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REVIEW ARTICLE

# Association of methylenetetrahydrofolate reductase (*MTHFR*) gene C677T polymorphism with autism: evidence of genetic susceptibility

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**Abstract** Autism (MIM 209850) is a heterogeneous neurodevelopmental disease that manifests within the first 3 years of life. Numerous articles reported that dysfunctional folate-methionine pathway enzymes may play an important role in the pathophysiology of autism. Methylenetetrahydrofolate reductase (*MTHFR*) is a critical enzyme of this pathway and *MTHFR* C677T polymorphism reported as risk factor for autism in several case control studies. However, controversial reports were also published. Hence the present meta-analysis was designed to investigate the relationship of the *MTHFR* C677T polymorphism with the risk of autism. Electronic databases were searched for case control studies with following search terms - '*MTHFR*', 'C677T', in combination with 'Autism'. Pooled OR with its corresponding 95 % CI was calculated and used as association measure to investigate the association between *MTHFR* C677T polymorphism and risk of autism. Total of thirteen studies were found suitable for the inclusion in the present meta-analysis, which comprises 1978 cases and 7257 controls. Meta-analysis using all four genetic models showed significant association between C677T polymorphism and autism ($OR_{Tvs.C}$ = 1.48; 95 % CI: 1.18–1.86; $P$ = 0.0007; $OR_{TT+CT\ vs.\ CC}$ = 1.70, 95 % CI = 0.96–2.9, $p$ = 0.05; $OR_{TT\ vs.\ CC}$ = 1.84, 95 % CI = 1.12–3.02, $p$ = 0.02; $OR_{CT\ vs.CC}$ = 1.60, 95 % CI = 1.2–2.1, $p$ = 0.003; $OR_{TT\ vs.CT+CC}$ = 1.5, 95 % CI = 1.02–2.2, $p$ = 0.03). In total 13 studies, 9 studies were from Caucasian population and 4 studies were from Asian population. The association between C677T polymorphism and autism was significant in Caucasian ($OR_{Tvs.C}$ = 1.43; 95 % CI = 1.1–1.87; $p$ = 0.009) and Asian population ($OR_{Tvs.C}$ = 1.68; 95 % CI = 1.02–2.77; $p$ = 0.04) using allele contrast model. In conclusion, present meta-analysis strongly suggested a significant association of the *MTHFR* C677T polymorphism with autism.

**Keywords** Autism · *MTHFR* · Meta-analysis · Homocysteine · Methylation · C677T polymorphism

## Introduction

Autism is a complex neurodevelopmental disorder marked by deficits in social communication and interaction and by restricted, repetitive behaviors, interests, or activities (Schendel et al. 2016). Centers for Disease Control and Prevention (CDC 2012) reported the prevalence rate of autism spectrum disorder (ASD) as 1 in 68 children (1 in 42 boys and 1 in 189 girls). A number of factors such as genetic, environmental, autoimmune function, oxidative stress, and inflammatory biomarkers have been implicated in the etiology of ASD (Ali et al. 2011). Family and twin studies have conclusively described autism as a highly heritable neuropsychiatry disorder (Bailey et al. 1995).

Folate plays an important role in neurological development because it act as a methyl group transporter (Meguid et al. 2015). Folate-methionine pathway is crucial for DNA methylation and synthesis and also for redox balance in cell. Several studies reported that folate-methionine pathway was defective in autism patients (Selhub and Rosenberg 1996; Barbato et al. 2007; Main et al. 2010). Folate facilitates methionine synthesis from homocysteine by acting as a cofactor for methylenetetrahydrofolate reductase (*MTHFR*) enzyme, which converts 5,10-methylenetetrahydrofolate (CH2THF) to 5-methyltetrahydrofolate (CH3THF). Sufficient folate and vitamin B reduce levels of homocysteine, while deficiency of B vitamins can cause hyperhomocysteinemia, (Flickera et al.

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2004; Almeida et al. 2005). Homocysteine in the body is metabolized along two pathways, remethylation to methionine or transsulfuration to cysteine (Miller 2003; James et al. 2004).

*MTHFR* is the crucial enzyme in folate-methionine metabolism and acts at the crossroads between DNA methylation and DNA synthesis. Decreased activity of *MTHFR* enzyme due to polymorphism may affect DNA synthesis and methylation (Ryan and Weir 2001; James et al. 2004) and favored increase levels of plasma homocysteine (Hcy) (James et al. 2004; Pasca et al. 2009). High levels of plasma Hcy and increased oxidative stress have generally been associated in the pathophysiology of many neuropsychiatric disorders including Autism (Suh et al. 2008; Tu et al. 2010).

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, resulted in substitution of alanine amino acid by valine at position 222 in MTHFR protein (Frosst et al. 1995). C677T polymorphism is associated with reduced enzyme activity (60 %) and results in approximately 20 % rise in serum Hcy levels (Frosst et al. 1995; Christensen et al. 1997; Devlin et al. 2006). *MTHFR* functions in dimeric form and flavin adenine dinucleotide (FAD) works as a co-factor for this enzyme, but variant *MTHFR* enzyme dissociates into monomers and its enzymatic activity reduces. Mutant T allele frequency range approximately from 0.24 to 0.44 in European and Caucasian populations, 0.06 in an African population, and 0.35 to 0.41 in Asian populations (Pepe et al. 1998; Botto and Yang 2000; Rai et al. 2012). The frequency of homozygosity (TT) ranges from 1 % in US African-American populations to more than 20 % in US Latinos; 5 % to 30 % in White populations in Europe and North America; 32.2 % in Mexico; 5.8 % in White Canadians in Alberta to 14.3 % in those in Quebec, Canada; 0.0 % in Sub-Saharan Africa; 10.7 % in Oceania; 11.5 % in Japanese and 16 % in Chinese (Botto and Yang 2000).

Several independent case control studies have investigated the role of the *MTHFR* C677T polymorphism in autism risk (Guo et al. 2012; Park et al. 2014), but the results are inconclusive. Hence, to estimate the overall risk of the C677T polymorphism for autism, a meta-analysis was performed on all published case-control studies.

## Method

Meta-analysis was carried out according to MOOSE guidelines (Stroup et al. 2000).

### Search strategy and identification of studies

Eligible studies were identified by searching PubMed, Google Scholar, Elsevier and Springer link databases. Following search terms were used: "*MTHFR*", "methylenetetrahydrofolate reductase", "C677T", and "polymorphism" in combination with "Autism" up to January 15, 2016.

### Inclusion/exclusion criteria

The inclusion criteria were as follows: studies should: 1) be original, 2) used case control approach, 3) be reported genotype frequency of cases and controls, and if not, the text provided data enabling such calculations, 4) used standard diagnostic criteria for autism patient diagnosis (Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) or the International Classification of Diseases (ICD)).

Studies were excluded if: 1) their sample was not independent from that investigated in another study, 2) incomplete raw data/information and not providing complete information for number of genotype and/or allele number calculation, 3) studies based on pedigree and 4) review, letter to editors and book chapters. If more than one study was published using the same dataset, the most recent study, or the study with the larger sample size, was selected.

### Data extraction

The following information was extracted from the each identified studies: the first author family name, year of publication, sample size, country name, ethnicity, genotyping method, the numbers of patients and controls, and *MTHFR* C677T genotypes information and frequencies of alleles in all study. If important information was not given in the article, the relevant information was obtained by contacting authors.

### Statistical analysis

The strength of association between the C677T polymorphism of *MTHFR* and autism risk was estimated by the odds ratio (OR) along with its 95 % confidence interval (CI). Heterogeneity among studies was examined with the $\chi^2$ test-based Q statistics (Lau et al. 1997) and $P < 0.05$ was considered significant. To quantify heterogeneity '$I^2$' index was used, which calculated as the percentage of the total variability in a set of effect sizes due to true heterogeneity (Higgins and Thompson 2002) and '$I^2$' > 50 % is considered high heterogenity (Whitehead 2002). Fixed and random-effects summary ORs were calculated using the Mantel-Haenszel and DerSimonian and Laird methods (Mantel and Haenszel 1959; DerSimonian and Laird 1986), random-effect summary ORs was used, when there was higher heterogeneity. The pooled ORs were performed for the allele contrasts/additive model (T versus C), homozygote model (TT versus CC), recessive model (TT versus CT+ CC), dominant model (TT + CT versus CC), and co-dominant/heterozygote model (CT

versus CC). Control population of each study was tested for Hardy-Weinberg Equilibrium (HWE) using the $\chi^2$ test.

Sensitivity analysis was performed to evaluate the stability of the results by removing the studies not in Hardy–Weinberg equilibrium (HWE), and studies with small sample size. Cumulative meta-analysis was performed to see the effect of subsequent addition of each study. The publication bias was evaluated by a Begg's test and Egger's linear regression test (Begg and Mazumdar 1994; Egger et al. 1997). All statistical analysis was undertaken using MIX program version 1.7 (Bax et al. 2006). *P* values were two-tailed with a significance level of 0.05.

## Results

The literature search and detailed study selection procedures were presented in Fig. 1. The search strategy retrieved 28 studies from PubMed, Google Scholar, Elsevier and Springer Link databases. However, most of them were excluded after reviewing titles and abstracts, leaving 19 for full text review. Finally, 13 studies (Boris et al. 2004; James et al. 2006; Mohammad et al. 2009; Pasca et al. 2009; dos Santos et al. 2010; Liu et al. 2011; Schmidt et al. 2011; Guo et al. 2012; Divyakolu et al. 2013; Park et al. 2014; Sener et al. 2014; Shawky et al. 2014; Meguid et al. 2015) were included in present meta-analysis. The studies were published between 2004 and 2015. All thirteen studies were performed in different countries like- America (Boris et al. 2004; James et al. 2006; Schmidt et al. 2011), Brazil (dos Santos et al. 2010), Canada (Liu et al. 2011), China (Guo et al. 2012), India (Mohammad et al. 2009; Divyakolu et al. 2013), Romania (Pasca et al. 2009; Park et al. 2014), Turkey (Sener et al. 2014) and Egypt (Shawky et al. 2014; Meguid et al. 2015).

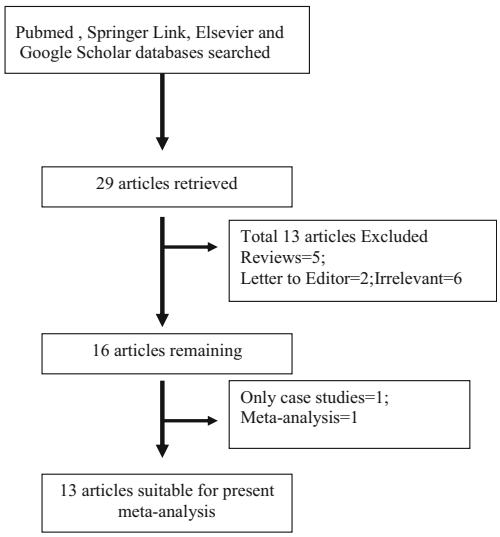


**Fig. 1** Flow chart of study selection

### Characteristic of included studies

The number of cases varied from 20 (Shawky et al. 2014) to 356 (James et al. 2006), and the number of controls varied from 22 (Shawky et al. 2014) to 5389 (Boris et al. 2004) (Table 1). ORs for more than one were reported in ten studies and two studies did not show significant any association (Schmidt et al. 2011; Park et al. 2014). In all thirteen studies, total cases were 1978 with CC (788), CT (925) and TT (265), and controls were 7257 with CC (3466), CT (2975), and TT (816) genotypes. In controls genotypes, percentage of CC, CT and TT were 47.76 %, 40.99 %, and 11.24 % respectively. In total cases, genotype percentage of CC, CT, and TT was 39.83 %, 46.76 % and 13.39 % respectively. Frequencies of CC genotype and C allele were highest in both cases and controls (Table 1). Except one study (Boris et al. 2004), control populations of all studies were in Hardy-Weinberg equilibrium.

### Meta-analysis

Meta-analysis with allele contrast showed significant association with both fixed effect ($OR_{Tvs.C}$ = 1.37; 95 % CI: 1.25–1.50; $P < 0.0001$) and random effect ($OR_{Tvs.C}$ = 1.48; 95 % CI: 1.18–1.86; $P = 0.0007$) models (Table 2; Fig. 2). Higher heterogeneity was found so random effect model was applied. C677T polymorphism had a significant association with susceptibility to autism in other four genetic models also (for TT + CT vs. CC (dominant model): OR =1.7, 95 % CI = 0.96–2.9, $p = 0.05$; for TT vs. CC (homozygote model): OR =1.84, 95 % CI = 1.12–3.02, $p = 0.01$(Figure3); for CT vs. CC (co-dominant/heterozygote model): OR = 1.6, 95 % CI = 1.2–2.1, $p = 0.003$; for TT vs. CT + CC (recessive model): OR = 1.5, 95 % CI = 1.0–2.2, $p = 0.03$).

### Subgroup analysis

The association between the C677T polymorphism and autism was further stratified by ethnicity. As shown in Table 2 and Fig. 3, in Asian populations C677T polymorphism significantly increased the risk of autism only in allele contrast and co-dominant models (T vs. C: OR =1.68,95%CI = 1.02–2.77, $P = 0.04$; for TT vs. CC: OR = 1.68, 95%CI = 0.69–4.08, $P = 0.24$; for TT + CT vs. *CC*: OR =2.78, 95%CI = 0.53–14.56, $p = 0.22$; for CT vs. *CC*: OR =1.66, 95%CI = 1.00–2.76, $P = 0.04$; for TT vs. *CT* + *CC*: OR =1.56, 95%CI = 0.61–3.95, $P = 0.35$). Results of the meta-analysis of nine Caucasian studies showed significant association between C677T polymorphism and autism was found in allele contrast and co-dominant genetic models (T vs. C: OR =1.43,95%CI = 1.1–1.87, $P = 0.009$; for CT vs. *CC*: OR =1.48, 95%CI = 1.03–2.10, $P = 0.03$) (Table 2; Fig. 4).

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

| Study | Country | Case | Control | Case Genotype | | | Control Genotype | | | HWE *p*-value |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | CC | CT | TT | CC | CT | TT | |
| Boris et al. 2004 | USA | 168 | 5389 | 35 | 94 | 39 | 2570 | 2213 | 606 | 0.000 |
| James et al. 2006 | USA | 356 | 205 | 134 | 176 | 46 | 93 | 90 | 22 | 0.97 |
| Mohammas et al. 2009 | USA | 138 | 138 | 98 | 35 | 5 | 120 | 18 | 0 | 0.41 |
| Pasca et al. 2009 | Romania | 39 | 80 | 21 | 14 | 4 | 46 | 28 | 6 | 0.55 |
| dos Santos et al. 2010 | Brazil | 151 | 100 | 60 | 68 | 23 | 45 | 41 | 14 | 0.35 |
| Liu et al. 2011 | Canada | 205 | 384 | 68 | 98 | 39 | 177 | 166 | 41 | 0.82 |
| Schmidt et al. 2011 | USA | 294 | 180 | 128 | 133 | 33 | 74 | 77 | 29 | 0.24 |
| Guo et al. 2012 | China | 186 | 186 | 79 | 77 | 30 | 87 | 83 | 16 | 0.54 |
| Divyakolu et al. 2013 | India | 50 | 50 | 27 | 22 | 1 | 42 | 8 | 0 | 0.53 |
| Park et al. 2014 | Korea | 249 | 423 | 76 | 136 | 37 | 139 | 204 | 80 | 0.73 |
| Sener et al. 2014 | Turkey | 98 | 70 | 44 | 51 | 3 | 37 | 33 | 0 | 0.09 |
| Shawky et al. 2014 | Egypt | 20 | 22 | 7 | 10 | 3 | 16 | 6 | 0 | 0.45 |
| Meguid et al. 2015 | Egypt | 24 | 30 | 11 | 11 | 2 | 20 | 8 | 2 | 0.36 |

## Heterogeneity and sensitivity analysis

A true heterogeneity existed between studies for allele contrast model ($P_{heterogeneity}$ = <0.0001, Q = 58.97, df = 11, $I^2$ = 81.3 %, $t^2$ = 0.11), dominant model ($P_{heterogeneity}$ = <0.0001, Q = 147.34, df = 11, $I^2$ = 92.9 %, $t^2$ = 0.638). The '$I^2$' value of more than 50 % for between studies comparison in both allele and genotype analysis shows high level of true heterogeneity. In allele contrast meta-analysis, high heterogeneity was observed in Asian ($P_{heterogeneity}$ = 0.0004, $I^2$ = 83.57 %) and Caucasian ($P_{heterogeneity}$ = <0.0001, $I^2$ = 84.32 %) population studies.

In overall allele contrast meta-analysis, sensitivity analysis performed by exclusion of the studies in which control population was not in Hardy Weinberg equilibrium and studies with small sample size. Control population of one study

**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 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) |
|---|---|---|---|---|---|
| Total studies | | | | | |
| Allele Contrast (T vs C) | 1.37(1.25–1.50),<0.0001 | 1.48(1.18–1.86),0.0007 | <0.0001 | 79.37 | 0.31 |
| Co-dominant (CT vs CC) | 1.6(1.3–1.8),<0.001 | 1.6(1.2–2.1),0.003 | 0.0004 | 71.88 | 0.6 |
| Homozygote (TT vs CC) | 1.7(1.36–2.0),<0.0001 | 1.84(1.12–3.02),0.01 | <0.0001 | 75.84 | 0.5 |
| Dominant (TT + CT vs CC) | 1.2(1.0–1.4),0.009 | 1.7(0.96–2.9),0.06 | <0.0001 | 92.9 | 0.02 |
| Recessive (TT vs CT + CC) | 1.6(1.3–1.9),<0.0001 | 1.5(1.0–2.2),0.03 | 0.009 | 60.41 | 0.97 |
| Asian studies | | | | | |
| Allele Contrast (T vs C) | 1.22(1.03–1.45),0.02 | 1.68(1.02–2.77),0.04 | 0.0004 | 83.57 | 0.30 |
| Co-dominant (CT vs CC) | 1.4(1.10–1.78),0.005 | 1.66(1.00–2.76),0.04 | 0.01 | 72.08 | 0.09 |
| Homozygote (TT vs CC) | 1.26(0.87–1.8),0.22 | 1.68(0.69–4.08),0.24 | 0.04 | 61.81 | 0.23 |
| Dominant (TT + CT vs CC) | 1.28(1.04–1.57),0.01 | 2.78(0.53–14.56),0.22 | <0.0001 | 97.76 | 0.16 |
| Recessive (TT vs CT + CC) | 1.1(0.78–1.53),0.58 | 1.56(0.61–3.95),0.35 | 0.02 | 68.65 | 0.30 |
| Caucasian Studies | | | | | |
| Allele Contrast (T vs C) | 1.44(1.29–1.60),<0.0001 | 1.43(1.1–1.87),0.009 | <0.0001 | 79.3 | 0.97 |
| Co-dominant (CT vs CC) | 1.55(1.3–1.85),<0.0001 | 1.48(1.03–2.10),0.03 | 0.002 | 73.2 | 0.63 |
| Homozygote (TT vs CC) | 1.83(1.4–2.34),<0.0001 | 1.69(0.89–3.21),0.11 | <0.0001 | 83.98 | 0.43 |
| Dominant (TT + CT vs CC) | 0.9(0.77–1.05),0.16 | 0.95(0.75–1.19),0.64 | 0.08 | 46.51 | 0.06 |
| Recessive (TT vs CT + CC) | 1.47(1.17–1.83),0.0007 | 1.38(0.89–2.1),0.15 | 0.003 | 71.71 | 0.36 |

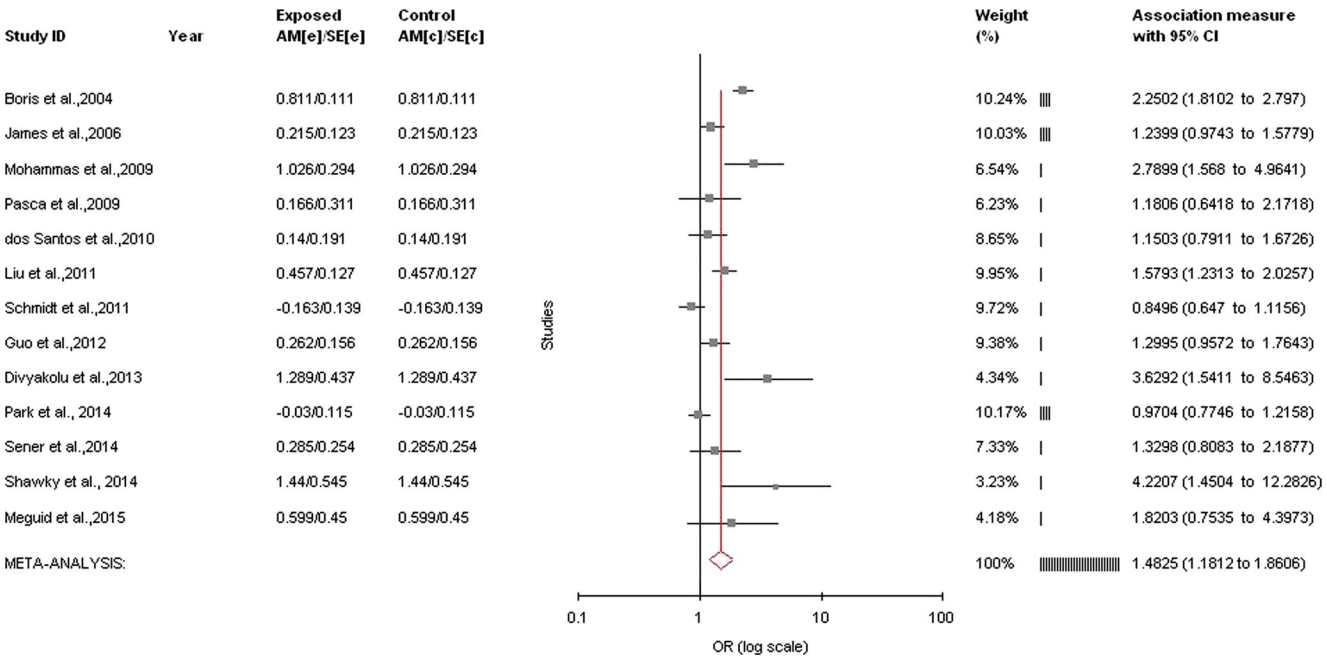


**Fig. 2** Forest plot of total studies of allele contrast (T vs C) genetic model

(Boris et al. 2004) were not in HW equilibrium and sample size in three studies was less than 50 (Pasca et al. 2009, $n = 39$; Shawky et al. 2014, $n = 20$; Meguid et al. 2015, $n = 24$). Exclusion of these four studies decreased heterogeneity and ($p = 0.0001$, $I^2 = 76.08$ %) also decreased odds ratio (OR =1.3;95%CI = 1.0–1.67;$P = 0.02$).

### Publication bias

Publication bias was not observed in additive/allele contrast, co-dominant, homozygote and recessive models (Begg's $p = 0.195$, Egger's $p = 0.34$ for T vs. C; Begg's $p = 0.458$, Egger's $p = 0.5$ for TT vs. CC; Begg's $p = 0.026$, Egger's $p = 0.6$ for CT vs. CC; and Begg's $p = 0.987$, Egger's $p = 0.97$ for TT vs. CC + CT) of overall meta-analysis (Table 2). Publication bias was not observed in subgroup analysis based on ethnicity as Asian and Caucasian populations (Tables 2) by using of Begg's and Egger's test. Funnel plots were showed in Fig. 5.

## Discussion

MTHFR enzyme is important enzyme of folate-methionine pathway and is involved in to two important processes - DNA methylation and DNA synthesis. It converts 5,10-methylenetetrahydro folate (5,10-methylene THF) in to 5-methyltetrahydrofolate (5-methyl THF), which donates methyl group for the remethylation of Hcy in to methionine. Methionine is converted in to S-adenosylmethionine (SAM) which is the main methyl group donor in the cellular methylation reaction of DNA, RNA, proteins and lipid. Under the condition of folate deficiency and/or hypofunctional *MTHFR* may result in less conversion of 5, 10-methylene THF to less 5-methyl THF, and less conversion of Hcy to methionine and consequently increased the concentration of plasma Hcy (James et al. 2006; Melnyk et al. 2011) which may contribute independently to the cause of autism.

Normal activity of *MTHFR* is required for normal genome methylation and imprinting. Genetic, epigenetic and

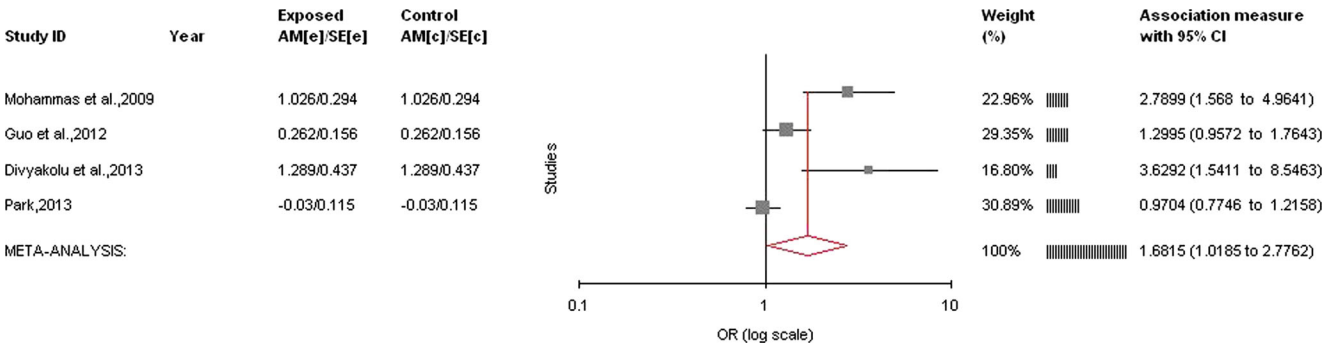


**Fig. 3** Forest plot of Asian studies of allele contrast (T vs C) genetic model

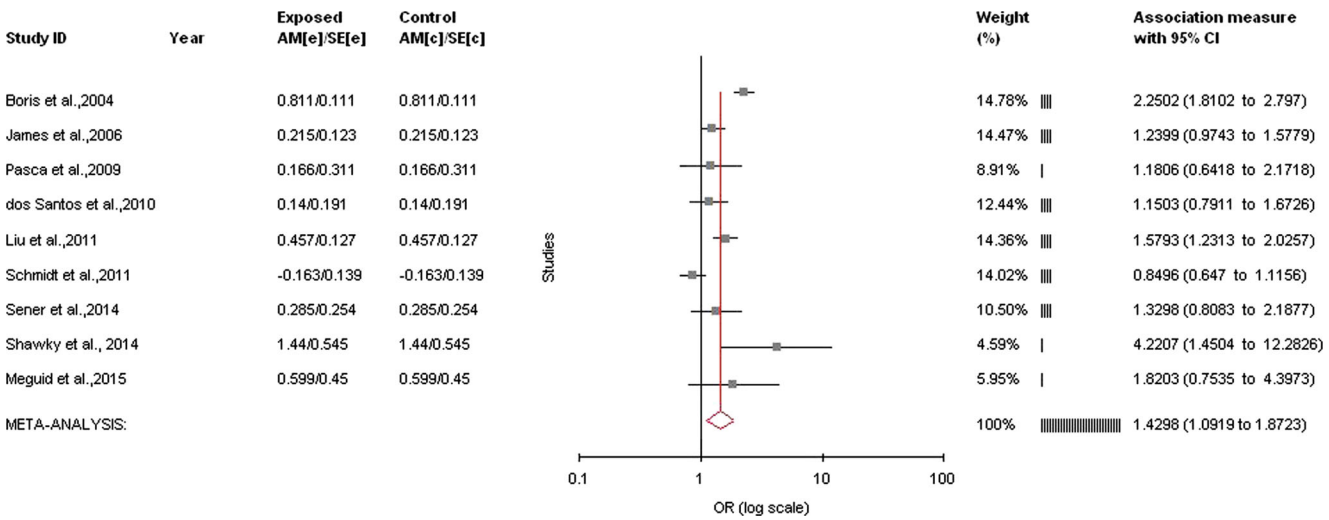


**Fig. 4** Forest plot of Caucasian studies of allele contrast (T vs C) genetic model

environment factors play role in autism rate and symptom severity (Schaevitz and Berger-Sweeney 2012). The DNA methylation or epigenetic programming is essential for gene imprinting and cell differentiation during embryogenesis (Li 2002). Most critical period of epigenetic programming are prenatal and early post natal, when DNA methylation is essential for development of normal brain and neuron networks (Schaevitz and Berger-Sweeney 2012). The main neuroanatomical abnormalities in autistic children at birth are mainly in hippocampal and prefrontal cortex regions and following abnormalities were reported like (i) overgrowth of some specific regions of the brain (Anagnostou and Taylor 2011; Courchesne et al. 2007), (ii) network of long distance connections were underdeveloped (Wass 2011) and (iii) networks of short range were over developed (Wass 2011).

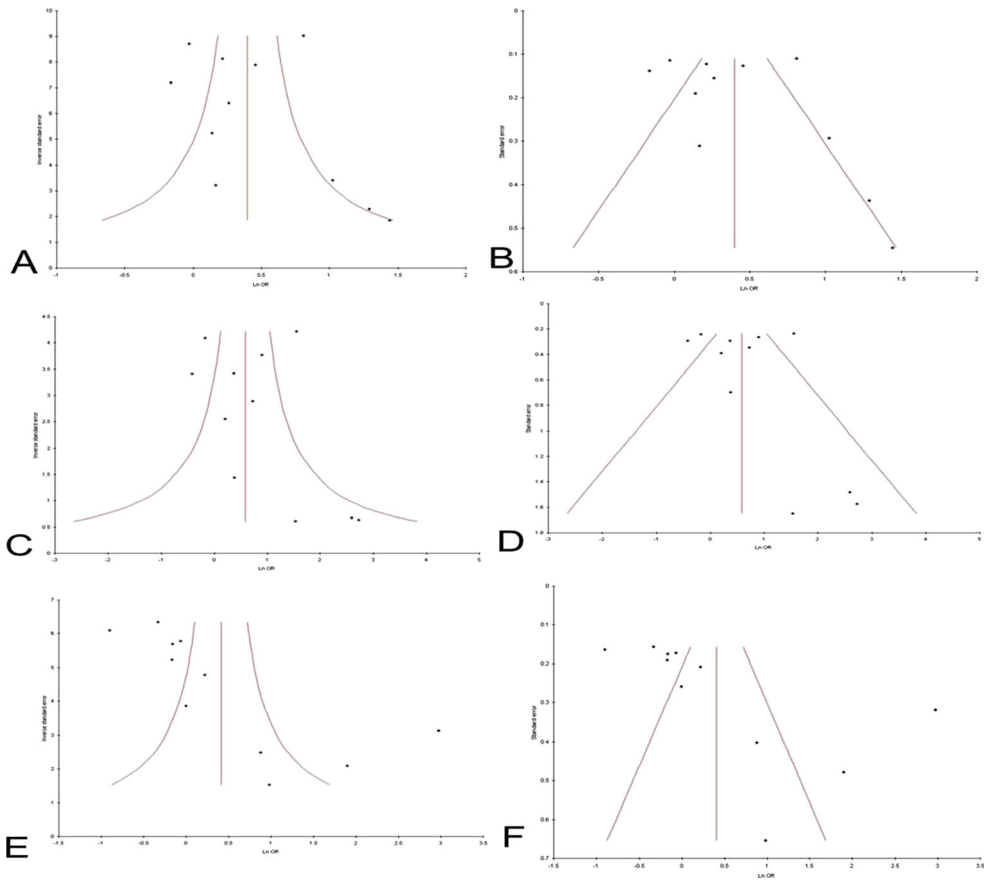


**Fig. 5** Funnel plot of total studies A. precision vs log OR of T vs C model, B. SE vs log OR of T vs C model,C. precision vs log OR of TT vs CC model, D. SE vs log OR of TT vs CC model, E. precision vs log OR of TT+CT vs CC model, F. SE vs log OR of TT+CT vs CC model

The epigenetic mechanism controls several processes during neurodevelopment which occurs prenatally and early postnatal up to 2 years of age like (i) establishment of neuron networks, (ii) selected cell death, (iii) synaptogenesis and (iv) pruning of inappropriate dendritic arbors and synapses etc. High concentration of Hcy and its metabolites inhibit activity of methyl transferases like Catechol-O-methyl transferase (COMT) (Schatz et al. 1981) and experiments on animal models have showed that COMT activity is high during early embryogenesis at the time of development of sympathetic nervous system (Ignarro and Shideman 1968). COMT degrades dopamine neurotransmitter by transferring methyl group from SAM to dopamine. Excess dopamine inhibits expression of brain derived neurotrophic factor (BDNF) (Fumagalli et al. 2003), which is essential for normal brain development (Schmidt et al. 2011). Abnormal methylation due to variant *MTHFR* enzyme reduced the activity of COMT and increased the concentration of dopamine, which consequently inhibit the synthesis of BDNF and abnormal neurodevelopment is resulted.

Meta-analysis is a statistical procedure for combining the results of several individuals studies to produce a pooled estimate for summarizing inconsistent results from different studies (Munafo and Flint 2004). Several meta-analyses were published accessing MTHFR as risk factor to various diseases/disorders like- neural tube defects (Yadav et al. 2015), down syndrome (Rai et al. 2014), cleft lip and palate (Zhao et al. 2014), cardiovascular disease (Gao et al. 2014), anxiety (Peerbooms et al. 2011), schizophrenia (Hu et al. 2014), depression (Rai 2014a), bipolar disorder (Rai 2011; Hu et al. 2014), Alzheimer's disease (Zhang et al. 2010) and cancer (Rai 2014b).

Author identified two meta-analyses (Frustaci et al. 2012; Pu et al. 2013) concerning similar topic during the literature search. Frustaci et al. (2012) conducted a meta-analysis on MTHFR C677T and autism risk based on six publications (Boris et al. 2004; dos Santos et al. 2010; James et al. 2006; Liu et al. 2011; Mohammad et al. 2009; Pasca et al. 2009) and reported significant association. Pu et al. (2013) included only eight studies in their meta-analysis with 1672 case and 6762 control subjects. They reported random effect (OR = 1.42; 95 % CI = 1.09–1.85) models of meta-analysis showed significantly increased autism risk in the presence of mutant T allele. There are several published studies which were not included in these previous meta-analyses. Hence comprehensive meta-analysis with the largest number of studies and number of samples was conducted to investigate the possible relationship between *MTHFR* C677T polymorphism and the risk of Autism. The quality of meta-analysis is compromised by presence of publication bias, sampling method and variations in genetic background of the subjects due to different ethnicity. However to minimize these limitation, author tried to use appropriate inclusion and exclusion criteria, performed sensitivity analysis and subgroup analysis to reduce selection bias and to lower heterogeneity, (Zhang et al. 2014) but failed to minimize the heterogeneity.

There were few limitations in present meta-analysis: (i) used crude ORs in the pooled analysis without adjustment, (ii) some studies with small sample sizes were also included in the meta-analysis, (iii) did not done subgroup analysis by diagnostic subgroups because of the lack of information, (iv) in the subgroup analyses, the number of studies in Asian subgroup was relatively small, which could lead to a lack of sufficient statistical power to explore the true association, (v) analyses stratified by other related susceptible factors, such as maternal infection, smoking, drink abuse status during pregnancy have not been conducted in the present study due to unavailability of sufficient data, and (vi) due to lack of data, gene–gene and gene–environment interactions could not be included. Along with limitations, present meta-analysis had some strength also like substantial number of cases and controls were pooled from different studies, which significantly increased the statistical power of the analysis.

Results of present meta-analysis suggested that C677T polymorphism of MTHFR gene was a risk factor for autism susceptibility in overall population as well as, Asian and Caucasian populations. Further autism is a multifactorial disease; therefore, single susceptibility gene polymorphisms might have modest effects. More case control studies stratified by environmental exposure, or other risk factors, should be performed in the future to better understand the role of the *MTHFR* C677T polymorphism in the pathogenesis of autism.

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