MTHFR polymorphisms and cognitive ageing in the ninth decade: the Lothian Bith Cohort 1921

MTHFR polymorphisms and cognitive ageing in the ninth decade: the Lothian Birth Cohort 1921

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ABSTRACT

Low blood levels of B vitamins have been implicated in age-associated cognitive impairment. The present study investigated the association between genetic variation in folate metabolism and age-related cognitive decline in the ninth decade of life. Both the 677C>T (rs1801133) polymorphism and the scarcely-studied 1298A>C (rs1801131) polymorphism of the *MTHFR* gene were assessed in relation to cognitive change over 8 years in older community-dwelling individuals. *MTHFR* genotype was determined in 476 participants of the Lothian Birth Cohort 1921, whose intelligence was measured in childhood in the Scottish Mental Survey of 1932. Cognitive performance on the domains of verbal memory, abstract reasoning, and verbal fluency was assessed at mean age 79 (n = 476), and again at mean ages of 83 (n = 275) and 87 (n = 180). Using linear mixed models, the *MTHFR* 677C>T and 1298A>C variants were not associated with the rate of cognitive change between 79 and 87 years, neither in the total sample, nor in a subsample of individuals with erythrocyte folate levels below the median. *APOE* E4 allele carrier status did not interact with *MTHFR* genotype in affecting change in cognitive performance over 8 years. A joint analysis investigating the combined effect of both polymorphisms did not reveal any significant results. In conclusion, *MTHFR* 677C>T and 1298A>C polymorphisms were not associated with individual change in cognitive functioning in the ninth decade of life. Although polymorphisms in the *MTHFR* gene may cause disturbances in folate metabolism, they do not appear to be accompanied by changes in cognitive functioning in old age.

INTRODUCTION

Low blood levels of B vitamins, such as folate and vitamin B12, have been implicated in age-associated cognitive impairment (Clarke *et al*., 2007, Kado *et al*., 2005). A low folate status may result from poor dietary intake, or from genetic disturbances in folate metabolism. For example, the common 677C>T (rs1801133) and 1298A>C (rs1801131) polymorphisms of the 5,10-methylenetetrahydrofolate reductase (*MTHFR*) gene may mimic dietary folate deficiency by reducing folate metabolism (Frosst *et al*., 1995, Weisberg *et al*., 1998). The non-synonymous *MTHFR* 677C>T and 1298A>C polymorphisms are associated with decreased MTHFR activity (Frosst *et al*., 1995, Weisberg *et al*., 1998), resulting in reduced availability of 5-methyltetrahydrofolate for the conversion of homocysteine into methionine. Both polymorphisms have been found to increase homocysteine levels, although the effects of the 1298A>C polymorphism are somewhat less pronounced than those associated with the 677C>T polymorphism (Friedman *et al*., 1999).

In addition, by reducing the availability of methyl donors, *MTHFR* 677C>T and 1298A>C polymorphisms may impair DNA methylation processes (Castro *et al*., 2004, Friso *et al*., 2002), which play a key role in regulating gene expression. As both DNA hypomethylation and elevated homocysteine levels have been related to deficits in cognitive functioning (Levenson & Sweatt, 2005, Mattson & Shea, 2003, Zhao *et al*., 2003), we hypothesized that the *MTHFR* 677C>T and 1298A>C polymorphisms might confer increased susceptibility to cognitive impairment and age-related cognitive decline.

To date, a number of studies have examined the associations between the *MTHFR* 677C>T polymorphism and cognitive functioning in older individuals (Almeida *et al*., 2005, Bathum *et al*., 2007, Durga *et al*., 2006, Elkins *et al*., 2007,

Gussekloo *et al*., 1999, Visscher *et al*., 2003), albeit with mixed results. The possible relationship between the 1298A>C polymorphism and cognitive performance, however, has scarcely been investigated (De Lau *et al*., 2008).

It should be noted that most of the previous studies were cross-sectional. Few studies have addressed the relationship between *MTHFR* genotype and longitudinal change in cognitive functioning in the ageing population (Bathum *et al*., 2007, Elkins *et al*., 2007). Whereas the *MTHFR* 677TT genotype was associated with greater annual cognitive decline in a large population-based sample of older women (Elkins *et al*., 2007), no such associations were found in community-dwelling nonagenarians whose cognitive performance was assessed over 5 years (Bathum *et al*., 2007).

In some of the above-mentioned studies, possible associations between *MTHFR* genotype and cognitive performance might have been obscured by not taking into account individual variation in folate status, because phenotypic expression of the *MTHFR* 677TT genotype is most pronounced when folate status is low (Girelli *et al*., 1998).

In addition, few of the earlier studies controlled for prior cognitive ability, which may confound the relationship between *MTHFR* polymorphisms and age-related cognitive change, because childhood mental ability accounts for about half of the variance associated with cognitive ability in adulthood (Deary *et al.*, 2004).

Furthermore, *APOE* E4 carrier status has been suggested to interact with a low B vitamin status to increase individual vulnerability to cognitive impairment (Bunce *et al.*, 2004, Shea *et al.*, 2004). However, the putative interactions between *MTHFR* genotype and *APOE* E4 carrier status have rarely been studied.

The objective of the present study was, therefore, to examine the longitudinal associations between the *MTHFR* 677C>T and 1298A>C polymorphisms and key

domains of cognitive functioning in the healthy ageing population, while taking into account folate status, prior cognitive ability, and *APOE* E4 allele carrier status. We assessed cognitive performance three times between mean ages of 79 and 87 in a sample of older individuals whose mental ability was measured in childhood: the Lothian Birth Cohort 1921 (Deary *et al.*, 2004). By using a cohort with very little age variation, any problems associated with heterogeneity of *MTHFR*'s effect on cognitive performance at different ages were excluded.

MATERIALS AND METHODS

Study population

The study population was the Lothian Birth Cohort 1921. This cohort consisted of 550 surviving participants of the Scottish Mental Survey 1932, which tested cognitive ability in almost all children born in 1921 and attending school in Scotland in June 1932 (Scottish Council for Research in Education, 1933). Participants living in Edinburgh and the surrounding areas were recruited for medical and cognitive retesting via general practitioner's patient lists and advertisements in the media, as described in detail elsewhere (Deary *et al.*, 2009, Deary *et al.*, 2004).

All participants who had completed the initial reassessments at mean age 79 years (old age baseline), excluding those who had withdrawn or were known to have died, were invited to participate in the follow-up measurements at mean age 83. In addition, all participants who had completed the assessments at mean age 83, excluding those who had withdrawn or were known to have died, were invited to take part in the follow-up measurements at mean age 87. Of the 454 participants invited to take part at age 83, 335 agreed to participate, and 321 were tested. At mean age 87, 268 participants were invited, and 207 were tested (Gow *et al.*, in press). Reasons for not attending included withdrawal (n = 16 at age 83; n = 4 at age 87), inability or refusal to participate (n = 80 at age 83; n = 42 at age 87), having moved away (n = 13 at age 83; n = 4 at age 87), exclusion due to dementia or memory problems (n = 3 at age 87), and death (n = 10 at age 83; n = 8 at age 87) (Gow *et al.*, 2008, Starr *et al.*, 2010). Of the total sample of 550 individuals, 229 had died during the course of the study. At baseline, all participants lived independently in the community and most were in good general health.

Baseline data on cognitive functioning were lacking for two individuals. At baseline, five participants reported a history of dementia-related illness, and nine individuals scored < 24 on the Mini-Mental State Examination (MMSE) (Folstein *et al.*, 1975), which may suggest possible dementia. These individuals were excluded from statistical analysis, along with 27 participants who maintained contact with the study and developed symptoms of dementia during follow-up. *MTHFR* genotyping was successful for all but 31 of the 507 participants with valid cognitive data at baseline. The resulting study sample consisted of 476 individuals (192 men, 284 women) at baseline, 275 individuals (118 men, 157 women) at mean age 83, and 180 individuals (77 men, 103 women) at mean age 87.

The Multi-Centre Research Ethics Committee for Scotland and the Lothian Research Ethics Committee approved the study. All participants gave written, informed consent.

Cognitive tests

In the Scottish Mental Survey of 1932, a version of the Moray House Test No. 12 (MHT) was administered as a measure of general cognitive ability at age 11 years (Scottish Council for Research in Education, 1933). Although it is primarily focused on verbal reasoning, this test also assesses numerical, spatial, and general reasoning. The MHT, consisting of 71 numbered items, 75 items in total, is to be completed within 45 min, and has a maximum test score of 76. Raw MHT test scores were corrected for age in days at the time of testing and converted to IQ-type scores (mean = 100, SD = 15).

At each wave of testing in old age, a battery of cognitive tests was administered to assess the important cognitive domains of verbal fluency, verbal

memory, and abstract reasoning. The Verbal Fluency Test (Lezak, 1995) provides a measure of executive functioning. Participants are required to name as many words as possible beginning with the letter C in 1 min. This process is repeated for the letters F and L. The overall test score is the total number of correct words named, excluding proper names, numbers, and repeated words. Verbal declarative memory was assessed by means of the Logical Memory subtest from the Wechsler Memory Scale–Revised (Wechsler, 1987). Two short stories, each containing twenty-five memory items, are read aloud. Immediately after each story, the participants recall as much of the story as possible ('immediate recall'). After a delay of about 25 min, the participants are again asked to recall as much as they can ('delayed recall'). The total score is the sum of the two immediate and two delayed recall scores. Maximum possible overall test score is 100. Raven's Standard Progressive Matrices (Raven *et al.*, 1977) was used to measure abstract reasoning. The test consists of sixty items, each presenting a pattern that needs to be completed. Participants complete as many items as possible in 20 min. The outcome measure is the total number of correctly completed items.

Genotyping

MTHFR genotype was determined at as described previously (Houlihan *et al*., 2010). In short, for 542 of the 550 participants in the LBC1921, genomic DNA was isolated from whole blood by standard procedure at Medical Research Council Technology, Western General Hospital, Edinburgh. Sixteen samples failed quality control preceding the genotyping procedure. The remaining 526 samples were genotyped by the Wellcome Trust Clinical Research Facility (WTCRF) Genetics Core with the Illumina Human610-Quadv1 chip. These samples were then subjected to quality-control procedures. All individuals were checked for disagreement between

genetic and reported gender (n = 1). Relatedness between participants was investigated and, for any related pair of individuals, one was removed (n = 1). Samples with a call rate ≤ 0.95 (n = 5), and those showing evidence of non-Caucasian descent by multidimensional scaling, were also removed (n = 2). The *MTHFR* 677C>T and 1298A>C polymorphisms met the following conditions: call rate ≥ 0.98, minor allele frequency ≥ 0.01, and Hardy-Weinberg equilibrium test with $p \geq 0.001$. In total, 517 samples remained.

APOE genotype was determined by PCR amplification of a 227-bp fragment of the *APOE* gene containing two polymorphic sites that account for the three alleles, E2, E3, and E4 (Wenham *et al.*, 1991), followed by restriction digest with *Cfo*I and electrophoresis in 4% NuSieve gel.

Blood measurements

Venous blood samples were collected at baseline and at mean age 87. The blood samples were processed and stored at -80°C until analysed. Erythrocyte folate concentrations were measured by immunoassay (Advia Centaur Immuno Assay System, Siemens Healthcare Diagnostics, Deerfield, IL). Data on erythrocyte folate status were available for 399 individuals at baseline, and 128 individuals at mean age 87.

Lifestyle and health-related variables

Body mass index (BMI, kg/m^2), smoking status (current smoking, yes/no), and alcohol consumption (standard units per week) were recorded at baseline. Physical activity, recorded as the frequency (days/month) of sport or physical exercise lasting at least 20 min at a time, was assessed at baseline. As these lifestyle and health-related

variables may confound the associations between folate metabolism and cognitive performance (Feng *et al.*, 2006, Kado *et al.*, 2005, Koike *et al.*, 2008), they were included as covariates in the statistical models to reduce residual variation in the outcome measures.

Statistical analysis

Normality of data distributions was ascertained by means of normal Q-Q plots. The *MTHFR* genotypes were defined as rare variant (677TT or 1298CC) or common variant (677CC/677CT or 1298AA/1298AC). A recessive genetic model was used to test the associations between *MTHFR* genotype and cognitive performance, as previous studies have indicated that plasma homocysteine concentrations were significantly increased in persons with the *MTHFR* 677TT rare variant, but not in persons with the 677CC and 677CT common variants (Frosst *et al.*, 1995, Weisberg *et al.*, 1998).

The cross-sectional and longitudinal associations between the *MTHFR* polymorphisms and cognitive functioning were assessed by means of linear mixed models (Verbeke & Molenberghs, 2000). This analysis method takes into account the intraindividual correlation between repeated measurements and allows the inclusion of participants with incomplete data at follow-up. Changes over time within individuals, i.e. the longitudinal effect, are distinguished from differences among individuals at baseline, i.e. the cross-sectional effect. Akaike's Information Criterion indicated that a Toeplitz covariance structure best fitted the data. Separate models were fitted for the *MTHFR* 677C>T and 1298A>C genotypes in relation to each of the dependent cognitive variables, i.e. Verbal Fluency, Logical Memory, and Raven's Matrices. Time (measured in years since baseline, which is acceptable because there

is very little age variation among the participants within each wave) was included to estimate the change in cognitive performance between 79 and 87 years. The main effect of *MTHFR* genotype represents the cross-sectional association between *MTHFR* genotype and cognitive performance at baseline. The longitudinal effect of *MTHFR* genotype was estimated by the two-way interaction between time and *MTHFR* genotype, which represents the rate of change in cognitive performance over 8 years as a function of *MTHFR* genotype. The statistical models were adjusted for a number of covariates in three consecutive steps. In Model 1, the analyses were corrected for the demographic variables age (in days), age^2 (to test for non-linear effects of age), and sex (Model 1). Age was centered around the mean to reduce the correlation between the terms for age and age^2. In Model 2, the analyses were additionally adjusted for age 11 MHT IQ to reduce the confounding effect of prior cognitive ability on age-related change in cognitive performance. In Model 3, the lifestyle and health-related variables BMI, smoking status, alcohol consumption, and physical activity were entered (Model 3).

The analyses were repeated in a subsample of individuals with erythrocyte folate concentrations below the median, i.e. 314 μg/L (n = 203), as low folate concentrations may increase the phenotypic expression of the genotypes studied (Girelli *et al.*, 1998). In addition, the analyses were stratified by *APOE* E4 carrier status (defined as E4+ or E4–, depending on the presence or absence of at least one E4 allele) to investigate whether the cross-sectional and longitudinal associations between the *MTHFR* polymorphisms and cognitive functioning differed between carriers and non-carriers of the *APOE* E4 allele.

In secondary analyses, linear mixed models, corrected for demographic and health-related variables, were performed for both the *MTHFR* 677C>T and 1298A>C

polymorphisms to examine the possibility of a linear trend in cognitive performance associated with the presence of zero, one, or two variant alleles. In addition, as the *MTHFR* 677C>T and 1298A>C polymorphisms are in linkage disequilibrium (Stegmann *et al.*, 1999), linear mixed models, corrected for demographic and health-related variables, were performed to investigate the combined effect of these polymorphisms on cognitive performance. To this end, the combined *MTHFR* 677C>T/1298A>C genotypes were classified as 677CC/1298AA, 677CC/1298AC, 677CC/1298CC, 677CT/1298AA, 677CT/1298AC, or 677TT/1298AA.

Statistical differences were considered significant at p-values < 0.05. All analyses were performed using SPSS 16.0 (SPSS Inc., Chicago, IL).

RESULTS

Descriptives

Table 1 shows the baseline characteristics of the study sample. Chi-square tests indicated that the *MTHFR* 677C>T and 1298A>C genotypes were in Hardy-Weinberg equilibrium ($p = 0.797$ and $p = 0.982$, respectively). The allele frequencies were as follows: 677C = 0.65, 677T = 0.35, 1298A = 0.71, and 1298C = 0.29. These frequencies are comparable to those reported in other population-based samples (Elkins *et al.*, 2007, Weisberg *et al.*, 1998). Distribution of the combined 677C>T/1298A>C genotypes was as follows: 677CC/1298AA, $n = 67$ (14.1%); 677CC/1298AC, $n = 97$ (20.4%); 677CC/1298CC, $n = 38$ (8.0%); 677CT/1298AA, $n = 112$ (23.5%); 677CT/1298AC, $n = 99$ (20.8%); and 677TT/1298AA, $n = 63$ (13.2%). Individuals with the rare variant of one polymorphism (677TT or 1298CC) were always homozygous wildtype for the other polymorphism (1298AA or 677CC, respectively); none of the participants had more than two variant alleles. Erythrocyte folate concentrations did not differ significantly between individuals homozygous for the rare 677T or 1298C polymorphism and individuals carrying the common polymorphisms ($p = 0.079$ and $p = 0.440$, respectively). Independent samples *t* tests and Chi-square tests indicated that the covariates did not differ between individuals with the 677TT or 1298CC genotype and the other participants, except for age at baseline, which was higher in individuals with the 1298CC genotype as compared with the rest of the sample (mean ± SD = 79.3 ± 0.6 and 79.0 ± 0.6, respectively; $p = 0.004$). Participants homozygous for the 677C>T or 1298A>C rare variant did not differ from the other participants in terms of cognitive test performance at baseline or at follow-up (Tables 2 and 3).

Logistic regression analysis revealed that dropout was not associated with *MTHFR* 677C>T or 1298A>C genotype ($p = 0.544$ and $p = 0.570$, respectively). However, lower cognitive scores at baseline significantly predicted dropout at follow-up ($p < 0.01$ for each of the three cognitive measures). Chi-square tests indicated that participants who were lost to follow-up did not differ from the remaining participants in terms of demographic or health-related characteristics, except for smoking, which significantly increased the likelihood of dropping out ($p = 0.023$).

Associations between *MTHFR* polymorphisms and cognitive decline

Tables 4 and 5 show the results of the linear mixed model analyses. Cognitive performance on the domains of verbal fluency and abstract reasoning declined by 2.0% and 13.3%, respectively, between 79 and 87 years, while Logical Memory scores did not show a significant decline over the 8-year follow-up period. For verbal fluency, the effect of time was statistically significant in the models with *MTHFR* 677C>T genotype as the independent variable (Table 4), but not in the models with *MTHFR* 1298A>C genotype as the independent variable (Table 5). This discrepancy can be explained by the fact that part of the variance associated with time was accounted for by the interaction term for time × genotype; the main effect of time was similar in both models when no interaction term was included, i.e. parameter estimate (95% CI) = -0.20 (-0.38, -0.03), $p = 0.025$ in the fully adjusted models.

The *MTHFR* 677C>T and 1298A>C polymorphisms were not significantly associated with individual change in cognitive performance between 79 and 87 years on any of the domains measured. Stratifying the analyses by *APOE* E4 carrier status did not yield any significant results, indicating that *APOE* E4 allele did not modify the putative longitudinal associations between *MTHFR* genotype and cognitive

performance. Performing separate analyses for individuals with a low folate status did not reveal any associations between *MTHFR* polymorphisms and individual variation in the rate of cognitive change. Post-hoc analyses using linear mixed models with erythrocyte folate concentration as the independent variable revealed that folate status was not directly associated with individual change in cognitive performance between 79 and 87 years.

Linear mixed models investigating a linear trend in cognitive performance associated with the number of variant alleles did not reveal any significant results (data not shown), implying that the *MTHFR* 677C>T or 1298A>C polymorphisms did not show a dose-effect relationship with individual change in cognitive performance between mean ages of 79 and 87 years. When the combined effect of both polymorphisms was examined, no significant associations were identified (Table 6), indicating that the two polymorphisms did not interact in affecting age-related cognitive decline over 8 years.

DISCUSSION

We showed that the *MTHFR* 677C>T and 1298A>C polymorphisms were not associated with individual change in cognitive performance on the domains of verbal fluency, verbal memory, and abstract reasoning between 79 and 87 years in older community-dwelling individuals. The present results suggest that altered folate metabolism due to reduced MTHFR activity does not influence cognitive performance or age-related cognitive decline in the ninth decade in the healthy ageing population.

Our findings are in line with earlier studies reporting the lack of a relationship between *MTHFR* genotype and cognitive performance in older adults (Almeida *et al.*, 2005, Bathum *et al.*, 2007, De Lau *et al.*, 2008, Gussekloo *et al.*, 1999, Visscher *et al.*, 2003). However, Elkins *et al.* (2007) found that the *MTHFR* 677TT genotype was related to decreased information processing speed and executive functioning, as well as a small excess annual decline in global cognitive functioning in women aged 65 years or older. In contrast, Durga and colleagues (2006) reported a positive cross-sectional association between the 677TT genotype and sensorimotor speed in community-dwelling individuals aged 50 to 70 years.

An important difference between the two above-mentioned studies and our study is the age range of the study samples, as the participants in the studies by Elkins et al. and Durga et al. were considerably younger than those included in the present study. Interestingly, it has been found that the effect of the *MTHFR* 677C>T polymorphism on homocysteine levels decreases with advancing age (Husemoen *et al.*, 2003), implying that phenotypic expression of the 677TT genotype is weaker in older adults as compared with younger individuals. It might be hypothesized that the relationship between *MTHFR* genotype and cognitive performance may be more

prominent in younger populations, thereby leading to contrasting results when different age groups are compared. The interaction between *MTHFR* genotype and age seems to offer a reasonable explanation for the lack of a significant relationship between *MTHFR* polymorphisms and cognitive performance in the present study. In this respect, it is worth noting that most of the other studies reporting null findings on the relationship between *MTHFR* genotype and cognitive performance were also performed in study populations with a higher mean age than the samples used by Elkins *et al.* and Durga *et al.* (Almeida *et al.*, 2005, Bathum *et al.*, 2007, Gussekloo *et al.*, 1999).

Although phenotypic expression of the *MTHFR* 677TT genotype may decrease with advancing age, it may increase in the presence of a low folate status (Girelli *et al.*, 1998). Therefore, we performed secondary analyses to determine whether *MTHFR* genotype was associated with cognitive performance in individuals with erythrocyte folate concentrations below the median. However, no significant associations were identified, which might have been related to the small number of participants with erythrocyte folate levels indicative of folate deficiency, i.e. below 140 μg/L (n = 14).

The present findings might imply that the *MTHFR* 677C>T and 1298A>C polymorphisms do not influence homocysteine levels or DNA methylation capacity to such an extent that cognitive performance is affected in the ninth decade. On the other hand, it might also be argued that these polymorphisms may simultaneously exert detrimental as well as beneficial effects, thereby causing no detectable changes in cognitive performance. For example, the *MTHFR* 677C>T polymorphism may have a negative impact on cognitive functioning by elevating homocysteine concentrations (Frosst *et al*., 1995) and impairing DNA methylation (Castro *et al*., 2004, Friso *et al*.,

2002), but it might also exert positive effects by increasing the availability of 5,10-methylenetetrahydrofolate for DNA synthesis (Skibola *et al.*, 1999).

The present results did not offer support for the hypothesis that *APOE* E4 allele carrier status, which has been shown to increase the risk of cognitive impairment in older individuals (Caselli *et al.*, 2009, Deary *et al.*, 2002), might modify the putative associations between *MTHFR* genotype and cognitive performance. Our findings are in concordance with previous research, indicating that *APOE* E4 carrier status and disturbances in folate metabolism might represent two distinct routes to cognitive impairment (Gottfries *et al.*, 2001). In a similar vein, Religa and colleagues (2003) reported that the association between *APOE* E4 carrier status and Alzheimer's disease was unrelated to homocysteine levels, folate status, and *MTHFR* genotype. Furthermore, a case-control study by Brunelli *et al.* (2001) showed that *MTHFR* genotype was not associated with Alzheimer's disease and did not interact with *APOE* E4 allele carrier status in mediating Alzheimer's disease risk.

Previously, we found that folate status was positively correlated with cognitive performance in healthy ageing individuals (Duthie *et al*., 2002, Starr *et al*., 2005). However, we did not find any evidence for a cross-sectional relationship between the *MTHFR* 677C>T polymorphism on the one hand and cognitive performance in old age or lifetime cognitive change on the other (Visscher *et al*., 2003). The present study further extends these observations by showing that neither the 677C>T nor the 1298A>C polymorphism were related to individual variation in the rate of cognitive decline between 79 and 87 years.

A common limitation of longitudinal studies is selective dropout. Statistical testing indicated that dropout was not related to *MTHFR* genotype in the present study. This finding was further supported by the fact that the *MTHFR* allele

frequencies in our study were comparable to those reported for younger populations (Friedman *et al.*, 1999, Frosst *et al.*, 1995, Weisberg *et al.*, 1998). However, dropout was significantly associated with a lower level of cognitive performance at baseline. The use of a statistical method that allows for the inclusion of participants with missing data at follow-up enabled us to compensate, at least in part, for this selection bias.

Another potential limitation of longitudinal studies is the repeated administration of cognitive tests, which may introduce learning effects. Learning effects on cognitive tests might be considered a disadvantage, as they may obscure age-related cognitive decline. In contrast to verbal fluency and abstract reasoning, which showed a gradual decline over 8 years of follow-up, verbal memory performance did not show a significant decline between 79 and 87 years in our study. This may be due to the effects of procedural learning. However, it is unlikely that learning effects on the Logical Memory test might have contributed to the lack of significant results in the present study, as longitudinal variability in cognitive performance is generally well preserved, thereby allowing for the detection of individual differences in the rate of age-associated cognitive change.

The present study was characterized by substantial dropout (62%), due to the old age of the study participants. Although we used a the statistical method that allowed for the inclusion of individuals with missing data at follow-up, thereby preventing a large decrease in statistical power, we cannot rule out completely that our study might not have been able to detect very modest associations between *MTHFR* genotype and individual change in cognitive performance over 8 years.

The strengths of our study were its longitudinal design, the use of sensitive, domain-specific cognitive tests, the narrow chronological age range of the sample,

and the adjustment for a number of potential confounders. In addition, not only did we investigate the relationship between cognitive performance and the common *MTHFR* 677C>T polymorphism, we also assessed the rarely-studied *MTHFR* 1298A>C polymorphism. Furthermore, correcting the analyses for childhood intelligence enabled us to examine individual variation in the rate of cognitive change in old age, whilst eliminating the confounding influence of the stable trait of general mental ability (Deary *et al.*, 2004).

We conclude that the *MTHFR* 677C>T and 1298A>C polymorphisms are not associated with individual change in cognitive functioning in the ninth decade of the lifecourse. Although polymorphisms in the *MTHFR* gene may cause disturbances in folate metabolism, they do not appear to be accompanied by changes on the level of cognitive functioning in later life. Future studies are necessary to further investigate the extent to which genetic and environmental factors underlying individual differences in folate metabolism may be involved in age-related cognitive decline.

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TABLE 1 Participant characteristics at baseline

	Total sample
n	476
Age (years)	79.0 ± 0.6
Female	284 (59.7%)
Age 11 IQ[a]	100.9 ± 14.4
BMI (kg/m^2)	26.3 ± 4.1
Current smoker	32 (6.7%)
Alcohol consumption (standard units/week)[b]	1.0 (0.5; 7.0)
Physical activity (days/month)[b]	2.0 (0.0; 10.0)
Erythrocyte folate (μg/L)	344.2 ± 155.3
MTHFR 677C>T genotype (% CC / CT / TT)	42.4 / 44.3 / 13.2
MTHFR 1298A>C genotype (% AA / AC / CC)	50.8 / 41.2 / 8.0
APOE E4 carrier status	126 (26.5%)

Values represent *n* (%) or means ± SD. BMI, body mass index; *MTHFR*, 5,10-methylenetetrahydrofolate reductase; *APOE*, apolipoprotein E.

[a] Based on Moray House Test No. 12 score, corrected for age at the time of testing.

[b] Median value (interquartile range) is given because of skewed data distribution.