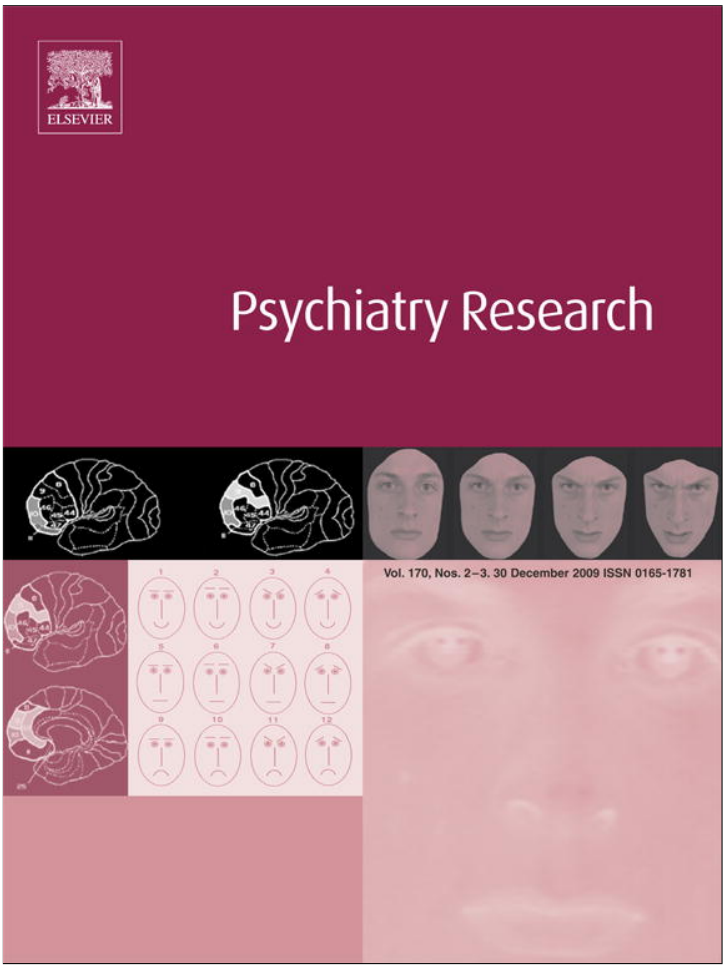

Psychiatry Research 170 (2009) 168–171

Contents lists available at ScienceDirect

Psychiatry Research

journal homepage: www.elsevier.com/locate/psychres

The *844ins68* polymorphism of the cystathionine beta-synthase gene is associated with schizophrenia

Vera Golimbet *, Galina Korovaitseva, Lilia Abramova, Vasily Kaleda

Mental Health Research Center, Russian Academy of Medical Sciences, Moscow, Russia

ARTICLE INFO

Article history:
Received 21 September 2007
Received in revised form 30 May 2008
Accepted 20 July 2008

Keywords:
Homocysteine
Case control study
Family-based study

ABSTRACT

A subtle genetic defect in homocysteine metabolism is thought to play an etiologic role in schizophrenia. Cystathionine-beta-synthase (CBS) is a key enzyme related to homocysteine levels. The aim of the present study was to search for association between the *844ins68* polymorphism of the *CBS* gene and schizophrenia in a large Russian sample using case-control and family-based designs. The sample comprised 1135 patients, 626 controls and 172 families. There was a trend for association between the *844ins68* polymorphism and schizophrenia in the case-control study, with higher frequency of the insertion in the control group. The FBAT revealed a statistically significant difference in transmission of alleles from parents to the affected proband, with preferential transmission of the variant without insertion. When the sample of patients was stratified by sex and forms of schizophrenia, the significantly lower frequency of insertion was observed in the group of female patients with chronic schizophrenia ($n = 180$) as compared to psychiatrically well women. The insertion variant has been reported earlier to be related to decreased levels of homocysteine and thus thought to play a protective role. In conclusion, our study revealed a possible relation of the *CBS 844ins68* polymorphism to schizophrenia.

1. Introduction

It has repeatedly been reported that a significant proportion of patients with schizophrenia have increased homocysteine levels that are unrelated to psychopharmacological medication or nutrient deficiency in folate or cobalamin (Regland et al., 1995; Susser et al., 1998; Muntjewerff et al., 2003). An excess of homocysteine is known to exert a toxic effect on the cell and induce such processes as oxidative stress, aberrant DNA methylation, DNA strand breakage, and apoptosis (Yi et al., 2000; Mattson and Shea, 2003) which are thought to be implicated in the etiology and pathogenesis of schizophrenia. On the basis of these data, Brown and Susser (2005) suggested a hypothesis that a subtle genetic defect in the homocysteine metabolism might play an etiologic role in schizophrenia.

The level of homocysteine can be reduced by the conversion of this amino acid either to methionine or to cysteine. A key enzyme for methionine metabolism is methylenetetrahydrofolate reductase (MTHFR) and that for cysteine is cystathionine-beta-synthase (CBS). CBS irreversibly removes homocysteine from the methionine cycle by transsulfuration to cystathionine. Under conditions of folate deficiency, this pathway becomes more important. There are several explanations for the relationship between CBS and schizophrenia. A deficiency of CBS leads to homocystinuria, an inherited human disease characterized by mental retardation, seizures, psychiatric disturbances, skeletal abnormalities, and vascular disorders. Case reports have long suggested a predisposition to schizophrenia (for review see Picker and Coyle, 2005). Animal studies revealed that CBS was associated with the generation and/or differentiation of the radial glia/astrocyte lineage cells in the developing CNS (Enokido et al., 2005). It is thought that a glia/astrocyte dysfunction may be involved in the pathogenesis of schizophrenia.

Molecular genetic studies have been largely focused on the *MTHFR 677C>T* polymorphism, which has been reported to be found to be associated with schizophrenia (see a meta-analysis of Muntjewerff et al., 2006). At the same time, the *CBS* gene, though its role appears to be no less important for the metabolism of homocysteine, has not been tested for association with the disease so far. The *844ins68* is a common polymorphism in the general population that consists of a 68-bp insertion duplicating the 3¢ splice site of intron 7 and the 5′-end of exon 8. The frequency of insertion is estimated between 5 and 10% in Caucasians (Tsai et al., 1996; De Stefano et al., 1998), it is absent among Asians (Pepe et al., 1999), and it has a much higher prevalence among blacks (Franco et al., 1998). An insertion variant is thought to be linked with increased activity of the enzyme since it has been associated with decreased levels of plasma homocysteine (Tsai et al., 1999; Wang et al., 1999; Dekou et al., 2001).

The aim of the present study was to search for association between the *844ins68* polymorphism of the *CBS* gene and schizophrenia in a large sample of Russian patients and controls. To our knowledge, this study is the first one, at this writing, to explore a *CBS* polymorphism in relation to schizophrenia. Based on the previous findings that carriers of the

* Corresponding author. Mental Health Research Center, Russian Academy of Medical Sciences, Zagorodnoe sh. 2/2, 117152 Moscow, Russia. Tel.: +7 495 9529040; fax: +7 495 9528940.
E-mail address: golimbet@mail.ru (V. Golimbet).

0165-1781/$ – see front matter © 2008 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.psychres.2008.07.007

insertion variant had a decreased homocysteine level, we hypothesized that the frequency of this variant would be lower in patients as compared to controls.

2. Methods

2.1. Subjects

Patients were recruited from clinical departments of the Mental Health Research Center. A diagnosis was been made according to ICD-10 diagnostic criteria on the basis of the Mini International Neuropsychiatric Interview (M.I.N.I., version 5.0) responses and medical records. Once established by a psychiatrist, a diagnosis was confirmed by a senior researcher. The total sample comprised 1135 patients with schizophrenia (broad definition), 450 men, 685 women, aged 16–70 years, mean age 38 ± 14.1 years, age-at-disease-onset 26.4 ± 10.8 years. The diagnosis of schizophrenia (F20) was assigned to 997 patients and schizoaffective disorder (F25) to 138 patients. Patients with schizophrenia were divided into two groups, with chronic or continuous (item F20.00) and episodic course (items F20.01 – 04). A course type was defined basing on medical records. Clinical symptoms were assessed with the Positive and Negative Syndrome Scale (PANSS).

Families were ascertained through the clinical departments of the Mental Health Research Center where the probands were admitted. Parents of each patient participating in the study were asked to meet a psychiatrist. A diagnosis of psychosis was excluded based on the results of this interview. Parents who were not diagnosed with psychiatric disorder were asked to complete self-rated psychological tests, the Schizotypal Personality Questionnaire (SPQ-74) and the Minnesota Multiphasic Personality Inventory (MMPI). Those who met the inclusion criteria on the basis of psychological examination (T-scores less than 70 on scale F (MMPI) and total scores less than 40 on the SPQ-74) were selected for the molecular-genetic study. A total of 172 nuclear families (585 persons) participated in the family-based analysis. While parent's DNA was used only in the family-based study, probands were also included in the case–control analysis. The sample comprised 91 men and 81 women; 160 patients with schizophrenia and 12 with schizoaffective disorder.

Controls were recruited by word of mouth from the community. The sample included students, researchers, hospital staff, employees of various Moscow companies, and pensioners. Each participant was asked to complete the SPQ-74 and the MMPI. Inclusion criteria were MMPI T-scores less than 70 on scale F and total scores less than 40 on the SPQ-74. Finally the control sample comprised 626 psychiatrically healthy individuals (328 men, 298 women), aged 16–78 years, mean age 42 ± 12.9 years. Fifty-eight subjects from the original sample were not included.

The case and control samples were not age- and sex-matched. Though mean ages of both groups seemed similar (38 (14.1) and 42 (12.9) years, respectively), statistical comparison revealed significant differences ($t = 5.8$; $df = 1759$; $P < 0.0001$) between them, the control group being older. The proportion of women in the case sample was larger than in the controls (60% vs. 47%, $X^2 = 7.5$, $df = 1$, $P = 0.006$).

Each participant gave written informed consent. The design of the study has been approved by the Ethical Committee of the Mental Health Research Center.

2.2. Genotyping

The participants donated venous blood and DNA was extracted using the phenol-chloroform method. The primer sequences to amplify the *844ins68* polymorphism in the exon 8 and flanking intron 7 of the *CBS* gene were: forward — 5′-GTTGTTAACGGCGG-TATTGG-3′ and reverse — 5′-GTTGTCTGCTCCGTCTGGTT-3′. PCR reaction was carried out in a reaction volume of 20 μl containing 100 ng of genomic DNA, 10 pmoles of each primer, 0.5 U Taq polymerase (Helicon, Russia), 200 μM of each dNTP and 2.5 mM $MgCl_2$. After an initial denaturation at 95 °C for 2 min, amplification was performed as follows: 30 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 25 s and extension at 72 °C for 25 s with a final extension for 5 min at 72 °C. PCR products were separated by 8% polyacrylamide gel electrophoresis at 240 V for 1 h, stained with ethidium bromide and visualized in UV light. The resulting fragments were 252-bp in the presence and 184-bp in the absence of insertion.

2.3. Statistics

The X^2-test and the odds ratio (OR) test were used for comparing allele and genotype frequency. A null hypothesis of the absence of association was tested.

A family-based association test (FBAT) was applied to test the 844ins68 locus for linkage in the presence of association/linkage disequilibrium. To estimate the difference between clinical subgroups by PANSS scores, analysis of variance (ANOVA_ with subgroups and sex as between-subject factors followed by multiple comparison testing was used. Two-tailed tests were employed throughout. Power calculations were made with PAWE software (Gordon et al., 2002). We used a genetic model-free method.

3. Results

The genotype distribution in each group was in accordance to the Hardy–Weinberg equilibrium. Genotype and allele frequencies for the control group and patients with schizophrenia are presented in Table 1. Because the frequency of the Ins+/Ins+ genotype was very low in both groups, we further compared frequencies of the combined Ins+/Ins+ and Ins+/Ins− genotypes versus the Ins−/Ins− genotype. There was a trend for significant difference between allele and genotype frequencies in patients and controls in the case–control study, with higher frequencies of an allele with insertion ($\chi^2 = 2.8$; $df = 1$; $P = 0.09$; OR 1.27, 95% CI 0.9–1.7) and genotypes containing one or two copies of insertion ($\chi^2 = 3.2$; $df = 1$; $P = 0.07$; OR 1.27, 95% CI 0.9–1.7) in the control group. The FBAT revealed a statistically significant difference ($P = 0.03$) in transmission of alleles from parents to the affected proband, with preferential transmission of the variant without insertion (*Ins*−). Therefore, the null hypothesis of no association and no linkage between the marker and affected status has been rejected. The allele frequencies and number of transmissions are presented in Table 2.

The trend for association in the case–control analysis may be the result of ethnical stratification as well as of clinical heterogeneity of schizophrenia and sex differences that often play a mediating role in association studies of psychiatric disorders. Therefore, the sample has been stratified by sex, diagnosis (schizophrenia or schizoaffective disorder) and disease course (chronic or episodic). Because earlier studies revealed that homocysteine levels might differ in male and female patients with different ages (Levine et al., 2002), we compared groups in the following age ranges: 18–29, 30–39, 40–49, 50–59 and 60–70 years.

No difference in the frequency of insertion between groups of different ages was found in comparison with sex- and age-matched controls (data not presented). The genotype distribution in male and female groups with different types of psychosis is shown in Table 3. The *CBS 844ins68* polymorphism was not associated with schizoaffective psychosis. The association was found between the *CBS 844ins68* polymorphism and chronic schizophrenia (ICD-10 F20.00) only in female patients, with the frequency of insertion variants being significantly lower as compared to the female controls ($\chi^2 = 3.9$; $df = 1$; $P = 0.02$; OR 2.1, 95% CI 1.1–4). The allele-wise comparison revealed that frequency of insertion in this group was also lower ($\chi^2 = 4.1$; $df = 1$; $P = 0.04$; OR 1.8, 95% CI 1.0–3.4). The power of the study was 0.89 (allelic test) and 0.82 (genotypic test).

When compared with other groups by PANSS scores, the female group of patients with chronic schizophrenia had the highest total scores ($P < 0.0001$) on the positive, negative and general psychopathology subscales. ANCOVA with PANSS scores as dependent variables and sex and age as covariates did not reveal an effect of genotype on symptom severity ($F = 0.6$; $df = 2,1130$; $P = 0.54$) (Table 4).

Table 1
Allele and genotype frequencies (%) of the *CBS 844ins68* polymorphism in patients with schizophrenia and psychiatrically healthy controls.

Allele *Ins+*	Group	Allele *Ins−*	*Ins+ Ins+*	*Ins+ Ins−*	*Ins− Ins−*	Hardy–Weinberg equilibrium
5.3	Patients $n = 1135$	94.7	0.2 (3)	10.2 (112)	89.6 (1020)	$\chi^2 = 0.08$; $df = 1$; $P = 0.78$
6.5*	Controls $n = 626$	93.5	0.2 (1)	12.8 (80)*	87.1 (545)	$\chi^2 = 1.22$; $df = 1$; $P = 0.29$

( ) — number of subjects.
*— frequency of an allele with insertion was higher in the controls as compared to the patients on the trend level ($\chi^2 = 2.8$; $df = 1$; $P = 0.09$; OR 1.27, 95% CI 0.9–1.7).
**— frequency of the Ins+/Ins− genotype was higher in the controls as compared to the patients on the trend level ($\chi^2 = 3.2$; $df = 1$; $P = 0.07$; OR 1.3, 95% CI 0.97–1.78).

Table 2
Family-based association test of *844ins68* alleles with schizophrenia in nuclear families.

CBS allele	Allele frequency	*S*	E (*S*)	*P*	*Z*-score
Ins+	0.062	14	21	0.03	−2.16
Ins−	0.938	66	59	0.03	2.16

S, statistical score for the observed number of transmissions.
E (*S*), statistical score for the expected number of transmissions.

Table 3
Frequency of the *CBS 844ins68* genotypes in male and female patients with different types of psychosis.

	Males				Females			
	Frequency of *CBS 844ins68* genotypes							
Type of schizophrenia	Ins+ Ins+	Ins+ Ins−	Ins− Ins−	Total	Ins+ Ins+	Ins+ Ins−	Ins− Ins−	Total
Schizophrenia (chronic course)	0 (0)*	11 (18)	89 (146)	100 (164)	0.5 (1)	6.3 (12)	93.2 (167)*,**	100 (180)
Schizophrenia (episodic course)	0 (0)	9.8 (22)	90.2 (202)	100 (224)	0.5 (2)	10.9 (47)	88.6 (380)	100 (429)
Schizoaffective disorder	0 (0)	9.7 (6)	90.3 (56)	100 (62)	0 (0)	9.2 (7)	90.8 (69)	100 (76)
Controls	0.3 (1)	11.5 (38)	88.2 (289)	100 (328)	0 (0)	14.1 (42)	85.9 (256)	100 (298)

*— frequency (%), number of patients in parentheses.
**— In female patients with chronic course of schizophrenia, frequency of the insertion variant was significantly lower as compared to psychiatrically well women ($\chi^2 = 3.9$; $df = 1$; $P = 0.02$; OR 2.1, CI 95% 1.1–4.0).

4. Discussion

4.1. The CBS 844ins68 polymorphism and risk for schizophrenia

We compared the large groups of patients with schizophrenia and controls and revealed that the *CBS 844ins68* polymorphism was associated with schizophrenia only in the family-based analysis, though there was a suggestive association in the case–control study. In families, a variant with insertion was transmitted less often to the schizophrenic proband than the variant without insertion. In general, the results obtained have confirmed our hypothesis that the frequency of insertion is lower in schizophrenic patients compared with the control group. It has been shown earlier that healthy individuals with an insertion allele have lower median homocysteine than individuals homozygous for the common allele without insertion (Dekou et al., 2001). Given that schizophrenic patients revealed elevated levels of homocysteine (Levine et al., 2005; Neeman et al., 2005), we can suggest that a variant with insertion is more favorable from a metabolic point of view. A protective effect of insertion has been reported for some other pathologies, e.g. in colorectal cancer (Shannon et al., 2002), vascular thromboembolic disease (Zhang and Dai, 2001), and CNS demyelination in X-linked adrenoleukodystrophy (Linnebank et al., 2006). This effect may be explained by the fact that increased plasma homocysteine is linked to DNA hypomethylation in lymphocytes through conversion to *S*-adenosylhomocysteine, a potent inhibitor of DNA methyltransferase (Yi et al., 2000). Since the insertion is associated with lowered homocysteine levels, it may protect against altered DNA methylation that appears to play a critical role in mediating differential regulation of genes. Of note, methylation deficiency is thought to be involved in the pathogenesis of schizophrenia (Regland et al., 1995; Mattson and Shea, 2003).

4.2. A role of sex in the homocysteine metabolism

The association between the *844ins68* polymorphism and schizophrenia was found only in the group of female patients with a chronic course of disease. The frequency of insertion in this group was lower compared to the controls. The finding is supported by several lines of evidence for the role of sex in the homocysteine metabolism. The difference between men and women may be related to the more rapid methionine cycling in women that may result in a greater proportion of homocysteine being diverted to cystathionine (Wilcken and Gupta, 1979; Fukagawa et al., 2000). Kemperman et al. (2006) revealed that the variance of homocysteine in patients with schizophrenia might be explained by different mechanisms. In male patients, it was dependent on folate, a cofactor required for conversion of homocysteine to methionine, and in female patients, on vitamin B6, which activates CBS. Also effects of estrogen on the homocysteine metabolism cannot be ruled out (Giltay et al., 2003; Shah et al., 2006). Changes in endogenous estrogen levels are inversely associated with changes in serum homocysteine. It should be mentioned that in the earlier studies of schizophrenic populations higher homocysteine levels were reported for young male patients under 50 years old (Levine et al., 2005; Nevo et al., 2006) and older female patients, aged 50–60 years old (Levine et al., 2002). However, in a recent study (Haidemenos et al., 2007) increased homocysteine levels were revealed both in young males and in young and older females. Of note, our sample comprised 85 (47%) women with chronic schizophrenia aged in the above-mentioned range, which might explain the low frequency of protective variant with insertion in this group. We did not, however, find any differences in the frequency of insertion between the female subgroup aged between 50–60 years and sex- and age-matched controls.

In our study women with chronic schizophrenia had the highest scores on ratings of clinical symptomatology in comparison with male patients, though some previous research reported that men tended to have more severe forms of schizophrenia (Leung and Chue, 2000). This may be again explained by the fact that our female sample comprised a high percentage of women aged above 50 years. And women with schizophrenia in their later years often have a renewed onset of psychotic symptoms and a worse course of the disease that may be due to the lack of protective effects of estrogen (Häfner, 2003). The ANCOVA revealed that CBS genotype did not contribute to the variance of clinical symptoms in measured with the PANSS in either the male or female patients. The results are in line with those obtained using χ^2 tests, i.e. no association has been found between the CBS polymorphism and clinical types of schizophrenia classified by the severity of PANSS symptoms. As reported above, the association has been found only between the *844ins68* polymorphism of the CBS gene and schizophrenia with chronic course in female patients as compared to the controls.

4.3. Limitations and strength of the study

There are some limitations to our study. Although the sample of patients with schizophrenia was large, it was not clinically homogenous,

Table 4
Scores (mean, SD) for positive, negative and general psychopathological symptoms subscales of the PANSS in different clinical groups of schizophrenic patients.

Type of schizophrenia	Schizophrenia, chronic course $n = 344$		Schizophrenia, episodic course $n = 653$		Schizoaffective disorder $n = 138$	
Subscale (scores)	Men ($n = 164$)	Women ($n = 180$)	Men ($n = 224$)	Women ($n = 429$)	Men ($n = 62$)	Women ($n = 76$)
Positive symptoms subscale	21.9 (7.4)	26.2 (6.8)*	20.9 (7.5)	21.7 (8.1)	18.8 (8.5)	19.8 (8.2)
Negative symptoms subscale	26.2 (5.7)	31.0 (6.0)*	23.7 (5.9)	24.5 (7.9)	14.9 (5.8)	15.1 (5.9)
General psychopathological symptoms subscale	45.5 (12.1)	55.1 (11.1)*	45.9 (10.9)	48.7 (11.8)	41.1(12.6)	44.1 (12.8)

*$P < 0.0001$.

i.e., it included patients with different forms of schizophrenia and patients with schizoaffective disorder. We should also mention that the association between the *844ins68* polymorphism and chronic schizophrenia was found in a relatively small group of patients (180 people) and we cannot exclude an inflation of type I error. Also we were not able to confirm the effects of sex and clinical course of schizophrenia in the family-based study because of the reduced sample sizes. The strength of the study is that the results obtained are in the predicted direction, though the study was exploratory. Although there is growing evidence for impairment of homocysteine metabolism in schizophrenia, the CBS gene polymorphism, to the best of our knowledge, has been first tested for association with schizophrenia in the present study. Obviously, the effect of the *844ins68* polymorphism is rather small. And in future studies it would be worthwhile to search for its association with schizophrenia in combinations with other polymorphisms within the CBS gene as well as with other genes involved in the homocysteine metabolism and use the more targeted groups of patients, e.g., post-menopausal female patients with poor vitamin B_6 status.

In conclusion, we revealed the possible relation of the *CBS 844ins68* polymorphism to schizophrenia, with the frequency of the protective allele with insertion being the lowest in female patients with chronic schizophrenia.

References

Brown, A.S., Susser, E.S., 2005. Homocysteine and schizophrenia: from prenatal to adult life. Progress in Neuro-Psychopharmacology & Biological Psychiatry 29, 1175–1180.

Dekou, V., Gudnason, V., Hawe, E., Miller, G.J., Stansbie, D., Humphries, S.E., 2001. Gene–environment and gene–gene interaction in the determination of plasma homocysteine levels in healthy middle-aged men. Thrombosis and Haemostasis 85, 67–74.

De Stefano, V., Dekou, V., Nicaud, V., Chasse, J.F., London, J., Stansbie, D., Humphries, S.E., Gudnason, V., 1998. Linkage disequilibrium at the cystathionine beta synthase (CBS) locus and the association between genetic variation at the CBS locus and plasma levels of homocysteine. The Ears II Group. European Atherosclerosis Research Study. Annals of Human Genetics 62, 481–490.

Enokido, Y., Suzuki, E., Iwasawa, K., Namekata, K., Okazawa, H., Kimura, H., 2005. Cystathionine beta-synthase, a key enzyme for homocysteine metabolism, is preferentially expressed in the radial glia/astrocyte lineage of developing mouse CNS. The FASEB Journal 9, 1854–1856.

Franco, R.F., Elion, J., Lavinha, J., Krishnamoorthy, R., Tavella, M.H., Zago, M.A., 1998. Heterogeneous ethnic distribution of the 844ins68 in the cystathionine beta-synthase gene. Human Heredity 48, 338–342.

Fukagawa, N.K., Martin, J.M., Wurthmann, A., Prue, A.H., Ebenstein, D., O'Rourke, B., 2000. Sex-related differences in methionine metabolism and plasma homocysteine concentrations. The American Journal of Clinical Nutrition 72, 22–29.

Giltay, E.J., Verhoef, P., Gooren, L.J., Geleijnse, J.M., Schouten, E.G., Stehouwer, C.D., 2003. Oral and transdermal estrogens both lower plasma total homocysteine in male-to-female transsexuals. Atherosclerosis 168, 139–146.

Gordon, D., Finch, S., Nothnagel, M., Ott, J., 2002. Power and sample size calculations for case–control genetic association tests when errors present: application to single nucleotide polymorphisms. Human Heredity. 54, 22–33.

Häfner, H., 2003. Gender differences in schizophrenia. Psychoneuroendocrinology 28, 17–54.

Haidemenos, A., Kontis, D., Gazi, A., Kallai, E., Allin, M., Lucia, B., 2007. Plasma homocysteine, folate and B12 in chronic schizophrenia. Progress in Neuro-Psychopharmacology & Biological Psychiatry 31, 1289–1296.

Kemperman, R.F.J., Veurink, M., Van Der Wal, T., Knegtering, H., Bruggeman, R., Fokkema, M.R., Kema, I.P., Korf, J., Muskiet, F.A.J., 2006. Low essential fatty acid and B-vitamin status in a subgroup of patients with schizophrenia and its response to dietary supplementation. Prostaglandins, Leukotrienes, and Essential Fatty Acids 74, 75–85.

Leung, A., Chue, P., 2000. Sex differences in schizophrenia, a review of the literature. Acta Psychiatrica Scandinavica. Supplementum 401, 3–38.

Levine, J., Stahl, Z., Ami, B., Slava, S., Ruderman, G.V., Belmaker, R.H., 2002. Elevated homocysteine levels in young male patients with schizophrenia. American Journal of Psychiatry 159, 1790–1792.

Levine, J., Sela, B.A., Osher, Y., Belmaker, R.H., 2005. High homocysteine serum levels in young male schizophrenia and bipolar patients and in an animal model. Progress in Neuro-Psychopharmacology & Biological Psychiatry 29, 1181–1191.

Linnebank, M., Semmler, A., Kleijer, W.J., van der Sterre, M.L., Gartner, J., Fliessbach, K., Sokolowski, P., Kohler, W., Schlegel, U., Klockgether, T., Wanders, R.J., Schmidt, S., Wullner, U., Kemp, S., 2006. The cystathionine beta-synthase variant c.844_845ins68 protects against CNS demyelination in X-linked adrenoleukodystrophy. Human Mutation 27, 1063–1064.

Mattson, M.P., Shea, T.B., 2003. Folate and homocysteine metabolism in neural plasticity and neurodegenerative disorders. Trends in Neurosciences 26, 137–146.

Muntjewerff, J.W., van der Put, N., Eskes, T., Ellenbroek, B., Steegers, E., Blom, H., Zitman, F., 2003. Homocysteine metabolism and B-vitamins in schizophrenic patients: low plasma folate as a possible independent risk factor for schizophrenia. Psychiatry Research 121, 1–9.

Muntjewerff, J.W., Kahn, R.S., Blom, H.J., den Heijer, M., 2006. Homocysteine, methylenetetrahydrofolate reductase and risk of schizophrenia: a meta-analysis. Molecular Psychiatry 11, 143–149.

Neeman, G., Blanaru, M., Bloch, B., Kremer, I., Ermilov, M., Javitt, D.C., Heresco-Levy, U., 2005. Relation of plasma glycine, serine, and homocysteine levels to schizophrenia symptoms and medication type. American Journal of Psychiatry 162, 1738–1740.

Nevo, G.A., Meged, S., Sela, B.A., Hanoch-Levi, A., Hershko, R., Weizman, A., 2006. Homocysteine levels in adolescent schizophrenia patients. European Neuropsychopharmacology 16, 588–591.

Pepe, G., Camacho Vanegas, O., Rickards, O., Giusti, B., Comeglio, P., Brunelli, T., Marcucci, R., Prisco, D., Gensini, G.F., Abbate, R., 1999. World distribution of the T833C/844INS68 CBS in cis double mutation: a reliable anthropological marker. Human Genetics 104, 126–129.

Picker, J.D., Coyle, J.T., 2005. Do maternal folate and homocysteine levels play a role in neurodevelopmental processes that increase risk for schizophrenia? Harvard Review of Psychiatry 13, 197–205.

Regland, B., Johansson, B.V., Grenfeldt, B., Hjelmgren, L.T., Medhus, M., 1995. Homocysteinemia is a common feature of schizophrenia. Journal of Neural Transmission. General Section 100, 165–169.

Shah, S., Bell, R.J., Davis, S.R., 2006. Homocysteine, estrogen and cognitive decline. Climacteric 9, 77–87.

Shannon, B., Gnanasampanthan, S., Beilby, J., Iacopetta, B., 2002. A polymorphism in the methylenetetrahydrofolate reductase gene predisposes to colorectal cancers with microsatellite instability. Gut 50, 520–524.

Susser, E., Brown, A.S., Klonowski, E., Allen, R.H., Lindenbaum, J., 1998. Schizophrenia and impaired homocysteine metabolism: a possible association. Biological Psychiatry 44, 141–143.

Tsai, M.Y., Bignell, M., Schwichtenberg, K., Hanson, N.Q., 1996. High prevalence of a mutation in the cystathionine beta-synthase gene. American Journal of Human Genetics 59, 1262–1267.

Tsai, M.Y., Yang, F., Bignell, M., Aras, O., Hanson, N.Q., 1999. Relation between plasma homocysteine concentration, the 844ins68 variant of the cystathionine beta-synthase gene, and pyridoxal-5′-phosphate concentration. Molecular Genetics and Metabolism 67, 352–356.

Wang, X.L., Duarte, N., Cai, H., Adachi, T., Sim, A.S., Cranney, G., Wilcken, D.E., 1999. Relationship between total plasma homocysteine, polymorphisms of homocysteine metabolism related enzymes, risk factors and coronary artery disease in the Australian hospital-based population. Atherosclerosis 146, 133–140.

Wilcken, D., Gupta, V.J., 1979. Cysteine-homocysteine mixed disulphide: differing plasma concentrations in normal men and women. Clinical Science (Colch.) 57, 211–215.

Yi, P., Melnyk, S., Pogribna, M., Pogribny, I.P., Hine, R.J., James, S.J., 2000. Increase in plasma homocysteine associated with parallel increases in plasma *S*-adenosylhomocysteine and lymphocyte DNA hypomethylation. The Journal of Biological Chemistry 275, 29318–29323.

Zhang, G., Dai, G., 2001. Gene polymorphisms of homocysteine metabolism-related enzymes in Chinese patients with occlusive coronary artery or cerebral vascular diseases. Thrombosis Research 104, 187–195.