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ORIGINAL ARTICLE

The *GRM7* gene, early response to risperidone, and schizophrenia: a genome-wide association study and a confirmatory pharmacogenetic analysis

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The search for biomarkers of response to antipsychotic medications is hindered by difficulties inherent in the topic or related to persistent methodological difficulties, such as high rates of anticipated discontinuation and consequent distortions in the imputation of missing data. Because early response to antipsychotics represents a sufficiently reliable index of the subsequent treatment response in patients with schizophrenia, we undertook a real-world, genome-wide association study (GWAS) with the aim of identifying genetic predictors of response to risperidone after 2 weeks in 86 patients with schizophrenia. Limited to the associations reaching significance in the GWAS, confirmatory analysis relative to risperidone response over 9 months was also designed involving 97 patients (European only) enroled in the CATIE (Clinical Antipsychotic Trials of Intervention Effectiveness) genetic substudy. The GWAS revealed a significant association (false discovery rate 0.02) of the single-nucleotide polymorphism rs2133450 inside the *GRM7* gene with Emsley's positive domain derived from the positive and negative syndrome scale (PANSS). Patients with the rs2133450 CC genotype presented poorer improvement in the positive domain over 2 weeks, with odds ratios of 12.68 (95% CI, 3.51–45.76) and 6.95 (95% confidence interval (CI), 2.37–20.37) compared with patients with the AA and AC genotypes, respectively. Compared with A homozygotes, rs2133450 C homozygotes enroled in the CATIE-derived confirmatory analysis showed less improvement in Emsley's positive, excited and depression domains, positive and general PANSS subtypes, and total PANSS after 9 months of treatment with risperidone. The original GWAS and the CATIE-derived confirmatory analysis support the proposal that the rs2133450 may have translational relevance as a predictor of response to risperidone.

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INTRODUCTION

A tailored approach to the treatment of schizophrenia relies on solid foundations. Since Bleuler,[1] a great deal of evidence has underlined that schizophrenia label applies to a heterogeneous group of disorders characterized by differences in the clinical picture and pathophysiologic correlates. Furthermore, individual first- and second-generation antipsychotics present small but robust distinctive patterns of efficacy[2–4] as a result of appreciable class- and drug-specific pharmacokinetic and pharmacodynamic differences.[5–7] These independent studies provide evidence that the response to antipsychotic medications is moderated in the real world by significant interindividual variability.

Despite a sound premise, the goal of individualized interventions for people with schizophrenia remains at the pioneering stage because reliable predictors of treatment response are rare.[8] Early response to antipsychotics represents a reasonable exception to this general picture because sufficient evidence[2,4,9–22] indicates that clinical response after 2 weeks of treatment is an acceptable indicator of subsequent pharmacological outcome. In the clinical realm, the early response paradigm represents an appreciable although partial progress; it restricts but does not eliminate the risk of adverse events as a consequence of treatments that will be ineffective.

Nevertheless, the early response end point has been poorly utilized in the search for biomarkers of response to antipsychotics, probably because international guidelines[23–25] support a delayed therapeutic effect of these drugs despite experimental evidence to the contrary.[26–28] The case of genetic markers of response to antipsychotics is paradigmatic in this regard; the abundant literature on predictors of response at 4, 6 or more weeks of treatment[29–34] is in sharp contrast to the lack of genome-wide association studies (GWAS) and the negligible number of reports[35–38] challenging associations between early response to antipsychotics and single-nucleotide polymorphisms (SNPs).

Genes and early antipsychotic response (GEAR) is a multistep project designed to fill this gap. This study refers to the first completed section, the Risperidone Genome-Wide trial (GEAR-RisGW), a hypothesis-free study to search for associations between SNPs and response to risperidone after 2 weeks in patients with acute schizophrenia, and considering the dimensional perspective. The validity of the option for a dimensional approach seems *a posteriori* weighted by the demonstration that both schizophrenia diagnosis[39] and response to antipsychotics[40] are well accounted

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for by hierarchical models involving multiple symptom domains. The original experimental design also implied that, in the presence of significant GWAS associations, the SNPs of interest were challenged in an independent confirmatory analysis derived from the database of the optional Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) genetic substudy[41]

MATERIALS AND METHODS

The GEAR-RisGW pharmacogenomic trial

Study design. The GEAR-RisGW trial had an open label, flexible dose, quasi-naturalistic design based on procedures typical of the clinical routine adopted at the Brescia University and Spedali Civili Psychiatric Unit. The unique differences included the involvement of two investigators for the clinical assessments, independence between the treating physician and the investigator, preclusion of combining different antipsychotics, and genetic tests.

Given the strict overlap with daily practice, informed consent was restricted to acceptance of genotyping and the use of individual data for research purposes. In agreement with the indications posited by the Ethic Committee of the Brescia Fatebenefratelli Hospital 'San Giovanni di Dio' (act no. 3/2004 of 01/22/2004), before genotyping, the patients were requested to sign a consent form after the aims of the study were explained and assurances on anonymity were given.[42]

Subjects. Eligibility for the study was restricted to consenting men and women aged between 18 and 65 years who were hospitalized for exacerbation of psychotic symptoms, judged suitable for treatment with risperidone, and satisfied a set of predefined criteria as follows: Caucasians of Italian descent for at least two generations; unrelated to other prospective participants; and, in the opinion of the treating physician, have a level of understanding and enough attention to give valid consent, and score at least 3 in the Clinical Global Impression-Severity scale.[43] Prospective participants also had to satisfy the DSM-IV-TR criteria[44] for schizophrenia or a schizophreniform disorder at study entry. For patients with an initial diagnosis of schizophreniform disorder, definitive inclusion was restricted *post hoc* to patients with an unequivocal transition to schizophrenia at the 6-month re-evaluation.

Exclusion criteria were as follows: the presence of other DSM-IV-TR Axis 1 comorbid disorders; a history of intolerance to risperidone or paliperidone, that is, the emergence of adverse reactions severe enough in previous episodes to preclude treatment continuation or require a dose reduction; concomitant use of antidepressants or mood stabilizers; diagnosis of severe, unstable medical conditions; and in the opinion of the treating physician, it would be inappropriate to abruptly discontinue non-allowed therapies.

Treatments. Similar to the clinical routine, an independent physician was responsible for the medical decisions on the basis of personal judgment acting in the best interest of the patient.

During the 2-week study period, the dose of risperidone was adjusted within the range of 3–13 mg/day, with a possible 2 mg titration in the first 2 days of treatment. Benzodiazepines for insomnia or anxiety and anticholinergic medications for parkinsonian symptoms were allowed.

Assessments. As described elsewhere,[45] the diagnostic procedures and acquisition of the key demographic and clinical data were based on detailed interviews with the patient and revision of all medical records. When required, supplementary interviews with close relatives and a DSM-IV-TR adjusted version of the Standardized Clinical Interview for DSM-IV Axis 1 Disorders, Clinician Version,[46] were also carried out.

Changes in symptom severity were monitored by administering the positive and negative syndrome scale (PANSS)[47] at baseline and after 2 weeks of treatment. Patients were also grouped into positive, negative, or mixed schizophrenia subtypes according to their PANSS profile.[48,49]

Adherence to medication was certified by trained nurses by direct and protracted observation that the patients did not hide pills in their mouth. This method, recommended as one of the most accurate,[50] is inconvenient in the clinics but, because of the short duration of the trial, was judged to be acceptable.

A unique team of experienced psychiatrists was in charge of data collection, after dedicated training and demonstration of valid inter-rater reliability. The diagnosis was posited by two physicians in the team who independently evaluated potential participants in rotation. In presence of discordance, a joint revision of all the information was carried out with an independent referee (ES) who made the final decision, after discussion.

The same two physicians who administered the PANSS at baseline were involved the interviews. For each interview, divergence in PANSS total score of $< 3\%$ was allowed between the evaluators. With greater deviations, the raters re-analysed the scores item by item until consensus was reached. No time window was set for administration of the post-baseline PANSS and the scores represented the mean of two independent evaluations.

The patients enroled in the study were re-analysed *a posteriori* in order to check the compatibility of the original DSM-IV-TR diagnosis with the DSM-5 criteria for schizophrenia.[51]

The clinical investigators made their assessments in reciprocal blind fashion with the laboratory team.

Efficacy measures. Efficacy was evaluated using a PANSS-derived battery, which comprised the total scale, the positive, negative, and general subscales, and the seven dimensions of psychopathology from a factor analysis.[52] Changes from baseline score after 2 weeks in individual components of the PANSS-derived battery represented the primary efficacy measures. The proportion of patients who improved by 20% or more in the different components of the PANSS-derived battery at the 2-week end point was the secondary efficacy measure.

In order to facilitate comparability with the current literature on the efficacy of antipsychotic medications, PANSS items were scaled according to the conventional 1–7 rating whenever absolute changes from the baseline score were considered. A 0–6 rating system was preferred for grouping patients according to the good/poor responder dichotomy because the literature[16,53–55] indicates that this is the standard for correct estimates of percentage changes in PANSS.

SNP array genotyping. Genomic DNAs were purified from peripheral blood using a standard salting out method and genotyped by Affymetrix Human Mapping GeneChip 6.0 arrays (Affymetrix, Santa Clara, CA, USA), incorporating 2 million probes, half of which are polymorphic. DNA was processed according to the instructions provided in the Affymetrix Genome-wide Human SNP *Nsp*/*Sty* 6.0 Assay Manual.

Intensity data were acquired by an initial array analysis using the Affymetrix GeneChip Command Console Software (AGCC). The genotypes were established by analysing the AGCC probe cell intensity data with the GenotypeConsole 3.01 (GTC3.01). The risk for genotype errors was reduced using quality thresholds. Twenty patients with a quality control value $\leqslant 0.4$ were discarded from the SNP array analysis in agreement with the Affymetrix specifications but their data were retained for the study on the top hit SNPs, using a real-time method.

Quality controls of the structure of the population. The chp.files generated by the Birdseed v2 algorithm implemented in GTC 3.01 were imported into the SNP & Variation Suite (SVS) 7 software for statistical analyses (Golden Helix SVS, Bozeman, MT, USA). Data cleaning was performed as follows: SNPs on sex chromosomes, those with a call rate $< 95\%$, a minor allele frequency < 0.1, and a Hardy–Weinberg *P*-value $< 1 \times 10^{-7}$ (cutoff obtained by correcting the nominal *P*-value of 0.05 for the number of SNPs analysed) were excluded from the initial set of 906 599 SNPs. Also SNPs with a frequency of 0–5 for the rare homozygous genotype were discarded to reduce the effects of false positives related to outliers. After the cleaning process, 350 796 SNPs were retained.

The risk for spurious associations due to population stratification was controlled using an enhanced version of EIGENSTRAT contained in SVS 7 (Bozeman, MT, USA). The first 20 principal components had eigenvalues ranging from 1.14 to 1.06 and the scatterplot of the subjects showed that they were clustered together; therefore, the sample population was considered homogeneous.

Genotype imputation and bioinformatics analyses. Data from the 1000 Genomes Project acted as reference samples for imputing SNPs potentially providing regulatory function and mapped close to those that provided the best *P*-value in the experimental cohort. BEAGLE software[56] was used for imputation. A low imputation quality ($r^2 < 0.3$), a minor allele frequency < 0.1, a Hardy–Weinberg *P*-value $< 1 \times 10^{-7}$, and/or an absolute frequency of 0–5 for the rare homozygous genotype implied removal of the SNP from subsequent linear regression analyses and linkage disequilibrium (LD) estimates.

In order to assess their potential functionality, the SNPs included in the same LD block as the SNPs with the best *P*-value were uploaded in the Regulome database (http://regulomedb.org/).[57]

Real-time analysis. An Applied Biosystems TaqMan SNP Genotyping Assay (C__16103928_10; Carlsbad, CA, USA) was used to validate genotype calls for the SNPs with the best *P*-value. The same assay was used to genotype the 20 patients for whom it was not possible to perform SNP array analysis.

CATIE-derived confirmatory analysis

The CATIE-derived confirmatory analysis was designed with the purpose of confirming in an independent sample population the GEAR-RisGW results that eventually reached the significance but over a longer time period (9 months).

The most salient characteristics that distinguished the CATIE-derived confirmatory analysis from the GEAR-RisGW trial were as follows: a multicentre, randomized double-blind design; lower risperidone dose range (1.5–6 mg day^{-1}); outpatient status of the participants; inclusion independent of symptom severity. Furthermore, therapy was started irrespective of exacerbation of psychotic symptoms and a large time window on either side of the scheduled PANSS interview was allowed.

The genotypes relative to the patients included in the CATIE-derived confirmatory analysis were extrapolated with permission from the CATIE genetic substudy database.[41] In analogy with the GEAR-RisGW trial, the evaluation was exclusive to participants classified as European only. Furthermore, the analyses were restricted to the first 9 months of therapy because this time interval was judged to offer better linearity of symptom improvement. Changes in PANSS scores from baseline were extrapolated applying linear mixed models for repeated measures to the 1–7 ratings reported in the original database.

Statistical plan

GWAS. In the GEAR-RisGW trial, the influence of SNPs on changes after 2 weeks for each component of the PANSS-derived battery was explored by challenging the dependent variable, symptom improvement, with the basic allele test implemented in SVS 7 software. The basic allele test assumes that alleles rather than genotypes represent the statistical units of reference. Consequently, the genotypes AA, Aa, and aa are resolved into pairs of alleles (*A* and *A*, *A* and *a*, or *a* and *a*, respectively) and the same value of the dependent variable is attributed to both alleles of the pair.

The advantage of the model is that the number of observations is doubled and the power of the analysis is increased; the disadvantage is that genotype-specific information is ignored. The level of significance was set for a nominal *P*-value $< 5 \times 10^{-8}$ and false discovery rate < 0.05.

According to the G*Power Calculator software,[58] in our cohort of 106 patients, the power was $>90\%$ (for $\alpha = 1 \times 10^{-7}$) for detecting $R^2 \geqslant 0.34$ (large effect).

In the presence of significant results, supplementary analyses of variance were carried out in order to establish the moderating effect played by the genotype. The baseline score of the component of the PANSS battery and belonging to the positive, negative or mixed subtype of schizophrenia were included in the analyses in order to correct for eventual additive or interactive effects on the drug response phenotype. SPSS software (SPSS, Chicago, IL, USA) was used for the analyses of variance.

Whenever a significant association between a gene variant and an early response in a component of the PANSS battery occurred, the sensitivity, the specificity, the number needed to treat and the odds ratio (OR) were calculated.

All statistical tests used met relative assumptions (for example, distribution, sample size).

CATIE-derived extrapolation analyses. The score components of the PANSS-derived battery, coded as variations from the baseline value, were modelled using linear mixed models. These models included as covariates the genotype (with the AA genotype as the reference value), the occurrence of a visit (labelled as 'time') coded as time elapsed from the first visit, an interaction term for genotype and time, and the baseline score of components of the PANSS-derived battery investigated time by time. Whenever a switch from risperidone to another antipsychotic medication occurred, the patient was considered a dropout and the reason for the dropout was inserted in the model, coding the occurrence of adverse events as yes (any unacceptable event) or no. The genotype effects were estimated at 270 days after the first visit. All the models included both a random intercept and a random time effect (slope).

All the analyses were performed using R-version 3.1.0,[59] lme4 packages,[60] and the lmerTest.[61]

RESULTS

GEAR-RisGW pharmacogenomic study

One hundred and six patients were involved in the GEAR-RisGW trial. Middle-aged, male, chronic patients with severe psychotic exacerbation characterized by mixed symptoms prevailed in the sample (Table 1). When re-evaluated *a posteriori*, the entire study cohort fulfilled the DSM-5 criteria for schizophrenia. Seventy-four per cent of the patients were supplemented, generally for a few days, with benzodiazepines and 3% received adjunctive anticholinergic medications during the study period. After a 2-day titration, the mean daily dose of risperidone was 6.8 ± 1.9 mg.

Eighty-six patients were included in the analysis that explored the association between gene polymorphisms and early response to risperidone. Only one SNP inside the metabotropic glutamate receptor 7 (*GRM7*) gene, the rs2133450, reached the threshold for a significant genome-wide association with a component of the PANSS-derived battery, the Emsley's positive dimension; four supplementary SNPs inside the same gene exceeded the 0.05 false discovery rate threshold (Figure 1, Supplementary Figure 1, Table 2).

The TaqMan SNP Genotyping Assay confirmed the results of the chip analysis and allowed the addition of 20 patients for whom it was impossible to perform SNP array analysis. In the regression analyses applied to the total sample ($n = 106$), rs2133450 C homozygous patients showed less improvement than individuals presenting the most frequent AA genotype and had a trend for poorer response in the other components of the PANSS battery, in particular total PANSS and PANSS-positive subtype (Table 3).

Application of Emsley's positive dimension to the good/poor 2-week responder dichotomy led to a 71.7% responder rate, with shifts to 88.6, 81.0 and 37.9% when rs2133450 AA, AC or CC genotypes were considered. In particular, compared with AA patients, the OR for CC patients was 12.68 (95% confidence interval (CI), 3.51–45.76) for poor early response, with an number needed to treat of 2, and a specificity and sensitivity of 81.8 and 73.8%, respectively. Furthermore, C homozygous patients had a lower chance of a good response than AC patients (OR, 6.95 (95% CI 2.37–20.37); number needed to treat, 2; specificity, 69.2%; and sensitivity, 75.6%).

The imputation of other 1828 SNPs covering the entire *GRM7* gene showed that rs2133450 maps in an LD block that spans over 155 kb (February 2009, GrCh_37, chr3, 7 262 169–7 372 399), overlaps exons 3 and 4, and includes 176 SNPs after imputation (Supplementary Figure 2). None of the imputed SNPs inside the LD block was a coding variant. Therefore, the Regulome database was checked to investigate whether some of the imputed *GRM7* SNPs affect the binding site of regulatory elements (Supplementary Table 1): three SNPs had a Regulome score of 3, a value indicating indirect evidence of some regulatory effect; another SNP scored 6, a value that suggests minimal evidence of an effect on binding; both rs2133450 and the remaining 4 SNPs in complete LD with it had no score due to the absence of data.

CATIE-derived confirmatory analysis

Ninety-seven DSM-IV, European only patients with schizophrenia who were randomized to risperidone and participated in the optional genetic CATIE substudy were included in the confirmatory *ad hoc* analysis to challenge whether rs2133450 exerts some

Table 1. Key demographic and clinical features of patients enroled in GEAR-RisGW trial and CATIE-derived confirmatory analysis

Baseline characteristics	*GEAR-RisGW trial*	*CATIE-derived confirmatory analysis*
Total sample, *N*	106	97
Diagnostic criteria	DSM-IV-TR, DSM-5	DSM-IV
Diagnoses	Schizophrenia	Schizophrenia and schizoaffective disorder
Age (years), mean (s.d.)	40.2 (12.7)	42.8 (12.7)
Gender (M), *n* (%)	68 (64.2)	76 (78.3)
Age at disease onset (years), mean (s.d.)	25.4 (8.1)	ND
Subtype[a] *%*		
Negative	9.4	2.7
Mixed	67.0	59.8
Positive	23.6	17.5
PANSS total score,[b] mean (s.d.)	106.9 (16.1)	76.7 (16.7)
PANSS-positive subscale score,[b] mean (s.d.)	28.4 (6.2)	18.3 (5.9)
PANSS negative subscale score,[b] mean (s.d.)	27.1 (6.6)	20.0 (6.3)
PANSS general subscale score,[b] mean (s.d.)	51.4 (9.2)	38.4 (8.2)
Emsley's negative factor score,[b] mean (s.d.)	26.8 (7.1)	19.7 (6.5)
Emsley's positive factor score,[b] mean (s.d.)	33.4 (6.2)	19.6 (6.2)
Emsley's disorganized factor score,[b] mean (s.d.)	16.3 (4.3)	12.5 (3.9)
Emsley's excited factor score,[b] mean (s.d.)	13.5 (4.6)	7.1 (2.9)
Emsley's motor factor score,[b] mean (s.d.)	4.9 (1.9)	3.8 (1.7)
Emsley's depressive factor score,[b] mean (s.d.)	2.9 (1.6)	5.8 (2.2)
Emsley's anxiety factor score,[b] mean (s.d.)	9.2 (3.1)	8.2 (2.5)

Abbreviations: CATIE, clinical antipsychotic trials of intervention effectiveness; GEAR, genes and early antipsychotic response; ND, not defined; PANSS, positive and negative syndrome scale. [a]According to Lindermayer *et al*. [b]PANSS items scored according to the 1–7 rating.

moderating role on the response to risperidone 9-months after the start of the therapy.

The sample mainly consisted of middle-aged, male, moderately ill outpatients with mixed symptoms (Table 1).

The rs2133450 SNP was directly genotyped in the CATIE cohort, with a call rate $>95\%$. The application of linear mixed models to data recorded in the original database showed that rs2133450 A homozygotes responded better than C homozygotes in Emsley's positive, excited, and depression domains, positive and general PANSS subtypes, and total PANSS. Homozygotes for the A allele improved also more than heterozygotes in Emsley's excited domain, total PANSS and general PANSS subtype (Tables 4 and 5).

DISCUSSION

To summarize, the exploratory GEAR-RisGW trial and the confirmatory CATIE-derived analysis conjunctly indicate that the CC genotype of a SNP inside the *GRM7* gene, the rs2133450, hinders improvement in Emsley's positive dimension by the second week of treatment with risperidone and that this unfavourable moderation not only persists over time but even extends, at the 9-month end point, to other psychopathological domains and, more importantly, to total PANSS.

The experimental design included several novel features. No GWAS has challenged early response to antipsychotics and then immediately partnered by a confirmatory analysis. Similarly, no study on a candidate biomarker of response to risperidone has involved patients with DSM-IV-TR schizophrenia who were *a posteriori* certified to also fulfil the DSM-5 criteria for the disorder. Furthermore, unlike most studies on predictors of treatment response, the GEAR-RisGW trial ensured strict monitoring of medication taking behaviour, thus plausibly avoiding the risks of interference from unrecognized poor treatment adherence on correct assessment of poor and good responders.[62] Strong control of medication seems especially crucial in the initial stages of the therapy, given the report that, compared with early responders, early non-responders perceive antipsychotics as less beneficial and consequently are at increased risk for poor medication adherence.[9]

The study also provides some general added value. For example, in the GEAR-RisGW trial, the contemporaneous use of DSM-IV-TR and DSM-5 criteria should facilitate comparisons with future studies and eventual translation of current results to a DSM-5-oriented psychiatric practice. Facilitated transfer of the results into the clinical routine is also supported by the demonstration that the real-world prerequisite was satisfied: at the 2-week end point, the risperidone dose and the rate of patients with an improvement of 20% or more in total PANSS found in the experimental sample population were close to the values ($7.2\,mg\,day^{-1}$ and 68%) for patients of the Brescia Psychiatric Department who, due to an exacerbation of psychosis, were hospitalized in the 3 previous years and routinely treated with the same antipsychotic medication. The real-world nature of the trial is further supported by the close overlap between almost three-quarters of the GWAS-RisGW patients, who received supplementation with benzodiazepines for a few days and the 71% observed routinely in the department during the first 2 weeks of hospitalization. A high prevalence of acute, severely ill patients with schizophrenia treated also with benzodiazepines during the early phase of their hospitalization seems to be a common phenomenon.[63,64] In contrast, the addition of benzodiazepines to therapy is decidedly rarer in moderately ill patients as was the case in the CATIE[65] population. The demonstration that the association between the CC genotype of the rs2133450 gene and poor response to risperidone was present in sample populations with remarkable differences in the prescription of benzodiazepines strongly supports the inference that the use of these medications had no relevant impact on the association itself. The fact that the prescription of benzodiazepines was unrelated to the rs2133450 genotype also applies.

With regard to the core results, the demonstration of an appreciable involvement of rs2133450 in moderating response to risperidone, although new, was not completely unexpected. A discrete body of evidence accumulated over the years has

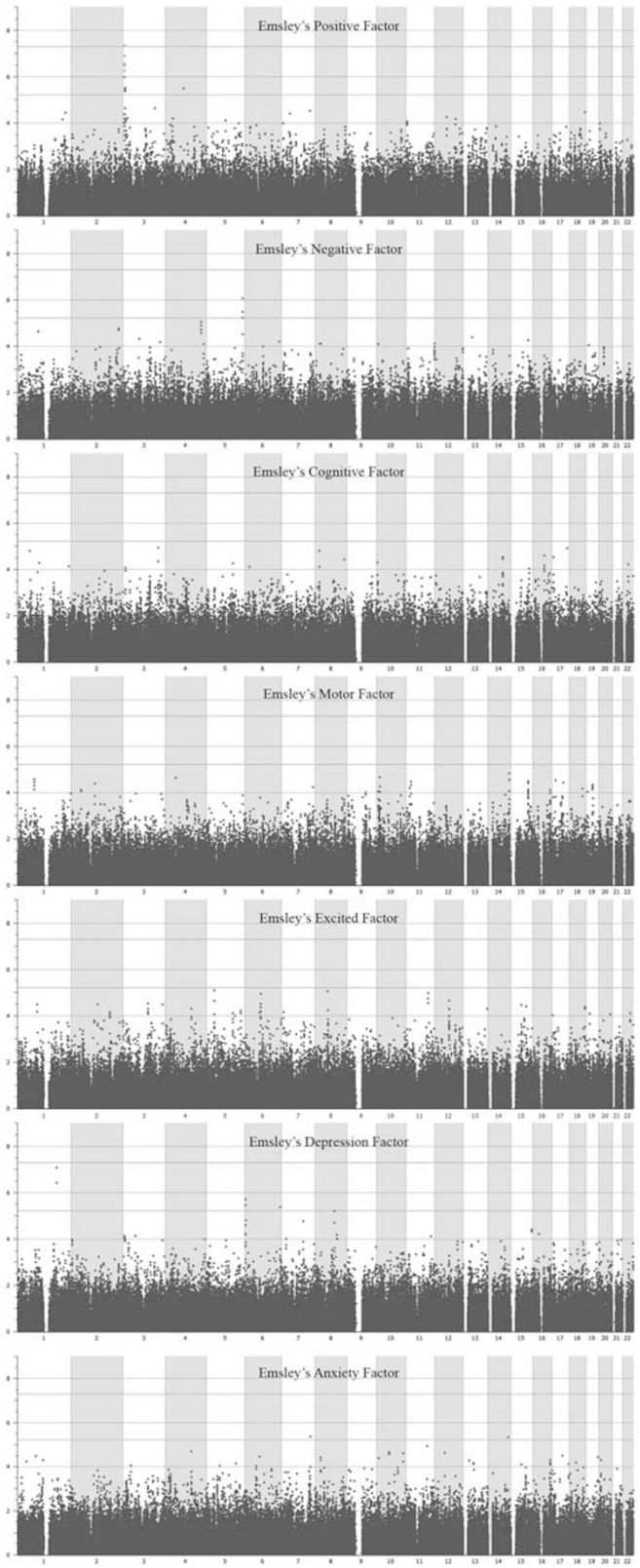

Figure 1. Manhattan plots of linear logistic –log10 *P*-values for the seven factors of the Emsley factorial model. Chromosomes are shown alternatively shaded for clarity. *P*-value thresholds of 5×10^{-8} and 6.09×10^{-6} are highlighted by solid horizontal lines.

associated schizophrenia risk not only with different metabotropic genes in general[66] but also with *GRM7* gene in particular.[67–69] Furthermore, increased expression of the mGlur7 protein in the dorsolateral prefrontal cortex of patients with schizophrenia has been reported[70] and a CATIE substudy[71] has shown that the greatest hits for 3-month PANSS improvement involved another *GRM7* SNP, rs7627369. This result, however, has appreciable limitations because the association did not survive after correction for multiple comparisons and another CATIE substudy failed to replicate the observation.[72]

In general, good concordance between the GWAS results and those of the confirmatory analysis makes it plausible that the action of rs2133450 is in large measure unaffected by symptom severity because patients enroled in the GEAR-RisGW trial had higher baseline PANSS scores than individuals entered in the CATIE substudy.

The effect of rs2133450 on symptom improvement seems subject to a risperidone dose-related ascending gradient of the SNP effect: the moderation exerted by the SNP was decidedly more pronounced in the GWAS population, which received a higher mean dose of risperidone than the maximum allowed for patients in the CATIE study.[65]

It is also likely that rs2133450 affects the response to risperidone according to a dimension-specific timing because the shift in the end point between the 2 weeks of the GWAS to the 9 months of the confirmatory analysis extended the influence of the SNP on symptom improvement from the initial Emsley's positive domain to other PANSS-derived dimensions and total PANSS. This proposal seems in agreement with the indication[73] that the antipsychotic medication for patients with predominantly negative symptoms should only be switched after an extended period of use.

The magnitude of the negative influence played by the CC genotype on the possibility of early improvement in Emsley's positive dimension may seem surprising. The observed OR and the number needed to treat exceeded the corresponding values relative to the superiority of antipsychotics over placebo in short-term trials.[74] Furthermore, very high levels of variance in treatment response explained by a single gene must always be regarded with caution because response to antipsychotics represents the phenotypic expression of a complex trait sustained by multiple determinants and moderators of genetic and non-genetic origin. From a critical perspective, the large CIs of the ORs are compatible with the hypothesis that the real impact of the SNP on early response differs discretely from the actual value observed. It is also true that the small sample size of the GWAS could have increased the risk for false positive results. However, the longer time results of the CATIE-derived confirmatory analysis suggest, although with a lower level of significance, that *GRM7* really does play a role in risperidone response. Some other considerations contribute to make the relevant power of the association between rs2133450 genotype and early improvement in Emsley's positive domain less surprising. For example, the dimensional approach clusters relatively homogeneous domains of psychopathology and thus should offer improved protection against confounding effects of symptom-specific differences in response to antipsychotics. Also the reduction of the follow-up to 2 weeks and rigorous control of medication adherence should have contributed to zero occurrence of early withdrawals and thus prevented distortions related to imputation of missing data, bypassing the limits of representativeness typical of special populations generated by early exclusion of the less improved and/or the more intolerant patients. Being primarily based on the results of previous cycles of therapies with antipsychotics, the treating physician's decision to initiate risperidone might also have led to the selection of a special GWAS population. However, the major influence on the magnitude of the association between rs2133450 and early

Table 2. GEAR-RisGW: GRM7 SNPs associated (FDR < 0.05) with 2-week change in Emsley's positive dimension

SNP	*Position*	*dd*		*Dd*		*DD*		*Regression P*	*FDR*[a]
		Average	*N*	*Average*	*N*	*Average*	*N*		
rs2133450	**Chr3: 7,336,452**	**−3.68**	**25**	**−7.52**	**33**	**−9.89**	**28**	**4.33×10^{-8}**	**0.02**
rs12637466	Chr3: 7,321,909	−3.24	21	−7.40	35	−9.76	29	1.25×10^{-7}	0.02
rs6766479	Chr3: 7,324,186	−3.58	24	−7.79	33	−9.77	26	2.63×10^{-7}	0.03
rs41412146	Chr3: 7,341,024	−3.80	25	−7.66	32	−9.64	28	3.13×10^{-7}	0.03
rs1400165	Chr3: 7,268,362	−3.26	19	−7.35	34	−9.58	31	5.36×10^{-7}	0.04

Abbreviations: FDR, false discovery rate; GEAR, genes and early antipsychotic response; N, number of subjects; SNP, single-nucleotide polymorphism. Average symptom improvement measured as differences between scores after 2 weeks of treatment and the baseline values: dd, rare homozygous genotype; Dd, heterozygous genotype; and DD, most frequent homozygous genotype. [a]Higlighted in bold is SNP with a significant association.

Table 3. GEAR-RisGW: *P*-values from univariate analysis of variance for rs2133450 SNP (genotype model)[a]

rs2133450 genotypes	*Mean symptom improvement (s.d.)*[b]			*Genotype P-value*
	AA (N = 35)	*AC (N = 42)*	*CC (N = 29)*	
PANSS total	−31.74 (13.44)	−24.33 (11.44)	−18.17 (10.25)	1.84×10^{-5}
PANSS-positive subscale	−10.60 (5.15)	−9.24 (4.03)	−5.90 (4.54)	1.70×10^{-5}
PANSS negative subscale	−5.31 (3.71)	−3.17 (3.39)	−3.10 (2.09)	0.0111
PANSS general subscale	−15.83 (7.80)	−11.93 (6.62)	−9.17 (6.00)	0.0002
Emsley's positive factor	−9.80 (5.30)	−8.07 (4.39)	−4.14 (3.79)	1.13×10^{-6}
Emsley's negative factor	−6.80 (4.25)	−3.93 (3.86)	−4.17 (2.75)	0.0049
Emsley's disorganized factor	−3.49 (2.66)	−2.69 (2.18)	−2.62 (2.30)	0.1147
Emsley's excited factor	−6.40 (3.85)	−5.12 (2.98)	−4.14 (3.56)	0.0036
Emsley's motor factor	−1.00 (1.35)	−0.45 (1.17)	−0.45 (0.87)	0.1517
Emsley's depression factor	−0.49 (1.07)	−0.55 (1.15)	−0.24 (0.91)	0.8899
Emsley's anxiety factor	−3.77 (2.21)	−3.52 (2.54)	−2.41 (2.41)	0.0134

Abbreviations: GEAR, genes and early antipsychotic response; PANSS, positive and negative syndrome scale; SNP, single-nucleotide polymorphism. [a]Covariates appearing in the model are Subtype and T0 outcome . [b]Mean symptom improvement measured as differences between scores after 2 weeks of treatment and the baseline values.

Table 4. Effect of rs2133450 SNP genotype on 9-month symptom improvement in 'European only' CATIE patients randomized to risperidone: linear mixed model results relative to Emsley's domains of psychopathology

	Emsley's PANSS-derived dimensions													
	Positive		*Negative*		*Disorganized*		*Excited*		*Motor*		*Depressive*		*Anxiety*	
	Estimate	P-*value*	*Estimate*	P-*value*	*Estimate*	P-*value*	*Estimate*	P-*value*	*Estimate*	P-*value*	*Estimate*	P-*value*	*Estimate*	P-*value*
Intercept	3.922	0.008	5.017	0.001	3.623	2.29×10^{-4}	2.133	0.000	0.944	0.007	1.513	0.007	2.457	3.13×10^{-4}
AC genotype[a]	1.668	0.098	2.064	0.071	−0.115	0.860	1.087	0.032	0.517	0.104	0.529	0.214	0.472	0.284
CC genotype[a]	2.346	0.044	1.218	0.349	0.608	0.416	1.609	0.006	0.345	0.340	0.971	0.047	0.336	0.503
Time[b]	−0.574	0.038	0.032	0.928	−0.107	0.463	−0.227	0.055	0.019	0.819	−0.255	0.003	−0.148	0.212
Side effects[c]	0.921	0.429	0.201	0.863	0.165	0.828	0.726	0.195	0.062	0.857	0.481	0.405	0.871	0.145
Baseline	−0.420	2.57×10^{-10}	−0.368	2.88×10^{-9}	−0.404	3.83×10^{-9}	−0.540	5.06×10^{-13}	−0.376	3.97×10^{-8}	−0.484	2.04×10^{-8}	−0.465	6.87×10^{-9}
AC * time[d]	0.512	0.158	−0.140	0.763	−0.205	0.295	0.260	0.099	0.014	0.897	0.063	0.575	0.066	0.676
CC * time[d]	0.581	0.164	−0.117	0.827	0.109	0.627	0.412	0.025	−0.034	0.787	0.097	0.453	0.136	0.453

Abbreviations: CATIE, clinical antipsychotic trials of intervention effectiveness; PANSS, positive and negative syndrome scale; SNP, single-nucleotide polymorphism. [a]Vs AA genotype. [b]Visit occurrence coded as time elapsed (days) from the baseline visit. [c]Side effects coded as yes (any unacceptable effect present) or no. [d]Interaction term for genotype and time.

improvement in Emsley's positive domain plausibly resides in placing the study end point at 2 weeks; studies on the trajectories of response to antipsychotic medications are in agreement that rapid responders may represent a specific endophenotype.[14,20,75–78]

Whether rs2133450 is a response marker with drug or class specificity is an unanswered question because the GWAS and the confirmatory analysis only challenged risperidone. Therefore, generalization of the current results to patients with schizophrenia treated with other antipsychotics is not possible.

Table 5. Effect of rs2133450 genotype on 9-month symptom improvement in 'European only' CATIE patients randomized to risperidone: linear mixed model results relative to total PANSS, and positive, negative and general PANSS subscales

	PANSS							
	Total scale		*Positive subscale*		*Negative subscale*		*General subscale*	
	Estimate	P-*value*	*Estimate*	P-*value*	*Estimate*	P-*value*	*Estimate*	P-*value*
(Intercept)	10.458	0.055	2.965	0.046	6.203	0.000	0.813	0.087
AC Genotype[a]	6.451	0.046	2.032	0.058	1.330	0.222	2.984	0.050
CC Genotype[a]	7.816	0.035	2.550	0.037	0.682	0.582	4.471	0.011
Time[b]	−1.083	0.201	−0.537	0.050	−0.007	0.980	−0.679	0.088
Side Effects[c]	2.573	0.433	0.387	0.739	−0.069	0.952	2.380	0.174
Baseline	−0.300	5.16×10^{-6}	−0.381	5.27×10^{-8}	−0.391	9.33×10^{-10}	−0.310	8.25×10^{-6}
AC * time[d]	0.568	0.612	0.490	0.174	−0.209	0.594	0.275	0.600
CC * time[d]	1.231	0.342	0.683	0.102	−0.089	0.844	0.664	0.273

Abbreviations: CATIE, clinical antipsychotic trials of intervention effectiveness; PANSS, positive and negative syndrome scale. [a]Vs AA genotype. [b]Visit occurence coded as time elapsed (days) from the baseline visit. [c]Side effects coded as yes (any unacceptable effect present) or no. [d]Interaction term for genotype and time.

The pathways underlying the association between the rs2133450 CC genotype and poor response to risperidone remain substantially undefined. Metabotropic receptors are peripheral to the direct targets of risperidone action and none of the imputed SNPs inside the LD block mapping rs2133450 is an encoding variant. However, the Regulome database supported some modulatory effect of the LD block on the expression of the *GRM7* gene and sparse evidence from the literature[70,79–82] offers some clues for generating hypotheses (Supplementary Table 2).

The current results indicate that the moderating effect of rs2133450 on the response to risperidone is consistent and plausible enough. Nevertheless, prospective studies to replicate these findings and new dedicated research on relevant but so far untouched issues are required before conclusions can be drawn on the translational relevance of the SNP as a predictor of the short- to medium-term efficacy of risperidone and a biomarker of an endophenotype or subphenotype of schizophrenia. However, one fact has emerged: when the quality of evidence and the effect size of the association between the CC genotype and a poor early response in Emsley's positive domain were aggregated according to a quantitative hierarchical level of evidence schema, the sum score was 6, a value reported for only one out of 257 predictive biomarkers.[8]

CONFLICT OF INTEREST

In the past 3 years, ES has received funding for research, advisory board membership and sponsored lectures from the following private companies: Angelini, Chiesi, Content Rd Net srl, EDRA LSWR, Eli Lilly, Health and Publishing Services srl, Janssen-Cilag, Lundbeck, McCann, Otsuka, Pfizer, Roche, Servier, Stroder, Sunovion, Takeda and Valeas. He is not a shareholder in any of these corporations. In the past 3 years, AV has received funding for research, advisory board membership and sponsored lectures from Astra Zeneca Pharmaceuticals, Eli Lilly, Janssen-Cilag, Lundbeck, Pfizer, Otsuka, Sanofi and Stroder. He is not a shareholder in any of these corporations. PV has received funding for research and sponsored lectures from Abbott, AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, Eli Lilly, Innova Pharma, Janssen-Cilag, Lundbeck, Otsuka, Pfizer and Wyeth Lederle. He is not a shareholder in any of these corporations. The authors declare no conflicts of interest.

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Supplementary Information accompanies the paper on the The Pharmacogenomics Journal website (http://www.nature.com/tpj)