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Effect of the methylenetetrahydrofolate reductase gene polymorphisms on homocysteine, folate and vitamin B12 in patients with bipolar disorder and relatives

Zeynep Ozbek [a], Cem Ismail Kucukali [b,*], Elif Ozkok [c], Nurcan Orhan [c], Makbule Aydin [c], Gamze Kilic [d], Ali Sazci [e], Ihsan Kara [c]

[a] Department of Biochemistry, Eyup State Hospital, Istanbul, Turkey
[b] Department of Neurology, Istanbul Erenkoy Psychiatric and Neurological Disorders Hospital, Istanbul, Turkey
[c] Department of Neuroscience, Istanbul University Institute for Experimental Medicine, Istanbul, Turkey
[d] Department of Genetics, St. Jude Children's Research Hospital, Memphis, TN 38105, USA
[e] Department of Medical Biology and Genetics, Faculty of Medicine, University of Kocaeli, Umuttepe, 41380 Kocaeli, Turkey

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ABSTRACT

We investigated the effect of polymorphic variants of c.1298A>C (Glu429Ala) and c.677C>T (Ala222Val) in methylenetetrahydrofolate (MTHFR) gene on the total homocysteine (tHcy), folate and B12 levels in patients with bipolar disorder, first-degree relatives of patients, and controls. The c.677C>T and c.1298A>C polymorphisms in MTHFR were determined by polymerase chain reaction-restriction fragment length polymorphism in 197 bipolar patients, 278 relatives and 238 controls. tHcy and folate and vitamin B12 levels were measured by Fluorescence Polarization Immunoassay and Electrochemiluminescence, respectively. The tHcy was significantly increased in patients and relatives. In contrast, folate and B12 were significantly lower in patients and relatives. Gender was not considered as a significant determinant in the multivariate analysis. Genotypes of c.1298A>C and c.677C>T were correlated with tHcy, folate and B12. Patients and relatives carrying TT and/or AA and AC genotypes had elevated tHcy and reduced folate and B12 levels. High tHcy but low folate and vitamin B12 levels may be a risk factor for development of bipolar disorder.

1. Introduction

Bipolar disorder (BD) is a psychiatric disease that is characterized by mood alteration associated with recurrent depression and mania in lifetime (Swann et al., 2005). The elucidation of etiology and pathophysiology of this disease is extremely important to establish treatment and prevention strategy (Mamdani et al., 2003; Murray et al., 2004). Twin studies and family investigation revealed that multiple environmental and genetic factors have an effect on the development of this disease (Licinio, 2002). Although some genes have been associated with psychiatric disorders, less is known about their interaction with the environment in disease development. Pharmacogenetics and genetic testing have the potential to play key roles in the future of clinical psychiatry. Recently, there has been considerable interest in the role of folic acid and vitamin B12 for the normal function of the brain (Bottiglieri, 1996). Early studies showed that a deficiency in these vitamins is associated with a number of neurological and psychiatric disorders including depression and cognitive dysfunction (Reynolds, 1976; Carney et al., 1990; Bottiglieri et al., 2001). Several commonly occurring polymorphic mutations in folate metabolizing enzymes have been reported which are associated with reduced enzyme activity indicating that there may be a genetic–nutrient interaction that can predispose an individual to hyperhomocysteinemia (Carmel et al., 2003; Cortese and Motti, 2001). A significant proportion of patients with schizophrenia were reported to have increased tHcy levels that were unrelated to psychopharmacological medication or nutrient deficiency in folate or cobalamin (Regland et al., 1994; Regland et al., 1995).

More recent studies interested in patients with the MTHFR deficiency often show psychiatric manifestations, suggesting that the enzyme may be involved in the pathogenesis of psychiatric conditions such as schizophrenia and mood disorders (Picker and Coyle, 2005). The 5, 10-MTHFR gene is located at the end of the short arm of chromosome 1 (1p36.3). The enzyme plays a central role in folate metabolism by irreversibly converting 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the predominant circulating form of folate. 5-Methyltetrahydrofolate donates a methyl group to homocysteine in the generation of *S*-adenosylmethionine (SAM), a major

Abbreviations: BD, Bipolar disorder; BPRS, Brief Psychiatric Rating Scale; FPIA, Fluorescence Polarization Immunoassay; MTHFR, methylenetetrahydrofolate; SAM, *S*-adenosylmethionine; SADS-L, Schedule for Affective Disorders and Schizophrenia-Lifetime; tHcy, total homocysteine; TDT, transmission disequilibrium test.

* Corresponding author. Tel.: +90 2163689697; fax: +90 2163560496.
E-mail address: cemsmile@hotmail.com (C.I. Kucukali).

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Table 1
Clinical characteristics and biochemical parameters of patients with bipolar affective disorders, their relatives and controls

Parameters	Patients (n=197)	Relatives (n=278)	Controls (n=238)	p value[a]	Power[b,c]
Age (year)	40.55±12.23[†]	45.07±14.87*	41.31±12.69		0.09, 0.82
Gender (female/male)	123/74	154/124	171/67	0.001[§]	0.55/0.55, 0.97/0.97
Homocysteine (μmol/L)	14.99±7.43**	13.84±5.44**	9.64±2.18	0.000[§]	1.00, 1.00
Folate (nmol/L)	12.18±4.45**,[†]	14.50±5.16*	15.99±5.65	0.000[§]	0.99, 0.90
Vitamin B12 (pmol/L)	222.21±130.63*[‡]	185.70±81.64**	266.89±142.83	0.000[§]	0.94, 1.00

*p<0.01, **p<0.001, compared with controls.
[‡]p<0.01, compared with relatives, [†]p<0.001, compared with relatives.
[§]p values were significant after Bonferroni correction for three groups (significant p<0.0167).
[a] Comparisons were evaluated with Kruskal–Wallis test among three groups according to homocysteine, folate, and vitamin B12 levels.
[b] Patients compared with controls.
[c] Relatives compared with controls.

source of methyl groups in the brain (Nishimura et al., 1985). c.677C>T transition is a common mutation in the coding region of the MTHFR gene that causes an alanine to valine (Al222Val) amino-acid substitution (Frosst et al., 1995). Another common polymorphism of the MTHFR gene is located at position c.1298A>C (Glu429Ala) (Weisberg et al., 1999). The c.1298A>C polymorphism in combination with the c.677C>T base pair change has been associated with MTHFR activity and tHcy levels (van der Put et al., 1998).

Since the rate of bipolar disorders in first-degree relatives of bipolar probands is higher than in the general population, and since genetic polymorphisms that alter enzymes involved in tHcy metabolism, such as MTHFR, and vitamin deficiency can result in variation in tHcy (Winokur et al., 1982; Jacques et al., 1996), we investigated the effect of polymorphic variants of c.1298A>C and c.677C>T of MTHFR, on the homocysteine, folate and vitamin B12 levels in patients with bipolar disorder, their unaffected first-degree relatives and healthy normal controls.

2. Methods

2.1. Subjects

Seven hundred and thirteen subjects were included in the study: 197 unrelated bipolar patients, 278 first-degree relatives of patients with bipolar disorder and 238 normal healthy controls. Patients and their healthy first-degree relatives (brother, sister and offspring, parent) were recruited from Erenkoy Psychiatric and Neurological Disorders Hospital. Diagnoses in all cases were made based on case records and clinical assessments by consensus of two experienced psychiatrists according to DSM-IV criteria (American Psychiatric Association, 1994). None of the subjects had significant neurological comorbidity, epilepsy, mental retardation or a history of substance abuse. For clinical evaluation, Brief Psychiatric Rating Scale (BPRS) (Overal and Gorhan, 1962), A Rating Scale for Mania (Young et al., 1978), and Extrapyramidal Symptom Rating Scale (Chouinard and Ross-Chouinard, 1984) were applied to all of the subjects. None of the subjects presented with current or past history of cardiovascular disease, endocrinological and metabolic disease, or a family history of coronary heart disease. Subjects with normal glucose and lipid profile were selected for study. The number of subjects included to the study was low because of these strict selection criteria. Relative subjects were selected from mentally healthy parents, offspring, sisters or brothers of patients with bipolar disorder. Relatives consist of mother or father or both parents of 50 patients (50 subjects), offsprings of 80 patients (103 subjects; 38 male and 65 female) and siblings of 67 patients (125 subjects; 61 male and 64 female). All patients received an antipsychotic medication. Of the 197 patients, 17% received an atypical antipsychotic, 65% received typical antipsychotic and 18% received an atypical antipsychotic in combination with typical antipsychotic. Healthy and unrelated volunteers without psychiatric disorders were selected as a control group. Control group and first-degree relatives of bipolar patients were administered Schedule for Affective Disorders and Schizophrenia-Lifetime Version (SADS-L) (Endicott and Spitzer, 1978) interviews. The study population was selected from Turkish population who live in Istanbul. The study was approved by the Medical Ethics Committee of Istanbul Medical Faculty, and all participants, patients, controls and unaffected family members gave written informed consent.

2.2. Genotyping

DNA was extracted from peripheral blood by salting-out method (Miller et al., 1988). The c.677C>T (rs1801133) and c.1298A>C (rs1801131) polymorphisms in MTHFR gene were determined by polymerase chain reaction-restriction fragment length polymorphism using HinfI and MboII restriction enzymes, respectively (Kara et al., 2003; Weisberg et al., 2001). The amplified and digested PCR products were analyzed by ethidium bromide staining in 2% agarose gel. Each gel was read by 2 observers unaware of the subject's status.

2.3. Biochemical analysis

Venous blood samples were collected after an overnight fast. tHcy, serum folate, and vitamin B12 levels were determined in all subjects. Blood samples for measurement of tHcy concentrations in serum were drawn in vacutainer tubes. The sera were separated and immediately stored at −20 °C. Total Hcy concentrations were measured by Fluorescence Polarization Immunoassay (FPIA), the AxSYM system Abbot Laboratories, Chicago, IL, in Centro Laboratory (Ueland et al., 1993). Folate and vitamin B12 levels were determined in serum using Electrochemiluminescence Immunoassay analyzers, Modular Analytics E170 (Elecsys module), Roche Diagnostic GmbH, Mannheim, Germany. All biochemical parameters were measured twice.

2.4. Statistical analysis

Statistical analyses were performed using the SPSS software package, revision 12.0. Distributions of allele and genotypes were compared by chi-square test. Linkage disequilibrium between c.677C>T and c.1298A>C polymorphisms in MTHFR gene was assessed using D' and r^2 values generated through the use of the program Haploview (Barrett et al., 2005). The transmission disequilibrium test

Table 2
Distributions of genotypes and alleles of MTHFR in patients, their relatives and controls

Genotypes		Patients (n=197)	Relatives (n=278)	Controls (n=238)	p values
		n (%)	n (%)	n (%)	
c.677C>T					
	CC	104 (52.8)	137 (49.3)	116 (48.7)	
	CT	76 (38.6)	119 (42.8)	97 (40.8)	
	TT	17 (8.6)	22 (7.9)	25 (10.5)	>0.05
Alleles	C	284 (0.72)	393 (0.71)	329 (0.69)	>0.05
	T	110 (0.28)	163 (0.29)	147 (0.31)	>0.05
c.1298A>C					
	AA	91 (46.2)	120 (43.2)	113 (47.5)	
	AC	84 (42.6)	134 (48.2)	101 (42.4)	
	CC	22 (11.2)	24 (8.6)	24 (10.1)	>0.05
Alleles	A	266 (0.68)	374 (0.67)	327 (0.69)	>0.05
	C	128 (0.32)	182 (0.33)	149 (0.31)	>0.05