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ORIGINAL ARTICLE

Pharmacogenetic study of antipsychotic induced acute extrapyramidal symptoms in a first episode psychosis cohort: role of dopamine, serotonin and glutamate candidate genes

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This study investigated whether the risk of presenting antipsychotic (AP)-induced extrapyramidal symptoms (EPS) could be related to single-nucleotide polymorphisms (SNPs) in a naturalistic cohort of first episode psychosis (FEP) patients. Two hundred and two SNPs in 31 candidate genes (involved in dopamine, serotonin and glutamate pathways) were analyzed in the present study. One hundred and thirteen FEP patients (43 presenting EPS and 70 non-presenting EPS) treated with high-potency AP (amisulpride, paliperidone, risperidone and ziprasidone) were included in the analysis. The statistical analysis was adjusted by age, gender, AP dosage, AP combinations and concomitant treatments as covariates. Four SNPs in different genes (*DRD2*, *SLC18A2*, *HTR2A* and *GRIK3*) contributed significantly to the risk of EPS after correction for multiple testing ($P < 1 \times 10^{-4}$). These findings support the involvement of dopamine, serotonin and glutamate pathways in AP-induced EPS.

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INTRODUCTION

Antipsychotics (AP) use in schizophrenia, although effective in treating positive symptoms, is limited by their adverse effects. First generation AP are typically defined by their adverse effect profiles with marked extrapyramidal symptoms (EPSs). Conversely, second generation antipsychotics (SGAs) have been associated with metabolic side effects.[1] However, recent clinical trials and meta-analyses have shown that, although SGAs cause fewer EPSs than first generation AP, differences in EPS risk are less marked than originally thought.[1,2]

EPSs are serious, debilitating and stigmatizing adverse effects that frequently require additional pharmacotherapy.[3] The two types of EPSs are as follows: early acute EPS, which most often develops at the start of therapy or after a dose increase; and late-onset EPS, which presents as tardive dyskinesia (TD) after prolonged treatment. Acute EPSs include akathisia (restlessness and pacing), acute dystonia (sustained abnormal postures and muscle spasms, particularly of the head and neck) and parkinsonism (tremor, muscle rigidity and bradykinesia). Although these side effects usually respond to dose reduction or to additional pharmacological treatment, they are a major cause of poor adherence to AP treatment.[3]

Several risk factors for EPSs have been described, including the following: the type and dose of AP or the combination of different APs; age (younger age for acute dystonia and older age for parkinsonism) and gender (male for acute dystonia, but female for parkinsonism); personal history of substance abuse or a family history of movement disorders; and disease-related factors such as cognitive deficits and early onset.[3] In addition, there is an association between EPS and genetic markers in candidate genes related to neurotransmission. This includes dopaminergic,[4–10] serotonergic[11–16] and more recently glutamatergic[17–20] transmission, although no single factor has been able to predict EPSs.[21] Three non-hypothesis driven genome-wide association studies in patients with AP-induced EPSs were published that revealed different genetic variants.[22–24] These still need confirmation.

To date, analyses have largely been conducted in patients with chronic schizophrenia and may not be applicable to patients with first episode psychosis (FEP), given that these patients have little or no prior AP exposure, tend to be treated with lower AP doses, and appear to be more sensitive to developing EPSs.[25] Therefore, the main aim of this study was to investigate whether the risk of presenting EPS could be related to genetic polymorphisms in candidate dopaminergic, serotonergic and glutamatergic genes in a naturalistic cohort of FEP patients.

MATERIALS AND METHODS

Sample

The PEPs study (phenotype – genotype and environmental interaction. Application of a predictive model in first psychotic episodes) was a multicenter, prospective, longitudinal, naturalistic, follow-up study

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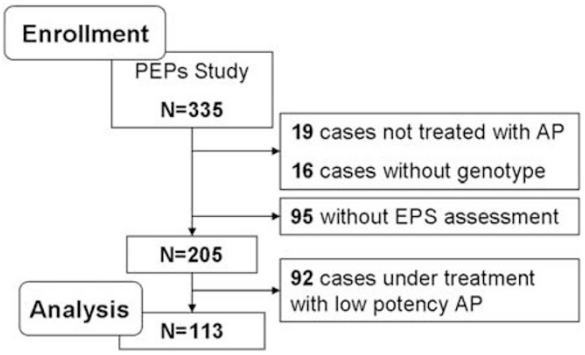

Figure 1. Consolidated Standards of Reporting Trials flow diagram.

designed to evaluate the clinical, neuropsychological, neuroimaging, biochemical and genetic variables.[26] It uses a sample of 335 patients with FEP. Participants were recruited from 16 centers in Spain.

The inclusion criteria for patients were: age between 7 and 35 years, presence of first psychotic symptoms (positive symptoms or disorganization) of at least 1 week duration in the last 12 months and speak Spanish correctly. The exclusion criteria for patients were: (1) mental retardation according to DSM-IV criteria,[27] (2) history of head trauma with loss of consciousness and (3) presence of an organic disease with mental repercussions.

As this was a naturalistic study, there were no specific guidelines for treatments (drugs and/or psychotherapy). Treatment with AP did not exceed 12 months at study entry. All scales included in the PEPs project protocol, except those self-administered, were administered by expert clinicians. The rationale for these criteria and the complete clinical protocol used in the PEPs project was previously published elsewhere.[26]

Figure 1 summarizes the Consolidated Standards of Reporting Trials flow diagram of the present study. SGAs are a heterogeneous group, with different potency to induce EPS. In patients treated with low-potency SGAs, the effect of genetic polymorphisms in the appearance of EPS could be masked (patients with a high-risk genotype for EPS will not suffer EPS if treated with APs that rarely induced this side effect). For this reason, in order to avoid the confounding effect of AP potency and to obtain a more homogenous group, 113 patients treated with high-potency SGAs (amisulpride, paliperidone, risperidone and ziprasidone) were included and 92 patients treated with low-potency SGAs (aripiprazole, clozapine, olanzapine and quetiapine) were excluded. The distinction between low- and high-potency SGAs was based on the findings of a recently published meta-analysis.[1]

The study was approved by the investigation ethics committees of all participating clinical centers. Informed consent was obtained from all participants. For participants aged 18 years or younger, parents or legal guardians gave written informed consent before their participation in the study, and they consented to participate themselves. A specific informed consent form was completed for the genetic investigation.

Extrapyramidal symptoms assessment

In order to assess in detail the adverse drug reactions, two procedures were followed: (a) spontaneous reports of Adverse Drug Reactions; (b) systematic assessment of the effects targeted (like metabolic syndrome, cardiotoxicity or EPSs) from physical examination (electrocardiogram, antipsychotic plasmatic levels and general blood tests) and two scales administrated in every follow-up visit (baseline, 2 and 6 moths): the Scale of the Udvalg for Kiniske Undersogelser,[28] a comprehensive rating scale designed to assess general side effects of psychotropic drugs; and the Simpson – Angus Scale,[29] included to evaluate the extrapyramidal side effects. Investigators also reported any specific treatment or change in the prescription due to Adverse Drug Reactions appearance, including antipsychotic discontinuation, dose reduction or start of an anticholinergic drug.

According to our previous studies,[5,6,8] EPSs were considered present when Simpson – Angus Scale values were >3 during the observational period (at baseline and at 2 and 6 months) or EPSs were recorded as a spontaneous adverse reaction in the pharmacovigilance database during the observational period (at baseline and at 2 and 6 months). Patients without EPS (Simpson – Angus Scale <3 or without reporting EPS during the observational period) were taken as controls.

Calculations of prescribed daily dose

After the inclusion of the patient in the PEPs project, all psychotropic drugs prescribed to every patient were recorded, independently of the dose and separating different formulations of the same substance. Thus a number expressing the sum of concomitant prescriptions for each treatment day were obtained. The prescribed daily dose for a drug was defined as the daily dose of a drug formulation, oral or injectable, calculated separately for each treatment day of an individual patient, who were treated with this particular drug formulation for at least 3 consecutive days (irrespective of the dose). Different formulations of the same drug were separated. The prescribed daily dose of Long Acting Injectable Antipsychotics was calculated by dividing the given dose by the number of days until the next depot injection. In order to compare the different AP between them, the prescribed daily doses of AP were converted to an estimated equivalent amount of chlorpromazine following the international consensus.[30] Baseline polypharmacy was registered considering simultaneous treatment in the same patient with one antipsychotic together with an antidepressant, an anticholinergic drug, a mood stabilizer, a benzodiazepine or another antipsychotic used at the same time.

The pharmacological information showed in the present study was recorded as follows: for those patients presenting EPS, we showed the treatment and dosage under use in the moment that the EPS were recorded; for those patients without EPS, we showed the treatment with the AP with higher potency at the higher dosage used during the observational period.

Candidate genes selection

Candidate genes were included in the present study if: (1) they belong to one of the pathways selected for the present study (dopamine, serotonin or glutamate neurotransmission) including neurotransmitter receptors and downstream signaling proteins, transporters and genes involved in neurotransmitter metabolism; and (2) they have been associated with AP response in previous association studies, mainly with AP–induced movement disorders (EPS and TD), but also with AP effectiveness or other AP side effects. We also included two genes associated with AP-induced movement disorders through genome-wide association studies. Supplementary Table S1 included the complete gene list and the rationale for its inclusion in the present study.

Single-nucleotide polymorphism selection, genotyping and quality control

Two hundred and two single-nucleotide polymorphisms (SNPs) (Supplementary Table S2) were selected in candidate gene regions (covering target loci and upstream and downstream regions) following one of the following three strategies: (1) tagging analysis (as implemented in Haploview 4.2) at an r^2 threshold of 0.8 to capture 98% of the most common HapMap phase II variants based on the CEU panel (minor allele frequency >0.1) (range 91–100% for individual genes); (2) suspected SNP functionality according to data published in Ensembl (http://www.ensembl.org), dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/) and PupaSuite 3 (http://pupasuite.bioinfo.cipf.es/) databases, with a validated minor allele frequency >0.1 in the Caucasian population; or (3) a previous association reported in the literature, either with FEP or other psychiatric diseases, when the statistical power was sufficient to achieve the reported odds ratio or relative risk. When tagging strategies using HapMap provided >15 tagged SNPs, selection followed strategies (2) and (3). SNPs were genotyped by the GoldenGate assay with the Veracode genotyping system (Illumina, San Diego, USA) at the Madrid Node of the Spanish National Genotyping Center (CeGen).

Single-nucleotide polymorphism and haplotype analysis

To estimate the independent contribution of each SNP to the disease susceptibility, genotype frequencies were assessed by multivariate logistic regression analyses via the SNPassoc R package.[31] Each model was adjusted by age, gender, AP dosage, AP combinations and concomitant treatments as covariates. Hardy–Weinberg equilibrium, linkage disequilibrium (LD) and haplotype block structures were evaluated by Haploview software v.3.2 (http://broad.mit.edu/mpg/haploview). To estimate the significance of the best result for the single SNP-based and haplotype-

based analyses 10 000 permutations were performed (as implemented in SNPassoc R package). According to the permutation test, significance level was adjusted at 4×10^{-4}. However, as four genetic models were tested (codominant, dominant, recessive and overdominant), we divided the empirical *P*-value by four, leading a significance threshold of $1x10^{-4}$.

Interaction analysis using entropy-based measures

We used entropy-based measures of information gain (IG) to test the SNP × SNP interaction and the interaction with risk factors related to EPS (age, gender, AP dosage, AP combinations and concomitant treatments). Only those SNPs achieving a *P*-value $<4\times10^{-4}$ (the empirical *P*-value obtained after permutation test) were included in this analysis. Entropy is a measurement of the uncertainty of a random variable, or a measurement of the dispersion, as, for example, the variance.[32] The joint entropy is the amount of uncertainty (information) that a variable (SNP or EPS risk factor) provides about other (EPS status). The IG is the information that two variables considered jointly (SNP and/or EPS risk factor) provided about other (EPS status). Entropy-based IG was estimated for individual attributes (SNP main effect) and each pairwise combination of attributes (that is, SNP × SNP or SNP × EPS risk factors). Pairs of attributes were sorted, and those with the highest IG, or percentage of entropy removed, were selected. The algorithms for entropy-based measures of IG were implemented in the Orange machine learning software package version 2.7 (http://orange.biolab.si/download/).[32]

Statistical analysis

Sample size and statistical power were calculated using Quanto1.2 software (http://hydra.usc.edu/gxe). Given the sample size, and assuming a 5% level of significance, we were able to detect odds ratio values of >3.0 with >70% statistical power when polymorphisms with allele frequencies of >0.1 were analyzed. Statistical analyses were performed in SPSS version 17 (SPSS Inc., Chicago, Ill, USA). Normal distributions of the data were confirmed using Shapiro – Wilk test, and equality of the variance between groups was assessed by means of Levene's test. For comparing two groups, a two-tailed Student's *t* test was used.

RESULTS

The demographic, clinical and pharmacological data of the participants are summarized in Table 1. The cases (EPS) and controls (non-EPS) were evenly distributed within each cohort in terms of gender, smoking habits, substance abuse, ethnicity and diagnosis. Some differences existed in age, AP dosage (daily AP dosage was calculated as the Chlorpromazine Equivalent Daily Dose) and the use of other treatments, although only age reached statistical significance. In the statistical analysis, those variables that could be related to the appearance of EPS, such as age, gender, AP dosage, AP combinations and concomitant treatments were used as covariates.

Of the 202 SNPs studied, four (rs9567733 (*HTR2A*), rs363341 (*SLC18A2*), rs1334802 (*GRIK3*) and rs1124491 (*DRD2*)) contributed significantly and independently to the risk of EPS after correction for multiple testing (significance threshold $P<1\times10^{-4}$) (Supplementary Table S2 include a summary of the statistics for each SNP included in the analysis). Table 2 summarizes the genotype models and statistics of the significant associations. None of the haplotypes constructed for each gene were significantly associated with EPS; nor were the haplotype blocks that included SNPs significantly associated with these diseases.

We tested and computed the entropy-based measures of IG for each SNP with empirical *P*-values $<4\times10^{-4}$ (rs1334802 (*GRIK3*), rs7544500 (*HSPG2*), rs363341 (*SLC18A2*), rs1124491 (*DRD2*), rs9567733 (*HTR2A*) and rs8045712 (*GRIN2A*)) (Supplementary Table S2), and for the IG of each pairwise SNP × SNP interaction and SNP × risk factor interaction. We included the following risk factors: age, gender, AP dosage, AP combinations and concomitant treatments. Figure 2a shows the five SNPs with IG > 2.0% (rs1334802 (*GRIK3*), rs7544500 (*HSPG2*), rs363341 (*SLC18A2*), rs1124491 (*DRD2*) and rs8045712 (*GRIN2A*)) and, also the interaction with AP dosage, the only risk factor showing IG > 2.0%.

Table 1. Demographic and clinical characteristics of participants

	No EPS	*EPS*	
N	70	43	
Gender, male (%)	46 (65.7)	32 (74.4)	$X^2_1=0.94$, $P=0.40$
Age, mean (s.d.)	24.4 (6.6)	21.9 (6.1)	$t_{111}=1.95$, $P=0.05$
Ethnicity			
Caucasian, *N* (%)	59 (84.3)	39 (90.7)	$X^2_1=0.21$, $P=0.64$
Diagnostic			
Affective, *N* (%)	12 (17.1)	4 (9.3)	$X^2_1=0.21$, $P=0.64$
Non-affective, *N* (%)	58 (82.9)	39 (90.7)	
Toxic habits			
Tobacco, *N* (%)	45 (64.2)	23 (53.5)	$X^2_1=1.29$, $P=0.32$
Alcohol, *N* (%)	26 (37.1)	14 (32.5)	$X^2_1=0.25$, $P=0.69$
Cannabis, *N* (%)	26 (37.1)	18 (41.9)	$X^2_1=0.25$, $P=0.68$
Cocaine, *N* (%)	6 (8.6)	4 (9.3)	$X^2_1=0.01$, $P=1.00$
Sedative agents, *N* (%)	9 (12.8)	3 (7.0)	$X^2_1=0.97$, $P=0.53$
Psycho stimulants, *N* (%)	2 (2.8)	2 (4.6)	$X^2_1=1.91$, $P=0.38$
Antipsychotic[a]			$X^2_4=6.13$, $P=0.19$
Amisulpride, *N* (%)	6 (8.6)	1 (2.3)	$X^2_1=1.67$, $P=0.19$
Paliperidone, *N* (%)	17 (24.3)	6 (13.9)	$X^2_1=1.39$, $P=0.23$
Risperidone, *N* (%)	42 (60.0)	30 (69.7)	$X^2_1=0.39$, $P=0.52$
Risperidone LAI, *N* (%)	4 (5.7)	6 (13.9)	$X^2_1=2.04$, $P=0.15$
Ziprasidone, *N* (%)	1 (1.4)	0 (0.0)	$X^2_1=0.61$, $P=0.43$
Antipsychotic dose, mean (s.d.)[b]	625.2 (464.2)	756.8 (452.2)	$t_{111}=1.47$, $P=0.14$
Antipsychotic combination, *N* (%)	27 (38.5)	17 (39.5)	$X^2_1=0.10$, $P=0.91$
Other treatments, *N* (%)	48 (68.5)	24 (55.8)	$X^2_1=1.87$, $P=0.22$
Lithium, *N* (%)	5 (7.1)	1 (2.3)	$X^2_1=1.22$, $P=0.26$
Antiepileptic, *N* (%)	9 (12.8)	5 (11.6)	$X^2_1=0.03$, $P=0.84$
Antidepressant, *N* (%)	14 (20.0)	6 (13.9)	$X^2_1=0.69$, $P=0.41$
Anxiolitic, *N* (%)	32 (45.7)	17 (39.5)	$X^2_1=0.44$, $P=0.52$
Acute extrapyramidal symptoms			
Parkinsonism, *N* (%)	—	26 (60.4)	
Acute dystonia, *N* (%)	—	6 (13.9)	
Akathisia, *N* (%)	—	7 (16.3)	
Undefined, *N* (%)	—	4 (9.3)	
SAS total, mean (s.d.)	1.6 (2.0)	6.4 (5.4)	$t_{111}=6.02$, $P<0.001$
Other adverse reactions (%)	16 (22.8)	4 (9.3)	$X^2_1=2.76$, $P=0.09$

Abbreviations: AP, antipsychotics; EPS, extrapyramidal symptoms; LAI, long acting injection; SAS, Simpson – Angus Scale . [a]For those patients treated with an AP combination, the AP with the higher Chlorpromazine Equivalent Daily Dose value is listed. [b]For those patients treated with an AP combination, the sum of the Chlorpromazine Equivalent Daily Dose of each AP in the combination is calculated.

We tested if protein – protein interactions existed between the genes that were significantly associated with EPS. To this end, we used the STRING database (http://string-db.org/) (Figure 2b).

According to the PupaSuite software (http://pupasuite.bioinfo.cipf.es), rs1124491 (*DRD2*) could introduce changes in splicing regulation. In addition, the PupaSuite software notes that the rs7544500 (*HSPG2*), which is located in the 5′ region of the gene, could introduce changes in the transcription factor binding site.

DISCUSSION

In the present study, we found several significant associations between AP-induced acute EPS in FEP patients and gene variants directly related to dopamine (*DRD2* and *SLC18A2*), serotonin (*HTR2A*) and glutamate neurotransmission (*GRIK3*). In addition,

Table 2. Genotype analysis of the four significantly ($P < 1 \times 10^{-4}$) associated with AP-induced EPS

SNP	*No EPS*	*%*	*EPS*	*%*	*OR*	*95% CI*	P-*value*
rs1334802 (GRIK3)							
A/A	59	84.3	28	65.1	1.00		6×10^{-5}
A/G	11	15.7	15	34.9	2.87	1.17 – 7.06	
rs363341 (SLC18A2)							
C/C -C/T	68	97.1	37	86.0	1		5×10^{-5}
T/T	2	2.9	6	14.0	5.51	1.10 – 28.70	
rs1124491 (DRD2)							
G/G	48	70.6	37	90.2	1		1×10^{-4}
A/G-AA	20	29.4	4	9.8	0.26	0.08 – 0.82	
rs9567733 (HTR2A)							
A/A	37	52.9	16	38.1	1.00		4×10^{-5}
A/G	28	40.0	19	45.2	1.57	0.69 – 3.59	
G/G	5	7.1	7	16.7	3.24	1.56 – 11.75	

Abbreviations: AP, antipsychotics; CI, confidence interval; EPS, extrapyramidal symptoms; OR, odds ratio; SNP, single-nucleotide polymorphism. For each SNP, the best genotype model (dominant, codominant, overdominant or recessive) is shown. Each model was adjusted by age, gender, AP dosage, AP combinations and concomitant treatments as covariates.

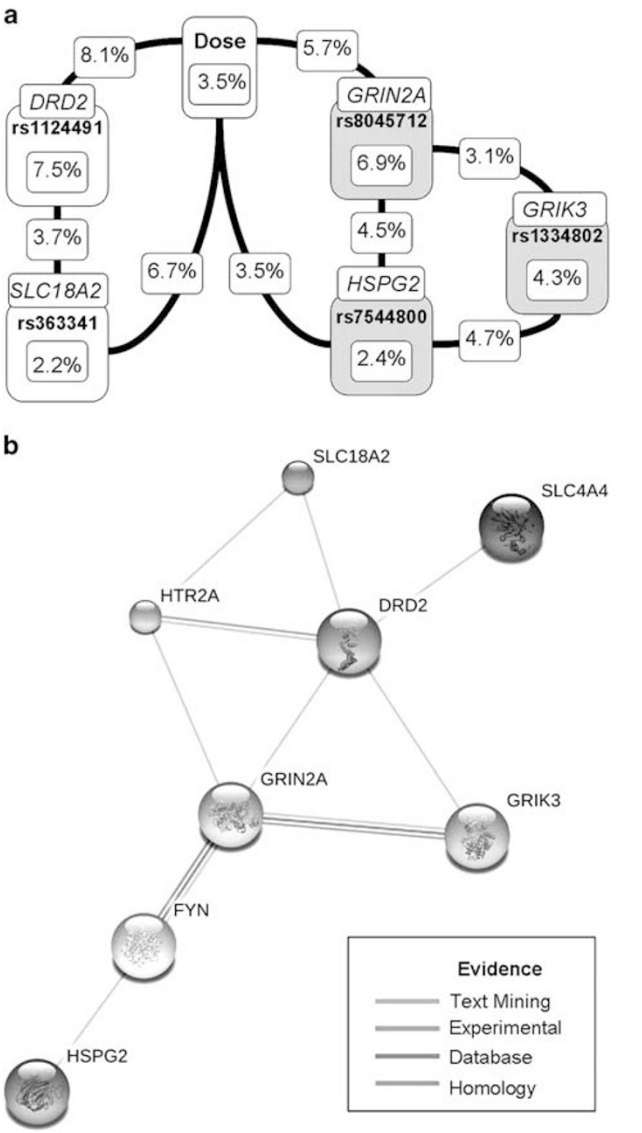

Figure 2. (**a**) Interaction graph of the five single-nucleotide polymorphisms (SNPs) showing gene × gene and gene × dosage interactions with entropy-based measures of information gain > 2%. The figure shows the entropy values removed by each SNP and dosage (main effect) and each pairwise comparison (gene × gene and gene × dosage). (**b**) Protein – protein interactions among the seven genes with significant associations with AP-induced EPS, according to the STRING database.

we identified an interaction among genes related to dopamine (*DRD2* and *SLC18A2*) and glutamate (*GRIK3*, *GRIN2A* and *HSPG2*) with AP dosage.

The antidopaminergic effects of APs are thought to be their main mechanism of action, and dopamine D2 receptor blockade is a property of all known AP. Most pharmacogenetic studies to date have examined the 3' Taq1A polymorphism (rs1800497) in *DRD2*. Possibly due to LD at another site (or sites) within *DRD2*, the minor T allele (that is, the A1 allele) at rs1800497 has also been associated with a 40% reduction in striatal D2 receptor density based on both *in vitro* assays and *in vivo* imaging studies. However, previous studies have reported inconsistent findings; although some have indicated that the A1 allele conferred susceptibility to EPSs,[9,11,33] other authors have been unable to replicate the results.[4,6,10,12,34,35] Moreover, several authors identified A2, rather than A1, as a risk allele for TD.[36,37] In our study, non-significant results were obtained with the Taq1A polymorphism (rs1800497). However, rs1124491, the significant SNP in *DRD2* associated with EPS in our study, is in high LD with rs1800497 and could be the functional SNP that is in LD with rs1800497, and could therefore explain the observed association between rs1800497 and *DRD2* density. Differences in LD patterns between both SNPs in different populations could explain the controversial results described so far.

The *SLC18A2* gene encodes the vesicular monoamine transporter 2 a target of the inhibitor tetrabenazine, which is used for the treatment of a number of hyperkinetic movement disorders, including TD.[38] The rs2015586 marker in the *SLC18A2* gene was the top finding in a large association study of 128 candidate genes associated with TD in the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) sample.[39] Recently, Zai *et al.*[40] found a significant association between *SLC18A2* tag SNPs and TD, as well as significant interactions between these polymorphisms and a functional polymorphism in *DRD2*.[40] Our study is the first to identify genetic variants in *SLC18A2* as susceptibility markers of EPS, and confirms the statistical interaction with *DRD2* observed in TD.

Several studies have focused on polymorphisms in *HTR2A*, with a particular emphasis on the functional −1438A/G SNP in the promoter region of the gene. This is in high LD with SNP 102T/C and has been demonstrated to influence promoter activity. Another frequently studied polymorphism in *HTR2A* is His452Ty. Regarding EPS, only one study identified a significant association with the 102C allele,[12] whereas several authors have failed to identify any significant association between *HTR2A* variants and EPS.[41-43] The SNP associated with the development of EPS in our sample (rs9567733) is localized in the 3′ end of the gene in a haplotype block that influences serotonin transporter binding potential.[44]

Recently, the glutamate system has emerged as a key factor in the pathophysiology of AP-induced movement disorders. Genome-wide association mapping of inbred mice treated with haloperidol identified candidate glutamate receptor genes (*Grin1* and *Grin2a*) involved with EPS and the direct target of AP, *Drd2*.[45] In the CATIE sample, a number of glutamate system genes, namely *GRM7*, *GRM8* and *GRIA3*, showed a statistical trend for association

with TD.[39] In a larger sample from Siberia ($n = 574$), the authors reported that the *GRIN2A* rs1345423 marker was associated with TD.[46] Although this result was partially confirmed in a Caucasian sample ($n = 207$), the observed association did not survive multiple testing corrections.[42] *GRIK3*, another gene for glutamate receptors, was also associated with AP-induced EPS in the present study. Two studies have failed to report associations using functional polymorphisms.[17,20] To our knowledge, this is the first study that has identified *GRIK3* genetic variants as being associated with the development of EPSs.

A recent genome-wide association screening of TD in Japanese patients with schizophrenia implicated the rs2445142 marker in the heparan sulfate proteoglycan 2 (*HSPG2*) gene.[47] Additional gene expression analyses in human post-mortem brains and a rodent model revealed that the risk allele was significantly associated with increased *HSPG2* expression, which may protect against the development of TD via a cholinergic or basic fibroblast growth factor mediated neuroprotective mechanism.[47] These initial findings with *HSPG2* were more recently supported by a re-analysis of the CATIE genome-wide dataset, as well as an Israeli Jewish sample.[48] However, negative results were also obtained in independent populations.[42] According to HapMap data, both the rs7544500 SNP associated with EPS in the present study, and the rs2445142 marker related to TD in genome-wide association studies are in high LD; this could explain the observed associations of rs2445142 with increased *HSPG2* expression.

Finally, we also tested if gene × gene interactions existed among the genes with significant associations with EPS. We identified strong interactions between genes related to dopamine (*DRD2* and *SLC18A2*), which is consistent with recent literature.[40] We also observed strong interactions between glutamate receptors (*GRIN2A* and *GRIK3*) and the *HSPG2* gene. Dopamine interactive genes and glutamate interactive genes showed no interactions between each other, but both systems interacted with dosage. As a result, we obtained a six-factor interaction, including significant SNPs in *DRD2*, *SLC18A2*, *GRIN2A*, *GRIK3* and *HSPG2*, as well as dosage. To give biological plausibility to the statistical interaction identified in our study, we confirm that these genes conform to an interactive network. However, these interactions were mostly identified by the literature and without experimental evidence of the interaction in the database.

Our study has a number of limitations. The main one is the sample size, which limits its statistical power and makes it difficult to detect small or modest effects of common variants. We achieved sufficient statistical power to identify a number of significant associations; however, it was not sufficient for us to state conclusively that the genes that did not yield significant results made no contribution to EPS, because the discrete odds ratios for association of their SNPs may not have been identified in our small sample. It should be noted that our sample comprised patients with FEP, representing a homogeneous clinical population from a relatively small number of sites integrated in a research network. In addition, the sample selected for these analyses only included patients treated with high-potency SGAs and controlled for several confounding factors such as age, gender, dosage, AP combinations and concomitant treatments. Another limitation is that our study used a candidate gene strategy. A disadvantage of this approach is that the objects of the study are limited by our current understanding of the molecular mechanisms involved in the pathology of FEP; therefore, this method cannot identify hitherto unsuspected predictor genes.[49] Moreover, among the selected genes, our study was limited by our current knowledge of their genetic variability that is available in public databases. Given our incomplete knowledge of the pathophysiology of EPS, other candidate genes could be considered in the pathways selected in this study and elsewhere.[49] Finally, we did not consider any pharmacokinetic variables in our study because, as we have demonstrated previously,[50] clinicians practice intuitive pharmacogenetics with dose titration strategies, which masks the effect of genetic variability in drug-metabolizing enzymes on EPS risk when naturalistic cohorts are studied.

In conclusion, and taking into account the study limitations, we have identified several genetic associations with the risk of developing AP-induced EPS. Specifically, we have confirmed the involvement of dopamine, serotonin and glutamate pathways. Further studies are needed to confirm the clinical applicability of our data.

CONFLICT OF INTEREST

AA has received a trainee research staff grant awarded by the University of Barcelona (APIF-UB grants). AB has received grants from the Spanish Ministry of Science and Innovation (FIS). AL has received grants from the Spanish Ministry of Science and Innovation (FIS). AM has received financial support to attend meetings, travel support and served as a speaker from Otsuka and Janssen-Cilag. EV has received grants and served as consultant, advisor or CME speaker for the following entities: AstraZeneca, Bristol-Myers Squibb, Ferrari, Forest Research Institute, Gideon Richter, GlaxoSmithKline, Janssen, Lundbeck, Otsuka, Pfizer, Roche, Snafu-Aventis, Servier, Shire, Sunovion, Takeda, the Brain and Behaviour Foundation, the Spanish Ministry of Science and Innovation (CIBERSAM), the Seventh European Framework Programme (ENBREC) and the Stanley Medical Research Institute. FC has received financial support to attend meetings, travel support and served as advisor or speaker for the following entities: Lilly, Janssen-Cilag, Lundbeck, Otsuka, the Spanish Ministry of Science and Innovation (CIBERSAM) and the Ministry of Science (Carlos III Institute). IB has received grants from CIBERSAM, Fundación Alicia Koplowitz and Institute de Salad Carlos III, and has received honoraria as a speaker for Janssen, as well as support from Otsuka for attending some conferences. MB has been a consultant for, received grant/research support and honoraria from, and been on the speakers/advisory board of ABBiotics, Adamed, Almirall, AMGEN, Eli Lilly, Ferrari, Forum Pharmaceuticals, Gideon, Hersill, Janssen-Cilag, Lundbeck, Otsuka, Pfizer, Roche and Servier, and has obtained research funding from the Spanish Ministry of Health, the Spanish Ministry of Science and Education, the Spanish Ministry of Economy and Competitiveness, Centro de Investigación Biomédica en Red de Salud Mental (CIBERSAM), by the Government of Catalonia, Secretaria d'Universitats i Recerca del Departament d'Economia i Coneixement, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS) and the 7th Framework Program of the European Union. MB has been a consultant for, received grant/research support and honoraria from, and been on the speakers/advisory board of Adamed, Ferrer, Janssen-Cilag, Lundbeck, Otsuka and Pfizer. The remaining authors declare no conflict of interest.

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