European Journal of Human Genetics (2008) 16, 1075–1082

www.nature.com/ejhg

ARTICLE

Catechol *O*-methyl transferase and dopamine D2 receptor gene polymorphisms: evidence of positive heterosis and gene–gene interaction on working memory functioning

M Florencia Gosso*[,1,2,3], Eco JC de Geus[1,3], Tinca JC Polderman[1,4], Dorret I Boomsma[1,2,3], Peter Heutink[1,2,3] and Danielle Posthuma[1,3]

[1]*Department of Biological Psychology, Vrije Universiteit, Amsterdam, The Netherlands;* [2]*Department of Clinical Genetics, Section of Medical Genomics, VU Medical Center, Vrije Universiteit, Amsterdam, The Netherlands;* [3]*Center for Neurogenomics and Cognitive Research – CNCR, Vrije Universiteit, Amsterdam, The Netherlands;* [4]*Department of Child and Adolescent Psychiatry, Erasmus University Rotterdam, Rotterdam, The Netherlands*

The *COMT* Val[108/158]Met polymorphism has been extensively studied in relation to individual differences in working memory (WM) performance. The present study tested the association of the *COMT* Val[108/158]Met polymorphism with WM performance in two independent family-based Dutch samples: 371 children (mean age 12.4 years) and 391 adults (mean age 36.2 years). A significant association was found between the *COMT* polymorphism and WM scores in the combined adult and young cohorts. The association reflected positive heterosis such that the Met/Met and Val/Val homozygotes did not perform as well as the Met/Val heterozygotes on the WM tasks. A secondary analysis was conducted in which a *DRD2*-tagging SNP (rs2075654) was tested for an interactive effect with the *COMT* polymorphism on WM performance. A significant interactive effect of the *DRD2* and *COMT* genes was found such that heterosis was present only in the *DRD2* genotype that has been linked to lower receptor density. Our results support previous findings that WM performance needs an optimal level of dopamine signaling within the PFC. This optimum level depends on enzymatic activity controlling dopamine level as well as dopamine receptor sensitivity, both of which may differ as a function of age and genotype. We conclude that the effects of a single polymorphism in a dopaminergic gene on a well-defined cognitive trait may easily remain hidden if the interaction with age and other genes in the pathway are not taken into account.
European Journal of Human Genetics (2008) **16,** 1075–1082; doi:10.1038/ejhg.2008.57; published online 2 April 2008

Keywords: genetic association; epistasis; cognitive ability

Introduction

Working memory (WM) is a fundamental component of human intelligence.[1,2] It refers to the processes that support the short-term maintenance or manipulation of relevant information in the presence of distracting irrelevant information. Nonhuman primates physiology and human functional imaging studies support a critical involvement of the prefrontal cortex (PFC) in WM.[3,4] The PFC is the primary target of extensive dopamine (DA)

*Correspondence: Dr M Florencia Gosso, Department of Biological Psychology, Vrije Universiteit, Van der Boechorststraat 1, Amsterdam 1081 BT, The Netherlands.
Tel: +31 20 5988793; Fax: +31 20 5988832;
E-mail: mf.gosso@vumc.nl
Received 21 August 2007; revised 5 January 2008; accepted 5 February 2008; published online 2 April 2008

projections from the midbrain, and several lines of evidence suggest that the frontal DA level is a critical modulator of WM performance.[1,5] Hence, genes involved in dopaminergic pathway metabolism have been of particular interest to explain individual differences in WM performance.[6–11] Amongst these, the catechol-*O*-methyltransferase (*COMT*) gene has been studied the most extensively. The *COMT* gene is located on chromosome 22q11, and contains six exons.[12] It is involved in enzymatic activity that degrades DA, norepinephrine, and epinephrine.[13] Two promoters encoding different isoforms, a membrane-bound *COMT* (MB-*COMT*) and a soluble *COMT* (S-*COMT*), are known. Both transcripts start at exon 3.[14] Differential expression and activity profiles have been well characterized, MB-*COMT* is predominantly expressed in brain neurons,[15] whereas S-*COMT* is predominantly expressed in other tissues, such as liver, blood, and kidney.[14] The human MB-*COMT* plays a crucial role in regulation of DA signaling at the PFC level. It contains a common functional single-nucleotide polymorphism (SNP) (rs4680) that substitutes a *Val* for a *Met* residue at codon 158 (see Figure 1).

The *Met* allele encodes an enzyme with relatively lower activity,[16] and is thought to be specific to humans; as no equivalent polymorphism has been found in any other species.[17] Decrease in enzyme activity present in *Met/Met* individuals, compared to individuals homozygous for the *Val* allele, leads to a relatively higher DA availability, whereas *Met/Val* heterozygous display an intermediate enzyme activity.[18] Because frontal DA level is a critical modulator of WM processes, the decreased *COMT* activity of *Met* carriers might be beneficial to their cognitive performance. In line with this, Savitz *et al*[10] found that 20 of the 26 studies on the association between the *COMT* Val$^{108/158}$Met polymorphism and cognitive function reported a significant association. All but two of these studies suggested that the low-activity *Met* allele yields better performance on cognitive tasks that have a WM component. However, these studies were often based on small and/or clinical samples (ADHD in children; schizophrenia in adults) and no significant association to WM was found in a much larger sample of healthy adult males.[19] In addition, Mattay *et al*[20] have shown that the role of the *COMT* Val$^{108/158}$Met polymorphism in PFC function, particularly in WM performance, is not straightforward. Although homozygous individuals for the *Met* allele perform significantly better than individuals homozygous for the *Val* allele, when the *Met* homozygotes are given DA agonists their response actually deteriorates. In contrast, the response of the 'dopamine-poor' *Val* homozygotes improves with DA agonists. This suggests that the relation between DA availability at the PFC level as indexed by *COMT* activity and WM performance is not linear, but instead follows an inverted-U shape.

DA signaling, furthermore, is not only dependent on the availability of DA, but also on the efficiency of the DA receptor and its downstream signaling cascade. Because of their importance in reward processing, dopaminergic receptors, in particular the DA *D2* receptor gene (*DRD2*), have been studied extensively in addiction research.[21] The *DRD2* gene is located on chromosome 11 at q22-q23 (see Figure 2). A *DRD2 Taq* IA variant, a restriction fragment length polymorphism (RFLP), located on the 3′ untranslated region (3′UTR) of the *DRD2* gene, is associated with altered receptor density.[22] Individuals with the A1 allele show a 30–40% reduction in *D2* DA receptor density compared with those homozygous for the A2 allele.[22,23]

Recently, Reuter *et al*[24] conducted an association analysis using an adult cohort enriched for *COMT* and *DRD2* homozygotes. They found a significant interaction between *DRD2* Taq IA and *COMT* Val$^{108/158}$Met polymorphisms, and performance on response interference on the Stroop color–word conflict task. *Met* homozygotes performed better than *Val* allele carriers, but only if they had the *DRD2* genotype associated with low receptor density. In fact, *Met* homozygotes also bearing two *DRD2* A2 alleles showed a significantly worse performance compared to all other genotypes. Although response interference and WM are not unitary constructs, we showed a significant correlation ($r=-0.26$, $P<0.05$) between these two measures of PFC function.[25] Hence, we hypothesize that a *COMT* by *DRD2* interaction may also be found for WM performance.

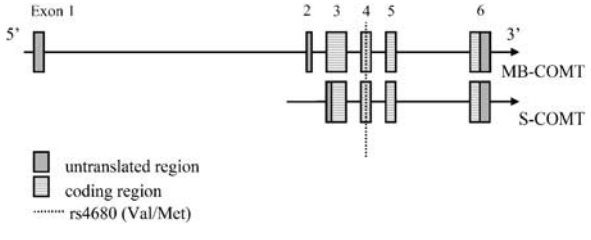

Figure 1 *COMT* gene and functional single-nucleotide polymorphism (SNP) rs4680 on 22q11.21.

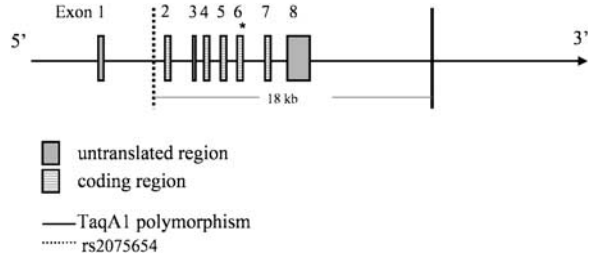

Figure 2 *DRD2* gene and tagging SNP rs2075654 near the restriction site Taq A1 on 11q23. * indicates splicing site at exon 6.

In the present study, which included 762 genotyped subjects, from two independent family-based Dutch samples of 371 (mean age 12.4 years) and 391 (mean age 36.2 years) subjects, respectively, our principal goal was to test for association of the *COMT* Val$^{108/158}$Met polymorphism with WM performance. The use of a family-based sample made it possible to test for association in a combined within- and between-family design to estimate genetic effects, which are free from spurious effects of population stratification.[26] As a secondary analysis, we tested for an interactive effect of the *COMT* Val$^{108/158}$Met polymorphism and genetic variation in the *DRD2* gene on WM performance comparable to the effect reported by Reuter *et al*[24] for Stroop interference. Because DA receptor sensitivity has been shown to decline with aging in both animal[27] and human studies,[28] our analyses will allow this interaction to be different in children and adults.

Materials and methods

Subjects

All twins and their siblings were part of two larger cognitive studies and were recruited from the Netherlands Twin Registry.[29] Informed consent was obtained from the participants (adult cohort) or from their parents if they were under 18 years of age (young cohort). The current study was approved by the institutional review board of the VU University Medical Center. None of the individuals tested suffered from severe physical or mental handicaps, as assessed through standard questionnaire.

Young cohort

The young cohort consisted of 177 twin pairs born between 1990 and 1992, and 55 siblings,[30,31] of which 371 were available for genotyping. The genotyped twins were 12.4 (SD = 0.9) years of age and the siblings were between 8 and 15 years of age at the time of testing. There were 35 monozygotic male twin pairs (MZM), 28 dizygotic male twin pairs (DZM), 48 monozygotic female twin pairs (MZF), 23 dizygotic female twin pairs (DZF), 26 dizygotic opposite-sex twin pairs (DOS), 24 male siblings and 24 female siblings, and 3 subjects form incomplete twin pairs (1 male, 2 females). Participation in this study included a voluntary agreement to provide buccal swabs for DNA extraction.

Adult cohort

A total of 793 family members from 317 extended twin families participated in the adult cognition study.[32] Participation in this study did not automatically include DNA collection, however, part of the sample, 276 subjects returned to the lab to provide blood samples, 115 provided buccal swabs through the biobanking project[33] for DNA extraction. Mean age of the genotyped sample was 36.2 years (SD = 12.6). There were 25 monozygotic male twin pairs (MZM), 15 dizygotic male twin pairs (DZM), 1 DZM triplet, 20 monozygotic female twin pairs (MZF), 28 dizygotic female twin pairs (DZF), 23 dizygotic opposite-sex twin pairs (DOS), 29 female siblings, 28 male siblings, and 109 subjects from incomplete twin pairs (41 males, 68 females).

Cognitive testing

WM tasks were assessed in the young cohort, using the Dutch adaptation of the Wechsler Intelligence Scale for Children-Revised (WISC-R)[34] consisting of two subtests: arithmetic and digit span. WM performance was indexed as the sum score of the two subtests and corrected for age and sex. The Dutch adaptation of the Wechsler Adult Intelligence Scale III-Revised (WAIS-IIIR)[35] was used to assess WM performance in the adult cohort and consisted of two subtests taxing WM (arithmetic and letter–number sequencing). WM was indexed as the sum score of arithmetic and letter-number sequencing and corrected for age and sex.

DNA collection and genotyping

DNA isolation from buccal swabs was performed using a chloroform/isopropanol extraction[36] DNA was extracted from blood samples using the salting out protocol.[37] Zygosity was assessed using 11 polymorphic microsatellite markers (Het > 0.80). Genotyping was performed blind to familial status and phenotypic data. Both MZ twins of a pair were included, serving as additional quality control on genotyping. *COMT* genotyping was performed using fluorogenic probes in the high-throughput 5′-nuclease assay and following manufacturer's recommendations (TaqMan, PE Applied Biosystems, Foster city, CA, USA). For *DRD2*, instead of the A1 allele of the Taq IA polymorphism, a *tag*-SNP (rs2075654) lying 18 kb downstream of the Taq IA variant was genotyped. LD between rs2075654 and Taq IA was calculated using the CEPH population, which is presumably of similar genetic ancestry to the Dutch population. LD between these two polymorphisms was high ($r^2 = 0.65$, LOD score 14.14). In view of the LD between the tag-SNP and *Taq* IA, we will refer to the T allele as 'A1'. *DRD2*-SNP genotyping was performed as part of a SNPlex assay, which included multiple other genes, following a tagging approach.[38] The SNPlex assay was conducted following the manufacturer's recommendations (Applied Biosystems). Here we focus on the *DRD2* gene only. Results on cognitive effects of two other genes are described elsewhere.[39,40]

Statistical analyses

Allele frequencies of the *COMT* Val$^{108/158}$Met and *DRD2* A1/A2 polymorphisms were estimated in both cohorts using Haploview (http://www.broad. mit.edu/mpg/haploview), in which a Hardy–Weinberg test is implemented, based on an exact calculation of the probability of observing a certain number of heterozygotes conditional

on the number of copies of the minor SNP allele. Family-based genetic association tests were conducted using the program QTDT (http://www.sph.umich.edu/csg/abecasis/QTDT/), which implements the orthogonal association model proposed by Abecasis *et al*[41] (see also Fulker *et al*[42] extended by Posthuma *et al*[43]) This model allows one the decomposition of the genotypic association effect into orthogonal between- (β_b) and within- (β_w) family components and can incorporate fixed effects of covariates and can also model the residual sib-correlation as a function of polygenic or environmental factors. MZ twins can be included and are modeled as such, by adding zygosity status to the data file. They are not informative to the within-family association component (unless they are paired with non-twin siblings) but are informative for the between family component. The between-family association component is sensitive to population admixture, whereas the within-family component is significant only in the presence of true association. Testing for the equality of the β_b and β_w effects serves as a test of population stratification. If population stratification acts to create a false association, the test for association using the within-family component (β_b) is still valid and provides a conservative test of association. If this test is not significant, the between- and within-family effects are equal and the more powerful association test that uses the whole population at once can be applied. The residual sib correlation was modeled as a function of residual genetic variance and non-shared environmental variance. The *DRD2* genotype was recoded into carriers (A1+) *versus* non-carriers (A1−). We used one-sided hypothesis testing for the interaction effects as our hypotheses specify the direction of genetic effects.

Results

In total, 762 subjects were available for SNP genotyping. Based on blind controls and intrapair MZ comparisons, a low genotyping error rate was found (0.015%). For the total sample, the success rate was 98.5 and 93.4% for the *COMT* $Val^{108/158}Met$ polymorphism in the young and adult cohort, respectively. For *DRD2* rs2075654, success rates were 97 and 100%, 365 adults and 360 children had genotype data for both *COMT* and *DRD2*. The distribution of genotype and allele frequencies of the *COMT* and *DRD2* polymorphisms as well as means, standard deviations, and standard errors for WM performance are provided in Table 1. Phenotypic means are provided for the complete phenotypic sample as well as for the genotyped subsamples.

Stratification

Tests for the presence of population stratification were not significant at the 0.05 level (*COMT*: $\chi^2_{(1360)}=0.937$, $P=0.33$; $\chi^2_{(1360)}=0.23$, $P=0.64$; $\chi^2_{(1724)}=1.516$, $P=0.22$; for children, adults and the combined sample, respectively) (*DRD2*: $\chi^2_{(1355)}=0.220$, $P=0.64$; $\chi^2_{(1388)}=0.00$, $P=0.9976$; $\chi^2_{(1748)}=0.084$, $P=0.77$; for children, adults and the combined sample, respectively), indicating that genotypic effects within families were not significantly different from those observed between families, suggesting that the more powerful population-based association test can be meaningfully interpreted for both *COMT* and *DRD2*.

COMT polymorphism

WM performance was compared across the three possible genotype groups (Met/Met, Met/Val, and Val/Val) suggesting

Table 1 Means and standard deviations WM scores for *COMT* and *DRD2* genotypes

Cohort	*Genotype*	*N*	*Frequency*	*WM mean*	*Standard deviation*	*Standard error of mean*
Young						
Total phenotypic sample		407		22.88	4.85	0.24
Genotyped sample		371		22.90	4.89	0.25
COMT	Met/Met	117	0.32	22.51	4.96	0.46
$N=365$	Met/Val	175	0.48	23.49	5.06	0.38
	Val/Val	73	0.20	22.27	4.33	0.51
DRD2	A1A1	9	0.03	23.98	2.08	0.69
$N=360$	A1A2	94	0.26	23.12	5.10	0.53
	A2A2	257	0.71	22.78	4.88	0.30
Adult						
Total phenotypic sample		793		109.91	15.89	0.56
Genotyped sample		391		109.92	15.80	0.80
COMT	Met/Met	112	0.31	108.48	15.73	1.49
$N=365$	Met/Val	168	0.46	111.51	14.89	1.16
	Val/Val	85	0.23	107.77	17.02	1.84
DRD2	A1A1	16	0.04	104.96	14.66	3.67
$N=391$	A1A2	109	0.27	110.76	14.25	1.36
	A2A2	266	0.69	110.36	16.32	1.00

Note: The WM scores of children and adults are based on different subtests. Although these index the same theoretical construct, the WM scores cannot be compared directly across age cohorts.

a positive heterosis pattern in both the young and the adult cohorts (see Table 1). Heterosis refers to a situation in which a given trait is significantly greater (or lesser) in individuals heterozygous at a specific gene marker than those homozygous for either allele. We tested for heterosis by adding a non-additive (dominance) genetic component to the population-based analysis in QTDT and testing whether the heterozygous genotypes were associated with better WM performance. A significant heterosis effect was found for the association between *COMT* Val$^{108/158}$Met polymorphism and WM in the adult cohort ($\chi^2_{(1360)} = 4.80$, $P = 0.014$) even after correction for multiple testing. In the young cohort, the association did not reach significance ($\chi^2_{(1360)} = 1.54$, $P = 0.107$), although the effects were in the same direction as in the adult cohort. The strongest effect was found in the combined sample ($\chi^2_{(1724)} = 5.70$, $P = 0.008$).

DRD2 polymorphism

No significant main effect was found on the *DRD2* rs2075654 *tag*-SNP and WM performance ($\chi^2_{(1,356)} = 0.42$ $P = 0.52$; $\chi^2_{(1,391)} = 0.04$ $P = 0.84$; and $\chi^2_{(1749)} = 0.10$ $P = 0.75$, for children, adults, and the combined sample, respectively).

COMT and *DRD2* interaction

Figure 3 plots WM performance against six possible combined genotype groups (Met/Met,A−; Val/Met,A−; Val/Val,A−; Met/Met;A+; Val/Met,A+ and Val/Val,A+). The figure suggests that the heterosis found for the *COMT* gene is entirely limited to subjects with the *DRD2* A+ genotype, the genotype previously linked to the reduced receptor density. In support of this, a significant interaction effect was detected between the *DRD2* and *COMT* polymorphisms in the combined sample ($\chi^2_{(1699)} = 2.72$, one-sided $P = 0.050$), which seemed confined to the adult cohort (see Table 2).

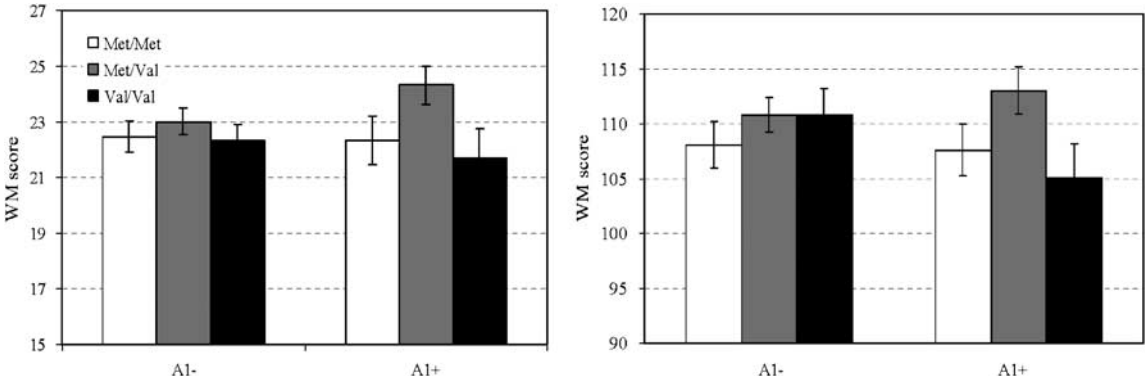

Figure 3 Working memory (WM) means plotted for the six combined genotype groups of the *COMT* and *DRD2* genes for young (left panel) and adult (right panel) cohort. Bars denote standard error. *Note*: A+ denotes carriers of the A1 allele, whereas A- denotes non-carriers.

Table 2 Results of population-based gene–gene interaction analysis – for *COMT* Val$^{108/158}$Met, and *DRD2* rs2075654 (tagging the TaqA1 polymorphism) for the young, adult, and combined cohorts

Cohort	*Model*	*2 lnlk*	*df*	*vs*	χ^2	P-*value*[a]
Young	1. Full model including main effects *COMT* and *DRD2*+additive and heterosis interaction effects	952.64	350			
	2. Test for significance of heterosis interaction *COMT* × *DRD2*	953.70	351	1	1.06	0.152
	3. Test for significance of additive interaction *COMT* × *DRD2*	954.32	352	2	0.62	0.216
Adult	1. Full model including main effects *COMT* and *DRD2*+additive and heterosis interaction effects	926.38	340			
	2. Test for significance of heterosis interaction *COMT* × *DRD2*	927.62	341	1	1.24	0.133
	3. Test for significance of additive interaction *COMT* × *DRD2*	929.16	342	2	1.54	0.107
All	1. Full model including main effects *COMT* and *DRD2*+additive and heterosis interaction effects	1881.9	698			
	2. Test for significance of heterosis interaction *COMT* × *DRD2*	1884.62	699	1	2.72	0.050
	3. Test for significance of additive interaction *COMT* × *DRD2*	1886.32	700	2	1.7	0.096

[a]One-sided *P*-value.
Note: Model 1 = full model, includes a grand mean, main additive and heterosis effects of *COMT*, a main effect of A1 carriers *versus* non carriers of *DRD2*, and additive and heterosis interaction of *COMT* genotypes × *DRD2* A1 carriers *versus* non-carriers. Model 2 = model 1, except the heterosis interaction. Model 3 = model 2, except the additive interaction.

Discussion

In the present study, we tested the association of the *COMT* Val$^{108/158}$Met polymorphism with WM performance. A significant association was found in the combined sample with stronger contribution from the adult than the young cohort. The association reflected positive heterosis such that the *Met/Val* heterozygotes performed better than both *Met/Met* and *Val/Val* homozygotes on the WM tasks. An age-dependent positive heterosis pattern has previously been reported in a longitudinal study by Harris*et al*.[44] The *COMT* genotype was not associated with childhood intelligence measured at the age of 11 years in the Scottish Mental Survey of 1932. At the age of 79 years, *COMT* genotype was significantly related to differences in verbal declarative memory and to scores on the personality traits of intellect/imagination. For both traits, the elderly Val/Met heterozygotes had higher scores than both homozygous groups, which echo the pattern of heterosis on WM found in adult cohort. Because the *COMT* polymorphism has been hypothesized to have a nonlinear effect on DA availability in the prefrontal cortex,[20] the finding of heterosis is in keeping with the idea that the relationship between DA signaling and cognitive performance follows an inverted U-shaped curve, with both suboptimal and supraoptimal DA activity, impairing prefrontal function.[45] Burst firing of VTA neurons causes synaptic DA release in pyramidal cells in the PFC. Because these cells contain little DA transporter (*DAT*), most DA diffuses out of the synaptic cleft to bind to extrasynpatic *D1* receptors, where it is inactivated by *COMT*.[46] The higher activity Val allele decreases extrasynaptic DA levels and, therefore, *D1* activation, shifting the balance in favor of intrasynaptic *D2* receptor activation.[46,47] Cognitive performance may be critically dependent on the *D1/D2* binding ratio, with a relative lack of *D1* signaling causing impulsivity, distractibility, and poor WM performance with schizophrenia at the extreme end.[47] A relative lack of *D2* signaling, on the other hand, may fail to signal the presence of reward information, a signal that is required to engage the PFC in updating its WM system.[48]

The above suggests that the optimum level of DA signaling depends not simply on frontal DA availability, but on its combination with *D2* receptor sensitivity. Therefore, individual differences in DA availability as well as *D2* receptor sensitivity may come into play during the performance of WM tasks. We tested this expectation in a secondary analysis in which a *DRD2* tagging SNP (rs2075654) was tested for an interactive effect with the *COMT* polymorphism. No significant main effect on WM was found for the rs2075654 *tag*-SNP in *DRD2*. However, in the combined cohort, the *DRD2* and *COMT* polymorphisms had a significant interactive effect on WM performance. The interaction suggested that the *Met/Val* heterozygotes perform better than both *Met/Met* and *Val/Val* homozygotes only when they carry one or two A1 alleles. The A1 alleles have been associated with lower receptor density, suggesting that the U-curve-shaped effect of DA availability on WM performance disappears when receptor density is high. Such a pattern has been previously reported by Reuter *et al*,[24] who reported a significant interactive effect between the *DRD2 Taq* IA and the *COMT* polymorphisms on the amount of response interference in the Stroop color–word conflict task. Inspection of Figure 3 suggests that the interaction is stronger in the adult than in the young cohort. Although *p*-values in neither cohort reach formal significance levels, this age difference may be real. Evidence for age-related changes regarding DA metabolism within the PFC has been postulated in both animal[27] and human studies,[28] with increased DA metabolism (eg, *MAO*, *COMT*) thought to be present at a more mature age.[49] Furthermore, several lines of evidence showed a decrease of DA receptors with age.[50–52] When age-related changes in overall levels of DA availability and DA receptor sensitivity are superimposed on the influence of genetic polymorphisms on these levels, a different change in overall DA signaling may occur with age in the various *COMT–DRD2* haplotypes. When we add to this that there may be an inverted U-curved relation between DA signaling and WM performance, interactions between age, *DRD2*, and *COMT* genes should be the rule rather than the exception.

Clearly, full genetic contribution to dopaminergic variation in frontal executive function will rely on far more complex interactions between multiple receptor (eg, *DRD1*, *DRD2*, and *DRD4*), transporters and enzymatic polymorphisms (eg, *DAT*, *COMT*, and *MAO)*.[9,53,54] Further studies systematically involving such interactions are needed to obtain a clearer overview of the dopaminergic pathway. At the same time, denser SNP coverage of the area under study is needed in genes like *DRD2* to reveal the true functional variants, which while tagged, are still undiscovered. In summary, our results are in keeping with previous findings suggesting that WM performance needs an optimal level of DA signaling within the PFC. This optimum level depends on enzymatic activity controlling DA level as well as on DA receptor sensitivity, both of which may differ as a function of age and genotype. We conclude that the effects of a single polymorphism in a dopaminergic gene on a well-defined cognitive trait may easily remain hidden if the interaction with age and other genes in the pathway are not taken into account.

Acknowledgements

This study was supported by the Universitair Stimulerings Fonds (Grant number 96/22), the Human Frontiers of Science Program (Grant number rg0154/1998-B), the Netherlands Organization for Scientific Research (NWO) Grants 904-57-94 and NWO/SPI 56-464-14192. This study was also supported by the Centre for Medical Systems Biology (CMSB), a center of excellence approved by the Netherlands Genomics Initiative/Netherlands Organization for

Scientific Research (NWO). We thank the families from the Netherlands Twin Registry (NTR) who participated in this study.

References

1 Conway ARA, Cowan N, Bunting MF, Therriault DJ, Minkhoff SRB: A latent variable analysis of working memory capacity, short-term memory capacity, processing speed, and general fluid intelligence. *Intelligence* 2002; **30**: 163–183.
2 Colom R, Rebollo I, Palacios A, Juan-Espinosa M, Kyllonen PC: Working memory is (almost) perfectly predicted by *g*. *Intelligence* 2004; **32**: 277–296.
3 Curtis CE, D'Esposito M: Persistent activity in the prefrontal cortex during working memory. *Trends Cogn Sci* 2003; **7**: 415–423.
4 Fuster JM: Network memory. *Trends Neurosci* 1997; **20**: 451–459.
5 Gibbs SE, D'Esposito M: Individual capacity differences predict working memory performance and prefrontal activity following dopamine receptor stimulation. *Cogn Affect Behav Neurosci* 2005; **5**: 212–221.
6 Brunner HG, Nelen M, Breakefield XO, Ropers HH, van Oost BA: Abnormal behavior associated with a point mutation in the structural gene for monoamine oxidase A. *Science* 1993; **262**: 578–580.
7 Harmer CJ, McTavish SF, Clark L, Goodwin GM, Cowen PJ: Tyrosine depletion attenuates dopamine function in healthy volunteers. *Psychopharmacology (Berl)* 2001; **154**: 105–111.
8 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* 2002; **159**: 652–654.
9 Williams GV, Castner SA: Under the curve: critical issues for elucidating D1 receptor function in working memory. *Neuroscience* 2006; **139**: 263–276.
10 Savitz J, Solms M, Ramesar R: The molecular genetics of cognition: dopamine, COMT and BDNF. *Genes Brain Behav* 2006; **5**: 311–328.
11 Reuter M, Hennig J: Association of the functional catechol-O-methyltransferase VAL158MET polymorphism with the personality trait of extraversion. *Neuroreport* 2005; **16**: 1135–1138.
12 Grossman MH, Emanuel BS, Budarf ML: Chromosomal mapping of the human catechol-O-methyltransferase gene to 22q11.1-q11.2. *Genomics* 1992; **12**: 822–825.
13 Axelrod J: O-Methylation of epinephrine and other catechols *in vitro* and *in vivo*. *Science* 1957; **126**: 400–401.
14 Tenhunen J, Salminen M, Lundstrom K, Kiviluoto T, Savolainen R, Ulmanen I: Genomic organization of the human catechol O-methyltransferase gene and its expression from two distinct promoters. *Eur J Biochem* 1994; **223**: 1049–1059.
15 Matsumoto M, Weickert CS, Akil M *et al*: Catechol O-methyltransferase mRNA expression in human and rat brain. Evidence for a role in cortical neuronal function. *Neuroscience* 2003; **116**: 127–137.
16 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* 1995; **34**: 4202–4210.
17 Palmatier MA, Kang AM, Kidd KK: Global variation in the frequencies of functionally different catechol-O-methyltransferase alleles. *Biol Psychiatry* 1999; **46**: 557–567.
18 Boudikova B, Szumlanski CL, Maidak B, Weinshilboum RM: Human liver catechol-O-methyltransferase pharmacogenetics. *Clin Pharmacol Ther* 1990; **48**: 381–389.
19 Stefanis NC, Van Os J, Avramopoulos D *et al*: Variation in catechol-o-methyltransferase val158 met genotype associated with schizotypy but not cognition: a population study in 543 young men. *Biol Psychiatry* 2004; **56**: 510–515.
20 Mattay VS, Goldberg TE, Fera F *et al*: Catechol O-methyltransferase val158-met genotype and individual variation in the brain response to amphetamine. *Proc Natl Acad Sci USA* 2003; **100**: 6186–6191.
21 Comings DE, Blum K: Reward deficiency syndrome: genetic aspects of behavioral disorders. *Prog Brain Res* 2000; **126**: 325–341.
22 Thompson J, Thomas N, Singleton A *et al*: D2 dopamine receptor gene (DRD2) Taq1 A polymorphism: reduced dopamine D2 receptor binding in the human striatum associated with the A1 allele. *Pharmacogenetics* 1997; **7**: 479–484.
23 Pohjalainen T, Rinne JO, Nagren K *et al*: The A1 allele of the human D2 dopamine receptor gene predicts low D2 receptor availability in healthy volunteers. *Mol Psychiatry* 1998; **3**: 256–260.
24 Reuter M, Peters K, Schroeter K *et al*: The influence of the dopaminergic system on cognitive functioning: a molecular genetic approach. *Behav Brain Res* 2005; **164**: 93–99.
25 Stins JF, Polderman JC, Boomsma DI, de Geus EJ: Response interference and working memory in 12-year-old children. *Child Neuropsychol* 2005; **11**: 191–201.
26 Laird NM, Lange C: Family-based designs in the age of large-scale gene-association studies. *Nat Rev Genet* 2006; **7**: 385–394.
27 Lee JJ, Chang CK, Liu IM, Chi TC, Yu HJ, Cheng JT: Changes in endogenous monoamines in aged rats. *Clin Exp Pharmacol Physiol* 2001; **28**: 285–289.
28 Kaasinen V, Rinne JO: Functional imaging studies of dopamine system and cognition in normal aging and Parkinson's disease. *Neurosci Biobehav Rev* 2002; **26**: 785–793.
29 Boomsma DI, de Geus EJ, Vink JM *et al*: Netherlands twin register: from twins to twin families. *Twin Res Hum Genet* 2006; **9**: 849–857.
30 Polderman TJC, Gosso MF, Posthuma D *et al*: A longitudinal twin study on IQ, executive functioning, and attention problems during childhood and early adolescence. *Acta Neurol Belg* 2006; **106**: 191–207.
31 Polderman TJC, Stins JF, Posthuma D, Gosso MF, Verhulst FC, Boomsma DI: The phenotypic and genotypic relation between working memory speed and capacity. *Intelligence* 2006; **34**: 549–560.
32 Posthuma D, Luciano M, Geus EJ *et al*: A genomewide scan for intelligence identifies quantitative trait loci on 2q and 6p. *Am J Hum Genet* 2005; **77**: 318–326.
33 Hoekstra C, Meijer P, Kluft C *et al*: Genetics of dizygotic twinning: a feasibility study for a biobank. *Twin Res* 2004; **7**: 556–563.
34 Wechsler D: Wechsler intelligence scale for children-revised (WISC-R). *Lisse, Swets & Zeitlinger* 1986.
35 Wechsler D WAIS-III Wechsler Adult Intelligence Scale San Antonio, Texas, Psychological Corporation, 1997.
36 Meulenbelt I, Droog S, Trommelen GJ, Boomsma DI, Slagboom PE: High-yield noninvasive human genomic DNA isolation method for genetic studies in geographically dispersed families and populations. *Am J Hum Genet* 1995; **57**: 1252–1254.
37 Miller SA, Dykes DD, Polesky HF: A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988; **16**: 1215.
38 Hirschhorn JN, Daly MJ: Genome-wide association studies for common diseases and complex traits. *Nat Rev Genet* 2005; **6**: 95–108.
39 Gosso MF, de Geus EJ, van Belzen MJ *et al*: The SNAP-25 gene is associated with cognitive ability: evidence from a family-based study in two independent Dutch cohorts. *Mol Psychiatry* 2006; **11**: 878–886.
40 Gosso MF, van Belzen M, de Geus EJ *et al*: Association between the CHRM2 gene and intelligence in a sample of 304 Dutch families. *Genes Brain Behav* 2006; **5**: 577–584.
41 Abecasis GR, Cardon LR, Cookson WO: A general test of association for quantitative traits in nuclear families. *Am J Hum Genet* 2000; **66**: 279–292.

42 Fulker DW, Cherny SS, Sham PC, Hewitt JK: Combined linkage and association sib-pair analysis for quantitative traits. *Am J Hum Genet* 1999; **64**: 259–267.
43 Posthuma D, de Geus EJ, Boomsma DI, Neale MC: Combined linkage and association tests in mx. *Behav Genet* 2004; **34**: 179–196.
44 Harris SE, Wright AF, Hayward C, Starr JM, Whalley LJ, Deary IJ: The functional COMT polymorphism, Val 158 Met, is associated with logical memory and the personality trait intellect/imagination in a cohort of healthy 79 year olds. *Neurosci Lett* 2005; **385**: 1–6.
45 Cools R, Robbins TW: Chemistry of the adaptive mind. *Philos Transact A Math Phys Eng Sci* 2004; **362**: 2871–2888.
46 Bilder RM, Volavka J, Lachman HM, Grace AA: The catechol-O-methyltransferase polymorphism: relations to the tonic-phasic dopamine hypothesis and neuropsychiatric phenotypes. *Neuropsychopharmacology* 2004; **29**: 1943–1961.
47 Winterer G, Weinberger DR: Genes, dopamine and cortical signal-to-noise ratio in schizophrenia. *Trends neurosci* 2004; **27**: 683–690.
48 Weinberger DR, Egan MF, Bertolino A *et al*: Prefrontal neurons and the genetics of schizophrenia. *Biol Psychiatry* 2001; **50**: 825–844.
49 Gottfries CG: Neurochemical aspects on aging and diseases with cognitive impairment. *J Neurosci Res* 1990; **27**: 541–547.
50 Wong DF, Wagner Jr HN, Dannals RF *et al*: Effects of age on dopamine and serotonin receptors measured by positron tomography in the living human brain. *Science* 1984; **226**: 1393–1396.
51 Suhara T, Fukuda H, Inoue O *et al*: Age-related changes in human D1 dopamine receptors measured by positron emission tomography. *Psychopharmacology (Berl)* 1991; **103**: 41–45.
52 Volkow ND, Ding YS, Fowler JS *et al*: Dopamine transporters decrease with age. *J Nucl Med* 1996; **37**: 554–559.
53 Tsai SJ, Yu YW, Lin CH, Chen TJ, Chen SP, Hong CJ: Dopamine D2 receptor and *N*-methyl-*D*-aspartate receptor 2B subunit genetic variants and intelligence. *Neuropsychobiology* 2002; **45**: 128–130.
54 Bertolino A, Blasi G, Latorre V *et al*: Additive effects of genetic variation in dopamine regulating genes on working memory cortical activity in human brain. *J Neurosci* 2006; **26**: 3918–3922.