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(54) Title: METHODS FOR SELECTING ANTIDEPRESSANT DRUG THERAPY TO TREAT DEPRESSION

(57) Abstract: The present invention provides methods for selecting therapy and/or predicting a positive response to an antidepressant drug therapy, such as an antidepressant drug monotherapy, in a subject with depression, e.g., major depressive disorder. The present invention also provides methods for determining a good prognosis of a depressed subject receiving an antidepressant drug.



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METHODS FOR SELECTING ANTIDEPRESSANT DRUG THERAPY TO TREAT DEPRESSION

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 61/951,468, filed March 11, 2014, the disclosure of which is herein incorporated by reference in its entirety for all purposes.

REFERENCE TO A "SEQUENCE LISTING," A TABLE, OR A COMPUTER PROGRAM LISTING APPENDIX SUBMITTED ON A COMPACT DISK

[0002] This application includes a Sequence Listing as a text file named SEQ_LISTING_88473-027310PC-937333.txt created March 03, 2015 and containing 4,000 bytes. The material contained in this text file is incorporated by reference in its entirety for all purposes.

BACKGROUND OF THE INVENTION

[0003] Recent estimates indicate that more than 19 million Americans over the age of 18 experience a depressive illness each year. While significant advances in the treatment of depression have been made in the past decade, as many as 29% to 46% of patients with depression taking an antidepressant are still partially or totally resistant to the treatment, despite adequate dosage, duration, and compliance. In addition, those who suffer from treatment-resistant depression have almost no alternatives. Approximately 50% of patients with major depressive disorder and receiving antidepressant drug have no response or partial response. Furthermore, the presence of residual symptoms is also associated with a higher risk of recurrence, more chronic depressive episodes and a shorter duration between episodes.

[0004] Clinical guidelines for treating depression recommend four possible strategies for managing non-response or partial response. These include: increasing the dose of the antidepressant drug; replacing the drug with a different antidepressant drug; augmenting the antidepressant therapy with a non-antidepressant agent; or combining the initial antidepressant with a second antidepressant. As not every treatment regimen is effective for each individual, there is a strong need to identify predictive markers that can facilitate the

selection of an appropriate and effective treatment regimen for depression. The present invention satisfies this need and provides related advantages as well.

BRIEF SUMMARY OF THE INVENTION

[0005] In one aspect, the present invention provides a method for selecting a suitable treatment for an individual diagnosed with depression. The method comprises (a) detecting the presence or absence of the following single nucleotide polymorphisms (SNPs) in a sample obtained from the individual: (i) a SNP at position 27 of SEQ ID NO:1 comprising at least one thymine “T” allele or the complement thereof for MTHFR (rs 1801133); (ii) a SNP at position 27 of SEQ ID NO:2 comprising at least one guanine “G” allele or the complement thereof for MTR (rs1805087); (iii) a SNP at position 27 of SEQ ID NO:3 comprising at least one thymine “T” allele or the complement thereof for GCH1 (rs8007267); and (iv) a SNP at position 27 of SEQ ID NO:4 comprising at least one guanine “G” allele or the complement thereof for COMT (rs4680); and (b) selecting a treatment comprising an antidepressant drug if the absence of the SNPs is detected. The method provided herein can further comprise administering the antidepressant drug to the individual.

[0006] In some embodiments, the antidepressant drug comprises a selective serotonin reuptake inhibitor (SSRI). The SSRI can be selected from the group consisting of fluoxetine, citalopram, paroxetine, escitalopram, sertraline and a combination thereof. In some embodiments, the selected treatment consists of an antidepressant drug monotherapy if the absence of the SNPs is detected.

[0007] In some embodiments, the depression is major depressive disorder (MDD). In some embodiments, the individual is an adult (*e.g.*, not a child). The individual can be a human adult. In some instances, the individual is obese. Obesity can be determined (*e.g.*, identified) if the individual has one of the following conditions: (a) a BMI value greater than 30 kg/m²; b) a waist circumference greater than 40 inches in men, or greater than 35 inches in women; c) a waist-hip ratio above 0.95 for men or above 0.80 for women; or (d) a body fat percentage of at least about 25% in men or at least about 32% in women.

[0008] In some embodiments, the individual is a drug naïve individual. In other embodiments, the individual is resistant (*e.g.*, has no response or a negative response) to at least one antidepressant drug that is different than the selected antidepressant drug. In such a case, the selected antidepressant drug to which the individual has not developed resistance can be recommended or administered. In yet other embodiments, the individual has a partial

response to at least one antidepressant drug. In such a case, a different or an additional antidepressant drug can be selected, recommended or administered.

[0009] In some embodiments, the sample from the individual is selected from the group consisting of a blood sample, a serum sample, a plasma sample, a urine sample, a buccal sample, a saliva sample and a combination thereof.

[0010] In a second aspect, the present invention provides a method for predicting a positive response to an antidepressant drug therapy in an individual having depression. The method comprises (a) detecting the presence or absence of the following single nucleotide polymorphisms (SNPs) in a sample obtained from the individual: (i) a SNP at position 27 of SEQ ID NO:1 comprising at least one thymine "T" allele or the complement thereof for MTHFR (rs 1801133); (ii) a SNP at position 27 of SEQ ID NO:2 comprising at least one guanine "G" allele or the complement thereof for MTR (rs1805087); (iii) a SNP at position 27 of SEQ ID NO:3 comprising at least one thymine "T" allele or the complement thereof for GCH1 (rs8007267); and (iv) a SNP at position 27 of SEQ ID NO:4 comprising at least one guanine "G" allele or the complement thereof for COMT (rs4680); and (b) predicting a likelihood of positive response to the antidepressant drug therapy in the individual if the absence of the SNPs is detected. In some embodiments, step (b) also comprises predicting a likelihood of non-response to a folate drug if the presence of at least one of the SNPs is detected. In some embodiments, the method further includes administering an antidepressant drug to the individual.

[0011] In some embodiments, the antidepressant drug therapy comprises a selective serotonin reuptake inhibitor (SSRI). The SSRI can be selected from the group consisting of fluoxetine, citalopram, paroxetine, escitalopram, sertraline and a combination thereof. In some embodiments, the antidepressant drug therapy consists of an antidepressant drug monotherapy if the absence of the SNPs is detected.

[0012] In some embodiments, the depression is major depressive disorder (MDD). In some embodiments, the individual is an adult (*e.g.*, not a child). The individual can be a human adult. In some instances, the individual is obese. Obesity can be determined (*e.g.*, identified) if the individual has one of the following conditions: (a) a BMI value greater than 30 kg/m²; b) a waist circumference greater than 40 inches in men, or greater than 35 inches in women; c) a waist-hip ratio above 0.95 for men or above 0.80 for women; or (d) a body fat percentage of at least about 25% in men or at least about 32% in women.

[0013] In some embodiments, the individual is a drug naïve individual. In other embodiments, the individual is resistant (*e.g.*, has no response or a negative response) to at least one antidepressant drug that is different than the antidepressant drug predicted to have a positive response. In such a case, the antidepressant drug predicted to have a positive response to which the individual has not developed resistance can be selected, recommended or administered. In yet other embodiments, the individual has a partial response to at least one antidepressant drug. In such a case, a different or an additional antidepressant drug can be selected, recommended or administered.

[0014] The sample used in the method described above can be selected from the group consisting of a blood sample, a serum sample, a plasma sample, a urine sample, a buccal sample, a saliva sample and a combination thereof.

[0015] In a third aspect, the present invention provides a method for determining the prognosis of an individual diagnosed with depression and receiving an antidepressant drug therapy. The method comprises (a) detecting the presence or absence of the following single nucleotide polymorphisms (SNPs) in a sample obtained from the individual: (i) a SNP at position 27 of SEQ ID NO:1 comprising at least one thymine “T” allele or the complement thereof for MTHFR (rs 1801133); (ii) a SNP at position 27 of SEQ ID NO:2 comprising at least one guanine “G” allele or the complement thereof for MTR (rs1805087); (iii) a SNP at position 27 of SEQ ID NO:3 comprising at least one thymine “T” allele or the complement thereof for GCH1 (rs8007267); and (iv) a SNP at position 27 of SEQ ID NO:4 comprising at least one guanine “G” allele or the complement thereof for COMT (rs4680); and (b) predicting a good prognosis for the individual receiving the antidepressant drug therapy if the absence of the SNPs is detected.

[0016] In some embodiments, the method also includes predicting a poor prognosis for the individual receiving the antidepressant drug therapy if the presence of any one of the SNPs is detected. In some cases, a prediction of poor prognosis also includes recommending that the individual receive a non-antidepressant drug. A non-antidepressant drug can be a folate-containing compound.

[0017] In some embodiments, the antidepressant drug therapy comprises a selective serotonin reuptake inhibitor (SSRI). The SSRI can be selected from the group consisting of fluoxetine, citalopram, paroxetine, escitalopram, sertraline and a combination thereof. In

some embodiments, the antidepressant drug therapy consists of an antidepressant drug monotherapy if the absence of the SNPs is detected.

[0018] In some embodiments, the depression is major depressive disorder (MDD). In some embodiments, the individual is an adult (*e.g.*, not a child). The individual can be a human adult. In some instances, the individual is obese. Obesity can be determined (*e.g.*, identified) if the individual has one of the following conditions: (a) a BMI value greater than 30 kg/m²; b) a waist circumference greater than 40 inches in men, or greater than 35 inches in women; c) a waist-hip ratio above 0.95 for men or above 0.80 for women; or (d) a body fat percentage of at least about 25% in men or at least about 32% in women.

[0019] In some embodiments, the sample obtained from the individual is selected from the group consisting of a blood sample, a serum sample, a plasma sample, a urine sample, a buccal sample, a saliva sample and a combination thereof.

[0020] Other objects, features, and advantages of the present invention will be apparent to one of skill in the art from the following detailed description.

DETAILED DESCRIPTION OF THE INVENTION

I. Introduction

[0021] The present invention is based, in part, upon the surprising discovery that the therapeutic efficacy of drug monotherapy for the treatment of depression, *e.g.*, major depressive disorder (MDD), can be substantially improved by detecting the genotype of certain markers in a biological sample from an individual. The present invention provides methods for selecting an antidepressant drug therapy for the individual. The present invention also provides methods for predicting whether an individual will respond to an antidepressant drug. In addition, the present invention provides methods for determining the prognosis of a depressed patient receiving an antidepressant drug. In some instances, the methods described herein utilize multiple genetic and/or other markers to provide an indication of a patient's projected response to an antidepressant drug monotherapy. In some instances, the use of the markers provides the ability to distinguish prospective responders from non-responders. In other instances, the markers enable the determination of patients who are resistant to antidepressant therapy.

II. Definitions

[0022] As used herein, the following terms have the meanings ascribed to them unless specified otherwise.

[0023] The term “depression” refers to a mental state of depressed mood characterized by feelings of sadness, despair and discouragement. In some instances, depression is a clinical symptom, and can include, but not limited to, major depressive disorder (including single episode and recurrent), unipolar depression, treatment-refractory depression, resistant depression, anxious depression and dysthymia (also referred to as dysthymic disorder). Further, the term “depression” can encompass any major depressive disorder, dysthymic disorder, mood disorders due to medical conditions with depressive features, mood disorders due to medical conditions with major depressive-like episodes, substance-induced mood disorders with depressive features and depressive disorder not otherwise specific as defined by their diagnostic criteria, as listed in the American Psychiatric Association’s Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) or any later edition thereof, or the World Health Organization's International Statistical Classification of Diseases and Related Health Problems (ICD-10).

[0024] The term “sample” includes any biological specimen obtained from an individual. Suitable samples for use in the present invention include, without limitation, whole blood, plasma, serum, cerebrospinal fluid, saliva, urine, stool, tears, any other bodily fluid, tissue samples (*e.g.*, biopsy), and cellular extracts thereof (*e.g.*, red blood cellular extract). In a preferred embodiment, the sample is a blood, plasma or serum sample. The use of samples such as serum, saliva, and urine is well known in the art (see, *e.g.*, Hashida *et al.*, *J. Clin. Lab. Anal.*, 11:267-86 (1997)). One skilled in the art will appreciate that samples such as blood, plasma or serum samples can be diluted prior to the analysis of marker levels.

[0025] The term “marker” or “biomarker” includes any biochemical marker, serological marker, protein markers, genetic marker, metabolic markers, or other clinical characteristic that can be used in the selection of therapy for depression, in the prediction of the probable course and outcome of depression, and/or in the prediction of the likelihood of recovery from the disorder. In some embodiments, the markers are utilized in combination with one or more (*e.g.*, a plurality of) statistical analyses to aid or provide a prognosis of depression in an individual. In other embodiments, the markers are used to aid or provide a prediction of

therapeutic response to a treatment (*e.g.*, antidepressant drug therapy or monotherapy) in an individual diagnosed with depression (*e.g.*, major depressive disorder).

[0026] The present invention relies, in part, on determining the presence (or absence) or level (*e.g.*, concentration) of at least one marker in a sample obtained from an individual. As used herein, the term “detecting the presence of at least one marker” includes determining the presence of each marker of interest by using any quantitative or qualitative assay known to one of skill in the art. In certain instances, qualitative assays that determine the presence or absence of a particular trait, variable, genotype, and/or biochemical or serological substance (*e.g.*, protein or antibody) are suitable for detecting each marker of interest. In certain other instances, quantitative assays that determine the presence or absence of DNA, RNA, protein, antibody, or activity are suitable for detecting each marker of interest. As used herein, the term “detecting the level of at least one marker” includes determining the level of each marker of interest by using any direct or indirect quantitative assay known to one of skill in the art. In certain instances, quantitative assays that determine, for example, the relative or absolute amount of DNA, RNA, protein, antibody, or activity are suitable for detecting the level of each marker of interest. One skilled in the art will appreciate that any assay useful for detecting the level of a marker is also useful for detecting the presence or absence of the marker.

[0027] The term “gene” refers to the segment of DNA involved in producing a polypeptide chain; it includes regions preceding and following the coding region, such as the promoter and 3'-untranslated region, respectively, as well as intervening sequences (introns) between individual coding segments (exons).

[0028] The term “genotype” refers to the genetic composition of an organism, including, for example, whether a diploid organism is heterozygous or homozygous for one or more variant alleles of interest.

[0029] The term “polymorphism” refers to the occurrence of two or more genetically determined alternative sequences or alleles in a population. A “polymorphic site” refers to the locus at which divergence occurs. Preferred polymorphic sites have at least two alleles, each occurring at a particular frequency in a population. A polymorphic locus may be as small as one base pair (*i.e.*, single nucleotide polymorphism or SNP). Polymorphic markers include restriction fragment length polymorphisms, variable number of tandem repeats (VNTR's), hypervariable regions, minisatellites, dinucleotide repeats, trinucleotide repeats,

tetranucleotide repeats, simple sequence repeats, and insertion elements such as Alu. The first identified allele is arbitrarily designated as the reference allele, and other alleles are designated as alternative alleles, “variant alleles,” or “variances.” The allele occurring most frequently in a selected population is sometimes referred to as the “wild-type” allele. Diploid organisms may be homozygous or heterozygous for the variant alleles. The variant allele may or may not produce an observable physical or biochemical characteristic (“phenotype”) in an individual carrying the variant allele. For example, a variant allele may alter the enzymatic activity of a protein encoded by a gene of interest.

[0030] The term “single nucleotide polymorphism (SNP)” and variants thereof refers to a change of a single nucleotide with a polynucleotide, including within an allele. This can include the replacement of one nucleotide by another, as well as deletion or insertion of a single nucleotide. Most typically, SNPs are biallelic markers although tri- and tetra-allelic markers can also exist. By way of non-limiting example, a nucleic acid molecule comprising SNP AC/CC may include a C allele and an A allele at the polymorphic position, or two C alleles at the polymorphic position. For combinations of SNPs, the term “haplotype” is used, *e.g.* the genotype of the SNPs in a single DNA strand that are linked to one another. In some embodiments, the term “haplotype” can be used to describe a combination of SNP alleles, *e.g.*, the alleles of the SNPs found together on a single DNA molecule. In further embodiments, the SNPs in a haplotype can be in linkage disequilibrium with one another.

[0031] The term “SAM” refers to S-adenosyl methionine, commonly known as SAM, or SAM-e, or AdoMet, which is a natural compound found in all living cells. It is one of the most used enzymatic substrates in biochemical reactions. S-Adenosyl methionine is a common cosubstrate involved in methyl group transfers. It is made from adenosine triphosphate (ATP) and methionine by methionine adenosyltransferase. SAM is used in metabolic pathways such as transmethylation, transsulfuration, and aminopropylation.

[0032] The term “SAH” refers to S-adenosylhomocysteine which is formed by the demethylation of S-adenosyl-L-methionine. The relative levels of S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH) in blood, plasma or serum can be used to predict metabolic changes associated with nutritional and chronic disease states, such as cardiovascular disease, some cancers and neuropsychiatric disease.

[0033] The term “SAM/SAH ratio” refers to the relative levels of S-adenosyl methionine to S-adenosylhomocysteine. In some instances, a decrease in the SAM/SAH ratio is associated with increased homocysteine in serum and an increase in SAH.

[0034] The term “4-HNE” refers to 4-hydroxynonenal, or 4-hydroxy-2-nonenal which is an α , β -unsaturated hydroxyalkenal that is produced by lipid peroxidation in cells. 4-HNE is the primary α , β -unsaturated hydroxyalkenal formed in this process. It is found throughout body tissue, and in higher quantities during oxidative stress due to the increase in the lipid peroxidation chain reaction in stress events. It appears to play a key role in cell signal transduction in a variety of pathways from cell cycle events to cellular adhesion. 4-HNE is also considered as possible causal agents of numerous diseases, such as chronic inflammation, neurodegenerative diseases, adult respiratory distress syndrome, atherogenesis, diabetes and different types of cancer.

[0035] The term “hsCRP” refers to the high-sensitivity c-reactive protein which is found in, e.g., blood, serum, and plasma. Elevated levels of hsCRP in serum and plasma have been detected in patients with symptoms of depression, as well as patients with an inflammatory disease. For example, it has been reported that major depression is associated with increased levels of hsCRP and inflammatory markers such as IL-6 and TNF- α (see, e.g., Dinan TG. *Current Opinion Psychia.*, 2009, 22(1):32-6).

[0036] The term “individual,” “subject,” or “patient” are used interchangeably and typically includes humans, but also includes other animals such as, e.g., other primates, rodents, canines, felines, equines, ovines, porcines, and the like.

[0037] The term “naïve individual” refers to a subject who has not received a therapeutic drug for the treatment of a disease/disorder, e.g., depression. In some embodiments, the naïve patient with depression has not been administered an SSRI drug, a folate drug or other drug used to treat depression.

[0038] The term “folate drug,” “folate monotherapy” or “folate treatment” refers to a compound containing an effective amount of at least one folate for use in the methods described herein. Folate is a form of the water-soluble vitamin B9. The term “folate” encompasses the naturally-occurring form of folate, folic acid (also known as vitamin B9 or folacin) and metabolites or derivatives thereof such as methylfolate, tetrahydrofolate, and methyltetrahydrofolate. The term “folate” can also refer to both pteronic acid monoglutamate (folic acid) and reduced forms such as dihydrofolates and tetrahydrofolates, e.g. 5-

formyltetrahydrofolic acid, 5-methyltetrahydrofolic acid, 5,10-methylenetetrahydrofolic acid, 5,10-methenyltetrahydrofolic acid, 10-formyltetrahydrofolic acid and tetrahydrofolic acid, polyglutamates thereof, optical isomers thereof (*e.g.*, optically pure natural isomers thereof, and also mixtures of optical isomers such as racemic mixtures), derivatives thereof, 5 pharmaceutically acceptable salts and esters thereof, glucosamine salts thereof, and galactosamine salts thereof.

[0039] The term “antidepressant” or “antidepressant drug” refers to any pharmaceutical agent which treats depression. In some embodiments, the antidepressant drug administered to the subject in accordance with the methods described herein can be any conventional 10 pharmaceutical agent which is commonly indicated for treating depression. Examples of antidepressant drugs include, but are not limited to, selective serotonin reuptake inhibitors (SSRIs), serotonin and dopamine reuptake inhibitors (SDRIs), serotonin-norepinephrine reuptake inhibitors (SNRIs), serotonin-noradrenaline-dopamine reuptake inhibitors (SNDRI), norepinephrine-dopamine reuptake inhibitors (NDRIs), norepinephrine 15 (noradrenaline) reuptake inhibitors (NRIs), monoamine oxidase inhibitors (MAOIs), selective serotonin reuptake enhancers (SSREs), melatonergic agonists, tryptamines, tricyclic antidepressants, and atypical antidepressants.

[0040] The term “drug monotherapy” refers to a treatment regimen that includes the use of one therapeutic agent (*e.g.*, drug) or a single class of therapeutic agents during the course of 20 therapy. The agent(s) can be administered once or repeatedly during the course of therapy. One skilled in the art will appreciate that the drug monotherapy described herein can be administered alone or as part of a combined therapeutic approach with psychotherapy, psychodynamic therapy, cognitive behavior therapy and combinations thereof.

[0041] The term “antidepressant drug monotherapy” refers to a drug treatment regimen 25 consisting of a single antidepressant drug (*e.g.*, an SSRI drug) or a single class of antidepressant drugs (*e.g.*, a combination of SSRIs) during the course of therapy. For instance, one or more antidepressant drugs can be used. The antidepressant drug(s) can be administered once or repeated during the course of therapy.

[0042] The terms “treatment” and “treating” as used herein, with respect to treatment of a 30 disease, refers to preventing the progression of the disease, altering the course of the disorder (for example, but are not limited to, slowing the progression of the disorder), partially reversing a symptom of the disorder or reducing one or more symptoms and/or one or more

biochemical markers in a subject, preventing one or more symptoms from worsening or progressing, promoting recovery, and/or improving prognosis.

[0043] The term “treatment regimen” refers to a clinically relevant course of treatment for reducing (decreasing) at least one symptom associated with a disease/disorder, and/or
5 improving the course of the disease/disorder.

[0044] The term “negative response” includes a worsening of a disorder or condition in a patient receiving therapy, such that the patient experiences increased or additional signs or symptoms of the disorder.

[0045] The term “positive response” includes an improvement in a patient with a disorder
10 or condition, such that the therapy alleviates signs or symptoms of the disorder.

[0046] The term “poor prognosis” refers to an unlikely prospect of survival and/or recovery from a disease. A poor prognosis can correspond to a higher likelihood that a patient will not survive or will not recover from a specific disease or disorder than the patient surviving or recovering. In some instances, a poor prognosis for depression can include progressive
15 decline of mental state, increased number of depressed episodes, increased duration of depressed episodes, increased number of depressed episodes, increased severity of depression and the like.

[0047] The term “good prognosis” includes a likely prospect of survival and/or recovery from a disease. A good prognosis can correspond to a higher likelihood that a patient will
20 survive or will recover from a specific disease or disorder than the patient not surviving or not recovering. In some instances, a good prognosis for depression can include progressive improvement in mental state as assessed, for example, according to the HAM-D28 scale, decreased number of depressed episodes, decreased duration of depressed episodes, decreased number of depressed episodes, decreased severity of depression and the like.

25 III. Detailed Description of the Embodiments

A. Diagnosing Depression

[0048] The methods described herein are directed to individuals who are diagnosed with depression, *e.g.*, major depressive disorder, or are at risk for depression. Depression can be diagnosed using standard clinical criteria, *e.g.*, the DSM-IV-TR system. For example, the
30 DSM-IV system for diagnosing MDD requires the presence of at least five out of the ten depressive symptoms including depressed mood or irritable, decreased interest or pleasure,

significant weight change (5%) or change in appetite, change in sleep (*e.g.*, insomnia or hypersomnia), change in activity, fatigue or loss of energy, guilt or worthlessness, diminished concentration and suicidality. In addition, the symptoms should be represent for at least two weeks and each symptom should be at sufficient severity for nearly every day.

5 [0049] Generally, depression is evaluated by a clinician using, *e.g.*, the criteria listed in the DSM-IV or efficacy measures (neuropsychological assessments) such as the Hamilton Depression Rating Scale (HAMD-28 or HAMD-7), the Clinical Global Impression (CGI) Scale, the Montgomery-Åsberg Depression Rating Scale (MADRS), the Beck Depression Inventory (BDI), the Zung Self-Rating Depression Scale, the Wechsler Depression Rating
10 Scale, the Raskin Depression Rating Scale, the Inventory of Depressive Symptomatology (IDS), and the Quick Inventory of Depressive Symptomatology (QIDS). For example, measurable lessening of depression (*e.g.*, clinical improvement) includes any clinically significant decline in a measurable marker or symptom, such as measuring markers for depression in the blood, *e.g.*, red blood cell folate, serum folate, serum MTHF, or assessing
15 the degree of depression, *e.g.*, using a neuropsychological assessment.

[0050] For example, a score of 0-7 on HAMD is typically considered to be normal. Scores of 20 or higher indicate moderate, severe, or very severe depression. Questions 18-21 may be recorded to give further information about the depression (such as whether diurnal l variation or paranoid symptoms are present), but are not necessary part of the scale. Thus, a reduction
20 of symptoms can be considered clinically relevant if, *e.g.*, the HAMD score is decreased to under, *e.g.*, 20.

[0051] A good prognosis for patients diagnosed with depression can include recovery to a non-depressive state, significant and lasting improvement in mental health, fewer intermittent depressive episodes compared to baseline (*e.g.*, after initial episode or time of diagnosis),
25 good compliance with prescribed treatment, higher chance of recovery than no recovery, less frequent depressive episodes compared to baseline (*e.g.*, after initial episode or time of diagnosis), less severe depression as determined by a clinical scale, decreased duration of depressive episodes, early remission as defined as a patient's no longer meeting the criteria for depression according to the DSM, consistency of remission, good clinical response as
30 measured by, *e.g.*, HDRS, CGI-S, and the Hamilton Anxiety Rate Scale (HARS), and improved quality of life after initial depressive episode as measured by a clinical scale.

[0052] A poor prognosis can include an absence of a good prognosis, a low chance or probability (*e.g.*, <20%) of recovery, no improvement in mental health, poor compliance with prescribed treatment, a higher chance of no recovery than recovery, more frequent depressive episodes compared to baseline, more severe depression than initial depressive episode, no remission, no response or partial response to drug therapy, relapse that occurs within 6 months after either response or remission, worsening of quality of life after initial depressive episode, increased duration of actual depressive episodes, change from less severe depression to more severe depression (*e.g.*, from full remission to poor remission, from mild depression to moderate depression, from moderate depression to severe depression without psychotic features or with psychotic features), and progression from single episode to recurrent episodes.

[0053] In some embodiments, a good response to a drug therapy for depression includes a patient achieving remission in the first period, *e.g.*, 6 months, of treatment and remaining in remission until the end of follow-up, *e.g.*, 12 months. In some embodiments, no response or non-response represents an absence of a good response.

[0054] According to the DSM, a patient is diagnosed with major depressive disorder if the patient endorses (exhibits or agrees to having) five of nine criteria symptoms, with one of the five being either depressed mood or loss of interest or pleasure. The other criteria symptoms can include significant weight loss or gain, or decrease or increase in appetite nearly every day; fatigue or loss of energy nearly every day; insomnia or hypersomnia nearly every day; psychomotor agitation or retardation nearly every day; insomnia or hypersomnia nearly every day; feelings of worthlessness or guilt nearly every day; diminished ability to think or concentrate, or indecisiveness nearly every day; and recurrent thought of death or suicidal intent. The symptoms of MDD must be present for a duration of 2 weeks or longer, represent a change from previous functioning, and cause clinically significant distress or impairment in social, occupational or other important areas of functioning. The DSM-IV determines severity of MDD as mild, moderate or severe based on the number of criteria symptoms, the severity of the symptoms, and the degree of functional disability and distress. A mild depressive episode can be characterized by the presence of only five or six depressive symptoms and either mild disability or the capacity to function normally but with substantial and unusual effort. Moderate episodes have a severity that is intermediate between mild and severe. Severe episodes can include the presence of most of the criteria symptoms and observable disability.

[0055] Provided herein are methods for predicting a positive response to an antidepressant drug therapy in a patient diagnosed with depression, such as MDD. Also provided are methods for determining if a patient diagnosed with depression and receiving an antidepressant drug therapy will have a good prognosis.

5 **B. Selecting Antidepressant Drug Therapy**

[0056] In some embodiments, the method of selecting a suitable treatment for an individual with depression is based on the determination that the individual does not carry any of the following SNPs: a SNP at position 27 of SEQ ID NO:1 comprising at least one thymine “T” allele or the complement thereof for MTHFR (rs 1801133); a SNP at position 27 of SEQ ID NO:2 comprising at least one guanine “G” allele or the complement thereof for MTR (rs1805087); a SNP at position 27 of SEQ ID NO:3 comprising at least one thymine “T” allele or the complement thereof for GCH1 (rs8007267); and a SNP at position 27 of SEQ ID NO:4 comprising at least one guanine “G” allele or the complement thereof for COMT (rs4680) (Table 1). If all the SNPs are absent, the selected or recommended treatment can include an antidepressant drug. In some instances, the treatment consists of an antidepressant drug monotherapy.

Table 1. SNPs for MTHFR, MTR, GCH1 and COMT genes.

SEQ ID NO:	SNP ID	Gene	Sequence
SEQ ID NO:1	rs1801133	human MTHFR gene	CTTGAAGGAGAAGGTGTCTGCGGGAG[C/T] CGATTTCATCATCACGCAGCTTTTC
SEQ ID NO:2	rs1805087	human MTR gene	GGAAGAATATGAAGATATTAGACAGG[A/G] CCATTATGAGTCTCTCAAGGTAAGT
SEQ ID NO:3	rs8007267	human GCH1 gene	CAATAGGAGCGTGTGTTTGAACAGTA[C/T]A CGCCAAACTTCAGTCATTCAAGTA
SEQ ID NO:4	rs4680	human COMT gene	CCCAGCGGATGGTGGATTTCGCTGGC[A/G]T GAAGGACAAGGTGTGCATGCCTGA

[0057] In other embodiments, a positive response to an antidepressant drug therapy is predicted if the individual does not carry any of the following SNPs: a SNP at position 27 of SEQ ID NO:1 comprising at least one thymine “T” allele or the complement thereof for MTHFR (rs 1801133); a SNP at position 27 of SEQ ID NO:2 comprising at least one guanine “G” allele or the complement thereof for MTR (rs1805087); a SNP at position 27 of SEQ ID NO:3 comprising at least one thymine “T” allele or the complement thereof for GCH1

(rs8007267); and a SNP at position 27 of SEQ ID NO:4 comprising at least one guanine “G” allele or the complement thereof for COMT (rs4680). In some instances, the antidepressant drug therapy consists of an antidepressant drug monotherapy.

[0058] The individual can be diagnosed with a type of depression such as major depressive disorder (MDD). In some embodiments, the depressed individual is obese. Obesity can be determined if the individual has a BMI value greater than 30 kg/m², a waist circumference greater than 40 inches in men or greater than 35 inches in women, a waist-hip ratio above 0.95 for men or above 0.80 for women, or a body fat percentage (%) of at least about 25% in men or at least about 32% in women. In some embodiments, the individual is depressed and is obese. In other embodiments, the individual is depressed and not obese. In yet other embodiments, the individual has MDD and is obese. Additionally, the individual can be an adult or a non-adult (*e.g.*, child, toddler, adolescent, or teenager).

[0059] In some embodiments, the individual has not received an antidepressant drug. In other embodiments, the individual has not received any therapy for depression. The individual can be treatment-naïve. In other instances, the individual is resistant or refractory to a drug therapy, such as an antidepressant drug. In some embodiments, the individual is resistant to an SSRI. If the individual is resistant to one or more antidepressant drugs and does not carry any of the SNPs described herein, an antidepressant drug that the individual is not resistant to can be selected, recommended or administered. For instance, if the individual is resistant to an SSRI, an SNRI drug can be given. Alternatively, if the individual is resistant to a particular SSRI drug, a different SSRI drug can be given.

[0060] In some embodiments, the antidepressant drug monotherapy includes one or more (*e.g.*, at least 1, 2, 3, 4, 5, 6 or more) antidepressant drugs from a single (*i.e.*, the same) class of antidepressants. In other embodiments, the antidepressant drug monotherapy includes one or more (*e.g.*, at least 1, 2, 3, 4, 5, 6 or more) antidepressant drugs from one or more (*e.g.*, at least 1, 2, 3, 4, 5, 6 or more) different classes of antidepressants. In yet other embodiments, the antidepressant drug monotherapy excludes all non-antidepressant drugs such as folate-containing compounds.

C. Selecting Folate Adjunctive Therapy

[0061] In some embodiments, the method of selecting folate adjunctive therapy for depression is based on the determination that the subject carries both folate-responsive markers: a SNP in the MTHFR gene (identified by rs1801133) comprising at least one

thymine "T" allele or the complement thereof, and a SNP in the MTR gene (identified by rs1805087) comprising at least one guanine "G" allele or the complement thereof. In other embodiments, the method of selecting a suitable treatment for a subject with depression is based on the determination that the subject carries both folate-responsive markers: a SNP in the GCH1 gene (identified by rs8007267) comprising at least one thymine "T" allele or the complement thereof, and a SNP in the COMT gene (identified by rs4680) comprising at least one guanine "G" allele or the complement thereof. In some embodiments, if it is determined that the subject does not carry a T allele at rs1801133, a G allele at rs1805087, a T allele at rs8007267, and a G allele at rs4680, a folate-containing compound is not selected, recommended or administered, and an antidepressant drug monotherapy is selected, recommended or administered for the treatment of depression.

[0062] In some embodiments, the method for predicting a positive response to an antidepressant drug comprises detecting the absence of the MTHFR variant allele, the MTR variant allele, the GCH1 variant allele and the COMT variant allele. In some embodiments, the absence of the SNPs MTHFR 677 CT/TT, MTR 2756 AG/GG, GCH1 TC/TT and COMT Val158Met AG/GG indicate that the subject will have a positive response to the antidepressant drug. In addition, the subject is predicted to have a likelihood of non-response to a folate-containing compound if the presence of one or more of the following SNPs are detected: SNPs MTHFR 677 CT/TT, MTR 2756 AG/GG, GCH1 TC/TT and COMT Val158Met AG/GG. In some instances, it is recommended that the depressed subject receive antidepressant monotherapy.

[0063] In some embodiments, the subject has not received any drug therapy for depression prior to the drug selection. In other embodiments, the subject is resistant to an antidepressant drug. If drug resistance is known or detected, a different antidepressant drug in the same class or different class can be selected for the subject.

[0064] In some embodiments, the method for determining a good prognosis of a depressed subject receiving an antidepressant drug comprises detecting the absence of the MTHFR variant allele, the MTR variant allele, the GCH1 variant allele and the COMT variant allele. In some embodiments, the absence of MTHFR 677 CT/TT, MTR 2756 AG/GG, GCH1 TC/TT and COMT Val158Met AG/GG indicates that the subject will have a good prognosis on antidepressant monotherapy. In yet other embodiments, the presence of the SNP pair of MTHFR 677 CT/TT and MTR 2756 AG/GG and the presence of the SNP pair of GCH1

TC/TT and COMT Val158Met AG/GG indicates that the subject will have a poor prognosis on antidepressant monotherapy.

D. Genetic Markers

[0065] The determination of the presence or absence of allelic variants such as SNPs in the methylenetetrahydrofolate reductase (MTHFR) gene is particularly useful in the present invention. As used herein, the term “MTHFR variant” or variants thereof includes a nucleotide sequence of a MTHFR gene containing one or more changes as compared to the wild-type MTHFR gene or an amino acid sequence of a MTHFR polypeptide containing one or more changes as compared to the wild-type MTHFR polypeptide sequence. For instance, the absence of the MTHFR variant allele refers to the absence of at least one thymine “T” allele or the complement thereof at the SNP locus for the MTHFR gene corresponding to rs1801133 or at position 27 of SEQ ID NO:1. The absence of the MTHFR variant allele refers to the presence of two cytosine “C” alleles or the complement thereof at the SNP locus for the MTHFR gene corresponding to rs1801133 or at positions 27 of SEQ ID NO:1. The presence of the MTHFR variant allele can refer to the presence of at least one thymine “T” allele or the complement thereof at the SNP locus for the MTHFR gene corresponding to rs1801133 or at position 27 of SEQ ID NO:1. The presence of the heterozygous or homozygous MTHFR variant can be denoted as MTHFR 677 CT/TT. MTHFR, also known as EC 1.5.1.20, has been localized to human chromosome 1 (1p.36.22).

[0066] Gene location information for MTHFR is set forth in, *e.g.*, GeneID: 4524. The mRNA (coding) and polypeptide sequences of human MTHFR are set forth in, *e.g.*, NM_005957.4 and NP_005948.3, respectively. In addition, the sequence of human chromosome 1, GRCh38 primary reference assembly, which includes MTHFR, is set forth in, *e.g.*, GenBank Accession No. NC_000001.11. Furthermore, the sequence of MTHFR from other species can be found in the GenBank database.

[0067] The rs1801133 SNP that finds use in the methods of the present invention is located at nucleotide position 677 of NM_005957.4, as a C to T transition. The presence/absence of the MTHFR rs1801133 SNP and other variants can be detected, for example, by allelic discrimination assays or sequence analysis.

[0068] The determination of the presence or absence of allelic variants such as SNPs in the 5-methyltetrahydrofolate-homocysteine methyltransferase (MTR) gene is particularly useful in the present invention. As used herein, the term “MTR variant” or variants thereof includes

a nucleotide sequence of a MTR gene containing one or more changes as compared to the wild-type MTR gene or an amino acid sequence of a MTR polypeptide containing one or more changes as compared to the wild-type MTR polypeptide sequence. For instance, the absence of the MTR variant allele refers to the absence of at least one guanine “G” allele or the complement thereof at the SNP locus for the MTR gene corresponding to rs1805087 or at position 27 of SEQ ID NO:2. The absence of the MTR variant allele can refer to the presence of two adenine “A” alleles or the complement thereof at the SNP locus for the MTR gene corresponding to rs1805087 or at position 27 of SEQ ID NO:2. The presence of the MTR variant allele can refer to the presence of at least one guanine “G” allele or the complement thereof at the SNP locus for the MTR gene corresponding to rs1805087 or at position 27 of SEQ ID NO:2. The presence of the heterozygous or homozygous MTR variant can be denoted as MTR 2756 AG/GG. MTR, also known as EC 2.1.1.13, has been localized to human chromosome 1 (1q43).

[0069] Gene location information for MTR is set forth in, *e.g.*, GeneID: 4548. The mRNA (coding) and polypeptide sequences of human MTR are set forth in, *e.g.*, EMBL No. AAI36441.1 and UniProt No. Q99707, respectively. In addition, the sequence of human chromosome 1, GRCh38 primary reference assembly, which includes MTR, is set forth in, *e.g.*, GenBank Accession No. NC_000001.11. Furthermore, the sequence of MTR from other species can be found in the GenBank database.

[0070] The rs1805087 SNP that finds use in the methods of the present invention is located at nucleotide position 2756 of AAI36441.1, as an A to G transition. The presence/absence of the MTR rs1805087 SNP and other variants can be detected, for example, by allelic discrimination assays or sequence analysis.

[0071] The determination of the presence or absence of allelic variants such as SNPs in the GTP cyclohydrolase 1 (GCH1) gene is particularly useful in the present invention. As used herein, the term “GCH1 variant” or variants thereof includes a nucleotide sequence of a GCH1 gene containing one or more changes as compared to the wild-type GCH1 gene or an amino acid sequence of a GCH1 polypeptide containing one or more changes as compared to the wild-type GCH1 polypeptide sequence. For instance, the absence of the GCH1 variant allele refers to the absence of at least one thymine “T” allele or the complement thereof at the SNP locus for the GCH1 gene corresponding to rs8007267 or at position 27 of SEQ ID NO:3. The absence of the GCH1 variant allele can refer to the presence of two cytosine “C” alleles

or the complement thereof at the SNP locus for the GCH1 gene corresponding to rs8007267 or at position 27 of SEQ ID NO:3. The presence of the GCH1 variant allele can refer to the presence of at least one thymidine “T” allele or the complement thereof at the SNP locus for the GCH1 gene corresponding to rs8007267 or at position 27 of SEQ ID NO:3. The presence of the heterozygous or homozygous GCH1 variant can be denoted as GCH1 TC/TT. GCH1, also known as EC 3.5.4.16, has been localized to human chromosome 14 (14q22.2).

[0072] Gene location information for GCH1 is set forth in, *e.g.*, GeneID: 2643. The mRNA (coding) and polypeptide sequences of human GCH1 are set forth in, *e.g.*, NCBI RefSeq No. NM_001024024.1 and NP_001019195, respectively. In addition, the sequence of human chromosome 14, GRCh38 primary reference assembly, which includes GCH1, is set forth in, *e.g.*, GenBank Accession No. NC_000014.8. Furthermore, the sequence of GCH1 from other species can be found in the GenBank database.

[0073] The rs8007267 SNP that finds use in the methods of the present invention is located in the GCH1 gene as a C to T transition. The presence/absence of the GCH1 rs8007267 SNP and other variants can be detected, for example, by allelic discrimination assays or sequence analysis.

[0074] The determination of the presence or absence of allelic variants such as SNPs in the catechol-O-methyltransferase (COMT) gene is particularly useful in the present invention. As used herein, the term “COMT variant” or variants thereof includes a nucleotide sequence of a COMT gene containing one or more changes as compared to the wild-type COMT gene or an amino acid sequence of a COMT polypeptide containing one or more changes as compared to the wild-type COMT polypeptide sequence. For instance, the absence of the COMT variant allele refers to the absence of at least one guanine “G” allele or the complement thereof at the SNP locus for the COMT gene corresponding to rs4680 or at position 27 of SEQ ID NO:4. The absence of the COMT variant allele can refer to the presence of two adenine “T” alleles or the complement thereof at the SNP locus for the COMT gene corresponding to rs4680 or at position 27 of SEQ ID NO:4. The presence of the heterozygous or homozygous COMT variant can be denoted as COMT Val158Met AG/GG. COMT, also known as EC 2.1.1.6, has been localized to human chromosome 22 (22q11.21).

[0075] Gene location information for COMT is set forth in, *e.g.*, GeneID: 1312. The mRNA (coding) and polypeptide sequences of human COMT are set forth in, *e.g.*, NCBI RefSeq No. NM_000754.3 and NP_000745, respectively. In addition, the sequence of human chromosome 22, GRCh38 primary reference assembly, which includes COMT, is set forth in, *e.g.*, GenBank Accession No. NC_000022.11. Furthermore, the sequence of COMT from other species can be found in the GenBank database.

[0076] The rs4680 SNP that finds use in the methods of the present invention is located in the COMT gene as an A to G transition. The presence/absence of the COMT rs4680 SNP and other variants can be detected, for example, by allelic discrimination assays or sequence analysis.

[0077] In some aspects, provided herein is a method for determining whether a subject is homozygous for a polymorphism, heterozygous for a polymorphism, or lacking the polymorphism altogether (*i.e.* homozygous wild-type) is encompassed. As an exemplary embodiment only, provided herein is a method to detect the C to T variance at position 27 of SEQ ID NO:1 (corresponding to a SNP of the MTHFR gene; rs1801133), for instance, a method for determining the allele (*e.g.*, heterozygous for the C- and T-alleles, homozygous for the C-allele, or homozygous for the T-allele) at the SNP loci.

E. Methods for Detecting SNPs

[0078] A variety of means can be used to genotype an individual at a polymorphic site in a gene or any other genetic marker described herein to determine whether a sample (*e.g.*, a nucleic acid sample) contains a specific variant allele or haplotype. For example, enzymatic amplification of nucleic acid from an individual can be conveniently used to obtain nucleic acid for subsequent analysis. The presence of a specific variant allele or haplotype in one or more genetic markers of interest can also be determined directly from the individual's nucleic acid without enzymatic amplification. In preferred embodiments, an individual is genotyped at the *MTHFR*, *MTR*, *GCHI* and *COMT* loci.

[0079] Genotyping of nucleic acid from an individual, whether amplified or not, can be performed using any of various techniques. Useful techniques include, without limitation, polymerase chain reaction (PCR) based analysis, sequence analysis, and electrophoretic analysis, which can be used alone or in combination. As used herein, the term "nucleic acid" means a polynucleotide such as a single- or double-stranded DNA or RNA molecule including, for example, genomic DNA, cDNA and mRNA. This term encompasses nucleic

acid molecules of both natural and synthetic origin as well as molecules of linear, circular, or branched configuration representing either the sense or antisense strand, or both, of a native nucleic acid molecule. It is understood that such nucleic acids can be unpurified, purified, or attached, for example, to a synthetic material such as a bead or column matrix.

5 **[0080]** Material containing nucleic acid is routinely obtained from individuals. Such material is any biological matter from which nucleic acid can be prepared. As non-limiting examples, material can be whole blood, serum, plasma, urine, feces, saliva, synovial fluid, tears, mucus, a cheek swab, sputum, or other bodily fluid or tissue that contains nucleic acid. In one embodiment, a method of the present invention is practiced with whole blood, serum,
10 plasma, urine, saliva or a buccal sample, which can be obtained readily by non-invasive means and used to prepare genomic DNA. In another embodiment, genotyping involves amplification of an individual's nucleic acid using the polymerase chain reaction (PCR). Use of PCR for the amplification of nucleic acids is well known in the art (*see, e.g.,* Mullis *et al.* (Eds.), *The Polymerase Chain Reaction*, Birkhäuser, Boston, (1994)). In yet another
15 embodiment, PCR amplification is performed using one or more fluorescently labeled primers. In a further embodiment, PCR amplification is performed using one or more labeled or unlabeled primers that contain a DNA minor groove binder.

[0081] Any of a variety of different primers can be used to amplify an individual's nucleic acid by PCR in order to determine the presence of a variant allele in one or more genes or
20 loci or other genetic marker in a method of the invention. Non-limiting examples of loci include the human *MTHFR*, *MTR*, *GCHI* and *COMT* loci. For example, the PCR primers can be used to amplify specific regions of the *MTHFR* locus, such as the polymorphism at rs1801133. As understood by one skilled in the art, additional primers for PCR analysis can be designed based on the sequence flanking the polymorphic site(s) of interest in the *MTHFR*
25 locus or other genetic markers (*e.g.,* the *MTR* locus at rs1805087, *GCHI* locus at rs8007267 and *COMT* locus at rs4680). As a non-limiting example, a sequence primer can contain from about 15 to about 30 nucleotides of a sequence upstream or downstream of the polymorphic site of interest in the *MTHFR* locus, *MTR* locus, *GCHI* locus or *COMT* locus. Such primers generally are designed to have sufficient guanine and cytosine content to attain a high melting
30 temperature which allows for a stable annealing step in the amplification reaction. Several computer programs, such as Primer Select, are available to aid in the design of PCR primers.

[0082] A TaqMan[®] allelic discrimination assay available from Applied Biosystems can be useful for genotyping an individual at a polymorphic site and thereby determining the presence of a particular variant allele or haplotype in the *MTHFR* locus, *MTR* locus, *GCHI* locus or *COMT* locus. In a TaqMan[®] allelic discrimination assay, a specific fluorescent dye-labeled probe for each allele is constructed. The probes contain different fluorescent reporter dyes such as FAM[™] and VIC[™] to differentiate amplification of each allele. In addition, each probe has a quencher dye at one end which quenches fluorescence by fluorescence resonance energy transfer. During PCR, each probe anneals specifically to complementary sequences in the nucleic acid from the individual. The 5' nuclease activity of Taq polymerase is used to cleave only probe that hybridizes to the allele. Cleavage separates the reporter dye from the quencher dye, resulting in increased fluorescence by the reporter dye. Thus, the fluorescence signal generated by PCR amplification indicates which alleles are present in the sample. Mismatches between a probe and allele reduce the efficiency of both probe hybridization and cleavage by Taq polymerase, resulting in little to no fluorescent signal. Those skilled in the art understand that improved specificity in allelic discrimination assays can be achieved by conjugating a DNA minor groove binder (MGB) group to a DNA probe as described, *e.g.*, in Kuttyavin *et al.*, *Nuc. Acids Research* 28:655-661 (2000). Minor groove binders include, but are not limited to, compounds such as dihydrocyclopyrroloindole tripeptide (DPI3).

[0083] Sequence analysis can also be useful for genotyping an individual according to the methods described herein to determine the presence of a particular variant allele or haplotype in the *MTHFR* locus at rs1801133 or the other genetic markers (*e.g.*, the *MTR* locus at rs1805087, *GCHI* locus at rs8007267 and *COMT* locus at rs4680). As is known by those skilled in the art, a variant allele of interest can be detected by sequence analysis using the appropriate primers, which are designed based on the sequence flanking the polymorphic site of interest in the *MTHFR*, *MTR*, *GCHI* and *COMT* loci or other genetic markers. Additional or alternative sequence primers can contain from about 15 to about 30 nucleotides of a sequence that corresponds to a sequence about 40 to about 400 base pairs upstream or downstream of the polymorphic site of interest in one or more of the *MTHFR*, *MTR*, *GCHI* and *COMT* loci, or another genetic marker. Such primers are generally designed to have sufficient guanine and cytosine content to attain a high melting temperature which allows for a stable annealing step in the sequencing reaction.

[0084] The term "sequence analysis" includes any manual or automated process by which the order of nucleotides in a nucleic acid is determined. As an example, sequence analysis

can be used to determine the nucleotide sequence of a sample of DNA. The term sequence analysis encompasses, without limitation, chemical and enzymatic methods such as dideoxy enzymatic methods including, for example, Maxam-Gilbert and Sanger sequencing as well as variations thereof. The term sequence analysis further encompasses, but is not limited to, capillary array DNA sequencing, which relies on capillary electrophoresis and laser-induced fluorescence detection and can be performed using instruments such as the MegaBACE 1000 or ABI 3700. As additional non-limiting examples, the term sequence analysis encompasses thermal cycle sequencing (*see*, Sears *et al.*, *Biotechniques* 13:626-633 (1992)); solid-phase sequencing (*see*, Zimmerman *et al.*, *Methods Mol. Cell Biol.* 3:39-42 (1992); and sequencing with mass spectrometry, such as matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (*see*, MALDI-TOF MS; Fu *et al.*, *Nature Biotech.* 16:381-384 (1998)). The term sequence analysis further includes, but is not limited to, sequencing by hybridization (SBH), which relies on an array of all possible short oligonucleotides to identify a segment of sequence (*see*, Chee *et al.*, *Science* 274:610-614 (1996); Drmanac *et al.*, *Science* 260:1649-1652 (1993); and Drmanac *et al.*, *Nature Biotech.* 16:54-58 (1998)). One skilled in the art understands that these and additional variations are encompassed by the term sequence analysis as defined herein.

[0085] Electrophoretic analysis also can be useful in genotyping an individual according to the methods of the present invention to determine the presence of a particular variant allele or haplotype in the *MTHFR*, *MTR*, *GCH1* and *COMT* loci, or another genetic marker. "Electrophoretic analysis" as used herein in reference to one or more nucleic acids such as amplified fragments includes a process whereby charged molecules are moved through a stationary medium under the influence of an electric field. Electrophoretic migration separates nucleic acids primarily on the basis of their charge, which is in proportion to their size, with smaller molecules migrating more quickly. The term electrophoretic analysis includes, without limitation, analysis using slab gel electrophoresis, such as agarose or polyacrylamide gel electrophoresis, or capillary electrophoresis. Capillary electrophoretic analysis generally occurs inside a small-diameter (50-100 μ m) quartz capillary in the presence of high (kilovolt-level) separating voltages with separation times of a few minutes. Using capillary electrophoretic analysis, nucleic acids are conveniently detected by UV absorption or fluorescent labeling, and single-base resolution can be obtained on fragments up to several hundred base pairs. Such methods of electrophoretic analysis, and variations thereof, are well

known in the art, as described, for example, in Ausubel *et al.*, *Current Protocols in Molecular Biology* Chapter 2 (Supplement 45) John Wiley & Sons, Inc. New York (1999).

[0086] Restriction fragment length polymorphism (RFLP) analysis can also be useful for genotyping an individual according to the methods of the present invention to determine the presence of a particular variant allele or haplotype in the *MTHFR*, *MTR*, *GCHI* and *COMT* loci or other genetic marker (*see*, Jarcho *et al.* in Dracopoli *et al.*, *Current Protocols in Human Genetics* pages 2.7.1-2.7.5, John Wiley & Sons, New York; Innis *et al.*, (Ed.), *PCR Protocols*, San Diego: Academic Press, Inc. (1990)). As used herein, “restriction fragment length polymorphism analysis” includes any method for distinguishing polymorphic alleles using a restriction enzyme, which is an endonuclease that catalyzes degradation of nucleic acid following recognition of a specific base sequence, generally a palindrome or inverted repeat. One skilled in the art understands that the use of RFLP analysis depends upon an enzyme that can differentiate a variant allele from a wild-type or other allele at a polymorphic site.

[0087] In addition, allele-specific oligonucleotide hybridization can be useful for genotyping an individual in the methods described herein to determine the presence of a particular variant allele or haplotype in the *MTHFR*, *MTR*, *GCHI* and *COMT* loci, or another genetic marker. Allele-specific oligonucleotide hybridization is based on the use of a labeled oligonucleotide probe having a sequence perfectly complementary, for example, to the sequence encompassing the variant allele. Under appropriate conditions, the variant allele-specific probe hybridizes to a nucleic acid containing the variant allele but does not hybridize to the one or more other alleles, which have one or more nucleotide mismatches as compared to the probe. If desired, a second allele-specific oligonucleotide probe that matches an alternate (*e.g.*, wild-type) allele can also be used. Similarly, the technique of allele-specific oligonucleotide amplification can be used to selectively amplify, for example, a variant allele by using an allele-specific oligonucleotide primer that is perfectly complementary to the nucleotide sequence of the variant allele but which has one or more mismatches as compared to other alleles (Mullis *et al.*, *supra*). One skilled in the art understands that the one or more nucleotide mismatches that distinguish between the variant allele and other alleles are often located in the center of an allele-specific oligonucleotide primer to be used in the allele-specific oligonucleotide hybridization. In contrast, an allele-specific oligonucleotide primer to be used in PCR amplification generally contains the one or more nucleotide mismatches that distinguish between the variant and other alleles at the 3' end of the primer.

[0088] A heteroduplex mobility assay (HMA) is another well-known assay that can be used for genotyping in the methods of the present invention to determine the presence/absence of a particular variant allele or haplotype in the *MTHFR*, *MTR*, *GCHI* and *COMT* loci, or another genetic marker. HMA is useful for detecting the absence of a variant allele since a DNA duplex carrying a mismatch has reduced mobility in a polyacrylamide gel compared to the mobility of a perfectly base-paired duplex (*see*, Delwart *et al.*, *Science*, 262:1257-1261 (1993); White *et al.*, *Genomics*, 12:301-306 (1992)).

[0089] The technique of single strand conformational polymorphism (SSCP) can also be useful for genotyping in the methods described herein to determine the presence/absence of a particular variant allele or haplotype in the *MTHFR*, *MTR*, *GCHI* and *COMT* loci, or another genetic marker (*see*, Hayashi, *Methods Applic.*, 1:34-38 (1991)). This technique is used to detect variant alleles based on differences in the secondary structure of single-stranded DNA that produce an altered electrophoretic mobility upon non-denaturing gel electrophoresis. Variant alleles are detected by comparison of the electrophoretic pattern of the test fragment to corresponding standard fragments containing known alleles.

[0090] Denaturing gradient gel electrophoresis (DGGE) can also be useful in the methods of the invention to determine the presence/absence of a particular variant allele or haplotype in the *MTHFR*, *MTR*, *GCHI* and *COMT* loci, or another genetic marker. In DGGE, double-stranded DNA is electrophoresed in a gel containing an increasing concentration of denaturant; double-stranded fragments made up of mismatched alleles have segments that melt more rapidly, causing such fragments to migrate differently as compared to perfectly complementary sequences (*see*, Sheffield *et al.*, "Identifying DNA Polymorphisms by Denaturing Gradient Gel Electrophoresis" in Innis *et al.*, *supra*, 1990).

[0091] Other molecular methods useful for genotyping an individual are known in the art and useful in the methods of the present invention. Such well-known genotyping approaches include, without limitation, automated sequencing and RNase mismatch techniques (*see*, Winter *et al.*, *Proc. Natl. Acad. Sci.*, 82:7575-7579 (1985)). Furthermore, one skilled in the art understands that, where the presence/absence of multiple variant alleles is to be determined, individual variant alleles can be detected by any combination of molecular methods. *See*, in general, Birren *et al.* (Eds.) *Genome Analysis: A Laboratory Manual* Volume 1 (Analyzing DNA) New York, Cold Spring Harbor Laboratory Press (1997). In

addition, one skilled in the art understands that multiple variant alleles can be detected in individual reactions or in a single reaction (a “multiplex” assay).

[0092] In view of the above, one skilled in the art realizes that the methods of the present invention provide information regarding the likelihood of a positive response to antidepressant drug therapy in patients with depression, *e.g.*, major depressive disorder. Also, the methods provide information regarding a good prognosis of a depressed subject receiving antidepressant drug therapy, *e.g.*, antidepressant drug monotherapy (*e.g.*, by determining the absence of the variant alleles of genes such as, but not limited to, the *MTHFR*, *MTR*, *GCHI* and *COMT* loci) can be practiced using one or any combination of the well-known genotyping assays described above or other assays known in the art.

[0093] The presence or absence of a variant allele in a genetic marker can be determined using an assay described herein. Assays that can be used to determine variant allele status include, but are not limited to, electrophoretic analysis assays, restriction length polymorphism analysis assays, sequence analysis assays (*e.g.*, Sanger sequencing, pyrosequencingTM and next-generation sequencing), hybridization analysis assays, PCR analysis assays, allele-specific hybridization, oligonucleotide ligation allele-specific elongation/ligation, allele-specific amplification, single-base extension, molecular inversion probe, invasive cleavage, selective termination, restriction length polymorphism, sequencing, single strand conformation polymorphism (SSCP), single strand chain polymorphism, mismatch-cleaving, denaturing gradient gel electrophoresis, melting-curve FRET hybridization, fluorescent polarization, INVADER[®] assay, SNP microarrays, and combinations thereof. These assays have been well-described and standard methods are known in the art. See, *e.g.*, Ausubel *et al.*, Current Protocols in Molecular Biology, John Wiley & Sons, Inc. New York (1984-2008), Chapter 7 and Supplement 47; Theophilus, *et al.*, "PCR Mutation Detection Protocols," Humana Press, (2002); Innis *et al.*, PCR Protocols, San Diego, Academic Press, Inc. (1990); Maniatis, *et al.*, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Lab., New York, (1982); Ausubel *et al.*, Current Protocols in Genetics and Genomics, John Wiley & Sons, Inc. New York (1984-2008); and Ausubel *et al.*, Current Protocols in Human Genetics, John Wiley & Sons, Inc. New York (1984-2008); all incorporated herein by reference in their entirety for all purposes.

F. Statistical Algorithms

[0094] In some aspects, the present invention provides methods for selecting treatment for depression or determining the likelihood of a positive response or a good prognosis to treatment with antidepressant drug therapy (*e.g.*, antidepressant drug monotherapy) by detecting the absence of the *MTHFR*, *MTR*, *GCHI* and *COMT* variant alleles (*e.g.*, SNPs) and applying a statistical analysis such as quantile analysis or a learning statistical classifier system to the genotype(s) detected at the *MTHFR*, *MTR*, *GCHI* and *COMT* loci and/or to the presence of one or more clinical factors, *e.g.*, drug response. In certain embodiments, the use of statistical analyses in the methods of the present invention advantageously provide improved sensitivity, specificity, negative predictive value, positive predictive value, and/or overall accuracy for selecting treatment for depression or predicting or identifying the probability that a subject with depression will respond to treatment with an antidepressant drug therapy.

[0095] The term “statistical analysis” or “statistical algorithm” or “statistical process” includes any of a variety of statistical methods and models used to determine relationships between variables. In the present invention, the variables are the presence, level, or genotype of at least one marker of interest. Any number of markers can be analyzed using a statistical analysis described herein. For example, the presence or level of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, or more markers can be included in a statistical analysis. In one embodiment, logistic regression is used. In another embodiment, linear regression is used. In certain preferred embodiments, the statistical analyses comprise a quantile measurement of one or more markers, *e.g.*, within a given population, as a variable. Quantiles are a set of “cut points” that divide a sample of data into groups containing (as far as possible) equal numbers of observations. For example, quartiles are values that divide a sample of data into four groups containing (as far as possible) equal numbers of observations. The lower quartile is the data value a quarter way up through the ordered data set; the upper quartile is the data value a quarter way down through the ordered data set. Quintiles are values that divide a sample of data into five groups containing (as far as possible) equal numbers of observations. The present invention can also include the use of percentile ranges of marker levels (*e.g.*, tertiles, quartile, quintiles, *etc.*), or their cumulative indices (*e.g.*, quartile sums of marker levels to obtain quartile sum scores (QSS), *etc.*) as variables in the statistical analyses (just as with continuous variables).

[0096] In some embodiments, the statistical analyses comprise one or more learning statistical classifier systems. As used herein, the term “learning statistical classifier system” includes a machine learning algorithmic technique capable of adapting to complex data sets (e.g., panel of markers of interest) and making decisions based upon such data sets. In some
5 embodiments, a single learning statistical classifier system such as a decision/classification tree (e.g., random forest (RF) or classification and regression tree (C&RT)) is used. In other embodiments, a combination of 2, 3, 4, 5, 6, 7, 8, 9, 10, or more learning statistical classifier systems are used, preferably in tandem. Examples of learning statistical classifier systems include, but are not limited to, those using inductive learning (e.g., decision/classification
10 trees such as random forests, classification and regression trees (C&RT), boosted trees, *etc.*), Probably Approximately Correct (PAC) learning, connectionist learning (e.g., neural networks (NN), artificial neural networks (ANN), neuro fuzzy networks (NFN), network structures, perceptrons such as multi-layer perceptrons, multi-layer feed-forward networks, applications of neural networks, Bayesian learning in belief networks, *etc.*), reinforcement
15 learning (e.g., passive learning in a known environment such as naïve learning, adaptive dynamic learning, and temporal difference learning, passive learning in an unknown environment, active learning in an unknown environment, learning action-value functions, applications of reinforcement learning, *etc.*), and genetic algorithms and evolutionary programming. Other learning statistical classifier systems include support vector machines
20 (e.g., Kernel methods), multivariate adaptive regression splines (MARS), Levenberg-Marquardt algorithms, Gauss-Newton algorithms, mixtures of Gaussians, gradient descent algorithms, and learning vector quantization (LVQ).

[0097] Random forests are learning statistical classifier systems that are constructed using an algorithm developed by Leo Breiman and Adele Cutler. Random forests use a large
25 number of individual decision trees and decide the class by choosing the mode (*i.e.*, most frequently occurring) of the classes as determined by the individual trees. Random forest analysis can be performed, e.g., using the RandomForest software available from Salford Systems (San Diego, CA). See, e.g., Breiman, *Machine Learning*, 45:5-32 (2001); and at the website stat-www.berkeley.edu/users/breiman/RandomForests/cc_home.htm, for a
30 description of random forests.

[0098] Classification and regression trees represent a computer intensive alternative to fitting classical regression models and are typically used to determine the best possible model for a categorical or continuous response of interest based upon one or more predictors.

Classification and regression tree analysis can be performed, *e.g.*, using the C&RT software available from Salford Systems or the Statistica data analysis software available from StatSoft, Inc. (Tulsa, OK). A description of classification and regression trees is found, *e.g.*, in Breiman *et al.* "Classification and Regression Trees," Chapman and Hall, New York (1984); and Steinberg *et al.*, "CART: Tree-Structured Non-Parametric Data Analysis," Salford Systems, San Diego, (1995).

[0099] Neural networks are interconnected groups of artificial neurons that use a mathematical or computational model for information processing based on a connectionist approach to computation. Typically, neural networks are adaptive systems that change their structure based on external or internal information that flows through the network. Specific examples of neural networks include feed-forward neural networks such as perceptrons, single-layer perceptrons, multi-layer perceptrons, backpropagation networks, ADALINE networks, MADALINE networks, Learnmatrix networks, radial basis function (RBF) networks, and self-organizing maps or Kohonen self-organizing networks; recurrent neural networks such as simple recurrent networks and Hopfield networks; stochastic neural networks such as Boltzmann machines; modular neural networks such as committee of machines and associative neural networks; and other types of networks such as instantaneously trained neural networks, spiking neural networks, dynamic neural networks, and cascading neural networks. Neural network analysis can be performed, *e.g.*, using the Statistica data analysis software available from StatSoft, Inc. See, *e.g.*, Freeman *et al.*, In "Neural Networks: Algorithms, Applications and Programming Techniques," Addison-Wesley Publishing Company (1991); Zadeh, Information and Control, 8:338-353 (1965); Zadeh, "IEEE Trans. on Systems, Man and Cybernetics," 3:28-44 (1973); Gersho *et al.*, In "Vector Quantization and Signal Compression," Kluwer Academic Publishers, Boston, Dordrecht, London (1992); and Hassoun, "Fundamentals of Artificial Neural Networks," MIT Press, Cambridge, Massachusetts, London (1995), for a description of neural networks.

[0100] Support vector machines are a set of related supervised learning techniques used for classification and regression and are described, *e.g.*, in Cristianini *et al.*, "An Introduction to Support Vector Machines and Other Kernel-Based Learning Methods," Cambridge University Press (2000). Support vector machine analysis can be performed, *e.g.*, using the SVM^{light} software developed by Thorsten Joachims (Cornell University) or using the LIBSVM software developed by Chih-Chung Chang and Chih-Jen Lin (National Taiwan University).

[0101] As used herein, the term “sensitivity” refers to the probability that a predictive method of the present invention gives a positive result when the sample is positive, *e.g.*, having the predicted therapeutic response to an antidepressant drug monotherapy in an individual with depression. Sensitivity is calculated as the number of true positive results divided by the sum of the true positives and false negatives. Sensitivity essentially is a measure of how well the present invention correctly identifies those with depression who have the predicted therapeutic response to an antidepressant drug monotherapy. The statistical methods and models can be selected such that the sensitivity is at least about 60%, and can be, *e.g.*, at least about 65%, 70%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%.

[0102] The term “specificity” refers to the probability that a predictive method of the present invention gives a negative result when the sample is not positive, *e.g.*, not having the predicted therapeutic response to an antidepressant drug monotherapy in an individual with depression. Specificity is calculated as the number of true negative results divided by the sum of the true negatives and false positives. Specificity essentially is a measure of how well the present invention excludes those with depression who do not have the predicted therapeutic response to an antidepressant drug monotherapy. The statistical methods and models can be selected such that the specificity is at least about 60%, and can be, *e.g.*, at least about 65%, 70%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%.

[0103] As used herein, the term “negative predictive value” or “NPV” refers to the probability that an individual identified as having depression is not having the predicted therapeutic response to an antidepressant drug monotherapy. Negative predictive value can be calculated as the number of true negatives divided by the sum of the true negatives and false negatives. Negative predictive value is determined by the characteristics of the diagnostic or prognostic method as well as the prevalence of the disease in the population analyzed. The statistical methods and models can be selected such that the negative predictive value in a population having a disease prevalence is in the range of about 70% to about 99% and can be, for example, at least about 70%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%.

[0104] The term “positive predictive value” or “PPV” refers to the probability that an individual identified as having depression is having the predicted therapeutic response to an antidepressant drug monotherapy. Positive predictive value can be calculated as the number of true positives divided by the sum of the true positives and false positives. Positive predictive value is determined by the characteristics of the predictive method as well as the prevalence of the disease in the population analyzed. The statistical methods and models can be selected such that the positive predictive value in a population having a disease prevalence is in the range of about 70% to about 99% and can be, for example, at least about 70%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%.

[0105] Predictive values, including negative and positive predictive values, are influenced by the prevalence of the disease in the population analyzed. In the present invention, the statistical methods and models can be selected to produce a desired clinical parameter for a clinical population with a particular depression prevalence. For example, statistical methods and models can be selected for a depression prevalence of up to about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, or 70%, which can be seen, *e.g.*, in a clinician’s office such as a psychiatrist’s office or a general practitioner’s office.

[0106] As used herein, the term “overall agreement” or “overall accuracy” refers to the accuracy with which a method of the present invention predicts response to an antidepressant drug monotherapy. Overall accuracy is calculated as the sum of the true positives and true negatives divided by the total number of sample results and is affected by the prevalence of depression in the population analyzed. For example, the statistical methods and models can be selected such that the overall accuracy in a patient population having a disease prevalence is at least about 40%, and can be, *e.g.*, at least about 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%.

G. Physical Biomarkers

[0107] In some embodiments, a physical biomarker, *e.g.*, obesity indicator, can be measured, wherein obesity is defined as, *e.g.*, a BMI value of at least about 30 kg/m² or

greater; a waist circumference greater than 40 inches (or greater than 120 cm) in men, or greater than 35 inches (or greater than 88 cm) in women; a waist-hip ratio above 0.95 for men or above 0.80 for women; and/or a body fat percentage of at least about 25% in men or at least about 32% in women. In some embodiments, if a subject is obese and does not carry at least one thymidine allele at rs1801133, at least one guanine allele at rs1805087, at least one thymine allele at rs800726 and at least one guanine at rs4680, an antidepressant drug is selected, recommended and/or administered to treat depression.

[0108] In some embodiments, the methods provided herein can comprise determining if the human subject is obese or not. If the human subject is determined to be obese, then the human subject is selected for and optionally administered with a treatment regimen comprising an effective amount of a folate drug. Methods of determining obesity in a human subject are known in the art and can include, but are not limited to, body mass index (BMI) measurement, measurement of abdominal fat (*e.g.*, by waist circumference or waist-hip ratio), measurement of body fat, skinfold thickness, underwater weighing (densitometry), air-displacement plethysmography, computerized tomography (CT) and magnetic resonance imaging (MRI), and dual energy X-ray absorptiometry (DEXA), and any combinations thereof.

[0109] In some embodiments, the methods also include measuring the level of metabolites, such as SAM, SAH and/or 4-HNE, in a sample, *e.g.*, blood serum, plasma, or CSF sample, from a subject having or at risk for depression. Levels of metabolites (*e.g.*, SAM, SAH, and/or 4-HNE) can be detected by any known methods in the art. For example, mass spectrometry (MS) can be used, *e.g.*, the biological sample is infused directly into the mass spectrometer which provides both separation and detection of metabolites (*e.g.*, SAM, SAH, and/or 4-HNE). In some instances, the target metabolite (*e.g.*, SAM, SAH and/or 4-HNE) can be optionally separated (*e.g.*, prior to detection) from a biological sample by gas chromatography (GC), *e.g.*, when interfaced with mass spectrometry (GC-MS), and/or high performance liquid chromatography (HPLC), and/or capillary electrophoresis (CE). Further details about detecting SAM and SAH, including immunoassays for determining SAM, SAH and/or ratios thereof are described in U.S. Pat. App. No.: US 2009/0263879, which is incorporated herein by reference.

H. Antidepressant Drugs

[0110] In some embodiments, a patient is selected to receive an antidepressant drug therapy, such as an antidepressant drug monotherapy. In some instances, the monotherapy includes receiving one or more drugs from a single class of antidepressants. In other instances, the antidepressant drug monotherapy includes receiving one or more (*e.g.*, 1, 2, 3, 4, 5, 6 or more) drugs from one or more (*e.g.*, 1, 2, 3, 4, 5, 6 or more) different classes of antidepressants. In yet other instances, the antidepressant drug monotherapy is a selective serotonin reuptake inhibitor (SSRI). In yet other instances, the antidepressant drug monotherapy is from a non-SSRI class of antidepressants.

[0111] In some embodiments, if the subject is resistant to a specific antidepressant drug or

a specific class of antidepressant drugs, the subject can be administered a different antidepressant drug from the same class or from a different class of antidepressant drugs. For instance, if the subject is resistant to an SSRI drug (*e.g.*, fluoxetine), another SSRI drug (*e.g.*, citalopram) or an SNRI or SDRI drug may be selected or recommended for the subject and/or administered.

[0112] In other embodiments, the subject is not resistant to an antidepressant drug or a class of antidepressant drugs. In some instances, the subject has never received an antidepressant drug. In other instances, the subject has never received any drug therapy for the treatment of depression.

[0113] Various types or classes of antidepressants are known and commercially available. Non-limiting examples of antidepressants include serotonin reuptake inhibitors (SSRIs), serotonin and dopamine reuptake inhibitors (SDRIs), serotonin-norepinephrine reuptake inhibitors (SNRIs), serotonin-noradrenaline-dopamine reuptake inhibitors (SNDRI), noradrenergic and specific serotonergic anti-depressants (NASSAs), norepinephrine-dopamine reuptake inhibitors (NDRIs), norepinephrine (noradrenaline) reuptake inhibitors (NRIs), monoamine oxidase inhibitors (MAOIs), selective serotonin reuptake enhancers (SSREs), melatonergic agonists, tryptamines, tricyclic antidepressants (TCAs), and atypical antidepressants. In some embodiments, an SSRI, SDRI, SNRI, SNDRI, NASSA, NDRI, NRI, MAOI, SSRE, TCA or an atypical antidepressant is selected for, recommended to or

paroxetine, escitalopram, sertraline, or a combination thereof is selected for the subject who does not carry these SNPs. In some instances, the subject is selected to receive one or more SSRIs, *e.g.*, 1, 2, 3, 4, 5 or more SSRIs.

[0114] SSRIs act to prevent the reuptake of serotonin by the presynaptic neuron, thereby
 5 maintaining high levels of serotonin in the synapse. Examples of SSRIs include, but are not limited to, the following (trade names in parentheses): alaproclate; amoxapine; citalopram (such as CELEXA[®], CIPRAMIL[®], EMOCAL[®], SEPRAM[®] and SEROPRAM[®]); clomipramine; dapoxetine; duloxetine (*e.g.*, CYMBALTA[®]); escitalopram oxalate (*e.g.*, LEXAPRO[®], CIPRALEX[®] and ESERTIA[®]); femoxetine; fenfluramine; fluoxetine (*e.g.*,
 10 PROZAC[®], FONTEX[®], SEROMEX[®], SERONIL[®], SARAFEM[®], FLUCTIN[®] (EUR), and FLUOX[®] (NZ)); fluvoxamine maleate (*e.g.*, LUVOX[®], FAVERIN[®], and DUMYROX[®]); indalpine; milnacipran; norfenfluramine; olanzapine; paroxetine (*e.g.*, PAXIL[®], SEROXAT[®], AROPAX[®], DEROXAT[®], REXETIN[®], XETANOR[®], and PAROXAT[®]); sertraline (*e.g.*, ZOLOFT[®], LUSTRAL[®] and SERLAIN[®]); trazodone (*e.g.*, DESYREL[®],
 15 MOLIPAXIN[®], TRITTICO[®], THOMBRAN[®], TRIALODINE[®], TRAZOREL[®], TRITICUM[®], and TRAZONE[®]); venlafaxine and zimelidine. Additional examples of SSRIs and/or SRIs include, without limitations, citalopram, R-fluoxetine, nefazodone, imipramine, imipramine N-oxide, desipramine, pirandamine, dazepinil, nefopam, befuraline, fezolamine, cianoimipramine, litoxetine, cericlamine, seproxetine, WY 27587, WY 27866, imeldine,
 20 ifoxetine, tiflucarbine, viqualine, bazinaprine, YM 922, S 33005, F 98214TA, OPC 14523, cyanodothepine, trimipramine, quinupramine, dothiepin, , nitroxazepine, McN 5652, McN 5707, Ol 77, Org 6582, Org 6997, Org 6906, amitriptyline, amitriptyline N-oxide, nortriptyline, CL 255. 663, pirlindole, indatraline, LY 113.821, LY 214.281, CGP 6085 A, RU 25.591, napamezole, diclofensine, trazodone, EMD 68.843, BMY 42.569, NS 2389,
 25 sercloremine, nitroquipazine, ademethionine, sibutramine and clovoxamine.

[0115] Bupropion, *e.g.*, WELLBUTRIN[®], is a non-limiting example of a serotonin and dopamine reuptake inhibitor (SDRI). Non-limiting examples of SNRIs include: venlafaxine (EFFEXOR XR[®], EFFEXOR[®]); desvenlafaxine (PRISTIQ[®]); sibutramine (MERIDIA[®], REDUCTIL[®]); nefazodone (SERZONE[®]); milnacipran (DALCIPRAN[®], IXEL[®]); duloxetine (CYMBALTA[®]); and, bicifadine. Non-limiting examples of SNDRI include: tesofensine, brasofensine; NS2359; Nomifensine; Venlafaxine (EFFEXOR[®]) and Sibutramine (MERIDIA[®]/REDUCTIL[®]). A non-limiting example of a NASSA includes mirtazapine (AVANZA[®], ZISPIN[®], REMERON[®]). NRIs include, without limitation, atomoxetine,

maprotiline, nisoxetine, reboxetine, viloxazine and TCAs/Tetras (such as AMITRIPTYLINE[®], AMOXAPINE[®], BUTRIPTYLINE[®], DESIPRAMINE[®]/LOFEPRAMINE[®], DIBENZEPIN[®], DOSULEPIN[®], DOXEPIN[®], IMIPRAMINE[®], IPRINDOLE[®], MELITRACEN[®], NORTRIPTYLINE[®], OPIPRAMOL[®],
5 PROTRIPTYLINE[®], TRIMIPRAMINE[®], and MAPROTILINE[®]). Non-limiting examples of MAOIs include: iproclozide, iproniazid, isocarboxazid, nialamide, pargyline, phenelzine, rasagiline, selegiline, toloxatone, tranylcypromine, RIMAs (brofaromine, beta-carbolines (harmaline) and moclobemide). A non-limiting example of a SSRE includes tianeptine (STABLON[®], COAXIL[®], and TATINOL[®]). A non-limiting example of a TCA includes
10 desipramine (NORPRAMIN[®] and PERTOFRANEIS[®]).

[0116] Additional antidepressants that can be used in the invention described herein can include, but are not limited to, natural products such as Kava-Kava, and St. John's Wort; dietary supplements such as s-adenosylmethionine; neuropeptides such as thyrotropin-releasing hormone; compounds targeting neuropeptide receptors such as neurokinin receptor
15 antagonists; and hormones such as triiodothyronine.

[0117] One skilled in the art would be able to readily determine recommended dosage levels for known and/or marketed antidepressant drugs by consulting appropriate references such as drug package inserts, FDA guidelines, and the Physician's Desk Reference. For example, the antidepressant drug dose can range from 0.1 mg/day to about 1000 mg/day,
20 from about 0.5 mg/day to about 500 mg/day, from about 1 mg/day to about 400 mg/day, from about 5 mg/day to about 300 mg/day, or from about 10 mg/day to about 200 mg/day. One of skill in the art can readily adjust dosage for each different antidepressant drug, depending on a number of factors such as types and/or potency of antidepressants, severity of depression, physical condition of a subject (*e.g.*, ages, genders, and weights), administration routes, other
25 medications taken by a subject, and any combinations thereof.

[0118] In some embodiments, the therapeutically effective amount of the antidepressant drug monotherapy is sufficient to increase the degree of improvement in at least one neuropsychological test, *e.g.*, as measured by HAMD-17, HAMD-28 or other efficacy measures, by at least about 5%, at least about 10%, at least about 20%, at least about 30%, at
30 least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, or at least about 90%, as compared to the degree of improvement obtained in the absence of the antidepressant drug. In some embodiments, the therapeutically effective

amount of the antidepressant drug monotherapy is sufficient to increase the degree of improvement in at least one neuropsychological test, *e.g.*, as measured by HAMD-17, HAMD-28 or other efficacy measures, by at least about 1-fold, at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold or more, as compared to the degree of improvement obtained in the absence of the antidepressant drug.

I. Folate Containing Compounds

[0119] Any art-recognized folate drug, *e.g.*, folate-comprising compound, can be selected and/or optionally administered to a human subject selected to carry at least one or more of the SNP described herein. In some embodiments, the folate drug comprises L-methylfolate. In some embodiments, the folate drug comprises 6(S)-5-methyltetrahydrofolate (also known as 6(S)-5-MTHF).

[0120] The folate drug can include at least one (including at least two, at least three or more) alkaline metal or alkaline earth metal salt of folate, *e.g.*, but not limited to, a calcium salt of folate. In some embodiments, the folate drug is methyl folate, also known as Me-THF, N5-Methyl-THF, MTHF, 5-MTHF, L-methylfolate, and Levomefolic acid, or a pharmaceutically acceptable salt thereof (*e.g.*, sodium salt, potassium salt, magnesium salt, calcium salt, glucosamine salt, or galactosamine salt). Methyl folate calcium salt is available by prescription in the United States as DEPLIN[®] (L-methylfolate calcium salt). Methyl folate calcium salt is also available outside of the United States as METAFOLIN[®], BODYFOLIN[®], and NUTRIFOLIN[®].

[0121] In some embodiments, the folate drug can include at least one (including at least two, at least three or more) glucosamine salt and/or galactosamine salt of folate (including, *e.g.*, folic acid and reduced folate, *e.g.*, but not limited to, tetrahydrofolate, and derivatives thereof). Examples of glucosamine-folate and/or galactosamine-folate and derivatives thereof, *e.g.*, disclosed in U.S. Patent No. 7,947,662, can be administered to a human subject according to the methods described. In one embodiment, QUATREFOLIC[®] (Gnosis S.p.A, Milan, IT) or N-[4-[[[(6S)-2-amino-1,4,5,6,7,8-hexahydro-5-methyl-4-oxo-6-pteridiny]methyl]amino]benzoyl]-L-glutamic acid, glucosamine salt can be administered to a human subject according to the methods described herein.

[0122] Additional examples of folates or folate-comprising compounds that can be administered to a subject according to the method provided herein include, but not limited to, the ones described in the U.S. Patent Nos. 4,336,185; 6,921,754; and 7,947,662; and U.S. Pat.

App. Publication No. US 2008/0064702, the disclosures of which are incorporated are herein incorporated by reference for all purposes.

[0123] The effective amount of folate for use in the treatment methods described herein can vary, depending upon the types of folate, severity of depression, physical conditions of a subject (*e.g.*, ages, genders, weights). In some embodiments, the therapeutically effective amount of the folate containing compound monotherapy is sufficient to increase the degree of improvement in at least one neuropsychological test, *e.g.*, as measured by HAMD-17, HAMD-28 or other efficacy measures, by at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, or at least about 90%, as compared to the degree of improvement obtained in the absence of the folate drug. In some embodiments, the therapeutically effective amount of the folate drug monotherapy is sufficient to increase the degree of improvement in at least one neuropsychological test, *e.g.*, as measured by HAMD-17, HAMD-28 or other efficacy measures, by at least about 1-fold, at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold or more, as compared to the degree of improvement obtained in the absence of the folate drug.

J. Pharmaceutical Compositions and Administration Thereof

[0124] According to the methods of the present invention, the therapeutic drugs described herein (*e.g.*, antidepressant drugs) to treat depression are administered to a subject by any convenient means known in the art. The methods of the present invention can be used to predict the therapeutic efficacy of an antidepressant drug in a subject having depression, *e.g.*, major depressive disorder. The methods of the present invention can also be used to predict the response of the subject to treatment with a drug monotherapy consisting of an antidepressant drug. The methods of the invention can also be used to select a suitable antidepressant drug for the treatment of depression. One skilled in the art will appreciate that the drug monotherapy described herein can be administered alone or as part of a combined therapeutic approach with psychotherapy, psychodynamic therapy, cognitive behavior therapy and combinations thereof.

[0125] The drug monotherapy described herein can be administered via a single dosage form. For example, the single dosage form can be administered as a single tablet, pill, capsule for oral administration or a solution for parenteral administration. Alternatively, the drug can be administered as separate compositions, *e.g.*, as separate tablets or solutions. The

length of time between administrations of a sub-dose of a drug can be adjusted to achieve the desired therapeutic effect.

[0126] In some embodiments, a dose of an antidepressant or a pharmaceutically acceptable salt thereof suitable for administration to a human is in the range of about 0.01 to 50 mg per kilogram body weight of the recipient per day, or in the range of 0.1 to 5 mg per kilogram body weight per day. In certain embodiments, the desired dose can be presented as one single unit dosage form, *e.g.*, containing about 1 mg to about 500 mg, or about 5 mg to about 300 mg. In other embodiments, the desired dose can be presented in two, three, four, five or more sub-doses administered at appropriate intervals throughout the day. These sub-doses can be administered in unit dosage forms, for example, containing about 0.1 mg to about 100 mg or about 1 mg to about 50 mg.

[0127] For oral administration, the therapeutically effective dose can be in the form of tablets, capsules, emulsions, suspensions, solutions, syrups, sprays, lozenges, powders, and sustained-release formulations. Suitable excipients for oral administration include pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, gelatin, sucrose, magnesium carbonate, and the like.

[0128] The pharmaceutically acceptable composition can be delivered via injection. These routes for administration (delivery) include, but are not limited to, subcutaneous or parenteral including intravenous, intraarterial, intramuscular, intraperitoneal, intramyocardial, and infusion techniques. In one embodiment, the pharmaceutical acceptable composition is in a form that is suitable for injection. In another embodiment, the pharmaceutical composition is formulated for delivery by a catheter.

[0129] When administering a pharmaceutical composition parenterally, it can be generally formulated in a unit dosage injectable form (solution, suspension, emulsion). The pharmaceutical formulations suitable for injection include sterile aqueous solutions or dispersions. The carrier can be a solvent or dispersing medium containing, for example, water, cell culture medium, buffers (*e.g.*, phosphate buffered saline), polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol, and the like), suitable mixtures thereof. In some embodiments, the pharmaceutical carrier can be a buffered solution (*e.g.* PBS). In some embodiments, the pharmaceutical composition can be formulated in an emulsion or a gel.

[0130] In some embodiments, the drug monotherapy (*e.g.*, antidepressant drug) is formulated in slow-release or sustained release composition. As used herein, the term “sustained release” or “sustained delivery” refers to continual delivery of a therapeutic agent *in vivo* over a period of time following administration. For example, sustained release can occur over a period of at least about 1 hour, at least about 2 hours, at least about 3 hours, at least about 4 hours, at least about 5 hours, at least about 6 hours, at least about 9 hours, at least about 12 hours, at least about 16 hours, at least about 24 hours following administration. In some embodiments, sustained release can occur over a period of at least about 1 day, at least about 2 days, at least about 3 days, at least about 4 days, at least about 5 days, at least about 6 days, at least about 7 days following administration. In some embodiments, the release of the drug monotherapy from a drug-delivery system can be steady state (zero-order kinetics) with at least about 30% (*e.g.*, including at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95% or more) of the drug compound released between about 3-6 hours post administration, or between about 4-5 hours post administration. In one embodiment, the release of the drug compound from a drug-delivery system can be steady state (zero-order kinetics) with substantially full release (*e.g.*, ~100%) of the drug released between about 3-6 hours post administration, or between about 4-5 hours post administration. In some embodiments, the drug compound can be released from a drug-delivery system at a rate that is slow enough not to overload the intestinal absorption capacity of a patient’s duodenum.

[0131] In some embodiments, a drug delivery system can comprise the drug compound encapsulated in polymer-based particles. For example, the folate-containing polymer-based particles can be filled into capsules or single-dose sachets for additional control of release.

[0132] Controlled-release (*e.g.*, sustained release) drug delivery systems for different administration methods (*e.g.*, oral administration, injection, implantation, and inhalation) are known in the art and can be adopted to deliver a drug monotherapy for the treatment methods described herein. *See, e.g.*, International Pat. App. Nos. WO 2012/111961 (oral formulation), WO 2012/131678 (injectable formulation); U.S. Pat. App. Nos. US 2012/0258161 (implantable formulation), US 2001/0038854, US 2001/0033866; and U.S. Pat. No. 8,268,347 (inhalation formulation), the disclosures of which are hereby incorporated by reference in their entirety for all purposes, for various types of drug-delivery systems to deliver an active agent via various administration routes.

[0133] A pharmaceutically acceptable carrier includes a pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, manufacturing aid (*e.g.*, lubricant, talc magnesium, calcium or zinc stearate, or steric acid), or solvent encapsulating material, involved in carrying or transporting the subject compound from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be “acceptable” in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient. Some examples of materials which can serve as pharmaceutically-acceptable carriers include: (i) sugars, such as lactose, glucose and sucrose; (ii) starches, such as corn starch and potato starch; (iii) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, methylcellulose, ethyl cellulose, microcrystalline cellulose and cellulose acetate; (iv) powdered tragacanth; (v) malt; (vi) gelatin; (vii) lubricating agents, such as magnesium stearate, sodium lauryl sulfate and talc; (viii) excipients, such as cocoa butter and suppository waxes; (ix) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (x) glycols, such as propylene glycol; (xi) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol (PEG); (xii) esters, such as ethyl oleate and ethyl laurate; (xiii) agar; (xiv) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (xv) alginic acid; (xvi) pyrogen-free water; (xvii) isotonic saline; (xviii) Ringer's solution; (xix) ethyl alcohol; (xx) pH buffered solutions; (xxi) polyesters, polycarbonates and/or polyanhydrides; (xxii) bulking agents, such as polypeptides and amino acids (xxiii) serum component, such as serum albumin, HDL and LDL; (xxiv) C2-C12 alcohols, such as ethanol; and (xxv) other non-toxic compatible substances employed in pharmaceutical formulations. Wetting agents, coloring agents, release agents, coating agents, sweetening agents, flavoring agents, perfuming agents, preservative and antioxidants can also be present in the formulation. Pharmaceutically acceptable carriers can vary depending on the administration route and formulation.

[0134] Additionally, various additives which enhance the stability, sterility, and isotonicity of the compositions, including antimicrobial preservatives, antioxidants, chelating agents, and buffers, can be added. Prevention of the action of microorganisms can be ensured by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, and the like. In many cases, it may be desirable to include isotonic agents, for example, sugars, sodium chloride, and the like.

[0135] The compositions can also contain auxiliary substances such as wetting or emulsifying agents, pH buffering agents, gelling or viscosity enhancing additives,

preservatives, colors, binders, and the like, depending upon the route of administration and the preparation desired. Standard texts, such as "REMINGTON'S PHARMACEUTICAL SCIENCE", 17th edition, 1985, incorporated herein by reference, may be consulted to prepare suitable preparations, without undue experimentation. With respect to compositions described herein, however, any vehicle, diluent, or additive used should have to be biocompatible with the antidepressant, the folate drug, or a pharmaceutically acceptable salt thereof.

[0136] The pharmaceutical compositions can be isotonic, *i.e.*, they can have the same osmotic pressure as blood and lacrimal fluid. The desired isotonicity of the compositions of the composition described herein can be accomplished using sodium chloride, or other pharmaceutically acceptable agents such as dextrose, boric acid, sodium tartrate, propylene glycol or other inorganic or organic solutes. In one embodiment, sodium chloride is used in buffers containing sodium ions.

[0137] Viscosity of the compositions can be maintained at the selected level using a pharmaceutically acceptable thickening agent. In one embodiment, methylcellulose is used because it is readily and economically available and is easy to work with. Other suitable thickening agents include, for example, xanthan gum, carboxymethyl cellulose, hydroxypropyl cellulose, carbomer, and the like. The preferred concentration of the thickener will depend upon the agent selected. The important point is to use an amount which will achieve the selected viscosity. Viscous compositions are normally prepared from solutions by the addition of such thickening agents.

[0138] Typically, any additives (in addition to the antidepressant and/or folate-comprising compound) can be present in an amount of 0.001 to 50 wt % solution in phosphate buffered saline, and the active ingredient is present in the order of micrograms to milligrams to grams, such as about 0.0001 to about 5 wt %, about 0.0001 to about 1 wt %, about 0.0001 to about 0.05 wt % or about 0.001 to about 20 wt %, about 0.01 to about 10 wt %, and about 0.05 to about 5 wt %. For any therapeutic composition to be administered to a subject with compression, and for any particular method of administration, it is preferred to determine toxicity, such as by determining the lethal dose (LD) and LD50 in a suitable animal model *e.g.*, rodent such as mouse; and, the dosage of the composition(s), concentration of components therein and timing of administering the composition(s), which elicit a suitable

response. Such determinations do not require undue experimentation from the knowledge of the skilled artisan.

[0139] The compositions described herein can be prepared by mixing the ingredients following generally-accepted procedures. For example, the ingredients can be mixed in an appropriate pharmaceutically acceptable carrier and the mixture can be adjusted to the final concentration and viscosity by the addition of water or thickening agent and possibly a buffer to control pH or an additional solute to control tonicity. Generally the pH can vary from about 3 to about 7.5. In some embodiments, the pH of the composition can be about 6.5 to about 7.5. Compositions can be administered in dosages and by techniques well known to those skilled in the medical and veterinary arts taking into consideration such factors as the age, sex, weight, and condition of the particular patient, and the composition form used for administration (*e.g.*, liquid).

[0140] A subject can also be monitored at periodic time intervals to assess the efficacy of a certain therapeutic regimen. For example, the activation states of certain signal transduction molecules may change based on the therapeutic effect of treatment with one or more of the drugs described herein to treat depression. The subject can be monitored to assess response and understand the effects of certain drugs or treatments in an individualized approach. Additionally, subjects who initially respond to a specific drug for depression may become refractory to the drug, indicating that these subjects have developed acquired drug resistance. These subjects can be discontinued on their current therapy and an alternative treatment prescribed in accordance with the methods of the present invention.

[0141] In some embodiments, for adjunctive therapy a dose of the folate containing compound for administration to a human can be in the range of about 0.01 to about 50 mg per kilogram body weight of the recipient per day, in the range of about 0.05 to about 5 mg per kilogram body weight per day, or in the range of about 0.1 to about 1 mg per kilogram body weight per day. In certain embodiments, the desired dose can be presented as one single unit dosage form, *e.g.*, containing about 0.5 mg to about 500 mg, about 5 mg to about 250 mg, about 10 mg to about 100 mg, or about 10 mg to about 50 mg. In some embodiments, one single unit dosage form can provide about 1 mg to about 70 mg folate, about 5 mg to about 60 mg folate, or from about 7 mg to about 50 mg folate. In other embodiments, one single unit dosage form can provide about 15 mg to about 50 mg folate. In yet other embodiments, one single unit dosage form can provide about 20 mg folate. In other embodiments, the

desired dose can be presented in two, three, four, five or more sub-doses administered at appropriate intervals throughout the day. These sub-doses can be administered in unit dosage forms, for example, containing about 0.1 mg to about 250 mg, about 1 mg to about 100 mg, about 2 mg to about 20 mg, or about 2 mg to about 10 mg.

- 5 [0142] The effective amount of folate administered to a selected human subject for the treatment of depression as described herein is significantly higher than the typical amount taken as a dietary supplement (between 50-600 µg/day). In some embodiments, the effective amount of folate administered to a selected human subject is at least about 2-fold, at least about 5-fold, at least about 10-fold, at least about 25-fold, at least about 50-fold, at least about 100-fold, at least about 250-fold, at least about 500-fold, at least about 1000-fold or more
10 than the typical amount taken as a dietary supplement.

IV. Example

[0143] The following example is offered to illustrate, but not to limit, the claimed invention.

15 Example 1. Method for Selecting SSRI Monotherapy for Patients with Depression.

- [0144] This example illustrates a method for selecting an effective treatment for a patient with depression. This example also illustrates a method for identifying patients with depression, *e.g.*, major depressive disorder (MDD), who are likely to respond to antidepressant drug monotherapy, *e.g.*, SSRI monotherapy, and patients who will likely not
20 respond to such therapy. This example shows that patients that do not carry the SNPs MTHFR 677 CT/TT (rs18001133), MTR 2756 AG/GG (rs1805087), GCH1 TC/TT (rs8007267) and COMT Val58Met AG/GG (rs4680) positively responded to antidepressant drug monotherapy. Furthermore, this example shows that patients who responded to adjunctive therapy of an SSRI in combination with a folate-containing drug are positive for
25 the MTHFR and MTR SNPs and/or the GCH1 and COMT SNPs.

- [0145] The analysis was performed on results from exploratory analyses of a multi-center, 60-day, randomized, double-blind trial of 15 mg L-methylfolate as adjunctive therapy for patients with SSRI-resistant MDD (*see, e.g.*, Papakostas *et al.*, *Am J Psychiatry*, 169:1267-1274 (2012)). The study was divided into two, 30-day phases (phases I and II), according to
30 the sequential parallel comparison design (SPCD) of Fava *et al.*, *Psychother Psychosom*, 72:115-127 (2003).

Methods

Patient Selection

[0146] Patients were included in the study if they met the following criteria: 1) age 18-65 years and meeting the DSM-IV criteria for a current episode of MDD if they had a Quick Inventory of Depressive Symptoms-Self Report (QIDS-SR) score ≥ 12 at screening and baseline visits; 2) receiving an SSRI drug during the current episode of MDD for ≥ 8 weeks at adequate doses (defined as 20 mg/day or more of fluoxetine, citalopram, or paroxetine, 10 mg/day or more of escitalopram, and 50 mg/day or more of sertraline) as assessed using the Massachusetts General Hospital (MGH) Antidepressant Treatment Response Questionnaire (ATRQ) (Chandler *et al.*, *CNS Neurosci Ther.*, 16:322-325 (2010)); and 3) maintaining a stable SSRI dose for the past 4 weeks. Patients were excluded if they had failed more than 2 adequate antidepressant trials during the current episode. Patients who demonstrated $\geq 25\%$ decrease in depressive symptoms on the QIDS-SR total score from screening to baseline were also excluded. Eligibility was assessed during the screening and baseline visits, which occurred within 14 days of each other. Patients eligible during the baseline visit were enrolled in the study using the SPCD previously described.

Study Procedures

[0147] Patients were randomized to one of three treatment groups where they received placebo-placebo (SSRI-SSRI), placebo-L-methylfolate 15 mg/day (SSRI-L-methylfolate), or L-methylfolate-L-methylfolate 15 mg/day during phases I and II using a randomization code generated by the primary study center. Each phase was 30 days in duration. Study visits occurred every 10 days during which the concomitant SSRI doses remained constant, and patients unable to tolerate the study medications were withdrawn from the study. Patients and investigators were blinded to study assignment.

[0148] Patients were assessed at each study visit with the Hamilton Depression Rating Scale (HDRS). In addition, symptom response was evaluated with the HAM-D-28 (McIntyre *et al.*, *J Psychiatry Neurosci*, 27:235-239 (2002)), the Cognitive and Physical Function Questionnaire (CPFQ) (Fava *et al.*, *Psychother Psychosom*, 78:91-97 (2009)), and the Clinical Global Impression Scale (CGI-S) (Guy W. Ecdeu. Assessment Manual for Psychopharmacology—Revised (DHEW Publ. No. ADM 76-338). Rockville, MD, U.S. Department of Health, Education, and Welfare, Public Health Service, Alcohol, Drug Abuse, and Mental Health Administration, NIMH Psychopharmacology Research Branch, Division

of Extramural Research Programs (1976)). Height and weight were measured, and BMI was calculated in kg/m^2 . Baseline blood samples were collected to assess baseline levels of plasma hsCRP, 4-hydroxy-2-nonenal (4-HNE), and low S-adenosylmethionine/S-adenosylhomocysteine (SAM/SAH) ratio. Also assessed were genetic polymorphisms for a) the C677T (677 CT/TT; rs1801133) genotype for methylenetetrahydrofolate reductase (MTHFR); b) the A2756G (2756 AG/GG; rs1805087) genotype for methionine synthase (MTR); c) the TC/TT (rs8007267) genotype for GTP cyclohydrolase 1 (GCH1); and d) the Val158Met AG/GG (rs4680) genotype for catechol-O-methyltransferase (COMT).

Assay Methods

[0149] The presence of genetic polymorphisms was performed on DNA purified from whole blood using a DNeasy blood and tissue kit (Qiagen Inc, Valencia, CA). Genotyping was conducted using the MassArray platform (Sequenom, Inc., San Diego, CA).

Statistical Analyses

[0150] For exploratory analyses, the pooled treatment effect was assessed by average differences in mean changes from baseline to endpoint for L-methylfolate and placebo groups, pooled across the two phases of the study, consistent with the SPCD of Fava *et al.*, 2003. The effect of biomarkers on the response on the HDRS-28 with L-methylfolate compared to placebo was stratified by BMI (≥ 30 or $< 30 \text{ kg/m}^2$), hsCRP level (median baseline value $\geq$ or $< 2.25 \text{ mg/L}$), SAM/SAH ratio (median baseline value $\geq$ or < 2.71), and 4-HNE level (median baseline level $\geq$ or $< 3.28 \text{ } \mu\text{g/mL}$). Further, the presence of SNP genotypes was measured. Elevated BMI, low ratio of SAM/SAH, elevated plasma levels of hsCRP, and 4-HNE, and molecular polymorphisms were evaluated as predictors of a greater pooled (phases I and II according to SPCD) drug/placebo difference.

[0151] A standard SPCD analysis approach was employed in order to analyze the study efficacy data. Specifically, an intent-to-treat/last observation carried forward (ITT/LOCF) approach was employed for patients treated with L-methylfolate during phase I. The phase II dataset of interest was limited to patients treated with placebo during phase I and who completed phase I, who did not experience a clinical response on the HDRS during phase I and entered phase II. The LOCF approach was applied to the dataset for phase II, with the final visit of phase I/first visit of phase II serving as the new baseline visit. The ITT/LOCF data comparing L-methylfolate and placebo during phase I were combined with the data comparing L-methylfolate and placebo in phase II according to the model for SPCD and were

analyzed using the standard approach using a weight ($w=0.50$) and a randomization fraction ($a=0.333$).

[0152] Dichotomous measures were analyzed, while seemingly unrelated regression analysis, controlling for baseline scores, was employed for the comparison of continuous outcomes (Tamura *et al.*, *Clin Trials*, 4:309-317 (2007)). All tests were conducted as two-tailed, with alpha set at 0.05. Pooled mean changes from baseline to endpoint for L-methylfolate vs. placebo on the HAMD-28 were stratified for each biomarker and genetic marker. Treatment effect, effect size (difference between means divided by a standard deviation), and 95% confidence intervals (CI) were calculated for each biomarker. In addition, within group analyses, HAMD -28 response rate (at least 50% reduction from baseline), odds ratio, and number needed to treat were determined. Within group analyses were conducted separately for individuals who received L-methylfolate (in phase I or as placebo non-responders in phase II) or placebo (in phase I or as placebo non-responders in phase II) with the biomarker or genetic marker status as exposure. Because individuals were not randomized based on their biomarkers status, the within-group analyses adjusted for potential confounders including age, sex, race, and BMI as well as baseline level of HDRS-28. Adjustment was made using linear regression for continuous HDRS-28 and through propensity score stratified analysis for binary outcomes (to decrease the number of predictors in the final model).

Results

[0153] Overall, 74 patients provided data, and 61 (81.3%) completed the study. Detailed results from the primary analysis of the study (efficacy, safety, tolerability of 15 mg L-methylfolate versus placebo) have been published (*see, e.g., Papakostas et al., Am J Psychiatry*, 169:1267-1274 (2012)). For some analyses, results from both phase I and phase II of the study were pooled according to the SPCD method. Pooled (phases I and II) mean change from baseline was significantly greater with adjunctive L-methylfolate 15 mg/day than placebo for HDRS-28 (-6.8 ± 7.2 vs. -3.7 ± 6.5 , $p=0.017$).

[0154] Patients who were negative for both SNP pairs, *e.g.*, did not carry the MTHFR, MTR, GCH1 and COMT SNPs, responded to SSRI monotherapy, while those patients who were positive for either of the SNP pairs did not (-8.3 ± 6.6 vs. -1.5 ± 5.9). In addition, marker negative patients did not have an increased positive response to adjunctive therapy of SSRI and folate drug compared to SSRI alone (-7.1 ± 7.6 vs. -8.3 ± 6.6). *See, e.g., Table 2.*

[0155] Results from phase I showed that the pooled mean change from baseline with L-methylfolate vs. placebo on the HDRS-28 was significantly greater ($p<0.001$) in patients positive for one of the SNP pairs (*e.g.*, MTHFR CT/TT and MTR AG/GG or GCH1 TC/TT and COMT GG).

- 5 [0156] Patients carrying either SNP pair (heterozygous for the MTHFR and MTR alleles, or heterozygous for the GCH1 allele and homozygous for the COMT allele) and receiving L-methylfolate showed a significant clinical improvement (*e.g.*, a decrease in at least one symptom of depression). The improvement was statistically significant compared to the marker positive patients who did not receive the folate drug (-15.4 ± 7.2 vs -1.5 ± 5.9 , $p<0.001$;
10 Table 2). In addition, marker positive patients who received the folate drug showed a greater improvement compared to the marker negative patients also receiving the folate drug (-15.4 ± 7.2 vs -7.1 ± 7.6 ; Table 2).

Table 2. Effect of L-methylfolate Therapy in Patients with SNP Biomarker Pairs (MTHFR CT/TT and MTR AG/GG or GCH1 TC/TT and COMT GG) in Phase I of the Study

Response	Marker Positive + Treatment	Marker Positive + Placebo	p-value	Marker Negative + Treatment	Marker Negative + Placebo	p-value
HAMD-28 Mean baseline	26 (4.2)	27 (4.8)		27.2 (3.8)	26.1 (4.1)	
Mean change	-15.4 (7.2)	-1.5 (5.9)	<0.001	-7.1 (7.6)	-8.3 (6.6)	
Patients (n)	5	12		11	31	

15

- [0157] The data demonstrates that an SSRI monotherapy can be administered to a patient with depression who does not carry either of the SNP combinations. The patients who did not carry the MTHFR, MTR, GCH1 and COMT SNPs had a greater mean change from baseline when administered an SSRI monotherapy (placebo in this study) than those patients
20 who carried either the MTHFR and MTR SNP pairs or the GCH1 and COMT SNP pairs (-8.3 ± 6.9 vs. -3.8 ± 6.4 ; Table 3). The SNP marker negative patients exhibited a positive response to the SSRI drug.

- [0158] Also, the data indicates that a folate-containing drug can be administered to a patient who carries either SNP combination for the treatment of depression. Further analysis
25 revealed that patients who were positive for the MTHFR and MTR combination or the SNP combination of GCH1 TC/TT and COMT AG/GG showed a statistically significant clinical improvement if they received a folate drug (-11.1 ± 9.8 vs -3.8 ± 6.4 , $p<0.001$; Table 3).

Table 3. Effect of L-methylfolate Therapy in Patients with SNP Biomarker Pairs (MTHFR CT/TT and MTR AG/GG or GCH1 TC/TT and COMT AG/GG) in Phase I of the Study

Response	Marker Positive + Treatment	Marker Positive + Placebo	p-value	Marker Negative + Treatment	Marker Negative + Placebo	p-value
HAMD-28 Mean baseline	26.3 (4.4)	26.9 (4.4)		27.4 (2.9)	26 (5)	
Mean change	-11.1 (9.8)	-3.8 (6.4)	<0.001	-7.9 (6)	-8.3 (6.9)	
Patients (n)	9	18		7	25	

[0159] The data also shows that patients with the MTHFR CT/TT, MTR AG/GG, GCH1 TC/TT and COMT AG/GG (or COMT GG) genotypes are resistant to SSRI therapy (Tables 1 and 2). When these patients were given the SSRI drug, they failed to respond and showed no change or very little change in their current episode of depression (-1.5 ± 5.9 in Table 2 and -3.8 ± 6.4 in Table 3). On the other hand, patients who do not have the MTHFR CT/TT, MTR AG/GG, GCH1 TC/TT, and COMT AG/GG (or COMT GG) genotypes are responsive to SSRI therapy. When these patients received SSRI monotherapy, they had a positive response and showed an alleviation of at least one symptom of depression, as reflected in a decrease in HAMD-28 score from baseline.

[0160] Comparison of pool mean change from baseline with L-methylfolate vs. placebo (SSRI drug alone) shows that the patients who are negative for the SNP pairs did not respond to L-methylfolate but responded to an SSRI (Table 2 and Table 3). These patients had a better response to SSRI monotherapy than adjunctive therapy. The marker negative patient showed greater responsiveness to the SSRI and clinical improvement compared to the marker positive patients (-8.3 ± 6.6 vs. -1.5 ± 5.9 in Table 2 and -8.3 ± 6.9 vs. -3.8 ± 6.4 in Table 3). The data indicates that an SSRI drug can be administered as an effective treatment of depression in patients who do not carry the MTHFR CT/TT and MTR AG/GG SNP combination, the GCH1 TC/TT and COMT GG SNP combination, or the GCH1 TC/TT and COMT AG/GG SNP combination.

[0161] Similar results were seen in the analysis of the phase II data (Tables 4 and 5). Patients in the phase II subset of the study included patients treated with placebo during phase I and who completed the phase but did not experience a clinical response on the HAMD-28.

Table 4. Effect of L-methylfolate Therapy in Patients with SNP Biomarker Pairs (MTHFR CT/TT and MTR AG/GG or GCH1 TC/TT and COMT GG) in Phase II of the Study

Response	Marker Positive + Treatment	Marker Positive + Placebo	p-value	Marker Negative + Treatment	Marker Negative + Placebo	p-value
HAMD-28 Mean baseline	31 (0)	20.3 (5.9)		27.2 (3.8)	26.1 (4.1)	
Mean change	-16 (0)	-2.3 (4.3)		-7.1 (7.6)	-8.3 (6.6)	
Patients (n)	1	6		11	31	

5 Table 5. Effect of L-methylfolate Therapy in Patients with SNP Biomarker Pairs (MTHFR CT/TT and MTR AG/GG or GCH1 TC/TT and COMT AG/GG) in Phase II of the Study

Response	Marker Positive + Treatment	Marker Positive + Placebo	p-value	Marker Negative + Treatment	Marker Negative + Placebo	p-value
HAMD-28 Mean baseline	25.7 (5.5)	21.3 (5.4)		21.7 (5.4)	21.9 (4.4)	
Mean change	-10 (6)	-1.9 (4.7)		-1.9 (6.9)	-2.9 (5.6)	
Patients (n)	3	8		11	7	

[0162] Marker positive patients showed a positive clinical response to folate drug therapy and failed to respond to SSRI drug treatment (-16 vs. -2.3±4.3 in Table 4; -10±6 vs. -1.9±4.7 in Table 5). Patients who did not carry the SNP markers MTHFR CT/TT, MTR AG/GG, 10 GCH1 TC/TT and COMT GG had a clinical response (positive response) to SSRI drug and no response to folate drug (-8.3±6.6 vs. -7.1±7.6 in Table 4).

[0163] In summary, the SNPs MTHFR CT/TT, MTR AG/GG, GCH1 TC/TT and COMT AG/GG are useful for determining or predicting whether a depressed patient will respond to SSRI monotherapy or L-methylfolate adjunctive therapy. For example, L-methylfolate 15 adjunctive therapy, and not SSRI monotherapy, should be selected for a patient who is positive for one of the SNP pairs, *e.g.*, (a) heterozygous or homozygous for the thymine “T” allele or complement thereof for the MTHFR SNP rs1801133, and heterozygous or homozygous for the guanine “G” allele or complement thereof for the MTR SNP rs1805087; or (b) heterozygous or homozygous for the thymine “T” allele or complement thereof for the 20 GCH1 SNP rs8007267, and heterozygous or homozygous for the guanine “G” allele or complement thereof for the COMT SNP rs4680. This patient is likely to respond to L-methylfolate adjunctive therapy, and not to respond to SSRI monotherapy. In other words,

this patient is predicted to have a good prognosis with L-methylfolate adjunctive therapy, but is predicted to have a bad or poor prognosis with SSRI monotherapy due to a lack of response to SSRI drugs.

[0164] A patient negative for both SNP pairs should receive SSRI monotherapy. This patient is unlikely to respond to L-methylfolate adjunctive therapy and is predicted to have a positive response to SSRI monotherapy. Furthermore, a patient who is negative for the MTHFR, MTR, GCH1 and COMT SNPs is likely to have a good prognosis if administered an SSRI monotherapy. The SNP combinations described herein can be used to predict, determine, or select an effective therapy for patients with depression, including drug-naïve patients (*e.g.*, patients who have never been treated with SSRI drug therapy) and SSRI-resistant patients.

[0165] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, one of skill in the art will appreciate that certain changes and modifications may be practiced within the scope of the appended claims. In addition, each reference provided herein is incorporated by reference in its entirety to the same extent as if each reference was individually incorporated by reference.

WHAT IS CLAIMED IS:

1 1. A method for selecting a suitable treatment for an individual diagnosed
2 with depression, said method comprising:

3 (a) detecting the presence or absence of the following single nucleotide
4 polymorphisms (SNPs) in a sample obtained from the individual:

5 (i) a SNP at position 27 of SEQ ID NO:1 comprising at least one
6 thymine "T" allele or the complement thereof for MTHFR (rs 1801133);

7 (ii) a SNP at position 27 of SEQ ID NO:2 comprising at least one
8 guanine "G" allele or the complement thereof for MTR (rs1805087);

9 (iii) a SNP at position 27 of SEQ ID NO:3 comprising at least one
10 thymine "T" allele or the complement thereof for GCH1 (rs8007267); and

11 (iv) a SNP at position 27 of SEQ ID NO:4 comprising at least one
12 guanine "G" allele or the complement thereof for COMT (rs4680); and

13 (b) selecting a treatment comprising an antidepressant drug if the absence of
14 the SNPs is detected.

1 2. The method of claim 1, wherein the selected treatment consists of an
2 antidepressant drug monotherapy if the absence of the SNPs is detected.

1 3. The method of claim 1 or 2, wherein the antidepressant drug is a
2 selective serotonin reuptake inhibitor (SSRI).

1 4. The method of claim 3, wherein the selective serotonin reuptake
2 inhibitor (SSRI) is selected from the group consisting of fluoxetine, citalopram, paroxetine,
3 escitalopram, sertraline and a combination thereof.

1 5. The method of any one of claims 1 to 4, further comprising
2 recommending therapy with or administering said antidepressant drug to the individual.

1 6. The method of any one of claims 1 to 5, wherein the depression is
2 major depressive disorder.

1 7. The method of any one of claims 1 to 6, wherein the individual is an
2 adult.

1 8. The method of any one of claims 1 to 7, wherein the individual is
2 obese.

1 9. The method of claim 8, wherein obesity is determined if the individual
2 has one of the following conditions:

3 (a) a BMI value greater than 30 kg/m²;

4 (b) a waist circumference greater than 40 inches in men, or greater than 35
5 inches in women;

6 (c) a waist-hip ratio above 0.95 for men or above 0.80 for women; and

7 (d) a body fat percentage of at least about 25% in men or at least about 32% in
8 women.

1 10. The method of any one of claims 1 to 9, wherein the sample is selected
2 from the group consisting of a blood sample, a serum sample, a plasma sample, a urine
3 sample, a buccal sample, a saliva sample and a combination thereof.

1 11. The method of any one of claims 1 to 10, wherein the individual is a
2 drug naïve individual.

1 12. The method of any one of claims 1 to 10, wherein the individual is
2 resistant to at least one antidepressant drug that is different than the selected antidepressant
3 drug.

1 13. A method for predicting a positive response to an antidepressant drug
2 therapy in an individual having depression, said method comprising:

3 (a) detecting the presence or absence of the following single nucleotide
4 polymorphisms (SNPs) in a sample obtained from the individual:

5 (i) a SNP at position 27 of SEQ ID NO:1 comprising at least one
6 thymine "T" allele or the complement thereof for MTHFR (rs 1801133);

7 (ii) a SNP at position 27 of SEQ ID NO:2 comprising at least one
8 guanine "G" allele or the complement thereof for MTR (rs1805087);

9 (iii) a SNP at position 27 of SEQ ID NO:3 comprising at least one
10 thymine "T" allele or the complement thereof for GCH1 (rs8007267); and

11 (iv) a SNP at position 27 of SEQ ID NO:4 comprising at least one
12 guanine "G" allele or the complement thereof for COMT (rs4680); and

13 (b) predicting a likelihood of positive response to the antidepressant drug
14 therapy in the individual if the absence of the SNPs is detected.

1 14. The method of claim 13, wherein the antidepressant drug therapy
2 consists of an antidepressant drug monotherapy.

1 15. The method of claim 13 or 14, wherein step (b) comprises predicting a
2 likelihood of non-response to a folate drug if the presence of at least one of the SNPs is
3 detected.

1 16. The method of any one of claims 13 to 15, further comprising
2 recommending therapy with or administering said antidepressant drug to the individual.

1 17. The method of any one of claims 13 to 16, wherein the antidepressant
2 drug is a selective serotonin reuptake inhibitor.

1 18. The method of claim 17, wherein the selective serotonin reuptake
2 inhibitor (SSRI) is selected from the group consisting of fluoxetine, citalopram, paroxetine,
3 escitalopram, sertraline and a combination thereof.

1 19. The method of any one of claims 13 to 18, wherein the individual is a
2 drug naïve individual.

1 20. The method of any one of claims 13 to 15, wherein the individual is
2 resistant to an antidepressant drug.

1 21. The method of claim 20, further comprising recommending therapy
2 with or administering an antidepressant drug to which the individual has not developed
3 resistance.

1 22. The method of any one of claims 13 to 20, wherein the depression is
2 major depressive disorder.

1 23. The method of any one of claims 13 to 22, wherein the sample is
2 selected from the group consisting of a blood sample, a serum sample, a plasma sample, a
3 urine sample, a buccal sample, a saliva sample and a combination thereof.

1 24. A method for determining the prognosis of an individual diagnosed
2 with depression and receiving an antidepressant drug therapy, said method comprising:

3 (a) detecting the presence or absence of the following single nucleotide
4 polymorphisms (SNPs) in a sample obtained from the individual:

5 (i) a SNP at position 27 of SEQ ID NO:1 comprising at least one
6 thymine "T" allele or the complement thereof for MTHFR (rs 1801133);

7 (ii) a SNP at position 27 of SEQ ID NO:2 comprising at least one
8 guanine "G" allele or the complement thereof for MTR (rs1805087);

9 (iii) a SNP at position 27 of SEQ ID NO:3 comprising at least one
10 thymine "T" allele or the complement thereof for GCH1 (rs8007267); and

11 (iv) a SNP at position 27 of SEQ ID NO:4 comprising at least one
12 guanine "G" allele or the complement thereof for COMT (rs4680); and

13 (b) predicting a good prognosis for the individual receiving the antidepressant
14 drug therapy if the absence of the SNPs is detected.

1 25. The method of claim 24, wherein the antidepressant drug therapy
2 consists of an antidepressant drug monotherapy.

1 26. The method of claim 24 or 25, wherein the antidepressant drug is a
2 selective serotonin reuptake inhibitor.

1 27. The method of claim 26, wherein the selective serotonin reuptake
2 inhibitor (SSRI) is selected from the group consisting of fluoxetine, citalopram, paroxetine,
3 escitalopram, sertraline and a combination thereof.

1 28. The method of any one of claims 24 to 27, further comprising
2 predicting a poor prognosis for the individual receiving the antidepressant drug therapy if the
3 presence of any one of the SNPs is detected.

1 29. The method of claim 28, further comprising recommending that the
2 individual receive a non-antidepressant drug.

1 30. The method of any one of claims 24 to 29, wherein the depression is
2 major depressive disorder.

- 1 31. The method of any one of claims 24 to 30, wherein the sample is
2 selected from the group consisting of a blood sample, a serum sample, a plasma sample, a
3 urine sample, a buccal sample, a saliva sample and a combination thereof.

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2015/051741

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/68
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12Q G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, FSTA, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2013/074676 A2 (GEN HOSPITAL CORP [US]; PAMLAB L L C [US]) 23 May 2013 (2013-05-23) claims 1, 4x., 4xvi; 5x., 5xvi. -----	1-13,15, 16,18, 19,21, 23,24, 28,29,31
A	GEORGE I PAPAKOSTAS ET AL: "Article L-Methylfolate as Adjunctive Therapy for SSRI-Resistant Major Depression: Results of Two Randomized, Double-Blind, Parallel-Sequential Trials", AM J PSYCHIATRY, vol. 169, 1 December 2012 (2012-12-01), pages 1267-1274, XP055196927, the whole document ----- -/--	1-31



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

29 June 2015

Date of mailing of the international search report

09/07/2015

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INTERNATIONAL SEARCH REPORT

International application No

PCT/IB2015/051741

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CHEE NG ET AL: "Pharmacogenetic polymorphisms and response to escitalopram and venlafaxine over 8?weeks in major depression", HUMAN PSYCHOPHARMACOLOGY: CLINICAL AND EXPERIMENTAL, vol. 28, no. 5, 1 September 2013 (2013-09-01), pages 516-522, XP055196975, ISSN: 0885-6222, DOI: 10.1002/hup.2340 the whole document	1-31
A	MISCHOULON DAVID ET AL: "Prevalence of MTHFR C677T and MS A2756G polymorphisms in major depressive disorder, and their impact on response to fluoxetine treatment", CNS SPECTRUMS, MBL COMMUNICATIONS, NEW YORK, NY, US, vol. 17, no. 2, 1 June 2012 (2012-06-01), pages 76-86, XP009168179, ISSN: 1092-8529, DOI: 10.1017/S1092852912000430 the whole document	1-31
A	DE MATTIA E ET AL: "C677T and A1298C MTHFR polymorphisms, a challenge for antifolate and fluoropyrimidine-based therapy personalisation", EUROPEAN JOURNAL OF CANCER, PERGAMON, vol. 45, no. 8, 1 May 2009 (2009-05-01), pages 1333-1351, XP026056385, ISSN: 0959-8049, DOI: 10.1016/J.EJCA.2008.12.004 [retrieved on 2009-01-12] the whole document	1-31
A	US 2008/261220 A1 (CRACAUER RAYMOND F [US] ET AL) 23 October 2008 (2008-10-23) par. 1224	1-31

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB2015/051741

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
- a. ☒ forming part of the international application as filed:
- ☒ in the form of an Annex C/ST.25 text file.
- ☐ on paper or in the form of an image file.
- b. ☐ furnished together with the international application under PCT Rule 13~~ter~~.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
- c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
- ☐ in the form of an Annex C/ST.25 text file (Rule 13~~ter~~.1(a)).
- ☐ on paper or in the form of an image file (Rule 13~~ter~~.1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/IB2015/051741

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2013074676	A2	23-05-2013	
		AU 2012340015 A1	22-05-2014
		CA 2855640 A1	23-05-2013
		CN 104053785 A	17-09-2014
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		US 2013267523 A1	10-10-2013
		WO 2013074676 A2	23-05-2013

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