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Regular Article

Association analysis of the catechol-O-methyltransferase /methylenetetrahydrofolate reductase genes and cognition in late-onset depression

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Aims: Increasing evidence suggests that the catechol-O-methyltransferase (COMT) gene might be associated with cognition in patients with mental disorders and healthy people. The metabolic pathways of COMT and methylenetetrahydrofolate reductase (MTHFR) are closely interconnected. In this study, we aimed to examine whether the COMT-MTHFR genotype interacted with cognitive function in late-onset depression (LOD) patients and COMT Val/Val homozygous individuals who also carried the MTHFR T allele and had poor neuropsychological test performance.

Methods: Ninety-seven unrelated LOD patients who met DSM-IV criteria for major depressive disorder were recruited for the study and 103 normal controls were recruited from the local community. All of these patients and 44 normal controls completed a series of neuropsychological tests. Patients and normal controls were genotyped for COMT (rs4680) and MTHFR (rs1801133) variants using polymerase chain reaction-restriction fragment length polymorphism.

Results: There were no significant differences in the frequencies of the single alleles and genotypes of two polymorphisms between LOD patients and normal controls. No main effects of COMT or MTHFR genotype on any neuropsychological test performance were observed. There was a significant interactive effect of COMT Val158Met and MTHFR C677T polymorphisms on the Symbol Digit Modalities Test independent of diagnosis ($P < 0.05$). After controlling for covariates, the subjects with COMT Met/ Met and MTHFR C/C genotype had better Symbol Digit Modalities Test performance.

Conclusions: The results suggest no major effect of COMT or MTHFR on cognitive function alone. However, an interaction of COMT Val158Met and MTHFR C677T polymorphisms may be associated with cognitive function. Further studies in a large sample are needed to replicate the genetic role in the LOD patients.

Key words: cognitive function, catechol-O-methyltransferase, late-onset, major depressive disorder, methylenetetrahydrofolate reductase.

LATE-ONSET DEPRESSION (LOD) is one of the most common psychiatric disorders for elderly adults and one major cause of death and disability.[1] The prevalence of depression in the elderly has been estimated as 6.5–9%.[2] Some studies suggest that apart from more severe cognitive impairments in the acute phase of illness, LOD patients have also more residual cognitive deficits after the remission of depressive

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symptoms than younger depressed patients.[3,4] Furthermore, LOD patients with cognitive impairment have increased risk of conversion to dementia, and it might be a frequent prodrome for the development to dementia.[5] So it is very significant to make the cause of cognitive dysfunction clear in LOD.

Animal studies and human neuroimaging studies suggest that dopamine plays a major role in the regulation of cognitive function in the prefrontal cortex. Catechol-O-methyltransferase (COMT) is an important enzyme involved in the degradation of released dopamine. The functional polymorphism Val108/158Met of the COMT gene (rs4680) has been indicated to influence enzyme activity, and the Val allele results in approximately three- to fourfold higher activity than the Met allele.[6] Different enzyme activity affects dopamine concentrations, which result in the alteration of cognition. For example, some studies showed the Val/Val genotype carriers had greater percentage of preservative errors on the Wisconsin Card Sorting Test, which reflected executive function in schizophrenia disorders, as well as the healthy individuals.[7–9] Other studies found that the Met allele was associated with better executive function and memory tasks.[9,10] However, Strauss *et al*. failed to implicate COMT in memory performance,[11] and another recent study also did not find that COMT Val108/158Met polymorphism was associated with cognition in older depressed populations.[12] These inconsistent results might be because COMT activity is influenced by other genetic variants.

A common single nucleotide polymorphism in a coding region of the methylenetetrahydrofolate reductase (MTHFR) gene, C677T (rs1801133), has been shown to associate with enzyme activity, namely, the T allele causes a 35% reduction in MTHFR activity.[13] Several studies reported that the MTHFR C677T variant was relevant to both risk of depression and cognitive abilities.[14–16] COMT function, such as its transcription and its degradation of dopamine via transmethylation, is mostly responsible for folate metabolism, which is regulated by the activity of MTHFR. For this reason, genetic variation in MTHFR could affect COMT function, and accordingly affect cognition by regulating dopamine levels. Further, a recent study indicated that an interaction between COMT Val108/158Met and MTHFR C677T polymorphisms was associated with executive function in schizophrenia patients, and suggested that the MTHFR T allele may exacerbate the low-dopamine state of patients who also carry the COMT Val allele by diminishing promoter methylation, increasing COMT expression and further reducing dopamine signaling.[17] Even though the metabolic pathways of COMT and MTHFR are interconnected, little is known about the association between the polymorphisms and cognitive function in LOD patients. In this study, we aimed to explore whether there is an interaction between these polymorphisms and cognition, and if the subjects with COMT Val/Val and MTHFR T/T genotype show poorer psychocognitive performance.

METHODS

Subjects

A total of 97 unrelated acute depressive inpatients were recruited from the Affiliated Brain Hospital of Nanjing Medical University from December 2008 to August 2010. They were diagnosed according to the diagnostic criteria for major depressive disorder (MDD) (DSM-IV Axis I)[18] using a Structured Clinical Interview for DSM-IV by two experienced psychiatrists. The age of first episode for all patients was 55 years or older (33 men and 64 women, mean age 67.36 ± 5.94). In addition, all patients had scores of ≥17 on the 17-item Hamilton Depression Rating Scale (HDRS),[19] and Mini-Mental State Examination (MMSE)[20] scores were >24. Exclusion criteria included other major psychiatric disorders, neurodegenerative illness, severe physical illnesses and other medical illness impairing cognitive function.

The 103 normal controls (30 men and 73 women; mean age [66.78 ± 6.94]), who were free of a history of any psychiatric illness and major physical illness, were enrolled from the general community. All controls had HDRS scores <7 and MMSE scores >24.

Neuropsychological assessment

All patients experienced a series of neuropsychological tests via standardized administration by two senior psychiatrists, who had received uniform neuropsychological tests training. Cognitive assessments were completed once for 45 min in a quiet consulting room. Also, this assessment occurred before all patients started antidepressant treatment. The patients had to be free of taking any antidepressants and/or mood stabilizers at least 2 weeks before the initiation of the therapy. The neuropsychological battery comprises the MMSE, the Rey Auditory Verbal Learning Test (RAVLT),[21] the Symbol Digit Modalities

Test (SDMT),[22] Verbal Fluency Test (animal category),[23] Verbal Fluency Test (verb category),[23] Digit Span Test (DST),[24] and the Trail-making Test A and B (TMT).[25]

The MMSE is widely used to test overall cognitive function. The test includes 19 items that assess orientation to place and time, learning and memory, construction ability, attention, and calculation skills. The total scores of the MMSE are associated with education years. The possible range of scores is 0–30 and the subjects who have undergone greater than 6 years' education score more than 24, which represents no cognitive impairment.

The RAVLT measures short-term and long-term verbal memory. The examiner reads a list of 12 words three times, and the subject is requested to repeat these in a loose order. After the examiner reads the words each time, the subject is requested to quickly recall the words without any suggestion. After 5 min and 20 min, the subject is again asked to repeat the words, respectively. Scores are calculated from the sum of all correct recallable words.

The SDMT is a measure of complex attention and concentration requiring the subject to correlate symbols with numbers and quickly generate and write the number when shown the symbol. Scores are calculated from the sum of all correct numbers during 90 s.

The Verbal Fluency Test (VFT) is used to measure semantic verbal fluency, reflecting executive cognition. The subject is required to say as many animal words or verbs representing an act as possible beginning from the same semantic category in 60 s. Scores are calculated from the sum of all acceptable words.

DST tests short-term memory, working memory and attention from the Wechsler Adult Intelligence Scale. During the forward DST task, subjects are asked to remember a series of digits and repeat them back in the same order, and they are asked to repeat the digits in reverse order during the backward DST task.

TMT A assesses psychomotor slowness. In the task, the subject must connect encircled numbers in ascending order as quickly as possible. TMT B measures cognitive set shifting and requires the alternation between numbers and letters in ascending order. Scores are calculated from the sum of spent time (s).

Genotyping

Genomic DNA was obtained from 250-uL EDTA-anticoagulated venous blood using a DNA extract kit (Tiangen, Beijing, China) according to the manufacturer's recommendations. Allele status for COMT (rs4680) and MTHFR (rs1801133) was determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Forward and reverse primers' sequences were as follows: F: 5′-TCG TGG ACG CCG TGA TTC AGG-3′, R: 5′-ACA ACG GGT CAG GCA TGC A-3′(rs4680), and F: 5′-AGG AGA AGG TGT CTG CGGGAG-3, R: 5′-CCC TCA CCT GGA TGG GAA AGAT-3′(rs1801133).

PCR were performed in a 25-μL volume containing 1 μL of 100 ng/μL DNA, 12.5 μL 2 × Taq Master Mix (Bioedify Nanjing), 2 μL primer and 9.5 μL distilled water. DNA was amplified at the thermal cycling, which includes an initial denaturation of 5 min at 95°C followed by 32 cycles of denaturating at 95°C (30 s), annealing at 56.5°C (40 s) and extension at 72°C (50 s), with a final extension at 72°C for 5 min. Then, the 164 bp PCR product of SNP (rs4680) was digested with NlaIII (New England Biolabs, Beverly, MA, USA) at 37°C for 4 h and run in 8% polyacrylamide gel at 120 V for 2 h generating different restriction patterns for the Val/Val genotype (114 bp + 81 bp + 22 bp) and the Met/Met genotype (96 bp + 81 bp + 22 bp + 18 bp). The PCR product of SNP (rs1801133) was cut with TAQI (New England Biolabs) at 37°C overnight and run in 2% agarose gels at 100 V for 45 min, yielding different digested products for the CC genotype (198 bp) and the TT genotype (175 bp + 23 bp). Genotypes were identified from a gel imaging system by at least two researchers. Additionally, ambiguous or unidentifiable results would be reamplified and samples that continued to amplify poorly were excluded from the study.

Statistical analysis

Statistical analysis was conducted with SPSS for Windows 13.0 (Chicago, IL, USA). Baseline information between cases and controls was compared with the *t*-test for continuous data and the χ^2-test for categorical data. Correlations between the HDRS and the scores of neuropsychological tests in LOD patients were analyzed by Spearman's correlation. The Hardy–Weinberg equilibrium test, linkage disequilibrium statistics, allele and genotype frequencies were calculated by using the SHEsis program (Bio-X Life Science Research Center of Shanghai JiaoTong University, Shanghai, China).[26] The MTHFR variable was dichotomized, with the T carrier as one group and CC as the

other group. Similarly, the COMT variable was divided into Val/Val homozygotes and Met66 carriers.

General linear models were fit to test for the effect that the subjects' status (patients or controls) and MTHFR genotype and COMT genotype had on the cognitive tasks as outcome variables. The predictor variables for the models with cognitive tasks as outcomes included the main effects of depressive diagnosis, MTHFR genotype, COMT genotype, a depression diagnosis by the MTHFR gene interactive effect, a depression diagnosis by MTHFR gene interactive effect and the COMT gene by MTHFR gene interactive effect. The models were fit, including age, education, and sex as covariates and each cognitive measure as the dependent variable. The criterion for the statistical significance was set at $P < 0.05$ for all the tests.

Ethics

The study was approved by the medical ethics committee. All subjects signed a written informed consent after the programme had been carefully explained.

RESULTS

The demographic, allele and genotype frequency of SNP of the study subjects are shown in Table 1. There were no significant differences in sex, age and years of education between LOD patients and control subjects between the groups. Correlation analysis indicated that the total scores of HDRS were negatively associated with forward DST score in LOD patients ($r = -0.211$, $P = 0.038$). There were no significant differences in genotype and allele frequency in MTHFR or in COMT genotype between the patients and the controls.

After controlling for age, education and sex, there were significant differences in scores for HDRS, MMSE, RAVLT delayed recall, SDMT, Digit Span Test, and TMT A between LOD patients and controls (all $P < 0.01$) (Table 2).

Table 3 and Table 4 display statistical analyses testing for differences in cognition based on genotype and diagnoses. In general linear models, the relation between genotype and neurocognitive task were tested among all participants after adjusting age, sex, and education. Neither main effects of COMT genotype nor interactive effects of between COMT genotype and depression diagnosis were found to be significantly related to any neurocognitive task. In addition, we did not find a main effect of MTHFR genotype or MTHFR genotype by diagnosis interaction on each cognitive test performance in models.

However, a significant interaction was found between COMT-MTHFR genotype and SDMT performance ($F = 10.542$, $P = 0.001$, $\eta^2 = 0.076$), which

Table 1. Demographics, HDRS scores, allele and genotype frequencies of SNP between case and control samples

	Case group ($n = 97$)	Control group ($n = 103$)	t or χ^2	P
Mean age in years	67.36 ± 5.94	66.78 ± 6.94	0.627	0.531
Sex (male)	33 (34.0%)	30 (29.1%)	0.555	0.456
School education in years	9.59 ± 3.08	10.28 ± 4.18	−1.341	0.182
HDRS	31.43 ± 4.35	2.39 ± 3.52	42.064	0.000
Allele frequency of rs4680				
Val (*n*, %)	103 (53.1%)	123 (59.7%)	1.779	0.182
Met (*n*, %)	91 (46.9%)	83 (40.3%)		
Genotype frequency of rs4680				
Val/Val (*n*, %)	21 (21.6%)	35 (34.0%)	3.768	0.052
Met carrier (*n*, %)	76 (78.4%)	68 (66.0%)		
Allele frequency of rs1801133				
C (*n*, %)	105 (54.1%)	121 (58.7%)	0.865	0.352
T (*n*, %)	89 (45.9%)	85 (41.3%)		
Genotype frequency of rs1801133				
C/C (*n*, %)	19 (19.6%)	28 (27.2%)	1.604	0.205
T carrier (*n*, %)	78 (80.4%)	75 (79.6%)		

COMT, catechol-O-methyltransferase; HDRS, Hamilton Depression Rating Scale; MTHFR, methylenetetrahydrofolate reductase; SNP, single nucleotide polymorphisms.

Table 2. Neuropsychological data of the groups

	Case group ($n = 97$)	Control group ($n = 44$)	F	*P*
Mean age in years	67.36 ± 5.94	65.09 ± 7.48	1.775	0.08
Sex (male)	33 (34.0%)	21 (47.7%)	2.407	0.121
School education in years	9.59 ± 3.08	13.41 ± 3.10	−6.798	0.000
MMSE	27.27 ± 2.46	29.50 ± 0.90	7.695	0.006
RAVLT delayed recall	4.91 ± 1.76	6.95 ± 1.75	17.82	0.000
SDMT	17.46 ± 6.26	40.09 ± 10.32	153.89	0.000
Verbal Fluency Test (animal)	13.41 ± 3.90	20.68 ± 4.15	43.68	0.000
Verbal Fluency Test (verb)	10.29 ± 3.66	19.00 ± 5.33	66.083	0.000
Digit Span forward	6.97 ± 1.53	8.61 ± 1.33	14.266	0.000
Digit Span backward	3.91 ± 0.90	5.20 ± 1.11	17.826	0.000
Trail-making Test A (s)	118.70 ± 48.75	71.68 ± 21.91	9.581	0.002
Trail-making Test B (s)	206.48 ± 84.47	141.94 ± 42.65	2.397	0.124

Age, sex and education were included as covariates; MMSE, Mini-Mental State Exam; RAVLT, Rey Auditory Verbal Learning Test; SDMT, Symbol Digit Modalities Test.

contributed to 7.6% of the variance in SDMT score adjusted by age, education, sex and depression diagnosis. To further examine this interaction, we compared adjusted means across groups, and individuals with Val/Met genotype who also carried MTHFR TT genotype exhibited significantly higher SDMT scores (Table 5).

DISCUSSION

Major depressive disorder is a clinically heterogeneous disorder resulting from an interaction of multiple genes with environmental factors.[27,28] The evidence from family and twin studies shows that genetic factors play a significant role in the pathogenesis of MDD.[29] In this study, we examined two SNP (rs4680 in COMT gene and rs1801133 in MTHFR gene) in LOD patients, which have been previously found to confer susceptibility to MDD.[15,30] Our results did not support the role of the Val158Met polymorphism or MTHFR in the risk for LOD. Similar to our study, Potter *et al*. and Hong *et al*. did not find significant association between COMT Val158Met or MTHFR C677T polymorphism and

Table 3. Main effect of COMT, depression diagnosis and interactive effect of COMT genotype and depression diagnosis

	Case group		Control group		Main effect of COMT		Main effect of diagnosis		Effect of diagnosis × COMT	
	Val/Val ($n = 21$)	Met carrier ($n = 76$)	Val/Val ($n = 16$)	Met carrier ($n = 28$)	F	*P*	F	*P*	F	*P*
MMSE	27.81 ± 2.34	27.13 ± 2.48	29.63 ± 0.72	29.43 ± 0.99	1.021	0.314	4.653	0.033	0.644	0.424
RAVLT delayed recall	5.00 ± 1.70	4.88 ± 1.79	6.81 ± 1.42	7.03 ± 1.93	0.101	0.751	13.583	0.000	0.469	0.495
SDMT	15.86 ± 5.72	17.91 ± 6.37	38.31 ± 10.12	41.11 ± 10.48	3.671	0.057	139.887	0.000	0.300	0.585
Verbal Flunce Test (animal)	14.00 ± 4.17	13.25 ± 3.85	20.19 ± 4.73	20.96 ± 3.84	0.020	0.889	32.297	0.000	1.581	0.211
Verbal Flunce Test (verb)	9.95 ± 4.25	10.38 ± 3.51	19.13 ± 5.68	18.93 ± 5.22	0.052	0.820	58.281	0.000	0.065	0.799
Digit Span forward	6.95 ± 1.40	6.97 ± 1.57	9.13 ± 1.20	8.32 ± 1.34	1.709	0.193	14.848	0.000	1.689	0.196
Digit Span backward	4.00 ± 1.00	3.88 ± 0.88	5.38 ± 1.31	5.11 ± 0.99	0.891	0.347	14.849	0.000	0.023	0.880
Trail Making Test A (seconds)	133.37 ± 66.29	114.64 ± 42.36	69.63 ± 22.49	72.86 ± 21.89	1.405	0.238	12.458	0.001	1.414	0.237
Trail Making Test B (seconds)	225.67 ± 109.70	201.17 ± 76.11	138.35 ± 35.36	143.99 ± 46.80	0.866	0.354	3.541	0.062	0.745	0.390

Age, sex and education were included as covariates; COMT, catechol-O-methyltransferase; MMSE, Mini-Mental State Exam; MTHFR, methylenetetrahydrofolate reductase; RAVLT, Rey Auditory Verbal Learning Test; SDMT, Symbol Digit Modalities Test.

Table 4. Main Effect of MTHFR, depression diagnosis and interactive effect of MTHFR genotype and depression diagnosis

	Case group		Control group		Main effect of MTHFR		Main effect of diagnosis		Effect of diagnosis × MTHFR	
	C/C ($n = 19$)	T carrier ($n = 78$)	C/C ($n = 13$)	T carrier ($n = 31$)	F	*P*	F	*P*	F	*P*
MMSE	27.21 ± 2.53	27.28 ± 2.45	29.39 ± 1.19	29.55 ± 0.77	0.089	0.766	6.244	0.014	0.001	0.972
RAVLT delayed recall	5.00 ± 1.86	4.88 ± 1.75	6.31 ± 1.65	7.23 ± 1.75	1.338	0.249	10.619	0.001	2.299	0.132
SDMT	18.21 ± 7.78	17.28 ± 5.88	39.58 ± 10.85	40.31 ± 9.22	0.388	0.535	131.402	0.000	0.196	0.659
Verbal Flunce Test (animal)	14.42 ± 4.83	13.17 ± 3.64	19.62 ± 4.48	21.13 ± 3.98	0.090	0.764	27.692	0.000	2.906	0.091
Verbal Flunce Test (verb)	11.11 ± 4.64	10.09 ± 3.39	18.46 ± 4.84	19.23 ± 5.58	0.010	0.919	47.325	0.000	0.937	0.335
Digit Span forward	7.26 ± 1.73	6.90 ± 1.48	8.69 ± 1.38	8.58 ± 1.34	0.390	0.533	10.137	0.002	0.122	0.728
Digit Span backward	3.95 ± 1.08	3.89 ± 0.86	5.15 ± 0.90	5.23 ± 1.20	0.095	0.759	13.773	0.000	0.055	0.816
Trail Making Test A (seconds)	123.77 ± 65.83	117.48 ± 44.08	76.68 ± 19.47	69.59 ± 22.83	0.767	0.383	8.339	0.005	0.001	0.976
Trail Making Test B (seconds)	219.38 ± 119.19	203.33 ± 74.32	138.71 ± 42.16	143.29 ± 43.47	0.298	0.586	3.320	0.075	0.639	0.426

Age, sex and education were included as covariates; COMT, catechol-O-methyltransferase; MMSE, Mini-Mental State Exam; MTHFR, methylenetetrahydrofolate reductase; RAVLT, Rey Auditory Verbal Learning Test; SDMT, Symbol Digit Modalities Test.

geriatric depression, respectively.[12,31] The reason for this discrepancy may be due to the differences of sample size and ethnic factor.

In line with our previous studies of cognitive function in LOD patients, we found that LOD patients performed significantly worse than controls in neurocognition tests except TMT-B.[32] Several studies also suggested that some cognitive deficits were related to late-life depression, consisting of impairment in executive function, attention, episodic memory, working memory and information processing, which was consistent with our results.[33,34] Therefore, cognitive deficits may be a common symptom in LOD patients. In addition, our data also showed that HDRS score was negatively associated with score of forward DST, which is concerned with short-term attention and memory capability.[35] Belanoff *et al.* also found verbal learning and memory performance

Table 5. Interactive effect of MTHFR and COMT genotype and neuropsychological test performance

	COMT genotype				Effect of COMT × MTHFR	
	Val/Val		Met carrier			
MTHFR genotype	C/C ($n = 11$)	T carrier ($n = 26$)	C/C ($n = 21$)	T Carrier ($n = 83$)	F	*P*
MMSE	28.36 ± 2.50	28.69 ± 1.83	27.95 ± 2.29	27.69 ± 2.50	0.475	0.492
RAVLT delayed recall	5.36 ± 1.91	5.96 ± 1.77	5.62 ± 1.88	5.42 ± 2.11	1.064	0.304
SDMT	22.64 ± 13.11	26.81 ± 14.02	30.19 ± 14.31	22.63 ± 12.08	10.542	0.001
Verbal Fluency Test (animal)	16.18 ± 3.92	16.88 ± 5.91	16.71 ± 5.98	14.98 ± 4.89	1.696	0.195
Verbal Fluency Test (verb)	13.18 ± 6.23	14.23 ± 6.96	14.57 ± 5.85	12.20 ± 5.38	2.501	0.116
Digit Span forward	7.73 ± 1.27	7.96 ± 1.87	7.90 ± 1.94	7.19 ± 1.51	1.953	0.165
Digit Span backward	4.64 ± 1.29	4.58 ± 1.36	4.33 ± 1.11	4.18 ± 1.05	0.015	0.903
Trail-making Test A (s)	113.85 ± 84.13	102.41 ± 49.22	99.79 ± 37.03	104.31 ± 43.54	0.828	0.364
Trail-making Test B (s)	204.05 ± 135.80	181.08 ± 74.88	177.48 ± 82.99	187.88 ± 71.65	1.260	0.264

Age, sex, depression diagnosis and education were included as covariates. COMT, catechol-O-methyltransferase; MMSE, Mini-Mental State Exam; MTHFR, methylenetetrahydrofolate reductase; RAVLT, Rey Auditory Verbal Learning Test; SDMT, Symbol Digit Modalities Test.

related to depressive severity.[36] A recent meta-analysis showed that psychomotor speed and memory functioning were associated with illness severity, whereas attention and executive functioning were more likely trait markers in first-episode MDD.[37] The above results suggested that depressive severity might affect some cognitive domains but not all cognitive domains.

To our knowledge, the effect of genetic factors on the development of cognition is well established.[38] In the present study, we did not observe a major role for COMT and MTHFR genotype alone in any cognitive performance. After controlling for age, sex and education, the depressive diagnosis accounted for the most variance of neuropsychological test performance. But it is worth noting that a significant association between COMT-MTHFR genotype interaction and SDMT performance was found, regardless of the diagnosis of depression. Adjusted by age, sex and education, the interaction with SDMT still persists, and can explain 7.6% of the variance in the scores of SDMT. Further, after comparing adjusted means across groups we found that the COMT Met carriers with MTHFR C/C genotype performed better than other genotype carriers in SDMT, which reflects associative recognition, recall, working memory and selective attention functions.[39] Again, evidence for association of COMT Val158Met polymorphism and cognition has remained controversial. Some studies pointed out that the Met allele of COMT contributes to better cognitive function in schizophrenia patients as well as in normal controls,[6,8] whereas results given by other researchers suggested that they are not significantly correlated with cognitive in elderly depressed patients.[12] Our findings may in part account for the reason for the relation of COMT polymorphism, which was supposed to contain a mixture of MTHFR C/C and T carriers.

Our studies indicated a significant interaction between COMT and MTHFR genotype, and the COMT Met carriers with MTHFR C/C performed better than other genotype carriers in SDMT performance. Similarly, Roffman *et al*. observed that an interaction between COMT and MTHFR polymorphisms was related to executive function in schizophrenia patients, wherein COMT Val/Val individuals who also carried MTHFR T allele showed a significantly higher percentage of perseverative errors than other genotype carriers.[17] We speculated that the MTHFR C/C genotype was related to much higher enzymic activity that would lead to hypermethylation of the COMT promoter region, decreased COMT expression and increased dopamine concentrations.[17] Meanwhile, the MTHFR C allele would further magnify the effect of COMT Val158Met polymorphism on dopamine catabolism.[40] In addition, a recent study found no major role for COMT and MTHFR genotype alone and a significant interaction of COMT and MTHFR genotype for putamen volume in depressed and nondepressed subjects.[41] Tunbridge *et al*. reported that the high activity of Val allele of COMT interacting with the low activity T allele of MTHFR could increase plasma total homocysteine levels, which have been linked with stroke and Alzheimer's disease.[42] Their report was partly consistent with our results. The above findings show that gene–gene interactions may be an independent influential factor of cognition abilities in adults.

This is an exploratory study, and the findings should be interpreted cautiously, bearing in mind the following limitations. First, there was a relatively small sample size in our study compared to other gene–gene interaction studies. Second, the relations among MTHFR genotype, COMT genotype and cognition in older adults were very complicated. This needs further study integrating structural and functional imaging to ascertain the causative mechanism that COMT and MTHFR genetic variations affect individual differences in cognition, and to validate whether the effect of MTHFR genotype depends on methylation of COMT promoter region. Third, although we assess cognition with several tests that are thought sensitive to cognitive impairment in depression, other neuropsychological tests in cognition, including the Wisconsin Card Sorting Test and N-back, which are positively associated with COMT Val158Met polymorphism, have not been used for our study.[8,9] Also, the effects of COMTVal158Met or MTHFR C677T on cognition may not be sensitive to all cognitive tasks. Finally, besides MTHFR C677T, additional genetic variants, like the dopamine transporter polymorphism, have also been found to have a significant interactive effect with COMT Val108/158Met on working memory.[43] Thereby, with regard to the study examining the association of gene and cognition, other genes should be considered in gene–gene interaction models.

Conclusion

The present result suggests depression may contribute significantly to a difference in cognition between

LOD patients and controls, but an interaction of COMT and MTHFR genotype itself may also be an independent influential factor in cognition in older adults. These preliminary findings should be replicated in large prospective clinical trials in future work.

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