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- (71) Applicants: **UNIVERSITAT DE BARCELONA** [ES/ES]; Centro de Patentes UB, Baldiri i Reixac 4, E-0802 Barcelona (ES). **HOSPITAL CLÍNIC DE BARCELONA** [ES/ES]; C/ Villarroel, 170, E-08036 Barcelona (ES). **INSTITUT D'INVESTIGACIONS BIOMÈDIQUES AUGUST PI I SUNYER** [ES/ES]; C/ Villarroel, 170, E-08036 Barcelona (ES). **CENTRO DE INVESTIGACIÓN BIOMÉDICA EN RED (CIBER)** [ES/ES]; Instituto de Salud Carlos III, C/ Monforte de Lemos, 3-5 - Pabellón 11, E-28029 Madrid (ES).
- (72) Inventors: **MAS HERRERO, Sergi**; Universitat de Barcelona, Unitat de Farmacologia - Facultat de Medicina, C/ Casanova 143 (planta 0), E-08036 Barcelona (ES). **GASSÓ ASTORGA, Patricia**; C/ París 130 - 4º 1ª, E-08036 Barcelona (ES). **MALAGELADA GRAU, Cristina**; C/ Enamorats nº 34-36 - 5è4a, E-08013 Barcelona (ES). **BERNARDO ARROYO, Miquel**; Hospital Clinic de Barcelona, C/ Villarroel 170, escala 12 planta 0, E-08036 Barcelona (ES). **LAFUENTE FLO, Amalia**; Universitat de Barcelona, Unitat de Farmacologia - Facultat de Medicina, C/ Casanova 143, E-08036 Barcelo (ES).
- (74) Agent: **ALCONADA RODRIGUEZ, Agustín**; ABG Patentes, S.L., Avda. de Burgos, 16D, Edificio EUROMOR, E-28036 Madrid (ES).
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(54) Title: METHOD FOR PREDICTING THE ONSET OF EXTRAPYRAMIDAL SYMPTOMS (EPS) INDUCED BY AN ANTI-PSYCHOTIC-BASED TREATMENT

(57) Abstract: The invention relates to methods for predicting the onset of extrapyramidal symptoms (EPS) induced by an anti-psychotic-based treatment as well as methods for providing personalized medicine to patients based on the sequence of several SNPs associated with the onset of EPS. The invention relates as well to kits for carrying out the diagnostic and predictive medicine methods.



**METHOD FOR PREDICTING THE ONSET OF EXTRAPYRAMIDAL
SYMPTOMS (EPS) INDUCED BY AN ANTIPSYCHOTIC-BASED
TREATMENT**

5 FIELD OF THE INVENTION

The invention relates to methods for predicting the onset of extrapyramidal symptoms (EPS) induced by an antipsychotic-based treatment as well as methods for providing personalized medicine to patients. The invention relates as well to kits for
10 carrying out the diagnostic and predictive medicine methods.

BACKGROUND OF THE INVENTION

Antipsychotic treatment-emergent extrapyramidal symptoms (EPS) are frequent
15 and serious acute adverse reactions of antipsychotic drugs, the signs of which may develop within few days of starting medication. EPS is a complex phenotype that includes several recognized syndromes namely parkinsonism, akathisia, acute dystonia and dyskinesia. While the situation has improved since the pre-clozapine era, the EPS problem has by no means gone away; for example, in CATIE (Comparative
20 Effectiveness of Antipsychotic Medications in Patients with Schizophrenia), a large effectiveness trial, 10.5% of patients stopped their assigned medication for EPS-related reasons.

Even though the exact mechanism underlying EPS is not clear, striatal dopamine
25 D2 receptor (DRD2) blockade is believed to be the principal cause. The essential control of motor activity is assumed by the basal ganglia, whose main constituent is the striatum. In the dorsal striatum, dopamine regulates the motor activity by whether interacting with dopamine D1 receptor (DRD1) or DRD2, resulting in two different projecting pathways (“direct and “indirect” pathways) and most importantly in opposite stimulation of the thalamus. According to this model, dopamine promotes motor activity
30 by increasing the activity of the direct pathway and, concomitantly by inhibiting the indirect pathway.

Several studies have tried to identify potential risk factors for developing EPS, such as younger age, male gender and psychiatric diagnosis, especially mood disorders. Pharmacogenetics markers have been also tested (Zhang and Malhotra, 2011, Expert Opin Drug Metab Toxicol 7:9-37). Although some genetic variant may have significant effects on EPS appearance (Gassó et al., 2009, Pharmacogenomics J 9:404-10; Greenbaum et al., 2009, Pharmacogenomics J 9:103-10) no single factor can predict this phenomenon.

Almoguera et al. [2012, Pharmacogenomics J. doi: 10.1038/tpj.2011.57 (Epub ahead of print Jan 3 2012)] describes the association of genetic variants in several genes with adverse effects caused by risperidone in patients with schizophrenia. All analyzed gene variants had been previously described in relation to the ability to predict patient response to risperidone or adverse effects. Almoguera et al. further disclose that ADRB2 gene variants 16Gly, SLC6A4 L/S, SLC6A4 S/S and DRD3 9Gly correlate with the onset of sexual adverse events, somnolence, EPS and weight gain in patients treated with risperidone.

WO 2011/148379 describes genotypes associated with resistance to parkinsonism and other antipsychotic-induced EPS, in particular the rs12678719 SNP in gene *ZFPM2* and the rs4606 SNP in gene *RGS2*.

WO 2007/144874 describes genotypes associated with resistance to EPS induced by antipsychotics, especially the rs2179652, rs1933695, rs2746073, rs4606, rs1819741 and rs1152746 SNPs. Furthermore, this document identifies genotypes associated with a predisposition to the onset or aggravation of EPS. Gene variants associated with EPS are in *RGS2* gene.

Xu et al. (2007, J.Clin.Psychiatry, 68:1358-67) describes the identification of the rs3803300 SNP in gene *AKT1* and of a haplotype consisting of 5 SNPs in said gene that are associated with the onset of schizophrenia. However, neither the rs3803300 SNP nor the haplotype show any relationship with the onset of EPS.

Thus, there is still a need in the art for a method that allows to predict the onset EPS induced by an antipsychotic-based treatment.

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BRIEF DESCRIPTION OF THE INVENTION

The authors of the present invention have identified for the first time a set of single nucleotide polymorphisms (SNPs) which provides a reliable method for the prediction of the onset of extrapyramidal symptoms (EPS) in patients undergoing treatment with antipsychotics. For instance, as shown in example 1 of the application, some allelic combinations of the rs1130214, rs456998, rs7211818 and rs1053639 SNPs predict a high risk of the subject to develop EPS, whereas other allelic combinations predict a low risk of the subject to develop EPS.

Thus, in a first aspect, the invention relates to a method for predicting the onset of extrapyramidal symptoms (EPS) induced by an antipsychotic-based treatment in a subject, comprising

- i) determining the sequence of the rs1130214, rs456998, rs7211818 and rs1053639 single nucleotide polymorphisms (SNPs) in a sample comprising genetic material from said subject, and
- ii) predicting the risk of said subject to develop EPS based on the sequence of said SNPs.

In another aspect, the invention relates to a method for selecting a subject suffering from a disease treatable with antipsychotics to receive a low DRD2 blockade potency antipsychotic-based therapy, comprising

- i) determining the sequence of the rs1130214, rs456998, rs7211818 and rs1053639 SNPs in a sample comprising genetic material from said subject, and
- ii) selecting said subject to receive a low DRD2 blockade potency antipsychotic-based therapy based on the sequence of said SNPs.

In another aspect, the invention relates to a method for selecting a suitable antipsychotic-based therapy to treat a subject suffering from a disease treatable with antipsychotics, comprising

- i) determining the sequence of the rs1130214, rs456998, rs7211818 and rs1053639 SNPs in a sample comprising genetic material from said subject, and
- ii) selecting a suitable antipsychotic-based therapy based on the sequence of said SNPs,

wherein said antipsychotic is selected from the group consisting of a low DRD2 blockade potency antipsychotic-based therapy and any antipsychotic-based therapy.

In another aspect, the invention relates to a kit comprising reagents suitable for determining the sequence of the rs1130214, rs456998, rs7211818 and rs1053639 SNPs,
5 wherein said reagents comprise DNA or RNA probes.

In another aspect, the invention relates to the use of a kit comprising reagents suitable for determining the sequence of the rs1130214, rs456998, rs7211818 and rs1053639 SNPs for predicting the onset of EPS induced by an antipsychotic-based treatment in a subject based on the sequence of the rs1130214, rs456998, rs7211818 and
10 rs1053639 SNPs.

In another aspect, the invention relates to a low DRD2 blockade potency antipsychotic for use in the treatment of a disease treatable with antipsychotics in a subject, wherein said subject has been selected using the method for selecting a subject suffering from a disease treatable with antipsychotics to receive a low DRD2 blockade
15 potency antipsychotic-based therapy.

In another aspect, the invention relates to the use of the rs1130214, rs456998, rs7211818 and rs1053639 SNPs for predicting the onset of EPS induced by an antipsychotic-based treatment in a subject, for selecting a subject suffering from a disease treatable with antipsychotics to receive a low DRD2 blockade potency
20 antipsychotic-based therapy or for selecting a suitable antipsychotic-based therapy to treat a subject suffering from a disease treatable with antipsychotics.

DETAILED DESCRIPTION OF THE INVENTION

25 The authors of the present invention have identified for the first time a set of single nucleotide polymorphisms (SNPs) which provides a reliable method for the prediction of the onset of extrapyramidal symptoms (EPS) in patients undergoing treatment with antipsychotics.

The inventors performed a study where 241 psychiatric patients receiving an
30 antipsychotic-based therapy were screened for nine SNPs, and studied their contribution to the risk of EPS. They found that the combination of the rs1130214, rs456998, rs7211818 and rs1053639 SNPs was statistically significant. This finding opens the

door to new genetic predictors of EPS in the treatment of diseases treatable with antipsychotics, helping the physician in the design of individualized therapy of subjects. Based on these findings, the inventors have developed the methods of the present invention which will be described now in detail.

5 For the avoidance of doubt, the methods of the invention do not involve diagnosis practiced on the human or animal body. The methods of the invention are preferably conducted on a sample that has previously been removed from the subject. The kits of the invention, described hereunder, may include means for extracting the sample from the subject.

10

Method for predicting the onset of EPS induced by an antipsychotic-based treatment in a subject

In a first aspect, the invention relates to a method (hereinafter referred to as the
15 “first method of the invention”) for predicting the onset of EPS induced by an antipsychotic-based treatment in a subject, comprising

- i) determining the sequence of the rs1130214, rs456998, rs7211818 and rs1053639 single nucleotide polymorphisms (SNPs) in a sample comprising genetic material from said subject, and
- 20 ii) predicting the risk of said subject to develop EPS based on the sequence of said SNPs.

The term “predicting the onset of extrapyramidal symptoms (EPS) induced by an antipsychotic-based treatment”, is used herein to refer to the likelihood that a patient will develop EPS as a consequence of a treatment based on antipsychotics. The
25 predictive methods of the present invention can be used clinically to make treatment decisions by choosing the most appropriate treatment modalities for any particular patient. The predictive methods of the present invention are valuable tools in predicting if a patient is likely to develop EPS respond favorably to a treatment regimen, such as an antipsychotic-based treatment. The prediction may include prognostic factors.

30 As it will be understood by those skilled in the art, the prediction, although preferred to be, need not be correct for 100% of the subjects to be evaluated. The term, however, requires that a statistically significant portion of subjects can be identified as

having an increased probability of having a given outcome. Whether a subject is statistically significant can be determined without further ado by the person skilled in the art using various well known statistic evaluation tools, e.g., determination of confidence intervals, p-value determination, cross-validated classification rates and the like etc. Details are found in Dowdy and Wearden, Statistics for Research, John Wiley & Sons, New York 1983. Preferred confidence intervals are at least 50%, at least 60%, at least 70%, at least 80%, at least 90% or at least 95%. The p-values are, preferably, 0.01, 0,005 or lower.

The term “extrapyramidal symptoms” or “EPS” or “extrapyramidal side-effects (EPSE)”, as used herein, refers to the various effects on motor control, including acute dystonic reactions, pseudoparkinsonism, akathisia (an inability to sit still), trembling, involuntary repetitive body movements (tardive dyskinesia), , suffered as a result of the intake of antipsychotic drugs. EPS are normally rated using the Simpson-Angus Rating Scale. This scale contains 10 items: gait, arm dropping, shoulder shaking, elbow rigidity, wrist rigidity, leg pendulousness, head dropping, glabella tap, tremor, and salivation. Each item is rated between 0 and 4. A total score is obtained by adding the items and dividing by 10. Scores of up to 0.3 are considered within the normal range.

The term “treatment” or “therapy” includes any process, action, application, therapy, or the like, wherein a subject, including a human being, is provided medical aid with the object of improving the subject's condition, directly or indirectly, or slowing the progression of a condition or disorder in the subject, or ameliorating at least one symptom of the disease or disorder under treatment. The term “antipsychotic-based treatment” or “antipsychotic-based therapy”, as used herein, refers to any treatment or therapy that comprises at least one antipsychotic.

The term “antipsychotic” or “neuroleptic”, as used herein, refers to a psychiatric medication primarily used to manage psychosis (including delusions or hallucinations, as well as disordered thought), and is increasingly being used in the management of non-psychotic disorders. Antipsychotics are designed with the Anatomical Therapeutic Chemical (ATC) code N05A. Antipsychotics are broadly divided into two groups, the typical or first-generation antipsychotics and the atypical or second-generation antipsychotics. The typical antipsychotics are classified according to their chemical structure while the atypical antipsychotics are classified according to their

pharmacological properties. These include serotonin-dopamine antagonists (see dopamine antagonist and serotonin antagonist), multi-acting receptor-targeted antipsychotics (MARTA, those targeting several systems), and dopamine partial agonists, which are often categorized as atypicals.

5 Recently, a new classification system is being used, wherein antipsychotics are divided according to their potency in blocking the dopaminergic receptor D2 (DRD2), i.e. antipsychotics are divided into low, medium and high DRD2 blockade potency antipsychotics. Low DRD2 blockade potency antipsychotics are known in the art to have a lower probability to induce EPS in subjects, where as medium and high DRD2
10 blockade potency antipsychotics are known in the art to have a higher probability to induce EPS in subjects. Non-limitative examples of low DRD2 blockade potency antipsychotics include clozapine, ziprasidone, quetiapine and olanzapine. Non-limitative examples of medium DRD2 blockade potency antipsychotics include amisulpride, risperidone and zuclopinixol. Non-limitative examples of high DRD2
15 blockade potency antipsychotics include haloperidol and chlorpromazine (Gardner et al., 2005, Can Med Assoc J 172:1703-11).

 The term “subject”, as used herein, refers to an individual, such as a human, a nonhuman primate (e.g. chimpanzees and other apes and monkey species); farm animals, such as birds, fish, cattle, sheep, pigs, goats and horses; domestic mammals,
20 such as dogs and cats; laboratory animals including rodents, such as mice, rats and guinea pigs. The term does not denote a particular age or sex. In a particular embodiment of the invention, the subject is a mammal. In a preferred embodiment of the invention, the subject is a human. In a more preferred embodiment of the invention, the subject has been diagnosed with a disease treatable with antipsychotics.

25 As the person skilled in the art will recognize, an antipsychotic-based treatment is not only aimed at treating a disorder or disease that presents psychosis, and it can be used to treat other kinds of disorders or diseases. Thus, in another particular embodiment of the first method of the invention, the subject suffers a disease treatable with antipsychotics. As used herein, “disease treatable with antipsychotics” refers to any
30 disease or disorder which condition can be improved, which progression can be slowed, or which at least one symptom can be ameliorated under treatment with antipsychotics. Non-limitative examples of diseases treatable with antipsychotics include

schizophrenia, schizoaffective disorder, acute psychotic disorder, delusional disorder, schizotypal personality disorder, bipolar disorder, obsessive compulsive disorder, personality disorder, psychotic depression, conduct disorder, cognitive deficits, nausea and vomiting, and Alzheimer disease.

5 In a first step, the first method of the invention comprises the determination of the sequence of the rs1130214, rs456998, rs7211818 and rs1053639 single nucleotide polymorphisms (SNPs) in a sample comprising genetic material from said subject.

The term “single nucleotide polymorphism” or “SNP”, as used herein, refers to a variation in the nucleotide sequence of a nucleic acid that occurs in a single nucleotide
10 (A, C, T or G), wherein each possible sequence is present in a proportion equal to or greater than a 1% of the population. The SNPs are typically named using the accession number in the SNP database (dbSNP) at National Center for Biotechnology Information (NCBI) accessible at <http://www.ncbi.nlm.nih.gov/projects/SNP/>. In general, SNPs represent one of the most common forms of genetic variations. These polymorphisms
15 appear when a single nucleotide in the genome is altered (such as via substitution, addition or deletion). Each version of the sequence with respect to the polymorphic site is referred to as an allele of the polymorphic site. SNPs tend to be evolutionary stable from generation to generation and, as such, can be used to study specific genetic abnormalities throughout a population. If SNPs occur in the protein coding region, it
20 can lead to the expression of a variant, sometimes defective, form of the protein that may lead to development of a genetic disease. Some SNPs may occur in non-coding regions, but nevertheless, may result in differential or defective splicing, or altered protein expression levels. SNPs can therefore serve as effective indicators of a genetic disease. SNPs can also be used as diagnostic and/or prognostic tools for identifying
25 individuals with a predisposition for a disease or for a fast evolution of the disease, genotyping the individual suffering from the disease, and facilitating drug development based on the insight revealed regarding the role of target proteins in the pathogenesis process. Each version of the sequence with respect to the SNP is referred to as an allele of the SNP.

30 The term “allele”, as used herein, relates to one of two or more forms of a gene, locus or genetic polymorphism. Sometimes, different alleles can result in different traits; however, other times, different alleles will have the same result in the expression

of a gene. Most multicellular organisms have two sets of chromosomes, that is, they are diploid. These chromosomes are referred to as homologous chromosomes. Diploid organisms have one copy of each gene (and one allele) on each chromosome. If both alleles are the same, they are homozygotes. If the alleles are different, they are heterozygotes.

The term “sample” or “biological sample”, as used herein, refers to biological material isolated from a subject. The biological sample contains any biological material suitable for detecting the desired SNP and can comprise cell and/or non-cell material of the subject. In the present invention, the sample comprises genetic material, e.g., DNA, genomic DNA (gDNA), complementary DNA (cDNA), RNA, heterogeneous nuclear RNA (hnRNA), mRNA, etc., from the subject under study. The sample can be isolated from any suitable tissue or biological fluid such as, for example blood, saliva, plasma, serum, urine, cerebrospinal liquid (CSF), feces, a buccal or buccal-pharyngeal swab, a surgical specimen, and a specimen obtained from a biopsy. Methods for isolating cell and tissue samples are well known to those skilled in the art. In a particular embodiment, the sample is selected from the group consisting of blood, urine, saliva, serum, plasma, a buccal or buccal-pharyngeal swab, hair, a surgical specimen, and a specimen obtained from a biopsy. In preferred embodiment, the sample is selected from blood, hair, urine and saliva.

The terms “determining the sequence of an SNP” or “detecting an SNP” are used indistinctly in the present invention, and refer to the determination of the sequence of a particular SNP in both alleles of the subject under study. The determination of the sequence of the SNP can be performed by means of multiple processes known by the person skilled in the art.

In some embodiments, for example, when the determination of the sequence of the SNPs is carried out in a sample from whole blood, it may be used directly for the detection of said SNPs. In other embodiments, the nucleic acid is extracted from cells which are present in a biological fluid (e.g., whole blood, saliva, synovial fluid, etc.) as an initial step, and, in such cases, the total nucleic acid extracted from said samples would represent the working material suitable for subsequent amplification. Isolating the nucleic acid of the sample can be performed by methods known by the person skilled in the art. Said methods can be found, for example, in Sambrook et al., 2001.

“Molecular cloning: a Laboratory Manual”, 3rd ed., Cold Spring Harbor Laboratory Press, N.Y., Vol. 1-3. Further, as mentioned above, in some embodiments, generation of nucleic acids for analysis from samples requires nucleic acid amplification. Many amplification methods rely on an enzymatic chain reaction such as, for example, a
5 polymerase chain reaction (PCR), a ligase chain reaction (LCR), or a self-sustained sequence replication, rolling-circle amplification assays, etc.; this list is merely illustrative and in no way limiting. Methods for amplifying nucleic acid are described in Sambrook et al., 2001 (cited at supra).

After isolating, and amplifying (if necessary), the nucleic acid, the sequences of the
10 different SNPs of the invention are detected. Those skilled in the art will readily recognize that the analysis of the nucleotides present in one or several of the SNPs, or polymorphisms, disclosed herein in a patient's nucleic acid can be done by any method or technique capable of determining nucleotides present in a SNP or polymorphism. For instance, one may detect SNPs in the first method of the invention by performing
15 sequencing, mini-sequencing, hybridization, restriction fragment analysis, oligonucleotide ligation assay, allele-specific PCR, or a combination thereof. As such, systems and methods for the detection of SNPs, in general include, but are not limited to, nucleic acid sequencing, hybridization methods and array technology (e.g. technology available from Aclara BioSciences, Affymetrix, Agilent Technologies,
20 Illumina Inc., etc); also techniques based on mobility shift in amplified nucleic acid fragments, Single Stranded Conformational Polymorphism (SSCP), denaturing gradient gel electrophoresis (DGGE), Chemical Mismatch Cleavage (CMC), Restriction Fragment Polymorphisms (RFLPs), and WAVE analysis can be used (Methods Mol. Med. 2004; 108: 173-88), and the like. Of course, this list is merely illustrative and in
25 no way limiting. Those skilled in the art may use any appropriate method to achieve such detection. As it is obvious in the art, the sequence of said SNPs can be determined from either nucleic acid strand or from both strands. In the present invention, the sequences of said SNPs are determined from both strands.

In another particular embodiment, the determination of the sequence of said SNPs
30 is carried out by real-time PCR.

The SNPs used in the present invention are identified below:

- rs1130214 is located in the *AKT1* gene and corresponds to SEQ ID NO: 1;
CTGGGGTTTCTCCCAGGAGGTTTTTG [G/T] GCTTGCGCTGGAGGGCTCTGGACTC
- rs456998 is located in the *FCHSD1* gene and corresponds to SEQ ID NO: 2;
CATTCTATTATGCTCATAATAAAAAAT [G/T] TACTGAGGACTCTATGCCAGAAATT
- 5 - rs7211818 is located in the *RPTOR* gene and corresponds to SEQ ID NO: 3; and
AAAGCAGAAGGAAAGAAATAACAAAC [A/G] GCAGAAATCAATAAAAATAGAGTACA
- rs1053639 is located in the *DDIT4* gene and corresponds to SEQ ID NO: 4.
GAGGCAGGAGCTGAGGGACTGATTCC [A/T] GTGGTTGGAAAAC TGAGGCAGCCAC

10 In a second step, the first method of the invention comprises the prediction of the risk of said subject to develop EPS based on the sequence of the rs1130214, rs456998, rs7211818 and rs1053639 SNPs.

In this connection, the invention provides not only some specific SNPs which in combination are significantly associated with the prediction of the onset of EPS induced
15 by an antipsychotic-based treatment in a subject, but also the corresponding allelic combinations for high and low risk to develop EPS of said SNPs, which are mentioned in Tables 1 and 2. Thus, in a particular embodiment, the presence of one allelic combination according to Table 1 is indicative that that there is a high risk of the subject to develop EPS, In another particular embodiment, the presence of one allelic
20 combination according to Table 2 is indicative that there is a low risk of the subject to develop EPS.

Table 1: Allelic combinations that predict a high risk to develop EPS

rs1130214	rs456998	rs7211818	rs1053639	Predicted Status
1	0	1	0	EPS
1	0	1	1	EPS
1	2	1	0	EPS
1	2	1	2	EPS
1	2	0	2	EPS
1	2	0	1	EPS
1	2	2	1	EPS
0	0	0	0	EPS

0	2	1	1	EPS
0	2	0	0	EPS
0	2	2	2	EPS
0	1	1	0	EPS
0	1	0	1	EPS
0	1	2	1	EPS
2	0	1	0	EPS
2	0	1	2	EPS
2	0	1	1	EPS
2	0	0	1	EPS
2	2	0	2	EPS

For each of the SNPs: 0 = Homozygosis for allele 1; 1 = Heterozygosis for allele 1/allele 2; 2= Homozygosis for allele 2.

rs1130214: - allele 1= G; allele 2=T;

- genotype 0= GG; genotype 1=GT; genotype 2= TT

5 rs456998: - allele 1= T; allele 2=G;

- genotype 0= TT; genotype 1=GT; genotype 2= GG

rs7211818: - allele 1= A; allele 2=G;

- genotype 0= AA; genotype 1=AG; genotype 2= GG

rs1053639: - allele 1= T; allele 2=A;

10 - genotype 0= TT; genotype 1=AT; genotype 2= AA

Table 2: Allelic combinations that predict a low risk to develop EPS

rs1130214	rs456998	rs7211818	rs1053639	Predicted Status
1	0	0	1	No-EPS
1	2	1	1	No-EPS
1	2	0	0	No-EPS
1	1	1	0	No-EPS
1	1	1	2	No-EPS
1	1	1	1	No-EPS
1	1	0	0	No-EPS
1	1	0	2	No-EPS
1	1	0	1	No-EPS
1	1	2	0	No-EPS
0	0	1	0	No-EPS
0	0	1	2	No-EPS
0	0	1	1	No-EPS
0	0	0	2	No-EPS
0	0	0	1	No-EPS
0	2	1	0	No-EPS
0	2	0	2	No-EPS
0	2	0	1	No-EPS
0	1	1	1	No-EPS
0	1	0	0	No-EPS
0	1	0	2	No-EPS
0	1	2	2	No-EPS
2	2	0	0	No-EPS
2	2	0	1	No-EPS
2	2	2	2	No-EPS
2	1	0	0	No-EPS
2	1	0	1	No-EPS

For each of the SNPs: 0 = Homozygosis for allele 1; 1 = Heterozygosis for allele 1/allele 2; 2= Homozygosis for allele 2.

rs1130214: - allele 1= G; allele 2=T;

- rs456998: - genotype 0= GG; genotype 1=GT; genotype 2= TT
 - allele 1= T; allele 2=G;
 - genotype 0= TT; genotype 1=GT; genotype 2= GG
- rs7211818: - allele 1= A; allele 2=G;
 5 - genotype 0= AA; genotype 1=AG; genotype 2= GG
- rs1053639: - allele 1= T; allele 2=A;
 - genotype 0= TT; genotype 1=AT; genotype 2= AA

The expression “risk to develop EPS”, as used herein, refers to the predisposition,
 10 susceptibility or likelihood of a subject being treated with an antipsychotic-based
 therapy to develop EPS. The risk to develop EPS generally implies that there is a high
 or a low risk. In this respect, a subject being treated with an antipsychotic-based therapy
 at high risk to develop EPS is a subject who presents one allelic combination according
 to Table 1. Thus, a subject at high risk to develop EPS has at least a 50%, or at least a
 15 60%, or at least a 70%, or at least a 80%, or at least a 90%, or at least a 95%, or at least
 a 97%, or at least a 98%, or at least a 99%, or at least a 100% probability to develop
 EPS. Similarly, a subject being treated with an antipsychotic-based therapy at low risk
 to develop EPS is a subject who presents one allelic combination according to Table 2.
 Thus, a subject at low risk to develop EPS has at least a 0%, or at least a 1%, or at least
 20 a 2%, or at least a 3%, or at least a 5%, or at least a 10%, or at least a 20%, or at least a
 30%, or at least a 40%, or at least a 49% probability to develop EPS.

In general, the expression “predicting the risk”, “prediction of the risk”, or similar,
 relates to the risk that a subject being treated with an antipsychotic-based therapy to
 develop EPS either high or low. As it will be understood by those skilled in the art, the
 25 prediction (or the risk), although preferred to be, need not be correct for 100% of the
 subjects to be evaluated. The term, however, requires that a statistically significant
 portion of subjects can be identified as having an increased probability of developing
 EPS. Whether a subject is statistically significant can be determined without further ado
 by the person skilled in the art by using various well known statistic evaluation tools,
 30 e.g., determination of confidence intervals, p-value determination, Student's t-test,
 Mann-Whitney test, etc. Details can be found in Dowdy and Wearden, Statistics for
 Research, John Wiley & Sons, New York 1983. Preferred confidence intervals are at

least 50%, at least 60%, at least 70%, at least 80%, at least 90% at least 95%. The p-values are, preferably 0.1, 0.05, 0.02, 0.01 or lower.

5 Method for selecting a subject suffering from a disease treatable with antipsychotics to receive a low DRD2 blockade potency antipsychotic-based therapy

The person skilled in the art will realize that the predictive value of the rs1130214, rs456998, rs7211818 and rs1053639 SNPs can be further put in practice to select those patients that are at high risk to develop antipsychotic-induced EPS to receive therapy
10 with a lower probability to induce EPS, i.e. a low DRD2 blockade potency antipsychotic-based therapy. Thus, in another aspect, the invention relates to a method for selecting a subject suffering from a disease treatable with antipsychotics to receive a low DRD2 blockade potency antipsychotic-based therapy (hereinafter referred to as the “second method of the invention”), comprising

- 15 i) determining the sequence of the rs1130214, rs456998, rs7211818 and rs1053639 SNPs in a sample comprising genetic material from said subject, and
ii) selecting said subject to receive a low DRD2 blockade potency antipsychotic-based therapy based on the sequence of said SNPs.

20 The terms “subject”, “antipsychotics”, “disease treatable with antipsychotics”, “low DRD2 blockade potency antipsychotic-based therapy”, “SNP”, “rs1130214”, “rs456998”, “rs7211818”, “rs1053639”, and “sample comprising genetic material”, and their particulars have been described in detail in the context of the first method of the invention and are used with the same meaning in the context of the second method
25 according to the invention.

In a first step, the second method of the invention comprises determining the sequence of the rs1130214, rs456998, rs7211818 and rs1053639 SNPs in a sample comprising genetic material from said subject. The particulars of determining the sequence of SNPs have been described in detail in the context of the first method of the
30 invention as well as the particulars of the SNPs to be detected and are applied with same meaning in the context of the second method according to the invention.

In a second step, the second method of the invention comprises selecting said subject to receive a low DRD2 blockade potency antipsychotic-based therapy based on the sequence of said SNPs.

In a particular embodiment of the second method of the invention, the subject is
5 selected to receive a low DRD2 blockade potency antipsychotic-based therapy if the presence of one allelic combination according to Table 1 is detected in the sample. In a preferred embodiment, the low DRD2 blockade potency antipsychotic is selected from the group consisting of clozapine, ziprasidone, quetiapine and olanzapine.

In another particular embodiment, the low DRD2 blockade potency antipsychotic-
10 based therapy additionally comprises an adjuvant antiparkinsonian. As used herein, the term “adjuvant” or “adjuvant therapy” refers to any type of treatment of disease treatable with antipsychotics given as additional treatment, usually to decrease the probability of the antipsychotics to induce EPS. The aim of such an adjuvant treatment is to improve the risk of the subject receiving therapy to develop EPS.

15 As used herein, the term “antiparkinsonian” refers to a type of drug which is intended to treat and relieve the symptoms of Parkinson's disease (PD) or Parkinsonism. Most of these drugs act by either increasing dopamine activity or reducing acetylcholine activity in the central nervous system. Examples of antiparkinsonians include dopaminergic precursors, selective monoamine oxidase b inhibitors, catechol-o-methyl
20 transferase (COMT) inhibitors, dopamine receptor agonists, and anticholinergics. In a preferred embodiment, the antiparkinsonian is an anticholinergic. As used herein, the term “anticholinergic” refers to a class of drugs that inhibit parasympathetic nerve impulses by selectively blocking the binding of the neurotransmitter acetylcholine to its receptor in nerve cells. The nerve fibers of the parasympathetic system are responsible
25 for the involuntary movements of smooth muscles present in the gastrointestinal tract, urinary tract, lungs, etc. Anticholinergics are classified according to the receptors that are affected into antimuscarinic agents, which operate on the muscarinic acetylcholine receptors, and antinicotinic agents, which operate on the nicotinic acetylcholine receptors. Examples of anticholinergics include, but are not limited to, benztropine,
30 ipratropium, oxitropium, tiotropium, glycopyrrolate, oxybutinin, tolterodine, chlorphenamine, diphenhydramin, dimenhydrinate, bupropion, hexamethonium, tubocurarine, dextromethorphan, mecamlamine, and doxacurium.

Method for selecting a suitable antipsychotic-based therapy to treat a subject suffering from a disease treatable with antipsychotics

5 The present invention also contemplates the selection of personalized therapies in accordance with the allelic combinations of the rs1130214, rs456998, rs7211818 and rs1053639 SNPs present in the subject to be treated.

 Thus, in another aspect, the present invention relates to a method for selecting a suitable antipsychotic-based therapy to treat a subject suffering from a disease treatable
10 with antipsychotics (hereinafter referred to as the “third method of the invention”), comprising

- i) determining the sequence of the rs1130214, rs456998, rs7211818 and rs1053639 SNPs in a sample comprising genetic material from said subject, and
- 15 ii) selecting a suitable antipsychotic-based therapy based on the sequence of said SNPs,

wherein said antipsychotic is selected from the group consisting of a low DRD2 blockade potency antipsychotic-based therapy and any antipsychotic-based therapy.

 The terms “subject”, “antipsychotic”, “antipsychotic-based therapy”, “disease
20 treatable with antipsychotics”, “low DRD2 blockade potency antipsychotic-based therapy”, “SNP”, “rs1130214”, “rs456998”, “rs7211818”, “rs1053639”, and “sample comprising genetic material”, and their particulars have been described in detail in the context of the first method of the invention and are used with the same meaning in the context of the third method according to the invention.

25 In a first step, the third method of the invention comprises determining the sequence of the rs1130214, rs456998, rs7211818 and rs1053639 SNPs in a sample comprising genetic material from said subject. The particulars of determining the sequence of SNPs have been described in detail in the context of the first method of the invention as well as the particulars of the SNPs to be detected and are applied with same
30 meaning in the context of the third method according to the invention.

 In a second step, the third method of the invention comprises selecting a suitable antipsychotic-based therapy based on the sequence of said SNPs, wherein said

antipsychotic is selected from the group consisting of a low DRD2 blockade potency antipsychotic-based therapy and any antipsychotic-based therapy.

In a particular embodiment, the presence of one allelic combination according to Table 1 is indicative that said subject is selected to receive a low DRD2 blockade
5 potency antipsychotic-based therapy, and the presence of one allelic combination according to Table 2 is indicative that the subject is selected to receive any antipsychotic-based therapy.

In a preferred embodiment, the low DRD2 blockade potency antipsychotic is selected from the group consisting of clozapine, ziprasidone, quetiapine and olanzapine.

10 In another preferred embodiment, the low DRD2 blockade potency antipsychotic-based therapy additionally comprises an adjuvant antiparkinsonian. In a more preferred embodiment, the adjuvant antiparkinsonian is an anticholinergic. The terms “adjuvant” and “antiparkinsonian” and their particulars have been described in detail in the context of the second method of the invention and are used with the same meaning in the
15 context of the third method according to the invention.

The term “any antipsychotic-based therapy”, as used herein, refers to a therapy based on an antipsychotic indistinctly selected from the group consisting of a low DRD2 blockade potency antipsychotic, a medium DRD2 blockade potency antipsychotic, and a high DRD2 blockade potency antipsychotic. Thus, in a preferred
20 embodiment, the any antipsychotic-based therapy is selected from the group consisting of a low DRD2 blockade potency antipsychotic, a medium DRD2 blockade potency antipsychotic and a high DRD2 blockade potency antipsychotic. In a more preferred embodiment, the low DRD2 blockade potency antipsychotic is selected from the group consisting of clozapine, ziprasidone, quetiapine and olanzapine. In another more
25 preferred embodiment, the medium DRD2 blockade potency antipsychotic is selected from the group consisting of amisulpride, risperidone and zuclophenixol. In another more preferred embodiment, the high DRD2 blockade potency antipsychotic is selected from the group consisting of haloperidol and chlorpromazine.

30 Kits of the invention

The present invention also contemplates the preparation of kits for use in accordance with the present invention.

Thus, in another aspect, the present invention relates to a kit (hereinafter referred to as the “kit of the invention”), comprising reagents suitable for determining the sequence
5 of the rs1130214, rs456998, rs7211818 and rs1053639 SNPs, wherein the reagents of the kit comprise DNA or RNA probes.

Suitable kits include various reagents for use in accordance with the present invention in suitable containers and packaging materials, including tubes, vials, and shrink-wrapped and blow-molded packages. Additionally, the kits of the invention can
10 contain instructions for the simultaneous, sequential or separate use of the different components which are in the kit. Said instructions can be in the form of printed material or in the form of an electronic support capable of storing instructions such that they can be read by a subject, such as electronic storage media (magnetic disks, tapes and the like), optical media (CD-ROM, DVD) and the like. Additionally or alternatively, the
15 media can contain Internet addresses that provide said instructions.

Materials suitable for inclusion in an exemplary kit in accordance with the present invention comprise one or more of the following: gene specific PCR primer pairs (oligonucleotides) that anneal to DNA or cDNA sequence domains that flank the
20 rs1130214, rs456998, rs7211818 and rs1053639 SNPs; reagents capable of amplifying a specific sequence domain in either genomic DNA or cDNA without the requirement of performing PCR; reagents required to discriminate between the various possible alleles in the sequence domains amplified by PCR or non-PCR amplification (e.g., restriction endonucleases, oligonucleotide that anneal preferentially to one allele of the polymorphism, including those modified to contain enzymes or fluorescent chemical
25 groups that amplify the signal from the oligonucleotide and make discrimination of alleles more robust); or reagents required to physically separate products derived from the various alleles (e.g. agarose or polyacrylamide and a buffer to be used in electrophoresis, HPLC columns, SSCP gels, formamide gels or a matrix support for MALDI-TOF).

30 Specifically contemplated are kits comprising two or more allele-specific oligonucleotides or oligonucleotide pairs, wherein each of the allele-specific oligonucleotide or oligonucleotide pair is directed to one of the rs1130214, rs456998,

rs7211818 and rs1053639 SNPs. It will be appreciated that in this context the term “directed to” means an oligonucleotide or oligonucleotide pair capable of identifying the allele present at the SNP. By way of illustration, the present invention contemplates a kit comprising a probe set, comprising a plurality of oligonucleotide probes that
5 interrogate the rs1130214, rs456998, rs7211818 and rs1053639 SNPs, wherein said oligonucleotide probes make up at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80% or at least 90% of the oligonucleotide probes in the probe set.

In a particular embodiment, the kit includes a set of at least four oligonucleotide
10 probes, each oligonucleotide probe specific to one allele of the rs1130214, rs456998, rs7211818 and rs1053639 SNPs, wherein said oligonucleotide probes make up at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80% or at least 90% of the oligonucleotide probes in the probe set. In a preferred embodiment, the kit includes a set of four oligonucleotide probes, each specific to one
15 allele of the rs1130214, rs456998, rs7211818 and rs1053639 SNPs.

In a particular embodiment, the kit includes a set of at least four oligonucleotide pair probes, each oligonucleotide pair probe specific to one allele of the rs1130214, rs456998, rs7211818 and rs1053639 SNPs, wherein said oligonucleotide pair probes make up at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least
20 60%, at least 70%, at least 80% or at least 90% of the oligonucleotide probes in the probe set. In a preferred embodiment, the kit includes a set of four oligonucleotide pair probes, each specific to one allele of the rs1130214, rs456998, rs7211818 and rs1053639 SNPs.

In another aspect, the present invention relates to the use of a kit according to the
25 invention for predicting the onset of EPS induced by an antipsychotic-based treatment in a subject based on the sequence of the rs1130214, rs456998, rs7211818 and rs1053639 SNPs. The particulars of the kit according to the invention have been described in detail in the context of the kit of the invention and are applied with same meaning in the context of the uses of said kit.

30

Personalized therapies of the invention

The second method of the invention defined above also allows providing personalized therapies to patients suffering a disease treatable with antipsychotics. In particular, patients which are considered as having a high risk to develop EPS will most likely benefit from an antipsychotic-based therapy known to have a lower probability to induce EPS. Conversely, patients showing low risk to develop EPS may do with any antipsychotic-based therapy.

Thus, in another aspect, the invention relates to a low DRD2 blockade potency antipsychotic for use in the treatment of a disease treatable with antipsychotics in a subject (hereinafter referred to as "personalised therapy of the invention"), wherein said subject is selected according to the second method of the invention.

The particulars of the second method of the invention have been described in detail in the context of said second method of the invention, and are applied with same meaning in the context of the personalised therapy according to the invention.

The terms "subject", "antipsychotic", "treatment", "disease treatable with antipsychotics", and "low DRD2 blockade potency antipsychotic" and their particulars have been described in detail in the context of the first method of the invention and are used with the same meaning in the context of the personalised therapy according to the invention.

In a particular embodiment, the low DRD2 blockade potency antipsychotic is selected from the group consisting of clozapine, ziprasidone, quetiapine and olanzapine.

In another particular embodiment, the low DRD2 blockade potency antipsychotic-based therapy additionally comprises an adjuvant antiparkinsonian. In a more preferred embodiment, the adjuvant antiparkinsonian is an anticholinergic. The terms "adjuvant" and "antiparkinsonian" and their particulars have been described in detail in the context of the second method of the invention and are used with the same meaning in the context of the personalised therapy of the invention.

Uses of the invention

In another aspect, the invention relates to the use of the rs1130214, rs456998, rs7211818 and rs1053639 SNPs for predicting the onset of EPS induced by an

antipsychotic-based treatment in a subject (hereinafter referred to as the “first use of the invention”).

In a particular embodiment of the first use of the invention, the presence of one allelic combination according to Table 1 is indicative that that there is a high risk of the
5 subject to develop EPS.

In another particular embodiment of the first use of the invention, the presence of one allelic combination according to Table 2 is indicative that there is a low risk of the subject to develop EPS.

In another aspect, the invention relates to the use of the rs1130214, rs456998,
10 rs7211818 and rs1053639 SNPs for selecting a subject suffering from a disease treatable with antipsychotics to receive a low DRD2 blockade potency antipsychotic-based therapy (hereinafter referred to as the “second use of the invention”).

In a particular embodiment of the second use of the invention, the subject is selected to receive a low DRD2 blockade potency antipsychotic-based therapy if the
15 presence of one allelic combination according to Table 1 is detected in the sample.

In another aspect, the invention relates to the use of the rs1130214, rs456998, rs7211818 and rs1053639 SNPs for selecting a suitable antipsychotic-based therapy to treat a subject suffering from a disease treatable with antipsychotics (hereinafter referred to as the “third use of the invention”).

20 In a particular embodiment of the third use of the invention, the presence of one allelic combination according to Table 1 is indicative that said subject is selected to receive a low DRD2 blockade potency antipsychotic-based therapy.

In another particular embodiment of the first use of the invention, the presence of one allelic combination according to Table 2 is indicative that the subject is selected to
25 receive any antipsychotic-based therapy.

The terms and particulars of the first, second and third methods of the invention have been described in detail in the context of the methods of the invention and are used with the same meaning in the context of the uses according to the invention.

The invention is detailed below by means of the following examples which are merely illustrative and by no means limiting for the scope of the invention.

EXAMPLES

5

Materials and methods

Subjects

Sample size calculations assuming a 5% level of significance, 80% power and a
10 EPS risk (odds ratio) of 2 associated with carrying the studied alleles (allelic
frequencies > 0.1). Calculations were performed with Quanto1.2 software
(<http://hydra.usc.edu/gxe>).

A cohort of 321 psychiatric inpatients receiving antipsychotic therapy was recruited
consecutively in the Psychiatry Service of the Hospital Clínic (Barcelona, Spain) over a
15 period of three years (2002-2004). 241 subjects of this cohort participated in the
pharmacogenetic study of EPS presented here. Subjects were diagnosed according to
DSM-IV criteria: 184 subjects were diagnosed with schizophrenia (n=125) and related
disorders (n=22 schizoaffective disorder; n=27 acute psychotic disorder; n=9 delusional
paranoid disorder; n=1 schizotypal disorder); 40 were diagnosed as having bipolar
20 disorders; and 17 had other diagnoses (including personality disorder, psychotic
depression, behavior disorder, mild cognitive impairment and obsessive-compulsive
disorder). Acute EPS induced by antipsychotic medication was evaluated using the
Simpson-Angus Scale⁴⁸. 69 patients presenting EPS (Simpson-Angus >3) during the
hospitalization period and/or a history of movement disorders were considered as cases.
25 172 patients without EPS (Simpson-Angus ≤3) at the time of the study or previously
were taken as controls. A full description of these populations can be found in previous
studies (Gassó et al., 2009, cited *supra*; Mas et al., 2012, *Pharmacogenomics J* 12:255–
9). Written informed consent and whole blood samples were obtained from each
subject. The study was approved by the Ethics Committee of the Hospital Clínic.

30 To obtain independent data sets for prediction construction and predictor
evaluation, the data set was split into a training population, and a validation population.
To this end, division was made according to antipsychotic treatment: a cohort of

patients treated with risperidone (n=114, 39 cases and 75 controls) (cohort 1) was used to train the data and test the best predictor; a second cohort (n=127, 30 cases and 97 controls) (cohort 2) with the rest of patients treated with other antipsychotic different than risperidone (haloperidol n=27; clozapine n=24; amisulpride n=3; olanzapine n=34; 5 zuclopentixol n=6; ziprasidone n=10; quetiapine n=22) was used to validate the predictor. As a final step the authors of the present invention tested the predictor in the whole cohort, referred as cohort 3 (n=241) (cohort1+cohort3).

SNPs selection and Genotyping

10 SNPs of the different nine genes participating in the regulation of mTOR pathway genes were selected according to the following strategy: first, a literature research was conducted using several databases (PubMed, Ensembl, Genetic Association Database, SZGene, PDGene, AlzGene, MSGene) to find SNPs in those genes which were associated with mental disorders; second, if no SNP could be found, SNPs associated 15 with other diseases were looked for since this association could mean a functional change in the protein; third, if for some gene no SNP could be selected from the previous steps, a search was performed using PupaSuit database to detect SNPs with predicted functionality; finally the type (coding synonymous, coding non-synonymous, intron, mRNA utr) and frequency of the SNP were checked with the NCBI's SNP 20 database (<http://www.ncbi.nlm.nih.gov/snp>). A frequency lower than 10% lead to exclusion. The selected SNPs are specified in Table 3.

Polymorphisms (Table 3) were detected with real-time PCR by TaqMan allelic discrimination pre-designed assays (TaqMan-SNP genotyping assays; probe C_32127211_20 to detect rs7874234, probe C_3282533_10 to detect rs13335638, probe 25 C_11647371_10 to detect rs2024627, probe C_26352825_10 to detect rs1130214, probe C_31167105_10 to detect rs456998, probe C_8701299_10 to detect rs1801582, probe C_2747617_30 to detect rs3737597, probe C_1971465_10 to detect rs7211818, probe C_9596692_10 to detect rs1053639), from Applied Biosystems according to the manufacturer's guidelines (Applied Biosystems, Foster City, California).

30

Table 3: List of selected genes of mTOR pathway and SNPs with corresponding alleles and functionality information, together with the p-value for the Hardy-Weinberg

equilibrium, the allelic frequencies in cases (EPS) and controls (No-EPS), and the p-value of the allelic association analysis adjusted by sex and age in Cohort 1.

Gene	SNP	Allele ¹	Functionality related to	H-W ²	EPS ³	No- EPS ⁴	p- value ⁵
TSC1	rs7874234	<u>C</u> /T	Increased transcription	0.307	0.26	0.23	0.605
TSC2	rs13335638	<u>C</u> /T	High conservative region	0.301	0.25	0.23	0.595
mTOR	rs2024627	T/ <u>C</u>	High conservative region	0.326	0.26	0.24	0.825
AKT1	rs1130214	G/ <u>T</u>	Increased transcription	0.541	0.33	0.35	0.859
FCHSD1	rs456998	<u>G</u> /T	Cisplatin-induced cytotoxicity	0.357	0.45	0.49	0.797
PARK2	rs1801582	C/ <u>G</u>	Sporadic Parkinson's disease	0.587	0.20	0.22	0.664
DISC1	rs3737597	C/ <u>T</u>	Schizophrenia	1.0	0.04	0.02	0.422
Raptor	rs7211818	A/ <u>G</u>	Bladder cancer risk	0.601	0.28	0.19	0.091
DDIT4	rs1053639	<u>A</u> /T	Regulatory region	0.708	0.44	0.42	0.812

¹Underlined the associated allele.

²p-value for the Hardy-Weinberg equilibrium in cases and controls.

5 ³Allele frequency in cases (EPS).

⁴Allelic frequency in controls (No-EPS).

⁵After bonferroni correction, significant pvalue<0.005.

Statistics

10 To estimate the independent contribution of each SNP to EPS risk, genotype frequencies were assessed by means of multivariate methods based on logistic regression analysis, using the SNPassoc R package. Also, the SNP data were checked for any departure from Hardy-Weinberg equilibrium in both populations, cases and controls. Then gene-gene interactions were analyzed by multifactor dimensionality reduction (MDR), as described elsewhere, using the MDR 2.0 software available at the
15 open-source MDR project (www.epistasis.org/software.html). First, using Cohort 1 all possible SNP combinations were constructed by testing all possible two- to four-loci interactions using 10-fold cross-validation in an exhaustive search (the sample was split

in ten parts, using nine parts to train the data, and one part to test. The process was repeated ten times, using each time a different part to test). As outcome parameters, the authors of the present invention considered cross-validation consistency (measures the consistency of the identification of the variations selected on the basis of the best model applied to computation during the 10-fold cross-validation), testing balanced accuracy (measures the degree to which interaction accurately predicts case-control status and is the mean of the testing performed in the 10-fold cross-validation (label 1 indicates good prediction by the model; label 0.5 indicates that the model was no better than chance in selecting cases from controls) and statistical significance (the p-value of the best model was corrected for multiple testing by 10,000 permutations with the MDR Permutation Testing Module 1.0). Second, a new multilocus attribute with the best model obtained was created. This is the model with the best outcome parameters described above. The new attribute was constructed and re-analyzed in order to calculate the statistics in the Cohort 1 whole dataset, obtaining the odds-ratio and its confidence interval, p-value, sensitivity (TP/TP+FN, measures the ability to correctly predict EPS cases), specificity (TN/TN+FP, measures the ability to correctly reject no-EPS controls), accuracy (TP+TN/TP+TN+FP+FN, measures the ability to correctly predict EPS and no-EPS patients) and precision (TP/TP+FP, measure the truly predicted EPS cases). Thirdly, the constructed attribute was validated in Cohort 2, and Cohort 3 obtaining the same statistics as in cohort 1 whole dataset; odds-ratio and its confidence interval, p-value, sensitivity, specificity, accuracy and precision.

Results

None of the nine SNPs studied alone contributes significantly to the risk of EPS when were tested in Cohort 1. Table 3 summarizes the allelic frequencies in cases and controls, the logistic regression analysis adjusted by sex and age, and also the p-value for the Hardy-Weinberg equilibrium.

The results of the exhaustive MDR analysis are given in Table 4. A four-way model including rs1130214 (AKT1), rs456998 (FCHSD1), rs7211818 (Raptor) and rs1053639 (DDIT4) variants had the best overall performances (testing accuracy 0.660) and a cross-validation consistency of 10/10 (permutation testing $p < 0.0001$).

Table 4: Results of the multifactor dimension reduction analysis used in Cohort 1 showing the best model of all possible one- to four-loci interactions and their outcome parameters, including training and testing accuracy, 10-fold cross-validation consistency and model p-value.

Model	Training Accuracy ¹	Testing Accuracy ²	CVC ³	p-value ⁴
rs456998	0.602	0.514	5/10	>0.05
rs1130214 rs456998	0.656	0.525	6/10	>0.05
rs1130214 rs456998 rs1053639	0.725	0.550	6/10	>0.05
rs1130214 rs456998 rs7211818 rs1053639	0.837	0.660	10/10	<0.0001

5 ¹Accuracy in the 10-fold training dataset.

²Accuracy in the 10-fold testing dataset.

³10-fold cross-validation consistency.

⁴p-value of the best model corrected by 10,000 permutations.

10 A multilocus attribute with the four SNPs identified was constructed and tested in the whole dataset of cohort 1. Two types of attributes were identified, i.e. predisposing (Table 1) and non-predisposing (Table 2). Carriers of the predisposing attribute (78.9 % of cases vs. 11.8% of controls) were 27 times more likely to suffer EPS than those without the attribute (OR 27.91; 95% CI 9.81-79.39; p-value<0.0001). The constructed
 15 predisposing genetic attribute correctly predicts 97 of 114 patients (85,1% accuracy), including 30 of 39 cases with EPS (76.9% sensitivity), and 67 of 75 controls without EPS (89.3% specificity). The attribute predicted 38 cases, 30 true positives and 8 false positives (78.9% precision) (Table 5).

20 Table 5: Whole dataset statistics after the application of predictors in Cohort 1, Cohort 2 and Cohort 3.

	Cohort 1	Cohort 2	Cohort 3
Accuracy	0.85	0.80	0.84
Sensitivity	0.76	0.70	0.85

Specificity	0.89	0.90	0.83
Precision	0.78	0.70	0.67

As testing a predictor in the same population used to training the data is overoptimistic, the authors of the present invention used cohort 2 to independently validate the constructed attribute. When the attribute was tested in the whole data set of cohort 2, accuracy decreased to 73.23%. As cohort 2 was formed by patients treated with different types of antipsychotic (in contraposition to cohort 1, which includes only patients in treatment with risperidone), a new variable was included in the model to account for this variability. Antipsychotics were categorized according to their potency to blockade DRD2. When this new variable (antipsychotic potency) was added to the predisposing genetic attribute identified in cohort 1, accuracy in cohort 2 increased up to 80.36% (109 patients correctly predicted of 127) (Table 5); carriers of the new predisposing attribute (70.0 % of cases vs. 9.27% of controls) were 22 times more likely to suffer EPS than those without the attribute (OR 22.81; 95% CI 8.06-64.50; p-value<0.0001).

The new predictor that includes the four SNPs (identified in cohort 1) and the antipsychotic potency (included in the analysis in cohort 2) was validated in cohort 3 (cohort 1 plus cohort 3). Carriers of the predisposing attribute (85.5 % of cases vs. 16.3% of controls) were 30 times more likely to suffer EPS than those without the attribute (OR 30.24; 95% CI 13.86-66.39; p-value<0.0001). As could be observed in Table 3 similar values of accuracy (203 cases correctly predicted of 241), sensitivity (59 of 69 cases with EPS correctly predicted) and specificity (149 controls without EPS of 172 correctly predicted) were obtained with cohort 3. Precision was diminished in cohort 3 respect cohort1 (from 78.95% to 67.82%).

CLAIMS

1. Method for predicting the onset of extrapyramidal symptoms (EPS) induced by an antipsychotic-based treatment in a subject, comprising
 - 5 i) determining the sequence of the rs1130214, rs456998, rs7211818 and rs1053639 single nucleotide polymorphisms (SNPs) in a sample comprising genetic material from said subject, and
 - ii) predicting the risk of said subject to develop EPS based on the sequence of said SNPs.
- 10 2. Method according to claim 1, wherein the presence of one allelic combination according to Table 1 is indicative that there is a high risk of the subject to develop EPS, or wherein the presence of one allelic combination according to Table 2 is indicative that there is a low risk of the subject to develop EPS.
- 15 3. Method for selecting a subject suffering from a disease treatable with antipsychotics to receive a low DRD2 blockade potency antipsychotic-based therapy, comprising
 - 20 i) determining the sequence of the rs1130214, rs456998, rs7211818 and rs1053639 SNPs in a sample comprising genetic material from said subject, and
 - ii) selecting said subject to receive a low DRD2 blockade potency antipsychotic-based therapy based on the sequence of said SNPs.
- 25 4. Method according to claim 3, wherein the subject is selected to receive a low DRD2 blockade potency antipsychotic-based therapy if the presence of one allelic combination according to Table 1 is detected in the sample.
- 30 5. Method for selecting a suitable antipsychotic-based therapy to treat a subject suffering from a disease treatable with antipsychotics, comprising

- i) determining the sequence of the rs1130214, rs456998, rs7211818 and rs1053639 SNPs in a sample comprising genetic material from said subject, and
 - ii) selecting a suitable antipsychotic-based therapy based on the sequence of said SNPs,
- 5 wherein said antipsychotic is selected from the group consisting of a low DRD2 blockade potency antipsychotic-based therapy and any antipsychotic-based therapy.
6. Method according to claim 5, wherein the presence of one allelic combination according to Table 1 is indicative that said subject is selected to receive a low
10 DRD2 blockade potency antipsychotic-based therapy, and wherein the presence of one allelic combination according to Table 2 is indicative that the subject is selected to receive any antipsychotic-based therapy.
- 15 7. Method according to any of claims 5 or 6, wherein the any antipsychotic-based therapy is selected from the group consisting of a low DRD2 blockade potency antipsychotic, a medium DRD2 blockade potency antipsychotic and a high DRD2 blockade potency antipsychotic.
- 20 8. Method according to any of claims 3 to 7, wherein the low DRD2 blockade potency antipsychotic-based therapy additionally comprises an adjuvant antiparkinsonian, preferably an anticholinergic.
9. Kit comprising reagents suitable for determining the sequence of the rs1130214,
25 rs456998, rs7211818 and rs1053639 SNPs, wherein said reagents comprise DNA or RNA probes.
10. Use of a kit according to claim 9 for predicting the onset of EPS induced by an antipsychotic-based treatment in a subject based on the sequence of the rs1130214,
30 rs456998, rs7211818 and rs1053639 SNPs.
11. Low DRD2 blockade potency antipsychotic for use in the treatment of a disease treatable with antipsychotics in a subject, wherein said subject is selected by a

method according to claims 3 or 4 or wherein the subject is characterized in that a sample containing genetic material isolated from said subject shows one allelic combination according to Table 1 in the rs1130214, rs456998, rs7211818 and rs1053639 single nucleotide polymorphisms (SNPs).

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12. The low DRD2 blockade potency antipsychotic for use in the treatment of a disease treatable with antipsychotics according to claim 11, wherein said treatment additionally comprises an adjuvant antiparkinsonian, preferably an anticholinergic.

10 13. Use of the rs1130214, rs456998, rs7211818 and rs1053639 SNPs for predicting the onset of EPS induced by an antipsychotic-based treatment in a subject, for selecting a subject suffering from a disease treatable with antipsychotics to receive a low DRD2 blockade potency antipsychotic-based therapy, or for selecting a suitable antipsychotic-based therapy to treat a subject suffering from a disease treatable with antipsychotics

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14. Use according to claim 13, wherein the presence of one allelic combination according to Table 1 is indicative that there is a high risk of the subject to develop EPS, or wherein the presence of one allelic combination according to Table 2 is
20 indicative that there is a low risk of the subject to develop EPS, or wherein the subject is selected to receive a low DRD2 blockade potency antipsychotic-based therapy if the presence of one allelic combination according to Table 1 is detected in the sample, or wherein the presence of one allelic combination according to Table 1 is indicative that said subject is selected to receive a low DRD2 blockade
25 potency antipsychotic-based therapy, or wherein the presence of one allelic combination according to Table 2 is indicative that the subject is selected to receive any antipsychotic-based therapy.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP2014/051369

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 and necessary to the claimed invention, the international search was carried out on the basis of:
- a. (means)
- on paper
- in electronic form
- b. (time)
- in the international application as filed
- together with the international application in electronic form
- subsequently to this Authority for the purpose of search
2. In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2014/051369

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
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, 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	DATABASE GeO [Online] 23 June 2008 (2008-06-23), Illumina: "Illumina HumanHap550-Duov3 Genotyping BeadChip (HumanHap550-2v3_B)", XP002695524, retrieved from NCBI Database accession no. GPL6982 the whole document	9
X	WO 2009/092032 A1 (SUREGENE LLC [US]; BRENNAN MARK DAVID [US]; RAMSEY TIMOTHY LYNN [US]) 23 July 2009 (2009-07-23) examples 2-7 ----- -/--	11,12

Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents :

<p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier application or patent but published on or after the international filing date</p> <p>"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)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p>	<p>"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</p> <p>"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</p> <p>"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</p> <p>"&" document member of the same patent family</p>
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Date of the actual completion of the international search 17 February 2014	Date of mailing of the international search report 26/02/2014
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Cornelis, Karen

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2014/051369

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	B ALMOGUERA ET AL: "Association of common genetic variants with risperidone adverse events in a Spanish schizophrenic population", THE PHARMACOGENOMICS JOURNAL, vol. 13, no. 2, 3 January 2012 (2012-01-03), pages 197-204, XP055059603, ISSN: 1470-269X, DOI: 10.1038/tpj.2011.57 the whole document	1-14
A	----- WO 2011/148379 A2 (HADASIT MED RES SERVICE [IL]; LERER BERNARD [IL]; GREENBAUM LIOR [IL];) 1 December 2011 (2011-12-01) examples 4-8 -----	1-14

INTERNATIONAL SEARCH REPORT

Information on patent family members

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			WO	2009092032 A1	23-07-2009

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