CLAIMS

1. An *in vitro* 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, wherein the level of miR-452 is compared to reference levels of miR-452 and wherein the body fluid sample is a sample of blood, plasma or serum.

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 claim 1, 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.

5. The method according to claim 1, 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.

6. The method according to claim 1, 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.

7. The method according to claim 1, 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.

8. 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.

9. The method according to claim 8, wherein TBT and NTBT subjects have the following liver biopsy-derived grades:

a) TBT1:

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)

NTBT1:

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) TBT2:

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)

NTBT2:

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.

10. 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.

11. The method according to any one of claims 1 to 10, 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.

12. 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 8.

13. Use of a miR-452 inhibitory compound chosen from the list including short- or small-interfering RNA ("siRNA"), antagomirs, antisense nucleic acids, circular RNA, artificial miRNA sponges and ribozymes in the preparation of a medicament for treating a subject having NAFLD, NASH and/or liver fibrosis.

14. Use of miR-452 inhibitory compound according to claim 13, wherein the subject was diagnosed or classified to-be-treated according to any one of claims 1 to 9.

15. An in vitro 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

(i) 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,

(ii) combining these measures for generating mathematical models (algorithms) through bioinformatic approaches for obtaining a NAFLD, NASH and/or liver fibrosis score with high diagnostic/monitoring/prognostic/predictive performances for assessment of NALFD, NASH, Active-NASH or liver fibrosis in a subject.

16. The method according to claim 15, for the diagnosis of NASH or for the classification of a subject as a receiver or non-receiver of a treatment for NASH.

17. A kit comprising means for determining the level of:

(i) hsa-miR-452-5p, and

(ii) at least one blood, serum or plasma circulating marker of liver damage selected from the group comprising apoptosis markers, inflammatory markers, lipid oxidation products, adipocytokines and hormones, fatty acid binding protein (FABP)4, fibroblast growth factor (FGF21)), lysosomal enzymes.

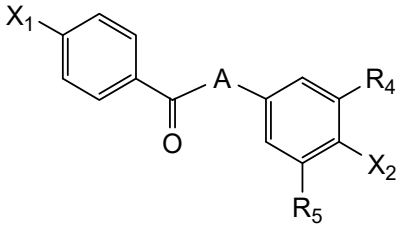
18. A kit comprising means for determining the level of:

(i) hsa-miR-452-5p, and

(ii) at least one biomarker selected from the group consisting of: alpha 2 macroglobulin (A2M), glycated haemoglobin (HbA1c), fasting glucose level, fructosamine level, N-terminal pro-peptide of collagen type III (PIIINP) and YKL-40.

19. The kit according to claim 17 or 18, for use in the method according to any one of claims 1 to 16.

20. 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 16, wherein said anti-NASH 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;

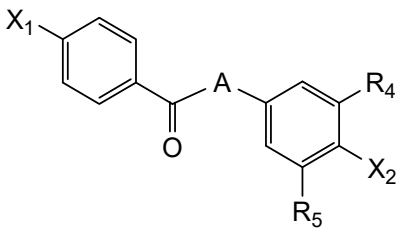
or a pharmaceutically acceptable salt thereof.

21. The use according to claim 20, wherein the anti-NASH 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.

22. The use according to claim 20 or 21, wherein the anti-NASH molecule is Elafibranor.

23. The use of Elafibranor and another anti-NASH molecule selected from nitazoxanide, vitamin E or pioglitazone, obeticholic acid, selonsertib, saroglitazar and cenicrivoc in the manufacture of a medicament for treating NASH, wherein Elafibranor is administered in combination with another anti-NASH molecule.

24. 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 16, wherein said anti-fibrotic 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.

25. The use according to claim 24, wherein the anti-fibrotic 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.

26. The use according to claims 24 or 25, wherein the anti-fibrotic molecule is Elafibranor.

27. The use of Elafibranor and another anti-fibrotic molecule selected from nitazoxanide, vitamin E or pioglitazone, obeticholic acid, selonsertib, saroglitazar and cenicrivoc in the manufacture of a medicament for treating liver fibrosis, wherein Elafibranor is administered in combination with another anti-fibrotic molecule.

28. The use according to any one of claims 24 or 25, wherein the anti-fibrotic molecule is nitazoxanide.

29. The use of nitazoxanide and another anti-fibrotic molecule selected from vitamin E or pioglitazone, obeticholic acid, elafibranor, selonsertib, saroglitazar and cenicrivoc in the manufacture of a medicament for treating liver fibrosis, wherein nitazoxanide is administered in combination with another anti-fibrotic molecule.