Putative Role of the COMT Gene Polymorphism (Val158Met) on Verbal Working Memory Functioning in a Healthy Population

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Working memory has been described as a neurocognitive probe of prefrontal brain functioning. Genetic variability related with catechol-O-methyltransferase (COMT) gene (Val158Met polymorphism) has received increasing attention as a possible modulator of working memory tasks in both schizophrenic patients and healthy subjects, although inconsistencies across studies have been found. This may be related to the existence of different working memory components, processes and modalities, which may have different sensitivities to subtle changes in dopamine levels and, therefore, the effect of the underlying COMT Val158Met genetic variability. To test this out, a large sample of 521 healthy individuals from the general population were tested on the WCST and three working memory tasks that cover the assessment of verbal and spatial working modalities as well as different components and processes (Letter and Number Sequencing, CPT-IP, Backwards Visual Span). All individuals were genotyped for the rs4680 (Val158Met) polymorphism at the COMT gene. Met carriers showed near-significant better performance in the LNS compared with Val/Val individuals (F = 3.9, df = 1, P = 0.046). Moreover, the analysis for linear trend found that Met allele carriers showed significantly better performance than Val/Val individuals (B = 0.58, P = 0.031), although evidence for a linear trend was not found. None of the WCST indices differed among genotypes. Consistent with the hypothesis that Val158Met polymorphism (COMT gene) might account for individual differences on dopamine-dependent prefrontally related neurocognitive functions, the Letter-Number Sequencing task, which requires not only maintenance but also active manipulation of information seemed to be more sensitive to the disadvantageous Val/Val genotype in a large non-clinical sample.

KEY WORDS: working memory; catechol-O-methyltransferase; genetic variability; executive function


INTRODUCTION

Working memory, the process of actively holding information “on-line” in the mind’s eye and manipulating it in the service of guiding behavior [Baddeley, 1992] is one of the most important subordinate components of executive functions [Tranel et al., 1994]. Working memory deficits seem to be key in schizophrenia, and have also been found, to a lesser degree, in healthy relatives of schizophrenia patients [Kremen et al., 1994; Hoff et al., 2005; Snitz et al., 2006; Kremen et al., 2007].

Dopamine levels in prefrontal cortex (PFC) are critical for intact prefrontally dependent cognitive functions such as working memory [Rotaru et al., 2007]. The enzyme catechol-O-methyltransferase (COMT), which main target is to degrade dopamine (DA) from the synaptic cleft, has attracted increasing attention as a possible modulator of prefrontal functions in both schizophrenia patients and healthy subjects [Meyer-Lindenberg et al., 2006; Tunbridge et al., 2006].

The COMT gene maps at 22q11 [Grossman et al., 1992]. A functional polymorphism consisting of a transition of guanine to adenine at codon 158 leading to a substitution of Val to Met has been reported [Lachman et al., 1996]. The Met allele results in a three to fourfold lower enzymatic activity than Val allele. As the alleles present codominance, heterozygous individuals show enzyme activity that is midway between homozygous individuals [Mannisto and Kaakkola, 1999; Weinshilboum et al., 1999].

In a seminal study by Egan et al. [2001], it was reported that the Val allele causes reduced performance in executive functioning as measured by the Wisconsin Cart Sorting Test (WCST) both in schizophrenic patients and controls, suggesting that COMT genetic variability was modulating not only pathological prefrontal function but also the normal one. In this sense, the association between the Met/Met genotype and...
better WCST performance has been further replicated in healthy individuals [Joobe et al., 2002; Malhotra et al., 2002; Mattay et al., 2003; Rosa et al., 2004; Bruder et al., 2005], even though other studies have not found significant results among genotypes [Bilder et al., 2002; Tsai et al., 2003; see review Barnett et al., 2007].

Most studies thus far have used the WCST as a measure of prefrontal cognition, and few have analyzed whether COMT genotypes are related to a specific subcomponent of executive functions such as working memory. Research has gradually shifted attention to some of the individual components embedded in the broad construct of executive functions in order to disentangle the specific cognitive processes that may underlie the association between COMT Val158Met genotypes and WCST performance. In this sense, and focusing on working memory, there is a body of evidence that suggest that Met allele carriers show better performance than Val carriers, both within affected subjects and within healthy individuals [Bilder et al., 2002; Goldberg et al., 2003; Diamond et al., 2004; Diaz-Asper et al., 2008]. However, a recent study based on a large cohort in the general population has not replicated these findings [Stefanis et al., 2004]. Such heterogeneity of results could be due to the use of different working memory tests (e.g., N-Back tasks, Dots mixed tasks, etc.), which may require slightly different types of cognitive demands [Wager and Smith, 2003]. To our knowledge, only a recent study has analyzed the association between COMT and different working memory processes (simple retention of information, demands on memory for temporal order of verbal information, “online” update and manipulation of information) tapped by four tasks [Bruder et al., 2005]. These last authors described that Met/Met subjects showed the best performance only on the task that demanded active manipulation of information [Letter-Number Sequencing (LNS) test]. This finding makes it attractive to speculate that different working memory demands may respond differentially to subtle changes in dopamine levels and, accordingly, present different sensitivity to the effect of the underlying COMT genetic variation. However, as outlined in a recent the review by Savitz et al. [2006], the specific cognitive functions that are sensitive to dopaminergic modulation and to COMT genotype remain still unknown.

The aim of the present study is to relate COMT genotypes with (i) executive functioning, (ii) verbal and spatial working memory domains, and (iii) three different types of working memory processes (sustaining and updating, reversing order, and manipulating information), in a sample of non-clinical subjects, that is, without the impact of confounding variables such as the presence of symptoms or the effects of medication. It was hypothesized a dose–response relationship between Val158Met polymorphism in those tasks involving high-order components of processing, such as active mental manipulation of the information, irrespective of processing modality (verbal vs. spatial).

METHODS

Sample

Participants were recruited from the campus of the University Jaume I in Castelló (Spain), and from University offices and community technical schools from the metropolitan area of Barcelona (Spain). Participants were 521 healthy subjects (247 male/274 female), with a mean age of 22.8 years (SD = 3.3, range 18–55); and 13.8 (SD = 2.2) years of education. Exclusion criteria were any major medical illness that could affect brain function, current substance abuse, neurological conditions, history of head injury, and personal history of psychiatric medical treatment.

Ethical approval was obtained from Spanish local research ethic committees. All participants provided a complete written informed consent before inclusion in the study.

Measures

Neurocognitive Measures. Estimated general intelligence was assessed by Block Design and Information subtests of the Wechsler Adult Intelligence Scale-III (WAIS-III) [Wechsler, 1999].

Several tasks were used to tap different processes of working memory. Firstly, D-prime indices (shapes and numbers indices to tap, respectively, spatial and verbal processes) of the Continuous Performance Test-Identical Pairs version (CPT-IP) [Cornblatt et al., 1989] were used. The IP version of the CPT demands working memory as participants must continuously update the stimuli presented on screen at a pace of 1 per second and determine whether consecutive stimuli are identical, in which case they must provide a response [van den Bosch et al., 1996]. Secondly, upholding and reordering information was assessed with Backwards Visual Span subtest of the Wechsler Memory Scale Revised (WMS-R); [Wechsler, 1987]. Thirdly, active manipulation of information was assessed with the LNS subtest of the WAIS-III; [Wechsler, 1999]. Finally, working memory modality was recorded by two verbal (number d’ and LNS) and two spatial tasks (shapes d’ and Backward Visual Span).

The Wisconsin Card Sorting Test (WCST) [Heaton, 1981] was used to measure executive functioning; the indices included in the analyses were number of perseverative errors (PE) and categories completed (CC).

Laboratory Methods. Genomic DNA was extracted from saliva samples using the Collection Kit BucalAmp DNA extraction kit (Epicentre, ECOGEN, Barcelona, Spain). The SNP rs4680 (Val158Met) of the COMT gene was genotyped using Applied Biosystems (AB) TaqMan technology. AB assay-on-demand service was used to order the probes. Genotype determinations were performed blind to clinical condition. Randomized individuals were re-tested for their genotypes in order to confirm the pattern reproducibility.

Statistical Analysis

Chi-squared tests were performed to confirm presence or absence of allele or genotype associations. A principal component analysis was carried out to explore the latent structure proposed above of the neurocognitive measures used in this study. Additionally, composite indices of verbal and spatial working memory were created as the sum of standardized scores of verbal or spatial working memory tests divided by the number of indices used for each domain. The effect of COMT genotype on neurocognitive performance was estimated using ANOVAs. Finally, regression analyses were performed to test the hypothesized dose–response relationship between COMT genotype on prefrontal functioning.

Analyses were performed using SPSS for windows [Inc., 2003], STATA 9.1 [StataCorp., 2005] and EpiInfo [CDC, 1996].

RESULTS

Genotype distribution of the Val158Met polymorphism in our Spanish healthy population was 153 Val/Val subjects (29.4%), 250 Val/Met subjects (48%), and 118 Met/Met subjects (22.6%). Allele distribution was found to be 53.5% for the Val158 allele and 46.6% for the Met158 allele. The genotype distribution of the polymorphism was within the Hardy–Weinberg (χ² = 0.31, df = 2, P = 0.85), and were similar to those frequencies described in European Caucasian population [Rosa et al., 2004].
No significant differences were found among genotype frequencies by age \( (P = 0.49, \text{df} = 2, P = 0.61) \), sex \( (\chi^2 = 0.10, \text{df} = 2, P = 0.95) \), years of education \( (P = 1.36, \text{df} = 2, P = 0.26) \) or estimated IQ \( (P = 0.87, \text{df} = 2, P = 0.42) \).

A principal component analysis was carried out with the six neurocognitive indices. It yielded two principal factors with an eigenvalue greater than 1, which explained 62.3% of the total variability. The first factor, which accounted for 35.5% of the variance, grouped all working memory measures: d's (load 0.69, d’s (load 0.75), visual backward span (load 0.62) and LNS (load 0.67). The second factor, which accounted for 26.8% of the variance, was composed by the two WCST executive functioning indices (PE and CC loading –0.95 and 0.95, respectively). No statistical differences were found among the three COMT genotypes for either the working memory \( (F = 0.19, \text{df} = 2, P = 0.83) \) or the executive factor \( (F = 0.62, \text{df} = 2, P = 0.54) \). COMT genotypes did not also differ on the composite indices of either verbal \( (F = 0.66, \text{df} = 2, P = 0.52) \) or spatial \( (F = 0.7, \text{df} = 2, P = 0.93) \) working memory modalities.

The comparison of COMT genotypes on individual neurocognitive measures are shown in Table I. The effect of COMT genotypes on LNS approached statistical significance \( (F = 2.35, \text{df} = 2, P = 0.09) \). None of the WCST indices differed among genotypes. However, when we grouped the Met carriers \( (\text{Met/Met and Val/Met}) \) and compared them to the Val/Val homozygous group, Met carriers showed near-significant better performance on the LNS compared to Val/Val individuals \( (F = 3.9, \text{df} = 1, P = 0.046) \). Moreover, this trend remained close to significant \( (F = 3.9, \text{df} = 1, P = 0.049) \) when sex was included as a covariate.

It is supposed that heterozygotes present intermediate values in between homozygotes so the effect of allelic dosage was analyzed for each neurocognitive variable. A main effect of COMT genotype on LNS was found \( (\text{Val/Met}: B = 0.33, \text{df} = 1, P = 0.685; \text{Met/Met}: B = -0.93 P = 0.345; \text{using Val/Val as reference category}) \), indicating that Met allele carriers showed significantly better performance than Val/Val individuals, although evidence for a linear trend was not found. No other significant linear effects of COMT genotype on neurocognitive variables were found. Finally, a non-significant pattern of dose-response relationship between COMT genotypes and PE was found \( (\text{Val/Met}: B = -0.33 P = 0.685; \text{Met/Met}: B = -0.93 P = 0.345; \text{using Val/Val as reference category}) \).

**DISCUSSION**

A near significant effect was found between COMT genotypes on the LNS in a large sample of Spanish healthy individuals. That is, Val homozygotes showed poorer performance on the LNS test than Met allele carriers. These findings support the effect of COMT genotypes on high-order components of prefrontally related cognitive processing (i.e., mental manipulation of information). However, the magnitude of the effect was not as strong as expected. Interestingly, additional analyses showed that Val/Val individuals differed significantly from the Val/Met group, although there was no evidence of a dose–response relationship between Val loading and LNS performance. The other working memory tests, the CPT-IP d'prime indices and the visual backward span, were not related to COMT genotypes in this non-clinical sample. Moreover, COMT genotypes did not differ on composite measures of working memory and, as expected, there were no differences according to verbal or spatial modalities. Finally, executive indices (PE and CC) were not related to COMT genotypes. It should be reminded that no sociodemographic or general cognitive ability differences can account for these results, given that there were no differences among genotypes on these potentially confounding variables.

Consistent with previous reports, Val allele was associated with an inefficient prefrontal function in healthy population [Egan et al., 2001]. Moreover, it has been recently reported that this pattern seems to be present not only in healthy individuals but also in extreme phenotypes such as schizophrenia [Bertolino et al., 2006].

The LNS task, which demands not only maintenance but also an active process of manipulation (i.e., reordering of information according to two different rules, numeric and alphabetic) seemed to be more sensitive to dopaminergic modulation than the other working memory tasks, as also found by Bruder et al. [2005]. A recent report showed that relatives of schizophrenic patients showed an intermediate performance on the LNS test, between that of their affected siblings and healthy controls [Barrantes-Vidal et al., 2007]. Therefore, the LNS task might be a better candidate to become a putative neurocognitive endophenotype for schizophrenia spectrum disorders.

However, and consistent with the report by Bruder et al. [2005], our results did not support the expected allelic dosage effect across the COMT genotypes on LNS performance. Interestingly, this analysis pointed out that the greatest slope on LNS performance is placed between the Val/Val group and the heterozygote group. The differences between Val homozygotes and Met carriers to face up with subtle dopaminergic changes would be apparent when performing specific cognitive processes, such as an active mental manipulation of information.

Our results are not consistent with previous studies in which Met/Met individuals presented significantly better WCST executive functioning performance (i.e., less PE in the WCST)
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[Egan et al., 2001; Malhotra et al., 2002; Rosa et al., 2004; Bruder et al., 2005; Barnett et al., 2007]. Interestingly, although not statistically significant, a tendency for an allelic dosage was seen between COMT genotypes and PE, Val/Val individuals performed worst and Met/Met ones showed the best performance, with heterozygotes showing an intermediate performance. The fact that multiple subprocesses are involved in a successful WCST performance (attention, problem solving, working memory, information maintenance, abstraction, set-shifting, or inhibition) may contribute to explain the lack of power in the detection of stronger differences between groups, as well as the inconsistencies between different studies. Furthermore, a new potential source of functional heterogeneity related to COMT genetic variability could be contributing to these inconsistencies [Nackley et al., 2006]. A recent study carried out by Diaz-Asper et al. [2008] showed that the effect of haplotype variation in COMT, including the Val158Met polymorphism on prefrONTAL functioning, seems to be critical for pathologic phenotypes such as schizophrenia. Thus, additional genetic variability in COMT might explain some of the inconsistencies found when relating the COMT Val158Met polymorphism and pre-frontal function in extreme phenotypes such as schizophrenia [see review: Barnett et al., 2007]. Future studies taking into account this new source of variability may help to clarify this issue.

Our study presents some limitations. Firstly, our results would not be statistically significant if multiple testing correction was carried out. However, this correction is overly strict and conservative in the context of the present study since the selection of the genetic polymorphism and the analyses performed did have a directional hypothesis based on previous findings [Egan et al., 2001; Bruder et al., 2005]. Secondly, our sample contained a large proportion of college undergraduates, which might imply that the range of scores is biased towards an overrepresentation of good performers. This might involve that in a more heterogeneous community sample, with a wider variability in terms of years of education and cognitive abilities, we might have detected a stronger association between COMT and cognitive functioning. In any case, the nature of the sample would have decreased the strength of the association, but not yielded a false association.

In conclusion, consistent with the hypothesis that Val158-Met polymorphism (COMT gene) might account for subtle individual differences in dopamine-dependent prefrontally related neurocognitive functions, the LNS task, which requires individual differences in dopamine-dependent prefrontally involved in a successful WCST performance (attention, problem solving, working memory, information maintenance, abstraction, set-shifting, or inhibition) may contribute to explain the lack of power in the detection of stronger differences between groups, as well as the inconsistencies between different studies. Furthermore, a new potential source of functional heterogeneity related to COMT genetic variability could be contributing to these inconsistencies [Nackley et al., 2006]. A recent study carried out by Diaz-Asper et al. [2008] showed that the effect of haplotype variation in COMT, including the Val158Met polymorphism on prefrontal functioning, seems to be critical for pathologic phenotypes such as schizophrenia. Thus, additional genetic variability in COMT might explain some of the inconsistencies found when relating the COMT Val158Met polymorphism and pre-frontal function in extreme phenotypes such as schizophrenia [see review: Barnett et al., 2007]. Future studies taking into account this new source of variability may help to clarify this issue.

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