CLAIMS

1. A method for diagnosing a human subject's Autism Spectrum Disorder (ASD) health state or change in ASD health state or identifying a human subject's risk of ASD, the method comprising: comparing quantifying data comprising one or more accurate masses listed in Table 2 of a sample from the human subject to corresponding data obtained from one or more reference samples;
wherein the human subject's ASD health state or change in ASD health state for or risk of ASD is based on a difference in intensity of the one or more accurate masses between the sample from the human subject and one or more reference samples.

2. A method for diagnosing a human subject's ASD health state or change in ASD health state or identifying a human subject's risk of ASD, the method comprising the steps of:

a) analyzing a sample obtained from a patient to obtain quantifying data for one or more accurate masses;

b) comparing the quantifying data for said one or more accurate masses to corresponding data obtained from one or more reference samples; and

c) using said comparison to diagnose the human subject's ASD health state or change in ASD health state based on the differences between the quantifying data and the corresponding data of the one or more accurate masses;

wherein the one or more accurate masses is listed in Table 2.

3. A method for diagnosing a human subject's ASD health state or change in ASD health state or identifying a human subject's risk of ASD, the method comprising the steps of:

a) analyzing a sample obtained from a patient to obtain quantifying data for one or more ethanolamine phospholipids;

b) comparing the quantifying data for said one or more ethanolamine phospholipids to corresponding data obtained from one or more reference samples; and

c) using said comparison to diagnose the human subject's ASD health state or change in ASD health state

4. The method of claim 3, wherein the one or more ethanolamine phospholipids is listed in any one of Tables 3 to 10.

5. The method of claim 1, 2 or 3, wherein the determination of the ASD health state or change in ASD health state comprises the determination of: the presence or absence of ASD, the biochemical ASD phenotype of the subject, an elevated risk of ASD, or a positive, negative, or nil effect of an ASD therapeutic strategy on the subject's underlying biochemical ASD phenotype.

6. The method of claim 5, wherein the biochemical ASD phenotype is characterized as any one of:

a) elevated levels of saturated or monounsaturated very long chain fatty acid (VLCFA)-containing ethanolamine phospholipids;

b) elevated levels of docosahexaenoic acid (22:6, DHA)-containing ethanolamine phospholipids;

c) elevated levels of polyunsaturated VLCFA-containing ethanolamine phospholipids;

d) decreased levels of 18:3, 20:3, 22:3, or 24:3- containing ethanolamine phospholipids and

e) combinations thereof.

7. The method of claim 1, 2 or 3, wherein the sample is whole blood, plasma, serum, or a subfraction of whole blood.

8. The method of claim 2, wherein step a) comprises the extraction of the accurate masses into an organic solvent.

9. The method of claim 2, wherein step a) comprises the extraction of the accurate masses into an aqueous solvent.

10. The method of claim 2, wherein step a) comprises analyzing the sample by mass spectrometry.

11. The method of claim 10, wherein the mass spectrometer is selected from the group consisting of: a Fourier transform ion cyclotron resonance, time of flight, orbitrap, quadrupole and triple quadrupole mass spectrometer.

12. The method of claim 11, wherein the mass spectrometer is a triple quadrupole mass spectrometer.

13. The method of claim 1, 2 or 3, wherein the reference sample is taken from a non-ASD subject.

14. The method of claim 1, 2 or 3, wherein the reference sample is taken from one or more ASD subjects not on a therapeutic regimen.

15. The method of claim 1, 2 or 3, wherein the reference sample is one or more samples taken from the human at a pre-therapy stage or at an earlier-therapy stage.

16. The method of claim 1 or 2, wherein the human subject is diagnosed with ASD based on having either: elevated levels of ethanolamine phospholipids containing saturated or monounsaturated very long chain fatty acids (VLCFA); docosahexaenoic acid (22:6, DHA); VLCFA DHA precursor (24:5, 24:6); catabolic products of DHA beta-oxidation (20:6), polyunsaturated VLCFA; decreased levels of ethanolamine phospholipids containing 18:3, 20:3, 22:3, 24:3 fatty acids or combinations thereof.

17. The method of claim 16, wherein the human subject is diagnosed with ASD based on having a metabolic abnormality described by one or more metabolite listed in

any one of tables 11-18 having a statistically significant ($p<0.05$) change relative to the corresponding data of the reference sample from a non-ASD subject.

18. A method for diagnosing a human subject's ASD health state or change in ASD health state or identifying a human subject's risk of ASD, the method comprising the steps of:

a) analyzing a sample obtained from a patient to obtain quantifying data for one or more of the following:

saturated or monounsaturated very long chain fatty acid (VLCFA)-containing ethanolamine phospholipids;

docosahexaenoic acid (22:6, DHA)-containing phospholipids;

DHA precursor (24:5, 24:6)-containing phospholipids;

catabolic products of DHA beta-oxidation (20:6)-containing phospholipids;

polyunsaturated VLCFA-containing phospholipids; and

combinations thereof;

b) comparing the quantifying data to corresponding data obtained from one or more reference samples; and

c) using said comparison to diagnose the human subject's ASD health state or change in ASD health state based on having one of the following characterizations when compared to the corresponding data obtained from one or more reference samples:

elevated levels of saturated or monounsaturated very long chain fatty acid (VLCFA)-containing ethanolamine phospholipids;

elevated levels of docosahexaenoic acid (22:6, DHA)-containing ethanolamine phospholipids;

elevated levels of DHA precursor (24:5, 24:6)-containing ethanolamine phospholipids;

elevated levels of ethanolamine phospholipids containing catabolic products of DHA beta-oxidation (20:6);

elevated levels of polyunsaturated VLCFA-containing ethanolamine phospholipids;

decreased levels of ethanolamine phospholipids containing LCFA containing three double bonds (18:3, 20:3, 22:3, 24:3)

or combinations thereof.

19. The method of claim 2, wherein the method is for monitoring an ASD therapy, wherein step b) comprises:

comparing the quantifying data for the one or more accurate masses to corresponding data obtained from one or more reference samples collected from non-ASD subjects and/or with previous collected quantifying data from a pre-therapy stage or an earlier therapy stage of the patient;

and step c) comprises:

c) using said comparison to determine the biochemical effect of the therapy on the human subject;

wherein the one or more accurate masses is listed in Table 2.

20. The method of claim 19, wherein the therapy is a carnitine therapy.

Claims

1. A method for diagnosing a human subject's Autism Spectrum Disorder (ASD) health state or change in ASD health state or identifying a human subject's risk of ASD, the method comprising the steps of:

a) analyzing at least one blood sample from said subject using an analytic device or system comprising a mass spectrometer; to obtain quantifying data for one or more than one metabolite marker;

b) comparing the quantifying data for said one or more than one metabolite marker to corresponding data obtained from one or more than one reference blood sample to identify an increase or decrease in the level of said one or more than one metabolite marker in said blood sample; and

c) using said increase or decrease in the level of said one or more than one metabolite marker to diagnose the human subject's ASD health state or change in ASD health state, or to identify the risk of ASD in said subject,

wherein the one or more than one metabolite marker comprises one or more than one molecule selected from the group consisting of ethanolamine phospholipids; docosahexaenoic acid (DHA)-containing phospholipids; DHA precursor-containing phospholipids; catabolic products of DHA beta-oxidation-containing phospholipids; polyunsaturated very long chain fatty acids (VLCFA) containing phospholipids; and combinations thereof, and

wherein the human subject is diagnosed with ASD based on having: elevated levels of ethanolamine phospholipids containing saturated or monounsaturated VLCFA, docosahexaenoic acid (22:6, DHA), VLCFA DHA precursor (24:5, 24:6), catabolic products of DHA beta-oxidation (20:6), or polyunsaturated VLCFA; decreased levels of ethanolamine phospholipids containing 18:3, 20:3, 22:3, 24:3 fatty acids; or combinations thereof.

2. The method of claim 1, wherein said method comprises monitoring an ASD therapy in the human subject, and wherein said increase or decrease in the level of said one or more than one metabolite marker is used in step (c) to determine whether the therapy is improving the biochemical state of the subject.

3. The method of claim 2, wherein the therapy is a carnitine therapy.

4. The method of claim 1, wherein the one or more than one metabolite marker is selected from the group consisting of: PtdEtn 16:0/18:0, PtdEtn 16:0/20:0, PtdEtn 16:0/22:0, PtdEtn 16:0/24:0, PtdEtn 16:0/26:0, PtdEtn 16:0/28:0, PtdEtn 16:0/30:0, PtdEtn 16:0/32:0, PtdEtn 16:0/34:0, PtdEtn 16:0/36:0, PtdEtn 16:0/38:0, PtdEtn 16:0/40:0, PtdEtn 18:0/18:0, PtdEtn 18:0/20:0, PtdEtn 18:0/22:0, PtdEtn 18:0/24:0, PtdEtn 18:0/26:0, PtdEtn 18:0/28:0, PtdEtn 18:0/30:0, PtdEtn 18:0/32:0, PtdEtn 18:0/34:0, PtdEtn 18:0/36:0, PtdEtn 18:0/38:0, PtdEtn 18:0/40:0, PtdEtn 16:0/18:1, PtdEtn 16:0/20:1, PtdEtn 16:0/22:1, PtdEtn 16:0/24:1, PtdEtn 16:0/26:1, PtdEtn 16:0/28:1, PtdEtn 16:0/30:1, PtdEtn 16:0/32:1, PtdEtn 16:0/34:1, PtdEtn 16:0/36:1, PtdEtn 16:0/38:1, PtdEtn 16:0/40:1, PtdEtn 18:0/18:1, PtdEtn 18:0/20:1, PtdEtn 18:0/22:1, PtdEtn 18:0/24:1, PtdEtn 18:0/26:1, PtdEtn 18:0/28:1, PtdEtn 18:0/30:1, PtdEtn

18:0/32:1, PtdEtn 18:0/34:1, PtdEtn 18:0/36:1, PtdEtn 18:0/38:1, PtdEtn 16:0/40:1, PtdEtn 18:0/18:1, PtdEtn 18:0/20:1, PtdEtn 18:0/22:1, PtdEtn 18:0/24:1, PtdEtn 18:0/26:1, PtdEtn 18:0/28:1, PtdEtn 18:0/30:1, PtdEtn 18:0/32:1, PtdEtn 18:0/34:1, PtdEtn 18:0/36:1, PtdEtn 18:0/38:1, PtdEtn 18:0/40:1, PtdEtn 16:0/18:2, PtdEtn 16:0/20:2, PtdEtn 16:0/22:2, PtdEtn 16:0/24:2, PtdEtn 16:0/26:2, PtdEtn 16:0/28:2, PtdEtn 16:0/30:2, PtdEtn 16:0/32:2, PtdEtn 16:0/34:2, PtdEtn 16:0/36:2, PtdEtn 16:0/38:2, PtdEtn 16:0/40:2, PtdEtn 18:0/18:2, PtdEtn 18:0/20:2, PtdEtn 18:0/22:2, PtdEtn 18:0/24:2, PtdEtn 18:0/26:2, PtdEtn 18:0/28:2, PtdEtn 18:0/30:2, PtdEtn 18:0/32:2, PtdEtn 18:0/34:2, PtdEtn 18:0/36:2, PtdEtn 18:0/38:2, PtdEtn 18:0/40:2, PtdEtn 16:0/18:3, PtdEtn 16:0/20:3, PtdEtn 16:0/22:3, PtdEtn 16:0/24:3, PtdEtn 16:0/26:3, PtdEtn 16:0/28:3, PtdEtn 16:0/30:3, PtdEtn 16:0/32:3, PtdEtn 16:0/34:3, PtdEtn 16:0/36:3, PtdEtn 16:0/38:3, PtdEtn 16:0/40:3, PtdEtn 18:0/18:3, PtdEtn 18:0/20:3, PtdEtn 18:0/22:3, PtdEtn 18:0/24:3, PtdEtn 18:0/26:3, PtdEtn 18:0/28:3, PtdEtn 18:0/30:3, PtdEtn 18:0/32:3, PtdEtn 18:0/34:3, PtdEtn 18:0/36:3, PtdEtn 18:0/38:3, PtdEtn 18:0/40:3, PtdEtn 16:0/20:4, PtdEtn 16:0/22:4, PtdEtn 16:0/24:4, PtdEtn 16:0/26:4, PtdEtn 16:0/28:4, PtdEtn 16:0/30:4, PtdEtn 16:0/32:4, PtdEtn 16:0/34:4, PtdEtn 16:0/36:4, PtdEtn 16:0/38:4, PtdEtn 16:0/40:4, PtdEtn 18:0/20:4, PtdEtn 18:0/22:4, PtdEtn 18:0/24:4, PtdEtn 18:0/26:4, PtdEtn 18:0/28:4, PtdEtn 18:0/30:4, PtdEtn 18:0/32:4, PtdEtn 18:0/34:4, PtdEtn 18:0/36:4, PtdEtn 18:0/38:4, PtdEtn 18:0/40:4, PtdEtn 16:0/20:5, PtdEtn 16:0/22:5, PtdEtn 16:0/24:5, PtdEtn 16:0/26:5, PtdEtn 16:0/28:5, PtdEtn 16:0/30:5, PtdEtn 16:0/32:5, PtdEtn 16:0/34:5, PtdEtn 16:0/36:5, PtdEtn 16:0/38:5, PtdEtn 16:0/40:5, PtdEtn 18:0/20:5, PtdEtn 18:0/22:5, PtdEtn 18:0/24:5, PtdEtn 18:0/26:5, PtdEtn 18:0/28:5, PtdEtn 18:0/30:5, PtdEtn 18:0/32:5, PtdEtn 18:0/34:5, PtdEtn 18:0/36:5, PtdEtn 18:0/38:5, PtdEtn 18:0/40:5, PtdEtn 16:0/20:6, PtdEtn 16:0/22:6, PtdEtn 16:0/24:6, PtdEtn 16:0/26:6, PtdEtn 16:0/28:6, PtdEtn 16:0/30:6, PtdEtn 16:0/32:6, PtdEtn 16:0/34:6, PtdEtn 16:0/36:6, PtdEtn 16:0/38:6, PtdEtn 16:0/40:6, PtdEtn 18:0/20:6, PtdEtn 18:0/22:6, PtdEtn 18:0/24:6, PtdEtn 18:0/26:6, PtdEtn 18:0/28:6, PtdEtn 18:0/30:6, PtdEtn 18:0/32:6, PtdEtn 18:0/34:6, PtdEtn 18:0/36:6, PtdEtn 18:0/38:6, PtdEtn 18:0/40:6, PlsEtn 16:0/18:1, PlsEtn 16:0/18:2, PlsEtn 16:0/18:3, PlsEtn 16:0/20:4, PlsEtn 16:0/22:6, PlsEtn 18:0/18:1, PlsEtn 18:0/18:2, PlsEtn 18:0/18:3, PlsEtn 18:0/20:4, PlsEtn 18:0/22:6, PlsEtn 18:1/18:1, PlsEtn 18:1/18:2, PlsEtn 18:1/18:3, PlsEtn 18:1/20:4, PlsEtn 18:1/22:6 and combinations thereof.

5. The method of claim 4, wherein the human subject is diagnosed with ASD based on a statistically significant ($p<0.05$) increase or decrease in the level of said one or more than one metabolite marker relative to the corresponding data of the reference blood sample from a non-ASD subject.

6. The method of claim 1, wherein the blood samples are analyzed by MS/MS transition.

7. The method of claim 6, wherein the one or more than one metabolite marker is characterized by a MS/MS transition selected from the group consisting of: 718.5/255.2, 746.6/255.2, 774.6/255.2, 802.6/255.2, 830.7/255.2, 858.7/255.2, 886.7/255.2, 914.8/255.2, 942.8/255.2, 970.8/255.2, 998.9/255.2, 1026.9/255.2, 746.6/283.2, 774.6/283.2, 802.6/283.2, 830.7/283.2, 858.7/283.2, 886.7/283.2, 914.8/283.2, 942.8/283.2, 970.8/283.2, 998.9/283.2, 1026.9/283.2, 1054.9/283.2, 716.5/255.2, 744.6/255.2, 772.6/255.2, 800.6/255.2, 828.6/255.2, 856.7/255.2, 884.7/255.2, 912.7/255.2, 940.8//255.2, 968.8/255.2, 996.8/255.2, 1024.9/255.2, 744.6/283.2, 772.6/283.2, 800.6/283.2, 828.6/283.2, 856.7/283.2, 884.7/283.2, 912.7/283.2, 940.8/283.2, 968.8/283.2, 996.8/283.2, 1024.9/283.2, 1052.9/283.2, 714.5/255.2, 742.5/255.2,

770.6/255.2, 798.6/255.2, 826.6/255.2, 854.7/255.2, 882.7/255.2, 910.7/255.2, 938.8/255.2, 966.8/255.2, 994.8/255.2, 1022.9/255.2, 742.5/283.2, 770.6/283.2, 798.6/283.2, 826.6/283.2, 854.7/283.2, 882.7/283.2, 910.7/283.2, 938.8/283.2, 966.8/283.2, 994.8/283.2, 1022.9/283.2, 1050.9/283.2, 712.5/255.2, 740.5/255.2, 768.6/255.2, 796.6/255.2, 824.6/255.2, 852.6/255.2, 880.7/255.2, 908.7/255.2, 936.7/255.2, 964.8/255.2, 992.8/255.2, 1020.8/255.2, 740.5/283.2, 768.6/283.2, 796.6/283.2, 824.6/283.2, 852.6/283.2, 880.7/283.2, 908.7/283.2, 936.7/283.2, 964.8/283.2, 992.8/283.2, 1020.8/283.2, 1048.9/283.2, 738.5/255.2, 766.5/255.2, 794.6/255.2, 822.6/255.2, 850.6/255.2, 878.7/255.2, 906.7/255.2, 934.7/255.2, 962.8/255.2, 990.8/255.2, 1018.8/255.2, 766.5/283.2, 794.6/283.2, 822.6/283.2, 850.6/283.2, 878.7/283.2, 906.7/283.2, 934.7/283.2, 962.8/283.2, 990.8/283.2, 1018.8/283.2, 1046.9/283.2, 736.5/255.2, 764.5/255.2, 792.6/255.2, 820.6/255.2, 848.6/255.2, 876.6/255.2, 904.7/255.2, 932.7/255.2, 960.7/255.2, 988.8/255.2, 1016.8/255.2, 764.5/283.2, 792.6/283.2, 820.6/283.2, 848.6/283.2, 876.6/283.2, 904.7/283.2, 932.7/283.2, 960.7/283.2, 988.8/283.2, 1016.8/283.2, 1044.8/283.2, 734.5/255.2, 762.5/255.2, 790.5/255.2, 818.6/255.2, 846.6/255.2, 874.6/255.2, 902.7/255.2, 930.7/255.2, 958.7/255.2, 986.8/255.2, 1014.8/255.2, 762.5/283.2, 790.5/283.2, 818.6/283.2, 846.6/283.2, 874.6/283.2, 902.7/283.2, 930.7/283.2, 958.7/283.2, 986.8/283.2, 1014.8/283.2, 1042.8/283.2, 700.5/281.2, 698.5/279.2, 696.5/277.2, 722.5/303.2, 746.5/327.2, 728.5/281.2, 726.5/279.2, 724.5/277.2, 750.5/303.2, 774.5/327.2, 726.5/281.2, 724.5/279.2, 722.5/277.2, 748.5/303.2, 772.5/327.2, and combinations thereof.

8. The method of claim 7, wherein the human subject is diagnosed with ASD based on a statistically significant ($p<0.05$) increase or decrease in the level of said one or more than one metabolite marker relative to the corresponding data of the reference blood sample from a non-ASD subject.

9. The method of claim 1, wherein the analytic device or system further comprises a chromatography column, and the blood samples are analyzed by liquid chromatography (LC) and MS/MS transition.

10. The method of claim 1, further comprising:

analyzing at least one blood sample from said subject to obtain quantifying data for one or more than one internal control metabolite; and

obtaining a ratio for each of the levels of said one or more than one metabolite marker to the level obtained for the one or more than one internal control metabolite;

wherein the comparing step (b) comprises comparing each ratio to one or more corresponding ratios obtained for the one or more than one reference blood sample.

11. The method of claim 10, wherein the internal control metabolite is cholic acid.

12. The method of claim 1, wherein the determination of the ASD health state or change in ASD health state comprises the determination of: the presence or absence of ASD, the biochemical ASD phenotype of the subject, an elevated risk of ASD, or a positive, negative, or nil effect of an ASD therapeutic strategy on the subject's underlying biochemical ASD phenotype.

13. The method of claim 12, wherein ASD is detected by measuring any one of the following biochemical ASD phenotypes:

a) elevated levels of saturated or monounsaturated very long chain fatty acid (VLCFA)-containing ethanolamine phospholipids;

b) elevated levels of docosahexaenoic acid (22:6, DHA)-containing ethanolamine phospholipids;

c) elevated levels of polyunsaturated VLCFA-containing ethanolamine phospholipids;

d) decreased levels of 18:3, 20:3, 22:3, or 24:3-containing ethanolamine phospholipids; and

e) combinations thereof.

14. The method of claim 1, wherein the blood sample is whole blood, plasma, serum, or a subfraction of whole blood.

15. The method of claim 1, wherein the mass spectrometer of the analytic device or system is selected from the group consisting of: a Fourier transform ion cyclotron resonance, time of flight, orbitrap, quadrupole and triple quadrupole mass spectrometer.

16. The method of claim 1, wherein a liquid/liquid extraction is performed on the blood samples whereby non-polar metabolites are dissolved in an organic solvent and polar metabolites are dissolved in an aqueous solvent.

17. The method of claim 16, wherein the analytic device or system analyzes the extracted samples by positive or negative electrospray ionization, positive or negative atmospheric pressure chemical ionization, or a combination thereof.

18. The method of claim 1, wherein the reference blood sample is taken from a non-ASD subject, one or more ASD subjects not on a therapeutic regimen, or from the human subject at a pre-therapy stage or at an earlier-therapy stage.