

Association of methylenetetrahydrofolate reductase gene 677C>T polymorphism with post-stroke depression risk and antidepressant treatment response in Han Chinese

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Abstract

Objectives: To investigate the association of methylenetetrahydrofolate reductase gene 677C>T polymorphism with post-stroke depression risk and antidepressant treatment response in Han Chinese.

Methods: This cross-sectional study was conducted at the Department of Neurology and Neurosurgery, the Second Affiliated Hospital of Nanjing Medical University and Nanjing Brain Hospital, China, between February 2010 and December 2014. It comprised patients who had experienced first-episode acute stroke. Psychological assessment was performed at day 7, 1 month, 3 months and 6 months after stroke. Post-stroke depression patients were characterised by clinical response to anti-depressive pharmacological treatment measured by intra-individual changes based on the 17-item Hamilton rating scale for depression scores.

Results: Of the total 292 patients, 11(3.76%) were lost during 6-month follow-up. Our final analysis comprised 106(36.3%) post-stroke depression patients and 175(59.93%) post-stroke depression-free patients. The 677T allele and 677C/T genotype of methylenetetrahydrofolate reductase gene 677C>T polymorphism, which were over-represented in depression patients and were respectively associated with 1.82-fold ($p=0.001$) and 3.65-fold ($p<0.001$) increased risk of post-stroke depression relative to the 677C allele and 677C/C genotype. No significant association was observed between this polymorphism and treatment response ($p>0.05$).

Conclusions: Mutation of methylenetetrahydrofolate reductase gene 677C>T polymorphism was found to be significantly associated with the increased risk of post-stroke depression, but not with treatment response.

Keywords: Post-stroke depression, Antidepressant treatment response, Methylenetetrahydrofolate reductase, Polymorphism. (JPMA 68: 888; 2018)

Introduction

Post-stroke depression (PSD) is a common neuropsychiatric complication of stroke.¹ Generally, PSD is defined as a condition with different degrees of PSD symptoms that persist for more than 2 weeks. The overall incidence rate of PSD is 40%-50%, and the acute period incidence rate is approximately 33%.^{2,3} Although great endeavours have been made over the past decade, unravelling the pathogenesis of PSD still remains a challengeable task. It is widely recognised that PSD is primarily caused by social psychology, neural anatomy, and neurobiology factors.⁴ A review has underscored that homocysteine (Hcy) is not only associated with stroke but also with resultant depression.⁵ For example, serum levels of total Hcy were observed to be significantly higher in depressed patients than normal controls.⁶ Specifically, the degree of depression increased with the increase of total blood Hcy levels.⁷

From a biological viewpoint, Hcy methylation is influenced by several rate-limiting enzymes, and thereof methylenetetrahydrofolate reductase (MTHFR) is a key enzyme. The gene encoding human MTHFR is mapped on chromosome 1p36.3, and it includes 11 exons and 10 introns with complementary deoxyribonucleic acid (cDNA) spanning 212 kb.⁸ The genomic sequence of MTHFR gene is polymorphic, and one polymorphism in the 4th exon, 677C>T (rs1801133), has been widely evaluated, as its mutation can cause decreased enzyme activity and lead to increased blood Hcy levels consequently. A study found that carriers of MTHFR gene 677T/T genotype had significantly higher levels of blood Hcy than those with the 677C/C genotype.⁹ Another study in an elderly population from Norway revealed a close relation between 677T/T genotype and depression.⁸ On the basis of above observations, it is reasonable to hypothesise that MTHFR gene 677C>T polymorphism might be involved in the development of PSD. To test this hypothesis, the current study was planned to investigate the association of MTHFR gene 677C>T polymorphism with PSD risk and antidepressant treatment response.

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Patients and Methods

This hospital-based case-control association study was conducted at the Department of Neurology and Neurosurgery, the Second Affiliated Hospital of Nanjing Medical University and Nanjing Brain Hospital, China, between February 2010 and December 2014. Approval was obtained from the institutional ethics committee, and written informed consent was obtained from all the subjects.

Stroke was diagnosed by neurologists according the criteria formulated by the World Health Organisation (WHO),^{10,11} and was further confirmed through magnetic resonance imaging (MRI).

Eligibility criteria¹² limited the sample to subjects age 18 years or above who had been admitted into hospital within 72 hours of stroke onset, and whose clinical data and blood samples were available for analysis, and the subjects themselves available for a minimum follow-up of 6 months. Patients were excluded if they had a history of stroke or other central nervous system (CNS) diseases, such as epilepsy, dementia, substance abuse disorders, schizophrenia, delusional disorder, psychotic disorders, bipolar disorder, or personality disorder diagnosed by Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV).

Psychological assessment was performed by a trained psychologist at approximately day 7, 1 month, 3 months and 6 months after stroke onset. PSD was diagnosed based on the DSM-IV classification system criteria¹³ for major depressive disorder (MDD). At the time of recruitment, all patients had scores of ≥ 17 on the 17-item Hamilton rating scale for depression (HAMD). Clinical response was defined as at least 50% reduction of HAMD scores after 6 weeks of antidepressant treatment at adequate doses for the current major depressive episode compared to the baseline.¹⁴ Patients with HAMD scores less than 8 were considered to be in remission.

On the basis of a recent meta-analysis on MTHFR gene 677C>T polymorphism and depression in the Chinese

population,¹⁵ the overall frequency of mutant 677T allele was estimated to be 39.7% in control groups and the 677T allele was associated with 1.52-fold increased risk of depression. Our current sample had 90.3% power to detect the expected 52% increase in risk of PSD for MTHFR gene 677C>T polymorphism at a two-sided alpha of 0.05.

Genomic DNA was obtained from 250 μ L EDTA-anticoagulated venous blood using a DNA extraction kit (Tiangen Biotech (Beijing) Co., Ltd.) based on the manufacturer's recommendations. The genotypes of MTHFR gene 677C>T polymorphism was determined by polymerase chain reaction (PCR) — restriction fragment length polymorphism (RFLP). Forward and reverse primer sequences were 5'-AGG AGA AGG TGT CTG CGG GAG-3' and 5'-CCC TCA CCT GGA TGG GAA AGA T-3', respectively. PCR reactions were performed in a 25 μ L volume containing 1 μ L of 100 ng/L DNA, 12.5 μ L 2 \times Taq Master Mix (Bioedify Nanjing), 2 μ L primer, and 9.5 μ L distilled water. DNA was amplified during thermal cycling, which included an initial denaturation at 95°C for 5 minutes, followed by 32 denaturation cycles at 95°C for 30 seconds, annealing at 56.5°C for 40 seconds, and extension at 72°C for 50 seconds, with a final extension at 72°C for 5 minutes. The PCR products were then cut with TaqI (New England Biolabs) at 37°C overnight, and were run in 2% agarose gels at 100 V for 45 minutes.

Comparisons of allele and genotype counts between PSD patients and PSD-free patients were performed using the chi-square test. Hardy-Weinberg equilibrium was tested using the chi-square test as well. The power to detect statistical significance was calculated using Power and Sample Size Calculations (PS) software, version 3.0. Risk estimates expressed as odds ratio (OR) and its 95% confidence interval (95% CI) were calculated using the UNPHASED programme. Multiple comparisons were corrected by the Bonferroni method. All tests were two-tailed, and statistical significance was defined as $p < 0.05$.

Results

Of the 292 patients recruited, 11(3.76%) were lost to

Table-1: Genotype and allele distributions of MTHFR gene 677C>T polymorphism between patients with and without PSD.

Allele or Genotype	n (%)		OR (95% CI)	P-value	P for HWE
	PSD patients (n=106)	PSD-free patients (n=175)			
677C	125 (59.1)	253 (72.4)	Reference group		0.59
677T	87 (40.9)	97 (27.6)	1.82 (1.27;2.60)	0.001	
677C/C	25 (23.9)	90 (51.6)	Reference group		
677C/T	75 (70.4)	73 (41.6)	3.65 (2.11;6.32)	<0.001	
677T/T	6 (5.7)	12 (6.8)	1.80 (0.61;5.28)	0.61	

Abbreviations: MTHFR, methylenetetrahydrofolate reductase; PSD, post-stroke depression; OR, odds ratio; 95% CI, 95% confidence interval; HWE, Hardy-Weinberg equilibrium.

Table-2: Genotype and allele distributions of MTHFR gene 677C>T polymorphism between responders and non-responders.

Allele or Genotype	n (%)		OR (95% CI)	P-value	P for HWE
	Responders (n=84)	Non-responders (n=22)			
677C	100 (59.5)	25 (56.8)	Reference group		0.59
677T	68 (40.5)	19 (43.2)	0.89 (0.45?1.75)	0.88	
677C/C	20 (23.8)	5 (22.7)	Reference group		
677C/T	60 (71.4)	15 (68.2)	1.00 (0.32?3.10)	0.77	
677T/T	4 (4.8)	2 (9.1)	0.50 (0.07?3.55)	0.88	

Abbreviations: MTHFR, methylenetetrahydrofolate reductase; OR, odds ratio; 95% CI, 95% confidence interval; HWE, Hardy-Weinberg equilibrium.

follow-up. The final analysis involved a total of 281(96.23%) patients of whom 106(36.3%) were diagnosed to have PSD, and 175(59.93%) were free of PSD. Of those diagnosed with PSD, 57(53.77%) were males and 49(46.22%) were females having an overall mean age of 62.5±10.4 years. Of the PSD-free patients, 102(58.25%) were males and 73(41.71%) were females, having an overall mean age of 63.6±10.2 years. Age and gender were frequency-matched between the two groups ($p=0.54$ and $p=0.39$). The mean level of HAMD was 26.31±9.33 in PSD patients. Of the PSD patients, 84(79.2%) were classified as responders and 22(20.8%) as non-responders after 6-month's follow-up.

The genotype distributions of MTHFR gene 677C>T polymorphism were in Hardy-Weinberg equilibrium in PSD-free patients ($p>0.05$).

Genetic frequencies of this polymorphism and associated risk prediction for PSD were noted (Table-1). The mutant 677T allele of this polymorphism accounted for 87(40.9%) of PSD patients and 97(27.6%) of PSD-free patients. The frequencies of 677T/T, 677T/C and 677C/C genotypes were 6(5.7%), 75(70.4%) and 25(23.9%) in PSD patients and 12(6.8%), 73(41.6%) and 90(51.6%) in PSD-free patients. The power to detect allele differences between the two groups was estimated to be 88%. The 677T allele and 677C/C genotype were significantly associated with 1.82-fold (95% CI: 1.27-2.60, $p=0.001$) and 3.65-fold (95% CI: 2.11-6.32, $p<0.001$) increased risk of PSD relative to the 677C allele and 677C/C genotype, respectively, even after the Bonferroni correction.

There was no detectable significance for the allele and genotype comparisons of MTHFR gene 677C>T polymorphism between responders and non-responders in PSD patients ($p>0.05$) (Table-2).

Discussion

Via a candidate gene approach, this association study has identified the mutation of MTHFR gene 677C>T

polymorphism to be significantly associated with the increased risk of PSD in Han Chinese, which supports the hypothesis that MTHFR gene is involved in the development of PSD. However, our findings did not support the susceptibility of this polymorphism to antidepressant treatment response after 6-month follow-up. To the best of our knowledge, this is the first report that has interrogated the association between MTHFR gene 677C>T polymorphism and PSD risk.

It is widely recognised that MTHFR is a key enzyme involved in folate metabolism and DNA methylation, and it plays a central role in Hcy homeostasis. Growing evidence indicates that MTHFR genetic defects can lead to hyperhomocysteinemia.⁵ Until now, dozens of mutations have been identified in MTHFR gene, and an exonic polymorphism, 677C>T is the widest researched locus. The mutation of this polymorphism can result in the conversion of valine to alanine at codon 222, and is linked to the reduced enzyme activity of MTHFR. In the current literature, the association between MTHFR gene 677C>T polymorphism and depression has been widely evaluated, but it still remains a subject of debate. Some studies have shown that this polymorphism may be causally related to depression.^{16,17} By contrast, a study found no significant association between 677C>T polymorphism and MDD risk in both males and females.¹⁸ Another study also reported that 677C/T genotype, folate deficiency, and Hcy were not associated with cognition or depression in either ethnicity-pooled or stratified analysis.¹⁹ In view of these conflicting findings, we propose that the MTHFR gene-depression relationship may be contingent on the diversities in race and/or region. Extending previous observations, we designed a case-control association study, and for the first time found that the 677T allele and 677C/T genotype were overrepresented in PSD patients and they were associated with the significantly increased risk of having PSD in Han Chinese.

From a biochemical viewpoint, there is evidence that

the rise in blood Hcy levels observed in PSD patients is ascribed to methylation failure of Hcy to methionine due to a supply shortage of methyl groups from methyl folate or lack of vitamin B12 cofactor for this methylation reaction.⁶ Methionine is the precursor of S-adenosylmethionine (SAMe), the methyl donor in a host of methylation reactions in the central nervous system involving monoamines and various neurotransmitters. So, increased Hcy levels in depression can serve as an indication of functional folate and/or B12 deficiency, which ultimately cause an imbalance at the monoamine or neurotransmitter level.²⁰ Thus far, the mechanism underlying the association between MTHFR gene and PSD remains unclear, and it is reasonable to expect that, 677C>T mutation, if involved, can alter Hcy metabolism-related enzyme activity, regulate circulating Hcy levels and ultimately involve in the development of PSD.

Besides, in the present study we have examined the possible association between MTHFR gene 677C>T polymorphism and antidepressant treatment response, yet no hint of significance was attained, in agreement with the findings from a study which reported that this polymorphism did not affect the antidepressant response of fluoxetine treatment.²¹ Contrastingly, other studies indicated that MTHFR gene 677C>T polymorphism may have various effects on therapeutic response to different treatments in MDD patients.^{22,23} In addition, the findings of a recent randomised, double-blind, placebo-controlled study using a combination of reduced B-vitamins and micronutrients to treat MDD, supported the Hcy-depression theory and the therapeutic benefits of reduced B vitamins as monotherapy for MDD, especially hinging on the 677C>T genotype.²⁴ To avoid chance findings due to limited sample sizes of the present study, especially in treatment response analysis, we agree that confirmation in a large, well-designed study is critical.

Finally, several limitations deserve special considerations. First, this study was cross-sectional in design, which precluded addressing the time course of an association between MTHFR gene 677C>T polymorphism and PSD risk, as well as its treatment response. Second, this study was carried out in a monohospital, and generalisation of our findings to the more general population should be interpreted cautiously, as a recent meta-analysis indicated that MTHFR gene 677C>T polymorphism was associated with depression varied across different geographic locations of China.¹⁵ Third, only one polymorphism was genotyped in MTHFR gene, and its contribution to PSD risk and

treatment response may be small. Further studies covering the whole MTHFR gene with haplotype tags are required to confirm the involvement of this gene in the development of PSD, and also its interaction with other genes and/or environmental factors is encouraging.²⁵

Conclusion

The study provided evidence that the mutation of MTHFR gene 677C>T polymorphism was significantly associated with the increased risk of PSD, but not with antidepressant treatment response in Han Chinese. Additional studies are warranted to replicate our findings as well as elucidate the biological and clinical relevance between MTHFR gene and PSD.

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Conflict of Interest: None.

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