American Journal of Epidemiology
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Vol. 165, No. 1
DOI: 10.1093/aje/kwj347
Advance Access publication October 30, 2006

Human Genome Epidemiology (HuGE) Review

Methylenetetrahydrofolate Reductase (*MTHFR*) Genetic Polymorphisms and Psychiatric Disorders: A HuGE Review

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Received for publication March 8, 2006; accepted for publication May 23, 2006.

The authors performed a meta-analysis of studies examining the association between polymorphisms in the 5,10-methylenetetrahydrofolate reductase (*MTHFR*) gene, including *MTHFR* C677T and A1298C, and common psychiatric disorders, including unipolar depression, anxiety disorders, bipolar disorder, and schizophrenia. The primary comparison was between homozygote variants and the wild type for *MTHFR* C677T and A1298C. For unipolar depression and the *MTHFR* C677T polymorphism, the fixed-effects odds ratio for homozygote variants (TT) versus the wild type (CC) was 1.36 (95% confidence interval (CI): 1.11, 1.67), with no residual between-study heterogeneity ($I^2 = 0\%$)—based on 1,280 cases and 10,429 controls. For schizophrenia and *MTHFR* C677T, the fixed-effects odds ratio for TT versus CC was 1.44 (95% CI: 1.21, 1.70), with low heterogeneity ($I^2 = 42\%$)—based on 2,762 cases and 3,363 controls. For bipolar disorder and *MTHFR* C677T, the fixed-effects odds ratio for TT versus CC was 1.82 (95% CI: 1.22, 2.70), with low heterogeneity ($I^2 = 42\%$)—based on 550 cases and 1,098 controls. These results were robust to various sensitively analyses. This meta-analysis demonstrates an association between the *MTHFR* C677T variant and depression, schizophrenia, and bipolar disorder, raising the possibility of the use of folate in treatment and prevention.

anxiety; bipolar disorder; depression; folic acid; genetics; methylenetetrahydrofolate reductase (NADPH2); *MTHFR*; schizophrenia

Abbreviations: CI, confidence interval; HuGE, Human Genome Epidemiology; MTHFR, methylenetetrahydrofolate reductase; OR, odds ratio.

Editor's note: *This article also appears on the website of the Human Genome Epidemiology Network (http://www.cdc.gov/genomics/hugenet/).*

GENE

The 5,10-methylenetetrahydrofolate reductase (*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-methylenetetrahydrofolate, the predominant circulating form of folate. 5-Methylenetetrahydrofolate donates a methyl group to homocysteine in the generation of *S*-adenosylmethionine, a major source of methyl groups in the brain (1).

GENE VARIANTS

Two common single nucleotide polymorphisms in *MTHFR* have been reported, a C→T transition at nucleotide 677 in exon 4 and an A→C transversion in exon 7 at

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position 1298. Both of these polymorphisms are functional and result in diminished enzyme activity. For the C677T polymorphism, homozygote variants have 30 percent enzyme activity in comparison with homozygotes for the wild-type C allele, while heterozygotes retain 65 percent of wild-type *MTHFR* enzyme activity (2). The consequences of the C677T polymorphism have been demonstrated in population studies, where lower levels of red blood cell folate, plasma folate, and vitamin B_{12} have been reported among nondiseased persons with the 677 TT genotype in comparison with persons with other genotypes (3). The 1298 polymorphism has been less extensively studied; however, it is known that persons with the 1298 CC genotype have approximately 60 percent of the enzyme activity of those with the common AA genotype (4).

The frequency of the C677T allele is subject to considerable ethnic and geographic variation, with a high allele frequency being reported in California Hispanics and a low allele frequency in US Blacks. There is also marked variation in the frequency of C677T homozygote variants between populations. The highest frequency (>20 percent) is found among US Hispanics, Colombians, and Amerindians in Brazil; conversely, in Black populations, less than 2 percent have the variant genotype (5). Among White populations in Europe, North America, and Australia, the frequency ranges from 8 percent to 20 percent, although interestingly in Europe there seems to be a drift in the occurrence of the homozygote variant from north to south. The prevalence of the A1298C homozygote variant genotype ranges from 7 percent to 12 percent in White populations from North America and Europe. Lower frequencies have been reported in Hispanics (4–5 percent), Chinese (1–4 percent), and Asian populations (1–4 percent). (For more information, see the previously published Human Genome Epidemiology (HuGE) reviews by Botto and Yang (5), Robein and Ulrich (6), and Sharp and Little (7).)

DISEASES

The diseases covered in this review are the common functional psychiatric disorders: major unipolar depression, anxiety disorders, bipolar affective disorder, and schizophrenia (8). Organic disorders and disorders of addiction are not covered in this review. Common functional psychiatric disorders contribute 15 percent of the global burden of disease (9), and each has been shown to have both a genetic etiology and an environmental etiology (10, 11).

Depression is the most common psychiatric disorder, with a 12-month incidence of 6–12 percent (12, 13). It is characterized by low mood and biologic disturbances of sleep, volition, and physical and mental activity (14).

Anxiety disorders are common, with an annual incidence of 5 percent (15). They are characterized by excessive, inappropriate, and disabling anxiety, accompanied by autonomic symptoms and catastrophic thoughts (14).

Schizophrenia is the most common functional psychotic illness, with a lifetime incidence of 1–2 percent (14). It is characterized by disorders of thought and volition and by abnormal perceptions (hallucinations) and beliefs (delusions) (14).

Bipolar disorder (manic depressive illness) is much less common than unipolar depression, with an annual incidence of 0.5 percent (15). It is characterized by alternating and severe disorders of mood (elation and depression), with accompanying shifts in energy and volition (14).

Functional psychiatric disorders, including depression (16), schizophrenia (17), and bipolar disorder (18), have been linked to low folate levels (19) and defective folate metabolism (16). The most extensively studied link is between a low folate level and depression, where an association has been demonstrated and a therapeutic role for folate has been proposed (16). However, evidence of association comes from either case-control studies, which do not control for important confounding factors (20, 21), or cross-sectional studies, where the direction of causality is impossible to establish (22). In contrast, associations with functional *MTHFR* polymorphisms are less susceptible to confounding or reverse causality.

We carried out a meta-analysis to examine whether genetic polymorphisms in *MTHFR* were associated with common psychiatric disorders.

MATERIALS AND METHODS

We conducted a systematic review and meta-analysis in accordance with the guidelines provided by the Human Genome Epidemiology Network (HuGE Net) (23–25).

Inclusion criteria

We included all studies which examined an association between genetic polymorphisms of *MTHFR* and a functional psychiatric disorder. Nonfamilial cross-sectional, case-control, and cohort studies were included. The presence of a psychiatric disorder was established by means of any standardized method, including a validated diagnostic instrument or a standardized diagnostic interview (8, 14). Studies using self-reported diagnoses were included, but the robustness of all analyses was tested to assess their impact.

Search strategy

The following databases were searched from their inception to May 15, 2006, with no language restrictions: MEDLINE, EMBASE, PsycINFO, BIOSIS, HuGE Net, Genetics Abstracts, and Science Direct. We used exploded subject headings, synonyms, and free text terms for folate, *MTHFR*, *MTHFR* polymorphisms (C677T and A1298C), and psychiatric disorders. Reference lists were also scrutinized for relevant studies.

Data extraction

Two investigators independently extracted the following data from each publication: author; country of origin; selection and characteristics of cases and controls; demographic information; racial descent of the study population; numbers of eligible and genotyped cases and controls; and numbers of cases and controls for each *MTHFR* genotype. Particular care was taken to avoid ''double counting'' of evidence in

cases where papers had the same authors. We also examined matching and blinding to the clinical status of the subjects. We sought assistance from authors in situations where data were missing, ambiguous, or incomplete.

Meta-analysis

Our primary analysis for *MTHFR* C677T compared the homozygote variant with the homozygote wild type (TT vs. CC) for patients versus controls. We also examined C-versus-T allele frequency and contrasted persons who were homozygous wild-type (CC) with heterozygotes (CT). In addition, we tested for deviations from Hardy-Weinberg equilibrium in control populations, using the exact method (26).

We combined odds ratios using both fixed-effects and random-effects models (27). In subgroup analyses, we estimated race-specific odds ratios for each allele contrast. We also performed cumulative meta-analysis (28) to evaluate whether the association changed over time. We estimated between-study heterogeneity using the I^2 statistic (29). As a guide, I^2 values of 25 percent may be considered ''low,'' values of 50 percent may be considered ''moderate,'' and values of 75 percent may be considered ''high'' (29).

Genetic association studies may be especially susceptible to the selective publication of positive findings on gene associations (30). To test for publication bias, we applied funnel plot analysis and a regression test (31). We evaluated whether the summary results were different when the analyses were limited to studies with rigorous selection of cases (those that confirmed the diagnosis using a standardized interview) or when we excluded studies that were in Hardy-Weinberg disequilibrium (30). We also adjusted our meta-analyses for control group Hardy-Weinberg disequilibrium according to a recently introduced method (32).

Where analyses were stratified by race, quality, disequilibrium, or diagnostic method, we tested for relations between study-level variables and relations between odds ratios by means of logistic meta-regression (33), using a permutation test (with 1,000 Monte Carlo simulations) to calculate p values and to reduce the chance of spurious false-positive findings (34). The amount of heterogeneity explained by the use of predictive covariates was examined by reductions in the I^2 inconsistency statistic.

All analyses were conducted in Stata, version 8 (Stata Corporation, College Station, Texas), using the *metan*, *metabias*, *metareg*, *metacum*, and *genhw* commands.

META-ANALYSIS RESULTS

Depression

Searches identified 10 studies (11,709 participants; 1,280 cases of depression, 10,429 controls) meeting our inclusion criteria. All studies examined the *MTHFR* C677T polymorphism (22, 35–43), and one study (38) also examined the *MTHFR* A1298C polymorphism.

Three studies (22, 41, 43) were cross-sectional population-based studies, and seven studies (22, 35–42) used a case-control design. All case-control studies used clinician-diagnosed cases of major unipolar depression according to accepted criteria and unrelated control subjects. Three studies screened controls for either current or previous depression (36, 39, 42). One cross-sectional study (43) used patient self-report and current use of antidepressants as a method of case identification, in combination with self-report on a non-specific depression/anxiety question on the EuroQol instrument (44). All studies employed polymerase chain reaction methods in genotyping (see tables 1 and 2 for study details).

MTHFR C677T meta-analysis. Ten studies allowed us to make all planned comparisons of alleles and genotypes (following the inclusion of unpublished data provided by the authors of two studies (39, 41)). With our primary analysis, there was an increased risk of depression among persons with the homozygote variant TT genotype, with no discernible statistical between-study heterogeneity (fixed-effects odds ratio (OR) $OR_{TTvCC} = 1.36$, 95 percent confidence interval (CI): 1.11, 1.67; $I^2 = 0$ percent) (table 3 and figure 1). We found a smaller magnitude of association for heterozygotes that was of borderline significance and subject to greater between-study heterogeneity (fixed-effects $OR_{CTvCC} = 1.10$, 95 percent CI: 0.96, 1.25; $I^2 = 49$ percent). Depressed subjects showed a significantly increased frequency of the T allele (fixed-effects $OR_{TvC} = 1.14$, 95 percent CI: 1.04, 1.26; $I^2 = 0$ percent). A cumulative meta-analysis by year of publication showed that the association between depression and *MTHFR* C677T has remained significant over time, but the magnitude of association has been reduced with the emergence of more recent studies (figure 2).

Sensitivity analyses. Stratification by ethnic subtype showed that there was little variation between Asian and European populations and no reduction in overall heterogeneity (for European studies, $OR_{TTvCC} = 1.35$, 95 percent CI: 1.08, 1.68 ($I^2 = 0$ percent); for Asian studies, $OR_{TTvCC} = 1.43$, 95 percent CI: 0.84, 2.44 ($I^2 = 16$ percent) (table 3); logistic meta-regression beta coefficient = 0.076, $p = 0.81$). No studies were in Hardy-Weinberg disequilibrium. The one study which did not employ a standardized diagnostic interview or validated screening test (43) was also the largest and most influential study. The overall homozygote meta-analysis was robust to the inclusion and exclusion of this study (with the study, $OR_{TTvCC} = 1.36$, 95 percent CI: 1.11, 1.67; without the study, $OR_{TTvCC} = 1.37$, 95 percent CI: 1.03, 1.82). All meta-analysis results were stable when we adjusted for Hardy-Weinberg disequilibrium (table 3). Funnel plot analysis indicated no evidence of publication bias (table 3 and figure 3).

MTHFR A1298C polymorphism. One European study (38) reported a positive association with *MTHFR* A1298C when homozygote variants were compared with the homozygote wild type (CC vs. AA) ($OR_{CCvAA} = 3.25$, 95 percent CI: 1.19, 8.88). No association with depression was observed for heterozygotes compared with the homozygous wild-type group ($OR_{CCvAC} = 1.10$, 95 percent CI: 0.84, 1.43). This study was in Hardy-Weinberg disequilibrium ($p = 0.02$) (table 4).

Schizophrenia

Our searches identified 12 studies (6,125 participants; 2,762 cases of schizophrenia, 3,363 controls) meeting our

TABLE 1. Racial origin and case/control characteristics of participants in studies of methylenetetrahydrofolate reductase (*MTHFR*) genetic polymorphisms

First author and year (ref. no.)	Country	Racial descent	Cases	Controls/comparators
			Depression	
Tan 2004 (36)	Singapore (Chinese)	Asian	DSM-IV* (14) major depression. No other psychiatric comorbidity ($n = 88$).	Racially matched unrelated hospital employees with no psychiatric history ($n = 120$)
Chen 2005 (37)	Taiwan	Asian	DSM-IV diagnosis of major depression made by Geriatric Mental State—late onset (>60 years). No other psychiatric comorbidity ($n = 39$).	Age-matched unrelated controls ($n = 20$)
Reif 2005 (38)	Germany	European	Consecutive female inpatients with ICD-10* (8) major depression ($n = 45$)	Nonmatched healthy blood donors from the same region. Not screened for depression ($n = 284$).
Hickie 2001 (39)	Australia	European	DSM-IV major depression. Mean age 55 years ($n = 78$).	Nonmatched healthy unrelated controls recruited by newspaper and screened for current and previous depression ($n = 22$)
Arinami 1997 (40)	Japan	Asian	DSM-III* (68) depression. Persons with heart disease excluded. Mean age 54 years ($n = 32$).	Nonmatched unrelated controls. Mean age 49 years. Screening for previous history unclear ($n = 419$).
Kunugi 1998 (35)	Japan	Asian	DSM-IV major depression diagnosed by two psychiatrists. Mean age 55 years ($n = 71$).	Nonmatched healthy controls. Not formally screened for psychiatric disorder. Mean age 31 years ($n = 258$).
Almeida 2005 (41)	Australia	European	Beck Depression Inventory score $\geq$13 ($n = 42$)	Nonmatched unrelated controls with Beck Depression Inventory score <13. No screening for previous history ($n = 198$).
Bjelland 2003 (22)	Norway	European	HADS* depression score $\geq$8 ($n = 243$)	Nonmatched unrelated controls with HADS depression score <8. No screening for previous history ($n = 4{,}806$).
Kelly 2004 (42)	Northern Ireland	European	ICD-10 depression diagnosed by clinician. Mean age 48 years ($n = 100$).	Age- and sex-matched unrelated controls screened for previous and current depression ($n = 89$)
Lewis 2006 (43)	England	European	Women with self-reported history of ever having been diagnosed with depression ($n = 545$)	Women with no self-report of depression ($n = 2{,}942$)
			Schizophrenia	
Muntjewerff 2003 (45)	Netherlands	European	DSM-IV schizophrenia diagnosed by clinician. Males and females. Mean age 39 years ($n = 35$).	Unrelated healthy hospital employee controls. Mean age 36 years ($n = 104$).
Tan 2004 (36)	Singapore (Chinese)	Asian	DSM-IV schizophrenia diagnosed by clinician. No other psychiatric comorbidity ($n = 236$).	Racially matched unrelated hospital employees with no psychiatric history ($n = 120$)
Joober 2000 (46)	Canada	European	DSM-IV schizophrenia diagnosed by clinician. No other psychiatric comorbidity ($n = 90$).	Racially matched unrelated controls with no psychiatric history ($n = 105$)

Table continues

inclusion criteria. All of the studies examined the *MTHFR* C677T polymorphism (35, 36, 40, 45–52), and two studies also examined the *MTHFR* A1298C polymorphism (47, 48).

All studies used a case-control design, with standardized clinician-diagnosed cases of schizophrenia and unrelated control subjects. In three studies, investigators took explicit steps to match participants on the basis of age, sex, and race (36, 47, 48). These investigators also took explicit steps to exclude current or previous history of schizophrenia, in two cases verifying this over three generations (47, 48). Participation rates among cases and controls were not reported in any detail. Polymerase chain reaction methods were used in all genotyping (see tables 1 and 2 for study details).

MTHFR C677T meta-analysis. Twelve studies allowed us to make all planned comparisons of alleles and genotypes. With our primary analysis, there was an increased risk of schizophrenia among homozygote variants (TT), with low statistical between-study heterogeneity (fixed-effects $OR_{TTvCC} = 1.44$, 95 percent CI: 1.21, 1.70; $I^2 = 42$ percent) (table 3 and figure 1). A nonsignificant association was observed for heterozygotes (fixed-effects $OR_{CTvCC} = 1.07$, 95 percent CI: 0.96, 1.20; $I^2 = 41$ percent). Subjects with schizophrenia showed a significantly increased frequency of the T allele (fixed-effects $OR_{TvC} = 1.17$, 95 percent CI: 1.08, 1.26; $I^2 = 46$ percent). A cumulative meta-analysis showed that a moderate and significant association between

TABLE 1. Continued

First author and year (ref. no.)	Country	Racial descent	Cases	Controls/comparators
Sazci 2003 (47)	Turkey	European	DSM-IV schizophrenia diagnosed by clinician ($n = 130$)	Age-, sex-, and racially matched unrelated healthy controls with no history of schizophrenia over three generations ($n = 226$)
Sazci 2005 (48)	Turkey	European	DSM-IV schizophrenia diagnosed by clinician ($n = 297$)	Age-, sex-, and racially matched unrelated healthy controls ($n = 341$)
Virgos 1999 (49)	Spain	European	DSM-IV schizophrenia diagnosed by clinician ($n = 210$)	Nonmatched healthy controls ($n = 218$)
Muntjewerff 2005 (50)	Netherlands	European	DSM-IV schizophrenia diagnosed by clinician. Males and females. Mean age 39 years ($n = 254$).	Unrelated healthy hospital employee controls. Mean age 36 years ($n = 414$).
Yu 2004 (51)	China	Asian	DSM-IV schizophrenia diagnosed by clinician ($n = 230$)	Nonmatched healthy controls ($n = 251$)
Yu 2004 (51)	Scotland	European	DSM-IV schizophrenia diagnosed by clinician ($n = 426$)	Nonmatched healthy controls ($n = 628$)
Kunugi 1998 (35)	Japan	Asian	DSM-IV schizophrenia diagnosed by two psychiatrists. Mean age 48 years ($n = 343$).	Nonmatched healthy controls. Not formally screened for psychiatric disorder. Mean age 31 years ($n = 258$).
Arinami 1997 (40)	Japan	Asian	DSM-III schizophrenia. Persons with heart disease excluded. Mean age 45 years ($n = 297$).	Nonmatched unrelated controls. Mean age 49 years. Screening for previous history unclear ($n = 419$).
Kempisty 2006 (52)	Poland	European	DSM-IV schizophrenia diagnosed by two psychiatrists. Mean ages 30 years and 33 years in males and females, respectively ($n = 200$).	Nonmatched unrelated controls (blood donors). Mean ages 45 years and 40 years in males and females, respectively. No screening for psychiatric disorder ($n = 300$).
			Bipolar disorder	
Kunugi 1998 (35)	Japan	Asian	DSM-IV bipolar disorder diagnosed by two psychiatrists. Mean age 48 years ($n = 143$).	Nonmatched healthy controls. Not formally screened for psychiatric disorder. Mean age 31 years ($n = 258$).
Arinami 1997 (40)	Japan	Asian	DSM-III bipolar disorder. Persons with heart disease excluded. Mean age 54 years ($n = 40$).	Nonmatched unrelated controls. Mean age 49 years. Screening for previous history unclear ($n = 419$).
Tan 2004 (36)	Singapore (Chinese)	Asian	DSM-IV bipolar disorder diagnosed by clinician. No psychiatric comorbidity ($n = 167$).	Racially matched unrelated hospital employees with no psychiatric history ($n = 120$)
Kempisty 2006 (52)	Poland	European	DSM-IV bipolar disorder diagnosed by two psychiatrists. Mean ages 44 years and 46 years in males and females, respectively ($n = 200$).	Nonmatched unrelated controls (blood donors). Mean ages 45 years and 40 years in males and females, respectively. No screening for psychiatric disorder ($n = 300$).
			Anxiety	
Bjelland 2003 (22)	Norway	European	HADS anxiety score ≥ 8 ($n = 621$)	Nonmatched unrelated controls with HADS anxiety score <8. No screening for previous history ($n = 6{,}185$).

* DSM-IV, *Diagnostic and Statistical Manual of Mental Disorders*, Fourth Edition; ICD-10, *International Classification of Diseases*, Tenth Revision; DSM-III, *Diagnostic and Statistical Manual of Mental Disorders*, Third Edition; HADS, Hospital Anxiety and Depression Scale.

schizophrenia and *MTHFR* C677T has remained over time (figure 4).

Sensitivity analyses. Stratification by ethnic subtype showed that there was little variation between Asian and European pooled populations (for European populations, $OR_{TTvCC} = 1.46$, 95 percent CI: 1.18, 1.82 ($I^2 = 53$ percent); for Asian populations, $OR_{TTvCC} = 1.40$, 95 percent CI: 1.07, 1.83 ($I^2 = 26$ percent) (table 3); logistic meta-regression beta coefficient = –0.02, $p = 0.94$). One study was in Hardy-Weinberg disequilibrium (45); removal of this study did not alter the overall result (for studies in Hardy-Weinberg equilibrium, $OR_{TTvCC} = 1.41$, 95 percent CI: 1.19, 1.67 ($I^2 = 41$ percent)). The overall meta-analysis results were stable when we adjusted for Hardy-Weinberg disequilibrium (table 3). There was no evidence of small-study bias or publication bias for any comparisons.

MTHFR A1298C meta-analysis. From two studies (47, 48), there was an increased risk of schizophrenia among persons with the homozygote variant CC genotype, with no between-study heterogeneity (fixed effects $OR_{CCvAA} = 1.64$, 95 percent CI: 1.05, 2.54; $I^2 = 0$ percent). This association was not found for heterozygotes when they were compared with the homozygous wild-type group (fixed-effects $OR_{ACvAA} = 1.10$, 95 percent CI: 0.84, 1.43; $I^2 = 0$ percent).

TABLE 2. Distribution of C677T genotypes in association studies of methylenetetrahydrofolate reductase (*MTHFR*) genetic polymorphisms and susceptibility to psychiatric disorders

First author and year (ref. no.)	CC				CT				TT				T allele				Hardy-Weinberg *p* value*
	No. of cases	%	No. of controls	%	No. of cases	%	No. of controls	%	No. of cases	%	No. of controls	%	No. of cases	%	No. of controls	%	
Depression																	
Tan 2004 (36)	49	55.7	80	66.1	34	38.6	34	28.1	5	5.7	7	5.8	44	25.0	47	19.6	0.16
Chen 2005 (37)	22	56.4	11	55.0	15	38.5	9	45.0	2	5.1	0	0.0	19	24.4	9	22.5	0.53
Reif 2005 (38)	23	50.0	75	42.6	17	37.0	80	45.5	6	13.0	21	11.9	29	31.5	122	34.7	1.00
Hickie 2001 (39)	33	44.0	12	54.5	33	44.0	9	40.9	9	12.0	1	4.5	5	34.0	1	25.0	1.00
Arinami 1997 (40)	9	28.1	154	36.8	14	43.8	214	51.1	9	28.1	51	12.2	32	50.0	316	37.7	0.10
Almeida 2005 (41)	13	31.0	85	42.9	26	61.9	87	43.9	3	7.1	26	13.1	32	38.1	139	35.1	0.64
Bjelland 2003 (22)	127	52.5	3,381	49.7	85	35.1	2,864	42.1	30	12.4	561	8.2	145	30.0	3,986	29.3	0.19
Kelly 2004 (42)	30	30.0	40	44.9	56	56.0	37	41.6	14	14.0	12	13.5	84	42.0	61	34.3	0.48
Kunugi 1998 (35)	30	42.3	95	36.8	31	43.7	129	50.0	10	14.1	34	13.2	51	35.9	197	38.2	0.43
Lewis 2006 (43)	221	40.6	1,344	45.7	251	46.1	1,269	43.1	73	13.4	329	11.2	397	36.4	1,927	32.7	0.26
Schizophrenia																	
Muntjewerff 2003 (45)	15	44.1	45	46.4	16	47.1	35	36.1	3	8.8	17	17.5	22	32.3	69	35.6	0.05
Tan 2004 (36)	136	57.6	80	66.1	84	35.6	34	28.1	16	6.8	7	5.8	116	24.6	48	19.8	0.25
Joober 2000 (46)	30	28.6	41	45.6	52	49.5	36	40.0	23	21.9	13	14.4	98	46.7	62	34.4	0.35
Sazci 2003 (47)	59	45.4	106	46.9	49	37.7	103	45.6	22	16.9	17	7.5	93	35.8	137	30.3	0.27
Sazci 2005 (48)	144	48.5	161	47.2	115	38.7	156	45.7	38	12.8	24	7.0	191	32.2	204	29.9	0.47
Virgos 1999 (49)	81	38.6	79	36.2	98	46.7	106	48.6	31	14.8	33	15.1	160	38.0	172	39.4	0.89
Muntjewerff 2005 (50)	112	44.1	212	51.2	111	43.7	166	40.1	31	12.2	36	8.7	173	34.1	238	28.7	0.72
Yu 2004 (51)	199	46.7	306	48.7	186	43.7	260	41.4	41	9.6	62	9.9	268	31.5	384	30.6	0.57
Yu 2004 (51)	91	39.6	85	33.9	96	41.7	126	50.2	43	18.7	40	15.9	182	39.6	206	41.0	0.60
Kunugi 1998 (35)	121	35.3	95	36.8	168	49.0	129	50.0	54	15.7	34	13.2	276	40.2	197	38.2	0.43
Arinami 1997 (40)	96	32.3	154	36.8	138	46.5	214	51.1	63	21.2	51	12.2	264	44.4	316	37.7	0.10
Kempisty 2006 (52)	113	56.5	210	70.0	68	34.0	79	26.3	19	9.5	11	3.7	106	26.5	101	16.8	0.30
Bipolar disorder																	
Kunugi 1998 (35)	41	28.7	95	36.8	74	51.7	129	50.0	28	19.6	34	13.2	130	45.5	197	38.2	0.43
Arinami 1997 (40)	15	37.5	154	36.8	20	50.0	214	51.1	5	12.5	51	12.2	30	37.5	316	31.7	0.10
Tan 2004 (36)	99	59.3	80	66.1	60	35.9	34	28.1	8	4.8	7	5.8	76	22.7	48	19.8	0.25
Kempisty 2006 (52)	108	54.0	210	70.0	73	36.5	79	26.3	19	9.5	11	3.7	111	27.5	101	16.8	0.30
Anxiety																	
Bjelland 2003 (22)	308	49.6	3,073	49.7	263	42.4	2,601	42.1	50	8.1	511	8.3	363	29.2	3,623	29.3	0.19

* *p* value from test for Hardy-Weinberg equilibrium.

TABLE 3. Summary odd ratios from meta-analysis of the methylenetetrahydrofolate reductase (*MTHFR*) C677T polymorphism and risk of psychiatric disorders

Comparison and racial descent	No. of studies	Total sample size	No. of cases	No. of controls	OR_{fixed}*	95% CI*	I^2 (%)	OR_{random}	95% CI	OR_{fixed} adjusted for Hardy-Weinberg equilibrium	95% CI	Small-study bias (31) *p* value
						Depression						
T versus C	10	11,709	1,280	10,429	1.14	1.04, 1.26	0	1.14	1.04, 1.26	1.15	1.04, 1.26	0.62
Asian	4	1,047	230	817	1.19	0.93, 1.52	22	1.21	0.90, 1.61	1.18	0.93, 1.51	
European	6	10,662	1,050	9,612	1.14	1.03, 1.26	0	1.14	1.03, 1.26	1.14	1.03, 1.26	
TT versus CC	10	11,709	1,280	10,429	1.36	1.11, 1.67	0	1.36	1.11, 1.67	1.37	1.12, 1.68	0.79
Asian	4	1,047	230	817	1.43	0.84, 2.44	16	1.48	0.80, 2.75	1.38	0.81, 2.36	
European	6	10,662	1,050	9,612	1.35	1.08, 1.68	0	1.35	1.08, 1.68	1.37	1.10, 1.71	
CT versus CC	10	11,709	1,280	10,429	1.10	0.96, 1.25	49	1.13	0.89, 1.43	1.09	0.95, 1.24	0.73
Asian	4	1,047	230	817	1.08	0.76, 1.53	21	1.08	0.72, 1.62	1.13	0.79, 1.60	
European	6	10,662	1,050	9,612	1.10	0.95, 1.27	64	1.16	0.84, 1.60	1.08	0.93, 1.25	
						Schizophrenia						
T versus C	12	6,125	2,762	3,363	1.17	1.08, 1.26	46	1.18	1.06, 1.32	1.17	1.08, 1.26	0.47
Asian	4	2,155	1,106	1,049	1.15	1.01, 1.31	35	1.15	0.98, 1.35	1.15	1.01, 1.31	
European	8	3,970	1,656	2,314	1.18	1.07, 1.30	57	1.21	1.04, 1.41	1.18	1.07, 1.30	
TT versus CC	12	6,125	2,762	3,363	1.44	1.21, 1.70	42	1.47	1.16, 1.86	1.41	1.19, 1.67	0.98
Asian	4	2,155	1,106	1,049	1.40	1.07, 1.83	26	1.38	1.00, 1.91	1.35	1.04, 1.76	
European	8	3,970	1,656	2,314	1.46	1.18, 1.82	53	1.54	1.10, 2.16	1.45	1.17, 1.80	
CT versus CC	12	6,125	2,762	3,363	1.07	0.96, 1.20	41	1.08	0.93, 1.26	1.09	0.98, 1.22	0.36
Asian	4	2,155	1,106	1,049	1.00	0.83, 1.21	42	1.00	0.78, 1.29	1.07	0.88, 1.29	
European	8	3,970	1,656	2,314	1.11	0.97, 1.27	44	1.13	0.93, 1.37	1.11	0.96, 1.27	
						Bipolar disorder						
T versus C	4	1,648	550	1,098	1.41	1.19, 1.68	54	1.37	1.05, 1.78	1.41	1.18, 1.68	NA*
Asian	3	1,148	350	798	1.23	0.99, 1.51	0	1.23	0.99, 1.52	1.23	0.99, 1.52	
European	1	500	200	300	1.90	1.40, 2.58		1.90	1.40, 2.56	1.88	1.39, 2.56	
TT versus CC	4	1,648	550	1,098	1.82	1.22, 2.70	42	1.72	0.99, 3.01	1.90	1.27, 2.84	NA
Asian	3	1,148	350	798	1.44	0.90, 2.31	0	1.45	0.90, 2.33	1.46	0.90, 2.34	
European	1	500	200	300	3.36	1.54, 7.31		3.36	1.54, 7.31	4.07	1.78, 9.29	
CT versus CC	4	1,648	550	1,098	1.45	1.14, 1.86	0	1.46	1.14, 1.86	1.44	1.13, 1.83	NA
Asian	3	1,148	350	798	1.28	0.94, 1.74	0	1.28	0.94, 1.74	1.31	0.96, 1.78	
European	1	500	200	300	1.80	1.21, 2.66		1.80	1.21, 2.66	1.67	1.13, 2.47	
						Anxiety disorders						
T versus C	1	6,806	621	6,185	1.00	0.88, 1.13		1.00	0.88, 1.13	1.00	0.88, 1.13	NA
TT versus CC	1	6,806	621	6,185	0.98	0.71, 1.34		0.98	0.71, 1.34	0.95	0.69, 1.29	NA
CT versus CC	1	6,806	621	6,185	1.01	0.85, 1.20		1.01	0.85, 1.20	1.03	0.87, 1.23	NA

* OR, odds ratio; CI, confidence interval; NA, not applicable.

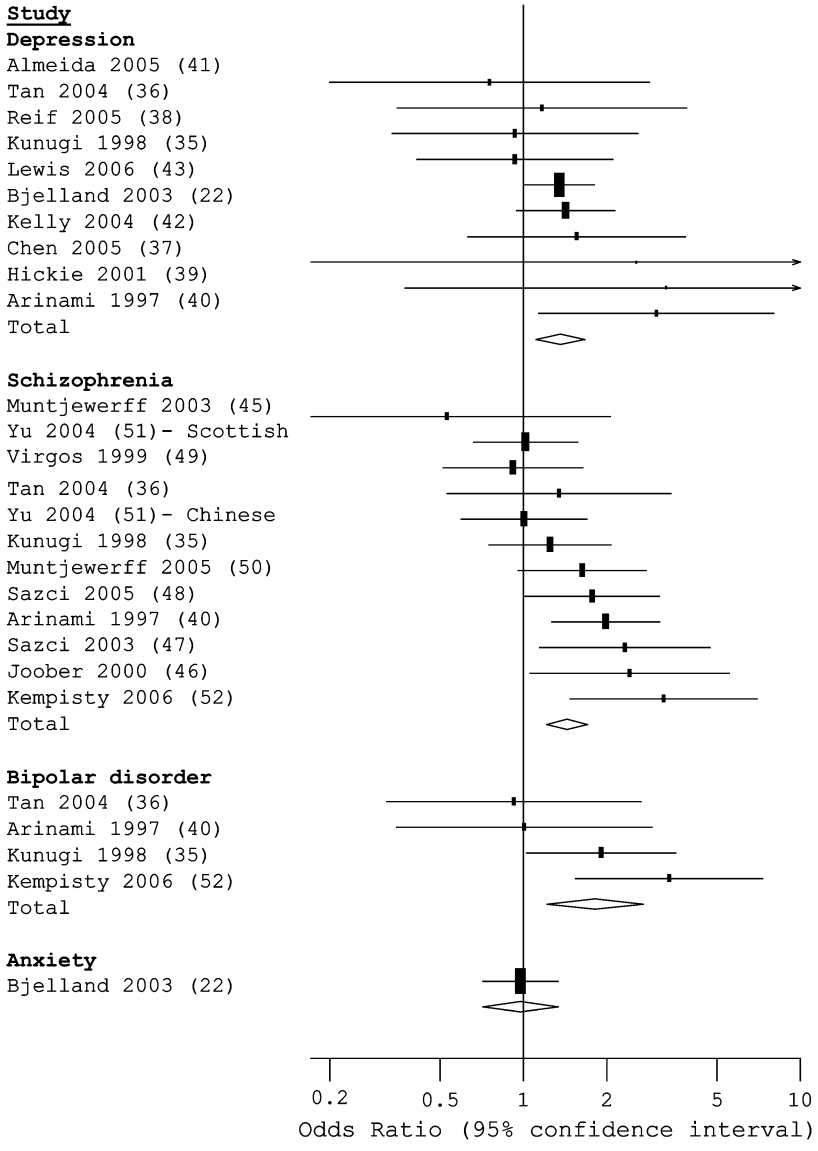

FIGURE 1. Results from fixed-effects meta-analysis of homozygous (TT vs. CC) genotypes of the methylenetetrahydrofolate reductase (*MTHFR*) C677T polymorphism and psychiatric disorders. Reference numbers are given in parentheses. For study details and locations, see tables 1 and 2.

Bipolar disorder

Our searches identified four studies of the *MTHFR* C677T polymorphism and bipolar disorder (35, 36, 40, 52) (1,648 participants; 550 cases of bipolar disorder, 1,098 controls). All studies used a case-control design, with clinician-diagnosed cases of bipolar disorder and unrelated control subjects. Tan et al. (36) reported taking explicit steps to provide racial matching and to exclude current or previous history of bipolar disorder. All studies employed polymerase chain reaction methods in genotyping (tables 1 and 2).

MTHFR C677T meta-analysis. There was an increased risk of bipolar disorder associated with the homozygote variant genotype (fixed-effects $OR_{TTvCC} = 1.82$, 95 percent CI: 1.22, 2.70; $I^2 = 42$ percent) (table 4 and figure 1). The frequency of the T allele was increased among persons with bipolar disorder (fixed-effects $OR_{TvC} = 1.41$, 95 percent CI: 1.19, 1.68; $I^2 = 54$ percent) (table 3).

Sensitivity analyses. All studies but one evaluated persons of Asian origin, and all showed a positive trend. No studies were in Hardy-Weinberg disequilibrium. The overall meta-analysis results were stable when adjusted for Hardy-Weinberg disequilibrium. There were insufficient studies to construct funnel plots, and the presence of publication bias could not be excluded (table 3).

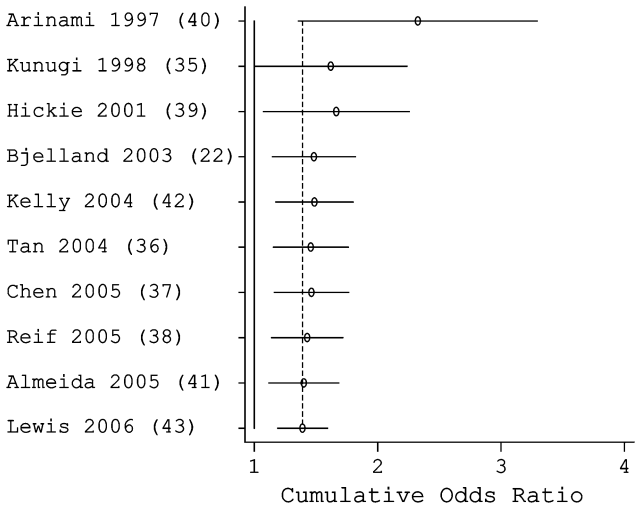

FIGURE 2. Results from cumulative fixed-effects meta-analysis of homozygous (TT vs. CC) genotypes of the methylenetetrahydrofolate reductase (*MTHFR*) C677T polymorphism in depressed and nondepressed populations, by year of publication. Reference numbers are given in parentheses. For study details and locations, see tables 1 and 2.

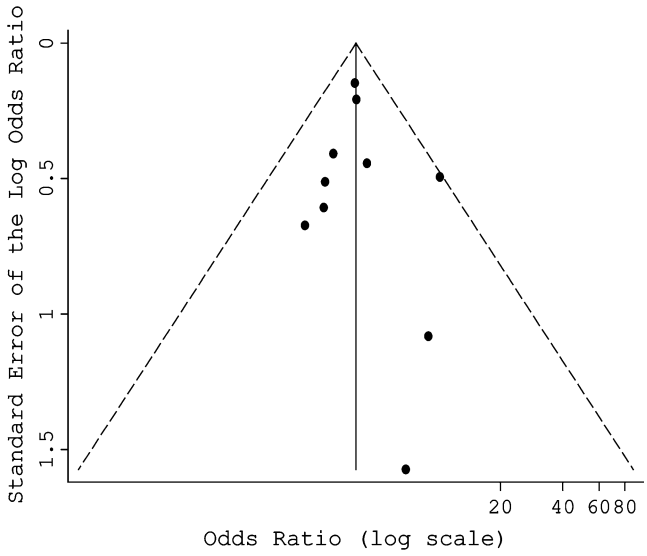

FIGURE 3. Funnel plot of effect size versus inverse standard error for homozygous (TT vs. CC) genotypes of the methylenetetrahydrofolate reductase (*MTHFR*) C677T polymorphism in depressed and control populations.

Anxiety disorders

One large ($n = 6{,}806$) population-based cross-sectional study (22) examined the risk of anxiety, using a validated self-report questionnaire. No association between *MTHFR* C677T and anxiety disorders was found ($OR_{TTvCC} = 0.98$, 95 percent CI: 0.71, 1.34) (tables 1 and 3).

DISCUSSION

Main findings

To our knowledge, this is the first general overview of associations between *MTHFR* polymorphisms and psychiatric disorders. This is a rapidly evolving area of interest, and our meta-analysis represents the most up-to-date quantitative synthesis of available results from gene-psychiatric disorder association studies. In the future, this review will be updated as a mini-HuGE review in line with emerging evidence.

Our main finding was that the *MTHFR* C677T polymorphism is associated with major depression (OR = 1.36), schizophrenia (OR = 1.44), and bipolar disorder (OR = 1.82). The magnitudes of the associations were moderate but statistically significant. Emerging evidence of an association with the *MTHFR* A1298C polymorphism was also found. However, in line with other HuGE reviews, associations with this variant have been less extensively researched. Further research on disease associations with this *MTHFR* polymorphism is needed.

Our findings were based upon several gene-association studies and several thousand participants and were robust to each of the planned sensitivity analyses used. There was

TABLE 4. Distribution of A1298C genotypes in association studies of methylenetetrahydrofolate reductase (*MTHFR*) genetic polymorphisms and susceptibility to psychiatric disorders

First author and year (ref. no.)	CC				CT				TT				T allele				Hardy-Weinberg *p* value*
	No. of cases	%	No. of controls	%	No. of cases	%	No. of controls	%	No. of cases	%	No. of controls	%	No. of cases	%	No. of controls	%	
Depression																	
Reif 2005 (38)	16	34.8	75	40.8	21	45.7	96	52.2	9	19.6	13	7.1	39	54.9	122	44.8	0.02
Schizophrenia																	
Sazci 2003 (47)	57	43.8	114	50.4	59	45.4	93	41.2	14	10.8	19	8.4	87	43.3	131	36.5	1.00
Sazci 2005 (48)	130	43.8	159	46.6	129	43.4	155	45.5	38	12.8	27	7.9	205	44.1	209	39.7	0.25

* *p* value from test for Hardy-Weinberg equilibrium.

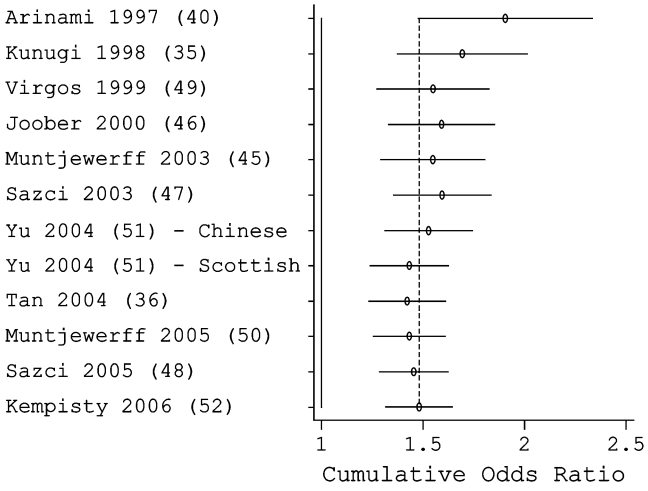

FIGURE 4. Results from cumulative fixed-effects meta-analysis of homozygous (TT vs. CC) genotypes of the methylenetetrahydrofolate reductase (*MTHFR*) C677T polymorphism in persons with schizophrenia and control populations, by year of publication. Reference numbers are given in parentheses. For study details and locations, see tables 1 and 2.

no evidence of publication bias, and we found low between-study heterogeneity. When we examined Hardy-Weinberg equilibrium, the majority of studies showed no deviation, and the overall results were robust to the exclusion of disequilibrium studies and statistical adjustment for control-group disequilibrium, using a recently introduced test (32). Of particular interest was the finding that our results were consistent across different racial groups, irrespective of allele frequency—a finding that is in line with those for other gene-disease associations (53).

Limitations of the epidemiologic evidence

When we examined the design and methodological aspects of these studies, we found that the majority employed a case-control design. Few investigators took explicit steps to provide racial matching or to exclude participants with a previous, current, or family history of the disorder. Participation rates for cases and controls were rarely reported. However, nonparticipation and selection bias are much less of a problem in gene-association studies than in classical epidemiologic designs, since nonparticipation is rarely related to genotype (54). Among large-scale population-level studies, cases were necessarily less rigorously defined. When we took steps to examine the robustness of our overall results to the inclusion and exclusion of these studies, we found that all significant gene-disease associations remained.

Possible mechanisms of gene-disorder association

Folate and *MTHFR* polymorphisms are related to neural tube defects (55) and have now been implicated in the pathogenesis of several diseases and disorders, including leukemia (6), colorectal cancer (7), cardiovascular disease (56), and other congenital abnormalities (5).

The 1-carbon cycle/folate metabolic pathway is complex and regulates not only nucleotide synthesis but also DNA methylation. 5-Methyltetrahydrofolate is the predominant circulating form of folate, and it donates a methyl group to homocysteine in the generation of *S*-adenosylmethionine, a major source of methyl groups in the brain (1). MTHFR is a critical component of the 1-carbon cycle, and the *MTHFR* polymorphisms C677T and A1298C affect both nucleotide synthesis and DNA methylation (57). This forms a plausible biologic explanation for potential associations between genetic variation in folate metabolism and both depression and schizophrenia (16). The *MTHFR* C677T polymorphism is associated with a reduction in the bioavailability of folate and folate metabolites and ''mimics'' low dietary folate intake (3).

Depression in association with reduced folate has been demonstrated within observational epidemiologic studies (22), but a causal link has been difficult to establish because of confounding and reverse causality (58). Estimates of association between low folate and depression range from an odds ratio of 12 in case-control studies measuring serum folate levels (59) to an odds ratio of 1.6 in a large cross-sectional study estimating dietary folate intake from food diaries (60). In contrast to traditional epidemiologic approaches, the choice of bioassay method, reverse causality, and confounding of genotype-depression associations are unlikely to influence the association between *MTHFR* polymorphisms and functional psychiatric disorders (54).

A possible gene-environment association between impaired folate metabolism and dietary folate might also exist, although we did not explicitly address this question. Three studies within this review (22, 37, 41) measured both *MTHFR* polymorphisms and folate levels. In case-control studies (37, 41), the results were inconclusive. In one cross-sectional study (22), the authors reported on the association of folate levels with *MTHFR* polymorphisms but did not explicitly examine gene-environment interaction. Similarly, there are several possible genes which also work in the folate/1-carbon pathway and which might interact with *MTHFR* polymorphisms (57). We did not set out to examine these interactions, nor did we find any studies which did so. Gene-environment and gene-gene interactions require examination in more detail; future updates of this HuGE review will explicitly address these issues.

Schizophrenia is increasingly considered to be a neurodevelopmental disorder (61), with in-utero exposures and epigenetic mechanisms such as DNA methylation being important in its etiology (62, 63). DNA methylation is a critical epigenetic modification of the genome that controls many biologic processes, including embryonic development, X-chromosome inactivation, imprinting, and gene expression. Incorrect methylation patterns can affect embryogenesis, leading to developmental malformations and embryonic death. Although these patterns are established during early life, they are not fixed, and gradual hypomethylation of the genome occurs in most tissues with age, together with aberrant hypermethylation of gene promoter regions. Thus, the correct development of DNA methylation patterns is

important not only for early life but also for long-term health benefits, including neurologic disease susceptibility.

Methylation is genetically predetermined, either by imprinting or by inheritance of genes which influence methylation, such as *MTHFR* and other genes involved in the 1-carbon cycle (64). Methyl groups required for methylation are synthesized de novo or are supplied in the diet, primarily from folate. Thus, methylation may be modified by gene-exposure interactions occurring during development. This link between folate, folate metabolism, and DNA methylation therefore provides a plausible biologic mechanism for the observed association between *MTHFR* and schizophrenia.

Recently, Coppen and Bolander-Gouaille (16) recommended the use of folate in the treatment and prevention of depression as a population-level strategy. The results of the present review also raise the possibility of folate supplementation's playing a protective role in the development of schizophrenia, when taken during pregnancy. Positive results of population-level folate fortification strategies in preventing neural tube defects have been found (65), and studies examining the potential of folate in reducing schizophrenia risk are required. Mandatory food fortification is used in several countries (including the United States, Canada, and Australia) (66, 67). The cost-effectiveness and safety of this approach might be additionally supported by the demonstration of a further gene-environment association for folate, folate metabolism, and depression.

ACKNOWLEDGMENTS

Conflict of interest: none declared.

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