Determining the Association between Methylenetetrahydrofolate Reductase (MTHFR) Gene Polymorphisms and Genomic DNA Methylation Level: A Meta-Analysis

Li Wang, Shaofang Shangguan, Shaoyan Chang, Xin Yu, Zhen Wang, Xiaolin Lu, Lihua Wu, and Ting Zhang*

Background: The methylenetetrahydrofolate reductase (*MTHFR*) polymorphism is a risk factor for neural tube defects. C677T and A1298C *MTHFR* polymorphisms produce an enzyme with reduced folate-related one carbon metabolism, and this has been associated with aberrant methylation modifications in DNA and protein. Methods: A meta-analysis was conducted to assess the association between *MTHFR* C677T/A1298C genotypes and global genomic methylation. Results: Eleven studies met the inclusion criteria. Of these, 10 were performed on C677T *MTHFR* genotypes and 6 were performed on A1298C *MTHFR* genotypes. Our results did not indicate any correlation between global methylation and *MTHFR* A1298C, C677T polymorphisms. Conclusion: The results of our study provide evidence to assess the global methylation modification alterations of *MTHFR* polymorphisms among individuals. However, our data did not found any conceivable proof supporting the hypothesis that common variant of *MTHFR* A1298C, C677T contributes to methylation modification.

Introduction

Folate deficiency has been linked to many different pathologies, including cancer and birth defects (Czeizel and Dudas, 1992; Bhargava and Tyagi, 2014). Whereas some underlying mechanisms have been proposed, there are many more to be determined. It appears that folic acid and other one-carbon intermediates may determine clinical programming effects by means of DNA and protein methylation modifications (Lamprecht and Lipkin, 2003; Stover, 2009; Bailey et al., 2010).

The enzyme methylenetetrahydrofolate reductase (MTHFR) is the rate-limiting enzyme in the folate metabolism cycle. It catalyzes the reduction of 5,10 methylene tetrahydrofolate (THF) to 5-methyl THF; the 5-methyl THF form of the vitamin converts the amino acid homocysteine to methionine. Methionine is further converted and provides a methyl group that is important in a variety of biological reactions, including the methylation of DNA.

Because of the important role of the *MTHFR* gene in one carbon metabolism, *MTHFR* gene polymorphisms were hypothesized to play an essential role in inherited DNA methylation profiles through folic acid metabolism (Christensen et al., 2010; Kim et al., 2010; Wallace et al., 2010). *MTHFR* single nucleotide polymorphisms (SNPs) can decrease the function of the enzyme and, therefore, the body's ability to methylate. At least 23 polymorphisms in the *MTHFR* gene have been identified and are thought to be associated with an increased susceptibility to neural tube defects (NTDs), occlusive vascular disease, Alzheimer's disease and other forms of dementia, colon cancer, and acute leukemia (Posey et al., 1996; Martinez de Villarreal et al., 2001; Cunha et al., 2002; Sailasree et al., 2010; Saberi et al., 2012; de Arruda et al., 2014).

Two of these polymorphisms (*MTHFR* C677T and A1298C) are well understood and have been tested in a variety of diseases. The common 677 C>T and 1298 A>C polymorphisms in the *MTHFR* gene are hypothesized to cause production of a thermolabile enzyme with reduced function, or to affect the conversion of MTHF to BH4, and eventually cause genomic DNA hypomethylation (Yamada et al., 2001). Some studies have explained the association between *MTHFR* polymorphisms and types of cancer by suggesting that hypomethylation is induced by the polymorphism and consequently activates proto-oncogenes (Izmirli, 2012). However, other studies have provided inconsistent results on the relationship between *MTHFR* polymorphisms and genomic DNA hypomethylation in

---

TABLE 1. *Methylation Levels among MTHFR Gene Polymorphism in Shanxi Province, China*

	Global Methylation level%[a] (n[b])	LINE-1 Methylation level%[a] (n[b])
A1298C aa	6.24 ± 2.46 (22)/7.48 ± 1.30 (5)	59.10 ± 4.98 (20)/54.58 ± 5.69 (6)
A1298C ac	6.02 ± 1.97 (5)/5.84 ± 4.03 (4)	57.36 ± 3.76 (4)/54.43 ± 5.41 (4)
A1298C cc	3.58 ± 1.95 (2)/5.63 ± 2.89 (4)	60.13 ± 2.47 (2)/58.92 ± 7.89 (3)
C677T cc	5.35 ± 1.97 (8)/6.76 (1)	59.80 ± 2.70 (7)/53.86 (1)
C677T ct	6.42 ± 2.73 (12)/6.17 ± 3.15 (8)	59.51 ± 4.07 (11)/54.91 ± 6.16 (8)
C677T tt	6.00 ± 2.51 (8)/6.80 ± 2.56 (4)	58.59 ± 6.71 (7)/55.54 ± 5.91 (4)

[a]Methylation level shows as methylation level in controls/methylation level in NTDs.
[b]Number of samples shown in bracket.

patients (Arakawa et al., 2012; Hanks et al., 2013; de Arruda et al., 2014). Until now, no specific meta-analyses have been carried out to explore the relationship between *MTHFR* genotype and global methylation levels. Given the important role of *MTHFR* in the folate metabolism cycle, understanding the magnitude of the impact that C677T and A1298C *MTHFR* polymorphisms may have on methylation modifications could be critical in assessing the population-level risk of tumors and birth defects. Therefore, the current meta-analysis was carried out to estimate the magnitude of association of the most common *MTHFR* polymorphisms (C677T and A1298C) with global DNA methylation.

Materials and Methods

DNA METHYLATION AND MTHFR GENOTYPING ANALYSIS
Experimental data in this study were obtained and extracted from our own lab, all samples came from Shanxi province, China. Global DNA methylation was detected with the Methylamp Global DNA Methylation Quantification Ultra Kit, and LINE-1 methylation analysis was performed using Sequenom MassARRAY platform, as previously described (Wang et al., 2010). *MTHFR* variant calling was performed as published before (Yu et al., 2014).

LITERATURE SEARCH
We performed a literature search in PubMed, Embase, and Medline using the following terms, "*MTHFR*," "global methylation/genomic methylation/global DNA methylation/genomic DNA methylation/5-methylcytosine," and "polymorphism/SNP/genotype/gene polymorphism/genetic risk," limiting our search to publications in the English language and on human studies up to December 31, 2015. A second search was then performed using the same terms and by assigning all terms within Title/Abstract. The results were compared with the first result to make sure no related literature was missed. For studies involving LINE_1 methylation levels as a marker of global methylation, a further search was carried out using the following terms: "*MTHFR*,"

AND "polymorphism/SNP/genotype/gene polymorphism/genetic risk," and "LINE-1 methylation/long interspersed nuclear element methylation." References of included literature were checked and related literatures were included. These steps were performed independently by two researchers (L.W. and Y.X.) and were cross-verified. Any differences in opinion were resolved through team discussion.

INCLUSION CRITERIA AND EXCLUSION CRITERIA
As we did not expect many data from randomized controlled trials, we included both cohort and case–control studies. We included studies that compared global DNA methylation levels and LINE-1 methylation levels in human cases. Only novel data were included. Studies were excluded if there was no control group, for example, case series and case reports. Data from populations with extra folate intervention were excluded because folate could provide more methyl groups. Review papers were excluded. Animal and cell model studies were also excluded.

DATA EXTRACTION
For each study, information such as: authors, year of publication, country of origin, sample type, number of cases with gender, study design, genotyping, methylation level, and analysis method was extracted. In some cases, additional folate or folate analogues were used in the design, and these data were not included in the meta-analysis. Some studies that presented DNA methylation results without mean methylation or without standard deviation or variance were excluded in this analysis. Data with only 95% confidence interval were transferred to standard deviation in hands by calculator tools in Review Manager 5.1. All the findings were then grouped by a specific data extraction template.

META-ANALYSIS
Review Manager 5.1 software was used to conduct meta-analyses. Considering methylation levels were measured using different methods, a random effects model was used in all analyses of the mean methylation level; where
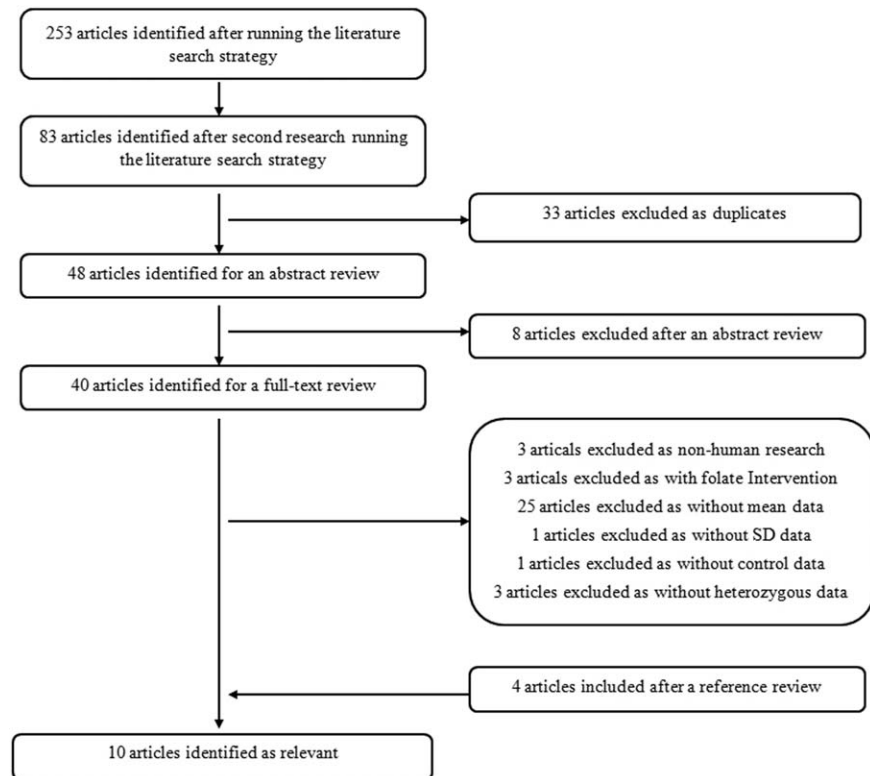
feasible, a fixed effect model was performed when the study heterogeneity was less than 50%. Stratified analyses were performed by source of case samples (peripheral blood or not).

Results

GENOMIC DNA METHYLATION AND LINE-1 METHYLATION AMONG MTHFR GENE POLYMORPHISMS IN DISEASE TISSUES

MTHFR gene polymorphisms and methylation modifications are involved the pathogenesis of NTDs. To assess if the *MTHFR* genotype affects global methylation, our previous data were extracted to compare methylation levels with C677T and A1298C *MTHFR*. Both genomic DNA methylation and LINE-1 methylation were compared in brain tissue of control and a NTD fetus. As shown in the results in Table 1, no significant difference in methylation levels was found among *MTHFR* polymorphisms.

CHARACTERISTICS OF THE STUDIES

The literature search identified a total of 253 potential relevant articles, with 83 articles remaining after the second search. Of these, 33 articles were excluded as duplicates and 8 articles were excluded after reading the title or abstract because of obvious irrelevance. In addition, 3 articles were excluded as nonhuman research; 3 articles were excluded because they had folate intervention; 25 articles were excluded because they lacked mean data; 1 article was excluded because it lacked standard deviation

data; 1 article was excluded because it did not have any control data; 1 article was excluded because there were no heterozygous data; 2 articles were excluded because there were no separately heterozygous data; 4 articles were included after a reference review.

In total, 13 articles met all the primary inclusion criteria, and 12 studies for *MTHFR* C677T, 7 studies for *MTHFR* A1298C were included in the final meta-analysis. A flow chart describing the process of study inclusion/exclusion is displayed in Figure 1. Selected characteristics of these studies related to the association with methylation are summarized in Table 2. Tested sample types include peripheral blood cells and normal disease tissues. The mean age of the total population ranged from 0 to 88 years. Several methylation assay methods were used, including the Methyl Flash Methylated DNA Quantification Kit, Global DNA Methylation Quantification Ultra Kits, [3H]methyl-SAM assay, LC-MS/LC mCyt/mgDNA, in vitro methyl acceptance assay,[3H]-Dctp assay, high-performance capillary electrophoresis, Global DNA Methylation Quantification Ultra Kit, and Epityper MassArray (Sequenom) assay.

META-ANALYSIS RESULTS

For C677T *MTHFR* genotyping and methylation assays, the meta-analysis included pooled data from 1147 individuals. Overall, this meta-analysis showed that the C677T *MTHFR*

TABLE 2. *Characteristics of the Studies Included in the Meta-analysis of MTHFR Gene Polymorphism with Genomic DNA Methylation Level*

Study	Geography	Design	Sample size (male/female)	Age (Mix–max)	Studied SNP	Samples	Method
Alexandra S. Weiner (Weiner et al., 2013)	Russian	Cohort	160 (0/160)	31.6[a]	C677T	Leukocytes	Methyl Flash Methylated DNA Quantification Kit
Xiaoli Chen (Chen et al., 2010)	China	Case–control	65 (30/35)	0[b]	C677T	Brain tissue	Global DNA Methylation Quantification Ultra Kits
Francesco Graziano (Graziano et al., 2006)	Italy	Case–control	164 (83/81)	32–79	C677T	Peripheral blood	[3H]methyl-SAM
Simonetta Friso (Friso et al., 2005)	Italy	Cohort	198 (\)	\	A1298C	Lymphocytes	LC-MS mCyt/mgDNA
M Pufulete (Pufulete et al., 2005)	UK	Cohort	65 (33/35)	36–78	C677T A1298C	Normal mucosa tissue	in vitro methyl acceptance assay
Simonetta Friso (Friso et al., 2002)	Italy	Cohort	292 (231/61)	59.82[a]	C677T	Peripheral blood	Liquid chromatography
Lori Lathrop Stern (Stern et al., 2000)	USA	Cohort	19 (8/11)	25-75	C677T	Peripheral blood	[3H]methyl-SAM
Sabrina Narayanan (Narayanan et al., 2004)	Scotland.	Case-control	199 (100/99)	32–88	C677T A1298C	Lymphocytes	[3H]methyl-SAM
R Castro, (Castro et al., 2004)	Portuguese	Cohort	96 (37/59)	20–69	C677T A1298C,	Peripheral blood	[3H]-Dctp
Maria F. Paz (Paz et al., 2002)	Barcelona	Case-control	57(\)	\	C677T A1298C	Peri-carcinous tissue	high-performance capillary electrophoresis
Li Wang(unpublished)	China	Cohort	30 (11/19)	0#	C677T A1298C	Brain tissue	Global DNA Methylation Quantification Ultra Kits, MassArray(Sequenome)

[a]Average age.
[b]Fetus aborted.

polymorphism was not associated with global methylation level (for TT vs. CC: $z = 0.25$; $p = 0.80$; for CT vs. CC: $z = 1.04$; $p = 0.30$). Heterogeneity between studies was observed in the overall comparisons as well as in subgroup analyses (Fig. 2). Publication bias was explored using funnel plots; results are shown in Supplementary Figure S1, which is available online. Similarly, no associations were found in subgroup analyses when sample type was limited to peripheral blood cells (Fig. 3).

Another meta-analysis for A1298C *MTHFR* genotyping and global methylation assays included pooled data from 1053 individuals. Similarly, this meta-analysis showed that when a random effects model was used in overall and subgroup analyses. Publication bias was explored using funnel plots, results were shown in Supplementary Figure S1. *MTHFR* A versus C allele polymorphism was not associated with global methylation levels (data not shown). As the

results show in Figure 2, no correlation was found between *MTHFR* 1298 A versus C alleles. Heterogeneity between studies was observed in the overall comparisons as well as in subgroup analyses when *MTHFR* 1298AC and CC groups were compared. However, no associations were found (Figs. 2 and 3).

Discussion

To our knowledge, this is the first meta-analysis investigating the association between *MTHFR* polymorphisms and global DNA methylation levels. No significant evidence was found to indicate that global DNA methylation level was regulated by *MTHFR* polymorphisms in individuals, even in samples originating from peripheral blood cells.

In recent years, population and experimental studies have provided evidence to support the role of folate
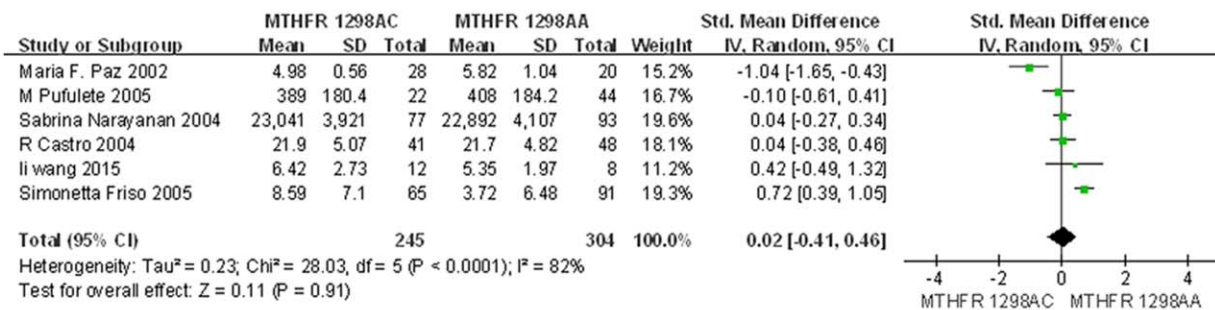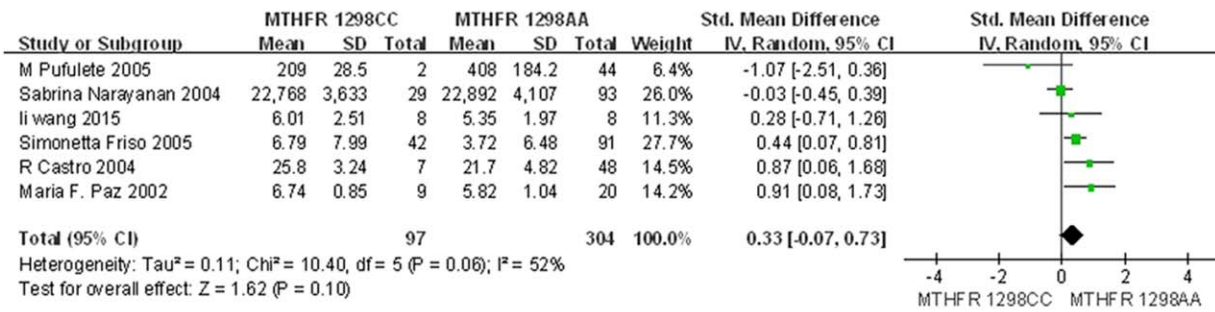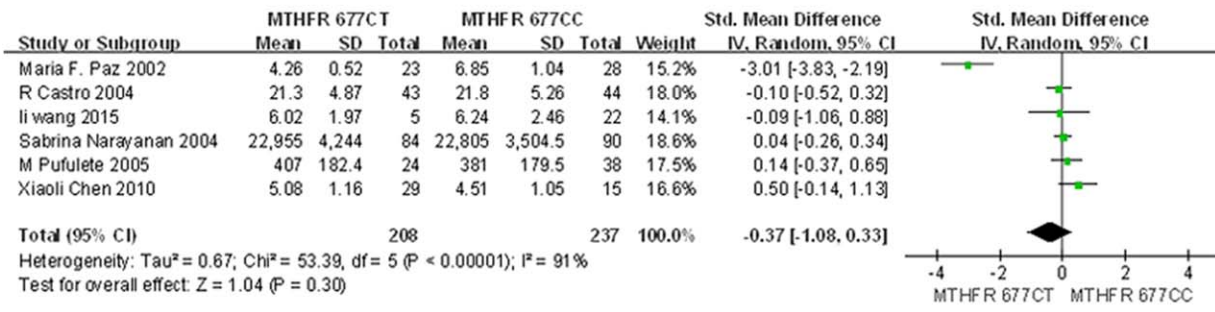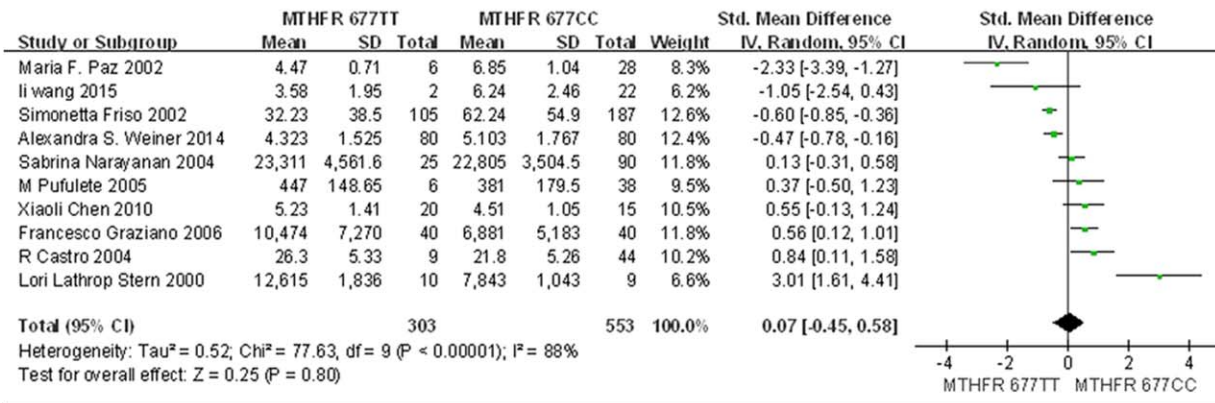
Study or Subgroup	MTHFR 677TT Mean	SD	Total	MTHFR 677CC Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
Maria F. Paz 2002	4.47	0.71	6	6.85	1.04	28	8.3%	-2.33 [-3.39, -1.27]
li wang 2015	3.58	1.95	2	6.24	2.46	22	6.2%	-1.05 [-2.54, 0.43]
Simonetta Friso 2002	32.23	38.5	105	62.24	54.9	187	12.6%	-0.60 [-0.85, -0.36]
Alexandra S. Weiner 2014	4.323	1.525	80	5.103	1.767	80	12.4%	-0.47 [-0.78, -0.16]
Sabrina Narayanan 2004	23,311	4,561.6	25	22,805	3,504.5	90	11.8%	0.13 [-0.31, 0.58]
M Pufulete 2005	447	148.65	6	381	179.5	38	9.5%	0.37 [-0.50, 1.23]
Xiaoli Chen 2010	5.23	1.41	20	4.51	1.05	15	10.5%	0.55 [-0.13, 1.24]
Francesco Graziano 2006	10,474	7,270	40	6,881	5,183	40	11.8%	0.56 [0.12, 1.01]
R Castro 2004	26.3	5.33	9	21.8	5.26	44	10.2%	0.84 [0.11, 1.58]
Lori Lathrop Stern 2000	12,615	1,836	10	7,843	1,043	9	6.6%	3.01 [1.61, 4.41]
Total (95% CI)			**303**			**553**	**100.0%**	**0.07 [-0.45, 0.58]**

Heterogeneity: Tau² = 0.52; Chi² = 77.63, df = 9 (P < 0.00001); I² = 88%
Test for overall effect: Z = 0.25 (P = 0.80)

Study or Subgroup	MTHFR 677CT Mean	SD	Total	MTHFR 677CC Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
Maria F. Paz 2002	4.26	0.52	23	6.85	1.04	28	15.2%	-3.01 [-3.83, -2.19]
R Castro 2004	21.3	4.87	43	21.8	5.26	44	18.0%	-0.10 [-0.52, 0.32]
li wang 2015	6.02	1.97	5	6.24	2.46	22	14.1%	-0.09 [-1.06, 0.88]
Sabrina Narayanan 2004	22,955	4,244	84	22,805	3,504.5	90	18.6%	0.04 [-0.26, 0.34]
M Pufulete 2005	407	182.4	24	381	179.5	38	17.5%	0.14 [-0.37, 0.65]
Xiaoli Chen 2010	5.08	1.16	29	4.51	1.05	15	16.6%	0.50 [-0.14, 1.13]
Total (95% CI)			**208**			**237**	**100.0%**	**-0.37 [-1.08, 0.33]**

Heterogeneity: Tau² = 0.67; Chi² = 53.39, df = 5 (P < 0.00001); I² = 91%
Test for overall effect: Z = 1.04 (P = 0.30)

Study or Subgroup	MTHFR 1298CC Mean	SD	Total	MTHFR 1298AA Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
M Pufulete 2005	209	28.5	2	408	184.2	44	6.4%	-1.07 [-2.51, 0.36]
Sabrina Narayanan 2004	22,768	3,633	29	22,892	4,107	93	26.0%	-0.03 [-0.45, 0.39]
li wang 2015	6.01	2.51	8	5.35	1.97	8	11.3%	0.28 [-0.71, 1.26]
Simonetta Friso 2005	6.79	7.99	42	3.72	6.48	91	27.7%	0.44 [0.07, 0.81]
R Castro 2004	25.8	3.24	7	21.7	4.82	48	14.5%	0.87 [0.06, 1.68]
Maria F. Paz 2002	6.74	0.85	9	5.82	1.04	20	14.2%	0.91 [0.08, 1.73]
Total (95% CI)			**97**			**304**	**100.0%**	**0.33 [-0.07, 0.73]**

Heterogeneity: Tau² = 0.11; Chi² = 10.40, df = 5 (P = 0.06); I² = 52%
Test for overall effect: Z = 1.62 (P = 0.10)

Study or Subgroup	MTHFR 1298AC Mean	SD	Total	MTHFR 1298AA Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
Maria F. Paz 2002	4.98	0.56	28	5.82	1.04	20	15.2%	-1.04 [-1.65, -0.43]
M Pufulete 2005	389	180.4	22	408	184.2	44	16.7%	-0.10 [-0.61, 0.41]
Sabrina Narayanan 2004	23,041	3,921	77	22,892	4,107	93	19.6%	0.04 [-0.27, 0.34]
R Castro 2004	21.9	5.07	41	21.7	4.82	48	18.1%	0.04 [-0.38, 0.46]
li wang 2015	6.42	2.73	12	5.35	1.97	8	11.2%	0.42 [-0.49, 1.32]
Simonetta Friso 2005	8.59	7.1	65	3.72	6.48	91	19.3%	0.72 [0.39, 1.05]
Total (95% CI)			**245**			**304**	**100.0%**	**0.02 [-0.41, 0.46]**

Heterogeneity: Tau² = 0.23; Chi² = 28.03, df = 5 (P < 0.0001); I² = 82%
Test for overall effect: Z = 0.11 (P = 0.91)

FIGURE 2. *Forest plot analyses for MTHFR A1298C and MTHFR C677T genotyping and global methylation. The random effects odds ratio with the corresponding 95% confidence interval (CI) is shown. Total indicates the total number of samples. Tau2, the estimated between-study heterogeneity variance of log odds ratios; Chi², chi-squared test, it assesses whether observed differences in results are compatible with chance alone; df, degrees of freedom, the percentage of the variability in effect estimates that is due to heterogeneity rather than sampling error (chance); I², heterogeneity index (0–100).*
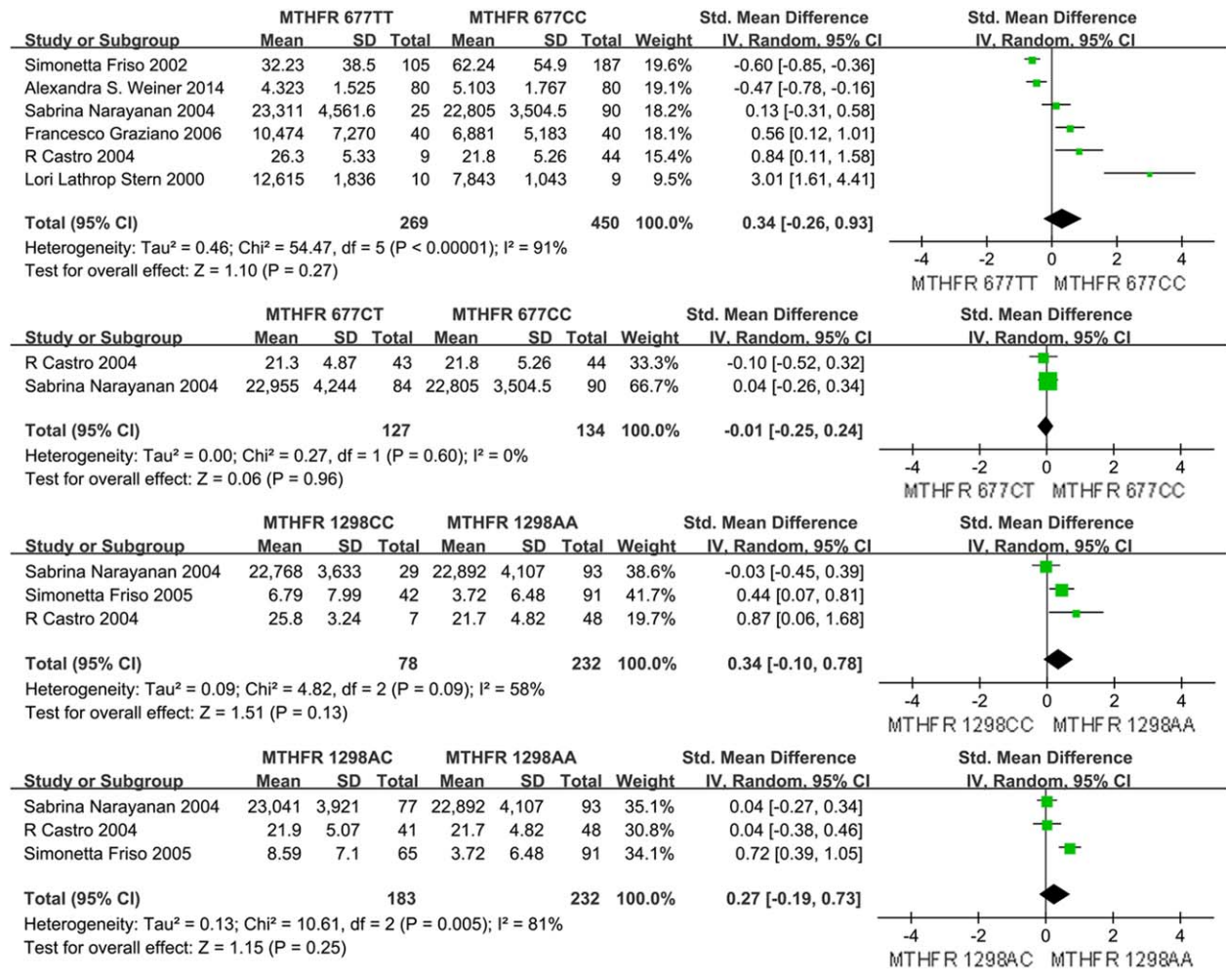
FIGURE 3. *Meta-analysis for MTHFR A1298C & C677T polymorphism and global methylation level in peripheral blood cells. The random effects odds ratio with the corresponding 95% confidence interval (CI) is shown. Total indicates the total number of samples. Tau2, the estimated between-study heterogeneity variance of log odds ratios; Chi^2, chi-squared test, it assesses whether observed differences in results are compatible with chance alone; df, degrees of freedom, the percentage of the variability in effect estimates that is due to heterogeneity rather than sampling error (chance); I^2, heterogeneity index (0–100).*

metabolism in healthy development, through regulation of methylation modifications of the genome (Crott et al., 2008; Wang et al., 2010; Crider et al., 2012; Serra-Juhe et al., 2015). *MTHFR* genotypes are believed to alter folate metabolism in cells (Kim et al., 2010; Tsang et al., 2015) and subsequently modify methylation. Although several hypotheses have been proposed, as yet there is no clear evidence for a link between *MTHFR* polymorphisms and DNA methylation status. Our data come from high risk NTD regions, and no correlation was observed between *MTHFR* genotype and methylation modification. For this reason, it is not possible to assess if *MTHFR* genotypes could genuinely affect methylation modification by folate metabolism and increase the risk of diseases.

MTHFR C677T is one of the most common polymorphisms connected with birth defects and cancer across ethnic groups and regions. The *MTHFR* 677TT polymorphism leads to the amino acid alanine being replaced by valine (p.Ala222- Val) and the production of a thermolabile variant of *MTHFR* with 30% less enzyme activity (Sharp and Little, 2004). However, no alteration in global DNA methylation with *MTHFR* C677T polymorphism was observed in this meta-analysis.

Another common polymorphism is *MTHFR* A1298C. The C polymorphism leads to glutamic acid being replaced by alanine (p. Glu429- Ala) and affects the conversion of MTHF to BH4 (Yamada et al., 2001). Our results indicated no correlation was observed between global methylation and *MTHFR* A1298C polymorphism.

Given that methods for methylation assays have greatly changed over the years with the development in technology, all data included in this study were obtained from different methods and the random effect was taken as priority method for analysis. However, for a continuous variable evaluation, incorporating random effects increased the likelihood of accounting for inter-study heterogeneity and addressed the potential correlation among results reported.

Methylation modification can be tissue specific, and so to remove biases induced by different tissue samples, studies in peripheral blood cells were analyzed for further comparison. However, there was still no alteration in global DNA methylation, even if the sample type was limited to the same origin.

Our study is subject to some limitations. The results were based on data abstracted from previous publications; however, this does not necessarily eliminate the robust estimate of association, and instead it allowed us to estimate the genetic effect of *MTHFR* in a larger sample size and thereby increased its power. Besides, differences between assay methods occur because global methylation modifications are represented by different forms, such as mCyt, methyl, or LINE-1 methylation. It is difficult to avoid the limited literature used in this study.

In conclusion, the results of our study provide evidence to assess the global methylation modification alterations of *MTHFR* among individuals. Whereas it is possible that the common variant *MTHFR* A1298C, C677T did not contribute to methylation modification it might still predispose to disease. Further studies should be conducted to identify and understand methylation regulation as a result of *MTHFR* polymorphisms.

Acknowledgments

References

Arakawa Y, Watanabe M, Inoue N, et al. 2012. Association of polymorphisms in DNMT1, DNMT3A, DNMT3B, MTHFR and MTRR genes with global DNA methylation levels and prognosis of autoimmune thyroid disease. Clin Exp Immunol 170:194–201.

Bailey RL, Mills JL, Yetley EA, et al. 2010. Unmetabolized serum folic acid and its relation to folic acid intake from diet and supplements in a nationally representative sample of adults aged > or =60 y in the United States. Am J Clin Nutr 92:383–389.

Bhargava S, Tyagi SC. 2014. Nutriepigenetic regulation by folate-homocysteine-methionine axis: a review. Mol Cell Biochem 387:55–61.

Castro R, Rivera I, Ravasco P, et al. 2004. 5,10-methylenetetrahydrofolate reductase (MTHFR) 677C–>T and 1298A–>C mutations are associated with DNA hypomethylation. J Med Genet 41:454–458.

Chen X, Guo J, Lei Y, et al. 2010. Global DNA hypomethylation is associated with NTD-affected pregnancy: a case-control study. Birth Defects Res A Clin Mol Teratol 88:575–581.

Christensen BC, Kelsey KT, Zheng S, et al. 2010. Breast cancer DNA methylation profiles are associated with tumor size and alcohol and folate intake. PLoS Genet 6:e1001043.

Crider KS, Yang TP, Berry RJ, Bailey LB. 2012. Folate and DNA methylation: a review of molecular mechanisms and the evidence for folate's role. Adv Nutr 3:21–38.

Crott JW, Liu Z, Keyes MK, et al. 2008. Moderate folate depletion modulates the expression of selected genes involved in cell cycle, intracellular signaling and folate uptake in human colonic epithelial cell lines. J Nutr Biochem 19:328–335.

Cunha AL, Hirata MH, Kim CA, et al. 2002. Metabolic effects of C677T and A1298C mutations at the MTHFR gene in Brazilian children with neural tube defects. Clin Chim Acta 318:139–143.

Czeizel AE, Dudas I. 1992. Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. N Engl J Med 327:1832–1835.

de Arruda IT, Persuhn DC, de Oliveira NF. 2014. The MTHFR C677T polymorphism and global DNA methylation in oral epithelial cells. Genet Mol Biol 36:490–493.

Friso S, Choi SW, Girelli D, et al. 2002. A common mutation in the 5,10-methylenetetrahydrofolate reductase gene affects genomic DNA methylation through an interaction with folate status. Proc Natl Acad Sci U S A 99:5606–5611.

Friso S, Girelli D, Trabetti E, et al. 2005. The MTHFR 1298A>C polymorphism and genomic DNA methylation in human lymphocytes. Cancer Epidemiol Biomarkers Prev 14:938–943.

Graziano F, Kawakami K, Ruzzo A, et al. 2006. Methylenetetrahydrofolate reductase 677C/T gene polymorphism, gastric cancer susceptibility and genomic DNA hypomethylation in an at-risk Italian population. Int J Cancer 118:628–632.

Hanks J, Ayed I, Kukreja N, et al. 2013. The association between MTHFR 677C>T genotype and folate status and genomic and gene-specific DNA methylation in the colon of individuals without colorectal neoplasia. Am J Clin Nutr 98:1564–1574.

Izmirli M. 2012. A literature review of MTHFR (C677T and A1298C polymorphisms) and cancer risk. Mol Biol Rep 40:625–637.

Kim JW, Park HM, Choi YK, et al. 2010. Polymorphisms in genes involved in folate metabolism and plasma DNA methylation in colorectal cancer patients. Oncol Rep 25:167–172.

Lamprecht SA, Lipkin M. 2003. Chemoprevention of colon cancer by calcium, vitamin D and folate: molecular mechanisms. Nat Rev Cancer 3:601–614.

Martinez de Villarreal LE, Delgado-Enciso I, Valdez-Leal R, et al. 2001. Folate levels and N(5),N(10)-methylenetetrahydrofolate reductase genotype (MTHFR) in mothers of offspring with neural tube defects: a case-control study. Arch Med Res 32:277–282.

Narayanan S, McConnell J, Little J, et al. 2004. Associations between two common variants C677T and A1298C in the methylenetetrahydrofolate reductase gene and measures of folate metabolism and DNA stability (strand breaks, misincorporated uracil, and DNA methylation status) in human lymphocytes in vivo. Cancer Epidemiol Biomarkers Prev 13:1436–1443.

Paz MF, Avila S, Fraga MF, et al. 2002. Germ-line variants in methyl-group metabolism genes and susceptibility to DNA methylation in normal tissues and human primary tumors. Cancer Res 62:4519–4524.

Posey DL, Khoury MJ, Mulinare J, et al. 1996. Is mutated MTHFR a risk factor for neural tube defects? Lancet 347:686–687.

Pufulete M, Al-Ghnaniem R, Rennie JA, et al. 2005. Influence of folate status on genomic DNA methylation in colonic mucosa of subjects without colorectal adenoma or cancer. Br J Cancer 92: 838–842.

Saberi S, Zendehdel K, Jahangiri S, et al. 2012. Impact of methylenetetrahydrofolate reductase C677T polymorphism on the risk of gastric cancer and its interaction with Helicobacter pylori infection. Iran Biomed J 16:179–184.

Sailasree R, Nalinakumari KR, Sebastian P, Kannan S. 2010. Influence of methylenetetrahydrofolate reductase polymorphisms in oral cancer patients. J Oral Pathol Med 40:61–66.

Serra-Juhe C, Cusco I, Homs A, et al. 2015. DNA methylation abnormalities in congenital heart disease. Epigenetics 10:167–177.

Sharp L, Little J. 2004. Polymorphisms in genes involved in folate metabolism and colorectal neoplasia: a HuGE review. Am J Epidemiol 159:423–443.

Stern LL, Mason JB, Selhub J, Choi SW. 2000. Genomic DNA hypomethylation, a characteristic of most cancers, is present in peripheral leukocytes of individuals who are homozygous for the C677T polymorphism in the methylenetetrahydrofolate reductase gene. Cancer Epidemiol Biomarkers Prev 9:849–853.

Stover PJ. 2009. One-carbon metabolism-genome interactions in folate-associated pathologies. J Nutr 139:2402–2405.

Tsang BL, Devine OJ, Cordero AM, et al. 2015. Assessing the association between the methylenetetrahydrofolate reductase (MTHFR) 677C>T polymorphism and blood folate concentrations: a systematic review and meta-analysis of trials and observational studies. Am J Clin Nutr 101:1286–1294.

Wallace K, Grau MV, Levine AJ, et al. 2010. Association between folate levels and CpG Island hypermethylation in normal colorectal mucosa. Cancer Prev Res (Phila) 3:1552–1564.

Wang L, Wang F, Guan J, et al. 2010. Relation between hypomethylation of long interspersed nucleotide elements and risk of neural tube defects. Am J Clin Nutr 91:1359–1367.

Weiner AS, Boyarskikh UA, Voronina EN, et al. 2013. Methylenetetrahydrofolate reductase C677T and methionine synthase A2756G polymorphisms influence on leukocyte genomic DNA methylation level. Gene 533:168–172.

Yamada K, Chen Z, Rozen R, Matthews RG. 2001. Effects of common polymorphisms on the properties of recombinant human methylenetetrahydrofolate reductase. Proc Natl Acad Sci U S A 98:14853–14858.

Yu Y, Wang F, Bao Y, et al. 2014. Association between MTHFR gene polymorphism and NTDs in Chinese Han population. Int J Clin Exp Med 7:2901–2906.