Angiotensin-Converting Enzyme Gene Polymorphism and Carotid Artery Wall Thickness

A Meta-Analysis

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Background and Purpose—Many studies have investigated the association between the angiotensin-converting enzyme (ACE) gene insertion/deletion (I/D) polymorphism and carotid artery intima-media thickness (IMT); however, most studies were small and conducted in selective samples. The aim of this study was to evaluate this association by performing a meta-analysis on published articles.

Methods—We searched Medline for articles studying the association between the ACE I/D polymorphism and carotid IMT. Twenty-six studies were found; 23 articles containing 9833 subjects were qualified to enter the meta-analysis. We classified those articles on the basis of their samples into high-risk and low-risk populations and white and Asian ethnic groups. IMT was used as a continuous variable, and data were analyzed with the Cochrane Review Manager.

Results—A significant positive association was present between the D allele and common carotid IMT (weighted mean difference between DD and II genotypes, 0.23 mm \(\times 10^{-1}\); \(P<0.01\)). The association was stronger among high-risk populations. The point estimates of DD versus II were higher than those of ID versus II.

Conclusions—Our meta-analysis showed evidence of a positive association between the D allele of the ACE gene and common carotid IMT. The overall results were concordant in both ethnic groups. (Stroke. 2003;34:1634-1639.)

Key Words: angiotensins ■ carotid arteries ■ genetics ■ meta-analysis

The angiotensin-converting enzyme (ACE), a key enzyme in the renin-angiotensin system, plays an important role in vascular wall homeostasis.\(^1\) –\(^3\) Regulation of circulation and probably tissue ACE activity are under strong genetic control.\(^4\)–\(^5\) 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 \textit{alu} repeat (287 bp)\(^6\) in intron 16 of the ACE gene; the D allele results from the absence of the above insertion. It has been shown that higher serum ACE activity is present in subjects with the D compared with the I allele.\(^4\)–\(^5\)

Numerous studies have reported a relation between the D allele and cardiovascular diseases,\(^6\)–\(^9\) but findings have been controversial.\(^1\) –\(^3\) Additionally, results from a large number of studies on the association of the ACE polymorphism with carotid artery intima-media thickness (IMT), which was used as a measure of atherosclerosis, have been controversial. Some studies found a positive association between the D allele and IMT,\(^1\) –\(^3\) whereas others failed to find such a relation.\(^2\)–\(^3\) However, the studies were conducted in selective and heterogeneous groups from low-risk populations to hypertensive or diabetic patients. Furthermore, the sample sizes of these studies have been relatively small. The aim of our study was to evaluate the effect of this polymorphism on carotid IMT by performing a meta-analysis using all studies published until October 2002.

Materials and Methods

Identification of Studies

We searched Medline for all publications relating to association studies using the ACE I/D polymorphism and carotid IMT. References from retrieved publications were checked for any additional studies. Twenty-six articles were identified.\(^1\) –\(^3\) Two studies that used family-based design were excluded.\(^2\) –\(^3\) One study did not report detailed statistics for IMT measurement\(^2\) and therefore was not included in the meta-analysis. Furthermore, 2 case-control studies did not report detailed results of their controls groups,\(^1\) –\(^3\) and only case groups were included.

In total, 23 articles were entered into the meta-analysis, from which 17 studies have been conducted in white populations. Most of the sample sizes were comprised of <500 subjects. Three studies used the case-control design,\(^1\) –\(^3\) and 6 other studies included only individuals at high risk of atherosclerosis.\(^1\) –\(^3\) In total, there were 1448 patients with hypertension, type I diabetes, type II diabetes, or cerebrovascular or arterial occlusive diseases. Other studies used either a random sample of the general population or relatively healthy subjects.

Four studies used samples that included only men.\(^1\) –\(^3\) Among other studies that used both sexes, 3 studies performed comparisons...
between men and women in regard to an association between the polymorphism and carotid IMT. None found any evidence that the association may be different between 2 sexes. Therefore, both sexes were included in the meta-analysis.

Measurements of carotid artery IMT were done in all the studies by means of B-mode ultrasound examinations. Participants were examined in the supine position with slight extension of the neck, and IMT measurements were performed with a 5- to 10-MHz transducer along the segment 10 to 30 mm proximal to the origin of the carotid bulb. Some studies measured IMT in several arterial sites, and various cut points were used to convert the IMT from a continuous variable to a nominal variable. Therefore, to make the studies more comparable, we used IMT measurement of the far wall of the common carotid artery as a continuous variable whenever several statistics were presented in original articles.

In most of the studies, the genotype frequencies were consistent with Hardy-Weinberg equilibrium (HWE). Deviations from HWE were found in some studies among high-risk populations. Because deviation may occur in diseased populations if there is an association between disease and the allele, which may have been the case in the present studies, we did not exclude them from the meta-analysis.

**Statistical Analysis**

We entered the available data in the Cochrane Review Manager (RevMan, version 4.1.1) and analyze them with Metaview 4.1. The method of moments proposed by DerSimonian and Laird has been used to calculate the weighted mean differences (WMDs) in a random-effects model for the pooled data. We used the funnel plot to examine publication bias of reported associations.

In addition to the total group, we analyzed the studies using the high-risk populations separately from low-risk/general populations because it has been shown that the association with the gene could be different because of risk factor profiles. Furthermore, we classified the studies into studies of Asians and whites because genotype frequencies and prevalence of cardiovascular diseases are reported to be different among ethnic groups. The effect of the ACE gene variant was assessed by use of comparisons between DD and II, DD and ID, and ID and II.

When the SE was reported in the original article, the value of the SD has been calculated, and all descriptive data are expressed as mean±SD. The mean differences are presented in 10⁻¹ mm with the 95% confidence interval (CI) for each difference. Probability values are presented with 2 decimal places.

**Results**

In total, the studies contained 9833 subjects. Details of the individual studies are shown in Table 1. Mannami et al from Japan and Hung et al from Australia used the largest sample...
sized. Almost all studies used middle-aged samples of the population.

The total frequency of the D allele was 46.2%. There was a high range for the DD genotype frequencies: from 13.0% in Mannami et al.35 to 43.1% in Nergizoglu et al. 19 Table 2 shows the allele frequencies and distribution of genotypes in the individual studies. The lowest frequencies for the DD genotype have been observed in Asian populations.

The carotid IMTs in different studies according to the 3 ACE genotype groups are presented in Table 3. Only 1 study reported a positive effect of the I allele on carotid IMT.39 This study introduced a highly significant heterogeneity (P<0.002) and was excluded from the rest of the analysis.

Overall, there was a significant positive association between the D allele and carotid IMT (WMD=0.23 mm×10−1 between DD and II, P<0.01) (Table 4). Among high-risk populations, this WMD was significant in both whites (WMD=0.49 mm×10−1, P<0.01) and Asians (WMD=1.74 mm×10−1, P<0.01). In low-risk/general populations, however, the association was positively significant only among whites (WMD=0.14 mm×10−1, P=0.02).

Table 4 shows the WMDs of carotid IMT and corresponding 95% CIs between the genotypes. The point estimates of the difference between DD and II were generally higher than the point estimates of ID versus II. The mean differences between DD and II in the individual studies and the weighted pooled data are shown in Figure 1.

Figure 2 presents the funnel plot for 2 different ethnic subgroups. The plot for whites is quite symmetric, but this is not the case for Asians, suggesting a publication bias in these populations.

**Discussion**

In this study, we pooled the data of >9800 subjects from available published studies to compute an estimate of the association between the I/D polymorphism of the ACE gene locus and the common carotid IMT. We calculated the WMD between every 2 genotypes and presented the differences among whites and Asians in low-risk and high-risk groups separately. The significant WMDs between DD and II among both high-risk and low-risk populations support the positive association between the D allele and carotid IMT. Although we could not show the difference between DD and ID, the point estimates for the mean differences between DD and II were generally higher

<table>
<thead>
<tr>
<th>Study</th>
<th>Frequency of D Allele.* %</th>
<th>Genotype Frequency, n (%)</th>
<th>P (HWE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-risk/general populations</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Arnett et al25</td>
<td>56.4</td>
<td>88 (17.8)</td>
<td>256 (51.7)</td>
</tr>
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<td>Balkestein et al21</td>
<td>45.8</td>
<td>116 (30.5)</td>
<td>180 (47.4)</td>
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<tr>
<td>Castellano et al14</td>
<td>64.2</td>
<td>23 (12.3)</td>
<td>88 (47.1)</td>
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<td>23 (9.6)</td>
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<tr>
<td>Ferrieres et al27</td>
<td>59.2</td>
<td>70 (19.7)</td>
<td>150 (42.3)</td>
</tr>
<tr>
<td>Girerd et al26</td>
<td>59.0</td>
<td>57 (16.8)</td>
<td>165 (48.5)</td>
</tr>
<tr>
<td>Huang et al29</td>
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<td>42 (19.2)</td>
<td>100 (45.7)</td>
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<td>228 (20.6)</td>
<td>535 (48.4)</td>
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<td>33 (22.0)</td>
<td>80 (53.3)</td>
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<td>87 (37.0)</td>
<td>116 (49.4)</td>
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<td>86 (46.7)</td>
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<td>31 (20.9)</td>
<td>55 (37.2)</td>
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<tr>
<td>Markus et al29 (cases)</td>
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<td>18 (17.8)</td>
<td>47 (46.5)</td>
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<td>65 (37.1)</td>
<td>69 (39.4)</td>
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<tr>
<td>Kagawa et al18 (cases)</td>
<td>37.8</td>
<td>147 (41.3)</td>
<td>149 (41.9)</td>
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</tbody>
</table>

*Allele frequencies are recalculated using the genotype frequencies.
than those of the difference between ID and II, suggesting an allele-dose effect of the allele.

In a few studies, the genotype frequencies were not consistent with HWE. Almost all those studies used high-risk populations, and deviation from HWE is expected among such populations. Laboratory error is not very likely because genotyping has been performed using standard protocols in all the studies and overdetection of the D allele has been corrected by performing a second polymerase chain reaction. The most likely explanation is a higher selection of the DD genotype because of the background diseased populations. There was still 1 study among low-risk populations that did not follow the HWE. However, we considered excluding these studies from our meta-analysis, and the results did not materially change.

Most of the studies used similar transducers (7.5 MHz) to measure IMT at the same segment of the common carotid artery (10 mm proximal to the carotid bulb). On the other hand, any possible measurement error will be presented equally in all 3 genotypes, and because we used WMDs in our analysis, it is not likely to induce an error in our results. The study by Sass et al. had the smallest SD of IMT measure-
ments and consequently gained a relatively large weight in the meta-analysis, whereas it comprised only $\approx 2\%$ of the total sample size. Excluding this study did not materially change the results, ruling out the possibility of overweighting effect of this study on final findings.

It is important to mention that there is a large variability of case selection among the high-risk group. However, the fact that all of those study populations are at high risk of atherosclerosis means that they share some genetic-environmental backgrounds that make them susceptible for the disorder; in this sense, they may be considered homogeneous. Possible explanations for the greater gene effect observed in this group could be the interaction between those background factors and the ACE gene, as well as the greater variation in IMT among them.

An important source of bias in every meta-analysis is publication bias because the likelihood of publishing a study could be related to the results of that study. However, among our meta-analysis, there have been many studies published with negative findings.\textsuperscript{21-23,25-30,32-35} Although the funnel plot for Asians is not symmetric, the overall results of both ethnic groups are concordant, indicating that this bias cannot affect the final result.

On the other hand, funnel plot asymmetry is not always caused by publication bias. True heterogeneity may also lead to funnel plot asymmetry. For example, significant difference may be seen only in high-risk individuals, and these high-risk people are usually more likely to be included in small studies. This is particularly true in our meta-analysis because all the significant associations in Asians have been observed among the studies from high-risk populations. Language bias or citation bias also could be an important source in this group of studies, meaning that the studies without significant findings are preferentially published in languages other than English and less likely to be cited in other articles. Finally, it is possible, of course, that an asymmetrical funnel plot arises simply by chance.

In summary, we found evidence of a positive association between the presence of the D allele of the ACE gene with common carotid IMT in a meta-analysis of 23 published articles (9833 subjects) that was stronger among high-risk populations.

**Acknowledgment**

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References

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