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Genetic polymorphisms of methylenetetrahydrofolate reductase (MTHFR) gene and susceptibility to depression in Asian population: a systematic meta-analysis

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Abstract

Genetic association studies on MTHFR C677 T polymorphism and depression have been repeatedly performed over the last two decades and results are generally inconsistent. The aim of the present study was to assess the risk of MTHFR C677T polymorphism for depression in Asian population. The author performed a meta-analysis and pooled data from individual case-control studies that examined the association between C677T polymorphism and depression (meta-analysis: 13 studies, 1,120 cases and 1,688 controls).The pooled Odd Ratios (OR) were estimated by both fixed effects and random effects models. Overall, there was an association between MTHFR C677T polymorphism and increased risk of depression under five genetic models (OR T vs. C=1.44, 95% CI= 1.56-1.78, P=0.001; OR TT vs. CC= 1.78, 95% CI 1.17– 2.69, P=0.006; OR CT vs CC=1.102, 95% CI=0.91-1.32,P=0.31; OR TT vs. CT+CC=1.73, 95% CI= 0.87-3.41, P=0.11; OR TT+CT vs. CC=1.26, 95% CI=0.96-1.64, P=0.08). Sensitivity analysis suggested exclusion of any single study did not alter the overall pooled Ors. In conclusion results of present meta-analysis supports that there is a significant association between MTHFR C677T polymorphism and depression risk, and MTHFR 677T allele contributes to increased risk of depression in Asian individuals.

Key words: Meta-analysis, Depression, MTHFR, C677T, Genotype, Polymorphism, Asian population.

Introduction

Neuropsychiatric diseases are complex genetic disorders and family, twin and adoption studies indicate that simple Mendelian genetics with one gene–one phenotype are not applicable to psychiatric diseases. Mood disorders are among the most prevalent forms of mental illness. Epidemiologic studies show that roughly 40%-50% of the risk for depression is genetic (1). This makes depression a highly heritable disorder, at least as heritable as several common complex medical conditions like-type II diabetes, hypertension, asthma etc. Although a possible role of nutritional factors in the pathogenesis of neuropsychiatric disorders has long been debated, clinical studies have shown an inverse relationship between folate status and depression (2-4). Such a relationship has been inferred from studies showing increased frequency of folate deficiency among depressed patients, more severe and prolonged (5) depressive episodes and weaker treatments with low folate status (5-7) and enhanced antidepressed response with folic acid supplementation (5,7,8).

The principal biochemical role of folate in mammals is in mediating the transfer of single-carbon molecules for various biological reactions (9). Folate plays an integral role in DNA synthesis and methylation, and as an epigenetic regulator of gene expression, DNA integrity and stability (9). The intracellular coenzymatic form of folate is 5, 10-methylenetetrahydrofolate, which is required for de novo purine synthesis (9) and also in the remethylation of homocysteine to methionine. Methionine is the precursor of S-adenoysylmethionine (SAM), which is the primary methyl group donor for most biological methylation reactions, including DNA methylation (10). Methylenetetrahydrofolate reductase (MTHFR) is one of the enzymes involved in this metabolic pathway. MTHFR catalyzes the irreversible conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate and directs the flux of intracellular folate toward the conversion of homocysteine to methionine at the expense of nucleotide synthesis (11). C677T (Ala222Val) is clinically important and common polymorphisms have been described for this enzyme (12). The MTHFR gene variants are reported to be associated with the risk of certain human diseases/ disorders, including cardiovascular disorders, cancers, neural tube defects, Down syndrome and pregnancy complications and psychiatric disorders etc. (12-20). There is marked variation in the frequency of C677T allele between populations (21-23). The highest frequency (>20%) is found among US Hispanics, Colombians and Amerindians in Brazil, conversely in Black populations, less than 2 percent have the variant genotype (24). Case–control studies that investigated the association between depression and the C677T polymorphismso far provided controversial or inconclusive results. Each study involved small numbers of cases and controls, and data interpretation was complicated by the fact that different populations and sampling strategies were used. To shed some light on these controversial results, as well as to decrease the uncertainty of the effect size of estimated risk, a meta-analysis of all available Asian studies relating the C677T polymorphism of the MTHFR gene to the risk of developing depression was carried out.

Methods

Study identification

A literature search for all studies reporting on the association between MTHFR C677T genotype and de-

Table 1. Characteristics of thirteen studies included in the present meta-analysis.

S. No.	Study	Country	Case	Control	Year	References
1	Arinami et al.,1997	Japan	32	419	1997	Am J Med Genet 74:526–528
2	Kunugi et al.,1998	Japan	71	258	1998	Mol Psychiatry 3:435–437.
3	Tan et al.,2004	Singapore	88	120	2004	Psychiatr Genet 14:227–231
4	Chen et al.,2005	China	39	20	2005	Am J Geriatr Psychiatry 13:872 -875
5	Yuan et al.,2007	China	60	71	2007	Chin J Geriatrics 26:767–9.
6	Yuan et al.,2008	China	116	80	2008	Acta Neuropsychiatrica 20: 251–255
7	Zhao et al.,2008	China	77	85	2008	Chin J Nerv Ment Dis 34:378–80.
8	Hong et al.,2009	China	178	85	2009	Am J Geriatr Psychiatry 17:847–55.
9	Kim et al.,2009	South Korea	63	458	2009	Psychosom. 71, 286–291
10	Yang et al.,2009	China	100	100	2009	Chin J Behav Med Brain Sci 18:398–400.
11	Cao et al.,2010	China	50	59	2010	Guangdong Med J 31:2946–9.
12	Feng et al.,2010	Chian	152	152	2010	Basic Clin Med 30: 811–4.
13	Quiao et al.,2012	China	94	98	2012	J Psychiatry 24:92–4.

Table 2. The distributions of MTHFR C677T genotypes and allele frequencies in depression cases and controls.

Study ID	Genotype						Alleles			
	CC		CT		TT		C		T	
	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control
Arinami,1997	9	154	14	214	9	51	32	522	32	316
Kunugi,1998	30	95	31	129	10	34	90	319	51	197
Tan,2004	49	80	34	33	5	7	132	193	44	47
Chen,2005	22	11	15	9	2	0	59	31	19	9
Yuan,2007	22	27	27	38	11	15	49	92	92	68
Yuan,2008	46	27	48	38	22	15	92	140	68	92
Zhao,2008	12	21	37	48	28	16	61	90	93	80
Hong,2009	75	32	84	44	19	9	234	108	122	62
Kim,2009	16	84	28	248	19	126	69	416	66	500
Yang,2009	33	52	50	40	17	8	116	144	84	58
Cao,2010	9	24	23	27	18	8	41	75	59	43
Feng,2010	32	51	66	81	54	20	130	183	174	121
Quiao,2012	24	36	43	45	27	17	91	117	97	79

pression was conducted using the electronic databases PubMed, Google Scholar and Springer link up to October, 2013. The search strategy included the keywords ‘‘depression’’, ‘‘polymorphism’’, “methylenetetrahydrofolate reductase” and ‘‘MTHFR’’. Eligible studies had to meet all of the following criteria: (1) published in a peer-reviewed journal, (2) contained independent data, (3) presented sufficient data to calculate the odds ratio (OR) with a confidence interval and a P-value, (4) were association studies, (5) described the relevant genotyping protocols, (6) they properly diagnosed patients according to DSM-IV criteria and (7) they used healthy individuals as controls.

Data extraction

Following informations were extracted from each study- first authors name, journal name, year of publication, country name, number of cases and controls. When a study reported results on different subpopulations according to ethnicity, author considered each subpopulation as a separate study in the meta-analysis.

Meta-analysis

The meta-analysis examined the overall association of the 677T allele with the risk of depression relative to allele C. The associations were indicated as odds ratios (OR) with the corresponding 95% confidence interval (CI). Then, based on the individual OR, a pooled OR was estimated. Heterogeneity between studies was tested using the Q-statistic, which is a weighted sum of squares of the deviations of individual study OR estimates from the overall (pooled) estimate (25) and quantified with the I2 metric. I2 takes values between 0 and 100% with higher values denoting greater degree of heterogeneity (26,27). The pooled OR was estimated using fixed effects (FE) (28) and random effects (29) models. Random effects modeling assume a genuine diversity in the results of various studies, and it incorporates to the calculations a between-study variance. Hence, when there is heterogeneity between studies, then the pooled OR is preferably estimated using the random effects model.

Publication bias

Publication bias was investigated with the funnel plot. Funnel plot asymmetry was further assessed by the method of Egger’s linear regression test (30). All analyses were performed using the computer program MIX version 1.7 (31). A p value less than 0.05 was considered statistically significant, and all the p values were two sided.

Results

Characteristics of included studies

Thirteen studies, summarized in Table 1, reported the association of SNP C677T polymorphism in the MTHFR gene with depression and included in the present meta-analysis (32-44). The studies were carried out in Japan (32,33), Singapore (34), Taiwan (35), China (36-39,41-44), and Korea (40). In all thirteen studies, total cases were 1120 with CC (379), CT (500) and

TT (241), and controls were 1688 with CC (694), CT (994), and TT (326). In controls genotypes percentage of CC, CT and TT were 41.11%, 58.88% and 19.31% respectively. In total cases genotype percentage of CC, CT, and TT was 33.84%, 44.64% and 21.52 % respectively. Frequency of CC genotype was highest in both cases and controls (Table 2). Except three studies (33,39,40), OR was above one in all included studies. Author has assessed whether the frequencies of CC, CT and TT genotypes among controls in individual studies were consistent with the expected distribution (that is in Hardy-Weinberg equilibrium) by using the X2 test. Genotypes were in Hardy-Weinberg equilibrium in all controls.

Meta-analysis

The main analysis for investigating the association of the C677T allele T and the risk of developing depression relative to the allele C showed significant heterogeneity (Phet=0.0001, I2= 68.68%) between the 13 studies; the fixed and random effects pooled OR were significant (ORFE=1.4, 95% CI=1.25-1.58, p<0.0001; OR RE=1.44, 95%CI=1.56-1.78, p=0.001) (Table3; Figure 1). The genotype differences for the homozygote (TT vs. CC) revealed significant heterogeneity (Phet= 0.02; I2= 60.83%) and a significant association in both fixed and random effects pooled OR (ORFE = 1.75, 95% CI= 1.37-2.23, p<0.0001; ORRE= 1.79, 95%CI= 1.17-2.69, p=0.006) (Figure 2). The recessive model for allele T (TT vs CT+CC) produced the same pattern of genotypic association as found for the homozygote frequencies, and found significant heterogeneity, (Phet<0.0001; I2=90.38), overall fixed effects OR=1.19(95%CI=1.00-1.43;p=0.04) and random effect OR= 1.73 (95%CI=0.87-3.41; p=0). The dominant model for the effect of T allele in the main analysis

Table 3. Summary estimates for the odds ratio (OR) of MTHFR C677T in various allele/genotype contrasts, the significance level (p value) of heterogeneity test (Q test), and the I^2 metric and publication bias p-value (Egger Test).

Genetic Models	Fixed effect OR (95% CI), p	Random effect OR (95% CI), p	Heterogeneity p-value (Q test)	I^2 (%)	Publication Bias (p of Egger's test)
Allele Contrast (T vs C)	1.41(1.251-1.585),<0.0001	1.44(1.56-1.783),0.001	0.0001	68.68	0.57
Co-dominant (Ct vs CC)	1.10(0.92-1.326), 0.3013	1.10 (0.878-1.381),0.4025	0.14	30.16	0.73
Homozygoote (TT vs CC)	1.75(1.372-2.235),<0.0001	1.78(1.174-2.692),0 006	0.002	60.83	0.74
Dominant (TT+CT vs CC)	1.25(1.052-1.490),0.011	1.26(0.969-1.640), 0.084	0.01	53.45	0.57
Recessive (TT vs CT+CC)	1.19(1.00-1.431),0.049	1.73(0.871-3.416),0.117	<0.001	90.38	0.25

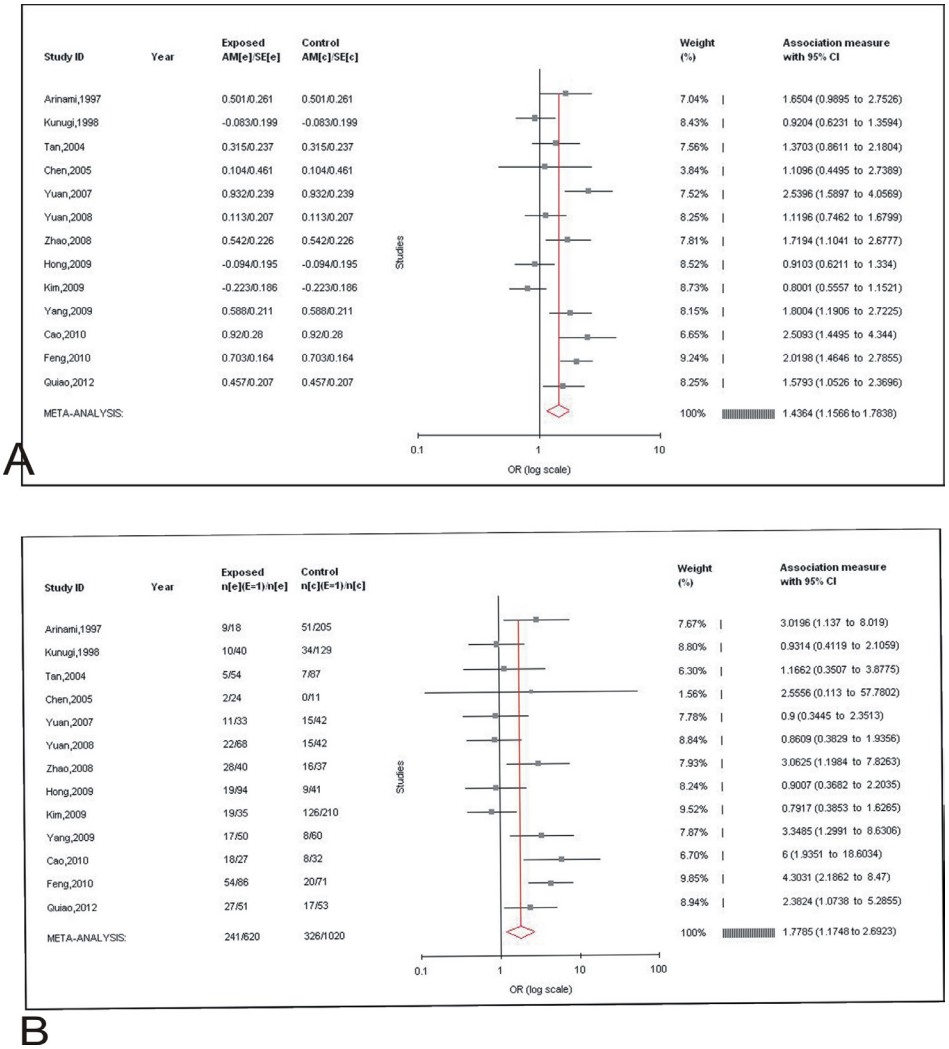
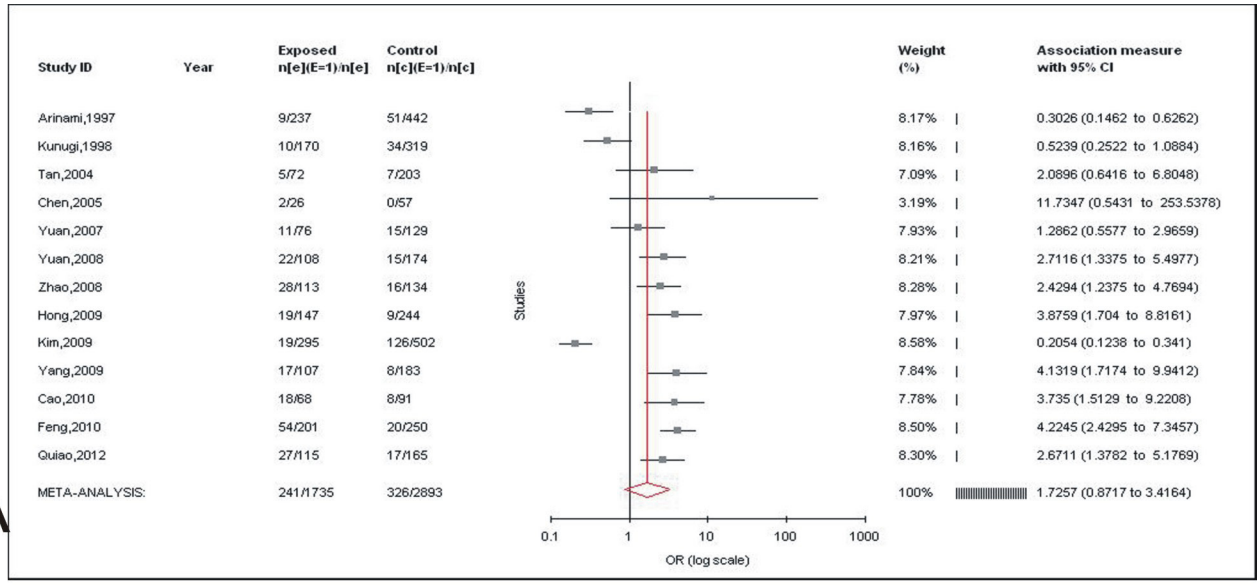
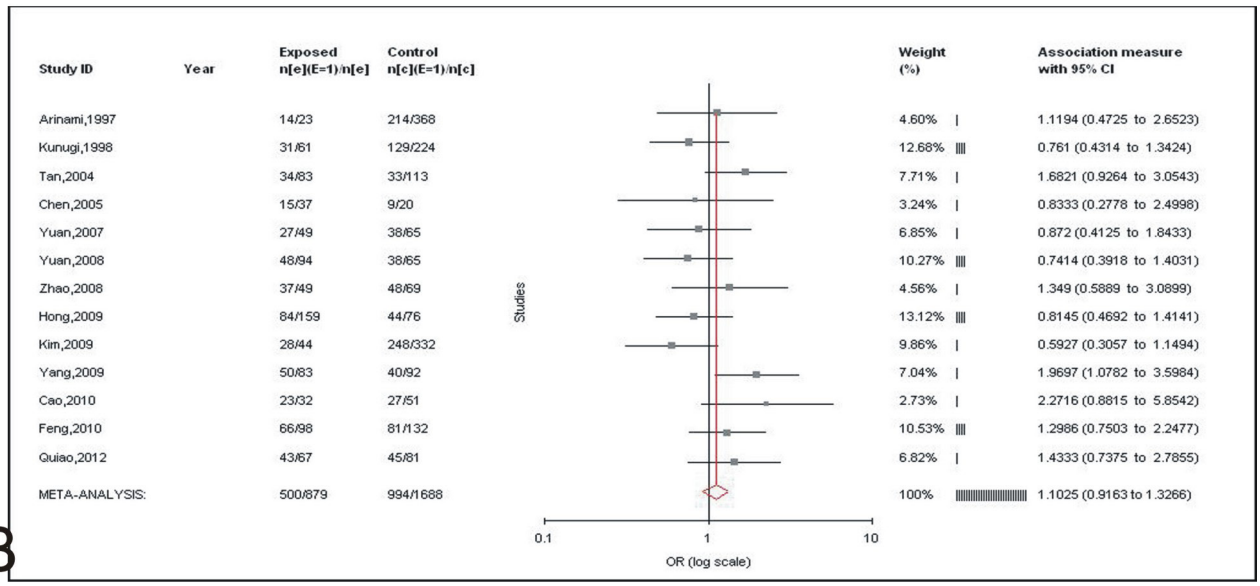


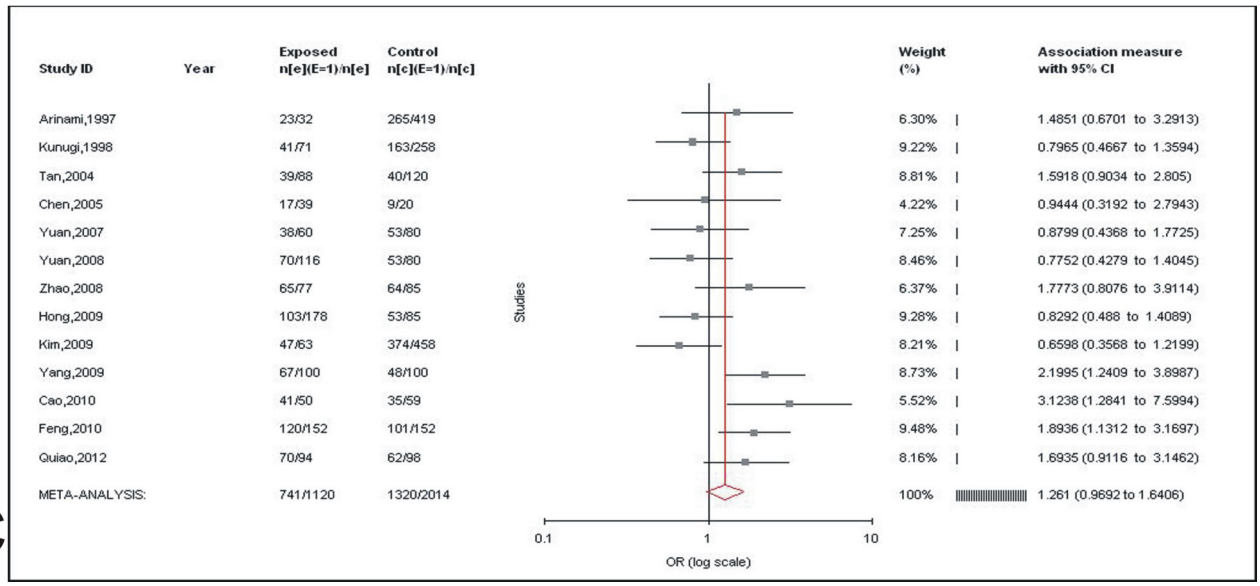
Figure 1. A. Forest plots for the association between MTHFR C677T polymorphism and depression for additive model (T vs C) with fixed effect model, **B.** for homozygote model (TT vs. CC).



A



B



C

Figure 2. A. Forest plots for the association between MTHFR C677T polymorphism and depression for dominant model (TT+CT vs. CC), **B.** co-dominant model (CT vs. CC) and **C.** recessive model (TT vs. CT+TT).

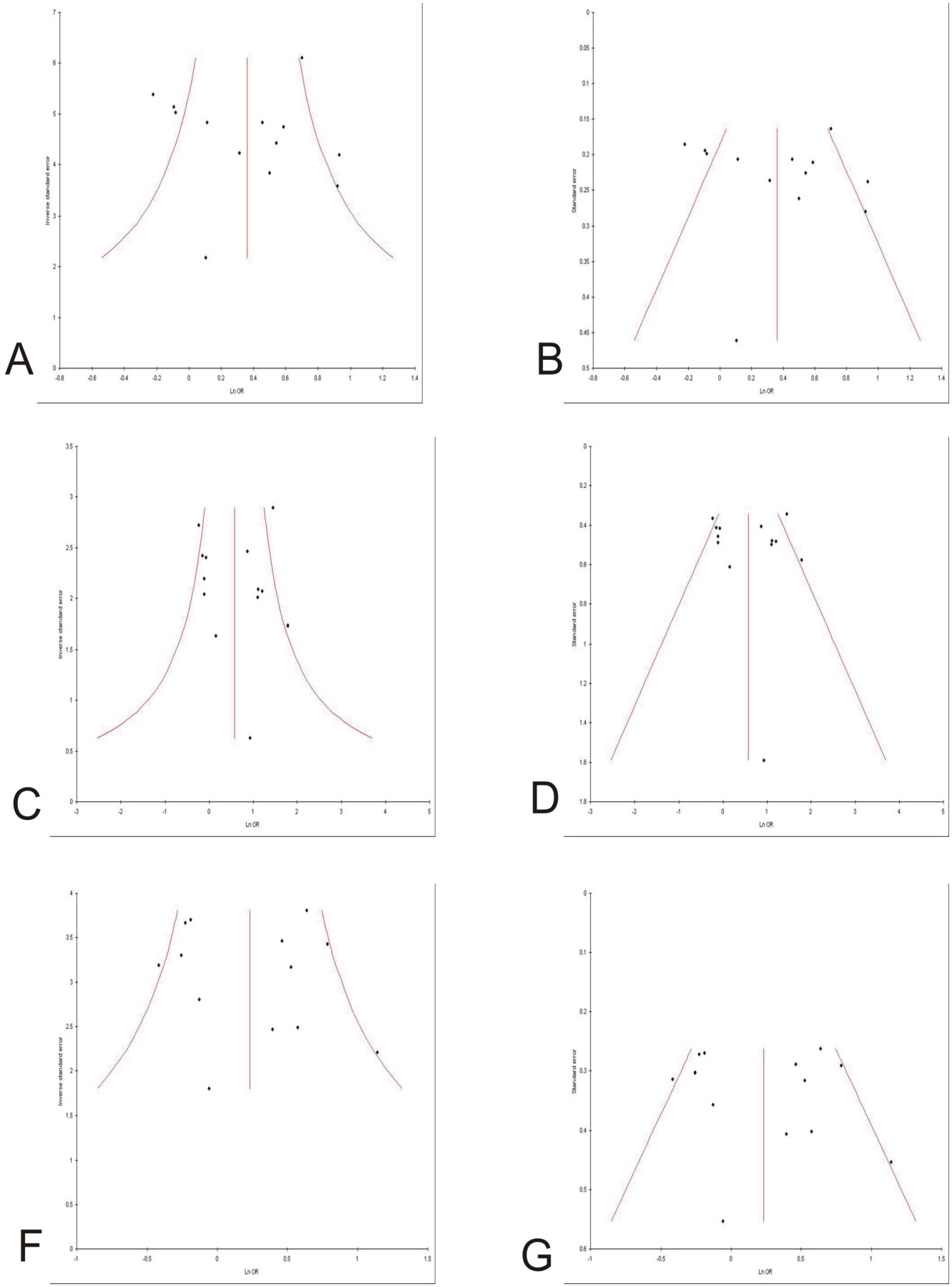


Figure 3. Funnel plots A. precision versus OR (T vs C), **B.** standard error versus OR (T vs C), **C.** precision versus OR (TT vs CC) **D. s**tandard error versus OR (TT vs. CC), **E.** precision versus OR (TT+CT vs CC) and **F.** standard error versus OR (TT+CT vs CC).

showed significant marginal association with fixed effects model (OR= 1.25; 95%CI= 1.05-1.49; p=0.01) and insignificant association with random effects (OR= 1.26; 95% CI= 0.96-1.64; p=0.08) with moderate heterogeneity (Phet=0.01; I2=53.45) (Figure 3).

Publication bias

Funnel plot and Egger’s test were used to assess the publication bias in this meta-analysis. Funnel plots’ shape of all contrasts did not reveal obvious evidence of asymmetry, and all the P values of Egger’s test were

more than 0.05, providing statistical evidence for the funnel plots' symmetry (Table 3;Figure 4).

Discussion

Folate deficiency due to low dietary, or impaired absorption and metabolism or due to dysfunctional MTHFR enzyme, may result in decreased DNA synthesis, increased DNA strand breaks, impaired DNA repair, enhanced mutagenesis and alterations in DNA methylation patterns. All of these events have been implicated in neurodevelopment (45-47). It has also been reported that disturbances in the folate-dependent one carbon metabolism may contribute to neurodegenerative diseases including depression (48). A low concentration of SAM is associated with reduced activity of SAM-dependent methyltransferases, including DNA methyltransferase and COMT (49). MTHFR plays a central role in balancing DNA synthesis (which involves methylenetetrahydrofolate) and DNA methylation (which involves methyltetrahydrofolate). Heterozygosity and homozygosity for MTHFR C677T polymorphism resulted in a lower MTHFR activity (50) leads to hyperhomocysteinemia. High concentrations of homocysteine are toxic not only for vascular endothelial cells but also to neuronal cells (51). An adequate amount of folate is required to maintain low levels of homocysteine in the central nervous system and brain especially developing brain may be particularly vulnerable to high level of homocysteine in the blood because it lacks two major metabolic pathways for its elimination: betaine remethylation and transsulfuration (52). The possible mechanism underlying MTFHR C677T polymorphism and depression association is that the genetic and environmental factors elevate homocysteine levels, which cause vascular disease of the brain, and /or transmitter alterations, which causes depression (53).

Meta-analysis is a powerful tool for analyzing cumulative data of studies where the individual sample sizes are small and the statistical power low. Present meta-analysis has included data for the C677T MTHFR polymorphism from over 1120 subjects who were suffering with depression, along with a 1688 controls. Author found an association of this polymorphism with depression, but there was statistically significant heterogeneity in the results of different studies. Several meta-analysis studies were published to illustrate the utility of the technique in identifying genes of small effects like MTHFR with phenotypes like cancer (54,55), myocardial infarction (56), NTD (57), Down syndrome (58,59), Stroke (60), Migraine (61), Schizophrenia (20,62), bipolar disorder (63), Alzheimers disease (64), recurrent pregnancy loss (65) and orofacial cleft (66) etc.

Author identified four meta-analysis (20,49,67,68) published between 2006 to 2013 concerning similar topic during the literature search; all four examined the effect of MTHFR C677T on depression risk. In 2006, Zintzaras (67) performed a meta-analysis based on five studies and did not find significant association between MTHFR polymorphisms and depression risk (OR=1.15; 95% CI=0.97–1.36). In 2007, another meta-analysis aggregated with ten studies and found slight significant relationship (OR=1.14; 95%CI= 1.04-1.26) (49). Peerbooms et al (20) included seventeen studies of depression in their combined meta-analysis of major psychiatric disorder including 26 studies of schizophrenia, bipolar and depression. In a recent meta-analysis (68), authors included twenty six studies in their meta-analysis on the association between MTHFR gene polymorphisms and depression, including 4992 depression cases and 17,082 controls and found statistical association with additive dominant model: OR = 1.19, 95%CI = 1.07–1.32. There are several newly published studies available but not included in the previous meta-analyses. In addition these meta-analyses included mixed population. So author conducted a meta-analysis with single ethnic population i.e Asian population and found strong significant association between C677T polymorphism and depression risk in Asian population. Depression risk in Asian population could be ascribed to the folate metabolism profile and dietary structure of different regions.

There were few limitations in the present meta-analysis: i) used crude ORs in the pooled analysis without adjustment; ii) relatively small sample sizes of some studies included in the analysis. Present meta-analysis had some strength. First, only Asian studies were included, second substantial number of cases and controls were pooled from different studies, which significantly increased the statistical power of the analysis and third, no publication biases were detected; indicating that the whole pooled results may be unbiased.

In conclusion, pooled analysis of data from thirteen separate studies indicates that the MTHFR 677TT genotype is associated with significant risk of depression in Asian population. This association was complicated by between study heterogeneity. Future large-scale, population-based association studies are required to investigate potential gene–gene and gene–environment interactions involving the MTHFR C677T polymorphism in determining depression risk in Asian population.

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References

1. Kendler, J.S., Neale, M., and Kessler R, et al. The lifetime history of major depression in women: reliability of diagnosis and heritability. *Arch. Gen. Psych.* 1993, **50**:863-70.

2. Alpert, J.E, Mischoulon, D. and Nierenberg, A.A. et al. Nutrition and depression: focus on folate. *Nutrition* 2000,**16**: 544–6.

3. Bottiglieri, T. Homocysteine and folate metabolism in depression. *Progn Neuro-Psychpharma. Biol. Psychia.* 2005,**29**:1103-1112. doi: 10.1016/j.pnpbp.2005.06.021

4. Kim, J.M., Stewart, R., and Kim, S.W., et al. The predictive value of folate, vitamin B12, homocysteine levels in late-life depression. *Br. J. Psychiatry* 2008, **192**:268–74. doi: 10.1192/bjp.bp.107.039511

5. Bjelland, I., Tell, G.S., Vollset, S.E., Refsum, H., and Ueland, P.M. Folate, vitamin B12, homocysteine, and the MTHFR 677C → T polymorphism in anxiety and depression: the Hordaland homocysteine study. *Arch. Gen. Psychiatry* 2003, **60**:618–26. doi: 10.1001/archpsyc.60.6.618

6. Carney, M.W., Chary, T.K., Laundy, M., et al. Red cell folate concentrations in psychiatric patients. *J. Affect. Disord.* 1990, **19**:207–13. doi: 10.1016/0165-0327(90)90093-N
7. Coppen, A., and Bolander-Gouaille, C. Treatment of depression: time to consider folic acid and vitamin B12. *J. Psychopharmacol.* 2005, **19**:59–65. doi: 10.1177/0269881105048899
8. Abou-Saleh, M.T., and Coppen, A. Serum and red blood cell folate in depression. *Acta. Psychiatr. Scand.* 1989, **80**:78–82. doi: 10.1111/j.1600-0447.1989.tb01303.x
9. Wagner, C. Biochemical role of folate in cellular metabolism. In: Bailey LB (ed) Folate in health and disease. Marcel Dekker Inc, New York, 1995, 23–42.
10. Selhub, J., Miller, J.W. The pathogenesis of homocysteinemia: interruption of the coordinate regulation by Sadenosylmethionine of the remethylation and transsulfuration of homocysteine. *Am. J. Clin. Nutr.* 1992, **55**:131–138.
11. Ueland, P.M., Hustad, S., Schneede, J., Refsum, H., and Vollset, S.E. Biological and clinical implications of the MTHFR C677T polymorphism. *Trends Pharmacol. Sci.* 2001, **22**: 195–201. doi: 10.1016/S0165-6147(00)01675-8
12. Frosst, P., Blom, H.J., Milos, R., Goyette, P., Sheppard, C.A., and Matthews, R.G., et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat. Genet.* 1995, **10**:111–3. doi: 10.1038/ng0595-111
13. Van der Put, N.M.J., Eskes, T.K.A.B., and Blom, H.J. Is the common 677CT mutation in the methylenetetrahydrofolate reductase gene a risk factor for neural tube defects? A meta-analysis. *Q. J. Med.* 1997, **90**: 111-115.
14. Hobbs, C.A., Sherman, S.I., Yi, P., Hopkins, S.E., Torfs, C.P., and Hine, R.J., et al. Polymorphism in genes involved in folate metabolism as maternal risk factors for Down syndrome. *Am. J. Hum. Genet*. 2000, **67**: 623-630. doi: 10.1086/303055
15. Nelen, W.L., Steegers, E.A., Eskes, T.K., and Blom, H.J. Genetic risk factor for unexplained recurrent early pregnancy loss. *Lancet* 1997, 350-861. doi: 10.1016/S0140-6736(97)24038-9
16. Robien, K., and Ulrich, C.M. 5,10- Methylenetetrahydrofolate reductase polymorphisms and leukemia risk: a HuGE minireview. *Am. J. Epidemiol.* 2003, **157**:571-82. doi: 10.1093/aje/kwg024
17. Sharp, L., and Little, J. Polymorphism in genes involved in folate metabolism and colorectal neoplasia: a HuGE review. *Am. J. Epidemiol.* 2004, **159**:423-43. doi : 10.1093/aje/kwh066

18. Lee, H.C., Jeong, Y.M., Lee, S.H., Cha, K.Y., Song, S.H., and Kim, N.K., et al. Association study of four polymorphisms in three folate-related enzyme genes with non-obstructive male infertility. *Hum. Reprod.* 2006; **21**: 3162–70. doi: 10.1093/humrep/del280
19. Mitraoui, N., Zammiti, W., Ghazouani, L., Braham, N., Saidi, S., and Finan, R.R., et al. Methylenetetrahydrofolate reductase C677T and A1298C polymorphism and changes in homocysteine concentrations in women with idiopathic recurrent pregnancy loss. *Reproduction* 2006, **131**: 395-401. doi: 10.1530/rep.1.00815
20. Peerbooms, O.L., Van, O.S.J., Drukker, M., Kenis, G., Hoogveld, L., and de Hert, M., et al. Meta-analysis of MTHFR gene variants in schizophrenia, bipolar disorder and unipolar depressive disorder: evidence for a common genetic vulnerability? *Brain Behav. Immun.* 2011, **25**: 1530–43. doi: 10.1016/j.bbi.2010.12.006
21. Hegele, R.A, Tully, C., Young, T.K., and Connelly, P.W. V677 mutation of methylenetetrahydrofolate reductases and cardiovascular disease in Canadian Inuit. *Lancet*. 1997,**349**:1221-2. doi: 10.1016/S0140-6736(05)62414-2
22. Pepe, G., Venegas, O.C., Giusti, B., Brunelli, T., Marcucci, R., and Attanasio, M. et al. Heterogeneity in world distribution of thermolabile C677T mutation in 5,10-methylenetetrahydrofolate reductase. *Am. J. Hum. Genet.* 1998, **63**:917-20. doi: 10.1086/302015
23. Rai, V., Yadav, U., and Kumar, P. Prevalence of methylenetetrahydrofolate reductase C677T polymorphism in eastern Uttar Pradesh. *Indian J. Hum. Genet.* 2012; **18:** 43-6. doi: 10.4103/0971-6866.96645
24. Botto, L.D., and Yang, Q. 5,10-Methylenetetrahydrofolate reductase gene variants and congenital anomalies: a HuGE review. *Am. J. Epidemiol.* 2000, **151**: 862 - 877. doi: 10.1093/oxfordjournals.aje.a010290
25. Zintzaras, E., and Ioannidis, J.P. Heterogeneity testing in meta-analysis of genome searches. *Genet. Epidemiol.* 2005, **28**:123–137. doi: 10.1002/gepi.20048
26. Zintzaras, E., and Hadjigeorgiou, G.M. The role of G196A polymorphism in the brain-derived neurotrophic factor gene in the cause of Parkinson’s disease: a meta-analysis. *J. Hum. Genet.* 2005, **50**:560–566. doi: 10.1007/s10038-005-0295-z
27. Higgins, J.P., and Thompson, S.E. Quantifying heterogeneity in a meta-analysis. *Stat. Med.* 2002, **21**:1539–1558. doi: 10.1002/sim.1186
28. Mantel, N., and Haenszel, W. Statistical aspects of the analysis of data from retrospective studies of disease. *J. Natl. Cancer Inst*. 1959, **22**: 719-48.
29. DerSimonian, R., and Laird, N. Meta-analysis in clinical trials. *Control Clin. Trials* 1986, **7**: 177–88. doi: 10.1016/0197-2456(86)90046-2
30. Egger, M., Davey Smith, G., Schneider, M., and Minder, C. Bias in meta-analysis detected by a simple, graphical test. *B.M.J.* 1997, **315**:629–34. doi: 10.1136/bmj.315.7109.629
31. Bax, L., Yu, L.M., Ikeda, N., Tsuruta, H., and Moons, K.G. Development and validation of MIX: comprehensive free software for meta-analysis of causal research data. *BMC Med. Res. Methodol* 2006, **6**: 50.
32. Arinami, T., Yamada, N., and Yamakawa-Kobayashi, K., et al. Methylenetetrahydrofolate reductase variant and schizophrenia/depression. *Am. J. Med. Genet.* 1997, **74**:526–8. doi: 10.1002/(SICI)1096-8628(19970919)74:5%3C526::AID-AJMG14%3E3.0.CO;2-E
33. Kunugi, H., Fukuda, R., Hattori, M., Kato, T., Tatsumi, M., and Sakai, T., et al. C677T polymorphism in methylenetetrahydrofolate reductase gene and psychoses. *Mol. Psychiatry* 1998, **3**:435–7. doi: 10.1038/sj.mp.4000390
34. Tan, E.C., Chong, S.A., Lim, L.C., Chan, A.O., Teo, Y.Y., and Tan, C.H., et al. Genetic analysis of the thermolabile methylenetetrahydrofolate reductase variant in schizophrenia and mood disorders. *Psychiatr. Genet.* 2004, **14**:227–31. doi: 10.1097/00041444-200412000-00012
35. Chen, C.S., Tsai, J.C., Tsang, H.Y., Kuo, Y.T., Lin, H.F., and Chiang, I.C., et al. Homocysteine levels, MTHFR C677T genotype, and MRI hyperintensities in late-onset major depressive disorder. *Am. J. Geriatr. Psychiatry* 2005, **13**:869–75. doi: 10.1176/appi.ajgp.13.10.869
36. Yuan, Y.G., Ye, Q., Chen, Y., Zhang, S.N., Li, H.L., and Lu, R., et al. Association of methylenetetrahydrofolate reductase gene polymorphism and plasma homocysteine with senile depression and Alzheimer disease. *Chin. J. Geriatrics*. 2007, **26**:767–9.

37. Yuan, Y.G., Zhang, Z.J., and Li, J.J. Plasma homocysteine but not MTHFR gene polymorphism is associated with geriatric depression in the Chinese population. *Acta. Neuropsychiatr.* 2008, **20**:251–5. doi: 10.1111/j.1601-5215.2008.00290.x
38. Zhao, G.Q., Jiao, Z.A., Wang, S.M., Wang, S.X., and Shi, H. Association of homocysteine and methylenetetrahydrofolate reductase gene polymorphism with major depression. *Chin. J. Nerv. Ment. Dis.* 2008, **34**:378–80.
39. Hong, E.D., Taylor, W.D., McQuoid, D.R., Potter, G.G., Payne,

M.E., and Ashley-Koch, A., et al. Influence of the MTHFR C677T polymorphism on magnetic resonance imaging hyperintensity volume and cognition in geriatric depression. *Am. J. Geriatr. Psychiatry* 2009, **17**:847–55.

40. Kim, J.M., Stewart, R., Kim, S.W., Yang, S.J., Shin, I.S., and Yoon, J.S. Modification by two genes of associations between general somatic health and incident depressive syndrome in older people. *Psychosom. Med.* 2009, **71**:286–91. doi : 10.1097/PSY.0b013e3181990fff

41. Yang, D.Y., Lu, X.B., Xue, L.Q., Yang, M.X., Cheng, D.M., and Zhou, Y.M. Association of methylenetetrahydrofolate reductase gene polymorphism with depression. *Chin. J. Behav. Med. Brain Sci.* 2009, **18**:398–400.

42. Cao, L., Liu, Z.H., and Zhao, L.X. Association of methylenetetrahydrofolate reductase gene polymorphism and plasma homocysteine with post-stroke depression. *Guangdong Med. J.* 2010, **31**:2946–9.

43. Feng, L.G., Shao, C.Q., Liu, Y.H., Tao, Y.H., and Hao, X.L. The detection of gene polymorphisms of MTHFR C677T and A1298C in severe depression patients. *Basic Clin.Med.* 2010, **30**: 811–4.

44. Quiao, J., Zhao, H.F., Zhu, X.H., and Geng, D.Q. The study of MTHFR gene polymorphism in depression. *J. Psychiatry* 2012, **24**:92–4.

45. Duthie, S.J., Narayanan, S., Brand, G.M., Pirie, L., and Grant, G. Impact of folate deficiency on DNA stability. *J. Nutr.* 2002, **132**: 2444S-2449S.

46. Castro, R., Rivera, I., and Ravasco, P., et al. 5,10-methylenetetrahydrofolate reductase (MTHFR) 677C/T and 1298A/C mutations are associated with DNA hypomethylation. *J. Med. Genet.* 2004, **41**:454–8. Doi: 10.1136/jmg.2003.017244

47. Friso, S., Choi, S.W., Girelli, D., mason,J.B., Dolnikowski, G.G., and Bagley, P.J., et al. A common mutation in the 5,10-methylenetetrahydrofolate reductase gene affects genomic DNA methylation through an interaction with folate status. *Proc. Nat.l Acad. Sci U S A.* 2002, **99**:5606–11. Doi: 10.1073/pnas.062066299

48. Frankenburg, F.R. The role of one-carbon metabolism in schizophrenia and depression. *Harv. Rev. Psychiatry* 2007, **15**:146–160. doi: 10.1080/10673220701551136

49. Gilbody, S., Lewis, S., and Lightfoot, T. Methylenetetrahydrofolate reductase (MTHFR) genetic polymorphisms and psychiatric disorders: a HuGE review. *Am. J. Epidemiol*. 2007, **165**: 1–13. doi: 10.1093/aje/kwj347

50. van der Put, N.M., Gabreëls, F., Stevens, E.M., Smeitink, J.A., Trijbels, F.J., and Eskes, T.K., et al. A second common mutation in the methylenetetrahydrofolate reductase gene: an additional risk factor for neural-tube defects? *Am. J. Hum. Genet.* 1998, **62**:1044-51. doi: 10.1086/301825

51. Mattson, M.P., and Shea, T.B. Folate and homocysteien metabolism in neural plasticity and neurodegenerative disorders. *Trends. Neurosci.* 2003, **26**:137-146. doi: 10.1016/S0166-2236(03)00032-8

52. Sachdev, P.S. Homocysteine and brain atrophy. *Pro. Neuropsychopharmacology Biol. Psychiatry* 2005, **29**: 1152-61. doi: 10.1016/j.pnpbp.2005.06.026

53. Folstein, M., Liu, T., Peter, I., Buell, J., Arsenault, L., and Scott, T., et al. The homocysteine hypothesis of depression. *Am. J. Psychiatry* 2007, **164**:861–7. doi: 10.1176/appi.ajp.164.6.861

54. Yang, Z., Zhang, X.F., Liu, H.X., Hao, Y.S., Zhao, C.L. MTHFR C677T polymorphism and colorectal cancer risk in Asians, a meta-analysis of 21 studies. *Asian Pac. J. Cancer Prev.* 2012, **13**:1203–8. doi: 10.7314/APJCP.2012.13.4.1203

55. Rai, V. The Methylenetetrahydrofolate Reductase C677T Polymorphism and Breast Cancer Risk in Asian Populations. *Asian Pac. J. Cancer Prev.* 2014, 15: 5853-5860.

56. Xuan, C., Bai, X.Y., Gao, G., Yang, Q., and He, G.W. Association between polymorphism of methylenetetrahydrofolate reductase (MTHFR) C677T and risk of myocardial infarction: A meta-analysis for 8,140 cases and 10,522 controls. *Arch. Medl Res.* 2011, **42**: 677e685. doi: 10.1016/j.arcmed.2011.11.009

57. Zhang, T., Lou, J., Zhong, R., Wu, J., Zou, L., and Sun, Y., et al. Genetic Variants in the Folate Pathway and the Risk of Neural Tube Defects: A Meta-Analysis of the Published Literature. *Plos one* 2013, **8**(4): e59570. doi: 10.1371/journal.pone.0059570

58. Zintzaras, E. Maternal gene polymorphisms involved in folate metabolism and risk of Down syndrome offspring: a meta-analysis. *J. Hum. Genet.* 2007, **52**:943–53. doi: 10.1007/s10038-007-0202-x

59. Wu, X., Wang, X., Chan, Y., Jia, S., Luo, Y., and Tang, W. Folate metabolizing gene polymorphisms MTHFR C677T and A1298c and risk for Down syndrome offspring: a meta-analysis. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 2013, **167**: 154-159. doi: 10.1016/j.ejogrb.2012.11.022

60. Yadav, S., Hasan, N., Marjot, T., Khan, M.S., Prasad, K., Bentley, P., and Sharma, P. Detailed Analysis of Gene Polymorphisms Associated with Ischemic Stroke in South Asians. *Plos one* 2013, **8**: e57305. doi: 10.1371/journal.pone.0057305

61. Schürks, M., Rist, P.M,. and Kurth, T. 5-HTTLPR Polymorphism in the Serotonin Transporter Gene and Migraine: A Systematic Review and Meta-Analysis. *Cephalalgia* 2010, **30**(11): 1296–1305.

62. Kim, S.G., Song, J.Y., Joo, E.J., Jeong, S.H., Kim, S.H., and Lee, K.Y., et al. No association of functional polymorphisms in methylenetetrahydrofolate reductase and the risk and minor physical anomalies of schizophrenia in Korean population. *J. Korean Med. Sci.* 2011, **26(10)**: 1356-63. doi: 10.3346/jkms.2011.26.10.1356

63. Rai, V. Evaluation of methylenetetrahydrofolate reductase gene variant (C677T) as risk factor for bipolar disorder. *Cell. Mol. Biol* 2011, **57**: OL1558-OL1566. doi: 10.1170/180

64. Zhang, M.Y., Miaoa, L., Li, Y.S., and Hub, G.Y. Meta-analysis of the methylenetetrahydrofolate reductase C677T polymorphism and susceptibility to Alzheimer’s disease. *Neuroscience Res.* 2010, **68**: 142–150. doi: 10.1016/j.neures.2010.06.011. E

65. Rai, V. Methylenetetrahydrofolate reductase gene A1298C polymorphism and susceptibility to recurrent pregnancy loss: a meta-analysis. *Cell. Mol. Biol.* 2014, **60**: 27-34. doi: 10.14715/cmb/2014.60.2.5

66. Rai, V. Maternal methylenetetrahydrofolate reductase (MTHFR) gene A1298C polymorphism and risk of nonsyndromic Cleft lip and/or Palate (NSCL/P) in offspring: A meta-analysis. *Asian J. Medical Sci*. 2014, **6**:16-21. Doi: 10.3126/ajms.v6i1.10281

67. Zintzaras, E. C677T and A1298C methylenetetrahydrofolate reductase gene polymorphisms in schizophrenia, bipolar disorder and depression: a meta-analysis of genetic association studies. *Psychiatr. Genet.* 2006, **16**(3):105-115. doi: 10.1097/01.ypg.0000199444.77291. e2

68. Wu, Y.L., Ding, X.X., Sun, Y.H., Yang, H.Y., Chen, J., and Zhao, X., et al. Association between MTHFR C677T polymorphism and depression: An updated meta-analysis of 26 studies. *Prog. Neuro-Psychopharm. Biol. Psychiatry* 2013, **46**: 78–85. doi: 10.1016/j.pnpbp.2013.06.015.