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The C677T variant in *MTHFR* modulates associations between blood-based and cerebrospinal fluid biomarkers of neurodegeneration

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The C677T functional variant in the methylene-tetrahydrofolate reductase (*MTHFR*) gene results in reduced enzymatic activity and elevated blood levels of homocysteine. Plasma levels of apolipoprotein E (ApoE) are negatively correlated with cerebral amyloid burden, but plasma homocysteine concentrations are associated with increased amyloid-β (Aβ) deposition in the brain. Here, we sought to determine whether associations between low plasma ApoE levels and elevated in-vivo amyloid burden were modulated by carrying the C677T variant. We tested this hypothesis in a large sample of elderly participants from the Alzheimer's Disease Neuroimaging Initiative. We used general linear models to examine associations between plasma homocysteine concentrations, circulating ApoE levels, cerebrospinal fluid concentrations of Aβ, and their modulation by *MTHFR* and *ApoE* genotype. Age, sex, and dementia status were included as covariates in all analyses. Higher circulating levels of ApoE predicted increased cerebrospinal fluid concentrations of Aβ, indicating lower in-vivo burden, in C-allele carriers, but not in homozygotes at the C677T variant, who showed significant elevations in plasma homocysteine levels. This modulation by the *MTHFR* genotype did not remain significant after controlling for *ApoE* genotype. In T-homozygotes who do not carry the ApoE-ε4 allele, the relationship between low plasma ApoE levels and an increased risk of dementia is likely obscured by the presence of elevated plasma homocysteine. This report suggests the value of genotyping patients at the C677T functional variant when using plasma ApoE levels as a preclinical biomarker for Alzheimer's disease. *NeuroReport* 27:948–951

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Introduction

Methylene-tetrahydrofolate reductase (MTHFR) is involved in the conversion of the amino acid homocysteine into methionine. The C677T functional variant in the *MTHFR* gene (minor allele frequency = 0.245), which codes for a heat-sensitive variant characterized by reduced activity of the MTHFR enzyme, leads to elevated levels of homocysteine in the blood [1]. Hyperhomocysteinemia is associated with higher rates of several age-related disorders, such as cardiovascular diseases [2,3], including vascular dementia [4]. We previously reported that older adults with elevated homocysteine levels had more pronounced regional brain atrophy [5] and thinner cortical gray matter [6] on 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) [7], and most recently expanded these findings by providing evidence for these associations across the dementia spectrum of normal cognitive aging, MCI, and Alzheimer's disease (AD) [8].

Low cerebrospinal fluid (CSF) levels of amyloid-β_{1-42} ($A\beta_{1-42}$) indicate the sequestration of Aβ in amyloid plaques in the brain and elevated in-vivo amyloid burden [9]. This plaque burden is associated with AD, vascular dementia, and other degenerative brain disorders [10], including Parkinson's disease [11], and also correlates with higher brain atrophy rates in healthy older adults [12]. CSF $A\beta_{1-42}$ may serve as one preclinical – and potentially predictive – biomarker for age-related cognitive decline and accelerated brain aging.

Apolipoprotein E (ApoE) is involved in $A\beta_{1-42}$ clearance [13] and plasma levels of this protein are negatively correlated with cerebral amyloid burden as measured by neuroimaging [14,15]. In fact, plasma ApoE levels are being proposed as a novel preclinical biomarker for AD [16]. However, as carriers of the C677T variant have

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increased plasma homocysteine levels [1,17] and as higher plasma homocysteine concentrations are associated with increased $A\beta_{1-42}$ deposition in the brain [18, 19], we hypothesized that the link between elevated plasma ApoE levels and reduced cerebral amyloid burden may not hold in carriers of this variant, with important implications for the use of plasma ApoE as a preclinical marker of neurodegeneration in these individuals.

Materials and methods

We tested this hypothesis in a large sample of elderly individuals from the Alzheimer's Disease Neuroimaging Initiative (ADNI). ADNI participants were recruited from 58 sites across North America. The study was carried out according to the Good Clinical Practice guidelines, the Declaration of Helsinki, and US 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 at *http://adni.loni.usc.edu*. To avoid the known effects of population stratification on genetic analysis [20], we only included non-Hispanic White patients identified by self-report and confirmed by multidimensional scaling analysis [21]. The ADNI cohort included multiple diagnostic groups: patients with AD, patients with MCI, and healthy elderly [cognitively normal (CON)] participants.

The ADNI sample was genotyped using the Illumina 610-Quad BeadChip (Illumina, San Diego, California, USA). Our analyses focused on the C677T functional variant in the *MTHFR* gene at the rs1801133 locus. *ApoE* genotyping was performed separately, on DNA samples obtained from participants' blood, using an *ApoE* genotyping kit, as described in *http://www.adni-info.org/scientists/ADNIStudyProcedures.aspx*. Plasma homocysteine levels (pg/ml) and ApoE concentrations (mg/dl) were extracted from blood samples collected using standard venipuncture protocols. CSF samples were obtained through lumbar puncture after an overnight fast. Samples from various sites were transferred, on dry ice, to the ADNI Biomarker Core Laboratory at the University of Pennsylvania Medical Center, where $A\beta_{1-42}$ concentrations were measured with a multiplex immunoassay platform under the guidance of Drs Leslie Shaw and John Trojanowski [22].

In the ADNI public database (*http://www.adni.loni.usc.edu*), plasma levels of homocysteine were available for 732 individuals (average age $\pm$ SD = 75.51 $\pm$ 6.80 years; 436 men/296 women, including 173 AD, 355 MCI, and 204 CON); plasma ApoE levels for 517 participants (average age $\pm$ SD = 75.18 $\pm$ 7.30 years; 321 men/196 women, including 110 AD, 353 MCI, and 54 CON); and CSF $A\beta_{1-42}$ concentrations were accessible for 384 patients (average age $\pm$ SD = 75.09 $\pm$ 7.00 years; 231 men/153 women, including 100 AD, 181 MCI, and 103 CON). We used general linear models to examine associations between plasma homocysteine concentrations, circulating ApoE levels, CSF $A\beta_{1-42}$ concentrations, and their modulation by *MTHFR* and *ApoE* genotype. Age, sex, and dementia status were included as covariates in all analyses. The effect of the C677T variant on plasma homocysteine levels was additionally examined using a one-way analysis of variance with a Bonferroni post-hoc test, and the association between plasma ApoE concentrations and CSF levels of $A\beta_{1-42}$ in C-allele carriers was also investigated using a two-tailed bivariate Pearson correlation. All statistical analyses were carried out in SPSS 23.0 (SPSS Inc., Chicago, Illinois, USA).

Results

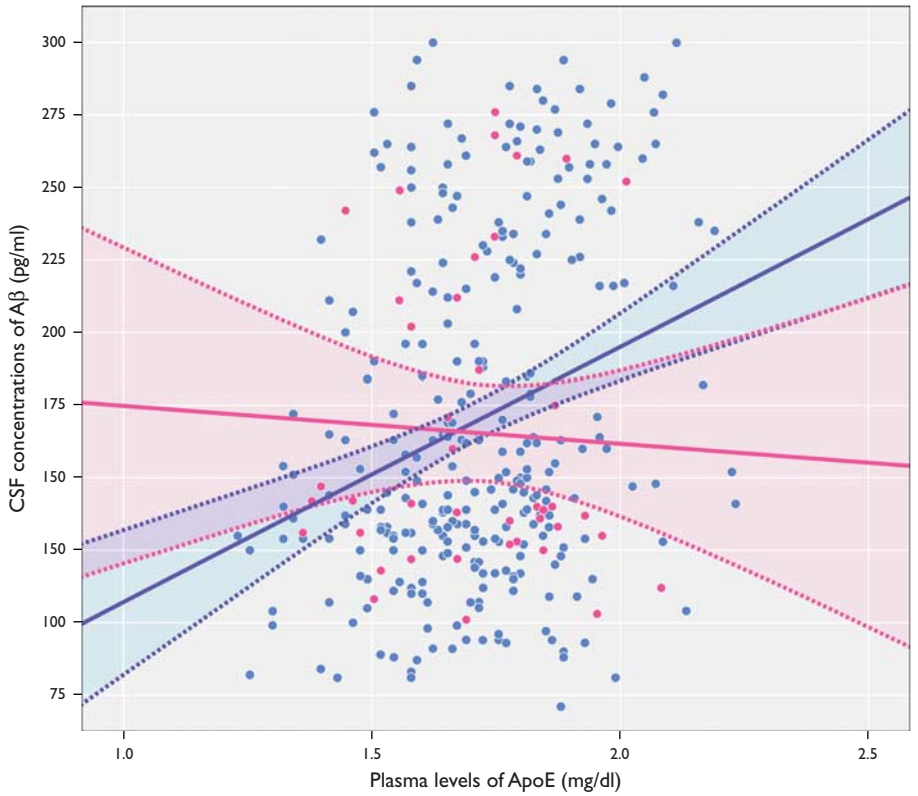
As expected, the C677T variant was associated with significant elevations in plasma homocysteine levels, after controlling for age, sex, and dementia status ($P < 0.001$, F-ratio = 10.375). A one-way analysis of variance also showed significant effects of the C677T variant on plasma concentrations of homocysteine ($P < 0.001$, F-ratio = 10.705). However, a Bonferroni post-hoc test showed that levels did not differ significantly between carriers of a single T-allele and C-homozygotes. We found a significant association between higher circulating levels of ApoE and increased concentration of $A\beta_{1-42}$ in the CSF, indicating a reduced in-vivo amyloid burden – in C-allele carriers, after controlling for age, sex, and dementia status ($P = 0.047$, F-ratio = 3.997). However, in T-homozygotes (who show significant elevations in plasma homocysteine levels), ApoE concentrations in the blood were not a significant predictor of CSF levels of $A\beta_{1-42}$ ($P = 0.409$, F-ratio = 0.699), after controlling for age, sex, and diagnosis.

Figure 1 shows the significant positive correlation (Pearson's $r = 0.289$, $P < 0.001$) between plasma ApoE concentrations and CSF levels of $A\beta_{1-42}$ in C-allele carriers, which we failed to observe in T-homozygotes (Pearson's $r = -0.059$, $P = 0.711$). As the ApoE4 isotope leads to increased Aβ-peptide deposition [23] and as the ε4 allele is associated with lower plasma ApoE levels [14–16], this modulation by the *MTHFR* genotype did not remain significant after introducing *ApoE* genotype as an additional covariate in the statistical models.

Conclusion

This study is the first to show that the C677T variant in *MTHFR* modulates associations between blood-based and CSF biomarkers of neurodegeneration. Low levels of ApoE, a plasma protein involved in $A\beta_{1-42}$ clearance [13], are associated with higher amyloid burden in the brain [14,15], and with an increased risk of dementia, independent of *ApoE* genotype [14–16]. Here, higher plasma ApoE levels predicted increased concentrations of $A\beta_{1-42}$ in the CSF, indicating decreased in-vivo

Fig. 1



Higher plasma ApoE levels are associated with increased CSF $A\beta_{1-42}$ concentrations in C-allele carriers (blue), but not in T-homozygotes (pink). *x*-axis: plasma ApoE levels; *y*-axis: CSF levels of $A\beta_{1-42}$. Each dot represents a single patient's plasma and CSF concentrations of these biomarkers. Solid lines indicate regression lines. Dotted lines represent 95% confidence intervals for the mean. $A\beta_{1-42}$, amyloid-β_{1-42}; ApoE, apolipoprotein E; CSF, cerebrospinal fluid.

amyloid burden [9], in C-allele carriers, but not in individuals with the TT genotype. The modulation of the relationship between plasma ApoE and CSF $A\beta_{1-42}$ by *MTHFR* genotype did not remain significant after controlling for *ApoE* genotype, suggesting that, although plasma homocysteine promotes amyloid deposition in the brain [18,19], its contribution toward cerebral amyloid burden may be weaker than that of the ApoE4 isotype, which leads to a reduction in the $A\beta_{1-42}$ clearance rate [13].

Consistent with published findings [1,17], we found that T-homozygotes showed significant elevations in plasma homocysteine levels. As elevated plasma homocysteine concentrations are associated with increased $A\beta_{1-42}$ deposition in the brain [18,19], it is possible that high homocysteine may offset the protective effects of elevated plasma ApoE levels in homozygotes at the C677T variant (who showed no correlation between plasma ApoE and CSF concentrations of $A\beta_{1-42}$). Although genome-wide association studies provide no evidence that the C677T variant is an independent risk factor for AD, homocysteine itself is clearly associated with some of the hallmarks of dementia, including cerebral amyloid aggregation [19]. Consequently, the relationship between low plasma ApoE levels and increased risk of AD may be obscured by the presence of elevated plasma homocysteine in T-homozygotes at this functional variant.

Our finding that the C677T functional variant in *MTHFR* affects the relationship between low plasma ApoE and increased amyloid deposition in the aging human brain is descriptive in nature and future investigations will need to clarify the precise biological mechanisms underlying these observations. Nonetheless, our results have important practical and clinical implications as plasma levels of ApoE are being proposed as a promising new and easily accessible preclinical biomarker for AD [16]. In T-homozygotes who do not carry the ApoE-ε4 allele, high plasma ApoE may not be an accurate predictor of low cerebral amyloid burden. This report therefore highlights the importance of genotyping patients at the C677T variant in *MTHFR* when using plasma ApoE levels as a circulatory biomarker for degenerative brain disorders.

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Conflicts of interest

There are no conflicts of interest.

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