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Polymorphism of Angiotensin Converting Enzyme, Angiotensinogen, and Apolipoprotein E Genes in a Japanese Population With Cerebrovascular Disease

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The homozygous deletion allele of the angiotensin converting enzyme gene (ACE/DD), homozygous threonine allele of the angiotensinogen gene (AGN/TT), and the ϵ4 allele of the apolipoprotein E gene (apoE/ϵ4) are reported to be associated with ischemic heart disease. Cerebrovascular disease (CVD) is another atherosclerotic disease; and the effects of these polymorphisms on CVD have been confusing. In this study, we investigated whether ACE/DD, AGN/TT, and apoE/ϵ4 genotypes are associated with CVD and whether genetic risk is enhanced by the effect of one upon another. We ascertained these genotypes in patients with cerebral infarction (n = 55) and cerebral hemorrhage (n = 38), diagnosed by brain computed tomography. Control subjects for the infarction group and the hemorrhage group were randomly selected from 583 subjects matched for age, gender, and history of hypertension with patients. Frequency of ACE/DD genotype was higher in the patients with infarction than in the controls (χ^2 = 6.1, P < .05). The AGN/TT genotype was not associated with either infarction or hemorrhage, but it increased the relative risk for cerebral infarction in the subjects with ACE/DD genotype (χ^2 = 8.0, P < .01, odds ratio; 11.7, 95% confidence intervals: 1.4 to 96.0). There was no significant association between apoE/ϵ4 and CVD. These results suggest that ACE/DD predicts cerebral infarction, but not cerebral hemorrhage, and that AGN/TT enhances the risk for cerebral infarction associated with ACE/DD. Am J Hypertens 1997;10:1391–1395 © 1997 American Journal of Hypertension, Ltd.

KEY WORDS: **Polymorphism, genetics, angiotensin converting enzyme, angiotensinogen, apolipoprotein E, cerebrovascular disease.**

Cerebrovascular disease is a multifactorial disease caused by the interactions of several genetic and environmental factors, as with ischemic heart disease. Recent advances in genetic epidemiology have revealed that some genetic variants increase the risk for myocardial infarction. The genes of angiotensin converting enzyme (ACE),[1–4] angiotensinogen (AGN),[5–7] and apolipoprotein E (apoE)[8–11] have been extensively examined. A homozygous deletion allele in intron 16 of the ACE gene (ACE/DD) has been reported to be associated with an increase in the incidence of ischemic

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heart disease and left ventricular hypertrophy.[12–14] A homozygous molecular variant of the AGN gene, with threonine instead of methionine at position 235 (AGN/TT), is known to be one of the inherited predisposing factors for essential hypertension[15,16] and myocardial infarction.[5–7] Furthermore, we have reported that polymorphism of the apoE gene (ϵ2, ϵ3, and ϵ4 code for three apoE protein isoforms) is another inherited risk factor for ischemic heart disease.[8–11,17]

In general, cerebrovascular disease (CVD) and ischemic heart disease have risk factors in common, such as hypertension, hyperlipidemia, and smoking; and both types of diseases are pathologically based on atherosclerosis. However, genetic risk factors in CVD have not been extensively studied as compared with those involved in ischemic heart disease. The genetic polymorphism of ACE/DD and of AGN/TT are suggested to be involved in atherosclerosis via activation of angiotensin generation.[1,15,18,19] However, several reports on the effect of ACE/DD and AGN/TT on the incidence of CVD have shown conflicting results.[20–24] The apoE/ϵ4 allele also influences atherogenesis indirectly by an effect on circulating levels of low density lipoprotein cholesterol and apolipoprotein B.[9,25] A recent report, however, showed no association between apoE/ϵ4 and CVD in whites.[26] Therefore, we investigated whether the gene polymorphisms of ACE, AGN, and apoE associated with the incidence of CVD as well as ischemic heart disease in Japanese. Ethnic difference is an important factor in evaluating genetic risk. Furthermore, analysis of three genes in one population would be informative in optimizing our understanding of interaction among genetic effects of three genes.

METHODS

Subjects and Measurements Patients with documented CVD were identified from clinical records from July 1992 to December 1995 of six hospitals in Osaka, Japan. Patients aged younger than 30 and older than 80 years were excluded. Subjects with risk factors for cardiogenic stroke, such as atrial fibrillation, valvular heart disease, endocarditis, acute myocardial infarction, dilated cardiomyopathy, and arteriosclerosis obliterans, were also excluded. Final diagnosis of CVD was confirmed on brain computed tomography or brain magnetic resonance imaging. We applied the criteria of National Institute for Neurological Disorders and Stroke.[27] We identified 93 patients with CVD, including cerebral infarction (n = 55) and cerebral hemorrhage (n = 38). Subjects without a history of CVD or transient ischemic attack were recruited from individuals attending Sakuragaoka Hospital (Hyogo, Japan) undergoing general check-ups from September 1991 to March 1993 (total number: 3920; men, 3103, women, 817). A total of 99 control subjects were randomly recruited from this population, matched with study patients for age, gender, and history of hypertension. The existence of hypertension in CVD patients and in control subjects was matched because AGN/TT is a genetic risk factor for hypertension, which influences the incidence of CVD. All cases and controls (all Japanese) gave informed consent before participating in the research protocol, which was approved by the ethics committee of each hospital.

Determination of Genotypes DNA was extracted from 10 mL of whole blood using SepaGene (Sanko Junyaku Co., Tokyo, Japan). All polymerase chain reactions to detect polymorphism of the ACE, AGN, and apoE genes were carried out with 100 ng of genomic DNA as a template, using a DNA Thermal Cycler PJ 2000 (Perkin Elmer Co., Norwalk, CT). Determination of ACE gene polymorphism followed the protocol of Tiret et al[28] with minor modifications.[11] PCR products were separated by 2% agarose gel electrophoresis and visualized by ethidium bromide staining. The protocols of genotyping of AGN and apoE gene were taken from those of Russ[29] and Emi,[30] respectively. Each PCR product was digested with proper restriction enzymes (Takara Shuzo Co. Ltd., Osaka, Japan) for 3 h and then electrophoresed in 2% agarose or 10% polyacrylamide gel.

Statistical Analysis For all statistical analyses, we used a computer software application, StatView (Abacus Concepts, Inc., Berkeley, CA). A χ^2 analysis was performed to assess the statistical differences in the genotype distribution and other characteristics between the two groups. Allele frequencies were deduced from genotype frequencies.

RESULTS

The characteristics of the patients with cerebral infarction or cerebral hemorrhage and those of control subjects are summarized in Table 1.

The genotype distribution of each gene in patients and control subjects did not deviate significantly from Hardy-Weinberg equilibrium. The distributions of genotypes and alleles of the ACE, AGN, and apoE genes were not different between CVD patients and control subjects. Frequencies of ACE/DD, AGN/TT, and apoE/ϵ4, which are reported to be genetic risk factors for ischemic heart disease, were not different between patients and control subjects.

The genetic analyses for ischemic infarction and cerebral hemorrhage were separately performed. In comparing the ischemic infarction group with the matched control group, the genotype distribution of the ACE gene was found to be significantly different between the two groups (Table 2). The frequency of subjects with ACE/DD was higher in the infarction

TABLE 1. CHARACTERISTICS OF PATIENTS AND CONTROL SUBJECTS

Parameter	Patients	Control
Infarction group		
n	55	61
Age	66 ± 14	67 ± 8
Percent male	45	49
Percent hypertension	65	65
Hemorrhage group		
n	38	38
Age	63 ± 10	63 ± 10
Percent male	68	68
Percent hypertension	86	86

Hypertension is defined as systolic blood pressure ≥160 or diastolic blood pressure ≥95 mm Hg, or receiving hypertensive medication. Values are means ± SD.

TABLE 3. DISTRIBUTION OF ANGIOTENSINOGEN GENOTYPES IN CEREBRAL INFARCTION AND CEREBRAL HEMORRHAGE AND IN CONTROL GROUPS

Subjects	*MM*	*MT*	*TT*	
Infarction group				
Patients	7	18	30	NS
Controls	3	31	27	
Hemorrhage group				
Patients	2	17	19	NS
Controls	2	18	18	

NS, not significant.

group than in the control group ($\chi^2 = 5.6$; $P < .05$; odds ratio, 3.2; 95% confidence interval, 1.2 to 8.4). In the infarction group, the frequency of the ACE/DD genotype did not differ between hypertensive (*II+ID/DD* = 24/12) and normotensive (*II+ID/DD* = 15/4) subjects, although the statistical power was very weak. The cerebral hemorrhage group showed no difference from the matched control group in the distribution of ACE genotypes (Table 2). The distribution of genotypes and alleles of the AGN and apoE genes were not associated with either cerebral infarction or cerebral hemorrhage (Tables 3, 4). Of interest, AGN/TT genotype appeared to increase the relative risk for cerebral infarction in the subjects with ACE/DD ($\chi^2 = 8.0$; $P < .01$; odds ratio, 11.7; 95% confidence interval, 1.4 to 96.0) (Table 5). The apoE gene did not interact with the other two genes in regard to the risk for CVD.

DISCUSSION

The present study demonstrated that the renin-angiotensin system related genes were associated with the incidence of cerebral infarction but not with that of cerebral hemorrhage. The differences between these two diseases may be due to their different pathogeneses. Cerebral hemorrhage is mainly induced by rupture of a small aneurysm, which is not related to the atheromatous change. In contrast, most cerebral infarctions are related to atherosclerosis of the cerebral arteries. Furthermore, the common and major pathological changes in ischemic heart disease and cerebral infarction, are atherosclerosis and thrombogenesis in the artery. These findings suggest that the association of the ACE/DD genotype with the incidence of both cerebral infarction and ischemic heart disease may be related to vascular atherogenesis and thrombogenesis.

Of interest, the combined analysis of the AGN/*TT* and ACE/*DD* genotypes further enhanced the predictability of cerebral infarction. We reported a similar enhancement of the predictability with the combined analysis of these genotypes in myocardial infarction.[6] Furthermore, both genotypes are reported to be involved in an increase of angiotensin II generation, not only in the circulation[1,15] but also in local tissues.[18,19] Recent investigations have revealed that angiotensin II contributes to atherosclerotic changes and plaque rupture via several mechanisms such as vasoconstriction, vascular smooth muscle cell growth, thrombogenesis, and antifibrinolysis. These findings further support the theory that the AGN/*TT* and ACE/*DD* genotypes

TABLE 2. DISTRIBUTION OF ANGIOTENSIN CONVERTING ENZYME GENOTYPES IN CEREBRAL INFARCTION AND CEREBRAL HEMORRHAGE AND IN CONTROL GROUPS

	Genotype			Statistics	
Subjects	*II*	*ID*	*DD*	*II* v *ID* v *DD*	*II+ID* v *DD*
Infarction group					
Patients	15	24	16	$\chi^2 = 7.3$, $P < .05$	$\chi^2 = 5.6$, $P < .05$
Controls	14	40	7		OR = 3.16
Hemorrhage group					
Patients	18	17	3	NS	NS
Controls	11	22	5		

OR, odds ratio; NS, not significant.

TABLE 4. DISTRIBUTION OF APOLIPOPROTEIN E ALLELE FREQUENCIES IN CEREBRAL INFARCTION AND CEREBRAL HEMORRHAGE AND IN CONTROL GROUPS

Subjects	ϵ2	ϵ3	ϵ4	
Infarction group				
Patients	0.02	0.89	0.09	NS
Controls	0.06	0.90	0.04	
Hemorrhage group				
Patients	0.07	0.86	0.07	NS
Controls	0.06	0.85	0.09	

NS, not significant.

contribute to vascular atherogenesis and thrombogenesis via activation of angiotensin II production.

The apparent lack of association of the single genetic variant of AGN with cerebral infarction might be affected by matching the hypertension history between cases and controls, as the AGN gene is known to be strongly correlated with hypertension.[15,16] Another interpretation is that patients with cerebral infarction contained a subgroup, those with subcortical infarction, which is known to be associated with the necrosis of the blood vessels and severity of hypertension rather than with atheromatous changes. However, we could not perform analysis using only patients with atheromatous infarction, due to the small number of subjects and low statistical power.

Another gene analyzed, the apoE/ϵ4 allele, had no association with either cerebral infarction or cerebral hemorrhage, although this gene is reported to be associated with atherosclerotic disease of the heart, such as myocardial infarction, silent myocardial ischemia and restenosis after coronary angioplasty, and carotid artery atherosclerosis. However, the role of apoE polymorphism in ischemic stroke is still controversial.[26,31–33] There are two reports demonstrating significant association between apoE/ϵ4 and cerebral infarction.[31–32] However, it has been pointed out that there was a lower frequency of ϵ4 allele in the control groups of these researchers compared with that in the general white population.[30] The allele frequency in our study did not deviate from that of other studies in Japan. Further studies are required to clarify whether variation of the genetic risk of the renin-angiotensin system and apoE between cerebral infarction and ischemic heart disease might be explained by organ specificity in the function of angiotensin II and apoE.

TABLE 5. COMBINED ANALYSIS OF ANGIOTENSIN CONVERTING ENZYME GENOTYPES AND ANGIOTENSINOGEN GENOTYPES IN CEREBRAL INFARCTION AND CEREBRAL HEMORRHAGE AND IN CONTROL GROUPS

Subjects	*DD* and *TT*	Other Genotypes	
Infarction group			
Patients	9	46	$\chi^2 = 8.0$, $P < .01$
Controls	1	60	OR = 11.7
Hemorrhage group			
Patients	2	36	NS
Controls	2	36	

OR, odds ratio; NS, not significant.

Although hypertension is the main risk factor for CVD, a recent study[34] revealed that there were trait loci contributing not to hypertension but to CVD in stroke-prone spontaneously hypertensive rats, and that these loci could account for 28% of the overall phenotypic variance. Our results are highly consistent with their report[34] that cerebral infarction is not only a complication of hypertension but is also an independent vascular disease, influenced in part by genetic factors. It is not even known whether the ACE/DD and AGN/TT polymorphisms are causative variants or just markers of another functional variant. Further studies are necessary to determine the genetic locus responsible for CVD, and whether the AGN and ACE genes themselves, and not other genes beside them, confer susceptibility to cerebrovascular events. Although there is no direct evidence showing that the ACE/DD and AGN/TT genotypes could influence cerebral infarction due to activation of angiotensin II production in cerebral arteries, it may be useful to introduce genetic pharmacology for evaluation of the effects of ACE inhibitors on the prevention of cerebral infarction as well as on myocardial infarction.

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