CLAIMS

1. A method for diagnosing or monitoring a non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH) and/or liver fibrosis, and/or for determining the efficacy of a treatment of a NAFLD, NASH and/or liver fibrosis, comprising determining the level of miR-452 in a body fluid sample of said subject.

2. The method according to claim 1, wherein miR-452 is hsa-miR-452, in particular hsa-miR-452-5p, hsa-miR-452-3p.

3. The method according to claim 1 or 2, comprising determining the level of hsa-miR-452-5p.

4. The method according to any one of claims 1 or 3, wherein the body fluid sample is a sample of blood, plasma or serum.

5. The method according to any one of claims 1 to 4, wherein the level of miR-452 is compared to reference levels of miR-452.

6. The method according to claim 5, wherein a level of miR-452 that is higher than reference levels obtained in samples from healthy subjects with no hepatic steatosis indicates the presence of NAFLD.

7. The method according to claim 20, wherein a level of said at least one microRNA, that is higher than a reference level obtained in samples from healthy subjects with no hepatic steatosis, indicates the presence of NAFL.

8. The method according to claim 5, wherein a level of miR-452 that is higher than reference range levels obtained in samples from a non-NASH subject indicates the presence of NASH defined as at least one point in steatosis, lobular inflammation and hepatocyte ballooning score.

9. The method according to claim 5, wherein a level of miR-452 that is higher than a reference range level obtained in samples from subjects without Active-NASH indicates the presence of Active-NASH defined as NAS ≥ 4 with at least one point in steatosis, one point in lobular inflammation and one point in the hepatocyte ballooning scores.

10. The method according to claim 5, wherein a level of miR-452 that is higher than reference levels obtained in samples from subjects with no or minimal liver fibrosis (F = 0 or 1) indicates the presence of a significant (F = 2), moderate (F = 3) or severe (F = 4) liver fibrosis.

11. The method according to any one of the preceding claims, comprising the classification of a subject as being potential receiver (TBT) or non-receiver (NTBT) of a treatment for NASH and/or liver fibrosis, based on the detection of an increased level of miR-452 relative to a reference level of miR-452 measured in NTBT subjects.

12. The method according to claim 11, wherein TBT and NTBT subjects have the following liver biopsy-derived grades:

a) <u>TBT1:</u>
steatosis score ≥ 1
hepatocyte ballooning score ≥ 1
lobular inflammation score ≥ 1
NAS (NAFLD Activity Score) ≥ 4
fibrosis stage ≥ 1 (such as a fibrosis stage equal to 1, 2, 3 or 4 3)
<u>NTBT1:</u>
differs from TBT1 in a at least one point lesser grade in any of steatosis score, hepatocyte ballooning score, lobular inflammation score, NAS or fibrosis stage;

or

b) <u>TBT2:</u>
steatosis score ≥ 1
hepatocyte ballooning score ≥ 1
lobular inflammation score ≥ 1
NAS (NAFLD Activity Score) ≥ 4
fibrosis stage ≥ 2 (such as a fibrosis stage equal to 2, 3 or 4, in particular 2 or 3)
<u>NTBT2:</u>
differs from TBT in a at least one point lesser grade in any of steatosis score, hepatocyte ballooning score, lobular inflammation score, NAS or fibrosis stage;

or

c) TBT7:

steatosis score ≥ 1

hepatocyte ballooning score ≥ 1

lobular inflammation score ≥ 1

NAS (NAFLD Activity Score) ≥ 4

fibrosis stage fibrosis stage = 1b, 1c, 2, 3 or 4

NTBT7:

differs from TBT in a at least one point lesser grade in any of steatosis score, hepatocyte ballooning score, lobular inflammation score, NAS or fibrosis stage.

13. The method according to any one of the preceding claims, comprising monitoring the evolution of NAFLD activity, NASH activity or liver fibrosis in the subject based on the evolution of the level of miR-452 in samples collected two or more times apart from the same subject.

14. The method according to any one of claims 1 to 13, for evaluating the efficiency of a treatment of NAFLD, NASH or liver fibrosis, based on the evolution of the level of miR-452 in samples collected two or more times apart from the same treated subject.

15. An anti-NAFLD, anti-NASH or anti-fibrosis compound for use in treating a subject having NAFLD, NASH or liver fibrosis respectively, wherein the subject is diagnosed or classified as a to-be-treated patient because of NAFLD, NASH or liver fibrosis according to any one of claims 1 to 11, and said compound is to be administered to said diagnosed subject.

16. A miR-452 inhibitory compound for use in treating a subject having NAFLD, NASH and/or liver fibrosis.

17. Use of an anti-NAFLD, anti-NASH or anti-fibrosis compound in the preparation of a medicament for treating a subject having NAFLD, NASH or liver fibrosis respectively, wherein the subject is diagnosed or classified as a to-be-treated patient because of NAFLD, NASH or liver fibrosis according to any one of claims 1 to 11.

18. Use of a miR-452 inhibitory compound in the preparation of a medicament for treating a subject having NAFLD, NASH and/or liver fibrosis.

19. The miR-452 inhibitory compound for use according to claim 16 or the use according to claim 18, wherein the subject was diagnosed or classified to-be-treated according to any one of claims 1 to 12.

20. A method for the diagnosis of non-alcoholic steatohepatitis (NASH) and/or for determining the activity, the stage, or the severity of NASH in a subject, and/or for the classification of a subject as a receiver or non receiver of a treatment for NASH, and/or for the evaluation of the efficacy of a medical treatment, and/or for the determination of the progression or the regression of the pathology in NASH patients, and/or for the classification of a patient as a potential responder or non responder to a medical treatment, and/or for the prediction of disease outcome for a patient, and/or for the identification of surrogate markers of clinical relevant outcomes, comprising the measure of the level of blood, serum or plasma circulating hsa-miR-452 and at least one other blood, serum or plasma circulating marker of liver damage.

21. The method according to claim 20, for the diagnosis of NASH or for the classification of a subject as a receiver or non-receiver of a treatment for NASH.

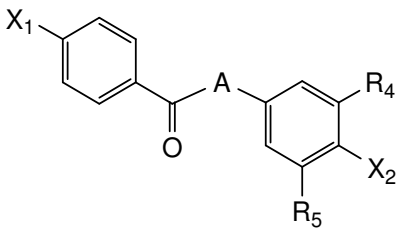
22. A kit comprising means for determining the level of:
(i) hsa-miR-452 (in particular hsa-miR-452-5p), and
(ii) at least one blood, serum or plasma circulating marker of liver damage.

23. The kit according to claim 22, for use in the method according to any one of claims 1 to 21.

24. An anti-NASH molecule for use in a method for the treatment of NASH, wherein said anti-NASH molecule is to be administered to a patient in need thereof diagnosed with NASH or classified according to the method of any one of claims 1 to 21.

25. Use of an anti-NASH molecule in the preparation of a medicament for the treatment of NASH, wherein said anti-NASH molecule is to be administered to a patient in need thereof diagnosed with NASH or classified according to the method of any one of claims 1 to 21.

26. The anti-NASH molecule for use according to claim 24 or the use according to claim 25, wherein said molecule is of formula (I):




wherein:

X1 represents a halogen, a R1, or G1-R1 group;

A represents a CH=CH or a CH2-CH2 group;

X2 represents a G2-R2 group;

G1 and G2, identical or different, represent an atom of oxygen or sulfur;

R1 represents a hydrogen atom, an unsubstituted alkyl group, an aryl group or an alkyl group that is substituted by one or more halogen atoms, an alkoxy or an alkylthio group, cycloalkyl groups, cycloalkylthio groups or heterocyclic groups;

R2 represents an alkyl group substituted by at least a –COOR3 group, wherein R3 represents a hydrogen atom, or an alkyl group that is substituted or not by one or more halogen atoms, cycloalkyl groups, or heterocyclic groups.

R4 and R5, identical or different, representing an alkyl group that is substituted or not by one or more halogen atoms, cycloalkyl groups, heterocyclic groups;

or a pharmaceutically acceptable salt thereof.

27. The anti-NASH molecule for use or the use according to any one of claims 24 to 26, wherein the molecule is selected from the group consisting of 1-[4-methylthiophenyl]-3-[3,5-dimethyl-4-carboxydimethylmethyloxy phenyl]prop-2-en-1-one, 1-[4-methylthiophenyl]-3-[3,5-dimethyl-4-isopropyloxy carbonyldimethylmethyloxyphenyl]prop-2-en-1-one, 1-[4-methylthiophenyl]-3-[3,5-dimethyl-4-tertbutyloxycarbonyldimethylmethyloxyphenyl] prop-2-en-1-one, 1-[4-trifluoromethylphenyl]-3-[3,5-dimethyl-4-tertbutyloxycarbonyl dimethylmethyloxyphenyl]prop-2-en-1-one, 1-[4-trifluoromethylphenyl]-3-[3,5-dimethyl-4-carboxydimethylmethyloxyphenyl]prop-2-en-1-one, 1-[4-trifluoromethyl oxyphenyl]-3-[3,5-dimethyl-4-tertbutyloxycarbonyldimethylmethyloxy phenyl] prop-2-en-1-one, 1-[4-trifluoromethyloxyphenyl]-3-[3,5-dimethyl-4-carboxydimethylmethyl oxyphenyl]prop-2-en-1-one, 2-[2,6-dimethyl-4-[3-[4-(methylthio)phenyl]-3-oxo-propyl] phenoxy]-2-methylpropanoic acid, and 2-[2,6-dimethyl-4-[3-[4-(methylthio) phenyl]-3-oxo-propyl]phenoxy]-2-methyl-propanoic acid isopropyl ester; or a pharmaceutically acceptable salt thereof, in particular 1-[4-methylthiophenyl]-3-[3,5-dimethyl-4-carboxydimethylmethyloxy phenyl]prop-2-en-1-one or a pharmaceutically acceptable salt thereof.

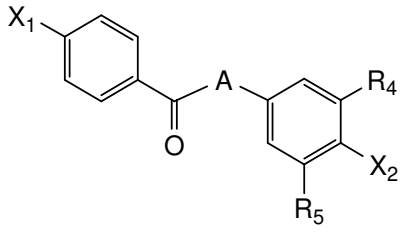
28. The molecule for use or the use according to any one of claims 24 to 27, wherein the anti-NASH molecule is Elafibranor.

29. The molecule for use or the use according to claim 28, wherein Elafibranor is combined to another anti-NASH molecule selected from nitazoxanide, vitamin E or pioglitazone, obeticholic acid, selonsertib, saroglitazar and cenicrivoc.

30. An anti-fibrotic molecule for use in the treatment of liver fibrosis, wherein said anti-fibrotic molecule is to be administered to a patient in need thereof diagnosed with liver fibrosis or classified as a to-be-treated patient or classified as a receiver of a treatment of liver fibrosis according to the method of any one of claims 1 to 21.

31. Use of an anti-fibrotic molecule in the preparation of a medicament for the treatment of liver fibrosis, wherein said anti-fibrotic molecule is to be administered to a patient in need thereof diagnosed with liver fibrosis or classified as a to-be-treated patient or classified as a receiver of a treatment of liver fibrosis according to the method of any one of claims 1 to 21.

32. The anti-fibrotic molecule for use according to claim 30 or the use according to claim 31, wherein said molecule is of formula (I):




wherein:

X1 represents a halogen, a R1, or G1-R1 group;

A represents a CH=CH or a CH2-CH2 group;

X2 represents a G2-R2 group;

G1 and G2, identical or different, represent an atom of oxygen or sulfur;

R1 represents a hydrogen atom, an unsubstituted alkyl group, an aryl group or an alkyl group that is substituted by one or more halogen atoms, an alkoxy or an alkylthio group, cycloalkyl groups, cycloalkylthio groups or heterocyclic groups;

R2 represents an alkyl group substituted by at least a –COOR3 group, wherein R3 represents a hydrogen atom, or an alkyl group that is substituted or not by one or more halogen atoms, cycloalkyl groups, or heterocyclic groups.

R4 and R5, identical or different, representing an alkyl group that is substituted or not by one or more halogen atoms, cycloalkyl groups, heterocyclic groups;

or a pharmaceutically acceptable salt thereof.

33. The anti-fibrotic molecule for use or the use according to any one of claims 28 to 31, wherein the molecule is selected from the group consisting of 1-[4-methylthiophenyl]-3-[3,5-dimethyl-4-carboxydimethylmethyloxy phenyl]prop-2-en-1-one, 1-[4-methylthiophenyl]-3-[3,5-dimethyl-4-isopropyloxy carbonyldimethylmethyloxyphenyl]prop-2-en-1-one, 1-[4-methylthiophenyl]-3-[3,5-dimethyl-4-tertbutyloxycarbonyldimethylmethyloxyphenyl] prop-2-en-1-one, 1-[4-trifluoromethylphenyl]-3-[3,5-dimethyl-4-tertbutyloxycarbonyl dimethylmethyloxyphenyl]prop-2-en-1-one, 1-[4-trifluoromethylphenyl]-3-[3,5-dimethyl-4-carboxydimethylmethyloxyphenyl]prop-2-en-1-one, 1-[4-trifluoromethyl oxyphenyl]-3-[3,5-dimethyl-4-tertbutyloxycarbonyldimethylmethyloxy phenyl] prop-2-en-1-one, 1-[4-trifluoromethyloxyphenyl]-3-[3,5-dimethyl-4-carboxydimethylmethyl oxyphenyl]prop-2-en-1-one, 2-[2,6-dimethyl-4-[3-[4-(methylthio)phenyl]-3-oxo-propyl] phenoxy]-2-methylpropanoic acid, and 2-[2,6-dimethyl-4-[3-[4-(methylthio) phenyl]-3-oxo-propyl]phenoxy]-2-methyl-propanoic acid isopropyl ester; or a pharmaceutically acceptable salt thereof, in particular 1-[4-methylthiophenyl]-3-[3,5-dimethyl-4-carboxydimethylmethyloxy phenyl]prop-2-en-1-one or a pharmaceutically acceptable salt thereof.

34. The molecule or the use according to any one of claims 30 to 32, wherein the anti-fibrotic molecule is Elafibranor.

35. The molecule or the use according to claim 34, wherein the molecule is combined to another anti-fibrotic molecule selected from nitazoxanide, vitamin E or pioglitazone, obeticholic acid, selonsertib, saroglitazar and cenicrivoc.

36. The molecule or use according to any one of claims 30 to 32, wherein the anti-fibrotic molecule is nitazoxanide.

37. The molecule or the use according to claim 36, wherein the molecule is combined to another anti-fibrotic molecule selected from vitamin E or pioglitazone, obeticholic acid, elafibranor, selonsertib, saroglitazar and cenicrivoc in the preparation of said combination medicament.