Asian Journal of Psychiatry 46 (2019) 54–61

Contents lists available at ScienceDirect

Asian Journal of Psychiatry

journal homepage: www.elsevier.com/locate/ajp

Association of MTHFR 677C > T and 1298A > C polymorphisms with susceptibility to autism: A systematic review and meta-analysis

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ARTICLE INFO

Keywords:
Autism
MTHFR Gene
Folate
Polymorphism
Meta-analysis

ABSTRACT

Several studies have investigated association of MTHFR 677C > T and 1298A > C polymorphisms with risk of autism, but they have reported controversial and inconclusive results. The present meta-analysis was designed to evaluate association of MTHFR 677C > T and 1298A > C polymorphisms with risk of autism. A comprehensive literature search was done in PubMed, EMBASE, and CNKI databases to identify all eligible publications up to April 01, 2019. Finally, 25 case-control studies including 18 studies on MTHFR 677C > T and 7 studies on MTHFR 1298A > C polymorphism were selected. Overall, a significant association was found between MTHFR 677C > T and an increased risk of autism under all five genetic models (T vs. C: OR = 1.483, 95% CI 1.188–1.850, p ≤ 0.001; TT vs. CC: OR = 1.834, 95% CI 1.155–2.913, p = 0.010; TC vs. CC: OR = 1.512, 95% CI 1.101–2.078, p = 0.011; TT + TC vs. CC: OR = 1.632, 95% CI 1.261–2.113, p ≤ 0.001; and TT vs. TC + CC: OR = 1.427, 95% CI 1.002–2.032, p = 0.049). However, no significant association was found between MTHFR 1298A > C and autism risk. Stratified analyses showed that MTHFR 677C > T and 1298A > C polymorphisms are involved in genetic susceptibility of autism by ethnicity. Results of this meta-analysis indicated that MTHFR 677C > T polymorphism may be associated with increased risk of autism in overall and by ethnicity, while MTHFR 1298A > C was reported to be significantly associated with the risk of autism only in Caucasians. MTHFR polymorphisms could be used as a diagnostic marker for autism with respect to ethnicity background.

1. Introduction

Autism Spectrum Disorders (ASDs) and autism are both general terms for a group of heterogeneous neurological disorder, characterized by three core symptoms: difficulties in social interaction, verbal and nonverbal communication, and repetitive behaviors (Maximo et al., 2014; Wing, 1981). Recent statistics from the Centers for Disease Control (CDC) indicate that ASDs currently affect approximately 1 in 68 children in the United States, and boys are five times more likely to be diagnosed with an ASD compared to girls (Christensen et al., 2016; Liu et al., 2010). Autism symptoms exhibited by individuals on the spectrum tend to vary in severity and pattern with some individuals having severe impairment while others have only minor impairment (Landa, 2008; Ozonoff et al., 2008). Impairment existing in reciprocal social and communication skills, language development is abnormal, and individuals with autism engage in behaviors or have interests restricted in repertoire (Kazdoba et al., 2015; Young et al., 2003).

Exact molecular and biochemical mechanism of autism is not well understood (Eissa et al., 2018; Petinou and Minaidou, 2017). While some researchers support genetics hypothesis, others support the hypothesis that factors in the environment such as intrauterine or postnatal exposure to mercury may trigger autism (Chaste and Leboyer,

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https://doi.org/10.1016/j.ajp.2019.09.016
Received 13 April 2019; Received in revised form 21 September 2019; Accepted 21 September 2019

2012; Dietert et al., 2011; Muhle et al., 2004). Therefore, autism is believed to have a biological, genetic, and possibly an environmental basis (Bonora et al., 2005; Muhle et al., 2004). To date, more than 100 causative genes caring deleterious mutations in approximately 10–25% of autistic patients have been identified (Bourgeron, 2016). Methylenetetrahydrofolate Reductase (MTHFR) is one of good potential candidate of susceptibility genes, which has been studied so far (Rai, 2016). MTHFR enzyme is essential for DNA synthesis and methylation. Human MTHFR gene is located on chromosome 1 at 1p36.3, containing 11 exons and spanning 2.2 kb (Abedinzadeh et al., 2015; Kamali et al., 2018). MTHFR gene polymorphisms are thought to contribute to a variety of neurological diseases such as autism (Divyakolu et al., 2013). Currently, there are several common variants in MTHFR gene regulating transcription and production of MTHFR and subsequently causing elevated plasma level of homocysteine such as MTHFR 677C > T and 1298A > C (Kamali et al., 2018; Pu et al., 2013; Rai, 2016). Prevalence of the two polymorphisms varies in different geographical regions and ethnic groups (Abedinzadeh et al., 2015; Kamali et al., 2018).

Folate plays an important role in neurological development, because it acts as a methyl group transporter (Abbasi et al., 2018). In the past decades, several studies have indicated that low blood levels of folate and vitamin B12, and elevated homocysteine levels were associated with neurodevelopmental symptoms, especially cognitive decline, in psychogeriatric and psychiatric patients (Divyakolu et al., 2013). To date, several epidemiological studies have been performed to evaluate associations of MTHFR 677C > T and 1298A > C polymorphisms with susceptibility to autism (Pu et al., 2013; Rai, 2016). However, results were conflicting and inconclusive, presumably due to limited sample size of single study or different characteristics among studies such as ethnicity, genotyping methods, sources of controls, and possible selection bias. Meta-analysis is a widely used statistical method in medical research, especially about a topic under extensive study, for which controversial results have been reported. Therefore, to provide a more comprehensive assessment of associations of MTHFR 677C > T and 1298A > C polymorphisms with autism risk, herein a systematic review and meta-analysis of all eligible studies was carried out.

2. Materials and methods

2.1. Identification and selection of the studies

A comprehensive literature search was performed in PubMed, Web of Science, EMBASE, Scopus, Google Scholar, Cochrane Library, Scientific Information Database (SID), Wan Fang, Chinese National Knowledge Infrastructure (CNKI), Chinese Biomedical Literature (CBM) databases to identify all relevant studies evaluated association of MTHFR 677C > T and 1298A > C polymorphisms with risk of autism up to April 01, 2019. The following terms and keywords were used: (''Autism Spectrum Disorders'' OR ''Autism'' OR ''ASD'') AND (''Methylenetetrahydrofolate Reductase'' OR ''MTHFR'') AND (''MTHFR 677C > T'' OR ''MTHFR Ala222Val'' OR''MTHFR 1298A > C'' OR ''MTHFR Glu222Val'' OR ''rs1801133'' OR ''rs1801131'') AND (''Polymorphism'' OR ''Genotype'' OR ''Variant'' OR ''Mutation'' OR ''SNPs'' OR ''Allele''). In addition, reference lists of retrieved case-control studies, reviews, and previous meta-analyses were hand-searched for collecting other relevant studies missed in electronic search.

2.2. Inclusion and exclusion criteria

Studies included in this meta-analysis met inclusion criteria as follows: 1) a case-control or cohort design; 2) evaluation of association between MTHFR 677 C > T and 1298 A > C polymorphisms and autism; 3) sufficient published data for calculating odds ratios (ORs) with their 95% confidence intervals (95% CIs); and 4) diagnosis of ASD patients based on ICD-10, DSM-IV, or DSM-5 criteria. Accordingly, main reasons for exclusion were: 1) not designed as case-control or cohort studies; 2) lack of providing numbers of genotypes and other essential data; 3) case only studies; 4) linkage studies, twin and family -based studies; 5) abstracts, reviews, case reports, editorials, comments, animal studies; posters; and 5) overlapping data and duplicate of previous studies. If multiple studies from the same case series were available, the one including the most individuals was used in the analysis.

2.3. Data extraction

Original data were independently extracted by two investigators carefully from each of published studies. In case of disagreement, consensus was obtained on every item by joint review of the study through consultation with a third author. The following data was collected from each study: first author, year of publication, country of origin, ethnicity (Caucasian, Asian, African, and Mixed), total number of cases and controls, frequencies of genotypes and alleles for MTHFR 677C > T and 1298A > C polymorphisms, genotyping methods, Minor Allele Frequencies (MAFs), and p-value for Hardy-Weinberg Equilibrium (HWE) in controls.

2.4. Statistical methods

Strength of association of MTHFR 677C > T and 1298A > C polymorphisms with risk of autism was estimated by crude Odds Ratios (ORs) with corresponding 95% Confidence Intervals (CIs). The Z-test was used to determine significance of pooled OR, and a p-value of 0.10 was considered statistically significant. Pooled ORs were determined for MTHFR 677C > T polymorphism under five genetic models, i.e., allele (T vs. C), homozygote (TT vs. CC), heterozygote (TC vs. CC), dominant (TT + TC vs. CC), and recessive (TT vs. TC + CC), and possible association for MTHFR 1298A > C polymorphism was assessed under five genetic models, i.e., allele (C vs. A), homozygote (CC vs. AA), heterozygote (CA vs. AA), dominant (CC + AC vs. AA), and recessive (CC vs. AC + AA). Between-study heterogeneity was assessed by a Chi -Square- based Q-statistic test, in which p-value < 0.05 was considered statistically significant. In addition, the effect of heterogeneity was quantified using I^2, in which I^2 values of 25, 50, and 75% were considered as nominally low, moderate, and high estimates, respectively. When Q-test and I^2 value indicated a lack of heterogeneity, a fixed-effects model (Mantel-Haenszel method) was adopted to evaluate pooled ORs. Otherwise, a random-effects model (DerSimonian and Laird method) was applied. The Chi-Square test was applied to establish whether genotype distribution was compatible with Hardy-Weinberg Equilibrium (HWE) in controls for each study, in which P-value < 0.05 was representative of HWE-deviation. To explore source of between-study heterogeneity, subgroup analyses by ethnicity and genotyping method was performed. Sensitivity analysis was performed to investigate whether single studies included in the meta-analysis significantly altered pooled results. In addition, sensitivity analysis was performed by excluding HWE-violating studies. All statistical analyses were performed using Comprehensive Meta-Analysis (CMA) software version 2.0 (Biostat, USA). All p-values were two-sided, and p-values less than 0.05 were considered statistically significant.

3. Results

3.1. Characteristics of the studies

A flow chart describing process of inclusion/exclusion of the studies is presented in Fig. 1. A total of 137 publications were obtained as a result of initial search of databases. Among these studies, in the first screening, 29 publications were excluded, as they were duplicates, leaving 108 studies for further selection. Among these publications, 83 studies were excluded because they were review papers, case reports, previous meta-analyses, other polymorphisms of MTHFR gene, and

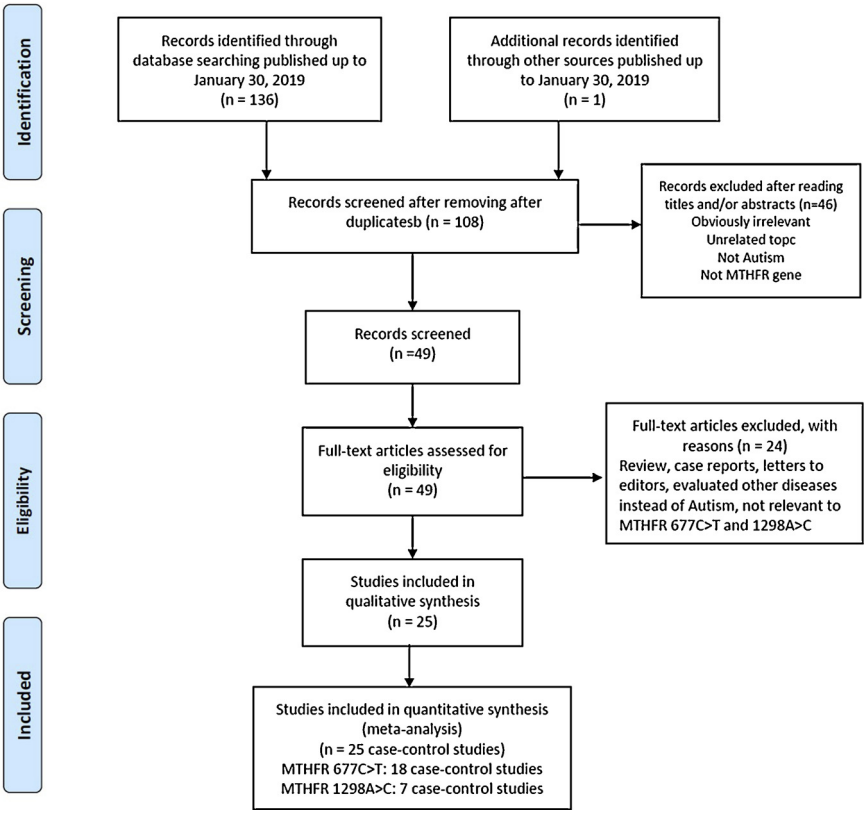

Fig. 1. Flow diagram of selection of studies included in the current meta-analysis.

Table 1
General characteristics of studies included in this meta-analysis.

First Author	Country (Ethnicity)	Genotyping Method	Case/Control	Cases					Controls					MAFs	HWE
				Genotype			Allele		Genotype			Allele			
MTHFR 677C > T				CC	CT	TT	C	T	CC	CT	TT	C	T		
Boris 2004	USA(Caucasian)	PCR	168/5389	35	94	39	164	172	2570	2213	606	7353	3425	0.317	0.001
James 2006	USA(Caucasian)	RT-PCR	356/205	134	176	46	444	268	93	90	22	276	134	0.326	0.974
Mohammad 2009	USA(Caucasian)	PCR-RFLP	138/138	98	35	5	231	45	120	18	0	258	18	0.065	0.412
Pasca 2009	Romania(Caucasian)	RT-PCR	39/80	21	14	4	56	22	46	28	6	120	40	0.250	0.551
dos Santos 2010	Brazil(Latinos)	PCR	151/100	60	68	23	188	114	45	41	14	131	69	0.345	0.353
Liu 2011	Canada(Caucasian)	TaqMan	205/384	68	98	39	234	176	177	166	41	520	248	0.322	0.823
Schmidt 2011	USA(Caucasian)	TaqMan	294/180	128	133	33	389	199	74	77	29	225	135	0.375	0.240
Guo 2012	China(Asian)	PCR-RFLP	186/186	79	77	30	235	137	87	83	16	257	115	0.309	0.542
Dong 2012	China(Asian)	PCR-RFLP	98/70	52	29	17	133	86	60	7	3	127	13	0.092	≤0.001
Divyakolu 2013	India(Asian)	PCR-RFLP	50/50	27	22	1	76	24	42	8	0	92	8	0.080	0.538
Park 2014	Korea(Asian)	NS	249/423	76	136	37	288	210	139	204	80	482	364	0.430	0.737
Shawky 2014	Egypt(African)	PCR-RH	20/22	7	10	3	24	16	16	6	0	38	6	0.136	0.458
Agam 2014	USA(Caucasian)	TaqMan	13/32	4	7	2	15	11	16	13	4	45	21	0.318	0.596
Sener 2014	Turkey(Caucasian)	PCR-RFLP	98/70	44	51	3	139	57	37	33	0	107	33	0.235	0.009
Meguid 2015	Egypt(African)	PCR-RFLP	24/30	11	11	2	33	15	20	8	2	48	12	0.020	0.361
El-baz 2017	Egypt(African)	PCR-RFLP	31/39	12	15	4	39	23	35	4	0	74	4	0.051	0.735
Zhang 2018	China(Asian)	TaqMan	201/200	68	101	32	237	165	71	86	42	228	170	0.425	0.099
Delshadpour 2018	Iran(Asian)	PCR-RFLP	171/198	87	78	6	252	90	108	87	3	303	93	0.234	0.001
MTHFR 1298A > C				**AA**	**AC**	**CC**	**A**	**C**	**AA**	**AC**	**CC**	**A**	**C**		
Boris 2004	USA(Caucasian)	PCR	168/159	93	65	10	251	85	70	75	14	215	103	0.323	0.331
James 2006	USA(Caucasian)	RT-PCR	356/204	175	147	34	497	215	103	77	24	283	125	0.306	0.109
Mohammad 2009	USA(Caucasian)	PCR-RFLP	138/138	35	59	44	129	147	48	32	58	128	148	0.536	0.001
Liu 2011	Canada(Caucasian)	RT-PCR	205/382	109	81	15	299	111	170	175	37	515	249	0.325	0.404
Schmidt 2011	USA(Caucasian)	TaqMan	296/177	160	117	19	437	155	89	76	12	254	100	0.282	0.430
Park 2014	Korea(Asian)	NS	236/423	147	75	14	369	103	298	114	11	710	136	0.160	0.980
El-baz 2017	Egypt(African)	PCR-RFLP	31/39	7	13	11	27	35	31	7	1	69	9	0.115	0.450

Abbreviations: PCR-RFLP: Restriction Fragment Length Polymorphism; RT-PCR: Real-Time PCR; RH: Reverse Hybridization; NS: Not Stated; MAF: Minor Allele Frequency; HWE: Hardy–Weinberg Equilibrium.

related to other diseases. Finally, a total of 25 case-control studies in 18 publications with 3891 autism cases and 9279 controls were obtained (Agam et al., 2014; Boris et al., 2004; Delshadpour et al., 2017; Divyakolu et al., 2013; Dong et al., 2012; El-baz et al., 2017; Guo et al., 2012; James et al., 2006; Liu et al., 2011; Mohammad et al., 2009; Park et al., 2014; Paşca et al., 2009; Rehab Khalil et al., 2015; Santos et al., 2010; Schmidt et al., 2012; Sener et al., 2014; Shawky et al., 2014; Zhang et al., 2018). Included studies were published between 2004 and 2018, and they were written in English. Main characteristics of selected studies are summarized in Table 1. Among those studies, there were 18 case-control studies with 2492 autism cases and 7796 controls on MTHFR 677C > T polymorphism and 7 case-control studies with 1399 autism cases and 1483 controls on MTHFR 1298A > C polymorphism. Among included studies for MTHFR 677C > T polymorphism, 8 studies were conducted in Caucasians with 1311 cases and 6478 controls, 3 in Asians with 955 cases and 704 controls, 3 studies in African with 75 cases and 91 controls, and one study in mixed populations (Latinos) with 151 cases and 100 controls. For MTHFR A1298C polymorphism, 5 studies were conducted in Caucasians with 1163 and 1060 controls, one study in Asians with 236 and 423 controls, and one study in Africans with 31 and 39 controls. Countries of these studies included USA, Romania, Brazil, Canada, China, India, Korea, Egypt, Turkey, and Iran. Genotyping was performed using five different methods including PCR, RT-PCR, PCR-RFLP, TaqMan, and PCR-RH. Table 1 also displays distributions of MTHFR 677C > T and 1298A > C polymorphisms genotypes, minor allele frequency and p-value for HWE in controls. Genetic distributions of control groups in all studies were consistent with HWE except for 3 studies for MTHFR 677C > T, and one for MTHFR 1298A > C (Table 1).

3.2. Quantitative data syntheses

3.2.1. MTHFR 677C > T polymorphism

Table 2 listed the main results of the meta-analysis of MTHFR 677C > T polymorphism with autism risk. When all the eligible studies were pooled into the meta-analysis of MTHFR 677C > T polymorphism, significantly increased risk of autism was observed under all five genetic models, i.e., allele (T vs. C: OR = 1.644, 95% CI 1.302–2.075, p ≤ 0.001, Fig. 2A), homozygote (TT vs. CC: OR = 1.987, 95% CI 1.291–3.059, p = 0.002), heterozygote (TC vs. CC: OR = 1.513, 95% CI 1.092–2.096, p = 0.013), dominant (TT + TC vs. CC: OR = 1.600, 95% CI 1.116–2.292, p = 0.011) and recessive (TT vs. TC + CC: OR = 1.483, 95% CI 1.058–2.078, p = 0.022). When stratified by ethnicity, there was a significant association between MTHFR 677C > T polymorphism and an increased risk of autism in Caucasians (T vs. C: OR = 1.478, 95% CI = 1.103–1.979, p = 0.009; TT vs. CC: OR = 2.061, 95% CI = 1.067–3.984, p = 0.031; and TT + TC vs. CC: OR = 1.670, 95% CI = 1.163–2.397, p = 0.005), Asians (T vs. C: OR = 1.639, 95% CI = 1.056–2.543, p = 0.027 and TC vs. CC: OR = 1.509, 95% CI = 1.039–2.193, p = 0.031), and Africans (T vs. C: OR = 4.170, 95% CI 1.490–11.670, p = 0.007; TT vs. CC: OR = 7.353, 95% CI 1.646–32.842, p = 0.009; TC vs. CC: OR = 4.545, 95% CI 2.195–9.409, p p ≤ 0.001; and TT + TC vs. CC: OR = 5.052, 95% CI 2.502–10.197, p ≤ 0.001).

3.2.2. MTHFR 1298A > C polymorphism

Table 3 listed the main results of the meta-analysis of MTHFR 1298A > C polymorphism with autism risk. The overall analyses suggested there was no a significant associations between the MTHFR 1298A > C polymorphism and risk of autism in all five genetic models,

Table 2
Meta-analysis results for association of MTHFR 677C > T and 1298A > C polymorphisms with autism risk.

Subgroup	Genetic Model	Type of Model	Heterogeneity		Odds Ratio				Publication Bias	
			I^2 (%)	P_H	OR	95% CI	Z_{test}	P_{OR}	P_{Beggs}	P_{Eggers}
MTHFR 677C > T										
Overall	T vs. C	Random	84.28	≤0.001	1.644	1.302-2.075	4.177	≤0.001	0.041	0.051
	TT vs. CC	Random	72.61	≤0.001	1.987	1.291-3.059	3.120	0.002	0.150	0.158
	TC vs. CC	Random	82.49	≤0.001	1.513	1.092-2.096	2.488	0.013	0.150	0.157
	TT + TC vs. CC	Random	86.72	≤0.001	1.600	1.116-2.292	2.559	0.011	0.150	0.625
	TT vs. TC + CC	Random	62.27	≤0.001	1.483	1.058-2.078	2.288	0.022	0.324	0.173
By Ethnicity										
Caucasian	T vs. C	Random	81.24	≤0.001	1.478	1.103-1.979	2.620	0.009	0.710	0.894
	TT vs. CC	Random	77.86	≤0.001	2.061	1.067-3.984	2.151	0.031	1.000	0.920
	TC vs. CC	Random	84.59	≤0.001	1.413	0.875-2.283	1.412	0.158	0.901	0.835
	TT + TC vs. CC	Random	76.32	≤0.001	1.670	1.163-2.397	2.780	0.005	0.536	0.938
	TT vs. TC + CC	Random	63.27	0.008	1.536	0.966-2.440	1.816	0.069	1.000	0.907
Asian	T vs. C	Random	89.04	≤0.001	1.639	1.056-2.543	2.205	0.027	0.060	0.021
	TT vs. CC	Fixed	66.46	0.011	1.598	0.857-2.977	1.475	0.140	0.452	0.096
	TC vs. CC	Random	68.73	0.007	1.509	1.039-2.193	2.160	0.031	0.259	0.006
	TT + TC vs. CC	Random	93.35	≤0.001	0.986	0.417-2.329	−0.032	0.974	1.000	0.770
	TT vs. TC + CC	Random	68.32	0.007	1.389	0.764-2.526	1.077	0.281	0.452	0.111
African	T vs. C	Random	67.11	0.048	4.170	1.490-11.670	2.720	0.007	0.296	0.188
	TT vs. CC	Fixed	43.50	0.170	7.353	1.646-32.842	2.613	0.009	0.296	0.034
	TC vs. CC	Fixed	30.23	0.239	4.545	2.195-9.409	4.078	≤0.001	1.000	0.674
	TT + TC vs. CC	Fixed	53.17	0.118	5.052	2.502-10.197	4.520	≤0.001	1.000	0.499
	TT vs. TC + CC	Fixed	3.24	0.356	3.564	0.821-15.473	1.696	0.090	1.000	0.104
MTHFR 1298A > C										
Overall	C vs. A	Fixed	64.88	0.014	0.943	0.770-1.156	−0.563	0.574	0.452	0.751
	CC vs. AA	Fixed	45.76	0.101	0.915	0.692-1.210	−0.621	0.534	1.000	0.763
	CA vs. AA	Fixed	73.66	0.002	1.038	0.750-1.436	0.223	0.823	1.000	0.399
	CC + CA vs. AA	Fixed	69.27	0.006	0.978	0.737-1.297	−0.155	0.877	1.000	0.882
	CC vs. CA + AA	Fixed	37.84	0.154	0.830	0.640-1.078	−1.395	0.163	0.452	0.241
By Ethnicity										
Caucasian	C vs. A	Fixed	0.00	0.458	0.865	0.758-0.986	−2.165	0.030	0.462	0.800
	CC vs. AA	Fixed	0.00	0.705	0.797	0.593-1.073	−1.494	0.135	0.462	0.287
	CA vs. AA	Random	74.54	0.003	0.984	0.677-1.431	−0.083	0.934	0.806	0.233
	CC + CA vs. AA	Random	58.37	0.048	0.893	0.679-1.174	−0.809	0.419	0.806	0.480
	CC vs. CA + AA	Fixed	0.00	0.934	0.735	0.558-0.968	−2.189	0.029	0.999	0.536

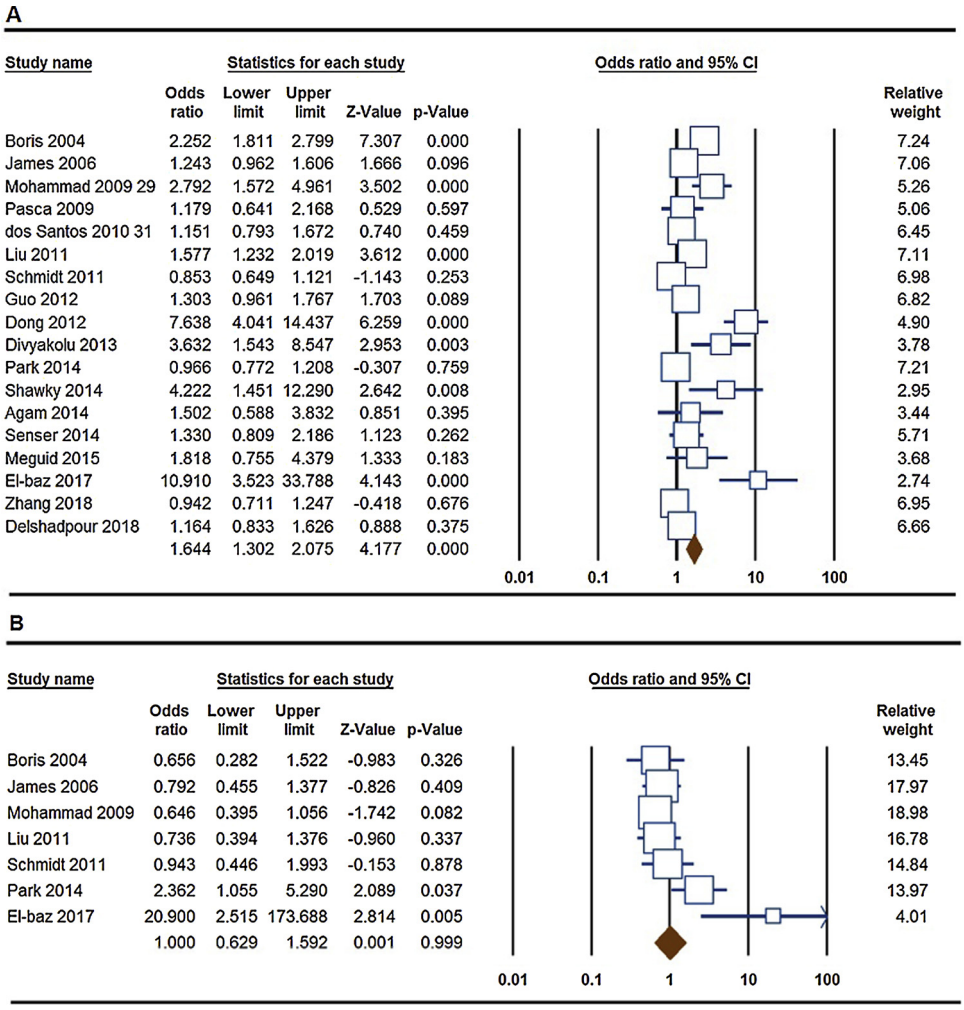

Fig. 2. Forest plots for the association of MTHFR 677C > T and 1298A > C polymorphisms with risk of autism risk. **A:** MTHFR 677C > T (allele model; T vs. C), **B:** MTHFR 1298A > C (recessive model; CC vs. CA + AA).

i.e., allele (C vs. A: OR = 0.943, 95% CI 0.770–1.156, p = 0.574), homozygote (CC vs. AA: OR = 0.915, 95% CI 0.692–1.210, p = 0.534), heterozygote (CA vs. AA: OR = 1.038, 95% CI 0.750–1.436, p = 0.823), dominant (CC + CA vs. AA: OR = 0.978, 95% CI 0.737–1.297, p = 0.877) and recessive (CC vs. CA + AA: OR = 0.830, 95% CI 0.640–1.078, p = 0.163). However, when stratified by ethnicity, a significant association between MTHFR 1298A > C polymorphism and an increased risk of autism in Caucasians under all two genetic models, i.e., allele (C vs. A: OR = 0.865, 95% CI = 0.758-0.986, p = 0.030) and recessive (CC vs. CA + AA: OR = 0.735, 95% CI = 0.558-0.968, p = 0.029, Fig. 2B).

3.3. Between-study heterogeneity test and sensitivity analysis

Between-study heterogeneity test showed that there was a significant heterogeneity in terms of MTHFR 677C > T polymorphism in overall population (Table 2). Thus, source of heterogeneity was assessed by meta-regression analysis via stratification by ethnicity. However, it was found that ethnicity did not contribute to substantial heterogeneity in the current meta-analysis (data not shown). Furthermore, a sensitivity analysis was performed to assess the influence of included studies on pooled ORs by sequential omission of individual studies. However, corresponding pooled ORs for MTHFR 677C > T and 1298A > C polymorphisms were not materially altered by removing any individual study (data not shown).

3.4. Publication bias

The Begg's funnel plot and Egger's test were used to assess potential publication bias in available literature. Visual inspection of funnel plot did not indicate any evidence of funnel plot asymmetry (Table 2). Additionally, results of Egger's test revealed no significant publication bias for both MTHFR 677C > T and 1298A > C polymorphisms in overall population under all five genetic models. For example, Fig. 3 shows shape of the funnel plot for association between MTHFR 677C > T polymorphism and autism risk under dominant model (TT + TC vs. CC: $P_{Beggs} = 0.150$ and $P_{Eggers} = 0.625$).

3.5. Minor Allele Frequencies (MAFs)

Minor Allele Frequency (MAF) of MTHFR 677C > T and 1298A > C polymorphisms are summarized in Table 1. MAFs of MTHFR 677C > T in healthy controls varied from 0.065 to 0.375 in the Caucasian, from 0.080 to 0.430 in the Asians, and were from 0.020 to 0.136 in the African (Egyptian) population. However, frequency of MTHFR 1298A > C allele ranged from 0.282 to 0.536 among Caucasian population and 0.160 in Asian population.

4. Discussion

Autism is a biologically heterogeneous neurodevelopmental disorder characterized by persistent deficits in social communication,

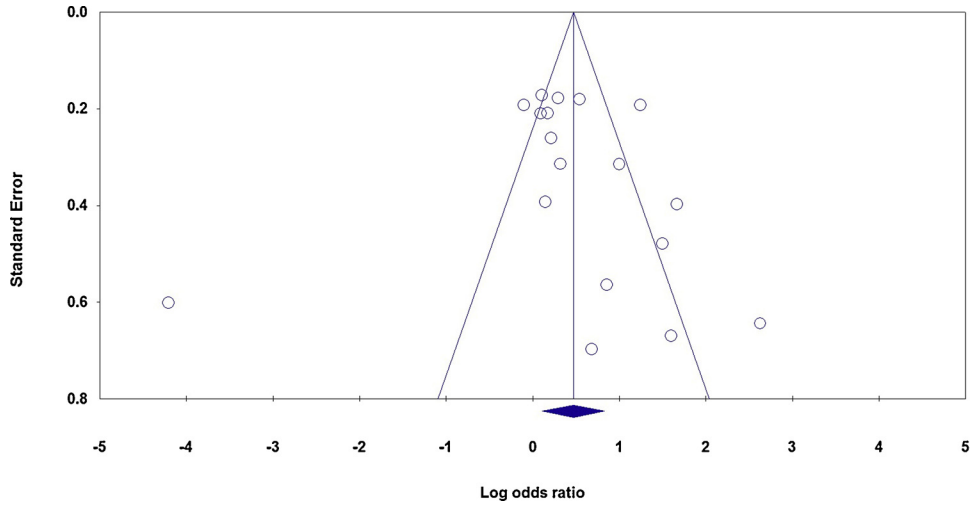

Fig. 3. Begg's funnel plot of publication bias test for the association of MTHFR 677C > T polymorphism with autism risk under dominant model (TT + TC vs. CC).

social interaction and restricted, repetitive patterns of behavior, and sensory alterations (Ocakoğlu et al., 2018). In the past decades, the concept of autism has been changed by several modifications in the diagnostic criteria (Gyawali and Patra, 2019). Despite these progress in the g diagnostic criteria, the etiology of autism is still unknown (Eissa et al., 2018). Genetic variants in folate/homocysteine pathway may play a role in development of autism. Normal activity of MTHFR is necessary for normal genome methylation and imprinting (Park et al., 2014; Pu et al., 2013; Rai, 2016). DNA methylation or epigenetic programming is essential for gene imprinting and cell differentiation during embryogenesis for development of normal brain and neuronal networks. Emerging evidences have shown that DNA methylation defects might be correlated with neurodevelopmental disorders such as autism, and role of MTHFR gene in folate metabolism may contribute to epigenetic mechanisms modifying complex gene expression, thus causing development of autism (Sener et al., 2014). Associations between MTHFR polymorphisms and susceptibility to autism have remained poorly elucidated owing to conflicting data generated by independent studies. To resolve this controversy, the present meta-analysis involving 25 case-control studies was conducted to investigate association of MTHFR 677C > T and 1298A > C polymorphisms with autism risk. In this meta-analysis, a significant association was found between MTHFR 677C > T polymorphism and autism risk under all five genetic models. When stratified by ethnicity, an association was found between MTHFR 677C > T polymorphism and autism risk in Caucasians, Asians, and Africans. However, pooled results showed that MTHFR 1298A > C polymorphism was not significantly associated with autism risk, although a significant association was found in Caucasians by subgroup analysis. Our results showed that MTHFR polymorphisms are predictors of autism and might be used clinically in diagnosis of autism. Therefore, it can be assumed that folic acid and methyl B12 supplementations might be useful for the children with MTHFR polymorphisms (Shaik Mohammad et al., 2016).

Two published meta-analyses were found on the same topic during literature search. In 2016, Rai et al., in a meta-analysis of 13 studies with 1978 cases and 7257 controls evaluated association between MTHFR 677C > T polymorphism and autism risk. Their results suggested that MTHFR 677C > T was associated with increased autism risk in overall and by ethnicity among Asians and Caucasians (Rai, 2016). Pu et al., performed meta-analysis based on 8 case-control studies with 1672 cases and 6760 controls to evaluate association of MTHFR 677C > T and 1298A > C polymorphisms with risk of autism. They found that both MTHFR 677C > T and 1298A > C polymorphisms were significantly associated with increased risk of autism (Pu et al., 2013). Inconsistence with Pu et al., we have found that MTHFR 1298A > C polymorphism was not significantly associated with autism in overall population. In addition, previous meta-analyses results about MTHFR polymorphisms and autism risk essentially have remained an open field, as their selected number of studies was considerably smaller than that needed for achievement of robust and powerful conclusions, so, herein a comprehensive meta-analysis was performed with the largest number of studies on both MTHFR 677C > T and 1298A > C polymorphisms. Moreover, their meta-analyses did not address associations of MTHFR 677C > T and 1298A > C polymorphisms in the African populations. Additionally, new epidemiological studies have recently been conducted to estimate associations of MTHFR 677C > T and 1298A > C polymorphisms with autism in Asians and Caucasians and provide new evidences, that were not included in the past meta-analyses.

Between-study heterogeneity, which may have been introduced by a poorly defined study base, is a potential concern in a meta-analysis (Kamali et al., 2017; Sadeghiyeh et al., 2017; Sobhan et al., 2018). Various factors such as population stratification, source of controls, population size, deviation from HWE, and other covariates could be source of heterogeneity (Gohari et al., 2016). In the current meta-analysis, an obvious between-study heterogeneity was observed for MTHFR 677C > T polymorphism in overall estimations under all five genetic models. Through subgroup analysis, it was found that source of heterogeneity was mainly from Asian and Caucasians, indicating that ethnicity playing an important role for between-study heterogeneity in this meta-analysis. In addition, sensitivity analysis was performed by excluding HWE-violating studies to determine the effect of those studies on between -study heterogeneity. However, between-study heterogeneity did not significantly reduce by sensitivity analysis.

Main strengths of the current meta-analysis were absence of publication bias and pooled data of studies from different ethnicities. Moreover, herein, a large sample size was assessed for MTHFR 677C > T and 1298A > C polymorphisms and potential sources of heterogeneity in meta-analysis, which significantly increased power of this meta-analysis. Despite clear strengths of this meta-analysis, there were some limitations associated with this meta-analysis, which should be considered. First, although all published studies currently available on MTHFR 1298A > C polymorphism were pooled, but it is believed that our results did not have enough power to provide a confirmed conclusion due to small sample size. Second, because most of included studies on MTHFR 1298A > C polymorphism were performed on Caucasians, results must be carefully interpreted. Further, studies concerning populations in other areas such as Africa and North America are needed to resolve ethnic variation-produced biases. Third, the present analysis was based on unadjusted estimates because most studies had

not provided adjusted data. More precise analysis on factors such as age, gender, lifestyle factors, and environmental factors should be conducted if possible. Finally, gene-gene, gene-environment, MTHFR 677C > T and 1298A > C polymorphisms or even other SNPs of MTHFR gene associations were not evaluated in this meta-analysis due to lack of relevant data.

5. Conclusions

Results of the present meta-analysis indicated that MTHFR 677C > T polymorphism was significantly associated with an increased risk of autism in overall population and by ethnicity, while MTHFR 1298A > C polymorphism was not associated with autism risk in overall. However, stratification analysis indicated that MTHFR 1298A > C polymorphism might be a susceptible factor for autism in Caucasians. Further well-designed studies with large sample size are required to evaluate our results and potential gene-gene and gene-environment interactions in development of autism.

Funding

No specific funding was obtained to support the conduct of this study.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

Acknowledgment

Not applicable.

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