

comparing the measured levels of the analytes in the blood sample, the serum sample or the plasma sample with one or more reference samples, wherein the references are representative of matched human subjects; and

identifying the subject as having atherosclerotic coronary artery disease or as having a coronary atherosclerotic plaque if measured levels of the analytes in the blood sample, the serum sample or the plasma sample are increased or decreased relative to the amounts of the analytes in the reference samples.

Any of the panels above may further include the analysis of age and gender.

In another example, the disclosure provides a method comprising measuring by mass spectrometry the levels of each of a plurality of metabolites in a blood sample obtained from a human subject, wherein the plurality of metabolites is selected from the group consisting of (i) valylleucine, glutamate, acisoga, urate, glucuronate, fucose, butyrylcarnitine (C4) and mannose; (ii) cisoga, o.cresol.sulfate, threonate and cysteine.glutathione.disulfide; (iii) N.acetylphenylalanine, N.acetylleucine, valylleucine, xylitol, 2.hydroxyhippurate (salicylurate), glycylphenylalanine, serylleucine and fucose; (iv) glutamate, acisoga, valylleucine, mannose, glucuronate, urate, valerylcarnitine (C5) and 12.HETE; m(v) o.cresol.sulfate, acisoga, cysteine.glutathione.disulfide, glucose, 1.nonadecanoylglycerophosphocholine (19:0) and threonate; (vi) N.acetylphenylalanine, N.acetylleucine, glycylphenylalanine, valylleucine, xylitol, 12.HETE and 2.hydroxyhippurate (salicylurate); (vii) N.acetylthreonine, tigloylglycine, glycerate, valerylcarnitine (C5), threonate, 2.hydroxyhippurate (salicylurate), salicylate and tartronate (hydroxymalonated); (viii) N.acetyl glycine and 3.ethylphenylsulfate; (viv) serotonin (5HT), N.acetylputrescine, leucylleucine, propionylglycine (C3), cholate, glycocholate.sulfate, asparagine and 3.ethylphenylsulfate; (x) acisoga, mannose, valerylcarnitine (C5), 1.linoleoyl.GPE (18.2), glyoursodeoxycholate, threonate, 2.hydroxyhippurate (salicylurate) and salicylate; (xi) N.acetyl glycine, threonate, 2.hydroxyhippurate (salicylurate), 3.ethylphenylsulfate, salicylate; and(xii) asparagine, taurine, acisoga, 1.oleoylglycerol

(18:1), cholesterol, 2.hydroxyhippurate (salicylurate), 6.oxopiperidine.2.carboxylic.acid, and propionylglycine (C3).

In one example, the disclosure provides methods for evaluating a human subject for having ASCAD or as having a coronary atherosclerotic plaque which comprises measuring the levels of each analyte within a panel of analyte biomarkers in a biological sample obtained from a subject, wherein the panel of analyte biomarkers is selected from the group consisting of:

- (xiii) a panel of biomarkers comprising valylleucine, isoleucylvaline, glutamine-leucine, X-12212, glucuronate and glutamate;
- (xiv) a panel of biomarkers comprising glucose, gamma-glutamyl transferase, uric acid, low density lipoprotein cholesterol, triglycerides, lipoprotein (a) mass, alkaline phosphatase, and apolipoprotein B;
- (xv) a panel of biomarkers comprising glutamate, X-16132, isoleucylalanine, isoleucylphenylalanine, lipoprotein (a) mass, small dense low density lipoprotein cholesterol, X-12212. ADSGEGDFXAEGGGVR (SEQ ID NO: 1), glutamine, leucine, and threonylleucine;
- (xvi) a panel of biomarkers comprising CER22:1, HexCER18:1, TG15:0, SM16:0, CER20:0, DHC20:1, and CER18:0;
- (xvii) a panel of biomarkers comprising CER20:0, apolipoprotein B, SM16:0, lipoprotein (a) mass, DHC20L1, alkaline phosphatase, and CER18:0;
- (xviii) a panel of biomarkers comprising glutamate, X-16132, isoleucylalanine, Lipoprotein (a) mass, X-12212, and glutamine-leucine;
- (xix) a panel of biomarkers comprising oleic2, N-terminal pro-brain natriuretic peptide, lipoprotein (a) mass, insulin, glycated hemoglobin A1C, cismontaotl, small dense low density lipoprotein cholesterol, behenic2, adiponectin, and gamma-glutamyl transferase;
- (xx) a panel of biomarkers comprising isoleucylalanine, glucuronate, glycine, isoleucylphenylalanine, mannose, X-21452, 1-oleoylglycerophosphoglycerol, X-21335, 7-methylxanthine, X-12729, acisoga, alpha-hydroxyisovaleroyl carnitine, 2-aminoadipate, X-18914, tigloylglycine, and pyridoxal;
- (xxi) a panel of biomarkers comprising isoleucylalanine, glycine, isoleucylphenylalanine, oleic2, X-21335, methylxanthine, aminoadipate, mannose, acisoga, arachidonoyl.GPE.20.4, and triglycerides;

(xxii) a panel of biomarkers comprising DHC18:0, DG18:1n9, total diacylglycerol, TG20:3n9, CE20:4n6, CER18:1, DHC20:1, CE18:2n6, PL18:2n6, SM18:0, FA14:1n5, PL15:0, and CE16:0;

(xxiii) a panel of biomarkers comprising glycated hemoglobin A1C, total diacylglycerol, cismontotl, lipoprotein (a) mass, TG20.3n9, oleic2, DHC18.1, N-terminal pro-brain natriuretic peptide, gamma-glutamyl transferase, and CER18;

(xxiv) a panel of biomarkers comprising isoleucylalanine, glycine, cismontotl, lipoprotein (a) mass, X-21452, isoleucylphenylalanine, glycated hemoglobin A1C, X-21335, X7.methylxanthine, acisoga and X-12729; wherein the levels, including relative levels, or the occurrences of the plurality of analyte biomarkers are indicative of having atherosclerotic coronary artery disease (ASCAD) or the presence of a coronary atherosclerotic plaque.

Also provided in examples of the disclosure are panels of biomarkers useful for evaluating a human subject for having ASCAD or as having a coronary atherosclerotic plaque. By a “panel of biomarkers” it is meant a collection, or combination, of two or more molecular entities, e.g. two, three, four, five, or more than five entities, whose representation in a sample is associated with ASCAD or a coronary atherosclerotic plaque. The panel of biomarkers described herein may be used to diagnose ASCAD or identify the presences of a coronary atherosclerotic plaque., to provide a prognosis to a patient having ASCAD or a coronary atherosclerotic plaque, to provide a prediction of the responsiveness of a patient with ASCAD or a coronary atherosclerotic plaque to a medical therapy, to monitor a patient having ASCAD or a coronary atherosclerotic plaque, to treat a patient having ASCAD or a coronary atherosclerotic plaque, etc.

In some examples, the methods further comprise of performing a comparison between the measured levels of the analytes in the biological sample with one or more reference samples, said references being representative of matched (e.g., age, gender, etc.) human subjects.

In some examples, the methods further include identifying a subject as having ASCAD or as having a coronary atherosclerotic plaque if the measured levels of the analytes in the biological sample are increased or decreased relative to the amounts of the analytes in the reference samples.

In some examples, the methods include instructing a healthcare professional (e.g., a physician, physician assistant, nurse practitioner, nurse and case

manager) to complete a non-invasive cardiovascular evaluation on a subject identified as having atherosclerotic coronary artery disease or as having a coronary atherosclerotic plaque. The further non-invasive cardiovascular evaluation can confirm whether the subject has or does not have atherosclerotic coronary artery disease or a coronary atherosclerotic plaque.

In some examples, the methods further comprise of performing a non-invasive cardiovascular evaluation on a subject identified as having ASCAD or as having a coronary atherosclerotic plaque to confirm the subject does or does not have atherosclerotic coronary artery disease or a coronary atherosclerotic plaque. The non-invasive cardiovascular evaluation can be a procedure selected from the group consisting of cardiovascular computed tomography (CT) imaging, an exercise stress test, a pharmacologic stress test, myocardial perfusion imaging, stress echocardiography, and cardiovascular magnetic resonance imaging.

In yet another example, the methods further comprise selectively administering a composition comprising an effective amount of a therapeutic agent to a subject identified as having atherosclerotic coronary artery disease or as having a coronary atherosclerotic plaque to treat the subject, wherein the therapeutic agent is selected from the group consisting of a statin, cholesterol absorption inhibitors, niacin-derivatives, omega-3-fatty acid compounds, bile acid sequestrants, PCSK9 inhibitor, anti-platelet agents, aldosterone blockers, angiotensin-converting enzyme (ACE) inhibitors, angiotensin-receptor blockers (ARBs), beta blockers, diuretics, digitalis, hydralazine and nitrates, warfarin and aspirin.

In one aspect, there is provided use of a composition comprising an effective amount of a therapeutic agent selected from the group consisting of a statin, cholesterol absorption inhibitors, niacin-derivatives, omega-3-fatty acid compounds, bile acid sequestrants, anti-platelet agents, aldosterone blockers, angiotensin-converting enzyme (ACE) inhibitors, angiotensin-receptor blockers (ARBs), beta blockers, diuretics, digitalis, hydralazine and nitrates, warfarin and aspirin in the manufacture of a medicament for treating atherosclerotic coronary artery disease or coronary atherosclerotic plaque in a subject that has been identified by the method disclosed herein as having atherosclerotic coronary artery disease or as having coronary atherosclerotic plaque.

For subjects identified as having ASCAD or as having a coronary atherosclerotic plaque, the methods may further comprise selecting a treatment plan for a subject. For example, the methods may further comprise selecting a treatment plan for a subject, which comprises selectively administering a composition comprising an effective amount of a therapeutic agent, and/or performing a non-invasive cardiovascular evaluation, which comprises, for example, performing a procedure selected from the group consisting of cardiovascular computed tomography (CT) imaging, an exercise stress test, a pharmacologic stress test, myocardial perfusion imaging, stress echocardiography, and cardiovascular magnetic resonance imaging. The treatment plan can comprise prescribing to the subject therapeutic lifestyle changes to improve cardiovascular health.

In some examples, the level of each analyte is measured using mass spectrometry (MS) analysis. The MS analysis method can be liquid chromatography-tandem mass spectrometry (UPLC-MS/MS), chromatography-mass spectrometry (GC-MS), or nuclear magnetic resonance (NMR).

In some example, the methods disclosed herein further comprise selecting a subject identified as having ASCAD or as having a coronary atherosclerotic plaque for treatment. In some examples, the treatment comprises selectively administering a composition comprising an effective amount of a therapeutic agent selected from the group consisting of a statin, cholesterol absorption inhibitors, niacin-derivatives, omega-3-fatty acid compounds, bile acid sequestrants, PCSK9 inhibitors, anti-platelet agents, aldosterone blockers, angiotensin-converting enzyme (ACE) inhibitors, angiotensin-receptor blockers (ARBs), beta blockers, diuretics, digitalis, hydralazine and nitrates, warfarin and aspirin to a subject identified as having atherosclerotic coronary artery disease or as having a coronary atherosclerotic plaque to treat the subject. In some examples, the treatment comprises performing a non-invasive cardiovascular evaluation on a subject identified as having atherosclerotic coronary artery disease or as having a coronary atherosclerotic plaque to confirm whether the subject does or does not have atherosclerotic coronary artery disease or a coronary atherosclerotic plaque. In some examples, the treatment plan comprises prescribing therapeutic lifestyle changes to improve cardiovascular health.

The disclosure also provides a kit for evaluating a human subject for having ASCAD or as having a coronary atherosclerotic plaque. The kits include reagents suitable for determining levels of a plurality of analytes in a test sample (.e.g., reagents suitable for determining levels of the metabolomic biomarkers disclosed herein); optionally one or more control samples comprising predetermined levels of the same analytes, wherein comparison of the levels of the analytes in a test sample with levels in the control samples identifies a subject as having atherosclerotic coronary artery disease or as having a coronary atherosclerotic plaque; and instructions for use of the kit in the method described herein.

In one example, there is provided a kit for evaluating a human subject for having atherosclerotic coronary artery disease (ASCAD) or as having a coronary atherosclerotic plaque, the kit comprising:

reagents suitable for determining levels of a plurality of analytes in a test sample, wherein the plurality of analytes comprise:

a panel of biomarkers comprising valylleucine, glutamate, acisoga, urate, glucuronate, fucose, butyrylcarnitine (C4) and mannose;

optionally one or more control samples comprising predetermined levels of the same analytes, wherein comparison of the levels of the analytes in a test sample with levels in the control samples identifies a subject as having atherosclerotic coronary artery disease or as having a coronary atherosclerotic plaque; and

instructions for use of the kit in the method of the disclosure.

As used herein, the term “biological sample” or “sample” refers to a sample obtained or derived from a subject. By way of example, the sample may be selected from the group consisting of body fluids, blood, whole blood, plasma, serum, mucus secretions, urine or saliva. In some examples the sample is, or comprises a blood

sample. The preferred biological source for detection of the biomarkers is a blood sample, a serum sample or a plasma sample.

The term “subject” as used herein refers to a mammal. A subject therefore refers to, for example, dogs, cats, horses, cows, pigs, guinea pigs, humans and the like. When the subject is a human, the subject may be referred to herein as a patient. The subject can be symptomatic (e.g., the subject presents symptoms associated with atherosclerotic CAD, such as, for example, chest pain, angina, angina equivalent, dyspnea, or dyspnea on exertion; and/or presents risk factors associated with coronary artery disease, such as, for example, male gender, hypertension, dyslipidemia, diabetes, post-menopausal state in females, smoking, or a family history of coronary artery disease), or the subject can be asymptomatic (e.g., the subject does not present symptoms associated with atherosclerotic CAD).

As used herein, “obtain” or “obtaining” can be any means whereby one comes into possession of the sample by “direct” or “indirect” means. Directly obtaining a sample means performing a process (e.g., performing a physical method such as extraction) to obtain the sample. Indirectly obtaining a sample refers to receiving the sample from another party or source (e.g., a third party laboratory that directly acquired the sample). Directly obtaining a sample includes performing a process that includes a physical change in a physical substance, e.g., a starting material, such as a blood, e.g., blood that was previously isolated from a patient. Thus, obtain is used to mean collection and/or removal of the sample from the subject. Furthermore, “obtain” is also used to mean where one receives the sample from another who was in possession of the sample previously.

In some examples, the reference sample is obtained from at least one individual not suffering from a cardiovascular disease. In some other examples, the reference sample is obtained from at least one individual previously diagnosed as having a cardiovascular disease (e.g., atherosclerotic coronary artery disease (ASCAD) or a coronary atherosclerotic plaque). In some examples, the reference sample comprises a predetermined, statistically significant reference analyte levels.

In some examples, the determining step is performed using a gradient boosting algorithm. In some examples, the determining step is performed using generalized linear modeling.

In yet another example, the methods further comprise modifying the subject’s clinical record to identify the subject as having atherosclerotic coronary

What is claimed is:

1. A method for evaluating a human subject for having atherosclerotic coronary artery disease (ASCAD) or as having a coronary atherosclerotic plaque, the method comprising:

measuring the levels of each analyte within a panel of analyte biomarkers in a blood sample, a serum sample or a plasma sample that has been obtained from the subject, wherein the panel of analyte biomarkers comprises valylleucine, glutamate, acisoga, urate, glucuronate, fucose, butyrylcarnitine (C4) and mannose;

comparing the measured levels of the analytes in the blood sample, the serum sample or the plasma sample with one or more reference samples, wherein the references are representative of matched human subjects; and

identifying the subject as having atherosclerotic coronary artery disease or as having a coronary atherosclerotic plaque if measured levels of the analytes in the blood sample, the serum sample or the plasma sample are increased or decreased relative to the amounts of the analytes in the reference samples.

2. The method of claim 1, wherein the levels are measured using mass spectrometry (MS) analysis, optionally wherein the MS analysis comprises liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) or gas chromatography-mass spectrometry (GC-MS).

3. The method of claim 1 or 2, wherein the subject has atherosclerosis, optionally wherein the subject presents with symptoms of atherosclerotic CAD comprising chest pain, angina, angina equivalent, dyspnea, or dyspnea on exertion, and optionally wherein the subject has no previous history of coronary artery disease.

4. The method of any one of claims 1 to 3, wherein the subject presents with risk factors associated with coronary artery disease selected from the group consisting of male gender, hypertension, dyslipidemia, diabetes, and a family history of coronary artery disease.

5. The method of any one of claims 1 to 4, wherein the reference sample is one that has been obtained from at least one individual not suffering from a

cardiovascular disease, or wherein the reference sample comprises predetermined, statistically significant reference analyte levels.

6. The method of any one of claims 1 to 5, further comprising modifying the subject's clinical record to identify the subject as having atherosclerotic coronary artery disease or as having a coronary atherosclerotic plaque, optionally wherein the clinical record is stored in a computer readable medium.

7. Use of a composition comprising an effective amount of a therapeutic agent selected from the group consisting of a statin, cholesterol absorption inhibitors, niacin-derivatives, omega-3-fatty acid compounds, bile acid sequestrants, anti-platelet agents, aldosterone blockers, angiotensin-converting enzyme (ACE) inhibitors, angiotensin-receptor blockers (ARBs), beta blockers, diuretics, digitalis, hydralazine and nitrates, warfarin and aspirin in the manufacture of a medicament for treating atherosclerotic coronary artery disease or coronary atherosclerotic plaque in a subject that has been identified by the method of claim 1 as having atherosclerotic coronary artery disease or as having coronary atherosclerotic plaque.