Meta-Analysis of the ACE Gene Polymorphism in Cerebral Infarction

Hong-miao Tao, Bei Shao, Guo-zhong Chen

ABSTRACT: Background: The angiotensin-1 converting enzyme (ACE) gene is known to have two polymorphic alleles insertion/deletion (I/D). People with the DD genotype have been shown to be at greater risk of cerebral infarction, but only in some studies. Identification of cerebral infarction susceptibility genes and quantification of associated risks have been hampered by conflicting results from underpowered case-control studies. This meta-analysis was made to look specifically into the genetics of cerebral infarction among Han Chinese population. Methods: Genetic associations studies published from January 1, 1990 to December 30, 2007 were collected from databases of MEDLINE, EMBASE, CBM and CNKI. Data were extracted using standardised forms and pooled odds ratios (ORs) with 95% confidence intervals (CIs) were calculated. Results: Twenty-nine original case-control studies of Han Chinese population, comprising 3654 patients with cerebral infarction and 3058 controls were included in the meta-analysis. Using the random effects model, the pooled ORs of ACE DD genotype VS ID+II was 1.91 (95% CI 1.56 to 2.34, P<0.00001). Conclusions: These data suggest that the ACE DD genotype may be a risk factor for cerebral infarction in Han Chinese population. A large scale case-control study is needed to clarify the functional effect of the polymorphism of the ACE I/D gene in the pathogenesis of cerebral infarction in Han Chinese population.

Original Article

Ischemic stroke is a leading cause of death and disability worldwide.1 Despite recent advances in acute stroke therapy, effective prevention is an important strategy to reduce the overall burden of stroke worldwide. Established causal risk factors such as hypertension and smoking are estimated to account for 50% of vascular disease risk. Therefore the identification of novel markers of stroke risk is of key importance, both for risk prediction and potential modification to reduce future events. The angiotensin-converting enzyme (ACE), a key enzyme in the renin-angiotensin system, plays an important role in vascular wall homeostasis.2-4 Regulation of circulation and probably tissue ACE activity are under strong genetic control. The ACE gene located on chromosome 17q23 has an insertion/deletion (I/D) polymorphism in the noncoding region of the gene. The insertion that gives rise to the I allele is an Alu repeat 4 in intron 16 of the ACE gene; the D allele results from the absence of the above insertion. Alu repeats are transponson related repeats about 250-300 base pairs long and unique to the genome of primates and have been implicated in various diseases. It has been shown that higher serum ACE activity is present in subjects...
with the D compared with the I allele. Numerous studies have reported a positive or null relation between the D allele and cerebrovascular diseases, but findings have been controversial. The sample sizes of these studies in Han Chinese population have been relatively small. Identification of stroke susceptibility genes and quantification of associated risks have been hampered by conflicting results from underpowered case-control studies. One independent meta-analysis suggested 58% increase in risk of cerebral infarction in Han Chinese population. That meta-analysis included only 2066 individuals. With the publication of several more recent studies, the present meta-analysis is needed. To clarify the varying results of ACE I/D, we have undertaken a meta-analysis of ACE polymorphism and cerebral infarction among Han Chinese population.

**METHODS**

**Data sources**

Electronic databases including MEDLINE, EMBASE, CBMDisc (Chinese Biomedical Literature analysis and retrieval system for compact disc) and CNKI (China National Knowledge Infrastructure) were searched from January 1, 1990 to December 30, 2007 for all case-control studies evaluating ACE gene polymorphism and cerebral infarction in Han Chinese population. The Medical Subject Headings terms and text words used for the search were: cerebrovascular disease, stroke, brain infarction, cerebral ischemia, angiotensin converting enzyme genes, polymorphism(s), mutation. All languages were searched. The references of all computer identified publications were searched for any additional studies, and the MEDLINE option related articles was used for all the relevant articles. In addition, a search to identify previous meta-analyses in stroke was also performed.

**Study selection**

The internationally recognized diagnostic criterion of cerebral infarction was applied. Neuroimaging (magnetic resonance imaging or computed tomography) had been used to confirm the diagnosis of cerebral infarction.

To be included in the meta-analysis, studies had to meet the following criteria: (1) the design had to be a case-control study; (2) the outcome had to be cerebral infarction; (3) there had to be at least two comparison groups (cerebral infarction v control groups). Participants could be of any age; and (4) the genotype frequencies in the control group were consistent with Hardy-Weinberg equilibrium (HWE). Studies were excluded if one of the following existed: (1) hemorrhagic stroke; (2) the genotype frequency was not reported; (3) there was insufficient information for extraction of data; (4) the genotype frequencies in the control group were inconsistent with HWE; or (5) for duplicate publications, the smaller data set was discarded.

**Data extraction**

From each study, the following information was abstracted: first author, journal, year of publication, ethnicity of the study population, demographics, cerebral infarction definition; clinical characteristics, matching, validity of the genotyping method, and the number of cases and controls for ACE I/D genotype. Data were extracted independently and in duplicate by two investigators. The results were compared and the disagreements were resolved by consensus.

**Statistical analysis**

We examined the contrast of DD versus (DI + II). Data were analyzed using Review Manager, version 4.2. Heterogeneity among studies was examined with the Q, and I² statistics. A P value of <0.1 was considered significant for the Q statistic; and I² was interpreted as the proportion of total variation contributed by between-study variation. Based on the test of heterogeneity, a pooled odds ratio (OR) was calculated using fixed (Mantel-Haenszel) and random-effects models (DerSimonian and Laird), along with the 95% confidence interval (CI) to measure the strength of the genetic association. Visual funnel plot inspection and Egger regression tests were performed with SPSS version 12.0 to examine for publication bias. For the Egger test the significance level was set at 0.1.

**RESULTS**

**Characteristics of included studies**

Twenty-nine original case-control studies of Han Chinese population, comprising 3654 patients with cerebral infarction and 3058 controls were included in the meta-analysis, and are profiled in the Table. Specific matching for age and sex was described in seven studies. None of the studies included in the meta-analysis stated that genotyping was performed blinded to clinical status. Polymerase chain reaction (PCR) based genotyping method was mentioned in each study. The distribution of the genotypes in the control group was consistent with HWE (P>0.05).

**Association between ACE I/D and cerebral infarction**

Thirteen studies found a positive association between ACE I/D and cerebral infarction. Sixteen other studies reported a null association. There was significant heterogeneity among individual estimates of the ORs (Q test P<0.0001; I²=57.3%) and the original data were combined by means of random effect model. As shown in Figure 1, there was a statistically significant increase (91%) of risk to cerebral infarction in DD genotype, compared with ID + II (OR=1.91, 95% CI 1.56 to 2.34, P<0.00001).

**Sensitivity analysis**

As shown in Figure 2, the sensitivity analysis performed after excluding ten studies with relatively small sample size gave a pooled OR of 1.70 (95% CI 1.34 to 2.17, P<0.0001). These results were similar to the results of the meta-analysis before the sensitivity analysis (OR=1.91, 95% CI 1.56 to 2.34, P<0.0001). The sensitivity analysis did not alter the pattern of results. So it suggested that the combined results of meta-analysis were reliable.

**Publication bias**

Figure 3 showed that the distribution of the ORs from individual studies in relation to their respective standard deviation was symmetric in funnel plot. This was supported by...
Table: Characteristics and genotype information of published studies about the association between ACE I/D gene polymorphism and cerebral infarction

<table>
<thead>
<tr>
<th>No</th>
<th>References</th>
<th>Geographical Location</th>
<th>Study Num</th>
<th>Phenotype Type</th>
<th>OR (odds ratio)</th>
<th>Weight</th>
<th>OR (random)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bi X^27</td>
<td>Heilongjiang</td>
<td>DIS 65</td>
<td>26 29 10</td>
<td>0.629</td>
<td>0.37</td>
<td>YES</td>
</tr>
<tr>
<td>2</td>
<td>Song X^28</td>
<td>Liaoning</td>
<td>DIS 117</td>
<td>46 34 37</td>
<td>0.549</td>
<td>0.46</td>
<td>YES</td>
</tr>
<tr>
<td>3</td>
<td>Xu Y^29</td>
<td>Zhejiang</td>
<td>DIS 65</td>
<td>15 23 27</td>
<td>0.419</td>
<td>YES</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>He Y^30</td>
<td>Shanghai</td>
<td>DIS 134</td>
<td>43 69 22</td>
<td>0.584</td>
<td>0.42</td>
<td>YES</td>
</tr>
<tr>
<td>5</td>
<td>Chen X^31</td>
<td>Shandong</td>
<td>DIS 55</td>
<td>25 17 13</td>
<td>0.603</td>
<td>0.40</td>
<td>YES</td>
</tr>
<tr>
<td>6</td>
<td>Qiu X^32</td>
<td>Zhejiang</td>
<td>DIS 116</td>
<td>13 56 47</td>
<td>0.356</td>
<td>0.65</td>
<td>YES</td>
</tr>
<tr>
<td>7</td>
<td>Shi G^33</td>
<td>Heilongjiang</td>
<td>DIS 60</td>
<td>12 25 41</td>
<td>0.30</td>
<td>0.70</td>
<td>YES</td>
</tr>
<tr>
<td>8</td>
<td>Zhang C^34</td>
<td>Shandong</td>
<td>DIS 58</td>
<td>12 24 22</td>
<td>0.419</td>
<td>0.58</td>
<td>YES</td>
</tr>
<tr>
<td>9</td>
<td>Cui T^35</td>
<td>Hubei</td>
<td>DIS 58</td>
<td>17 32 9</td>
<td>0.575</td>
<td>0.43</td>
<td>YES</td>
</tr>
<tr>
<td>10</td>
<td>Zhang X^36</td>
<td>Zhejiang</td>
<td>DIS 165</td>
<td>71 76 18</td>
<td>0.660</td>
<td>0.34</td>
<td>YES</td>
</tr>
<tr>
<td>11</td>
<td>Zhao Y^37</td>
<td>Beijing</td>
<td>DIS 294</td>
<td>39 114 112</td>
<td>0.370</td>
<td>0.63</td>
<td>YES</td>
</tr>
<tr>
<td>12</td>
<td>Fan G^38</td>
<td>Shandong</td>
<td>DIS 72</td>
<td>33 29 10</td>
<td>0.603</td>
<td>0.40</td>
<td>YES</td>
</tr>
<tr>
<td>13</td>
<td>Yao L^39</td>
<td>Heilongjiang</td>
<td>DIS 60</td>
<td>12 25 41</td>
<td>0.30</td>
<td>0.70</td>
<td>YES</td>
</tr>
<tr>
<td>14</td>
<td>Zhang J^40</td>
<td>Shanghai</td>
<td>DIS 325</td>
<td>80 135 110</td>
<td>0.456</td>
<td>0.55</td>
<td>YES</td>
</tr>
<tr>
<td>15</td>
<td>Gong Y^41</td>
<td>Shanghai</td>
<td>DIS 126</td>
<td>26 43 57</td>
<td>0.382</td>
<td>0.62</td>
<td>YES</td>
</tr>
<tr>
<td>16</td>
<td>He J^42</td>
<td>Inner Mongolia</td>
<td>DIS 68</td>
<td>13 30 25</td>
<td>0.419</td>
<td>0.59</td>
<td>YES</td>
</tr>
<tr>
<td>17</td>
<td>Jiang H^43</td>
<td>Hubei</td>
<td>DIS 58</td>
<td>17 32 9</td>
<td>0.575</td>
<td>0.43</td>
<td>YES</td>
</tr>
<tr>
<td>18</td>
<td>Pan Q^44</td>
<td>Guangdong</td>
<td>DIS 323</td>
<td>67 122 134</td>
<td>0.406</td>
<td>0.60</td>
<td>YES</td>
</tr>
<tr>
<td>19</td>
<td>Yu Y^45</td>
<td>Heilongjiang</td>
<td>DIS 36</td>
<td>20 12 4</td>
<td>0.720</td>
<td>0.28</td>
<td>YES</td>
</tr>
<tr>
<td>20</td>
<td>Zhang Y^46</td>
<td>Jiangsu</td>
<td>DIS 99</td>
<td>21 43 35</td>
<td>0.439</td>
<td>0.57</td>
<td>YES</td>
</tr>
<tr>
<td>21</td>
<td>Li X^47</td>
<td>Tianjin</td>
<td>DIS 85</td>
<td>29 39 17</td>
<td>0.578</td>
<td>0.43</td>
<td>YES</td>
</tr>
<tr>
<td>22</td>
<td>Huo Y^48</td>
<td>Beijing</td>
<td>DIS 100</td>
<td>11 47 42</td>
<td>0.349</td>
<td>0.66</td>
<td>YES</td>
</tr>
<tr>
<td>23</td>
<td>Luo Y^49</td>
<td>Tianjin</td>
<td>DIS 80</td>
<td>22 35 23</td>
<td>0.505</td>
<td>0.50</td>
<td>YES</td>
</tr>
<tr>
<td>24</td>
<td>Yi X^50</td>
<td>Ningxia</td>
<td>DIS 96</td>
<td>14 27 13</td>
<td>0.370</td>
<td>0.63</td>
<td>YES</td>
</tr>
<tr>
<td>25</td>
<td>Chen D^51</td>
<td>Beijing</td>
<td>DIS 150</td>
<td>64 20 60</td>
<td>0.635</td>
<td>0.35</td>
<td>YES</td>
</tr>
<tr>
<td>26</td>
<td>Cui J^52</td>
<td>Guangdong</td>
<td>DIS 114</td>
<td>29 61 24</td>
<td>0.524</td>
<td>0.48</td>
<td>YES</td>
</tr>
<tr>
<td>27</td>
<td>Gu G^53</td>
<td>Jiangsu</td>
<td>DIS 42</td>
<td>17 12 13</td>
<td>0.550</td>
<td>0.45</td>
<td>YES</td>
</tr>
<tr>
<td>28</td>
<td>Li C^54</td>
<td>Guangdong</td>
<td>DIS 454</td>
<td>102 197 155</td>
<td>0.440</td>
<td>0.56</td>
<td>YES</td>
</tr>
<tr>
<td>29</td>
<td>Lv J^55</td>
<td>Liaoning</td>
<td>DIS 163</td>
<td>40 67 56</td>
<td>0.450</td>
<td>0.55</td>
<td>YES</td>
</tr>
</tbody>
</table>

DIS (disease): patient with cerebral infarction; Ctrl: control group; HWE: Hardy-Weinberg Equilibrium

Figure 1: Forrest plot on the correlation of ACE D/I polymorphism and cerebral infarction in Han Chinese population.

Figure 2: Sensitivity analysis ACE D/I polymorphism and cerebral infarction in Han Chinese population.
Figure 3: Funnel plot on the correlation between ACE D/I polymorphism and cerebral infarction in Han Chinese population.

Egger regression test (P=0.801), which failed to detect significant bias.

DISCUSSION

The meta-analysis of relationship between gene polymorphism and disease showed that there was 91% increased risk of cerebral infarction for the variant homozygotes (DD) compared with the(DI+II) genotypes. The data from this meta-analysis clearly suggest that the ACE DD genotype is a genetic contributor to overall cerebral infarction susceptibility in Han Chinese population. We did not find evidence of publication bias either funnel plot visual inspection or Egger regression test. It suggested the combined results of meta-analysis were reliable by sensitivity analysis.

The result of this meta-analysis is the same with which of the Ariyaratnam R et al.36 ACE I/D polymorphism with a overall OR for the nine studies (a total of 3,572 individuals) of 1.90 (95% CI 1.23 to 2.93) in Chinese. They only selected English-language articles for review and the genotype frequencies in control groups were not consistent with HWE. So control groups of our meta-analysis were more representative. Furthermore, we searched studies published in all language to eliminate language bias. One more earlier meta-analysis performed by Zhang et al.5 suggested 58% increase in risk of cerebral infarction in Han Chinese population. That meta-analysis included only 2066 individuals. With the publication of more recent studies, the result of this meta-analysis was more convincing, similar to a meta-analysis on Caucasians.37 The overall OR estimate for the five studies (1196 cases and 722 controls) is 1.31 (95% CI 1.06 to 1.62; P=0.01). It suggests that ACE genetic associations studies to date for cerebral infarction among persons of Han Chinese population are similar to Caucasians. Another similar meta-analysis on all ethnic populations was performed by Juan et al.38 The ACE I/D polymorphism was evaluated in 11 studies (2990 cases and 11305 controls) and a summary OR of 1.21 (95% CI 1.08 to 1.35). Might the differences in genetic effects among different ethnic populations for complex disorders such as stroke be overstated? The result should be validated by more studies on different ethnic populations.

The mechanisms underlying positive associations between the ACE I/D alleles and disease are not yet clear. Plasma and intracellular levels of ACE have been shown to be partly determined by the presence of the ACE I/D polymorphism in healthy individuals and in patients with stroke.38-42 Individuals homozygous for the D allele have a 56% increase in ACE activity compared with I allele homozygotes.43 The ACE converts angiotensin I to angiotensin II, which is known to be involved in vascular hypertrophy, vasoconstriction, and atherosclerotic processes.44 Also, ACE is responsible for degradation of bradykinin, a vasoactive peptide that has been suggested to stimulate vasodilator nitric oxide production.44

As with all meta-analysis, our analysis has limitations that must be considered when interpreting the findings. As no prospective studies have addressed our question, all included studies followed a retrospective case control design. Insufficient data were available for direct analysis of the influence of DD genotype on either stroke subtype or on stroke risk for different level of ACE. Only three studies7,10,28 explored the correlation between ACE gene and subtype of cerebral infarction. Two studies28 showed ACE gene might be an independent risk factor for both lacunar stroke and cerebral embolism. Another study28 showed ACE ID/DD genotypes didn’t represented a significantly increased risk for either large-artery atherothromboembolic stroke or small-vessel thrombotic stroke. Only three studies16,18,25 described the influence of DD genotype on stroke risk for different level of ACE. A few reviewed studies presented detailed information about interaction between ACE gene polymorphism and other factors such as age,30 gender,14 plasma lipid,22 smoking and alcohol consumption.28 Zhang et al.16 reported that the ACE gene polymorphism might be related to position of damage. The mechanism remains unclear.

Based on known or presumed mechanisms of disease pathophysiology, candidate gene strategies provide a useful approach for evaluating gene-disease associations. However, candidate gene case-control studies have been criticized because of a lack of replication. Firstly, if another variant in or near the ACE I/D gene was the causal variant, the true association could easily be missed. Different linkage disequilibrium patterns with the functional variant may lead to variable results in different populations. Secondly, several cerebral infarction association studies have shown inconsistent results in Han Chinese population. Small sample size, study design flaws, population stratification, genotyping error, and other biases may be common reasons for the observed discrepancies between studies of genetic risks. Specific environmental exposures such as smoking or hypertension are another confounding factor for cerebral infarction with a strong gene environmental interaction in explaining the inconsistencies among observational studies. Interactions with other genetic or environmental factors have been discussed in a few studies.

Our data suggest that the ACE DD genotype may be a risk factor in the aetiology of cerebral infarction in Han Chinese population. The pooled ORs in this study of the ACE DD genotype suggest a modest but definite genetic effect. A large scale case-control study needs to be assessed to help answer the
question of whether or not the polymorphism of the ACE I/D gene confers susceptibility to cerebral infarction.

Acknowledgment

The authors thank Dr. Shao for her valuable contribution and kindly revising the manuscript.

References