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One-carbon metabolism gene polymorphisms are associated with cognitive trajectory among African-American adults

May A. Beydoun [a,*], Salman M. Tajuddin [a], Danielle Shaked [a,b], Hind A. Beydoun [c], Michele K. Evans [a], Alan B. Zonderman [a]

[a] Laboratory of Epidemiology and Population Sciences, National Institute on Aging, NIA/NIH/IRP, Baltimore, MD, USA
[b] Department of Psychology, University of Maryland Baltimore County, Catonsville, MD, USA
[c] Department of Medicine, Johns Hopkins School of Medicine, Baltimore, MD, USA

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ABSTRACT

The sex-specific link between longitudinal annual rate of cognitive change (LARCC) and polymorphisms in one-carbon metabolism enzymatic genes remains unclear, particularly among African-American adults. We tested associations of 14 single nucleotide polymorphisms (SNPs) from *MTHFR*, *MTRR*, *MTR*, and *SHMT* genes and select *MTHFR* haplotypes and latent classes (SNPHAP/SNPLC) with LARCC. Up to 797 African-American participants in the Healthy Aging in Neighborhoods of Diversity across the Life Span study (age: 30–64 y, 52% women) had 1.6–1.7 (i.e., 1 or 2) repeated measures (follow-up time, mean = 4.69 y) on 9 cognitive test scores, reflecting verbal and visual memory, verbal fluency, psychomotor speed, attention, and executive function: California Verbal Learning Test–immediate recall (CVLT-List A), CVLT-DFR (delayed free recall), Benton Visual Retention Test (BVRT), Animal Fluency (AF), Digits Span Forward and Backward tests, and Trail Making Test parts A and B (Trails A and B). Multiple linear mixed-effects and multiple linear regression models were conducted. Overall, *MTHFR* SNPs rs4846051(A1317G, G>A) and rs1801131(A1298C, G>T) were associated with slower and faster declines on AF, respectively, whereas rs2066462(C1056T, A>G) was related to slower decline on Trails B (executive function). Among men, rs4846051(A1317G, G>A) was linked to faster decline on BVRT (visual memory), whereas rs2066462(C1056T, A>G) and rs9651118(C>T) were associated with slower decline on CVLT-List A and rs9651118(C>T) with faster decline on CVLT-DFR. Among women, a slower decline on the domain "verbal memory/fluency" was observed with rs1801133(C677T, A>G). *MTHFR*$_2$ *SNPHAP* [rs1801133(C677T, A>G)/rs1801131(A1298C, G>T): GG] was associated with slower decline on AF among women, whereas *MTHFR*$_3$ *SNPHAP*(AT) was linked with slower decline on CVLT-List A among men but faster decline on "verbal memory/fluency" among women. Similar patterns were observed for *MTHFR* SNPLCs. In sum, *MTHFR* gene variations can differentially impact longitudinal changes in multiple cognitive domains among African-American adults.

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1. Introduction

As the world's population ages, dementia from all causes is estimated to have a prevalence of 4.7% among older adults aged ≥60 year (Sosa-Ortiz et al., 2012) with 4.6–7.7 million cases added annually worldwide and region-specific incidence rates ranging between 3.5 and 10.5 per 1000 (Ferri et al., 2005; Prince et al., 2013; Sosa-Ortiz et al., 2012). Alzheimer's disease (AD) accounts for 60%–80% of all dementias (Sosa-Ortiz et al., 2012). Known to be a neurodegenerative disorder with multifactorial etiology, AD manifests itself with progressive episodic memory deterioration followed by impairment in several domains of cognition (Lindeboom and Weinstein, 2004). Two key hallmarks of AD are progressive β-amyloid deposition in the brain—"the amyloid cascade hypothesis"—(Hardy and Selkoe, 2002) and neurofibrillary tangles arising from hyperphosphorylated tau (Turner, 2003). AD is also the leading cause of disability in old age (Helmer et al., 2006) and the sixth leading cause of death in the United States (Alzheimer's Association, 2016). Around 5.4 million Americans currently live with AD, a continuously rising number expected to reach 13.8 million by 2050 (Alzheimer's Association, 2016). Although an effective treatment is yet to be discovered, important genetic risk factors were identified for late-onset AD, such as the APOE4 genotype (Bertram et al., 2007),

* Corresponding author at: NIH Biomedical Research Center, National Institute on Aging, IRP 251 Bayview Blvd. Suite 100 Room #:04B118, Baltimore, MD 21224, USA. Tel.: +1 4105588648; fax: +410 558 8236.
E-mail address: baydounm@mail.nih.gov (M.A. Beydoun).

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as well as mid-life modifiable risk factors including education, smoking, physical inactivity, depression, mid-life obesity, hypertension, type 2 diabetes, antioxidants (e.g., vitamin E), n-3 fatty acids, and homocysteine (Barnes and Yaffe, 2011; Beydoun et al., 2014).

In fact, elevated total homocysteine (tHcy) plasma concentration, a sulfur amino acid, is an established risk factor for cardiovascular and cerebrovascular disease (Refsum et al., 1998). The latter could mediate tHcy-dementia relationship in old age (Ford et al., 2012a; Haan et al., 2007; Kim et al., 2008; Ravaglia et al., 2005; Seshadri et al., 2002; Zylberstein et al., 2011). Specifically, tHcy was directly linked to greater white matter hyperintensities and faster brain atrophy (Bleich and Kornhuber, 2003; den Heijer et al., 2003; Dufouil et al., 2003; Sachdev et al., 2002; Scott et al., 2004).

Although the risk of homocysteinemia increases with age and diminished renal function, B-vitamin dietary intakes can influence that risk by converting tHcy into methionine and cysteine through one-carbon metabolism (OCM) cycles (Bottiglieri, 2005; Troesch et al., 2016). Thus, B-vitamin interventions can potentially reduce plasma tHcy, with evidence of an inverse relationship in plasma between tHcy and folate/B-12 (Selhub et al., 1993). Importantly, folate and vitamins B-6 and B-12 may impede cognitive decline and delay dementia onset (Duthie et al., 2002; Feng et al., 2006; Kado et al., 2005; Mooijaart et al., 2005; Ramos et al., 2005; Ravaglia et al., 2005; Tucker et al., 2005), with studies suggesting an antagonistic interaction between vitamin B-12 and tHcy in plasma with respect to age-related cognitive decline (Haan et al., 2007; Li et al., 2008; Vidal et al., 2008). Furthermore, in vitro studies show that tHcy has neurotoxic and excitotoxic properties (Kruman et al., 2000; Parsons et al., 1998), suggesting direct and adverse influences on brain function.

OCM enzymatic genetic polymorphisms play an equally important role as B-vitamins in determining tHcy plasma concentrations. Mutations in methylenetetrahydrofolate reductase (MTHFR), the most widely studied OCM enzyme, (e.g., *MTHFR* C677T, A1793G, and A1298C) are risk factors for hyperhomocysteinemia because of encoding of an enzyme with reduced activity (Rozen, 1997). Thus, MTHFR and its pathological sequelae are also risks for AD, with some studies indicating synergistic interaction between hyperhomocysteinemia or hypertension and "T" allele of C677T mutation in adverse cognitive outcomes (Bottiglieri et al., 2001; Cai et al., 2016; Deshmukh et al., 2009; Elkins et al., 2007; Ford et al., 2012b; Guenther et al., 1999; Kageyama et al., 2008; Polito et al., 2016; Rajagopalan et al., 2012; Religa et al., 2003; Roussotte et al., 2017; Schiepers et al., 2011; Schwahn and Rozen, 2001; Troesch et al., 2016; Tsai et al., 2011; Wakutani et al., 2004; Weisberg et al., 1998; Yamada et al., 2001). Other relevant OCM mutations include methionine synthase, *MTR* (e.g., A2756G) (Chen et al., 1997; Leclerc et al., 1996; Troesch et al., 2016), methionine synthase reductase, *MTRR* (e.g., A66G, C574T) (Olteanu et al., 2002; Troesch et al., 2016), cystathionine β-synthase (CBS) (e.g., 68 bp insertion at exon 8, G9276A, and 31 bp variable number of tandem repeats) (Barbaux et al., 2000; Nienaber-Rousseau et al., 2013; Olteanu et al., 2002; Sebastio et al., 1995; Troesch et al., 2016), and serine hydroxymethyltransferase (*SHMT*) (e.g., C1420T) (Lievers et al., 2001; Troesch et al., 2016). OCM-related genes are understudied in relation to cognitive outcomes, particularly among middle-aged African-American adults. To date, only one cross-sectional study tested these associations among African-American adults, advocating the need to have longitudinal studies that target this sociodemographic group (Moorthy et al., 2012). In addition, gender differences in OCM resulting in higher levels of tHcy among men suggest that genetic factors linked to this metabolic pathway can alter cognitive trajectories differently between men and women (Sadre-Marandi et al., 2018).

This study tested associations of selected OCM enzymatic gene single nucleotide polymorphisms (SNPs), SNP latent classes (SNPLCs), and SNP haplotypes (SNPHAPs) with longitudinal changes in cognitive performance in a large sample of African-American urban adults. Individual SNPs, such as *MTHFR* (C677T, rs1801133), *MTR* (A2756G, rs1805087), and *MTRR*(A66G, rs1801394; C574T, rs1532268), known to be associated with increased serum tHcy (Chen et al., 1997; Leclerc et al., 1996; Olteanu et al., 2002; Rozen, 1997; Troesch et al., 2016) are expected to be directly linked with age-related cognitive decline. In an attempt to replicate findings from a previous study (Wakutani et al., 2004), we specifically hypothesize that the "GG" *MTHFR* SNPHAP [rs1801133(C677T, A>G)/rs1801131(A1298C, G>T)] is associated with slower age-related cognitive decline.

2. Methods

2.1. Database

Initiated in 2004, Healthy Aging in Neighborhoods of Diversity across the Life Span (HANDLS) is an on-going population-based cohort study. Household screenings from an area probability sample of 13 neighborhoods in Baltimore City, Maryland, were conducted. Neighborhoods were contiguous groups of census tracts with high participant yield filling a 4-way factorial design: race by sex by age by socioeconomic status (below or above 125% of the federal poverty level). After residential dwellings were identified within selected neighborhoods, doorstep interviews were conducted to select one or 2 eligible persons in each household to become HANDLS participants. Eligible persons were 30–64 years old, self-identified as white or African American, capable of providing informed consent, performed ≥5 tests, and present valid photo identification. Pregnancy, being within 6 months of active cancer treatment, or self-identifying as multiethnic were key reasons for exclusion (Evans et al., 2010). Written informed consent was obtained after reviewing a layman term protocol booklet and a video detailing procedures and future recontacts. HANDLS was approved by the National Institute on Environmental Health Sciences, National Institutes of Health Institutional Review Board.

Our present study analyzed longitudinal data from 2 HANDLS visits, selecting the sample of African-American participants with repeated cognitive data and complete baseline covariate and genetic data. Time between examination visits 1 (wave 1: 2004–2009) and 2 (also known as wave 3: 2009–2013; National Institute on Aging, 2004) ranged from <1 year to ~8 year, with a mean of 4.64 ± 0.93 year.

2.2. Study subjects

HANDLS recruited 3720 (mean ± standard deviation [SD] age of 48.3 ± 9.4 years, 45.3% men, and 59.1% African Americans and 40.9% white). Of 2198 African Americans, baseline HANDLS participants, 1024 had complete genetic data. Nine cognitive test scores were used in this study as was done previously (Beydoun et al., 2012). Cognitive data completeness at either visit among African Americans ranged between 1392 for California Verbal Learning Test–delayed free recall (CVLT-DFR) and 1472 for Benton Visual Retention Test (BVRT), with an average of 1.7 visits/participant, excluding participants with missing baseline smoking status. Mixed-effects regression models for predicting the annualized rates of cognitive change assumed missingness at random (Ibrahim and Molenberghs, 2009) and used full information on repeated cognitive tests. The final analytic sample size ranged between 660 participants for CVLT-DFR and 797 participants for the verbal fluency test-categorical (Fig. 1).

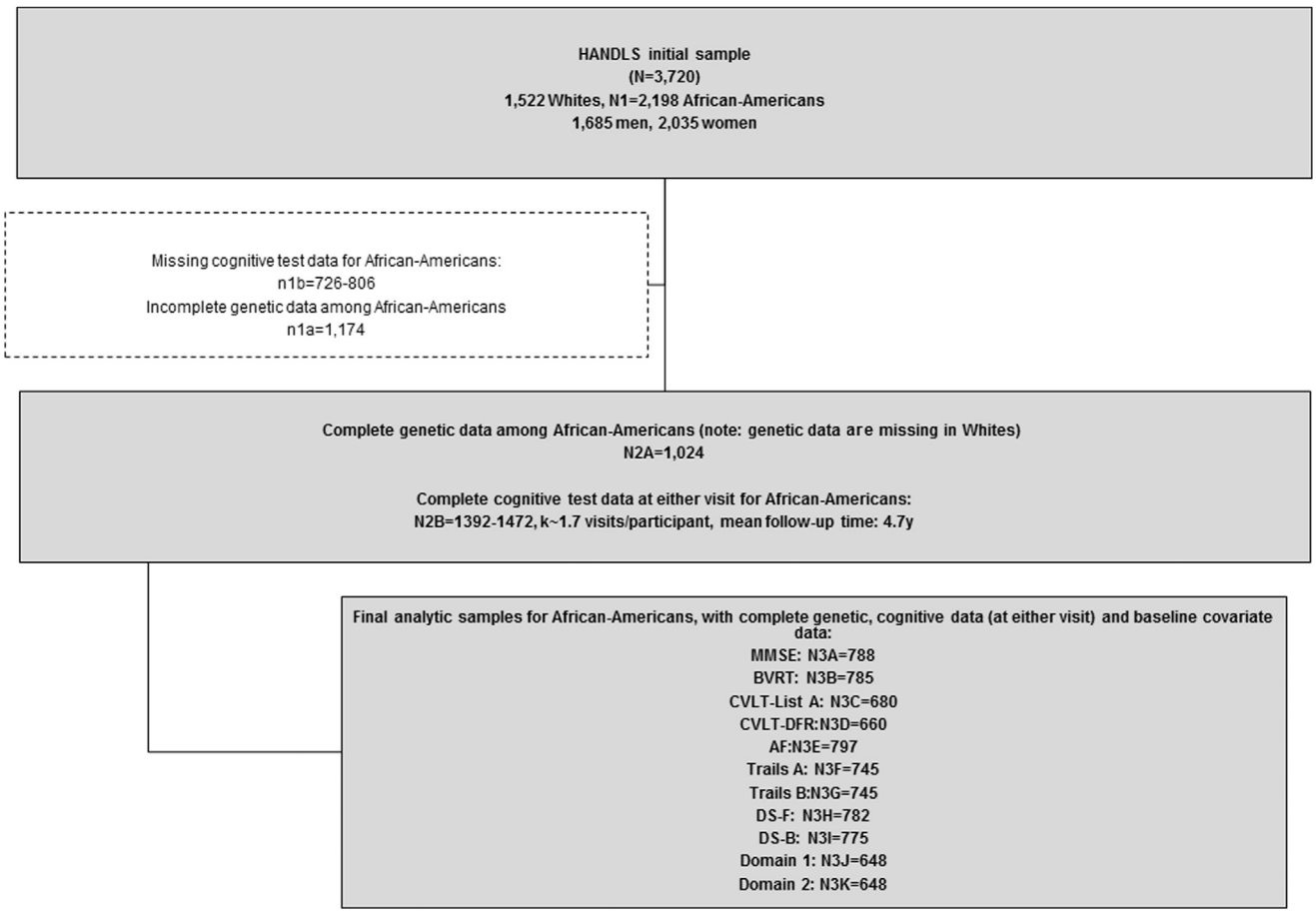

Fig. 1. Participant flowchart. Abbreviations: AF, Animal Fluency; BVRT, Benton Visual Retention Test; CVLT-List A, California Verbal Learning Test immediate recall; CVLT-DFR, California Verbal Learning Test–delayed free recall; DS-B, Digits Span Backward; DS-F, Digits Span Forward; HANDLS, Healthy Aging in Neighborhoods of Diversity across the Life Span; MMSE, Mini–Mental State Examination; Trails A and B, Trail Making Test, parts A and B.

2.3. Cognitive assessment

Cognitive assessment included 6 tests with 9 test scores covering 7 domains (mental status, attention, learning/memory, executive function, visuospatial/visuoconstruction ability, psychomotor speed, and language/verbal): Mini–Mental State Examination (MMSE) [mental status], CVLT immediate recall (List A) and CVLT-DFR [learning/memory, language/verbal], Digits Span Forward and Backward tests (DS-F and DS-B) [attention and working memory], BVRT [figural memory and visuoconstructional abilities], Animal Fluency (AF) [semantic verbal fluency], and Trail Making Test, parts A and B (Trails A and B) [attention and executive functioning]. BVRT and Trails A and B were coded as higher scores reflecting poorer performance (Supplemental Method 1). Multiple linear mixed-effects regression models with quadratic age added among fixed effects were conducted for cognitive score estimation at specific ages (Beydoun et al., 2012) and for prediction of annualized rate of cognitive change at mean follow-up age, which was termed longitudinal annual rate of cognitive change (LARCC). The LARCC of 8 of 9 cognitive test scores (excluding MMSE) was entered into a factor analytic model (Sharma, 1996):

$$\mathrm{LARCC_i} = \sum_{j=1}^{k} \lambda_{ij} \times Domain_j + \varphi_i$$

where LARCCi is the standardized z-score for each cognitive test LARCC, λ_{ij} is the factor loading for each LARCC and each factor, $\mathrm{Domain_j}$ is the standardized z-score for each factor j, and φ_i is the residual error. Two factors were extracted based on the eigenvalue >1 rule, and their factor loadings were rotated using varimax orthogonal rotation, creating domain-specific LARCCs that were uncorrelated. Those factors were interpreted and labeled based on loadings ($\lambda_{ij} \geq 0.40$). Domains were labeled as domain 1 [verbal memory(+) and fluency(+)] and domain 2 [visual(−)/working memory(+) and executive function(−)], where "+" indicates slower decline with higher score and "−" faster decline with higher score. Most $\mathrm{LARCC_i}$ factor loadings were elevated for a single domain of 2, creating an easily interpretable simple structure. Pearson's correlation between extracted domains 1 and 2 was weak ($r = 0.12$). In addition, the MMSE LARCCs, which was not included in the factor analysis, were inversely correlated with domain 2 ($r = -0.66$), whereas being weakly correlated with domain 1 ($r = -0.03$) (see Supplemental Method 2).

All HANDLS participants completed informed consent after probing for protocol understanding. Participants were administered mental status tests, which they completed successfully, with a low score resulting from poor literacy skills rather than signs of dementia.

2.4. OCM enzyme SNPs, SNPLCs, and SNPHAPs

HANDLS participants were genotyped using Illumina 1M genotyping arrays. A total of 1024 individuals were successfully

genotyped and passed genotype quality control criteria. Details are provided in Supplemental Method 3.

SNP selection was based on previously published genome-wide association studies relating cognitive function, cognitive decline, or dementia to gene polymorphisms involved in the OCM (Coppede, 2010; Porter et al., 2016; Rai, 2016, 2017; Sun et al., 2015; Troesch et al., 2016) and as an attempt to replicate a previous case-control study that examined associations between *MTHFR* haplotypes and AD (Wakutani et al., 2004). All available *MTHFR* SNPs were screened for imputation quality and minor allele frequency (MAF). SNPs with MAF < 0.05 were excluded. Our database included the most selected SNPs from each target genes. For *MTHFR*, 10 SNPs were selected with MAF > 0.05: rs4846049 (T>G, MAF = 0.472), rs1476413 (T>C, MAF = 0.170), rs4846051 (A1317G, G>A, MAF = 0.323), rs1801131 (A1298C, G>T, MAF = 0.175), rs2066462 (C1056T, A>G, MAF = 0.095), rs1801133 (C677T, A>G, MAF = 0.088), rs1703796 (T>C, MAF = 0.092), rs9651118 (C>T, MAF = 0.058), rs17367504 (G>A, MAF = 0.108), and rs2066470 (A>G, MAF = 0.071). Two *MTRR* SNPs were selected: rs1801394 (G>A, MAF = 0.284) and rs1532268 (T>C, MAF = 0.259). Finally, *MTR* and *SHMT* included only 1 SNP each, rs1805087 (G>A, MAF = 0.261) and rs1979277 (A>G, MAF = 0.362), respectively. Excluded SNPs were included in *MTHFR* rs2274976 and rs17375901 (MAF < 0.05) and in *SHMT* rs5742905 (MAF < 0.05 and $R^2 = 0.007$).

MTHFR SNPLCs were extracted using latent class analysis (PROC LCA in SAS, version 9.3) (Iivonen et al., 2004; Lanza et al., 2007), using an additive mode of inheritance (i.e., SNPs: 0/1/2). Model fit was determined based on reduced Bayesian information criterion, allowing for 10% difference between more- versus less-parsimonious model (range of latent class numbers: 4–8) (Beydoun et al., 2012, 2013, 2017).

Similarly, *MTHFR* SNP haplotypes (SNPHAP) were considered among key predictors. Using Haploview, version 4.2 (Barrett et al., 2005; Wigginton et al., 2005), we extracted 3 distinctive and common SNPHAP with frequency >5% based on 2 commonly studied *MTHFR* SNPs, namely rs1801133(C677T, A>G)/rs1801131(A1298C,G>T): $MTHFR_1$: GT, $MTHFR_2$: GG, and $MTHFR_3$: AT, coded as 0 = having no $MTHFR_x$ haplotype; 1 = having one allele carrying the $MTHFR_x$ haplotype; and 2 = having 2 alleles carrying the $MTHFR_x$ haplotype.

2.5. Covariates

Three sets of covariates were assessed as potential confounders: (1) sociodemographic factors—baseline age, sex, educational attainment (years of schooling), and smoking status as a lifestyle-related factor (never, former or current smoker); (2) self-reported history of type 2 diabetes, hypertension, cardiovascular disease (stroke, congestive heart failure, nonfatal myocardial infarction, or atrial fibrillation), and dyslipidemia at first-visit; and (3) measured first-visit body mass index (BMI in kg/m^2). Right and left sitting systolic and diastolic blood pressure levels were averaged. Blood pressure was measured noninvasively using brachial artery auscultation with an aneroid manometer, a stethoscope, and an inflatable cuff. Following an overnight fast (8–12 hours) and consenting, blood was drawn and collected from an antecubital vein. Serum total cholesterol, high-density lipoprotein-cholesterol (HDL-C), and glucose were assessed using a spectrophotometer (Olympus 5400). First-visit blood pressure (systolic and diastolic in mm Hg), plasma total cholesterol and HDL-C, and fasting blood glucose (in mg/dL) were only analyzed for descriptive purposes (Beydoun et al., 2012, 2017). Serum folate and B-12 were measured at baseline using immunoassays. [https://www.questdiagnostics.com/testcenter/BUOrderInfo.action?tc=7065&labCode=AMD&searchString=Folate,%20Serum]. Although those measures were not included in our key models given their potential mediating role in the association between one-carbon genetic risk markers and cognitive decline, they are presented for descriptive purposes by sex and MTHFR SNP (C667T).

2.6. Statistical analysis

For each selected SNP, Hardy-Weinberg equilibrium was assessed with an exact test, whereas pairwise linkage disequilibrium was computed and visualized with Haploview, version 4.2 (Barrett et al., 2005; Wigginton et al., 2005). Weighted participant study characteristics were estimated and compared by sex and *MTHFR* C667T (0 = low risk, 1 = medium risk, and 2 = high risk) SNP, using design-based *F*-test. Moreover, unweighted analyses were also conducted for LARCCs to obtain their respective standard deviations and aid in interpreting effect sizes of genetic markers on cognitive change. Study characteristics were also compared according to completeness in genetic data, although not fully presented. The association between MTHFR C667T genotype and LARCCs was additionally age and sex adjusted.

Associations of *MTHFR, MTR, MTRR,* and *SHMT* SNPs and of *MTHFR* SNPLC and SNPHAP with LARCCs were examined by multivariable-adjusted ordinary least squares (OLS) that did not take into account sampling design complexity. SNPs (wild type [w]/variant [v]) were operationalized as genotypes, comparing 2 variant genotypes (wv, vv) with wild-type genotype (ww) and as dosage of variant allele (v), using an additive mode of inheritance model. *P*-trend was estimated when testing the linear relationship between haplotype dosage (0, 1, 2 copies) and cognitive outcomes. Effect sizes were interpreted as the effect of one additional allele copy on the LARCC and was compared to 1 SD of LARCC in the descriptive part of the analysis. An effect >10% of 1 SD was considered appreciable.

Nonrandom selection of participants with genetic data was accounted for using a 2-stage Heckman selection model (Heckman, 1979). At stage 1, a probit model estimated using inverse Mills ratio was derived from predicted probability of being selected, conditional on covariates in that model (Beydoun et al., 2010). At stage 2, inverse Mills ratio was included in OLS regression models, thus adjusting for related selection bias. Sex-stratified analyses were conducted, with effect modification by sex formally tested by including interaction terms.

Type I error of 0.05 was considered for all analyses, and *p*-values between 0.05 and 0.10 were considered as borderline significant for main effects, whereas a *p*-value below 0.10 was considered significant for interaction terms (Selvin, 2004), before multiple testing adjustments. Correction for multiple testing was done using familywise Bonferroni procedure, taking only the cognitive outcome into account. Specifically, a family was defined as a cognitive test, assuming their content-wise independence though not necessarily in their degree of correlation (Hochberg and Tamhane, 1987). Within each cognitive test, there were generally 2 test scores for which correction for multiple testing was required. This was the case for CVLT-DFR and CVLT-List A; Trails A and Trails B; and DS-F and DS-B. For these cognitive tests, statistical significance criterion for *p*-values and *p*-values for trend was reduced to $p = 0.05/2 = 0.025$ (marginal significance: $p = 0.10/2 = 0.05$). For MMSE (a measure of global mental status), BVRT, AF, domains 1 and 2, no correction was needed, an approach taken in our previous study (Beydoun et al., 2012). Selected findings were illustrated using plotted predictive margins with 95% confidence interval (CI) from the associated OLS models. All analyses (except for LCA [SAS ver. 9.3] and haplotype extraction [Haploview, version 4.2]) were performed using Stata, version 15.0 (STATA, 2015).

3. Results

3.1. Study sample characteristics

Baseline study sample characteristics are presented in Table 1, comparing the sample by sex and MTHFR C667T genotype (from 0 = low risk to 2 = highest risk). Sample characteristics by completeness in genetic data are also analyzed but not fully presented. Except for the LARCCs for which samples were specific to the cognitive tests, comparisons were made for the sample that was complete on MMSE LARCC as the main criterion for selection (N = 648–797 for both sexes). More than half of the sample consisted of women. Comparable sociodemographic, lifestyle, and health-related characteristic distributions were observed by genetic data availability, except for systolic blood pressure being higher and cognitive decline on BVRT, CVLT-List A, and DS-B being faster among those with "complete genetic data" ($p < 0.05$). Within the latter group, whereas smoking was more prevalent among men, women had higher cardiovascular disease prevalence, higher mean BMI, and HDL-C level. Nevertheless, cognitive decline was consistently faster among men for most cognitive tests. Most notably, the "verbal memory and fluency" domain 1 indicated an overall faster decline among men. When comparing the low-risk with highest-risk group for the MTHFR C667T genotype, the highest-risk group had a lower mean BMI, total cholesterol, HDL-C, fasting glucose, and most importantly a lower level of serum folate ($p < 0.001$). In addition, cognitive decline was found to be faster in this group for both domains of cognition. However, after adjustment for age and sex, only domain 2 remained significantly associated with the high-risk group (vs. lowest risk), mainly driven by a faster decline on Trails A and B and BVRT (data not shown).

All examined SNPs were in Hardy-Weinberg equilibrium ($p > 0.002$). Variants within each *MTHFR* and *MTRR* gene were deemed in low linkage equilibrium ($r^2<0.30$). Genotypic frequencies suggested that one genotype in each SNP had a prevalence of >45% and thus was dominant compared with other genotypes (Fig. 2, Figs. S1-S3). Table 2 presents *MTHFR* SNPLC (determined by LCA) and SNPHAP [overall frequency and for 0, 1, or 2 copies] distributions. Although SNPLCs yield mutually exclusive categories, SNPHAPs are independent and nonmutually exclusive, reflecting allelic combinations for the selected individuals.

3.2. MTHFR SNPs and their associations with LARCC

Supplemental Table 1 presents findings from multiple OLS models examining associations between *MTHFR* SNPs (entered alternatively, models A-J) and LARCC, overall and stratifying by sex. After adjustment for multiple testing, overall, rs4846051(A1317G, G>A) and rs1801131(A1298C, G>T) were associated with slower ($\beta = +0.0023 \pm 0.0014$, $p = 0.014$) and faster ($\beta = -0.0027 \pm 0.0011$, $p = 0.036$) decline on AF (SD = 0.023 for AF, Table 1), respectively. The latter association was also found among women ($\beta = -0.0042 \pm 0.0018$, $p = 0.017$). Among men, rs4846051(A1317G, G>A) was associated with a faster decline on the BVRT ($\beta = +0.0049 \pm 0.0024$, $p = 0.042$), whereas rs2066462(C1056T, A>G) and rs9651118(C>T) were associated with a slower decline on CVLT-List A ($\beta = +0.0013 \pm 0.0005$, $p = 0.005$; $\beta = +0.0012 \pm 0.0005$, $p = 0.020$, respectively). However, those latter SNPs, particularly rs9651118(C>T), were linked to a faster decline on CVLT-DFR, among men ($\beta = -0.0021 \pm 0.0009$, $p = 0.023$). When examining further those associations among women, rs1801133(C677T, A>G) was linked to a slower decline on cognitive domain 1, ($\beta = +0.0840 \pm 0.0364$, $p = 0.022$) combining mainly longitudinal changes in tests of verbal memory and verbal fluency. This net effect is the equivalent of an increase by 15% of 1 SD (SD = 0.55 among women) in domain 1 and thus is considered to be appreciable.

3.3. MTHFR and MTRR SNPLC and their associations with LARCC: sex-stratified findings

In Table 3, we present findings from OLS regression models whereby SNPLC predicted LARCC among men and women, separately. Following multiple-testing adjustments, among men, *MTRR*: rs1532268(T>C) was associated with faster decline on BVRT ($\beta = +0.0066 \pm 0.0027$, $p = 0.013$), whereas *MTRR*: rs1801394(G>A) was associated with slower decline on DS-B ($\beta = +0.0031 \pm 0.0012$, $p = 0.014$). Compared with *MTHFR4* SNPLC, *MTHFR5* was associated with faster decline on CVLT-List A in men ($\beta = -0.0015 \pm 0.0006$, $p = 0.018$). Among women, *MTRR*: rs1532268(T>C) was associated with slower decline on Trails B ($\beta = -0.3948 \pm 0.1689$, $p = 0.020$), as reflected by an inverse relationship with domain 2 ($\beta = -0.1136 \pm 0.00574$, $p = 0.049$). Furthermore, among women, compared with $MTHFR_4$, $MTHFR_1$ was associated with slower decline on AF ($\beta = +0.0075 \pm 0.0033$, $p = 0.023$). No significant associations were detected between *MTR*/*SHMT* SNPs and LARCCs.

3.4. MTHFR SNPHAP and their associations with LARCC: sex-stratified findings

Table 4 displays findings when testing net associations between each of the 2 MTHFR SNPHAPs on the LARCC, adjusting for other SNPs and potentially confounding covariates. After multiple-testing adjustments, $MTHFR_2$ *SNPHAP* [rs1801133(C677T, A>G)/rs1801131(A1298C, G>T): GG] was associated with slower decline on AF among women, ($\beta = +0.0040 \pm 0.0018$, $p = 0.024$), whereas $MTHFR_3$ *SNPHAP*(AT) was linked with slower decline on CVLT-List A among men ($\beta = +0.0012 \pm 0.0005$, $p = 0.019$) but faster decline on "verbal memory/fluency" among women ($\beta = -0.092 \pm 0.038$, $p = 0.017$). Fig. 3 shows the predictive margins from OLS regression model with AF LARCC as the outcome and $MTHFR_2$ SNPHAP (GG). It is clear from this figure that the potential protective effect of the GG haplotype was only found among women. A sensitivity analysis, which included 10 population stratification principal components into the main models, did not change the main associations of interest observed in the reduced models.

4. Discussion

4.1. Key findings

This study tested associations between OCM enzymatic gene variations and cognitive performance change over ~5 y mean follow-up among 660–797 African-American HANDLS participants. Overall, *MTHFR* SNPs rs4846051(A1317G, G>A) and rs1801131(A1298C, G>T) were associated with slower and faster declines on AF, respectively, whereas rs2066462(C1056T, A>G) was related to slower decline on Trails B (executive function). Among men, rs4846051(A1317G, G>A) was linked to faster decline on BVRT (visual memory), whereas rs2066462(C1056T, A>G) and rs9651118(C>T) were associated with slower decline on CVLT-List A and rs9651118(C>T) with faster decline on CVLT-DFR. Among women, a slower decline on the domain "verbal memory/fluency" was observed with rs1801133(C677T, A>G). $MTHFR_2$ *SNPHAP* [rs1801133(C677T, A>G)/rs1801131(A1298C, G>T): GG] was associated with slower decline on AF among women, whereas $MTHFR_3$ *SNPHAP*(AT) was linked with slower decline on CVLT-List A among men but faster decline on domain 1 among women, mainly driven by a decline on DS test scores. Similar patterns were observed for *MTHFR* SNPLCs.

Table 1
Baseline study sample characteristics, genetic SNPs, and predicted LARCC by availability, sex, and MTHFR C667T SNP among eligible African-American participants (n = 788; HANDLS study)[e]

Characteristics	By sex				p[a]	*By MTHFR*: rs1801133(C677T, G>A)			p (1 vs. 0)[b]	p (2 vs. 0)[b]	Total with genetic data	
	Men		Women			*0: Low risk %*, Mean ± SE	*1: Medium risk*	*2: High risk*				
	n	%, Mean ± SE	*n*	%, Mean ± SE		83.3	15.3	1.3			*n*	%, Mean ± SE
Baseline study sample characteristics												
Female, %		—		—		52.2	55.0	10.7	0.15		788	52.1
Baseline age (y)	349	48.6 ± 0.8	439	47.0 ± 0.8	0.18	47.8 ± 0.6	47.7 ± 1.5	43.3 ± 2.9	0.94	0.091	788	47.8 ± 0.57
Education, years	349	12.6 ± 0.2	439	12.6 ± 0.2	0.85	12.6 ± 0.2	12.5 ± 0.3	12.4 ± 0.4	0.79	0.56	788	12.60 ± 0.16
Smoking status, %	349		439		0.025				0.039		788	
Never/former		42.2		56.4		51.5	43.4	3.9				49.6
Current		57.8		43.5		48.5	56.6	96.1				50.4
Type 2 diabetes, %	349	12.8	439	15.8	0.43	12.5	25.4	0.0	0.17		788	14.3
Hypertension, %	349	36.3	439	47.9	0.06	43.2	40.4	5.8	0.16		788	42.3
Cardiovascular disease, %[c]	349	10.3	439	18.1	0.022	14.6	17.1	3.9	0.60		788	14.8
Dyslipidemia, %	349	23.2	439	24.5	0.80	22.3	33.5	8.4	0.17		788	23.9
Body mass index, kg m^{-2}	349	27.4 ± 0.5	439	31.6 ± 0.8	<0.001	29.8 ± 0.6	29.3 ± 1.0	22.3 ± 0.8	0.68	<0.001	788	29.6 ± 0.5
Systolic blood pressure (mm Hg)	341	121.4 ± 1.4	427	122.7 ± 1.6	0.55	121.7 ± 1.2	124.5 ± 2.9	120.6 ± 2.6	0.37	0.68	768	122.1 ± 1.1
Diastolic blood pressure (mm Hg)	337	79.1 ± 0.8	418	76.4 ± 1.3	0.07	77.7 ± 0.9	77.7 ± 1.4	77.2 ± 1.6	0.97	0.75	755	77.7 ± 0.8
Serum total cholesterol level (mg/dL)	336	185.8 ± 5.0	424	187.2 ± 3.5	0.83	185.4 ± 2.9	198.7 ± 9.5	116.9 ± 7.0	0.18	<0.001	760	186.5 ± 3.0
Serum HDL-C (mg/dL)	336	49.9 ± 1.9	424	57.2 ± 1.7	0.004	54.1 ± 1.5	52.7 ± 1.9	40.9 ± 2.0	0.55	<0.001	760	53.7 ± 1.3
Fasting plasma glucose (mg/dL)	336	104.5 ± 3.4	424	103.9 ± 3.1	0.88	103.0 ± 2.3	112.0 ± 7.7	88.7 ± 1.9	0.26	<0.001	760	104.2 ± 2.3
Serum folate (ng/mL)	338	15.9 ± 1.9	424	13.5 ± 0.5	0.23	14.8 ± 1.1	14.2 ± 0.9	9.5 ± 0.5	0.66	<0.001	762	14.7 ± 1.0
Serum vitamin B-12 (pg/mL)	338	527 ± 19	424	594 ± 25	0.032	570 ± 19	531 ± 30	442 ± 107	0.27	0.19	762	562 ± 16
Genetic SNPs												
MTHFR: rs4846049(T>G)	349	0.89 ± 0.07	439	0.83 ± 0.06	0.48	0.73 ± 0.05	1.48 ± 0.07	2.00 ± 0.00	<0.001	<0.001	788	0.86 ± 0.05
MTHFR: rs1476413(T>C)	349	1.56 ± 0.06	439	1.59 ± 0.05	0.77	1.52 ± 0.04	1.85 ± 0.04	2.00 ± 0.00	<0.001	<0.001	788	1.58 ± 0.04
MTHFR: rs4846051(A1317G, G>A)	349	1.36 ± 0.05	439	1.38 ± 0.05	0.78	1.31 ± 0.04	1.65 ± 0.06	2.00 ± 0.00	<0.001	<0.001	788	1.37 ± 0.04
MTHFR: rs1801131(A1298C, G>T)	349	1.56 ± 0.05	439	1.57 ± 0.05	0.87	1.51 ± 0.04	1.86 ± 0.04	2.00 ± 0.00	<0.001	<0.001	788	1.57 ± 0.04
MTHFR: rs2066462(C1056T, A>G)	349	1.79 ± 0.04	439	1.74 ± 0.05	0.44	1.73 ± 0.04	1.92 ± 0.03	2.00 ± 0.00	<0.001	<0.001	788	1.76 ± 0.03
MTHFR: rs1801133(C677T, A>G)	349	1.81 ± 0.05	439	1.83 ± 0.03	0.67	2.00 ± 0.00	1.00 ± 0.00	0.00 ± 0.00	<0.001	<0.001	788	1.82 ± 0.03
MTHFR: rs1703796(T>C)	349	1.83 ± 0.03	439	1.76 ± 0.04	0.22	1.77 ± 0.03	1.93 ± 0.03	2.00 ± 0.00	<0.001	<0.001	788	1.80 ± 0.03
MTHFR: rs9651118(C>T)	349	1.90 ± 0.02	439	1.88 ± 0.02	0.46	1.89 ± 0.02	1.88 ± 0.04	2.00 ± 0.00	0.77	<0.001	788	1.89 ± 0.01
MTHFR: rs17367504 (G>A)	349	1.74 ± 0.04	439	1.72 ± 0.05	0.81	1.70 ± 0.04	1.92 ± 0.03	2.00 ± 0.00	<0.001	<0.001	788	1.73 ± 0.03
MTHFR: rs2066470(A>G)	349	1.87 ± 0.03	439	1.81 ± 0.04	0.20	1.82 ± 0.03	1.93 ± 0.03	2.00 ± 0.00	0.005	<0.001	788	1.84 ± 0.03
MTRR: rs1801394 (G>A)	349	1.32 ± 0.06	439	1.37 ± 0.05	0.47	1.35 ± 0.04	1.39 ± 0.08	0.96 ± 0.05	0.63	<0.001	788	1.35 ± 0.04
MTRR: rs1532268 (T>C)	349	1.39 ± 0.06	439	1.57 ± 0.05	0.041	1.47 ± 0.05	1.61 ± 0.07	1.10 ± 0.13	0.097	0.003	788	1.48 ± 0.04
MTR: rs1805087(G>A)	349	1.46 ± 0.05	439	1.47 ± 0.06	0.86	1.48 ± 0.04	1.35 ± 0.10	1.90 ± 0.13	0.22	<0.001	788	1.47 ± 0.04
SHMT: rs1979277(A>G)	349	1.18 ± 0.07	439	1.27 ± 0.06	0.35	1.24 ± 0.05	1.11 ± 0.12	1.92 ± 0.09	0.34	<0.001	788	1.23 ± 0.05
Predicted LARCC[d]												
MMSE	349	−0.046 ± 0.002 (SD: 0.022)	439	−0.034 ± 0.002 (SD: 0.018)	<0.001	−0.040 ± 0.001 (SD: 0.020)	−0.040 ± 0.003 (SD: 0.024)	−0.034 ± 0.001 (SD: 0.010)	0.87	0.005	788	−0.040 ± 0.001 (SD: 0.021)
BVRT	350	+0.218 ± 0.003 (SD: 0.045)	432	+0.173 ± 0.004 (SD: 0.046)	<0.001	+0.195 ± 0.003 (SD: 0.050)	+0.193 ± 0.007 (SD: 0.053)	+0.222 ± 0.005 (SD: 0.037)	0.22	0.77	782	+0.195 ± 0.003 (SD: 0.050)
CVLT-List A	295	−0.292 ± 0.002 (SD: 0.018)	385	−0.270 ± 0.002 (SD: 0.019)	<0.001	−0.281 ± 0.002 (SD: 0.021)	−0.278 ± 0.003 (SD: 0.019)	−0.283 ± 0.003 (SD: 0.014)	0.27	0.57	680	−0.281 ± 0.001 (SD: 0.021)
CVLT-DFR	284	−0.138 ± 0.001 (SD: 0.008)	376	−0.119 ± 0.001 (SD: 0.009)	<0.001	−0.128 ± 0.001 (SD: 0.012)	−0.127 ± 0.001 (SD: 0.012)	−0.134 ± 0.002 (SD: 0.009)	0.60	0.002	660	−0.128 ± 0.001 (SD: 0.012)
AF	356	−0.067 ± 0.002 (SD: 0.022)	441	−0.045 ± 0.002 (SD: 0.020)	<0.001	−0.056 ± 0.002 (SD: 0.023)	−0.057 ± 0.003 (SD: 0.020)	−0.049 ± 0.002 (SD: 0.010)	0.67	0.016	797	−0.056 ± 0.002 (SD: 0.023)
Trails A	326	+0.745 ± 0.072 (SD: 1.023)	419	+0.855 ± 0.117 (SD: 1.224)	0.43	+0.774 ± 0.078 (SD: 1.080)	+0.945 ± 0.182 (SD: 1.230)	+0.993 ± 0.192 (SD: 3.720)	0.39	0.24	745	+0.803 ± 0.071 (SD: 1.140)
Trails B	326	+4.865 ± 0.261 (SD: 2.607)	419	+4.137 ± 0.194 (SD: 2.600)	0.026	+4.309 ± 0.157 (SD: 2.549)	+4.856 ± 0.310 (SD: 2.884)	+10.515 ± 1.587 (SD: 4.037)	0.115	<0.001	745	+4.480 ± 0.163 (SD: 2.625)

(*continued on next page*)

Table 1 (*continued*)

Characteristics	By sex				p^a	By *MTHFR*: rs1801133(C677T, G>A)			p (*1 vs. 0*)[b]	p (*2 vs. 0*)[b]	Total with genetic data	
	Men		Women			*0: Low risk* %, Mean ± SE	*1: Medium risk*	*2: High risk*				
	n	%, Mean ± SE	*n*	%, Mean ± SE		83.3	15.3	1.3			*n*	%, Mean ± SE
DS-F	351	−0.030 ± 0.001 (SD: 0.011)	431	−0.015 ± 0.001 (SD: 0.010)	<0.001	−0.023 ± 0.001 (SD: 0.013)	−0.020 ± 0.001 (SD: 0.0124)	−0.032 ± 0.003 (SD: 0.010)	0.10	0.002	782	−0.022 ± 0.001 (SD: 0.013)
DS-B	351	−0.027 ± 0.002 (SD: 0.016)	424	−0.017 ± 0.001 (SD: 0.017)	<0.001	−0.023 ± 0.001 (SD: 0.018)	−0.018 ± 0.002 (SD: 0.018)	−0.030 ± 0.002 (SD: 0.013)	0.048	0.001	775	−0.022 ± 0.001 (SD: 0.018)
Cognitive domain 1	277	−0.848 ± 0.062 (SD: 0.55)	371	+0.674 ± 0.048 (SD: 0.55)	<0.001	−0.034 ± 0.08 (SD: 0.91)	+0.021 ± 0.108 (SD: 0.79)	−0.514 ± 0.046 (SD: 0.31)	0.69	<0.001	648	−0.033 ± 0.070 (SD: 0.89)
Cognitive domain 2	277	−0.002 ± 0.081 (SD: 0.86)	371	−0.128 ± 0.087 (SD: 0.87)	0.29	−0.088 ± 0.07 (SD: 0.86)	−0.037 ± 0.130 (SD: 0.93)	+0.462 ± 0.112 (SD: 0.64)	0.73	<0.001	648	−0.069 ± 0.061 (SD: 0.87)

Key: AF, Animal Fluency; BMI, body mass index (calculated as weight in kg/square of height in meters); BVRT, Benton Visual Retention Test; CVLT-DFR, California Verbal Learning Test–delayed free recall; CVLT-List A, California Verbal Learning Test–immediate recall; DS-B, Digits Span Backward; DS-F, Digits Span Forward; HDL-C, high-density lipoprotein cholesterol; MMSE, Mini–Mental State Examination; Trails A and B, Trail Making Test parts A and B; SD, standard deviation; SE, standard error of the mean; HANDLS, Healthy Aging in Neighborhoods of Diversity across the Life Span; LARCC, longitudinal annual rate of cognitive change; *MTHFR*, methylenetetrahydrofolate reductase; SHMT, serine hydroxymethyltransferase; SNP, single nucleotide polymorphism.

[a] *p*-value for null hypothesis of no difference between sexes among those with complete genetic data. Note that this analysis was done on African-American participants with complete baseline covariates, including baseline MMSE scores.

[b] *p*-value for null hypothesis of no difference between MTHFR C667T SNPs (0: low risk, 1: medium risk, 2: high risk) using design-based *F*-test.

[c] Reported any of the following conditions at first visit: stroke, congestive heart failure, nonfatal myocardial infarction, or atrial fibrillation.

[d] Cognitive scores were predicted at mean age at follow-up before onset of dementia or for all time points using a linear mixed model controlling for sex, race/ethnicity, education (y), and smoking status, with age (centered at 50 y) added among the fixed effect variables to allow for quadratic nonlinear change, whereas age (centered at 50 y) was added to the random effects to allow for individual-level variation in slopes. The slope or annual rate of change was predicted from these models at the mean age at follow-up (i.e., between age 50 and individual mean age of follow-up for each cognitive test). Using factor analysis, 2 factor scores were estimated and were labeled as LARCC in the following domains—Domain 1: "Verbal memory and fluency" and Domain 2: "Visual/working memory and executive function" (see Supplemental Method 1).

[e] Sociodemographic, lifestyle, and health-related factors are presented among participants with complete data on those variables, as well as complete data on MMSE LARCC. LARCC measures are presented for eligible subjects with complete data on covariates entered into subsequent models and complete data on each of the cognitive test scores at either baseline or follow-up wave. Unreliable data from each cognitive test score were excluded. Serum folate and vitamin B-12 were not included into main models as they could be mediators.

4.2. Previous studies

The positive association between tHcy and adverse cognitive outcomes, including dementia, mild cognitive impairment, and AD, were previously shown in several studies (Annerbo et al., 2005; Blasko et al., 2008; Gabryelewicz et al., 2007; Haan et al., 2007; Kim et al., 2007; Quadri et al., 2004, 2005). Other studies testing associations between tHcy and cognitive domains of verbal memory (Mooijaart et al., 2005; Schafer et al., 2005) and executive function (Elias et al., 2006; Eussen et al., 2007; Garcia et al., 2004; Mooijaart et al., 2005; Schafer et al., 2005) among others have reported positive findings as well (Elias et al., 2006; Eussen et al., 2007; Feng et al., 2006; Schafer et al., 2005). A recent review and meta-analysis of modifiable risk factors for cognitive decline and impairment concluded that elevated tHcy was associated with an average 93% increase in the risk of incident AD (hazard ratio = 1.93, 95% CI: 1.50–2.49) (Beydoun et al., 2014).

For most selected gene polymorphisms, evidence of an effect on tHcy serum concentration is inconsistent (Chango et al., 2000; Chen et al., 2001; de Lau et al., 2010; Fredriksen et al., 2007; Gaughan et al., 2001; Hanson et al., 2001; Jacques et al., 2003; Ravaglia et al., 2004; von Castel-Dunwoody et al., 2005; Yates and Lucock, 2003) except for the *MTHFR* 677TT variant, which was linked with an increased tHcy level in multiple recent studies (de Lau et al., 2010; Elkins et al., 2007; Ford et al., 2012b; Fredriksen et al., 2007; Polito et al., 2016; Tsai et al., 2011; Ueland et al., 2000). Results were inconclusive when examining *MTHFR* 1298A>C, *MTR* 2756A>G, and *CBS* 844ins68 in relation to cognition (Barbaux et al., 2000; Ravaglia et al., 2004; Schiepers et al., 2011). Nevertheless, MTHFR 677TT dosage (C>T) was associated with AD risk in a recent meta-analysis, with a pooled OR of 1.29 (95% CI: 1.07, 1.56) (Rai, 2017), and several cross-sectional and longitudinal studies have found that "TT" was linked to faster decline or poorer performance on global mental status (Elkins et al., 2007; Ford et al., 2012b; Tsai et al., 2011) and on domains of psychomotor speed (Elkins et al., 2007), executive function (Elkins et al., 2007; Polito et al., 2016), short-term memory (Tsai et al., 2011), and concentration/mental manipulation (Tsai et al., 2011). The "C" allele of *MTHFR* 1298(A>C) was also recently linked with lower abstraction ability (Cai et al., 2016). Our haplotype analysis, which included only 677A/G-1298G/T, gave similar results as a previous study that indicated that Haplotype C(677G-1298G-1793C) of the *MTHFR* gene is protective against the development of AD (Wakutani et al., 2004). In fact, our study concluded that the supposedly "protective" haplotype "GG" was associated with slower decline on a test of verbal fluency among women. Nevertheless, further large studies are needed to replicate our findings and that of others (Wakutani et al., 2004).

4.3. Biological pathways

The OCM is a complex metabolic pathway in which folate's active form (tetrahydrofolate [THF]) transfers methyl groups by acting as a cofactor to specific enzymes (Troesch et al., 2016). The OCM consists of a series of interrelated cycles known as methionine, thymidylate, and purine cycles (Troesch et al., 2016). The neurotoxic substance tHcy is metabolized by either entering the methionine or the thymidylate cycle. A negative feedback loop exists, whereby under low levels of methionine, tHcy is remethylated into methionine, through a methyl group's transfer from MTHF to tHcy by MTR, producing THF and methionine (Shane, 2008; Troesch et al., 2016). Methionine is further metabolized into *S*-adenosylmethionine (SAM), the principal methyl donor in DNA methylation, and the synthesis of phospholipids, myelin, and neurotransmitters (Shane, 2008; Troesch et al., 2016).

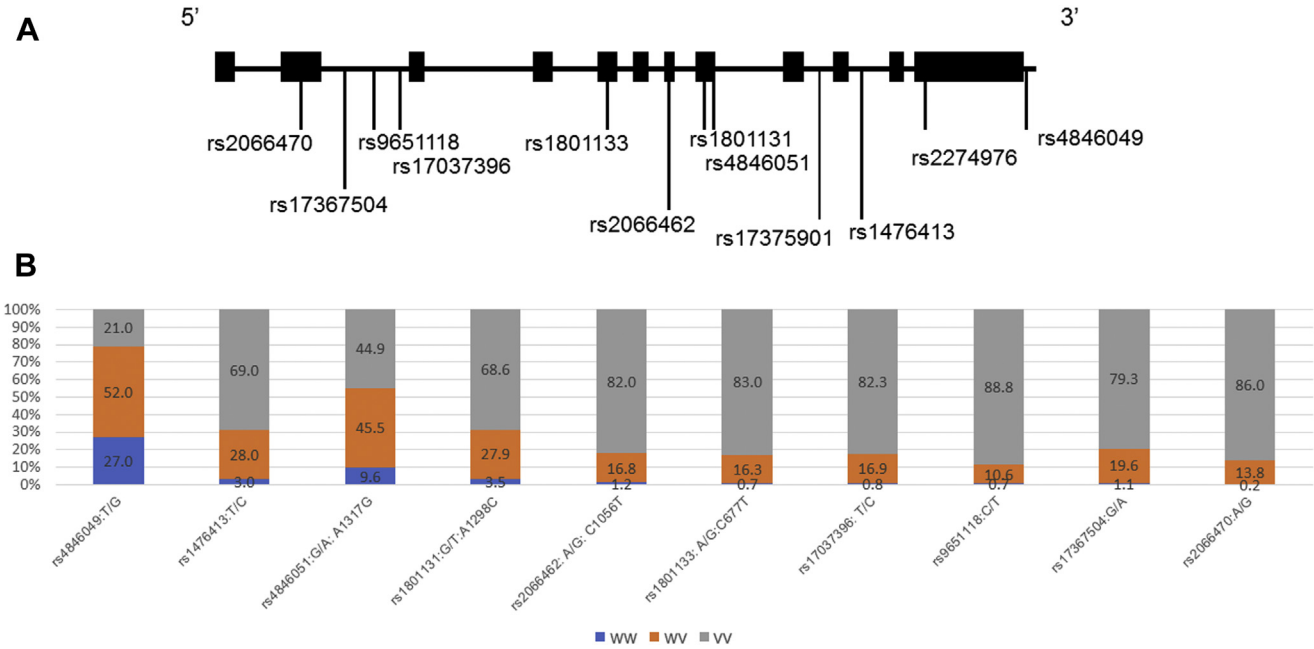

Fig. 2. (A) Schematic representation of the *MTHFR* gene. The SNP and gene coordinates are based on NCBI build 37 (hg19, May 2013, Phase 3 of the 1000 Genomes Project). The *MTHFR* gene on chromosome 1 composed of 12 exons and 20.4 kilobase pairs in size. (B) Genotype frequencies (%) of selected *MTHFR* SNPs of original sample with complete genetic data (n = 1024). Abbreviations: hg, human genome; *MTHFR*, methylenetetrahydrofolate reductase; RefSeq, reference sequence; SNP, single nucleotide polymorphism; vv, variant-variant; wv, wild type–variant; ww, wild type–wild type. *Note*: *MTHFR* SNPs are in the direction v → w: 0 = vv, 1 = wv, and 2 = ww.

Following transfer of a methyl group from SAM, S-adenosylhomocysteine (SAH) is hydrolyzed back into tHcy (Selhub, 1999; Troesch et al., 2016). Serine and THF react together to form glycine and 5,10-methylene-THF with the help of SHMT (Troesch et al., 2016). Closing the cycle is the reduction of 5,10-methylene-THF to 5-MTHF, a reaction catalyzed by the enzyme MTHFR (Troesch et al., 2016). Under conditions where methionine is abundant and tHcy is accumulating, the latter condenses with nonessential amino acid serine to form cystathionine and then cysteine (Shane, 2008; Troesch et al., 2016), a reaction termed "transsulfuration pathway," which requires 2 vitamin B6-dependent enzymes (CBS and cystathionase) (Shane, 2008; Troesch et al., 2016).

In fact, many of the OCM enzymes depend on vitamins B-2, B-6, folate (B-9), and B-12. For instance, MTR depends on the active form of vitamin B12, or methylcobalamin (Shane, 2008; Troesch et al., 2016), acting as a methyl carrier and making it essential for the OCM cycle (Troesch et al., 2016). Vitamin B12 is activated back into methylcobalamin through MTRR by remethylation with one-carbon units from SAM (Ludwig and Matthews, 1997; Troesch et al., 2016). Furthermore, MTRR is a flavoprotein (Leclerc et al., 1998; Troesch

Table 2
Findings from latent class analysis and haplotype analysis: definitions and distributions of SNPLC and SNPHAP for the selected *MTHFR* SNPs (n = 1024)

Characteristics	SNP haplotypes (SNPHAP)		SNP latent classes (SNPLC)	
	Definitions	%	Definitions	%
MTHFR	rs1801133(C677T, A>G)/rs1801131(A1298C, G>T)		rs4846049(T>G)/rs1476413(T>C)/rs4846051(A1317G, G>A) /rs1801131(A1298C, G>T)/rs2066462(C1056T, A>G) /rs1801133(C677T, A>G)/rs1703796(T>C)/rs9651118(C>T) /rs17367504 (A>G)/rs2066470(A>G)	
Overall	*MTHFR*$_1$: GT	73.7	*MTHFR*$_1$: TT/–/-A/**–**/–/**GG**/-C/TT/–/GG	10.7
	MTHFR$_2$: GG	17.3	*MTHFR*$_2$: T-/CC/–/**TT**/GG/**GG**/CC/TT/-A/GG	11.4
	MTHFR$_3$: AT	8.8	*MTHFR*$_3$: -G/-C/AA/**-T**/GG/**–**/CC/–/-A/GG	19.8
			MTHFR$_4$: TG/-C/-A/**TT**/GG/**-G**/CC/-T/-A/GG	34.4
			MTHFR$_5$: TG/-C/-A/**GT**/-G/**-G**/-C/-T/-A/GG	9.6
			MTHFR$_6$: –/–/-A/**–**/–/**-G**/T-/-T/–/–	14.1
Allele copies				
MTHFR$_1$				
0		4.2		
1		41.6		
2		54.2		
MTHFR$_2$				
0		71.2		
1		25.3		
2		3.5		
MTHFR$_3$				
0		85.6		
1		13.7		
2		0.7		

Key: *MTHFR*, methylenetetrahydrofolate reductase; SNP, single nucleotide polymorphism; SNPLC, single nucleotide polymorphism latent class; SNPHAP, single nucleotide polymorphism haplotype.

Table 3
MTHFR, *MTRR* SNPLC, *MTR*, and *SHMT* SNPs' associations with predicted LARCC by sex: multiple OLS regression analysis (n = 648–788; HANDLS study)

Characteristics	Predicted LARCC[d]					
	Men			Women		
	n	β ± SE[a]	*p*	n	β ± SE[a]	*p*
MMSE						
MTHFR$_1$ vs. *MTHFR*$_4$	349	+0.0022 ± 0.0031	0.46	439	−0.0010 ± 0.0022	0.66
MTHFR$_2$ vs. *MTHFR*$_4$	349	−0.0003 ± 0.0033	0.92	439	−0.0012 ± 0.0021	0.56
MTHFR$_3$ vs. *MTHFR*$_4$	349	+0.0014 ± 0.0026	0.61	439	−0.0002 ± 0.002	0.92
MTHFR$_5$ vs. *MTHFR*$_4$	349	−0.0005 ± 0.0031	0.88	439	+0.0030 ± 0.0025	0.23
MTHFR$_6$ vs. *MTHFR*$_4$	349	+0.0015 ± 0.0031	0.62	439	−0.0001 ± 0.0020	0.95
MTRR: rs1801394(G>A)	349	+0.0002 ± 0.0015	0.91	439	+0.0008 ± 0.0010	0.43
MTRR: rs1532268(T>C)	349	−0.0012 ± 0.0016	0.47	439	−0.0001 ± 0.0010	0.89
MTR: rs1805087(G>A)	349	−0.0006 ± 0.0015	0.67	439	+0.0008 ± 0.0010	0.46
SHMT: rs1979277(A>G)	349	−0.0012 ± 0.0014	0.36	439	−0.0000 ± 0.0009	0.99
BVRT						
MTHFR$_1$ vs. *MTHFR*$_4$	350	+0.0066 ± 0.0050	0.19	432	+0.0017 ± 0.0051	0.74
MTHFR$_2$ vs. *MTHFR*$_4$	350	+0.0027 ± 0.0056	0.63	432	+0.0054 ± 0.0048	0.26
MTHFR$_3$ vs. *MTHFR*$_4$	350	+0.0043 ± 0.0045	0.34	432	+0.0065 ± 0.0042	0.12
MTHFR$_5$ vs. *MTHFR*$_4$	350	+0.0064 ± 0.0052	0.23	432	+0.0088 ± 0.0056	0.12
MTHFR$_6$ vs. *MTHFR*$_4$	350	+0.0072 ± 0.0053	0.17	432	+0.0027 ± 0.0044	0.55
MTRR: rs1801394(G>A)	350	−0.0014 ± 0.0026	0.59	432	+0.0025 ± 0.0023	0.29
MTRR: rs1532268(T>C)	350	+0.0066 ± 0.0027	0.013[b,c]	432	−0.0031 ± 0.0024	0.20
MTR: rs1805087(G>A)	350	+0.0000 ± 0.0026	0.97	432	−0.0009 ± 0.0024	0.71
SHMT: rs1979277(A>G)	350	+0.0001 ± 0.0023	0.96	432	+0.0011 ± 0.0021	0.59
CVLT-List A						
MTHFR$_1$ vs. *MTHFR*$_4$	295	−0.0008 ± 0.0006	0.21	385	+0.0003 ± 0.0006	0.59
MTHFR$_2$ vs. *MTHFR*$_4$	295	−0.0009 ± 0.0007	0.16	385	−0.0010 ± 0.0006	0.080
MTHFR$_3$ vs. *MTHFR*$_4$	295	−0.0004 ± 0.0005	0.39	385	−0.0002 ± 0.0005	0.66
MTHFR$_5$ vs. *MTHFR*$_4$	295	−0.0015 ± 0.0006	0.018[b,c]	385	+0.0006 ± 0.0007	0.39
MTHFR$_6$ vs. *MTHFR*$_4$	295	−0.0014 ± 0.0007	0.047	385	−0.0002 ± 0.0005	0.70
MTRR: rs1801394(G>A)	295	−0.0002 ± 0.0003	0.45	385	+0.0004 ± 0.0003	0.20
MTRR: rs1532268(T>C)	295	−0.0006 ± 0.0003	0.073[c]	385	+0.0002 ± 0.0003	0.51
MTR: rs1805087(G>A)	295	−0.0002 ± 0.0003	0.54	385	−0.0001 ± 0.0003	0.66
SHMT: rs1979277(A>G)	295	−0.0006 ± 0.0003	0.054[c]	385	+0.0005 ± 0.0002	0.074
CVLT-DFR						
MTHFR$_1$ vs. *MTHFR*$_4$	284	+0.0011 ± 0.0011	0.30	376	+0.0001 ± 0.0012	0.93
MTHFR$_2$ vs. *MTHFR*$_4$	284	+0.0019 ± 0.0011	0.10	376	+0.0006 ± 0.0011	0.55
MTHFR$_3$ vs. *MTHFR*$_4$	284	+0.0008 ± 0.0009	0.41	376	+0.0001 ± 0.0009	0.96
MTHFR$_5$ vs. *MTHFR*$_4$	284	+0.0013 ± 0.0011	0.24	376	−0.0011 ± 0.0012	0.38
MTHFR$_6$ vs. *MTHFR*$_4$	284	+0.0023 ± 0.0012	0.057	376	−0.0002 ± 0.0010	0.85
MTRR: rs1801394(G>A)	284	+0.0001 ± 0.0006	0.88	376	−0.0008 ± 0.0005	0.10
MTRR: rs1532268(T>C)	284	+0.0005 ± 0.0006	0.38	376	+0.0000 ± 0.0006	0.92
MTR: rs1805087(G>A)	284	+0.0004 ± 0.0006	0.43	376	−0.0004 ± 0.0005	0.49
SHMT: rs1979277(A>G)	284	−0.0003 ± 0.0005	0.57	376	−0.0008 ± 0.0004	0.11
AF						
MTHFR$_1$ vs. *MTHFR*$_4$	356	+0.0012 ± 0.0037	0.74	441	+0.0075 ± 0.0033	0.023[b]
MTHFR$_2$ vs. *MTHFR*$_4$	356	−0.0031 ± 0.0040	0.44	441	−0.0028 ± 0.0030	0.36
MTHFR$_3$ vs. *MTHFR*$_4$	356	+0.0020 ± 0.003	0.54	441	+0.0005 ± 0.0027	0.85
MTHFR$_5$ vs. *MTHFR*$_4$	356	+0.0046 ± 0.0039	0.24	441	+0.0042 ± 0.0036	0.24
MTHFR$_6$ vs. *MTHFR*$_4$	356	+0.0051 ± 0.0038	0.18	441	+0.0022 ± 0.0028	0.43
MTRR: rs1801394(G>A)	356	−0.0005 ± 0.0018	0.77	441	−0.0016 ± 0.0015	0.28
MTRR: rs1532268(T>C)	356	+0.0016 ± 0.0019	0.40	441	−0.0002 ± 0.0015	0.90
MTR: rs1805087(G>A)	356	−0.0019 ± 0.0018	0.31	441	−0.0019 ± 0.0015	0.23
SHMT: rs1979277(A>G)	356	−0.0015 ± 0.0017	0.37	441	+0.0006 ± 0.0014	0.65
Trails A						
MTHFR$_1$ vs. *MTHFR*$_4$	326	−0.0453 ± 0.1931	0.82	419	+0.3476 ± 0.2127	0.10
MTHFR$_2$ vs. *MTHFR*$_4$	326	−0.1714 ± 0.2107	0.42	419	+0.2525 ± 0.1969	0.20
MTHFR$_3$ vs. *MTHFR*$_4$	326	−0.0440 ± 0.1665	0.79	419	−0.0839 ± 0.1738	0.63
MTHFR$_5$ vs. *MTHFR*$_4$	326	−0.1429 ± 0.1952	0.46	419	−0.1884 ± 0.2331	0.42
MTHFR$_6$ vs. *MTHFR*$_4$	326	+0.0323 ± 0.1919	0.87	419	−0.1076 ± 0.1868	0.57
MTRR: rs1801394(G>A)	326	+0.0724 ± 0.0951	0.45	419	−0.0786 ± 0.0960	0.41
MTRR: rs1532268(T>C)	326	+0.0387 ± 0.0991	0.70	419	−0.1347 ± 0.1009	0.18
MTR: rs1805087(G>A)	326	+0.0975 ± 0.0950	0.31	419	−0.1186 ± 0.0994	0.23
SHMT: rs1979277(A>G)	326	+0.0308 ± 0.0863	0.72	419	−0.1161 ± 0.0887	0.19
Trails B						
MTHFR$_1$ vs. *MTHFR*$_4$	326	+0.4533 ± 0.3958	0.25	419	+0.5724 ± 0.3586	0.11
MTHFR$_2$ vs. *MTHFR*$_4$	326	+0.1398 ± 0.4350	0.75	419	−0.0050 ± 0.3291	0.99
MTHFR$_3$ vs. *MTHFR*$_4$	326	+0.3257 ± 0.3434	0.34	419	−0.0488 ± 0.2937	0.87
MTHFR$_5$ vs. *MTHFR*$_4$	326	+0.1402 ± 0.4006	0.73	419	−0.1132 ± 0.3931	0.77
MTHFR$_6$ vs. *MTHFR*$_4$	326	+0.2980 ± 0.3939	0.45	419	+0.5033 ± 0.3145	0.11
MTRR: rs1801394(G>A)	326	+0.0254 ± 0.1958	0.90	419	+0.1122 ± 0.1633	0.49
MTRR: rs1532268(T>C)	326	+0.2369 ± 0.2046	0.25	419	−0.3948 ± 0.1689	0.020[b,c]
MTR: rs1805087(G>A)	326	+0.0409 ± 0.1951	0.83	419	−0.2059 ± 0.1673	0.22
SHMT: rs1979277(A>G)	326	+0.0967 ± 0.1772	0.58	419	−0.2441 ± 0.1502	0.11
DS-F						
MTHFR$_1$ vs. *MTHFR*$_4$	351	+0.0006 ± 0.0016	0.72	431	+0.0020 ± 0.0015	0.19

Table 3 (*continued*)

Characteristics	Predicted LARCC[d]					
	Men			Women		
	n	β ± SE[a]	*p*	n	β ± SE[a]	*p*
MTHFR$_2$ vs. *MTHFR*$_4$	351	−0.0005 ± 0.0018	0.79	431	−0.0010 ± 0.0014	0.49
MTHFR$_3$ vs. *MTHFR*$_4$	351	+0.0003 ± 0.0014	0.83	431	−0.0016 ± 0.0012	0.18
MTHFR$_5$ vs. *MTHFR*$_4$	351	+0.0010 ± 0.0017	0.53	431	−0.0003 ± 0.0016	0.84
MTHFR$_6$ vs. *MTHFR*$_4$	351	−0.0011 ± 0.0017	0.52	431	+0.0015 ± 0.0013	0.28
MTRR: rs1801394(G>A)	351	+0.0002 ± 0.0008	0.78	431	+0.0005 ± 0.0007	0.50
MTRR: rs1532268(T>C)	351	+0.0005 ± 0.0008	0.54	431	−0.0008 ± 0.0007	0.28
MTR: rs1805087(G>A)	351	−0.0016 ± 0.0008	0.048	431	−0.0004 ± 0.0007	0.61
SHMT: rs1979277(A>G)	351	+0.0002 ± 0.0007	0.75	431	−0.0003 ± 0.0006	0.67
DS-B						
MTHFR$_1$ vs. *MTHFR*$_4$	351	+0.0045 ± 0.0024	0.066	424	+0.0007 ± 0.0027	0.80
MTHFR$_2$ vs. *MTHFR*$_4$	351	+0.0022 ± 0.0027	0.42	424	−0.0006 ± 0.0024	0.80
MTHFR$_3$ vs. *MTHFR*$_4$	351	+0.0003 ± 0.0021	0.89	424	−0.0020 ± 0.0021	0.35
MTHFR$_5$ vs. *MTHFR*$_4$	351	+0.0028 ± 0.0025	0.27	424	+0.0015 ± 0.0028	0.60
MTHFR$_6$ vs. *MTHFR*$_4$	351	+0.0008 ± 0.0025	0.76	424	+0.0013 ± 0.0023	0.57
MTRR: rs1801394(G>A)	351	+0.0031 ± 0.0012	0.014[b,c]	424	−0.0004 ± 0.0012	0.77
MTRR: rs1532268(T>C)	351	+0.0013 ± 0.0013	0.31[c]	424	−0.0025 ± 0.0012	0.045
MTR: rs1805087(G>A)	351	−0.0017 ± 0.0012	0.16	424	+0.0001 ± 0.0012	0.96
SHMT: rs1979277(A>G)	351	−0.0020 ± 0.0011	0.068	424	−0.0013 ± 0.0011	0.23
Cognitive domain 1						
MTHFR$_1$ vs. *MTHFR*$_4$	277	+0.0462 ± 0.0578	0.43	371	+0.0566 ± 0.0516	0.27
MTHFR$_2$ vs. *MTHFR*$_4$	277	+0.0249 ± 0.0590	0.67	371	−0.0543 ± 0.0478	0.26
MTHFR$_3$ vs. *MTHFR*$_4$	277	+0.0343 ± 0.0483	0.48	371	−0.0674 ± 0.0420	0.11
MTHFR$_5$ vs. *MTHFR*$_4$	277	+0.0530 ± 0.0588	0.37	371	−0.0579 ± 0.0579	0.32
MTHFR$_6$ vs. *MTHFR*$_4$	277	+0.0813 ± 0.0615	0.19	371	+0.0137 ± 0.0450	0.76
MTRR: rs1801394(G>A)	277	+0.0018 ± 0.0293	0.95	371	−0.0090 ± 0.0233	0.70
MTRR: rs1532268(T>C)	277	+0.0058 ± 0.0300	0.85	371	−0.0081 ± 0.0251	0.75
MTR: rs1805087(G>A)	277	−0.0338 ± 0.0284	0.24	371	−0.0212 ± 0.0245	0.39
SHMT: rs1979277(A>G)	277	−0.0230 ± 0.0257	0.37	371	−0.0261 ± 0.0211	0.22
Cognitive domain 2						
MTHFR$_1$ vs. *MTHFR*$_4$	277	+0.1849 ± 0.1203	0.13	371	+0.0765 ± 0.1179	0.52
MTHFR$_2$ vs. *MTHFR*$_4$	277	+0.0194 ± 0.1226	0.87	371	−0.0285 ± 0.1092	0.79
MTHFR$_3$ vs. *MTHFR*$_4$	277	+0.1168 ± 0.1005	0.25	371	−0.0385 ± 0.0959	0.69
MTHFR$_5$ vs. *MTHFR*$_4$	277	+0.0333 ± 0.1224	0.79	371	+0.0138 ± 0.1322	0.92
MTHFR$_6$ vs. *MTHFR*$_4$	277	+0.1740 ± 0.1278	0.18	371	+0.0912 ± 0.1028	0.38
MTRR: rs1801394(G>A)	277	+0.0072 ± 0.0609	0.91	371	+0.0253 ± 0.0531	0.64
MTRR: rs1532268(T>C)	277	+0.0523 ± 0.0623	0.40	371	−0.1136 ± 0.0574	0.049[b]
MTR: rs1805087(G>A)	277	−0.0757 ± 0.0591	0.20	371	−0.0742 ± 0.0560	0.19
SHMT: rs1979277(A>G)	277	+0.0128 ± 0.0535	0.81	371	−0.0464 ± 0.0483	0.34

Key: AF, Animal, Fluency; BMI, body mass index (calculated as weight in kg/square of height in meters); BVRT, Benton Visual Retention Test; CVLT-DFR, California Verbal Learning Test—delayed free recall; CVLT-List A, California Verbal Learning Test—immediate recall; DS-B, Digits Span Backward; DS-F, Digits Span Forward; HANDLS, Healthy Aging in Neighborhoods of Diversity across the Life Span; LARCC, longitudinal annual rate of cognitive change; MMSE, Mini—Mental State Examination; MTHFR, methylenetetrahydrofolate reductase; MTR, methionine synthase; MTRR, methionine synthase reductase; OLS, ordinary least square; SHMT, serine hydroxymethyltransferase; SNP, single nucleotide polymorphism; SNPLC, SNP latent class; Trails A and B, Trail Making Test parts A and B.

[a] Based on multiple OLS regression models with outcome being cognitive annual rate of change. The model controlled for first-visit age, mean age at follow-up, education, first-visit smoking status, first-visit self-reported type 2 diabetes, hypertension, cardiovascular disease, and BMI. The 10 principal components obtained with multidimensional scaling (see Supplemental Method 3) were also added in a separate sensitivity analysis.

[b] Significant main effects after familywise Bonferroni correction: $p < 0.05$ for MMSE, BVRT, AF, and cognitive domains and $p < 0.025$ for other cognitive tests.

[c] $p < 0.05$ for null hypothesis that sex × SNPLC or sex × SNP interaction term = 0 in a model where main effect of sex was added.

[d] Cognitive scores were predicted at mean age of follow-up using a linear mixed model controlling for sex, race/ethnicity, education (y), and smoking status, with age (centered at 50 y) added among the fixed effect variables to allow for quadratic nonlinear change, whereas age (centered at 50 y) was added to the random effects to allow for individual-level variation in slopes. The slope or annual rate of change was predicted from these models at the mean age at follow-up. Using factor analysis, 2 factor scores were estimated and were labeled as LARCC in the following domains: Domain 1: "Verbal memory and fluency" and Domain 2: "Visual/working memory and executive function" (Supplemental Method 1). See Table 2 for more details on definition the SNP latent classes.

et al., 2016), thus depending on vitamin B-2 for its activity. Each of SHMT's 4 subunits uses vitamin B-6's active form as a cofactor (Renwick et al., 1998; Troesch et al., 2016). Finally, MTHFR uses flavin adenine dinucleotide (FAD), a cofactor derived from vitamin B-2 (Leclerc et al., 1998; Troesch et al., 2016). An imbalance in any one B-vitamin may alter OCM cycles and tHcy homeostasis (Troesch et al., 2016).

4.4. Strengths and limitations

Our study has several strengths, including a relatively large sample, a longitudinal study design, and the use of advanced statistical techniques by conducting both multiple linear mixed-effects regression models and OLS multiple linear regression analyses to test associations of OCM SNPs, SNPHAPs, and SNPLCs, with the annual rates of change in cognitive performance. Although used less frequently than haplotype analysis, LCA was conducted to examine clustering of genotypes within the key enzymes involved in the OCM cycles and the effect of that clustering on cognitive change over time.

Nevertheless, our study has notable limitations. First, the final analytic sample may have been selected in a non-random manner by oversampling certain groups from the African-American participants. A 2-stage Heckman selection model accounted for these biases (Heckman, 1979). Second, baseline age and follow-up durations varied among participants rendering data structure unbalanced. Mixed-effects regression models were therefore used to predict cognitive test scores and annual rates of change at specific

Table 4
MTHFR SNP haplotypes (SNPHAP: [rs1801133(C677T, A>G)/rs1801131(A1298C, G>T)]) and their associations with predicted LARCC by sex: multiple OLS regression analysis (n = 648–788; HANDLS study)

Characteristics	Predicted LARCC[a]					
	Men			Women		
	n	β ± SE[b]	p	n	β ± SE[b]	p
MMSE: Models A-C						
MTHFR$_1$: *GT*	349	+0.0008 ± 0.0016	0.61	439	+0.0002 ± 0.0011	0.89
MTHFR$_2$: *GG*	349	−0.0014 ± 0.0017	0.41	439	−0.0000 ± 0.0012	0.99
MTHFR$_3$: *AT*	349	−0.0024 ± 0.0025	0.33	439	−0.0009 ± 0.0016	0.59
BVRT: Models A-C						
MTHFR$_1$: *GT*	350	−0.0032 ± 0.0027	0.24	432	−0.0008 ± 0.0026	0.75
MTHFR$_2$: *GG*	350	+0.0039 ± 0.0028	0.16	432	−0.0005 ± 0.0028	0.86
MTHFR$_3$: *AT*	350	−0.0040 ± 0.0042	0.35	432	+0.0015 ± 0.0038	0.69
CVLT-List A: Models A-C						
MTHFR$_1$: *GT*	295	+0.0001 ± 0.0003	0.67	385	−0.0005 ± 0.0003	0.15
MTHFR$_2$: *GG*	295	−0.0006 ± 0.0003	0.11	385	+0.0001 ± 0.0003	0.79
MTHFR$_3$: *AT*	295	+0.0012 ± 0.0005	0.019[c]	385	+0.0006 ± 0.0005	0.23
CVLT-DFR: Models A-C						
MTHFR$_1$: *GT*	284	−0.0001 ± 0.0006	0.88	376	+0.0005 ± 0.0006	0.42
MTHFR$_2$: *GG*	284	+0.0006 ± 0.0008	0.36	376	−0.0001 ± 0.0006	0.53
MTHFR$_3$: *AT*	284	−0.0012 ± 0.0009	0.18	376	−0.0008 ± 0.0009	0.37
AF: Models A-C						
MTHFR$_1$: *GT*	356	−0.0010 ± 0.0019	0.59	441	−0.0023 ± 0.0016	0.17
MTHFR$_2$: *GG*	356	−0.0002 ± 0.0020	0.91	441	+0.0040 ± 0.0018	0.024[c,d]
MTHFR$_3$: *AT*	356	−0.0006 ± 0.0031	0.85	441	−0.0030 ± 0.0024	0.23
Trails A: Models A-C						
MTHFR$_1$: *GT*	326	−0.178 ± 0.100	0.071	419	−0.0225 ± 0.108	0.84
MTHFR$_2$: *GG*	326	+0.0594 ± 0.1034	0.57	419	+0.0429 ± 0.1187	0.72
MTHFR$_3$: *AT*	326	+0.2762 ± 0.151	0.068	419	+0.0272 ± 0.0157	0.86
Trails B: Models A-C						
MTHFR$_1$: *GT*	326	−0.3788 ± 0.2025	0.062	419	−0.1663 ± 0.1813	0.36
MTHFR$_2$: *GG*	326	+0.1050 ± 0.2135	0.62	419	+0.2170 ± 0.1992	0.28
MTHFR$_3$: *AT*	326	+0.5935 ± 0.3102	0.057	419	−0.1958 ± 0.2675	0.46
DS-F: Models A-C						
MTHFR$_1$: *GT*	351	+0.0001 ± 0.0009	0.91	431	−0.0003 ± 0.0008	0.74
MTHFR$_2$: *GG*	351	−0.0007 ± 0.0009	0.42	431	+0.0013 ± 0.0008	0.12
MTHFR$_3$: *AT*	351	+0.0006 ± 0.0013	0.65	431	−0.0022 ± 0.0011	0.052
DS-B: Models A-C						
MTHFR$_1$: *GT*	351	−0.0012 ± 0.0013	0.36	424	+0.0004 ± 0.0013	0.78
MTHFR$_2$: *GG*	351	+0.0012 ± 0.0013	0.35	424	+0.0017 ± 0.0014	0.24
MTHFR$_3$: *AT*	351	−0.0007 ± 0.0021	0.72	424	−0.0039 ± 0.0019	0.045
Cognitive domain 1: Models A-C						
MTHFR$_1$: *GT*	277	−0.0032 ± 0.031	0.92	371	−0.0364 ± 0.0263	0.88
MTHFR$_2$: *GG*	277	−0.0007 ± 0.0327	0.98	371	+0.0467 ± 0.0284	0.10
MTHFR$_3$: *AT*	277	−0.0123 ± 0.0461	0.79	371	−0.092 ± 0.0383	0.017[c,d]
Cognitive domain 2: Models A-C						
MTHFR$_1$: *GT*	277	−0.0651 ± 0.0636	0.31	371	−0.0161 ± 0.0596	0.79
MTHFR$_2$: *GG*	277	+0.0539 ± 0.0682	0.43	371	+0.0566 ± 0.0647	0.38
MTHFR$_3$: *AT*	277	+0.0124 ± 0.0962	0.90	371	−0.0928 ± 0.0872	0.29

Key: AF, Animal Fluency; BMI, body mass index (calculated as weight in kg/square of height in meters); BVRT, Benton Visual Retention Test; CVLT-DFR, California Verbal Learning Test—delayed free recall; CVLT-List A, California Verbal Learning Test—immediate recall; DS-B, Digits Span Backward; DS-F, Digits Span Forward; HANDLS, Healthy Aging in Neighborhoods of Diversity across the Life Span; LARCC, longitudinal annual rate of cognitive change; MMSE, Mini–Mental State Examination; MTHFR, methylenetetrahydrofolate reductase; MTR, methionine synthase; MTRR, methionine synthase reductase; OLS, ordinary least square; SHMT, serine hydroxymethyltransferase; SNP, single nucleotide polymorphism; SNPHAP, SNP haplotypes; Trails A and B, Trail Making Test parts A and B.

[a] Cognitive scores were predicted at mean age of follow-up using a linear mixed model controlling for sex, race/ethnicity, education (y), and smoking status, with age (centered at 50 y) added among the fixed effect variables to allow for quadratic nonlinear change, whereas age (centered at 50 y) was added to the random effects to allow for individual-level variation in slopes. The slope or annual rate of change was predicted from these models at the mean age at follow-up. Using factor analysis, 2 factor scores were estimated and were labeled as LARCC in the following domains: Domain 1: "Verbal memory and fluency" and Domain 2: "Visual/working memory and executive function" (Supplemental Method 1). See Table 2 for more details on definition the SNP haplotypes.

[b] Based on multiple OLS regression models with outcome being cognitive annual rate of change. Each model included one SNPHAP (Model A: SNPHAP$_1$, Model B: SNPHAP$_2$, and Model C: SNHAP$_3$) and controlled for first-visit age, mean age at follow-up, education, first-visit smoking status, first-visit self-reported type 2 diabetes, hypertension, cardiovascular disease, and BMI. The 10 principal components obtained with multidimensional scaling (see Supplemental Method 3) were also added in a separate sensitivity analysis.

[c] Significant main effects after familywise Bonferroni correction: $p < 0.05$ for MMSE, BVRT, AF, and cognitive domains and $p < 0.025$ for other cognitive tests.

[d] $p < 0.05$ for null hypothesis that sex × SNPHAP interaction term = 0 in a model where main effect of sex was added.

ages where data was most dense to estimate the LARCC. Those models assume missingness at random, even though informative censoring may occur. However, due to a relatively younger age distribution in our study, dropout due to cognitive impairment is less likely than for older study populations (Ibrahim and Molenberghs, 2009). The younger baseline age group, with only 1.7 repeats on average and 5 y follow-up may also limit the ability to find clinically significant cognitive change. Our main OLS regression models were additionally controlled for both first-visit and mean age at follow-up. Third, serum tHcy was not available at the time of the analysis to examine tHcy-gene interaction and its potential role in mediating the association between *MTHFR* gene polymorphisms and age-related cognitive decline. Moreover, such interaction (as well as potential mediating role) can only be tested in larger samples with higher statistical power. Finally, positive findings may have been due to chance, residual confounding by key unmeasured

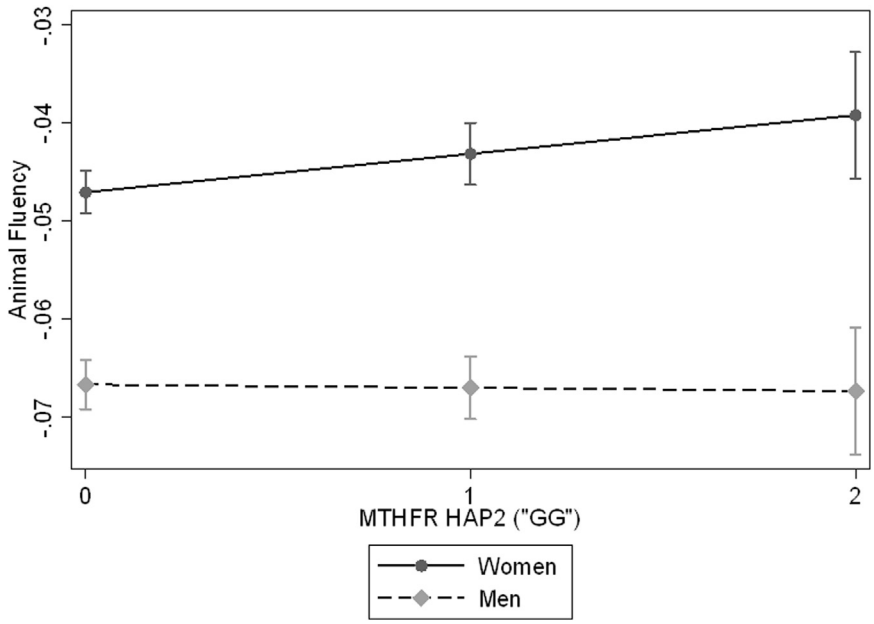

Fig. 3. Predictive margins with 95% CI for Animal Fluency LARCC by *MTHFR*$_2$ *SNPHAP* [rs1801133(C677T, A>G)/rs1801131(A1298C, G>T): GG], for men and women: Multiple OLS regression models. Abbreviations: LARCC, longitudinal annual rate of cognitive change; OLS, ordinary least square; *MTHFR*, methylenetetrahydrofolate reductase; SNPHAP, single nucleotide polymorphism haplotype. Note: Predictive margins estimated from the multiple linear regression model with Animal Fluency LARCC as the main outcome and MTHFR$_2$ SNPHAP interacted with sex. For list of covariates adjusted for, see Table 4.

factors or selection bias due to unequal probability of selection from the initial study sample of African Americans, whereas negative findings may be due to low statistical power. Some of the positive findings were statistically but not necessarily clinically significant. For instance, as the CVLT-List A score declines on average by 0.280 units per year with an SD of 0.018, the difference between 2 SNPLCs (MTHFR5 vs. MTHFR4) was −0.0015, indicating ∼8.3% SD in change, a weak net effect. Thus, those findings should be interpreted with caution until they are replicated elsewhere on comparable adult populations.

5. Conclusions

In summary, OCM enzymatic gene variations can alter age-related cognitive trajectories among African-American urban adults, specifically in domains of visual and verbal memory and in verbal fluency. Future studies should examine associations of those SNPs, SNPLCs, and SNPHAPs with incident dementia, AD, and mild cognitive impairment in comparable populations, using a longitudinal study with an extended follow-up period.

Disclosure

None.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.neurobiolaging.2019.05.013.

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Supplemental Figures 1-3

Fig S1. (A)

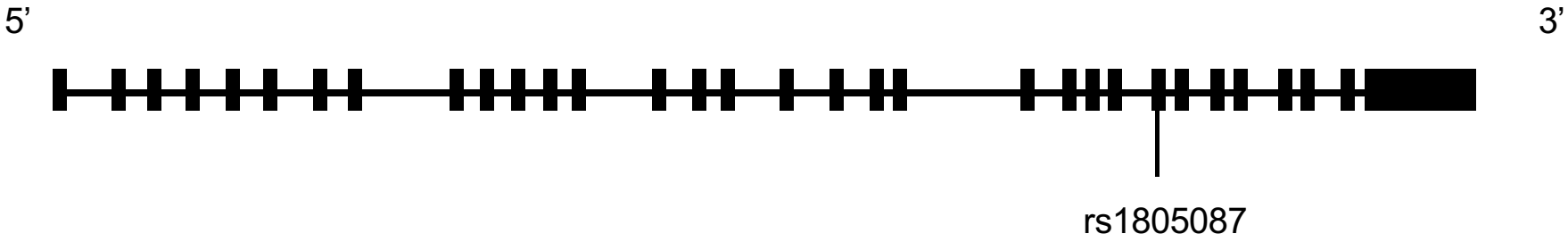


Fig S1. (B)

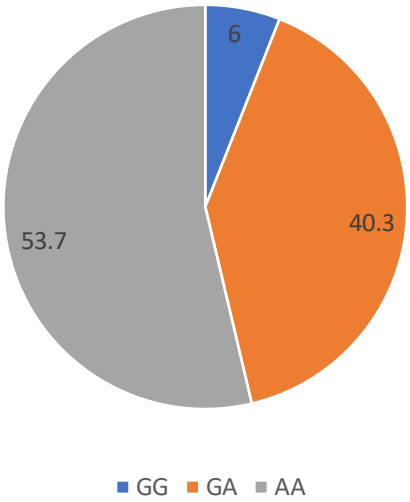


Fig S2. (A)

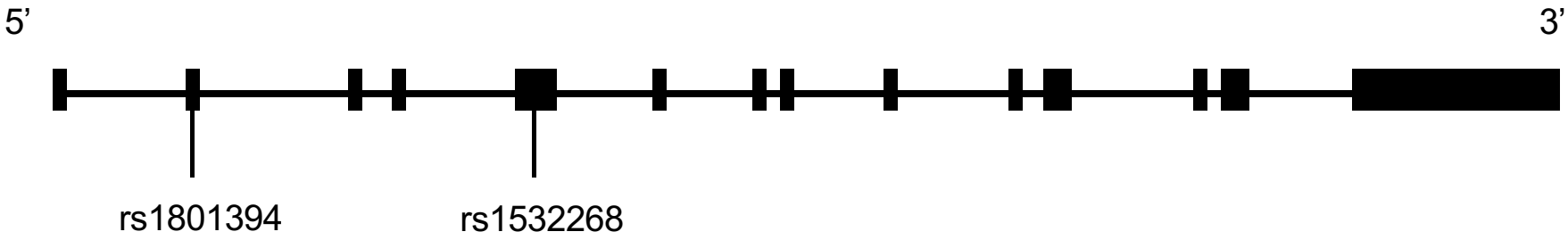


Fig S2. (B)

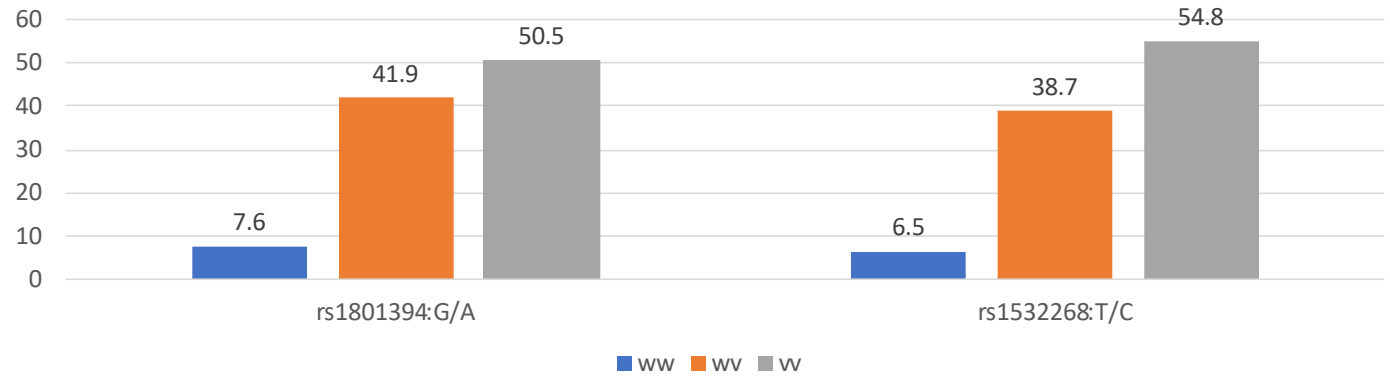


Fig S3. (A)

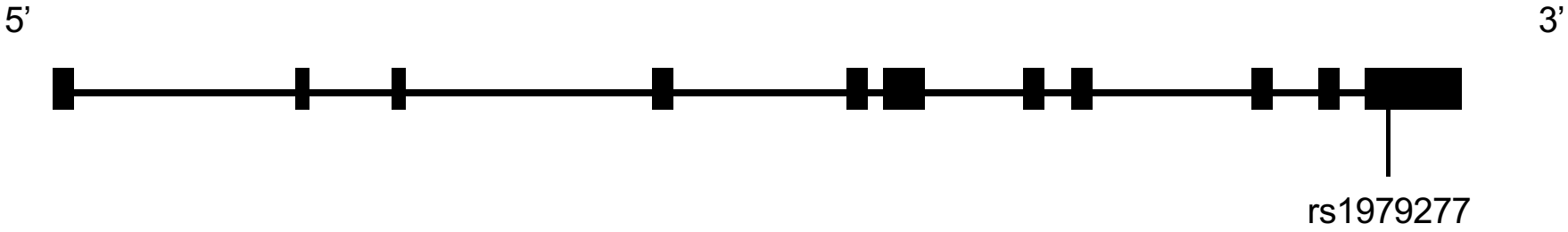
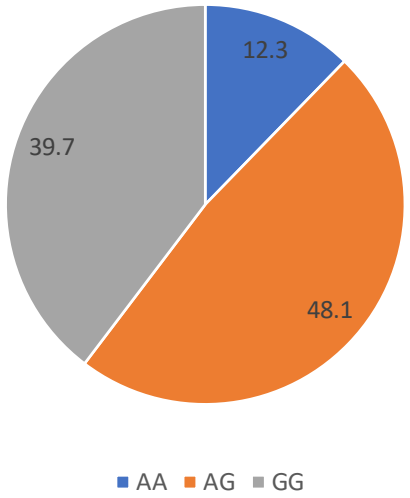


Fig S3. (B)



Supplemental Method 1: Description of Cognitive Tests

Mini-Mental State Examination (MMSE)

The MMSE (Folstein, et al., 1975) is a brief mental status test and global cognitive functioning measuring orientation, concentration, immediate and delayed memory, language and constructional praxis. Scores range from 0 to 30, with higher scores indicating better cognitive performance.

California Verbal Learning Test (CVLT)

The CVLT (Delis, et al., 1988) is a 16-item shopping list measuring verbal learning and memory. A modified version of the CVLT was used with three, rather than five, list A learning trials. Cued recall was not administered. Variables of interest in this study were total correct for List A sum across trials 1-3 and List A long-delay free recall. Scores ranged from 0 to 48 for List A sum and 0 to 16 for List A long-delay free recall. Higher scores indicate better verbal memory. The CVLT is described in detail elsewhere (Delis, et al., 1988).

Benton Visual Retention Test (BVRT)

The BVRT (Benton, 1974) is a test of short-term figural memory and visuo-constructional abilities. Administration A, Form D was used. Two trained examiners independently scored the BVRT using a modified error scoring system, based on the BVRT Manual scoring. A consensus was achieved for discrepancies in scoring. If a consensus between the two examiners could not be reached, a research psychologist assigned the score. Scores were total errors, such that higher values indicate poorer visual memory.

Digit Span Forward and Backward (DS-F and DS-B)

The Wechsler Adult Intelligence Scale, Revised(Wechsler, 1981) Digit Span Forward and Backward are tests of attention and executive functioning, specifically working memory. They were administered according to standard instructions, and the total score was the total number correct for each test.

Animal Fluency (AF)

Animal fluency, a measure of semantic verbal fluency, requires participants to generate as many animals as possible for 60 seconds. Higher scores indicate better verbal fluency, with the total number of words, minus intrusions and perseverations analyzed.

Trail Making Tests A and B (Trails A and Trails B)

Trailmaking test A and B(Reitan, 1992) are tests of attention and executive functioning, respectively, specifically cognitive control and visuo-motor scanning. Participants were instructed to draw lines between consecutive numbers (Trails A) or alternate between numbers and letter (Trails B) as fast as they could while a stop watch recorded time. When errors were committed the participant corrected the error by returning to his/her last correct response and continued from there. The stop-watch ran while corrections were made. Scores reflected time to completion (in seconds) separately for Trails A and B. Higher scores indicate poorer performance.

Supplemental References:

Benton, A.L. 1974. Revised visual retention test (fifth edition). The Psychological Corportation, New York.

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Supplemental Method 1: Linear mixed models for prediction of cognitive performance, factor analysis of LARCC

A standard taxonomy of models (Singer and Willet, 2003) was used, starting from the unconditional means model (Model A), unconditional growth model (Model B), growth model with level-2 controlled effects of other factors namely sex, race/ethnicity, education and smoking status (Model C), growth model with level-2 controlled effects of other factors, adding a squared-age term that would allow the rate of change to vary with time (Model D). In all models, age was centered at 50 years, while education was centered at 16 years. The following equations apply to each of the models considered:

Model	Level-1 model	Level-2 model	Composite model
A	$Y_{ij} = \pi_{0i} + \varepsilon_{ij}$	$\pi_{0i} = \gamma_{00} + \zeta_{0i}$	$Y_{ij} = \gamma_{00} + (\zeta_{0i} + \varepsilon_{ij})$
B	$Y_{ij} = \pi_{0i} + \pi_{1i} Age_{50} + \varepsilon_{ij}$	$\pi_{0i} = \gamma_{00} + \zeta_{0i}$ $\pi_{1i} = \gamma_{10} + \zeta_{1i}$	$Y_{ij} = \gamma_{00} + \gamma_{10} Age_{50} + (\zeta_{0i} + \zeta_{1i} Age_{50} + \varepsilon_{ij})$
C	$Y_{ij} = \pi_{0i} + \pi_{1i} Age_{50} + \varepsilon_{ij}$	$\pi_{0i} = \gamma_{00} + \sum_{k=1}^{7} \gamma_{0k} Z_{ik} + \zeta_{0i}$ $\pi_{1i} = \gamma_{10} + \sum_{k=1}^{7} \gamma_{1k} Z_{ik} + \zeta_{1i}$	$Y_{ij} = \gamma_{00} + \sum_{k=1}^{7} \gamma_{0k} Z_{ik} + \gamma_{10} Age_{50} + \sum_{k=1}^{7} \gamma_{1k} Z_{ik} Age_{50} + (\zeta_{0i} + \zeta_{1i} Age_{50} + \varepsilon_{ij})$
D	$Y_{ij} = \pi_{0i} + \pi_{1i} Age_{50} + \varepsilon_{ij}$	$\pi_{0i} = \gamma_{00} + \sum_{k=1}^{7} \gamma_{0k} Z_{ik} + \zeta_{0i}$ $\pi_{1i} = \gamma_{10} + \sum_{k=1}^{8} \gamma_{1k} Z_{ik} + \zeta_{1i}$	$Y_{ij} = \gamma_{00} + \sum_{k=1}^{7} \gamma_{0k} Z_{ik} + \gamma_{10} Age50 + \sum_{k=1}^{8} \gamma_{1k} Z_{ik} Age_{50} + (\zeta_{0i} + \zeta_{1i} Age_{50} + \varepsilon_{ij})$

Notations: Y_{ij} is the response variable for each individual "i" and age at visit "j". π_{0i} is the level-1 intercept for individual i; π_{1i} is the level-1 slope for individual i; γ_{00} is the level-2 intercept of the

random intercept π_{0i}; γ_{10} is the level-2 intercept of the slope π_{1i}; Z_{ik} is a vector of fixed covariates for each individual i that are used to predict level-1 intercepts and slopes; ζ_{0i} and ζ_{1i} are level-2 disturbances; ε_{ij} is the within-person level-1 disturbance. In model D, an additional Z_{ik} variable is added for Age_{50}, to account for quadratic age changes in the fixed effects portion of the model, which increased the number of k terms from 7 to 8 between models C and D.

Model D's improvement in fit compared to the simpler models was evaluated using Deviance, AIC and BIC statistics as well as pseudo-R^2. In addition, residuals were plotted against predicted values to assess their normality. It is worth noting that the models were fit using the entire HANDLS cohort with complete data on either waves 1 or 3 on cognitive tests was used to improve reliability of predicted estimates. Finally, empirical Bayes estimators of outcomes Y_{ij} were predicted from Model D at specific ages using the following method, after estimating the random effects (ζ_{0i} for the intercept and ζ_{1i} for the slope) for each individual *i*:

Intercept $$\pi_{0i} = \gamma_{00} + \sum_{k=1}^{7} \gamma_{0k} Z_{ik} + \zeta_{0i}$$

Slope $$\pi_{1i} = \gamma_{10} + \sum_{k=1}^{8} \gamma_{1k} Z_{ik} + \zeta_{1i}$$

Prediction $$Y_{ij} = \pi_{0i} + \pi_{1i}(Age_{50})_i$$

where $(Age_{50})_i$ is assigned individual mean age at follow-up values centered at age 50, thus positive values if Age>50 and negative values if Age<50.

Y_{ij} in this case is the cognitive score for a specific test j and individual i. Slopes π_{1i} were estimated for each test j and individual i, taking into account non-linear changes with age (i.e. the age-square term) at individual-level mean follow-up age and those were labeled as LARCC (Longitudinal annual rate of cognitive change) and interpreted as annual rate of change in each cognitive score at mean follow-up age.

Following this estimation, LARCC for each cognitive test score were entered into a factor analysis model as measured variables (Sharma, 1996) in which a number of common factors were extracted based on common variance, factor loadings estimated and the residual variance labeled as uniqueness for each LARCC. The common factor model can be summarized as follows:

$$LARCC_i = \sum_{j=1}^{k} \lambda_{ij} * Domain_j + \varphi_i$$

Where LARCCi is the standardized z-score for each cognitive test LARCC, λ_{ij} is the factor loading for each LARCC and each factor, $Domain_j$ is the standardized z-score for each factor j, and φ_i is the residual error, the squared value of which is the uniqueness. The sum of squared factor loadings for each $LARCC_i$ is the communality or the common variance that is accounted for by the extracted factors.

An eigenvalue>1 rule was used and the scree plot was observed to determine the adequate number of extracted factors that would produce the best model fit. The factor loadings were then rotated using varimax orthogonal rotation and the factors were interpreted, and cognitive domains labeled accordingly, with cutoff point of 0.40 or more for significant loading. The factor scores (*z*-scores) were predicted and used as markers of LARCC for specific cognitive domains.

Appendix Table 1. Varimax rotated two-factor solution of LARCC, using nine cognitive test scores LARCC as measured variables.

	Factor loadings, λ_{ij}		Uniqueness, φ_i
$LARCC_i$	Domain 1	Domain 2	
BVRT	-0.26	+0.64*	0.52
CVLT-List A	+0.71*	0.17	0.47
CVLT-DR	+0.81*	+0.13	0.32
AF	+0.55*	+0.23	0.64
Trails A	-0.011	+0.27	0.93
Trails B	+0.15	+0.64*	0.56
DS-F	+0.55*	+0.49*	0.45
DS-B	+0.26	+0.67*	0.48
Eigenvalue	2.31	1.31	
% var explained	0.65	0.37	

Note: See list of abbreviations.

*Factor loading>0.40. Domains were labeled as follows: "Domain 1: "Verbal memory and fluency", Domain 2: "Visual/working memory and executive function", based on the combination of significantly high factor loadings and the corresponding measured variables or $LARCC_i$. With the exception of Trails A and DS-F, all $LARCC_i$ factor loadings were significant only for one of the two domains, creating a relatively simple structure that is easy to label and interpret. The labels were determined based on the nature of the cognitive test, as described in OSM 1.

Supplemental References:

Sharma, S. 1996. Applied multivariate techniques. Wiley, USA.
Singer, J.D., Willet, J.B. 2003. Applied Longitudinal Data Analysis: Modeling change and event occurrence. Oxford University Press, New York.

Supplemental Method 3: Genetic data quality control

Sample quality control inclusion criteria were: **(1)** concordance between self-reported sex and X-chromosome estimated sex; **(2)** sample call rate >95%, **(3)** concordance between self-reported African ancestry and ancestry estimated using genotyped SNPs, and **(4)** proportional sharing of genotypes < 15% between samples, excluding close relatives from the final sample. SNPs in HANDLS were selected when the following criteria were met: **(1)** Hardy-Weinberg equilibrium p-value (HWE $P > 10^{-7}$); **(2)** Missing by haplotype $P > 10^{-7}$; **(3)** Minor allele frequency>0.01, and **(4)** SNP call rate >95%. Quality control and data management for each genotype was conducted using PLINKv1.06.(Purcell, et al., 2007) Cryptic relatedness was estimated via pairwise identity by descent analyses in PLINK and confirmed using RELPAIR.(Epstein, et al., 2000) STRUCTUREv2.3 (Falush, et al., 2003,Falush, et al., 2007,Pritchard, et al., 2000) and multidimensional scaling (MDS) function in PLINKv1.06 were applied to determine ancestry among HANDLS participants. HANDLS participants with component vector estimates consistent with the HapMap African ancestry samples for the first 4 component vectors were included. Moreover, in a sensitivity analysis, we adjusted for all the first 10 principal components obtained from genotype data with MDS to control for residual effects of population structure.(Price, et al., 2006). SNPs that passed quality control criteria were used for genotype imputation with MACH and minimac software (http://www.sph.umich.edu/csg/abecasis/mach/). The 1000 Genomes Project phase 1 alpha freeze multiethnic panel were used as a reference population for genotype imputation. SNPs with imputation quality measure of $R^2<0.3$ or minor allele frequency of <1% were excluded from further analyses.

SNP	Al1	Al2	MAF	R-square	Note
rs4846049	G	T	0.47229	0.97729	
rs2274976	C	T	0.03507	0.99117	low frequency variant
rs1476413	C	T	0.16954	0.99418	
rs17375901	C	T	0.02913	0.9407	low frequency variant
rs4846051	A	G	0.32311	0.99914	
rs1801131	T	G	0.17517	0.99638	
rs2066462	G	A	0.09509	0.98793	
rs1801133	G	A	0.08838	0.99645	
rs17037396	C	T	0.09229	1.00048	
rs9651118	T	C	0.0584	0.83601	
rs17367504	A	G	0.10864	0.99913	
rs2066470	G	A	0.0708	1.00049	
rs1801394	A	G	0.28493	0.97601	
rs1532268	C	T	0.25864	0.99988	
rs1805087	A	G	0.26124	1.00044	
rs1979277	G	A	0.36154	0.98763	
rs5742905	A	G	0.00003	0.00746	low quality SNP, consider excluding any snp with R-square of < 0.30

SNP ID	Chr	basepair position	Ref	Alt	Function	SNP location with gene	Gene	Exonic Function	Amino Acid change
rs4846049	1	11850365	G	T	UTR3		MTHFR		
rs2274976	1	11850927	C	T	exonic	exon 12	MTHFR	nonsynonymous	R -> Q
rs1476413	1	11852300	C	T	intronic		MTHFR		
rs17375901	1	11852516	C	T	intronic		MTHFR		
rs4846051	1	11854457	A	G	exonic	exon 8	MTHFR	synonymous	F -> F
rs1801131	1	11854476	T	G	exonic	exon 8	MTHFR	nonsynonymous	E -> A
rs2066462	1	11854896	G	A	exonic	exon 7	MTHFR	synonymous	S -> S
rs1801133	1	11856378	G	A	exonic	exon 5	MTHFR	nonsynonymous	A -> V
rs17037396	1	11862047	C	T	intronic		MTHFR		
rs9651118	1	11862214	T	C	intronic		MTHFR		
rs17367504	1	11862778	A	G	intronic		MTHFR		
rs2066470	1	11863057	G	A	exonic	exon 2	MTHFR	synonymous	P -> P
rs1805087	1	2.37E+08	A	G	exonic	exon 25	MTR	nonsynonymous	D -> G
rs1801394	5	7870973	A	G	exonic	exon 2	MTRR	nonsynonymous	I -> M
rs1532268	5	7878179	C	T	exonic	exon 5	MTRR	nonsynonymous	S -> L
rs1979277	17	18232096	G	A	exonic	exon 11	SHMT	nonsynonymous	L -> F
rs5742905	21	44483184	A	G	exonic	exon 7	CBS	nonsynonymous	I -> T

Source: https://www.ncbi.nlm.nih.gov/pubmed/20601685

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Epstein, M.P., Duren, W.L., Boehnke, M. 2000. Improved inference of relationship for pairs of individuals. Am J Hum Genet 67(5), 1219-31. doi:S0002-9297(07)62952-8 [pii]
10.1016/S0002-9297(07)62952-8.

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Pritchard, J.K., Stephens, M., Donnelly, P. 2000. Inference of population structure using multilocus genotype data. Genetics 155(2), 945-59.

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10.1086/519795.

Table S1. Selected *MTHFR* gene single nucleotide polymorphisms' (SNP) associations with predicted longitudinal annual rate of cognitive change (LARCC): Multiple OLS regression analysis, (*n*=648-788); HANDLS study

	Predicted LARCC[1]								
	Total			Men			Women		
	n	β±SE	P	n	β±SE	P	n	β±SE	P
MMSE: Models A-J									
rs4846049(T>G)	788	-0.0003±0.0008	0.73	349	-0.0003±0.0013	0.85	439	+0.0002±0.0009	0.83
rs1476413(T>C)	788	-0.0007±0.0010	0.49	349	-0.0007±0.0017	0.68	439	-0.0002±0.0013	0.90
rs4846051(A1317G, G>A)	788	+0.0000±0.0008	0.95	349	-0.0003±0.0014	0.81	439	+0.0008±0.0010	0.44
rs1801131(A1298C,G>T)	788	-0.0005±0.0010	0.62	349	-0.0002±0.0017	0.90	439	-0.00028±0.0012	0.82
rs2066462(C1056T,A>G)	788	+0.0002±0.0013	0.90	349	+0.0003±0.0023	0.91	439	+0.0003±0.0015	0.83
rs1801133(C677T,A>G)	788	***+0.0024±0.0013***	***0.078***	349	***+0.0045±0.0024***	***0.054***	439	+0.0003±0.0016	0.86
rs1703796(T>C)	788	-0.0008±0.0013	0.57	349	-0.0020±0.0024	0.40	439	-0.0001±0.0015	0.96
rs9651118(C>T)	788	+0.0001±0.0016	0.96	349	+0.0025±0.0026	0.34	439	-0.0028±0.0020	0.17
rs17367504 (G>A)	788	-0.0005±0.0012	0.64	349	-0.0006±0.0020	0.75	439	-0.00037±0.0015	0.81
rs2066470(A>G)	788	-0.0002±0.0016	0.87	349	-0.0011±0.0027	0.69	439	+0.00023±0.0018	0.90
BVRT: Models A-J									
rs4846049(T>G)	782	+0.0011±0.0015	0.48	350	+0.0006±0.0022	0.78	432	+0.0012±0.0022	0.58
rs1476413(T>C)	782	-0.0019±0.0020	0.34	350	-0.0043±0.0028	0.15	432	-0.0007±0.0028	0.82
rs4846051(A1317G, G>A)	782	+0.0027±0.0016	0.10	350	**+0.0049±0.0024**	**0.042**[3]	432	+0.0012±0.0023	0.60
rs1801131(A1298C,G>T)	782	-0.0022±0.0019	0.25	350	***-0.0051±0.0028***	***0.064***	432	-0.0002±0.0028	0.95
rs2066462(C1056T,A>G)	782	***-0.0043±0.0026***	***0.094***	350	***-0.0072±0.0039***	***0.067***	432	-0.0031±0.0035	0.38
rs1801133(C677T,A>G)	782	-0.0011±0.0027	0.67	350	+0.0009±0.0040	0.82	432	-0.0025±0.0036	0.49
rs1703796(T>C)	782	-0.0000±0.0026	1.00	350	-0.0033±0.0041	0.42	432	+0.0024±0.0035	0.49
rs9651118(C>T)	782	-0.0018±0.0032	0.57	350	-0.0062±0.0043	0.15	432	+0.0032±0.0047	0.49
rs17367504 (G>A)	782	-0.0013±0.0024	0.58	350	-0.0046±0.0033	0.17	432	+0.0020±0.0034	0.55
rs2066470(A>G)	782	-0.0020±0.0030	0.52	350	-0.0041±0.0046	0.37	432	+0.0005±0.0041	0.90
CVLT-List A: Models A-J									
rs4846049(T>G)	680	+0.0006±0.0019	0.73	295	+0.0003±0.0003	0.28	385	+0.0000±0.0002	0.86
rs1476413(T>C)	680	+0.0001±0.0002	0.78	295	**+0.0007±0.0004**	**0.047**	385	-0.0002±0.0003	0.50
rs4846051(A1317G, G>A)	680	-0.0001±0.0002	0.77	295	-0.0002±0.0003	0.50	385	+0.0001±0.0003	0.67
rs1801131(A1298C,G>T)	680	+0.0001±0.0002	0.71	295	**+0.0007±0.0003**	**0.048**[4]	385	-0.0002±0.0003	0.49
rs2066462(C1056T,A>G)	680	+0.0003±0.0003	0.29	295	**+0.0013±0.0005**	**0.005**[3,4]	385	-0.0002±0.0004	0.71
rs1801133(C677T,A>G)	680	**-0.0007±0.0003**	**0.028**	295	-0.0008±0.0005	0.10	385	-0.0007±0.0004	0.10
rs1703796(T>C)	680	+0.0002±0.0003	0.64	295	+0.0007±0.0005	0.15	385	-0.0001±0.0004	0.79
rs9651118(C>T)	680	+0.0004±0.0004	0.29	295	**+0.0012±0.0005**	**0.020**[3,4]	385	-0.0003±0.0006	0.55

rs17367504 (G>A)	680	+0.0003±0.0003	0.29	295	**+0.0009±0.0004**	**0.034**	385	-0.0001±0.0004	0.76
rs2066470(A>G)	680	+0.0003±0.0004	0.50	295	+0.0006±0.0006	0.27	385	+0.0000±0.0005	0.96
CVLT-DFR: Models A-J									
rs4846049(T>G)	660	-0.0002±0.0003	0.52	284	-0.0006±0.0005	0.20	376	-0.0001±0.0005	0.84
rs1476413(T>C)	660	-0.0001±0.0004	0.82	284	-0.0009±0.0006	0.15	376	+0.0002±0.0006	0.75
rs4846051(A1317G, G>A)	660	+0.0000±0.0004	0.95	284	-0.0000±0.0005	0.95	376	-0.0000±0.0005	1.00
rs1801131(A1298C,G>T)	660	-0.0001±0.0004	0.84	284	-0.0007±0.0006	0.28	376	+0.0001±0.0006	0.80
rs2066462(C1056T,A>G)	660	-0.0003±0.0006	0.54	284	***-0.0015±0.0008***	***0.079***	376	+0.0002±0.0008	0.84
rs1801133(C677T,A>G)	660	+0.0008±0.0006	0.17	284	+0.0009±0.0009	0.29	376	+0.0008±0.0008	0.32
rs1703796(T>C)	660	-0.0002±0.0006	0.71	284	-0.0014±0.0009	0.12	376	+0.0004±0.0007	0.62
rs9651118(C>T)	660	-0.0011±0.0007	0.12	284	**-0.0021±0.0009**	**0.023**[3]	376	+0.0000±0.0010	0.98
rs17367504 (G>A)	660	-0.0001±0.0005	0.83	284	-0.0008±0.0007	0.26	376	+0.0005±0.0008	0.50
rs2066470(A>G)	660	-0.0004±0.0007	0.60	284	-0.0014±0.0010	0.18	376	+0.0003±0.0009	0.76
AF: Models A-J									
rs4846049(T>G)	797	+0.0006±0.0010	0.56	356	+0.0019±0.0016	0.27	441	-0.0003±0.0013	0.81
rs1476413(T>C)	797	***-0.0013±0.0011***	***0.083***	356	-0.0003±0.0021	0.88	441	-0.0043±0.0018	0.016
rs4846051(A1317G, G>A)	797	**+0.0023±0.0014**	**0.014**[3]	356	***+0.0033±0.0017***	***0.054***	441	+0.0024±0.0015	0.10
rs1801131(A1298C,G>T)	797	**-0.0027±0.0011**	**0.036**[3]	356	-0.0014±0.0020	0.51	441	**-0.0042±0.0018**	**0.017**[3]
rs2066462(C1056T,A>G)	797	***-0.0031±0.0017***	***0.070***	356	-0.0041±0.0028	0.14	441	-0.0026±0.0022	0.23
rs1801133(C677T,A>G)	797	+0.0002±0.0018	0.92	356	-0.0027±0.0029	0.34	441	+0.0023±0.0023	0.32
rs1703796(T>C)	797	-0.0009±0.0017	0.61	356	+0.0001±0.0029	0.96	441	-0.0019±0.0022	0.38
rs9651118(C>T)	797	-0.0016±0.0021	0.45	356	-0.0013±0.0031	0.68	441	-0.0022±0.0029	0.47
rs17367504 (G>A)	797	***-0.0027±0.0016***	***0.094***	356	-0.0032±0.0025	0.19	441	-0.0025±0.0022	0.25
rs2066470(A>G)	797	-0.0017±0.0020	0.41	356	-0.0030±0.0033	0.37	441	-0.0010±0.0026	0.69
Trails A: Models A-J									
rs4846049(T>G)	745	-0.0747±0.0616	0.23	326	-0.0119±0.0841	0.89	419	***-0.1679±0.0897***	***0.062***
rs1476413(T>C)	745	-0.0365±0.0800	0.65	326	-0.1008±0.1050	0.34	419	-0.0234±0.1199	0.84
rs4846051(A1317G, G>A)	745	-0.0606±0.0657	0.36	326	+0.0277±0.0895	0.76	419	-0.1486±0.0959	0.12
rs1801131(A1298C,G>T)	745	-0.0128±0.0781	0.87	326	-0.0639±0.1028	0.54	419	-0.0128±0.1170	0.91
rs2066462(C1056T,A>G)	745	+0.0936±0.1041	0.37	326	+0.0031±0.1468	0.98	419	+0.1193±0.1490	0.42
rs1801133(C677T,A>G)	745	-0.1243±0.1044	0.24	326	***-0.2629±0.1435***	***0.068***	419	+0.0262±0.1509	0.86
rs1703796(T>C)	745	+0.0245±0.1051	0.82	326	-0.1839±0.1501	0.22	419	+0.1385±0.1471	0.35
rs9651118(C>T)	745	+0.0722±0.1247	0.56	326	+0.1172±0.1590	0.46	419	+0.0312±0.1929	0.87
rs17367504 (G>A)	745	+0.0508±0.0960	0.60	326	-0.0707±0.1253	0.57	419	+0.1520±0.1446	0.29
rs2066470(A>G)	745	-0.0381±0.1211	0.75	326	***-0.2917±0.1665***	***0.081***	419	+0.1433±0.1731	0.41
Trails B: Models A-J									
rs4846049(T>G)	745	-0.0473±0.1123	0.67	326	+0.0592±0.1731	0.73	419	-0.1910±0.1508	0.21

rs1476413(T>C)	745	-0.1352±0.1460	0.36	326	-0.0346±0.2162	0.87	419	-0.3010±0.2009	0.14
rs4846051(A1317G, G>A)	745	+0.0607±0.1196	0.61	326	+0.1639±0.1842	0.37	419	-0.0514±0.1612	0.75
rs1801131(A1298C,G>T)	745	-0.1919±0.1423	0.18	326	-0.1389±0.2114	0.51	419	-0.2981±0.1961	0.13
rs2066462(C1056T,A>G)	745	**-0.4304±0.1894**	**0.023**[3]	326	-0.4644±0.2994	0.12	419	***-0.4403±0.2494***	***0.078***
rs1801133(C677T,A>G)	745	-0.3008±0.1906	0.12	326	**-0.5992±0.2946**	**0.043**	419	+0.0254±0.2543	0.92
rs1703796(T>C)	745	-0.1435±0.1917	0.46	326	-0.0707±0.3086	0.82	419	-0.1867±0.2472	0.45
rs9651118(C>T)	745	+0.2975±0.2273	0.19	326	+0.2198±0.3274	0.50	419	+0.3867±0.3236	0.23
rs17367504 (G>A)	745	***-0.3074±0.1749***	***0.079***	326	-0.1298±0.2570	0.61	419	***-0.4661±0.2422***	***0.055***
rs2066470(A>G)	745	-0.3357±0.2206	0.13	326	-0.0849±0.3430	0.81	419	***-0.4828±0.2901***	***0.097***
DS-F: Models A-J									
rs4846049(T>G)	782	+0.0000±0.0005	0.92	351	+0.0005±0.0007	0.49	431	-0.0005±0.0006	0.41
rs1476413(T>C)	782	-0.0005±0.0006	0.42	351	+0.0001±0.0009	0.88	431	-0.0013±0.0008	0.13
rs4846051(A1317G, G>A)	782	-0.0002±0.0005	0.74	351	+0.0001±0.0008	0.93	431	-0.0004±0.0006	0.58
rs1801131(A1298C,G>T)	782	-0.0005±0.0006	0.44	351	+0.0003±0.0009	0.74	431	***-0.0014±0.0008***	***0.084***
rs2066462(C1056T,A>G)	782	-0.0001±0.0008	0.54	351	+0.0008±0.0012	0.51	431	-0.0011±0.0010	0.28
rs1801133(C677T,A>G)	782	+0.0003±0.0008	0.74	351	-0.0012±0.0013	0.33	431	+0.0017±0.0011	0.11
rs1703796(T>C)	782	+0.0003±0.0008	0.75	351	+0.0015±0.0013	0.25	431	-0.0010±0.0010	0.35
rs9651118(C>T)	782	+0.0005±0.0010	0.60	351	-0.0010±0.0014	0.46	431	+0.0022±0.0014	0.11
rs17367504 (G>A)	782	-0.0008±0.0007	0.30	351	+0.0000±0.0011	0.99	431	***-0.0017±0.0010***	***0.093***
rs2066470(A>G)	782	-0.0006±0.0009	0.49	351	+0.0010±0.0014	0.47	431	***-0.0022±0.0012***	***0.073***
DS-B: Models A-J									
rs4846049(T>G)	775	-0.0006±0.0008	0.43	351	-0.0013±0.0011	0.22	424	-0.0003±0.0011	0.75
rs1476413(T>C)	775	-0.0012±0.0010	0.22	351	-0.0013±0.0014	0.35	424	-0.0020±0.0014	0.17
rs4846051(A1317G, G>A)	775	+0.0002±0.0008	0.81	351	-0.0000±0.0012	0.99	424	+0.0006±0.0011	0.63
rs1801131(A1298C,G>T)	775	-0.0012±0.0010	0.21	351	-0.0016±0.0013	0.23	424	-0.0016±0.0014	0.25
rs2066462(C1056T,A>G)	775	-0.0014±0.0013	0.78	351	-0.0007±0.0018	0.70	424	***-0.0030±0.0018***	***0.093***
rs1801133(C677T,A>G)	775	+0.0016±0.0013	0.24	351	-0.0001±0.0019	0.96	424	***+0.0036±0.0018***	***0.053***
rs1703796(T>C)	775	+0.0008±0.0013	0.56	351	+0.0021±0.0019	0.28	424	-0.0007±0.0018	0.71
rs9651118(C>T)	775	+0.0005±0.0016	0.76	351	-0.0035±0.0021	0.11[4]	424	***+0.0044±0.0023***	***0.062***
rs17367504 (G>A)	775	-0.0009±0.0012	0.44	351	-0.0017±0.0016	0.28	424	-0.0005±0.0018	0.77
rs2066470(A>G)	775	-0.0006±0.0015	0.67	351	+0.0010±0.0022	0.64	424	-0.0023±0.0021	0.28
Cognitive domain 1: Models A-J									
rs4846049(T>G)	648	-0.0067±0.0158	0.67	277	-0.0016±0.0245	0.95	371	-0.0159±0.0217	0.47
rs1476413(T>C)	648	-0.0226±0.0215	0.29	277	-0.0198±0.0340	0.56	371	-0.0344±0.0287	0.23
rs4846051(A1317G, G>A)	648	+0.0066±0.0171	0.70	277	+0.0116±0.0259	0.66	371	+0.0037±0.0236	0.88
rs1801131(A1298C,G>T)	648	-0.0244±0.0209	0.24	277	-0.0010±0.0323	0.76	371	-0.0441±0.0282	0.12
rs2066462(C1056T,A>G)	648	-0.0164±0.0279	0.56	277	-0.0264±0.0461	0.57	371	-0.0245±0.0362	0.50

rs1801133(C677T,A>G)	648	+0.0443±0.0276	0.11	277	-0.0085±0.0441	0.85	371	**+0.0840±0.0364**	**0.022**[3]
rs1703796(T>C)	648	-0.0148±0.0279	0.60	277	-0.0146±0.0493	0.77	371	-0.0233±0.0345	0.50
rs9651118(C>T)	648	-0.0264±0.0320	0.41	277	***-0.0826±0.0470***	***0.080***	371	+0.0165±0.0454	0.72
rs17367504 (G>A)	648	-0.0303±0.0252	0.23	277	-0.0271±0.0378	0.47	371	-0.0400±0.0346	0.25
rs2066470(A>G)	648	-0.0371±0.0325	0.25	277	-0.0429±0.0534	0.42	371	-0.0387±0.0417	0.35
Cognitive domain 2: Models A-J									
rs4846049(T>G)	648	+0.0161±0.0350	0.65	277	-0.0241±0.0512	0.64	371	-0.0094±0.0491	0.85
rs1476413(T>C)	648	-0.0347±0.0475	0.47	277	-0.0102±0.0709	0.89	371	-0.0791±0.0650	0.23
rs4846051(A1317G, G>A)	648	+0.0442±0.0376	0.24	277	***+0.0941±0.0539***	***0.082***	371	+0.0010±0.0534	0.99
rs1801131(A1298C,G>T)	648	-0.0536±0.0461	0.25	277	-0.0672±0.0672	0.32	371	-0.0676±0.0639	0.29
rs2066462(C1056T,A>G)	648	-0.0677±0.0614	0.27	277	-0.0923±0.0961	0.34	371	-0.0950±0.0817	0.25
rs1801133(C677T,A>G)	648	+0.0126±0.0611	0.84	277	-0.0388±0.0921	0.67	371	+0.0609±0.0831	0.46
rs1703796(T>C)	648	-0.0084±0.0617	0.89	277	-0.0111±0.1030	0.91	371	-0.0208±0.0782	0.79
rs9651118(C>T)	648	-0.0249±0.0707	0.73	277	***-0.1778±0.0980***	***0.071***	371	+0.0980±0.1026	0.34
rs17367504 (G>A)	648	***-0.0947±0.0555***	***0.088***	277	***-0.1322±0.0785***	***0.093***	371	-0.0728±0.0786	0.36
rs2066470(A>G)	648	-0.1138±0.0716	0.11	277	-0.1038±0.1114	0.35	371	-0.1140±0.0943	0.23

Abbreviations: AF=Animal Fluency; BMI=body mass index (calculated as weight in kg/square of height in meters); BVRT=Benton Visual Retention test; CVLT-List A=California Verbal Learning Test, List A; CVLT-DFR=California Verbal Learning Test, Delayed Free Recall; DS-B=Digits Span Backwards; DS-F=Digits Span Forward; MMSE=Mini-Mental State Examination; MTHFR=Methylenetetrahydrofolate Reductase; *MTR*=Methionine synthase; *MTRR*=methionine synthase reductase; OLS=Ordinary Least Square; *SHMT*= Serine Hydroxymethyltransferase ; SNP=Single Nucleotide polymorphism; Trails A and B= Trailmaking test, parts A and B; Note that each SNP is denoted by an rs number followed by the polymorphism in which one nucleotide is replaced by another (e.g. C/T or G/A).

[1] Cognitive scores were predicted at mean age of follow-up using a linear mixed model controlling for sex, race/ethnicity, education (years), and smoking status , with age (centered at 50y) added among the fixed effect variables to allow for quadratic non-linear change, while age (centered at 50) was added to the random effects to allow for individual-level variation in slopes. The slope or annual rate of change was predicted from these models at the mean age at follow-up. Using factor analysis, two factor scores were estimated and were labeled as LARCC in the following domains: Domain 1: "Verbal memory and fluency", Domain 2: "Visual/working memory and executive function" (Supplemental method 1).

[2] Based on multiple OLS regression models with outcome being cognitive annual rate of change and main exposures being each of 10 *MTHFR* , adjusting for the *MTR*, *MTRR* and *SHMT* SNPs. The model also controlled for first-visit age, mean age at follow-up, education, first-visit smoking status, first-visit self-reported type 2 diabetes, hypertension, cardiovascular disease and BMI. The 10 principal components obtained from the genotype data with multidimensional scaling analysis (Supplemental method 3) were also added in a separate sensitivity analysis.

[3]Significant main effects after familywise Bonferroni correction: $p<0.05$ for MMSE, BVRT, AF and cognitive domains and $p<0.025$ for other cognitive tests.

[4] $P<0.05$ for interaction term sex×SNP