Contribution of the MTHFR gene to the causal pathway for depression, anxiety and cognitive impairment in later life

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Abstract

Homocysteine (Hcy) is harmful to neurons and blood vessels, including the cerebral microvasculature. It is possible that such effects contribute to the cascade of events that leads to cognitive decline, dementia, and depression in later life. Hcy is produced during the metabolism of the essential amino-acid methionine, which also involves a methyl group transfer derived from folate and choline metabolism. Its plasma level can be influenced by factors such as age, vitamin deficiency, renal function, and a common mutation in the methylenetetrahydrofolate reductase (MTHFR) gene, where cytosine is replaced by thymidine (C→T) at nucleotide position 677. Subjects with the TT genotype have higher homocysteine levels and may be particularly prone to experiencing depression as a result of high plasma Hcy and dysfunction of methylation metabolic pathways critical to the synthesis of noradrenaline and serotonin. We designed the present study to investigate whether older women with the TT genotype would have higher depression and lower cognitive scores than women with CT and CC genotypes. A total of 240 community-dwelling women aged 70 years or over volunteered to take part in the study – 29 carried the TT genotype, 113 the CT and 98 the CC genotype. The Beck Depression Inventory (BDI) score for subjects with the TT genotype was statistically similar to the other groups (P=0.609). Plasma Hcy showed a modest and significant correlation with BDI scores (r=0.21) that was independent from age, B 12 and folate levels. There was no association between beck anxiety inventory (BAI) scores and MTHFR genotype or homocysteine levels. The cognitive assessment of participants included measures of verbal memory, memory for faces, verbal fluency, visuo-spatial abilities and the cognitive section of the Cambridge Examination For Mental Disorders Of The Elderly (CAMCOG) – MTHFR genotype had no clear association with cognitive scores. These results indicate that, in isolation, the MTHFR C677T gene variation does not play an important role in the modulation of mood and cognitive performance in later life.

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1. Introduction

The pathological mechanisms that lead to the expression of depression and dementia in later life remain largely unknown, but both disorders have been associated with cerebrovascular disease [9,12,22,30] and high plasma homocysteine (Hcy) [2,6,11,26]. Homocysteine is a sulphur containing amino-acid formed during the metabolism of the essential amino-acid methionine and sits at the intersection of three important metabolic pathways: re-methylation, trans-methylation and transsulphuration (folate cycle). By receiving a methyl group from 5′-methyltetrahydrofolate, Hcy can be re-methylated to methionine, which is also the immediate precursor of S-adenosylmethionine (SAM), the universal methyl donor of numerous methylation reactions that are crucial to the synthesis of DNA, proteins, phospholipids and various neurotransmitters and polyamines. In the brain, SAM is directly involved in the synthesis and metabolism of dopamine, noradrenaline and serotonin, which are neurotransmitters postulated to play an important role in the pathogenesis of depression and anxiety [1]. High
plasma homocysteine commonly results from vitamin deficiency (folate, B_12 and B_6 – approximately 2/3 of cases), ageing, renal impairment, or a common genetic mutation to the methylenetetrahydrofolate reductase (MTHFR) gene, in which cytosine is replaced by thymidine (C→T) at base position 677 [20].

Methylenetetrahydrofolate reductase catalyses the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which re-methylates Hcy to methionine. The T allele and TT genotype, which occur with a frequency of 45 and 11%, respectively [32], are associated with reduced enzyme activity and consequent elevation of serum Hcy concentrations. This effect appears to manifest most frequently in the setting of low folate status, and has been linked to increased risk of ischaemic heart disease and stroke [20]. It is conceivable, therefore, that the MTHFR genotype plays an important role in the modulation of mood and cognitive function in humans. We designed the present study to investigate the association between MTHFR genotype, depressive and anxiety symptoms and cognitive function in a community-dwelling cohort of older Australian women. We hypothesized that TT carriers would present higher depression and lower cognitive scores than women with CT or CC genotype.

2. Methods

This cross-sectional study recruited community-dwelling women aged 70 years and over living in the metropolitan area of Perth, Western Australia. They were volunteers recruited through advertisement in the local press for an ongoing project designed to investigate mood and well-being amongst older women. Subjects were excluded from participating in the study if they had: (1) Cambridge Examination For Mental Disorders Of The Elderly (CAMCOG) lower than 70, (2) non-English speaking background, (3) severe sensory impairment, (4) history of strokes, (5) history of current or previous hazardous drinking, and (6) used hormone replacement therapy during the six months prior to assessment.

2.1. Clinical assessment

All participants were interviewed with the Cambridge Examination for Mental Disorders of the Elderly (CAMDEX-R) [25], a reliable semi-structured interview designed to assess mental state and cognitive functioning of older adults. Depressive symptoms were assessed with the Beck Depression Inventory (BDI) [4], which is a self-rating scale designed to evaluate the severity of depression in clinical and research settings. It includes 21 questions with possible ratings ranging from 0 to 3. The BDI has high internal consistency (0.86 or greater) and is sensitive to change in the severity of depression. Scores of 13 or more indicate the presence of clinically significant depressive symptoms.

The Beck Anxiety Inventory was used for the assessment of anxiety symptoms (BAI) [3]. This self-rating scale includes 21 items describing common symptoms of anxiety that can be rated according to their intensity from 0 to 3. Internal consistency (α = 0.92) and test-retest reliability ratings (r = 0.75) are high, and so are different measures of validity. Scores of 13 or more indicate the presence of clinically significant anxiety symptoms.

2.2. Cognitive assessment

The neuropsychological assessment of participants included the investigation of specific and general measures of function. The CAMCOG [25] is divided into several subsections measuring various aspects of cognitive functioning: orientation, language, memory, attention and concentration, praxis, perception, calculation, and executive functions. The total score can range from 0 to 105, and is highly correlated with the MMSE total score (which can also be computed from the CAMCOG). Reported test-retest reliability scores are greater than 0.8.

Faces measures, immediate and delayed memory for faces [35]. Subjects are presented a sequence of 25 faces and subsequently asked to recognize, from amongst a total of 50 faces, the faces presented immediately and after a delay of approximately 25 min.

Word lists (WL) [35] measures immediate and delayed memory for verbal material. Subjects are presented with a list of 12 semantically unrelated words, and then asked to recall as many words as possible. This procedure is repeated for three additional trials for a total of four learning trials. Then, the examiner reads a new word list and asks the subject to recall the words on this list and, subsequently, on the first list. In WL II, the examinee is asked to recall the first list or words (delayed recall). Then the examiner reads a list of 24 words and asks the examinee to identify each word as either one he or she was asked to remember of a new word (recognition). For the purposes of this study, we computed and analyzed the ‘recall total score’ (sum of trials one to four), ‘recognition total score’ and ‘percent retention’ [(delayed recall)/word list recall for trial 4] × 100.

Verbal Paired Associates (VPA) [35] uses the same test procedures described for WL, and produces measures of immediate and delayed cued recall for semantically unrelated pairs of words; i.e., during the recall phase of the test, the examiner reads aloud one word of the pair and the examinee is asked to recall the word that was associated with the former. As previously described for word lists, the performance of subjects on this test was summarized by the recall total score, recognition total score and percent retention.

Block design (BD) [34] is a constructional test in which the subject is presented with four or nine colored blocks. The aim is to use the blocks to construct replicas of 10 designs printed in a booklet. This is a sensitive test of visuospatial
organism, with the total possible score ranging from 0 to 68 points.
Finally, verbal fluency (VF) was investigated by asking subjects to name as many words as possible starting with the letters f, a and s (1 min each). The VF total score represents the sum of the number of words produced for each one of the three trials.

2.3. Biochemical and genetic analysis

Blood, from fasting subjects, was collected for serum, with plasma (EDTA tubes) being separated within a maximum period of 1 h. Total plasma homocysteine (Hcy) was measured by fluorescence polarization immunoassay on an IMx analyzer [21]. Serum folate was measured by the ion-capture enzyme immunoassay, and B12 (cyanocobalamin) was measured by microparticle enzyme immunoassay, both on an AxSYM analyser. The interassay coefficient of variation (CV) for these tests ranges from 4 to 6%.

Genomic DNA was isolated from nucleated blood cells by use of a Triton X-100 method, and the nt 677 C → T mutation was determined by use of the polymerase chain reaction (PCR) and Hinfl restriction enzyme digestion as described by Frosst et al. [15]. Hinfl digestion (1.5 U/25 μL reaction mixture) was performed directly in the PCR tube at 37 °C for 4 h before analysis of restriction fragments by polyacrylamide gel electrophoresis (PAGE; 12% T, 3.3% C) as previously described [32]. Allele frequencies were estimated by gene counting and observed numbers of each genotype were compared with those expected under Hardy–Weinberg equilibrium.

2.4. Statistical analysis

Data were analyzed with the statistical package ‘Stata, release 7’. Descriptive statistics were used to determine frequencies, means, 95% confidence intervals of the mean (CI), and distribution of data. The frequency distribution on contingency tables was investigated using the chi-square method of Pearson (χ 2). On contingency tables was investigated using the chi-square (CI), and distribution of data. The frequency distribution of BDI and BAI scores according to MTHFR genotype. There was no difference between TT carriers and the remainder of the sample on either the BDI or BAI scores greater or equal to 13, indicating the presence of clinically significant depressive symptoms. Similarly, Beck Anxiety Inventory (BAI) scores ranged from 0 to 64 (mean ± S.D. = 7.6 ± 5.8). Forty-two women had scores greater or equal to 13, indicating the presence of clinically significant depressive symptoms. Similarly, Beck Anxiety Inventory (BAI) scores ranged from 0 to 29 (6.0 ± 6.4), with 35 subjects scoring 13 or more. Fig. 1 shows the distribution of BDI and BAI scores according to MTHFR genotype. There was no difference between TT carriers and the remainder of the sample on either the BDI or BAI scores (7.1 ± 6.0 versus 7.7 ± 5.8, t = 0.53, P = 0.597; 5.7 ± 6.4 versus 6.0 ± 6.4, t = 0.25, P = 0.801, respectively). In addition, only 3/29 TT women scored 13 or more on the BDI compared to 39/211 of non-TT carriers (χ 2 = 1.17, P = 0.279). A similar frequency distribution was observed for subjects with BAI scores greater or equal to 13 (4/29 versus 1/211; χ 2 = 0.02, P = 0.898).

Beck Depression Inventory (BDI) scores showed a modest, but significant direct correlation with Hcy levels (Pearson’s r = 0.24, P < 0.001), and women with clinically significant depression (BDI ≥ 13) had higher Hcy levels (12.4 ± 4.5 versus 10.9 ± 4.5, t = 2.30, P = 0.022 after natural logarithmic transformation of Hcy levels). We also found that the association between Hcy and the MTHFR-TT genotype could not be explained by folate and B12 levels alone (ANCOVA F = 4.27, d.f. = 1, P = 0.040 after natural logarithmic transformation; F = 4.45, d.f. = 3, P = 0.005 for total model). A total of 14.6% of women in our sample had Hcy ≥ 15 μmol/L (high plasma homocysteine).

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Fig. 1. Scatterplot distribution of depression and anxiety scores according to MTHFR genotype. Data points are jittered 2.5% to enable display of overlap in the distribution of scores.

Table 1
Cognitive scores of community-dwelling older women according to their MTHFR genotype

<table>
<thead>
<tr>
<th>Cognitive scores (mean ± S.D.)</th>
<th>MTHFR genotype</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TT (n = 29)</td>
<td>CT (n = 113)</td>
<td>CC (n = 98)</td>
</tr>
<tr>
<td>CAMCOG</td>
<td>94.1 ± 5.2</td>
<td>94.9 ± 4.8</td>
<td>94.9 ± 5.3</td>
</tr>
<tr>
<td>Verbal fluency (FAS)</td>
<td>43.5 ± 11.4</td>
<td>42.2 ± 14.9</td>
<td>44.8 ± 12.1</td>
</tr>
<tr>
<td>Faces-immediate recall</td>
<td>33.5 ± 4.9</td>
<td>35.4 ± 4.3</td>
<td>35.6 ± 4.3</td>
</tr>
<tr>
<td>Faces-delayed recall</td>
<td>34.2 ± 5.1</td>
<td>34.5 ± 4.7</td>
<td>34.4 ± 4.4</td>
</tr>
<tr>
<td>Block design</td>
<td>28.0 ± 7.7</td>
<td>28.4 ± 9.1</td>
<td>28.7 ± 6.0</td>
</tr>
<tr>
<td>WL – total recall</td>
<td>30.4 ± 6.0</td>
<td>30.2 ± 6.4</td>
<td>30.3 ± 6.3</td>
</tr>
<tr>
<td>WL – delayed recall</td>
<td>5.9 ± 3.8</td>
<td>6.0 ± 3.0</td>
<td>5.7 ± 3.3</td>
</tr>
<tr>
<td>WL – percentage retention</td>
<td>60.1 ± 37.0</td>
<td>64.1 ± 28.9</td>
<td>58.2 ± 31.1</td>
</tr>
<tr>
<td>VPA – total recall</td>
<td>16.1 ± 7.7</td>
<td>17.3 ± 8.0</td>
<td>17.3 ± 7.8</td>
</tr>
<tr>
<td>VPA – delayed recall</td>
<td>5.2 ± 2.4</td>
<td>5.3 ± 2.3</td>
<td>5.4 ± 2.4</td>
</tr>
<tr>
<td>VPA – percentage retention</td>
<td>89.6 ± 38.9</td>
<td>95.0 ± 37.3</td>
<td>92.9 ± 32.8</td>
</tr>
</tbody>
</table>

WL: words list subtest of the Wechsler Memory Scale-III (Wechsler, 1987); VPA: Verbal Paired Associates subtest of the Wechsler Memory Scale-III (Wechsler, 1987).

Table 2
Plasma homocysteine, B12 and folate levels according to MTHFR genotype

<table>
<thead>
<tr>
<th>MTHFR genotype</th>
<th>Statistic</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homocysteine (mean ± S.D.)</td>
<td>F = 2.82</td>
<td>0.062</td>
</tr>
<tr>
<td>B12 (mean ± S.D.)</td>
<td>χ² = 1.84</td>
<td>0.594</td>
</tr>
<tr>
<td>Folate (mean±S.D.)</td>
<td>χ² = 4.44</td>
<td>0.108</td>
</tr>
</tbody>
</table>

F statistic from oneway analysis of variance; χ² statistic from Kruskal-Wallis oneway analysis of variance.

* After natural logarithmic transformation.
In addition, we did not find that the cognitive scores were associated with plasma Hcy rather than low B12 or folate levels in our sample, which suggests that depression in later life is independent from B12, folate, age and CAMCOG scores (partial correlation r = 0.23, P = 0.001). The association between presenting clinically significant depressive symptoms (BDI score > 13) and plasma Hcy remained after adjustment for positive history of hypertension, TT genotype and age in a logistic regression analysis (OR = 1.1, 95% CI = 1.0–1.2; z = 2.02, P = 0.044).

Table 1 summarizes the cognitive performance of subjects according to their MTHFR genotype – there was no obvious association between MTHFR genotype and cognitive scores. In addition, we did not find that cognitive scores were associated with Hcy, B12 or folate levels (P > 0.30 for all correlations). Table 2 summarizes the association between MTHFR genotype and plasma Hcy, B12 and folate levels.

4. Discussion

The results of the present study indicate that MTHFR polymorphism is unlikely to play a direct role in the modulation of mood and cognitive performance of healthy older women, but confirm the existence of an association between plasma Hcy and depression in later life. Bottiglieri et al. [6] had previously shown, in a case-control study, that older adults with depression have higher Hcy levels than normal controls or patients with neurological illnesses. A more recent nested case-control investigation using subjects from the Rotterdam Study cohort confirmed and extended these findings to older adults – elderly people with a depressive disorder (n = 112) have higher Hcy levels than controls (n = 416) [31]. Previously reported data from epidemiological surveys indicate that low vitamin B12 and, to some extent, folate levels are associated with depression in later life [23,31]. We were unable to replicate such findings in our sample, which suggests that depression in later life is associated with plasma Hcy rather than low B12 or folate levels. A large cross-sectional Norwegian study (n = 5948) has also found that subjects with depression, as defined by a score greater than 7 on the depression subscale of the Hospital Depression and Anxiety Scale, were more likely to have high plasma Hcy, but not low plasma B12 or folate [5]. Taken together, these findings indicate that the higher Hcy levels observed amongst older women are unlikely to be simply the result of poorer self-care and insufficient diet (i.e., a consequence of depression).

The frequency distribution of MTHFR genotypes in our sample is remarkably similar to that described in previously published population-based studies [19,32,5], as is the frequency of high plasma Hcy [5], which suggests that recruitment bias cannot adequately explain our results (although we cannot completely dismiss such a possibility). In addition, the present study confirmed the findings of previous reports indicating that TT subjects have higher Hcy levels than CT or CC subjects [7]. Of interest, the report from the Hoedland Homocysteine Study [5] suggested that subjects with clinically significant depressive symptoms were more likely to carry the MTHFR-TT genotype, although our analysis of their data for women aged 70–74 years only showed that the TT genotype was not associated with increased risk of depression (OR = 1.3, 95% CI = 0.4–3.2).

The mechanisms that underlie the association between plasma Hcy and depression remain largely unknown, but it is possible that such an association is at least partly mediated by cerebrovascular illness in the form of strokes [20] or white matter disease [13]. The latter could include mechanisms involving disturbed cellular methylation, which are critical to the synthesis and metabolism of norepinephrine, serotonin and dopamine. Our recent finding [19] of increased red cell folate content among people with TT genotype is consistent with the accumulation of folate in the 5,10-methylene form at the expense of 5-methyl-THF – this may have important consequences for cellular methylation reactions. This ‘folate trap’ is presumably due to the metabolic block produced by the defective MTHFR activity in TT homozygosity and has been observed by others [33]. There is abundant evidence that the reduced levels of 5-methyl-THF in TT homozygotes, particularly when associated with low folate intake, leads to reduced levels of S-adenosyl-methionine (SAM), the universal methyl donor in more than 100 methylation reactions [16,18,24]. Indeed, the current view of cellular methylation status places preeminence on the ratio of SAM to S-adenosyl-homocysteine (SAH) – the latter is the immediate metabolic precursor of Hcy and is formed from SAM by transmethylation. S-Adenosyl-homocysteine is a potent inhibitor of SAM-dependent methylation reactions, and conditions that prevent the cellular removal of Hcy, such as renal failure, favor the formation of SAH via the SAH hydrolase reaction [10].

The cognitive assessment of our subjects included a general measure of cognitive function (CAMCOG), as well as tests assessing immediate and delayed recall for verbal material (Words list and Verbal Paired Associates) and faces (Faces), visuospatial abilities (Block design) and verbal fluency (FAS). There was no obvious association between MTHFR genotype and cognitive performance. In addition, we did not find that the cognitive scores were associated with Hcy, B12 or folate levels. Previous studies, have described that high total plasma Hcy is associated with increased risk of Alzheimer’s disease [11,26] and strokes [20]. High plasma homocysteine, and low B12 and folate levels, have also been associated with poorer cognitive performance in some cross-sectional studies [8,27], but their contribution to the observed variance is small [14]. Results from the Rotterdam Study, however, are consistent with our findings and show that plasma Hcy is not associated with either cognitive impairment (MMSE < 26) or decline (drop of 1 point per year on the MMSEI) [17]. Our data further indicate that neither the MTHFR gene nor the plasma levels of Hcy, folate and B12 are significantly associated with...
cognitive function in older women. We acknowledge that the exclusion of subjects with cognitive impairment from our sample, and the relatively narrow range of cognitive scores may have decreased the power of this study to detect such an effect. In fact, definitive proof of Hcy or SAM/SAH’s role in cognitive decline and depression will require prospective clinical trials using agents that lower these metabolites by folate dependent and independent means, as well as direct cerebrospinal fluid assessment of methylator status, folate, B12, B6, SAM, SAH and Hcy.

In summary, results of the present study indicate that the MTHFR gene does not directly modulate mood or cognitive function in older women. Our results, could also be interpreted as indicating that other genes and environmental factors interact to regulate the plasma level of Hcy[28,29], as well as the risk of depression and cognitive impairment in the elderly. This possibility would suggest that environmental risk factors, once detected, may be amenable to change and, as a consequence, have the potential to prevent depression in later life.

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References


