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Meta-Analysis

Association of C677T polymorphism (rs1801133) in MTHFR gene with depression

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Abstract: Depression is one of the mental disorders with a state of low mood and aversion to activities that exerts a negative effect on a person's thoughts and behavior. Genetic association studies on MTHFR C677T polymorphism and depression have been repeatedly performed over the last two decades, but results are inconsistent. The aim of the present study was to assess the relationship between MTHFR C677T polymorphism and depression by literature review and meta-analysis. Four electronic databases, PubMed, Google Scholar, Science direct and Springer Link were searched for case control articles focusing on MTHFR C677T polymorphism and the risk of depression. A total of 30 studies including 4,802 cases and 17,362 controls were involved in present meta-analysis. When all the eligible studies were pooled into this meta-analysis, significant association between depression risk and MTHFR C677T polymorphism was found in three genetic models (Additive model: OR T vs C= 1.20, 95 % CI= 1.00-1.34, p=0.0004; homozygote model: OR TT vs.CC=1.37, 95% CI= 1.13-1.65, p=0.0004; dominant model: OR TT+CT vs CC=1.13, 95 % CI= 0.99-1.28, p=0.04), while meta-analysis with other two genetic models did not show association with other two genetic models (recessive model: OR TT vs CT+CC= 1.36, 95 % CI = 0.91-2.04, p=0.13; co-dominant model: OR CT vs CC=1.00, 95 % CI=0.93-1.08,p=0.84). Present meta-analysis supports that there is a meager significant association between MTHFR C677T polymorphism and depression risk.

Key words: Meta-analysis; Depression; MTHFR; C677T; Genotype; Polymorphism.

Introduction

Depression is one of the most common psychiatric disorders causing impairment in several domains of daily life. The etiology of depression remains unexplained. It is a clinically heterogeneous disorder thought to result from an interaction of multiple genes with environmental influences and developmental epigenetic components. Epidemiologic studies show that roughly 40%-50% of the risk for depression is genetic (1). Neuroimaging and postmortem studies in patients with depression have demonstrated that decreased hippocampal volume (an average of 9% across all studies), atrophy of existing neurons and decreased neurogenesis may contribute to the pathophysiology of depression.

A possible role of nutritional factors in the pathogenesis of neuropsychiatric disorders has long been debated and epidemiologic studies have suggested that folate deficiency may increase the risk for several psychiatric disorders like-schizophrenia, anxiety, bipolar disorder and depression(2,3). Published case-control studies on folate status and depression demonstrate strong relationship (4). The main role of folate is in mediating transfer of one carbon for various cellular reactions (5). Folate plays an integral role in DNA synthesis, methylation, integrity and stability (5,6). Methylenetetrahydrofolate reductase (MTHFR) is an important enzyme of folate metabolic pathway and play a vital role in one carbon metabolism by regulating the channeling of one carbon units between DNA synthesis and DNA methylation (7).

MTHFR enzyme converts 5,10-methylene tetrahydrofolate to 5-methyl tetrahydrofolate which donates methyl group for methionine synthesis (8). MTHFR gene is located at chromosome 1.p36.3 and composed of 2.2 kilobases with a total of 11 exons (9). Several polymorphisms have been described so far in MTHFR gene, only two C677T and A1298C polymorphisms are clinically important and intensively investigated. C677T (ala222val) has a profound effect on the activity of enzyme, producing more labile forms with reduced activity (10) and increased blood homocysteine levels (11,12).

There is marked variation in the frequency of mutant T allele between populations, range approximately from 0.24 to 0.44 in Caucasian populations, 0.06 in an African population, and 0.35 to 0.41 in Asian populations (13-16). C677T polymorphism is reported risk factor for several neuropsychiatric and neurodegenerative diseases, including schizophrenia (17,18), bipolar disorder (19), autism (20), anxiety (21), Alzheimers disease (22) and Parkinson disease (23).

Several case-control studies have demonstrated a positive relationship between depression and MTHFR C677T polymorphism (24-27) but such relationship have not been confirmed in some other studies (28,29). These inconsistent results may be owing to control selection, ethnic heterogeneity and population substructure. The aim of the present study was to systematically review and perform a meta-analysis on studies that have investigated the association between the C677T polymorphism in the MTHFR gene and depression.

Materials and Methods

Meta-analysis was carried out according to MOOSE guidelines (30).

Study identification

Four databases: PubMed (http://www.ncbi.nlm.nih.gov/pubmed/), Google Scholar (scholar.google.co.in), Science direct (www.sciencedirect.com) and Springer Link (www.linkspringer.com) were searched for the case control studies up to December, 2015. The search strategy included the keywords ‘‘depression’’, ‘‘polymorphism’’, “methylene tetrahydrofolate reductase”, ‘‘MTHFR’’ and C677T. Studies that reported previously published data were excluded.

Inclusion criteria

Eligible studies had to meet all of the following criteria: (1) Study should be published, (2) Study should have sufficient data to calculate the odds ratio (OR) with confidence interval, (3) Depression patients were diagnosed by psychiatrist according to the Diagnostic and Statistical Manual of Mental Disorders IV criteria (DSM-IV) or the International Statistical Classification of Diseases and Related Health Problems 10th Revision (ICD-10).

Data extraction

For each included study, the following data were extracted: (i) first author(s) family name (ii) journal name (iii) year of publication; (iv) country of origin (v) ethnicity (vi) case and control sample size, and (vii) diagnostic criteria for depression.

Meta-analysis

The strength of association between the MTHFR C677T polymorphisms and depression risk was evaluated by OR with 95% CI according to allele contrast (T vs. C), homozygote (TT vs. CC), heterozygote (TC vs. CC), recessive (TT vs. TC+CC), and dominant (TT+TC vs. CC) models. The meta-analysis examined the overall association of the C677T allele T with the risk of depression relative to allele C. Pooled OR were analyzed by both fixed and random effects framework(31,32). The significance of the pooled OR was determined using a Z-test. Heterogeneity was quantified with the I^2 metric. I^2 takes values between 0 and 100% with higher values denoting greater degree of heterogeneity (33,34).

Publication bias

Publication bias was investigated by using the funnel plots; viz. funnel plot of standard error by log odds ratio and funnel plot of precision (1/standard error) by log odds ratio. Different statistical tests such as Begg and Mazumdar rank correlation (35) and Egger’s regression intercept(36) were adopted to assess the publication bias. All p values are two tailed with a significance level at 0.05. All statistical analyses were undertaken using the freely available program MIX version 1.7 (37).

Results

Characteristics of included studies

The flow chart of selection of studies and reasons for exclusion is presented in Figure 1. Four databases search based on the keywords, 181 articles were identified, of these, 10 articles were excluded for the following reasons: three comments, two editorials, sixteen letter to editor, twenty one reviews, seven case studies, twenty seven irrelevant , thirty three not case control study and, and forty two were duplicate. Twenty nine studies were found suitable for the inclusion in the present meta-analysis (21,24-29,38-59). One author studied two populations and both samples were considered separately. Hence total thirty studies were included in the present meta-analysis.

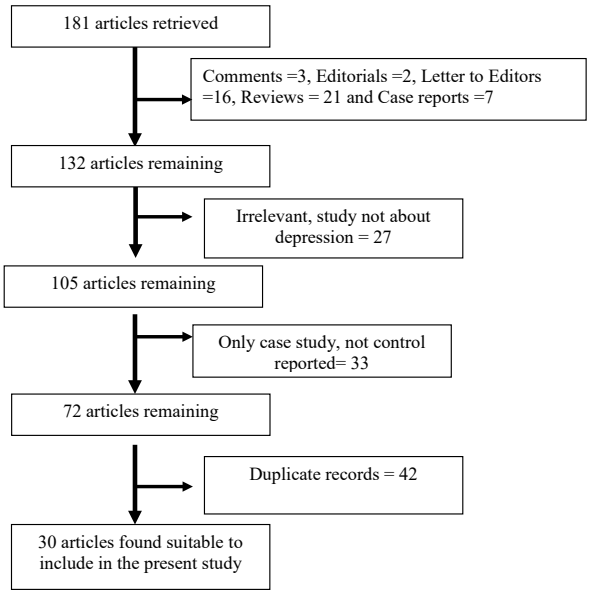

Figure 1. Flow diagram of study selection.

The studies were carried out in Japan (24,28), Australia (25,38,42), Norway (21), Singapore (29), Ireland (26), Germany (40), Taiwan (39), China (41,45,46,48,51,53,54,57), Korea (49), England (27,43), Czec Republic (52), Poland (44,56), Spain (47), Slovak Republic (58), South Africa (59), and USA (50,55). Genotypes were in Hardy-Weinberg equilibrium in all controls.

In all thirty studies, total cases were 4,802 with CC (2,020), CT (2,072) and TT genotypes(710), and controls were 17,362 with CC (7,842), CT (7,645), and TT (1,875). In controls genotypes percentage of CC, CT and TT were 45.17 %, 44.03% and 10.8% respectively. In total cases genotype percentage of CC, CT, and TT was 42.06%, 43.15% and 14.78% respectively (Table 1). The OR of twenty one studies out of total 30 studies for depression were above unity, and in nine studies OR were below one (28,40,42,43,47,48,49,50,56).

Meta-analysis

The main results of this meta-analysis and the heterogeneity test were shown in Tables 2.

Mutant allele showed significant association with depression in both fixed effect (OR=1.11, 95% CI = 1.05-1.17,p<0.0001) and random effect (OR= 1.20, 95 % CI= 1.00-1.34, p=0.0004) models (Table 2; Figure 2). In cumulative analysis using fixed and random effect models, the association of mutant ‘T’ allele with depression turned statistically significant with the addition of study of Almeida et al (38) and Lewis et al (27), and

Table 1. Characteristics of the eligible studies considered in the meta-analysis.

S. No.	Study	Ethnicity	Case/control	Case Genotypes CC/CT/TT	Control Genotypes CC/CT/TT	HWE
1	Arinami et al.,1997	Asian	32/419	9/14/9	154/214/51	0.37
2	Kunugi et al.,1998	Asian	71/258	30/31/10	95/129/34	0.34
3	Hickie et al.,2001	Caucasian	75/22	33/33/9	12/9/1	0.66
4	Bjelland et al.,2003	Caucasian	242/4752	127/85/30	2375/1996/381	0.17
5	Kelly et al.,2004	Caucasian	100/89	30/56/14	40/37/12	0.46
6	Tan et al.,2004	Asian	88/120	49/34/5	80/33/7	0.16
7	Almeida et al.,2005	Caucasian	42/198	13/26/3	85/87/26	0.61
8	Chen et al.,2005	Asian	39/20	22/15/2	11/9/0	0.19
9	Reif et al.,2005	Caucasian	47/47	24/15/8	75/80/21	0.96
10	Lewis et al.,2006	Caucasian	545/2942	221/251/73	1344/1269/329	0.26
11	Yuan et al.,2007	Asian	60/71	22/27/11	27/38/15	0.80
12	Almeida et al.,2008	Caucasian	513/3239	235/218/60	1423/1457/359	0.62
13	Gaysina et al.,2008, Men	Caucasian	242/371	119/92/31	152/174/45	0.65
14	Gaysina et al.,2008, Women	Caucasian	464/548	234/228/86	198/205/61	0.49
15	Slopien et al.,2008	Caucasian	83/89	26/38/19	46/36/7	0.99
16	Yuan et al.,2008	Asian	116/80	46/48/22	27/38/15	0.80
17	Zhao et al.,2008	Asian	77/85	12/37/28	21/48/16	0.21
18	Hernadez-Sanchez et al.,2009	Caucasian	21/21	9/8/4	11/8/2	0.75
19	Hong et al.,2009	Asian	178/178	75/84/19	32/44/9	0.28
20	Kim et al.,2009	Asian	63/458	16/28/19	84/248/126	0.05
21	Pan et al.,2009	Caucasian	170/83	72/79/19	30/44/9	0.22
22	Yang et al.,2009	Asian	100/100	33/50/17	52/40/8	0.93
23	Zeman et al.,2009	Caucasian	42/41	15/18/9	16/17/8	0.37
24	Cao et al.,2010	Asian	50/59	9/23/18	24/27/8	0.92
25	Feng et al.,2010	Asian	152/152	32/66/54	51/81/20	0.16
26	Lizer et al.,2011	Caucasian	82/74	31/34/17	33/28/13	0.11
27	Chojnicka et al.,2012	Caucasian	710/2547	342/300/68	1213/1081/253	0.000
28	Quiao et al.,2012	Asian	94/98	24/43/27	36/45/17	0.65
29.	Evinova et al.,2012	Caucasian	134/143	70/54/10	58/73/12	0.09
30	Delport et al.,2014	African	86/97	40/37/9	37/50/10	0.24

Table 2. 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: overall analysis, and subgroup analyses.

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)
All (30 studies)					
Allele Contrast (T vs C)	1.1(1.05-1.17),<0.0001	1.20(1.00-1.34),0.0004	<0.0001	66.83	0.07
Co-dominant (Ct vs CC)	1.0(0.932-1.088),0.849	1.03(0.917-1.170),0.565	0.013	40.85	0.216
Homozygote (TT vs CC)	1.28(1.14-1.14),<0.0001	1.37(1.13-1.65),0.0004	0.001	50.89	0.04
Dominant (TT+CT vs CC)	1.06(0.99-1.14),0.11	1.13(0.99-1.28),0.04	0.0006	52.75	0.061
Recessive (TT vs CT+CC)	0.66(0.60-0.73),<0.0001	1.36 (0.91-2.04),0.13	<0.0001	92.28	0.0006
Ethnicity					
Asian (13 studies)					
Allele Contrast (T vs C)	1.4(1.25-1.58),<0.0001	1.43(1.15-1.78),0.001	0.0001	68.68	0.57
Co-dominant (Ct vs CC)	1.1(0.91-1.32),0.30	1.1(0.87-1.32),0.40	0.14	30.16	0.73
Homozygote (TT vs CC)	1.75(1.37-2.23),<0.001	1.78(1.17-2.69),0.0004	0.022	60.38	0.75
Dominant (TT+CT vs CC)	1.25(1.05-1.49),0.01	1.26(0.97-1.64),0.047	0.01	53.45	0.58
Recessive (TT vs CT+CC)	1.2(1.00-1.43,0.04	1.72 (0.87-3.4),0.11	<0.0001	90.38	0.26
Caucasian (16 studies)					
Allele Contrast (T vs C)	1.04(0.97-1.1),0.22	1.05(0.96-1.15),0.33	0.02	44.52	0.46
Co-dominant (Ct vs CC)	0.97(0.89-1.1),0.54	0.98(0.84-1.13),0.03	0.009	51.51	0.59
Homozygote (TT vs CC)	1.16(1.03-1.31),0.02	1.17(1.00-1.36),0.04	0.26	16.1	0.30
Dominant (TT+CT vs CC)	1.00(0.93-1.1),0.81	1.02(0.89-1.2),0.03	0.007	52.38	0.41
Recessive (TT vs CT+CC)	0.60(0.54-0.67),<0.0001	1.25 (0.74-2.12),0.39	<0.0001	93.87	0.03

stayed significant thereafter.

Similar to allele meta-analysis, pooled odds ratio for homozygote model (TT vs CC) showed statistically significant association with depression adopting both fixed (OR= 1.28, 95% CI =1.14-1.46, p<0.0001) and random (OR= 1.37, 95% CI = 1.13-1.65, p=0.0004) effect models (Figure 3). Co-dominant (CT vs CC) and recessive (TT vs CT+CC) models did not show any association between C677T polymorphism and depression.

Except co-dominant model, higher significant heter-

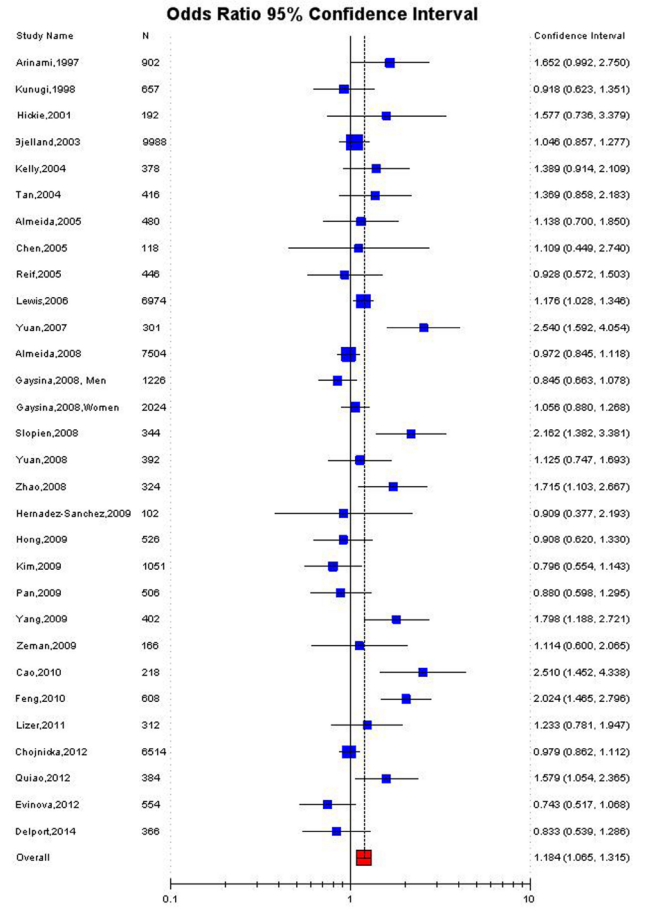

Figure 2. Forest plots for the association between MTHFR C677T polymorphism and depression for additive model (T vs C) with random effect model.

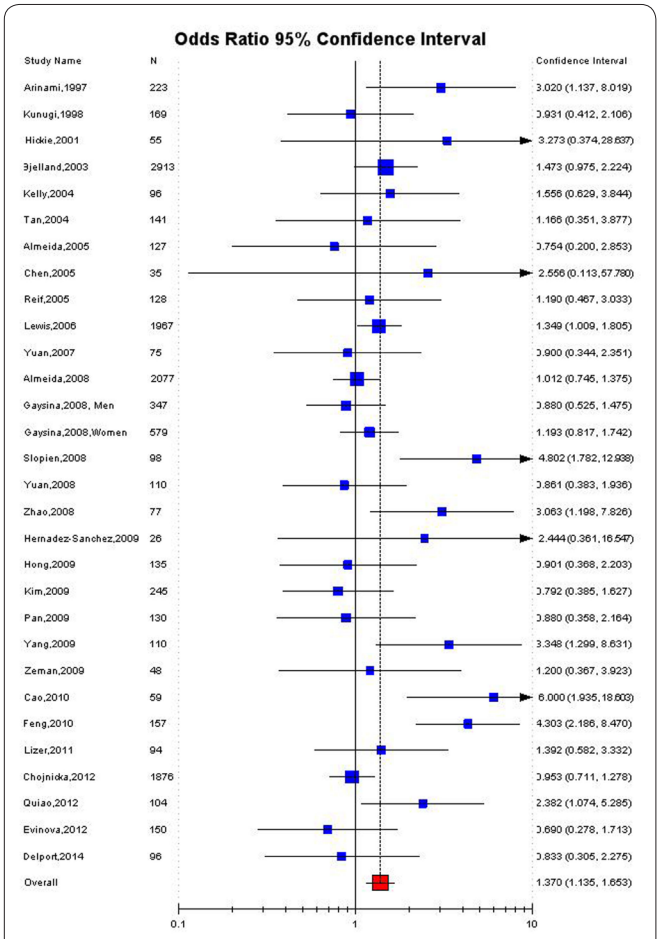

Figure 3. Forest plots for the association between MTHFR C677T polymorphism and depression for homozygote model (TT vs CC) with random effect model.

ogeneity was observed in other four models (For T vs C: $P_{heterogeneity}$<0.0001, I^2= 66.83; For TT vs CC: $P_{heterogeneity}$=0.001, I^2= 50.89%; For TT+CT vs CC: $P_{heterogeneity}$=0.0006, I^2= 52.75%: For TT vs CT+CC: $P_{heterogeneity}$<0.0001, I^2= 92.28).

Subgroup analysis

In total thirty studies , 13 studies were from Asian and 16 studies were from Caucasian populations. Allele contrast model of Asian studied showed strong significant association between C677T polymorphism and depression risk (OR= 1.43, 95% CI=1.15-1.78, p=0.001) (Figure 4), whereas allele contrast meta-analysis of Caucasian studies did not show any association (OR= 1.05, 95% CI=(0.96-1.15, p=0.22) (Figure 5).

Publication bias

Begg's funnel plot and the Egger's test were conducted to estimate the publication bias of articles. Both the results of Begg's and Egger's test did not show any evidence of publication bias (T vs C Begg's test, *P*= 0.002,Egger's test, *P*=0.0168; CT vs CC Begg's test, *P*=0.4411, Egger 's test, *P*=0.216; TT vs.CC Begg's test, *P*=0.0239, Egger 's test, *P*=0.0407; Dominant model TT+CT vs CC, Begg's test, *P*= 0.1056, Egger 's test, *P*=0.0608; Recessive model TT vs CT+CC, Begg's test, *P*=0.0381, Egger 's test, *P*=0.0006)(Table 2; Figure 6)

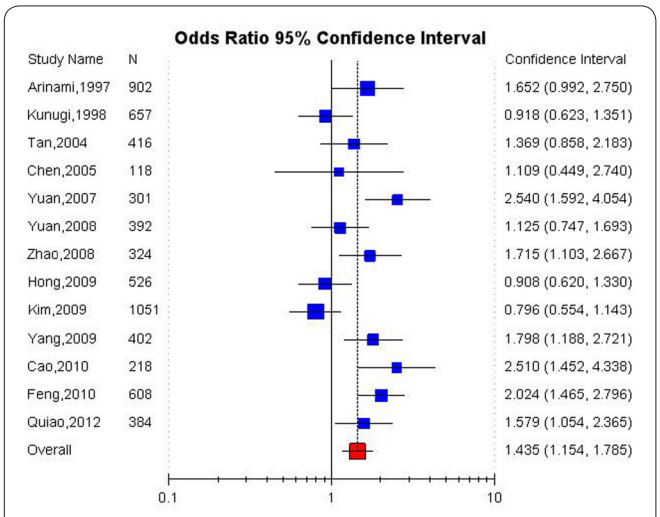

Figure 4. Forest plots for the association between MTHFR C677T polymorphism and depression for additive model (T vs C) of Asian studies with random effect model.

Discussion

Folate is a methyl donor during DNA methylation, as it provides substrate for MTHFR to convert 5,10-MTHF (methylene tetra hydro folate) to 5-MTHF and subsequently metabolise it to methionine(60). Methylation is genetically predetermined, either by imprinting or by inheritance of genes which influence methylation, such as MTHFR and other genes involved in the one-carbon cycle (61). Methyl groups required for methylation are synthesized de novo or are supplied in the diet, primar-

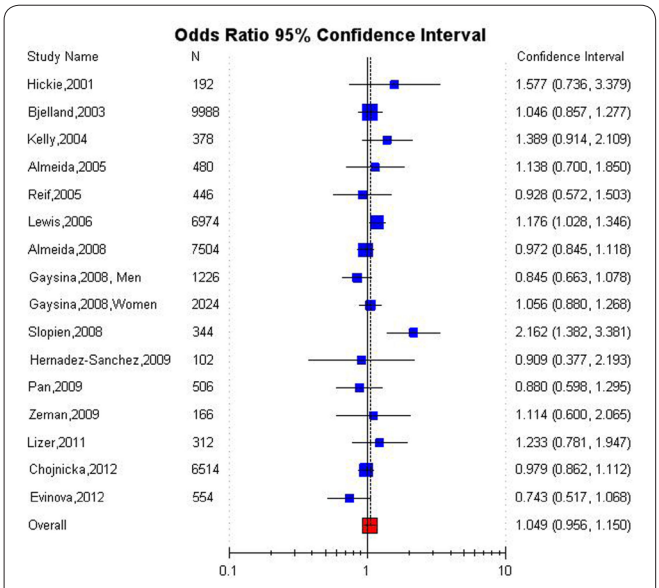

Figure 5. Forest plots for the association between MTHFR C677T polymorphism and depression for additive model (T vs C) of Caucasian studies with random effect model.

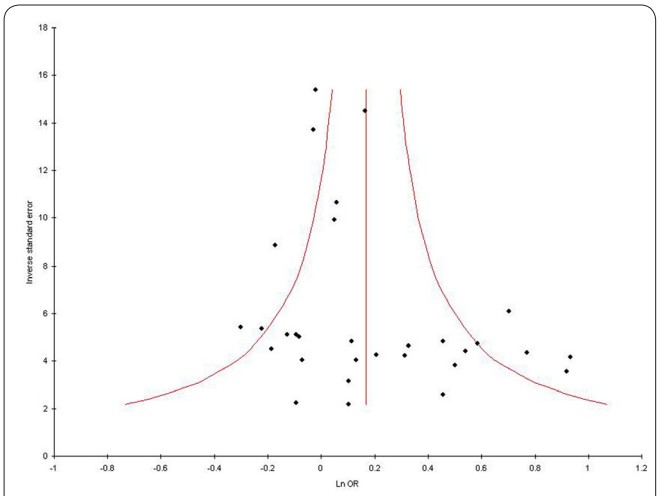

Figure 6. Funnel plot of precision versus OR for allele contrast (T vs. C).

ily from folate. Thus, methylation may be modified by gene-exposure interactions occurring during development.

Deficiency of B vitamins can cause hyperhomocysteinemia, which is associated with increased risk of heart disease, cognitive problems and mood disorders (42,62). Homocysteine has been implicated in amyloid buildup, DNA damage, mitochondrial dysfunction, nuclear disintegration, and apoptosis of neurons (63). Adequate supplies of SAM are crucial for maintenance of neurotransmitters and DNA synthesis (7,62).

Environmental and genetic risk factors may interact to cause disruption of the one-carbon metabolic pathway, resulting in elevated homocysteine levels implicated in many chronic diseases including depression(64).

Less activity of MTHFR and the ensuring increase in homocysteine can lead to severe metaboloic consequences. High concentrations of homocysteine is toxic not only for vascular endothelial cells but also to neuronal cells (65). Over the last decade, MTHFR polymorphisms and elevated total plasma homocysteine concentrations have been reported to be associated with a broad range of conditions e.g., with schizophrenia, bipolar disorder (3,66),(Muntjewerff et al, 2006; Gilbody et al, 2007), dementia, cognitive impairment (67,68), cardiovascular disease (69,70) and depression also. The homocysteine hypothesis is that genetic and environmental factors elevate homocysteine levels ,which cause vascular disease of the brain, and /or neurotransmitter alterations, which cause depression (71).

There is mounting evidence from several independent lines of investigation which supported the role of folate in depression -(i) folate deficiency is associated with a higher incidence of depression, (ii) low folate status is associated with reduced serotonergic and/or neurotransmitter function,(iii) low folate status is associated with poorer response to antidepressant medication, and (iv) folate supplementation as an adjuvant to antidepressant therapy can improve clinical outcome (2).

Several meta-analyses have convincingly proven the association between the MTHFR C677T mutation and depression (3,72,73). Zintzaras (72) 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). Gilbody et al (3) compiled ten studies in their meta-analysis and reported significant association with depression only in allele contrast model (OR= 1.14, 95% CI= 1.04-1.26). Wu et al (73)(2013) included 26 studies and reported significant association using all five genetic models (OR = 1.19, 95%CI = 1.07–1.32).

The contradictory results from individual case control studies on different populations and meta-analysis suggested that the role of C677T in susceptibility to depression might depend on ethnic or geographic factors (74). Gene–nutrient/environmental and gene–racial/ethnic interactions have been shown to affect the impact of these MTHFR genetic variants. Moreover, it is reported that the detrimental effects of the C677T mutation on enzymatic activity of MTHFR depends on status of folate (75,76).

Meta-analysis is an important statistical method, which quantitatively combines several small analysis of the same topic with low statistical power and small number of participants. Because of the large sample sizes, meta-analysis has more statistical power than a single study. Several meta-analysis illustrate the utility of the technique in identifying genes of small effects like MTHFR with phenotypes like NTD (77), Down syndrome (78), schizophrenia (79), bipolar (80), anxiety (3), epilepsy (81), Parkinson's (82), Alzheimer's disease (83) and cancer (84). This meta-analysis has included data for the C677T MTHFR polymorphism from over 4,802 subjects who were suffering with depression, along with 17,362 controls. Author found an association of this polymorphism with depression, but there was statistically significant heterogeneity in the results of different studies.

Strengths of this study include: (i) high sample sizes translating to robust statistical power of the component studies, (ii) highest number of studies were included (iii) controls were healthy and were matched to cases, and (iv) subgroup analysis was performed. However, there are still several limitations in present meta-analysis. (i) overall results were based on individual unadjusted ORs due to unavailable data, whereas a more precise esti-

mation should be adjusted by potentially influential factors including age, gender, and environmental factors, (ii) publication bias was observed except two genetic models, (iii) heterogeneity was present (iv) controls of three studies were not in HWE, were also included in the meta-analysis, (v) gene-gene interactions and gene environment interactions could not be considered owing to lack of data.

In conclusion, pooled analysis of data from thirty separate studies including individuals with different ethnicities indicated that the MTHFR 677TT genotype is associated with a modest, but significant, risk of depression. 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.

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