27 nucleotide pairs in length; or 21-23 nucleotide pairs in length.

In one example, each strand is independently no more than 30 nucleotides in length.

In one example, the sense strand is 21 nucleotides in length and the antisense strand is 23 nucleotides in length.

The region of complementarity may be at least 17 nucleotides in length; between 19 and 23 nucleotides in length; or 19 nucleotides in length.

In one example, at least one strand comprises a 3' overhang of at least 1 nucleotide. In another example, at least one strand comprises a 3' overhang of at least 2 nucleotides.

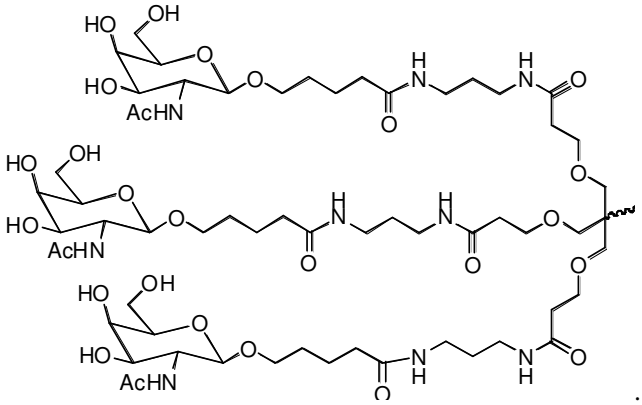
In one example, the dsRNA agent further comprises a ligand.

In one example, the ligand is conjugated to the 3' end of the sense strand of the dsRNA agent.

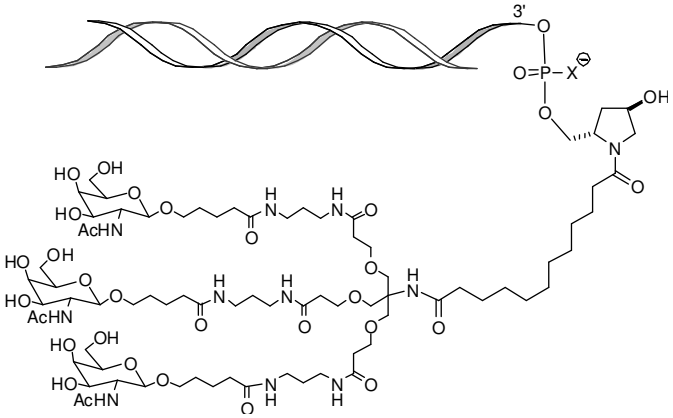
In one example, the ligand is an N-acetylgalactosamine (GalNAc) derivative.

In one example, the ligand is one or more GalNAc derivatives attached through a monovalent, bivalent, or trivalent branched linker.

In one example, the ligand is



In one example, the dsRNA agent is conjugated to the ligand as shown in the following schematic



and, wherein X is O or S.

In one example, the X is O.

In one example, the dsRNA agent further comprises at least one phosphorothioate or methylphosphonate internucleotide linkage.

In one example, the phosphorothioate or methylphosphonate internucleotide linkage is at the 3'-terminus of one strand, *e.g.,* the antisense strand or the sense strand.

In another example, the phosphorothioate or methylphosphonate internucleotide linkage is at the 5'-terminus of one strand, *e.g.,* the antisense strand or the sense strand.

In one example, the phosphorothioate or methylphosphonate internucleotide linkage is at the both the 5'- and 3'-terminus of one strand. In one example, the strand is the antisense strand.

In one example, the base pair at the 1 position of the 5′-end of the antisense strand of the duplex is an AU base pair.

The present disclosure also provides cells containing any of the dsRNA agents of the disclosure and pharmaceutical compositions comprising any of the dsRNA agents of the disclosure.

The pharmaceutical composition of the disclosure may include dsRNA agent in an unbuffered solution, *e.g.,* saline or water, or the pharmaceutical composition of the disclosure may include the dsRNA agent is in a buffer solution, *e.g.,* a buffer solution comprising acetate, citrate, prolamine, carbonate, or phosphate or any combination thereof; or phosphate buffered saline (PBS).

In one example, the present disclosure provides a method of inhibiting expression of a Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) gene in a cell. The method includes contacting the cell with any of the dsRNAs of the disclosure or any of the pharmaceutical compositions of the disclosure, thereby inhibiting expression of the PNPLA3 gene in the cell.

In one example, the cell is within a subject, *e.g.,* a human subject, *e.g.,* a subject having a Patatin-Like Phospholipase Domain Containing 3 (PNPLA3)-associated disorder, such as a Patatin-Like Phospholipase Domain Containing 3 (PNPLA3)-associated disorder selected from the group consisting of fatty liver (steatosis), nonalcoholic steatohepatitis (NASH), cirrhosis of the liver, accumulation of fat in the liver, inflammation of the liver, hepatocellular necrosis, liver fibrosis, obesity, or nonalcoholic fatty liver disease (NAFLD).

In one example, contacting the cell with the dsRNA agent inhibits the expression of PNPLA3 by at least 50%, 60%, 70%, 80%, 90%, or 95%.

In one example, inhibiting expression of PNPLA3 decreases PNPLA3 protein level in serum of the subject by at least 50%, 60%, 70%, 80%, 90%, or 95%.

In one example, the present disclosure provides a method of treating a subject having a disorder that would benefit from reduction in Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) expression. The method includes administering to the subject a therapeutically effective amount of any of the dsRNAs of the disclosure or any of the pharmaceutical compositions of the disclosure, thereby treating the subject having the disorder that would benefit from reduction in PNPLA3 expression.

In another example, the present disclosure provides a method of preventing at least one symptom in a subject having a disorder that would benefit from reduction in Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) expression. The method includes administering to the subject a prophylactically effective amount of any of the dsRNAs of the disclosure or any of the

pharmaceutical compositions of the disclosure, thereby preventing at least one symptom in the subject having the disorder that would benefit from reduction in PNPLA3 expression.

In one example, the disorder is a Patatin-Like Phospholipase Domain Containing 3 (PNPLA3)-associated disorder, *e.g.,* a Patatin-Like Phospholipase Domain Containing 3 (PNPLA3)-associated disorder is selected from the group consisting of fatty liver (steatosis), nonalcoholic steatohepatitis (NASH), cirrhosis of the liver, accumulation of fat in the liver, inflammation of the liver, hepatocellular necrosis, liver fibrosis, obesity, or nonalcoholic fatty liver disease (NAFLD).

In one example, the PNPLA3-associated disorder is NAFLD.

In one example, the subject is human.

In one example, the dsRNA agent is administered to the subject at a dose of about 0.01 mg/kg to about 50 mg/kg.

In one example, the dsRNA agent is administered to the subject subcutaneously.

In one example, the methods of the disclosure include further determining the level of PNPLA3 in a sample(s) from the subject.

In one example, the level of Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) in the subject sample(s) is a Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) protein level in a blood or serum sample(s).

In certain examples, the methods of the disclosure further comprise administering to the subject an additional therapeutic agent. In a further example, the additional therapeutic agent is selected from the group consisting of an HMG-CoA reductase inhibitor, a fibrate, a bile acid sequestrant, niacin, an antiplatelet agent, an angiotensin converting enzyme inhibitor, an angiotensin II receptor antagonist, an acylCoA cholesterol acetyltransferase (ACAT) inhibitor, a cholesterol absorption inhibitor, a cholesterol ester transfer protein (CETP) inhibitor, a microsomal triglyceride transfer protein (MTTP) inhibitor, a cholesterol modulator, a bile acid modulator, a peroxisome proliferation activated receptor (PPAR) agonist, a gene-based therapy, a composite vascular protectant, a glycoprotein Ilb/IIIa inhibitor, aspirin or an aspirin- like compound, an IBAT inhibitor, a squalene synthase inhibitor, a monocyte chemoattractant protein (MCP)-I inhibitor, or fish oil.

The present disclosure also provides kits comprising any of the dsRNAs of the disclosure or any of the pharmaceutical compositions of the disclosure, and optionally, instructions for use.

Brief Description of the Drawings

Figure 1 is a graph showing human PNPLA3 mRNA levels in mice (n = 3 per group) subcutaneously administered a single 3 mg/kg or 10 mg/kg dose of the indicated dsRNA duplexes, on day 7 or day14 post-dose. Human PNPLA3 mRNA levels are shown relative to control levels detected with PBS treatment.

Figure 2 is a graph showing human PNPLA3 mRNA levels in mice (n = 3 per group) subcutaneously administered a single dose of the indicated dsRNA duplexes, on day 7 post-dose as described in Example 3. Human PNPLA3 mRNA levels are shown relative to control levels detected with PBS treatment.

We claim:

1. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) in a cell,

wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region,

wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of nucleotides 1214-1234 of SEQ ID NO:1, and the antisense strand comprises at least 15 contiguous nucleotides from the corresponding nucleotide sequence of SEQ ID NO:2.

2. A double stranded ribonucleic acid (dsRNA) for inhibiting expression of Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) in a cell, wherein said dsRNA comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises a region of complementarity to an mRNA encoding PNPLA3, and wherein the region of complementarity comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the antisense nucleotide sequences in any one of Tables 2-11, 21, 24, 27, 30, 32, 33, 36, 37, 49 or 50.

3. A double stranded ribonucleic acid (dsRNA) for inhibiting expression of Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) in a cell,

wherein said dsRNA comprises a sense strand and an antisense strand forming a double stranded region,

wherein

a) the sense strand comprises at least 15 contiguous nucleotides differing by no more than three nucleotides from any one of the nucleotide sequence of nucleotides 677-721; 683-721; 773-817; 1185-1295; 1185-1241; 1202-1295; 1202-1241; 1255-1295; 1738-1792; 1901-1945; 1920-1945; 2108-2208; 2108-2166; 2108-2136, 2121-2166; 2121-2208; 2169-2208; 2176-2208; or 2239-2265 of SEQ ID NO: 1, and the antisense strand comprises at least 15 contiguous nucleotides from the corresponding nucleotide sequence of SEQ ID NO:2; or

b) the sense strand comprises at least 15 contiguous nucleotides differing by no more than three nucleotides from any one of the nucleotide sequence of nucleotides 574-596; 677-699; 683-705; 699-721; 773-795; 795-817; 1185-1207; 1192-1214; 1202-1224; 1208-1230; 1209-1231; 1210-1232; 1211-1233; 1212-1234; 1213-1233; 1214-1234; 1214-1236; 1215-1237; 1216-1238; 1219-1237; 1219-1241; 1255-1275; 1256-1276; 1257-1275; 1257-1277; 1258-1278; 1259-1279; 1260-1278; 1260-1280; 1261-1281; 1262-1282; 1263-1283; 1264-1282; 1264-1284; 1265-1285; 1267-1285; 1266-1286; 1266-1288; 1267-1285; 1267-1287; 1268-1290; 1269-1289; 1270-1290; 1271-1291; 1272-1292; 1273-1293; 1274-1294; 1275-1295; 1631-1653; 1738-1760; 1739-1761; 1740-

1760; 1740-1762; 1741-1763; 1744-1766; 1746-1766; 1750-1772; 1751-1773; 1752-1774; 1753-1775; 1754-1776; 1755-1777; 1756-1778; 1757-1779; 1758-1780; 1759-1781; 1760-1782; 1761-1783; 1762-1782; 1762-1784; 1763-1785; 1764-1786; 1765-1787; 1766-1786; 1766-1788; 1767-1787; 1768-1788; 1767-1789; 1769-1789; 1770-1788; 1770-1790; 1771-1791; 1772-1792; 1815-1837; 1901-1923, 1920-1942, 1923-1945; 2112-2130; 2169-2191; 2171-2191; 2176-2198, 2177-2199, 2178-2200; 2179-2201, 2180-2202; 2181-2203; 2183-2205; 2184-2206; 2186-2208; 2239-2261; 2241-2263; 2242-2264; or 2243-2265 of SEQ ID NO: 1, and the antisense strand comprises at least 15 contiguous nucleotides from the corresponding nucleotide sequence of SEQ ID NO:2.

4. The dsRNA agent of any one of claims 1-3, wherein the antisense strand comprises at least 15 contiguous nucleotides differing by no more than three nucleotides from any one of the antisense strand nucleotide sequences of a duplex selected from the group consisting of AD-517197.2; AD-517258.2; AD-516748.2; AD-516851.2; AD-519351.2; AD-519754.2; AD-519828.2; AD-520018.2; AD-520035.2; AD-520062.2; AD-520064.2; AD-520065.2; AD-520067.2; AD-75289.2; AD-520069.2; AD-520099.2; AD-67575.7; AD-520101.2; AD-1193323.1; AD-1193344.1; AD-1193350.1; AD-1193365.1; AD-1193379.1; AD-1193407.1; AD-1193421.1; AD-1193422.1; AD-1193429.1; AD-1193437.1; AD-1193443.1; AD-1193471.1; AD-1193481.1 and AD-67605.7.

5. The dsRNA agent of any one of claims 1-3, wherein the antisense strand comprises at least 15 contiguous nucleotides differing by nor more than three nucleotides from any one of the antisense strand nucleotide sequences of a duplex selected from the group consisting of AD-519345.1, AD-519346.1, AD-519347.1, AD-67554.7, AD-519752.3, AD-1010731.1, AD-1010732.1, AD-519343.1, AD-519344.1, AD-519349.1, AD-519350.1, AD-519753.2, AD-519932.1, AD-519935.2, AD-520018.6, AD-517837.2, AD-805635.2, AD-519329.2, AD-520063.2, AD-519757.2, AD-805631.2, AD-516917.2, AD-516828.2, AD-518983.2, AD-805636.2, AD-519754.7, AD-520062.2, AD-67575.9, AD-518923.3, AD-520053.4, AD-519667.2, AD-519773.2, AD-519354.2, AD-520060.4, AD-520061.4, AD-1010733.2, and AD-1010735.2.

6. The dsRNA agent of any one of claims 1-5, wherein the dsRNA agent comprises at least one modified nucleotide.

7. The dsRNA agent of any one of claims 1-6, wherein substantially all of the nucleotides of the sense strand; substantially all of the nucleotides of the antisense strand comprise a modification; or substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand comprise a modification.

8. The dsRNA agent of any one of claims 1-7, wherein all of the nucleotides of the sense strand comprise a modification; all of the nucleotides of the antisense strand comprise a modification; or all

of the nucleotides of the sense strand and all of the nucleotides of the antisense strand comprise a modification.

9. The dsRNA agent of any one of claims 6-8, wherein at least one of the modified nucleotides is selected from the group consisting of a deoxy-nucleotide, a 3'-terminal deoxy-thymine (dT) nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an unlocked nucleotide, a conformationally restricted nucleotide, a constrained ethyl nucleotide, an abasic nucleotide, a 2'-amino-modified nucleotide, a 2'-O-allyl-modified nucleotide, 2'-C-alkyl-modified nucleotide, 2'-hydroxly-modified nucleotide, a 2'-methoxyethyl modified nucleotide, a 2'-O-alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, a non-natural base comprising nucleotide, a tetrahydropyran modified nucleotide, a 1,5-anhydrohexitol modified nucleotide, a cyclohexenyl modified nucleotide, a nucleotide comprising a phosphorothioate group, a nucleotide comprising a methylphosphonate group, a nucleotide comprising a 5'-phosphate, a nucleotide comprising a 5'-phosphate mimic, a thermally destabilizing nucleotide, a glycol modified nucleotide (GNA), and a 2-O-(N-methylacetamide) modified nucleotide; and combinations thereof.

10. The dsRNA agent of any one of claims 6-8, wherein the modifications on the nucleotides are selected from the group consisting of LNA, HNA, CeNA, 2′-methoxyethyl, 2′-O-alkyl, 2′-O-allyl, 2′-C- allyl, 2′-fluoro, 2′-deoxy, 2'-hydroxyl, and glycol; and combinations thereof.

11. The dsRNA of any one of claims 6-8, wherein at least one of the modified nucleotides is selected from the group consisting of a deoxy-nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a glycol modified nucleotide (GNA), and, a vinyl-phosphonate nucleotide; and combinations thereof.

12. The dsRNA of any one of claims 6-8, wherein at least one of the modifications on the nucleotides is a thermally destabilizing nucleotide modification.

13. The dsRNA of claim 12, wherein the thermally destabilizing nucleotide modification is selected from the group consisting of an abasic modification; a mismatch with the opposing nucleotide in the duplex; and destabilizing sugar modification, a 2'-deoxy modification, an acyclic nucleotide, an unlocked nucleic acids (UNA), and a glycerol nucleic acid (GNA).

14. The dsRNA agent of any one of claims 1-13, wherein the double stranded region is 19-30 nucleotide pairs in length.

15. The dsRNA agent of claim 14, wherein the double stranded region is 19-25 nucleotide pairs in length.

16. The dsRNA agent of claim 14, wherein the double stranded region is 19-23 nucleotide pairs in length.

17. The dsRNA agent of claim 14, wherein the double stranded region is 23-27 nucleotide pairs in length.

18. The dsRNA agent of claim 14, wherein the double stranded region is 21-23 nucleotide pairs in length.

19. The dsRNA agent of any one of claims 1-18, wherein each strand is independently no more than 30 nucleotides in length.

20. The dsRNA agent of any one of claims 1-19, wherein the sense strand is 21 nucleotides in length and the antisense strand is 23 nucleotides in length.

21. The dsRNA agent of any one of claims 1-20, wherein the region of complementarity is at least 17 nucleotides in length.

22. The dsRNA agent of any one of claims 1-21, wherein the region of complementarity is between 19 and 23 nucleotides in length.

23. The dsRNA agent of any one of claims 1-22, wherein the region of complementarity is 19 nucleotides in length.

24. The dsRNA agent of any one of claims 1-23, wherein at least one strand comprises a 3' overhang of at least 1 nucleotide.

25. The dsRNA agent of any one of claims 1-23, wherein at least one strand comprises a 3' overhang of at least 2 nucleotides.

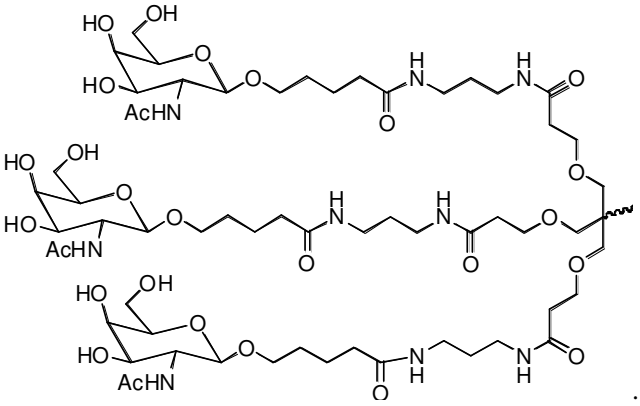
26. The dsRNA agent of any one of claims 1-25, further comprising a ligand.

27. The dsRNA agent of claim 26, wherein the ligand is conjugated to the 3' end of the sense strand of the dsRNA agent.

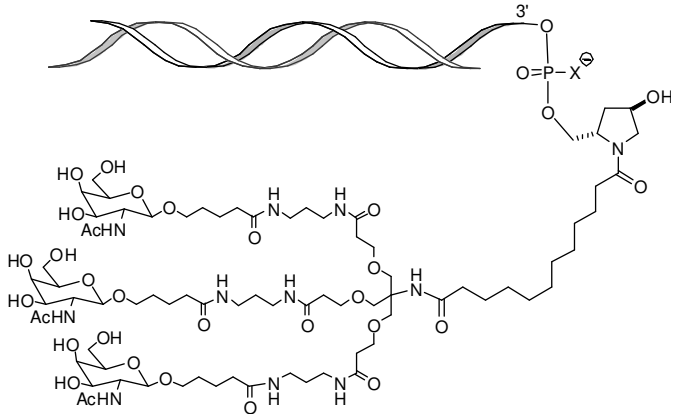
28. The dsRNA agent of claim 26 or 27, wherein the ligand is an N-acetylgalactosamine (GalNAc) derivative.

29. The dsRNA agent of any one of claims 26-28, wherein the ligand is one or more GalNAc derivatives attached through a monovalent, bivalent, or trivalent branched linker.

30. The dsRNA agent of claim 28 or 29, wherein the ligand is



31. The dsRNA agent of claim 30, wherein the dsRNA agent is conjugated to the ligand as shown in the following schematic



and, wherein X is O or S.

32. The dsRNA agent of claim 31, wherein the X is O.

33. The dsRNA agent of any one of claims 1-32, wherein the dsRNA agent further comprises at least one phosphorothioate or methylphosphonate internucleotide linkage.

34. The dsRNA agent of claim 33, wherein the phosphorothioate or methylphosphonate internucleotide linkage is at the 3'-terminus of one strand.

35. The dsRNA agent of claim 34, wherein the strand is the antisense strand.

36. The dsRNA agent of claim 34, wherein the strand is the sense strand.

37. The dsRNA agent of claim 33, wherein the phosphorothioate or methylphosphonate internucleotide linkage is at the 5'-terminus of one strand.

38. The dsRNA agent of claim 37, wherein the strand is the antisense strand.

39. The dsRNA agent of claim 37, wherein the strand is the sense strand.

40. The dsRNA agent of claim 33, wherein the phosphorothioate or methylphosphonate internucleotide linkage is at the both the 5'- and 3'-terminus of one strand.

41. The dsRNA agent of claim 40, wherein the strand is the antisense strand.

42. The dsRNA agent of any one of claims 1-41, wherein the base pair at the 1 position of the 5′-end of the antisense strand of the duplex is an AU base pair.

43. A cell containing the dsRNA agent of any one of claims 1-42.

44. A pharmaceutical composition for inhibiting expression of a gene encoding Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) comprising the dsRNA agent of any one of claims 1-42.

45. The pharmaceutical composition of claim 44, wherein dsRNA agent is in an unbuffered solution.

46. The pharmaceutical composition of claim 45, wherein the unbuffered solution is saline or water.

47. The pharmaceutical composition of claim 44, wherein said dsRNA agent is in a buffer solution.

48. The pharmaceutical composition of claim 47, wherein the buffer solution comprises acetate, citrate, prolamine, carbonate, or phosphate or any combination thereof.

49. The pharmaceutical composition of claim 48, wherein the buffer solution is phosphate buffered saline (PBS).

50. Use of the dsRNA agent of any one of claims 1-42, or the pharmaceutical composition of any one of claims 46-49 in the manufacture of a medicament for inhibiting expression of a Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) gene in a cell.

51. The use of claim 50, wherein the cell is within a subject.

52. The use of claim 51, wherein the subject is a human.

53. The use of claim 50, wherein the subject has a PNPLA3-associated disorder.

54. The use of claim 53, wherein the PNPLA3-associated disorder is selected from the group consisting of fatty liver (steatosis), nonalcoholic steatohepatitis (NASH), cirrhosis of the liver, accumulation of fat in the liver, inflammation of the liver, hepatocellular necrosis, liver fibrosis, obesity, and nonalcoholic fatty liver disease (NAFLD).

55. The use of any one of claims 50-54, wherein contacting the cell with the dsRNA agent inhibits the expression of PNPLA3 by at least 50%, 60%, 70%, 80%, 90%, or 95%.

56. The use of any one of claims 50-55, wherein inhibiting expression of PNPLA3 decreases PNPLA3 protein level in serum of the subject by at least 50%, 60%, 70%, 80%, 90%, or 95%.

57. Use of the dsRNA agent of any one of claims 1-42, or the pharmaceutical composition of any one of claims 44-49 in the manufacture of a medicament for treating a subject having a disorder that would benefit from reduction in Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) expression.

58. Use of the dsRNA agent of any one of claims 1-42, or the pharmaceutical composition of any one of claims 44-49 in the manufacture of a medicament for preventing at least one symptom in a subject having a disorder that would benefit from reduction in Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) expression.

59. The use of claim 57 or 58, wherein the disorder is a PNPLA3-associated disorder.

60. The use of claim 59, wherein the PNPLA3-associated disorder is selected from the group consisting of fatty liver (steatosis), nonalcoholic steatohepatitis (NASH), cirrhosis of the liver,

accumulation of fat in the liver, inflammation of the liver, hepatocellular necrosis, liver fibrosis, obesity, and nonalcoholic fatty liver disease (NAFLD).

61. The use of claim 59, wherein the PNPLA3-associated disorder is NAFLD.

62. The use of claim 59, wherein the subject is human.

63. The use of claim 57 or 58, wherein the administration of the agent to the subject causes a decrease in PNPLA3 protein accumulation.

64. The use of any one of claims 57-63, wherein the dsRNA agent is to be administered to the subject at a dose of 0.01 mg/kg to 50 mg/kg.

65. The use of any one of claims 57-64, wherein the dsRNA agent is to be administered to the subject subcutaneously.

66. The use of any one of claims 57-65, further comprising determining the level of PNPLA3 in a sample(s) from the subject.

67. The use of claim 66, wherein the level of PNPLA3 in the subject sample(s) is a PNPLA3 protein level in a blood or serum sample(s).

68. The use of any one of claims 57-67, further comprising that an additional therapeutic agent for treatment of a PNPLA3-associated disorder is to be administered to the subject.

69. A kit comprising the dsRNA agent of any one of claims 1-42 or the pharmaceutical composition of any one of claims 44-49.

70. A vial comprising the dsRNA agent of any one of claims 1-42 or the pharmaceutical composition of any one of claims 44-49.

71. A syringe comprising the dsRNA agent of any one of claims 1-42 or the pharmaceutical composition of any one of claims 44-49.