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Epistasis effects of *COMT* and *MTHFR* on inter-individual differences in mental health: Under the inverted U-shaped prefrontal dopamine model

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ABSTRACT

Higher cognitive performance, maintenance of mental health and psychological well-being require adequate prefrontal cortex (PFC) function. "Inverted U-shaped" dopamine model indicates optimal PFC dopamine level is important to attain its function while high or low levels have adverse effects. Catechol-O-methyltransferase (*COMT*) and methylenetetrahydrofolate reductase (*MTHFR*) may be involved in this complex non-linear PFC dopamine regulation. We addressed whether genetic variation reflecting COMT and MTHFR activities can explain the inter-individual mental health differences in healthy Japanese men ($n = 188$). The mental health was measured by Mental Health Inventory (MHI)-5 score. The rs4633–rs4818–rs4680 haplotypes were used to represent the multilevel COMT activities, while for *MTHFR*, the functional single polymorphism, rs1801133 (C677T), was used. We examined the effectiveness of haplotype-based association analysis of *COMT* on mental health together with studying its interaction with *MTHFR*-C677T. As a result, the relation between activity-ranked *COMT* genotype and MHI-5 score showed a tendency to fit into an "inverted U-shaped" quadratic curve ($P = 0.054$). This curvilinear correlation was significant in the subjects with *MTHFR*-CC ($P < 0.001$), but not with *MTHFR* T-allele carriers ($P = 0.793$). Our pilot study implies a potential influence of *COMT* and *MTHFR* genotypic combination on normal variation of mental health.

1. Introduction

The prefrontal cortex (PFC) is of significant importance in determining the nature of humans' higher cognitive and intellectual functioning such as working memory, executive function as well as psychological well-being [1–3]. Several studies have indicated that impaired PFC function is associated with various neuropsychological phenotypes ranging from normal variation to depression and negative moods [4,5]. Dopamine effects on PFC function follow an "inverted U-shaped" curve, where both deficient and excessive dopamine activity predicting impaired PFC function [6,7]. Moving beyond the dopamine hypothesis, a critical role of dopamine modulation by genetic polymorphisms in PFC function and psychiatric disorders has been vigorously investigated [8]. Among these, there has been extensive interest in the effect of the catechol-O-methyltransferase (*COMT*) gene on neuropsychological functions and phenotypes for decades [9,10].

COMT is a catecholamine-catabolizing enzyme that inactivates the catecholamine neurotransmitters (dopamine, epinephrine, and norepinephrine) by adding a methyl group donated by S-adenosyl methionine (SAM). Vanillylmandelic acid is an end-stage metabolite of catecholamine. The catecholaminergic pathway in both the central and peripheral nervous systems plays an essential role in response to environmental stimuli and stress [11]. The COMT enzyme is crucial for regulating frontal dopamine transmission, accounting for more than 60% of dopamine turnover in the PFC [12,13]. More recently, comprehensive *in vivo* studies in *Comt* knock-out mice suggest that COMT enzymes contribute about one half of the total dopamine degradation in the prefrontal cortex [14,15].

Abbreviations: MHI-5, five-item Mental Health Inventory; *COMT*, catechol-O-methyltransferase; *MTHFR*, methylenetetrahydrofolate reductase; PFC, prefrontal cortex; SNP, single nucleotide polymorphism.

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Given the preferential role of COMT in prefrontal dopamine degradation, a common functional single nucleotide polymorphism (SNP) at codon 158 in *COMT* (Val158Met polymorphism; rs4680 G > A) has influence on cognition by modulating dopamine signaling in the frontal lobes. This Met substitution displays a three- to fourfold reduction in catecholamine-degrading enzymatic activity compared to the original Val form. Thus Met carriers, having higher PFC dopamine levels [16], have shown greater cortical efficiency [9,17,18]. However, contradictory to this result, the observed effect of Met allele (low enzyme activity) on cognitive performance is relatively modest [19,20] or absent [21,22] in some studies.

Recent studies uncovered that the functional contribution of Val158Met is confounded by two synonymous SNPs, rs4633 and rs4818, those are located in the central region of *COMT*. The Val158Met SNP (rs4680) together with these two synonymous SNPs forms a haploblock (rs4633–rs4818–rs4680). The difference in this haplotype produces the difference in *COMT* mRNA secondary structure and stability [23,24]. And eventually, the difference of COMT enzymatic activity between the low- and the high-activity haplotypes is reported to be >18-fold [24]. Therefore, there is a need to evaluate COMT activity using haplotype-based analysis because most studies to date have analyzed only the single Val158Met polymorphism as a surrogate marker of COMT activity. Indeed, a recent large-scale cohort study of children by Barnett et al. showed that *COMT* haplotype analysis is more beneficial for prediction of normal cognitive variation in healthy children [25].

Based on haplotype study by Nackley et al., three major *COMT* haplotypes (CGG, TCA, CCG) represent high, intermediate and low activity, respectively [24]. It is noted here that the conventional high-activity 158Val (rs4680 G) variant needs to be re-evaluated as two distinct haplotypes: true high-activity haplotype CGG and low-activity haplotype CCG. The frequency of three major *COMT* haplotype each is considerably different between Caucasians and Japanese. Importantly, the ratio of low-activity haplotype CCG to the total 158Val type (conventionally assumed as high-activity) is almost the half (about 45–56%) in the Japanese population [26,27], but one-fifth in the Caucasian [24,27]. Therefore, haplotype analysis instead of Val158Met method may have a potential advantage to properly relate genotype with COMT activity in the Japanese population.

Furthermore, the effect of *COMT* on the PFC dopamine regulation would be modified by the methylenetetrahydrofolate reductase (*MTHFR*) genetic background. The thermolabile *MTHFR*-677T variant (Ala to Val substitution) shows a 35% reduction in MTHFR activity and methionine synthesis [28]. *MTHFR*-C677T has been known to interact with *COMT* and modify its enzymatic activity by modulating the availability of SAM, a methyl donor in the methylation reaction catalyzed by COMT [29,30]. Several studies have found epistatic interactions between *COMT* Val158Met and *MTHFR*-C677T in cognitive and neuropsychological functions [25,31]. They suggest that the beneficial or detrimental effect of COMT activity on dopamine homeostasis crucially depends on the *MTHFR* genotype.

In this study, we have examined whether *COMT* and *MTHFR* epistasis explains inter-individual mental health variation in healthy Japanese men. We have employed haplotypes that characterize COMT activities for association analysis. As a result, subjects with intermediate-activity *COMT* genotype have shown better mental health when they have *MTHFR*-CC genotype. Our study implies that *COMT* and *MTHFR* genotypic combination potentially influence mental health following the "inverted U-shaped" prefrontal dopamine model.

2. Materials and methods

2.1. Subjects

The present study was undertaken in an occupational cohort organized to investigate the association of lifestyle factors with metabolic syndrome according to different genetic factors in Japanese workers. It has been proposed that poor mental health could be one of the causes to exacerbate the metabolic syndrome. This occupational cohort comprised 320 healthy Japanese men (Table 1) [32]. Information about the age, current smoking, drinking, and energy intake of all participants was obtained by a self-report questionnaire. Medical history was acquired by interview. The study was approved by the ethics review committee of the Medical Research Institute, Tokyo Medical and Dental University (No. 5-2008), and written informed consent was obtained from all participants.

2.2. Genotyping

Genomic DNA was extracted from peripheral blood samples by the conventional methods. Genotyping for the three *COMT* polymorphisms (rs4633, rs4818, rs4680) and *MTHFR*-C677T (rs1801133) were performed by the TaqMan SNP genotyping assay (ABI) followed by allelic discrimination analysis using a sequence detection system (ABI PRISM 7900HT, USA, SDS software package version 2.2.1) with a successful genotype call rate of 99.9%.

2.3. Mental health assessment

For the assessment of mental health status, we adopted the five-item Mental Health Inventory (MHI-5), a brief questionnaire for measuring general mental health, including depression, anxiety, behavioral–emotional control, and general positive affect,

Table 1
General characteristics of participants (men).

Characteristics	All Subjects (n = 320) Mean ± SD	Subjects with MHI-5 data (n = 188) Mean ± SD	Subjects without MHI-5 data (n = 132) Mean ± SD
Age (years)	45.90 ± 11.61	45.16 ± 11.57	46.97 ± 11.61
BMI (kg/m^2)	23.43 ± 3.50	23.56 ± 3.46	23.23 ± 3.56
Systolic blood pressure (mmHg)	134.54 ± 17.90	133.95 ± 7.08	135.39 ± 19.06
Diastolic Blood pressure (mmHg)	81.66 ± 12.60	81.59 ± 12.55	81.75 ± 1 2.71
Serum homocysteine level (μmol/L)	10.35 ± 4.88	10.59 ± 5.59	10.00 ± 3.61
Serum folic acid level (nmol/L)	6.53 ± 3.36	6.55 ± 2.93	6.40 ± 3.92
MHI-5 score	NA	47.79 ± 10.36	NA
Smoking (%)	59.9	59.5	60.3
Drinking (%)	73.4	75.1	70.7

SD; standard deviation, BMI; body mass index.
NA; not available.

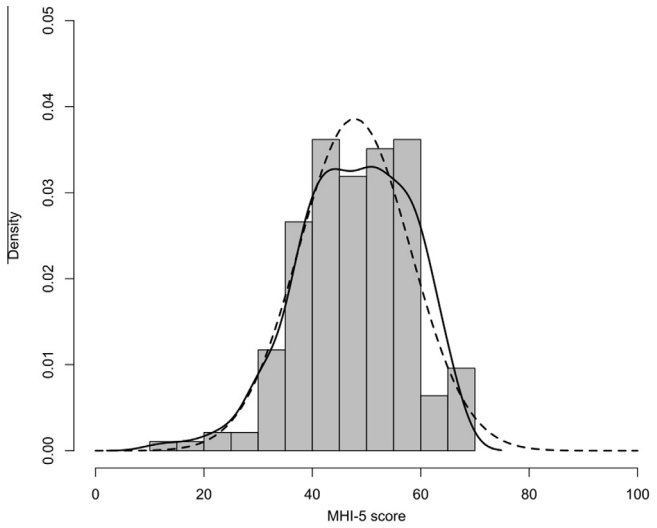

Fig. 1. MHI-5 scores are normally distributed. Mean MHI-5 score = 47.79 ± 10.34, Solid line: probability density distribution curve, Dotted line: normal distribution curve. One-sample Kolmogorov–Smirnov test: $D = 0.086$, P value = 0.124.

and has been proven reliable and valid for detecting depressive symptoms in the general population of Japan [33]. MHI-5 has been shown to have a high correlation with ZSDS (a self-rated depression scale) and has been commonly used to measure general mental health status worldwide [34]. Here, we assumed that the mental health status principally reflects PFC dopamine activity. The MHI-5 has five questions about feelings of depression and nervousness to be answered on a five-point scale ranging from "all of the time" to "none." The MHI-5 score ranges from 0 to 100 points, calculated by first reversing the coding of the two positively formulated questions and consequently summing the points of each item (Transformation of raw summated score: [(Raw scale score − Lowest possible score)/possible score range] × 100), with a higher score reflecting better mental health [33]. Our MHI-5 data show a Gaussian distribution (Fig. 1) and met the assumption of normality by one-sample Kolmogorov–Smirnov test ($P = 0.124$). The number of the subjects with complete MHI-5 data was 188. They were randomly chosen and there were no statistical differences in the general characteristics among the subjects with ($n = 188$) and without ($n = 132$) MHI-5 data, as shown in Table 1.

2.4. Single polymorphism association analysis

The allele frequency was determined by direct counting. Deviation of the genotype distribution from Hardy–Weinberg equilibrium was examined by chi-square test. Differences in the mean values of age, clinical characteristics, current smoking, drinking, and MHI-5 between the genotype groups were compared by analysis of variance (ANOVA) or chi-square test. In the single SNP analyses, the three *COMT* SNPs, rs4633, rs4818, and rs4680 (Val158Met) and *MTHFR*-C677T were independently tested for association with MHI-5 using one-way ANOVA (Table S1).

All association analyses were carried out using IBM SPSS Statistics version 19.0. P values < 0.05 were considered statistically significant.

2.5. Haplotype configuration

COMT haplotypes were configured from pair-wise linkage disequilibrium estimations by Haploview version 4.2 [35]. The haplotype analysis from the original genotyping results of 320 subjects identified eight haplotypes (Table 2). Referring to the classification by Nackley et al. three major haplotypes of *COMT* rs4633–rs4818–rs4680 (low: CGG; intermediate (Met): TCA; high: CCG) were identified. In addition, all the haplotypes carrying rs4680A (Met) were used as intermediate (Met) type [25]. Accordingly, the haplotypes were classified into four types such as Val-H (high), Val-L (low), Met, and Val-U (unknown). We used all those haplotypes except for "unknown" type for diplotype construction, which account for 90.7% of total (Table 2).

2.6. Diplotype reconstruction and association analysis

Table S2 shows the diplotypes reconstructed in 188 subjects with MHI-5 data. After exclusion of unknown activity (Val-U) haplotypes, a total of 161 subjects were assigned to one of the six possible diplotypes. Following the method by Barnett et al. we combined Val-H/Val-L in the Met/Met diplotype group and investigated the association of these five *COMT* diplotypes and MHI-5 [25]. First, MHI-5 scores were compared among the five diplotype groups by one-way ANOVA. Then we combined the highest and lowest COMT activity diplotypes into one group and the other three diplotypes (with intermediate activity) into another and compared these two groups for their association with MHI-5 by student's t-test. A power calculation using the G Power: 3 program [36] indicated that in the analysis of the *MTHFR*-CC group ($n = 52$), there was 98.1% power to detect the differences in MHI-5 scores between two COMT activity groups with an effect size of 1.32 at an alpha of 0.05.

3. Results

3.1. General characteristics of the participants

Table 1 shows the general characteristics of the participants. Genotyping data of total 320 subjects were used for *COMT* haplotype configuration. All genotype frequencies of the four SNPs

Table 2
Haplotypes frequencies.

	Haplotype sequence			Types: Val-H/Val-L/Val-U/Met	COMT activity	Frequency (%) $n = 320$
	rs4633	rs4818	rs4680 (Val/Met)			
1.	C	C	G	(Val-L)	Low	36.6
2.	C	G	G	(Val-H)	High	23.4
3.	T	C	A	(Met)	Met (intermediate)	17.3
4.	C	C	A	(Met)	Met	8.7
5.	T	C	G	(Val-U)	Unknown	7.2
6.	T	G	A	(Met)	Met	3.4
7.	T	G	G	(Val-U)	Unknown	2
8.	C	G	A	(Met)	Met	1.3

Eight haplotypes formed by 3 SNPs (rs4633, rs4818, rs4680) and their frequencies are shown. Three major haplotypes (1, 2, and 3) are reported to be associated with variations in COMT activity [24].
Val, valine; Met, methionine; L, low; H, High; U, unknown.

(*COMT*: rs4633, rs4818, rs4680; *MTHFR:* rs1801133) followed Hardy–Weinberg equilibrium. The association analysis with mental health status was performed for the subjects with complete Mental Health Inventory (MHI)-5 data ($n = 188$). There were no significant differences in general characteristics between the subjects with and without MHI-5 data.

3.2. Single SNP analysis revealed no association between COMT or MTHFR polymorphisms and MHI-5 score

There was no significant association found by single SNP analysis between the *COMT* polymorphisms (rs4633, rs4818, rs4680), *MTHFR*-C677T (rs1801133), and MHI-5 score. The results of ANOVA test, likelihood ratio test and Wald test are shown (Tables S1 and S3).

3.3. MHI-5 score was lower in the highest- and the lowest-activity COMT diplotypes

In ANOVA test for the association between *COMT* diplotypes and MHI-5, we detected no significant differences among five *COMT* diplotype groups ($P = 0.187$). However, of interest, the mean MHI-5 score was low in the extreme COMT activity diplotypes; the highest (Val-H/Val-H) (43.00 ± 15.60) and the lowest (Val-L/Val-L) (45.54 ± 8.56). Taking into account the role of COMT activity in PFC dopamine modulation, this finding suggests that the MHI-5 score reflects the "inverted U-shaped" correlation with COMT activity (Fig. S1).

To examine whether the association between MHI-5 score (dependent variable) and *COMT* diplotypes (explanatory variable) fit well in a curvilinear regression model, we compared the results between linear and quadratic regression analysis. No association was identified in linear regression analysis ($\beta = 0.102$, $t = 0.143$, $P = 0.886$). Although it was not statistically significant, the quadratic model was well fitted. (Model fit: $r^2 = 0.036$, $F = 2.978$, df = 2, 158, $P = 0.054$; linear term: $\beta = 0.102$, $t = 0.143$, $P = 0.886$; quadratic term: $\beta = -1.357$, $t = -2.436$, $P = 0.016$) (Fig. S1).

3.4. Nonlinear effects of COMT diplotypes on MHI-5 score within the context of MTHFR genotypes

We then examined whether the effect of *COMT* diplotypes on MHI-5 score is influenced by the *MTHFR*-C677T polymorphism. We classified the subjects into *MTHFR*-CC homozygotes and T-carrier groups and examined the association of COMT activity and MHI-5 score in each group. In the *MTHFR*-CC group ($n = 52$), statistical significance was observed in the quadratic model: $r^2 = 0.27$, $F = 9.10$, df = 2, 49, $P < 0.001$; quadratic term: $\beta = -3.53$, $t = -4.26$, $P < 0.001$), but not in linear model ($\beta = -0.15$, $t = -0.12$, $P = 0.902$) (Fig. 2A). This result indicates that the intermediate-activity COMT diplotypes had the best mental health status (high MHI-5 score) and that either too high or too low COMT activity would result in poor mental health status (low MHI-5 score). On the other hand, we could not find the association between *COMT* and mental health in the *MTHFR*-T carrier group ($n = 109$) (Quadratic model: $r^2 = 0.002$, $F = 0.17$, df = 2, 11, $P = 0.891$; linear term: $\beta = 0.24$, $t = 0.26$, $P = 0.79$; quadratic term: $\beta = -0.29$, $t = -0.21$, $P = 0.687$) (Fig. 2B).

As the MHI-5 score was strongly affected by both the highest and lowest COMT activity, we reclassified the five *COMT* diplotypes into two groups. The highest COMT activity Val-H/Val-H and lowest COMT activity Val-L/Val-L were classified into "Group-1" which represented the suboptimal COMT activity, the other three intermediate diplotype groups (Val-H/Met or Met/Met, Val-H/Val-L, Val-L/Met) into "Group-2", representing optimal COMT activity, and compared their association with MHI-5 score within the context of the *MTHFR* genotype. In *MTHFR*-CC subjects, the mean MHI-5 score in Group-2 (51.36 ± 7.68) was significantly higher than that in Group-1 (38.89 ± 10.95) ($P < 1.78 \times 10^{-5}$). On the contrary, there is no difference between two groups in *MTHFR*-T carrier subjects (Fig. 3).

4. Discussion

To the best of our knowledge, the current study reports the first case for an "inverted U-shaped" association between *COMT* diplotypes and mental health in a general population. We found that the *MTHFR*-CC subjects with intermediate-activity *COMT* diplotype showed a better mental health status as assessed as high MHI-5 score. Dopamine hypothesis suggests that an optimal functioning occurs within a narrow range of dopamine levels, and accordingly, both excessive (low COMT-activity Val-L/Val-L) and insufficient (high COMT-activity Val-H/Val-H) dopamine levels were associated with impaired mental health in our study (Figs. 2 and 3). The similar observation was reported in case of working memory performance of healthy children [25].

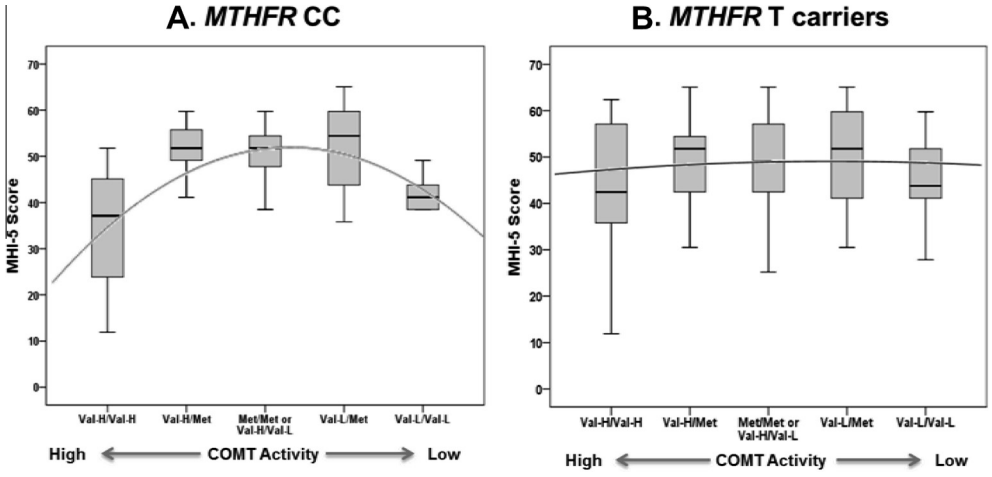

Fig. 2. Nonlinear association between MHI-5 score and activity-ranked *COMT* diplotypes in *MTHFR*-CC subjects. Diplotypes were ranked by COMT activity and sorted in descending order (from left to right). Solid curve indicates the regression curve of quadratic model. (A) *MTHFR*-CC ($n = 52$); the best-fit model illustrated is a nonlinear (quadratic) model of COMT activity associated with MHI-5 score (Quadratic model: $r^2 = 0.271$, $F = 9.102$, df = 2, 49, $P < 0.001$; linear term: $\beta = -0.148$, $t = -0.124$, $P = 0.902$; quadratic term: $\beta = -3.531$, $t = -4.264$, $P < 0.001$). (B) *MTHFR*-T carriers ($n = 109$); no significant association between MHI-5 and COMT activity was found in either the quadratic or linear model (Quadratic model: $r^2 = 0.002$, $F = 0.116$, df = 2, 106, $P = 0.891$; linear term: $\beta = 0.237$, $t = 0.263$, $P = 0.793$; quadratic term: $\beta = -0.288$, $t = -0.206$, $P = 0.687$).

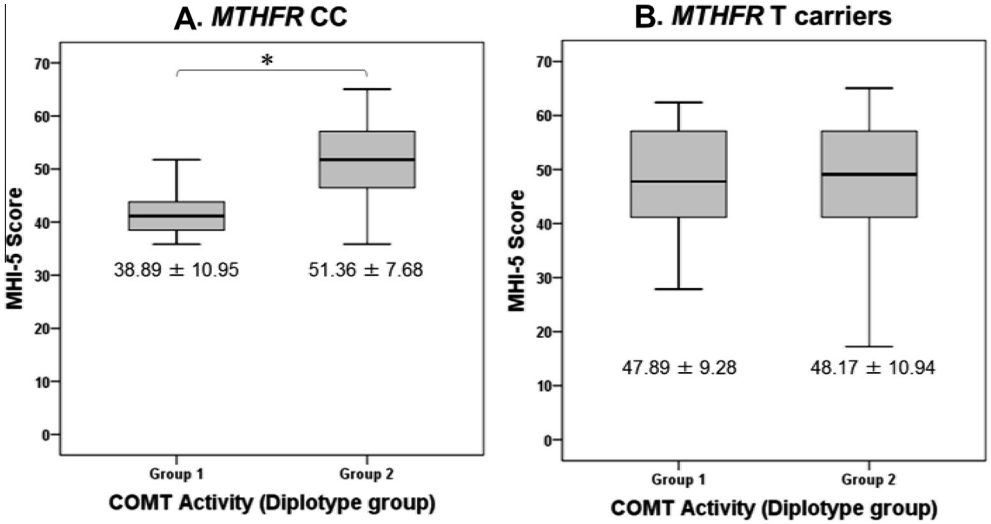

Fig. 3. Significant differences of the mean MHI-5 score between two *COMT* diplotype groups (Group-1 and Group-2) in *MTHFR*-CC subjects. Subjects were classified into *MTHFR*-CC homozygotes (A; $n = 52$) and T-carrier groups (B; $n = 109$). Subjects were further divided according to COMT activity. Group-1: suboptimal COMT activity, which was composed of two extremes of the least active and the most active *COMT* diplotype; Group-2: optimal COMT activity, which contained three intermediate-activity *COMT* diplotype groups. The asterisk (*) indicates P value < 1.78×10^{-5}.

Multi-population comparative analysis by Mukherjee et al. indicated that the diverse *COMT* haplotypic lineages exist among different ethnics group [37]. According to the haplotype frequency data, the haplotype-based analysis of *COMT* would be an effective tool to evaluate the level of COMT activity, especially in the Japanese population.

Previous studies have suggested that the epistatic interactions between *COMT* Val158Met and *MTHFR*-C677T may be involved in impaired cognitive function and psychiatric vulnerability [31,38]. MTHFR is crucially important in the pathway leading to generation of SAM, the major methyl donor for adequate methylation reactions [29,30]. Given the closely related biochemical pathway of MTHFR and COMT [30], it is plausible that in *MTHFR*-CC homozygous individuals, a sufficient amount of SAM may augment prefrontal dopamine signaling where COMT activity substantially contributes to determine dopamine levels [31]. In contrast, in hypoactive T-allele carriers, the range of COMT activities may be restricted due to low level of SAM, which may be insufficient to depict an "inverted U-shaped" curve (Fig. S2). Another speculation is that complex epigenetic regulation such as DNA methylation or histone modification would underpin the *MTHFR* × *COMT* interaction.

The present study has some limitations that the sample size for the current analyses was relatively small with only male subjects. Furthermore, the effect of the functional interaction between *MTHFR* and *COMT* on impaired PFC function needs to be compared between patients and healthy populations, since these epistatic effects are differentially observed for patients and controls [31]. Further large-scale studies in general healthy subjects and in patients, separately, are required to establish the contribution of *MTHFR* × *COMT* interaction on PFC dopamine function.

In conclusion, this pilot study in a Japanese general population suggested that the effect of variation in COMT activity on mental health is highly dependent on its haplotypes formed by a set of SNPs (rs4633–rs4818–rs4680), and that the effect of COMT activity on dopamine homeostasis is further influenced by the *MTHFR* genotype. As a result, the epistasis effects of *COMT* and *MTHFR* on inter-individual differences in mental health are consistent with the "inverted U-shaped" prefrontal dopamine model. A further replication study with a larger sample size is needed to confirm these findings.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.08.023.

References

[1] A.K. Barbey, R. Colom, J. Grafman, Dorsolateral prefrontal contributions to human intelligence, Neuropsychologia 51 (2013) 1361–1369.
[2] C.M. van Reekum, H.L. Urry, T. Johnstone, et al., Individual differences in amygdala and ventromedial prefrontal cortex activity are associated with evaluation speed and psychological well-being, J. Cogn. Neurosci. 19 (2007) 237–248.
[3] S. Funahashi, Prefrontal cortex and working memory processes, Neuroscience 139 (2006) 251–261.
[4] R. Aoki, H. Sato, T. Katura, et al., Relationship of negative mood with prefrontal cortex activity during working memory tasks: an optical topography study, Neurosci. Res. 70 (2011) 189–196.
[5] B.W. Dunlop, C.B. Nemeroff, The role of dopamine in the pathophysiology of depression, Arch. Gen. Psychiatry 64 (2007) 327–337.
[6] A.F. Arnsten, Catecholamine modulation of prefrontal cortical cognitive function, Trends Cogn. Sci. 2 (1998) 436–447.
[7] S. Vijayraghavan, M. Wang, S.G. Birnbaum, et al., Inverted-U dopamine D1 receptor actions on prefrontal neurons engaged in working memory, Nat. Neurosci. 10 (2007) 376–384.
[8] A. Meyer-Lindenberg, D.R. Weinberger, Intermediate phenotypes and genetic mechanisms of psychiatric disorders, Nat. Rev. Neurosci. 7 (2006) 818–827.
[9] V.S. Mattay, T.E. Goldberg, F. Fera, et al., Catechol O-methyltransferase val158-met genotype and individual variation in the brain response to amphetamine, Proc. Natl. Acad. Sci. U.S.A. 100 (2003) 6186–6191.
[10] L. Hosák, Role of the *COMT* gene Val158Met polymorphism in mental disorders: a review, Eur. Psychiatry 22 (2007) 276–281.
[11] R. Kvetnansky, E.L. Sabban, M. Palkovits, Catecholaminergic systems in stress: structural and molecular genetic approaches, Physiol. Rev. 89 (2009) 535–606.
[12] F. Karoum, S.J. Chrapusta, M.F. Egan, 3-Methoxytyramine is the major metabolite of released dopamine in the rat frontal cortex, J. Neurochem. 63 (1994) 972–979.

[13] S.R. Sesack, V.A. Hawrylak, C. Matus, et al., Dopamine axon varicosities in the prelimbic division of the rat prefrontal cortex exhibit sparse immunoreactivity for the dopamine transporter, J. Neurosci. 18 (1998) 2697–2708.
[14] L. Yavich, M.M. Forsberg, M. Karayiorgou, et al., Site-specific role of catechol-O-methyltransferase in dopamine overflow within prefrontal cortex and dorsal striatum, J. Neurosci. 27 (2007) 10196–10209.
[15] M. Käenmäki, A. Tammimäki, T. Myöhänen, et al., Quantitative role of COMT in dopamine clearance in the prefrontal cortex of freely moving mice, J. Neurochem. 114 (2010) 1745–1755.
[16] J. Chen, B.K. Lipska, N. Halim, et al., Functional analysis of genetic variation in catechol-O-methyltransferase (COMT): effects on mRNA, protein, and enzyme activity in postmortem human brain, Am. J. Hum. Genet. 75 (2004) 807–821.
[17] M.F. Egan, T.E. Goldberg, B.S. Kolachana, et al., Effect of *COMT* Val108/158 Met genotype on frontal lobe function and risk for schizophrenia, Proc. Natl. Acad. Sci. U.S.A. 98 (2001) 6917–6922.
[18] E.M. Tunbridge, P.J. Harrison, D.R. Weinberger, Catechol-o-methyltransferase, cognition, and psychosis: Val158Met and beyond, Biol. Psychiatry 60 (2006) 141–151.
[19] R.M. Bilder, J. Volavka, H.M. Lachman, A.A. Grace, The catechol-O-methyltransferase polymorphism: relations to the tonic-phasic dopamine hypothesis and neuropsychiatric phenotypes, Neuropsychopharmacology 29 (2004) 1943–1961.
[20] R. Joober, J. Gauthier, S. Lal, D. Bloom, et al., Catechol-O-methyltransferase Val-108/158-Met gene variants associated with performance on the Wisconsin Card Sorting Test, Arch. Gen. Psychiatry 59 (2002) 662–663.
[21] J.H. Barnett, P.B. Jones, T.W. Robbins, U. Müller, Effects of the catechol-O-methyltransferase Val158Met polymorphism on executive function: a meta-analysis of the Wisconsin Card Sort Test in schizophrenia and healthy controls, Mol. Psychiatry 12 (2007) 502–509.
[22] N. Smyrnis, D. Avramopoulos, I. Evdokimidis, et al., Effect of schizotypy on cognitive performance and its tuning by *COMT* val158 met genotype variations in a large population of young men, Biol. Psychiatry 61 (2007) 845–853.
[23] L. Diatchenko, G.D. Slade, A.G. Nackley, et al., Genetic basis for individual variations in pain perception and the development of a chronic pain condition, Hum. Mol. Genet. 14 (2005) 135–143.
[24] A.G. Nackley, S.A. Shabalina, I.E. Tchivileva, et al., Human catechol-O-methyltransferase haplotypes modulate protein expression by altering mRNA secondary structure, Science 314 (2006) 1930–1933.
[25] J.H. Barnett, J. Heron, D. Goldman, et al., Effects of catechol-O-methyltransferase on normal variation in the cognitive function of children, Am. J. Psychiatry 166 (2009) 909–916.
[26] K. Kamide, Y. Kokubo, J. Yang, et al., Association of genetic polymorphisms of *ACADSB* and *COMT* with human hypertension, J. Hypertens. 25 (2007) 103–110.
[27] G.A. Thorisson, A.V. Smith, L. Krishnan, L.D. Stein, The international HapMap project web site, Genome Res. 15 (2005) 1592–1593.
[28] K.J. Lievers, G.H. Boers, P. Verhoef, et al., A second common variant in the methylenetetrahydrofolate reductase (MTHFR) gene and its relationship to MTHFR enzyme activity, homocysteine, and cardiovascular disease risk, J. Mol. Med. (Berl.) 79 (2001) 522–528.
[29] P.T. Männistö, S. Kaakkola, Catechol-O-methyltransferase (COMT): biochemistry, molecular biology, pharmacology, and clinical efficacy of the new selective COMT inhibitors, Pharmacol. Rev. 51 (1999) 593–628.
[30] C. Sugden, One-carbon metabolism in psychiatric illness, Nutr. Res. Rev. 19 (2006) 117–136.
[31] J.L. Roffman, R.L. Gollub, V.D. Calhoun, et al., MTHFR 677C–>T genotype disrupts prefrontal function in schizophrenia through an interaction with COMT 158Val –>Met, Proc. Natl. Acad. Sci. U.S.A. 105 (2008) 17573–17578.
[32] N.C. Htun, K. Miyaki, Y. Song, et al., Association of the catechol-O-methyl transferase gene Val158Met polymorphism with blood pressure and prevalence of hypertension: interaction with dietary energy intake, Am. J. Hypertens. 24 (2011) 1022–1026.
[33] S. Yamazaki, S. Fukuhara, J. Green, Usefulness of five-item and three-item Mental Health Inventories to screen for depressive symptoms in the general population of Japan, Health Qual Life Outcomes 3 (2005) 48.
[34] J. Kristjánsdóttir, G.I. Olsson, C. Sundelin, T. Naessen, Could SF-36 be used as a screening instrument for depression in a Swedish youth population?, Scand J. Caring Sci. 25 (2011) 262–268.
[35] J.C. Barrett, B. Fry, J. Maller, M.J. Daly, Haploview: analysis and visualization of LD and haplotype maps, Bioinformatics 21 (2005) 263–265.
[36] F. Faul, E. Erdfelder, A.G. Lang, A. Buchner, G*Power3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences, Behav. Res. Methods 39 (2007) 175–191.
[37] N. Mukherjee, K.K. Kidd, A.J. Pakstis, et al., The complex global pattern of genetic variation and linkage disequilibrium at catechol-O-methyltransferase, Mol. Psychiatry 15 (2010) 216–225.
[38] O. Peerbooms, B.P. Rutten, D. Collip, et al., Evidence that interactive effects of *COMT* and *MTHFR* moderate psychotic response to environmental stress, Acta Psychiatr. Scand. 125 (2012) 247–256.