META-ANALYSIS

Large Meta-Analysis Establishes the ACE Insertion-Deletion Polymorphism as a Marker of Alzheimer’s Disease

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Apolipoprotein E ε4 (APOE*4) is the only fully established susceptibility allele for Alzheimer’s disease. One of the most studied candidates is the insertion (I)/deletion (D) polymorphism (indel) of the gene for angiotensin I-converting enzyme (ACE). This study aimed to clarify its association with Alzheimer’s disease. The meta-analysis included 39 samples, comprising 6,037 cases of Alzheimer’s disease and 12,099 controls, using mainly primary data. Potential interactions with gender, age, ethnic group, and carrier status of the apolipoprotein E ε4 allele were all examined. D homozygotes were at reduced risk of Alzheimer’s disease (odds ratio = 0.81, 95% confidence interval: 0.72, 0.90; corrected p = 0.0004); I homozygotes showed no association with Alzheimer’s disease, while heterozygotes were at increased risk. Although there were clear differences among the three ethnic groups examined (North Europeans, South Caucasians, and East Asians), in all groups D homozygotes were at reduced risk. These results confirm the association of the angiotensin I-converting enzyme indel with Alzheimer’s disease across diverse populations, although this is probably due to linkage disequilibrium with the true risk factor. Further, in North Europeans, both association and Hardy-Weinberg analysis suggested partial heterosis, that is, an increased risk for heterozygotes, due to a hidden interaction with another, as yet unknown, risk factor. This interaction warrants further investigation.

Alzheimer disease; heterogeneity; meta-analysis

Abbreviations: ACE, angiotensin I-converting enzyme (protein); ACE, angiotensin I-converting enzyme (gene); APOE*4, apolipoprotein E ε4 (allele).

In 1999, Kehoe et al. (1) first proposed an association with Alzheimer’s disease of the insertion (I)/deletion (D) polymorphism (indel) in intron 16 of the gene for angiotensin I-converting enzyme (ACE) on chromosome 17q23. The enzyme converts angiotensin I to angiotensin II, which is potently hypertensive. The D allele is associated with raised plasma levels of the enzyme (2, 3). Kehoe et al. (1) found that I positives, that is, DI + II, were at increased risk of
Alzheimer’s disease. The study comprised three samples, from Cardiff and London in Britain and Belfast in Northern Ireland, with strikingly similar results.

Since then, this potential association has been examined in over 40 samples (4, 5) (table 1) from around the world. Several studies replicated the report of increased risk for I positivies, just a few contradicted it, and many found no association with Alzheimer’s disease. One study suggested an effect of age on the association (6), and another proposed an influence of gender (7). An association with onset age of Alzheimer’s disease has also been proposed (8).

The association has recently been studied in the longitudinal, observational cohorts of the Oxford Project to Investigate Memory and Ageing (OPTIMA) and the Rotterdam Study (9). Both groups found a suggestion of a gender difference in their results, consistent with a previous report (7). We therefore undertook a meta-analysis of studies of the association of the ACE indel with Alzheimer’s disease, stratified by gender, by age, by ethnic group, and by apolipoprotein E ε4 allele (APOE*ε4) carrier status, using mainly primary data, supplied by authors.

Although three previous meta-analyses of ACE studies had been reported (10–12), subsequent studies have now made further data available. Compared even with the latest meta-analysis (12), 10 more samples from eight studies were available to us (9, 13–18, and the above study of the Oxford Project to Investigate Memory and Ageing). In addition, all previous meta-analyses were based on published data, that is, largely unstratified, and were thus limited in the interpretations they allowed. Using primary data, we were able to explore more deeply, searching for sources of heterogeneity. Two previous stratified meta-analyses of Alzheimer’s disease genes (19, 20), based on primary data, have both deepened our understanding.

Egger et al. (21) have stressed the need to examine sources of heterogeneity in meta-analyses of case-control studies. This has especially been the case for meta-analyses of genetic data, which are more likely than those of other types of data to have sources of heterogeneity. We also report the results of a meta-regression analysis on sources of heterogeneity in the meta-analysis of the ACE indel and Alzheimer’s disease.

### Table 1. Cohorts used in the meta-analysis of the ACE indel in Alzheimer’s disease

<table>
<thead>
<tr>
<th>Reference</th>
<th>Location of samples</th>
<th>Alzheimer’s disease (no.)</th>
<th>Controls (no.)</th>
<th>Alzheimer’s disease diagnosis †</th>
<th>Stratified data supplied and used ‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alvarez et al. (63)</td>
<td>Asturias, Spain</td>
<td>351</td>
<td>536</td>
<td>NINCDS-ADRDA probable</td>
<td>Yes</td>
</tr>
<tr>
<td>Buss et al. (40)</td>
<td>Hamburg, Germany; Basel, Switzerland; and Brescia, Italy</td>
<td>261</td>
<td>306</td>
<td>NINCDS-ADRDA</td>
<td>Yes</td>
</tr>
<tr>
<td>Camelo et al. (13)</td>
<td>Colombia</td>
<td>98</td>
<td>72</td>
<td>NINCDS-ADRDA probable</td>
<td>Yes</td>
</tr>
<tr>
<td>Carbonell et al. (64)</td>
<td>London, United Kingdom</td>
<td>80</td>
<td>65</td>
<td>NINCDS-ADRDA</td>
<td></td>
</tr>
<tr>
<td>Chapman et al. (65)</td>
<td>Tel Aviv, Israel</td>
<td>49</td>
<td>40</td>
<td>NINCDS-ADRDA probable</td>
<td></td>
</tr>
<tr>
<td>Cheng et al. (66)</td>
<td>Taipei, Taiwan</td>
<td>173</td>
<td>116</td>
<td>NINCDS-ADRDA probable</td>
<td></td>
</tr>
<tr>
<td>Crawford et al. (7)</td>
<td>Florida, United States</td>
<td>171</td>
<td>175</td>
<td>NINCDS-ADRDA</td>
<td></td>
</tr>
<tr>
<td>Farrer et al. (6)</td>
<td>Moscow, Russia</td>
<td>151</td>
<td>206</td>
<td>NINCDS-ADRDA</td>
<td></td>
</tr>
<tr>
<td>Farrer et al. (6)</td>
<td>Toronto, Canada; Miami, Florida; and Florence, Italy</td>
<td>236</td>
<td>169</td>
<td>NINCDS-ADRDA</td>
<td></td>
</tr>
<tr>
<td>Fernández-Novoa et al. (14)</td>
<td>Galicia, Spain</td>
<td>185</td>
<td>117</td>
<td>NINCDS-ADRDA and DSM-IV</td>
<td>Yes</td>
</tr>
<tr>
<td>Hu et al. (67)</td>
<td>Japan</td>
<td>132</td>
<td>148</td>
<td>NINCDS-ADRDA probable</td>
<td></td>
</tr>
<tr>
<td>Isbir et al. (68)</td>
<td>Turkey</td>
<td>35</td>
<td>29</td>
<td>NINCDS-ADRDA probable</td>
<td></td>
</tr>
<tr>
<td>Kehoe et al. (11)</td>
<td>Bristol and London, United Kingdom</td>
<td>296</td>
<td>95</td>
<td>CERAD or NINCDS-ADRDA</td>
<td>Yes</td>
</tr>
<tr>
<td>Kehoe et al. (1)</td>
<td>Cardiff, Wales</td>
<td>198</td>
<td>77</td>
<td>NINCDS-ADRDA</td>
<td></td>
</tr>
<tr>
<td>Kehoe et al. (1)</td>
<td>London, United Kingdom</td>
<td>201</td>
<td>205</td>
<td>NINCDS-ADRDA</td>
<td>Yes</td>
</tr>
<tr>
<td>Kehoe et al. (1)</td>
<td>Belfast, Northern Ireland</td>
<td>209</td>
<td>198</td>
<td>NINCDS-ADRDA and DSM-IV</td>
<td>Yes</td>
</tr>
<tr>
<td>Keikhaee et al. (15)</td>
<td>Iran</td>
<td>132</td>
<td>107</td>
<td>NINCDS-ADRDA or CERAD</td>
<td>Yes</td>
</tr>
<tr>
<td>Lond et al. (16)</td>
<td>Manchester, England</td>
<td>206</td>
<td>33</td>
<td>NINCDS-ADRDA probable</td>
<td>Yes</td>
</tr>
<tr>
<td>Lond et al. (16)</td>
<td>Scotland</td>
<td>167</td>
<td>357</td>
<td>NINCDS-ADRDA</td>
<td>Yes</td>
</tr>
<tr>
<td>Mattila et al. (69)</td>
<td>West Finland</td>
<td>77</td>
<td>67</td>
<td>CERAD or NINCDS-ADRDA</td>
<td>Yes</td>
</tr>
<tr>
<td>Monastero et al. (70)</td>
<td>Palermo, Italy</td>
<td>149</td>
<td>149</td>
<td>NINCDS-ADRDA</td>
<td></td>
</tr>
<tr>
<td>Myllykangas et al. (71)</td>
<td>Vantaa, Finland</td>
<td>121</td>
<td>75</td>
<td>CERAD</td>
<td></td>
</tr>
<tr>
<td>Narain et al. (10)</td>
<td>Cambridge, England</td>
<td>107</td>
<td>163</td>
<td>CERAD</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Table continues
studies. We therefore divided our analysis into two phases: first, a broad overall analysis and, second, the detailed examination of sources of heterogeneity. We aimed to discover whether the ACE indel is associated with Alzheimer’s disease and, if so, the nature of that association.

MATERIALS AND METHODS

Phase 1: broad analysis

In April 2003, following PubMed searches, the study of references and of abstracts, and communication with experts, we wrote to the authors of all association studies of the ACE indel and Alzheimer’s disease known to us, 33 studies in all, involving 41 samples (4, 5) (table 1), including seven samples from unpublished studies. The searches were updated in November 2004, without further data arising. For the stratified analyses, we used the raw data or stratified summaries received from authors on 26 samples. For the overall analyses, we also used published data, making 39 samples altogether (table 1). Very early onset cases (<50 years) and young controls (<50 years) were excluded, where identifiable, as were subjects with incomplete data. Distinct populations were treated separately, where possible. The large number of controls in the cohort of Sleegers et al. (9) had all been fully assessed (22). All samples had been genotyped for the ACE indel, except for the two of Prince et al. (23, 24), who had used the ACE rs4343 marker, which is in 85–90 percent linkage disequilibrium with the indel in North Europeans.

Phase 2: examination of heterogeneity

We examined six possible sources of heterogeneity. We performed analyses stratified by age, gender, and APOE*4 status. For the age stratifications (<65 years, 65–75 years, and >75 years), we divided the samples into two groups,
Data analysis and statistical methods

Three methods are commonly used to produce odds ratios in genetic association studies: method 1, allelic (i.e., \( D \) vs. \( I \) in this case); method 2, comparing each genotype with one other in turn (e.g., \( DD \) vs. \( DI \)); method 3, comparing each genotype in turn with the other two combined. All three methods are subject to bias, the degree of bias depending on how imperfectly the model fits reality. For instance, method 1 is best suited to testing a codominant model, where there is an allelic dose effect (e.g., \( DD > DI > II \)). Method 3, on the other hand, may be used to test either an \( I \)-dominant/\( D \)-recessive or a \( D \)-dominant/\( I \)-recessive model.

We chose to follow the hypothesis-generating study in ordering how the ages of cases had been defined, for example, at onset (first group) or at examination or death (second group). We also conducted analyses restricted to those of probable or definite Alzheimer’s disease according to criteria of the Consortium to Establish a Registry for Alzheimer’s Disease (CERAD) (25) or of the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer’s Disease and Related Disorders Association (NINCDS-ADRDA) (26) (26 full samples and two part samples). In separate analyses, we excluded eight samples where Hardy-Weinberg analysis and genotyping methods were compatible with mistyping. Underreporting of heterozygotes may occur unless either 5 percent dimethyl sulfoxide is included in the polymerase chain reaction of heterozygotes or underreporting may occur unless either 5 percent dimethyl sulfoxide is included in the polymerase chain reaction of heterozygotes may occur unless either 5 percent dimethyl sulfoxide is included in the polymerase chain reaction (27) or the \( DD \) genotype is confirmed by an insertion-specific second amplification (28). In further analyses, samples were placed where possible (34 of 39 samples) in one of three ethnic groups: North European (19 samples); South Caucasian (11 samples), defined here as from the Mediterranean or from the Middle East; and East Asian (four samples), from China or from Japan.

### TABLE 2. Odds ratios of Alzheimer’s disease for each ACE* genotype versus the other two in the meta-analysis †

<table>
<thead>
<tr>
<th>Comparison‡</th>
<th>Fixed effects§</th>
<th>Random effects§</th>
<th>Heterogeneity (p value)§</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odds ratio</td>
<td>95% confidence interval</td>
<td>Odds ratio</td>
</tr>
<tr>
<td>( DD ) versus (( DI + II ))</td>
<td>0.82</td>
<td>0.76, 0.89</td>
<td>0.81</td>
</tr>
<tr>
<td>( DI ) versus (( DD + II ))</td>
<td>1.12</td>
<td>1.04, 1.20</td>
<td>1.12</td>
</tr>
<tr>
<td>( II ) versus (( DD + DI ))</td>
<td>1.07</td>
<td>0.98, 1.17</td>
<td>1.07</td>
</tr>
</tbody>
</table>

* ACE, angiotensin I-converting enzyme gene.
† All analyses were based on 39 samples, comprising 6,037 cases of Alzheimer’s disease and 12,099 controls.
‡ \( D \) represents the deletion allele; \( I \) represents the insertion allele.
§ Fixed-effects odds ratios were by the method of Mantel and Haenszel (29), random-effects odds ratios by that of DerSimonian and Laird (30), and the heterogeneity test by that of Armitage (32).
¶ Not significant at 0.05.

Results

**Phase 1: broad analysis**

**Examination of bias.** We found no evidence of publication bias. Funnel plots for the comparisons most commonly made, that is, for \( D \) homozgyotes versus \( I \) positives (i.e., \( DI + II \)) and for \( I \) homozgyotes versus \( D \) positives, are shown in figure 1. These plots gave \( p = 0.19 \) and \( p = 0.21 \), respectively. Figure 2 shows the cumulative meta-analysis after each study from July 1998 to November 2004, for \( D \) homozgyotes versus \( I \) positives, that is, the comparison made in the hypothesis-generating study of Kehoe et al. (1). After only four of 39 cohorts had been studied, the odds ratio by DerSimonian and Laird (30) became significantly less than 1 and remained so thereafter. It changed very little from around 0.8 after 2001.
Risk of Alzheimer’s disease, by genotype. The odds ratios for each genotype versus the other two are shown in figures 3, 4, and 5 and are summarized in table 2. D homozygotes were at reduced risk of Alzheimer’s disease, with an overall odds ratio of 0.81 (95 percent confidence interval: 0.72, 0.90; corrected \( p = 0.0004 \)) by the method of DerSimonian and Laird (30). Heterozygotes were at increased risk of Alzheimer’s disease, with an overall odds ratio of 1.12 (95 percent confidence interval: 1.04, 1.22; corrected \( p = 0.006 \)). The odds ratio of Alzheimer’s disease for I homozygotes versus D positives did not differ significantly from 1. Two of these three results were heterogeneous.

Onset age of Alzheimer’s disease. Onset age data were available from 13 samples, comprising 2,309 cases of Alzheimer’s disease. Both stratified analyses and linear regression analysis were performed on these data and also on a subset of 10 North European samples, comprising 1,822 cases of Alzheimer’s disease. No effect of ACE*I carrier status on onset age of Alzheimer’s disease and, surprisingly, only a modest effect of APOE*4 carrier status on onset age were seen (data not shown).

Phase 2: examination of heterogeneity

We examined six potential sources of heterogeneity: age, gender, race, APOE*4 status, misdiagnosis, and mistyping.

Stratification by gender and by APOE*4 status. No significant effects were seen of gender or of APOE*4 status on the associations with Alzheimer’s disease, nor did stratification by either of these two factors remove the heterogeneity (data not shown).

Stratification by age. We calculated the odds ratios of Alzheimer’s disease for ACE*D homozygotes versus I positives when stratified by age (<65 years, 65–75 years, and >75 years). Rather little effect of age was seen on the association of D homozygotes with Alzheimer’s disease (data not shown), nor was there any reduction in heterogeneity on stratification by age.

Examination of diagnosis. We repeated the overall analyses of the three genotypes, restricting the data to the 28 samples that could be limited to the more rigorous diagnoses of probable or definite Alzheimer’s disease according to criteria of the Consortium to Establish a Registry for Alzheimer’s Disease (25) or the National Institute of
Neurological and Communicative Disorders and Stroke–Alzheimer’s Disease and Related Disorders Association (26). The odds ratios were similar to those shown in table 2, that is, significant odds ratios by the method of DerSimonian and Laird (30) of 0.77 for $D$ homozygotes and of 1.15 for heterozygotes and a nonsignificant odds ratio of 1.10 for $I$ homozygotes. Heterogeneity remained for $D$ homozygotes ($p = 0.005$) but was no longer significant ($p = 0.16$) for $I$ homozygotes.

Ethnic stratification. The samples were divided into three ethnic groups, where possible: North European (19 samples), South Caucasian (Mediterranean and Middle Eastern, 11 samples), and East Asian (Chinese and Japanese, four samples). This removed nearly all heterogeneity (table 3). Table 3 shows clear differences among the three ethnic groups. There was no overlap whatsoever in allelic frequencies among the three groups. North Europeans and East Asians differed in the odds ratios for $D$ homozygotes versus $I$ positives ($p = 0.0008$) and for $I$ homozygotes versus $D$ positives ($p = 0.0005$). South Caucasians also differed from East Asians in the odds ratio for $D$ homozygotes versus $I$ positives ($p = 0.0003$). $D$ homozygotes were at reduced risk in all groups, but particularly in East Asians. Heterozygotes were at increased risk in North Europeans. $I$ homozygotes were at higher risk in East Asians, except by Bayesian analysis. We further examined heterosis of 1.09 for $I$ homozygotes. Heterogeneity remained for $I$ homozygotes ($p = 0.02$) but was no longer significant for $D$ homozygotes ($p = 0.19$).
heterozygotes at greater risk) in North Europeans, by comparing DI with DD and with II in turn. We found that heterozygotes were at higher risk than D homozygotes (odds ratio = 1.25, 95 percent confidence interval: 1.08, 1.45) but not significantly higher than I homozygotes (odds ratio = 1.08, 95 percent confidence interval: 0.95, 1.22).

DISCUSSION

This was a large meta-analysis, by the standards of Alzheimer’s disease genetics, with 39 samples, comprising 6,037 cases of Alzheimer’s disease and 12,099 elderly controls (table 1). We found no evidence of publication bias.

ACE*D homozygotes were at reduced risk of Alzheimer’s disease (table 2) in each of the three main ethnic groups examined (table 3). I homozygotes were neutral as regards Alzheimer’s disease risk (table 2), except in East Asians, in whom I homozygotes may be at higher risk (table 3). Heterozygotes were at increased risk of Alzheimer’s disease overall (table 2) and mainly in North Europeans (table 3). This example of heterosis will be discussed below. Very similar results were achieved by three methods: fixed effects, random effects, and Bayesian random effects (refer to Materials and Methods and table 2). Only genotypic comparisons were made (refer to Materials and Methods), which showed that there was no allelic dose effect, except in East Asians (table 3), and that thus allelic comparisons would have proved less informative.

These findings of odds ratios close to 1, even though significantly different from 1 (tables 2 and 3), suggest either linkage disequilibrium with the true risk factor or confounding by a hidden interaction or both. The second possibility is discussed below. Regarding the former, Kehoe et al. (1) in their study of ACE haplotypes gave evidence that the indel may not be the actual risk factor but rather in linkage disequilibrium with a nearby functional polymorphism that is the true risk factor. ACE haplotype analysis has also proved of value with other phenotypes, such as enzyme levels (36), insulin levels, myocardial infarction, and obesity (37). Future Alzheimer’s disease studies may do better to examine polymorphisms in the regulatory regions, rather than the indel.


FIGURE 3. Odds ratios of Alzheimer’s disease for ACE*D homozygotes versus I positives. D represents the deletion allele, and I represents the insertion allele. Horizontal lines are 95% confidence intervals. OPTIMA, Oxford Project to Investigate Memory and Ageing; ACE, angiotensin I-converting enzyme gene. Additional study information is given in table 1.
The overall results were consistent with those of Kehoe et al. (1) from 1999 and also with those of the recent meta-analyses of Kehoe et al. (11) from 2003 and of Elkins et al. (12) from 2004. The latter report (12) also suggested an age difference, however, although actual ages were not available to them, only mean cohort ages, and they did not take into account the different ways of defining case ages (refer to Materials and Methods), a strongly confounding factor. Because of the latter problem, we cannot exclude the possibility that the association might be stronger in younger cases, for example, in those aged less than 65 years at onset or less than 75 years at death.

Heterogeneity

Our overall results showed heterogeneity (table 2). Heterogeneity may be due to differences in sample selection (e.g., in age, gender, or diagnosis) or in methods (e.g., of genotyping), or it may be due to real differences in populations (e.g., in race) or in interactions with other risk factors (e.g., APOE*4). We examined all of these potential sources of heterogeneity. We found little effect of age, gender, or APOE*4 status, thus confounding our initial hypothesis. Both diagnostic and genotyping differences contributed some heterogeneity. The former was mainly due to the diagnosis of “possible Alzheimer’s disease,” reported to have a poor record of confirmation at autopsy (38). The latter was largely due to certain genotyping methods that may lead to underreporting of heterozygotes (27, 28), as shown by Hardy-Weinberg analysis.

However, the greatest source of heterogeneity was ethnic differences. Ethnic stratification removed nearly all heterogeneity (table 3). There were clear contrasts among the three ethnic groups examined (North Europeans, South Caucasians, and East Asians) in allelic frequencies and in odds ratios (table 3). The latter might be due to differences in linkage disequilibrium with the true risk polymorphism (see above). Some of these ethnic differences had previously been pointed out by Panza et al. (17, 39), by Buss et al. (40), and by Elkins et al. (12). These contrasts highlight the

FIGURE 4. Odds ratios of Alzheimer’s disease for ACE heterozygotes versus all homozygotes. Horizontal lines are 95% confidence intervals. OPTIMA, Oxford Project to Investigate Memory and Ageing; ACE, angiotensin I-converting enzyme gene. Additional study information is given in table 1.
dangers of combining ethnic groups within a single cohort, even North and South Europeans, as indicated by Panza et al. (17, 39). Unfortunately, there was only one cohort of African origin, from the United States (41). Thus, no conclusions could be drawn about Africans.

Heterosis

Deviations from Hardy-Weinberg equilibrium are common in studies of the ACE indel, as previously pointed out by Buss et al. (40). This may be due to either mistyping or heterosis. Several cases of disequilibrium were indeed compatible with mistyping. Most examples of disequilibrium in North Europeans, however, were of an excess of heterozygotes in Alzheimer’s disease, which requires another explanation. We believe that explanation is heterosis. Heterosis occurs when heterozygotes show either a greater or lesser association with a given trait than does either group of homozygotes. In this case, in North Europeans, both an excess of heterozygotes in Alzheimer’s disease and an association of heterozygotes with Alzheimer’s disease were found. Comings and MacMurray (42) have suggested three explanations for heterosis, of which their second can be applied here. This proposes that heterosis may follow from the inadvertent combination of two unlike subsets due to a hidden interaction with another, unknown risk factor. To test this proposal, we constructed a mathematical model with two equal subsets based on a median split of an unknown factor X (table 4). The model illustrates how, although heterozygotes may have intermediate odds ratios in each subset, they can emerge with the highest odds ratio when the subsets are combined (i.e., heterosis). The overall results of this illustrative model (table 4) were very close to those of the present meta-analysis (table 2). We should stress, however, that heterosis was found only in North Europeans. Moreover, although heterozygotes may have intermediate odds ratios in each subset, they can emerge with the highest odds ratio when the subsets are combined (i.e., heterosis).

FIGURE 5. Odds ratios of Alzheimer’s disease for ACE I homozygotes versus D positives. D represents the deletion allele, and I represents the insertion allele. Horizontal lines are 95% confidence intervals. OPTIMA, Oxford Project to Investigate Memory and Ageing; ACE, angiotensin I-converting enzyme gene. Additional study information is given in table 1.
A hidden interaction

Partial heterosis in North Europeans suggests an interaction with another risk factor for Alzheimer’s disease, another potential source of heterogeneity. Candidates include cardiovascular risk factors, since many also contribute to Alzheimer’s disease risk and since the ACE indel has been associated with cardiovascular risk (43–47).

Biologic background

Full discussion of the biologic basis of these associations is outside the scope of this mainly analytical study. However, we note the associations of the ACE indel and of nearby polymorphisms with various phenotypes, including cardiovascular risk (37, 43–47). In addition, ACE protein levels are raised in certain brain regions with Alzheimer’s disease (48–50), the enzyme has been reported to modify β-amyloid aggregation (51), ACE inhibitors have been reported to maintain memory in aged rats (52) and in human patients (53), and brain-penetrating ACE inhibitors have been associated with both reduced incidence of Alzheimer’s disease in elderly hypertensive patients (54) and less cognitive decline in cases with mild-to-moderate Alzheimer’s disease (55). Sleegers et al. (9) have recently provided evidence from magnetic resonance imaging of increased atrophy in both the hippocampus and amygdala of nondemented women homozygous for the ACE*I allele, together with a modest but significant increase in risk of Alzheimer’s disease, independent of vascular factors. The D allele is associated with raised plasma levels of ACE (2), but that association is thought to be due to linkage disequilibrium with other nearby polymorphisms (3, 36). There is an

### TABLE 3. Odds ratios of Alzheimer’s disease for each ACE* genotype† in the meta-analysis, by ethnic group

<table>
<thead>
<tr>
<th></th>
<th>North Europeans</th>
<th>South Caucasians (Mediterranean and Middle East)</th>
<th>East Asians (China and Japan)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alzheimer’s disease cases (no./controls (no.))</td>
<td>3,380/9,361</td>
<td>1,350/1,591</td>
<td>589/587</td>
</tr>
<tr>
<td>D frequencies in controls (% (95% CI*))</td>
<td>53.0</td>
<td>52.2, 53.7</td>
<td>63.4</td>
</tr>
<tr>
<td>DD versus (DI + II) (OR*‡ (95% CI))</td>
<td>0.86 (a)§</td>
<td>0.71, 1.05</td>
<td>0.81</td>
</tr>
<tr>
<td>DI versus (DD + II) (OR‡ (95% CI))</td>
<td>1.11 (a)§</td>
<td>0.97, 1.28</td>
<td>1.11</td>
</tr>
<tr>
<td>II versus (DD + DI) (OR‡ (95% CI))</td>
<td>0.99 (a)§</td>
<td>0.85, 1.16</td>
<td>1.12</td>
</tr>
<tr>
<td>HW* excess (p value)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alzheimer’s disease Heterozygotes (0.0009)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls Equilibrium Homozygotes (0.01) Equilibrium</td>
<td></td>
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</tbody>
</table>

* ACE, angiotensin I-converting enzyme gene; CI, confidence interval; OR, odds ratio; HW, Hardy-Weinberg.
† D represents the deletion allele; I represents the insertion allele.
‡ All odds ratios shown were by the random-effects method of DerSimonian and Laird (30), which is generally more conservative than the fixed-effects method; all significant odds ratios were very closely replicated by the Bayesian random-effects method of Warn et al. (31), except for the II odds ratio for East Asians, whose result was 1.53 (95% CI: 0.96, 2.54) by the Bayesian method.
§ Heterogeneity was seen only in North European cohorts; it was removed upon exclusion of two cohorts with possible genotyping problems. Results for North Europeans are shown for all 19 cohorts (a) and after the removal of those two cohorts (b). North Europeans versus East Asians: OR for DD versus (DI + II), p = 0.0008; OR for II versus (DD + DI), p = 0.0005. South Caucasians versus East Asians: OR for DD versus (DI + II), p = 0.0003.

### TABLE 4. Model to show how heterosis may follow from the combination of two unlike subsets

<table>
<thead>
<tr>
<th>Model</th>
<th>Odds ratios for each ACE* genotype versus both others</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>DD</td>
</tr>
<tr>
<td>Low factor X†</td>
<td>0.50</td>
</tr>
<tr>
<td>High factor X‡</td>
<td>1.24</td>
</tr>
<tr>
<td>All</td>
<td>0.82</td>
</tr>
<tr>
<td>Present meta-analysis‡</td>
<td>0.81</td>
</tr>
</tbody>
</table>

* ACE, angiotensin I-converting enzyme gene.
† Factor X is hypothesized to interact with the ACE insertion-deletion polymorphism in Alzheimer’s disease risk; the two subsets (“low” and “high”) are based on a median split of values of factor X. The model was devised to give opposing results in each subset, while maintaining Hardy-Weinberg equilibrium, but to produce overall results similar to those of this meta-analysis.
‡ See table 2.
apparent conflict between the benefits of ACE inhibitor therapy and the associations of the indel with Alzheimer’s disease risk and pathology and with plasma levels of ACE. However, the influence of ACE polymorphisms on brain levels of the enzyme is not yet known and cannot be assumed to reflect the levels in plasma. This subject warrants fuller discussion than is possible here. A more detailed discussion is given by Kehoe (56), although it will need to be reconsidered and perhaps revised in the light of any interacting factor or factors that may emerge in future studies.

**Limitations of this meta-analysis**

The lack of information from some authors was a limitation, but we obtained full data on 84 percent of subjects. The quality of diagnosis and of genotyping varied among studies, but our overall results were not changed when these questions were taken into account. There was considerable heterogeneity in our initial results. However, we found the main sources of that heterogeneity and were able to remove it. Since cardiovascular factors are strong candidates for a potential interaction with ACE variants, the lack of data available to us on those factors was a limitation, which we hope will be overcome by future studies. Cardiovascular factors might also have influenced our results through selective mortality. However, D homozygotes have only a very slightly increased risk of cardiovascular complications (43–47, 57) and little evidence of any association with reduced life span (57–62). Thus, the influence of selective mortality, if any, is likely to be very small and not enough to provide an alternative explanation for our findings.

**Conclusions**

We suggest that this large study has established the ACE indel as a marker of Alzheimer’s disease risk, since we found no evidence of bias, we found and removed the main sources of heterogeneity, we excluded various potential interactions, and significant results remained in each of the three ethnic groups examined. The indel, however, is probably in linkage disequilibrium with the true risk polymorphism (11). There were marked contrasts among the three ethnic groups studied (table 3). We also found evidence of an interaction with another risk factor in North Europeans. Potential interactions with other factors than age, gender, and APOE*4 status should be examined, particularly interactions with cardiovascular risk factors.

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Conflict of interest: none declared.

**Note added in proof:** Two further association studies have been published since submission of this article: Kölsch et al. (Neurosci Lett 2005;377:37–9) and Zhang et al. (Dement Geriatr Cogn Disord 2005;20:52–6).

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