Original Article

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

Seyed Masoud Arzaghi, MD[1]
Arash Hossein-Nezhad, MD, PhD [2]
Seyed Vahid Shariat, MD[3]
Alireza Ghodsipour, MD [2]
Jamal Shams, MD [4]
Bagher Larijani, MD [2]

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,*

Iran J Psychiatry 2011; 6: 1-6

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 I disorders (SCID). A self-administered questionnaire, after education by researchers, providing information on demographic, socioeconomic, and psychosocial parameters; history of psychiatric disorders in first and second relatives; history of genetic or heritable diseases in participant or his/her family; history of head trauma and number of previous hospitalizations. None of the subjects had current and previous history of neurological problems, epilepsy, mental retardation, head trauma, cardiovascular, endocrinological or metabolic diseases.

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 or metabolic diseases, all were selected from the hospital staff and students of Iran University of medical sciences, with a similar socioeconomic background of the case group. Informed written consent was obtained from all the participants. 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

Groups	n	Genotype distribution absolute number (frequency)			Allele absolute number(frequency)	
		CC	CT	TT	T	C
Controls	94	54(57.4)	38(40.4)	2(2.1)	42(22.3)	146(77.7)
BID	90	52(57.8)	34(37.8)	4(4.4)	42(23.3)	138(76.7)
Schizophrenia	66	35(53)	27(40.9)	4(6.1)	35(26.5)	97(73.5)

BID: Bipolar Disorder Type I, Controls *vs.* BID: Chi-square P>0.05, Schizophrenia *vs.* BID: Chi-square P>0.05

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

	Relative Risk (95% confidence interval)	Odds Ratios (95% confidence interval)	P-Value
T *vs.* C Alleles	1.028(028-1.31)	1.058(0.65-1.72)	0.9
TT *vs.* (CT+TT) genotypes	1.55(0.49-4.85)	2.14(0.38-11.98)	0.4
TT *vs.* CC genotypes	1.52(0.48-4.81)	2.07(0.36-11.83)	0.6
CT *vs.* CC genotypes	0.96(0.72-1.28)	0.92(0.51-1.69)	0.87
(TT+CT) *vs.* CC genotypes	0.99(0.74-1.32)	0.99(0.54-1.77)	0.9

BID: Bipolar Disorder Type I

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

	Relative Risk (95% confidence interval)	Odds Ratios (95% confidence interval)	P-Value
T *vs.* C Alleles	1.05(0.92-1.20)	1.25(0.47-2.10)	0.42
TT *vs.* (CT+TT) genotypes	1.04(0.97-1.11)	2.96(0.52-16.70)	0.23
TT *vs.* CC genotypes	1.07(0.95-1.2)	3.08(0.53-17.76)	0.22
CT *vs.* CC genotypes	1.04(0.78-1.37)	1.09(0.57-2.10)	0.86
(TT+CT) *vs.* CC genotypes	1.08(0.81-1.44)	1.19(0.63-2.25)	0.62

In neither 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

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

This study was supported by a grant from the Neuroscience Research Center, subdivision of National Neuroscience Research Network, Shaheed Beheshti University of Medical Sciences, Tehran, Iran.

References

1. Godfrey PS, Toone BK, Carney MW, Flynn TG, Bottiglieri T, Laundy M, et al. Enhancement of recovery from psychiatric illness by methylfolate. Lancet 1990; 336: 392-395.
2. Catoni GL. S-Adenosylmethionine; a new intermediate formed enzymatically from L-methionine and adenosinetriphosphate. J Biol Chem 1953; 204: 403-416.
3. Anguelova M, Benkelfat C, Turecki G. A systematic review of association studies investigating genes coding for serotonin receptors and the serotonin transporter: II. Suicidal behavior. Mol Psychiatry 2003; 8: 646-653.
4. Mohammadi MR, Davidian H, Noorbala AA, Malekafzali H, Naghavi HR, Pouretemad HR, et al. An epidemiological survey of psychiatric disorders in Iran. Clin Pract Epidemiol Ment Health 2005; 1: 16.
5. Mamdani F, Jaitovich Groisman I, Alda M, Turecki G. Long-term responsiveness to lithium as a pharmacogenetic outcome variable: treatment and etiologic implications. Curr Psychiatry Rep 2003; 5: 484-492.
6. Cardno AG, Marshall EJ, Coid B, Macdonald AM, Ribchester TR, Davies NJ, et al. Heritability estimates for psychotic disorders: the Maudsley twin psychosis series. Arch Gen Psychiatry 1999; 56: 162-168.
7. Michelon L, Vallada H. [Genetics of bipolar disorder]. Rev Bras Psiquiatr 2004; 26 Suppl 3: 12-16.
8. Craddock N, Dave S, Greening J. Association studies of bipolar disorder. Bipolar Disord 2001; 3: 284-298.
9. Craddock N, Owen M. Chromosomal aberrations and bipolar affective disorder. Br J Psychiatry 1994; 164: 507-512.
10. Muntjewerff JW, Kahn RS, Blom HJ, den Heijer M. Homocysteine, methylenetetrahydrofolate reductase and risk of schizophrenia: a meta-analysis. Mol Psychiatry 2006; 11: 143-149.
11. Weinberger DR. Implications of normal brain development for the pathogenesis of schizophrenia. Arch Gen Psychiatry 1987; 44: 660-669.
12. Badner JA, Gershon ES. Meta-analysis of whole-genome linkage scans of bipolar disorder and schizophrenia. Mol Psychiatry 2002; 7: 405-411.
13. Lewis CM, Levinson DF, Wise LH, DeLisi LE, Straub RE, Hovatta I, et al. Genome scan meta-analysis of schizophrenia and bipolar disorder, part II: Schizophrenia. Am J Hum Genet 2003; 73: 34-48.
14. Kirov G, O'Donovan MC, Owen MJ. Finding schizophrenia genes. J Clin Invest 2005; 115: 1440-1448.
15. Maier W, Hofgen B, Zobel A, Rietschel M. Genetic models of schizophrenia and bipolar disorder: overlapping inheritance or discrete genotypes? Eur Arch Psychiatry Clin Neurosci 2005; 255: 159-166.
16. Goyette P, Sumner JS, Milos R, Duncan AM, Rosenblatt DS, Matthews RG, et al. Human methylenetetrahydrofolate reductase: isolation of cDNA, mapping and mutation identification. Nat Genet 1994; 7: 195-200.
17. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet 1995; 10: 111-113.
18. van der Put NM, Gabreels F, Stevens EM, Smeitink JA, Trijbels FJ, Eskes TK, et al. A second common mutation in the methylenetetrahydrofolate reductase gene: an additional risk factor for neural-tube defects? Am J Hum Genet 1998; 62: 1044-1051.
19. Lievers KJ, Boers GH, Verhoef P, den Heijer M, Kluijtmans LA, van der Put NM, et al. A second common variant in the methylenetetrahydrofolate reductase (MTHFR) gene and its relationship to MTHFR enzyme activity, homocysteine, and cardiovascular disease risk. J Mol Med 2001; 79: 522-528.
20. Rozen R. Molecular genetics of methylenetetrahydrofolate reductase deficiency. J Inherit Metab Dis 1996; 19: 589-594.
21. Matsushita S, Muramatsu T, Arai H, Matsui T, Higuchi S. The frequency of the

methylenetetrahydrofolate reductase-gene mutation varies with age in the normal population. Am J Hum Genet 1997; 61: 1459-1460.
22. Kang SS, Wong PW, Susmano A, Sora J, Norusis M, Ruggie N. Thermolabile methylenetetrahydrofolate reductase: an inherited risk factor for coronary artery disease. Am J Hum Genet 1991; 48: 536-545.
23. Kluijtmans LA, van den Heuvel LP, Boers GH, Frosst P, Stevens EM, van Oost BA, et al. Molecular genetic analysis in mild hyperhomocysteinemia: a common mutation in the methylenetetrahydrofolate reductase gene is a genetic risk factor for cardiovascular disease. Am J Hum Genet 1996; 58: 35-41.
24. van der Put NM, Steegers-Theunissen RP, Frosst P, Trijbels FJ, Eskes TK, van den Heuvel LP, et al. Mutated methylenetetrahydrofolate reductase as a risk factor for spina bifida. Lancet 1995; 346: 1070-1071.
25. van der Put NM, Eskes TK, Blom HJ. Is the common 677C-->T mutation in the methylenetetrahydrofolate reductase gene a risk factor for neural tube defects? A meta-analysis. QJM 1997; 90: 111-115.
26. Lee ES, Chen H, Soliman KF, Charlton CG. Effects of homocysteine on the dopaminergic system and behavior in rodents. Neurotoxicology 2005; 26: 361-371.
27. Picker JD, Coyle JT. Do maternal folate and homocysteine levels play a role in neurodevelopmental processes that increase risk for schizophrenia? Harv Rev Psychiatry 2005; 13: 197-205.
28. Andreoli VM, Maffei F. Letter: Blood-levels of S-adenosylmethionine in schizophrenia. Lancet 1975; 2: 922.
29. Olney JW. Role of excitotoxins in developmental neuropathology. APMIS Suppl 1993; 40: 103-112.
30. Muntjewerff JW, van der Put N, Eskes T, Ellenbroek B, Steegers E, Blom H, et al. Homocysteine metabolism and B-vitamins in schizophrenic patients: low plasma folate as a possible independent risk factor for schizophrenia. Psychiatry Res 2003; 121: 1-9.
31. Tan EC, Chong SA, Lim LC, Chan AO, Teo YY, Tan CH, et al. Genetic analysis of the thermolabile methylenetetrahydrofolate reductase variant in schizophrenia and mood disorders. Psychiatr Genet 2004; 14: 227-231.
32. Joober R, Benkelfat C, Lal S, Bloom D, Labelle A, Lalonde P, et al. Association between the methylenetetrahydrofolate reductase 677C-->T missense mutation and schizophrenia. Mol Psychiatry 2000; 5: 323-326.
33. Sazci A, Ergul E, Guzelhan Y, Kaya G, Kara I. Methylenetetrahydrofolate reductase gene polymorphisms in patients with schizophrenia. Brain Res Mol Brain Res 2003; 117: 104-107.
34. Sazci A, Ergul E, Kucukali I, Kara I, Kaya G. Association of the C677T and A1298C polymorphisms of methylenetetrahydrofolate reductase gene with schizophrenia: association is significant in men but not in women. Prog Neuropsychopharmacol Biol Psychiatry 2005; 29: 1113-1123.
35. Virgos C, Martorell L, Simo JM, Valero J, Figuera L, Joven J, et al. Plasma homocysteine and the methylenetetrahydrofolate reductase C677T gene variant: lack of association with schizophrenia. Neuroreport 1999; 10: 2035-2038.
36. Muntjewerff JW, Hoogendoorn ML, Kahn RS, Sinke RJ, Den Heijer M, Kluijtmans LA, et al. Hyperhomocysteinemia, methylenetetrahydrofolate reductase 677TT genotype, and the risk for schizophrenia: a Dutch population based case-control study. Am J Med Genet B Neuropsychiatr Genet 2005; 135B: 69-72.
37. Yu L, Li T, Robertson Z, Dean J, Gu NF, Feng GY, et al. No association between polymorphisms of methylenetetrahydrofolate reductase gene and schizophrenia in both Chinese and Scottish populations. Mol Psychiatry 2004; 9: 1063-1065.
38. Kunugi H, Fukuda R, Hattori M, Kato T, Tatsumi M, Sakai T, et al. C677T polymorphism in methylenetetrahydrofolate reductase gene and psychoses. Mol Psychiatry 1998; 3: 435-437.
39. Arinami T, Yamada N, Yamakawa-Kobayashi K, Hamaguchi H, Toru M. Methylenetetrahydrofolate reductase variant and schizophrenia/depression. Am J Med Genet 1997; 74: 526-528.
40. Kempisty B, Mostowska A, Gorska I, Luczak M, Czerski P, Szczepankiewicz A, et al. Association of 677C>T polymorphism of methylenetetrahydrofolate reductase (MTHFR) gene with bipolar disorder and schizophrenia. Neurosci Lett 2006; 400: 267-271.
41. Lee YS, Han DH, Jeon CM, Lyoo IK, Na C, Chae SL, et al. Serum homocysteine, folate level and methylenetetrahydrofolate reductase 677, 1298 gene polymorphism in Korean schizophrenic patients. Neuroreport 2006; 17: 743-746.
42. Philibert R, Gunter T, Hollenbeck N, Adams WJ, Bohle P, Packer H, et al. No association of the C677T methylenetetrahydrofolate reductase polymorphism with schizophrenia. Psychiatr Genet 2006; 16: 221-223.
43. Vilella E, Virgos C, Murphy M, Martorell L, Valero J, Simo JM, et al. Further evidence that hyperhomocysteinemia and methylenetetrahydrofolate reductase C677T and A1289C polymorphisms are not risk factors for schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry 2005; 29: 1169-1174.
44. Jonsson EG, Larsson K, Vares M, Hansen T, Wang AG, Djurovic S, et al. Two methylenetetrahydrofolate reductase gene (MTHFR) polymorphisms, schizophrenia and bipolar disorder: an association study. Am J Med Genet B Neuropsychiatr Genet 2008; 147B: 976-982.
45. Chen Z, Liu Y, Zhang D, Liu Z, Wang P, Zhou D, et al. C677T methylenetetrahydrofolate

reductase gene polymorphisms in bipolar disorder: an association study in the Chinese population and a meta-analysis of genetic association studies. Neurosci Lett 2009; 449: 48-51.
46. Reif A, Pfuhlmann B, Lesch KP. Homocysteinemia as well as methylenetetrahydrofolate reductase polymorphism are associated with affective psychoses. Prog Neuropsychopharmacol Biol Psychiatry 2005; 29: 1162-1168.
47. Ozbek Z, Kucukali CI, Ozkok E, Orhan N, Aydin M, Kilic G, et al. Effect of the methylenetetrahydrofolate reductase gene polymorphisms on homocysteine, folate and vitamin B12 in patients with bipolar disorder and relatives. Prog Neuropsychopharmacol Biol Psychiatry 2008; 32: 1331-1337.
48. Brattstrom L, Wilcken DE, Ohrvik J, Brudin L. Common methylenetetrahydrofolate reductase gene mutation leads to hyperhomocysteinemia but not to vascular disease: the result of a meta-analysis. Circulation 1998; 98: 2520-2526.
49. Friso S, Choi SW, Girelli D, Mason JB, Dolnikowski GG, Bagley PJ, et al. A common mutation in the 5,10-methylenetetrahydrofolate reductase gene affects genomic DNA methylation through an interaction with folate status. Proc Natl Acad Sci U S A 2002; 99: 5606-5611.
50. Goyette P, Sumner JS, Milos R, Duncan AM, Rosenblatt DS, Matthews RG, et al. Human methylenetetrahydrofolate reductase: isolation of cDNA mapping and mutation identification. Nat Genet 1994; 7: 551.
51. Goyette P, Christensen B, Rosenblatt DS, Rozen R. Severe and mild mutations in cis for the methylenetetrahydrofolate reductase (MTHFR) gene, and description of five novel mutations in MTHFR. Am J Hum Genet 1996; 59: 1268-1275.
52. Goyette P, Frosst P, Rosenblatt DS, Rozen R. Seven novel mutations in the methylenetetrahydrofolate reductase gene and genotype/phenotype correlations in severe methylenetetrahydrofolate reductase deficiency. Am J Hum Genet 1995; 56: 1052-1059.
53. Lucock M. Folic acid: nutritional biochemistry, molecular biology, and role in disease processes. Mol Genet Metab 2000; 71: 121-138.
54. Kempisty B, Sikora J, Lianeri M, Szczepankiewicz A, Czerski P, Hauser J, et al. MTHFD 1958G>A and MTR 2756A>G polymorphisms are associated with bipolar disorder and schizophrenia. Psychiatr Genet 2007; 17: 177-181.
55. Abdolmaleky HM, Thiagalingam S, Wilcox M. Genetics and epigenetics in major psychiatric disorders: dilemmas, achievements, applications, and future scope. Am J Pharmacogenomics 2005; 5: 149-160.
56. van Vliet J, Oates NA, Whitelaw E. Epigenetic mechanisms in the context of complex diseases. Cell Mol Life Sci 2007; 64: 1531-1538.
57. Singh SM, Murphy B, O'Reilly RL. Involvement of gene-diet/drug interaction in DNA methylation and its contribution to complex diseases: from cancer to schizophrenia. Clin Genet 2003; 64: 451-460.
58. Singh SM, McDonald P, Murphy B, O'Reilly R. Incidental neurodevelopmental episodes in the etiology of schizophrenia: an expanded model involving epigenetics and development. Clin Genet 2004; 65: 435-440.
59. Bjornsson HT, Fallin MD, Feinberg AP. An integrated epigenetic and genetic approach to common human disease. Trends Genet 2004; 20: 350-358.
60. Gilbody S, Lewis S, Lightfoot T. Methylenetetrahydrofolate reductase (MTHFR) genetic polymorphisms and psychiatric disorders: a HuGE review. Am J Epidemiol 2007; 165: 1-13.
61. Zintzaras E. C677T and A1298C methylenetetrahydrofolate reductase gene polymorphisms in schizophrenia, bipolar disorder and depression: a meta-analysis of genetic association studies. Psychiatr Genet 2006; 16: 105-115.