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REVIEW ARTICLE

Folate Pathway Gene Methylenetetrahydrofolate Reductase C677T Polymorphism and Alzheimer Disease Risk in Asian Population

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Abstract The association between methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism and susceptibility to Alzheimers disease (AD) was controversial in previous studies. The present meta-analysis was designed to investigate the association of MTHFR C677T polymorphism with AD. Nine studies were identified by search of PubMed, Google Scholar, Elsevier, Springer Link databases, up to January 2013. Odds ratios (ORs) with corresponding 95 % confidence interval (CI) were calculated using fixed effects model or random effects model. All statistical analysis was done by Mix version 1.7. MTHFR C677T polymorphism had a significant association with susceptibility to AD in all genetic models (for T vs C: OR 1.29, 95 % CI 1.15–1.44, $p < 0.0001$; for TT + CT vs CC: OR 1.38, 95 % CI 1.16–1.364, $p = 0.0002$; for TT vs CC: OR 1.60, 95 % CI 1.25–2.04, $p = 0.0001$; for CT vs CC: OR 1.28, 95 % CI 1.1–1.53, $p < 0.008$; for TT vs CT + CC: OR 1.37, 95 % CI 1.12–1.67, $p = 0.002$). Results from present meta-analysis supported that the MTHFR C677T polymorphism was associated with an increased risk of AD in Asian population.

Keywords Alzheimers disease · Neurodegeneration · MTHFR · C677T · Homocysteine · Folate

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Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder and is the leading cause of dementia in the elderly [1]. It is a genetically complex disorder that causes progressive memory loss in mid-to-late adult life. Some individuals inherit this form of dementia before the age of 65 (referred to as early-onset or familial AD); but most often, AD occurs late in life [2]. AD results from selective degeneration of neurons in neocortex, hippocampus, and basal forebrain cholinergic system. Affected regions show senile plaques, comprised of neurites displayed around extracellular deposits of beta-amyloid peptides; and many neurons develop neurofibrillary tangles were developed in neurons due to accumulation of abnormal intracytoplasmic filaments, composed of hyperphosphorylated tau protein [3]. In the quest for genetic risk factors for late-onset AD only APOE gene has been convincingly identified. Recently clinical and epidemiological studies show a relation between vascular disorders and late-onset AD [4]. Vascular risk factors such as diabetes, hyperlipidemia, high serum homocysteine (Hcy), hypertension and heart diseases are reportedly candidate risk factors for AD. Elevated levels of Hcy have been linked to several vascular diseases [5] and AD [6]. Cell culture experiments have shown that Hcy can cause brain damage through several mechanisms such as impairment of DNA repair, enhancement of beta-amyloid peptide generation and by sensitization of neurons to amyloid toxicity [7, 8].

There are nutritional, hormonal, and genetic determinants of Hcy level. Metabolism of Hcy requires enzymatic action, involving mainly the methylenetetrahydrofolate reductase (MTHFR) and vitamins, namely folic acid, B6, and B12. MTHFR mediates the irreversible conversion of 5,10-methylenetetrahydrofolate (5,10-MTHF) to

5-methyltetrahydrofolate (5-MTHF). Several MTHFR gene polymorphisms have been identified. A common genetic variation in the MTHFR gene, in which cytosine is replaced by thymidine at base position 677 [9] is associated with reduced enzyme activity (60 %) and results in approximately 20 % rise in serum Hcy levels [10]. The T-allele frequency ranges from 0.20 to 0.55 in Europeans, 0.11 to 0.35 in Americans, 0.063 to 0.094 in Africans, and from 0.04 to 0.38 in Asian population [11–13]. MTHFR functions in dimeric form and FAD works as a co-factor for this enzyme, but variant MTHFR (222 V) dissociates into monomers and its enzymatic activity reduces. It is established by docking study that the mutant enzyme (222 V) shows less affinity towards FAD than wild enzyme (222 A) [14]. Some studies showed that the T allele and TT genotype (or the T allele) increases Hcy levels (particular in folate deficiency state) [9, 15, 16], whereas others did not [17]. Its impact on AD development has also been controversial [18, 19]. The results of case control studies on role of MTHFR gene polymorphism in the pathogenesis of AD have been conflicting and inconclusive, hence present meta-analysis was designed to shed some light on the association of MTHFR C677T polymorphism and risk of AD disease in Asian population.

Methods

Identification of Studies

A systematic search was done on published literature using the keywords 'MTHFR and AD', 'folate metabolism and AD', 'MTHFR C677T polymorphism and AD' through 'Pubmed', 'Elsevier', 'Springer Link' and 'Google Scholar' up to January, 2014. Detailed information for each study on C677T polymorphism in AD such as the purpose and design of the study, presentation of the data, genotyping method used, inclusion and exclusion criteria of the cases and controls was collected.

Inclusion and Exclusion Criteria

The following inclusion criteria were set for the meta-analysis: (i) each study should be an independent case–control study; (ii) study should have enough information to calculate the odds ratio; (iii) inclusion of the patients was done according to the standard diagnosis parameter [Diagnostic and Statistical Manual of Mental Disorders (DSM-IV), Mini Mental State Examination (MMSE)]. The exclusion criteria included: (i) study not providing enough information (incomplete raw data) and (ii) not well-described.

Data Extraction

Genotype data for MTHFR C677T polymorphism related to AD was extracted. Following information were extracted from each included article- first author name, year of publication, ethnicity of study population, country name, number of cases and controls and allele and genotype frequency was collected.

Statistical Analysis

The present meta-analysis examined the overall association of mutant T allele with the risk of AD relative to the C allele. The strength of association between the MTHFR C677T polymorphisms and AD risk was evaluated by OR and 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. A pooled OR was estimated on the basis of the individual ORs. The OR was estimated by using fixed effects [20] and random effects [21] model depending upon heterogeneity. When there is considerable heterogeneity between studies then the pooled OR is preferably estimated using the RE model [22, 23]. The heterogeneity between studies was tested using the Q-statistic and heterogeneity between studies was quantified using the I^2 statistic [24]. If $I^2 > 50$ % then random effect model was used (which gives wider confidence intervals) otherwise fixed effect model applied. Genotype data for control population was analyzed for fitness in the Hardy–Weinberg Equilibrium. For this purpose, control data was analyzed using calculator available at http://ihg.gsf.de/cgi-bin/hw/hwa1.pl.

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/SE) by log odds ratio. Different statistical tests such as Begg and Mazumdar rank correlation [25] and Egger's regression intercept [26] 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 [27].

Result

Study characteristics were summarized in Table 1. Total nine articles that investigated the association between C677T polymorphism and AD were found suitable for the

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

Study	Country	Control	Case	Reference
Chapman et al. [18]	Israel	40	49	Stroke 29:1401–1404
KIDA et al. [28]	Japan	379	194	Psychogeriatrics 4:4–10
Wang et al. [19]	China	130	104	Stroke 29:1401–1404
Zhang et al. [29]	China	102	105	Chin Med Sci J 20:247–251
Keikhee et al. [30]	Iran	125	117	Neurochem Res 31:1079–1083
Kim et al. [31]	Korea	625	86	Int J Geriatr Psychiatry 23:454–459
Bi et al. [32]	China	375	386	Neurobiol Aging 30:1601–1607
Mansoori et al. [33]	India	120	80	Clin Neurol Neurosurg 115: 1693–1696
Divyakolu et al. [34]	India	50	25	Neurol Disord 2(1):1000142

inclusion in the present meta-analysis [18, 19, 28–34]. The studies were published between 1998 and 2013. All these nine studies were performed in different countries—China [19, 29, 32], India [33, 34], Iran [30], Israel [18], Japan [28], and Korea [31].

In all nine studies, total cases were 1146 with CC (385), CT (509) and TT (252), and controls were 1946 with CC (699), CT (879), and TT (368) genotypes. In controls genotypes, percentage of CC, CT and TT were 35.92, 45.17, and 18.91 % respectively. In total cases, genotype percentage of CC, CT, and TT was 33.59, 44.42 and 21.99 % respectively. Frequencies of CC and CT genotypes were highest in both cases and controls (Table 2). In cases and controls, the allele C was the most common. The genotype distributions among the controls of all studies followed Hardy–Weinberg equilibrium.

Meta-analysis

Meta-analysis with allele contrast showed significant association with fixed effect (OR_{TvsC} 1.29; 95 % CI 1.15–1.44; p = <0.0001; I^2 = 39.97 %; $P_{heterogeneity}$ = 0.10) (Fig. 1) and random effect model (OR_{TvsC} 1.34; 95 %CI 1.13–1.58; p = 0.0005) (Table 3).

There was evidence of association of the MTHFR TT genotype with the risk of AD relative to the MTHFR CC genotype with both fixed effect (OR_{TTvsCC} 1.60; 95 % CI 1.25–2.04; p = 0.0001; I^2 = 39.65 %; $P_{heterogeneity}$ = 0.10) (Fig. 2) and random effect (OR_{TTvsCC} 1.74; 95 % CI 1.2–2.5; p = 0.003) models. There was significant association of the MTHFR CT genotype with the risk of AD relative to the CC genotype using fixed and random effect models (OR_{CTvsCC} 1.28; 95 % CI 1.1–1.53; p = 0.008; I^2 = 0; $P_{heterogeneity}$ = 0.49). Dominant model (TT + CT vs CC) also showed significant association with fixed effect ($OR_{TT + CTvsCC}$ 1.38; 95 % CI 1.16–1.64; p = 0.0002; I^2 = 5.19 %; $P_{heterogeneity}$ = 0.39) and random effect ($OR_{TT + CTvsCC}$ 1.39; 95 % CI 1.16–1.66; p = 0.0004) models (Fig. 3). Recessive model also showed significant association with AD with fixed effect ($OR_{TTvsCT + CC}$ 1.37; 95 % CI 1.12–1.67; p = 0.002; I^2 = 46.03 %; $P_{heterogeneity}$ = 0.06) and random effect ($OR_{TTvsCT + CC}$ 1.48; 95 % CI 1.06–2.07; p = 0.02) models.

Publication bias

Begg's funnel plot and the Egger's test were conducted to estimate the publication bias of articles. Both the results of

Table 2 The distributions of MTHFR C677T genotypes and allele numbers in Alzheimer patients and healthy controls in Asian studies

Study ID	Genotype						Alleles			
	CC		CT		TT		C		T	
	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control
Chapman et al. [18]	12	15	31	16	6	9	53	46	43	34
KIDA et al. [28]	64	144	98	193	32	42	226	481	162	277
Wang et al. [19]	50	79	38	47	16	4	138	205	70	55
Zhang et al. [29]	39	34	45	49	21	19	123	117	87	87
Keikhee et al. [30]	57	84	44	33	16	8	158	201	76	49
Kim et al. [31]	11	122	43	332	32	171	65	576	107	674
Bi et al. [32]	82	90	179	172	125	113	343	352	429	398
Mansoori et al. [33]	51	89	26	29	3	2	128	207	32	33
Divyakolu et al. [34]	19	42	5	8	1	0	43	92	7	8

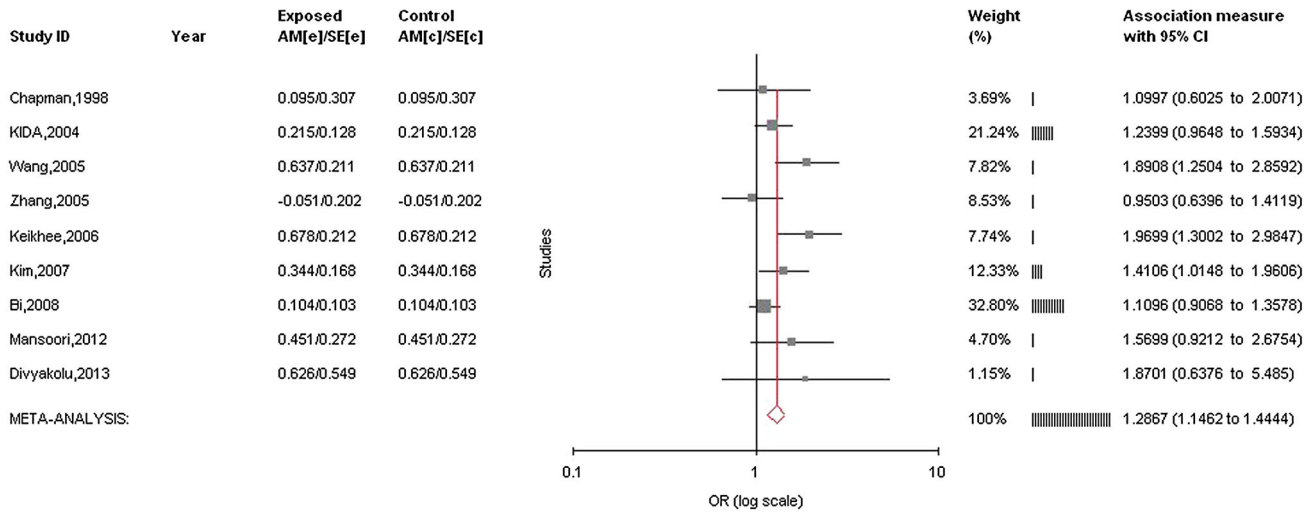

Fig. 1 Forest plots for the association between MTHFR C677T polymorphism and Alzheimers disease for allele contrast model (T vs C) with fixed effect model in Asian population

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), I^2 metric and publication bias p value (Egger Test) in Asian population

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)	1.29 (1.15–1.44), <0.0001	1.34 (1.13–1.58), 0.0005	0.10	39.97	0.20
Dominant (TT + CT vs CC)	1.38 (1.16–1.64), 0.0002	1.39 (1.16–1.66), 0.0004	0.39	5.19	0.26
Homozygote (TT vs CC)	1.60 (1.25–2.04), 0.0001	1.74 (1.2–2.5), 0.003	0.10	39.65	0.16
Co-dominant (CT vs CC)	1.28 (1.1–1.53), 0.008	1.28 (1.1–1.53), 0.009	0.49	0	0.22
Recessive (TT vs CT + CC)	1.37 (1.12–1.67), 0.002	1.48 (1.06–2.07), 0.02	0.06	46.03	0.23

Begg's and Egger's test did not show any evidence of publication bias (T vs C Begg's test, $p = 0.25$, Egger's test, $p = 0.20$; CT vs CC Begg's test, $p = 0.25$, Egger's test, $p = 0.22$; TT vsCC Begg's test, $p = 0.17$, Egger's test, $p = 0.16$; dominant model TT + CT vs CC, Begg's test, $p = 0.92$, Egger's test, $p = 0.26$; recessive model TT vs CT + CC, Begg's test, $p = 0.60$, Egger's test, $p = 0.23$)(Fig. 4).

Discussion

Folate is an essential ingredient of one-carbon metabolism, which is required for the remethylation of Hcy. Several epidemiological and experimental studies have linked folate deficiency and resultant increased Hcy levels with AD. Polymorphism of folate metabolizing gene MTHFR C677T may predispose individuals to elevated plasma levels of Hcy [35, 36]. MTHFR is an essential enzyme in the folate mediated one-carbon transfer reactions and any genetic deficiency in this enzyme could induce severe hyperhomocysteinemia [37]. Elevated Hcy has been reported to be a risk factor for several psychiatric and neurodevelopmental disorders like neural tube defects, depression, bipolar disorder and schizophrenia.

Hcy is implicated in increased oxidative stress, DNA damage, the triggering of apoptosis and excitotoxicity, all important mechanisms in neurodegeneration [37, 38]. It is rapidly taken up by neurons through a specific membrane transporter, leading to high intracellular levels of Hcy [38]. Brain may be particularly vulnerable to high level of Hcy in the blood because it lacks two major metabolic pathways for its elimination: betaine remethylation and transsulfuration [38]. Several mechanisms are proposed by which Hcy causes AD—(i) Hcy can induce calcium influx and oxidative stress, generate free oxygen radicals, accelerate DNA damage and eventually lead to neuron apoptosis [39], (ii) Hcy participates in the amyloid pathway that is involved in the pathophysiology of AD and (iii) Hcy impairs vascular endothelial function, stimulates vascular smooth muscle proliferation [39], breaks balance between coagulation and bleeding pathway and mediates thrombosis. Ultimately, these adverse effects reduce blood supply to the brain and accelerate the neurons apoptosis [39].

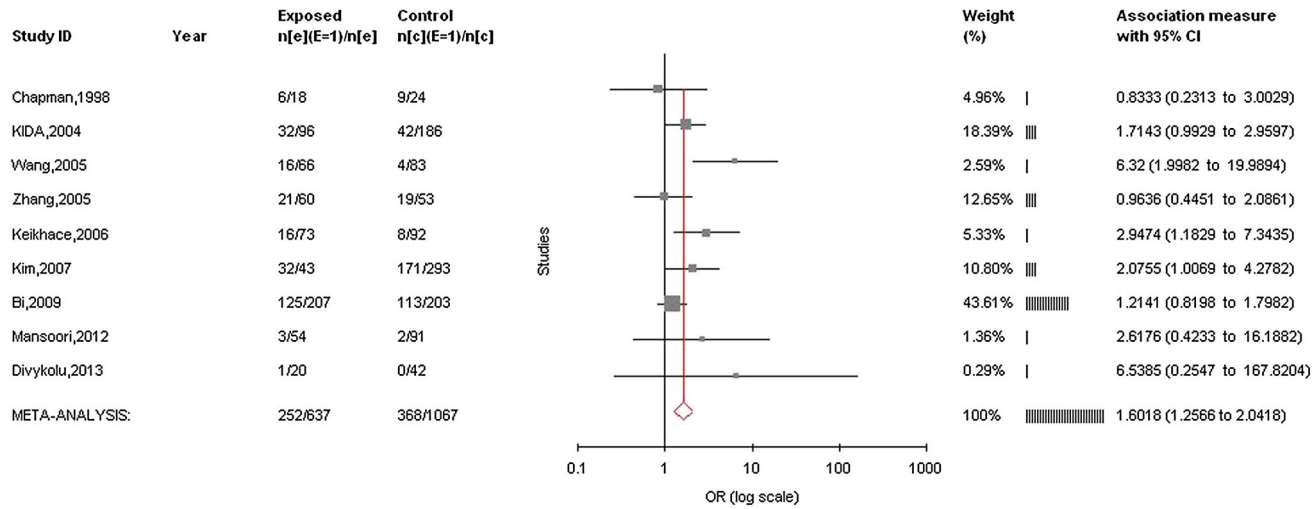

Fig. 2 Forest plots for the association between MTHFR C677T polymorphism and Alzheimers disease for homozygote model (TT vs CC) with fixed effect model in Asian population

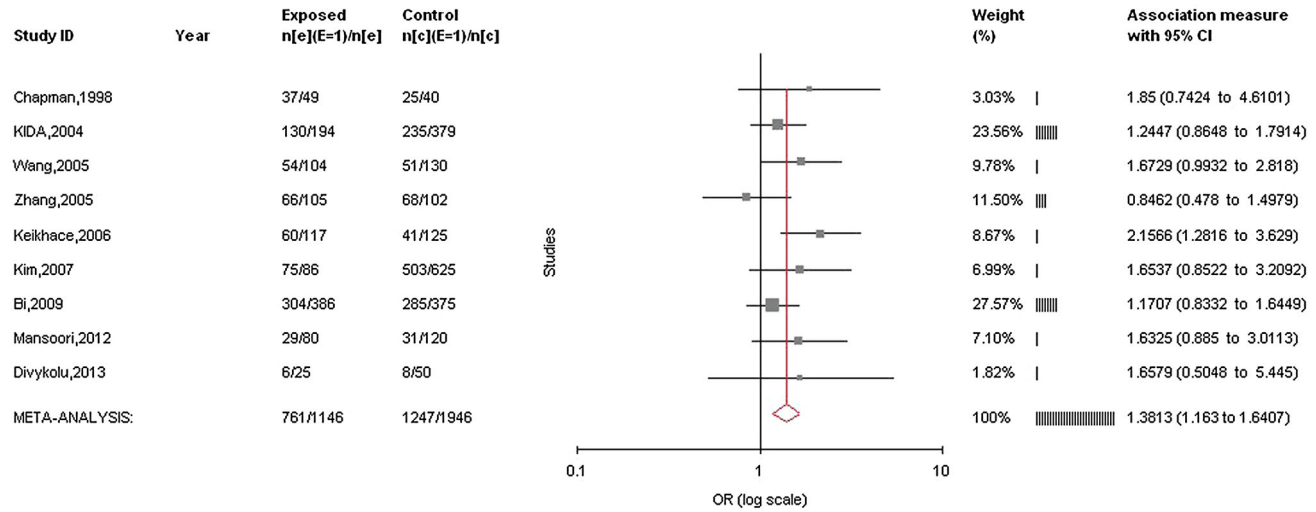

Fig. 3 Forest plots for the association between MTHFR C677T polymorphism and Alzheimers disease for dominant model (TT + CT vs CC) with fixed effect model in Asian population

Meta-analysis is usually used to combine comparative studies to enlarge the sample size and statistical power and reach more obvious conclusion. Numerous meta-analyses were published considering MTHFR C677T polymorphism as risk for several diseases like—neural tube defects [40], Down syndrome [41], cleft lip with/without palate [42], recurrent pregnancy loss [43], stroke [44], schizophrenia [45], bipolar [46], depression [45], migraine [47] and vascular dementia [48] etc.

Some limitations of present meta-analysis should be acknowledged like—(i) crude OR is used, (ii) study number (only nine studies) and sample size are small, (iii) controls were not uniform in all included studies, (iv) since only the papers published in English language were included in the present meta-analysis, selection bias may have occurred, (iv) other risk factors that have relativity to MTHFR gene or AD, such as hypertension, coronary artery disease and cancer should also be considered and (iv) gene–gene or gene-environment interactions may modify the AD risk and; however, such stratified analysis could not be performed owing to lack of data.

In conclusion, results of present meta-analysis support a strong overall significantly increased risk of AD associated with the C677T polymorphism. However, a very large number of subjects are needed to establish the genetic association between the C677T polymorphism and migraine.

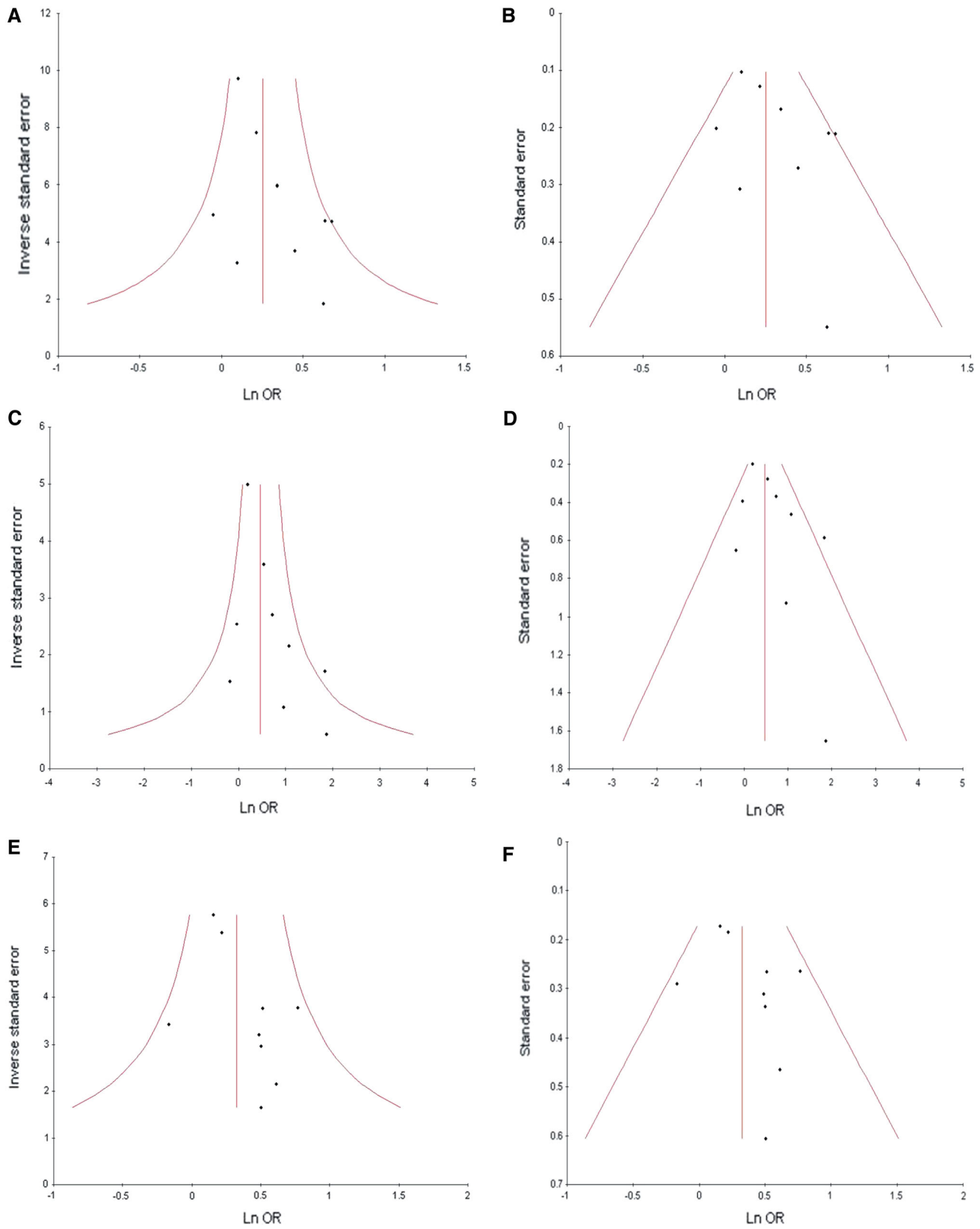

Fig. 4 Funnel plots. **a** Precision versus OR for additive model (T vs C), **b** SE versus OR for additive model (T vs C), **c** precision versus OR for TT versus CC, **d** SE versus OR for TT versus CC, **e** precision versus OR for TT + CT versus CC, **f** SE versus OR for TT + CT versus CC

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