Association between MTHFR Gene Polymorphisms and the Risk of Autism Spectrum Disorders: A Meta-Analysis

Danhua Pu, Yiping Shen, and Jie Wu

Methylenetetrahydrofolate reductase (MTHFR) is essential for DNA biosynthesis and the epigenetic process of DNA methylation, and its gene polymorphisms have been implicated as risk factors for birth defects, neurological disorders, and cancers. However, reports on the association of MTHFR polymorphisms with autism spectrum disorders (ASD) are inconclusive. Therefore, we investigated the relationship of the MTHFR polymorphisms (C677T and A1298C) and the risk of ASD by meta-analysis. Up to December 2012, eight case-control studies involving 1672 patients with ASD and 6760 controls were included for meta-analysis. The results showed that the C677T polymorphism was associated with significantly increased ASD risk in all the comparison models [T vs. C allele (frequency of allele): odds ratio (OR) = 1.42, 95% confidence interval (CI): 1.09–1.85; CT vs. CC (heterozygote): OR = 1.48, 95% CI: 1.09–2.00; TT vs. CC (homozygote): OR = 1.86, 95% CI: 1.08–3.20; CT+TT vs. CC (dominant model): OR = 1.56, 95% CI: 1.12–2.18; and TT vs. CC+CT (recessive model): OR = 1.51, 95% CI: 1.02–2.22], whereas the A1298C polymorphism was found to be significantly associated with reduced ASD risk but only in a recessive model (CC vs. AA+AC: OR = 0.73, 95% CI: 0.56–0.97). In addition, we stratified the patient population based on whether they were from a country with food fortification of folic acid or not. The meta-analysis showed that the C677T polymorphism was found to be associated with ASD only in children from countries without food fortification. Our study indicated that the MTHFR C677T polymorphism contributes to increased ASD risk, and periconceptional folic acid may reduce ASD risk in those with MTHFR 677C>T polymorphism. ***Autism Res*** *2013, 6: 384–392.* © 2013 International Society for Autism Research, Wiley Periodicals, Inc.

Keywords: methylenetetrahydrofolate reductase; polymorphism; autism spectrum disorders; folic acid; meta-analysis

Introduction

Autism spectrum disorders (ASD) form a heterogeneous group of neurodevelopmental disorders diagnosed in early childhood that are characterized by impairment in interpersonal social interaction, disrupted communication, and restricted repetitive and stereotyped patterns of behavior and interests [Committee on Children with Disabilities, 2001]. In 2008, the overall estimated prevalence of ASD among the 14 Autism and Developmental Disabilities Monitoring sites was 11.3 per 1000 (one in 88) children aged 8 years [Autism and Developmental Disabilities Monitoring Network Surveillance Year 2008 Principal Investigators & Centers for Disease Control and Prevention, 2012].

Based on the high concordance rate between monozygotic twins and the tenfold increase in ASD risk among siblings of affected children relative to the general population [Bailey et al., 1995], a strong genetic component (>90% of heritability) of ASD was widely accepted [Freitag, 2007; Lauritsen & Ewald, 2001]. However, a recent study also suggested a strong environmental contribution to the risk of ASD [Hallmayer et al., 2011]. It is conceivable that interactively, genetic, epigenetic, and environmental factors all play roles in the risk of ASD. Epigenetic and environmental factors are thought to be necessary to expose the genetic liability [Keller & Persico, 2003; Muhle, Trentacoste, & Rapin, 2004]. Especially, epigenetic modifications of DNA methylation and gene expression patterns are essential for brain development, brain growth, cognitive function, and behavior [McLellan et al., 2005].

The methylenetetrahydrofolate reductase (MTHFR), which is encoded by the MTHFR gene, is a critical enzyme that regulates the metabolism of folate and methionine, both of which are important factors in DNA methylation

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and nucleotide synthesis [Algasham, Ismail, Dewaidar, & Settin, 2009]. DNA methylation is responsible for a variety of functions, such as tissue-specific gene expression, chromatin structure, and imprinted gene expression. Two widely studied MTHFR polymorphisms (C677T and A1298C) that alter the enzyme activity have been implicated in neurological development. But current individual studies have produced inconsistent results regarding the role of these polymorphisms and the risk of ASD [Boris, Goldblatt, Galanko, & James, 2004; dos Santos, Longo, Brandalize, & Schüler-Faccini, 2010; Guo, Chen, Liu, Ji, & Yang, 2012; James et al., 2006; Liu et al., 2011; Mohammad et al., 2009; Paşca et al., 2009; Schmidt et al., 2011]. A single study may have been underpowered in clarifying the associations of MTHFR polymorphisms with ASD susceptibility. Therefore, a meta-analysis of all relevant published randomized and cohort studies was carried out to clarify the relationship between MTHFR polymorphism and ASD risk.

Materials and Methods

Identification and Eligibility of Relevant Studies

PubMed, EMBASE, and China National Knowledge Infrastructure were searched for all relevant reports (the last search update was December 31, 2012) using the search terms ("MTHFR" OR "methylenetetrahydrofolate reductase") AND ("autism" OR "autism spectrum disorders"). The search was limited to peer-reviewed, published articles. There was no language restriction. As a complementary approach, studies were also identified by a manual search of the original publications from the references of review articles. Of the articles with the overlapping data, we only selected the publication with the most extensive information. To be included in the meta-analysis, the identified articles had to meet all the following criteria: (a) there is quantitative information on the estimated risk of MTHFR C677T and/or A1298C polymorphism for autism, (b) used a case-control or cohort design, (c) contained complete information about all genotype frequency, and (d) clear definition of ASD. The exclusion criteria were as follows: (a) not for MTHFR polymorphism and autism research, (b) review articles, animal studies, simply commentaries, case reports, or unpublished reports, (c) reports containing no usable data, and (d) duplicate publications.

Data Extraction

All data were extracted independently by two authors (Pu D and Wu J). For each study, the following information was collected from the eligible studies: the first author's last name, year of publication, country, ethnicity, design type of study, diagnostic criteria for autism and/or ASD, and sample size of case and control groups. If allele frequencies were not given, they were calculated from the corresponding genotype distributions. For data not provided in published paper or supplemental materials, the relevant information was obtained by direct communications with the corresponding authors.

Statistical Analysis

Summary statistics were performed in the Review Manager 5.1 software [RevMan 5.1. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2011]. The strength of the association between the MTHFR C677T polymorphisms and ASD risk was measured by odds ratios (ORs) with their 95% confidence intervals (CIs). The statistical significance of the summary OR was determined with the *Z*-test. Five comparisons were performed: frequency of allele (T vs. C), dominant genetic model (CT + CC vs. TT), recessive genetic model (TT vs. CC + CT), heterozygote comparison (CT vs. CC), and homozygote comparison (TT vs. CC) of MTHFR C677T between two groups. The correlation of the MTHFR A1298C polymorphisms and ASD risk was also analyzed in the same manner. Considering that maternal folic acid intake is very important for the neurodevelopment, we stratified the data based on whether the participants were born in the countries with mandatory fortification of folic acid.

In consideration of the possibility of heterogeneity across the studies, a statistical test for heterogeneity was performed by an χ^2-based *Q*-test [Zamora, Abraira, Muriel, Khan, & Coomarasamy, 2006]. Statistical heterogeneity was assessed by measure of the I^2. An I^2 measurement greater than 50% was taken to indicate a substantial heterogeneity [The Cochrane Collaboration, 2011]. If substantial heterogeneity was detected, a random effect model was used instead of a fixed effect model. Stata software (version 11.0; StataCorp LP, College Station, TX, USA) was used to analyze publication bias, which was investigated with the funnel plot. Funnel plot asymmetry was further assessed by the method of Egger's linear regression test [Egger, Davey Smith, Schneider, & Minder, 1997]. The significance of the intercept was determined by the *t*-test, and a *P*-value less than 0.05 were considered statistically significant.

Results

Study Characteristics

With the search strategy applied, 17 published articles were identified that could possibly compare the association of MTHFR polymorphism and autism. There were eight studies (all in English) at last retrieved on the basis of the search criteria (Fig. 1, Tables S1 and S2), involving

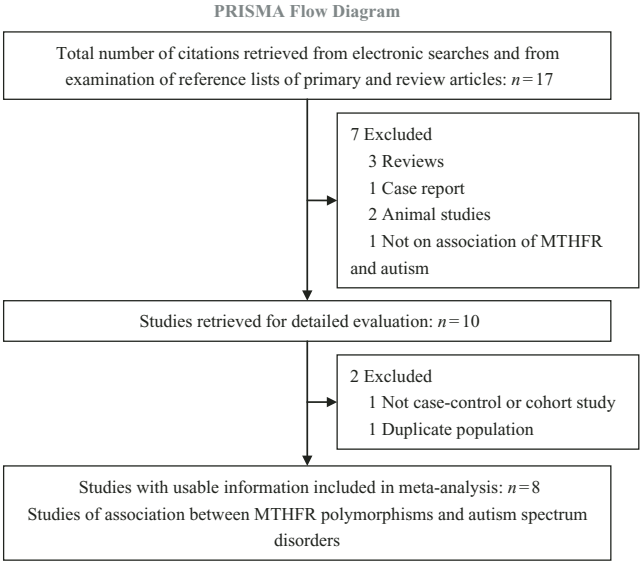

Figure 1. Studies identified with criteria for inclusion and exclusion.

1672 ASD patients and 6760 healthy controls in these studies [Boris et al., 2004; dos Santos et al., 2010; Guo et al., 2012; James et al., 2006; Liu et al., 2011; Mohammad et al., 2009; Paşca et al., 2009; Schmidt et al., 2011]. Three review articles, one case report paper and two animal studies were excluded, as well as one paper that did not study the polymorphism. One research designed in neither case-control nor cohort study, and another paper written by Schmidt et al. [2012], which enrolled the same participants as in their earlier publication [Schmidt et al., 2011], were also excluded. In Liu et al.'s [2011] study, ASD patients were classified into Simplex (SPX) and Multiplex (MPX) families. Patients from SPX families can be considered as idiopathic ASD, so they were included in the analysis. The distribution of genotypes in the controls of all studies was consistent with Hardy–Weinberg equilibrium. Detailed characteristics of each study are described in Table 1.

Meta-Analysis

In the meta-analysis, eight studies met our criteria for MTHFR C677T polymorphism, and three of the eight studies were eligible for MTHFR A1298C polymorphism meta-analysis. The distributions of MTHFR C677T and A1298C genotypes of the included studies are presented in Table 2. We used as the reference category the CC genotype in C677T polymorphism, and AA genotype in A1298C polymorphism.

Fixed-effects or random-effects models were used according to the heterogeneities in the comparisons (I2≤50%, fixed-effects models; I2>50%, random-effects models). As shown in Table 3 and Figure 2, MTHFR C677T polymorphism is significantly associated with autism risk in all five comparisons [frequency of T allele vs. C allele: OR = 1.42, 95% CI: 1.09–1.85, $P = 0.01$; heterozygote comparison (CT vs. CC): OR = 1.48, 95% CI: 1.09–2.00, $P = 0.01$; homozygote comparison (TT vs. CC): OR = 1.86, 95% CI: 1.08–3.20, $P = 0.03$; dominant model (TT+CT vs. CC): OR = 1.56, 95% CI: 1.12–2.18, $P = 0.009$; recessive model (TT vs. CT+CC): OR = 1.51, 95% CI: 1.02–2.22, $P = 0.04$].

Considering the condition of periconceptional folic acid intake, the data were reanalyzed by stratifying the participants based on whether they were born in a country with mandate food fortification of folic acid (the United States and Canada) or not (Brazil, India, Romania, and China). The results for studies from the United States and Canada indicated that there is no significant association of MTHFR C677T variants with autism risk (frequency of T allele vs. C allele: OR = 1.40, 95% CI: 0.94 2.10, $P = 0.1$; TT genotype vs. CC genotype: OR = 1.85, 95% CI: 0.80 4.25, $P = 0.15$), but the association was significant for studies from Brazil, India, Romania, and China (frequency of T allele vs. C allele: OR = 1.43, 95% CI: 1.02 2.01, $P = 0.04$; TT genotype vs. CC genotype: OR = 1.72, 95% CI: 1.07 2.76, $P = 0.03$) (Table 3 and Fig. 2).

In the analysis of the MTHFR A1298C polymorphism, only the recessive model comparison (CC vs. AA+AC: OR = 0.73, 95% CI: 0.56–0.97, $P = 0.03$) reached a significant difference, indicating that 1298CC genotype frequency was increased in the control group (Table 3, Fig. 3). No significant correlation of MTHFR A1298C polymorphism to ASD was found in the other four comparisons ($P > 0.05$) (Table 3).

In addition, two studies [James et al., 2006; Schmidt et al., 2011] carried out in the United States containing data of the MTHFR C677T polymorphism in mothers with autism children were also analyzed, and no significant difference between case and control groups ($P > 0.05$) was identified (Table S3).

Publication Bias

Begg's funnel plot and Egger's test were performed to evaluate the publication bias of literatures. As shown in Figure 4, the shape of the funnel plots was symmetrical in the comparison of allele frequencies (frequency of T allele vs. C allele). Then, the Egger's test was adopted to provide statistical evidence of funnel plot symmetry. The result still did not show any evidence of publication bias ($P = 0.796$).

Discussion

Several studies have investigated the association between candidate gene polymorphisms and the risk of autism

Table 1. Characteristics of Studies Included in the Meta-Analysis of MTHFR Polymorphisms between ASD Children and Controls

Author	Year	Study type	Country	Ethnicity	Diagnostic criteria	Genotyping method	Sample size	
							Cases	Controls
Boris et al.	2004	Case control	United States	Caucasian	DSM-IV	PCR DNA analysis	168	5389
James et al.	2006	Case control	United States	Caucasian-derived	DSM-IVE/ADOS/CARS	Real-time PCR	356	205
Mohammad et al.	2009	Case control	United States	Indian	DSM-IVE and ABC scoring	PCR-RFLP	138	138
Paşca et al.	2009	Case control	Romania	Caucasian	DSM-IVE	Real-time PCR	39	80
dos Santos et al.	2010	Case control	Brazil	European-derived	DSM-IVE/CARS	PCR	151	100
Liu et al.	2011	Case control	Canada	Caucasian	ASD-CARC and clinical criteria and (ADOS/ ADI-R/ PDDBI)	TaqMan	205	384
Schmidt et al.	2011	Case control	United States	Mixed	ADI-R and autism diagnostic observation schedule-generic	TaqMan	429	278
Guo et al.	2012	Case control	China	Chinese	ADI-R & autism diagnostic observation schedule	PCR-RFLP	186	186
Total							1672	6760

MTHFR, 5, 10-methylenetetrahydrofolate reductase; ASD, autism spectrum disorders; DSM-IVE, Diagnostic and Statistical Manual of Mental Disorder-Fourth Edition; ADOS, the Autism Diagnostic Observation Schedule; CARS, Childhood Autism Rating Scale; ABC, Autism Behavior Checklist; ASD-CARC, the Autism Spectrum Disorders-Canadian American Research Consortium; PDDBI, the PDD Behavior Inventory; ADI-R, Autism Diagnostic Interview-Revised; PCR-RFLP, polymerase chain reaction–restriction fragment length polymorphism.

Table 2. Distributions of MTHFR C677T/A1298C Genotypes of Eligible Studies Included in the Meta-Analysis

Article (MTHFR C677T)	Cases					Controls				
	CC	CT	TT	C allele	T allele	CC	CT	TT	C allele	T allele
Boris et al.	35	94	39	164	172	2570	2213	606	7353	3425
James et al.	134	176	46	444	268	93	90	22	276	134
Mohammad et al.	98	35	5	231	45	120	18	0	258	18
Paşca et al.	21	14	4	56	22	46	28	6	120	40
dos Santos et al.	60	68	23	188	114	45	41	14	131	69
Liu et al.	68	98	39	234	176	177	166	41	520	248
Schmidt et al.	128	133	33	389	199	74	77	29	225	135
Guo et al.	79	77	30	235	137	87	83	16	257	115
Total	623	695	219	1941	1133	3212	2716	734	9140	4184

Article (MTHFR A1298C)	Cases					Controls				
	AA	AC	CC	A allele	C allele	AA	AC	CC	A allele	C allele
Boris et al.	93	65	10	251	85	70	75	14	215	103
James et al.	175	147	34	497	215	103	77	24	283	125
Mohammad et al.	35	59	44	129	147	48	32	58	128	148
Liu et al.	109	81	15	299	111	170	175	37	515	249
Schmidt et al.	160	117	19	437	155	89	76	12	254	100
Total	572	469	122	1176	558	480	435	145	1141	625

MTHFR, 5, 10-methylenetetrahydrofolate reductase.

[Weiss, 2009]. For example, Guerini et al. [2011] reported that human leukocyte antigen (HLA) polymorphisms were associated with ASD in Italian children. Li et al. [2010] found that CNTNAP2 might be a susceptibility gene of ASD in the Chinese Han population. The association between MTHFR gene polymorphisms and ASD risk has been a topic of particular interest, but the results from individual studies had been inconsistent and sometimes with contradicting findings. In 2004, Boris et al. [2004] performed a case-control study and revealed a significant increase in the frequency of TT and CT genotype in ASD children compared with controls at position 677. They also found that CC genotype was more frequent in healthy control group than in autistic group at position 1298, suggesting a protective role of this polymorphism in autism. In another case-control study performed in

Table 3. Meta-Analysis of MTHFR C677T/A1298C Polymorphisms on Risk of Autism Spectrum Disorders

Comparisons	OR	95% CI	I^2 (%)	*P*
MTHFR C677T polymorphism and ASD (8 studies)				
Allele frequency comparison (T vs. C)	**1.42**	**1.09, 1.85**	**82**	**0.01**
Subgroup of mandatory fortification with folate	1.4	0.94, 2.10	91	0.1
Subgroup of no mandatory fortification	1.43	1.02, 2.01	58	0.04
Heterozygote comparison (CT vs. CC)	**1.48**	**1.09, 2.00**	**71**	**0.01**
Subgroup of mandatory fortification with folate	1.6	1.01, 2.53	83	0.05
Subgroup of no mandatory fortification	1.32	0.91, 1.92	39	0.14
Homozygote comparison (TT vs. CC)	**1.86**	**1.08, 3.20**	**79**	**0.03**
Subgroup of mandatory fortification with folate	1.85	0.80, 4.25	90	0.15
Subgroup of no mandatory fortification	1.72	1.07, 2.76	1	0.03
Dominant model analysis (CT+TT vs. CC)	**1.56**	**1.12, 2.18**	**77**	**0.009**
Subgroup of mandatory fortification with folate	1.65	0.97, 2.82	88	0.07
Subgroup of no mandatory fortification	1.44	0.99, 2.10	46	0.06
Recessive model analysis (TT vs. CT+CC)	**1.51**	**1.02, 2.22**	**65**	**0.04**
Subgroup of mandatory fortification with folate	1.43	0.82, 2.50	82	0.21
Subgroup of no mandatory fortification	1.61	0.98, 2.66	12	0.06
MTHFR A1298C polymorphism and ASD (5 studies)				
Allele frequency comparison (A vs. C)	0.86	0.68, 1.08	68	0.19
Heterozygote comparison (AC vs. AA)	0.98	0.68, 1.43	75	0.98
Homozygote comparison (CC vs. AA)	0.79	0.59, 1.07	0	0.13
Dominant model analysis (AC+CC vs. AA)	0.93	0.70, 1.23	60	0.61
Recessive model analysis (CC vs. AC+AA)	0.73	0.56, 0.97	0	0.03

ASD, autism spectrum disorders; MTHFR, 5, 10-methylenetetrahydrofolate reductase; OR, odds ratio; CI, confidence interval.
I^2, Cochran's χ^2-based Q-statistic test for assessing the heterogeneity (>50% indicates a substantial heterogeneity).
MTHFR C677T analysis is stratified by the studying country with or without mandatory fortification of folate.

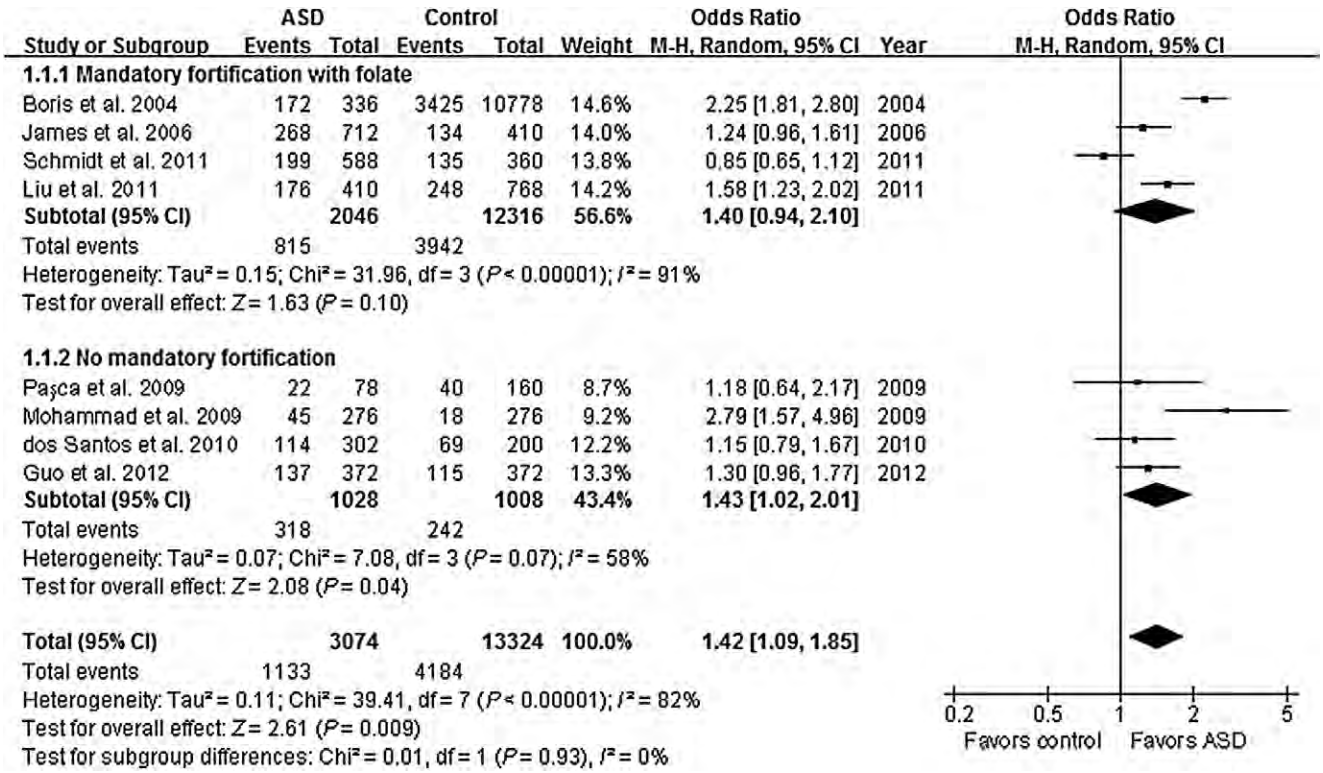

Figure 2. Association between MTHFR C677T polymorphism and autism spectrum disorders risk (frequency of T allele vs. C allele) stratified by mandatory folic acid fortification in study country.

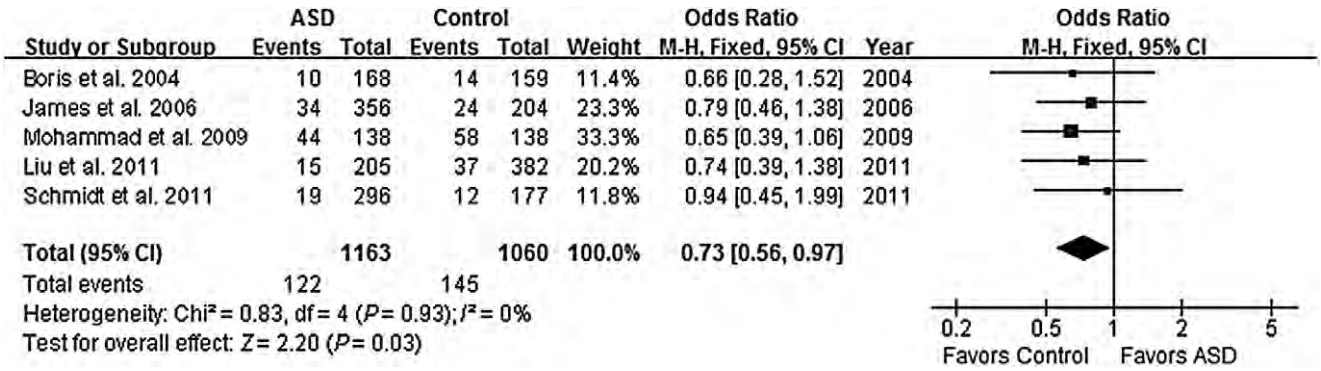

Figure 3. Association between MTHFR A1298C polymorphism and autism spectrum disorders risk (CC vs. AA+AC).

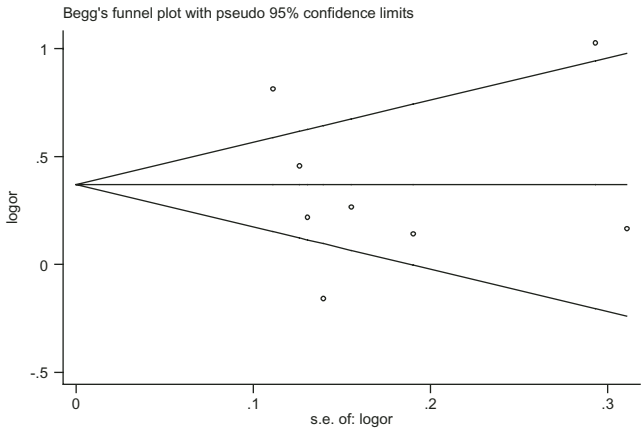

Figure 4. Begg's funnel plot for odds ratio of MTHFR C677T allele frequency comparison (T vs. C allele) in autism spectrum disorders.

India, Mohammad et al. [2009] found similar effect of the T allele on the risk of autism at position 677 but suggested that the C allele at position 1298 rather had an additive role for autism. Liu et al. [2011] studied the autism patients both in SPX (these patients were included in the meta-analysis) and MPX families, and interestingly they only found the association of T allele at position 677 in SPX cohort. The significantly higher frequency of 677TT/1298AA compounded genotypes and 677T-1298A haplotype in ASD group also suggested that the A allele at position 1298 in combination with the T allele at position 677 was associated with the increased risk for autism. Recently, a similar study with Chinese Han population also supported the notion that MTHFR C677T polymorphism was associated with increased risk for ASD [Guo et al., 2012].

However, in a study with predominately Caucasian individuals, James et al. [2006] revealed no association between MTHFR C677T and autism. A study carried out in South Brazil did not support the association either [dos Santos et al., 2010]. Paşca et al. [2009] attempted to study the C677T polymorphism in different subtypes of ASD (autistic disorder, Asperger's syndrome, and pervasive developmental disorders not otherwise specified). They found a normal distribution of the C677T polymorphism, but the study suffered from a low subject number, and the slightly increased frequency of the 677T allele was not conclusive. Schmidt et al. [2011, 2012] performed a study to determine whether maternal folic acid intake was in relation to the risk of ASD and developmental delay. MTHFR C677T and A1298C genotypes were examined in all the participants, and no marked difference was found in MTHFR C677T and A1298C polymorphisms between the ASD children and controls. However, interesting results were found that increased risk was associated with the MTHFR C677T maternal genotype when mothers did not take prenatal vitamins in the first month of pregnancy or before, and periconceptional intake of folic acid was associated with reduced risk of ASD in those mothers and children with susceptible genotypes.

Recently, 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; Paşca et al., 2009], of which three original publications support the association of MTHFR C677T with increased risk for ASD and the three others did not. The meta-analysis revealed that the distributions of TT, CT, and (TT + CT) genotypes were significantly increased compared with CC genotype in ASD patients (TT vs. CC: OR = 2.26; CT vs. CC: OR = 1.57; TT+CT vs. CC: OR = 1.66), thus supporting the association of C677T polymorphism with autism risk. We performed the most comprehensive meta-analysis based on all currently available data on the association between MTHFR polymorphism and ASD in eight publications [Boris et al., 2004; dos Santos et al., 2010; Guo et al., 2012; James et al., 2006; Liu et al., 2011; Mohammad et al., 2009; Paşca et al., 2009; Schmidt et al., 2011], in which we added two newest studies [Guo et al., 2012; Schmidt et al., 2011]. We confirmed that MTHFR C677T polymorphism was

significantly associated with increased ASD risk and further revealed that the association was significant in all five comparisons, including allele frequency, heterozygote, homozygote, dominant model, and recessive model (OR = 1.42, OR = 1.48, OR = 1.86, OR = 1.56, and OR = 1.51, respectively; $P < 0.05$), in which the strongest association was showed in homozygote comparison, namely the risk of developing ASD in children with TT genotype was 1.86 times higher than those with CC genotype. In addition, we also performed a meta-analysis on the association of MTHFR A1298C polymorphism with ASD based on the data from three studies [Boris et al., 2004; Liu et al., 2011; Mohammad et al., 2009]. Our meta-analysis result supports the protective effect of the C allele on ASD but only when it is at homozygous status (i.e. as 1298CC). To our knowledge, this is the first meta-analysis on the association of MTHFR A1298C polymorphism with ASD; since the numbers of study and individuals involved are limited, we await more data to confirm the conclusion.

Currently, the underlying mechanisms by which MTHFR 677T and/or 1298C allele increases or decreases ASD risk are not fully understood. Because the T allele of MTHFR at position 677 is known to decrease the enzymatic activity of the enzyme [Frosst et al., 1995], which plays an important role in folate-dependent, one-carbon metabolism, it is hypothesized that the association between MTHFR and ASD may be mediated by the folate level. The folate metabolic pathway is known to be important for neurodevelopmental processes; it has been suggested that ASD may be associated with metabolic abnormalities in the folate pathway [dos Santos et al., 2010; Mohammad et al., 2009; Paşca et al., 2009]. Studies have been carried out to evaluate the intake of folic acid and the risk for autism [Ali et al., 2011; Beard, Panser, & Katusic, 2011; Currenti, 2010; Rogers, 2008; Schmidt et al., 2011, 2012]. In Schmidt et al.'s study [2011, 2012], they enrolled 429 ASD patients and 278 healthy controls, and compared the folate intake by their mothers at periconceptional stage. They found that the mean folate intake at the first month of pregnancy was significantly greater for mothers of healthy children than for mothers of ASD children. Interestingly, they also found that the association was the strongest for mothers and/or children who possessed the MTHFR C677T variant genotypes. Ali et al. [2011] also found that autism children were presented with lower serum folate and vitamin B12 levels compared with controls. Our meta-analysis result in stratified populations further supported that the effect of folic acid on the ASD risk is dependent on the C677T status, which may explain why we have different conclusions on the effect of folic acid in ASD. If the genotype-dependent effect of folate intake with ASD is confirmed, it will be a useful indication for guiding the use of folate intake to benefit a certain population (mother and children).

Interestingly, some scientists reported that maternal folate intake might increase the risk of ASD [Beard et al., 2011; Currenti, 2010; Rogers, 2008].They considered that the enhancement of maternal folate status before and during pregnancy altered natural selection by increasing survival rates during pregnancy of infants possessing the MTHFR C677T polymorphism. Those infants with MTHFR polymorphisms and lower enzyme activity cannot maintain the higher folate status experienced in utero, thus leading to an increased number of cases of developmental disorders like autism [Currenti, 2010; Rogers, 2008]. The hypothesis was also somewhat documented by the recent study performed by Beard et al. [2011], in which high correlation between prenatal folic acid intake and increased incidence of autism [Pearson product moment correlation coefficient (r) = 0.87], and weak association between pediatric folic acid and autism incidence (r = 0.62), were discovered. In spite of this, it should be pointed out that these correlational/ecological studies based on overlapping trends in folic acid fortification and increased autism are of the weak epidemiological study design, and may be subject to ecological fallacy. Importantly, Haggarty et al. [2008] performed an observational perspective cohort study in which they found no evidence that supports the concern that folic acid fortification or supplement use in pregnancy causes selection of deleterious genotypes, including MTHFR C677T and A1298C.

Moreover, Rogers [2008] reported that the MTHFR polymorphic form in mothers may affect autism development in children. Therefore, we searched for the data on MTHFR variants of mothers with child with autism, and only two studies, which were performed in the United States, were identified [James et al., 2006; Schmidt et al., 2011]. Analyzed results do not show marked relationship between mother's MTHFR polymorphism and children's risk for developing ASD. More studies are needed.

Several limitations might be included in this study. First, the main limitation is the small study/sample size for meta-analysis. Second, we did not do the analysis stratified by the diagnostic subgroups because of the lack of information from primary literature. Third, analyses stratified by other related susceptible factors, such as sex and ethnicity [Autism and Developmental Disabilities Monitoring Network Surveillance Year 2008 Principal Investigators & Centers for Disease Control and Prevention. 2012], have not been conducted in the present study because insufficient data were available from the primary studies. Despite the limitations, the data of the present meta-analysis did show an association of MTHFR C677T polymorphism with increased susceptibility to ASD.

In conclusion, our analysis support the association of MTHFR C677T polymorphism with increased risk of ASD

and further pointed out that the possible modulating role of folate might be dependent on the MTHFR genotype status. Further studies are warranted to confirm the meta-analysis conclusion.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Characteristics of Included Studies
Table S2. Characteristics of Excluded Studies
Table S3. Meta-Analysis of MTHFR C677T Polymorphism on Risk of Mothers with Autism Spectrum Disorders Child