Gender effects of the COMT Val[158]Met genotype on verbal fluency in healthy adults

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Abstract. Cognitive performance in healthy individuals is associated with gender differences in specific tests; a female advantage has been demonstrated in language tests, whereas a male advantage has been demonstrated in spatial relation examinations. The prefrontal cortex (PFC) mediates important cognitive domains and is influenced by dopamine (DA) activity. The single nucleotide polymorphism (SNP) rs4680 in the catechol-O-methyltransferase (COMT) gene results in an amino acid substitution from valine (Val) to methionine (Met). The Met allele has been demonstrated to decrease COMT enzyme activity and improve PFC cognitive function. COMT regulates DA activity in the PFC and exhibits gender effects. The aim of the present study was to investigate the gender-specific effects of the COMT genotype on cognition in healthy young adults. Seventy-six healthy subjects were genotyped for COMT rs4680 and submitted to an extensive range of neuropsychological tests assessing aspects of PFC function. The COMT Met allele influenced the performance of executive function. The results revealed gender effects of the COMT rs4680 Met allele on verbal fluency, with positive effects in males and negative effects in females. This suggested that DA activity affects cognitive function in different ways, according to gender.

Introduction

Gender differences in behavior and cognitive performance involve cultural and biological factors. Numerous parameters of brain function and structure differ between males and females (1-3); however, the exact mechanisms involved and how they affect each cognitive domain remain unclear. The catechol-O-methyltransferase (COMT) enzyme has been implicated as a potential biological candidate involved in this gender dimorphism (4-6). To the best of our knowledge, there are a limited number of studies on this matter, the results of which are controversial.

Gender may impact cognitive function. Generally, females outperform males in their verbal abilities, while males outperform females in visuospatial tasks. Herlitz *et al* (7) investigated memory function and identified gender differences in the episodic memory that were in favor of females. Among individuals aged ≥85 years, females have demonstrated superior scores in cognitive speed and memory tasks, regardless of their often lower level of formal education (8). Halari *et al* (9) investigated whether sexually dimorphic cognitive performance in males and females was associated with sex hormones. Significant gender differences were observed, favoring males in the spatial and inhibition tasks, and favoring females in the verbal task (category fluency). However, no significant correlation was demonstrated between gender hormones and cognitive performance. van Hooren *et al* (10) analyzed the effects of age, education and gender on cognitive speed, verbal memory, executive function and verbal fluency in healthy older adults. A marked, age-related decrease in these tasks was identified. Education had a substantial effect on cognitive function; participants with a middle or high level of education demonstrated a superior performance in the cognitive tests (10). Additionally, females outperformed males in the verbal memory tasks (10).

The prefrontal cortex (PFC) is an important region of the brain for cognition, and is strongly influenced by dopamine (DA). COMT is regarded as an important regulator of PFC DA levels, whereas the DA transporter (DAT) is not as widely found in the PFC as it is in the striatum (11,12). COMT is a key enzyme that is specifically involved in the metabolic degradation of extraneuronal DA (13). With regard to gender differences, one study identified a 17% increase in COMT enzyme activity in healthy males compared with that in healthy females (11). However, certain studies have demonstrated similar COMT levels and expression in both genders (11,14,15), while others have revealed higher levels in

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females (16). Males and females also appear to differentially regulate the abundance of novel mRNA (17) or protein (18) isoforms of COMT, which may alter the enzyme activity without affecting the total level of COMT transcripts or proteins (4). These results are concordant with earlier findings demonstrating a 30% increase in the activity of the enzyme in males compared with that in females (19). A similar difference was described in studies using human erythrocytes (20-22); however, one study was not concordant (23).

Genetic studies have suggested that COMT enzyme activity may also vary considerably according to COMT single nucleotide polymorphisms (SNPs). The COMT gene SNP, rs4680, also known as Val^{158}Met, reduces the activity of the enzyme in Met carriers (Met^{+} individuals) (24). This, in turn, lowers the enzyme activity and presumably increases the PFC DA levels (24,25). COMT exerts a significant regulatory effect on cognitive function (26-29), which involves the well-established effects of PFC DA levels on working memory and executive function (11,30-32). It has been suggested that the COMT rs4680 SNP may exert a direct effect on cognition in schizophrenic patients and healthy controls (26-29). The COMT SNP rs4680 Met allele has been demonstrated to be correlated with higher PFC DA levels (24,25), and with superior performance in working memory, intelligence and executive function in numerous studies (11,28,33-36). However, a number of studies did not reveal similar cognitive results (37-39). Furthermore, the COMT Met allele has been demonstrated to modulate cognitive dysfunction across mood episodes of bipolar disorder (40).

Results of previous COMT studies specifically concerned with the effects of gender/genotype on cognition require further clarification. Only a limited number of studies have demonstrated a gender-by-genotype effect on cognition (4-6). Two studies addressing the association between gender/genotype and cognition have focused on children and elderly individuals (5,6). Barnett *et al* (5) performed a range of cognitive tests in >5000 children (age, 8-10 years), including tests for IQ, attention span and working memory. Subjects were genotyped for the COMT Val^{158}Met SNP, and the Met allele was found to be associated with improved function in several domains selectively among males. O'Hara *et al* (6) evaluated 163 older healthy adults and revealed that only Val/Val males performed superiorly in a test of delayed verbal recall, while a worse performance was observed in younger individuals.

Insufficient (hypodopaminergic) and excessive (hyperdopaminergic) DA receptor 1 (D1) stimulation has been demonstrated to impair PFC function in animal studies, resulting in cognitive dysfunction (41-43); however, the extension of these findings to healthy subjects remains speculative. Therefore, PFC cognition is hypothesized to depend on a specific level of DA in order to achieve optimal cognitive function (31,32). Given the limited data available on whether gender effects are associated with the COMT genotype interaction with cognition, the aim of the present study was to investigate this potential association in a homogeneous sample of healthy young adults. Based on studies demonstrating lower COMT activity in female COMT Met carriers, we hypothesized that females carrying the Met allele would have a lower cognitive performance due to altered PFC DA regulation.

Materials and methods

Subjects. Seventy-six healthy individuals (37 females and 39 males), aged 18-35 years (mean age, 23.2±3.26 years) with a mean schooling duration of 14.1±2.32 years and a mean IQ of 115.49±12.25, were recruited at the University of São Paulo (São Paulo, Brazil). Study participants were predominantly medical students. None of the subjects had a past history of or were currently suffering with a psychiatric disorder, according to the evaluation conducted by psychiatrists using the Mini International Neuropsychiatric Interview (MINI) (44). In addition, all subjects had no family history (amongst first-degree relatives) of mood or psychotic disorders, and had not used psychotropic substances or indulged in substance abuse within the last three months. Only females with a regular menstrual cycle, taking oral contraceptives, were included.

Neurocognitive assessments. A range of neurocognitive assessments were designed to assess the following domains: i) Attention, by the Wechsler Adult Intelligence Scale III [WAIS-III, including the Digit Span (DS) subtest] and the Trail Making Test, part A (TMT-A); ii) memory, using the immediate and delayed Logical Memory subtests of the Wechsler Memory Scale (WMS-LM1 and -LM2, respectively); iii) verbal fluency, by the Controlled Oral Word Association Test (FAS); and iv) executive function, assessed by the TMT-B (45-48). Experienced clinical neuropsychologists performed the neurocognitive assessments. Raw scores, corrected for demographic factors, were used given the absence of standardized scores for the Brazilian population.

Genotyping. DNA was extracted from the peripheral blood according to the salting out procedure (49), and was then genotyped for COMT rs4680 using quantitative (q)PCR allelic discrimination. PCR amplification for rs4680 was performed in 5-μl reactions with 5 ng template DNA, 1X TaqMan Universal Master mix (Applied Biosystems, Inc., Foster City, CA, USA), each primer and probe assay, and H_2O. Thermal cycling consisted of initial denaturation for 10 min at 95˚C, followed by 40 cycles of denaturation at 95˚C for 15 sec, and annealing at 60˚C for 1 min. The allele detection process and allelic discrimination were performed for 1 min at 60˚C on a 7500 Real-Time PCR system (Applied Biosystems, Inc.). Quality control of qPCR results was achieved by direct sequencing using an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Inc.).

Statistical analyses. Cognitive tests were stratified as a function of COMT rs4680 genotype (Met/Met, Val/Met and Val/Val) and functional allele [Met^{+} (Met/Met or Met/Val) or Met^{-} (Val/Val)]. The cognitive test results had a normal distribution, and parametric tests were used to analyze the data. The multivariate analysis of the covariance test was performed using the cognitive test results as factors, and age, gender, education, genotype and Met allele as covariates. COMT genotype and gender, as well as COMT Met allele and gender, interactions were also analyzed. $P<0.05$ was considered to indicate a statistically significant difference. All statistical analyses were performed using the PASW statistical software, version 18.0 (SPSS Inc., Chicago, IL, USA). Correction for multiple comparisons was performed using the Bonferroni test.

Table I. Sociodemographic and clinical variables by COMT genotype.

Variable	Met^+ (n=57)	Met^- (n=19)	P-value[a]
Age (years, mean ± SD)	23.37±3.40	23.53±3.20	0.85[b]
Gender (male/female)	26/31	13/6	0.11[c]
Years of education (mean ± SD)	14.19±2.31	13.82±2.43	0.99[b]

[a]Difference between the two groups (2-tailed P-value); [b]t-test; [c]χ^2 test. Significance level, P<0.05. COMT, catechol-O-methyltransferase; Met, methionine.

Table II. Multivariate analysis of cognitive tests, with age, education, gender, COMT allele Met and gender*COMT allele Met interaction as the cofactors.

Source	Dependent variable	B	F	P-value	Partial η^2 (%)	Observed power (a) (%)
Age	**WAIS-DS-FW**	**-0.19**	**5.638**	**0.020**	**7.66**	**64.84**
	WAIS-DS-BK	-0.09	0.971	0.328	1.41	16.3
	WAIS-DS	-0.23	2.475	0.120	3.51	34.16
	WMS-LM1	-0.10	0.179	0.673	0.26	7.02
	WMS-LM2	-0.11	0.222	0.639	0.33	7.51
	RCFT-COPY	-0.02	0.198	0.658	0.29	7.24
	RCFT-RECALL	-0.26	1.899	0.173	2.72	27.42
	TMT-A	**0.98**	**14.393**	**0.000**	**17.47**	**96.24**
	TMT-B	**2.50**	**5.565**	**0.021**	**7.57**	**64.27**
	FAS TOTAL	-0.38	1.983	0.164	2.83	28.42
	FAS F	-0.10	0.567	0.454	0.83	11.51
	FAS A	-0.16	1.805	0.184	2.59	26.31
	FAS S	-0.17	2.583	0.113	3.66	35.38
Education	WAIS-DS-FW	0.13	1.219	0.273	1.76	19.29
	WAIS-DS-BK	0.14	1.214	0.274	1.75	19.24
	WAIS-DS	0.26	1.605	0.209	2.31	23.93
	WMS-LM1	-0.17	0.281	0.598	0.41	8.18
	WMS-LM2	-0.23	0.498	0.483	0.73	10.70
	RCFT-COPY	0.06	1.123	0.293	1.62	18.14
	RCFT-RECALL	**0.56**	**4.367**	**0.040**	**6.03**	**53.99**
	TMT-A	-0.28	0.590	0.445	0.86	11.79
	TMT-B	2.03	1.862	0.177	2.67	26.99
	FAS TOTAL	0.26	0.491	0.486	0.72	10.63
	FAS F	0.10	0.323	0.572	0.47	8.67
	FAS A	0.13	0.590	0.445	0.86	11.79
	FAS S	-0.03	0.055	0.816	0.08	5.61
Gender	WAIS-DS-FW	0.10	0.122	0.727	0.18	6.37
	WAIS-DS-BK	0.78	1.415	0.238	2.04	21.64
	WAIS-DS	**0.98**	**6.885**	**0.011**	**9.19**	**73.46**
	WMS-LM1	-2.00	0.468	0.496	0.68	10.35
	WMS-LM2	-3.78	0.318	0.575	0.47	8.61
	RCFT-COPY	**0.25**	**4.328**	**0.041**	**5.98**	**53.63**
	RCFT-RECALL	-0.84	0.965	0.329	1.40	16.25
	TMT-A	1.42	1.336	0.252	1.93	20.70
	TMT-B	-0.34	3.315	0.073	4.65	43.45
	FAS TOTAL	2.77	0.729	0.396	1.06	13.43
	FAS F	0.19	0.639	0.427	0.93	12.36
	FAS A	0.99	0.166	0.685	0.24	6.86
	FAS S	1.11	1.092	0.300	1.58	17.76

Table II. Continued.

Source	Dependent variable	B	F	P-value	Partial η^2 (%)	Observed power(a) (%)
COMT allele Met	WAIS-DS-FW	-0.50	0.990	0.323	1.44	16.55
	WAIS-DS-BK	0.14	0.021	0.884	0.03	5.24
	WAIS-DS	3.62	2.076	0.154	2.96	29.51
	WMS-LM1	**7.35**	**5.424**	**0.023**	**7.39**	**63.16**
	WMS-LM2	5.74	2.799	0.099	3.95	37.82
	RCFT-COPY	0.46	0.018	0.893	0.03	5.20
	RCFT-RECALL	2.66	0.068	0.796	0.10	5.76
	TMT-A	-6.14	1.511	0.223	2.17	22.79
	TMT-B	**-32.86**	**5.121**	**0.027**	**7.00**	**60.68**
	FAS TOTAL	-4.78	0.014	0.907	0.02	5.15
	FAS F	-1.20	0.052	0.820	0.08	5.58
	FAS A	-0.95	0.200	0.656	0.29	7.25
	FAS S	-2.31	0.201	0.655	0.29	7.27
Gender*COMT interaction	WAIS-DS-FW	-0.23	0.035	0.852	0.05	5.39
	WAIS-DS-BK	-0.08	0.004	0.953	0.01	5.04
	WAIS-DS	-4.00	3.129	0.081	4.40	41.45
	WMS-LM1	-6.44	3.283	0.074	4.61	43.12
	WMS-LM2	-5.56	2.448	0.122	3.48	33.84
	RCFT-COPY	-0.84	1.722	0.194	2.47	25.32
	RCFT-RECALL	-4.56	2.429	0.124	3.45	33.63
	TMT-A	7.43	3.527	0.065	4.93	45.68
	TMT-B	29.03	3.184	0.079	4.47	42.05
	FAS TOTAL	**9.07**	**4.862**	**0.031**	**6.67**	**58.46**
	FAS F	1.96	0.986	0.324	1.43	16.50
	FAS A	2.74	2.167	0.146	3.09	30.58
	FAS S	**3.90**	**5.932**	**0.017**	**8.02**	**67.04**

COMT, catechol-O-methyltransferase; Met, methionine; WAIS-DS-FW/BK, Wechsler Adult Intelligence Scale III Forwards/Backwards Digit Span subtest; WMS-LM1/2, Wechsler Memory Scale immediate (1)/delayed (2) Logical Memory subtests; RCFT, Rey-Osterrieth Complex Figure Test; TMT-A/B, trail making test, part A/B; FAS F/A/S/TOTAL, Controlled Oral Word Association Test of words beginning with F/A/S/F+A+S. Bold text indicates P<0.05.

Ethics. The research ethics board of the Clinics Hospital of the University of São Paulo approved this study. Written informed consent was obtained from all subjects.

Results

No statistically significant differences in the sociodemographic data (age, gender and educational level) were observed among the COMT genotypes (Table I). Moreover, there were no differences in gender, age, education or IQ (Table I). The COMT genotype distributions in the experimental samples of males and females were identified to be in accordance with the Hardy-Weinberg equilibrium (χ^2=0.65), thus indicating representative samples. The allelic frequency of rs4680 was 51.3% for the Met allele and 48.7% for the Val allele.

A multivariate general linear test model, using age, educational level, gender, COMT rs4680 genotype and the interaction between COMT genotype and gender as covariates, was implemented.

Age negatively influenced cognitive performance on the WAIS-DS-FW (P=0.02, partial η^2, 7.66%), TMT-A (P<0.001, partial η^2, 17.4%) and TMT-B (P=0.02, partial η^2, 7.57%) (Table II). Gender influenced WAIS-DS (P=0.01, partial η^2, 9.1%) (Table II). Females performed better than males in both tests. The COMT genotype had no influence on cognitive performance after Bonferroni correction. Gender and COMT genotype interaction influenced the FAS total score (P=0.03, partial η^2, 6.67%) and FAS letter S (P=0.01, partial η^2, 8.0%) (Table III). Among males, Met homozygotes had the highest scores, Met heterozygotes had intermediate performance while Val homozygotes had the lowest scores. Among females, Val homozygotes had the highest scores, while Met homozygotes had the lowest scores (Fig 1).

Discussion

To the best of our knowledge, this is the first study to investigate the effect of the interaction between gender and the COMT rs4680 Met allele on verbal fluency, with opposite results identi-

Table III. Multivariate analysis of cognitive tests, with age, education, gender, COMT rs4680 genotype and gender*COMT rs4680 genotype interaction as the cofactors.

Source	Dependent variable	F	P-value	Partial η^2 (%)	Observed power (a) (%)
COMT rs4680 genotype	WAIS-DS-FW	1.043	0.358	3.06	22.50
	WAIS-DS-BK	0.251	0.779	0.75	8.79
	WAIS-DS	0.965	0.386	2.84	21.07
	WMS-LM1	2.776	0.070	7.76	52.91
	WMS-LM2	1.322	0.273	3.85	27.62
	RCFT-COPY	0.952	0.391	2.80	20.85
	RCFT-RECALL	1.882	0.160	5.39	37.80
	TMT-A	0.699	0.501	2.07	16.31
	TMT-B	2.579	0.083	7.25	49.76
	FAS TOTAL	0.272	0.763	0.82	9.13
	FAS F	2.008	0.142	5.73	40.04
	FAS A	0.225	0.799	0.68	8.39
	FAS S	0.123	0.885	0.37	6.81
Gender*COMT rs4680 genotype	WAIS-DS-FW	0.310	0.735	0.93	9.73
	WAIS-DS-BK	0.614	0.544	1.83	14.84
	WAIS-DS	2.105	0.130	6.00	41.76
	WMS-LM1	1.554	0.219	4.50	31.86
	WMS-LM2	1.436	0.245	4.17	29.70
	RCFT-COPY	2.200	0.119	6.25	43.39
	RCFT-RECALL	2.482	0.091	7.00	48.17
	TMT-A	2.569	0.084	7.22	49.59
	TMT-B	2.784	0.069	7.78	53.03
	FAS TOTAL	3.021	0.056	8.39	56.65
	FAS F	1.552	0.219	4.49	31.83
	FAS A	1.141	0.326	3.34	24.29
	FAS S	**3.442**	**0.038**	**9.44**	**62.63**

COMT, catechol-O-methyltransferase; WAIS-DS-FW/BK, Wechsler Adult Intelligence Scale III Forwards/Backwards Digit Span subtest; WMS-LM1/2, Wechsler Memory Scale immediate (1)/delayed (2) Logical Memory subtests; RCFT, Rey-Osterrieth Complex Figure Test; TMT-A/B, trail making test, part A/B; FAS F/A/S/TOTAL, Controlled Oral Word Association Test of words beginning with F/A/S/F+A+S. Bold text indicates P<0.05.

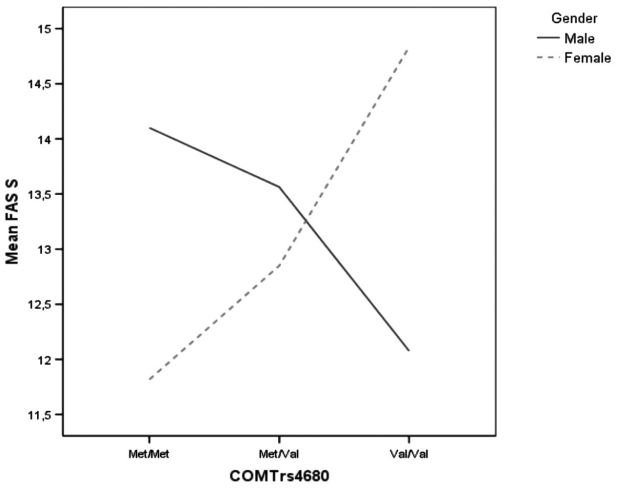

Figure 1. Graph demonstrating the effect of the COMT rs4680 genotype on Controlled Oral Word Association (of words beginning with S; FAS S) performance in males and females. COMT, catechol-O-methyltransferase.

fied for each gender. Female carriers of the Met allele had a lower performance than males in a verbal fluency test. In addition, subjects that were heterozygous (Val/Met) for COMT rs4680 had an intermediate performance in the verbal fluency test.

The results suggested a specific gender-dependent effect of Met^+ on verbal fluency, reinforcing the hypothesis that there is a distinct optimal DA activity/level for different components of cognition (28,50). Furthermore, the COMT Met allele negatively influenced cognitive performance (verbal fluency) in females compared with that in males (Fig. 1). Given that females have a lower COMT activity than males, *per se* (11,19), one possible explanation of our findings is that females carrying the low activity Met allele are likely to exceed the optimal PFC DA levels, thus receiving no benefit from its excessively high levels.

The effects of DA on neurocognition have been previously described. Several studies have demonstrated that lower doses of D1 agonists enhance working memory and

attention span (31,51), while higher levels of DA impair PFC function (52). Low doses of psychostimulants in hyperkinetic children have been demonstrated to be associated with significant improvements in short-term memory, whereas higher doses worsen cognitive performance (53). Similar dose-dependent effects were also observed in healthy volunteers using dextroamphetamine, a drug that potentiates dopaminergic activity (52). Studies on COMT functional SNPs and the differential effects of D1 and D2 receptor binding have also demonstrated cognitive decline to be associated with COMT activity. Healthy subjects carrying the Met$^+$ allele (rs4680) exhibited lower phasic and higher subcortical tonic DA transmission, which was proposed to be associated with an increase in central DA levels (28,54).

COMT enzyme activity appears to have a gender-specific effect; molecular postmortem studies have revealed that COMT enzyme activity in the PFC was 17% higher in males compared with that in females (11). These results are concordant with earlier findings demonstrating a 30% higher enzyme activity in males compared with females (19). A similar difference was described in studies using human erythrocytes (20-22); however, one study did not find differences between genders (23). Notably, 17-b-estradiol (E2) administration decreased COMT activity in the rat liver (55,56). Similarly, Xie *et al* (1999) demonstrated that there are two estrogen response elements in the COMT promoter, and that, at physiological concentrations, E2 inhibited COMT mRNA expression in cells expressing estrogen receptors, but not in cells that did not express these receptors. An estrogen-mediated decrease in COMT mRNA was also accompanied by a proportional decrease in COMT immunoreactivity and activity (55,56). In another study, the PFC DA levels were affected in male, but not female, COMT knockout mice (13). One possible explanation for this gender-distinct function is the bidirectional association between COMT and estrogen-related compounds, whereby COMT activity varies inversely according to estrogen levels (29). Moreover, estrogen levels affect the striatal dopaminergic system (57,58), and affect cognition (41-43,59,60). Overall, these findings may aid in the clarification of the role of gender-specific effects on COMT-modulated cognition.

Studies investigating the cognitive effects of DA in the PFC have mainly focused on the D1 receptor, the predominant type of receptor in the PFC. Hypodopaminergic and hyperdopaminergic D1 receptor stimulation have been demonstrated to impair PFC function (31,32,41-43), resulting in cognitive dysfunction. Therefore, PFC cognition may depend on an optimal level of DA to achieve normal function (31,32,51,52). These kinetics have been described by an inverted-U response function model in pharmacological studies (31,51,52). In this model, the effect of amphetamine and other drugs on cognition is described as an inverted-U shape, in which the peak is the threshold for maximum cognitive performance, with subsequent decline thereafter. We propose that the inverted-U shape model, which states that an optimal level of DA is required to benefit cognitive function, represents a plausible explanation as to why female Met allele carriers exhibit a worse performance in verbal fluency tests. The basis for this explanation is that females have lower COMT activity *per se*, which may be due to the effect of estrogen on this enzyme (29). Consequently, at least theoretically, male and female Met$^+$ subjects have different baseline DA levels.

The FAS is also considered to be a test of executive functions, including cognitive organization, initiation, maintenance of effort and the ability to conduct a non-routine search for words based on a specific first letter, rather than the lexical definition (61-63). This interpretation is consistent with studies demonstrating a worse performance in individuals with frontal lobe lesions (63,64), and sensitivity to cognitive dysfunction in disorders that affect executive functions (65). Effects of demographic variables are important to consider when interpreting FAS results. Age effects have not been identified in numerous studies (64,66,67); however, a number of studies have demonstrated modest age effects, with higher age predicting a worse performance (68). A higher level of education has been associated with improved FAS performance in several studies (64,67,69). In the present study, neither age nor education influenced FAS. However, with regard to gender, a superior FAS performance was observed in females. Previous studies have demonstrated controversial results; a number of studies identified superior FAS performance in females compared with males (66,70), while other studies did not observe a difference between the two genders (69,71).

Limitations of this study included the absence of information with regard to the menstrual cycle of participants. Furthermore, the use of oral contraceptives may have affected COMT activity.

To the best of our knowledge, this is the first study to identify a gender-specific effect of COMT SNP rs4680 on verbal fluency in young healthy subjects, with opposite effects on performance in each gender. The presence of the Met allele in female subjects was associated with worse verbal fluency; while in males, it was correlated with an improvement in this particular cognitive function. Our results suggested that DA activity affects cognitive function in different ways, according to gender, most likely due to COMT gender differences.

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