The ACE I allele is associated with increased risk for ruptured intracranial aneurysms

Mohammad Keramatipour, Robert S McConnell, Peter Kirkpatrick, Susan Tebbs, Robert A Furlong, David C Rubinsztein

Abstract
Genetic and environmental factors play roles in the aetiology of ruptured intracranial aneurysms. Hypertension has been reported as a risk factor for intracranial aneurysm haemorrhage. We have tested if genotypes at the angiotensin converting enzyme (ACE) gene locus are associated with ruptured intracranial aneurysms. The insertion/deletion polymorphism in the ACE gene was genotyped in 258 subjects presenting in East Anglia with ruptured intracranial aneurysms (confirmed at surgery or angiographically) and 299 controls from the same region. ACE allele frequencies were significantly different in the cases and the controls (alleles $\chi^2=6.67$, p=0.03). The I allele was associated with aneurysm risk (odds ratio for I allele versus D allele = 1.3 (95% CI=1.02–1.65); odds ratio for II versus DD genotype = 1.67 (95% CI=1.04–2.66)). The I allele at the ACE locus is over-represented in subjects with ruptured intracranial aneurysms. These data are supported by non-significant trends in the same direction in two previous smaller studies. Thus, this allele may be associated with risk for ruptured intracranial aneurysms. 

Keywords: ACE I allele; ruptured intracranial aneurysms

Cerebral aneurysm rupture is a major public health problem representing an important cause of mortality and long term morbidity. Rupture of a cerebral aneurysm causes subarachnoid haemorrhage with sudden death in up to 12% of patients, with a further 26% of patients dying within one month after admission to hospital. Intracranial aneurysm rupture is responsible for 22-25% of cerebrovascular deaths. Long term morbidity follows intracranial aneurysm rupture in 16% of cases. About 50% of patients admitted to hospital have a good outcome following treatment.

Several lines of evidence suggest that genetic factors may play a role in cerebral aneurysm formation and rupture. First, an increased risk of cerebral aneurysm formation is seen in some hereditary conditions, such as autosomal dominant polycystic kidney disease and Ehlers-Danlos syndrome. Second, familial clustering of cerebral aneurysm is well documented and first degree relatives of patients with cerebral aneurysms have been shown to be at four times greater risk of having or rupturing a cerebral aneurysm than the general population. Hypertension has been implicated as a risk factor for cerebral aneurysm by two large population based studies and in a family study. Blood pressure is partly regulated by angiotensin II, which is a potent arterial vasoconstrictor. Angiotensin II also increases aldosterone synthesis from the adrenal cortex and stimulates catecholamine synthesis and release from peripheral noradrenergic neurons. Angiotensin II is formed after cleavage of two carboxy-terminal amino acids from angiotensin I by angiotensin converting enzyme (ACE).

The D allele of the insertion/deletion (I/D) polymorphism in intron 16 of the ACE gene has been found to be associated with higher circulating ACE levels in humans. Although the D allele has been associated with a number of cardiovascular diseases, like myocardial infarction and left ventricular hypertrophy, these findings have not been consistently replicated. Furthermore, this locus is not clearly associated with essential hypertension in humans. Recently, the I allele has been associated with risk for Alzheimer’s disease, consistent with previous studies showing a depletion of the I allele in very elderly populations.

Two previous studies have considered this ACE polymorphism in the context of cerebrovascular haemorrhage. Catto et al studied 48 cases with intracerebral haemorrhage and 215 controls and Takenaka et al studied 83 cases with intracranial saccular aneurysms and 104 controls. Both studies showed a non-significant tendency for the I allele to be over-represented in cases. While Takenaka et al claimed that the DD genotype was significantly rarer in cases than controls (p=0.044), this p value did not take into account the multiple testing that should be considered when arbitrarily selecting the frequency of one out of three possible genotypes; one could have examined either the DD, DI, or II frequencies individually in cases and controls. Both studies showed a non-significant tendency for the I allele to be over-represented in cases. The overall genotype and allele frequencies in this study did not differ significantly in cases and controls (table 1). The difficulty in detecting effects in these studies may have been the result of type II errors, since the detection of small effects such as those expected in complex genetic diseases requires large samples.

Accordingly, we have tested this polymorphism in our cohort of 258 cases with ruptured intracranial aneurysms and 299 regionally matched controls.
**Table 1** Allele and genotype frequencies at the ACE locus in cases with aneurysmal intracranial haemorrhage and controls. Data are shown from Cambridge and Japan.\textsuperscript{21} In the Japanese study the excess of the I allele did not reach statistical significance ($\chi^2=1.96$, p=0.16) and the overall genotype distributions did not show significant differences between the cases and controls ($\chi^2=4.06$, p=0.13)

<table>
<thead>
<tr>
<th>Allele I</th>
<th>Allele II</th>
<th>Total</th>
<th>Genotype II</th>
<th>Total</th>
<th>Genotype II</th>
<th>Total</th>
<th>Genotype II</th>
<th>Total</th>
<th>Genotype II</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cambridge data</td>
<td>299 (100)</td>
<td>298 (100)</td>
<td>288 (48.2)</td>
<td>282 (54.7)</td>
<td>598 (100)</td>
<td>516 (100)</td>
<td>82 (27.4)</td>
<td>54 (20.9)</td>
<td>146 (48.8)</td>
<td>126 (48.8)</td>
</tr>
<tr>
<td>Japanese data\textsuperscript{21}</td>
<td>104 (100)</td>
<td>83 (100)</td>
<td>131 (63.0)</td>
<td>116 (69.9)</td>
<td>208 (100)</td>
<td>166 (100)</td>
<td>16 (15.4)</td>
<td>5 (6.0)</td>
<td>45 (43.3)</td>
<td>40 (48.2)</td>
</tr>
</tbody>
</table>

**Methods**

**PATIENT ASCERTAINMENT**

This study was performed with the approval of the local ethics committee and informed consent was obtained from participating patients. The cases were 258 subjects (80 males, 178 females; mean age 52.8 years (SD 12.9 years)) who presented to Addenbrooke’s Hospital with symptoms of intracranial haemorrhage and who had intracranial aneurysms confirmed angiographically or at surgery or both. The control samples comprised 297 subjects (126 males, 171 females; mean age 44.5 years (SD 13 years)) from an East Anglian population. These samples were anonymised and were obtained from the DNA Bank of the Molecular Genetics Laboratory, Addenbrooke’s Hospital. Referral patterns to this laboratory suggest that at least 97% of controls were white, about 80% had parents who were East Anglian, and virtually all of the remainder were from the UK. The surnames of the controls and the cases were screened to exclude those obviously of non-white origin. It is important to note that this region of the UK includes a large rural catchment area and is not characterised by the ethnic variation seen in many large cities.

ACE genotypes were determined using the “triple primer” method described by Evans et al.\textsuperscript{25} Meta-analysis and calculation of odds ratios with 95% confidence intervals was determined using unconditional logistic regression, with the general loglinear analysis option of SPSS version 6.1. This form of analysis can accommodate differences in control allele frequencies in different populations.

**Table 2** Odds ratios from the Cambridge study and from a meta-analysis of the Cambridge and Japanese\textsuperscript{21} data

<table>
<thead>
<tr>
<th>Odds ratio</th>
<th>Cambridge data</th>
<th>95% CI</th>
<th>Odds ratio</th>
<th>Cambridge data</th>
<th>Japanese data</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allele I vs D</td>
<td>1.30</td>
<td>1.02–1.65</td>
<td>1.31</td>
<td>1.06–1.62</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genotype II vs DD</td>
<td>1.67</td>
<td>1.04–2.66</td>
<td>2.46</td>
<td>0.84–1.88</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genotype DI vs DD</td>
<td>1.31</td>
<td>0.86–1.99</td>
<td>1.05</td>
<td>0.73–1.51</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Results**

The DD, DI, and II genotype frequencies in the Cambridge controls were 27.4%, 48.8%, and 23.7%, respectively, and were very similar to those expected under Hardy-Weinberg expectations (26.7%, 49.8%, and 23.1%, respectively; $\chi^2=0.082$, p=0.96). Allele frequencies differed significantly in the Cambridge patients with aneurysmal intracranial aneurysm versus controls (table 1) ($\chi^2=4.67$, p=0.03). The odds ratio for aneurysmal intracranial haemorrhage for the I allele versus the D allele was 1.30 (95% CI=1.02–1.65). The overall genotypes of the aneurysm cases were not significantly different from the control group ($\chi^2=4.6$, p=0.1). However, there was a tendency for the DD genotype to be under-represented and the DD genotype to be under-represented in cases versus controls, OR for DD $\div$ DD = 1.67 (95% CI=1.04–2.66) (table 2). The only previous analysis of this gene in patients with aneurysmal intracranial haemorrhage was the study of Takenaka et al.\textsuperscript{20} (table 1). A meta-analysis of their data in a Japanese sample together with our Cambridge sample showed a significant effect for alleles ($\chi^2=6.6$, p<0.02). The odds ratio for aneurysmal intracranial haemorrhage in this meta-analysis for the I allele versus the D allele was 1.31 (95% CI=1.06–1.62) (table 2). The meta-analysis of the genotype data did not yield a significant difference, but again there was a tendency for the DD genotype to be over-represented in aneurysm cases versus controls. The data from Catto et al\textsuperscript{20} were not included in the meta-analysis, as they analysed cases with intracranial haemorrhage, without specifying the diagnosis (aneurysms account for <1/3 of cases with intracranial haemorrhage).

**Discussion**

Multifactorial diseases are likely to involve a number of different contributing genes. Thus, the effect of any particular gene will be expected to be modest, contributing odds ratios of 2 or less.\textsuperscript{22} Our data suggest that the I allele at the ACE locus may be associated with the presentation of ruptured intracranial aneurysms. The findings in the Cambridge population show the same trends as observed in Japanese\textsuperscript{21} and in the study of Catto et al\textsuperscript{20}, who examined cases with intracranial haemorrhage, without specifying the diagnosis (aneurysms account for <1/3 of cases with intracranial haemorrhage).

It is interesting that the I allele is associated with increased risk for intracranial aneurysm rupture. Some studies have observed an association between the D allele and increased cardiovascular disease risk, but these findings are controversial. Conversely, the D allele has been associated with increased longevity in two


13 Cambien F, Costerousse O, Tirtl L, Pouzier O, Lecerf L, Froguel P, Lesueur-Ginot L, Cohen D. Genetic association of the I allele and ruptured aneurysmal haemorrhage via these peptides, and regulates vascular growth. Furthermore, as ACE degrades bradykinin and related kinins, it may modulate the risk of intracranial aneurysmal haemorrhage via these peptides, since kinins relax vascular smooth muscle. At present, it is difficult to predict if the putative associations of the ACE I allele with intracranial aneurysm and Alzheimer’s disease risk are mediated via the same physiological pathways. In summary, while ruptures of intracranial aneurysms are comparatively common and are associated with a modest familial risk, there are very few studies aiming to identify specific genetic risk factors. Our data implicate the ACE gene in the pathogenesis of this condition. Further large studies will be required to confirm this association.