Association of common genetic variants with risperidone adverse events in a Spanish schizophrenic population

B Almoguera1,2, R Riveiro-Alvarez1,2, J Lopez-Castroman3,4, P Dorado4,5, C Vaquero-Lorenzo2,6, J Fernandez-Piqueras2,6, A Llerena4,5, F Abad-Santos7,8, E Baca-Garcia3,5,9, R Dal-Re1,10,11, and C Ayuso1,2,11, Spanish Consortium of Pharmacogenetics Research in Schizophrenia12

1Department of Genetics and Genomics, CAIBER Unit, IIS-Fundación Jiménez Díaz, Madrid, Spain; 2CIBERER ISCIII, Madrid, Spain; 3Department of Psychiatry, IIS-Fundación Jiménez Díaz, Madrid, Spain; 4Department of Pharmacology, Universidad Autónoma de Madrid, CBMSO, CSIC, Madrid, Spain; 5Department of Clinical Pharmacology, CAIBER Unit, Hospital Universitario de la Princesa, Instituto de Investigación Sanitaria Princesa, Madrid, Spain; 6Department of Clinical Pharmacology, Universidad Autónoma de Madrid, CBMSO, CSIC, Madrid, Spain; 7Department of Biology, Universidad Autónoma de Madrid, CBMSO, CSIC, Madrid, Spain; 8Department of Preventive Medicine, Public Health and Medical Immunology and Microbiology, School of Health Sciences, Rey Juan Carlos University, Madrid, Spain

Correspondence:
Dr C Ayuso, Department of Genetics and Genomics, IIS-Fundacion Jimenez Diaz –CIBERER, Avda. Reyes Catolicos 2, Madrid 28040, Spain.
E-mail: cayuso@fjd.es

11These authors have contributed equally to this work.

12See Appendix.

Received 6 June 2011; revised 31 August 2011; accepted 7 November 2011; published online 3 January 2012

Risperidone non-compliance is often high due to undesirable side effects, whose development is in part genetically determined. Studies with genetic variants involved in the pharmacokinetics and pharmacodynamics of risperidone have yielded inconsistent results. Thus, the aim of this study was to investigate the putative association of genetic markers with the occurrence of four frequently observed adverse events secondary to risperidone treatment: sleepiness, weight gain, extrapyramidal symptoms and sexual adverse events. A series of 111 schizophrenia inpatients were genotyped for genetic variants previously associated with or potentially involved in risperidone response. Presence of adverse events was the main variable and potential confounding factors were considered. Allele 16Gly of ADRB2 was significantly associated with a higher risk of sexual adverse events. There were other non-significant trends for DRD3 9Gly and SLC6A4 S alleles. Our results, although preliminary, provide new candidate variants of potential use in risperidone safety prediction.

The Pharmacogenomics Journal (2013) 13, 197–204; doi:10.1038/tpj.2011.57; published online 3 January 2012

Keywords: pharmacogenetics; antipsychotic; risperidone; adverse events

Introduction

Since 1959 when Vogel coined the term pharmacogenetics, there is increasing evidence that therapeutic outcome is influenced genetically.1 One of the most important applications might be schizophrenia management because of the high prevalence of this illness (1% approximately across countries and cultures) and the non-compliance often seen with the antipsychotic treatment.2 Risperidone is a widely prescribed antipsychotic for the treatment of schizophrenia, with a relatively high rate of non-effectiveness or intolerable side effects. In fact, extensive research has been devoted to elucidate the genetic underpinnings of risperidone response to reduce its adverse effects.3
conflicting results, so no definitive genetic marker predictive of risperidone response exists currently.

Thus, the aim of this study was to examine the putative association of several genetic markers related to risperidone pharmacokinetics and pharmacodynamics or potentially involved, with four of the most frequent and discomforting adverse events secondary to risperidone treatment (sleepiness, weight gain, EPS and sexual dysfunction) in a sample of acutely ill schizophrenic inpatients.

Subjects and methods

Subjects
As a part of a larger pharmacogenetic schizophrenia project, 111 acutely ill schizophrenic patients were recruited at the Department of Psychiatry of Fundacion Jimenez Diaz Hospital between 2004 and 2009. All subjects were adult patients hospitalized in an Acute Psychiatric Unit, unrelated and Caucasian. DSM-IV diagnosis was obtained by means of a brief structured psychiatric interview, the Spanish version of the Mini International Neuropsychiatric Interview version 4.4 (MINI 4.4). The study was approved by the Research Ethics Committee of Fundacion Jimenez Diaz Hospital and conducted according to the tenets of the Declaration of Helsinki. All participants signed an informed consent form after the explanation of the study objective and procedures.

Clinical data
All subjects were inpatients with variable length of hospital stay. They were all treated with risperidone. Treatment dosage was established according to patient’s clinical state. Concurrent treatments, such as other antipsychotics, anticholinergics, antidepressants, antiepileptics or benzodiazepines, were used. Sex, age, risperidone dosages, length of hospitalization and concomitant treatments were considered as potential confounding factors for the association with the genetic variants. The main variables, sleepiness, weight gain, EPS and sexual adverse events, were assessed with the UKU scale at the hospital discharge by psychiatrists trained in the UKU evaluation.

Variant selection and genotyping
Genomic DNA was extracted from 7 ml of peripheral blood samples using an automatic DNA extractor (BioRobot EZ1, Qiagen, Hilden, Germany). Genes, variants and the rationale for the selection are summarized in Table 1. All genetic variants investigated were either previously related to risperidone outcome or potentially involved (Table 1). Some of them were included in PHARMAChip, a commercially available tool whose suitability in pharmacogenetic genotyping has been previously described, and others were determined by allelic discrimination or sequencing (Table 1). Allelic discrimination was performed using Taq-Man Pre-Designed SNP genotyping assays (Applied Biosystems, Foster City, CA, USA) in a LightCycler 480 (Roche Diagnostics, Mannheim, Germany). Sequencing was used to genotype BDNF Val66Met and to verify the truthfulness of the allelic discrimination results with a BigDye Terminator Cycle Sequencing Kit and an ABI Prism 3130x1 DNA sequencer (Applied Biosystems). Primer sequences and conditions are available upon request.

Data and statistical analysis
All genetic variants were tested for Hardy–Weinberg equilibrium (HWE) deviation using Pearson Chi-squared test (χ²). The association of the genetic variants with the dependent variable was performed through the codominant genetic model in all cases except CYP2D6, whose genotypes were grouped based on the number of functional alleles into poor metabolizers (two defective alleles), intermediate metabolizers (one defective allele), extensive metabolizers (two functional alleles) and ultra rapid metabolizers (more than two functional alleles), according to a previous report by Gaedigk et al.

The presence of all four adverse events (sleepiness, weight gain, EPS and sexual adverse events), notwithstanding their severity, was the main variable. EPS category consisted of the UKU items akathisia, hyperkinesia, hypokinesia, rigidity or tremor. The sexual adverse event category included the following items: diminished sexual desire, dry vagina and erectile, ejaculatory and orgasmic dysfunctions. For both EPS and sexual adverse effects, ‘presence’ was considered as the occurrence of at least one of the signs or symptoms.

For continuous variables, mean and standard deviations were calculated and also data distributions were tested for normality. In case of non-adjustment, they were categorized for the statistical analysis.

Logistic regression was used to examine the association of the genotypes with the presence of the adverse events, adjusting for covariates. The strength of the association was measured with the odds ratio (OR) and its 95% CI (confidence interval). The effect of the genetic variant on the outcome was adjusted by those demographic and clinical variables (covariates) either associated with the outcome (P<0.05) or modifying the risk (change in putative OR>20%) of the genetic variant on the outcome. Hence, the effect of the covariates on the risk to develop the adverse events was also investigated.

Because of the multiple testing, Bonferroni correction was applied and the cutoff value was set as P<0.003 (P=0.05/19 hypothesis tested).

Statistical analysis was carried out using the STATA v10 software (Stata Corp, TX, USA).

Results

Clinical and genetic data
None of the continuous variables were normally distributed, so they were categorized for the analysis as shown in Table 2. Age of the patients (45±13 years) was grouped into <40 years, 40–59 years and ≥60 years; and length of hospital stay (13±9 days) was transformed considering the median of data distribution (12 days).

Patients were prescribed oral (mean dosage = 8.1±3.3 mg) and intramuscular (25–100 mg biweekly) risperidone. 73% (n=81) were taking oral risperidone, 6.3% intramuscular
(n = 7) and 20.7% were taking both forms (n = 23). Risperidone dosages were grouped according to the terciles of data distribution (11 missing data), if administered orally, and in three categories in the case of intramuscular administration (2 missing data). Concomitant treatments were classified according to the therapeutic group (Table 2).

All genetic variants investigated were in HWE except ADRB2 16Gly (P = 0.002). When grouping CYP2D6 according to the number of defective alleles, 3 subjects were classified as poor metabolizers and 3 as ultra rapid metabolizers (0.294), 32 as intermediate metabolizers (0.314) and 64 as extensive metabolizers (0.628) (there were 9 subjects, which genotype was missing). For the X chromosome HTR2C −759C>T variant, CC accounted for 84 subjects (0.764) and allele T was present in 26 patients (0.236) (1 genotype was missing). Genotype frequencies of the remaining variants are summarized in Table 3. In one sample, no genotyping results for the PHARMAChip variants could be obtained because of the low quality of the DNA. Additionally, there were 5 missing genotypes for SLC6A4, also included in the array. For variants determined by allelic discrimination, there were 4 missing genotypes for ADRB1 −4884G>A, 9 for BDNF Val66Met and 3 in case of HTR1A −1019C>G.

Regarding the four adverse events investigated, the most frequent was sleepiness in 66 patients (56.7%), weight gain and EPS were observed in 40 patients each (36%) and 25 patients had sexual adverse events (22.5%).

### Table 1 Genetic variants included in the pharmacogenetic study

| Gene       | Gene name              | Genetic variant | Function                          | Previous associations ref.                   |
|------------|------------------------|-----------------|------------------------------------|--------------------------------------------|
| **Pharmacokinetics** |                        |                 |                                    |                                            |
| CYP2D6⁧  | Cytochrome P450 2D6    | *1, *2, *3, *4, *5, *6, *7, *8, *9, *10, *11, *14, *15, *17, *19, *20, *25, *26, *29, *30, *31, *35, *40, *41, *1XN, *2XN, *4XN, *10XN, *17XN, *35XN, *41XN | Risperidone metabolism | Risperidone plasma levels, effectiveness and adverse events¹⁸,¹⁹ |
| CYP3A4⁧  | Cytochrome P450 3A4    | *1B             | Risperidone metabolism             | Risperidone plasma levels and effectiveness²⁰ |
| MDR1⁧    | Multidrug resistance 1 | 3435C>T         | Risperidone active transporter     | Risperidone induced adverse events and response²¹,²² |
| **Pharmacodynamics** |                        |                 |                                    |                                            |
| ADRA1ᵇ   | Adrenergic receptor α1 | −4884A>G        | Risperidone target                 | Atypical antipsychotic response (negative findings)²³ |
| ADRB2ᵃ   | Adrenergic receptor β2 | Arg389Gly       | Members of the adrenergic pathway   | Potentially involved in risperidone response |
| BDNFᵇ    | Brain-derived neurotrophic factor | Arg16Gly       | Related to serotonin pathway        | Risperidone effectiveness and adverse events²⁴ |
| COMTᵇ    | Catechol-o-Methyl transferase | Val108Met     | Member of dopaminergic pathway      | Risperidone effectiveness²⁵ |
| DRD2ᵃ    | Dopamine receptor D2   | Taq1A           | Risperidone target                 | Risperidone effectiveness and adverse events²⁶ |
| DRD3ᵇ    | Dopamine receptor D3   | Ser9Gly         | Risperidone target                 | Risperidone effectiveness and adverse events²⁶ |
| GRIN2Bᵇ  | Glutamatergic receptor 2B | 2667C>T       | Member of glutamatergic pathway     | System previously associated to risperidone outcome²⁸ |
| HTR1Aᵇ   | Serotonin receptor 1A  | −1019C>G        | Member of serotoninergic pathway    | Effectiveness (negative findings)²⁶ |
| HTR2Aᵇ   | Serotonin receptor 2A  | 102C>T          | Risperidone target                 | Effectiveness and adverse events²⁷,²⁹ |
| HTR2Cᵇ   | Serotonin receptor 2C  | −759C>T         | Risperidone target                 | Effectiveness and adverse events²⁷ |
| HTR6ᵇ    | Serotonin receptor 6   | 267T>C          | Risperidone target                 | Effectiveness and adverse events²⁷ |
| SLC6A4ᵃ  | Serotonin transporter  | Promoter VNTR   | Member of serotoninergic pathway    | Effectiveness³⁰ |

Abbreviation: VNTR, variable number tandem repeat.

*Genotyped with PHARMAChip genotyping array.

ᵇAllelic discrimination or sequencing.
Genetic associations with adverse events

When the genotype significantly associated with the outcome was the heterozygous one, dominant genetic model was also evaluated. The only association that remained significant after Bonferroni correction was that of ADRB2 16Gly as a risk factor for developing risperidone-induced sexual adverse events (Table 4). Furthermore, as shown in Table 4, there were also some non-significant trends: DRD3 9Gly was present with a higher incidence in those patients who developed risperidone-induced sleepiness and, conversely, it appeared less frequently in patients with EPS. Additionally, allele S of SLC6A4 was underrepresented in patients with weight gain (S/S) and also in those with sexual adverse events (L/S).

Besides considering the demographic and clinical variables as potential confounding factors, it was also evaluated whether they could influence the development of the four adverse events investigated. Only anticholinergic consumption was associated with a higher incidence of weight gain (OR = 3.28; 95% CI: 1.02–10.54; P = 0.046).

Discussion

The present study sought to evaluate the possible association of genetic variants related to risperidone pharmacokinetics and pharmacodynamics, and others with potential involvement, with the risk of developing four of the most frequent adverse events secondary to risperidone treatment. The investigation of the genetic basis of risperidone safety has traditionally focused on weight gain and EPS, considered as the most handicapping adverse effects.32 However, to our knowledge, no report has examined sleepiness or sexual adverse events secondary to risperidone treatment.

Table 2 Frequency of patients according to the demographic and clinical variables

| Variable                          | Category | n (%) |
|-----------------------------------|----------|-------|
| Sex                               | Males    | 62 (55.8) |
|                                   | Females  | 49 (44.2) |
| Age                               | <40 years | 38 (34) |
|                                   | 40–59 years | 61 (55) |
|                                   | ≥60 years | 12 (11) |
| Hospital stay                     | ≤12 days | 57 (62) |
|                                   | >12 days | 35 (38) |
| Oral risperidone dosages          | <6mg per day | 44 (47) |
|                                   | 6–9mg per day | 28 (30) |
|                                   | >9mg per day | 21 (23) |
| Intramuscular risperidone dosages | 0mg      | 81 (73) |
|                                   | <50mg per 2 weeks | 9 (8.1) |
|                                   | ≥50mg per 2 weeks | 19 (17.1) |
| Concomitant treatments            | Anticholinergics | 21 (21) |
|                                   | Antidepressants | 10 (9) |
|                                   | Antiepileptics | 12 (10.8) |
|                                   | Atypical antipsychotics | 7 (6.3) |
|                                   | Benzodiazepines | 54 (48.6) |
|                                   | Classical antipsychotics | 12 (10.8) |
|                                   | Lithium | 2 (1.8) |

There were missing data for hospital stay (19) and risperidone dosages (11 for oral and 2 for intramuscular).

Table 3 Allelic and genotypic frequencies of the genetic variants investigated

| Genetic variant | Allele (N(p)) | Genotype (N (p)) |
|-----------------|--------------|-----------------|
|                 | A            | B               | AA              | AB             | BB              |
| ADR1 -563C>T    | 123 (0.554)  | 99 (0.446)      | 33 (0.297)      | 57 (0.513)     | 21 (0.189)      |
| ADR1 -4155C>G   | 109 (0.491)  | 113 (0.509)     | 26 (0.234)      | 57 (0.514)     | 28 (0.252)      |
| ADR1 -4884A>G   | 167 (0.780)  | 47 (0.220)      | 65 (0.607)      | 37 (0.346)     | 5 (0.047)       |
| ADRB1 Arg381Gly | 58 (0.264)   | 162 (0.736)     | 10 (0.091)      | 38 (0.345)     | 62 (0.564)      |
| ADRB2 Arg16Gly  | 169 (0.768)  | 51 (0.232)      | 75 (0.682)      | 19 (0.173)     | 16 (0.145)      |
| BDNF Val108Met  | 161 (0.789)  | 43 (0.211)      | 65 (0.637)      | 31 (0.304)     | 6 (0.059)       |
| DRD2 Taq1A      | 116 (0.527)  | 104 (0.473)     | 29 (0.264)      | 58 (0.527)     | 23 (0.209)      |
| CYP3A4 *1B      | 116 (0.527)  | 104 (0.473)     | 6 (0.054)       | 104 (0.945)    | 0 (0.000)       |
| DRD2 Taq1A      | 181 (0.815)  | 41 (0.185)      | 75 (0.676)      | 31 (0.279)     | 5 (0.045)       |
| DRD3 Ser9Gly    | 147 (0.668)  | 73 (0.332)      | 48 (0.436)      | 51 (0.464)     | 11 (0.100)      |
| GRIN2B 2664C>T  | 174 (0.791)  | 46 (0.209)      | 69 (0.627)      | 36 (0.327)     | 5 (0.045)       |
| HTR1A -1019C>G  | 103 (0.477)  | 113 (0.523)     | 29 (0.268)      | 45 (0.417)     | 34 (0.315)      |
| HTR2A His452Tyr | 196 (0.891)  | 24 (0.109)      | 86 (0.782)      | 24 (0.218)     | 0 (0.000)       |
| HTR2C 102C>T    | 123 (0.559)  | 97 (0.441)      | 38 (0.345)      | 47 (0.427)     | 25 (0.227)      |
| HTR2C -759C>T   | 179 (0.821)  | 39 (0.179)      | 80 (0.734)      | 19 (0.174)     | 10 (0.092)      |
| HTR6 267C>T     | 188 (0.847)  | 34 (0.153)      | 81 (0.730)      | 26 (0.234)     | 4 (0.036)       |
| MDR1 3435C>T    | 113 (0.514)  | 107 (0.486)     | 34 (0.309)      | 45 (0.409)     | 31 (0.282)      |
| SLC6A4 L/S      | 126 (0.613)  | 86 (0.387)      | 38 (0.358)      | 50 (0.472)     | 18 (0.170)      |

A refers to wild-type allele and B to the mutant one. For HTR2C -759C>T AA = CC (females)+C (males); AB = CT (females) and BB = TT (females)+T (males).

N = Absolute frequency and P = relative frequency.
adverse events, which are reported by patients to be as frequent and discomfiting as the former.2

This pharmacogenetic study describes for the first time the association between ADRB2 16Gly and a greater susceptibility of developing sexual adverse events in schizo-

...phrenic patients under risperidone treatment, considering potential demographic and clinical confounding factors. The elevated frequency of sexual dysfunction among schizophrenia patients treated with antipsychotics has been reported to reduce patients’ quality of life.33 Even though the incidence of these adverse events can reach as much as 50% of patients and that usually lead to treatment non-

...ion androgens, adrenergic and serotonergic, involved in processes like erection, ejaculation and orgasm.34 Therefore, the disruption of any of these systems might lead to sexual side effects and would support our findings about ADRB2 16Gly as a risk factor, which has been linked to lower β2 adrenergic receptor density and efficiency.35 It should be noted that although ADRB2 16Gly did not meet HWE it was not removed from the study to prevent from the possibility of being out of HWE because of a linkage with the disease. All patients were ascertained by their disease status and so, the subject selection was not random, which is one of the premises of HWE for genotypic frequencies to reach a fixed value.

We are aware that genotyping errors should also be considered as a cause of HWE departure. In this regard, two relatively recent reports have demonstrated the accuracy of PHARMACHip in genotyping, one of them performed by our group.16,17 Nevertheless, our previously mentioned study also reports this variant deviating from HWE in the Spanish control population.16 However, it has been demonstrated that this typically occurs in several European populations, suggesting this variant is subject to selective forces, such as parental selection or epistasis, leading to oscillations in genotype frequencies.36

Therefore, considering all the above explanations, although preliminary, this result should be taken into account and be further validated in an independent sample.

Although ADRB2 16Gly was the only variant that met Bonferroni cutoff value, there were some non-significant trends that should be highlighted. Although multiple comparison adjustment is necessary in order to avoid type-I error, it significantly increases type II and therefore the likelihood of false negatives, and Bonferroni correction is especially conservative in this regard. The consequence of the adjustment is a decrease in the statistical power, which was already low because of the population sample size, and the increase in the rate of false negative findings. Indeed, for the association of the event with the higher incidence and the genotype with the higher frequency, it is only possible to detect, with 80% statistical power, ORs greater than 4 (or lower than 0.25), which is higher than expected. It is suggested that the risk that genetic variants confer to the complex phenotypes, such as risperidone response, range from low to moderate.37 In consequence, it would require substantially larger sample sizes to detect associations with lower ORs, for genotypes with lower frequencies or for adverse events with lower incidence. In any case, the study was performed through a candidate gene approach, where the rationale for the variant selection was to be linked to risperidone pharmacokinetic and/or pharmacodynamic pathways. Additionally, among all possible variants, we chose from the literature those with the higher likelihood of being involved in the occurrence of the outcomes, because of the existence of previous positive findings. Hence, given that the study was hypothesis-driven and due to the small sample size of the population included, the non-significant trends found should not be completely ruled out, but considered as exploratory findings that need to be further confirmed.

One of those non-significant trends was the higher incidence of sleepiness in patients carrying allele 9Gly of DRD3. DRD3 Ser9Gly variant has been extensively studied with regard to many antipsychotics’ effectiveness and safety, but with inconsistent results.38,39 In the present study, the higher propensity observed to sleepiness among patients with the DRD3 9Gly allele suggests that disruption of the dopaminergic neurotransmission might contribute to the sleep disturbances experienced by schizophrenic patients, as it is well established that dopaminergic system participates in the wake-sleep cycle.40 Conversely, the same allele was linked to a lower incidence of EPS. Although the atypical antipsychotics are known to be safer concerning motor side effects compared with the classical ones, risperidone treatment is known to have a relatively high rate of EPS.32

Table 4 Association of the genetic variants with the adverse events

| Adverse event (n (%)) | Genetic variant | Genotypic frequencies (n (%)) | OR (95% CI) | P |
|-----------------------|-----------------|-------------------------------|-------------|---|
| **Sexual adverse events** |                 |                               |             |   |
| (25 (22.5%)) ADRB2 16Gly | 14 (56%) | 21 (25%) | 4.58 (1.72–12.20) | 0.002* |
| (25 (22.5%)) SLC6A4 L/S | 6 (25%) | 44 (53.6%) | 0.22 (0.06–0.75) | 0.015 |
| (66 (56.7%)) DRRD3 9Gly | 40 (64.5%) | 22 (45.8%) | 2.47 (1.00–6.09) | 0.050 |
| (40 (36%)) DRRD3 9Gly | 18 (45%) | 44 (62.8%) | 0.29 (0.11–0.79) | 0.015 |
| (40 (36%)) SLC6A4 S/S | 1 (2.7%) | 17 (24.6%) | 0.07 (0.01–0.66) | 0.020 |

Significant findings (*, P<0.003) and non-significant trends (P<0.05) from the genetic association study after adjusting with the potential confounding variables.

*P<0.003.
Whereas data on the possible relevance of the Ser9Gly variant of DRD3 in EPS are still controversial, our study reports a trend to a protective effect of the 9Gly allele. That effect could be explained by the role that D3 receptors have in motor control, with their agonists exerting an inhibitory effect. This, together with the higher affinity and effectiveness that dopamine shows for D3 receptors with the 9Gly variant could be in line with the observed effect.

On the other hand, patients carrying the SLC6A4 S/S genotype were less likely to gain weight secondarily to risperidone treatment. Currently, there is an increasing concern regarding weight gain in schizophrenia patients, as it significantly impacts the antipsychotic compliance. Although the exact mechanism for antipsychotic-related weight gain remains unclear, the serotonergic system has emerged as a strong candidate. Specifically and based on the large body of evidence that supports the role of serotonin in regulating feeding behavior, variants in genes involved in serotonergic neurotransmission, such as HTR2A, HTR2C, HTR6 or BDNF, have been previously associated with weight gain secondary to risperidone treatment. Despite our failure to replicate the above-mentioned associations, the lower incidence of weight gain in SLC6A4 S/S carriers is in consonance with the recent report by Bah et al., who found this genotype to be more prevalent in underweight control individuals.

Further, the L/S genotype was observed to predispose patients to lower incidence of sexual adverse events. As previously mentioned, the serotonergic system participates in normal sexual function and so carrying the SLC6A4 short allele could protect patients from developing sexual dysfunction secondary to risperidone treatment.

Overall, none of the previous associations were replicated. Strikingly, variants in CYP2D6 and MDR1, which have been reported to influence risperidone plasma concentrations, did not yield significant results. CYP2D6 is involved in the hydroxylation of risperidone to 9-OH risperidone and there is increasing evidence about the correlation between the number of functional CYP2D6 alleles and both risperidone and 9-OH risperidone plasma concentrations. Similarly, MDR1, involved in risperidone transportation through the blood–brain barrier, has been several times associated with risperidone plasma concentrations, and safety. MDR1 encodes P-glycoprotein, a drug transporter that works limiting risperidone entry to the brain. The 3435T allele of MDR1 results in lower P-glycoprotein expression, which may result in higher brain risperidone concentrations. According to their role in risperidone pharmacokinetics, it was expected for CYP2D6 and MDR1 to impact significantly the development of adverse events. However, once again, the small sample size studied could have been responsible for the negative findings.

The non-replication and the lack of association found for most of the genetic variants investigated point out the complexity of risperidone response, which is determined by the interaction of several genetic and environmental factors and whose contribution to the antipsychotic response is difficult to establish. In addition, there are other factors, intrinsic to psychiatric patients management, and hence, to pharmacogenetic studies on antipsychotics, that makes complex the search of the genetic basis of drug response. Such factors are the uncertainty of the psychiatric phenotype, the variety of treatments administered in psychiatric patients and the large number of confounding factors affecting the antipsychotic response, what conforms a heterogeneous subject sample. All these factors, typically common to all pharmacogenetic studies on antipsychotics, together with the small sample size here investigated, could have been responsible for the negative findings yielded in this study and for the general inconsistencies of pharmacogenetic studies in schizophrenia. The consequence is the inexistence of a definitive predictive factor of risperidone response until date.

In conclusion, although further replication in an independent sample with adequate statistical power is needed, this study provides new candidate genetic variants of potential use in risperidone safety prediction. Additionally, it points out the importance adrenergic, dopaminergic and serotonergic disruption might have in the risk of developing four of the most handicapping adverse events secondary to risperidone treatment, namely sleepiness, weight gain, EPS and sexual adverse events.

Conflict of interest
The authors declare no conflict of interest.

Acknowledgments
We especially thank all patients for their participation in this study. We also thank Ignacio Mahillo for his exceptional contribution with the statistical data and also Laia Jofré and Estibaliz Olano for their kind help with the chip genotyping and its interpretation. This study was supported by Fondo de Investigación Sanitaria (FIS) EC07/90393, EC07/90466 and EC07/90604. Berta Almoguera’s work is supported by a Rio Hortega Grant from Instituto de Salud Carlos III. Pedro Dorado is supported by Instituto de Salud Carlos III-FIS and European Union (FEDER) Grant CP06/0030. The contribution from B Almoguera is coordinated in the frame of the Iberoamerican Network of Pharmacogenetics (http://www.ribef.org).

References
1 Meyer UA. Pharmacogenetics - five decades of therapeutic lessons from genetic diversity. Nat Rev Genet 2004; 5: 669–676.
2 Lieberman JA, Stroup TS, McEvoy JP, Swartz MS, Rosenheck RA, Perkins DO et al. Effectiveness of antipsychotic drugs in patients with chronic schizophrenia. N Engl J Med 2005; 353: 1209–1223.
3 de Leon J, Sandson NB, Cozza KL. A preliminary attempt to personalize risperidone dosing using drug-drug interactions and genetics: part I. Psychosomatics 2008; 49: 258–270.
4 Gasso P, Mas S, Bernardo M, Alvarez S, Parellada E, Lafuente A. A common variant in DRD3 gene is associated with risperidone-induced extrapyramidal symptoms. Pharmacogenomics J 2009; 9: 404–410.
5 Yasui-Furukori N, Mihara K, Takahata T, Suzuki A, Nakagami T, De Vries R et al. Effects of various factors on steady-state plasma concentrations of risperidone and 9-hydroxyrisperidone: lack of impact of MDR-1 genotypes. Br J Clin Pharmacol 2004; 57: 569–575.
6 Smith RC, Segman RH, Golcer-Dubner T, Pavlov V, Lerer B. Allelic variation in ApoC3, ApoA5 and LPL genes and first and second generation antipsychotic effects on serum lipids in patients with schizophrenia. Pharmacogenomics J 2008; 8: 228–236.
18 Novalbos J, Lopez-Rodriguez R, Roman M, Gallego-Sandin S, Ochoa D, et al. Fijal BA, Kinon BJ, Kapur S, Stauffer VL, Conley RR, Jamal HH et al. Efficacy of the alpha1A-adrenergic receptor gene and antipsychotic-induced parkinsonism: protective role of a functional polymorphism in the 3′-untranslated region. Pharmacogenomics J 2009; 9: 103–110.

21 Kuzman MR, Medved V, Bozina N, Hotujac L, Sain I, Bilusic H. Relationship between response to risperidone in African-American and white patients with schizophrenia. Pharmacogenomics J 2009; 9: 311–318.

22 Xing Q, Gao R, Li H, Feng G, Lin Z et al. Genetic variants in the BDNF gene and therapeutic response to risperidone in schizophrenia patients: a pharmacogenetic study. Eur J Hum Genet 2010; 18: 707–712.

25 Gupta M, Bhatnagar P, Grover S, Kaur H, Baghel R, Bhasin Y et al. Association studies of catechol-O-methyltransferase (COMT) gene with schizophrenia and response to antipsychotic treatment. Pharmacogenomics 2009; 10: 385–397.

26 Ikeda M, Yamanouchi Y, Kinoshita Y, Kitajima T, Yoshimura R, Hashimoto S et al. Variants of dopamine and serotonin candidate genes as predictors of response to risperidone treatment in first-episode schizophrenia. Pharmacogenomics 2008; 9: 1437–1443.

27 Correia CT, Almeida JP, Santos PE, Sequeira AF, Marques CE, Miguel TS et al. Pharmacogenetics of risperidone therapy in autism: association analysis of eight candidate genes with drug efficacy and adverse drug reactions. Pharmacogenomics J 2010; 10: 418–430.

28 Greenbaum L, Smith RC, Rigbi A, Strous R, Telsh O, Kanyas K et al. Further evidence for the association of the RGS2 gene with antipsychotic-induced parkinsonism: protective role of a functional polymorphism in the 3′-untranslated region. Pharmacogenomics J 2009; 9: 93–100.

29 Kim B, Choi EY, Kim CY, Song K, Joo YH. Could HTR2A T102C and DRD3 9 SerGly predict clinical improvement in patients with acutely exacerbated schizophrenia? Results from treatment responses to risperidone in a naturalistic setting. Hum Psychopharmacol 2008; 23: 61–67.

30 Vazquez-Bourgon J, Arranz MJ, Mata I, Pelayo-Teran JM, Perez-Iglesias R, Medina-Gonzalez L et al. Serotonin transporter polymorphisms and early response to antipsychotic treatment in first episode of psychosis. Psychiatry Res 2010; 175: 189–194.

31 Gaedigk A, Simon SD, Pearce RE, Bradford LD, Kennedy MJ, Leeder JS. The CYP2D6 activity score: translating genotype information into a qualitative measure of phenotype. Clin Pharmacol Ther 2008; 83: 234–243.

32 Kapur S, Remington G. Atypical antipsychotics: new directions and new challenges in the treatment of schizophrenia. Annu Rev Med 2001; 52: 503–517.

33 Dossenbach N, Dyackova Y, Pirilid S, Anders M, Khalil A, Araszkiewicz A et al. Effects of atypical and typical antipsychotic treatments on sexual function in patients with schizophrenia: 12-month results from the Intermontiational Schizophrenia Outpatient Health Outcomes (IC-SOHO) study. Eur Psychiatry 2006; 21: 251–258.

34 Liu-Seleht H, Kison BJ, Tensant C, Sniadecki J, Volavka J. Sexual dysfunction in patients with schizophrenia treated with conventional antipsychotics or risperidone. Neuropsychiatr Dis Treat 2009; 5: 47–54.

35 Ellsworth DL, Coady SA, Chen W, Sirivasan SR, Elkasabany A, Gustaf J et al. Influence of the beta2-adrenergic receptor Arg16Gly polymorphism on longitudinal changes in obesity from childhood through young adulthood in a biracial cohort: the Bogalusa Heart Study. Int J Obes Relat Metab Disord 2002; 26: 928–937.

36 Cagliani R, Fumagalli M, Pozzoli U, Riva S, Comi GP, Torri F et al. Diverse evolutionary histories for beta-adrenoceptor genes in humans. Am J Hum Genet 2009; 85: 64–75.

37 Daly AK. Genome-wide association studies in pharmacogenomics. Nat Rev Genet 2010; 11: 241–246.

38 Szkerekas G, Keri S, Juhasz A, Rimanoczy A, Szendi I, Czaummer C et al. Role of dopamine D3 receptor (DRD3) and dopamine transporter (DAT) polymorphism in cognitive dysfunctions and therapeutic response to atypical antipsychotics in patients with schizophrenia. Am J Med Genet B Neuropsychiatr Genet 2004; 128B: 1–5.

39 Xuan J, Zhao X, He G, Yu L, Wang L, Tang W et al. Effects of the dopamine D3 receptor (DRD3) gene polymorphisms on risperidone response: a pharmacogenetic study. Neuropsychopharmacology 2008; 33: 305–311.

40 Govila I. Underlying brain mechanisms that regulate sleep-wakefulness cycles. International review of neurobiology 2010; 93: 1–21.

41 Gunes A, Scordo MG, Jaarson P, Dahl ML. Serotonin and dopamine receptor gene polymorphisms and the risk of extrapyramidal side effects in perphenazine-treated schizophrenic patients. Psychopharmacology 2007; 190: 479–484.

42 Suzuki M, Hurd YL, Sokoloff P, Schwartz JC, Sedgall G. D3 dopamine receptor mRNA is widely expressed in the human brain. Brain Res 1998; 779: 58–74.

43 Klimesch-Petersen T, Ljung E, Svensson K. Effects on locomotor activity after local application of D3 preferring compounds in discrete areas of the rat brain. J Neurotransm Gen Sect 1995; 102: 209–220.

44 Lundstrom K, Turpin MP. Proposed schizophrenia-related gene polymorphism: expression of the Ser9Gly mutant human dopamine D3 receptor with the Semliki Forest virus system. Biochem Biophys Res Commun 1996; 225: 1608–1672.
Appendix. The following members, listed in alphabetical order, constitute the ‘Spanish Consortium of Pharmacogenetics Research in Schizophrenia’:

Group 1: Departments of Genetics and Genomics and Psychiatry IIS - Fundacion Jimenez Diaz: Berta Almoguera, Carmen Ayuso (Coordinator), Enrique Baca-Garcia, Rafael Dal-Re (Senior Research Fellow Department of Preventive Medicine, Public Health and Medical Microbiology, School of Health Sciences, Rey Juan Carlos University, Alcorcon, Madrid, Spain); Jorge Lopez-Castroman, Rosa Riveiro-Alvarez, Maria Jose Trujillo, Cristina Villaverde (Berta Almoguera: balmoguera@fjd.es, Rosa Riveiro-Alvarez: rriveiro@fjd.es, Carmen Ayuso: cayuso@fjd.es, Rafael Dal-Re: rafael.dalre@urjc.es, Enrique Baca-Garcia: eb2452@columbia.edu, Jorge Lopez-Castroman: jorgecastroman@gmail.com).

Group 2: Department of Biology, Universidad Autonoma de Madrid, CBMSO, Madrid Spain: Montserrat Diaz, Pablo Fernandez-Navarro (associated researcher-Cancer and Environmental Epidemiology Unit, National Centre for Epidemiology, CIBERESP ISCIII Madrid, Spain), Jose Fernandez-Piqueras (principal investigator), Concepcion Vaquero-Lorenzo, (Jose Fernandez-Piqueras: jfpiqueras@cbm.uam.es, Concepcion Vaquero-Lorenzo: kasiopea72@yahoo.es).

Group 3: Department of Clinical Pharmacology, Hospital Universitario de la Princesa, Madrid, Spain: Francisco Abad-Santos (principal investigator), Teresa Cabaleiro, Rosario Lopez-Rodriguez, Jesus Novalbos, Dolores Ochoa, Manuel Roman, (Francisco Abad-Santos: fabad.hlpr@salud.madrid.org).

Group 4: CICAB, Clinical Research Centre, Extremadura University Hospital and Medical School, Badajoz, Spain: Alfredo de la Rubia (Merida Psychiatric Hospital), Jesus Covaleda (CJ Primary Care Center, SES), Pedro Dorado, Adrian Llerena (principal investigator), Eva M Peñas Lledo, (Adrian Llerena: allerena@unex.es, Pedro Dorado: pdorado@unex.es).