American Journal of Medical Genetics Part B (Neuropsychiatric Genetics) 147B:699–706 (2008)

No Association With the 5,10-Methylenetetrahydrofolate Reductase Gene and Major Depressive Disorder: Results of the Depression Case Control (DeCC) Study and a Meta-Analysis

D. Gaysina,[1,2*§] S. Cohen,[1§] N. Craddock,[3] A. Farmer,[1] F. Hoda,[1] A. Korszun,[4] M.J. Owen,[3] I.W. Craig,[1] and P. McGuffin[1]

[1]*MRC SGDP Centre, Institute of Psychiatry, King's College London, De Crespigny Park, London, UK*
[2]*Institute of Biochemistry and Genetics, Ufa Scientific Center, RAS, Ufa, Russia*
[3]*Department of Psychological Medicine, Cardiff University, School of Medicine, Heath Park, Cardiff, UK*
[4]*Centre for Psychiatry, Wolfson Institute of Preventive Medicine, Barts and The London, Queen Mary's School of Medicine, Charterhouse Square, London, UK*

Unipolar major depressive disorder (MDD) is a complex disorder thought to result from multiple genes in combination with environmental and developmental components. The 5,10-methylenetetrahydrofolate reductase gene (*MTHFR*) has been implicated in MDD in a meta-analysis of association studies and is within a linkage region suggested by a recent study of affected sib pairs. A single base mutation in the *MTHFR* gene (*C677T*) results in the production of a mildly dysfunctional thermolabile enzyme. The *MTHFR 677TT* genotype, and to a lesser extent the *677CT* genotype, is associated with a significant elevation in the circulating concentrations of homocysteine and a decrease in serum folate concentrations. This may parallel a similar reduction in 5-methyltetrahydrofolate in the CNS, leading to a potential reduction in monoamine neurotransmitter function and an elevated risk of depressive disorder. To test the hypothesis that the *MTHFR C677T* polymorphism is involved in the predisposition to MDD, we conducted an association study of 1,222 patients with recurrent MDD and 835 control subjects. This allows 99% power to detect an effect of the size reported in the study of Bjelland et al. [2003], however no significant differences in genotype or allele frequencies between depressive patients and controls were observed. This was the case in the sample as a whole, and when females and males were considered separately. Our findings suggest that the *MTHFR C677T* polymorphism is not involved in the etiology of clinically significant recurrent MDD.
© 2007 Wiley-Liss, Inc.

KEY WORDS: depression; folate; association; meta-analysis; MTHFR

Please cite this article as follows: Gaysina D, Cohen S, Craddock N, Farmer A, Hoda F, Korszun A, Owen MJ, Craig IW, McGuffin P. 2008. No Association With the 5,10-Methylenetetrahydrofolate Reductase Gene and Major Depressive Disorder: Results of the Depression Case Control (DeCC) Study and a Meta-Analysis. Am J Med Genet Part B 147B:699–706.

INTRODUCTION

The enzyme 5,10-methylenetetrahydrofolate reductase (MTHFR) is involved in 1-carbon metabolism and is responsible for the final step in the conversion of dietary forms of folate to 5-methyltetrahydrofolate. Several case-control studies have shown high prevalence of folate and vitamin B_{12} deficiency in depression, especially in cases with more severe forms, prolonged episodes, and poor treatment response [Alpert et al., 2000]. More recently total plasma homocysteine levels were shown to be a sensitive marker of folate and vitamin B_{12} deficiency, and higher concentrations of homocysteine were observed in depressed patients [Bottiglieri et al., 2000; Bjelland et al., 2003]. Folate, vitamin B_{12}, and homocysteine are involved in processes important for central nervous system function. Folate metabolism is linked to biopterin-dependent neurotransmitter synthesis [van Praag, 1982] and S-adenosylmethionine-dependent methylation of biogenic amines and phospholipids in the central nervous system [Bottiglieri et al., 1994]. Homocysteine or its metabolites may have a direct excitotoxic effect on the *N*-methyl-D-aspartate glutamate receptors, and may inhibit methylation processes in the central nervous system [Bottiglieri et al., 1994]. This biochemical knowledge alongside the clinical data suggests a possible role of 1-carbon metabolism in depression.

A recent genome-wide linkage analysis for recurrent major depressive disorder (MDD) presents suggestive evidence for female-specific linkage on chromosome 1p36 in the region where the *MTHFR* gene is located [McGuffin et al., 2005]. Further evidence from clinical, biochemical and genetic studies implicates the *MTHFR* gene (1p36.3) as a candidate gene for depression. A C → T transition at nucleotide 677 in exon 4 of the *MTHFR* gene (rs1801133) has been reported to result in the production of a mildly dysfunctional thermolabile enzyme [Ueland et al., 2001]. It has been shown that *677TT* homozygotes have 30% enzyme activity compared to homozygotes for the wild-type *677C* allele, while heterozygotes retain 65% of wild-type MTHFR enzyme activity [Rozen, 1996]. The *MTHFR 677TT* genotype is associated with a significant

[§]D. Gaysina and S. Cohen contributed equally to this study.

Grant sponsor: Medical Research Council (MRC).

*Correspondence to: D. Gaysina, MRC SGDP Centre, Institute of Psychiatry, King's College London, Box P080, De Crespigny Park, London SE5 8AF, UK. E-mail: dasha.gaysina@iop.kcl.ac.uk

Received 8 June 2007; Accepted 11 October 2007
DOI 10.1002/ajmg.b.30665

© 2007 Wiley-Liss, Inc.

elevation in circulating concentrations of homocysteine, and a decrease in serum folate concentrations. This may parallel a similar reduction in 5-methyltetrahydrofolate in the CNS and lead to a reduction in monoamine neurotransmitter function and an elevated risk of MDD. Indeed, lower levels of red blood cell folate, plasma folate, and vitamin B_{12} have been reported among non-diseased persons with the *677TT* genotype in comparison with persons possessing other genotypes [Molloy et al., 1997].

Previous studies have suggested an association between the *677TT* genotype of the *MTHFR* gene and depression [for review, see Gilbody et al., 2007]. Only two of these positive studies had large samples [Bjelland et al., 2003; Lewis et al., 2006], and these were cross-sectional population based studies rather than clinical case-control comparisons. Further to this, Bjelland et al. [2003] isolated their positive association to a subgroup exhibiting no or very low anxiety comorbid with their depression as measured by the Hospital Anxiety and Depression Scale (HADS). To test the hypothesis that the *MTHFR C677T* polymorphism is involved in the predisposition to MDD and if anxiety moderates this we conducted an association study of 1,237 patients with MDD and 858 controls. In addition to this we performed a meta-analysis that included our data, providing an overall estimate of association.

MATERIALS AND METHODS

Samples

The sample of 1,237 patients (men/women = 386/851) was recruited from three clinical sites: London, Cardiff, and Birmingham, UK. Mean age ± SD was 48.59 ± 11.71 years for men and 46.59 ± 12.31 years for women. Subjects were identified from psychiatric clinics, hospitals, and general medical practices, and from volunteers responding to media advertisements. Only subjects of white European ("Caucasian") parentage were included. Subjects were over the age of 18 and had experienced 2 or more episodes of unipolar depression of at least moderate severity separated by at least 2 months of remission as defined by the Diagnostic and Statistical Manual 4th edition operational criteria (DSM-IV), or the International Classification of Diseases 10th edition operational criteria (ICD10). All subjects were interviewed using the Schedules for Clinical Assessment in Neuropsychiatry (SCAN) [Wing et al., 1990]. The SCAN interview includes a rating of whether depression or anxiety is primary if both type of symptom co-occur.

Subjects were excluded if they, or a first-degree relative, ever fulfilled criteria for mania, hypomania, or schizophrenia. Subjects were also excluded if they experienced psychotic symptoms that were mood incongruent or present when there was no evidence of a mood disturbance. Other exclusion criteria were intravenous drug use with a lifetime diagnosis of dependency; depression occurring solely in relation to alcohol or substance abuse or depression only secondary to medical illness or medication, and a clear diagnosis of bipolar disorder, schizophrenia, schizo-affective disorder or acute or transient psychotic disorders in first or second-degree relatives.

Eight hundred and fifty-eight control subjects (men/women = 385/473) screened for lifetime absence of psychiatric disorder using a modified version of the Past History Schedule [McGuffin et al., 1986] were recruited. Mean age ± SD was 48.47 ± 6.92 years for men and 47.31 ± 9.23 years for women. Subjects were excluded if they or a first-degree relative ever fulfilled criteria for major depression, bipolar disorder or schizophrenia. Subjects were also excluded if they scored 10 or above on the Beck Depression Inventory (BDI) [Beck and Steer, 1984], did not return consent, or failed to return cheek swabs. The study was approved by the Local Ethical Committees in the three centers and informed written consent was obtained from all participants.

Genotyping

Interviewers obtained 25 ml of whole blood from unipolar depression patients at the time of interview, collected in 37.5 ml (EDTA containing) monovettes. In addition to this, drops of blood were placed on a Guthrie blood spot card. The blood samples were labeled with a unique ID bar code, gently mixed and stored frozen upright in a −20 degree centigrade freezer pending DNA extraction. Buccal mucosa swabs were collected for DNA of control subjects via the mail. The swab containers were labeled with a unique ID bar code pending DNA extraction. Genomic DNA was extracted by an in-house validated procedure from bloods and cheek swabs collected as described previously [Freeman et al., 1997, 2003].

Outsourced genotyping of the *MTHFR C677T* SNP was performed by KBioscience (Hoddesdon, Hertz, UK, www.kbioscience.co.uk). The genotyping system used is based on competitive allele specific PCR in a homogenous FRET format. Their technique, named KASPar, is patent pending and further information on the chemistry is now available on the web-site (http://www.kbioscience.co.uk/chemistry/kaspar_chemistry-intro.htm). The primers designed by KBioscience are:

Primer_Allele 1: GAAGGTGACCAAGTTCATGCTAAAGCTG-CGTGATGATGAAATCGA
Primer_Allele 2: GAAGGTCGGAGTCAACGGATTAAAGC-TGCGTGATGATGAAATCGG
Primer_Common: TTGAGGCTGACCTGAAGCACTTGA.

SNPviewer software utilizes a clustering algorithm to enable genotype calling. This can be downloaded from the KBioscience website by the customer so that genotype calls may be viewed and checked.

Genotyping was blind to all phenotypic information, including affected status. 835 controls (97.3%) and 1,222 cases (98.8%) were successfully genotyped. Five hundred and thirty randomly selected samples were re-genotyped using the same system (KBioscience), there was 1 clash (0.16%) and this sample was excluded from further analyses. We also used the TAQMAN® SNP genotyping system to perform in-house re-genotyping of 85 randomly selected samples with no clash observed (technical details are available on http://ihg.gsf.de/cgi-bin/hw/hwal.pl).

Statistics

To test for deviation from Hardy–Weinberg Equilibrium (HWE) the Pearson (χ^2) test statistic and an exact statistic were calculated with the computer program FINETTI (available on http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). Cases and controls were considered separately. The *P*-value for the exact test is calculated by generating the possible samples of genotype counts consistent with the observed allele counts, under a null hypothesis of HW equilibrium, calculating the appropriate Pearson (χ^2) test statistic for each sample. The exact *P*-value is calculated by comparing the value of the Pearson (χ^2) test statistic of the observed data with the distribution of the test statistic under the null hypothesis (http://www.meb.ki.se/genestat/tl/genass_ldmap/hwe_gterror/testing.htm).

Genotype and allele frequencies were assessed for association with MDD using standard contingency table analysis incorporating chi-squared test of independence, producing a χ^2 statistic with 1 or 2 degrees of freedom depending on the number of parameters and corresponding *P* values for allele and genotype distributions, respectively. Risk magnitudes were estimated by calculating odds ratios (OR) with 95%

confidence intervals (CI) using Woolf's method. Differences in mean levels of the BDI scale in different genotype groups of MDD patients were tested with analysis of variance. One-way ANOVA was run in SPSS for Windows (Version 13.0). The conventional α-level of 0.05 was specified as the significance threshold.

To calculate the power of our case-control sample size we used the PS program [Dupont and Plummer, 1990]. In this program the method of Schlesselman [1982] is used with independent case and control groups for analysis using an uncorrected Chi-squared test. When the case and control sample sizes are unequal, PS uses the generalization of Casagrande's method proposed by Fleiss [1981]. The alternative hypothesis is specified in terms of odds ratios.

A meta-analysis was conducted combining data extracted from the study by Gilbody et al. [2007] and our own results. In our meta-analysis we included case-control studies by Hickie et al. [2001], Kelly et al. [2004], Reif et al. [2005], and our group where the presence of recurrent depression was established using DSM-IV or ICD10 criteria, and where subjects were of white European origin. Our analysis compared *677TT* genotype frequency versus *677CC* genotype frequency for patients versus controls. We also examined C-versus-T allele frequency and contrasted persons who were homozygous wild-type (*677CC*) with heterozygotes (*677CT*). Odds ratios were combined using random-effects model. We estimated between-study heterogeneity using the I^2 statistics [Higgins et al., 2003]. Genetic association studies may be especially susceptible to the selective publication of positive findings on gene associations [Salanti et al., 2005], and so a funnel plot and Begg and Mazumdar's [1994] rank correlation test were used in order to assess publication bias. All analyses were conducted in Stata (Version 8.0) using the *metan* and *metabias* commands.

RESULTS

Genotyping results of the *MTHFR C677T* polymorphism are available for 835 controls (97.3%) and 1,222 cases (98.8%) of the DeCC sample. The distribution of genotype frequencies of the *C677T* polymorphism was in Hardy–Weinberg equilibrium (HWE) in the control subjects but not in the affected sample, specifically the females (Table I). There is close agreement between the two tests used to assess deviation from HWE.

No significant genotypic or allelic association was observed with the *C677T* SNP and recurrent unipolar depression in the sample as a whole, or when gender was considered (Table II). The analysis testing for association with the C677T genotype and the BDI scale showed no significant differences in genotype distribution and BDI scores amongst the depressive subjects (Table III). In view of a previous report that the association is seen in depression *without* anxiety [Bjelland et al., 2003] anxiety was factored into analyses. Differences in genotypic distribution between subjects with primary depression and subjects with primary anxiety in men were observed ($\chi^2 = 6.38$, $P = 0.041$), however no significant differences between patients of either group and unaffected controls were found (Table IV).

Three previous case-control studies, together with our results, were included in our meta-analysis [Hickie et al., 2001; Kelly et al., 2004; Reif et al., 2005]. The presence of depression in cases was established with DSM-IV criteria [Hickie et al., 2001] or ICD10 criteria [Kelly et al., 2004; Reif et al., 2005], and all subjects were of European origin: German [Reif et al., 2005], Northern Irish [Kelly et al., 2004], and Australian [Hickie et al., 2001]. There was no association with depression and the homozygote *677TT* genotype (random-effect $OR_{TTvCC} = 1.05$, 95% CI 0.81–1.35, $z = 0.34$, $P = 0.73$), and no discernible statistical between-study heterogeneity was observed ($I^2 = 0\%$; Table IV and Fig. 1). We also found no association with the heterozygotes (random-effect $OR_{CTvCC} = 1.07$, 95% CI: 0.69–1.68, $z = 0.31$, $P = 0.753$) although greater between-study heterogeneity was observed ($I^2 = 59.7\%$). There was no increased frequency of the *677T* allele detected in depressive subjects compared to controls ($OR_{TvC} = 1.05$, 95% CI 0.85–1.31, $z = 0.45$ $P = 0.65$), however some between-study heterogeneity was detected ($I = 31.1\%$). The funnel plot analysis indicates no evidence of publication bias.

DISCUSSION

We have performed the first large-scale case-control study investigating the association between the *MTHFR C677T* polymorphism and clinically significant recurrent unipolar depression. We failed to replicate previous reports of an association with the *MTHFR 677TT* genotype or *677T* allele with depression in population based [Bjelland et al., 2003; Lewis et al., 2006] and case-control studies [Arinami et al., 1997; Hickie et al., 2001; Kelly et al., 2004].

TABLE I. Tests for Deviation From Hardy–Weinberg Equilibrium in Investigated Groups

	Tests for deviation from Hardy–Weinberg equilibrium	
Groups	Controls	MDD patients
Men	n11 = 152 (153.96)	n11 = 172 (168.67)
	n12 = 174 (170.07)	n12 = 163 (169.67)
	n22 = 45 (46.96)	n22 = 46 (42.67)
	$P = 0.656$ (Pearson)	$P = 0.443$ (Pearson)
	$P = 0.734$ (Exact)	$P = 0.490$ (Exact)
Women	n11 = 198 (194.61)	n11 = 373 (357.08)
	n12 = 205 (211.77)	n12 = 350 (381.84)
	n22 = 61 (57.61)	n22 = 118 (102.08)
	$P = 0.491$ (Pearson)	$P = 0.016$ (Pearson)
	$P = 0.478$ (Exact)	$P = 0.018$ (Exact)
Total	n11 = 350 (348.58)	n11 = 545 (525.70)
	n12 = 379 (381.85)	n12 = 513 (551.61)
	n22 = 106 (104.58)	n22 = 164 (144.70)
	$P = 0.829$ (Pearson)	$P = 0.014$ (Pearson)
	$P = 0.821$ (Exact)	$P = 0.016$ (Exact)

n11, genotype CC (expected); n12, genotype CT (expected); n22, genotype TT (expected); *P* (Pearson), Pearson's goodness-of-fit Chi-square (df = 1); *P* (Exact), Exact test.

TABLE II. Association Analysis of the *MTHFR C677T* in Depression Case-Control Samples (Different Models of the *677T* Allele)

	Frequencies								
	Men			Women			Total		
Model	MDD patients (N = 381)	Controls (N = 371)	OR (95% CI)	MDD patients (N = 841)	Controls (N = 464)	OR (95% CI)	MDD patients (N = 1,222)	Controls (N = 835)	OR (95% CI)
T vs. C									
C	507 (66.5)	478 (64.4)	1	1,096 (65.2)	601 (64.8)	1	1,603 (65.6)	1,079 (64.6)	1
T	255 (34.5)	264 (35.6)	0.91 (0.74–1.13)	586 (34.8)	327 (35.2)	0.98 (0.83–1.16)	841 (34.4)	591 (35.4)	0.96 (0.84–1.09)
χ^2 (p), df = 1	0.74 (0.39)			0.04 (0.84)			0.42 (0.52)		
Codominant									
CC	172 (45.1)	152 (41.0)	1	373 (44.4)	198 (42.7)	1	545 (44.6)	350 (41.9)	1
CT	163 (42.8)	174 (46.9)	0.83 (0.61–1.12)	350 (41.6)	205 (44.2)	0.91 (0.71–1.54)	513 (41.9)	379 (45.4)	0.87 (0.72–1.05)
TT	46 (12.1)	45 (12.1)	0.9 (0.57–1.44)	118 (14.0)	61 (13.1)		164 (13.4)	106 (12.7)	0.99 (0.75–1.31)
χ^2 (p), df = 2	1.47 (0.48)			0.83 (0.66)			2.35 (0.31)		
Dominant (T allele)									
CC	172 (45.1)	152 (41.0)	1	373 (44.4)	198 (42.7)	1	545 (44.6)	350 (41.9)	1
TT-TC	209 (54.9)	219 (59.0)	0.84 (0.63–1.13)	468 (55.6)	266 (57.3)	0.93 (0.74–1.17)	677 (55.4)	485 (58.1)	0.89 (0.75–1.07)
χ^2 (p), df = 1	1.34 (0.25)			0.34 (0.56)			1.45 (0.23)		
Recessive (T allele)									
TC-CC	335 (87.9)	326 (87.9)	1	723 (86.0)	403 (86.9)	1	1,058 (86.5)	729 (87.3)	1
TT	46 (12.1)	45 (12.1)	0.99 (0.64–1.54)	118 (14.0)	61 (13.1)	1.08 (0.77–1.5)	164 (13.4)	106 (12.7)	1.07 (0.82–1.38)
χ^2 (p), df = 1	0.00 (1.00)			0.19 (0.66)			0.23 (0.63)		
Overdominant									
CC-TT	218 (57.2)	197 (53.1)	1	491 (58.4)	259 (55.8)	1	709 (58.0)	457 (54.7)	1
CT	163 (42.8)	174 (46.9)	0.85 (0.64–1.13)	350 (41.6)	205 (44.2)	0.9 (0.72–1.13)	513 (42.0)	379 (45.3)	0.87 (0.73–1.04)
χ^2 (p), df = 1	1.29 (0.26)			0.80 (0.37)			2.28 (0.13)		

TABLE III. *MTHFR C677T* Polymorphism and BDI—Depression Scale in MDD Subjects

	Men		Women		Total	
Genotypes	N = 361	Mean score of BDI ± SD	N = 790	Mean score of BDI ± SD	n = 1,151	Mean score of BDI ± SD
CC	164	20.24 ± 11.02	348	20.32 ± 12.653	512	20.29 ± 12.14
CT	153	20.85 ± 11.42	331	20.82 ± 11.874	484	20.83 ± 11.72
TT	44	20.75 ± 13.80	111	17.88 ± 12.132	155	18.70 ± 12.65
F (p)		0.12 (0.89)		2.43 (0.09)		1.85 (0.16)

To check for HWE in our sample two test statistics were applied. The Pearson (χ^2) test statistic is commonly used, however with smaller samples and rarer (or multiple) alleles the exact test is recommended [Emigh, 1980; Wigginton et al., 2005]. Although our mutant allele is not rare, and our sample is large, the division of our sample means smaller numbers, and so both statistics were checked. The two test statistics yielded *P*-values that were in close agreement although the exact test calculated a marginally higher *P*-value of 0.018 (see Table I) that is just above the significance threshold when Bonferroni correction is applied ($P \leq 0.016$). This correction may be considered overly conservative. Therefore, although our control sample is in HWE, the unipolar depression group significantly deviates from HWE. This can be isolated to our female case sample where there appears to be a deficiency in heterozygotes (see Table I). Deviation from HWE in the affected sample is not unexpected when there is association, however no significant association in the female cases is reported in this article. We shall discuss possible reasons for this deviation without association.

Genotyping error might account for HWE deviation, be it problematic calling due to the clustering algorithm, or unreliable data on specific plates. However our case and control samples were randomly mixed, and so deviation would not be restricted to just one affectation status (cases) or one gender (females). The reliability of the outsourced data was also checked using two independent genotyping systems: KBioscience re-genotyped 530 randomly selected samples, and 85 samples were repeated in-house. This yielded one genotype error (from KBioscience) and an error rate of just 0.16%. We feel confident that genotyping error does not explain the deviation from HWE observed in the female cases.

A second possible reason is population stratification as the frequency of the *677T* allele is subject to considerable ethnic and geographic variation [Botto and Yang, 2000]. Only unrelated subjects of white European parentage were included in our study. In Ireland and Britain previously reported frequencies of this genotype were 11% and 13%, respectively. This is comparable to frequencies observed in our control sample: 12.1% in men, and 14.0% in women. As our cases were recruited from three different sites (London, Cardiff, and Birmingham) genotypic distributions were compared across sites; no significant differences were found. Additionally no significant deviation from HWE was observed in these separate groups, although a border-line *P*-value of 0.05 was calculated in the Birmingham group with gender combined. The sensitivity of this marker highlights the ethnicity as a consideration in the design of genetic studies, however it seems

TABLE IV. Association Analysis of the *MTHFR C677T* With "Depression or Anxiety Primary" Scale

Genotypes/alleles	Controls	Depression primary	Anxiety primary
Men	N = 371	N = 242	N = 55
CC	152 (41.0)	119 (49.2)	20 (36.4)
CT	174 (46.9)	92 (38.0)	31 (56.4)
TT	45 (12.1)	31 (12.8)	4 (7.2)
χ^2 (p), df = 2		4.95 (0.08)[a]	2.13 (0.35)[a]
C	478 (64.4)	330 (68.2)	71 (64.5)
T	264 (35.6)	154 (31.8)	39 (35.5)
χ^2 (p), df = 1		1.84 (0.17)[a]	0.00 (0.98)[a]
Women	N = 464	N = 548	N = 120
CC	198 (42.7)	234 (42.7)	58 (48.3)
CT	205 (44.2)	228 (41.6)	51 (42.5)
TT	61 (13.1)	86 (15.7)	11 (9.2)
χ^2 (p), df = 2		1.51 (0.47)[a]	1.98 (0.37)[a]
C	601 (64.8)	696 (63.5)	167 (69.6)
T	327 (35.2)	400 (36.5)	73 (30.4)
χ^2 (p), df = 1		0.35 (0.56)[a]	1.97 (0.16)[a]
Total	N = 835	N = 790	N = 175
CC	350 (41.9)	353 (44.7)	78 (44.6)
CT	379 (45.4)	320 (40.5)	82 (46.8)
TT	106 (12.7)	117 (14.8)	15 (8.6)
χ^2 (p), df = 2		4.29 (0.12)[a]	2.36 (0.31)[a]
C	1,079 (64.6)	1,026 (64.9)	238 (68.0)
T	591 (35.4)	554 (35.1)	112 (32.0)
χ^2 (p), df = 1		0.04 (0.85)[a]	1.47 (0.23)[a]

[a]Compared to control group.

TABLE V. Distribution of the *MTHFR C677T* Genotypes and Alleles in Studies of MDD Including in Meta-Analysis of Random-Effect Model

	CC:CT:TT (N)				C:T (N)		
References	MDD patients	Controls	OR_{TTvCC} (95%CI)	OR_{TCvCC} (95%CI)	MDD patients	Controls	OR_{TvC} (95%CI)
Reif et al. [2005]	23:17:6	75:80:21	0.932 (0.34–2.59)	0.69 (0.34–1.40)	63:29	230:122	0.87 (0.53–1.42)
Hickie et al. [2001]	33:33:9	12:9:1	3.27 (0.37–28.63)	1.33 (0.50–3.59)	99:51	33:11	1.55 (0.72–3.31)
Kelly et al. [2004]	30:56:14	40:37:12	1.56 (0.63–3.84)	2.02 (1.08–3.79)	116:84	117:61	1.39 (0.91–2.11)
DeCC Study	545:513:164	351:379:106	0.99 (0.75–1.32)	0.87 (0.72–1.05)	1,603:841	1,079:591	0.96 (0.84–1.09)
Overall	631:619:193	478:505:140	1.05 (0.81–1.35)	1.07 (0.69–1.68)	1,881:1,005	1,459:785	1.05 (0.85–1.31)

unlikely to be the cause of the observed deviation from HWE in our female cases.

A third possibility is that the deviation from HWE could be a consequence of a true association of this SNP with another disease that is highly correlated with depression. Indeed it has been hypothesized that depression and vascular disease may be different manifestations of the same genetic substrates [Bondy et al., 2002]. Both of these conditions are a result of the interaction of multiple genetic factors and the environment, involving multiple genes with small interactive and additive effects. Gene effects can amplify the effect of environmental or metabolic factors on the final phenotype rather than directly affecting the risk of disease [Bondy et al., 2002]. We cannot exclude the possibility that previous positive findings could be the consequence of the comorbidity of depression with cardiovascular diseases. The *MTHFR C677T* SNP was shown to be associated with essential hypertension [Heux et al., 2004], coronary heart disease [Klerk et al., 2002], and migraine [Scher et al., 2006].

The final explanation relates to the functionality of the SNP as there is evidence that the functional effect of the *C677T* SNP is greater in individuals with plasma folate in the lower range, relative to those with higher levels of plasma folate [Jacques et al., 1996]. Further to this, artificial supplementation with folic acid reduces the circulating concentration of plasma homocysteine to a greater extent in *677TT* individuals than *677CT* or *677CC* individuals [Malinow et al., 1997], and can even reduce it to the level observed in *677CT* or *677CC* individuals [Nelen et al., 1998]. It has also been suggested that the functional effect of the *C677T* SNP may interact with environmental factors such as diet [Geisel et al., 2003]; this effect of diet may be moderated by folate levels, as fruits and vegetables provide a source of folate. It is feasible that in our sample there is an association with unipolar depression and the *C677T* genotype in the *MTHFR* gene but only in females

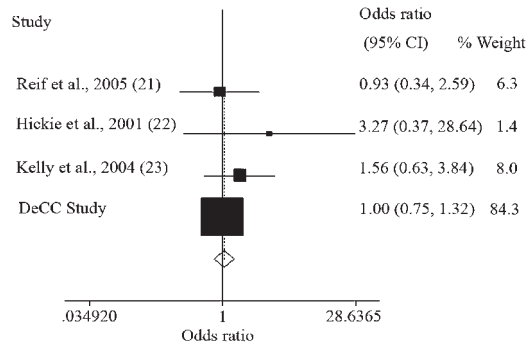

Fig. 1. Results from random effect meta-analysis for TT versus CC genotypes of the MTHFR C677T polymorphism in case-control studies of depressive disorder.

that have low folate levels. As these individuals are considered as a whole in our analyses, irrespective of folate status, this association may not be detected in case-control analysis, however could be enough to cause the female cases to not fit the model of HWE. Although a speculative hypothesis, this sex-specific association is parsimonious with the linkage peak reported in the DENT study in the region of the *MTHFR* gene in females [McGuffin et al., 2005]. Further to this, the largest randomized clinical trial to date reports a female-specific beneficial effect of folate supplementation in depression [Coppen and Bailey, 2000].

Our results are in disagreement with a range of studies reporting the significant association between depression and the *MTHFR C677T*. The most convincing results were obtained in two huge population based studies of Bjelland et al. [2003] and of Lewis et al. [2006]. However the design of these studies and the phenotypic definition of depression differ from that applied in the DeCC study. Lewis et al. [2006] the presence or absence of depression was established by self-report, and Bjelland et al. [2003] classified individuals with a score equal to or above 8 on the HADS as depressed. Our study enrolled subjects diagnosed with recurrent unipolar depression based on DSM-IV and ICD10 criteria. Strict exclusion criteria were applied in the recruitment of depressed patients. In the fore-mentioned studies [Bjelland et al., 2003; Lewis et al., 2006] controls were not screened for previous history of depression our control sample was thoroughly screened for previous and current depression.

Previous case-control reports have yielded inconsistent results [Arinami et al., 1997; Kunugi et al., 1998; Hickie et al., 2001; Kelly et al., 2004; Tan et al., 2004; Chen et al., 2005; Reif et al., 2005], possibly due to their small sample sizes that compromise the power of these studies. The sample size of our study is more than two thousand individuals (1,222 depressive patients and 835 controls), giving a statistical power of 99% to detect an odds ratio of 1.69 reported in the study by Bjelland et al. [2003] for *677TT* genotype.

One possible way to increase power of association analysis is to conduct a meta-analysis. A recent meta-analysis by Gilbody et al. [2007] supports the involvement of the *MTHFR C677T* polymorphism in unipolar depression: the fixed-effects odds ratio for the *677TT* variant versus the wild-type was 1.36. In this meta-analysis both population based cross-sectional studies and case-control studies were included. Of ten studies in total, six included samples of European origin, and four of Asian origin. The presence of depression was established by a variety of methods including self-report. We applied stricter criteria to select studies to include in our meta-analysis: only case-control studies limited to European ethnicity were included. The results of our meta-analysis do not support the association between *677TT* genotype or *677T* allele and depression. The power of this sample to detect possible association of the *677TT* genotype (taking an OR equal to 1.36) with depression is 77%, leaving

23% probability of a type II error. Our findings are in agreement with another meta-analysis that included five studies investigating non-bipolar depression [Zintzaras, 2006]; no association was observed between the *C677T* polymorphism and depression in Caucasians (random effects OR = 1.05).

Following the example of Bjelland et al. [2003] who report hyperhomocysteinemia and the *677TT* genotype to be significantly related to depression *without* comorbid anxiety disorder, we conducted an additional analysis dividing the sample of our patients according to whether depression or anxiety was primary. We failed to find any difference between the *C677T* genotypic distribution in the primary depression subgroup and the controls.

In conclusion, our results do not support a major role for the *MTHFR C677T* polymorphism in conferring susceptibility to recurrent unipolar depression in the British population as a whole. Further studies of the *MTHFR* gene are required in independent, adequately powered samples that also obtain data relating to relevant biochemical measures such as folate and homocysteine levels. This highly powered study emphasizes the importance of negative data being published enabling reference and consideration by the scientific community.

ACKNOWLEDGMENTS

Dr. Gaysina was supported by INTAS Postdoctoral Fellowship (Ref. Nr 04-83-3802) and Russian Science Support Foundation. Ms Cohen was supported by a Medical Research Council (MRC) UK PhD studentship. The depression case control collection (DeCC) was funded by the MRC UK.

REFERENCES

Alpert JE, Mischoulon D, Nierenberg AA, Fava M. 2000. Nutrition and depression: Focus on folate. Nutrition 16:544–546.

Arinami T, Yamada N, Yamakawa-Kobayashi K, Hamaguchi H, Toru M. 1997. Methylenetetrahydrofolate reductase variant and schizophrenia/depression. Am J Med Genet 74:526–528.

Beck AT, Steer RA. 1984. Internal consistencies of the original and revised Beck Depression Inventory. J Clin Psychol 40:1365–1367.

Begg CB, Mazumdar M. 1994. Operating characteristics of a rank correlation test for publication bias. Biometrics 50:1088–1101.

Bjelland I, Tell GS, Vollset SE, Refsum H, Ueland PM. 2003. Folate, vitamin B12, homocysteine, and the MTHFR 677C → T polymorphism in anxiety and depression: The Hordaland Homocysteine Study. Arch Gen Psychiatry 60:618–626.

Bondy B, Baghai TC, Zill P, Bottlender R, Jaeger M, Minov C, et al. 2002. Combined action of the ACE D- and the G-protein β3 T-allele in major depression: A possible link to cardiovascular disease? Mol Psychiatry 7:1120–1126.

Bottiglieri T, Hyland K, Reynolds EH. 1994. The clinical potential of ademetionine (S-adenosylmethionine) in neurological disorders. Drugs 48:137–152.

Bottiglieri T, Laundy M, Crellin R, Toone BK, Carney MW, Reynolds EH. 2000. Homocysteine, folate, methylation, and monoamine metabolism in depression. J Neurol Neurosurg Psychiatry 69:228–232.

Botto LD, Yang Q. 2000. 5,10-Methylenetetrahydrofolate reductase gene variants and congenital anomalies: A HuGE review. Am J Epidemiol 151:862–877.

Chen CS, Tsai JC, Tsang HY, Kuo YT, Lin HF, Chiang IC, et al. 2005. Homocysteine levels, MTHFR C677T genotype, and MRI hyperintensities in lateonset major depressive disorder. Am J Geriatr Psychiatry 13:869–875.

Coppen A, Bailey J. 2000. Enhancement of the antidepressant action of fluoxetine by folic acid: A randomised, placebo controlled trial. J Affect Disord 60:121–130.

Dupont WD, Plummer WD. 1990. Power and sample size calculations: A review and computer program. Control Clin Trials 11:116–1128.

Emigh TH. 1980. A comparison of tests for Hardy-Weinberg equilibrium. Biometrics 36:627–642.

Fleiss JL. 1981. Statistical methods for rates and proportions. 2nd edition. New York: John Wiley. pp 38–46.

Freeman B, Powell J, Ball D, Hill L, Craig I, Plomin R. 1997. DNA by mail: An inexpensive and noninvasive method for collecting DNA samples from widely dispersed populations. Behav Genet 27:251–257.

Freeman B, Smith N, Curtis C, Huckett L, Mill J, Craig IW. 2003. DNA from buccal swabs recruited by mail: Evaluation of storage effects on long-term stability and suitability for multiplex polymerase chain reaction genotyping. Behav Genet 33:67–72.

Geisel J, Hubner U, Bodis M, Schorr H, Knapp JP, Obeid R, et al. 2003. The role of genetic factors in the development of hyperhomocysteinemia. Clin Chem Lab Med 41:1427–1434.

Gilbody S, Lewis S, Lightfoot T. 2007. Methylenetetrahydrofolate Reductase (MTHFR) Genetic Polymorphisms and Psychiatric Disorders: A HuGE Review. Am J Epidemiol 165:1–13.

Heux S, Morin F, Lea RA, Ovcaric M, Tajouri L, Griffiths LR. 2004. The methylentetrahydrofolate reductase gene variant (C677T) as a risk factor for essential hypertension in Caucasians. Hypertens Res 27:663–667.

Hickie I, Scott E, Naismith S, Turner K, Scott E, Mitchell P, et al. 2001. Late-onset depression: Genetic, vascular and clinical contributions. Psychol Med 31:1403–1412.

Higgins JP, Thompson SG, Deeks JJ, Altman DG. 2003. Measuring inconsistency in meta-analyses. BMJ 327:557–560.

Jacques PF, Bostom AG, Williams RR, Ellison RC, Eckfeldt JH, Rosenberg IH, et al. 1996. Relation between folate status, a common mutation in methylenetetrahydrofolate reductase, and plasma homocysteine concentrations. Circulation 93:7–9.

Kelly CB, McDonnell AP, Johnston TG, Mulholland C, Cooper SJ, McMaster D, et al. 2004. The MTHFR C677T polymorphism is associated with depressive episodes in patients from Northern Ireland. J Psychopharmacol 18:567–571.

Klerk M, Verhoef P, Clarke R, Blom HJ, Kok FJ, Schouten EG, et al. 2002. MTHFR 677C → T polymorphism and risk of coronary heart disease: A meta-analysis. JAMA 288:2023–2031.

Kunugi H, Fukuda R, Hattori M, Kato T, Tatsumi M, Sakai T, et al. 1998. C677T polymorphism in methylenetetrahydrofolate reductase gene and psychoses. Mol Psychiatry 3:435–437.

Lewis SJ, Lawlor DA, Davey Smith G, Araya R, Timpson N, Day IN, Ebrahim S. 2006. The thermolabile variant of MTHFR is associated with depression in the British Women's Heart and Health Study and a meta-analysis. Mol Psychiatry 11:352–360.

Malinow MR, Nieto FJ, Kruger WD, Duell PB, Hess DL, Gluckman RA, et al. 1997. The effects of folic acid supplementation on plasma total homocysteine are modulated by multivitamin use and methylenetetrahydrofolate reductase genotypes. Arterioscler Thromb Vasc Biol 17: 1157–1162.

McGuffin P, Katz R, Aldrich J. 1986. Past and present state examination: The assessment of 'lifetime ever' psychopathology. Psychol Med 16:461–465.

McGuffin P, Knight J, Breen G, Brewster S, Boyd PR, Craddock N, et al. 2005. Whole genome linkage scan of recurrent depressive disorder from the depression network study. Hum Mol Genet 14:3337–3345.

Molloy AM, Daly S, Mills JL, Kirke PN, Whitehead AS, Ramsbottom D, et al. 1997. Thermolabile variant of 5,10-methylenetetrahydrofolate reductase associated with low red-cell folates: Implications for folate intake recommendations. Lancet 349:1591–1593.

Nelen WL, Blom HJ, Thomas CM, Steegers EA, Boers GH, Eskes TK. 1998. Methylenetetrahydrofolate reductase polymorphism affects the change in homocysteine and folate concentrations resulting from low dose folic acid supplementation in women with unexplained recurrent miscarriages. J Nutr 128:1336–1341.

Reif A, Pfuhlmann B, Lesch KP. 2005. Homocysteinemia as well as methylenetetrahydrofolate reductase polymorphism are associated with affective psychoses. Prog Neuropsychopharmacol Biol Psychiatry 29:1162–1168.

Rozen R. 1996. Molecular genetics of methylenetetrahydrofolate reductase deficiency. J Inherit Metab Dis 19:589–594.

Salanti G, Sanderson S, Higgins JP. 2005. Obstacles and opportunities in meta-analysis of genetic association studies. Genet Med 7:13–20.

Scher AI, Terwindt GM, Verschuren WM, Kruit MC, Blom HJ, Kowa H, et al. 2006. Migraine and MTHFR C677T genotype in a population-based sample. Ann Neurol 59:372–375.

Schlesselman JJ. 1982. Case-control studies: Design, conduct, analysis. New York: Oxford U. Press. pp 144–152.

Tan EC, Chong SA, Lim LC, Chan AO, Teo YY, Tan CH, et al. 2004. Genetic analysis of the thermolabile methylenetetrahydrofolate reductase variant in schizophrenia and mood disorders. Psychiatr Genet 14:227–231.

Ueland PM, Hustad S, Schneede J, Refsum H, Vollset SE. 2001. Biological and clinical implications of the MTHFR C677T polymorphism. Trends Pharmacol Sci 22:195–201.

van Praag HM. 1982. Depression. Lancet 2:1259–1283.

Wigginton JE, Cutler DJ, Abecasis GR. 2005. A note on exact tests of Hardy-Weinberg equilibrium. Am J Hum Genet 76:887–893.

Wing JK, Babor T, Brugha T, Burke J, Cooper JE, Giel R, et al. 1990. SCAN. Schedules for Clinical Assessment in Neuropsychiatry. Arch Gen Psychiatry 47:589–593.

Zintzaras E. 2006. C677T and A1298C methylenetetrahydrofolate reductase gene polymorphisms in schizophrenia, bipolar disorder and depression: A meta-analysis of genetic association studies. Psychiatr Genet 16:105–115.