Lack of an association between the angiotensin-converting enzyme insertion/deletion polymorphism and intracranial aneurysms in a Caucasian population in the United States

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Object. The identification of polymorphisms associated with an increase in the risk of developing disease is integral to the development of genetic biomarkers to identify individuals at risk. Based on reports indicating a role for angiotensin-converting enzyme (ACE) in the pathogenesis of intracranial aneurysms (IAs) as well as hypertension, an independent risk factor for IAs, the authors investigated the association between an insertion/deletion (I/D) polymorphism in the ACE gene and IAs in a Caucasian population in the US.

Methods. The patient population consisted of 162 randomly selected Caucasian patients who underwent surgical repair of an IA at Memorial–Hermann Hospital (Houston, TX) and had no family history of the disease. The ACE I/D polymorphism was typed using polymerase chain reaction amplification of genomic DNA, and allele and genotype frequencies were compared between the patients with IAs and 143 healthy Caucasian volunteers (control group) by performing logistic regression and chi-square tests.

The ACE I/D allele frequencies did not differ significantly between the patient and control populations. There were similar allele and genotype frequencies in male and female study participants in both patient and control populations. The authors found no evidence of an association between the allelic or genotypic distribution of the ACE I/D polymorphism and aneurysmal subarachnoid hemorrhage or unruptured IAs.

Conclusions. Contrary to findings in two European Caucasian populations (one British and one Polish), this polymorphism did not contribute to the risk of developing IAs in a Caucasian population in the US.

KEY WORDS • intracranial aneurysm • genetic • subarachnoid hemorrhage

The etiology and molecular pathogenesis of IAs are poorly understood. Rupture of these aneurysms results in high incidences of morbidity and mortality due to SAH.1,2 The surgical treatment of ruptured aneurysms is associated with poor outcomes compared with the outcomes of unruptured aneurysm repair.7,8 In addition, some incidences of morbidity and mortality are associated with the surgical treatment of unruptured aneurysms, making it critical to weigh the risk of rupture against the risks of surgery prior to treatment. An accurate understanding of the biological basis of the disease is beneficial to the development of technologies that aid in improving diagnosis and risk assessment as well as in improving prognosis by identifying aneurysms prone to rupture and by defining pathways for therapeutic drug-based intervention strategies.

Approximately 5% of IAs are associated with heritable connective tissue disorders such as Ehlers–Danlos syndrome Type IV, Marfan syndrome, autosomal dominant polycystic kidney disease, and neurofibromatosis Type 1.22 Between 5 and 20% of cases have been reported to occur in conjunction with a family history of aneurysms in the absence of inherited syndromes;11,13,17,23 however, the majority of IAs are sporadic, with no evidence of a family history of the disease. For these aneurysms, the risk of rupture is currently estimated based on the lesion’s size and location.21,26 The risk of rupture increases as an aneurysm enlarges, and is generally higher in lesions located in the posterior circulation than in those in the anterior circulation.21,22 There are no established molecular determinants that help risk assessment.

The etiology of sporadic IAs is clearly multifactorial, with genetic and environmental factors working in concert in the development and enlargement of IAs. Hypertension, which is also multifactorial in origin, has been reported to be a risk factor for IAs.4 Systemic blood pressure is regulated by a variety of controls, with the RAS playing a critical role.14 Angiotensin II is a major effector of RAS. Angiotensin II is generated from angiotensin I by ACE. At the genomic level, the presence (insertion [I] allele) or absence
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(duplication [D] allele) of a 287-bp Alu repeat polymorphism in intron 16 of the ACE gene has been reported to be correlated with levels of circulating ACE, with the C allele associated with higher levels of the enzyme, and with hypertension in some studies. In addition, ACE degrades the vasoactive hypotension-inducing peptide bradykinin, further contributing to an increase in blood pressure. Recent studies also support a role for ACE in intracellular signaling in endothelial cells through a C-Jun–mediated pathway, although the physiological impact of these findings remains to be elucidated. In summary, several studies support a role for ACE in blood pressure regulation and vascular function.

The I allele and the II genotype of ACE have been reported to be significantly associated with IAs in two European Caucasian populations, one British and one Polish. Additionally, reduced levels of components of the RAS (including ACE) have been observed in IA tissue by performing immunohistochemical studies and messenger RNA expression analyses, indicating that these reduced levels might be a constituent of the disease process in patients with IAs. Contrary to the implications of these studies, which imply a role for reduced ACE levels however, hypertension, postulated to be one of the consequences of higher ACE levels, has been found to be present more frequently in patients with IAs.

To assess the applicability of data obtained from the two reported European studies of Caucasian patients with IAs to other Caucasian populations, we tested the association between the ACE ID polymorphism and IAs in a Caucasian population in the US.

### Clinical Material and Methods

#### Sample Collection

After informed consent had been provided, peripheral blood, clinical information, and family histories were obtained from patients undergoing surgical repair of IAs at Memorial–Hermann Hospital (Houston, TX). Genomic DNA was extracted from blood samples according to the manufacturer’s protocol by using the PUREGENE genomic DNA isolation kit (Gentra Systems, Inc., Minneapolis, MN). The institutional review committee at the University of Texas Health Science Center–Houston approved this study.

#### Genotyping Studies

The ACE ID genotypes were determined based on amplification of DNA by using three primers (one located within the insertion for accurate typing of the I allele) followed by agarose gel electrophoresis. The primers and conditions used in this study have been described previously. The DD genotype generated a single 210-bp band, the II genotype generated a 498-bp, a 264-bp, and a 210-bp band. Amplified bands were electrophoresed on a 2% agarose gel in standard 0.5 T-Tris-acetate–ethylenediamine tetracetic acid buffer and were visualized by applying ethidium bromide stain.

#### Patient Population

The study population included patients who underwent surgical repair of an IA at Memorial–Hermann Hospital (Houston, TX) between August 2000 and September 2003. All consenting patients with IAs who underwent surgery at the hospital were evaluated for inclusion in the study and were interviewed by a research nurse. Patients with genetic defects that were associated with an increased incidence of IAs, including polycystic kidney disease, Turner syndrome, Noonan syndrome, connective tissue disorders (Marfan syndrome and Ehlers–Danlos syndrome Type IV), neurofibromatosis Type 1, or a family history of IAs, were excluded from this study. The patient population was limited to 162 Caucasian individuals residing in the US (Table 1). We did not have sufficient numbers of patients of other ethnic origins to perform association analyses. The mean age of the patients included in the study was 55.3 ± 12.98 years (standard deviation). The majority (72%) of patients had a ruptured aneurysm at presentation. Twenty-two percent of the patients had multiple aneurysms and 41% had a history of hypertension. Hypertension was noted when present as a prior medical diagnosis reported by the patient or a family member. Fifty-eight percent of hypertensive patients were on a regimen of prescription medication for hypertension when they presented for surgery. In the subgroup of patients with ruptured aneurysms, 21% had multiple aneurysms and 39% suffered from hypertension; in the subgroup of patients with intact aneurysms, 24% had multiple aneurysms and 48% suffered from hypertension. Thus, hypertension was not found to be more prevalent in patients with ruptured IAs in our study population. The control population consisted of 143 Caucasian volunteers residing in the US.

#### Statistical Analyses

The association between the ACE ID polymorphism and IAs was estimated by calculating the OR and the corresponding 95% CI and probability value. This was done by performing logistic regression with the aid of SAS software (version 8.2; SAS Institute, Cary, NC). Unconditional logistic regression analyses were performed using sex as a covariate to remove the possible confounding effect of that factor. In addition, allele and genotype frequencies in patients and healthy volunteers were compared using a standard contingency table chi-square test for independence. Statistical power at the significance level of 0.05 was determined by applying the methods of Casagrande, et al., and Dupont and Plummer. 

### Results

#### Case–Control Genetic Association Analysis

The frequencies of the ACE genotype were determined to be in Hardy–Weinberg equilibrium proportions in the control population (p = 0.13) by using the exact test as described by Guo and Thompson. Genotype and allele frequencies were not significantly different between patients and healthy volunteers (controls) (Table 2). The chi-square statistic for independence of genotypes between the two
groups was 0.34 (p = 0.84) and the same statistic for independence of allele frequencies was 0.29 (p = 0.63). Using the II genotype as a reference group, the OR for the heterozygote ID patient subgroup was 0.95 (95% CI 0.52–1.73, p = 0.56) and the OR for the homozygote DD subgroup was 0.84 (95% CI 0.43–1.62, p = 0.86). Thus, we did not find a significant increased risk of IAs associated with any allele or genotype.

To eliminate the possibility of overlooking an association between the ACE I/D polymorphism and aneurysmal SAH (the disease population in the two previous European Caucasian studies), we performed similar analyses in which we compared the subgroup of IA patients with ruptured aneurysms with the control group. The results of these analyses are presented in Table 3. The chi-square statistic for independence of genotypes was 0.20 (p = 0.91) and the same statistic for independence of allele frequencies was 0.15 (p = 0.72). Using the II genotype as a reference group, the OR for the heterozygote ID subgroup was 0.98 (95% CI 0.51–1.88, p = 0.85) and the OR for the homozygote DD subgroup was 0.87 (95% CI 0.43–1.80, p = 0.66). Thus, we did not find a significantly increased risk of aneurysmal SAH associated with any ACE I/D allele or genotype.

Based on our sample size (162 patients and 143 healthy volunteers) we have greater than 80% power at a significance level of 5% to detect an OR of 1.92. Similarly, the power was greater than 99% to detect an OR of 3 or more. The ORs for ACE associations from the two previous studies were 1.67 (British population) and 4.57 (Polish population). Given our sample the test power was greater than 60% to detect an OR of 1.67 and greater than 99% to detect an OR of 4.57. Additionally, in our association analysis we observed robustly nonsignificant results (p > 0.66) with no detectable trend toward significance, from which we infer a true lack of association between ACE genotypes and IA incidence in our population.

In keeping with the hypothesis that the D allele contributes to hypertension, we compared genotype and allele frequencies in patients with and without hypertension and performed logistic regression among patients with hypertension as an outcome variable. These results are presented in Table 4. It should be noted that there were very few patients in the reference group (II genotype). We found no statistically significant difference between groups with respect to allele frequencies ($\chi^2 = 0.69, p = 0.41$); however, the II genotype was underrepresented in hypertensive patients compared with normotensive patients. When we compared the II genotype with both genotypes containing a D allele (ID and DD) in the two patient subgroups, the difference in distributions approached significance (OR = 2.35, 95% CI 0.98–5.64, p = 0.06). On the other hand, our comparison of the II genotype with genotypes containing a D allele in patients with IAs and healthy volunteers did not yield a significant difference (p = 0.57). Thus, the D allele appears to contribute to the development of hypertension but did not contribute significantly to the development of IAs in our study.

### Discussion

Although the cause of IAs has long been known to be multifactorial, little is known about the molecular determinants that potentially could be used to assess and screen individuals for the disease. Findings of several studies support a role for the ACE DD genotype in cardiovascular and cerebrovascular disease, which perhaps is mediated by a

<table>
<thead>
<tr>
<th>TABLE 2</th>
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<tr>
<td>The ACE I/D polymorphism compared in 162 patients with IAs and 143 healthy volunteers*</td>
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<table>
<thead>
<tr>
<th>Factor</th>
<th>No. (%)</th>
<th>Chi-Square Test for Independence</th>
<th>Logistic Regression</th>
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<td></td>
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<tr>
<td></td>
<td>Patient Group</td>
<td>Control Group</td>
<td>$\chi^2$ Value</td>
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<tr>
<td>genotype</td>
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<td></td>
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<tr>
<td>II</td>
<td>33 (20.4)</td>
<td>27 (18.9)</td>
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</tr>
<tr>
<td>ID</td>
<td>85 (52.5)</td>
<td>73 (51.0)</td>
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<tr>
<td>DD</td>
<td>44 (27.2)</td>
<td>43 (30.1)</td>
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<tr>
<td>allele</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>I</td>
<td>151 (46.6)</td>
<td>127 (44.4)</td>
<td>0.29</td>
</tr>
<tr>
<td>D</td>
<td>173 (53.4)</td>
<td>159 (55.6)</td>
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* df = degree of freedom.

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<th>TABLE 3</th>
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<td>The ACE I/D polymorphism compared in 116 patients with ruptured IAs and 143 healthy volunteers</td>
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<table>
<thead>
<tr>
<th>Factor</th>
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<th>Chi-Square Test for Independence</th>
<th>Logistic Regression</th>
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<tr>
<td></td>
<td>Patient Group</td>
<td>Control Group</td>
<td>$\chi^2$ Value</td>
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<tr>
<td>genotype</td>
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<tr>
<td>II</td>
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<tr>
<td>ID</td>
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<tr>
<td>DD</td>
<td>32 (27.6)</td>
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<tr>
<td>I</td>
<td>107 (46.1)</td>
<td>127 (44.4)</td>
<td>0.15</td>
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<tr>
<td>D</td>
<td>125 (53.9)</td>
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The ACE insertion/deletion polymorphism in IA

### Table 4

<table>
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<tr>
<th>Factor</th>
<th>Hypertension Group</th>
<th>Normotension Group</th>
<th>χ² Value</th>
<th>df</th>
<th>p Value</th>
<th>OR</th>
<th>95% CI</th>
<th>p Value</th>
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<tbody>
<tr>
<td>genotype</td>
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<tr>
<td>II</td>
<td>8 (12.1)</td>
<td>23 (24.5)</td>
<td>3.78</td>
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<tr>
<td>ID &amp; DD</td>
<td>58 (87.9)</td>
<td>71 (75.5)</td>
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<td></td>
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<tr>
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<td></td>
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<td></td>
</tr>
<tr>
<td>I</td>
<td>57 (43.2)</td>
<td>90 (47.9)</td>
<td>0.69</td>
<td>1</td>
<td>0.41</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>75 (56.8)</td>
<td>98 (52.1)</td>
<td></td>
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Contribution to hypertension, itself a genetically heterogeneous, complex disease with well-documented variability in the contribution of the ACE I/D genotype to its etiology. Hypertension has been observed to be more prevalent in individuals with IAs; on average 45% of patients harboring these lesions exhibit hypertension, almost twice the general population incidence of 24%. Primate and rat models of cerebral aneurysm induction by ligation of extracerebral arteries and subsequent induction of hypertension have been described, further emphasizing the role of hypertension in cerebral aneurysms. Given that aneurysms arise from a combination of factors affecting hemodynamic flow and vascular wall strength and integrity, it has been hypothesized that hypertension contributes to aneurysm formation by inducing endothelial and/or intimal layer damage and consequent degenerative changes in the vessel wall.

In contrast to this hypothesis, the ACE II genotype was reported to be significantly associated with aneurysmal SAH in two European Caucasian populations. Additionally, investigators in a Japanese study reported that the frequency of the ACE DD genotype is significantly lower in patients with IAs, although no significant differences were noted for the II genotype. Nevertheless, we did not observe an association between either ruptured (equivalent to aneurysmal SAH) or intact IAs and any ACE I/D polymorphism genotype in the US Caucasian population we studied, despite a sample size comparable to those in previous studies.

The mechanism by which the ACE II genotype may contribute to IAs is unclear. The II genotype reportedly is associated with lower ACE levels. One explanation, supported by a single molecular study, is that reduced levels of ACE along with other components of the local RAS system might interfere with the appropriate remodeling of the injured vessel wall when needed. The authors of this study hypothesized that, normally, endothelial damage caused by increased hemodynamic stress in blood vessels (such as that caused by hypertension) induces proliferation of vascular smooth-muscle cells in response to upregulated local RAS components. In individuals in whom aneurysms develop this response is blunted due to lower expression levels of RAS components, however, and the resultant inadequate proliferation response by the smooth-muscle cells contributes to thinning of the medial layer, which leads to cerebral vessel dilation.

In light of these functional, albeit mechanistically incompletely defined considerations, it is important to assess the ACE I/D polymorphism in patients harboring IAs to determine the strength and prevalence of the association so that we can accurately incorporate this knowledge into improving patient care. Contrary to previous studies in two European Caucasian populations (one British and one Polish), we found no association between the ACE I/D polymorphism and IAs in a group of Caucasian patients in the US, although the allele and genotype frequencies we observed in our control population closely approximated those observed in previously reported Caucasian control populations (p ≥ 0.51). Our findings emphasize the etiological heterogeneity of IAs, even within ethnic groups. Because ACE I/D allele and genotype frequencies in our control population were similar to those observed in the European Caucasian control groups, epistatic gene–gene or gene–environment interactions could be the basis of the risk associated with the ACE I/D polymorphism. Alternately, the association found in European Caucasians could be the result of a variation that is in linkage disequilibrium with the I/D polymorphism in the European disease populations but not in the corresponding US population. Similar studies in additional Caucasian and other ethnic populations are needed to reach a definitive conclusion regarding the utility of this polymorphism in the assessment of risk in patients with IAs.

Conclusions

In summary the frequencies of ACE I/D alleles or genotypes did not differ significantly between the patient and control populations. Contrary to findings in two European Caucasian populations, the ACE I/D polymorphism did not contribute to the risk of developing IAs in a US Caucasian population.

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Drs. Pannu and Kim contributed equally to this study. The authors would like to thank the patients for their participation in these studies.

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