

(12) STANDARD PATENT
(19) AUSTRALIAN PATENT OFFICE

(11) Application No. **AU 2014336928 B2**

(54) Title
Genetic markers for antipsychotic induced weight gain and methods for use thereof

(51) International Patent Classification(s)
C12Q 1/68 (2006.01) **C40B 40/06** (2006.01)
C07H 21/00 (2006.01) **C40B 40/10** (2006.01)
C40B 30/04 (2006.01) **G01N 33/48** (2006.01)

(21) Application No: **2014336928** (22) Date of Filing: **2014.10.17**

(87) WIPO No: **WO15/054792**

(30) Priority Data

(31) Number (32) Date (33) Country
61/892,094 **2013.10.17** **US**

(43) Publication Date: **2015.04.23**

(44) Accepted Journal Date: **2020.07.16**

(71) Applicant(s)
Centre for Addiction and Mental Health

(72) Inventor(s)
Zai, Clement C.;Kennedy, James L.;Mueller, Daniel J.

(74) Agent / Attorney
Spruson & Ferguson, GPO Box 3898, Sydney, NSW, 2001, AU

(56) Related Art
WO 03039234 A2
BAUER, L.O. ET AL., "GABRA2 genotype, impulsivity and body mass", AM. J. ADDICT., (2012), vol. 21, pages 404 - 410



(51) International Patent Classification:

C12Q 1/68 (2006.01) *C40B 40/06* (2006.01)
C07H 21/00 (2006.01) *C40B 40/10* (2006.01)
C40B 30/04 (2006.01) *G01N 33/48* (2006.01)

(21) International Application Number:

PCT/CA2014/051000

(22) International Filing Date:

17 October 2014 (17.10.2014)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

61/892,094 17 October 2013 (17.10.2013) US

(71) Applicant: **CENTRE FOR ADDICTION AND MENTAL HEALTH** [CA/CA]; 33 Russell Street, Toronto, Ontario M5S 2S1 (CA).

(72) Inventors: **ZAI, Clement C.**; 250 College Street, Room R119, Toronto, Ontario M5T 1R8 (CA). **KENNEDY, James L.**; 250 College Street, Room 129, Toronto, Ontario M5T 1R8 (CA). **MUELLER, Daniel J.**; 250 College Street, Toronto, Ontario M5T 1R8 (CA).

(74) Agents: **SCHROEDER, Hans** et al.; Gowling Lafleur Henderson LLP, 160 Elgin Street, Suite 2600, Ottawa, Ontario K1P 1C3 (CA).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- with sequence listing part of description (Rule 5.2(a))

(54) Title: GENETIC MARKERS FOR ANTIPSYCHOTIC INDUCED WEIGHT GAIN AND METHODS FOR USE THEREOF

(57) Abstract: Provided is a method of predicting a subject's weight response to antipsychotic drug treatment by obtaining a biological sample comprising genomic DNA from the subject and determining the presence or absence of one or more polymorphisms in the *GABRA2* gene of the subject, wherein the presence of said one or more polymorphisms is predictive of the subject's weight change in response to antipsychotic drug treatment. The method also may comprise additional steps including treating the subject. Kits and components thereof are also provided.



Genetic Markers for Antipsychotic Induced Weight Gain and Methods for Use Thereof

FIELD OF INVENTION

[0001] The present invention relates to the use of genetic markers. More specifically, the present invention relates to genetic markers in *GABRA2* that are associated with antipsychotic induced weight gain and use thereof.

BACKGROUND OF THE INVENTION

[0002] Treatment of psychosis symptoms, for example schizophrenia (SCZ) symptoms with antipsychotics has been limited by poor efficacy and adverse reactions. This is especially true for second-generation antipsychotics, such as clozapine and olanzapine, where about 30% of treated patients experience significant weight gain. Antipsychotics are used to treat psychotic symptoms that are commonly observed in schizophrenia, bipolar disorder, and psychotic depression. They have been used increasingly to manage other psychiatric disorders, including bipolar manic and mixed episodes¹, major depressive disorder^{2,3}, autistic spectrum disorder^{4,5}, general anxiety disorder, obsessive-compulsive disorder, dementia⁶⁻⁸.

[0003] While the underlying mechanisms of antipsychotic response and adverse effects remain unclear, genetic factors appear to play a prominent role⁹⁻¹⁴.

[0004] There is increasing evidence for a role of gamma-aminobutyric acid (GABA) in the regulation of food intake. GABA is produced in many regions of the brain, including the (pro-opiomelanocortin) POMC and Agouti-related peptide (AGRP) neurons in the hypothalamus^{15,16}.

Diphtheria toxin-mediated ablation of GABA-secreting AGRP neurons induced an anorexic phenotype in mice (reviewed in¹⁷). Similarly, mice genetically deficient in GABA release from AGRP neurons were resistant to obesity induced by ghrelin¹⁸. The mechanism of this resistance could be through a decrease in food intake and an increase in energy expenditure in these AGRP GABA-deficient mice¹⁸. Conversely, administration of GABA agonists, including the benzodiazepine midazolam and L-838417, into the parabrachial nucleus in the brainstem, increased food intake¹⁹. Both GABA_A and GABA_B receptor agonists enhanced feeding in rodents and other animal models²⁰⁻²². The *GABRA2* gene, in particular, was one of the top

findings in a recent genome-wide meta-analysis of obesity²³, making it an appealing candidate gene for further investigation in obesity and related phenotypes.

[0005] There is also accumulating evidence for alterations in GABA neurotransmission by various antipsychotic drugs²⁴⁻²⁶. Clozapine and olanzapine, in particular, may exert their
5 anxiolytic activity by increasing GABA-ergic neurotransmission²⁷ through the allosteric action of neuroactive steroids including allopregnanolone at the GABA_A receptor²⁸. Olanzapine-induced weight gain and adiposity has been correlated to increased levels of the GABA synthesis enzyme GAD65²⁹.

[0006] The *GABRA2* gene (HGNC:4076), which is mapped to chromosomal region 4p12, codes
10 for the GABA_A receptor, alpha 2 subunit. While the *GABRA2* gene was implicated in obesity²³, it has not been investigated in relation to antipsychotic induced weight gain.

[0007] There is a need in the art for novel genetic markers. Further, there is a need in the art for novel genetic markers associated with antipsychotic-induced weight gain. Further, there is a need in the art for genetic diagnostic markers for antipsychotic-induced weight gain that provide
15 physicians and other health care professionals with the opportunity to generate educated decisions for prescribing medications for treatment of psychosis. Moreover, there is a need in the art for personalized medicine approaches that lower the risk of developing antipsychotic induced weight gain and related ailments such diabetes and cardiovascular disease.

20 SUMMARY OF THE INVENTION

[0008] The present invention relates to genetic markers. More specifically, the present invention relates to genetic markers in *GABRA2* that are associated with antipsychotic induced weight gain and use thereof.

[0009] As a convention, all references to nucleotide sequences herein are recited with respect to
25 the positive strand. As will be understood by a person of skill in the art, *GABRA2* gene is transcribed off the negative strand. Thus it is fully contemplated that the subject matter herein

may be practiced as outlined as recited or it may be practiced by employing/determining/analyzing the complement of the nucleotide sequences recited herein.

[0010] The following nucleotide sequences were examined in this study. The polymorphic sites are shown underlined in bold:

- 5 a) rs16859227
CCTTGGTTTTATACAAGCATGCAAAG**[C/T]**ATATAATAGAATCACATGGAAACAA
(SEQ ID NO:1),
- b) rs279858
ATTGTCATATTATGAGCTACTGATTT**[T/C]**TTCCCATTTGTGAAAAAAGGTATCTG
10 (SEQ ID NO:2);
- c) rs1442060
GTAAAGTGTACATCAATGCCATATC**[A/G]**TATTCTGTAGATGGCATGTTATCAT
(SEQ ID NO:3),
- d) rs3849591
15 CTCATTTCCTTGCTTCTAAGGTAGGG**[G/T]**TCATCAATTTATCTATCTCATGGGA
(SEQ ID NO:4),
- e) rs1442062
GAGAAGGTGAAATAGATTTAACAT**[A/G]**TATCAAATTAAGATTGCACCTTAAA
(SEQ ID NO:5),
- 20 f) rs16859354
TACAATATCTTGACTCAATGAGCTTC**[G/T]**AATCTTAATAAGGTAACAAGAGAAA
(SEQ ID NO:6),
- g) rs11503014
AAGCTATGGAGATTACTTCCTGGACT**[C/G]**TGTGTAGGACTTGATGATTGAGAGA
25 (SEQ ID NO:7),
- h) rs6856130
TCTGTTCTGTTTTATCTGAGGCGATA**[A/G]**AATCCAAACGTGCAACTTGAACAAC
(SEQ ID NO:8), or
- i) rs1372472
30 ATAAACTCTGGTAATTCAAACCAAA**[A/T]**ATTCCTCACTGAAAACATGCTTG
(SEQ ID NO:9).

[0011] According to the present invention there is provided a method of predicting a subject's weight change in response to antipsychotic drug treatment comprising,

- a) obtaining a biological sample comprising genomic DNA from the subject;
- b) determining the presence or absence of one or more polymorphisms in the *GABRA2* gene of the subject, wherein the presence of said one or more polymorphisms is predictive of the subject's weight change in response to antipsychotic drug treatment.

[0012] In a further embodiment, there is provided a method as described above, further comprising at least one step selected from the group consisting of a) treating the subject with one or more therapeutics based on the results obtained from said determining the presence or absence of one or more polymorphisms in the *GABRA2* gene b) advising and/or counseling the subject with respect to the results of determining the presence or absence of one or more polymorphisms in the *GABRA2* gene; c) transmitting, advising and/or conveying the results to a physician, medical service provider or other third party; d) treating the subject with one or more particular antipsychotic treatment(s) based on the results; e) treating the subject prior to, concurrently with or after antipsychotic treatment with one or more therapies or therapeutics to control weight gain; f) monitoring the subject's weight over a period of time; g) prescribing, recommending or subjecting the patient or subject to exercise or diet changes; h) monitoring the subject for metabolic syndrome, i) monitoring the subject for cardiovascular disease or symptoms thereof, or any combination of a-i) .

[0013] Also provided by the present invention is a method as described above, wherein the subject has been diagnosed with schizophrenia or schizoaffective disorder, is likely to develop schizophrenia or schizoaffective disorder, or exhibits one or more symptoms of schizophrenia or schizoaffective disorder. In a further embodiment, which is not meant to be limiting in any manner, it is also contemplated that the subject has not yet been diagnosed with schizophrenia or schizoaffective disorder before the method as described herein is performed.

[0014] According to a further embodiment, there is provided a method as described above wherein the one or more polymorphisms in the *GABRA2* gene are relative to:

a) rs16859227

CCTTGGTTTTATACAAGCATGCAAAG**[C/T]**ATATAATAGAATCACATGGAAACAA
(SEQ ID NO:1), or

b) rs279858

5 ATTGTCATATTATGAGCTACTGATTT**[T/C]**TTCCCATTGTGAAAAAAGGTATCTG
(SEQ ID NO:2);

wherein the polymorphic site is in brackets, underlined and in bold.

[0015] In a further embodiment, there is provided a method as described above, wherein at least one of the polymorphisms is defined by SEQ ID NO:1 or a variant or fragment thereof
10 comprising the polymorphic site. As indicated previously, the method also may be practiced by determining the presence or absence of the complement of the nucleotide sequence defined by SEQ ID NO:1 including the complement of the polymorphic site.

[0016] In a further embodiment, there is provided a method as described above, wherein at least one of the polymorphisms is defined by SEQ ID NO:2 or a variant or fragment thereof
15 comprising the polymorphic site. As indicated previously, the method also may be practiced by determining the presence or absence of the complement of the nucleotide sequence defined by SEQ ID NO:2 including the complement of the polymorphic site.

[0017] Also provided is a method as defined above, wherein the presence of the C allele (C/C genotype) of the rs16859227 polymorphism (SEQ ID NO:1) is associated with a higher
20 percentage weight gain in subjects. Also provided is a method as defined above, wherein the presence of two copies of the T allele (T/T genotype) of the rs279858 polymorphism (SEQ ID NO: 2) is associated with a higher percentage weight gain in subjects.

[0018] Also provided is a method as described above, wherein the sample is a blood sample.

[0019] Further provided is a kit comprising one or more of the following:

25 a) one or more primers to amplify a nucleotide sequence that comprises the polymorphism as defined in SEQ ID NOs:1-9, or a combination thereof;

b) one or more probes that hybridize to any one of SEQ ID NOs:1-9, over a region of nucleotides comprising the polymorphic site, wherein said probe hybridizes to a particular variant of the polymorphisms shown at the polymorphic site. Without wishing to be limiting in any manner, the probes may be labeled with an appropriate group, for example, a fluorescent tag, fluorophore, radioactive label or the like. Further, the one or more probes may be attached covalently or physically associated with a support for example, but not limited to a biochip, array, slide, multiwell plate, bead or the like. In an embodiment, which is not meant to be limiting in any manner, the probes may comprise an array of nucleic acids.

c) one or more reagents and/or products including, but not limited to, one or more buffers for performing PCR or probe hybridization, or any step in such as process as would be known to a person of skill in the art, one or more DNA amplifying enzymes, or any combination thereof;

d) one or more reagents, components and products for genotyping the polymorphisms as described herein, including, but not limited to those used in exonuclease assays, nucleotide sequencing, or any combination thereof;

e) one or more reagents, components or products for performing a DNA sequencing reaction that determines the sequence of a nucleotide sequence comprising any one of SEQ ID NOs: 1-9 or a combination thereof, and;

f) one or more sets of instructions for using the components as described herein, practicing the methods of the present invention as described herein, interpreting the data obtained from practicing the methods of the present invention or any combination thereof.

[0020] This summary of the invention does not necessarily describe all features of the invention.

DETAILED DESCRIPTION

[0021] The following description is of an illustrative embodiment.

[0022] The present invention provides genetic markers that can be used to predict a subject's susceptibility to weight change in response to antipsychotic drug therapy. As described in more detail below, specific polymorphisms in the *GABRA2* gene may be used to predict a subject's weight change in response to antipsychotic drug therapy. In a second embodiment, specific polymorphisms in the *GABRA2* gene may be used to assist in determining a treatment regimen for a subject diagnosed with schizophrenia or for a subject likely of developing schizophrenia. In a third embodiment, specific polymorphisms in the *GABRA2* gene may be used in treating a schizophrenic subject. In a fourth embodiment, there is provided a method of treating a subject with antipsychotic medication, wherein the method comprises identifying one or more specific polymorphisms in the *GABRA2* gene as part of the treatment regimen. Other embodiments are also provided as described herein.

[0023] The study described in the examples and as referred to herein and throughout investigated the effect of single nucleotide polymorphisms (SNPs) across the *GABRA2* gene on weight response to antipsychotic medication in multiple distinct schizophrenic populations. The subjects included 160 patients of European ancestry with DSM-III-R/IV diagnoses of schizophrenia or schizoaffective disorder. Results indicate that the T/T genotype of the rs279858 marker was associated with a higher percent weight change than the C-allele carrying genotypes (for example, either the T/C or C/C genotypes). The rs16859227 marker was also significantly associated with higher percent weight change in a subsample of schizophrenia or schizoaffective disorder subjects who were on clozapine or olanzapine medication. Results indicate that the C/C genotype of the rs16859227 marker was associated with a higher percent weight change than the T-allele carrying genotypes (for example, either the T/T or T/C genotypes). Other interesting results are also provided herein, particularly Tables 1 and 2.

[0024] According to an embodiment of the present invention, there is provided a method of predicting a subject's weight change in response to antipsychotic drug treatment comprising,

- a) obtaining a biological sample comprising genomic DNA from the subject;

b) determining the presence or absence of one or more polymorphisms in the *GABRA2* gene of the subject, wherein the presence of said one or more polymorphisms is predictive of the subject's susceptibility to weight change in response to antipsychotic drug treatment.

5 [0025] In a further embodiment, which is not meant to be limiting in any manner, the method may comprise one or more additional steps, for example, but not limited to advising and/or counseling the subject with respect to the results of determining the presence or absence of one or more polymorphisms in the *GABRA2* gene; transmitting, advising and/or conveying the results to a physician, medical service provider or other third party; treating the subject with one or
10 more particular antipsychotic treatment(s) based on the results; treating the subject prior to, concurrently with or after antipsychotic treatment with one or more therapies to control weight gain; monitoring the subject's weight over a period of time, monitoring the subject for metabolic syndrome or the development of metabolic syndrome which may include measuring blood lipid profiles, including triglycerol and triglycerides, blood glucose levels, body mass index (BMI)
15 and central obesity. As cardiovascular disease may result from metabolic syndrome, clinicians may also monitor for the development of heart disease. The following symptoms of heart disease may be monitored including elevated blood pressure, angina, heart failure, shortness of breath, rapid or irregular pulse, coughing and nausea, or any combination of the above. Based on the test, if for example a SCZ subject exhibits the T/T genotype for the rs279858 marker, more
20 frequent weight monitoring as well as the administration of an appetite suppressant or hypoglycemic drug, for example, but not limited to a sulfonylurea, thiazolidinedione, alpha glucosidase inhibitor, or metformin, a diet plan, an exercise regime, or their combinations in addition to antipsychotic medication may be recommended. Also, from the results provided, subjects exhibiting the T/T genotype for the rs279858 marker preferably are not treated with
25 second generation antipsychotics (especially those with higher propensity for weight gain: for example, clozapine, olanzapine) but should rather be treated with antipsychotics with lower propensity for weight gain³⁰ (including fluphenazine, aripiprazole, ziprasidone, haloperidol, loxapine, lurasidone, iloperidone, asenapine and molindone).

[0026] Thus, based on the genotype of the patient, a physician may wish to avoid the
30 prescription of antipsychotics that cause high or the highest level of weight gain, these include:

olanzapine and clozapine. Moderate risk medications such as paliperidone, perphenazine, thioridazine, chlorpromazine, risperidone and quetiapine may be prescribed with more frequent monitoring of metabolic syndrome and heart disease indices. Lastly, a physician may wish to choose a lower risk drug for induced weight gain, these drugs include: loxapine, iloperidone, asenapine, lurasidone, ziprasidone, aripiprazole, fluphenazine, and haloperidol.

[0027] As described above, but without wishing to be considered limiting, specific polymorphisms in the *GABRA2* gene may be used to assist in determining a treatment regimen for a subject diagnosed with schizophrenia (or schizoaffective disorder) or likely of developing schizophrenia (or schizoaffective disorder). For example, but not wishing to be considered limiting in any manner, the present invention provides a method of determining a treatment regimen for a subject diagnosed with schizophrenia or likely of developing schizophrenia comprising,

a) obtaining a biological sample comprising genomic DNA from the subject;

b) determining the presence or absence of one or more polymorphisms in the *GABRA2* gene of the subject, wherein the presence of said one or more polymorphisms is predictive of the subject's weight change in response to antipsychotic drug treatment, wherein

the presence of one or more *GABRA2* polymorphisms as described herein and/or the absence of one or more *GABRA2* polymorphisms as described herein define a treatment regimen for the subject.

[0028] In such an embodiment, the method may further comprise a step of treating the subject as described above, below or anywhere herein.

[0029] Further, as described above, specific polymorphisms in the *GABRA2* gene may be used in treating a schizophrenic subject or how to treat a subject that may be predisposed to schizophrenia. In such an embodiment, the present invention provides a method of treating a schizophrenic subject or a subject that may be predisposed to schizophrenia comprising,

a) obtaining a biological sample comprising genomic DNA from the subject;

b) determining the presence or absence of one or more polymorphisms in the *GABRA2* gene of the subject, wherein the presence of said one or more polymorphisms is predictive of the subject's weight change in response to antipsychotic drug treatment, wherein

5 the presence of one or more *GABRA2* polymorphisms as described herein and/or the absence of one or more *GABRA2* polymorphisms as described herein define a treatment regimen for the subject.

[0030] In such an embodiment, the method may further comprise a step of treating the subject as described above or anywhere herein.

10 [0031] By the term "one or more polymorphisms in the *GABRA2* gene" it is meant one or more polymorphisms in the nucleotide sequences as defined by:

a) rs16859227

CCTTGGTTTTATACAAGCATGCAAAG[C/T]ATATAATAGAATCACATGGAAACAA
(SEQ ID NO:1)

15 b) rs279858

ATTGTCATATTATGAGCTACTGATTT[T/C]TTCCCATTTGTGAAAAAAGGTATCTG
(SEQ ID NO:2)

c) rs1442060

20 GTAAAGTGTACATCAATGCCATATC[A/G]TATTCTGTAGATGGCATGTTATCAT
(SEQ ID NO:3),

d) rs3849591

CTCATTTCCCTTGCTTCTAAGGTAGGG[G/T]TCATCAATTTATCTATCTCATGGGA
(SEQ ID NO:4),

e) rs1442062

25 GAGAAGGTGAAATAGATTTAACAT[A/G]TATCAAATTAAGATTGCACCTTAAA
(SEQ ID NO:5),

f) rs16859354

TACAATATCTTGACTCAATGAGCTTC[G/T]AATCTTAATAAGGTAACAAGAGAAA
(SEQ ID NO:6),

30 g) rs11503014

AAGCTATGGAGATTACTTCCTGGACT[C/G]TGTGTAGGACTTGATGATTGAGAGA
(SEQ ID NO:7),

h) rs6856130

TCTGTTCTGTTTTATCTGAGGCGATA**A/G**AATCCAAACGTGCAACTTGAACAAC
(SEQ ID NO:8), or

i) rs1372472

5 ATAAAACTCTGGTAATTCAAACCAAA**A/T**ATTTCCTCACTGAAAACCTATGCTTG
(SEQ ID NO:9)

wherein the polymorphic site in each sequence is shown in bold, underlined brackets in relation to the nucleotide sequences upstream and downstream thereof. In a particularly preferred embodiment, one or more polymorphisms in the *GABRA2* gene comprises rs16859227, rs279858
10 or both. As indicated previously, the invention also may be practiced by determining the presence or absence of the complement of the nucleotide sequence defined by the SEQ ID NOs noted above, including the complement of the polymorphic site.

[0032] The present invention also contemplates one or more polymorphisms in one or more nucleotide sequences in the *GABRA2* gene which comprises between about 90% and 100%
15 sequence identity, for example, but not limited to 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% or 100% sequence identity with SEQ ID NOs:1-9, preferably SEQ ID NOs: 1, 2 or both SEQ ID NO:1 and SEQ ID NO: 2, and wherein the sequence also comprises the respective polymorphism as shown above in bold underlined brackets. For example, but not to be considered limiting in any manner, the first nucleotide shown in SEQ ID NO:1 is a “C”.
20 The present invention is meant to include a sequence that is substantially identical to SEQ ID NO:1 but that comprises, for example, but not limited to, an “A”, “G” or “T” at position number 1, as the variant nucleotide sequence exhibits more than 90% sequence identity with SEQ ID NO:1 and comprises the polymorphism shown in bold underlined brackets. The invention also may be practiced by determining the presence or absence of the complement of the nucleotide
25 sequence defined by the SEQ ID NOs noted above, including the complement of the polymorphic site.

[0033] To determine whether a nucleic acid exhibits similarity or a percentage identity with the sequences presented herein, oligonucleotide alignment algorithms may be used, for example, but not limited to a BLAST (GenBank URL: www.ncbi.nlm.nih.gov/cgi-bin/BLAST/, using default
30 parameters: Program: blastn; Database: nr; Expect 10; filter: default; Alignment: pairwise; Query genetic Codes: Standard(1)), BLAST2 (EMBL URL: <http://www.embl-heidelberg.de/Services/>

index.html using default parameters: Matrix BLOSUM62; Filter: default, echofilter: on, Expect:10, cutoff: default; Strand: both; Descriptions: 50, Alignments: 50), or FASTA, search, using default parameters. Polypeptide alignment algorithms are also available, for example, without limitation, BLAST 2 Sequences (www.ncbi.nlm.nih.gov/blast/bl2seq/bl2.html, using
5 default parameters Program: blastp; Matrix: BLOSUM62; Open gap (11) and extension gap (1) penalties; gap x_dropoff: 50; Expect 10; Word size: 3; filter: default).

[0034] An alternative indication that two nucleic acid sequences are substantially complementary to each other is that the two sequences hybridize to each other under moderately stringent, or preferably stringent, conditions. Hybridization to filter-bound sequences under
10 moderately stringent conditions may, for example, be performed in 0.5 M NaHPO₄, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65°C, and washing in 0.2 x SSC/0.1% SDS at 42°C for at least 1 hour (see Ausubel, et al. (eds), 1989, Current Protocols in Molecular Biology, Vol. 1, Green Publishing Associates, Inc., and John Wiley & Sons, Inc., New York, at p. 2.10.3). Alternatively, hybridization to filter-bound sequences under stringent conditions may, for
15 example, be performed in 0.5 M NaHPO₄, 7% SDS, 1 mM EDTA at 65°C, and washing in 0.1 x SSC/0.1% SDS at 68° C for at least 1 hour. Hybridization conditions may be modified in accordance with known methods depending on the sequence of interest (see Tijssen, 1993, Laboratory Techniques in Biochemistry and Molecular Biology -- Hybridization with Nucleic Acid Probes, Part I, Chapter 2 "Overview of principles of hybridization and the strategy of
20 nucleic acid probe assays", Elsevier, New York). Generally, but not wishing to be limiting, stringent conditions are selected to be about 5°C lower than the thermal melting point for the specific sequence at a defined ionic strength and pH. The present invention also contemplates nucleotide sequences which hybridize to a nucleotide sequence comprising or consisting of SEQ ID NO:1-9, preferably SEQ ID NOs:1-2 under stringent hybridization conditions.

[0035] In a preferred embodiment, the presence of a particular allele at the polymorphic site, for example, but not limited to as provided by SEQ ID NOs: 1-2 is determined in relation to the adjacent nucleotide sequence upstream and downstream from the polymorphic site, for example, but not limited to, about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 nucleotides upstream and/or about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 nucleotides downstream of the polymorphic site.
30 However, the present invention also contemplates that the presence of a particular allele may be

determined in relation to the nucleotide sequence comprising about 20, 25, 30, 50 or more nucleotides upstream (or any number therein between) and about 20, 25, 30, 50 and/or more nucleotides downstream (or any number therein between) of the polymorphic site as provided by SEQ ID NOs: 1-9, more preferably SEQ ID NOs: 1-2, respectively. The term “and/or” is used to specifically indicate that the number of continuous upstream and downstream nucleotides does not need to be the same. Other means and methods of comparing nucleotide sequences to determine if a particular polymorphism or group of polymorphisms is present in a subject, as would be known to a person of skill in the art may be employed in the practice of the present invention.

[0036] By the term “predicting a subject’s weight change in response” it is meant predicting if the subject is likely to gain weight with antipsychotic treatment in general, or with particular antipsychotic treatment, for example, but not limited to antipsychotics including clozapine and olanzapine.

[0037] In an embodiment of the present invention, but without wishing to be limiting in any manner, the method as described herein may be employed to determine a subject's weight change in response to antipsychotic medication, wherein at the time of screening the subject appears healthy. This information may be important when screening subjects that have a familial history of schizophrenia or other disorders with schizophrenic or psychotic symptoms, even though at the time of screening, the subject may have little or no symptoms of disease. Knowledge of how a subject is likely to respond to antipsychotic medication may be useful in developing treatment regimens if for example, the subject later develops schizophrenia or psychotic symptoms and requires treatment.

[0038] In an embodiment of the present invention, subjects from any ethnic race, age, gender or medical condition may be tested or screened to predict the subject’s weight change in response to antipsychotic drug treatment. In this regard, a healthy subject or a subject that does not have any symptoms of a disease or medical condition may be tested to determine weight change in response to antipsychotic medication. In this way, if treatment is ever needed, a proper drug and/or treatment regimen may be selected and/or administered to the subject. In a preferred embodiment, a subject diagnosed with a disorder with one or more psychotic symptoms,

schizophrenia, or schizoaffective disorder is tested to predict weight change in response to antipsychotic drug therapy, for example, but not limited to treatment with clozapine, olanzapine, risperidone, quetiapine, haloperidol, perphenazine, thioridazine, ziprasidone, aripiprazole, chlorpromazine, amisulpride, fluphenazine, molindone, loxapine, paliperidone, iloperidone, asenapine, lurasidone, or a combination thereof.

[0039] As described above, but without wishing to be limiting in any manner, the subject is diagnosed with schizophrenia or schizoaffective disorder. However, the subject that is tested may comprise an individual with one or more psychotic symptoms, schizophrenia symptoms, schizoaffective disorder symptoms or a combination thereof, for example, but not limited to as described in DSM-IV which is hereby incorporated by reference. The psychotic symptoms may comprise positive symptoms such as, but not limited to distortions or exaggerations of inferential thinking (i.e. delusions), perception (i.e. hallucinations), language and communication (disorganized speech) and behavioral monitoring (grossly disorganized or catatonic behavior) or any combination thereof. Further, the positive symptoms may comprise distinct dimensions, for example, psychotic dimensions including, but not limited to delusions and hallucinations and disorganization dimensions including, but not limited to disorganized speech and behavior. As described previously, it is also contemplated that the symptoms may comprise one or more negative symptoms, for example, but not limited to symptoms that reflect a diminution or loss of normal function (including but not limited to, loss of motivation, loss of social interest, loss of communication, or a combination thereof). Further, the subject may exhibit a combination of both positive and negative symptoms. In an embodiment of the invention, the subject that is tested has been diagnosed or is suspected of having schizophrenia or schizoaffective disorder.

[0040] Any human tissue or sample providing genomic DNA may be used for genotyping *GABRA2* polymorphisms, including but not limited to, blood, saliva, hair, spinal fluid, brain biopsy, cultured cells obtained from the subject, stool, urine, autopsy samples, or frozen sections taken for histological purposes. In certain examples, blood is obtained from a subject for assaying with respect to *GABRA2* polymorphisms. As an example, but without wishing to be limiting in any manner, venous blood is obtained from a subject using standard venipuncture techniques.

[0041] The DNA of the subject may be tested for the presence or absence of the single nucleotide polymorphisms (SNPs) by any suitable technique known in the art. Representative techniques that may be employed include without limitation PCR analysis, sequencing, 5' exonuclease fluorescence assay, probe hybridization or a combination thereof.

5 [0042] Polymorphisms may be genotyped using conventional techniques. For example, PCR using primers incorporating fluorescent probes is one suitable technique. Further, but not wishing to be considered limiting, primers having appropriate sequences upstream and downstream of the polymorphic site may be used to amplify the nucleotide regions comprising the polymorphisms.

10 [0043] Single nucleotide polymorphism (SNP) analysis is useful for detecting differences between alleles of the *GABRA2* gene. As described above, various methods exist in the art for genotyping nucleotide sequences including, but not limited to 5' exonuclease assays, sequencing, and the like. All such methods are meant to be encompassed herein. Further, various real-time PCR methods that can be used to detect SNPs, including, e.g., Taqman or molecular beacon-
15 based assays (U.S. Pat. Nos. 5,210,015; 5,487,972; and PCT WO 95/13399) are useful to monitor for the presence or absence of a SNP. Still other SNP detection methods are known in the art, including, without limitation, DNA sequencing, sequencing by hybridization, dot blotting, oligonucleotide array (DNA Chip) hybridization analysis.

[0044] Applied Biosystems, Inc (Foster City, CA) has developed several aspects of SNP
20 genotyping technology. In one well-used protocol, PCR amplification of a desired SNP region is conducted using targeting primers, including two allele-specific fluorogenic probes, each consisting of a different fluorescent reporter dye and a fluorescent quencher. Prior to PCR, proximity of the quencher to the fluorophore causes fluorescence resonance energy transfer (FRET), reducing the fluorescence from the reporter dye. During PCR, the 5' nuclease activity of
25 Taq digests the allele-specific probe bound to the region of the SNP, releasing the fluorescent dye from the quencher and allowing generation of a fluorescence signal.

[0045] The method of obtaining a sample and analyzing its DNA is not critical to the present invention and any methods may be used (e.g. Ausubel, et al. (eds), 1989, Current Protocols in Molecular Biology, Green Publishing Associates, Inc., and John Wiley & Sons, Inc., New York,

at p. 2.10.3, or Maniatis et al., in Molecular Cloning (A Laboratory Manual), Cold Spring Harbor Laboratory, 1982, p. 387 389). For example, which is not to be considered limiting in any manner, DNA may be extracted using a non-enzymatic high-salt procedure. Alternatively, the DNA may be analyzed *in situ* or present in bodily fluids and or tissues. Other methods of DNA analysis that are known to persons skilled in the art may also be used.

[0046] Several scientific collaborations have attempted to identify and/or classify SNPs for genomes of several species including *Homo sapiens*, *Arabidopsis thaliana*, *Caenorhabditis elegans*, *Ficedula albicollis*, *Ficedula hypoleuca*, *Gallus gallus*, *Mus musculus*, *Pan troglodytes*, *Plasmodium falciparum*, and *Rattus norvegicus*. For example, the HapMap project attempts to determine the common patterns of human DNA sequence variation (haplotypes). SNP genotypes, recombination rates and other types of information may be browsed at or downloaded from the HapMap website (www.hapmap.org). SNPs are typically identified by location within a nucleotide sequence, or by a database assigned reference SNP ID number (“rs” number). In addition to HapMap, SNPs may be searched using various other resources. For example, individual rs numbers of the SNPs that are known to be located in a sequence of interest may be obtained by conducting a Blast search at the UCSC Genome Bioinformatics Web Page (www.genome.ucsc.edu). Conversely, sequence and scientific literature information associated with a given rs number may be obtained by searching the dbSNP of the Entrez SNP search option provided by the NCBI web page (www.ncbi.nlm.nih.gov).

[0047] In an embodiment of the present invention, which is not meant to be considered limiting, there is provided a method of predicting a subject’s weight change in response to antipsychotic drug treatment comprising,

a) obtaining a biological sample from the subject;

b) determining the presence or absence of one or more polymorphisms in SEQ ID NO:1, SEQ ID NO:2, or a combination thereof, wherein,

for patients of European ancestry treated with clozapine or olanzapine, the presence of the C/C genotype of the rs16859227 polymorphism (SEQ ID NO:1) is associated with a higher percentage weight gain in subjects, and;

the presence of the T/T genotype of the rs279858 polymorphism (SEQ ID NO: 2) is associated with a higher percentage weight gain in subjects,

[0048] The present invention also contemplates products and kits for practicing the methods of the present invention. For example, a kit may comprise:

- 5 a) one or more primers to amplify a nucleotide sequence that comprises the polymorphism as defined in any one of SEQ ID NOs:1-9, preferably including SEQ ID NO 1 or 2, or a combination thereof;
- b) one or more probes that hybridize to any one of SEQ ID NOs:1-9, preferably including SEQ ID NO:1 or 2, or both SEQ ID NO:1 and SEQ ID NO:2 over a region of nucleotides comprising
10 the polymorphic site, wherein said probe hybridizes to a particular variant of the polymorphisms shown at the polymorphic site. Without wishing to be limiting in any manner, the probes may be labeled with an appropriate group, for example, a fluorescent tag, fluorophore, radioactive label or the like. Further, the one or more probes may be attached covalently or physically associated with a support for example, but not limited to a biochip, array, slide, multiwell plate, bead or the
15 like. In an embodiment, which is not meant to be limiting in any manner, the probes may comprise an array of nucleic acids.
- c) one or more reagents and/or products including, but not limited to, one or more buffers for performing PCR or probe hybridization, or any step in such a process as would be known to a person of skill in the art, one or more DNA amplifying enzymes, or any combination thereof;
- 20 d) one or more reagents, components and products for genotyping the polymorphisms as described herein, including, but not limited to those used in exonuclease assays, nucleotide sequencing, or any combination thereof;
- e) one or more reagents, components or products for performing a DNA sequencing reaction that determines the sequence of a nucleotide sequence comprising any one of SEQ ID NOs: 1-9,
25 preferably including SEQ ID NO:1 or 2, or both 1 and 2, or a combination thereof,

f) a gene chip or array comprising a plurality of nucleotide sequences comprising or consisting of SEQ ID NOs:1-9, preferably 1 and 2, preferably comprising nucleotide sequences only within the *GABRA2* gene, and;

g) one or more sets of instructions for using the components as described herein, practicing the methods of the present invention as described herein, interpreting the data obtained from practicing the methods of the present invention or;

h) any combination thereof.

[0049] Also provided by the present invention are individual components of the kit, for example, but not limited to any product, composition described in the kit or elsewhere in the application.

In a representative embodiment, the present invention provides one or more nucleic acid primers or probes.

[0050] The nucleic acid primers and probes may be of any suitable length for use in the methods of the present invention. Without wishing to be limiting in any manner, it is generally preferred that the primers and probes be between about 9 and about 100 nucleotides, for example, but not limited to about 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 23, 25, 27, 29, 30, 35, 40, 45, 50, 60, 70, 80, 90, about 100 nucleotides or any amount therein between. The length of the primers and probes may also be defined by a range of any two of the values provided above or any two values therein between. With respect to probes, it is generally preferred that the probe comprise at least one, more preferably 3 or more nucleotides on each side of the polymorphic site. It is also contemplated that one or more of the primers or nucleic acid probes may be labeled as is known in the art, for example, but not limited to, with a radioactive element or tag, fluorophore, or the like.

[0051] Also provided by the present invention is a microarray, gene chip or the like which comprises one or more nucleotide sequence(s) defined by SEQ ID NOs 1-9 or a fragment thereof which comprises the polymorphic site. Preferably the microarray or gene chip comprises nucleotide sequences defined by SEQ ID NOs:1, 2 or both 1 and 2. The microarray also may comprise the complement of the nucleotide sequences or a fragment thereof which comprises the polymorphic site. Preferably, the nucleotide sequences are of a length such as, but not limited to

7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 or more continuous nucleotides to permit strong hybridization under stringent hybridization conditions. In a preferred embodiment the microarray comprises or consists of one or more nucleotide sequences comprising polymorphic sites from the *GABRA2* gene as described herein. However, the microarray may comprise additional nucleotide sequences for other genes, for example, but not limited to those involved or implicated in the diagnosis or development of schizophrenia, schizoaffective disorder or the like.

[0052] The present invention will be further illustrated in the following examples.

Examples

[0053] **Clinical Diagnostic Criteria.** In total, 160 participants with psychotic symptoms were included in this study. Diagnosis for schizophrenia (SCZ) was assessed by the Structured Diagnostic Interviews for DSM-III-R and/or DSM-IV diagnoses (SCID-I, ^{31,32}), except for sample A where diagnoses were based on an interview assessing both DSM and ICD diagnoses. The inclusion criteria for adult probands were DSM-III-R/IV diagnosis of SCZ or schizoaffective disorder, with psychotic symptoms. A written informed consent was obtained after the complete study description was given to each participant, and the study has been approved by the Research Ethics Board. All subjects were self-reported as European Caucasians, and 92 of them were prescribed clozapine or olanzapine during this study period.

[0054] **Subjects:** Clinical and demographic variables for the total sample of European SCZ patients (N = 160) are listed in Table 1. **Sample A** (N = 93) was collected at the Charité University Medicine, Berlin, Germany. Patients 18–60 years old diagnosed with SCZ or schizoaffective disorder according to DSM-IV and ICD-10 criteria were included. This group of patients were treated with at least one of the following medications: clozapine, haloperidol, olanzapine, risperidone, fluphenazine, aripiprazole, quetiapine, ziprasidone, and/or amisulpride (more details have been described elsewhere; ³³). Patients from **Sample B** (N = 56) were recruited from Case Western Reserve University in Cleveland, Ohio or Hillside Hospital in Glen Oaks, New York. These patients received clozapine for treatment-refractoriness or intolerance to typical antipsychotic therapy according to criteria described elsewhere ³⁴. Clozapine serum levels were monitored during the course of the treatment to ascertain

compliance. Clinical response was assessed after 6 weeks using the Brief Psychiatric Rating Scale (BPRS)³⁵. Sample characterization has been described elsewhere³⁶. **Sample C** (N = 11) consists of inpatients who showed sub-optimal response to previous treatment, primarily defined by persistent positive symptoms and a poor level of functioning over the past two years. These participants were recruited at four psychiatric state hospitals (two in New York and two in North Carolina) and were randomly assigned to either clozapine or olanzapine in a 14-week, double-blinded study. Detailed clinical description of inclusion criteria, dosing schedules, assessment methods, and principal results describing antipsychotic efficacy was published elsewhere³⁷.

[0055] **Genotyping.** Venous blood was drawn from the probands in two 10cc EDTA tubes, and genomic DNA was extracted from blood lymphocytes using a high salt method³⁸. We selected single-nucleotide polymorphisms (SNPs) based on the minimum minor allele frequency of 0.20 using HapMap genotypes (Rel 28 Phase II+III, August10, on NCBI B36 assembly, dbSNP b126; URL: <http://hapmap.ncbi.nlm.nih.gov>). Specific SNPs were force-included based on previous studies. The SNPs rs279828³⁹⁻⁴², rs573400^{39, 42, 43}, rs11503014⁴³, rs279858 (Lys132Lys)^{40, 43-46}, rs16859227⁴³, and rs1372472⁴⁰ have been studied for possible association with alcoholism, nicotine dependence, and autism. The rs279871 marker has been associated with medial frontal brain activity in response to alcohol cue⁴⁷. Overall, the twelve genotyped markers would provide more than 99% coverage of common variations within and 10kb upstream and downstream of the *GABRA2* gene. We narrowed the number of analyzed SNPs to nine, because the rs279858 genotypes were highly correlated to genotypes of the rs573400, rs279871, and rs279828 markers in our sample ($r^2 > 0.80$).

[0056] **Statistical Analyses.** Statistical analyses of demographic variables, which included sex, age at recruitment, and duration of treatment, were performed across samples using Fisher's Exact tests, analysis of variance, or Kruskal-Wallis tests (Table 1). In terms of genetic analyses, the quantitative variable 'percent weight change' was analyzed using ANCOVA, with sex, treatment duration, and clozapine/olanzapine (yes/no) being included as covariates. We also analyzed the 'percent weight change' variable in a meta-analytic approach to take into account heterogeneity across the three patient sample groups using STATA version 8 (e.g.,⁴⁸). Analyses were done with all 160 patients with available clinical/weight data, as well as secondarily with the 92 patients receiving clozapine or olanzapine, the two antipsychotics with the highest

propensity for significant weight gain. Linkage disequilibrium and r^2 between marker pairs as determined by Haploview 4.1⁴⁹. We also performed haplotype analysis with covariates using UNPHASED version 3.1.5⁵⁰. We further performed an additional haplotype analysis using reconstructed haplotypes for each individual with PHASE⁵¹. Based on genotypic correlation among the tested SNPs, the effective number of independent markers was determined to be six; thus, we adjusted the significance threshold for multiple testing in the present study to 0.0085⁵².

RESULTS:

[0057] Table 2 presents the results from analyses of the percent weight change in antipsychotic-medicated SCZ patients of European ancestry. Genotype distributions did not deviate significantly from Hardy-Weinberg Equilibrium.

[0058] The rs279858 marker was positively associated with percent weight gain from the ANCOVA ($p < 0.05$). More specifically, the T/T genotype was associated with higher percent weight change than the C-allele carrying genotypes (ANCOVA $p = 0.009$). From the meta-analytic approach, the rs279858 marker (T/T homozygotes versus C allele genotype carriers) was statistically significant ($z = 3.80$; $p = 1.4 \times 10^{-4}$). The rs1442062 marker was also significant from the meta analysis, with the A-allele carriers being associated with less weight gain than the G/G homozygotes ($z = 5.55$; $p = 2.86 \times 10^{-8}$).

[0059] Regarding haplotypic analysis, we found a number of significant haplotypes using UNPHASED. The two-marker haplotype window across rs16859227 and rs279858 was significant ($p = 0.045$), with the C-T haplotype associated with higher percent weight change ($p = 0.015$; Estimated Additive Value: 0.057 [95% confidence interval: 0.011 to 0.103]). The two-marker haplotype window across rs279858 and rs1442060 was also significant ($p = 0.014$), with the T-A haplotype associated with higher percent weight change ($p = 0.014$; Estimated Additive Value: 0.070 [95% confidence interval: 0.014 to 0.126]) and the C-G haplotype associated with lower percent weight change ($p = 0.012$; Estimated Additive Value: -0.115 [95% confidence interval: -0.206 to -0.0232]). On an individual level, patients with at least one copy of the (rs279858-rs1442060) T-A haplotype appeared to experience higher percentage weight gain ($p = 0.008$; $b = 2.47 \pm 0.92$), and patients with at least one copy of the (rs279858-rs1442060) C-G haplotype appeared to experience lower percentage weight gain ($p = 0.017$; $b = -2.92 \pm 1.21$).

[0060] For patients treated with clozapine or olanzapine, the results with rs279858 were significant (ANCOVA $p=0.011$); these findings were similar to those from the overall sample. The meta-analysis of rs279858 across the three recruitment sites yielded statistically significant findings ($z=6.71$; $p=1.95 \times 10^{-11}$) that were more significant than those from the overall sample.

5 The *GABRA2* marker rs16859227 was also positive from the meta analysis ($z=9.36$; $p=7.97 \times 10^{-21}$), with the T-allele carriers associated with lower weight gain than C/C genotype carriers. Similarly, the rs1442062 A-allele carriers gained less weight on average than G/G homozygotes ($z=5.79$; $p=7.04 \times 10^{-9}$). Carriers of at least one copy of the G allele at rs11503014 gained more weight than C/C homozygotes ($z=2.10$; $p=0.036$), rs6856130 A/A homozygotes gained less weight than G-allele carriers ($z=2.20$; $p=0.028$), and rs1372472 T-allele carriers gained less weight than A/A genotype carriers ($z=3.32$; $p=9.0 \times 10^{-4}$).

[0061] Of all the single-marker tests, the rs279858 marker appeared to be the most consistently associated, with the T/T genotype being associated with higher percent weight gain. The two-marker haplotype window across rs16859227 and rs279858 was significant ($p=0.019$), with the C-T haplotype associated with higher percent weight change ($p=0.011$; Estimated Additive Value: 0.076 [95% confidence interval: 0.016 to 0.135]) and the T-C haplotype associated with lower percent weight change ($p=0.010$; Estimated Additive Value: -0.089 [95% confidence interval: -0.158 to -0.019]). The two-marker haplotype window across rs279858 and rs1442060 was nominally significant ($p=0.034$), with the T-A haplotype associated with higher percent weight change ($p=0.031$; Estimated Additive Value: 0.075 [95% confidence interval: 0.0057 to 0.145]). On an individual level, patients with at least one copy of the (rs16859227-rs279858) C-T haplotype appeared to experience higher percentage weight gain ($p=0.012$; $b=4.45 \pm 1.74$). Patients with at least one copy of the (rs279858-rs1442060) T-A haplotype appeared to experience higher percentage weight gain ($p=0.005$; $b=3.75 \pm 1.70$).

25

[0062] Table 1. Demographic information for the study sample of European ancestry.

| Samples | A(N=93) | B(N=56) | C(N=11) | p-value |
|-------------------------------------|-------------|------------|-------------|---------|
| Males/Females ^d | 56/37 | 35/21 | 11/0 | 0.037 |
| Age ^a | 32.14±11.98 | 33.37±7.45 | 42.15±4.83 | 0.044 |
| Study duration (weeks) ^a | 5.10±1.547 | 6.00±0.00 | 10.55±4.180 | <0.001 |

| | | | | |
|---------------------------------------|------------|------------|------------|-------|
| Percentage weight change ^c | 4.00±4.680 | 3.88±5.770 | 5.58±6.644 | 0.605 |
|---------------------------------------|------------|------------|------------|-------|

^c p-value from ANOVA.

^a p-value from Kruskal-Wallis tests.

^d p-values from Fisher's Exact Tests.

5 [0063] Table 2. The most significant findings from analysis of the nine *GABRA2* single-nucleotide polymorphisms (SNPs) in antipsychotic-induced weight gain in schizophrenia patients of European ancestry.

| SNP | Genotypes (test genotype(s) in bold) | Percentage weight change | Standard Deviation | Genotype <i>P</i> (all antipsychotics/ clozapine or olanzapine only) | <i>P</i> (all antipsychotics) Standardized Mean Difference (Confidence Interval) for rare allele-carrying genotypes | <i>P</i> (Clozapine/ Olanzapine only) Standardized Mean Difference (Confidence Interval) for rare allele-carrying genotypes |
|------------|--------------------------------------|--------------------------|----------------------|--|---|---|
| rs16859227 | T/T T/C C/C | 5.20 2.98 4.91 | 3.99 4.66 5.72 | 0.332/ 0.015 | 0.091 ^R -1.98 (-4.27, 0.32) | < 0.001 -3.01 (-3.64, -2.38) |
| rs279858 | T/T T/C C/C | 5.59 3.63 2.72 | 5.76 4.85 5.07 | 0.017/0.011 | < 0.001 ^R 2.18 (1.06, 3.31) | < 0.001 1.85 (1.31, 2.39) |
| rs1442060 | A/A A/G G/G | 4.25 4.48 2.95 | 5.76 5.26 4.62 | 0.236/0.516 | 0.939 ^R -0.091 (-2.42, 2.23) | 0.743 ^R 0.33 (-1.65, 2.32) |
| rs3849591 | T/T T/G G/G | 5.11 4.42 3.91 | 5.62 5.58 5.16 | 0.893/0.629 | 0.645 ^R 0.47 (-1.52, 2.45) | 0.530 ^R 0.65 (-1.38, 2.69) |
| rs1442062 | A/A A/G G/G | 5.60 3.32 4.56 | 5.87 5.04 5.39 | 0.578/0.630 | < 0.001 -0.97 (-1.31, -0.63) | < 0.001 -1.43 (-1.91, -0.95) |
| rs16859354 | T/T T/G G/G | 4.00 4.00 5.16 | 4.73 5.69 4.99 | 0.980/0.663 | 0.817 0.040 (-0.30, 0.38) | 0.899 0.0301 (-1.43, 0.50) |
| rs11503014 | G/G G/C C/C | 3.14 4.67 3.68 | 4.86 6.00 4.46 | 0.642/0.364 | 0.055 ^R 0.754 (-0.015, 1.52) | 0.036 0.48 (0.031, 0.93) |
| rs6856130 | A/A A/G G/G | 4.07 4.49 2.42 | 5.21 5.27 5.55 | 0.289/0.097 | 0.704 ^R -0.62 (-3.80, 2.56) | 0.028 ^R -1.28 (-2.43, -0.14) |
| rs1372472 | T/T | 5.71 | 5.42 | 0.385/0.651 | 0.409 ^R | < 0.001 |

| | | | | | | | | |
|--|-----|------|------|--|------------|----|-------|-----------|
| | T/A | 3.53 | 4.77 | | -0.55 | (- | -0.77 | (-1.23, - |
| | A/A | 4.32 | 5.69 | | 1.86,0.76) | | 0.32) | |

^c p-values from ANCOVA of percent weight change with sex, treatment duration, and clozapine/olanzapine (yes/no) as covariates.

^R random-effects model used.

[0064] The results provided suggest that variety of *GABRA2* SNPs may be employed as genetic markers for antipsychotic weight gain.

[0065] All citations are hereby incorporated by reference.

[0066] The present invention has been described with regard to one or more embodiments. However, it will be apparent to persons skilled in the art that a number of variations and modifications can be made without departing from the scope of the invention as defined in the claims.

[0067] REFERENCES:

1. Correll CU, Sheridan EM, DelBello MP. Antipsychotic and mood stabilizer efficacy and tolerability in pediatric and adult patients with bipolar I mania: a comparative analysis of acute, randomized, placebo-controlled trials. *Bipolar Disord* 2010; **12**(2): 116-141.
2. Komossa K, Depping AM, Gaudchau A, Kissling W, Leucht S. Second-generation antipsychotics for major depressive disorder and dysthymia. *Cochrane Database Syst Rev* 2010;(12): CD008121.
3. Spielmans GI, Berman MI, Linardatos E, Rosenlicht NZ, Perry A, Tsai AC. Adjunctive atypical antipsychotic treatment for major depressive disorder: a meta-analysis of depression, quality of life, and safety outcomes. *PLoS Med* 2013; **10**(3): e1001403.
4. Nurmi EL, Spilman SL, Whelan F, Scahill LL, Aman MG, McDougale CJ, *et al.* Moderation of antipsychotic-induced weight gain by energy balance gene variants in the RUPP autism network risperidone studies. *Transl Psychiatry* 2013; **3**: e274.
5. Zuddas A, Zanni R, Usala T. Second generation antipsychotics (SGAs) for non-psychotic disorders in children and adolescents: a review of the randomized controlled studies. *Eur Neuropsychopharmacol* 2011; **21**(8): 600-620.
6. Ballard C, Waite J. The effectiveness of atypical antipsychotics for the treatment of aggression and psychosis in Alzheimer's disease. *Cochrane Database Syst Rev* 2006;(1): CD003476.

7. Maher AR, Maglione M, Bagley S, Suttorp M, Hu JH, Ewing B, *et al.* Efficacy and comparative effectiveness of atypical antipsychotic medications for off-label uses in adults: a systematic review and meta-analysis. *JAMA* 2011; **306**(12): 1359-1369.
- 5 8. Maher AR, Theodore G. Summary of the comparative effectiveness review on off-label use of atypical antipsychotics. *J Manag Care Pharm* 2012; **18**(5 Suppl B): S1-20.
9. Arranz MJ, de Leon J. Pharmacogenetics and pharmacogenomics of schizophrenia: a review of last decade of research. *Mol Psychiatry* 2007; **12**(8): 707-747.
- 10 10. Muller DJ, Kennedy JL. Genetics of antipsychotic treatment emergent weight gain in schizophrenia. *Pharmacogenomics* 2006; **7**(6): 863-887.
11. Strange PG. Antipsychotic drugs: importance of dopamine receptors for mechanisms of therapeutic actions and side effects. *Pharmacol Rev* 2001; **53**(1): 119-133.
- 15 12. Vojvoda D, Grimmell K, Sernyak M, Mazure CM. Monozygotic twins concordant for response to clozapine. *Lancet* 1996; **347**(8993): 61.
- 20 13. Gebhardt S, Theisen FM, Haberhausen M, Heinzl-Gutenbrunner M, Wehmeier PM, Krieg JC, *et al.* Body weight gain induced by atypical antipsychotics: an extension of the monozygotic twin and sib pair study. *J Clin Pharm Ther* 2010; **35**(2): 207-211.
- 25 14. Lett TA, Wallace TJ, Chowdhury NI, Tiwari AK, Kennedy JL, Muller DJ. Pharmacogenetics of antipsychotic-induced weight gain: review and clinical implications. *Mol Psychiatry* 2011e.
- 30 15. Cone RD. Anatomy and regulation of the central melanocortin system. *Nat Neurosci* 2005; **8**(5): 571-578.
16. Hentges ST, Nishiyama M, Overstreet LS, Stenzel-Poore M, Williams JT, Low MJ. GABA release from proopiomelanocortin neurons. *J Neurosci* 2004; **24**(7): 1578-1583.
- 35 17. Wu Q, Palmiter RD. GABAergic signaling by AgRP neurons prevents anorexia via a melanocortin-independent mechanism. *Eur J Pharmacol* 2011; **660**(1): 21-27.
18. Tong Q, Ye CP, Jones JE, Elmquist JK, Lowell BB. Synaptic release of GABA by AgRP neurons is required for normal regulation of energy balance. *Nat Neurosci* 2008; **11**(9): 998-1000.
- 40 19. Soderpalm AH, Berridge KC. Food intake after diazepam, morphine or muscimol: microinjections in the nucleus accumbens shell. *Pharmacol Biochem Behav* 2000; **66**(2): 429-434.
- 45 20. Cooper SJ. Palatability-dependent appetite and benzodiazepines: new directions from the pharmacology of GABA(A) receptor subtypes. *Appetite* 2005; **44**(2): 133-150.

21. Duke AN, Platt DM, Cook JM, Huang S, Yin W, Mattingly BA, *et al.* Enhanced sucrose pellet consumption induced by benzodiazepine-type drugs in squirrel monkeys: role of GABAA receptor subtypes. *Psychopharmacology (Berl)* 2006; **187**(3): 321-330.
22. Ebenezer IS, Prabhaker M. The effects of intraperitoneal administration of the GABA(B) receptor agonist baclofen on food intake in CFLP and C57BL/6 mice. *Eur J Pharmacol* 2007; **569**(1-2): 90-93.
23. Willer CJ, Speliotes EK, Loos RJ, Li S, Lindgren CM, Heid IM, *et al.* Six new loci associated with body mass index highlight a neuronal influence on body weight regulation. *Nat Genet* 2009; **41**(1): 25-34.
24. Danovich L, Weinreb O, Youdim MB, Silver H. The involvement of GABA(A) receptor in the molecular mechanisms of combined selective serotonin reuptake inhibitor-antipsychotic treatment. *Int J Neuropsychopharmacol* 2011; **14**(2): 143-155.
25. Drew KL, O'Connor WT, Kehr J, Ungerstedt U. Regional specific effects of clozapine and haloperidol on GABA and dopamine release in rat basal ganglia. *Eur J Pharmacol* 1990; **187**(3): 385-397.
26. Vincent SL, Adamec E, Sorensen I, Benes FM. The effects of chronic haloperidol administration on GABA-immunoreactive axon terminals in rat medial prefrontal cortex. *Synapse* 1994; **17**(1): 26-35.
27. Marx CE, VanDoren MJ, Duncan GE, Lieberman JA, Morrow AL. Olanzapine and clozapine increase the GABAergic neuroactive steroid allopregnanolone in rodents. *Neuropsychopharmacology* 2003; **28**(1): 1-13.
28. Ugale RR, Hirani K, Morelli M, Chopde CT. Role of neuroactive steroid allopregnanolone in antipsychotic-like action of olanzapine in rodents. *Neuropsychopharmacology* 2004; **29**(9): 1597-1609.
29. Weston-Green K, Huang XF, Deng C. Alterations to melanocortinergic, GABAergic and cannabinoid neurotransmission associated with olanzapine-induced weight gain. *PLoS One* 2012; **7**(3): e33548.
30. De Hert M, Yu W, Detraux J, Sweers K, van Winkel R, Correll CU. Body weight and metabolic adverse effects of asenapine, iloperidone, lurasidone and paliperidone in the treatment of schizophrenia and bipolar disorder: a systematic review and exploratory meta-analysis. *CNS Drugs* 2012; **26**(9): 733-759.
31. Association AP. *Diagnostic and Statistical Manual of Mental Disorders. 4th ed. (DSM-IV)*. American Psychiatric Association: Washington, DC, 1994.

32. First MB, Gibbon M, Spitzer RL, Williams JBW. *Structured Clinical Interview for DSM-IV axis I disorders-Research Version (SCID-I/P, version 2.0, February, Final Version)*. American Psychiatric Press: Washington, DC., 1996.
- 5 33. Muller DJ, Zai CC, Sicard M, Remington E, Souza RP, Tiwari AK, *et al.* Systematic analysis of dopamine receptor genes (DRD1-DRD5) in antipsychotic-induced weight gain. *Pharmacogenomics J* 2010e.
- 10 34. Kane JM, Honigfeld G, Singer J, Meltzer H. Clozapine in treatment-resistant schizophrenics. *Psychopharmacol Bull* 1988; **24**(1): 62-67.
35. Overall JE, Gorham DR. The brief psychiatric rating scale. *Psychological Reports* 1962; **10**: 799-812.
- 15 36. Masellis M, Basile V, Meltzer HY, Lieberman JA, Sevy S, Macciardi FM, *et al.* Serotonin subtype 2 receptor genes and clinical response to clozapine in schizophrenia patients. *Neuropsychopharmacology* 1998; **19**(2): 123-132.
- 20 37. Volavka J, Czobor P, Sheitman B, Lindenmayer JP, Citrome L, McEvoy JP, *et al.* Clozapine, olanzapine, risperidone, and haloperidol in the treatment of patients with chronic schizophrenia and schizoaffective disorder. *Am J Psychiatry* 2002; **159**(2): 255-262.
- 25 38. Lahiri DK, Nurnberger JI, Jr. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. *Nucleic Acids Res* 1991; **19**(19): 5444.
39. Edenberg HJ, Dick DM, Xuei X, Tian H, Almasy L, Bauer LO, *et al.* Variations in GABRA2, encoding the alpha 2 subunit of the GABA(A) receptor, are associated with alcohol dependence and with brain oscillations. *Am J Hum Genet* 2004; **74**(4): 705-714.
- 30 40. Fehr C, Sander T, Tadic A, Lenzen KP, Anghelescu I, Klawe C, *et al.* Confirmation of association of the GABRA2 gene with alcohol dependence by subtype-specific analysis. *Psychiatr Genet* 2006; **16**(1): 9-17.
- 35 41. Enoch MA. The role of GABA(A) receptors in the development of alcoholism. *Pharmacol Biochem Behav* 2008; **90**(1): 95-104.
42. Haughey HM, Ray LA, Finan P, Villanueva R, Niculescu M, Hutchison KE. Human gamma-aminobutyric acid A receptor alpha2 gene moderates the acute effects of alcohol and brain mRNA expression. *Genes Brain Behav* 2008; **7**(4): 447-454.
- 40 43. Agrawal A, Pergadia ML, Saccone SF, Hinrichs AL, Lessov-Schlaggar CN, Saccone NL, *et al.* Gamma-aminobutyric acid receptor genes and nicotine dependence: evidence for association from a case-control study. *Addiction* 2008; **103**(6): 1027-1038.

44. Pierucci-Lagha A, Covault J, Feinn R, Nellissery M, Hernandez-Avila C, Oncken C, *et al.* GABRA2 alleles moderate the subjective effects of alcohol, which are attenuated by finasteride. *Neuropsychopharmacology* 2005; **30**(6): 1193-1203.
- 5 45. Bauer LO, Covault J, Harel O, Das S, Gelernter J, Anton R, *et al.* Variation in GABRA2 predicts drinking behavior in project MATCH subjects. *Alcohol Clin Exp Res* 2007; **31**(11): 1780-1787.
- 10 46. Ma DQ, Whitehead PL, Menold MM, Martin ER, Ashley-Koch AE, Mei H, *et al.* Identification of significant association and gene-gene interaction of GABA receptor subunit genes in autism. *Am J Hum Genet* 2005; **77**(3): 377-388.
- 15 47. Kareken DA, Liang T, Wetherill L, Dzemidzic M, Bragulat V, Cox C, *et al.* A polymorphism in GABRA2 is associated with the medial frontal response to alcohol cues in an fMRI study. *Alcohol Clin Exp Res* 2010; **34**(12): 2169-2178.
- 20 48. Zai GC, Zai CC, Chowdhury NI, Tiwari AK, Souza RP, Lieberman JA, *et al.* The role of brain-derived neurotrophic factor (BDNF) gene variants in antipsychotic response and antipsychotic-induced weight gain. *Prog Neuropsychopharmacol Biol Psychiatry* 2012; **39**(1): 96-101.
49. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005; **21**(2): 263-265.
- 25 50. Dudbridge F. Likelihood-based association analysis for nuclear families and unrelated subjects with missing genotype data. *Hum Hered* 2008; **66**(2): 87-98.
- 30 51. Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. *Am J Hum Genet* 2001; **68**(4): 978-989.
52. Li J, Ji L. Adjusting multiple testing in multilocus analyses using the eigenvalues of a correlation matrix. *Heredity (Edinb)* 2005; **95**(3): 221-227.
- 35

WHAT IS CLAIMED IS:

1. A method of predicting a subject's weight change in response to antipsychotic drug treatment, comprising determining the presence or absence of one or more polymorphisms in the *GABRA2* gene of the subject or the complement thereof, in a biological sample comprising a nucleic acid obtained from the subject, wherein the presence of said one or more polymorphisms is predictive of the subject's weight response to antipsychotic drug treatment, wherein the one or more polymorphisms in the *GABRA2* gene are relative to (a)-(f) or the complement thereof, wherein the polymorphic site is in brackets, underlined and in bold:

a) rs16859227

CCTTGGTTTTATACAAGCATGCAAAG**[C/T]**ATATAATAGAATCACATGGAAACAA
(SEQ ID NO:1);

b) rs279858

ATTGTCATATTATGAGCTACTGATTT**[T/C]**TTCCCATTTGTGAAAAAAGGTATCTG
(SEQ ID NO:2);

c) rs1442062

GAGAAGGTGAAATAGATTTAACAT**[A/G]**TATCAAATTAAGATTGCACCTTAAA
(SEQ ID NO:5);

d) rs11503014

AAGCTATGGAGATTACTTCCTGGACT**[C/G]**TGTGTAGGACTTGATGATTGAGAGA
(SEQ ID NO:7);

e) rs6856130

TCTGTTCTGTTTTATCTGAGGCGATA**[A/G]**AATCCAAACGTGCAACTTGAACAAC
(SEQ ID NO:8);

f) rs 13724 72

ATAAACTCTGGTAATTCAAACCAAA**[A/T]**ATTCCTCACTGAAAACCTATGCTTG
(SEQ ID NO:9);

wherein:

for (a) the presence of two copies of the C allele is associated with a higher percentage weight gain in subjects;

for (b) the presence of two copies of the T allele is associated with a higher percentage weight gain in subjects;

for (c) the presence of two copies of the G allele is associated with a higher percentage weight gain in subjects;

for (d) the presence of at least one copy of the G allele is associated with a higher percentage weight gain in subjects;

for (e) the presence of at least one copy of the G allele is associated with a higher percentage weight gain in subjects; or

for (f) the presence of two copies of the A allele is associated with a higher percentage weight gain in subjects.

2. The method of claim 1, wherein the subject has been diagnosed with schizophrenia or schizoaffective disorder, is likely to develop schizophrenia or schizoaffective disorder, or exhibits one or more symptoms of schizophrenia or schizoaffective disorder.

3. The method of claim 1, wherein said subject displays one or more psychotic symptoms or is at risk of displaying one or more psychotic symptoms.

4. The method of any preceding claim, wherein the one or more polymorphisms are relative to (a) and/or (b), or the complement thereof.

5. The method of claim 4, wherein the polymorphisms further include a polymorphism relative to one or more of (c)-(f), or the complement thereof.

6. The method of claim 1, wherein at least one of the polymorphisms is SEQ ID NO:1 or 2, or a variant or fragment thereof comprising the polymorphic site.

7. The method of any preceding claim, wherein the sample is a blood sample.

8. A method for treating a subject in need thereof with an antipsychotic drug in a manner that reduces the subject's risk of weight gain in response to treatment with the antipsychotic drug, the method comprising:

(i) determining the presence or absence of one or more polymorphisms in the *GABRA2* gene of the subject or the complement thereof, wherein the one or more polymorphisms in the *GABRA2* gene are relative to (a)-(f) or the complement thereof, wherein the polymorphic site is in brackets, underlined and in bold:

a) rs16859227

CCTTGGTTTTATACAAGCATGCAAAG**[C/T]**ATATAATAGAATCACATGGAAACAA
(SEQ ID NO:1);

b) rs279858

ATTGTCATATTATGAGCTACTGATTT**[T/C]**TTCCCATTTGTGAAAAAAGGTATCTG
(SEQ ID NO:2);

c) rs1442062

GAGAAGGTGAAATAGATTTAACAT**[A/G]**TATCAAATTAAGATTGCACCTTAAA
(SEQ ID NO:5);

d) rs11503014

AAGCTATGGAGATTACTTCCTGGACT**[C/G]**TGTGTAGGACTTGATGATTGAGAGA
(SEQ ID NO:7);

e) rs6856130

TCTGTTCTGTTTTATCTGAGGCGATA**[A/G]**AATCCAAACGTGCAACTTGAACAAC
(SEQ ID NO:8); or

f) rs1372472

ATAAACTCTGGTAATTCAAACCAAA[A/T]ATTTCCTCACTGAAAATATGCTTG
(SEQ ID NO:9),

(ii) identifying the subject as being at increased risk of weight gain in response to treatment with an antipsychotic drug wherein:

for (a) the presence of two copies of the C allele is associated with increased risk of weight gain;

for (b) the presence of two copies of the T allele is associated with increased risk of weight gain;

for (c) the presence of two copies of the G allele is associated with increased risk of weight gain;

for (d) the presence of at least one copy of the G allele is associated with increased risk of weight gain;

for (e) the presence of at least one copy of the G allele is associated with increased risk of weight gain; or

for (f) the presence of two copies of the A allele is associated with increased risk of weight gain, and

(iii) administering to the subject identified in (ii) an antipsychotic drug selected from the group consisting of paliperidone, perphenazine, thioridazine, chlorpromazine, risperidone and quetiapine.

9. The method of claim 8, wherein step (i) comprises determining the presence or absence of polymorphisms relative to at least two of (a)-(f), or the complement thereof.

10. The method of claim 9, wherein step (i) comprises determining the presence or absence of polymorphisms relative to (a) and (b), or the complement thereof.

11. The method of claim 9, wherein step (i) comprises determining the presence or absence of polymorphisms relative to (b) and (c), or the complement thereof.

12. The method of claim 8, wherein step (i) comprises determining the presence or absence of polymorphisms relative to (b), or the complement thereof.

13. The method of claim 8, wherein the method comprises determining a treatment regimen for the subject that avoids clozapine and olanzapine for the subject at increased risk of weight gain.

14. The method of claim 8, wherein the method comprises determining a treatment regimen for the subject that includes an antipsychotic selected from loxapine, iloperidone, asenapine, lurasidone, ziprasidone, aripiprazole, fluphenazine, and haloperidol for the subject at increased risk of weight gain.

15. The method of any preceding claim, wherein the subject is a human.

pctca2014051000-seql
SEQUENCE LISTING

<110> Centre for Addiction and Mental Health
<120> Genetic Markers for Antipsychotic Induced Weight Gain and Methods
for Use Thereof
<130> 08924607W0
<140>
<141>
<150> US 61/892,094
<151> 2013-10-17
<160> 9
<170> PatentIn version 3.5
<210> 1
<211> 53
<212> PRT
<213> homo sapien
<400> 1

Cys Cys Thr Thr Gly Gly Thr Thr Thr Thr Ala Thr Ala Cys Ala Ala
1 5 10 15

Gly Cys Ala Thr Gly Cys Ala Ala Ala Gly Cys Thr Ala Thr Ala Thr
20 25 30

Ala Ala Thr Ala Gly Ala Ala Thr Cys Ala Cys Ala Thr Gly Gly Ala
35 40 45

Ala Ala Cys Ala Ala
50

<210> 2
<211> 53
<212> PRT
<213> homo sapien
<400> 2

Ala Thr Thr Gly Thr Cys Ala Thr Ala Thr Thr Ala Thr Gly Ala Gly
1 5 10 15

Cys Thr Ala Cys Thr Gly Ala Thr Thr Thr Thr Cys Thr Thr Cys Cys
20 25 30

Cys Ala Thr Thr Gly Thr Gly Ala Ala Ala Ala Ala Ala Gly Gly Thr
35 40 45

Ala Thr Cys Thr Gly
50

<210> 3
<211> 53
<212> PRT

pctca2014051000-seql

<213> homo sapien

<400> 3

Gly Thr Ala Ala Ala Gly Thr Gly Thr Cys Ala Cys Ala Thr Cys Ala
1 5 10 15

Ala Thr Gly Cys Cys Ala Thr Ala Thr Cys Ala Gly Thr Ala Thr Thr
20 25 30

Cys Thr Gly Thr Ala Gly Ala Thr Gly Gly Cys Ala Thr Gly Thr Thr
35 40 45

Ala Thr Cys Ala Thr
50

<210> 4

<211> 53

<212> PRT

<213> homo sapien

<400> 4

Cys Thr Cys Ala Thr Thr Thr Cys Cys Thr Thr Gly Cys Thr Thr Cys
1 5 10 15

Thr Ala Ala Gly Gly Thr Ala Gly Gly Gly Gly Thr Thr Cys Ala Thr
20 25 30

Cys Ala Ala Thr Thr Thr Ala Thr Cys Thr Ala Thr Cys Thr Cys Ala
35 40 45

Thr Gly Gly Gly Ala
50

<210> 5

<211> 53

<212> PRT

<213> homo sapien

<400> 5

Gly Ala Gly Ala Ala Gly Gly Thr Gly Ala Ala Ala Thr Ala Gly Ala
1 5 10 15

Thr Thr Thr Ala Ala Cys Thr Cys Ala Thr Ala Gly Thr Ala Thr Cys
20 25 30

Ala Ala Ala Thr Thr Ala Ala Gly Ala Thr Thr Gly Cys Ala Cys Cys
35 40 45

Thr Thr Ala Ala Ala
50

<210> 6

<211> 53

pctca2014051000-seql

<212> PRT
<213> homo sapien

<400> 6

Thr Ala Cys Ala Ala Thr Ala Thr Cys Thr Thr Gly Ala Cys Thr Cys
1 5 10 15

Ala Ala Thr Gly Ala Gly Cys Thr Thr Cys Gly Thr Ala Ala Thr Cys
20 25 30

Thr Thr Ala Ala Thr Ala Ala Gly Gly Thr Ala Ala Cys Ala Ala Gly
35 40 45

Ala Gly Ala Ala Ala
50

<210> 7
<211> 53
<212> PRT
<213> homo sapien

<400> 7

Ala Ala Gly Cys Thr Ala Thr Gly Gly Ala Gly Ala Thr Thr Ala Cys
1 5 10 15

Thr Thr Cys Cys Thr Gly Gly Ala Cys Thr Cys Gly Thr Gly Thr Gly
20 25 30

Thr Ala Gly Gly Ala Cys Thr Thr Gly Ala Thr Gly Ala Thr Thr Gly
35 40 45

Ala Gly Ala Gly Ala
50

<210> 8
<211> 53
<212> PRT
<213> homo sapien

<400> 8

Thr Cys Thr Gly Thr Thr Cys Thr Gly Thr Thr Thr Thr Ala Thr Cys
1 5 10 15

Thr Gly Ala Gly Gly Cys Gly Ala Thr Ala Ala Gly Ala Ala Thr Cys
20 25 30

Cys Ala Ala Ala Cys Gly Thr Gly Cys Ala Ala Cys Thr Thr Gly Ala
35 40 45

Ala Cys Ala Ala Cys
50

<210> 9

pctca2014051000-seql

<211> 53
 <212> PRT
 <213> homo sapien

<400> 9

Al a Thr Al a Al a Al a Al a Cys Thr Cys Thr Gly Gly Thr Al a Al a Thr
 1 5 10 15

Thr Cys Al a Al a Al a Cys Cys Al a Al a Al a Al a Thr Al a Thr Thr Thr
 20 25 30

Cys Cys Thr Cys Al a Cys Thr Gly Al a Al a Al a Al a Cys Thr Al a Thr
 35 40 45

Gly Cys Thr Thr Gly
 50