C677T Methylenetetrahydrofolate Reductase (MTHFR) Gene Polymorphism in Schizophrenia and Bipolar Disorder: An Association Study in Iranian Population

Seyed Masoud Arzaghi, MD
Arash Hossein-Nezhad, MD, PhD
Seyed Vahid Shariat, MD
Ali Reza Ghodsipour, MD
Jamal Shams, MD
Bagher Larijani, MD

1 Psychosomatic Research Group, Endocrinology and Metabolism Research Center, Tehran University of Medical Sciences, Tehran, Iran
2 Endocrinology and Metabolism Research Center, Tehran University of Medical Sciences, Tehran, Iran
3 Mental Health Research Center, Tehran University of Medical Sciences, Tehran, Iran
4 Neuroscience Research Center, National Neuroscience Research Network, Shaheed Beheshti University of Medical Sciences, Tehran, Iran

Corresponding author:
Arash Hossein-Nezhad, MD, PhD
5th Floor, Dr. Shariati Hospital, North Kargar Avenue, Tehran 14114, Iran.
Tel: +98 (21) 88220037-38,
Fax: +98 (21) 88220052,
Email: ahosseinnezhad@tums.ac.ir

Objective: The methylenetetrahydrofolate reductase (MTHFR) gene polymorphism C677T is suspected to be a risk factor for psychiatric disorders, but it remains inconclusive whether the MTHFR polymorphism C677T is imputed to vulnerability to schizophrenia and bipolar disorder.

Method: We prompted impetus to appraise this polymorphism in an Iranian population. Therefore, 90 patients with bipolar disorder type I (BID), 66 patients with schizophrenia diagnosed according to DSM-IV criteria, and 94 unrelated controls with no history of psychiatric disorders were recruited for this study. Genotype distribution and allelic frequencies of C677T polymorphism were investigated.

Results: We found no robust differences between patients with BID and schizophrenia with control participants either for allele frequencies or genotype distribution of MTHFR C677T polymorphism. However, a trend toward an increased risk for T allele was observed in the BID patients [with odds ratio (OR) of 1.28 (CI 95%: 0.8-1.31), p>0.05].

Conclusion: However, the present and some previous studies failed to elucidate possible interaction between MTHFR C677T polymorphism and vulnerability to schizophrenia and bipolar disorder; still some associations have been revealed in performed meta-analyses that warrant further studies.

Keywords: Bipolar disorder, Methylenetetrahydrofolate reductase, Polymorphism, Schizophrenia,

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The methylenetetrahydrofolate reductase (MTHFR) reduces 5,10-methylenetetrahydrofolate to 5- methylenetetrahydrofolate, the main and active circulatory form of folate. 5- Methylenetetrahydrofolate plays a crucial role in one-carbon metabolism and DNA methylation. 5- Methylenetetrahydrofolate is necessary for methylation of homocysteine to methionine, the prerequisite of S- adenosylmethionine (SAM). (1, 2). This product is a great methyl group donor for several methylation reactions in the brain such as catechol-o- methyltransferase (COMT) metabolism (2, 3). Bipolar disorder (BD) is one of the major psychiatric disorders with rigorous life long disability and a great burden on the affected patients and the society. The prevalence of bipolar disorder was estimated to be 0.96% in a large population based study in Iran (4). Several family, twin and chromosomal studies suggest genetic predisposition as an etiopathogenesis factor for BD (3, 5-9).

Schizophrenia, another serious psychiatric disorder, affects 0.25% of the Iranian population (4), and numerous genetic studies have hitherto revealed noteworthy association (10-15). The MTHFR gene is located at the end of short arm of chromosome 1 (1p36.3) and two common single nucleotide polymorphism (SNPs) affecting enzyme activities have been reported: C677T and A1298C (2, 16-20). C677T mutation results in substitution of alanin with valine (A222V) and associates with decrease in enzyme activity, hyperhomocysteinemia, premature cardiovascular disease and neural tube defects (17, 3, 20-25). Hyperhomocysteinemia may induce toxic effects on dopaminergic neurons (26). MTHFR dysfunction has been associated with some psychiatric manifestation in more recent studies suggesting possible role in pathogenesis of psychiatric disorders (27). On the other hand, MTHFR C677T polymorphism may be linked to BD and schizophrenia via excitatory amino-acids hypothesis and/or low SAM plasma concentrations (28, 29). Numerous association...
studies from different societies and racial descents have focused on possible relations between C677T, and either schizophrenia (30-44) or BD (31, 38-40, 44-49), but results have not been consistent.

In the present study, we investigated MTHFR C677T polymorphism in schizophrenic and BID patients and control subjects in Iran.

Materials and Method

The study was performed on an Iranian population with the same ethnical background and included 90 patients with unrelated bipolar disorder type 1 (BID) (51 males and 39 females with Mean±SD age of 35±8 years), 66 unrelated schizophrenic patients (45 males and 21 female with Mean±SD age of 29±4 years) and 94 age and sex matched controls. Patients were recruited from the outpatient clinic and inpatients of Iran psychiatry hospital, Iran University of medical sciences, Tehran, Iran. Diagnosis in all cases were made based on clinical assessments by consensus of two experienced psychiatrists according to DSM-IV criteria using Structured Clinical Interview for DSM-IV Axis 1 disorders (SCID). A self-administered questionnaire, after education by researchers, providing information on demographic, socioeconomic, and psychosocial parameters; history of psychiatric disorders, neurological problems, mental retardation or metabolic diseases, all were selected as control subjects in Iran.

The control group included 94 persons (53 males and 41 females with Mean±SD age of 31±6 years). None of the controls had personal or familial history of major psychiatric disorders, neurological problems, mental retardation, head trauma, cardiovascular, endocrinological or metabolic diseases. The group and the controls on the mean age distribution.

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The study was accepted by the local ethics committee . At least 2 ml of saliva was collected from participants after washing the mouth and kept in a container until genomic DNA was extracted, using FlexiGen Kit (QIAGEN Inc. Valencia, CA), according to its protocol. The polymorphism was detected by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The target region was amplified by the PCR using the forward primer 5’-CTTTGAGGCTGACCTGAAGC-3’ and reverse primer, 5’-TCACAAAGCGGAA GAA TGTG-3’. PCR was performed in a total volume of 20 containing 200 ng genomic DNA, 0.5 pM of each primer, 0.2 mM dNTP, 2 mM MgCl2, 2 ml of 10 X buffer and 1 U of Taq DNA polymerase (MBI Fermentas, Vilnius, Lithuania). PCR conditions were as followed: initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 30 seconds, with a final extension at 72°C for 10 minutes. The PCR products were digested with 1 U BbsI ( Fermentas, Vilnius, Lithuania) for 16 h at 37°C using the recommended buffer. Then the digestion products were separated by 2.5% agarose gel electrophoresis stained with ethidium bromide and visualized under ultraviolet.

The statistical analyzes were performed with the software package SPSS 11.0. The Pearson Chi-square test was used to compare allele and genotypes distributions. The odds ratios (ORs) were estimated and expressed with 95% confidence interval (CI). Statistical significance was defined as p<0.05.

Results

A total number of 250 persons from an Iranian population were recruited (94 controls, 90 patients with BID, and 66 with schizophrenia). No significant differences were observed between the experimental group and the controls on the mean age distribution.

The genotype distributions of C677C, C677T, and T677T for patients with BID were evaluated to be 57.8%, 37.8%, and 4.4%; and for patients with schizophrenia they were 53%, 40.9%, and 6.1%, respectively. There was no significant difference between the patient group and controls in genotype distributions (Table 1).

The frequency of T allele for patients with BID and schizophrenia (Table 1) did not significantly differ from that of the controls.

The Relative Risks (RRs) and Odds Ratios (ORs) with 95% Confidence Intervals (CI 95%) of MTHFR C677T polymorphism in patients with BID and schizophrenia was calculated (Table 2 and 3).

Table 1. Genotype and alleles distribution in controls and patients with BID and Schizophrenia

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Genotype absolute number (frequency)</th>
<th>Allele absolute number(frequency)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CC (absolute number (frequency))</td>
<td>CT (absolute number (frequency))</td>
</tr>
<tr>
<td>Controls</td>
<td>94</td>
<td>54(57.4)</td>
<td>38(40.4)</td>
</tr>
<tr>
<td>BID</td>
<td>90</td>
<td>52(57.8)</td>
<td>34(37.8)</td>
</tr>
<tr>
<td>Schizophrenia</td>
<td>66</td>
<td>35(53)</td>
<td>27(40.9)</td>
</tr>
</tbody>
</table>

BID: Bipolar Disorder Type 1, Controls vs. BID: Chi-square P>0.05, Schizophrenia vs. BID: Chi-square P>0.05
In either BID nor schizophrenia, no significant association was observed between allele of C677T and the risk of developing illness, although a trend toward an increased risk for T allele was observed in the BID patients.

Discussion
There is a rapidly evolving area of interest in investigation of gene-psychiatric disorders worldwide and this study is now poised to discover multiple disease genes in the coming years. A vast majority of studies emphasized the role of biochemical abnormalities in vulnerability to neuropsychiatric conditions such as schizophrenia and bipolar disorder and folate, a major determinant of 1-carbon metabolic pathway, may play a crucial role in the liability to affliction.

Numerous case-control association studies investigated functional polymorphism C677T of MTHFR gene in patients with BID (31, 38-40, 44-47) and schizophrenia (30-44) from different racial descents all over the world. Notoriously discrepant and inconclusive results yielded that prompted impetus to appraise this polymorphism in an Iranian population.

In the present study, we found no impressive differences between patients with BID and controls. This is in concordance with the majority of the studies (31, 34, 39, 44-47) and in divergence with only one previous study (40). Likewise, we observed no robust significant differences when schizophrenic patients and controls were compared for C677T polymorphism in MTHFR gene. This results are in accordance with some (30, 31, 35-38, 41, 43, 44) and in contradiction with other previous studies (32-34, 39, 40, 42).

In summary, we found a great discrepancy in describing the contribution of MTHFR polymorphism to schizophrenia and to a lesser extent in bipolar disorder. These discrepancies, in part, may result from hidden population stratifications, explicitly, socio-economic status. Convincingly, dietary folate content has seminal effects on enzyme activity of MTHFR, typically, MTHFR T677T homozygote persons experience more defect in enzyme activity, in milieu of high homocysteine plasma levels, whenever they face lower folate levels than higher ones (44,48, 49), indicating possible compensatory effects of folate on defective enzyme activity. Of note, socio-economic and dietary adjustment should be considered in future studies.

On the other hand, heretofore, near 14 different polymorphisms in MTHFR gene have been identified, the less prevalent the mutation, have been revealed to engender the more severely deficient the enzyme activity (50-52); nonetheless, there are several possible genes impute playing a role in 1-carbon pathway in interaction with MTHFR polymorphism, as methylenetetrahydrofolate dehydrogenase and methionin synthase (53, 54). Therefore, one can not overlook the significance of this complexity. However, scrupulous detection of possible individual effects is formidable through association studies and warrants further well-designed investigations.

Nevertheless, schizophrenia and bipolar disorder are considered as polygenic conditions. In an additive model, the total genetic liability illness is then the sum of the probabilities contributed by all of the polygenes. Furthermore, as complex conditions, convey elaborate heterogeneity, multiple genes may combine to produce illness in a variety of different ways.

Besides, schizophrenia and bipolar disorder are the aftermath of a complex gene-environmental implication whose epigenetic factors have been proposed of noteworthy importance (55-58). DNA methylation, one of the epigenetic mechanisms, plays a critical role in modification of gene expression; in such a way that thoroughgoing DNA methylation is important not only for early in-utero life, but also

### Table 2. Odds Ratios and Relative Risk and 95% confidence intervals of MTHFR C677T polymorphism and BID

<table>
<thead>
<tr>
<th>Relative Risk (95% confidence interval)</th>
<th>Odds Ratios (95% confidence interval)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T vs. C Alleles 1.028(0.92-1.20)</td>
<td>1.058(0.65-1.72)</td>
<td>0.9</td>
</tr>
<tr>
<td>TT vs. (CT+TT) genotypes 1.55(0.49-4.85)</td>
<td>2.14(0.38-11.98)</td>
<td>0.4</td>
</tr>
<tr>
<td>TT vs. CC genotypes 1.52(0.48-4.81)</td>
<td>2.07(0.36-11.83)</td>
<td>0.6</td>
</tr>
<tr>
<td>CT vs. CC genotypes 0.96(0.72-1.28)</td>
<td>0.92(0.51-1.69)</td>
<td>0.87</td>
</tr>
<tr>
<td>(TT+CT) vs. CC genotypes 0.99(0.74-1.32)</td>
<td>0.99(0.54-1.77)</td>
<td>0.9</td>
</tr>
</tbody>
</table>

**BID:** Bipolar Disorder Type I

### Table 3. Odds Ratios and Relative Risk and 95% confidence intervals of MTHFR C677T polymorphism and Schizophrenia

<table>
<thead>
<tr>
<th>Relative Risk (95% confidence interval)</th>
<th>Odds Ratios (95% confidence interval)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T vs. C Alleles 1.05(0.92-1.20)</td>
<td>1.25(0.47-2.10)</td>
<td>0.42</td>
</tr>
<tr>
<td>TT vs. (CT+TT) genotypes 1.04(0.97-1.11)</td>
<td>2.96(0.52-16.70)</td>
<td>0.23</td>
</tr>
<tr>
<td>TT vs. CC genotypes 1.07(0.95-1.2)</td>
<td>3.08(0.53-17.76)</td>
<td>0.22</td>
</tr>
<tr>
<td>CT vs. CC genotypes 1.04(0.78-1.37)</td>
<td>1.09(0.57-2.10)</td>
<td>0.86</td>
</tr>
<tr>
<td>(TT+CT) vs. CC genotypes 1.08(0.81-1.44)</td>
<td>1.19(0.63-2.25)</td>
<td>0.62</td>
</tr>
</tbody>
</table>
throughout the life. Methylation may be influenced by some genes, including genes involved in 1-carbon pathway as MTHFR (59). Thus, more well-designed genetic studies should address the interaction between epigenetic mechanisms, MTHFR and both schizophrenia and bipolar disorder.

In this sense, one of the nearly all limitations of the previous studies, and of course, the present study, is dismissal of the role of environmental issues in case recruitment. Furthermore, the impact of psychotropic medications and eliciting drug abuse on gene expression may convey a subject for future studies to elucidate possible roles of the environmental issues. It is no surprise that future studies strive to elicit more hidden environmental factors which potentially affect genetic vulnerabilities in view of great discrepancies noted in heretofore studies.

However, the present and some previous studies failed to elucidate possible interaction between MTHFR C677T polymorphism and vulnerability to schizophrenia and bipolar disorder; still associations have been revealed in performed meta-analyses (60, 61) that warrant further studies with more precise methodology and larger populations.

Acknowledgment
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