Prospective Evaluation of the Angiotensin-Converting Enzyme Insertion/Deletion Polymorphism and the Risk of Stroke

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Background—The *D/I* polymorphism of the *ACE* gene has been studied in relation to a variety of cardiovascular disorders, including stroke. A number of small studies have been conducted, with inconsistent results. We investigated the association between *ACE* genotype and the incidence of stroke in a large, prospective, matched case-control sample from the Physicians' Health Study.

Methods and Results—In the Physicians' Health Study, 348 subjects who had been apparently healthy at enrollment suffered a stroke during 12 years of follow-up, as determined from medical records and autopsy. A total of 348 cases were matched by age, time of randomization, and smoking habit to an equal number of controls (who had remained free of stroke). The *D/I* polymorphism was determined by polymerase chain reaction. Data were analyzed for the entire nested case-control sample, and also among a subgroup without a history of hypertension or diabetes mellitus, considered to be at low conventional risk (207 cases and 280 controls). All observed genotype frequencies were in Hardy-Weinberg equilibrium. The relative risk associated with the *D* allele was 1.11 (95% CI, 0.90 to 1.37; $P=0.35$), assuming an additive model in the matched analysis. Additional analyses assuming dominant or recessive effects of the *D* allele, as well as the analysis after stratification for low-risk status, showed no material as a statistically significant association.

Conclusions—The results of this large, prospective study indicate that the *ACE D/I* gene polymorphism is not associated with subsequent risk of stroke. **(*Circulation*. 1999;99:340-343.)**

Key Words: angiotensin ■ enzymes ■ stroke ■ genetics

Despite marked declines in mortality, stroke remains a leading cause of morbidity and mortality in the United States. Strong evidence from twin and family studies shows that familial predisposition, in addition to such recognized risk factors as high blood pressure, smoking, diabetes, obesity, and advanced age, contributes to the pathogenesis of stroke.[1] Identification and characterization of gene variants that play such a role may allow improved prognostication, therapy, and prevention. A polymorphic marker associated with the gene encoding ACE has attracted widespread attention in recent years. This deletion/insertion (*D/I*) variant has consistently been shown to be associated with differential plasma and tissue ACE activities[2,3]; its possible association with cardiovascular disorders, however, remains inconclusive. An association between the *D* allele and stroke incidence has been reported by some, but not by others.[4–13] Most of the studies have been small, and all were retrospective. To overcome these limitations, we conducted a large, prospective, nested case-control study within the Physicians' Health Study.

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Methods

Study Subjects

The Physicians' Health Study is a randomized trial of aspirin and β-carotene initiated in 1982 in 22 071 male, predominantly white, US physicians 40 to 84 years of age at study entry.[14] Before randomization, 14 916 participants provided an EDTA-anticoagulated blood sample. All participants were free of prior history of stroke or transient ischemic attacks. Yearly follow-up questionnaires provide updated information on newly developed diseases. One subject was receiving ACE-inhibitor treatment at entry into the study, but this was discontinued after enrollment. Stroke was defined by the presence of a new focal neurological deficit, with

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TABLE 1. Characteristics of the Study Population

	All Subjects			Low-risk Subgroup		
Parameter	Cases	Controls	*P*	Cases	Controls	*P*
No. of subjects	338	338		207	280	
Age, y±SD	61.1±8.4	60.6±8.3	NS	60.5±8.8	59.8±8.3	NS
Diagnosis, %						
Ischemic stroke	80.2	N/A		78.3	N/A	
Hemorrhagic stroke	13.0	N/A		14.5	N/A	
Unknown	6.8	N/A		7.2	N/A	
BMI, kg/m^2±SD	25.5±3.2	24.9±2.9	<0.01	25.1±2.7	24.8±2.7	NS
Systolic BP, mm Hg±SD	134.6±12.8	129.4±12.1	<0.001	129.5±10.4	126.9±11.2	<0.05
Diastolic BP, mm Hg±SD	82.3±7.1	80.0±7.5	<0.001	79.9±6.6	78.8±7.0	NS
History of hypertension, %	35.0	15.8	<0.001	N/A	N/A	
History of diabetes, %	9.5	3.0	<0.001	N/A	N/A	
History of H-chol, %	13.3	9.2	NS	9.8	6.8	NS
Smoking history, %						
Never smoked	42.1	42.0	NS	41.1	41.8	NS
Former smoker	40.7	40.5	NS	43.5	40.4	NS
Current smoker	17.2	17.5	NS	15.4	17.8	NS

BMI indicates body mass index; BP, blood pressure; and H-chol, hypercholesterolemia.

symptoms and signs persisting for >24 hours,[14] and was ascertained from blinded review of medical records and autopsy results in 353 male subjects among those with stored blood samples. For 348 of the cases, a control matched by age, smoking history, and time of randomization into the study (to ensure comparable length of follow-up) was chosen among subjects without diagnosis of cerebrovascular diseases. In 10 subjects, we encountered difficulties in achieving reproducible, unambiguous genotyping results; they were excluded from the analyses.

ACE D/I Genotype Determination

Details of *ACE D/I* genotype determination have been described previously.[15] In brief, the *D* and *I* alleles were identified by polymerase chain reaction (PCR) amplification of the respective fragments from intron 16 of *ACE* and by subsequent electrophoretic size fractionation and ethidium bromide visualization. Because the *D* allele in heterozygotes is preferentially amplified, all *DD* genotype samples were subjected to a second independent PCR amplification with a primer pair that recognizes an insertion-specific sequence to ensure accurate genotyping. To confirm genotype assignment, the PCR procedure was performed on all samples on 2 separate occasions. PCR results were scored blinded as to case-control status.

Statistical Analysis

Allele and genotype frequencies among cases and controls were compared with values predicted by the Hardy-Weinberg equilibrium by the χ^2 test. ORs were calculated as a measure of association of genotype with stroke under assumptions of additive (assigning scores of 0, 1, and 2 for *II*, *DI*, and *DD*, respectively), dominant (with scores of 0 for *II* and 1 for *DI* and *DD* combined), or recessive (with scores of 0 for *II* and *DI* combined and 1 for *DD*) mode of inheritance. Because of the potential confounding effects of aspirin and β-carotene treatment, all analyses were adjusted for these variables. For each OR, we calculated 2-tailed probability value and 95% CI. We performed both matched-pair and unmatched analyses, with adjustments for possible confounding factors as appropriate (body mass index, diagnosis of hypertension, diabetes, and hypercholesterolemia) by unconditional logistic regression.[16] To directly examine a "low-risk" group, we repeated the analyses among the 207 cases and 280 controls who had no history of hypertension and/or diabetes mellitus. In addition, a subgroup analysis was carried out for ischemic stroke. A value of $P<0.05$ was considered as indicating a statistically significant effect.

Results

Characteristics of the Study Population

Baseline characteristics of stroke cases and controls are shown in Table 1. The data reflect the expected recognized risk factors, with higher prevalence of hypertension and diabetes mellitus among cases than controls.

Allele and Genotype Frequencies

Allele frequencies for *D* and *I* alleles were 0.58 and 0.42 in cases and 0.56 and 0.44 in controls, respectively (Table 2). Genotype frequencies did not deviate from the Hardy-Weinberg equilibrium in controls ($\chi^2_{2df}=0.91$, $P=0.64$), cases ($\chi^2_{2df}=0.13$, $P=0.94$), or the whole study group ($\chi^2_{2df}=0.89$, $P=0.64$).

Genotype-Stroke Correlations

No overall difference in genotype distribution was seen among cases and controls ($\chi^2_{2df}=1.01$, $P=0.60$). Logistic regression analysis, carried out under assumptions of additive (*DD* versus *DI* versus *II*), dominant (*DD* and *DI* versus *II*), or recessive (*DD* versus *DI* and *II*) mode of inheritance likewise failed to reveal a significant association between phenotype and genotype, both in the overall sample and in the low-risk subgroup from which subjects with either hypertension or diabetes had been excluded (Table 3). Restricting the analysis to cases with ischemic stroke only (n=271) and their matched controls yielded similar point estimates of relative risk associated with carrier status for the allelic variant (Table 3). Exclusion of hypertensive subjects only, but not of diabetics, resulted in a materially similar effect (data not

TABLE 2. Genotype Frequencies for the *ACE D/I* Polymorphism

	All Subjects				Low-Risk Subgroup			
	Cases		Controls		Cases		Controls	
Genotype	n	*f*	n	*f*	n	*f*	n	*f*
DD	117	0.34	110	0.32	70	0.34	98	0.35
DI	161	0.48	158	0.47	104	0.50	122	0.44
II	60	0.18	70	0.21	33	0.16	60	0.21
All	338	1.0	338	1.0	207	1.0	280	1.0

n indicates number of subjects; *f*, frequency.

shown), as did further adjustment for body mass index and for actual level of blood pressure.

Discussion

In this large, prospective investigation, we found no association between genetic variants of *ACE* and the risk of stroke. Prior studies of the *ACE D/I* polymorphism have all relied on retrospective analyses, and none have been as large as the present one. The results have been inconsistent, with some investigations reporting an association between the *D* allele and stroke[6,9,11] and others finding no such association.[4,5,7,8,12,13] There is some suggestion in the literature that the *ACE* polymorphism may be of relevance as a risk factor specifically in lacunar stroke, compared with any type of cerebrovascular disease; however, this notion is based on subgroup analysis of 18 patients,[6] whereas a larger study reported no such association.[13] Somewhat paradoxically, one of the articles reporting a weak association of the *DD* genotype with mortality from stroke (but not stroke per se) also observed significantly lower plasma ACE concentrations (which would classically be expected to be associated with the *II* genotype) among those stroke victims who died.[8] We believe that, on the basis of its size and prospective design, our study provides a more reliable assessment of the potential association between this marker and stroke. As with all "null" findings, the issue of statistical power to address the questions asked is of critical importance. On the basis of the size of our study, we can detect, with 80% probability, an OR of >1.30 for an association of the *D* allelic variant of *ACE* with stroke, assuming an additive model; for dominant or recessive models, the power is less. The results of this study do not rule out the possibility of a modest risk of stroke associated with the *ACE* genotypes, the possible masking of gene-environment interaction in a low-risk population, and the inherent weaknesses of the case-control association studies. The source of our study sample from among predominantly white male physicians imposes certain limitations regarding interpretation and extrapolation. Specifically, the incidence of cardiovascular disease in this study has been found to be considerably lower than in the North American population at large. It has been argued that this is due primarily to increased risk factor awareness and better medical care, factors expected to delay but not to remove the impact of inborn, genetically encoded predispositions. Indeed, it might therefore even be argued that in this particular sample, the influence of confounding environmental factors is minimized, raising the power to detect endogenous (eg, genetic) factors that contribute to the disease. Only in the exceptional case in which the phenotypic expression of a particular gene variant is critically dependent on the concomitant presence of a synergistically acting, nongenetic, conventional risk factor (ecogenetic interaction) would selection of low-conventional-risk subjects be potentially disadvantageous. Likewise, exclusion of subjects with well-appreciated risk factors for stroke, ie, hypertension and diabetes mellitus, should enhance the power to recognize other contributing factors; failure to observe an association in this group also argues for the robustness of our findings. Nevertheless, additional prospective studies among women and other ethnic groups will be needed. Finally, the majority of strokes in our study were ischemic; thus, extrapolations from this study to other types of stroke are probably not warranted.

TABLE 3. Odds Ratios of the *ACE D/I* Polymorphism for Stroke

	All Subjects			Low-Risk Subgroup			Ischemic Stroke		
Model Analysis	OR	95% CI	*P*	OR	95% CI	*P*	OR	95% CI	*P*
Matched data									
Additive	1.11	0.90–1.37	0.35	1.16	0.85–1.58	0.35	1.14	0.90–1.44	0.29
Dominant	1.22	0.83–1.79	0.32	1.58	0.88–2.82	0.12	1.37	0.89–2.11	0.16
Recessive	1.10	0.80–1.51	0.56	1.04	0.66–1.64	0.87	1.08	0.76–1.54	0.67
Unmatched data									
Additive	1.10	0.89–1.35	0.39	1.09	0.85–1.41	0.50	1.13	0.90–1.43	0.43
Dominant	1.21	0.82–1.77	0.33	1.45	0.91–2.33	0.12	1.31	0.87–1.98	0.20
Recessive	1.08	0.79–1.48	0.63	0.95	0.65–1.39	0.79	1.10	0.78–1.54	0.58

Additive, *DD* vs *DI* vs *II*; dominant, *DD* and *DI* vs *II*; recessive, *DD* vs *DI* and *II*.

In conclusion, this large, prospective, nested case-control study among middle-aged US men provides no evidence for an association between the *ACE D/I* polymorphism and risk of stroke. Our findings suggest that an important contribution of this gene to ischemic cerebrovascular accidents is unlikely and that the *ACE D/I* genotype will not be a useful tool for risk assessment or prognostication.

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