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# The C677T Variant in *MTHFR* Modulates Associations Between Brain Integrity, Mood, and Cognitive Functioning in Old Age

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## ABSTRACT

**BACKGROUND:** The C677T functional variant in the methylenetetrahydrofolate reductase (*MTHFR*) gene leads to reduced enzymatic activity and elevated blood homocysteine levels. Hyperhomocysteinemia has been linked with higher rates of cardiovascular diseases, cognitive decline, and late-life depression.

**METHODS:** Three-dimensional magnetic resonance imaging data were analyzed from 738 individuals (aged 75.5 ± 6.8 years; 438 men, 300 women), including 173 patients with Alzheimer's disease, 359 subjects with mild cognitive impairment, and 206 healthy older adults, scanned as part of the Alzheimer's Disease Neuroimaging Initiative.

**RESULTS:** We found that this variant associates with localized brain atrophy, after controlling for age, sex, and dementia status, in brain regions implicated in both intellectual and emotional functioning, notably the medial orbitofrontal cortices. The medial orbitofrontal cortex is involved in the cognitive modulation of emotional processes, and localized atrophy in this region was previously linked with both cognitive impairment and depressive symptoms. Here, we report that increased plasma homocysteine level mediates the association between *MTHFR* genotype and lower medial orbitofrontal volumes and that these volumes mediate the association between cognitive decline and depressed mood in this elderly cohort. We additionally show that vitamin $B_{12}$ deficiency interacts with the C677T variant in the etiology of hyperhomocysteinemia.

**CONCLUSIONS:** This study sheds light on important relationships between vascular risk factors, age-related cognitive decline, and late-life depression, and it represents a significant advance in our understanding of clinically relevant associations relating to *MTHFR* genotype.

***Keywords:*** Age-related cognitive decline, Brain atrophy, Homocysteine, Late-life depression, MRI, MTHFR

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Hyperhomocysteinemia, a metabolic anomaly involving elevated blood levels of the amino acid homocysteine, is associated with higher rates of numerous age-related disorders, such as cardiovascular diseases (1,2) including vascular dementia (3,4); cognitive decline (5–9); and depressed mood (10–12). Elevated plasma homocysteine levels may stem from the use of certain therapeutic drugs, from elevated alcohol ingestion, from intestinal malabsorption, or from impaired metabolism due to genetic alterations in metabolic enzymes, including methylenetetrahydrofolate reductase (MTHFR), most commonly when combined with insufficient dietary intake of B vitamins. Notably, homocysteine is recycled to methionine using vitamin $B_{12}$ as a cofactor, and MTHFR, the rate-limiting enzyme in the methyl cycle, catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a cosubstrate for homocysteine remethylation by methionine synthase. These relationships are illustrated in Figure 1.

We recently reported that older adults with higher homocysteine levels had more pronounced regional brain atrophy (13) and thinner cortical gray matter (14) on magnetic resonance imaging (MRI). We also found that the C677T variant in *MTHFR* was associated with smaller regional brain volumes in two independent elderly cohorts with mild cognitive impairment (MCI) (15). Increased susceptibility for cardiovascular diseases (16), which are strongly linked to both cognitive decline and depressive symptoms in old age (17), are also associated with the C677T variant.

Here, we first determined whether our previously reported associations between the T "risk" allele and more pronounced brain atrophy extended beyond individuals with MCI to both patients with dementia and healthy older adults. We further sought to model some of the mechanisms underlying relationships between brain integrity, clinical outcomes, and the genetic and environmental modulators of homocysteine metabolism. To this end, we first examined whether the effects of this *MTHFR* polymorphism on medial orbitofrontal volumes were mediated by its impact on plasma homocysteine levels. We also determined whether vitamin $B_{12}$ deficiency influenced the strength of the relationship between this variant and homocysteine levels. We then found that lower cognitive

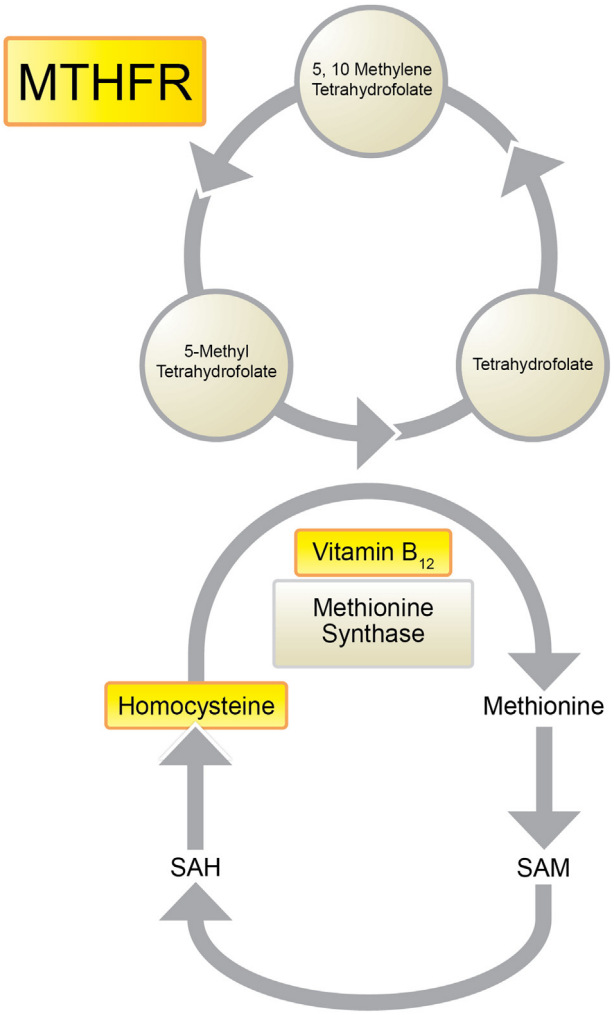


**Figure 1.** Simplified illustration of the one-carbon cycle. MTHFR, methylenetetrahydrofolate reductase; SAH, S-adenosyl-L-homocysteine; SAM, S-adenosyl-L-methionine.

performance and reduced medial orbitofrontal volumes were significant predictors of depressed mood and tested the hypothesis that compromised integrity in this brain region involved in the cognitive control of emotion may partially explain the relationship between cognitive impairment and depressive symptoms.

## METHODS AND MATERIALS

### Subjects

We analyzed a large sample of elderly individuals from the Alzheimer's Disease Neuroimaging Initiative (ADNI). The study was conducted according to the Good Clinical Practice guidelines, the Declaration of Helsinki, and U.S. 21 CFR Part 50 (Protection of Human Subjects) and Part 56 (Institutional Review Boards). Written informed consent was obtained from all participants before protocol-specific procedures were performed. All ADNI data are publicly available (http://adni.loni.usc.edu). To avoid the known effects of population stratification on genetic analysis (18), we included only non-Hispanic Caucasian subjects identified by self-report and confirmed by multidimensional scaling analysis (19). The ADNI cohort included multiple diagnostic groups: patients with Alzheimer's disease (AD), subjects with MCI, and healthy elderly (cognitively normal) participants. All subjects were administered the Mini–Mental State Examination (MMSE) (20) and the 15-item version of the Geriatric Depression Scale (GDS-15) (21). Our final analysis comprised 738 individuals (average age ± SD = 75.53 ± 6.78 years; 438 men, 300 women), including 173 AD, 359 MCI, and 206 healthy older adults. All participants received premortem clinical diagnoses, as described in detail in ADNI's General Procedures Manual (http://adni.loni.usc.edu/wp-content/uploads/2010/09/ADNI_GeneralProceduresManual.pdf). Table 1 illustrates demographic, cognitive, and mood data for all participants, stratified by genotype and substratified by diagnostic groups.

### Genotyping and Allele Frequency

The ADNI sample was genotyped using the Illumina 610-Quad BeadChip (Illumina, Inc., San Diego, CA). The only polymorphism examined in this study was the C677T functional variant in the *MTHFR* gene, at the rs1801133 locus. Allele frequency was computed from genotype frequency. Distributions of allele frequencies by diagnostic groups were evaluated by $\chi^2$ tests with a .05 significance level, using 3 × 2 and 2 × 2 contingency tables in SPSS, version 23.0 (IBM Corp., Armonk, NY).

### Neuroimaging

**Whole-Brain Analyses: Tensor-Based Morphometry.** The C677T polymorphism was analyzed for association with regional brain volumes in ADNI participants, as detailed below. Subjects were scanned using a standardized MRI protocol developed for this cohort (22,23). Briefly, high-resolution structural brain magnetic resonance images were acquired at 58 sites across North America, using 1.5T MRI scanners. A sagittal three-dimensional magnetization-prepared rapid gradient-echo sequence was used, optimized for consistency across sites (23) (repetition time/echo time = 2400/1000 ms; flip angle = 8°; field of view = 24 cm; final reconstructed voxel resolution = 0.9375 × 0.9375 × 1.2 $mm^3$). Image corrections were applied using a processing pipeline at the Mayo Clinic, consisting of 1) a procedure termed GradWarp to correct geometric distortion due to gradient nonlinearity (24), 2) a "B1-correction" to adjust for image intensity inhomogeneity due to B1 nonuniformity using calibration scans (23), 3) "N3" bias field correction for reducing residual intensity inhomogeneity (25), and 4) geometrical scaling, according to a phantom scan acquired for each subject (23), to adjust for scanner- and session-specific calibration errors. To adjust for global differences in brain positioning and scale, all subjects' scans were linearly registered to the stereotaxic space defined by the International Consortium for Brain Mapping template (ICBM-53) (26) using a nine-parameter transformation (three translations, three rotations, three scales) (27). We used standard trilinear interpolation and resampled the resulting aligned scans to have 1-mm isotropic voxels.