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Factors affecting cognitive remediation response in schizophrenia: The role of *COMT* gene and antipsychotic treatment

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ABSTRACT

Cognitive remediation is the best available tool to treat cognitive deficits in schizophrenia and has evidence of biological validity; however results are still heterogeneous and significant predictors are lacking. Previous studies showed that cognitive remediation is able to induce changes in PFC function and dopaminergic transmission and thus the study of possible sources of variability at these levels (i.e. antipsychotic treatments and genetic variability) might help to gain a deeper understanding of neurobiological correlates and translate into optimization and personalization of interventions. In the present study, we analyzed the interaction between pharmacological treatment (clozapine vs typical/atypical D2 blockers) and *COMT* rs4680 polymorphism on cognitive changes after cognitive remediation therapy, in a sample of 98 clinically stabilized patients with schizophrenia. The General Linear Model showed a significant interaction of pharmacological treatment and *COMT* polymorphism on the improvement in "Symbol Coding" subtest, a global measure of speed of processing. Post-hoc analysis revealed a significant difference between *COMT* genotypes, when treated with D2 blockers, with worse results among Val/Val patients.

These preliminary results suggest that genetic variability, influencing prefrontal dopamine, might affect individual capacity to improve with different patterns, depending on antipsychotic treatment.

1. Introduction

Cognitive deficits have been recognized for more than 20 years as core features of schizophrenia, critical determinants of functional outcome and key factors toward understanding the etiopathology (Keefe and Harvey, 2012). So far, rehabilitation interventions, such as cognitive remediation therapy (CRT), are the best available tools to treat cognitive deficits and improve general functioning in schizophrenia (Medalia and Choi, 2009; Wykes et al., 2011). However, the reported effects of CRT on cognitive outcomes at post-treatment and follow-up assessments are very heterogeneous and significant predictors are still lacking. This may also depend on the fact that little is known about the underlying neurobiological mechanisms (Wykes and Spaulding, 2011).

Recent studies proved that cognitive training is able to induce neurobiological changes. Data from functional neuroimaging showed that cognitive improvement was associated with increased task-related brain activation, mainly in frontal areas (Wykes et al., 2002; Haut et al., 2010; Bor et al., 2011; Penadés et al., 2013). An involvement of prefrontal dopamine bioavailability has been claimed to underlie the changes occurring with CRT.

Some studies suggested a role of genes affecting dopamine modulation on outcomes of cognitive remediation (Bosia et al., 2007; Pieramico et al., 2012; Panizzutti et al., 2013). Moreover, cognitive training effect has been associated to change in cortical dopamine D1 receptor binding. Specifically, the degree of improvement in working memory is associated to a decrease in D1 binding (McNab et al., 2009). Interestingly, catechol-O-methyltransferase (*COMT*) rs4680 polymorphism, regulating dopamine levels and affecting performance and neurophysiological response to tasks of prefrontal functions (Egan et al., 2001; Goldberg et al., 2003; Bertolino et al., 2006; Diaz-Asper et al., 2008; Green et al., 2013), has also been associated with D1 receptor expression. In particular,

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D1 receptor expression is higher among Val/Val homozygous, compared to carriers of the Met allele (Slifstein et al., 2008).

Another factor that needs to be taken into account is the pharmacological treatment. The effects of typical and atypical antipsychotics on cognition have been deeply investigated. Several studies suggested a superiority of atypical over typical (Keefe et al., 2004a; Rémillard et al., 2008), with effects also on electrophysiological marker of prefrontal brain function (Ehlis et al., 2007); however this was not confirmed in recent works (Keefe et al., 2007; Kucharska-Pietura et al., 2012). Indeed, also considering specific drug-related differences, the overall impact of antipsychotic treatment on cognitive functions is limited (Harvey and Keefe, 2001). However it can be hypothesized that the "cognitive potential" of drug treatment requires stimulation and training to benefit from, thus acting in synergy with cognitive remediation strategies. Commonly used antipsychotic drugs have direct and differentiated effects on the same biological mechanisms that are suggested to support cognitive remediation: PFC activity (Molina et al., 2008), dopaminergic transmission (Tauscher et al., 2004) and neurotrophic factors (Bai et al., 2003).

In this view clozapine, the prototype of atypical antipsychotics and still the gold standard for treatment of refractory schizophrenia, shows a peculiar profile. Clozapine displays some unique properties both in its receptorial binding profile, distinguished by a higher affinity for D1 than D2 and regional selectivity on D1 receptor, and neurophysiological effects. Possible reciprocal modulation of clozapine and *COMT* on CRT effect may be hypothesized. On the one hand, clozapine directly affects dopamine (DA) availability in the prefrontal cortex (Meltzer and Massey, 2011; Purkayastha et al., 2012) and has significantly higher affinity of for D1 binding site (Tauscher et al., 2004). On the other hand, *COMT* genotype is well known to regulate DA in the prefrontal cortex and significant differences in D1 expression have been reported between *COMT* genotypes (Slifstein et al., 2008). Previous studies, in patients treated with clozapine, also reported an association between *COMT* genotype and improvement in cognitive functions and negative symptoms, highly related to Prefrontal Cortex (PFC) dopaminergic activity (Weickert et al., 2004; Woodward et al., 2007).

These data provide the rationale to analyze the effects of clozapine alone, compared to several antipsychotics, even belonging to different classes, grouped together. Based on this evidence, we hypothesized that the heterogeneity of CRT response could partially rely on interaction between *COMT* genotype and antipsychotic treatment, as they both differentially affect dopamine transmission. In the present study we explore the possible role of *COMT* rs4680 polymorphism and pharmacological treatment, with particular attention to clozapine, in predicting CRT outcomes among patients with schizophrenia.

2. Methods

A sample of 98 Caucasian biologically unrelated outpatients were recruited at the San Raffaele Scientific Institute of Milan (Italy). Inclusion criteria were as follows: diagnosis of schizophrenia meeting DSM-IV-TR criteria, age from 18 to 65 years, I.Q. ≥ 70, treatment with a stable dose of the same antipsychotic in monotherapy since at least 3 months and good response to treatment (defined as a reduction of 30% or more in PANSS Total Score after 3 months of treatment) (Lin et al., 2013). Exclusion criteria were as follows: psychiatric comorbidities, concomitant psychiatric treatments except benzodiazepines, substance abuse, neurological disorders and brain injury. After a complete description of the study, informed consent to participation was obtained. The protocol followed the principles of the Declaration of Helsinki.

2.1. Genotyping

All patients underwent a venous blood sample for genotypic analysis of *COMT* rs4680 polymorphism. DNA was extracted from whole blood by manual extraction, using the "Illustra blood genomicPrep Midi Flow kit" (GE Healthcare, Milan, Italy). Polymerase chain reaction (PCR) was performed with the following primers: 5′-ACT GTG GCT ACT CAG CTG TG-3′, 5′-CCT TTT TCC AGG TCT GAC AA-3′. The PCR reaction was carried out by ABI 9700 PCR thermal-cycler (Applied Biosystems, APPLERA) in a 10 μl volume containing 150 ng of genomic DNA, 5 pmol of each primer, 10 nmol of dNTPs' mix, 10× HotMaster Taq Buffer and 0.5 U of HotMaster Taq DNA Polymerase (Eppendorf, Milan, Italy). The amplified fragment was then purified by Multi-Screen Colum Loader (MILLIPORE), filled up and packaged with Sephadex G-50 (Sigma-Aldrich's) to remove residual PCR reagents. An aliquot of purified PCR product was then used to perform sequencing reaction, using DYEnamic ET Dye Terminator Cycle Sequencing Kit (GE Healthcare, Milan, Italy). In its turn, sequencing reaction product, was purified following the above-mentioned protocol, to remove the excess of fluorescent dyes not, incorporated in the DNA fragment. The fragment was then sequenced by MegaBACE 500 genetic analyzer (GE Healthcare, Milan, Italy) under standard conditions.

2.2. Assessment

Basic clinical and demographic data were collected from clinical reports.

Psychopathology was assessed by means of Positive and Negative Syndrome Scale for Schizophrenia—PANSS (Kay et al., 1987), administered by trained psychiatrists.

The general intellectual level was evaluated with the Wechsler Adult Intelligence Scale – R (WAIS-R) (Wechsler, 2006), a standardized test designed to measure intelligence in adults.

Neuropsychological measures were evaluated at baseline and after completion of cognitive remediation therapy with the Brief Assessment of Cognition in Schizophrenia—BACS (Keefe et al., 2004b), a short battery of neuropsychological tests specifically designed in two versions (A and B) to evaluate patients before and after rehabilitation programs, without the results being influenced by recall.

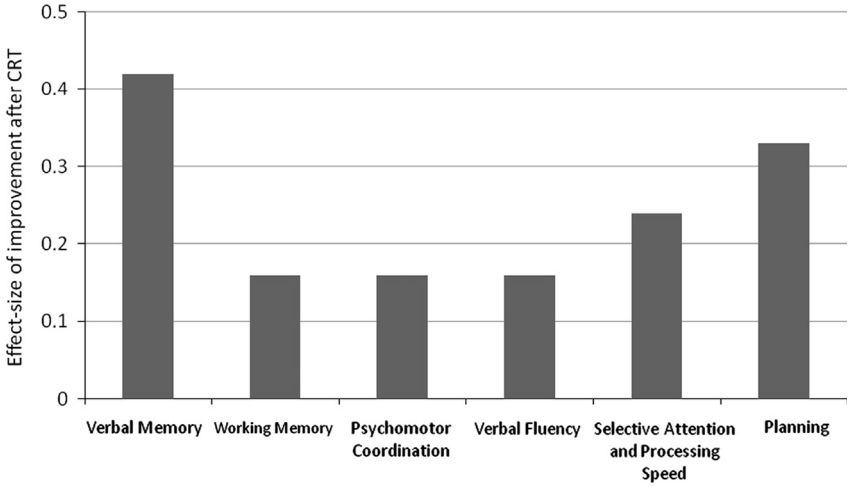

Fig. 1. Effect sizes of improvement after cognitive remediation for the different neuropsychological performances in the total sample ($n=98$).

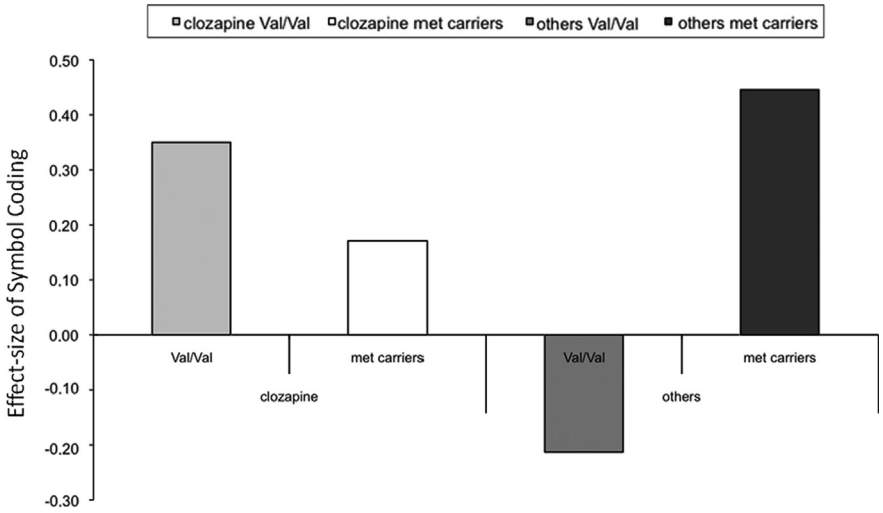

Fig. 2. Mean effect sizes of improvement in speed of processing, evaluated by means of Symbol Coding task, by COMT genotype and treatment (clozapine vs others) groups. Post-hoc Tukey Test shows a significant difference between COMT Met carriers and COMT Val/Val treated with antipsychotics other than clozapine ($p=0.01$), the latter showing worse performances.

Table 1
Demographic and clinical features of the sample, stratified by genotype and treatment groups (yrs = =years; S.D.=Standard Deviation).

	DEMOGRAPHIC AND CLINICAL FEATURES				
	Val/Val clozapine (Mean ± S.D.)	**Val/Val other treatments** (Mean ± S.D.)	**Met carriers clozapine** (Mean ± S.D.)	**Met carriers other treatments** (Mean ± S.D.)	**χ^2 or ANOVA**
Gender	M=7; F=2	M=10; F=5	M=20; F=13	M=23; F=18	$\chi 2=1.68$; $p=0.64$
Age (years)	36.2 ± 8.1	35.2 ± 10.4	33.5 ± 10.8	35.1 ± 9.4	$F=0.26$; $p=0.85$
Education (years)	11.5 ± 3.0	11.9 ± 2.6	11.6 ± 2.5	11.8 ± 2.4	$F=0.11$; $p=0.95$
Onset (years)	24.7 ± 8.8	21.6 ± 4.8	22.9 ± 6.4	23.4 ± 5.3	$F=0.53$; $p=0.66$
Duration of illness (years)	12.3 ± 8.4	12.1 ± 6.8	10.7 ± 9.2	11.7 ± 9.4	$F=0.12$; $p=0.95$
PANSS Positive Scale (score)	16.1 ± 5.2	18.3 ± 6.6	15.2 ± 5.3	15.3 ± 5.0	$F=0.90$; $p=0.44$
PANSS Negative Scale (score)	19.7 ± 7.7	26.2 ± 5.11	19.7 ± 7.7	21.6 ± 5.3	$F=2.55$; $p=0.06$
PANSS General Scale (score)	38.4 ± 7.1	36.8 ± 8.01	32.2 ± 10.6	36.0 ± 7.6	$F=1.40$; $p=0.25$
WAIS-R Total I.Q. (score)	87.8 ± 12.1	85.8 ± 16.5	84.5 ± 11.8	86.0 ± 11.0	$F=0.06$; $p=0.92$

It consists of the following tests: verbal memory (words recall), working memory (digit sequencing), token motor task (psychomotor speed and coordination), speed of processing (symbol coding), verbal fluency (semantic and letter production) and planning (Tower of London).

2.3. Cognitive remediation therapy

All patients underwent cognitive remediation therapy (CRT), added to standard rehabilitation therapy (SRT), including non cognitive subprograms of IPT (Brenner et al., 1994), social skills training and psychoeducation. The cognitive remediation protocol consisted of three sessions of 1 h each of function-specific computer-aided exercises a week (36 sessions). Computer-assisted neurocognitive exercise was performed employing the Cogpack Software (Marker, 1987–2007), the program was set for adaptive exercises, based on patients' performances during the course of the session. Sets of exercises were individually created for each patient, starting from baseline performances at neuropsychological assessment. Exercises were administered by trained psychologists whose role was to motivate patients and assist them in completing exercises and trying different strategies, without giving them the solutions to the exercises.

2.4. Data analysis

For genetic analysis, as in previous studies (Bosia et al., 2007), patients were divided into two groups: the homozygous for the Val allele vs carriers of, at least, one Met allele. The rationale is to avoid further levels of factors in the analysis, given the small sample size, and based also on previous studies showing no significant differences in cognitive performances between Met homozygous and Val/Met subjects (Bertolino et al., 2004; Rosa et al., 2004). To examine the effect of pharmacological treatment, the sample was divided into two groups: subjects treated with clozapine vs subjects treated with other drugs, characterized by higher dopamine D2 blocking activity. The rationale of this choice is explained in the introduction and further argued in the discussion. Demographic and clinical characteristics and basal neuropsychological measures were analyzed for group differences by means of analysis of variance (ANOVA) and χ^2 Test (for dichotomic variables).

To evaluate changes in cognition after CRT, for each BACS test we calculated an index value, determined by change in the test score divided by the standard error of the whole sample (mean between baseline and post-CRT). This value represents a proxy effect size measure of improvement (Wykes et al., 1999).

A General Linear Model Analysis, with genotype and treatment groups as categorical predictors, age and years of education as covariates and the effect size of improvement on BACS subtests as dependent variables was used to evaluate the effects of genotype and treatment on cognitive improvement after CRT. Post-hoc analyses were performed both with Fisher LSD Test and then with HSD Test for Unequal numbers, to be more conservative, given the unbalanced groups sizes.

3. Results

3.1. Descriptive analysis

The sample consisted of 98 patients, 60 males and 38 females. Ongoing pharmacological treatments were as follows: clozapine (43 subjects, mean dose 254.17 ± 123.42), risperidone (34 subjects, mean dose 4.43 ± 1.86), haloperidol (10 subjects, mean dose 3.94 ± 1.95), olanzapine (7 subjects, mean dose 13.33 ± 6.05) and paliperidone (4 subjects, mean dose 9.0 ± 3.0). DNA analysis showed the following genotypic distribution in Hardy–Weinberg equilibrium: 24 patients Val/Val, 50 Val/Met and 24 Met/Met.

We compared demographic and clinical variables between genotype and treatment groups separately and then stratified by genotype and treatment (four groups) with ANOVA or χ^2 test for dichotomic variables. The analyses did not show any significant differences among groups. Results, stratified by genotype and treatment, are reported in Table 1. We repeated these analyses also on neuropsychological measures, both at baseline and post-CRT and on the effect-size of improvement. The only significant effect was observed on improvement at Symbol Coding. Results are shown in Table 2 and Fig. 1. Moreover a χ^2 test showed no significant differences in genotype frequencies between gender and treatment groups.

3.2. General Linear Model analysis

The General Linear Model Analysis showed significant effects only in "Symbol Coding" BACS subtest, a task that evaluates selective attention and working memory, representing a global measure of speed of processing. The analysis, with *COMT* genotype and treatment group as categorical predictors and age and education as covariates, revealed a significant interaction effect of *COMT* genotype and treatment ($F=5.86$, $p=0.018$), while no effects were observed for age ($F=0.89$, $p=0.35$), education ($F=0.56$, $p=0.46$), *COMT* genotype ($F=1.92$, $p=0.17$) or treatment ($F=0.70$, $p=0.41$). Fisher's Post-hoc Test showed a significant difference between *COMT* Met carriers and *COMT* Val/Val treated with antipsychotics other than clozapine ($p=0.002$), the latter showing worse performances. Trends were also observed between *COMT* Val/Val treated with either clozapine or other antipsychotics ($p=0.06$), in favor of those treated with clozapine, and between *COMT* Val/Val treated with other antipsychotics and *COMT* Met carriers treated with clozapine ($p=0.07$). Post-hoc HSD for Unequal numbers confirmed a significant difference only between *COMT* Met carriers and *COMT* Val/Val treated with antipsychotics other than clozapine ($p=0.04$), the latter showing worse performances. Mean effect sizes of improvement in speed of processing, evaluated by means of Symbol Coding task, by COMT genotype and treatment (clozapine vs others) groups are shown in Fig. 2.

4. Discussion

Identification of predictors of response to CRT is of critical relevance for both clinical and research implications. It would allow to personalize interventions, maximizing the likelihood of successful improvement, and to point out the underlying biological factors. These may also contribute to gain a deeper knowledge into etiopathogenesis of the illness and represent fruitful targets for novel pharmacological agents.

To our knowledge, this is the first study investigating the effect of *COMT* rs4680 polymorphism on CRT in subjects affected by schizophrenia, taking also into account the effect of antipsychotic drugs, characterized by markedly different D1 and D2 receptorial binding profile. Results showed a statistically significant interaction of pharmacological treatment and *COMT* polymorphism on cognitive improvement, suggesting that the effect of *COMT* on CRT results may vary according to medication. We reported a significant difference between genotypes only among patients treated with more prominent D2 blocking antipsychotics (Met carriers obtaining a significantly greater effect size). The effect was specifically observed on the "Symbol Coding" subtest. This task involves integration of multiple component operations, relying mostly on effective connectivity among distributed brain networks, rather than specific subprocesses (Dickinson et al., 2007). Thus it requires high executive control that can be more strongly influenced by *COMT* genotype. Moreover the Symbol Coding task represents a measure of global speed of processing, a domain that

Table 2
Neuropsychological performances at basal evaluation and after Cognitive Remediation Therapy (CRT) and effect size of improvement, stratified by genotype and treatment groups.

	NEUROPSYCHOLOGICAL PERFORMANCES (raw scores)				
	Val/Val clozapine (Mean ± S.D.)	**Val/Val other treatments** (Mean ± S.D.)	**Met carriers clozapine** (Mean ± S.D.)	**Met carriers other treatments** (Mean ± S.D.)	**ANOVA**
Verbal memory					
Baseline	36.7 ± 6.5	30.6 ± 9.9	34.9 ± 13.3	36.6 ± 11.7	$F=1.00$; $p=0.40$
After CRT	39.5 ± 11.7	34.7 ± 10.1	39.5 ± 10.9	43.0 ± 10.4	$F=2.32$: $p=0.08$
Effect size	0.25	0.37	0.41	0.57	$F=0.42$; $p=0.73$
Working memory					
Baseline	17.8 ± 4.9	13.9 ± 6.9	17.0 ± 4.5	16.2 ± 4.0	$F=1.73$; $p=0.16$
After CRT	17.9 ± 4.2	15.1 ± 5.7	18.0 ± 3.9	16.9 ± 4.5	$F=1.50$; $p=0.22$
Effect size	0.01	0.13	0.11	0.07	$F=0.15$; $p=0.93$
Psychomotor speed/coordination					
Baseline	62.7 ± 24.2	61.6 ± 20.6	68.6 ± 14.7	65.6 ± 20.2	$F=0.51$; $p=0.67$
After CRT	70.7 ± 19.3	69.7 ± 10.34	65.8 ± 16.9	69.42 ± 18.2	$F=0.36$; $p=0.78$
Effect size	0.22	0.23	−0.05	0.09	$F=1.19$; $p=0.32$
Verbal fluency					
Baseline	37.4 ± 12.5	29.9 ± 12.2	37.6 ± 14.0	37.0 ± 12.1	$F=1.41$; $p=0.24$
After CRT	42.4 ± 11.1	36.3 ± 14.6	38.3 ± 10.4	37.9 ± 10.1	$F=0.57$; $p=0.64$
Effect size	0.41	0.52	0.05	0.08	$F=1.87$; $p=0.14$
Speed of processing					
Baseline	35.5 ± 11.4	35.3 ± 11.7	37.4 ± 11.7	36.8 ± 12.7	$F=0.12$; $p=0.94$
After CRT	39.5 ± 9.60	32.7 ± 13.2	39.3 ± 11.5	42.0 ± 10.7	$F=2.41$; $p=0.07$
Effect size	0.34	−0.22	0.19	0.44	$F=3.30$; $p=0.02$
Planning					
Baseline	13.1 ± 4.2	12.3 ± 5.8	13.3 ± 4.1	12.8 ± 3.5	$F=0.20$; $p=0.90$
After CRT	14.12 ± 2.5	14.1 ± 5.1	15.1 ± 7.0	14.1 ± 3.1	$F=0.26$; $p=0.85$
Effect size	0.03	0.24	0.46	0.28	$F=0.39$; $p=0.76$

usually shows only modest improvement after CRT (Wykes et al., 2011).

Few studies have investigated genetic correlates of CRT outcomes in schizophrenia, focusing on polymorphisms of *COMT*, a key regulator of prefrontal dopamine known to mediate some aspects of cognition. Bosia et al. (2007) observed a significant effect of *COMT* genotype on CRT response in a randomized study (CRT vs placebo), while two subsequent studies reported negative results (Bosia et al., 2007; Greenwood et al., 2011; Panizzutti et al., 2013). An effect of dopamine-related genes (*COMT* and *DRD3* polymorphisms) on cognitive improvement has been suggested also in healthy subjects through different types of interventions, namely combination training, aerobic exercise and transcranial direct current stimulation (Stroth et al., 2010; Pieramico et al., 2012; Plewnia et al., 2012).

These contradictory findings may rely, among other factors, on the medication regimen used in patients with schizophrenia, which was not taken into account in previous works, as the effect of *COMT* genotype may vary depending on treatment status. Several evidences suggest that pharmacological treatment may mediate the response to CRT and interact with *COMT* genetic variants. First, antipsychotics directly act on dopaminergic transmission, known to influence cognitive performances through a non-linear relationship, which is in turn modulated by *COMT* genotype. Secondly, it has been shown that antipsychotics drugs differentially affect PFC activity and changes at this level have been observed both within patient after CRT and between individuals carrying different *COMT* genotypic variants. Thirdly, a specific drug-related effect on neurotrophic factors (i.e. BDNF) has been reported, that are likely to influence also *COMT* expression, probably through epigenetic mechanisms, such as methylation processes. BDNF levels are critical for neuroplasticity and are suggested to be involved in the biological mechanisms underlying CRT. Moreover, a previous study exploring motor–cortex plasticity showed that the effects of a *BDNF* polymorphism were dependent on *COMT* Val/Met status (Witte et al., 2012). Even though several distinctions among treatments may be underpinned for each single molecule, we chose to compare the effect of clozapine with a group including different antipsychotics. Considering the above mentioned aspects, clozapine displays a peculiar profile that allows to distinguish it from other drugs. Beyond its affinity for dopamine D4 receptors, serotonin 5-HT2A receptor antagonism, effects on the noradrenergic system, and its relatively lower D2 receptors blockade compared to other antipsychotics, clozapine also shows a unique D1 and D2 receptor binding profile. Compared to other antipsychotics, the D1/D2 occupancy ratio is greater and it displays regional selectivity. Specifically co-expression of both D1 and D2 induces a significantly higher affinity of clozapine for D1 binding site, not affecting the affinity for D2 receptor (Tauscher et al., 2004; Chou et al., 2006; Faron-Górecka et al., 2008). Through partial agonist effect at the 5HT1A receptor, clozapine is also associated to increased DA release and heightened neuronal activity in the prefrontal cortex (Meltzer and Massey, 2011; Purkayastha et al., 2012). Moreover, different cerebral activity patterns during treatment with clozapine and other typical and atypical neuroleptics have been reported, suggesting that clozapine may facilitate activation of the regions involved in cognitive tasks (Molina et al., 2008). Finally, clozapine seems to exhibit distinctive effects on neurotrophins expression: animal studies showed that both haloperidol and risperidone treatment significantly decreased BDNF cortical concentrations (Angelucci et al., 2000), while clozapine increased BDNF expression in the rat hippocampus (Bai et al., 2003). This evidence was also supported by clinical finding of a positive correlation between serum BDNF and clozapine daily dose in a sample of patients with schizophrenia (Pedrini et al., 2011).

Given these evidences, we can make some speculations about biological mechanisms underlying our results. The improvement in cognition after CRT, that in *COMT* Val/Val patients is seen only when treated with clozapine, suggests that this drug may potentiated the DA transmission in *COMT* Val/Val genotype. On the one hand, *COMT* Val allele has been consistently associated to lower cognitive performance (Goldberg et al., 2003), probably as a result of the reduced PFC dopamine availability and D1 overexpression and the abnormalities of PFC activity (Slifstein et al., 2008). On the other, clozapine displays D1 blockade properties that may compensate the D1 overexpression associated with *COMT* Val/Val genotype.

Moreover clozapine has been reported to increase PFC activation, possibly restoring the PFC inefficiency observed among *COMT* Val/Val patients (Winterer and Goldman, 2003). Previous results showing worse cognitive performances among *COMT* Val/Val patients treated with CRT regardless of medication regimen (Bosia et al., 2007), or with clozapine without cognitive training (Woodward et al., 2007), may thus suggest that the synergy of both CRT and clozapine may be the best tool to treat cognitive deficit in *COMT* Val/Val patients. We can also hypothesize that the genotype-treatment effect that we observed on a behavioral measure, may reflect a direct activity of the drug on the same neurobiological mechanisms, modulated by *COMT* genetic variants and suggested to underlie the ability to restore cognitive deficit through CRT.

In sum, our results suggest the possibility that the rs4680 *COMT* polymorphism gene may differentially influence the response to CRT, depending on the antipsychotic treatment, particularly clozapine. Indeed, the differences in improvement seen among the treatment by genotype groups may be clinically meaningful, as they range from no improvement at all to medium-large gains in the advantageous subgroups.

However, there are criticisms that need to be addressed. First, we have a small sample size and we performed multiple tests without corrections. Second, we included only good responders to medications. This selection may reduce the generalizability of the findings; however in clinically stabilized patients with good response CRT response is likely to be maximed. Third, as in other studies (Greenwood et al., 2011; Panizzutti et al., 2013), we did not include in the analysis a control group, treated with placebo instead of CRT and therefore the changes in cognition caused by antipsychotics cannot be fully disentangled from those caused by CRT. However, to start CRT, all patients had to be good responders and treated with the same antipsychotic since at least three months. It is therefore unlikely that the changes in cognitions after CRT are caused by the antipsychotic treatment. Moreover, with respect to the antipsychotic effect, we did not observe differences in cognitive performance among treatment groups. Fourth, we cannot exclude that our result may depend also on other unexplored factors, such as different polymorphisms within the *COMT* gene or other functionally related genes and epigenetic mechanisms. Finally, in the present study we could not verify if the effect is maintained over time through follow-up evaluation and generalized to global functioning. The complimentary study of neurophysiologic and neurofunctional correlates would strengthen our findings. Although our data are preliminary and should be regarded mainly as hypothesis-generating, they need to be replicated in larger and more characterized samples, in order to potentially reach clinical translational relevance.

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