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(54) Benævnelse: **PHOSPHOETHANOLAMINE SOM BIOMARKØR FOR DEPRESSION**

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DESCRIPTION

[Cross Reference to Related Applications]

[0001] The present application claims the benefit of the priority to the Japanese Patent Applications No. 2009-187521 filed on August 12, 2009 and No. 2009-291029 filed on December 22, 2009.

[Technical field]

[0002] The present invention relates to biomarkers for diagnosing depression.

[Background Art]

[0003] The depression is one type of mood disorders, and its predominant symptoms are the "depressed mood" and "loss of interest or pleasure", but some patients complain other symptoms such as suppressed appetite, insomnia, fatigue, suicidal ideation etc. Its diagnosis is generally conducted based on the criterion under either ICD-10 (International Classification of Diseases) by WHO (World Health Organization) or "DSM-IV" by American Psychiatric Association.

[0004] However, the diagnosis of depression can be hardly objective because it depends on the impression by physician or psychologists, or impression by the patients or individuals complaining the symptom or stress. In fact, cases are often observed where symptoms are excessively reported either intentionally or unintentionally because of the disease gain in which a patient is somehow benefitted from the state of being sick, or otherwise symptoms are concealed for the purpose of avoiding prejudice or trouble from being known to be in depression. In such cases, accurate diagnosis of the disease is difficult, and determination of treatment method may become difficult, or even inappropriate treatment might be applied due to misdiagnosis.

[0005] Therefore, several methods have been tested to objectively assist the diagnosis by physician or psychologists. One example is the method in which the noise appearing in the symptom of a patient is excluded as much as possible by processing the results of diagnosis made by a physician or psychologist with a computer (WO2004/080312).

[0006] Several markers for depression are known, including macromolecular materials which have been reported in the method to measure the expression level of a gene (JP2008-253258) and the method to detect proteolytic products (JP2009-92550).

[0007] On the other hand, smaller compounds in living bodies are also tested as candidates for the depression marker, including those having been reported in the method to measure and analyze the contents of testosterone and cortisol (JP2007-24822) and the method to detect the degraded products of tryptophan *in vivo* (WO2006/105907), but none of them is brought into practice use so far.

[0008] In the article Modica-Napolitano et al., Biol. Psychiatry, 2004, 55, 273-277, it is suggested that the increased levels of phosphomonoesters (PME) observed in MRI studies of bipolar and depressed patients might contribute to an impairment of mitochondrial function and to the pathophysiology of affective disorders.
In the article Williamson et al., Prostaglandins Leukot. Essent. Fatty Acids 1996, 55 (1&2), 115-118 it is disclosed that schizophrenic patients show reduced levels of phosphomonoesters (PME) in the brain, as assessed by 31P MRS measurements, whereas those findings are less consistent in patients with affective disorders. In an assay with five patients with major depression, lower levels of PME and increased levels of phosphodiesters (PDE) were found in the left prefrontal cortex, compared to controls.
The US patent application US2007/0173901-A1 discloses an implantable device comprising a sensing channel for collecting values of one or more depression parameters, a circuitry for detecting depression based on the collected parameters, and a circuitry for causing delivery of anti-depressive therapy when depression is detected. The depression parameters include some blood chemical markers.

[Summary of Invention]

[Technical Problem]

[0009] The present invention is aimed to provide biomarkers for diagnosing depression.

[Solution to Problem]

[0010] The present invention relates to the use of phosphoethanolamine as a biomarker for diagnosing depression. Furthermore, the present invention provides a method of diagnosis of depression, a method for judging an effect of a drug for depression, and a recording medium readable by a computer as defined in the appended claims.

[Description of Embodiments]

[0011] Hereinafter the embodiments of the present invention accomplished based on the abovementioned findings are described in detail with reference to Examples. Note that the objective, characteristics, and advantages of the present invention as well as the idea thereof will be apparent to those skilled in the art from the descriptions given herein, and the present invention can be easily worked by a person skilled in the art based on these descriptions. It is to be understood that the embodiments and specific examples of the invention described hereinbelow are to be taken as preferred examples of the present invention. These embodiments and examples are presented only for illustrative or explanatory purposes, and are not intended to restrict the present invention thereto. It is apparent to those skilled in the art that various changes and modifications may be made based on the descriptions given herein within the scope of the present invention as defined in the appended claims.

== Depression to be Diagnosed Using Biomarker ==

[0012] As used herein, the "biomarkers for diagnosing depression" (hereinafter the term "biomarker for diagnosing" is also referred to as "diagnostic marker") include the biomarkers that can identify patients with depression with high probability (disease markers), as well as the biomarkers that can exclude healthy subjects from the patients with depression with high probability (health markers).

== Method for Measuring Content of Diagnostic Marker ==

[0013] The diagnostic marker according to the present invention is phosphoethanolamine. Whether or not a patient is afflicted with depression may be diagnosed by measuring a content of the diagnostic marker in a blood sample collected from the patient.

[0014] When the content of the diagnostic marker in the collected blood sample is to be measured, the blood sample may be preferably pretreated prior to the measurement. For example, a serum or plasma can be separated from the blood by incubation or centrifugation, and the separated serum or plasma may be preferably subjected to the measurement.

[0015] The content of a compound as the diagnostic marker in the collected blood sample may be measured by any known method. Examples of such method include, but not limited to, analysis of the mass of a target compound following isolation, such as high-performance liquid chromatography - mass spectrometer (LC-MS) and gas chromatography - mass spectrometer (GC-MS). When high-performance liquid chromatography is to be employed, any of those columns (ex. ion-exchange columns) capable of simultaneously analyzing multiple ionic metabolites may be preferably used.

[0016] Other methods for measuring the target compound include, but not limited to, those of measurement employing NMR analysis, those of measurement employing acid-base neutralization titration, those of measurement employing amino acid analyzer, those of measurement with enzymes, those of measurement using nucleic acid aptamars /peptide aptamers, those of measurement employing colorimetry, those of measurement only with high-performance liquid chromatography, those of measurement only with gas chromatography, and those of measurement only with mass spectrometer. When the high-performance liquid chromatography is to be employed, any of those columns (ex. ion-exchange columns) capable of simultaneously analyzing multiple ionic metabolites may be preferably used.

[0017] Use of capillary electrophoresis - time-of-flight mass spectrometer (CE-TOFMS) for the measurement is preferable in that it is capable of simultaneously analyzing the in-blood contents of all of the multiple compounds as the diagnostic markers. When CE-TOFMS is to be employed for the measurement, the pretreated serum or plasma may be further pretreated, preferably as follows.

[0018] The serum or plasma is mixed with an alcoholic solvent to terminate enzymatic reactions remaining in the serum or plasma. A preferable alcoholic solvent is methanol. The terminated serum or plasma is mixed with an organic solvent and water, and the mixture is subjected to phase separation and the organic phase containing liposoluble materials such as phospholipids etc. is removed. The organic solvent to be used may be of any type that can be separated from water by phase separation, and preferable examples are dichloromethane, chloroform, dichloroethane etc., and chloroform is particularly preferable. It is preferable that proteins are removed from the aqueous phase thus obtained. The method to remove proteins may be preferably ultrafiltration but not limited thereto. After removal of proteins, the alcoholic solvent remaining in the aqueous phase is preferably removed by distillation. The distillation methods for removal of the solvent include, but not limited to, natural drying, vacuum drying and centrifugal vacuum drying, but the centrifugal vacuum drying is preferable in that it can be conducted quickly and conveniently. Thus an aqueous solution can be prepared from the collected blood sample by removing insoluble materials, and this aqueous solution from which insoluble materials are removed may be preferably subjected to a measurement using CE-TOFMS.

[0019] The samples to be subjected to measurement with CE-TOFMS may also include an internal standard to provide criteria for measurements of electrophoresis time, content, etc. of compounds as the diagnostic marker. The internal standard can be any compound that does not interfere with the efficiency of electrophoresis or mass spectroscopy, and preferable examples include methionine sulfone and 10-camphor sulfonate (10-camphorsulfonic acid, CSA).

[0020] The capillary to be used in the capillary electrophoresis is preferably made of fused-silica. The internal diameter of the capillary is preferably no more than 100 μm to ensure better separation, and is most preferably 50 μm. The length of the capillary is preferably in the range of 50 cm to 150 cm.

[0021] Fractions containing the compound as the target diagnostic marker among those obtained by the capillary electrophoresis may be identified by any method, and examples include a method in which the electrophoretic migration time of the target compound is measured using a sample of the compound in advance, as well as a method in which relative values of the migration time are compared with that of an internal standard.

[0022] As the content of the compound as the target diagnostic marker, the peak area at the m/z of the target compound in the fraction identified to contain the compound is then measured. Each peak area can be normalized by comparing it with the peak area of the internal standard. The absolute concentration of the compound as the diagnostic marker in the collected blood sample can be calculated from the measured peak area by making a calibration curve using a sample of the target compound. The calibration curve is made preferably by the standard addition method rather than the standard solution method.

== Use of Diagnostic Marker ==

[0023] The diagnostic marker can be used in various embodiments of the present invention, including those illustrated below.

<Method for Diagnosing Depression>

[0024] First, a blood sample is collected from a human or non-human vertebrate. The collected blood sample may be used for diagnosing a disease by measuring the content of a diagnostic marker in the blood (hereinafter also referred to as a marker level). The in-blood content of a biomarker is preferably the absolute concentration of the biomarker, but may be any value that is correlated with the absolute concentration of the biomarker and can be used for comparison of the absolute concentrations among individuals, and examples include the relative concentration, the weight per unit volume, and the raw data from the measurement of absolute concentrations (for example, the value obtained by normalizing the peak area in a graph from CE-TOFMS measurement).

[0025] A threshold for the in-blood biomarker level is determined to distinguish diseased individuals of a vertebrate species from healthy individuals of the vertebrate species. For example, in-blood biomarker levels of plurality of diseased individuals and plurality of healthy individuals are measured to determine the threshold in advance. The threshold may be determined by any method known to those skilled in the art. For example, a threshold can be determined so that diseased individuals are included below the threshold (or above the threshold) at a first predetermined ratio and healthy individuals are included above the threshold (or below the threshold) at a second predetermined ratio. In an exemplary method, a statistical software such as JMP from SAS Inc. can be used to obtain a threshold value with which chi-square test yields the best result. A diagnostic marker is preferably provided with high values of both the first predetermined ratio and the second predetermined ratio; for example, the value may be no less than 70%, more preferably no less than 80%, even more preferably no less than 90%, and most preferably 100%. When both values are set high, specificity as well as sensitivity can be high. If it is impossible to set both of the first and second predetermined ratios high, the threshold may be determined so that either one of the specificity and the sensitivity becomes high. The values of both the specificity and sensitivity are high; for example, the value may be no less than 70%, more preferably no less than 80%, even more preferably no less than 90%, and most preferably 100%. As used herein, the specificity refers to a percentage value of (the number of healthy individuals above or below the threshold) / (the total number of healthy individuals), and the sensitivity refers to a percentage value of (the number of diseased individuals below or above the threshold) / (the total number of diseased individuals). Biomarkers having a high specificity can be suitably used as the biomarker for identifying a patient with depression (hereinafter referred to as a disease marker) because the probability of a patient being afflicted with depression is high if the level of such a biomarker of the patient is below (or above) the threshold. Biomarker having high sensitivity can be suitably used as the biomarker for excluding a healthy subject from patients with depression (hereinafter referred to as a health marker) because probability of a patient not being afflicted with depression is high if the level of such a biomarker is above (or below) the threshold.

[0026] The diagnostic marker according to the present invention, i.e., phosphoethanolamine, and also other diagnostic markers disclosed herein may be used as a disease marker as well as a health marker. Among them, phosphoethanolamine, taurine, aspartic acid, methionine, tyrosine, aminoadipic acid, histidine, isoleucine, ADP-RIBOSE, tyrosine, ATP, ADP, asparagine, AMP, serotonin, valine, tryptophan, kynurenine, phosphorylcholine, SDMA (symmetric dimethylarginine), piperidine, pipecolic acid, creatinine, and nicotinamide are effective as both of the disease marker and the health marker, and their plasma concentration in a subject vertebrate may be measured to make a diagnosis that the probability of the subject being either afflicted with depression or healthy is high if the concentration falls in such a certain range as illustrated in Table 1 for human subjects. Among the biomarkers, asparagine, phenylalanine, glucaric acid, hydroxyproline, cystathionine, alanine, aminoadipic acid, N-acetylaspartic acid, glutamic acid, trigonelline, creatine, guanidoacetic acid, sarcosine and betaine are suitably used as the disease markers, and their plasma concentration in a subject vertebrate may be measured to make a diagnosis that the probability of the subject being afflicted with depression is high if the concentration falls in such a certain range as illustrated in Table 1 for human subjects. Further, hypotaurine, phosphorylcholine, arginine, 3-aminobutyric acid, β-alanine, lysine, phosphocreatine, alanine, uric acid, choline, isocitric acid, leucine, malic acid, tyramine, valine, ADMA (asymmetric dimethylarginine), threonine, glyceric acid, serine, 2-methyl serine, sphingosine, homovanillic acid, sulfoxidated methionine, sphinganine, isobutyric acid are suitably used as the health markers, and their plasma concentration in a subject vertebrate may be measured to make a diagnosis that the probability of the subject being healthy is high if the concentration falls in such a certain range as illustrated for human subjects in either Table 1 derived from Example 1 or Table 2 derived from Example 2. When phosphoethanolamine is used as biomarker, the threshold is set to 2.41. The thresholds described herein are merely examples calculated from a particular population by a particular method, and they are likely to vary for different population.

[Table 1] (In the present disclosure biomarkers other than phosphoethanolamine are shown as comparative examples)

Biomarker	Threshold (μM)	Depressive	Healthy
		(Above or below)	
Phosphoethanolamine	1.99	Below	Above
Taurine	44.47	Below	Above
Hypotaurine	2.15	Below	Above
Aspartic acid	3.95	Below	Above
Methionine	20.33	Below	Above
Tyrosine	67.19	Below	Above
Phosphocholine	0.87	Below	Above
Arginine	89.27	Below	Above
Asparagine	36.26	Below	Above
3-aminobutyric acid	11.50	Above	Below
β-alanine,	4.47	Below	Above
Phenylalanine	43.27	Below	Above
Lysine	193.52	Below	Above
Phosphocreatine	0.93	Above	Below
Alanine	446.47	Below	Above
Uric acid	332.05	Below	Above
Choline	28.84	Below	Above
Isocitric acid	6.90	Below	Above
Leucine	188.26	Below	Above
Malic acid	2.08	Below	Above
2-aminoadipic acid	0.70	Below	Above
Tyramine	0.23	Above	Below
Valine	251.05	Below	Above
Glucaric acid	0.42	Above	Below
ADMA	0.33	Below	Above
Histidine	76.35	Below	Above
Isoleucine	30.59	Below	Above
Hydroxyproline	8.81	Below	Above
Cystathionine	5.63	Above	Below

Biomarker	Threshold (μM)	Depressive	Healthy
		(Above or below)	

[Table 2] (In the present disclosure biomarkers other than phosphoethanolamine are shown as comparative examples)

Biomarker	Threshold (μM)	Depressive	Healthy
		(Above or below)	
Phosphoethanolamine	2.06	Below	Above
Taurine	44.20	Below	Above
ADP-ribose	0.11	Below	Above
Aspartic acid	2.88	Below	Above
Tyrosine	66.85	Below	Above
ATP	1.76	Below	Above
Methionine	20.12	Below	Above
ADMA	0.33	Below	Above
Hypotaurine	2.15	Below	Above
ADP	0.93	Below	Above
Lysine	193.49	Below	Above
Phenylalanine	43.27	Below	Above
Asparagine	36.58	Below	Above
AMP	0.48	Below	Above
Serotonin	0.11	Below	Above
Histidine	90.39	Below	Above
Valine	223.95	Below	Above
Leucine	187.94	Below	Above
Alanine	362.35	Below	Above
Tryptophan	48.66	Below	Above
β-alanine	3.29	Below	Above
Aminoadipic acid	0.41	Below	Above
Kynurenine	1.17	Below	Above
Phosphorylcholine	0.59	Below	Above
Isoleucine	30.59	Below	Above
SDMA	0.42	Below	Above
Choline	28.84	Below	Above
Threonine	120.64	Below	Above
Glyceric acid	16.89	Below	Above
Isocitric acid	6.90	Below	Above
Serine	128.87	Below	Above
N-acetylaspartic acid	0.23	Below	Above
Malic acid	2.08	Below	Above
Glutamic acid	20.05	Below	Above
Trigonelline	0.18	Below	Above
Creatine	0.55	Below	Above
2-methyl serine	16.65	Below	Above
Sphingosine	13.38	Below	Above
Homovanillic acid	2.65	Below	Above
Piperidine	0.04	Below	Above
Sulfoxidated methionine	3.64	Below	Above
Pipecolic acid	1.10	Below	Above
Sphinganine	56605.00	Below	Above
y-butyrobetaine	2.72	Below	Above

Biomarker	Threshold (µM)	Depressive	Healthy
		(Above or below)	
Uric acid	332.05	Below	Above
Guanidoacetic acid	2.03	Below	Above
Isobutyric acid	2.40	Below	Above
Creatinine	60.58	Below	Above
Sarcosine	2.68	Below	Above
3-methyl butyric acid	0.62	Below	Above
Nicotinamide	0.26	Below	Above
Betaine	26.13	Below	Above
3-aminobutyric acid	12.24	Above	Below
Ornithine	90.34	Below	Above
Carnitine	43.03	Below	Above
Ethanolamine	11.11	Below	Above

[0027] These biomarkers may be used in combination to improve accuracy of diagnosis, and they may be combined in any combination. For example, when two kinds of disease markers are used, if only one of them is at a concentration indicating the probability of depression, then the subject may still be diagnosed as being afflicted with depression at a certain probability, but if both of the markers are at concentrations indicating the probability of depression, then the probability of depression would be higher than that in the case of the one marker. A health marker can be used in combination with a disease marker. For example, a subject of an animal individual is diagnosed using a health marker, and if the health marker is at a concentration indicating a state of "not being healthy", then the subject is further diagnosed by using a disease marker. If the disease marker is at a concentration indicating a state of "being afflicted", then the probability of being afflicted with depression is judged to be high, whereas if the disease marker is not at a concentration indicating the state of "being afflicted", then the probability of being healthy is judged to be high. In this way, the accuracy of diagnosis can be made higher than the case where only disease marker is used. When the health marker is at a concentration indicating a state of "being healthy", the subject may be diagnosed as being probably healthy, but when another diagnosis is made using a disease marker and if it is at a concentration indicating a state of "not being afflicted", then the probability of being healthy would be even higher. If the disease marker is at a concentration indicating a state of "being afflicted", then further diagnosis may be made, for example, by analyzing the distance of the concentration of the biomarkers from their respective thresholds, or judgement by using a third biomarker.

[0028] When identifying subjects with depression using plurality of biomarkers, the health marker may be used as a first biomarker with its threshold set at a higher level in order to increase the sensitivity, and subjects with high probability of being healthy may be excluded first. The threshold may be set at any high level, for example at about 90%, preferably at about 92%, and even more preferably at about 94%. According to this method, if the health marker at the first stage is proved to be at a concentration indicating a state of being "healthy", then the subject may be diagnosed as being healthy with high probability. When the health marker is not at the concentration indicating the state of being "healthy", the diagnostic marker may be used in the next step, and if the marker is at a concentration indicating the state of "being afflicted", then the probability of being afflicted with depression is judged to be high, whereas if the marker is not at the concentration indicating the state of "being afflicted", then the probability of being healthy is judged to be high. In this way, the accuracy of the judgement can be improved.

[0029] The diagnosis using these diagnostic markers may be combined with another conventional diagnostic method, such as an examination by interview or questionnaire.

[0030] The use of the diagnostic markers as described herein not only enables diagnosis with more easiness and higher accuracy at psychiatry, but also provides means for screening at the scenes such as health checkup where precise diagnosis cannot be conducted, clinical departments such as internal medicine and surgery other than psychiatry, and emergency conditions where verbal communication with a patient is not possible. In this way, it becomes possible to bring potential patients with depression who have been left off the psychiatric examination to the psychiatry department that should be the first place where they are to be treated.

[0031] By setting the threshold for the sensitivity of a marker at a very high level, the marker can be effectively used as a health marker. The threshold can be set at any high level, for example at about 90%, preferably at about 92%, and even more preferably at about 94%. At the scene of the health checkup where precise diagnosis cannot be conducted, a diagnosis using such a threshold may be employed as a first diagnosis. In this method, a subject who falls in the range which includes only small number of patients with depression but large number of healthy subjects is diagnosed as being healthy, whereas a patient who falls in the range which includes large number of patients with depression is diagnosed as being afflicted with depression at high probability, and then referred to a psychiatry department for specialized diagnosis. In this way, the ratio of patients with depression in the first diagnosis can be increased, thereby reducing the burden of second diagnosis at the psychiatry department. The depression to be diagnosed herein may be of any type that can be diagnosed by a conventional diagnostic method, and preferable examples are depressions that can be judged according to SCID Interview method. Patients having adjustment disorder are preferably excluded in advance by a conventional diagnostic method such as an examination by interview or questionnaire. Patients having anxiety disorder, personality disorders such as borderline personality disorder and depressive personality disorder or dysthymic disorders can be diagnosed, in which case the use of ADMA, hypotaurine, lysine, histidine, leucine, β-alanine, choline, threonine, glyceric acid, isocitric acid, serine, malic acid, 2-methyl serine, sphingosine, homovanillic acid, sulfoxidated methionine, sphinganine, phenylalanine, alanine, aminoadipic acid, N-acetylaspartic acid, glutamic acid, trigonelline, creatine, guanidoacetic acid, sarcosine, betaine, phosphoethanolamine, taurine, ADP-RIBOSE, aspartic acid, tyrosine, ATP, ADP, asparagine, AMP, serotonin, valine, tryptophan, kynurenine, phosphorylcholine, isoleucine, SDMA, piperidine, pipecolic acid, isobutyric acid, creatinine, nicotinamide and the like are preferable, and the threshold for them are preferably taken from those shown in Table 2, or thresholds obtained by the same method as used for Table 2. Patients not having anxiety disorder, personality disorder such as borderline personality disorder and depressive personality disorder, nor dysthymic disorder can be diagnosed by the marker according to the present invention or other markers disclosed herein, and the thresholds to be used for them can be taken from both of Table 1 and Table 2, but the thresholds shown in Table 1 or thresholds obtained by the same method as used for Table 1 is more preferable.

<Method for Judging Severity of Depression>

[0032] The plasma concentration of cystathionine is inversely proportional to the scale by CESD as well as to the number of diagnostic match with SCID, and therefore cystathionine is useful as a biomarker for judging the severity of depression in a patient with depression.

[0033] First, whether or not a subject animal is in depression is judged by using either the biomarker according to the present invention or other biomarkers disclosed herein, or any conventional diagnostic method such as the examination by interview or questionnaire. Then the plasma concentration of the patient with depression is examined, and if it is, for example, less than 18.36 μM, then a diagnosis of mild depression may be made, whereas if 18.36 μM or more, then diagnosis of severe depression may be made.

<Judgment of Drug Efficacy>

[0034] The effect of a therapeutic agent for certain disease often varies for different individuals. Therefore, knowing the efficacy of the therapeutic agent for a certain individual is quite beneficial, and it can be easily achieved by using the diagnostic marker according to the present invention. For example, blood samples are collected from a diseased vertebrate before and after administering a therapeutic agent for depression, and the content of a diagnostic marker in the collected blood samples is measured to make comparison of the content of the diagnostic marker between the blood samples before and after the administration of the therapeutic agent. A judgment for the therapeutic agent as being effective can be made if the content of the diagnostic marker comes closer to the range indicating the state as being healthy after the administration of the therapeutic agent. In this way, the diagnostic marker according to the present invention can be used to easily judge whether a therapeutic agent is effective.

== Computer for Utilizing Biomarker ==

[0035] After the content of a compound as the diagnostic marker in a blood sample collected from an individual of a vertebrate species is measured as described above, the result of the measurement may be transmitted to a computer, and the computer may utilize the results according to any of the methods described herein.

[0036] For example, a medical practitioner collects a blood sample from an individual of a vertebrate species and appropriately treats it, then loads the blood sample on an apparatus for measuring the biomarker. The computer causes an apparatus for measuring the biomarker to measure the content of the biomarker in the sample, and obtains the result of the measurement. Based on the plasma content of the marker thus obtained, the computer may then make a judgement as to whether the tested subject is either one or more selected from a group consisting of a healthy subject, not a healthy subject, a patient with depression and not a patient with depression. The computer may also make judgements regarding the severity of a depression, the efficacy of an antidepressant, and the like, similarly to the method described above. The computer may output the result of the judgements thus made, thereby enabling the medical practitioner to obtain information about the tested subject.

[0037] It should be noted that the program according to the present invention causes a computer to execute a method to utilize the plasma content of the marker thus obtained. It may also cause a computer to execute the step of causing the biomarker-measuring apparatus to measure the content of the biomarker in the sample.

[0038] The program may be recorded on a recording medium which is readable by a computer, and the recording medium may be any of a hard disk, CD, CD, DVD, USB memory, a floppy disk, and the like.

[Examples] (In the following examples data related to biomarkers other than phosphoethanolamine are shown as comparative examples)

[0039] Embodiments of the present invention and other background examples are hereinafter described by referring to examples, but the scope of the present invention is not to be limited to the examples described hereinbelow. In this example, it is demonstrated that patients with depression can be indeed distinguished from healthy subjects by using the marker according to the present invention and other markers disclosed herein on the patients who have been preliminary diagnosed as being depressed.

[Example 1]

[1] Diagnosis of Depressive Patients

[0040] Depressive patients were chosen from the outpatients at Hospital of National Center of Neurology and Psychiatry who have been diagnosed as being afflicted with depression by SCID (Structured Clinical Interview for Diagnosis) Interview Method mainly during their first to third examination, and who have agreed to volunteer under a predetermined procedure for informed consent as approved by Ethics Committee. The major depression (MD) that is complicated with disease in Axis I or Axis II was excluded. The SCID Interview Method stands for one type of differentiation diagnoses for psychiatric disorders, and is a structured interview to enable reproducible diagnosis under the DSM-IV criteria. The first-time diagnosis was conducted to obtain general information by questioning with regard to lifestyle including smoking habit, drug usage, chief complaint, menstruation and sleeping conditions as well as medical information such as anamnesis, family history, presence of physical disorders and test results for type-B hepatitis, type-C hepatitis, head CT, blood biochemistry, cardiography and radiographs. After informed consents were obtained, questionnaires were made for CESD (a scale for depression) and STAI (a score for anxiety). Missing answers were checked by conducting a direct interview with a research coordinator (a clinical psychologist). In order to obtain the plasma concentration of the markers, 14 mL of blood sample was collected from each of the patients, and then plasma was separated within 2 hours, and stored in liquid nitrogen until measurement. Excluded were the depressive patients being complicated with any of the anxiety disorders, patients with mild depression that is difficult to differentiate from the adjustment disorders, patients with depression accompanied by borderline personality disorder or any personality disorder, and patients having an episode of major depression during the development of dysthymic disorder, and the remaining Axis I patients and the patients of major depression (MD) having no complication with disease in Axis-II were chosen in the total number of 35 for the analyses.

[0041] Meanwhile, healthy subjects were recruited through advertisements etc. at National Institute of Mental Health and chosen from those who offered volunteering. Informed consents were first obtained at a laboratory of National Institute of Mental Health, and then the subjects were tested by questioning with regard to lifestyle including smoking habit, drug usage, chief complaint, menstruation, sleeping conditions, anamnesis, family history, presence of physical disorders, CESD and STAI. Those who scored 21 or more in CESD were excluded as not being healthy subjects only by questionnaire. Also, most of the subjects who turned out by the interview to be under treatment of any physical disorder were excluded. In the questionnaire, missing answers were checked by conducting a direct interview with a research coordinator (a clinical psychologist). The subjects diagnosed as being healthy were thus chosen in the total number of 41. In order to obtain the plasma concentrations of the markers, 14 mL of blood sample was collected from each of the subjects, and plasma was separated within 2 hours, and stored in liquid nitrogen until measurement.

[0042] Table 3 shows sexes, ages and results from the psychiatric tests (CESD, STAI) of the depressive patients as well as the healthy subjects.

[Table 3]

	Sex	Count	Age (SD)	CESD (SD)	STAI-S(SD)	STAI-T(SD)
Depressive group	Male	15	42.1(16.6)	27.7(11.7)	63.1(12.8)	53.5(6.7)
	Female	20	38.0(13.9)	33.7(6.8)	61.8(9.5)	53.5(9.6)
	Total	35	39.7(14.9)	30.9(9.6)	62.3(10.8)	53.5(8.4)
Healthy group	Male	17	45.5(11.4)	7.1(3.9)	42.1(5.8)	52.0(5.8)
	Female	24	30.5(12.1)	9.0(5.9)	42.3(7.9)	50.9(8.0)

	Sex	Count	Age (SD)	CESD (SD)	STAI-S(SD)	STAI-T(SD)
	Total	41	36.7(13.9)	8.2(5.2)	42.3(7.0)	51.4(2.8)

[0043] With regard to the distribution of the sex, a chi-square test showed no difference between the groups of depressive patients and healthy subjects. With regard to the age, a t-test showed no significant difference between the depressive patients and the healthy subjects, or between males or between females in each of the groups. No significant difference was found between both groups with regard to marital status, working status, body height, body weight, weight change in one month prior to the test, or smoking frequency, either.

[0044] In the results of CESD scale determined by a self-report in which the state of depressive symptom is represented, a t-test showed a significant difference at $p<0.01$ between the depressive patients and the healthy subjects, as well as a significant difference between males or between females in each of the groups. No significant difference was found between the males and the females in each of the groups. In the results of STAI-S score that represents the state of anxiety at the time of interview by a self-report, a t-test showed a significant difference at $p<0.05$ between the depressive patients and the healthy subjects, as well as a significant difference between males and between females in each of the groups. No significant difference was found between the males and the females in each of the groups. By contrast, in the results of STAI-T score that measures the innate likelihood of a subject to develop anxiety, no significant difference was found between the depressive patients and the healthy subjects, nor between males or between females in each of the groups. No significant difference was found between the males and the females in each of the group, either.

[0045] Followings are the psychosocial indexes that showed a significant difference between the two groups. Significantly higher frequency of insomnia symptoms of poor falling asleep and early morning awakening was found in the depressive group. BMI was slightly larger in the depressive group. Frequency for the alcohol intake was slightly higher in the healthy group. With regard to the level of educational status, while the depressive group included larger number of junior high school graduates, the healthy group included postgraduates, showing relatively lower educational status of the depressive group.

[0046] Use of medicine at the time of the test yielded a significant difference ($p < 0.01$) in the chi-square test as shown in Table 4 below. The medicine taken by healthy subjects were painkillers for headache, backache, etc. and antihypertensives, whereas the medicine taken by the depressive patients was mainly hypnotics and anxiolytics, and SSRI was taken by 7 patients.
[Table 4]

	Drug usage		
	Not in use	In use	Total
Depressive group	9	26	35
Healthy group	30	11	41
Total	38	37	76

[2] Preparation of Plasma Sample

[0047] In order to measure the amount in blood of the compound as one of the diagnostic markers by using capillary electrophoresis - time-of-flight mass spectrometer (CE-TOFMS), the plasma samples for CE-TOFMS measurement were prepared in the method described below.

[0048] Blood samples were collected from the patients under informed consent (conducted at National Center of Neurology and Psychiatry), and 100 µL each of plasma was prepared and put into a centrifuge tube. As an internal standard solution, 0.45 mL of methanol (Wako Pure Chemical, LC/MS grade) containing 10 µM of methionine sulfone and 10 µM of 10-camphor sulfonate (H3304-1002, purchased from Human Metabolome Technologies, Inc.) was added. After addition of 0.5 mL of chloroform (Wako Pure Chemical, reagent grade) and 200 µL of Milli-Q water, the sample was vigorously mixed by vortex for 30 sec., and centrifuged (4°C, 2300x g, 5 min). The aqueous phase was transferred to a ultrafiltration unit (Millipore, Ultrafree-MCPBCC centrifugal filter unit, 5kDa) and filtered by centrifugation (4°C, 9100x g, 2 to 4 hours) until almost no solution was remained in the filter cup. After the filter cup was removed, the filtrate was dried by centrifugation under vacuum. The dried material was redissolved in 50 µL of Milli-Q water containing the internal standard to prepare the sample for the measurement by CE-TOFMS.

[3] Method for CE-TOFMS Measurement and Analysis of Result

[0049] The CE-TOFMS measurement and the following data analyses were conducted on Agilent CE-TOF-MSD System (Agilent

Technologies Inc.) using fused silica capillaries.

[0050] The conditions for the CE-TOFMS measurement were as follows:

1. (A) Cation Mode

   Run buffer : Cation Buffer Solution (p/n : H3301-1001)

   Rinse buffer : Cation Buffer Solution (p/n : H3301-1001)

   Sample injection : Pressure injection 50 mbar, 10 sec

   CE voltage : Positive, 30 kV

   MS ionization : ESI Positive

   MS capillary voltage : 4,000 V

   MS scan range : m/z 50-1,000

   Sheath liquid : HMT Sheath Liquid (p/n : H3301-1020)

2. (B) Anion Mode

   Run buffer : Anion Buffer Solution (p/n : H3302-1021)

   Rinse buffer : Anion Buffer Solution (p/n : H3302-1022)

   Sample injection : Pressure injection 50 mbar, 25 sec

   CE voltage : Positive, 30 kV

   MS ionization : ESI Negative

   MS capillary voltage : 3,500 V

   MS scan range : m/z 50-1,000

   Sheath liquid : HMT Sheath Liquid (p/n : H3301-1020)

[0051] From the peaks detected by CE-TOFMS, m/z values, migration times (MT) and area values were obtained as the peak information. The peak area values obtained were converted to relative area values by Formula 1 below.
[Formula I]

$$\text{Relative area} = \frac{\text{Area of taget peak}}{\text{Area of internal standard}}$$

[4] Measurement of Plasma Concentration of Diagnostic Marker by CE-TOFMS and Analysis of Result

[0052] Blood samples were collected from the depressive patients and the healthy subjects, and the samples for the CE-TOFMS measurement were prepared from the plasma, and subjected to the measurement by CE-TOFMS under cation mode and anion mode. From these measurements, peak areas were obtained as the data representing the content of each compound in each of the samples.

[0053] From the data of peak areas, a value that yields the best chi-square value of judgement was chosen using the statistical software JMP from SAS Inc. as the threshold of the disease marker to distinguish depressive patients from healthy subjects. Also, using commercially available samples, a calibration curve was made and used to convert the thresholds thus obtained to absolute values (threshold (μM)) based on the concentration in the plasma. Table 5 shows the thresholds for the disease markers used and the numbers of the healthy subjects and depressive patients fall in the range above or below the thresholds, and the results of the calculation of specificity and sensitivity based on these values are shown in Table 6.
[Table 5]

		Above	Below				Above	Below
Phosphoethanolamine	Depressive	3	32		Uric acid	Depressive	0	35
	Healthy	33	8			Healthy	12	29
Taurine	Depressive	8	27		Choline	Depressive	3	32
	Healthy	35	6			Healthy	17	24
Hypotaurine	Depressive	2	33		Isocitric acid	Depressive	1	34
	Healthy	19	22			Healthy	13	28
Aspartic acid	Depressive	1	34		Leucine	Depressive	1	34
	Healthy	26	15			Healthy	16	25
Methionine	Depressive	6	29		Malic acid	Depressive	1	34
	Healthy	28	13			Healthy	13	28
Tyrosine	Depressive	1	34		Aminoadipic acid	Depressive	7	28
	Healthy	25	16			Healthy	25	16
Phosphocholine	Depressive	0	35		Tyramine	Depressive	35	0
	Healthy	12	29			Healthy	36	5
Arginine	Depressive	1	34		Valine	Depressive	5	30
	Healthy	17	24			Healthy	22	19
Aspartic acid	Depressive	16	19		Glucaric acid	Depressive	6	29
	Healthy	34	7			Healthy	0	41
3-aminobutyric acid	Depressive	33	2		ADMA	Depressive	0	35
	Healthy	24	17			Healthy	12	29
β-alanine	Depressive	1	34		Histidine	Depressive	11	24
	Healthy	13	28			Healthy	31	10
Phenylalanine	Depressive	21	14		Isoleucine	Depressive	7	28
	Healthy	39	2			Healthy	25	16
Lysine	Depressive	3	32		Hydroxyproline	Depressive	17	18
	Healthy	21	20			Healthy	28	13
Phosphocreatine	Depressive	35	0		Cystathionine	pressive	34	1
	Healthy	32	9			Healthy	34	7
Alanine	Depressive	1	34					
	Healthy	13	28					

[Table 6]

	Performance when used a sole marker				Depressive	Healthy
	Specificity	Sensitivity	Threshold	Threshold (μM)	(Above or below)	
Phosphoethanolamine	80.5%	91.4%	0.0059	1.99	Below	Above
Taurine	85.4%	77.1%	0.1133	44.47	Below	Above
Hypotaurine	46.3%	94.3%	0.0113	2.15	Below	Above
Aspartic acid	63.4%	97.1%	0.0316	3.95	Below	Above
Methionine	68.3%	82.9%	0.2743	20.33	Below	Above
Tyrosine	61.0%	97.1%	0.8549	67.19	Below	Above
Phosphocholine	29.3%	100.0%	0.0099	0.87	Below	Above
Arginine	41.5%	97.1%	1.6627	89.27	Below	Above
Asparagine	82.9%	54.3%	0.4149	36.26	Below	Above
3-aminobutyric acid	41.5%	94.3%	0.0038	11.50	Above	Below
β-alanine	31.7%	97.1 %	0.0398	4.47	Below	Above
Phenylalanine	95.1%	40.0%	1.0038	43.27	Below	Above
Lysine	51.2%	91.4%	2.5304	193.52	Below	Above
Phosphocreatine	22.0%	100.0%	0.0022	0.93	Above	Below
Alanine	31.7%	97.1 %	4.6678	446.47	Below	Above

	Performance when used a sole marker				Depressive	Healthy
	Specificity	Sensitivity	Threshold	Threshold (μM)	(Above or below)	
Uric acid	29.3%	100.0%	1.1712	332.05	Below	Above
Choline	41.5%	91.4%	0.5004	28.84	Below	Above
Isocitric acid	31.7%	97.1%	0.0462	6.90	Below	Above
Leucine	39.0%	97.1%	3.7470	188.26	Below	Above
Malic acid	31.7%	97.1%	0.0456	2.08	Below	Above
2-aminoadipic acid	61.0%	80.0%	0.0097	0.70	Below	Above
Tyramine	12.2%	100.0%	0.0034	0.23	Above	Below
Valine	53.7%	85.7%	4.5583	251.05	Below	Above
Glucaric acid	100.0%	17.1%	0.0021	0.42	Above	Below
ADMA	29.3%	100.0%	0.00113	0.33	Below	Above
Histidine	75.6%	68.6%	1.2351	76.35	Below	Above
Isoleucine	61.0%	80.0%	1.7544	30.59	Below	Above
Hydroxyproline	68.3%	51.4%	0.0849	8.81	Below	Above
Cystathionine	97.1%	17.1%	0.0008	5.63	Above	Below

[0054] Among the compounds listed, hypotaurine, phosphorylcholine, arginine, 3-aminobutyric acid, β-alanine, lysine, phosphocreatine, alanine, uric acid, choline, isocitric acid, leucine, malic acid, tyramine, valine, ADMA (asymmetric dimethylarginine) etc. have particularly high sensitivity, indicating their potential for excluding healthy subjects, and therefore they can be preferably used as health markers. Meanwhile, asparagine, phenylalanine, glucaric acid, hydroxyproline, cystathionine etc. have particularly high specificity, indicating their potential for finding patients, and therefore they can be preferably used as disease markers. In particular, the compounds having both the sensitivity and specificity over 60%, i.e. phosphoethanolamine, taurine, aspartic acid, methionine, tyrosine, aminoadipic acid, histidine, isoleucine etc. are useful as both the disease marker and the health marker, and each one of them may be used solely for diagnosis.

[0055] Since the contents of these compounds in blood can be easily measured all at once by simply analyzing a blood sample by CE-TOFMS, the results from plurality of these compounds can be quite easily combined to make diagnosis.

[5] Use of Health Marker for Effectively Excluding Healthy Subject

[0056] In this example, the abovementioned compounds were used as biomarkers wherein the threshold for their sensitivity as health markers was set to 94.3%. The numbers of depressive patients or healthy subjects included in the ranges above the threshold or below the threshold are shown in Table 7, and the results of the calculation for their specificity are shown in Table 8.

[Table 7]

Marker		Above	Below		Marker		Above	Below
Aspartic acid	Depressive	2	33		Uric acid	Depressive	2	33
	Healthy	28	13			Healthy	13	28
Methionine	Depressive	2	33		Choline	Depressive	2	33
	Healthy	19	22			Healthy	13	28
Tyrosine	Depressive	2	33		Isocitric acid	Depressive	2	33
	Healthy	26	15			Healthy	13	28
Phosphocholine	Depressive	2	33		Leucine	Depressive	2	33
	Healthy	16	25			Healthy	17	24
Arginine	Depressive	2	33		Malic acid	Depressive	2	33
	Healthy	17	24			Healthy	14	27
Asparagine	Depressive	2	33		2-aminoadipic acid	Depressive	2	33
	Healthy	13	28			Healthy	8	33
3-aminobutyric acid	Depressive	33	2		Tyramine	Depressive	33	2
	Healthy	24	17			Healthy	33	8

Marker		Above	Below		Marker		Above	Below
β-alanine	Depressive	2	33		Valine	Depressive	2	33
	Healthy	13	28			Healthy	12	28
Phenylalanine	Depressive	2	33		Glucaric acid	Depressive	33	2
	Healthy	15	26			Healthy	33	8
Lysine	Depressive	2	33		ADMA	Depressive	2	33
	Healthy	13	28			Healthy	15	26
Phosphocreatine	Depressive	33	2		Histidine	Depressive	2	33
	Healthy	26	15			Healthy	16	25
Alanine	Depressive	2	33		Isoleucine	Depressive	2	33
	Healthy	13	28			Healthy	16	25

[Table 8]

	Specificity when sensitivity is set to 94.3%			
	Specificity	Sensitivity	Threshold	Threshold (um)
Aspartic acid	68.3%	94.3%	0.0306	3.832
Methionine	46.3%	94.3%	0.2986	22.134
Tyrosine	63.4%	94.3%	0.8506	66.854
Phosphocholine	39.0%	94.3%	0.0087	0.761
Arginine	41.5%	94.3%	1.6627	89.272
Asparagine	31.7%	94.3%	0.5623	49.144
3-aminobutyric acid	41.5%	94.3%	0.0038	11.625
β-alanine	31.7%	94.3%	0.0398	4.469
Phenylalanine	36.6%	94.3%	1.4551	62.729
Lysine	31.7%	94.3%	2.8313	216.536
Phosphocreatine	36.6%	94.3%	0.0026	1.105
Alanine	31.7%	94.3%	4.6678	446.472
Uric acid	31.7%	94.3%	1.1507	326.248
Choline	31.7%	94.3%	0.5303	30.565
Isocitric acid	31.7%	94.3%	0.0462	6.899
Leucine	41.5%	94.3%	3.5795	179.846
Malic acid	34.1%	94.3%	0.0431	1.965
Aminoadipic acid	19.5%	94.3%	0.0144	0.702
Tyramine	19.5%	94.3%	0.0036	0.242
Valine	29.3%	94.3%	5.1873	285.691
Glucaric acid	19.5%	94.3%	0.0006	0.128
ADMA	39.0%	94.3%	0.0106	0.305
Histidine	36.6%	94.3%	1.4057	86.896
Isoleucine	39.0%	94.3%	2.1169	36.914

[0057] Determination of a threshold in this way makes it possible to seclude depressive patients to a range either above or below the threshold. Thus, the ratio of depressive patients among total subjects can be increased by adopting a range that contains a larger number of depressive patients.

[0058] Practically, in the case where the sensitivity is set to 94.3%, as long as the specificity is 5.7 (= 100-94.3)% or higher, this method can increase the ratio of depressive patients in a population regardless of the ratios of depressive patients and healthy subjects. All of the biomarkers listed in Table 8 have specificity of 5.7% or higher, and therefore are useful as the health markers.

[6] Diagnosis Using Plurality of Biomarkers

[0059] The accuracy of diagnosis can be improved by using plurality of biomarkers among the abovementioned compounds.

Effective combinations are considered to be, in particular, the combination of a disease marker and a health marker, the combination of a biomarker effective for both and a disease marker, the combination of a biomarker effective for both and a health marker, and the combination of two biomarkers effective for both. Their specific examples are as follows:

1. (1) Where the patients with aspartic acid less than 3.95μM and arginine less than 89.27μM are defined as depressive and the others are defined as healthy, the sensitivity is 94% and the specificity is 88%.
2. (2) Where the patients with aspartic acid less than 3.95μM and tyrosine less than 67.19μM are defined as depressive and the others are defined as healthy, the sensitivity is 94% and the specificity is 81%.
3. (3) Where the patients with tyrosine less than 67.19μM and glucaric acid 0.42μM or more are defined as depressive and the others are defined as healthy, the sensitivity is 94% and the specificity is 81%.
4. (4) Where the patients with tyrosine less than 67.19μM and 3-aminobutyric acid less than 11.50μM are defined as depressive and the others are defined as healthy, the sensitivity is 91% and the specificity is 85%.

Thus, the accuracy of diagnosis is improved in comparison to the case where each of the biomarkers is used alone.

[7] Biomarker for Judging Severity of Depression (Comparative example)

[0060] Similar to Example 4, the content of cystathionine in each sample from the depressive patient group was obtained in the form of peak area data, and was tested by Spearman's rank correlation coefficient in relation to the CESD scale as well as the number of diagnostic matches in SCID. Inverse correlation was found with CESD by r=-0.460 and p=0.039, and with SCID by r=-0.339 and p=0.049.

[0061] Then, the threshold of the cystathionine content between mildly depressive patients and severely depressive patients was analyzed, and it was found that the mildly depressive patients and severely depressive patients could be distinguished at the threshold of 0.00265 as shown in Table 9. In this example, those who scored 30 or less in CESD were defined as being afflicted with mild depression, whereas those of 31 or higher were defined as severe depression, and those who matched 5 to 6 items out of the 9 criteria in SCID were defined as mild depression, whereas those of 7 or more matches were defined as severe depression.

[Table 9]

	Above	Below
CESD - mild	4	14
CESD - severe	13	4
	Above	Below
SCID - mild	5	13
SCID - severe	8	9

[0062] Thus, cystathionine can be effectively used as a diagnostic marker for depression, in particular, the biomarker for judging the severity of depression.

[0063] It should be noted that the threshold could be converted to an absolute concentration of about 18.36 μM.

[Example 2]

[1] Diagnosis of Depressive Patients

[0064] In this example, the analyses were conducted on the patient group consisting of 34 Axis I patients including 3 depressive patients complicated with anxiety disorders other than adjustment disorder and 2 depressive patients complicated with borderline personality disorder, as well as the control group including 7 patients with mild depression which was difficult to distinguish from adjustment disorder, and 31 healthy subjects. The subjects in the patient group and the control group were chosen in the similar way to Example 1. Between both groups, no significant difference was found in terms of age, body height, body weight, BMI and sex. There was no significant difference in marital status, working status, weight change in one month prior to the test, or smoking frequency, either.

[2] Methods for Preparation of Plasma Sample, Measurement by CE-TOFMS and Analysis of Result

[0065] The methods for preparation of plasma samples from the abovementioned subjects to be tested and the measurement of each marker in the samples by CE-TOFMS were the same as those in Example 1.

[3] Measurement of Plasma Concentration of Diagnostic Marker by CE-TOFMS and Analysis of Result

[0066] The measurement of the concentration of diagnostic markers in the plasma of the subjects from the patient group and the control group, determination of the thresholds, and the analyses of the results were conducted in the similar way to Example 1.

[0067] Table 10 shows the results of the analyses for each of the diagnostic markers.
[Table 10]

Biomarker	Threshold	Threshold (concentration)	Below threshold		Above threshold		Sensitivity	Specificity
			Depression patient	Healthy subject	Depression patient	Healthy subject		
Phosphoethanolamine	0.0061	2.06	28	2	6	36	82.4%	94.7%
Taurine	0.1126	44.20	26	4	8	34	76.5%	89.5%
ADP-ribose	0.0005	0.11	32	11	2	27	94.1%	71.1%
Aspartic acid	0.023	2.88	21	3	13	35	61.8%	92.1 %
Tyrosine	0.8506	66.85	33	13	1	25	97.1%	65.8%
ATP	0.0063	1.76	28	6	6	32	82.4%	84.2%
Methionine	0.2714	20.12	29	13	5	25	85.3%	65.8%
ADMA	0.0113	0.33	34	24	0	14	100.0%	36.8%
Hypotaurine	0.0113	2.15	33	16	1	22	97.1%	57.9%
ADP	0.0049	0.93	28	8	6	30	82.4%	78.9%
Lysine	2.53	193.49	30	16	4	22	88.2%	57.9%
Phenylalanine	1.0038	43.27	14	1	20	37	41.2%	97.4%
Asparagine	0.4186	36.58	21	5	13	33	61.8%	86.8%
AMP	0.0019	0.48	30	14	4	24	88.2%	63.2%
Serotonin	0.0016	0.11	22	5	12	33	64.7%	86.8%
Histidine	1.4623	90.39	34	27	0	11	100.0%	28.9%
Valine	4.0663	223.95	21	6	13	32	61.8%	84.2%
Leucine	3.7406	187.94	32	21	2	17	94.1%	44.7%
Alanine	3.7883	362.35	19	6	15	32	55.9%	84.2%
Tryptophan	0.9408	48.66	26	11	8	27	76.5%	71.1 %
β-alanine	0.0293	3.29	30	17	4	21	88.2%	55.3%
Aminoadipic acid	0.0057	0.41	9	0	25	38	26.5%	100.0%
Kynurenine	0.0256	1.17	31	15	3	23	91.2%	60.5%
Phosphorylcholine	0.0067	0.59	24	11	10	27	70.6%	71.1%
Isoleucine	1.7544	30.59	27	14	7	24	79.4%	63.2%
SDMA	0.01	0.42	25	12	9	26	73.5%	68.4%
Choline	0.5004	28.84	31	21	3	17	91.2%	44.7%
Threonine	1.9038	120.64	34	28	0	10	100.0%	26.3%
Glyceric acid	0.0473	16.89	31	19	3	19	91.2%	50.0%
Isocitric acid	0.0462	6.90	32	22	2	16	94.1%	42.1 %
Serine	1.0526	128.87	29	17	5	21	85.3%	55.3%
N-acetylaspartic acid	0.0012	0.23	12	2	22	36	35.3%	94.7%
Malic acid	0.0456	2.08	32	22	2	16	94.1%	42.1 %
Glutamic acid	0.2428	20.05	13	3	21	35	38.2%	92.1%
Trigonelline	0.0034	0.18	12	1	22	37	35.3%	97.4%
Creatine	0.193	0.55	7	0	27	38	20.6%	100.0%
2-methyl serine	0.2012	16.65	29	21	5	17	85.3%	44.7%

Biomarker	Threshold	Threshold (concentration)	Below threshold		Above threshold			
			Depression patient	Healthy subject	Depression patient	Healthy subject	Sensitivity	Specificity
Sphingosine	13.3825	13.38	28	16	6	22	82.4%	57.9%
Homovanillic acid	0.0109	2.65	34	32	0	6	100.0%	15.8%
Piperidine	0.0006	0.04	23	10	11	28	67.6%	73.7%
Sulfoxidated methionine	0.0216	3.64	34	30	0	8	100.0%	21.1%
Pipecolic acid	0.0263	1.10	22	13	12	25	64.7%	65.8%
Sphinganine	56605	56605.00	28	16	6	22	82.4%	57.9%
γ-butyrobetaine	0.0671	2.72	34	30	0	8	100.0%	21.1%
Uric acid	1.1712	332.05	34	26	0	12	100.0%	31.6%
Guanidoacetic acid	0.0264	2.03	14	4	20	34	41.2%	89.5%
Isobutyric acid	0.0517	2.40	28	20	6	18	82.4%	47.4%
Creatinine	0.9358	60.58	24	15	10	23	70.6%	60.5%
Sarcosine	0.0304	2.68	16	6	18	32	47.1%	84.2%
3-methyl butyric acid	0.0472	0.62	29	20	5	18	85.3%	47.4%
Nicotinamide	0.0024	0.26	25	15	9	23	73.5%	60.5%
Betaine	0.6112	26.13	10	2	24	36	29.4%	94.7%
3-aminobutyric acid	0.004	12.24	34	30	0	8	100.0%	21.1%
Ornithine	1.5677	90.34	32	26	2	12	94.1%	31.6%
Carnitine	1.3303	43.03	26	18	8	20	76.5%	52.6%
Ethanolamine	0.0492	11.11	31	26	3	12	91.2%	31.6%

[0068] Among the compounds listed, ADMA, hypotaurine, lysine, histidine, leucine, β-alanine, choline, threonine, glyceric acid, isocitric acid, serine, malic acid, 2-methyl serine, sphingosine, homovanillic acid, sulfoxidated methionine, sphinganine, isobutyric acid etc. have particularly high sensitivity, indicating their potential for excluding healthy subjects, and therefore they can be preferably used as health markers. Meanwhile, phenylalanine, alanine, aminoadipic acid, N-acetylaspartic acid, glutamic acid, trigonelline, creatine, guanidoacetic acid, sarcosine, betaine etc. have particularly high specificity, indicating their potential for identifying patients, and therefore they can be preferably used as disease markers. In particular, the compounds having both the sensitivity and specificity over 60%, i.e. phosphoethanolamine, taurine, ADP-RIBOSE, aspartic acid, tyrosine, ATP, ADP, asparagine, AMP, serotonin, valine, tryptophan, kynurenine, phosphorylcholine, isoleucine, SDMA (symmetric dimethylarginine), piperidine, pipecolic acid, creatinine, nicotinamide etc. are useful as both the disease marker and the health marker, and each one of them may be used solely for diagnosis.

[4] Set of Health Marker and Its Threshold for Effectively Excluding Healthy Subject

[0069] This example demonstrates that health markers can effectively exclude healthy subjects.

[0070] When the threshold for each of the biomarkers was set so that their sensitivity becomes 94.1%, an exclusion of the healthy subjects was effectively achieved by excluding a population containing smaller number of depressive patients. The specificity was calculated at the threshold, and the results are shown in Table 11.

[Table 11]

Biomarker	Threshold	Threshold (concentration	Below threshold		Above threshold			
			Depression patient	Healthy subject	Depression patient	Healthy subject	Sensitivity	Specificity
ADP-Ribose	0.0005	0.11	2	32	23	15	94.1%	74.2%
Tyrosine	0.8506	66.85	2	32	22	16	94.1%	71.0%
Hypotaurine	0.0113	2.14	2	32	17	21	94.1%	54.8%
Methionine	0.3008	22.30	2	32	16	22	94.1%	51.6%
ADMA	0.0103	0.30	2	32	16	22	94.1%	51.6%
P-Alanine	0.0334	3.75	2	32	15	23	94.1%	48.4%

Biomarker	Threshold	Threshold (concentration	Below threshold		Above threshold		Sensitivity	Specificity
			Depression patient	Healthy subject	Depression patient	Healthy subject		
Aspartic acid	0.0354	4.42	2	32	15	23	94.1%	48.4%
Asparagine	0.5330	46.58	2	32	14	24	94.1%	45.2%
Taurine	0.1705	66.91	2	32	14	24	94.1%	45.2%
Leucine	3.7470	188.26	2	32	14	24	94.1%	45.2%
Uric acid	1.1407	323.41	2	32	14	24	94.1%	45.2%
Serotonin	0.0050	0.34	2	32	13	25	94.1%	41.9%
Malic acid	0.0456	2.08	2	32	13	25	94.1%	41.9%
Isocitric acid	0.0462	6.90	2	32	13	25	94.1%	41.9%
Lysine	2.8313	216.53	2	32	12	26	94.1%	38.7%
Histidine	1.4057	86.90	2	32	12	26	94.1%	38.7%
Choline	0.5303	30.56	2	32	12	26	94.1%	38.7%
Phenylalanine	1.4551	62.72	2	32	12	26	94.1%	38.7%
Glutamic acid	0.5787	47.79	2	32	12	26	94.1%	38.7%
Succinic acid	0.0390	8.36	2	32	12	26	94.1%	38.7%
Alanine	4.6678	446.47	2	32	11	27	94.1%	35.5%
Phosphoethanolamine	0.0106	3.58	2	32	11	27	94.1%	35.5%
Phosphorylcholine	0.0099	0.87	2	32	11	27	94.1%	35.5%
Serine	1.1198	137.10	2	32	11	27	94.1%	35.5%
Arginine	1.6627	89.27	2	32	11	27	94.1%	35.5%
Valine	5.3006	291.93	2	32	10	28	94.1%	32.3%
γ-Butyrobetaine	0.0652	2.64	2	32	10	28	94.1%	32.3%
Isoleucine	2.2144	38.61	2	32	10	28	94.1%	32.3%
N-Acetylaspartic acid	0.0019	0.37	2	32	10	28	94.1%	32.3%
Lactic acid	13.2742	4297.40	32	2	10	28	94.1%	32.3%
Ornithine	1.5677	90.34	2	32	10	28	94.1%	32.3%
Sphinganine 1p	281.4486	281.45	2	32	10	28	94.1%	32.3%
Sphingosine	1803.3079	1803.31	2	32	10	28	94.1%	32.3%
Sphingosine 1p	1803.3079	1803.31	2	32	10	28	94.1%	32.3%
Aminoadipic acid	0.0142	1.02	2	32	9	29	94.1%	29.0%
Phosphocreatine	0.0026	1.11	32	2	8	30	94.1%	25.8%
Tyramine	0.0037	0.25	32	2	7	31	94.1%	22.6%

[0071] Determination of a threshold in this way makes it possible to seclude depressive patients to a range either above or below the threshold. Thus, the ratio of depressive patients among total subjects can be increased by adopting the range which contains a larger number of depressive patients.

[0072] Practically, in the case where the sensitivity is set to 94.1%, as long as the specificity is 5.9 (= 100-94.1) % or higher, this method can increase the ratio of depressive patients in a population regardless of the ratios of depressive patients and healthy subjects. All of the biomarkers listed in Table 8 have specificity of 5.7% or higher, and therefore are useful as the health markers. All of the biomarkers listed in Table 11 have specificity of 5.9% or higher, and therefore are useful as the health markers.

[Example 3]

[1] Calculation of Plasma Marker Concentration Using Standard Addition Method

[0073] In Examples 1 and 2, the calibration curve was made from the commercially available samples as the standard solutions and used to convert the obtained thresholds to absolute values (threshold μM)) based on the plasma concentration (standard solution method). In this example, standard solutions were prepared at a predetermined series of concentrations, and for each of the samples, a series of the solutions added with each of the standard solutions were prepared to make a calibration curve, and then the plasma marker concentration of each of the samples in Example 2 was determined (standard addition method). Using the concentrations thus obtained, thresholds were determined in the similar way to Example 2, and the resulting threshold for phosphoethanolamine was 2.41 (μM),

[2] Change in Plasma Marker Concentration Before and After Treatment

[0074] The plasma concentrations (μM) of phosphoethanolamine was measured in 2 depressive patients, who were not included in the populations in Examples 1 and 2, before treatment (pretreatment) and after a diagnosis as remission of the depression by treatment using medicine such as SSRI for about 6 months (posttreatment). The concentrations were determined by the standard addition method.

[Table 12]

	Phosphoethanolamine		Taurine	
	Before treatment	After treatment	Before treatment	After treatment
Patient "A"	2.0	3.5	39.0	55.4
Patient "B"	2.2	3.8	44.6	100.1

[0075] Thus, the plasma concentration of phosphoethanolamine in both patients increased to a level comparable to that of the healthy subjects after the treatment. By using the threshold determined in [1] for example, both the Patient A and Patient B can be diagnosed as being afflicted with depression before the treatment, and as being healthy after the treatment, even using either of the markers.

[3] Validation of Threshold I

[0076] Plasma marker concentrations in 11 individuals independent of those tested in Examples 1 and 2 (of whom 6 have been definitively diagnosed as being healthy subjects and 5 as patients with depression) were measured in a similar way to [1] above, and diagnosis was made using the thresholds determined in [1].

[Table 13]

Subject	Definitive diagnosis	Marker diagnosis	
		Phosphoethanolamine	Taurine
1	Depression	Depressive	Depressive
2	Depression	Depressive	Depressive
3	Depression	Depressive	Depressive
4	Depression	Depressive	Healthy
5	Depression	Healthy	Healthy
6	Healthy	Healthy	Healthy
7	Healthy	Healthy	Healthy
8	Healthy	Healthy	Healthy
9	Healthy	Healthy	Healthy
10	Healthy	Healthy	Healthy
11	Healthy	Healthy	Healthy

[0077] Using the thresholds determined in [1], an appropriate diagnosis could be made for the healthy subjects with a probability of 100%. An appropriate diagnosis could be made for the patients with depression with a probability of 80% when using phosphoethanolamine.
Therefore, phosphoethanol amine is useful as diagnostic marker of depression.

[4] Validation of Threshold II

[0078] Plasma marker concentrations in 14 individuals independent of those tested in Examples 1 to 3 (All have panic disorder. Of them, 9 individuals have been definitively diagnosed as not being patients with depression [marked as healthy subjects in the table], and 5 as being patients of atypical depression) were measured in a similar way to (1) above, and diagnosis was made using the thresholds determined in (1).

[Table 14]

Subject	Definitive diagnosis	Marker diagnosis	
		Phosphoethanolamine	Taurine
1	Atypical depression	Depressive	Depressive
2	Atypical depression	Depressive	Depressive
3	Atypical depression	Depressive	Depressive
4	Atypical depression	Depressive	Healthy
5	Atypical depression	Depressive	Healthy
6	Healthy	Healthy	Depressive
7	Healthy	Healthy	Healthy
8	Healthy	Healthy	Healthy
9	Healthy	Healthy	Healthy
10	Healthy	Healthy	Healthy
11	Healthy	Healthy	Healthy
12	Healthy	Healthy	Healthy
13	Healthy	Healthy	Healthy
14	Healthy	Healthy	Healthy

[0079] Using the thresholds determined in [1], an appropriate diagnosis could be made for both the depressive patients and non-depressive patients with a probability of 100% when using phosphoethanolamine.

[0080] Therefore, phosphoethanolamine is useful as diagnostic marker of depression for the patients with atypical depression as well. Further, phosphoethanolamine can be used as a diagnostic marker even if depression is complicated with other disease (for example, panic disorder).

[Industrial Applicability]

[0081] The present invention can provide a biomarker for diagnosing depression and the diagnostic methods using the biomarker.

REFERENCES CITED IN THE DESCRIPTION

Patent documents cited in the description

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- **MODICA-NAPOLITANO et al.**Biol. Psychiatry, 2004, vol. 55, 273-277 [0008]
- **WILLIAMSON et al.**Prostaglandins Leukot. EssentFatty Acids, 1996, vol. 55, 1&2115-118 [0008]

PHOSPHOETHANOLAMINE SOM BIOMARKØR FOR DEPRESSION

PATENTKRAV

1. Brug af phosphoethanolamine som en biomarkør til diagnosticering af depression, hvori indholdet af phosphoethanolamine er bestemt i en blodprøve taget fra en person som skal testes.

2. En metode til diagnosticering af en patient med eller med mistanke om depression omfattende følgende trin:
måling af indholdet af phosphoethanolamine i en blodprøve taget fra patienten;
sammenligning af niveauet af phosphoethanolamine i blodprøven med en grænseværdi vejledende for en nedre grænse for niveauet af phosphoethanolamine i blodet hos et rask individ;
hvis såfremt niveauet af phosphoethanolamine i blodprøven er under grænseværdien så er patienten diagnosticeret som lidende af depression; og
hvori grænseværdien er 2,41µM.

3. En metode til at bedømme en effekt af et lægemiddel for en depression, metoden omfatter følgende trin:
måling af indhold af phosphoethanolamine i en blodprøve taget fra en patient som lider af depression
(a) før og efter indgivelse af lægemidlet til patienten eller
hvis såfremt indholdet af phosphoethanolamine i blodprøven er forøget efter indgivelse af lægemidlet sammenlignet med indholdet af phosphoethanolamine i blodprøven taget før indgivelse af lægemidlet, så er lægemidlet effektivt imod depression.

4. Et registrerbart medie som kan læses af en computer, mediet lagrer et program som bevirker at en computer kan udføre følgende trin:
fremskaffelse af indhold af phosphoethanolamine i en blodprøve taget fra en person som skal testes,
lave en bedømmelse baseret på indholdet af phosphoethanolamine, i forhold til hvorvidt personen er enten en patient med depression eller ikke en patient med depression, ved at sammenligne indholdet af phosphoethanolamine i forhold til en grænseværdi, og
udlæse resultatet af bedømmelsen;
hvori grænseværdien er 2,41µM.

5. Metoden i henhold til patentkrav 2 eller patentkrav 3, hvori depressionen er atypisk depression.

6. Brugen i henhold til patentkrav 1, hvori såfremt niveauet af phosphoethanolamine i blodprøven er under en grænseværdi betegnende en lavere grænse for niveauet af phosphoethanolamine i blodet i et raskt individ så er patienten diagnosticeret at have depression, og grænseværdien er 2,41µM.