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Methylenetetrahydrofolate reductase A1298C genetic variant & risk of schizophrenia: A meta-analysis

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***Background & objectives*: Methylenetetrahydrofolate reductase (MTHFR) is an important enzyme of folate metabolism, whose role in schizophrenia is debatable. Numerous case-control studies have investigated the association of *MTHFR* A1298C polymorphism with schizophrenia, but results are controversial. The aim of the present study was to find the association between *MTHFR* A1298C gene polymorphism and schizophrenia.**

***Methods*: PubMed, Google Scholar, Science Direct and Springer link databases were searched for case-control association studies in which *MTHFR* A1298C polymorphism was investigated as a risk factor for schizophrenia. In all, 19 studies with 4049 cases and 5488 controls were included in this meta-analysis. Odds ratios (ORs) with 95 per cent confidence intervals (CIs) were used as an association measure.**

***Results*: The results of meta-analysis reported a significant association between A1298C polymorphism and schizophrenia risk in overall comparisons in all genetic models (C vs. A: OR=1.13, 95% CI=1.01-1.27, *P*=0.02; CC vs. AA: OR=1.20, 95% CI=1.03-1.39, *P*=0.02; AC vs. AA: OR=1.13, 95% CI=1.03-1.23, *P*=0.009; AC+CC vs. AA: OR=1.14, 95% CI=1.02-1.24, *P*=0.002; CC vs. AA+AC: OR=1.17, 95% CI=1.01-1.35, *P*=0.04).**

***Interpretation & conclusions*: *MTHFR* A1298C polymorphism was found to be a risk factor for schizophrenia and might have played a significant role in the pathogenesis of schizophrenia.**

Key words A1298C - folate - homocysteine - meta-analysis - methylenetetrahydrofolate reductase - polymorphism - schizophrenia

Schizophrenia is a psychiatric disorder with the prevalence rate of 1 per cent[1,2]. According to the Diagnostic and Statistical Manual of Mental Disorders IV criteria (DSM-IV)[3], the diagnosis is based on symptoms such as delusions, hallucinations and affective flattening and alogia. The most widely considered hypothesis of schizophrenia is neurodevelopment hypothesis which integrates influences of environment and genes. The neurodevelopment hypothesis of schizophrenia postulates that altered biochemical pathways and defective neural circuitry during foetal brain development lead to cognitive and emotional defects in later part of life[4]. In the brain of schizophrenics, several neurotransmitter network systems have been found to be defective. Numerous schizophrenia candidate genes have been reported[4-7].

Folate is an essential nutrient required for *de novo* nucleotide synthesis and DNA methylation and it has been associated with DNA hypomethylation[8-10] and DNA strand breaks[11]. Methylenetetrahydrofolate (MTHFR) is an important enzyme of folate/homocysteine metabolism which catalyzes the synthesis of 5-methyltetrahydrofolate. 5-methyltetrahydrofolate donates methyl group for remethylation of homocysteine to methionine, which is a precursor of S-adenosylmethionine (SAM). SAM is the main methyl donor of several cellular methylation reactions. *MTHFR* polymorphism raises the dietary requirement for folic acid to maintain normal re-methylation of homocysteine to methionine[12]; consequently, low folate status in individuals with the *MTHFR* polymorphism results in an increase in homocysteine and a decrease in methionine levels. A deficiency in cellular folates and methyl-donors may be associated with abnormal methylation of DNA, proteins and neurotransmitters and DNA strand breaks[13-17]. If an adequate amount of folate is not available concentration of intracellular homocysteine is increased, and consequently, methionine synthesis is reduced[18]. SAM is the only source of methyl group for DNA methylation reactions in the brain, and this forms a plausible biologic explanation for potential associations between *MTHFR* gene polymorphism and schizophrenia risk[19].

In *MTHFR* gene, more than 40 polymorphisms have been reported, of which two mutations C677T[20] and A1298C[21,22] have been extensively studied. It has been reported that the C677T mutation decreases MTHFR activity and increases the plasma homocysteine level while the second polymorphism A1298C results in reduced enzymatic activity but to a lesser extent than the C677T mutation[22]. The *MTHFR* A1298C polymorphism is associated with a reduction in the bioavailability of cellular folate[23]. The mutant genotype (CC) prevalence ranges from 7 to 12 per cent in Caucasian, 4-5 per cent in Hispanic and 1-4 per cent in Asian populations[24,25].

Several case-control studies investigating the association between *MTHFR* gene A1298C polymorphism and schizophrenia have been published, but the results were conflicting. Some reported positive association[26-31] but others did not find any association between A1298C polymorphism and schizophrenia[32-35]. To shed some light on these controversial results, we performed a meta-analysis of published case-control studies, and a pooled estimate of the A1298C polymorphism as risk for schizophrenia was obtained for the allele contrast, homozygote, dominant, co-dominant and recessive models.

Material & Methods

Selection of studies: PubMed, Google Scholar, Science Direct and Springer Link databases were searched for the studies which investigated A1298C polymorphism as risk factor for schizophrenia up to June 2014. The articles were searched using the following terms - 'methylenetetrahydrofolate reductase,' 'MTHFR', 'A1298C' and 'Schizophrenia'. Studies published between 2003 and 2012 were included in the present meta-analysis. Eligible studies met the following criteria: (*i*) the study should be a case-control association study, (*ii*) patients were diagnosed by psychiatrists according to the DSM-IV, (*iii*) the study should report the sample size, distribution of *MTHFR* A1298C genotypes, or other information necessary for estimation of the odds ratio (OR) and 95 per cent confidence interval (CI), and (*iv*) the studies should be published. The following exclusion criteria were used: (*i*) studies containing duplicate data, (*ii*) no usable data reported, and (*iii*) case reports, letter to editor, book chapters or reviews.

Data extraction: The following information was extracted from each study - first author's family name, journal name, year of publication, country name, number of cases and controls and the distribution of genotypes. If any author reported the results on different populations, each population was included as an independent study. The allele numbers were calculated from the corresponding genotype distributions.

Statistical analysis: Pooled OR with its corresponding 95 per cent CI were calculated to investigate the association between *MTHFR* A1298C polymorphism and risk of schizophrenia for the allele contrast (C vs. A), homozygote (CC vs. AA), heterozygote (AC vs. AA), recessive (CC vs. AC+AA) and dominant (CC+AC vs. AA) models. The degree of heterogeneity between the studies was assessed by the Q-test based on Chi-squared statistic[36]. We also measured the effect of heterogeneity by another measure, $I^2=100\%\times(Q-df)/Q$[37]. I^2 ranges between 0 and 100 per cent, and I^2 values of 25, 50 and 75 per cent were defined as low, moderate and high estimates of heterogeneity, respectively. The pooled OR was estimated using both fixed effects (FE) and random effects models[38,39]. Whether the genotype distributions of controls were in Hardy–Weinberg

equilibrium (HWE) was evaluated in control genotypes of each study.

Publication bias: Publication bias was estimated by funnel plot and Egger's linear regression test[40]. All statistical analyses were done by software MIX version 1.7[41].

Results

Characteristic analysis of eligible studies: A total of 49 studies were retrieved after PubMed, Google Scholars, Science Direct and Springer Link databases. After reviewing abstracts of all retrieved studies, 24 were excluded and full text of the remaining 25 articles was considered. Of the 25 studies, four were review articles and one was not relevant for present meta-analysis, so five studies were excluded. A total of 19 studies (Fig. 1) that investigated the association between A1298C polymorphism and schizophrenia were found suitable for the inclusion in the present meta-analysis[26-35,42-47]. Two authors[26,33] studied different populations; we included data from each population as an independent study. All 19 studies were performed in different countries - Korea[29,45,46], China[26,30,47], Romania[34,44], Turkey[42,43], Bulgaria[35], Denmark[33], Germany[27], Norway[33], Poland[32], Scotland[26], Spain[28], Sweden[33] and Syria[31] (Table I). Except two studies[27,29], genotype distribution of control population in all studies was in HWE.

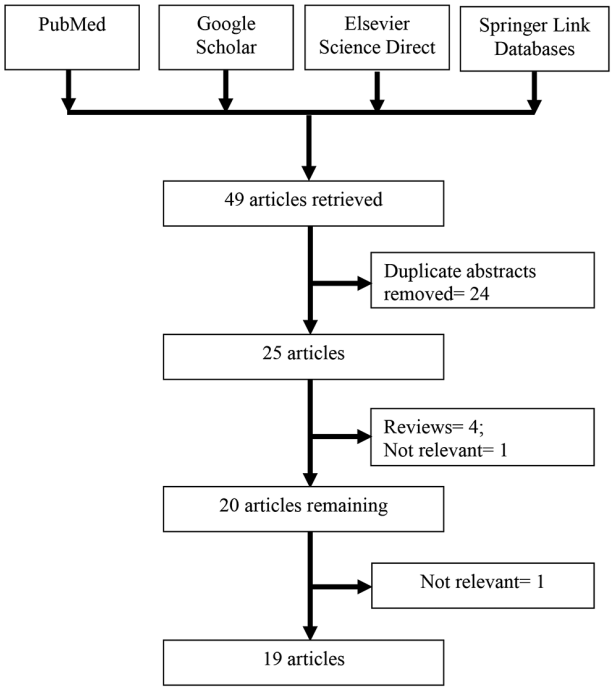

Fig. 1. Flow diagram showing selection of studies.

Table I. Details of included studied (n=19)

Study	Year	Country	Number of cases	Number of controls
Sazci *et al*[42]	2003	Turkey	130	226
Yu *et al*[26]	2004	China	230	251
Yu *et al*[26]	2004	Scotland	426	638
Reif *et al*[27]	2005	Germany	46	184
Sazci *et al*[43]	2005	Turkey	297	341
Vilella *et al*[28]	2005	Spain	158	234
Crisan[44]	2006	Romania	93	85
Lee *et al*[29]	2006	Korea	200	300
Kempisty *et al*[32]	2007	Poland	235	236
Jönsson *et al*[33]	2008	Denmark	382	1004
Jönsson *et al*[33]	2008	Norway	132	177
Jönsson *et al*[33]	2008	Sweden	233	293
Mavros *et al*[34]	2008	Romania	41	40
Betcheva *et al*[35]	2009	Bulgaria	181	183
Kang *et al*[45]	2010	Korea	360	348
Zhang *et al*[30]	2010	PR China	379	380
Kim *et al*[46]	2011	Korea	201	350
Lajin *et al*[31]	2012	Syria	85	126
Zhang *et al*[47]	2012	PR China	235	102

Superscript numerals denote reference numbers

In all 19 studies, total cases were 4049 with AA (2127), AC (1518) and CC (404), and controls were 5488 with AA (2950), AC (2045) and CC (493) genotypes. In controls' genotypes, the percentage of AA, AC and CC were 53.75, 37.26 and 8.98 per cent, respectively. In total cases, genotype percentages of AA, AC and CC were 52.53, 37.49 and 9.98 per cent, respectively (Table II).

The results of the overall meta-analysis and the heterogeneity test are shown in Table III. A1298C polymorphism was significantly associated with schizophrenia under all five models [C vs. A (allele contrast model): OR=1.13, 95% CI=1.02-1.26, *P*=0.02 (Fig. 2); CC vs. AA (homozygote model): OR=1.20, 95% CI=1.03-1.39, *P*=0.02 (Fig. 3); CC vs. AA+AC (recessive model): OR=1.17, 95% CI=1.01-1.35, *P*=0.04; AC vs. AA (co-dominant model): OR=1.13, 95% CI=1.03-1.23, *P*=0.009; CC+AC vs. AA (dominant model): OR=1.14, 95% CI=1.05-1.24, *P*=0.002) (Fig. 4)].

Table II. Methylenetetrahydrofolate reductase A1298C genotypes and allele number for cases and controls in all included studies

Study	Ethnicity	AA		AC		CC		A		C		HWE (*P*)
		Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	
Sazci *et al*[42]	Caucasian	57	114	59	93	14	19	173	321	87	131	0.99
Yu *et al*[26] (China)	Asian	130	154	78	81	22	16	338	389	122	113	0.23
Yu *et al*[26] (Scotland)	Caucasian	177	292	209	272	40	64	563	856	289	400	0.95
Reif *et al*[27]	Caucasian	16	75	21	96	9	13	53	246	39	122	0.01
Sazci *et al*[43]	Caucasian	130	159	129	155	38	27	389	473	205	209	0.2
Vilella *et al*[28]	Caucasian	76	124	68	97	14	13	220	345	96	123	0.28
Crisan *et al*[44]	Caucasian	54	73	39	12	0	0	147	158	39	12	0.48
Kempisty *et al*[32]	Caucasian	109	185	74	105	17	10	292	475	108	125	0.29
Lee *et al*[29]	Asian	157	145	7	14	71	77	321	304	149	168	<0.001
Jonsson *et al*[33] (Denmark)	Caucasian	172	462	168	419	47	123	512	1343	262	665	0.06
Jonsson *et al*[33] (Norway)	Caucasian	67	82	53	79	12	16	187	243	77	111	0.62
Jonsson *et al*[33] (Sweden)	Caucasian	100	122	100	129	33	42	300	373	166	213	0.4
Marvos *et al*[34]	Caucasian	26	17	13	20	2	3	65	54	17	26	0.37
Batcheva *et al*[35]	Caucasian	91	80	72	79	18	24	254	239	108	127	0.52
Kang *et al*[45]	Asian	248	239	105	100	7	9	601	578	119	118	0.7
Zhang *et al*[30]	Asian	230	260	127	108	22	12	587	628	171	132	0.84
Kim *et al*[46]	Asian	129	240	67	105	5	5	325	585	77	115	0.08
Lajin *et al*[31]	Asian	32	65	38	48	15	13	102	178	68	74	0.35
Zhang *et al*[47]	Asian	126	62	91	33	18	7	343	157	127	47	0.37

P value for Hardy–Weinberg equilibrium (HWE) in control group

Table III. Pooled odds ratio of methylenetetrahydrofolate reductase A1298C in different allele and genotype contrasts, *P* value of Q-test, the I^2 metric and *P* value of Egger test in all included studies

Studies	Genetic contrast	OR (95% CI), *P*		Heterogeneity *P* value (Q-test)	I^2 (%)	Publication bias (*P* of Egger's test)
		Fixed effect	Random effect			
All (19 studies)	Allele contrast (C vs. A)	1.12 (1.04-1.19), 0.001	1.13 (1.02-1.26), 0.02	0.002	54.39	0.26
	Co-dominant (AC vs. AA)	1.13 (1.03-1.23), 0.009	1.12 (0.99-1.27), 0.07	0.03	41.74	0.72
	Homozygote (CC vs. AA)	1.20 (1.03-1.39), 0.02	1.27 (1.03-1.56), 0.02	0.06	36.69	0.11
	Dominant (CC + AC vs. AA)	1.14 (1.05-1.24), 0.002	1.15 (1.01-1.29), 0.03	0.009	48.85	0.63
	Recessive (CC vs. AC + AA)	1.17 (1.01-1.35), 0.04	1.22 (1.02-1.47), 0.03	0.13	27.84	0.08
Asian (7 studies)	Allele contrast (C vs. A)	1.15 (1.03-1.29), 0.01	1.17 (0.99-1.37), 0.06	0.07	48.61	0.38
	Co-dominant (AC vs. AA)	1.18 (1.01-1.37), 0.04	1.18 (0.99-1.39), 0.05	0.36	9.51	0.46
	Homozygote (CC vs. AA)	1.22 (0.94-1.58), 0.13	1.34 (0.93-1.93), 0.11	0.15	36.99	0.18
	Dominant (CC+AC vs. AA)	1.17 (1.02-1.35), 0.02	1.18 (0.99-1.41), 0.06	0.17	33.91	0.51
	Recessive (CC vs. AC + AA)	1.19 (0.92-1.53), 0.17	1.22 (0.92-1.62), 0.16	0.35	10.48	0.25
Caucasian (12 studies)	Allele contrast (C vs. A)	1.10 (1.01-1.19), 0.02	1.12 (0.97-1.28), 0.11	0.004	59.66	0.46
	Co-dominant (AC vs. AA)	1.10 (0.99-1.23), 0.08	1.10 (0.92-1.30), 0.32	0.01	53.67	0.94
	Homozygote (CC vs. AA)	1.19 (0.99-1.43), 0.07	1.24 (0.96-1.62), 0.10	0.07	42.22	0.43
	Dominant (CC+AC vs. AA)	1.12 (1.01-1.24), 0.03	1.12 (0.94-1.34), 0.18	0.007	57.39	0.83
	Recessive (CC vs. AC + AA)	1.15 (0.97-1.37), 0.11	1.22 (0.95-1.56), 0.11	0.08	40.57	0.23

OR, odds ratio; CI, confidence interval

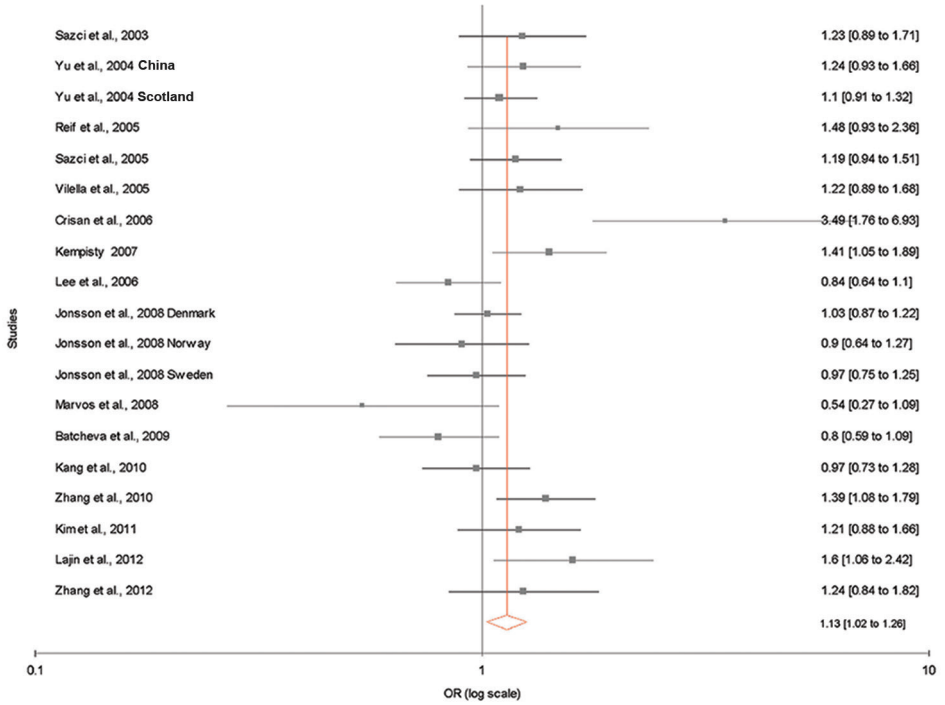

Fig. 2. Random effect forest plot showing a significant association between methylenetetrahydrofolate reductase (*MTHFR)* A1298C polymorphism and risk of schizophrenia using allele contrast model.

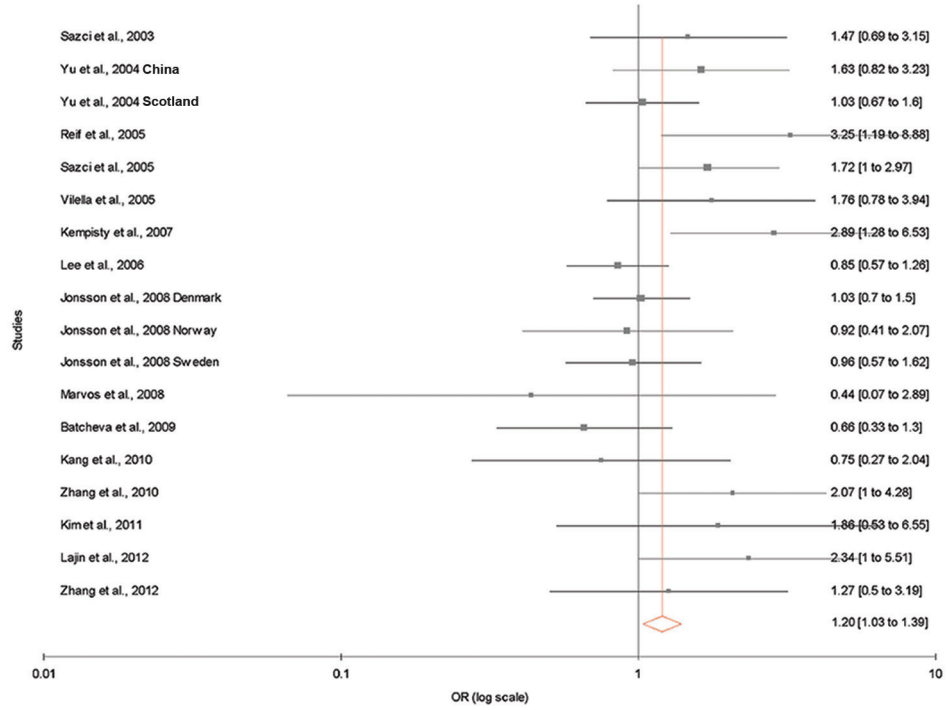

Fig 3. Fixed effect forest plot showing a significant association between methylenetetrahydrofolate reductase (*MTHFR*) A1298C polymorphism and risk of schizophrenia using homozygote model. Only 18 studies that had homozygous mutants were included.

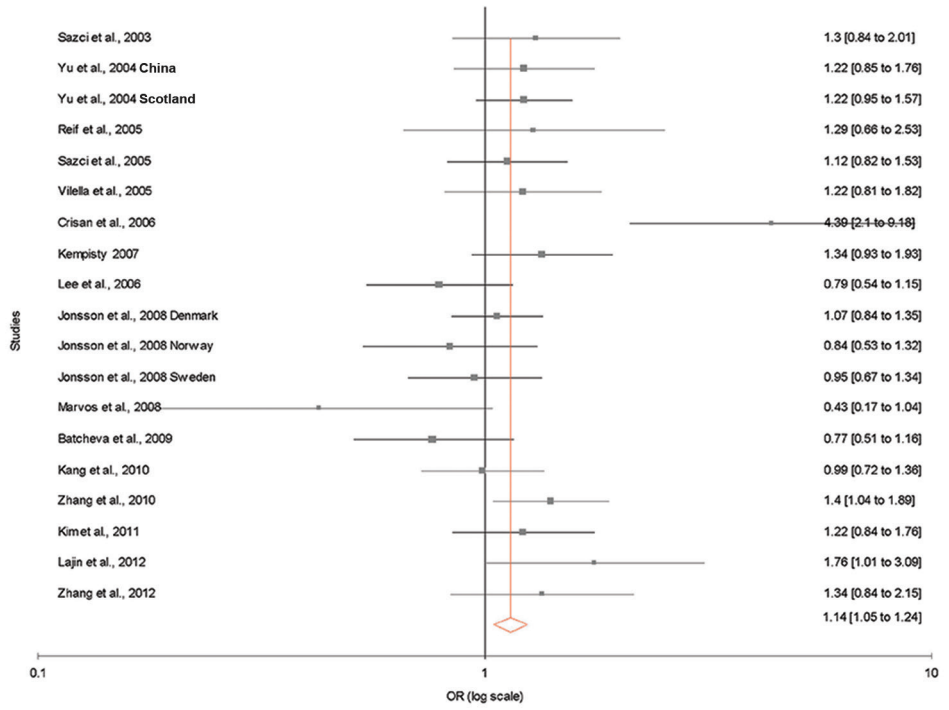

Fig. 4. Forest plots (random effect) showing no association between methylenetetrahydrofolate reductase A1298C polymorphism and risk of schizophrenia using dominant model (CC+AC vs. AA).

Subgroup analysis: Subgroup analysis based on ethnicity was performed. Of the 19 studies included in the present meta-analysis, seven were from Asian, and 12 from Caucasian populations. In Asian population, allele contrast meta-analysis showed significant association adopting fixed (OR=1.15; 95% CI=1.03-1.29; P=0.01; I^2=48.61%; $P_{heterogeneity}$=0.07; P_{Pb}=0.38) effect model. Combined mutant genotypes also showed significant association with fixed (OR=1.17; 95% CI=1.02-1.35; P=0.02) and random (OR=1.18; 95% CI=0.99-1.41; P=0.06) effect models. In this subgroup, heterogeneity between studies (I^2=33.91%; $P_{heterogeneity}$=0.17) was moderate and publication bias (P_{Pb}=0.51) was absent (Table III).

In Caucasian population, allele contrast meta-analysis showed significant association with FE model (OR=1.10; 95% CI=1.01-1.19; P=0.02) with significantheterogeneity(I^2=59.66%;$P_{heterogeneity}$=0.004). The combined mutant genotype showed significant associationwithFEmodel(OR=1.12;95%CI=1.01-1.24; P=0.03) with moderate heterogeneity (I^2=57.39%; $P_{heterogeneity}$=0.007) and no publication bias (P_{Pb}=0.23) (Table III).

Publication bias: Publication bias was not observed as funnel plots were symmetrical (Fig. 5A-F). In addition, results of Eggers also did not suggest any evidence of publication bias (P=0.77 for C vs. A with additive

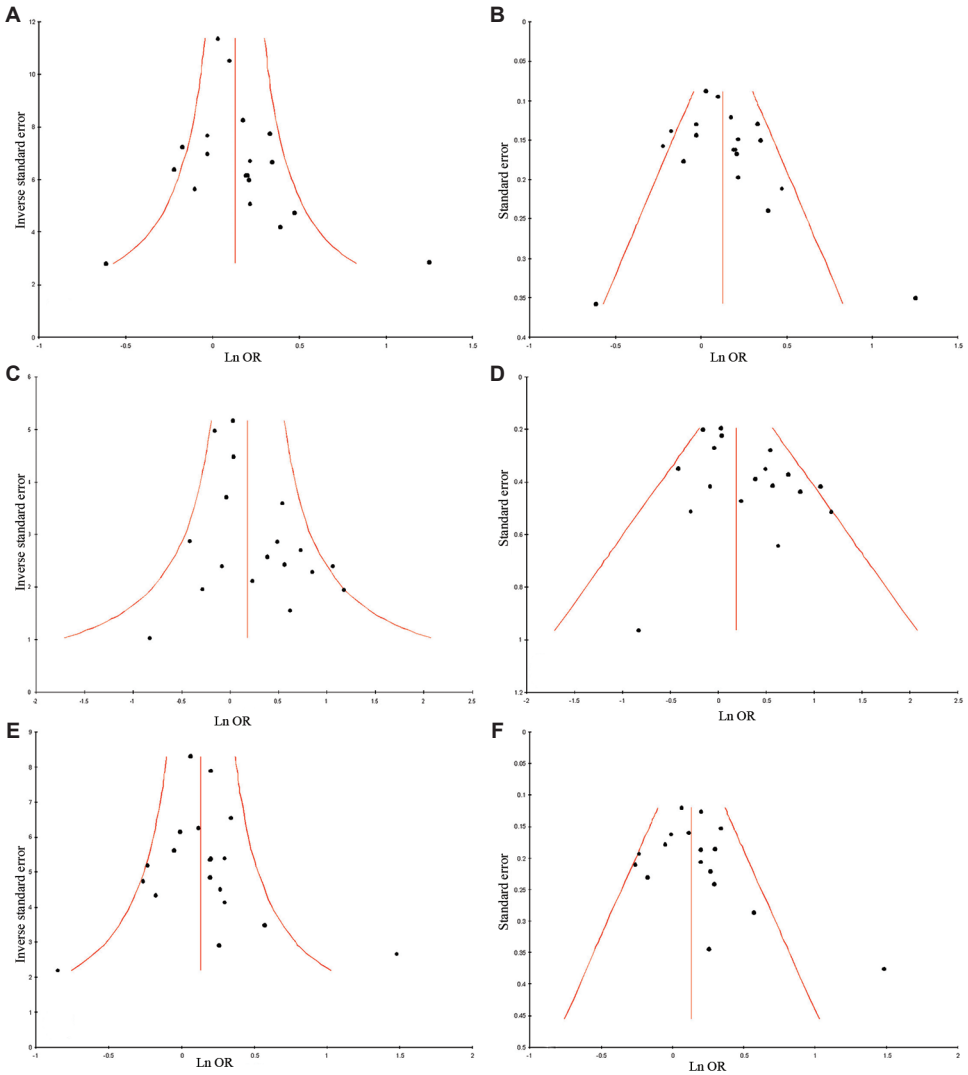

Fig. 5. (**A**) Funnel plot of precision by log odds ratio (allele contrast model); (**B**) Funnel plot of standard error by log odds ratio (allele contrast model); (**C**) Funnel plot of precision by log odds ratio (homozygote model); (**D**) Funnel plot of standard error by log odds ratio (homozygote model); (**E**) Funnel plot of precision by log odds ratio (dominant model); (**F**) Funnel plot of standard error by log odds ratio (dominant model).

model; P = 0.93 for AC vs. AA with co-dominant model; P=0.41 for CC vs. AA with homozygote model; P=0.66 for CC+AC vs. AA with dominant model; P=0.26 for CC vs. AA+AC with recessive model).

Discussion

Several obstetric complications such as foetal hypoxia, maternal stress, infection, bleeding during pregnancy and pre-eclampsia are the risk factors for psychiatric disorders in offspring[48-53]. In addition, perinatal folate deficiency and higher plasma concentration of homocysteine in mother have also been reported as a risk factor for schizophrenia[54]. Folate supplies the substrate for MTHFR reaction. During reduced serum folate and dysfunctional MTHFR, there is a profound deficit in MTHFR-dependent cellular and physiological processes. MTHFR enzyme activity is reduced in heterozygous (AC) and homozygous (CC) individuals, which leads to hyperhomocysteinemia[22].

Folate deficiency along with *MTHFR* polymorphism leads to DNA hypomethylation[55-58], and abnormal embryogenesis, which results into developmental malformation in foetus. The neurons in the developing brain are vulnerable to the effect of folate deficiency[59]. Two main metabolic pathways of homocysteine elimination, *i.e.*, betaine remethylation and transsulfuration are not found in brain[60,61]. Hence, MTHFR hypofunction/folate deficiency increases the concentration of homocysteine in brain which is toxic to neurons and blood vessels and can induce DNA strand breakage, oxidative stress and apoptosis[61-67].

Several meta-analyses have been published accessing folate pathway genes polymorphism as risk factor for various diseases/disorders such as neural tube defects[68], cleft lip and palate[69], Down syndrome[70], hyperurecemia[71], autism[72], bipolar disorder[73], depression[74], Alzheimer's disease[75], recurrent pregnancy loss[76] and cancer[77,78]. Twelve meta-analyses have been published regarding *MTHFR* polymorphisms and schizophrenia[33,46,72,79-87]. Of these, in only four meta-analyses *MTHFR* A1298C polymorphism has been investigated as a risk factor for schizophrenia[72,79,81,85]. Zintzaras[81] pooled nine studies for A1298C meta-analysis and reported that the A1298C association with schizophrenia was only marginally significant with a fixed effect (FE) OR of 1.16 (95% CI=1.09-1.331). Gilbody *et al*[72] performed a meta-analysis of only two studies examining the association between polymorphisms A1298C and schizophrenia and reported an association with fixed-effects [OR (CC vs. AA)=1.64 95% CI=1.04-2.54], with low heterogeneity (I^2=0%). Peerbooms *et al*[85] conducted a meta-analysis of 10 published case-control studies investigating associations between *MTHFR* A1298C and schizophrenia and reported that schizophrenia was not significantly associated with A1298C polymorphism. Hu *et al*[86] pooled 12 studies and reported a significant association between A1298C polymorphism and schizophrenia (OR=1.13, 95% CI 1.03-1.24).

The present meta-analysis had several strengths along with limitations. The main strength of the present meta-analysis was that the publication bias was not detected and pooled number of cases and controls from different studies significantly increased the statistical power of the analysis. The limitations of the present meta-analysis were (*i*) use of crude OR, (*ii*) small sample size (9537 samples), (*iii*) inclusion of studies with small sample size, (*iv*) only one gene *MTHFR* and one polymorphism (A1298C) of folate pathway considered, and (*v*) gene-gene interactions not considered.

In conclusion, the present meta-analysis indicated that A1298C polymorphism was associated with schizophrenia risk. In future, several case-control studies with larger samples size are required to investigate gene-gene and gene-environment interactions involving the *MTHFR* A1298C polymorphism to determine the susceptibility for schizophrenia.

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***Conflicts of Interest*:** None.

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