Genetic variation in folate metabolism is not associated with cognitive functioning or mood in healthy adults

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The present study examined the associations between genetic variation in folate metabolism on the one hand and cognitive functioning and mood on the other in healthy individuals. Two independent population-based samples were used, including 777 participants, aged 24-82 years, from the Maastricht Aging Study (MAAS); and 818 participants, aged 50-70 years, from the Folic Acid and Carotid Intima-Media Thickness (FACIT) study. Thymidylate synthase (TS) 2R→3R and serine hydroxymethyltransferase (SHMT1) 1420C→T polymorphisms were determined in both populations. In addition, the 5,10-methylenetetrahydrofolate reductase (MTHFR) 677C→T polymorphism was determined in the MAAS population. Cognitive performance was assessed in both populations using a neuropsychological test battery. In the MAAS population only, cognitive performance was retested after 12 years of follow-up (n = 612), and mood was measured at baseline (n = 772) and 12-year follow-up (n = 565) by means of the depression subscale of the Symptom Checklist 90. We found that in both study populations, cognitive performance was not associated with TS 2R→3R or SHMT1 1420C→T polymorphisms at baseline, after correction for age, sex, and level of education. The MTHFR 677C→T polymorphism was not associated with cognitive performance in the MAAS population. None of the polymorphisms in the MAAS population were related to mood at baseline or over 12 years. In conclusion, our findings do not support the involvement of genetic variation in folate metabolism in cognitive performance or mood in healthy individuals.

**Keywords:** folate metabolism; MTHFR; TS; SHMT1; cognitive performance; depressed mood
1. Introduction

Due to an increasing life expectancy, Western societies will be facing a steadily rising proportion of older individuals in the next decades. As impairments in cognitive functioning and mood state are highly prevalent in the ageing population, it is of growing interest to identify the underlying biological mechanisms.

Among the biological factors suggested to be involved in cognitive impairment and depressed mood is genetic variation in folate metabolism (Bjelland et al., 2003; Elkins et al., 2007). Genetically determined changes in the activity of enzymes that are involved in the folate-homocysteine cycle may contribute to disturbances in neurocognitive functioning by reducing the availability of methyl donors, such as S-adenosylmethionine, for methylation processes that play an essential role in neurotransmitter and phospholipid metabolism, stabilization of myelin, and regulation of gene expression (Fuso et al., 2005; Tchantchou et al., 2006). For example, the common 677C→T mutation of the gene encoding 5,10-methylenetetrahydrofolate reductase (MTHFR)\(^a\), which is associated with reduced enzyme activity, may lead to a decreased methylation capacity (Castro et al., 2004) (Figure 1). This polymorphism has been implicated in age-related cognitive decline and depressed mood, although results from population-based studies have yielded conflicting results. Whereas a number of studies have not found any relationship between the \textit{MTHFR} 677C→T polymorphism and cognitive performance (Almeida et

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\(^a\) \textit{Abbreviations}: APOE, apolipoprotein E; CST, Concept Shifting Test; FACIT, Folic Acid and Carotid Intima-Media Thickness study; LDST, Letter-Digit Substitution Test; MAAS, Maastricht Aging Study; \textit{MTHFR}, 5,10-methylenetetrahydrofolate reductase; SCLdep, depression subscale of the Symptom Checklist 90; \textit{SHMT1}, serine hydroxymethyltransferase; Str, speed on subtask of the Stroop Color-Word Interference Test; \textit{TS}, thymidylate synthase; WLT, Visual Verbal Word Learning Task; WLTdr, delayed recall score of the Visual Verbal Word Learning Task; WLTmax, maximum score of the Visual Verbal Word Learning Task; WLTtot, total score on the Visual Verbal Word Learning Task.
al., 2005; Bathum et al., 2007; De Lau et al., 2010; Gussekloo et al., 1999; Visscher et al., 2003) or depressive symptoms (Almeida et al., 2005; Gaysina et al., 2008). Several other studies have reported that the MTHFR 677TT genotype was predictive of decreased cognitive functioning (Elkins et al., 2007), greater age-related cognitive decline (Elkins et al., 2007), clinically diagnosed depression (Hickie et al., 2001; Kelly et al., 2004), or depressive symptoms as observed in the general population (Bjelland et al., 2003).

In contrast to the above-mentioned studies, we previously found that individuals carrying the MTHFR 677TT genotype showed better cognitive performance than CC homozygotes or CT heterozygotes in a large sample of older adults (Durga et al., 2006). We speculated that these positive effects might be related to a shift in the distribution of folate derivatives from methylated folates to formylated folates (Bagley and Selhub, 1998), which favors DNA synthesis and repair (Skibola et al., 2002) (Figure 1).

Evidence for a clinical effect of the MTHFR 677C→T polymorphism has been provided by epidemiological studies reporting a decreased risk of colon cancer in TT homozygotes (Férrandez-Peralta et al., 2010; Taioli et al., 2009), which has been suggested to be related to increased DNA synthesis and repair (Blount et al., 1997). Interestingly, as both increased DNA damage and deficient DNA repair processes have been implicated in cognitive disorders, such as Mild Cognitive Impairment and Alzheimer’s disease (Coppedè and Migliore, 2009), it is reasonable to hypothesize that the MTHFR 677TT genotype might also exert a protective effect on cognitive functioning.

DNA synthesis and fidelity may also be influenced by mutations in the gene encoding the enzyme thymidylate synthase (TS), which plays a critical role in
preventing chromosomal damage by lowering the incorporation of the abnormal nucleotide uracil in DNA (Horie et al., 1995). The presence of a triple versus double tandem repeat sequence in the regulatory region of the TS gene enhances its expression, thereby increasing DNA synthesis and repair (Horie et al., 1995). In addition, the 1420C→T polymorphism of the serine hydroxymethyltransferase (SHMT1) gene may result in a lower availability of folate derivatives for DNA synthesis (Hishida et al., 2003). Similar to the MTHFR 677TT genotype, both TS 3R and SHMT1 1420T variants have been associated with a reduced cancer risk (Hishida et al., 2003; Skibola et al., 2002).

On the functional level, the MTHFR 677C→T, TS 2R→3R, and SHMT1 1420C→T polymorphisms may be hypothesized to affect cognitive performance and mood state by influencing the fate of methyl donors. However, the associations between the TS and SHMT1 polymorphisms and cognitive functioning or mood have not yet been investigated. Therefore, the aim of the present study was to examine the associations between the MTHFR 677C→T, TS 2R→3R, and SHMT1 1420C→T polymorphisms on the one hand and cognitive functioning and mood on the other in healthy individuals.
2. Methods

2.1. Participants

The present study was carried out using data from two studies, the population-based Maastricht Aging Study (MAAS) and the Folic Acid and Carotid Intima-Media Thickness (FACIT) study. MAAS is a longitudinal research program investigating the determinants and consequences of cognitive aging (Jolles et al., 1995). Participants were randomly drawn from a register of family practices in the south of the Netherlands. Medically verified exclusion criteria at baseline were chronic neurological pathology, psychiatric disorders, mental retardation, and psychotropic drug use. The study population consisted of 1,823 participants, aged 24-81 years at baseline, and comprised four demographically identical panels, each stratified for age, sex, and level of occupational achievement. A detailed description of the MAAS study design can be found elsewhere (Jolles et al., 1995; Van Boxtel et al., 1998).

Between 1993 and 1995 (baseline), all participants completed a general health and lifestyle questionnaire and underwent an extensive medical and neuropsychological examination. Participation in the blood sampling procedure was voluntary; venous blood samples for genotyping were collected for 779 individuals. Twelve years after the baseline assessments, the participants were invited to take part in the follow-up examinations. During follow-up, 167 participants had dropped out due to various reasons, including death, illness, and refusal to participate. Demographical, neuropsychological, and genetic information was available for 777 individuals at baseline and 612 individuals at 12-year follow-up.

The FACIT study is a randomized, double-blind, placebo-controlled trial, originally designed to investigate the effects of 3-year folic acid supplementation on the risk of cardiovascular disease (Durga et al., 2007). The study population consisted
of 818 men and women from the Gelderland region in the Netherlands. Participants were recruited from blood bank registries as well as from municipal registries. At screening, individuals were included if between the ages of 50-70 years, and, specifically for women, had reached the menopause at least 2 years before. Exclusion criteria were plasma total homocysteine concentrations <13 µmol/L or >26 µmol/L, use of B-vitamin supplements or drugs that could affect atherosclerotic progression (e.g. lipid-lowering or hormone replacement therapies), or self-reported intestinal disease. Individuals with elevated homocysteine concentrations due to factors other than suboptimal folate concentrations, including serum vitamin B12 concentrations <200 pmol/L, self-reported medical diagnosis of renal or thyroid disorders, or self-reported use of medications that influence folate metabolism, were also excluded. Venous blood samples were collected for genotyping at baseline. Cognitive functioning was assessed at baseline and after 3 years by means of a neuropsychological test battery.

The MAAS study was approved by the Medical Ethics Committee of the Maastricht University Medical Centre, and the FACIT study by the Medical Ethics Committee of Wageningen University. Prior to enrolment all participants signed informed consent.

2.2. Cognitive functioning
Cognitive functioning was assessed by means of a neuropsychological test battery, consisting of five cognitive tests. The Visual Verbal Word Learning Task (WLT) was used to assess learning capacity, as well as recall and retrieval from long-term memory (Van der Elst et al., 2005). In three trials, fifteen commonly used monosyllabic words were visually presented in a fixed order at 2-s intervals.
Maximum and total immediate recall (WLTmax and WLTtot, respectively), as well as delayed recall after 20 min (WLTdr), were recorded.

The Stroop Color-Word Interference Test was used to test selective attention and interference susceptibility (Van der Elst et al., 2006d). Three subtasks were presented on separate test sheets containing four rows of ten columns of color names or colored patches. Participants were required to read aloud color names printed in black (subtask I), name the color of colored patches (subtask II), and name the ink color of color names printed in an incongruous color (subtask III). The outcome parameters were speed on subtask I (Str1) and subtask III (Str3).

The Concept Shifting Test (CST) is a test of behavioral planning and cognitive flexibility (Van der Elst et al., 2006b). Participants were asked to cross out sixteen items presented in small circles on a test sheet as fast as possible in the right order (1–2–3–4 [subtask A], A–B–C–D [subtask B], 1–A–2–B [subtask C]). In a final subtask (subtask O), individuals were instructed to cross out empty circles as fast as possible, in order to measure general motor speed. The outcome parameters were speed on subtask A (CSTa), subtask B (CSTb), subtask C (CSTc), and subtask O (CST0).

The Letter-Digit Substitution Test (LDST) was used to measure information processing speed and efficiency of operations in working memory (Van der Elst et al., 2006c). Participants were asked to replace letters presented on a test sheet by their corresponding digits, as indicated by a key showing nine numbers paired with different letters. The total number of correct substitutions completed within 90 s was recorded.

The Verbal Fluency Test measures the ability to recollect clusters of related words from encyclopedic memory (Van der Elst et al., 2006a). Participants were instructed to name as many animals as possible in 60 s. The outcome measure was the
total number of different animals named.

2.2.1. Data reduction

In order to limit the number of dependent variables and to improve the robustness of the underlying cognitive construct, the raw test scores of the WLT, the Stroop Color-Word Interference Test, and the CST were clustered into three a-priori defined composite performance indices (Lezak et al., 2004). The raw test scores were transformed into Z-scores by subtracting the mean score from the individual test score and dividing this by the mean SD \(Z = (x - \text{mean})/\text{SD}\). For the cross-sectional analyses, the means and SD’s of the baseline test scores were used to calculate the Z-scores per test. For the longitudinal analyses in the MAAS population, the grand means and SD’s of the pooled measurements (i.e. baseline and 12-year follow-up) were used to calculate the Z-scores for both time points, thereby creating test scores referring to the same Z-distribution, which enabled the inclusion of both baseline and follow-up scores in one statistical model. The Z-scores were averaged, resulting in the following composite scores: memory \([(Z_{\text{WLTmax}} + Z_{\text{WLTtot}} + Z_{\text{WLTdr}})/3]\), sensorimotor speed \([(Z_{\text{Str1}} + Z_{\text{CSTa}} + Z_{\text{CSTb}} + Z_{\text{CST0}})/4]\), and complex speed \([(Z_{\text{Str3}} + Z_{\text{CSTc}})/2]\). The signs of the two speed scores were inverted in order to reflect above normal performance when positive, and below normal when negative. Cognitive performance on the domains of information processing speed and word fluency was represented by the Z-score of the LDST test and the Z-score of the Verbal Fluency Test, respectively.

2.3. Mood

In the MAAS population, mood was assessed at baseline and after 12 years of follow-
up by means of the Dutch version of the depression subscale of the Symptom Checklist 90 (SCLdep), a multidimensional checklist for psychopathological complaints based on self-report (Arrindell and Ettema, 1986). The SCLdep consists of sixteen items, which are rated on a 5-point ordinal scale ranging from 1 (no complaints) to 5 (maximal complaints). Sum scores range from 16 to 80, with higher scores representing increased depressed mood. In total, 772 individuals completed the SCLdep at baseline, as well as 565 individuals at 12-year follow-up.

2.4. Genotyping
Genomic DNA was isolated from EDTA blood samples using an MN blood kit (Bioké, Leiden, The Netherlands). MTHFR 677C→T genotype was determined by polymerase chain reaction (PCR) with restriction fragment length polymorphism analysis with HinfI (Frosst et al., 1995). TS genotype was determined by PCR, followed by gel electrophoresis to show the presence of double (2R2R) or triple (3R3R) tandem repeats in the promoter region (Horie et al., 1995). SHMT1 1420C→T genotype was determined using fluorogenic probes in real-time PCR assay (Skibola et al., 2002). Apolipoprotein E (APOE) genotype was determined by PCR followed by restriction digestion with HhaI (Bekers et al., 2002).

2.5. Blood measurements
In the FACIT population, fasting venous blood samples were collected at baseline, directly processed, and stored at -80°C. Serum folate was measured using a chemiluminescent immunoassay (Immulite 2000, Diagnostic Products Corporation, Los Angeles, CA). Erythrocyte folate was determined in duplicate and the average was taken to reduce measurement error. Erythrocyte folate concentrations were
calculated by means of the following formula: (unadjusted erythrocyte folate/hematocrit) – ([1 – hematocrit]/hematocrit) × serum folate. Plasma total homocysteine was determined by high-performance liquid chromatography and fluorimetric detection, as described previously (Ubbink et al., 1991).

2.6. Education

Level of education was measured by classifying formal schooling according to the Dutch educational system (De Bie, 1987), and categorized into low, middle, or high, i.e. corresponding to primary education, junior vocational training, and senior vocational/academic training, respectively.

2.7. Statistical analysis

Normal probability plots were used to check whether the dependent variables were normally distributed. SCLdep scores showed a skewed distribution, which was corrected by log-transformation. Baseline data were used to assess the cross-sectional associations between the three genotypes and cognitive functioning or mood in both study populations. It should be noted that the cross-sectional relationship between MTHFR genotype and cognitive performance was not analyzed in the FACIT population, as these associations had been reported elsewhere (Durga et al., 2006).

Univariate ANCOVA were performed for each polymorphism in relation to each of the five cognitive performance indices, as well as mood. The analyses were corrected for age, sex, and level of education in order to reduce residual variance in the outcome measures, thereby increasing statistical power. Longitudinal analyses were restricted to the MAAS population, as the follow-up of the FACIT study involved 3-year supplementation with folic acid, which may influence both the
phenotypic expression of the polymorphisms studied (Girelli et al., 1998) and
cognitive performance (Durga et al., 2007). Power calculations showed that including
only the placebo group (n = 413) in the longitudinal analyses would not yield
sufficient statistical power. Repeated measures ANCOVA with time as a within-
subjects variable and genotype as between-subjects variable were used to investigate
the longitudinal associations between each of the three genotypes and cognitive
performance over 12 years of follow-up, which were represented by the time ×
genotype interaction. The analyses were adjusted for age, age$^2$ (to control for non-
linear effects of age), sex, and level of education. Similar analyses were performed
with mood as the dependent variable.

In secondary analyses, we stratified both study populations by $APOE$ E4
carrier status (defined as E4+ or E4−, depending on the presence of at least one E4
allele) to determine whether the cross-sectional and longitudinal associations between
the genotypes and cognitive functioning differed between carriers and non-carriers of
the $APOE$ E4 allele, as $APOE$ E4 carrier status may interact with a low folate status or
high homocysteine levels in increasing individual susceptibility for cognitive
impairment (Shea et al., 2004). In addition, the FACIT sample was stratified by folate
status (low-normal: erythrocyte folate <501 nmol/L, and high-normal: erythrocyte
folate ≥501 nmol/L) (Durga et al., 2006), as folate concentrations may influence
phenotypic expression of the genotypes studied (Girelli et al., 1998). The MAAS
sample was not stratified by folate status, as information on folate concentrations was
not available.

Homogeneity of error variances was ascertained by means of Levene’s test for
equality of error variances. Hardy-Weinberg equilibrium was assessed using Chi-
square tests. The statistical power of the cross-sectional and longitudinal analyses to
detect small effects ($f^2 = 0.02$) of each of the three polymorphisms on cognitive performance and mood in both populations was high, i.e. >0.90. Statistical differences were considered significant at $p$-values <0.05. All analyses were performed using SPSS 16.0 (SPSS Inc., Chicago, IL).
3. Results

Table 1 summarizes the baseline characteristics of the participants in both study populations. The \textit{MTHFR}, \textit{TS}, and \textit{SHMT1} allele frequencies were in Hardy-Weinberg equilibrium and were comparable to the frequencies reported in other healthy populations (Elkins et al., 2007; Skibola et al., 2002; Visscher et al., 2003).

At baseline, none of the genetic polymorphisms were associated with cognitive performance on any of the domains measured in the MAAS population (Table 2) or the FACIT population (Table 3). In addition, the different genotypes did not show any relationship with mood in the MAAS population (Table 2).

One sample \textit{t} tests indicated that cognitive performance in the MAAS population significantly declined over the 12-year follow-up period on the domains of sensorimotor speed (mean change ± SD = -0.29 ± 0.64, \( p < 0.001 \)), complex speed (mean change ± SD = -0.31 ± 0.67, \( p < 0.001 \)), information processing speed (mean change ± SD = -0.14 ± 0.55, \( p < 0.001 \)), and word fluency (mean change ± SD = -0.21 ± 0.87, \( p < 0.001 \)). Memory performance significantly improved (mean change ± SD = 0.32 ± 0.75, \( p < 0.001 \)), which is due to the effect of procedural learning. Depressive symptoms significantly increased over the 12-year follow-up period, as indicated by higher SCLdep scores (mean change ± SD = 1.42 ± 6.78, \( p < 0.001 \)). In the longitudinal analyses, we found no significant associations between the \textit{MTHFR} \(677C\rightarrow T\), \textit{TS} \(2R\rightarrow 3R\), and \textit{SHMT1} \(1420C\rightarrow T\) polymorphisms on the one hand and cognitive functioning and mood over 12 years on the other, which was revealed by the absence of significant time \(\times\) genotype interactions in repeated measures ANCOVA (Table 4).

To examine whether folate status might influence the associations between the \textit{TS} or \textit{SHMT1} polymorphisms and cognitive performance, the FACIT population was
stratified by erythrocyte folate concentration. The stratification procedure did not reveal any significant results (data not shown), suggesting that folate status did not modify the putative associations between the two genotypes and cognitive performance.

Both study populations were stratified by APOE E4 allele carrier status to determine whether possession of the APOE E4 allele influenced the associations between the three genotypes and cognitive performance. The results of the cross-sectional and longitudinal analyses in the MAAS sample did not differ between carriers and non-carriers of the APOE E4 allele (data not shown), indicating that this variable was no effect modifier in this study population. In the FACIT sample, however, the SHMT1 1420TT genotype was associated with decreased memory performance at baseline (mean ± SD = -0.33 ± 1.03) as compared with CC homozygotes (mean ± SD = 0.04 ± 0.90) and CT heterozygotes (mean ± SD = -0.04 ± 0.96) in non-carriers (p = 0.015), but not in carriers of the APOE E4 allele (p = 0.675).
4. Discussion

The present study showed that the *MTHFR* 677C→T, *TS* 2R→3R, and *SHMT1* 1420C→T polymorphisms were not related to cognitive performance or mood in two large samples of healthy individuals. The present findings are in line with other cross-sectional studies reporting the lack of any significant associations between *MTHFR* genotype and cognitive performance in older individuals (Almeida et al., 2005; Bathum et al., 2007; De Lau et al., 2010; Gussekloo et al., 1999; Visscher et al., 2003), and with a population-based study in 1,581 Danish nonagenarians showing no impact of the *MTHFR* 677TT genotype on cognitive decline over 5 years (Bathum et al., 2007).

In contrast to the present findings, Elkins et al. (2007) found that the *MTHFR* 677TT genotype was associated with decreased information processing speed, as well as greater annual cognitive decline as indicated by Mini-Mental State Examination score, in a US population-based sample of 6,653 older women. However, their longitudinal results should be interpreted with caution, as the Mini-Mental State Examination is a relatively crude indicator of cognitive functioning. Originally designed to screen for symptoms of cognitive impairment (Folstein et al., 1975), this test is fairly insensitive to individual variation in cognitive performance in healthy aging individuals.

The present results on mood are in line with a number of studies that found no significant relationship between the *MTHFR* 677C→T polymorphism and depressive symptoms (Almeida et al., 2005; Gaysina et al., 2008). In contrast, the *MTHFR* 677TT genotype was reported to be related to an increased risk of depression in a large population-based study of 5,948 older Norwegian individuals (Bjelland et al., 2003), a case-control study of 78 older Australian patients with late-onset depression.
and 22 healthy controls (Hickie et al., 2001), and a case-control study carried out in Northern Ireland, including 100 patients with major depressive disorder matched with 89 controls (Kelly et al., 2004). An important methodological difference between the present study and most of the above-mentioned studies is that we did not use a dichotomous measure of depressive symptoms. Instead, we measured depressive symptoms on a continuous scale, which not only increased statistical power, but also allowed for the detection of subclinical depressive symptoms, which are highly prevalent in the general population (Kessler et al., 1997). Although the studies comparing depressed persons with non-depressed individuals suggest that the MTHFR 677C→T polymorphism might increase the risk of depression, our results indicate that this polymorphism might not be related to subclinical depressive symptoms in the general population. Similar to our study, Almeida et al. (2005), who also used a continuous measurement of depressive symptoms, did not find any significant associations between MHTFR genotype and mood in a study sample of 240 community-dwelling women.

We previously found that the MTHFR 677TT genotype was associated with increased sensorimotor speed in the FACIT population (Durga et al., 2006). In the present study, we failed to replicate these findings in the MAAS population. These conflicting results could be attributable to differences in the age range of the two study populations. It might also be argued that the unequal sex distribution of the FACIT population might have contributed to the observed discrepancy in results. In addition, these contrasting findings might be related to the fact that, in contrast to the MAAS population, the FACIT population is not representative of the general population, as it included only individuals with plasma total homocysteine concentrations between 13 and 26 µmol/L. Thus, it cannot be ruled out that this
selection criterion might have biased the results obtained in the FACIT population. It should be noted, however, that it is unlikely that the lack of significant associations was due to insufficient statistical power, as our study had sufficient power to detect small associations in both study samples.

In our previous study, we showed that the MTHFR 677C→T polymorphism was associated with increased complex speed in individuals with erythrocyte folate concentrations below the median (Durga et al., 2006). As folate status may influence phenotypic expression of the polymorphisms studied (Girelli et al., 1998; Religa et al., 2003), it cannot be ruled out that the lack of stratification by folate concentrations in the MAAS population might have obscured any potential associations between the MTHFR 677C→T polymorphism and cognitive performance. With respect to the TS 2R→3R and SHMT1 1420C→T polymorphisms, however, it is not very likely that the null findings in the MAAS population may be accounted for by the lack of stratification by folate status, as the stratified analyses in the FACIT population suggest that folate status is not an effect modifier of the putative associations between these polymorphisms and cognitive performance.

The lack of significant associations in the present study might be due to the possibility that more than one genetic polymorphism might need to be present in order to significantly influence cognitive performance. However, there were too few individuals homozygous for either all three mutations (n = 3), or two out of three mutations studied (n = 56), to yield sufficient statistical power for detecting potential associations within these subsamples.

To investigate the possibility that an additional risk factor for cognitive impairment, such as APOE E4 carrier status (Caselli et al., 2009; Deary et al., 2002), must be present for cognitive effects of any of the genetic polymorphisms to become
apparent, we stratified our analyses by \textit{APOE} E4 allele carrier status. In the FACIT sample, we found that the \textit{SHMT1} 1420TT genotype was associated with decreased memory performance in non-carriers of the \textit{APOE} E4 allele. In the MAAS population, however, results did not differ between carriers and non-carriers of the \textit{APOE} E4 allele. Although the present findings did not unequivocally support the hypothesis that \textit{APOE} E4 carrier status may interact with \textit{MTHFR} 677C→T, \textit{TS} 2R→3R, or \textit{SHMT1} 1420C→T polymorphisms to influence cognitive functioning, they did suggest that the associations between the \textit{SHMT1} 1420TT genotype and memory performance might differ between carriers and non-carriers of the \textit{APOE} E4 allele. The biological mechanisms underlying such an interaction between the \textit{SHMT1} 1420C→T polymorphism and \textit{APOE} E4 carrier status remain to be identified.

Our study had several strengths, such as the use of two large study populations, the measurement of three different genotypes related to folate metabolism, and the inclusion of 12-year longitudinal data on cognitive performance and mood. To our knowledge, our study was the first to explore the \textit{TS} 2R→3R and \textit{SHMT1} 1420C→T polymorphisms in relation to cognitive functioning and mood.

Summarizing, the present study does not support the involvement of the \textit{MTHFR} 677C→T, \textit{TS} 2R→3R and \textit{SHMT1} 1420C→T polymorphisms, which are known to affect enzyme activity in folate metabolism, on the one hand and cognitive performance, age-related cognitive decline, or mood on the other in healthy individuals.

5. Conclusion

In the present study, we did not find evidence for the involvement of the \textit{MTHFR} 677C→T, \textit{TS} 2R→3R, or \textit{SHMT1} 1420C→T polymorphisms in cognitive functioning
or mood state in healthy adults. Our findings do not support the hypothesis that genetically determined variation in the activity of enzymes that may influence methylation capacity and DNA fidelity might play a role in depressed mood or age-related cognitive decline.
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