Association of gene polymorphism with genetic susceptibility to stroke in Asian populations: a meta-analysis

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Abstract Stroke is a heterogeneous multifactorial disease and is thought to have a polygenic basis. Case-control studies on gene sequence variations have identified a number of potential genetic predisposition factors, but due to the conflicting results, uncertainty remains on the effect of these polymorphisms on risk for the development of stroke. To qualitatively and quantitatively assess the risk associated with different gene polymorphisms for stroke in Asian populations, we comprehensively searched and identified all the studies of association. Clinically overt case-control studies were selected only if neuroimaging had been used as the confirmatory measure for diagnosis of stroke. We performed a meta-analysis of the three most investigated genes, viz., methylenetetrahydrofolate reductase (MTHFR), apolipoprotein E (ApoE) and angiotensin-converting enzyme (ACE). Statistically significant association with stroke were identified for C677T polymorphism of MTHFR [random effects odds ratio (OR) = 1.47, 95% confidence interval (95% CI) 1.19, 1.82; $P = 0.0004$] and marginally significant association was detected with allele ϵ 4 of ApoE (random effects OR = 1.47, 95% CI 1.00, 2.15; $P = 0.049$). The sensitivity analysis (exclusion of studies with controls not in Hardy-Weinberg equilibrium) revealed a significant association of stroke with the MTHFR C677T and ApoE ϵ 4 alleles but showed no association with ACE gene insertion/deletion polymorphism.

Keywords Stroke · MTHFR · ApoE · ACE · Polymorphism · Meta-analysis

Introduction

Stroke is the third leading cause of death and the largest cause of disability among older people worldwide (Feigin et al. 2003). According to the World Health Organization estimates, annually, 15 million people worldwide suffer a stroke. Of these, 5 million die and another 5 million are left permanently disabled. In 2002, nearly 5.5 million people died of stroke, and more than 50% of these deaths occurred in Asian countries such as India, Pakistan, Bangladesh, China, Korea and Japan (WHO report: http://www.who.int/cardiovascular_diseases/). Although there has been a decline in the incidence of stroke in the Western population during the past three decades (Heart Disease and Stroke Statistics-2003 Update, American Heart Association), the burden of this disease has increased and is expected to rise in the South Asian countries (Bulatao et al. 1992). In various epidemiological studies in families (Jousilahti et al. 1997; Liao et al. 1997) and in twins (Brass et al. 1992; Bak et al. 2002), genetic factors were found to be involved in the predisposition of stroke in conjunction with the other risk factors.

A candidate gene case-control approach, examining polymorphisms, is generally taken to investigate genetic risk factors for stroke. Since stroke is a heterogeneous multifactorial disease, there is a large number of candidate genes, which are involved in blood coagulation, blood pressure regulation, cholesterol metabolism, etc. (Hassan and Markus 2000; Casas

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et al. 2004). However, uncertainty arises about the nature and number of genes involved in the development of stroke, due to lack of reproducibility of genetic case–control studies (Casas et al. 2004). In the association studies, there are possibilities that some positive results might be spurious and some negative findings might be a consequence of low statistical power (Casas et al. 2004). It could be due to their small sample size or methodological shortcomings, such as the selection of an appropriate control group (Hassanand Markus 2000; Casas et al. 2004; Dichgans and Markus 2005). Meta-analysis might be a means of resolving disparate results. For example, pooling samples from several studies could produce greater power than from individual studies or might amplify trends for association in small individual studies (Casas et al. 2004). By increasing the statistical power, by taking into account all available published data, meta-analysis might be useful to identify the causative genes with reliability and to quantify with precision the genetic risks (Casas et al. 2004). We conducted a comprehensive meta-analysis of all genetic association studies in stroke in Asian populations and present our findings in this paper.

Materials and methods

Identification and selection of studies

A comprehensive literature search was conducted to collect data from all the studies that investigated association of genes with stroke in Asian populations. All the studies that were published before August 2006 were considered in the meta-analysis. We did extensive computer-based searches of PubMed to identify the case–control studies evaluating the role of any candidate gene in the development of stroke in Asian populations. The terms “stroke” and “polymorphism” were used as search criteria. The search results were limited to humans. Clinically overt case–control studies were selected only if neuroimaging [magnetic resonance imaging (MRI) or computed tomography (CT)] had been used as the confirmatory measure for diagnosis of stroke. Both ischaemic and haemorrhagic stroke types were considered for inclusion in the study, but other phenotypes, such as transient ischaemic attack (TIA), or cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), were excluded. A majority of the articles covered in the present study were written in English. Articles in other languages were considered if the abstracts of the reports were given in English and if

genotype frequencies and confirmation of diagnosis by neuroimaging were given. Those studies in which the genotype frequencies were not reported were excluded from our study.

Data extraction

We used a standard reporting form to extract data from each study that we included. We collected information on the name of the first author, year of publication, country, journal, racial descent of study population, demographics, number of cases and controls, genotype frequencies, allele frequencies (wherever available), and confirmation of diagnosis. Where allele frequencies were not given, they were calculated from the corresponding genotype frequencies of the case and the control groups.

Data synthesis and statistical analysis

Primary literature search generated 68 case–control studies of 30 candidate genes in which the presence or absence of stroke was determined in a dichotomous manner (Table 1). Methylenetetrahydrofolate reductase (MTHFR), apolipoprotein E (ApoE), and angiotensin-converting enzyme (ACE) were chosen for further analysis, as, on review, those three genes were found to be studied well in Asian populations. Ten studies of MTHFR (Morita et al. 1998; Zhang and Dai 2001; Wu et al. 2001; Li et al. 2002, 2003; Choi et al. 2003; Baum et al. 2004; Alluri et al. 2005; Fang et al. 2005; Gao et al. 2006), six studies for ApoE (Kokubo et al. 2000; Luthra et al. 2002; Um et al. 2003a; Jin et al. 2004; Lin et al. 2004; Gao et al. 2006), and six studies of ACE (Doi et al. 1997; Nakata et al. 1997; Um et al. 2001, 2003a; Li et al. 2002; Gao et al. 2006) gene polymorphisms were found to meet our selection criteria and were therefore included in the meta-analysis (Tables 2, 3 and 4). Because of unavailability of genotype data, two studies of ApoE polymorphism (Nakata et al. 1997; Chowdhury et al. 2001) were excluded for the present analyses. In one study, all the three polymorphisms were studied (Gao et al. 2006).

In the meta-analysis of MTHFR C677T polymorphism, the overall association of the T allele with risk of stroke was evaluated in comparison with the C allele. Also, the contrasts of homozygote TT versus CC, TT versus TC versus CC, TT versus (TC + CC), and (TT + TC) versus CC were examined.

In our study of ApoE polymorphisms, the risk of stroke associated with the ϵ 2 and ϵ 4 alleles relative to the ϵ 3 allele was examined. Furthermore, the contrasts of homozygotes ϵ 2 ϵ 2 and ϵ 4 ϵ 4 versus ϵ 3 ϵ 3, (ϵ 2 ϵ 2

Table 1 Candidate genes and stroke (*IS* ischaemic stroke, *CI* cerebral infarction, *CH* cerebral haemorrhage, *I/D* insertion/deletion)

Gene and polymorphism(s)	Populations	Cases	Control	Type	Reference
MTHFR 677 C/T	Japanese	256	325	IS	Morita et al. (1998)
	Chinese	102	100	IS	Zhang and Dai (2001)
	Japanese	77	229	IS	Wu et al. (2001)
	Chinese	143	154	IS	Li et al. (2002)
	Chinese	1,823	1,832	IS	Li et al. (2003)
	Korean	195	198	IS	Choi et al. (2003)
	Chinese	241	304	IS	Baum et al. (2004)
	Indian	75	49	IS	Alluri et al. (2005)
	Mongolian	64	22	CI + CH	Fang et al. (2005)
	Chinese	100	100	IS	Gao et al. (2006)
ApoE	Japanese	93	99	CI + CH	Nakata et al. (1997)
	Japanese	322	1,126	CI + CH	Kokubo et al. (2000)
	Bangladeshi	190	227	CI + CH	Chowdhury et al., (2001)
	Asian Indian	63	57		Luthra et al. (2002)
	Korean	365	319	CI	Um et al. (2003a)
	Chinese	226	201	CI	Jin et al. (2004)
	Taiwanese	227	112	IS	Lin et al. (2004)
	Chinese	100	100	IS	Gao et al. (2006)
ACE I/D	Japanese	181	271	IS	Doi et al. (1997)
	Japanese	93	99	CI + CH	Nakata et al. (1997)
	Korean	106	498	CI	Um et al. (2001)
	Chinese	143	154	IS	Li et al. (2002)
	Korean	365	319	CI	Um et al. (2003a)
	Chinese	100	100	IS	Gao et al. (2006)
Angiotensinogen 235 M/T	Japanese	93	99	CI + CH	Nakata et al. (1997)
	Korean	365	319	CI	Um et al. (2003a)
Apo H 341 G/A (88 Ser/Asn)	Chinese	260	100	CI	Xia et al. (2003)
Apo H 817 G/T (247 Leu/Val), 1080 C/T, 1025 G/C(316 Try/Ser)	Chinese	140	100	CH	Xia et al. (2004)
Cystathione beta-synthetase 844ins68	Chinese	102	100	IS	Zhang and Dai (2001)
Cystathione beta-synthetase 27796 T/C	Chinese	59	65	CI + CH	Fang et al. (2004)
eNOS 27 bp repeat in intron 4	Chinese	364	516	CI	Hou et al. (2001)
Fibrinogen -455 G/A	Japanese	85	170	IS	Nishiuma et al. (1998)
	Chinese	91	74 + 98	CI	Liu et al. (2002)
Fibrinogen 148 C/T	Chinese	100	100	IS	Gao et al. (2006)
IL 1 gene cluster	Korean	363	640	CI	Um et al. (2003b)
IL-1a-889	Korean	360	519	CI	Um et al. (2003c)
ILra, IL1 β	Korean	152	165	IS	Lee et al. (2004)
NADPH oxidase p22 PHOX 242 C/T	Japanese	226	301	CI + TIA	Ito et al. (2000a)
	Japanese	120	177	CI	Shimo-Nakanishi et al. (2004)
Methionine synthetase 919 D/G (2756 A/G)	Japanese	251	257	CI	Morita et al. (1999)
	Chinese	102	100	IS	Zhang and Dai (2001)
Lipoprotein lipase 447 Ser/Ter	Japanese	177	177	CI	Shimo-Nakanishi et al. (2001)
	Chinese	160	117	CI + CH	Zhao et al. (2003)
PAI -4G/5G	Chinese	54	83	CI	Zhan et al. (2003)
	Chinese	100	150	CI	Chen et al. (2003)
Paroxonase 1 55 M/L, 192 Q/R/spParoxonase 2 311 C/S	Japanese	235	431	CI	Imai et al. (2000)
Paroxonase 1 55 M/L, 192 Q/R	Japanese	112	106	CI	Ueno et al. (2003)
Notch3 6,746 T/C	Japanese	230	315	CI	Ito et al. (2002)
Platelet glycoprotein Ia 807 C/T, Ib 3,550 C/T, and IIIa Pl (A1/A2)	Taiwanese	157	157	CI	Chen et al. (2004)
GPIIb 1,018 C/T 145 Thr/Met	Japanese	200	317	CI	Sonoda et al. (2000)
	Chinese	100	100	IS	Gao et al. (2005)
Protein G 825 C/T	Chinese	990	1124	CI	Zhang et al. (2005)
TNF alpha -308 G/A	Korean	294	581	CI	Um et al. (2003d)
	Korean	366	610	IS	Um and Kim (2004)
	Korean	152	165	IS	Lee et al. (2004)
TNF β 252A/G	Korean	294	581	CI	Um et al. (2003d)
Toll like receptor 4 119C/A	Taiwanese	234	223	IS	Lin et al. (2005)
von Willebrand factor (vWF) sma 1 (intron 2)	Chinese	107	113	IS	Dai et al. (2001)

Table 1 continued

Gene and polymorphism(s)	Populations	Cases	Control	Type	Reference
PDE4D rs966221T/C	Japanese	208	270	CI	Nakayama et al. (2006)
Factor VII –401 G/T, –402 G/A, 5'F7A1/A2, IVS7, 353 R/Q	Pakistani	200	250	CI	Saleheen et al. (2005)
CD14 –260 C/T	Chinese	62	149	IS	Kang et al. (2004)
Apo(a) pentanucleotide TTTTA repeat (PNTR in promoter)	Japanese	235	309	IS	Ito et al. (2000b)
Apo-1/Fas promoter(CD95) –670 A/G	Korean	125	125	IS	Park et al. (2006)
Oestrogen receptor PvuII and XbaI	Chinese	1,825	1,817	IS + CH	Sun et al. (2003)
Plasma activating factor acetylhydrolase 279 val/phe	Chinese	91	103		Seo et al. (2002)
	Chinese	234	259	CI	Zhang et al. (2002)
	Japanese	120	134	CI	Hiramoto et al. (1997)

Table 2 Characteristics of studies of MTHFR C677T polymorphism and stroke (M/F male/female)

Population	Stroke type	Case	Control	Reference
Japanese	Ischaemic	256 patients (M/F = 123/133); mean age 70.3 ± 8.6 years	325 controls (M/F = 174/151); mean age 67.7 ± 7.5 years	Morita et al. (1998)
Chinese	Ischaemic	102 patients (M/F = 56/46); mean age 65 years (range 30–78)	100 controls (M/F = 70/30); average age 50 years (range: 22–70)	Zhang and Dai (2001)
Japanese	Ischaemic	77 patients (M/F = 49/28) mean age 61.4 ± 6.8 years	229 controls (M/F = 120/109); mean age 59.6 ± 6.9 years	Wu et al. (2001)
Chinese	Ischaemic	143 patients	154 controls	Li et al. (2002)
Chinese	Ischaemic and haemorrhagic	1823 patients (807 cerebral thrombosis, 513 lacunar infarction, 503 intracerebral haemorrhage); mean age 60.3 ± 9.4 years; 63.5% males	1832 controls; mean age 59.6 ± 8.8 years; 57.4% males	Li et al. (2003)
Korean	Ischaemic	195 patients (M/F = 106/89); mean age 61.4 ± 10.9 years	198 controls (M/F = 106/87); mean age 60.8 ± 11.2 years	Choi et al. (2003)
Chinese	Ischaemic	241 patients	304 controls	Baum et al. (2004)
Indian	Ischaemic	75 patients	49 controls	Alluri et al. (2005)
Mongolian	Ischaemic and haemorrhagic	86 subjects, 42 CI patients (M/ F = 24/18), mean age 66.5 ± 0.1 years; 22 CH patients (M/F = 14/8), mean age 64.1 ± 3.1 years	24 controls (M/F = 13/11) mean age 64.5 ± 8.3 years	Fang et al. (2005)
Chinese	Ischaemic	100 patients (M/F = 71/29); mean age 61.08 ± 10.77 years	100 controls (M/F = 71/29) mean age 60.90 ± 10.64 years	Gao et al. (2006)

+ $\varepsilon 2 \varepsilon 3$ + $\varepsilon 2 \varepsilon 4$) versus $\varepsilon 3 \varepsilon 3$, and ($\varepsilon 4 \varepsilon 4$ + $\varepsilon 2 \varepsilon 4$ + $\varepsilon 3 \varepsilon 4$) versus $\varepsilon 3 \varepsilon 3$, were statistically evaluated.

The insertion/deletion polymorphism of the ACE gene was studied in the meta-analysis. The overall association of the D allele of the ACE gene relative to I allele and the contrasts of DD versus II, DD versus ID versus II, DD versus (ID + II), (DD + ID) versus II, were examined. For each gene variant, we evaluated the associations by calculating the odds ratios (ORs), together with the 95% confidence interval (CI), to measure the strength of the genetic association. Based on the individual ORs, a pooled odds ratio was calculated.

Using the Q-statistic, which is a weighted sum of squares of the deviations of individual study OR estimates from the overall estimate, heterogeneity between studies was examined (Cochran 1954). Q follows a chi-square distribution with $r-1$ (r is the number of studies) degrees of freedom (*d.f.*), when the ORs were homogeneous (Zintzaras et al. 2005). The heterogeneity was considered statistically significant if the P value was <0.10 . Quantification of the heterogeneity was done with the I^2 metric ($I^2 = (Q-d.f.)/Q$), which is independent of the number of studies in the meta-analysis (Higgins and Thompson 2002). The I^2 values falls within the range 0–100%,

Table 3 Characteristics of studies of ApoE polymorphisms and stroke (M/F male/female)

Population	Stroke type	Case	Control	Reference
Japanese	Ischaemic and haemorrhagic	322 patients (58% males); 201 cerebral infarction, 84 intracerebral haemorrhage, 37 and subarachnoid haemorrhage; mean age 67.9±11.0 years	1,126 controls (29.7% males); mean age 64.3±10.5 years	Kokubo et al. (2000)
Asian Indian	Not mentioned	63 patients; mean age 56.4±13.1 years	57 controls; mean age 39.4±8 years	Luthra et al. (2002)
Korean	Ischaemic	365 patients (44.2% males); mean age 66.1±11.2 years	319 controls (47.5% males); mean age 67.2±9.8 years	Um et al. (2003a)
Chinese	Ischaemic	226 patients (M/F = 129/97); mean age 48.5±3.4 years	201 controls (M/F = 109/92); mean age 47.1±2.4 years	Jin et al. (2004)
Taiwan Chinese	Ischaemic	277 patients (M/F = 191/86); mean age 63.29±8.62 years	112 controls (M/F = 54/58); mean age 71.90±10.82	Lin et al. (2004)
Chinese	Ischaemic	100 patients (M/F = 71/29); mean age 61.08±10.77 years	100 controls (M/F = 71/29); mean age 60.90±10.64 years	Gao et al. (2006)

Table 4 Characteristics of studies of ACE insertion/deletion (I/D) polymorphism and stroke (M/F male/female)

Population	Stroke type	Case	Control	Reference
Japanese	Ischaemic	181 patients (M/F = 111/70); mean age 65.5 ± 10.3 years	271 controls (M/F = 167/104); mean age 61.1 ± 11.1 years	Doi et al. (1997)
Japanese	Cerebral infarction and haemorrhagic	55 infarction patients (%M = 45); mean age 66±14 years, 38 haemorrhagic patients (%M = 68); mean age 63±10 years	61 controls for the infarction group (%M = 49); mean age 67±8 years, 38 controls for the haemorrhagic group (%M = 68); mean age 63±10 years	Nakata et al. (1997)
Korean	Cerebral infarction	106 patients (M/F = 49/57); mean age 63.9 years	498 controls (M/F = 240/258); mean age 72.5 years	Um et al. (2001)
Chinese	Ischaemic	143 patients	154 controls	Li et al. (2002)
Korean	Cerebral infarction	365 infarction patients (%M = 44.2); mean age 66.1±11.2 years	319 controls (%M = 47.5); mean age 67.2±9.8 years	Um et al. (2003a)
Chinese	Ischaemic	100 patients (M/F = 71/29); mean age 61.08±10.77 years	100 controls (M/F = 71/29); mean age 60.90±10.64 years	Gao et al. (2006)

with higher values denoting greater degree of heterogeneity ($I^2 = 0$ –25%, no heterogeneity; $I^2 = 25$ –50%, moderate heterogeneity; $I^2 = 50$ –75%, large heterogeneity; $I^2 = 75$ –100%, extreme heterogeneity) (Zintzaras et al. 2005). The Mantel–Haenszel method was used to calculate the fixed-effect pooled ORs (Mantel and Haenszel 1959; Robins et al. 1986). The random-effects pooled ORs were calculated by the DerSimonian and Liard method (DerSimonian and Laird 1986). Heterogeneity between studies is expected, because the studies that are brought together by systematic reviews are both clinically and methodologically diverse (Higgins et al. 2002). Where there was heterogeneity in studies, a pooled OR was estimated by the random-effects model, because this model assumes a genuine diversity in the results of the studies, incorporates the calculations of between-study variability, and provides wider CIs (Whitehead 2002; Zintzaras et al. 2005). To examine the trend of pooled OR for the allele contrasts in time, we carried out

cumulative meta-analysis (Lau et al. 1992; Whitehead 1997) and recursive meta-analysis (Zintzaras et al. 2005). For assessment of publication bias for the allele contrast, the Egger regression test for funnel plot asymmetry (Egger et al. 1997; Ioannidis et al. 2003; Zintzaras et al. 2005) and the Begg–Mazumdar test, which is based on Kendall’s tau (Begg and Mazumdar 1994), were carried out. Sensitivity analyses were done to examine the effect of the studies with controls that are not in Hardy–Weinberg equilibrium by their exclusion (Zintzaras et al. 2005). Data were analysed with statistical analysis software StatsDirect, (version 2.5.7).

Results

The studies of MTHFR gene polymorphisms that were included in our meta-analysis were composed of 3,074 stroke cases and 3,315 healthy controls in total. The

prevalence of allele T was 44% and 38% for the case and the control groups, respectively. TT prevalence in the cases was 20%, while in controls it was 16%. The prevalence of CC among patients and controls was 33% and 41%, respectively. The proportion of the case and the control groups having CT genotype was 47% and 43%, respectively. The genotype and the allele frequencies of the individual studies are shown in Table 5. In the control group of one study (Li et al. 2003), the genotype distribution was not in Hardy–Weinberg equilibrium.

In all the studies of ApoE together, there were, in total, 1,353 stroke patients and 1,915 controls. The prevalence of allele ϵ 2 was 7% and 6% for the stroke cases and the control groups, while the prevalence of allele ϵ 4 for the same groups was 12% and 10%, respectively. In the cases and the controls, the prevalence of ϵ 2 ϵ 2 genotype was 2% and 1%, the prevalence of ϵ 4 ϵ 4 genotype was 2% and 1%, and the prevalence of ϵ 3 ϵ 3 genotype was 67% and 72%, respectively. The prevalence of ϵ 2 ϵ 3 genotype among the stroke patients and the healthy controls was both 9%, while for ϵ 2 ϵ 4 genotype it was 2% in both the groups. The ϵ 3 ϵ 4 genotype prevalence was 19% and 15% among the cases and the controls, respectively. The genotype and the allele frequencies of the individual studies are shown in Tables 6 and 7. Departure of the genotype frequencies from the Hardy–Weinberg equilibrium among controls was observed in four studies (Kokubo et al. 2000; Luthra et al. 2002; Um et al. 2003a; Lin et al. 2004).

In total, the studies of ACE gene polymorphism that were considered for our meta-analysis included 988 stroke patients and 1,441 control individuals. The prevalence of the D allele was 40% and 38% for the cases and the controls, respectively. The prevalence of DD genotype among patients and controls was 17%

and 15%, respectively, while the prevalence of II genotype for both the groups was 38%. The prevalence of heterozygotes ID among the case and the control groups was 45% and 47%, respectively. The genotype and the allele frequencies of the individual studies are shown in Table 8. Assessment of Hardy–Weinberg equilibrium for the genotypes among the controls revealed significant deviation in two studies (Nakata et al. 1997; Li et al. 2002).

Our results show that the carriers of the MTHFR T allele were 1.47-times more likely to develop stroke, according to the random effects pooled OR [95% CI (1.19, 1.82)]; $P = 0.0004$. However, extreme inter-study OR heterogeneity ($P < 0.0001$, $I^2 = 79\%$) was observed (Table 9; Fig. 1). With the exclusion of the study with controls not in Hardy–Weinberg equilibrium, i.e. in sensitivity analysis, the degree of heterogeneity between the studies did not decrease appreciably and remained extremely high ($P = 0.0002$, $I^2 = 73\%$). The fixed and the random effects ORs in the sensitivity analysis were significant: OR = 1.53 [95% CI (1.36, 1.72)]; $P < 0.0001$ and OR = 1.55 [95% CI (1.21, 1.99)]; $P = 0.0006$, respectively. The genotype contrast of the homozygotes as well as the additive, dominant and recessive models showed significant association, even in the sensitivity analyses. The random effects pooled OR of the allelic contrast was found to decline from 1.78 in 1998 (first study) to 1.39 in 2003 (six studies) and then increased to 1.47 in 2006, according to the cumulative and the recursive meta-analyses. Although the Begg–Mazumdar test, being of low power due to small numbers of studies, indicated low probability of publication bias ($P > 0.99$), the Egger test result was found to be significant ($P = 0.04$), suggesting the presence of publication bias.

In the meta-analysis of ApoE gene polymorphism (Table 10; Figs. 2 and 3), the association of allele ϵ 2

Table 5 The distribution of the methylenetetrahydrofolate reductase (MTHFR) genotypes and the allelic frequency for stroke patients and controls (values in parentheses are the corresponding percentages)

First author	Year	Population	Distribution of MTHFR genotypes						Frequency of MTHFR alleles			
			CC		CT		TT		C		T	
			Case	Control	Case	Control	Case	Control	Case	Control	Case	Control
Morita	1998	Japanese	80 (31)	153 (47)	121 (47)	139 (43)	55 (21)	33 (10)	281 (55)	445 (68)	231 (45)	205 (32)
Zhang	2001	Chinese	40 (40)	37 (37)	47 (46)	47 (47)	15 (14)	16 (16)	127 (62)	121 (60)	77 (38)	79 (40)
Wu	2001	Japanese	23 (30)	92 (40)	40 (52)	113 (50)	14 (18)	24 (10)	86 (56)	297 (65)	68 (44)	161 (35)
Li	2002	Chinese	58 (41)	97 (63)	65 (45)	49 (32)	20 (14)	8 (5)	181 (63)	243 (79)	105 (37)	65 (21)
Li	2003	Chinese	520 (29)	619 (34)	882 (48)	815 (44)	421 (23)	398 (22)	1922 (53)	2053 (56)	1724 (47)	1611 (44)
Choi	2003	Korean	62 (32)	73 (37)	97 (50)	100 (50)	36 (18)	25 (13)	221 (57)	246 (62)	169 (43)	150 (38)
Baum	2004	Chinese	125 (52)	195 (64)	98 (41)	95 (31)	18 (7)	14 (5)	348 (72)	485 (80)	134 (28)	123 (20)
Alluri	2005	Indian	50 (67)	48 (98)	23 (31)	1 (2)	2 (3)	0 (0)	123 (82)	97 (99)	27 (18)	1 (1)
Fang	2005	Mongolian	16 (26)	16 (67)	30 (48)	6 (25)	16 (26)	2 (8)	62 (50)	38 (79)	62 (50)	10 (21)
Gao	2006	Chinese	30 (30)	32 (32)	49 (49)	44 (44)	21 (21)	24 (24)	109 (55)	108 (54)	91 (45)	92 (46)

Table 6 The distribution of apolipoprotein E (ApoE) genotypes for stroke patients and controls (*values in parentheses are the corresponding percentages*)

First author	Year	Population	Distribution of ApoE genotypes									
			$\epsilon 2 \epsilon 2$		$\epsilon 2 \epsilon 3$		$\epsilon 2 \epsilon 4$		$\epsilon 3 \epsilon 3$		$\epsilon 3 \epsilon 4$	
			Case	Control	Case	Control	Case	Control	Case	Control	Case	Control
Kokubo	2000	Japanese	16 (5)	11 (1)	23 (7)	73 (6)	4 (1)	8 (1)	211 (66)	819 (73)	65 (20)	202 (18)
Luthra	2002	AsianIndian	1 (2)	3 (5)	8 (13)	10 (18)	1 (2)	1 (2)	26 (41)	35 (61)	17 (27)	4 (7)
Um	2003a	Korean	0 (0)	0 (0)	54 (15)	46 (14)	7 (2)	13 (4)	258 (71)	211 (66)	37 (10)	43 (14)
Jin	2004	Chinese	2 (1)	2 (1)	14 (6)	17 (8)	3 (1)	2 (1)	152 (67)	156 (78)	52 (23)	22 (11)
Lin	2004	TaiwanChinese	1 (0)	4 (4)	17 (6)	5 (4)	10 (4)	5 (4)	180 (65)	78 (70)	68 (25)	19 (17)
Gao	2006	Chinese	1 (1)	1 (1)	11 (11)	13 (13)	0 (0)	0 (0)	75 (75)	80 (80)	13 (13)	6 (6)
											3 (1)	13 (1)
											10 (16)	4 (7)
											9 (2)	6 (2)
											3 (1)	2 (1)
											1 (0)	1 (1)
											0 (0)	0 (0)

and the risk of developing stroke, in comparison with the $\epsilon 3$ allele, showed non-significant result, the random effects pooled OR = 0.97 [95% CI (0.62, 1.53)]; $P = 0.912$, while, for the allele $\epsilon 4$, the random effects pooled OR was marginally significant: OR = 1.47 [95% CI (1.00, 2.15)]; $P = 0.049$. High level of inter-study heterogeneity was found to exist in both cases ($P = 0.0007$, $I^2 = 77\%$) and ($P = 0.0015$, $I^2 = 74\%$), respectively. Taking into account only those studies with controls in Hardy–Weinberg equilibrium, we found that the $\epsilon 4$ versus $\epsilon 3$ allele contrast showed significant association, random effects OR = 2.09 [95% CI (1.37, 3.20)]; $P = 0.0006$. The genotype contrast $\epsilon 2 \epsilon 2$ versus $\epsilon 3 \epsilon 3$, under the fixed effects model, showed high significance, OR = 1.94 [95% CI (1.07, 3.51)]; $P = 0.018$, but was found to be non-significant in the random effects model, OR = 0.92 [95% CI (0.17, 4.78)]; $P = 0.92$. Large heterogeneity ($P = 0.003$, $I^2 = 75\%$) between the studies was found to exist. Although the odds ratios in the genotype contrast $\epsilon 4 \epsilon 4$ versus $\epsilon 3 \epsilon 3$ indicated increased risk for stroke in both the fixed effects and the random effects models, the results were not statistically significant, the fixed effects OR = 1.43 [95% CI (0.79, 2.61)]; $P = 0.304$, and the random effects OR = 1.41 [95% CI (0.76, 2.63)]; $P = 0.274$. In that genotype contrast, no inter-study heterogeneity was found to exist ($P = 0.545$, $I^2 = 0\%$). The genotype contrast ($\epsilon 4 \epsilon 4 + \epsilon 2 \epsilon 4 + \epsilon 3 \epsilon 4$) versus $\epsilon 3 \epsilon 3$ in the fixed effects model showed significant association, OR = 1.32 [95% CI (1.09, 1.60)]; $P = 0.004$, but the association according to the random effects model was marginally significant, OR = 1.56 [95% CI (0.99, 2.44)]; $P = 0.05$, although extreme heterogeneity ($P = 0.0007$, $I^2 = 76\%$) was found to exist between the studies. Cumulative and recursive meta-analysis for the allele contrast $\epsilon 2$ versus $\epsilon 3$ showed that the random effects pooled OR decreased from 2.15 in 2000 (first study) to 1.08 in 2004 (five studies), and then further decreased to 0.97 in 2006 (six studies), while an ascending trend was observed for the allele contrast $\epsilon 4$ versus $\epsilon 3$, where the random effects OR increased from 1.19 in 2000 (first study) to 1.47 in 2004 (five studies) but then remained unchanged till 2006 (six studies). The Egger test and the Begg–Mazumdar test results suggested a low probability of publication bias for the allele contrast $\epsilon 2$ versus $\epsilon 3$ ($P = 0.151$ and $P = 0.469$, respectively) and the allele contrast $\epsilon 4$ versus $\epsilon 3$ ($P = 0.168$ and $P = 0.272$, respectively).

In the meta-analysis of ACE insertion/deletion (I/D) polymorphism (Table 11; Fig. 4), the summary ORs under a fixed effects and a random effects model showed no statistically significant association of the D allele with the risk of developing stroke relative to

Table 7 The distribution of the apolipoprotein E allelic frequency for stroke patients and controls (*values in parentheses are the corresponding percentages*)

First author	Year	Population	Frequency of ApoE alleles					
			ϵ 2		ϵ 3		ϵ 4	
			Case	Control	Case	Control	Case	Control
Kokubo	2000	Japanese	59 (9)	103 (5)	510 (79)	1913 (85)	75 (12)	236 (11)
Luthra	2002	AsianIndian	11 (9)	17 (15)	77 (61)	84 (74)	38 (30)	13 (11)
Um	2003a	Korean	61 (8)	59 (9)	607 (83)	511 (80)	62 (8)	68 (11)
Jin	2004	Chinese	21 (5)	23 (6)	370 (82)	351 (87)	61 (13)	28 (7)
Lin	2004	TaiwanChinese	29 (5)	18 (8)	445 (80)	180 (80)	80 (15)	26 (12)
Gao	2006	Chinese	13 (7)	15 (8)	174 (87)	179 (90)	13 (7)	6 (3)

Table 8 The distribution of the angiotensin-converting enzyme (ACE) genotypes and the allelic frequency for stroke patients and controls (*values in parentheses are the corresponding percentages*)

First Author	Year	Population	Distribution of ACE genotypes						Frequency of ACE alleles			
			II		ID		DD		I		D	
			Case	Control	Case	Control	Case	Control	Case	Control	Case	Control
Doi	1997	Japanese	73 (40)	115 (42)	77 (43)	126 (46)	31 (17)	30 (11)	223 (62)	356 (66)	139 (38)	186 (34)
Nakata	1997	Japanese	33 (35)	25 (25)	41 (44)	62 (63)	19 (20)	12 (12)	107 (58)	112 (57)	79 (42)	86 (43)
Um	2001	Korean	42 (40)	185 (37)	48 (45)	237 (48)	16 (15)	76 (15)	132 (62)	607 (61)	80 (38)	389 (39)
Li	2002	Chinese	53 (37)	68 (44)	69 (48)	58 (38)	21 (15)	28 (18)	175 (61)	194 (63)	111 (39)	114 (37)
Um	2003a	Korean	128 (35)	116 (36)	166 (45)	154 (48)	71 (19)	49 (15)	422 (58)	386 (61)	308 (42)	252 (39)
Gao	2006	Chinese	42 (42)	42 (42)	47 (47)	44 (44)	11 (11)	14 (14)	131 (65)	128 (64)	69 (35)	72 (36)

Table 9 The distribution of the methylenetetrahydrofolate reductase (MTHFR) genotypes and the allelic frequency for stroke patients and controls (*HWE Hardy–Weinberg equilibrium*)

Genetic contrasts	Populations	Studies (n)	Alleles/genotypes (n)	Fixed effects [OR (95% CI)]	Random effects [OR (95% CI)]	I ² (%)	Q test P value
T versus C	All	10	12,778	1.28 (1.19, 1.37)	1.47 (1.19, 1.82)	79	<0.0001
	All in HWE	9	5,468	1.53 (1.36, 1.72)	1.55 (1.21, 1.99)	73	0.0002
TT versus CC	All	10	3,528	1.50 (1.29, 1.75)	1.91 (1.31, 2.77)	66	0.001
	All in HWE	9	1,570	2.11 (1.64, 2.72)	2.09 (1.39, 3.14)	53	0.03
TT versus TC versus CC	All	10	6,389	1.28 (1.15, 1.41)	1.41 (1.15, 1.72)	51	0.03
	All in HWE	9	2,734	1.53 (1.30, 1.81)	1.50 (1.21, 1.84)	30	0.181
TT versus (TC + CC)	All	10	6,389	1.25 (1.09, 1.42)	1.54 (1.13, 2.10)	59	0.009
	All in HWE	9	2,734	1.72 (1.36, 2.17)	1.68 (1.23, 2.30)	34	0.145
(TT + TC) versus CC	All	10	6,389	1.46 (1.31, 1.62)	1.64 (1.27, 2.11)	70	0.0004
	All in HWE	9	2,734	1.72 (1.47, 2.03)	1.74 (1.28, 2.37)	67	0.002

the I allele, OR = 1.06 [95% CI (0.94, 1.20)]; $P = 0.3$, for either model. No heterogeneity was observed between the six studies ($P = 0.842$, $I^2 = 0\%$). In the sensitivity analysis (exclusion of the studies in which there was no deviation from Hardy–Weinberg equilibrium among the controls), the fixed effects model and the random effects model both showed non-significant association, OR = 1.07 [95% CI (0.93, 1.24)]; $P = 0.3$, for either model. No inter-study heterogeneity ($P = 0.619$, $I^2 = 0\%$) was found to exist in this analysis. No significant association was found

either in the genotype contrast for homozygous alleles or with the additive or the dominant or the recessive models. In the cumulative and the recursive meta-analysis for the allelic contrast it was found that there was a slight decline in the random effects pooled OR, from 1.19 in 1997 (first study) to 1.06 in 2002 (four studies). After that, there was no further change in the random effects pooled OR till 2006 (six studies). Both the Egger test and the Begg–Mazumdar test results suggested a low probability of publication bias ($P = 0.134$ and $P = 0.136$, respectively).

Fig. 1 Results of published studies of the association between MTHFR C677T polymorphism and stroke in Asian populations. Each study is shown by an OR estimating the outcome of the comparison of the T allele against the C allele with the corresponding 95% CI. The random effects pooled ORs are shown. The size of the box is proportional to the weight of the study

Table 10 The distribution of the apolipoprotein E (ApoE) genotypes and the allelic frequency for stroke patients and controls (HWE Hardy–Weinberg equilibrium)

Genetic contrasts	Populations	Studies (n)	Alleles/ genotypes (n)	Fixed effects [OR (95% CI)]	Random effects [OR (95% CI)]	I ² (%)	Q test P value
ε 2 vs. ε 3	All	6	5,830	1.14 (0.92, 1.41)	0.97 (0.62, 1.53)	77	0.0007
	All in HWE	2	1,146	0.87 (0.54, 1.40)	0.87 (0.54, 1.41)	0	0.954
ε 4 vs. ε 3	All	6	6,107	1.28 (1.08, 1.52)	1.47 (1.00, 2.15)	74	0.0015
	All in HWE	2	1,182	2.09 (1.37, 3.20)	2.09 (1.37, 3.20)	0	0.893
ε 2 ε 2 vs. ε 3 ε 3	All	6	2,323	1.94 (1.07, 3.51)	0.92 (0.17, 4.78)	75	0.003
	All in HWE	2	469	1.04 (0.21, 5.20)	1.04 (0.21, 5.20)	0	0.982
ε 4 ε 4 vs. ε 3 ε 3	All	6	2,333	1.43 (0.79, 2.61)	1.41 (0.76, 2.63)	0	0.545
	All in HWE	1	468	1.54 (0.25, 9.34)	1.54 (0.25, 9.34)	NA	NA
(ε 2 ε 2 + ε 2 ε 3 + ε 2 ε 4) vs. ε 3 ε 3	All	6	2,668	1.10 (0.88, 1.38)	1.07 (0.77, 1.48)	44	0.114
	All in HWE	2	529	0.92 (0.55, 1.55)	0.92 (0.55, 1.55)	0	0.977
(ε 4 ε 4 + ε 2 ε 4 + ε 3 ε 4) vs. ε 3 ε 3	All	6	2,935	1.32 (1.09, 1.60)	1.56 (0.99, 2.44)	76	0.0007
	All in HWE	2	566	2.29 (1.45, 3.63)	2.29 (1.45, 3.63)	0	0.987

Discussion

In this comprehensive meta-analysis, two of the three candidate gene polymorphisms showed significant association with stroke in the Asian populations. The relationships between the gene variants analysed in our meta-analysis and stroke have been implicated in a large number of subjects in the association studies, but the individual studies were generally small and had several methodological limitations. Because of the contradictory results of the association studies of the gene variants and stroke in the Asian populations, a prospective study was necessary to evaluate the candidacy of those genes, with reliability, in the pathogenesis of stroke. Our study, in the context of the Asian populations, presented an overview of the

studies of the gene variants that have been examined for their association with stroke and evaluated and quantified the risks associated with the most studied polymorphisms for the development of stroke.

Of the three genes that have been studied extensively in Asian populations, MTHFR C677T polymorphism studies included the maximum number of cases (total number of stroke cases = 3,074). The MTHFR encoded product 5,10-methylenetetrahydrofolate reductase is a folate-dependent enzyme catalysing the rate-limiting step in the methylation of homocysteine to methionine. The C677T polymorphism in MTHFR results in the conversion of alanine to valine at amino acid 222 and was reported to be associated with decreased activity and, eventually, elevation of homocysteine levels (Kang et al. 1991;

Fig. 2 Results of published studies of the association between ApoE polymorphism and stroke in Asian populations. Each study is shown by an OR estimating the outcome of the comparison of the ϵ 2 allele against the ϵ 3 allele with the corresponding 95% CI. The random effects pooled ORs are shown. The size of the box is proportional to the weight of the study

Fig. 3 Results of published studies of the association between ApoE polymorphism and stroke in Asian populations. Each study is shown by an OR estimating the outcome of the comparison of the ϵ 4 allele against the ϵ 3 allele with the corresponding 95% CI. The random effects pooled ORs are shown. The size of the box is proportional to the weight of the study

Frosst et al. 1995). Elevated level of homocysteine causes endothelial dysfunction (Constans et al. 1999) and was found to be a risk factor for atherosclerosis and atherothrombosis (McCully 1969; Frosst et al. 1995). The C677T transition was reported to be associated with ischaemic stroke (Morita et al. 1998). Data from ten case-control studies of the MTHFR polymorphism and stroke were included for this meta-analysis. The studies were composed of a total of 3,074 stroke patients and 3,315 healthy controls. The overall results indicated a significant association of the C677T MTHFR polymorphism with stroke, but with large inter-study heterogeneity. Although a high level of

heterogeneity persisted, even in the sensitivity analysis, the results showed significant association. Our findings of the association of MTHFR C677T polymorphism in this meta-analysis are in agreement with the previously conducted meta-analyses where this polymorphism was found to be potentially involved in the development of stroke (Cronin et al. 2005; Casas et al. 2004; Kim and Becker 2003; Kelly et al. 2002).

The protein product of the apolipoprotein E (ApoE) gene is a glycoprotein with three common isoforms: E2, E3, and E4, encoded by the alleles ϵ 2, ϵ 3, and ϵ 4, respectively. The genotype ϵ 3 ϵ 3 occurs in approximately one-half to two-thirds in most popula-

Table 11 The distribution of the angiotensin-converting enzyme (ACE) genotypes and the allelic frequency for stroke patients and controls (HWE Hardy–Weinberg equilibrium)

Genetic contrasts	Populations	Studies (n)	Alleles/genotypes (n)	Fixed effects [OR (95% CI)]	Random effects [OR (95% CI)]	I ² (%)	Q test P value
D versus I	All	6	4,858	1.06 (0.94, 1.20)	1.06 (0.94, 1.20)	0	0.842
	All in HWE	4	3,880	1.07 (0.93, 1.24)	1.07 (0.93, 1.24)	0	0.619
DD versus II	All	6	1,300	1.17 (0.91, 1.51)	1.18 (0.91, 1.52)	0	0.685
	All in HWE	4	1,041	1.22 (0.91, 1.63)	1.22 (0.91, 1.63)	0	0.442
DD versus ID versus II	All	6	2,429	1.07 (0.90, 1.27)	1.07 (0.90, 1.28)	0	0.961
	All in HWE	4	1,940	1.08 (0.89, 1.32)	1.08 (0.89, 1.32)	0	0.822
DD versus (ID + II)	All	6	2,429	1.20 (0.95, 1.52)	1.19 (0.91, 1.56)	21	0.276
	All in HWE	4	1,940	1.24 (0.95, 1.62)	1.24 (0.95, 1.64)	4	0.374
(DD + ID) versus II	All	6	2,429	1.02 (0.86, 1.21)	1.02 (0.86, 1.21)	0	0.493
	All in HWE	4	1,940	1.02 (0.84, 1.25)	1.02 (0.84, 1.25)	0	0.919

Fig. 4 Results of published studies of the association between ACE I/D polymorphism and stroke in Asian populations. Each study is shown by an OR estimating the outcome of the comparison of the D allele against the I allele with the corresponding 95% CI. The random effects pooled ORs are shown. The size of the box is proportional to the weight of the study

tions (Sudlow et al. 2006). The major role of this protein is lipid transport and metabolism. It was found that the genotypes having the ϵ 4 allele are associated with increased cholesterol levels, in comparison with the ϵ 3 ϵ 3 genotype, while genotypes with the ϵ 2 allele were found to be associated with decreased levels (Eichner et al. 2002). The potential role of ApoE in the repair and regeneration of neurons and survival after brain injury was found in rodent models of head injury and ischaemic stroke (Graham et al. 1999; Horsburgh et al. 2000). Clinical studies have shown that genotypes with the ϵ 4 allele are associated with poor outcome after head injury (Waters and Nicoll 2005). According to these observations, it can be suggested that ApoE might influence outcome after acute stroke in humans (Waters and Nicoll 2005). Since different studies have produced conflicting results, there was the necessity of the conducting of a comprehensive meta-analysis. In

our study, the allele ϵ 4 was found to be associated with stroke, but high heterogeneity existed between the studies.

The function of the angiotensin-converting enzyme (ACE) is the conversion of angiotensin I to angiotensin II, which is involved in vasoconstriction, vascular hypertrophy, and atherosclerosis (Kim and Iwao 2000). ACE also inhibits the functions of bradykinin, a vasoactive peptide that is involved in vasodilatation, by its degradation (Kim and Iwao 2000). The ACE I/D polymorphism partly determines the plasma and intracellular levels of ACE in stroke patients and healthy individuals (Tiret et al. 1992; Sharma et al. 1994). Patients with a double deletion of a 287 bp in intron 16 (DD genotype) have higher plasma or tissue levels of ACE than do individuals with ID or II genotype (Rigat et al. 1990; Tiret et al. 1992). To explore whether I/D polymorphism within intron 16 of

the ACE gene confers susceptibility to stroke, we conducted a meta-analysis taking six studies. No statistically significant association with stroke was detected, either for the D allele or for the homozygous DD genotype. However, no heterogeneity was found to exist between the studies. Results did not change, either for the allele or for the genotypes, in the sensitivity analysis. Since the sample size was relatively small for ACE I/D polymorphism studies, these results should be considered with caution.

Although we tried to estimate a combined effect from groups of similar studies only in the Asian population, we found considerable heterogeneity among the studies of the MTHFR and ApoE polymorphisms. In the sensitivity analysis of ApoE polymorphism, the measures of heterogeneity dropped to null in most of the genotype and the allele contrasts. Although only two studies were considered for this analysis, the results indicate that the departure of the genotype frequencies from Hardy–Weinberg equilibrium in the control populations could be a possible cause for the inter-study heterogeneity. The sensitivity analysis of the MTHFR polymorphism, however, failed to change the effect of heterogeneity in an appreciable manner, except for the additive and the dominant models. Thus, in addition to the departure of the genotype frequencies from the Hardy–Weinberg equilibrium in the control populations, the cause of this observed heterogeneity could be attributable to the variations in genetic constitution and/or environmental characteristics across the populations or to the difference in the design and conduct of the studies. As evident from the observed results, homogeneity existed among the studies on ACE I/D polymorphism. It is interesting to note that many of the studies that were analysed had control groups whose genotype frequencies deviated significantly from the Hardy–Weinberg equilibrium (Li et al. 2003, 2002; Kokubo et al. 2000; Luthra et al. 2002; Um et al. 2003a; Lin et al. 2004; Nakata et al. 1997). Potential biases in the selection of control individuals, or genotyping errors, could be implicated in such deviations (Little et al. 2002).

Since we considered only the Asian population for our analyses, we looked for reports of other meta-analyses conducted with data from the European population to compare whether ethnic variations between these two populations have any effect on the association of the gene polymorphisms that we studied with the risk of developing stroke. The MTHFR C677T polymorphism was reported to be significantly associated with increased risk of stroke in the European population (Casas et al. 2004) and in the Asian as well as the European populations (Cronin et al. 2005). No

evidence of association was found to exist between the ApoE polymorphism and stroke in the European population (Casas et al. 2004), and a stronger association with stroke was found in the Asian populations than in white populations (Sudlow et al. 2006). Association of the ACE I/D polymorphism with increased risk of stroke in the European populations was evidenced from two meta-analyses (Casas et al. 2004; Sharma 1998). These reports, in conjunction with our results, suggest that ethnic variations among these two populations could be accounted for by the discordant findings on the roles ApoE and the ACE polymorphisms in the pathogenesis of stroke. Interestingly, no such discordance was observed in the case of the MTHFR polymorphism, and, in both the populations, this C677T polymorphism was found to be a potent risk factor for the development of stroke.

In conclusion, the evidence from these meta-analyses supports the notion of a role for the polymorphisms of MTHFR and ApoE genes in susceptibility to stroke. Meta-analysis of the ACE gene polymorphism has failed to provide evidence of increased risk for stroke. In our analyses we tested various allele and genotype contrasts for each of the three genes. However, such comparisons are not independent of each other and, therefore, might pose multiple-comparison problems. To confirm or refute these findings, additional larger studies with corrections for multiple comparisons are required.

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