Research Article
For reprint orders, please contact: reprints@futuremedicine.com

Pharmacogenomics

COMT Val158Met and *5-HT1A-R* -1019 C/G polymorphisms: effects on the negative symptom response to clozapine

Aim: Clozapine is still considered the gold standard for treatment-resistant schizophrenia patients; however, up to 40% of patients do not respond adequately. Identifying potential predictors of clinical response to this last-line antipsychotic could represent an important goal for treatment. Among these, functional polymorphisms involved in dopamine system modulation, known to be disrupted in schizophrenia, may play a role. We examined the *COMT* Val158Met polymorphism, which plays a key role in dopamine regulation at the prefrontal level, and the *5-HT1A-R* -1019 C/G polymorphism, a target of clozapine activity involved in the interaction between the serotonin and dopamine systems. **Materials & methods:** 107 neuroleptic-refractory, biologically unrelated Italian patients (70 males and 37 females) with a DSM-IV diagnosis of schizophrenia who were being treated with clozapine were recruited. Psychopathology was assessed by the Positive and Negative Symptoms Scale (PANSS) at the beginning of treatment, and at weeks 8 and 12. Genomic DNA was extracted from venous blood samples. *COMT* rs4680 (Val158Met) and *5-HT1A-R* rs6295 (-1019 C/G) polymorphisms were analyzed by PCR-based restriction fragment length and direct sequencing, respectively. **Results:** We found a significant effect of *COMT* and *5-HT1A-R* on the PANSS Negative Subscale variation, with greater improvement among *COMT* Val/Val and *5-HT1A-R* G/G subjects. **Conclusion:** The findings support the hypothesis that *COMT* rs4680 and *5-HT1A-R* rs6295 polymorphisms could influence the negative symptom response to clozapine, probably through modulation of the dopaminergic system.

Marta Bosia[1,2], Cristina Lorenzi*[,1], Adele Pirovano[3], Carmelo Guglielmino[1], Federica Cocchi[1], Marco Spangaro[1,3], Placido Bramanti[4], Enrico Smeraldi[1,3] & Roberto Cavallaro[1]

[1]Department of Clinical Neurosciences, I.R.C.C.S. San Raffaele Scientific Institute, Milan, Italy
[2]Center for Neurolinguistics & Theoretical Syntax (NeTS), Institute for Advanced Study, (IUSS), Pavia, Italy
[3]Università Vita-Salute San Raffaele, Milan, Italy
[4]I.R.C.C.S. Centro Neurolesi "Bonino Pulejo", Messina, Italy
*Author for correspondence:
Tel.: +39 02 2643 3331
Fax: +39 02 2643 3336
lorenzi.cristina@hsr.it

Original submitted 26 February 2014; Revision submitted 15 October 2014

Keywords: clozapine • genetic polymorphisms • negative symptoms • PANSS • Positive and Negative Symptoms Scale • psychopharmacogenetics • psychosis • schizophrenia

Clozapine, the first atypical antipsychotic drug, is well established for the treatment of schizophrenia, especially in cases of patients who are refractory and/or intolerant to conventional antipsychotics [1]. Nevertheless, clozapine is not free of side effects, and can even produce severe side effects that have to be carefully considered before administration [2].

Although it is the gold standard in cases of refractory schizophrenia, unfortunately, only between 30 and 60% of treatment-resistant patients show a good clinical response to clozapine [3], and they represent a particular subgroup of patients characterized by a more homogeneous biological substrate. Thus, identifying potential predictors of clinical response to this last-line antipsychotic could advance our understanding of the pathophysiology and represent an important goal for treatment. Clozapine displays high affinity for multiple receptors, including serotoninergic, dopaminergic and muscarinic receptors [4], and it has been reported to increase dopamine (DA) release and neuronal activation in the prefrontal cortex (PFC) through a mechanism involving 5-HT1A-R

Future Medicine part of fsg

binding [5–7]. Therefore, molecules that directly or indirectly regulate DA availability in the PFC may influence clozapine activity and genetic variability at this level could partially explain the heterogeneity observed in clinical response.

COMT, an enzyme that degrades DA, plays a major role in the regulation of DA levels in the PFC. The *COMT* gene presents a functional polymorphism (Val158Met, rs4680) consisting of an A to G substitution at codon 158, resulting in the presence of valine instead of methionine. This leads to a three- to four-times increase in enzymatic activity and therefore lower DA levels [8,9]. There is consensus in the literature of an association between the *COMT* Met allele and better PFC function, evaluated by both related performances through executive functions tests and regional activation through functional imaging studies [10,11]. Studies addressing symptom severity and clinical response to treatment in schizophrenia reported contradictory results, probably because of heterogeneity of the samples studied and variability of the assessments employed. The Val allele has been associated with more prominent negative symptoms [12–15], more severe psychotic symptoms and a poorer response to treatment [16]. By contrast, other studies have found an association between the Met allele and poor response to antipsychotics [17,18]. Furthermore, a lack of association between the *COMT* genotype and response to antipsychotic treatment has also been reported [19,20]. The *COMT* genotype has also been suggested to affect cognitive improvement after treatment, with the Met allele showing a better response [21–23].

At the crossroad between dopaminergic and serotoninergic transmission, the 5-HT1A-R contributes to clozapine activity at the PFC level [24] and has a direct effect on DA release [25]. The gene coding for 5-HT1A-R is located on the long arm of chromosome 5 (5q11.2–13) [26]. Extensive experimental literature suggests procognitive properties of 5-HT1A-R on PFC-related functions [27,28]. A functional polymorphism (rs6295) consisting of a C to G substitution at position -1019 in the promoter region of the *5-HT1A-R* gene was reported to modulate transcription rate [29]. This genetic variant has been associated to vulnerability to anxiety disorders, major depression, bipolar disorder and increased risk of suicidal behavior, even if negative findings have also been reported [30–32].

The reason for these nonhomogeneous outcomes could be ethnic differences among samples [33]. For example, although this polymorphism correlated with anxiety and depression [34–36] in Asians, the G allele was associated with improved treatment outcomes [37], whereas this finding was not confirmed in Caucasians.

Given its putative role in negative and cognitive features, some studies focused on the possible influence of the rs6295 polymorphism on symptomatology and antipsychotic drug response. A trend towards an effect of *5-HT1A-R* polymorphism on negative symptoms was suggested, with lower Positive and Negative Symptoms Scale (PANSS) scores among patients with the C/C genotype [38]. The polymorphism has also been associated with negative symptoms after treatment with risperidone, but not haloperidol: a greater improvement was observed among subjects with the C/C genotype [39,40]. In a sample of drug-naive patients with first-episode psychosis treated as standard care with different typical and atypical antipsychotics (not including clozapine), a significant effect of *5-HT1A-R* genotype was reported on negative and depressive features, with the C/C genotype showing substantial improvement [41]. Very recently, a significant effect of this polymorphism was also found on executive function improvement after cognitive remediation therapy [42]. Interestingly, this polymorphism is located within a CpG island rich region and thus represents a site for epigenetic control, regulating the gene transcriptional rate. Recently, Tang and colleagues reported a significant association between methylation status in the rs6295 region and clinical response in first-episode schizophrenia, affecting both the PANSS total score and negative factor [43].

Since both *COMT* and *5-HT1A-R* polymorphisms appear to influence the dopaminergic system, specifically in the PFC, we hypothesized that these genotypes could modulate response to clozapine, particularly in relation to negative symptoms.

Materials & methods

Sample & clinical assessments

The sample included 107 biologically unrelated subjects recruited from Italian patients followed by the Psychotic Disorders Centre of the Clinical Neurosciences Department of San Raffale-Turro Hospital in Milan, Italy. To be included, patients had to:

- Meet the -IV diagnostic criteria for schizophrenia;
- Be older than 18 years and younger than 65 years;
- Be eligible for treatment with clozapine;
- Show no evidence of substance dependence or abuse, comorbid diagnosis on axis I or II, epilepsy, or any other major neurological illness or perinatal trauma, or mental retardation.

Treatment with clozapine was started for indication of pharmaco-resistance, defined as nonresponse to at least 1-month treatment with a neuoleptic plus 1-month treatment with one second-generation anti-

psychotic [44]. All patients were treated with clozapine and no other concomitant antipsychotics and/or antidepressants were allowed. The dosage was titrated according to local guidelines up to 250 mg and further augmentations or reductions were made, when requested, on the basis of clinical response and plasma levels. Drug adherence was verified through the evaluation of clozapine plasma levels. A blood sample was taken intravenously from each recruited patient. Informed consent for participation to the study and for genetic analysis was obtained from all patients. The protocol followed the principles of the Declaration of Helsinki and it was accepted and approved by the local Ethical Committee.

Basic information, such as age, sex, education and duration of illness, were collected during the clinical evaluation and checked with available data from clinical records. Psychopathology was assessed by means of the PANSS for schizophrenia [45] at first administration of the antipsychotic, after 8 weeks of treatment and a follow-up assessment was also made after a further 4 weeks. Baseline, post-treatment and follow-up PANSS were administered by the psychiatrist who was in charge of the patient, blind to genotype. All the psychiatrists who did the assessments have previously been trained on PANSS rating and calibration. Inter-rater reliability of PANSS evaluations in terms of intraclass correlation coefficient for the PANSS total score ranged from 0.65 to 0.95.

DNA analysis

DNA was extracted from whole blood by manual extraction using the illustra blood genomicPrep Midi Flow kit (GE Healthcare, Milan, Italy). Genotyping of *COMT* rs4680 was performed using PCR-based restriction fragment length by the following primers: 5′ ACT GTG GCT ACT CAG CTG TG 3′ and 5′ CCT TTT TCC AGG TCT GAC AA 3′. The PCR reaction was performed by ABI 9700 PCR thermal-cycler (Applied Biosystems, APPLERA, Monza, Italy) as follows: after a first step at 94°C for 6 min, steps of 94°C for 70 s, 58°C for 25 s and 70°C for 70 s were carried out for 35 cycles. Then, a final extension step at 70°C for 10 min was added. The PCR product (169 bp) was digested using NlaIII (New England Biolabs, England, UK) and fragments were separated in 3% SeaKem® agarose gels (BMA, BioWhittaker Molecular Applications, Rockland, ME, USA). Depending on the presence of two or three restriction NlaIII sites, either three fragments (114, 29 and 26 bp; allele G or Val) or four fragments (96, 29, 26 and 18 bp; allele A or Met) were produced. All patients underwent a venous blood sample for genotypic analysis.

Genotyping of *5-HT1A* rs6295 was performed using direct sequencing. PCR was performed with the primers 5′-CCC AGA GTG GCA ATA GGA GA-3′ and 5′-CCG TTT TGT TGT TGT TGT CG-3′ using the ABI 9700 PCR thermal-cycler (Applied Biosystems, APPLERA) as follows: after a first step at 94°C for 5 min, steps of 94°C for 35 s, 62°C for 35 s and 70°C for 45 s were carried out for 35 cycles. Then, a final extension step at 70°C for 10 min was added. The PCR product was then used to perform sequencing reaction using the DYEnamic™ ET Dye Terminator Cycle Sequencing Kit (GE Healthcare). Next, the product was sequenced by the MegaBACE™ 500 genetic analyzer (GE Healthcare) under standard conditions.

Data analysis

Demographic and clinical characteristics at baseline were compared between genotypes using ANOVA, or chi-squared tests when appropriate. The effect of genotypes on psychopathological features was analyzed by means of repeated measures ANOVA, with PANSS Total score, Positive, Negative and General Subscales at enrollment and at weeks 8 and 12 as dependent measures, *COMT* and *5-HT1A-R* genotypes as categorical predictors and time as the fixed factor.

To better compare the magnitude of improvement in the Negative Symptoms Subscale of the PANSS between genotype groups, we calculated an index value, determined by the change in score between baseline and week 12, divided by the standard error of the whole sample at baseline and representing a proxy effect size of improvement measure, as described by Wykes [46]. To evaluate the additive effect of both genotypes, an exploratory analysis using a general regression model was then performed with a grouping variable for *COMT* and *5-HT1A-R* polymorphisms as a categorical predictor and the effect size of improvement in PANSS Negative Symptoms Subscale as a dependent variable. A *post-hoc* Tukey's test was used to detect differences between groups. In this case, because of the limited sample size, we divided *COMT* in Val/Val subjects versus Met carriers and *5-HT1A-R* in G/G homozygous versus carriers of the C allele, as already done in previous works [22,47]. G*Power Software version 3.1.7 was used for power analysis. All other analyses were performed using STATISTICA software (version 8).

Results

Descriptive analysis

The sample was composed of 107 patients (70 males and 37 females). DNA analysis showed the following allelic distribution for the *COMT* rs4680 polymorphism: 33 patients with Val/Val, 48 patients with Val/Met and 26 patients with Met/Met; for the *5-HT1A-R* rs6295 polymorphism: 22 patients with C/C, 64 patients with C/G and 21 patients

Table 1. Clinical and demographic characteristics between genotype groups for the *COMT* Val158Met polymorphism.

Characteristics	Genotype			ANOVA
	Val/Val (M = 23, F = 10)	Val/Met (M = 34, F = 14)	Met/Met (M = 13, F = 13)	
Age	38.09 ± 9.41	39.54 ± 10.24	38.42 ± 7.56	df = 2; F = 0.26; p = 0.79
Onset	23.73 ± 6.18	22.38 ± 5.01	23.42 ± 4.73	df = 2; F = 0.71; p = 0.49
Duration of illness	14.36 ± 8.10	17.17 ± 9.79	15.00 ± 6.42	df = 2; F = 1.19; p = 0.31
Education (years)	11.61 ± 2.75	10.83 ± 2.73	11.27 ± 2.81	df = 2; F = 0.79; p = 0.46
Clozapine dose (mg/day)	217.0 ± 111.1	227.77 ± 129.7	248.0 ± 103.6	df = 2; F = 0.79; p = 0.46
PANSS Total score	92.55 ± 17.41	87.46 ± 17.08	88.69 ± 22.51	df = 2; F = 0.75; p = 0.47
PANSS Positive	20.49 ± 6.06	21.40 ± 6.02	22.35 ± 8.10	df = 2; F = 0.58; p = 0.56
PANSS Negative	27.12 ± 5.52	24.17 ± 6.75	23.81 ± 7.61	df = 2; F = 2.49; p = 0.09
PANSS General	44.94 ± 10.50	41.89 ± 8.27	42.50 ± 11.29	df = 2; F = 0.99; p = 0.38

df: degrees of freedom; F: Female; M: Male.

with G/G. Genotypes of the *COMT* polymorphism were in Hardy–Weinberg equilibrium (HWE), while genotypes of *5-HT1A-R* polymorphism were not (p = 0.042). A Chi-square analysis did not show a significant difference in frequencies of alleles between males and females.

Characteristics and baseline psychopathological measures of each genotype group are shown in Tables 1 & 2. The one-way ANOVA did not show any significant difference between genotype groups, as reported in Tables 1 & 2.

Clinical response analysis

The repeated measures ANOVA showed a significant improvement over time for PANSS Total scores and each subscale: Positive, Negative and General (p < 0.000 for all). Significant main effects of genotype were observed only for the PANSS Negative Symptoms Subscale scores (df = 4; F = 5.15; p = 0.0006 for *COMT* polymorphism and df = 4; F = 2.80; p = 0.027 for *5-HT1A-R* polymorphism). The patterns of change in symptoms rating are reported in Figures 1 & 2 for *COMT* and *5-HT1A-R* genotype groups.

For these analyses, we had a power of 0.80 to detect a medium effect size (f = 0.25) at a type 1 error level of p < 0.05. The regression analysis with *COMT* and *5-HT1A-R* genotypes, as predictor, showed significant results (df = 3, R2 = 0.15, F = 5.84, p = 0.001), suggesting an additive effect of genotypes on the effect size of improvement in the PANSS Negative Symptoms Subscale. *Post-hoc* analysis with Tukey's test showed a significant difference between *COMT* Met carriers and *5-HT1A-R* C carriers and compared with both *COMT* Val/Val and *5-HT1A-R* C carriers (p = 0.01) and to *COMT* Val/Val and *5-HT1A-R* G/G (p = 0.04), the latter showing greater improvement.

Table 2. Clinical and demographic characteristics between genotype groups for the *5-HT1A-R* -1019 C/G polymorphism

Characteristics	Genotype			ANOVA
	C/C (M = 16, F = 6)	C/G (M = 38, F = 26)	G/G (M = 16, F = 5)	
Age	40.54 ± 8.37	39.28 ± 10.16	35.62 ± 6.99	df = 2; F = 1.71; p = 0.19
Onset	25.09 ± 6.82	22.53 ± 4.92	22.47 ± 4.40	df = 2; F = 2.09; p = 0.13
Duration of illness	15.45 ± 5.85	16.75 ± 9.84	13.14 ± 6.29	df = 2; F = 1.43; p = 0.24
Education (years)	10.73 ± 3.12	11.28 ± 2.75	11.33 ± 2.42	df = 2; F = 0.37; p = 0.69
Clozapine dose (mg/day)	226.3 ± 106.4	246.9 ± 114.6	200.3 ± 109.3	df = 2; F = 1.43; p = 0.24
PANSS Total score	88.91 ± 21.30	88.84 ± 18.53	91.24 ± 16.38	df = 2; F = 0.14; p = 0.87
PANSS Positive	21.10 ± 8.05	21.25 ± 6.20	21.90 ± 6.26	df = 2; F = 0.10; p = 0.91
PANSS Negative	23.23 ± 6.77	25.16 ± 7.02	26.33 ± 5.50	df = 2; F = 1.20; p = 0.30
PANSS General	44.60 ± 12.43	42.42 ± 9.21	43.00 ± 8.52	df = 2; F = 0.40; p = 0.67

df: Degrees of freedom; F: Female; M: Male.

Mean effect sizes of improvement in the PANSS Negative Symptoms Subscale among genotype groups are reported in Figure 3.

Discussion

To our knowledge, this is the first study assessing the influence of both *COMT* and *5-HT1A-R* polymorphisms on clinical response to clozapine. Clozapine, the gold standard for treatment-resistant schizophrenia, is characterized by a particular profile on dopaminergic system, known to be affected by *COMT* and *5-HT1A-R* genes .

At basal evaluation we did not find any difference between *COMT* or *5-HT1A-R* genotype groups for clinical and demographic characteristics and psychopathological assessment. This finding supports previous studies reporting a negative association between the *COMT* rs4680 polymorphism and clinical features [48] and only a trend for the *5-HT1A-R* polymorphism and negative symptomatology [38]. This suggests that the specific effects of single polymorphisms are likely to be very small and could differentially influence clinical symptomatology strongly depending on other underlying genetic factors.

It should be noted that our sample was not in HWE for the *5-HT1A-R* rs6295 polymorphism because of an excess of GG homozygous patients. However, deviations from HWE, when found in affected subjects, may indicate selection. The use of HWE in clinical samples is in fact a matter of debate; it has even been suggested that only healthy samples should be theoretically used when testing for deviations for HWE [49].

After 8 weeks of treatment with clozapine, we observed a global improvement in all psychopathological dimensions without significant differences between genotype groups, except for negative symptoms. The overall improvement was partially expected because the subjects were included from a naturalistic sample of patients who were resistant to neuroleptics and at least one atypical antipsychotic, representing a more

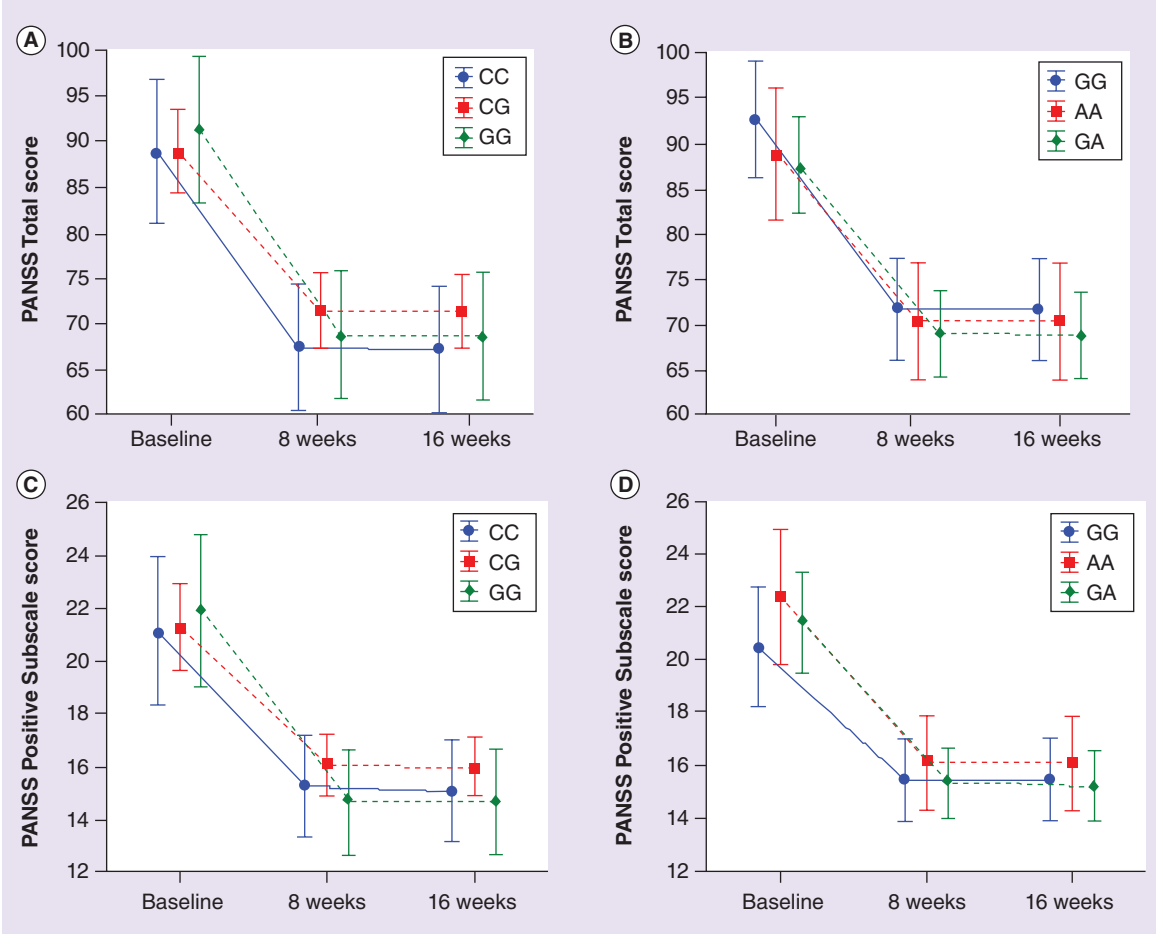

Figure 1. Trend analysis of PANSS Positive Subscale and PANSS Total Subscale at baseline, 8 weeks and 16 weeks in relation to rs6295 *5HT1A* genotype (CC, CG and GG) and to rs4680 *COMT* genotype (GG, AA and GA).
(A) *5HT1A* polymorphism and PANSS total score. **(B)** *COMT* polymorphism and PANSS total score. **(C)** *5HT1A* polymorphism and PANSS Positive Subscale. **(D)** *COMT* polymorphism and PANSS Positive Subscale.
PANSS: Positive and Negative Symptoms Scale.

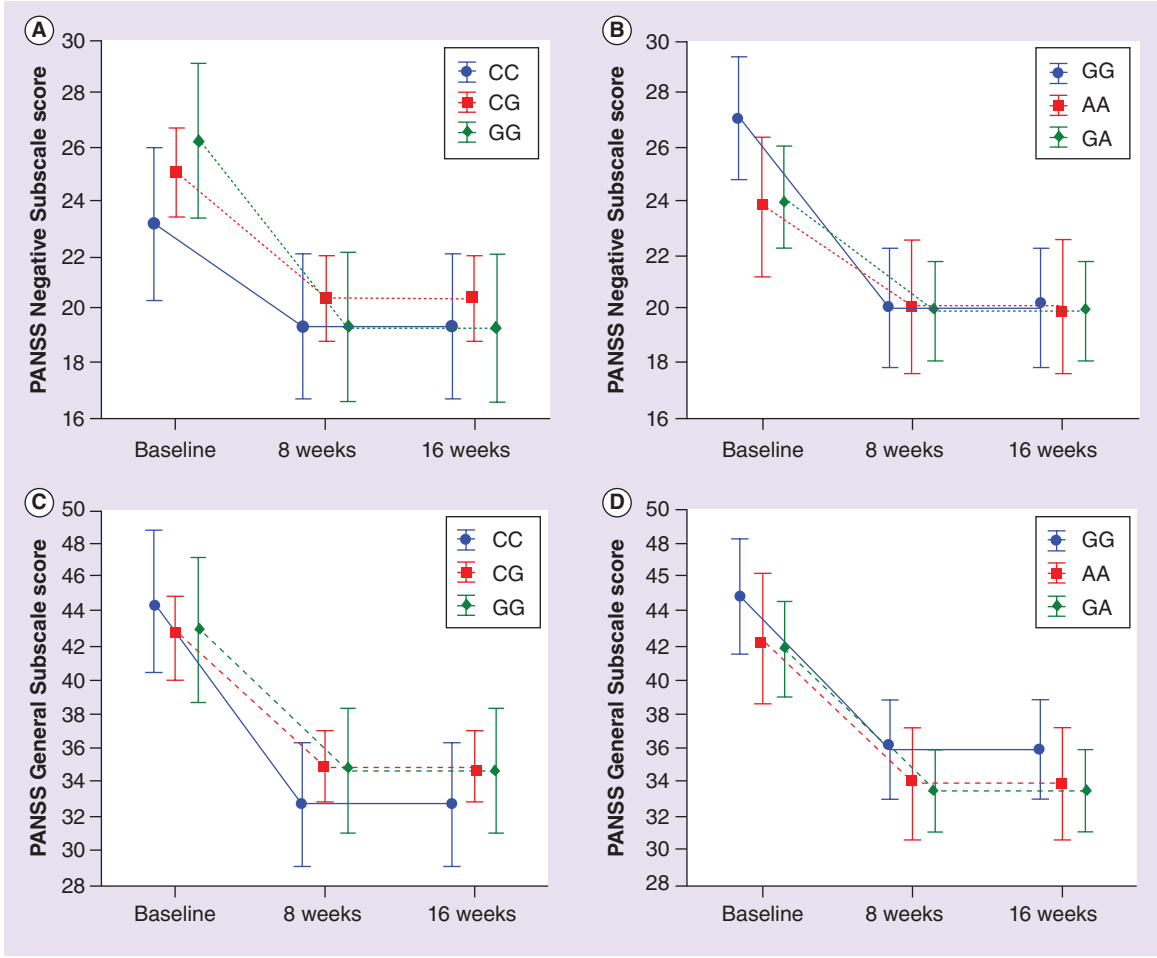

Figure 2. Trend analysis of PANSS Negative Subscale and PANSS General Subscale at baseline, 8 weeks and 16 weeks in relation to rs6295 *5HT1A* genotype (CC, CG and GG) and to rs4680 *COMT* genotype (GG, AA and GA). **(A)** *5HT1A* polymorphism and PANSS Negative Subscale. **(B)** *COMT* polymorphism and PANSS Negative Subscale. **(C)** *5HT1A* polymorphism and PANSS General Subscale. **(D)** *COMT* polymorphism and PANSS General Subscale. PANSS: Positive and Negative Symptoms Scale.

homogeneous subpopulation with a greater chance of responding to clozapine [44].

When comparing the changes in PANSS Negative Subscale, for the *COMT* genotype we observed a greater improvement in the Val/Val patients compared with both Val/Met and Met/Met, while for the *5-HT1A-R* polymorphism a significant difference was found between G/G and C/C subjects, with G/C subjects showing greater improvement. Thus, our results suggest that *COMT* rs4680 and *5-HT1A-R* rs6295 variations could influence response to clozapine. The effect is specific to negative symptoms, a core domain of schizophrenia, predictor of poor functional outcome and still poorly responsive to antipsychotic therapies [50]. Negative features are more closely related to dopaminergic activity at the PFC, putatively regulated by *COMT* and *5-HT1A-R* polymorphisms. Negative symptomatology has in fact been associated with a reduction in PFC activity [51] and worse performance in, for example, executive functions [52]. According to these data, among the psychopathological dimensions of schizophrenia, negative features appear the most likely to be modulated by *COMT* and *5-HT1A-R* functional polymorphisms in their response to pharmacological treatment.

Even if explanation of our findings is rather complex and may involve other unknown variables, some speculations can be made about the putative mechanisms underlying the observed effects. Regarding the *COMT* polymorphism, it could be hypothesized that clozapine may partially restore 'PFC inefficiency', which is more severe among Val/Val patients [9], through both a direct increase of PFC activation [7] and activity on D1 receptors [53], which are reported to be overexpressed among Val/Val subjects [54]. Similarly to *COMT* Val/Val, the *5-HT1A-R* G/G genotype seems to be disadvantageous, as it has been associated with more severe negative features [38] and cognitive

deficits [22]. The G allele, associated with an overexpression of *5-HT1A-R*, may lead to a decrease in PFC DA through inhibitory actions on pyramidal glutamatergic cells, as described for high-dose injection of *5-HT1A-R* agonists [5]. This genetic effect could be compensated for by clozapine treatment through its direct partial agonist activity on *5-HT1A-R* [55,56].

In the psychiatric literature much research has focused on *COMT* rs4680 and *5HT1A* rs6295 polymorphisms; nevertheless, this is the first study to analyze in schizophrenia the interaction that we believe exists between these SNPs in relation to the PANSS scales. It is well known that schizophrenia is a multifactorial and multigenic disease; for this reason, it is important to understand if and how different genetics markers may interact with each other to create a biological environment able to decrease or increase the disease susceptibility or the drug response. Our results support the hypothesis and suggest that *COMT* and *5-HT1A-R* genotypes could influence clozapine response and thus treatment choice, at least in patients with prominent negative features. However, other factors need to be taken into account. Among these, the epigenetic status of both investigated genes appears of relevance and may directly affect transcriptional levels, besides the DNA variants themselves. Clinical variables have also been reported to have a predictive effect on clozapine's response [57,58] and should be integrated with biological factors. Still, clinical features associated with better response to clozapine are generally derived from a relatively long disease course, while biological predictors could be more useful when choosing first-line treatment. Furthermore, the improvement in negative symptoms in patients treated with clozapine could be partly due to the improvement of the secondary negative symptoms, resulting from previous typical antipsychotic treatments. However, this effect would be observed in the whole sample, independently of the genetic variability.

This study has several limitations that need to be addressed. First of all, the relatively small sample size, which did not allow further analysis including analysis of other putatively influencing factors, such as gender. Moreover, since patients were included based on the criterion of pharmacoresistance, they could represent a specific subpopulation, a priori selected through the sequential treatment approach.

Conclusion

Our findings support the hypothesis that *COMT* rs4680 and *5-HT1A-R* rs6295 polymorphisms could influence the negative symptom response to clozapine, probably through modulation of the dopaminergic system. *COMT* Val/Val and *5-HT1A-R* G/G, the two genotypes previously reported to be associated with poorer response to antipsychotics [16,41] and hypothesized to be associated with lower PFC DA levels, seem to obtain a greater improvement when treated with clozapine, characterized by a different dopaminergic profile. Given the impact of negative symptoms on prognosis and func-

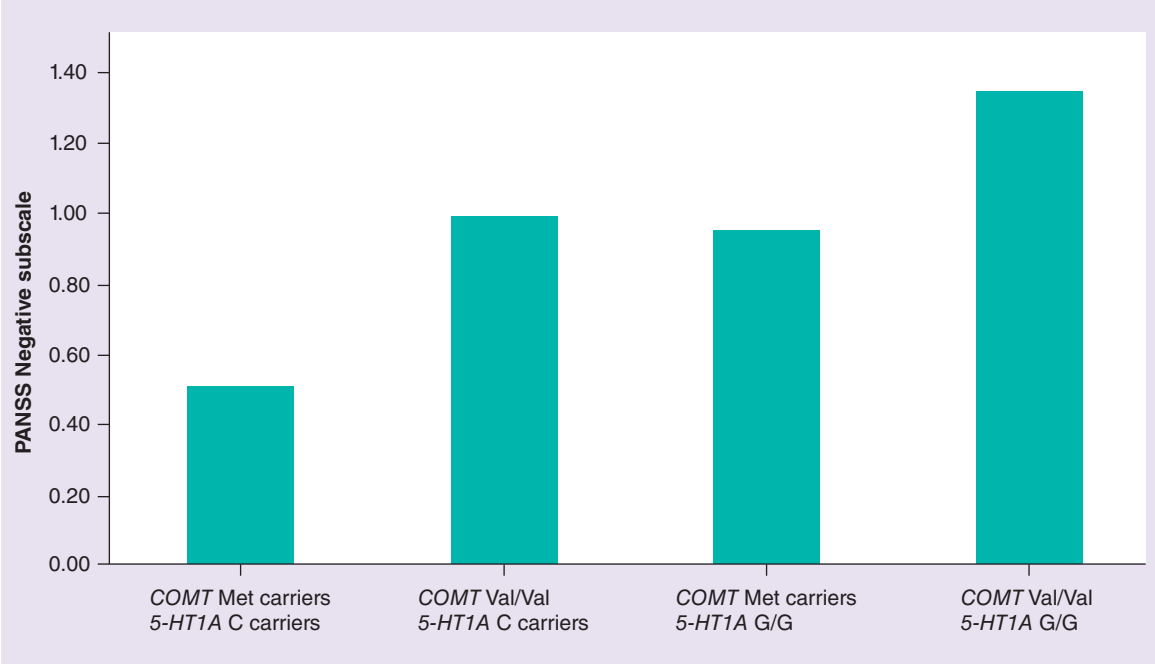

Figure 3. PANSS Negative Subscale: effect size of improvement at 12 weeks.
PANSS: Positive and Negative Symptoms Scale.

tional outcome [50], those genotypes could represent promising biological markers for treatment with clozapine. These findings may provide new perspectives on clozapine use, which is currently only allowed in cases of treatment resistance. It can be hypothesized that, in the future, clozapine could represent a first-line treatment in a group of patients selected on the basis of clinical and biological characteristics. Although these results are preliminary and need to be further replicated, they may help unravel the biological mechanisms underlying negative symptoms and pharmacological response.

Future perspective

To the best of our knowledge, this is the first paper investigating the synergic role of *COMT* rs4680 and *5-HT1A-R* rs6295 polymorphisms on negative symptomatology in treatment-resistant schizophrenia patients treated with clozapine. This pharmacogenetic approach could make important contributions with clinical relevance. On the one hand, it could contribute to an improved understanding of the biological mechanisms underlying the heterogeneity of clinical phenotypes. On the other hand, it may help unravel the neurobiological interface between symptoms and drugs in order to reach an individualized prediction of treatment response. This result should be added to previous findings in the context of a multivariate analysis, including gene variants and clinical features.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Executive summary

Background

- Clozapine is the gold standard for the treatment of pharmaco-resistant schizophrenia, despite up to 40% of patients not responding adequately to the treatment.
- Clinical studies widely suggest that prefrontal cortex (PFC) dysfunction occurs in schizophrenia.
- *COMT*, an enzyme involved in dopamine degradation, plays a major role in dopamine level regulation in the PFC.
- In the PFC, clozapine is able to increase dopamine release and neuronal activation through a mechanism involving 5-HT1A-R.

Aim of the study

- In the present paper, we examined the *COMT* rs4680 polymorphism, which has been proven to play a role in dopamine regulation at the PFC level, and the *5-HT1A-R* rs6295 variant, a target of clozapine activity involved in the interaction between the serotonin and dopamine systems.

Matherials & methods

- Neuroleptic-refractory patients diagnosed with schizophrenia (n = 107) were treated with clozapine and were assessed using the Positive and Negative Symptoms Scale at the beginning of treatment and, subsequently, at weeks 8 and 12.
- The patients were genotyped for *COMT* rs4680 and *5-HT1A-R* and rs6295 polymorphisms.

Results

- The paper supports the synergic influence of *COMT* rs4680 and *5-HT1A-R* and rs6295 polymorphisms on the negative symptom response to clozapine, probably through modulation of dopaminergic system.

Discussion & conclusion

- *COMT* and *5-HT1A-R* polymorphisms could direct treatment choice toward clozapine.
- Identification of potential predictors of clinical response to clozapine plays a key role in treating schizophrenia.

References

1 Grunder G, Hippius H, Carlsson A. The 'atypicality' of antipsychotics: a concept re-examined and re-defined. *Nat. Rev. Drug Discov.* 8(3), 197–202 (2009).

2 Raja M, Raja S. Clozapine safety, 40 years later. *Curr. Drug Saf.* 9(3), 163–195 (2014).

3 Chakos M, Lieberman J, Hoffman E, Bradford D, Sheitman B. Effectiveness of second-generation antipsychotics in patients with treatment-resistant schizophrenia: a review and

meta-analysis of randomized trials. *Am. J. Psychiatry* 158(4), 518–526 (2001).

4 Miller R. Mechanisms of action of antipsychotic drugs of different classes, refractoriness to therapeutic effects of classical neuroleptics, and individual variation in sensitivity to their actions: Part I. *Curr. Neuropharmacol.* 7(4), 302–314 (2009).

5 Diaz-Mataix L, Artigas F, Celada P. Activation of pyramidal cells in rat medial prefrontal cortex projecting to ventral tegmental area by a 5-HT1A receptor agonist. *Eur. Neuropsychopharmacol.* 16(4), 288–296 (2006).

6 Ettinger U, Williams Sc, Fannon D *et al.* Functional magnetic resonance imaging of a parametric working memory task in schizophrenia: relationship with performance and effects of antipsychotic treatment. *Psychopharmacology (Berl.)* 216(1), 17–27 (2011).

7 Purkayastha S, Ford J, Kanjilal B *et al.* Clozapine functions through the prefrontal cortex serotonin 1A receptor to heighten neuronal activity via calmodulin kinase II-NMDA receptor interactions. *J. Neurochem.* 120(3), 396–407 (2012).

8 Lotta T, Vidgren J, Tilgmann C *et al.* Kinetics of human soluble and membrane-bound catechol O-methyltransferase: a revised mechanism and description of the thermolabile variant of the enzyme. *Biochemistry* 34(13), 4202–4210 (1995).

9 Winterer G, Weinberger DR. Genes, dopamine and cortical signal-to-noise ratio in schizophrenia. *Trends Neurosci.* 27(11), 683–690 (2004).

10 Eisenberg DP, Sarpal D, Kohn PD *et al.* Catechol-O-methyltransferase valine(158)methionine genotype and resting regional cerebral blood flow in medication-free patients with schizophrenia. *Biol. Psychiatry* 67(3), 287–290 (2010).

11 Malhotra AK, Kestler LJ, Mazzanti C, Bates JA, Goldberg T, Goldman D. A functional polymorphism in the COMT gene and performance on a test of prefrontal cognition. *Am. J. Psychiatry* 159(4), 652–654 (2002).

12 Kang WS, Park JK, Park HJ *et al.* Association between catechol-O-methyltransferase Val158Met polymorphism and negative symptoms of schizophrenia in the Korean population. *Psychiatry Res.* 198(2), 338–339 (2012).

13 Pelayo-Teran JM, Crespo-Facorro B, Carrasco-Marin E *et al.* Catechol-O-methyltransferase Val158Met polymorphism and clinical characteristics in first episode non-affective psychosis. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 147B(5), 550–556 (2008).

14 Tsai SJ, Hong CJ, Yu YW, Chen TJ. Association study of catechol-O-methyltransferase gene and dopamine D4 receptor gene polymorphisms and personality traits in healthy young chinese females. *Neuropsychobiology* 50(2), 153–156 (2004).

15 Wang Y, Fang Y, Shen Y, Xu Q. Analysis of association between the catechol-O-methyltransferase (COMT) gene and negative symptoms in chronic schizophrenia. *Psychiatry Res.* 179(2), 147–150 (2010).

16 Molero P, Ortuno F, Zalacain M, Patino-Garcia A. Clinical involvement of catechol-O-methyltransferase polymorphisms in schizophrenia spectrum disorders: influence on the severity of psychotic symptoms and on the response to neuroleptic treatment. *Pharmacogenomics J.* 7(6), 418–426 (2007).

17 Anttila S, Illi A, Kampman O, Mattila KM, Lehtimaki T, Leinonen E. Interaction between NOTCH4 and catechol-O-methyltransferase genotypes in schizophrenia patients with poor response to typical neuroleptics. *Pharmacogenetics* 14(5), 303–307 (2004).

18 Inada T, Nakamura A, Iijima Y. Relationship between catechol-O-methyltransferase polymorphism and treatment-resistant schizophrenia. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 120B(1), 35–39 (2003).

19 Illi A, Kampman O, Hanninen K *et al.* Catechol-O-methyltransferase val108/158met genotype and response to antipsychotic medication in schizophrenia. *Hum. Psychopharmacol.* 22(4), 211–215 (2007).

20 Pelayo-Teran JM, Perez-Iglesias R, Vazquez-Bourgon J *et al.* Catechol-O-methyltransferase Val158Met polymorphism and negative symptoms after acute antipsychotic treatment in first-episode non-affective psychosis. *Psychiatry Res.* 185(1–2), 286–289 (2011).

21 Bertolino A, Caforio G, Blasi G *et al.* Interaction of COMT (Val(108/158)Met) genotype and olanzapine treatment on prefrontal cortical function in patients with schizophrenia. *Am. J. Psychiatry* 161(10), 1798–1805 (2004).

22 Bosia M, Bechi M, Marino E *et al.* Influence of catechol-O-methyltransferase Val158Met polymorphism on neuropsychological and functional outcomes of classical rehabilitation and cognitive remediation in schizophrenia. *Neurosci. Lett.* 417(3), 271–274 (2007).

23 Weickert TW, Goldberg TE, Mishara A *et al.* Catechol-O-methyltransferase val108/158met genotype predicts working memory response to antipsychotic medications. *Biol. Psychiatry* 56(9), 677–682 (2004).

24 Heusler P, Bruins Slot L, Tourette A, Tardif S, Cussac D. The clozapine metabolite N-desmethylclozapine displays variable activity in diverse functional assays at human dopamine D(2) and serotonin 5-HT(1)A receptors. *Eur. J. Pharmacol.* 669(1–3), 51–58 (2011).

25 Rollema H, Lu Y, Schmidt AW, Sprouse JS, Zorn SH. 5-HT(1A) receptor activation contributes to ziprasidone-induced dopamine release in the rat prefrontal cortex. *Biol. Psychiatry* 48(3), 229–237 (2000).

26 Kobilka BK, Frielle T, Collins S *et al.* An intronless gene encoding a potential member of the family of receptors coupled to guanine nucleotide regulatory proteins. *Nature* 329(6134), 75–79 (1987).

27 Meneses A. 5-HT system and cognition. *Neurosci. Biobehav. Rev.* 23(8), 1111–1125 (1999).

28 Steckler T, Sahgal A. Psychopharmacological studies in rats responding at touch-sensitive devices. *Psychopharmacology (Berl.)* 118(2), 226–229 (1995).

29 Lemonde S, Turecki G, Bakish D *et al.* Impaired trans-repression at a 5-HT1A receptor gene polimorphism associated with major depression and suicide. *J. Neurosci.* 23(25), 8788–8799 (2003).

30 Albert PR. Transcriptional regulation of the 5-HT1A receptor: implications for mental illness. *Philos. Trans. R Soc. Lond. B Biol. Sci.* 367(1601), 2402–2415 (2012).

31 Angles MR, Ocana DB, Medellin BC, Tovilla-Zarate C. No association between the HTR1A gene and suicidal behavior: a meta-analysis. *Rev. Bras. Psiquiatr.* 34(1), 38–42 (2012).

32 Gonzalez-Castro TB, Tovilla-Zarate CA, Juarez-Rojop I *et al.* Association of 5HTR1A gene variants with suicidal behavior: case-control study and updated meta-analysis. *J. Psychiatr. Res.* 47(11), 1665–1672 (2013).

33 Drago A, Ronchi DD, Serretti A. 5-HT1A gene variants and psychiatric disorders: a review of current literature and selection of SNPs for future studies. *Int. J. Neuropsychopharmacol.* 11(5), 701–721 (2008).

34 Gross C, Zhuang X, Stark K *et al.* Serotonin1A receptor acts during development to establish normal anxiety-like behaviour in the adult. *Nature* 416(6879), 396–400 (2002).

35 Lemonde S, Du L, Bakish D, Hrdina P, Albert PR. Association of the C(1019)G 5-HT1A functional promoter polymorphism with antidepressant response. *Psychopharmacology (Berl.)* 24, 24 (2004).

36 Strobel A, Gutknecht L, Rothe C *et al.* Allelic variation in 5-HT1A receptor expression is associated with anxiety- and depression-related personality traits. *J. Neural Transm.* 110(12), 1445–1453 (2003).

37 Kato M, Fukuda T, Wakeno M *et al.* Effect of 5-HT1A gene polymorphisms on antidepressant response in major depressive disorder. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 150B(1), 115–123 (2009).

38 Kim YK, Yoon HK. Effect of serotonin-related gene polymorphisms on pathogenesis and treatment response in Korean schizophrenic patients. *Behav. Genet.* 41(5), 709–715 (2011).

39 Mossner R, Schuhmacher A, Kuhn KU *et al.* Functional serotonin 1A receptor variant influences treatment response to atypical antipsychotics in schizophrenia. *Pharmacogenet. Genomics* 19(1), 91–94 (2009).

40 Wang L, Fang C, Zhang A *et al.* The -1019 C/G polymorphism of the 5-HT(1)A receptor gene is associated with negative symptom response to risperidone treatment in schizophrenia patients. *J. Psychopharmacol.* 22(8), 904–909 (2008).

41 Reynolds GP, Arranz B, Templeman LA, Fertuzinhos S, San L. Effect of 5-HT1A receptor gene polymorphism on negative and depressive symptom response to antipsychotic treatment of drug-naive psychotic patients. *Am. J. Psychiatry* 163(10), 1826–1829 (2006).

42 Bosia M, Bechi M, Pirovano A *et al.* COMT and 5-HT1A-receptor genotypes potentially affect executive functions improvement after cognitive remediation in schizophrenia. *Health Psychol. Behav. Med.* 2(1), 509–516 (2014).

43 Tang H, Dalton CF, Srisawat U, Zhang ZJ, Reynolds GP. Methylation at a transcription factor-binding site on the 5-HT1A receptor gene correlates with negative symptom treatment response in first episode schizophrenia. *Int. J. Neuropsychopharmacol.*17(4), 645–649 (2013).

44 Cavallaro R, Brambilla P, Smeraldi E. The sequential treatment approach to resistant schizophrenia with risperidone and clozapine: results of an open study with follow-up. *Hum. Psychopharmacol. Clin. Exp.* 13(2), 91–97 (1998).

45 Kay SR, Fiszbein A, Opler LA. The positive and negative syndrome scale (PANSS) for schizophrenia. *Schizophr. Bull.* 13(2), 261–276 (1987).

46 Wykes T, Reeder C, Corner J, Williams C, Everitt B. The effects of neurocognitive remediation on executive processing in patients with schizophrenia. *Schizophr. Bull.* 25(2), 291–307 (1999).

47 Bosia M, Anselmetti S, Bechi M *et al.* Effect of 5-HT1A-receptor functional polymorphism on Theory of Mind performances in schizophrenia. *Psychiatry Res.* 188(2), 187–190 (2011).

48 Strous RD, Lapidus R, Viglin D, Kotler M, Lachman HM. Analysis of an association between the COMT polymorphism and clinical symptomatology in schizophrenia. *Neurosci. Lett.* 393(2–3), 170–173 (2006).

49 Anderson CA, Pettersson FH, Clarke GM, Cardon LR, Morris AP, Zondervan KT. Data quality control in genetic case-control association studies. *Nat. Protoc.* 5(9), 1564–1573 (2010).

50 Lindenmayer JP, Czobor P, Volavka J *et al.* Effects of atypical antipsychotics on the syndromal profile in treatment-resistant schizophrenia. *J. Clin. Psychiatry* 65(4), 551–556 (2004).

51 Wang CS, Yang YK, Chen M, Chiu NT, Yeh TL, Lee IH. Negative symptoms and regional cerebral blood flow in patients with schizophrenia: a single photon emission computed tomography study. *Kaohsiung J. Med. Sci.* 19(9), 464–469 (2003).

52 O'Leary DS, Flaum M, Kesler ML, Flashman LA, Arndt S, Andreasen NC. Cognitive correlates of the negative, disorganized, and psychotic symptom dimensions of schizophrenia. *J. Neuropsychiatry Clin. Neurosci.* 12(1), 4–15 (2000).

53 Tauscher J, Hussain T, Agid O *et al.* Equivalent occupancy of dopamine D1 and D2 receptors with clozapine: differentiation from other atypical antipsychotics. *Am. J. Psychiatry* 161(9), 1620–1625 (2004).

54 Slifstein M, Kolachana B, Simpson EH *et al.* COMT genotype predicts cortical-limbic D1 receptor availability measured with [^{11}C]NNC112 and PET. *Mol. Psychiatry* 13(8), 821–827 (2008).

55 Millan MJ. Improving the treatment of schizophrenia: focus on serotonin (5-HT)(1A) receptors. *J. Pharmacol. Exp. Ther.* 295(3), 853–861 (2000).

56 Perry KW, Hemrick-Leucke S, Evans DC *et al.* Do atypical antipsychotic agents interact with 5-HT1A receptors and monoamine uptake sites *in vivo*. *Am. Soc. Neurosci. Abstr.* 24, 772 (1998).

57 Umbricht DS, Wirshing WS, Wirshing DA *et al.* Clinical predictors of response to clozapine treatment in ambulatory patients with schizophrenia. *J. Clin. Psychiatry* 63(5), 420–424 (2002).

58 Nielsen J, Nielsen RE, Correll CU *et al.* Predictors of clozapine response in patients with treatment-refractory schizophrenia: results from a Danish Register Study. *J. Clin. Psychopharmacol.* 32(5), 678–683 (2012).