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Association of the methylenetetrahydrofolate reductase gene A1298C polymorphism with stroke risk based on a meta-analysis

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ABSTRACT. Several independent studies have reported the role of the methylenetetrahydrofolate reductase gene (*MTHFR*) A1298C polymorphism in strokes, but the results are inconclusive. To derive a more precise estimation of the relationship, a meta-analysis was performed in the present study. In this meta-analysis, a total of 13 studies, including 1974 cases and 2660 controls, were selected to evaluate the possible association. Crude odds ratios (ORs) with 95% confidence intervals (CI) were used to assess the strength of the association in additive, dominant, and recessive models. The overall analysis showed that

MTHFR A1298C was associated with a significant increase in the risk of stroke in the heterozygote comparison (AC *vs* AA: OR = 1.17; 95%CI = 1.03-1.34) and in the dominant model (AC/CC *vs* AA: OR = 1.22; 95%CI = 1.01-1.49). Stratified analysis showed a significantly strong association between the *MTHFR* A1298C polymorphism and stroke risk in Asian populations (OR = 1.32 for AC *vs* AA; OR = 1.94 for CC *vs* AA; OR = 1.37 for AC/CC *vs* AA; OR = 1.33 for C *vs* A allele), but not in Caucasian populations. Additionally, the *MTHFR* 1298C allele was found to be a risk factor for developing ischemic strokes. However, no statistically significant increased risk of hemorrhagic stroke was found in any of the genetic models. In conclusion, this meta-analysis supported that the *MTHFR* A1298C polymorphism could be capable of increasing stroke susceptibility in Asian, but not in Caucasian, populations.

Key words: Stroke; Cerebrovascular disease; Meta-analysis; Methylenetetrahydrofolate reductase; Polymorphism

INTRODUCTION

Stroke ranks fourth among all causes of death after heart disease, cancer, and chronic lower respiratory disease (Roger et al., 2012). Established causal risk factors, such as hypertension and smoking, are estimated to account for approximately 50% of vascular disease risk (Cronin et al., 2005). Therefore, the identification of genetic contributors to strokes is of key importance, not only to explain or predict the minority of cases that occur in the absence of well-established risk factors, but also to account for the wide variability of stroke incidence within individuals who do harbor these common, acquired risk factors (Bentley et al., 2010).

The metabolism of folate is significant for the maintenance of genome integrity due to its role in DNA synthesis, repair, and methylation (Fowler, 2005). The enzyme 5,10-methylenetetrahydrofolate reductase (MTHFR) irreversibly catalyzes the reduction of 5,10-methylenetetrahydrofolate (5,10-methylene-THF) to 5-methyltetrahydrofolate (5-methyl-THF), which is used as a methyl donor in the synthesis of methionine from homocysteine (Toyoda et al., 2004). In turn, methionine provides the methyl group for the formation of *S*-adenosylmethionin, which is involved in numerous cellular reactions involving DNA, RNA, and histone methylation. A decreased pool of methionine might therefore affect DNA methylation. This is supported by the observation that some MTHFR variants are associated with DNA hypomethylation (Castro et al., 2004).

The *MTHFR* locus has been mapped to chromosome 1p36.3 in humans (Goyette et al., 1994). One of the most studied polymorphisms in *MTHFR* is an A-to-C transversion substitution at nucleotide 1298 (exon 7) that results in an amino acid substitution of glutamate (Glu) for alanine (Ala) at codon 429. Once this amino acid substitution takes place at the *S*-adenosylmethionine regulatory domain of *MTHFR*, the A1298C polymorphism generates a reduction in the enzyme activity (Pereira et al., 2006) that ensures elevation of the plasma homocysteine status (Kumar et al., 2005; Klai et al., 2011).

Case-control and prospective studies have demonstrated an association between moderate hyperhomocystinemia and the risk of ischemic (Li et al., 2003; Austin et al., 2004; Baum et al., 2004; Sazci et al., 2006) and hemorrhagic strokes (Aronis et al., 2002; Li et al., 2003).

These findings indicated that *MTHFR* deregulation could be involved in stroke risk due to its critical role in the modulation of plasma homocysteine status.

Several independent studies have investigated the role of the *MTHFR* A1298C polymorphism in stroke risk (Table 1) (Akar et al., 2001; Linnebank et al., 2005; Sun et al., 2005; Komitopoulou et al., 2006; Sazci et al., 2006; Nan et al., 2007; Sirachainan et al., 2008; Biswas et al., 2009; Morita et al., 2009; Sawula et al., 2009; Chen et al., 2010; Han et al., 2010; Hultdin et al., 2011), but the results are inconclusive, partially because of the possible small effect of the polymorphism on stroke risk and the relatively small sample size in each of the published studies. To estimate the overall risk of the A1298C polymorphism for strokes, as well as to quantify the potential between-study heterogeneity, we performed a meta-analysis on all eligible published case-control studies.

MATERIAL AND METHODS

Study selection

A literature search of the PubMed database was conducted by two independent investigators (Q.Q. Lv and J. Lu) to identify all articles that examined the association between *MTHFR* A1298C variants and strokes. The terms "methylenetetrahydrofolate reductase or MTHFR", "polymorphism or polymorphisms", and "stroke or cerebrovascular disease" were used as search criteria. All studies published before September 2012 were considered for initial screening. The Chinese Biomedical Literature Database (CBMDisc), which is the main Chinese medical literature retrieval system, was also used to search pertinent literature written in Chinese. References in the studies were also reviewed and manually searched to obtain additional studies. The inclusion criteria were as follows: i) study was conducted with human subjects; ii) a case-control study design was used; iii) a detailed genotype frequency of cases and controls was provided, and if not, the text provided data enabling such calculations. The exclusion criteria were i) reviews, case reports, or cross-sectional studies without controls; ii) duplicated studies; iii) study outcome being defined as other than stroke (e.g., cerebral sinovenous thrombosis, transient ischemic attack); iv) control group containing hemorrhagic stroke subjects; v) control group deviating from Hardy-Weinberg equilibrium. 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. Hence, only 13 studies qualifying our strict selection criteria were included in the final analysis (Figure 1).

Data extraction

Data from eligible studies were abstracted by two investigators independently (Q.Q. Lv and J. Lu). Discrepancies were resolved by discussion between the two investigators. If they could not reach a consensus, an expert was consulted to resolve the dispute. Information regarding the first author, year of publication, country of origin, ethnicity, number of cases and controls, genotype frequency for cases and controls, and minor allele frequency in the controls was collected. Different ethnicities were categorized as Asian and Caucasian.

Statistical analysis

The strength of association between the A1298C polymorphism of *MTHFR* and stroke

risk was estimated for each study by the odds ratio (OR) along with its 95% confidence interval (CI). Heterogeneity among studies was examined with the χ^2 test-based Q statistic (Lau et al., 1997) and $P < 0.05$ was considered significant. Fixed-effects summary ORs were calculated using the Mantel-Haenszel method (Mantel and Haenszel, 1959; DerSimonian and Laird, 1986), and the DerSimonian and Laird method was used to calculate random-effects summary ORs (DerSimonian and Laird, 1986). In case of heterogeneity, random-effect models are more appropriate; however, if heterogeneity is absent, both models provide nearly the same results. We first assessed the risks of the heterozygotes and variant homozygotes as compared to the wild-type homozygote, and then evaluated the risks of the combined variant homozygotes and heterozygotes versus the wild-type homozygote, and the variant homozygote versus the combined heterozygote and wild-type homozygote, assuming dominant and recessive effects of the variant allele, respectively. Meta regression was applied to illustrate potential causes for between-study heterogeneity. Publication bias was evaluated by Egger's regression asymmetry test and by examination of inverted funnel plots (Egger et al., 1997).

For all analyses performed, the statistical package Stata 12.0 (Stata, College Station, TX, USA) was used. Unless stated otherwise, all statistical evaluations were made assuming a two-sided test with a significance level of 0.05.

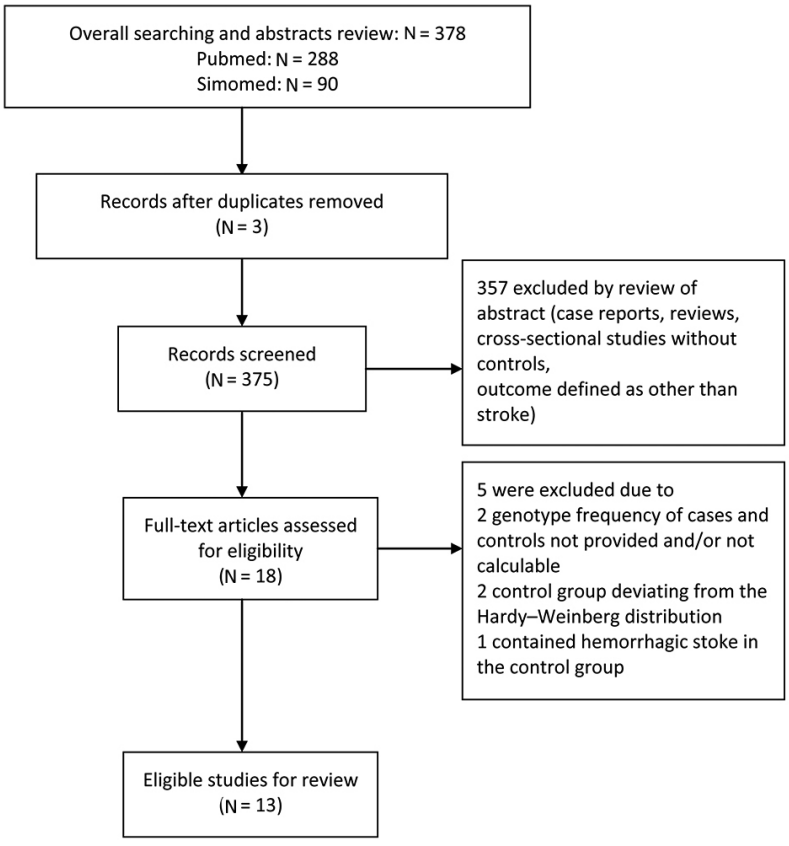

Figure 1. Flow diagram of the study selection process.

RESULTS

Study characteristics

The main characteristics of the studies are presented in Table 1. Thirteen studies with publication dates ranging from 2001 to 2011 were retrieved by our literature search strategy. Most reports presented demographic information regarding cases and controls. Specific matching for age and gender was described in seven studies (Sun et al., 2005; Sazci et al., 2006; Sirachainan et al., 2008; Biswas et al., 2009; Sawula et al., 2009; Chen et al., 2010; Hultdin et al., 2011).

There were seven studies of Caucasian populations and six studies of Asian populations. Five studies focused on pediatric strokes, and eight studies reported adult stroke data. Two studies contained case groups of both ischemic and hemorrhagic strokes, and 11 studies contained case groups of ischemic strokes only. In all studies, except two where such information was missing (Akar et al., 2001; Morita et al., 2009), genomic DNA was extracted from blood samples. Genotyping was performed consistently across studies with polymerase chain reaction (PCR). The distribution of genotypes in the controls was consistent with Hardy-Weinberg equilibrium for all selected studies.

Meta-analysis results

The evaluation of the association between the *MTHFR* A1298C polymorphism and stroke risk is presented in Table 2. Overall, there was large and significant between-study heterogeneity in the magnitude of the observed association between the presence of the A1298C polymorphism and stroke in the CC *vs* AA (P = 0.030), AC/CC *vs* AA (P = 0.029), CC *vs* AA/AC (P = 0.048), and C *vs* A (P = 0.001) comparisons. Thus, random-effect estimates were more appropriate for data synthesis. The result of the fixed-effect model was applied to the AC *vs* AA comparison (P = 0.224), as significant heterogeneity did not exist in the related 13 studies. The meta-analysis showed that *MTHFR* A1298C was associated with a significant increase in the risk of stroke in the heterozygote comparison (AC *vs* AA: OR = 1.17; 95%CI = 1.03-1.34; P = 0.224 for the heterogeneity test) and in the dominant model (AC/CC *vs* AA: OR = 1.22; 95%CI = 1.01-1.49; P = 0.029 for the heterogeneity test) (Figure 2 and Table 2).

The association between the A1298C polymorphism and stroke was further stratified by ethnicity. As shown in Table 2 and Figure 3, in Asian populations, we found that 1298C significantly increased the risk of stroke in all genetic models (AC *vs* AA: OR = 1.32; 95%CI = 1.09-1.61; P = 0.524 for the heterogeneity test; CC *vs* AA: OR = 1.94; 95%CI = 1.05-3.58; P = 0.396 for the heterogeneity test; dominant model, AC/CC *vs* AA: OR = 1.37; 95%CI = 1.13-1.65, P = 0.284 for the heterogeneity test; C *vs* A: OR = 1.33; 95%CI = 1.12-1.57; P = 0.130 for the heterogeneity test) (Figure 3 and Table 2) except for the recessive model (CC *vs* AA/AC: OR = 1.76; 95%CI = 0.96-3.23; P = 0.423 for the heterogeneity test). However, when the seven studies conducted in Caucasian populations were analyzed, no significant association was found between the polymorphism and the risk of stroke in any of the genetic models (Table 2).

Sub-group analysis was performed for ischemic and hemorrhagic stroke groups. A significant association between the A1298C polymorphism with ischemic stroke was observed in heterozygote comparisons (AC *vs* AA: OR = 1.19; 95%CI = 1.04-1.37; P = 0.254 for the heterogeneity test) and in the dominant model (AC/CC *vs* AA: OR = 1.25; 95%CI = 1.03-1.52, P = 0.031 for the heterogeneity test) (Figure 4 and Table 2). No significantly increased risk of hemorrhagic stroke was found in any of the genetic models.

Table 1. Main characteristics of all studies included in the meta-analysis.

First author	Year	Country	Ethnicity	Type of stroke	Age group	Case	Control	Case			Control				HWE[a] in control
								AA	AC	CC	AA	AC	CC	C allele (%)	
Akar N	2001	Turkey	White	ischemic	Children	46	68	13	27	6	31	32	5	42 (30.9)	0.399
Sun WP	2005	China	Asian	ischemic	Adults	97	94	55	40	2	60	31	3	37 (19.7)	0.676
Linnebank M	2005	Germany	White	ischemic	Adults	159	159	80	65	14	73	64	22	108 (34.0)	0.196
Komitopoulou A	2006	Greece	White	ischemic	Children	90	103	35	45	9	50	40	13	66 (32.0)	0.272
Sazci A	2006	Turkey	White	ischemic	Adults	92	259	36	37	19	130	108	21	150 (29.0)	0.828
Sazci A	2006	Turkey	White	hemorrhagic	Adults	28	259	16	9	3	130	108	21	150 (29.0)	0.828
Nan GX	2007	China	Asian	ischemic	Adults	100	100	56	40	4	64	33	3	39 (19.5)	0.609
Sirachainan N	2008	Thailand	Asian	ischemic	Children	51	169	22	19	3	82	69	13	95 (29.0)	0.774
Sawula W	2009	Poland	White	ischemic	Adults	131	64	53	57	18	26	22	11	44 (37.3)	0.119
Morita DC	2009	America	White	ischemic	Children	15	90	12	3	0	41	36	13	62 (34.4)	0.278
Biswas A	2009	India	Asian	ischemic	Children	58	58	38	14	6	50	7	1	9 (7.8)	0.232
Chen F	2010	China	Asian	ischemic	Adults	470	495	354	109	7	387	105	3	111 (11.2)	0.146
Han IB	2010	Korea	Asian	ischemic	Adults	264	234	179	80	5	182	51	1	53 (11.3)	0.193
Hultdin J	2011	Sweden	White	ischemic	Adults	314	767	135	142	37	328	346	93	532 (34.7)	0.904
Hultdin J	2011	Sweden	White	hemorrhagic	Adults	59	767	29	29	1	328	346	93	532 (34.7)	0.904

ND = not defined; [a]Hardy-Weinberg equilibrium (HWE) was calculated by goodness-of-fit chi-squared test.

Table 2. Main results of pooled ORs in the meta-analysis.

	Studies	AC *vs* AA			CC *vs* AA			AC/CC *vs* AA (dominant model)			CC *vs* AA/AC (recessive model)			C *vs* A		
		OR (95%CI)	P_h^a	P_h^b	OR (95%CI)	P_h^a	P_h^b	OR (95% CI)	P_h^a	P_h^b	OR (95% CI)	P_h^a	P_h^b	OR (95% CI)	P_h^a	P_h^b
Total	13	1.17 (1.03-1.34)	0.224		1.23 (0.81-1.88)[c]	0.030		1.22 (1.01-1.49)[c]	0.029		1.13 (0.76-1.66)[c]	0.048		1.17 (0.97-1.41)[c]	0.001	
Ethnicity																
Asian	6	1.32 (1.09-1.61)	0.524	0.116	1.94 (1.05-3.58)	0.396	0.126	1.37 (1.13-1.65)	0.284	0.045	1.76 (0.96-3.23)	0.423	0.154	1.33 (1.12-1.57)	0.130	0.016
White	7	1.06 (0.88-1.27)	0.193		1.04 (0.62-1.75)[c]	0.018		1.04 (0.88-1.23)	0.051		0.97 (0.61-1.56)[c]	0.027		1.03 (0.80-1.33)[c]	0.008	
Stroke type																
Ischemic	13	1.19 (1.04-1.37)	0.254	0.175	1.30 (0.84-2.01)[c]	0.024	0.304	1.25 (1.03-1.52)[c]	0.031	0.054	1.18 (0.79-1.75)[c]	0.044	0.876	1.19 (0.99-1.44)[c]	0.001	0.017
Hemorrhagic	2	0.86 (0.55-1.35)	0.514		0.42 (0.04-4.58)[c]	0.045		0.77 (0.49-1.19)	0.963		0.46 (0.03-6.15)[c]	0.027		0.73 (0.52-1.04)	0.448	
Age Group																
Children	5	1.36 (0.96-1.93)	0.070	0.302	1.22 (0.70-2.15)	0.123	0.564	1.30 (0.68-2.50)[c]	0.010	0.297	1.07 (0.62-1.86)	0.234	0.883	1.19 (0.68-2.10)[c]	0.003	0.383
Adults	8	1.14 (0.99-1.32)	0.589		1.19 (0.72-1.96)[c]	0.034		1.14 (0.99-1.31)	0.288		1.14 (0.70-1.85)[c]	0.029		1.14 (0.95-1.35)[c]	0.036	

CI = confidence interval; OR = odds ratio. Test for heterogeneity [a]in groups and [b]between groups. [c]Random-effects model was used when the P value for heterogeneity test was ≤0.05, otherwise the fixed-effect model was used. Only studies that provided specific data on subgroups of cases are included.

We also stratified the age of the case group. As shown in Table 2, no significant association was found between the polymorphism and the risk of stroke in any of the genetic models, either in adults or in children.

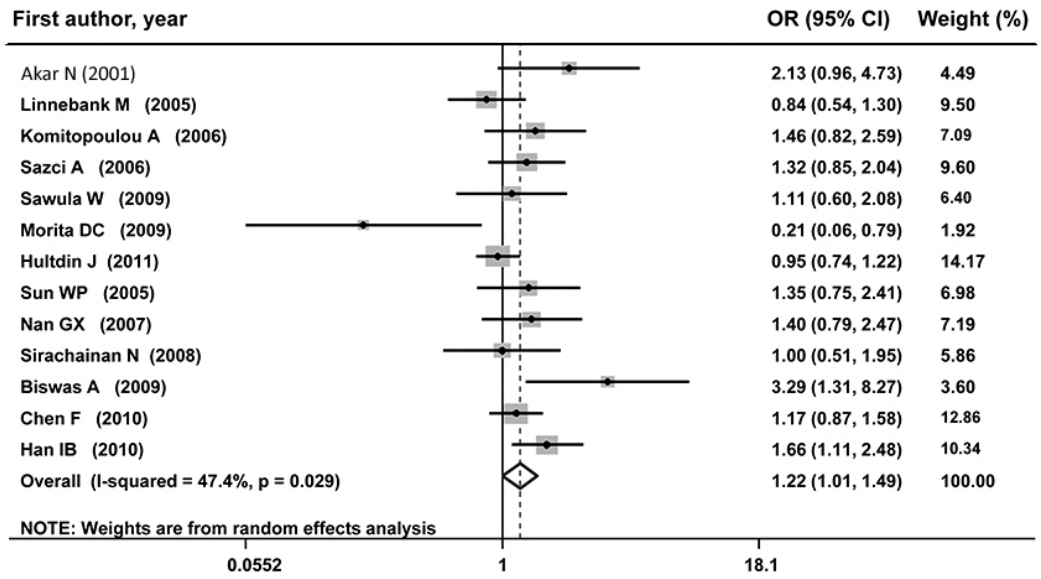

Figure 2. Forest plot of the *MTHFR A1298C* polymorphism and stroke in the dominant model.

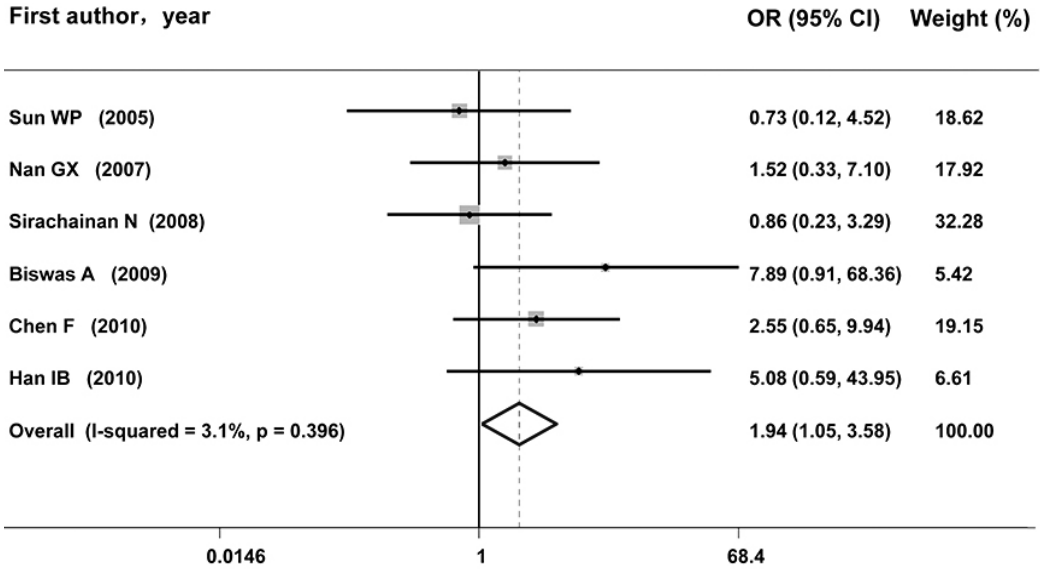

Figure 3. Forest plot of the *MTHFR A1298C* polymorphism and stroke in Asian populations in homozygote comparison.

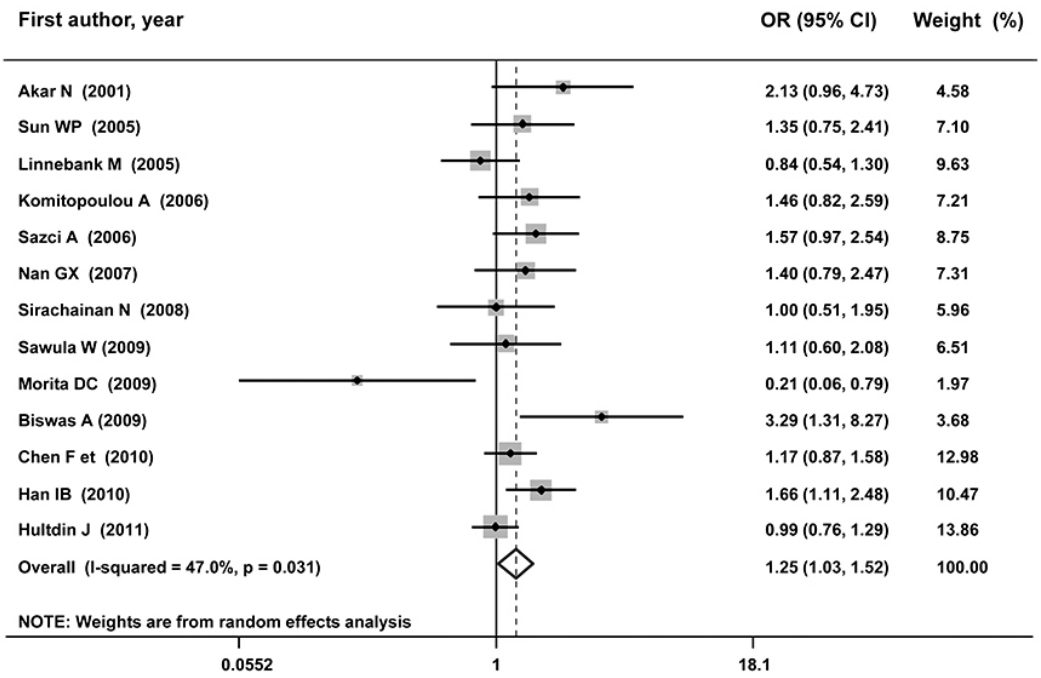

Figure 4. Forest plot of the *MTHFR A1298C* polymorphism and ischemic stroke in the dominant model.

Publication bias

Begg's funnel plot and the Egger test were performed to assess the publication bias of the literature. No obvious asymmetry was observed in any genetic model according to the visual assessment of the funnel plot (Figures not shown). In addition, there was no statistical evidence of publication bias among studies using Egger's regression test (P = 0.30 for AC *vs* AA; P = 0.36 for CC *vs* AA; P = 0.76 with the dominant model; P = 0.25 with the recessive model; and P = 0.67 for C *vs* A).

DISCUSSION

The association of the *MTHFR* A1298C genetic polymorphism with stroke has been investigated in several studies to date. However, the conclusions remain controversial. In order to form a more precise estimation of this correlation, we performed a meta-analysis of previously published studies.

The present meta-analysis included a total of 1974 cases and 2660 controls. In the overall analysis, *MTHFR* A1298C was associated with a significant increase in the risk of stroke in the AC *vs* AA and CC/AC *vs* AA comparisons. However, in the subgroup analysis of ethnicity, we found that although *MTHFR* 1298C had an effect on increasing the stroke risk in Asian populations, no such evidence was observed in Caucasian populations.

A subgroup analysis in the subjects showed that *MTHFR* A1298C was strongly asso-

ciated with ischemic strokes in the heterozygote comparison (AC *vs* AA) and in the dominant model (AC/CC *vs* AA), whereas no significant increased risk of hemorrhagic stroke was found in any of the genetic models.

In addition, we stratified age of the case group. No significant association was found between the polymorphism and stroke risk in any of the genetic models, either in adults or in children.

Previous reports have indicated that the *MTHFR* A1298C polymorphism is associated with elevated homocysteine status (Kumar et al., 2005). Thus, elevated homocysteine might cause either ischemic stroke through its coagulative effect or might cause hemorrhagic stroke by causing rupture of microaneurysms (Li et al., 2003). Nonetheless, in this meta-analysis, the *MTHFR* 1298C allele increased stroke risk in Asian populations. This susceptibility to stroke was not observed in Caucasian populations. This could be due to different genetic backgrounds, which cause ethnic differences in association studies. Likewise, environmental risk factors could also play important roles. It has been suggested that the progress of stroke is the outcome of the interaction between gene and environment. Evidence has showed that 1298 AC or CC genotypes were associated with tobacco and alcohol habits (Galbiatti et al., 2012). Additionally, previous studies have demonstrated that several polymorphisms in genes involved in the process of DNA synthesis were associated with strokes (Hamzi et al., 2011). For example, in the study of Szolnoki et al. (2006), although neither *MTHFR* C677T nor A1298C polymorphisms were found to be associated with ischemic stroke, the compound alleles of *MTHFR* 677T and 1298C had an increased risk factor of 3.39 (P = 0.001) for ischemic strokes. In another study by Sazci et al. (2006), the *MTHFR* T677T genotype and the T677T/A1298A compound genotype were significantly associated with hemorrhagic strokes, whereas the *MTHFR* C1298C genotype and the C677C/C1298C compound genotype were significantly associated with ischemic strokes. The difference in the results between Caucasian and Asian populations might also be explained by the presence of variants in other genes that were not investigated in the studies included in the meta-analysis. In summary, the negative association between the *MTHFR* genotyping and stroke in Caucasians as compared to Asians might be attributed to either environmental factors or other genetic factors.

Although the exact mechanism by which the *MTHFR* A1298C polymorphism affects stroke risk is not yet clear, some possible mechanisms have been put forward. MTHFR is the key rate-limiting enzyme required for the conversion of dietary folate to 5-methyltetrahydrofolate, the methyl group donor required for the remethylation of Hcy to methionine *in vivo* (Kelly et al., 2002). One of its functional polymorphisms, A1298C, has been known to be associated with reduced enzymatic activity and increased plasma total homocysteine (tHcy) concentrations (Kumar et al., 2005; Brustolin et al., 2010; Klai et al., 2011). Homocysteine acts as a toxin with respect to endothelial cells, enhances vascular smooth muscle cell proliferation, increases platelet aggregation, and acts on the coagulation cascade and fibrinolysis, thus directly inducing or acting in a synergistic manner with other factors in determining the appearance of atherosclerosis. In particular, it activates coagulation factors V, X, and XII; decreases the activation of protein C and cell-surface thrombomodulin; and modulates the binding of tissue plasminogen activator to its endothelial receptor, annexin II, which leads to the creation of a prothrombotic environment (Thambyrajah and Townend, 2000).

The mechanism of high tHcy underlying hemorrhagic strokes has not yet been fully elucidated. In a study by Aikawa et al. (1998), the levels and activities of tissue-destructive enzymes, such as MMP-9, were shown to increase in mice with hyperhomocysteinemia. Such

enzymes, which are present in atherosclerotic plaque, might promote lesion instability and rupture. It is also possible that elevated homocysteine status might initially cause an ischemic stroke and later result in a hemorrhagic stroke (Casas et al., 2005).

In addition, at the cellular level, folate deficiency and hyperhomocysteinemia could exert multiple detrimental effects. These include induction of DNA damage, uracil misincorporation into DNA, and altered patterns of DNA methylation. Low folate status and elevated homocysteine could increase the generation of reactive oxygen species and contribute to excitotoxicity and mitochondrial dysfunction, which might lead to apoptosis. Strong epidemiological and experimental evidence has linked derangements of one-carbon metabolism to vascular, neurodegenerative, and neuropsychiatric diseases, including strokes (Kronenberg et al., 2009). All of the above might partially explain the association between this polymorphism and stroke risk.

Several limitations were present in this meta-analysis. In the subgroup analyses, especially in the subgroup of hemorrhagic strokes and in strokes in children, the number of subjects in each subgroup was relatively small, which could lead to a lack of sufficient statistical power to explore the true association. Furthermore, the overall outcomes were based on individual unadjusted estimates, whereas a more precise evaluation should be adjusted by potentially suspected factors, including body mass index, smoking status, drinking status, and other lifestyle factors.

In conclusion, our meta-analysis suggested that the *MTHFR* A1298C polymorphism was strongly associated with stroke risk in Asian populations, whereas there was not enough evidence to confirm this association in Caucasian populations. Moreover, the *MTHFR* 1298C allele was highlighted as a risk factor for developing ischemic strokes. Additionally, stroke was shown to be a polygenic/multifactorial disease; therefore, single susceptibility gene polymorphisms might have modest effects. More studies or complete case-control studies, especially stratified by different ethnic backgrounds, environmental exposure, or other risk factors, should be performed in the future to better understand the role of the *MTHFR* A1298C polymorphism on the pathogenesis of strokes.

Conflicts of interest

The authors declare that they have no conflict of interest.

ACKNOWLEDGMENTS

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