[0003] Glycerol-3-phosphate acyltransferase, mitochondrial (GPAM, GPAT1), having a sequence as found in Genbank XM_005269998.1, is associated with non-alcoholic steatohepatitis (NASH). Missense mutations in GPAM associate with accumulation of excess liver fat and non-alcoholic fatty liver disease (NAFLD) related phenotypes (Jamialahmadi, O., et al., Exome-Wide Association Study on Alanine Aminotransferase Identifies Sequence Variants in the GPAM and APOE Associated With Fatty Liver Disease. Gastroenterology, 2021. 160(5): p. 1634-1646 e7).

[0004] Currently, NAFLD symptoms are managed via weight loss and treatment of any secondary conditions, as no pharmacologic treatments have been approved. Thus, there is a need for compositions and methods that treat NAFLD in affected individuals.

SUMMARY

[0005] The present disclosure provides an RNAi construct comprising a sense strand and an antisense strand, wherein the antisense strand comprises a region having a sequence that is complementary to a GPAM mRNA sequence, such as a GPAM mRNA sequence set forth in Table 1, and wherein the RNAi construct inhibits the expression of GPAM. In certain examples, the RNAi construct comprises a region having at least 15 contiguous nucleotides differing by no more than 3 nucleotides from an antisense sequence listed in Table 2. In some examples, the antisense strand hybridizes to a GPAM mRNA sequence listed in Table 1.

[0005a] In one aspect, the present disclosure provides an RNAi construct comprising a sense strand and an antisense strand, wherein the antisense strand comprises a region that is complementary to a glycerol-3-phosphate acyltransferase, mitochondrial (GPAM) mRNA sequence listed in Table 1, and wherein the RNAi construct inhibits the expression of GPAM.

[0005b] In another aspect, the present disclosure provides a composition comprising the RNAi construct of the disclosure and a pharmaceutically acceptable carrier, excipient, or diluent.

[0005c] In another aspect, the present disclosure provides use of the RNAi construct of the disclosure in the manufacture of a medicament for reducing the expression of GPAM in a patient in need thereof, wherein the medicament is to be administered to the patient.

[0005d] In another aspect, the present disclosure provides use of the composition of the disclosure in the manufacture of a medicament for reducing the expression of GPAM in a patient in need thereof, wherein the medicament is to be administered to the patient.

[0005e] In another aspect, the present disclosure provides an RNAi construct of the disclosure or a composition of the disclosure for use in therapy.

[0006] In some examples, the sense strand of the RNAi constructs described herein comprises a sequence that is sufficiently complementary to the sequence of the antisense strand to form a duplex region of about 15 to about 30 base pairs in length. In these and other examples, the sense and antisense strands each are about 15 to about 30 nucleotides in length. In some examples, the RNAi constructs comprise at least one blunt end. In other examples, the RNAi constructs comprise at least one nucleotide overhang. Such nucleotide overhangs may comprise at least 1 to 6 unpaired nucleotides and can be located at the 3' end of the sense strand, the 3' end of the antisense strand, or the 3' end of both the sense and antisense strand. In certain examples, the RNAi constructs comprise an overhang of two unpaired nucleotides at the 3' end of the sense strand and the 3' end of the antisense strand. In other examples, the RNAi constructs comprise an

overhang of two unpaired nucleotides at the 3' end of the antisense strand and a blunt end of the 3' end of the sense strand/5' end of the antisense strand.

[0007] The RNAi constructs of the disclosure may comprise one or more modified nucleotides, including nucleotides having modifications to the ribose ring, nucleobase, or phosphodiester backbone. In some examples, the RNAi constructs comprise one or more 2'-modified nucleotides. Such 2'-modified nucleotides can include 2'-fluoro modified nucleotides, 2'-O-methyl modified nucleotides, 2'-O-methoxyethyl modified nucleotides, 2'-O-allyl modified nucleotides, bicyclic nucleic acids (BNA), glycol nucleic acids (GNAs), inverted bases (e.g. inverted adenosine) or combinations thereof. In one particular example, the RNAi constructs comprise one or more 2'-fluoro modified nucleotides, 2'-O-methyl modified nucleotides, or combinations thereof. In some examples, all of the nucleotides in the sense and antisense strand of the RNAi construct are modified nucleotides.

[0008] In some examples, the RNAi constructs comprise at least one backbone modification, such as a modified internucleotide or internucleoside linkage. In certain examples, the RNAi constructs described herein comprise at least one phosphorothioate internucleotide linkage. In particular examples, the phosphorothioate internucleotide linkages may be positioned at the 3' or 5' ends of the sense and/or antisense strands.

[0009] In some examples, the antisense strand and/or the sense strand of the RNAi constructs of the disclosure may comprise or consist of a sequence from the antisense and sense sequences listed in Table 2. In certain examples, the RNAi construct may be any one of the duplex compounds listed in Table 2.

[0010] The disclosure also provides a composition comprising the aforementioned RNAi construct and a pharmaceutically acceptable carrier, excipient, or diluent, as well as methods of reducing the expression of GPAM in a patient in need thereof comprising administering to the patient the aforementioned RNAi construct or composition.

DETAILED DESCRIPTION

[0011] The present invention is based, in part, on the design and generation of RNAi constructs that target the GPAM gene and reduce expression of GPAM in liver cells. The specific inhibition of GPAM expression is useful for treating or preventing conditions

What is Claimed

1. An RNAi construct comprising a sense strand and an antisense strand, wherein the antisense strand comprises a region that is complementary to a glycerol-3-phosphate acyltransferase, mitochondrial (GPAM) mRNA sequence listed in Table 1, and wherein the RNAi construct inhibits the expression of GPAM.

2. The RNAi construct of claim 1, which comprises a region having at least 15 contiguous nucleotides differing by no more than 3 nucleotides from an antisense sequence listed in Table 2.

3. The RNAi construct of claim 1 or claim 2, wherein the antisense strand hybridizes to a GPAM mRNA sequence listed in Table 1.

4. The RNAi construct of any one of claims 1-3, wherein the sense strand comprises a sequence that is sufficiently complementary to the sequence of the antisense strand to form a duplex region of about 15 to about 30 base pairs in length.

5. The RNAi construct of claim 4, wherein the duplex region is about 17 to about 24 base pairs in length.

6. The RNAi construct of claim 4, wherein the duplex region is about 19 to about 21 base pairs in length.

7. The RNAi construct of claim 6, wherein the duplex region is 19 base pairs in length.

8. The RNAi construct of claim 6, wherein the duplex region is 20 base pairs in length.

9. The RNAi construct of claim 6, wherein the duplex region is 21 base pairs in length.

10. The RNAi construct of any one of claims 4-10, wherein the sense strand and the antisense strand are each about 15 to about 30 nucleotides in length.

11. The RNAi construct of claim 10, wherein the sense strand and the antisense strand are each about 19 to about 27 nucleotides in length.

12. The RNAi construct of claim 10, wherein the sense strand and the antisense strand are each about 21 to about 25 nucleotides in length.

13. The RNAi construct of claim 12, wherein the sense strand and the antisense strand are each about 21 to about 23 nucleotides in length.

14. The RNAi construct of any one of claims 1 to 13, which comprises at least one blunt end.

15. The RNAi construct of any one of claims 1 to 13, which comprises at least one nucleotide overhang of 1 to 4 unpaired nucleotides.

16. The RNAi construct of claim 15, wherein the nucleotide overhang has two unpaired nucleotides.

17. The RNAi construct of claim 15 or 16, wherein the RNAi construct comprises a nucleotide overhang at the 3' end of the sense strand, the 3' end of the antisense strand, or the 3' end of both the sense strand and the antisense strand.

18. The RNAi construct of any one of claims 15-17**,** wherein the nucleotide overhang comprises a 5'-UU-3' dinucleotide or a 5'-dTdT-3' dinucleotide.

19. The RNAi construct of any one of claims 1 to 18, wherein the RNAi construct comprises at least one modified nucleotide.

20. The RNAi construct of claim 19, wherein the modified nucleotide is a 2'-modified nucleotide.

21. The RNAi construct of claim 19, wherein the modified nucleotide is a 2'-fluoro modified nucleotide, a 2'-O-methyl modified nucleotide, a 2'-O-methoxyethyl modified nucleotide, a 2'-O-allyl modified nucleotide, a bicyclic nucleic acid (BNA), a glycol nucleic acid, an inverted base, or combinations thereof.

22. The RNAi construct of claim 21, wherein the modified nucleotide is a 2'-O-methyl modified nucleotide, a 2'-O-methoxyethyl modified nucleotide, a 2'-fluoro modified nucleotide, or combinations thereof.

23. The RNAi construct of claim 19, wherein all of the nucleotides in the sense and antisense strands are modified nucleotides.

24. The RNAi construct of claim 23, wherein the modified nucleotides are 2'-O-methylmodified nucleotides, 2'-fluoro modified nucleotides, or combinations thereof.

25. The RNAi construct of any one of claims 1 to 24, which comprises at least one phosphorothioate internucleotide linkage.

26. The RNAi construct of claim 25, wherein the RNAi construct comprises at least one phosphorothioate internucleotide linkages at the 3’ end of the sense strand.

27. The RNAi construct of claim 25, wherein the RNAi construct comprises at least one phosphorothioate internucleotide linkages at both the 3’ and 5’ ends of the sense strand.

28. The RNAi construct of any one of claims 1-27, wherein the antisense strand comprises a sequence selected from the antisense sequences listed in Table 2.

29. The RNAi construct of claim 28, wherein the sense strand comprises a sequence selected from the sense sequences listed in Table 2.

30. The RNAi construct of any one of claims 1-29, wherein the RNAi construct is any one of the duplex compounds listed in Table 2.

31. The RNAi construct of any one of claims 1-30, wherein the RNAi construct reduces the expression level of GPAM in liver cells following incubation with the RNAi construct as compared to the GPAM expression level in liver cells that have been incubated with a control RNAi construct.

32. The RNAi construct of claim 31, wherein the liver cells are HepG2cells.

33. The RNAi construct of any one of claims 1-32, wherein the RNAi construct inhibits at least 10% of GPAM expression at 5 nM in HepG2 cells *in vitro.*

34. The RNAi construct of any one of claims 1-33, wherein the RNAi construct inhibits GPAM expression in HepG2 cells with an IC50 of less than about 1 nM.

35. The RNAi construct of any one of claims 1-34, further comprising a ligand that binds to one or more proteins expressed on the surface of liver cells.

36. A composition comprising the RNAi construct of any one of claims 1-35 and a pharmaceutically acceptable carrier, excipient, or diluent.

37. Use of the RNAi construct of any one of claims 1-35 in the manufacture of a medicament for reducing the expression of GPAM in a patient in need thereof, wherein the medicament is to be administered to the patient.

38. Use of the composition of claim 36 in the manufacture of a medicament for reducing the expression of GPAM in a patient in need thereof, wherein the medicament is to be administered to the patient.

39. The use of claim 37 or claim 38, wherein the expression level of GPAM in hepatocytes is reduced in the patient following administration of the RNAi construct as compared to the GPAM expression level in a patient who did not receive the RNAi construct.

40. The use of any one of claims 37-39, wherein the patient suffers from nonalcoholic fatty liver disease (NAFLD).

41. The use of claim 40, wherein the patient suffers from non-alcoholic steatohepatitis (NASH).

42. An RNAi construct of any one of claims 1-35 or a composition of claim 36 for use in therapy.