Association of MTHFR C677T polymorphism with loneliness but not depression in cognitively normal elderly males

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HIGHLIGHTS

► MTHFR C677T polymorphism may be linked more to loneliness than depression in the cognitively normal elderly males.
► Subjects with MTHFR C/C genotype had higher loneliness scales than T-allele carriers.
► Three MTHFR genotypic groups did not differ in cognitive and depression rating.

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ABSTRACT

Methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism is involved in folate and homocysteine metabolism, and has been associated with geriatric disorders, including dementia and late-life depression. The present work aimed to investigate the effect of MTHFR C677T polymorphism on the presence of depression and loneliness in cognitively normal male subjects. A total of 323 cognitively normal male subjects were included in this study (mean age = 80.6; SD = 5.3). Depression was assessed by the Geriatric Depression Scale-Short Form (GDS-SF) and loneliness by UCLA loneliness scales. Analysis of variance (ANOVA) was used to test the between MTHFR genotype difference in depression and loneliness. Multiple regression was used to test the effect of MTHFR polymorphism on the loneliness, controlling for age, education, cognitive function, and depression. ANOVA showed a significant between-genotype difference in loneliness scores (P = 0.015), and post hoc comparisons showed that subjects with C/C genotype had significantly higher loneliness ratings, compared to those with C/T or T/T genotype. Regression analysis indicated that the effect of MTHFR polymorphism on loneliness was independent of age, education, cognitive function, and depression. Our findings suggest that MTHFR C677T polymorphism may be linked more to loneliness than depression in the cognitively normal elderly males, and may be implicated in the pathophysiology of late-life depression in relation to MTHFR genes.

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>50) [4]. However, the pathophysiological role of the homocysteine in depression remains elusive [5].

In human, the homocysteine plasma level can be influenced by various factors such as the vitamin deficiency, aging, and a common non-synonymous MTHFR C677T polymorphism [15]. The T allele at 677 position of MTHFR gene causes substitution of alanine to valine, resulting in reduced MTHFR enzyme activity and increased body homocysteine concentration [15]. The MTHFR C677T polymorphism has been found to be associated with many medical conditions, such as cancer and vascular diseases [3,14,21,23]. In 2001, Hickie et al. first demonstrated that patients with late-onset depression had an increased rate of the C677T MTHFR gene mutation, suggesting the MTHFR polymorphism may play a role in the pathogenesis of depression [13]. In line with their findings, a meta-analysis of ten studies, including 1280 cases of depression and 10,429 controls, demonstrated that the fixed-effect odds ratio was 1.36 (95% confidence interval: 1.11–1.67) to have depression for the homozygote variants (TT), compared to the wild type (CC) [12]. However, the association between MTHFR C677T polymorphism and late-onset depression was not replicated by another study [24]. In general population, Almeida et al. found that there were no differences in depression scores among the MTHFR C677T genotype groups in the older female population without dementia [1].

Loneliness and depression are very common in the elderly population [7,19]. Previously, we have found an association of CHRNA4 polymorphism with depression and loneliness in elderly males [25]. Because MTHFR C677T polymorphism has been reported to be associated with negative emotionality, the aim of this study is therefore to investigate the effect of MTHFR C677T gene polymorphism in depression and loneliness in older subjects without dementia. To minimize the confounding effects of gender, a homogenous group was constructed, composed entirely of healthy aged Han Chinese males.

A total of 323 males aged over 60 (mean age = 80.6; SD = 5.3) were recruited from a veteran housing in the northern Taiwan. Most participants were single and have stayed in the veteran housing for years. This study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board of Taipei Veterans General Hospital. Informed consent was obtained from all participants prior to commencement.

Each participant was evaluated initially by the self-reported current and past medical status and a review of participants’ medical records. Each participant received a neurological check-up and a diagnostic interview based on the Mini-International Neuropsychiatric Interview (MINI) by a trained assistant [22]. The MINI is a short diagnostic structured interview for 17 Diagnostic and Statistical Manual (DSM)-III-R Axis I psychiatric disorders. The validity and applicability of the MINI used by family medicine residents in primary health care had been investigated [8]. Concordance levels for any mental disorder, the broader current diagnostic categories and the most common specific diagnoses were analyzed. The findings suggested MINI comprehensibility and clinical relevance satisfactory (Kappa coefficients: 0.65–0.85; sensitivity: 0.75–0.92; specificity: 0.90–0.99). The Chinese version of the MINI has been tested and has good validity [18]. Subjects who had cognitive impairment or depression symptoms were further evaluated by a physician. Exclusion criteria included the following: (1) presence of psychotic disorders according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition; (2) severe medical illness (e.g., malignancy or heart failure); and (3) severe neurological disorders (e.g., stroke or Parkinson’s disease).

Depression was assessed by the Chinese version of the Geriatric Depression Scale-short form (GDS-SF) [17]. Scores on the GDS-SF showed a high validity in accordance with the original version [6]. The loneliness was assessed by the Loneliness Scale (University of California, Los Angeles, UCLA Version 3) [20]. The UCLA loneliness scale contains 20 items and participants rate how often they feel the way described in the items using a four point Likert scale ranging from never to often. Cognitive functions were assessed using the Mini-Mental Status Examination (MMSE) [9].

Peripheral venous blood was taken from the study subjects for genotyping of the MTHFR C677T polymorphisms. Genomic DNA was isolated using the PUREGENE DNA purification system (Gentra Systems, Minneapolis, MN, US). For DNA quality examination, all the samples were genotyped for 8 unrelated SNPs. The samples were diluted onto 96-well plates, and only the plate with an average successful genotyping rate greater than 95% for the 8 SNPs were used for further study. Primers and probes were designed with SpectroDESIGNER software (Sequenom, San Diego, US). A multiplex polymerase chain reaction was performed, and unincorporated double stranded nucleotide triphosphate bases (dNTPs) were dephosphorylated with shrimp alkaline phosphatase (Hoffman-LaRoche, Basel) followed by primer extension. The purified primer extension reaction was spotted on to a 384-element silicon chip (SpectroCHIP, Sequenom, San Diego, US) and analyzed in the Bruker Biflex III MALDI-TOF SpectroREADER mass spectrometer (Sequenom, San Diego, US). The resulting spectra were processed with SpectroTyPER (Sequenom, San Diego, US). All the experiments were done by investigators who were blind to the phenotypes.

Statistical analysis was conducted using SPSS v15.0 (SPSS Inc., Chicago, IL, USA). Based on genotype groups, demographic and clinical data were analyzed either by the Student’s t-test or one-way analysis of variance (ANOVA), followed by the post hoc least significance difference (LSD) multiple comparisons. Multiple linear regression was used to examine the effect of age, MMSE, GDS-S, years of education and MTHFR C677T on the loneliness rating score. Data are presented as mean (SD), with the level of significance set at P < 0.05 for all tests.

Demographic and assessment of depression, loneliness, and MMSE for subjects with the three MTHFR genotypes are presented in Table 1. Among them, 18 subjects (5.6%) were found to have major depression. The MTHFR genotype distribution (C/C: n = 118, 36.5%; C/T: n = 141, 43.7%; T/T: n = 64, 19.8%) was found to be in Hardy-Weinberg equilibrium. Three MTHFR groups did not differ in age, years of education, MMSE and GDS-SF rating.

ANOVA showed a significant between-genotype difference in loneliness scores (P = 0.015), and post hoc comparisons revealed that subjects with C/C genotype had a significantly higher loneliness scores than those with C/T or T/T genotype. C/T and T/T groups did not differ in loneliness scores, suggesting that the T-allele was dominant in the association with loneliness scores. Further analysis revealed significantly higher loneliness scores for the C/C group in comparison to the T-allele carriers (P = 0.006). Multiple regression analysis was performed with loneliness as the dependent variable and age, years of education, MMSE scores, GDS-SF scores and MTHFR genotypes (C/C vs. T-carriers) as the predictors. The regression model (R² = 0.39, F = 39.9, P < 0.001) showed that the MTHFR C677T genotype was still a significant predictor of the loneliness scale (β = –2.38, R² = 0.03, P = 0.008), controlling for age.
for age ($\beta = 0.08$, $R^2 = 0.006$, $P = 0.344$), MMSE ($\beta = -0.18$, $R^2 = 0.04$, $P = 0.009$), GDS-SF ($\beta = 1.89$, $R^2 = 0.35$, $P < 0.001$), and years of education ($\beta = 0.05$, $R^2 = 0.003$, $P = 0.610$).

The principle finding of this study is that the subjects with MTHFR C/C genotype had higher loneliness scales than T-allele carriers in cognitively normal elderly. To our knowledge, this is the first report demonstrated the association between MTHFR C677T polymorphism and loneliness in the cognitively normal elderly.

Although the MTHFR C677T polymorphism has been found to be associated with depression [13], this study showed only the effect of the MTHFR polymorphism on loneliness but not depression. In addition, the association of the MTHFR polymorphism with loneliness was independent of age, education, cognitive function, and the levels of depression. These findings are in line with a study in general population showing that the MTHFR C677T gene variation does not play a direct role in modulating depression mood in aged female subjects [1]. Previous study of loneliness and depression in the elderly showed that the two are strongly co-occurring with each other [19]. Intense loneliness might result in diminished feelings of self-worth, lack of confidence in interpersonal relationships, thus leading to the presence of depression. Our findings of the independent association between MTHFR polymorphism and loneliness may warrant the future investigation of the interaction between loneliness and depression in the longitudinal study.

The mechanisms of the association between MTHFR C677T polymorphism and loneliness were unclear. Previous study showed that the MTHFR C677T allele is correlated with reduced enzyme activity and increased plasma homocysteine [11]. Further study is needed to test the correlation between plasma homocysteine levels and loneliness. Furthermore, a prior study suggests that the interaction between MTHFR polymorphism and DNA methylation is under different folate status [10]. Therefore, MTHFR C677T polymorphism may be involved in the genesis of loneliness via the pathway of DNA methylation.

There are several limitations for this study. First, this study is limited by cross-sectional study design. Prospective study is more adequate to address the casual relation between MTHFR C677T polymorphism and geriatric depressive symptoms and loneliness. Second, plasma homocysteine and folate levels were not available in this study. Thus, we could not determine if the MTHFR genetic effect on loneliness in the elderly is related to homocysteine or folate levels. Third, our sample represents elderly veteran man dwelling in the veteran housing, and the findings of the association between the MTHFR C677T polymorphism and loneliness may need to be further validated in younger adults, females or other populations. Fourth, only MTHFR C677T polymorphism was tested in this study. Further study of other MTHFR polymorphisms, particularly functional ones, is needed to fully evaluate the role of MTHFR in the presence of loneliness. For example, the second most prevalent polymorphism in the MTHFR gene, which is also associated with reduced (35–40%) enzyme activity, is transversion of adenosine to cytosine at nucleotide position 1298 [27]. Finally, cystathionine beta synthase (CBS) is also a key enzyme that plays a critical role in homocysteine metabolism. The gene–gene interactions regarding the possible association of loneliness with specific mutations of MTHFR and CBS genes should be further examined in the future.

In conclusion, our findings suggest that MTHFR C677T polymorphism may be linked more to loneliness than depression in the cognitively normal elderly males. The role of loneliness in the pathophysiology of depression in relation to MTHFR polymorphism may be further explored in the longitudinal study.

Conflict of interest
No competing financial interests exist.

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References