Editorial Review

Angiotensin-converting enzyme gene polymorphism and cardiovascular disease

R. BUTLER, A. D. MORRIS and A. D. STRUTHERS
Department of Clinical Pharmacology, Ninewells Hospital and Medical School, Dundee DD1 9SY, U.K.

1. The crucial role played by the renin–angiotensin–aldosterone system in the cardiovascular system and the immense therapeutic potential of angiotensin-converting enzyme inhibitors and, more recently, angiotensin II receptor blocking agents, in both heart failure and post-myocardial infarction is becoming increasingly evident. Polymorphisms within the genes controlling this enzyme system are candidates for the elucidation of the pathogenesis of cardiovascular disease and this link is both intriguing and provocative. Recently, an association between a polymorphism of the angiotensin-converting enzyme gene and phenotypic expression of cardiovascular disease, namely myocardial infarction, was reported. Since then, several small case-controlled studies have confirmed an association with manifestations of ischaemic heart disease or various other cardiac end-points. However, in a large prospective study the angiotensin-converting enzyme gene conferred no appreciable risk.

2. Our aim was to review the evidence that links polymorphisms of the angiotensin-converting enzyme gene with cardiovascular disease. We searched the Medline database (1990–1997) using the key words myocardial infarction, ischaemic heart disease, angiotensin-converting enzyme and polymorphisms and performed a search of the reference citation of relevant articles. We selected clinical studies on cardiovascular disease related to the angiotensin-converting enzyme genotype.

3. Taken together, the available evidence supports the notion that the DD-angiotensin-converting enzyme genotype adversely influences specific cardiovascular diseases but appears to do so in specific geographical areas and in particular patient subgroups. It is not yet known whether it does this through an interaction with other genes or by as yet unexplained biochemical mechanisms.

4. We should regard the current data with the angiotensin-converting enzyme genotype as an intriguing clue in the pathogenesis of cardiovascular disease. However, the main factor against this potential benefit is that the impact of the DD genotype appears to be small and its clinical manifestations rather heterogeneous.

INTRODUCTION

The advent of large prospective trials showing substantial survival benefits of angiotensin-converting enzyme (ACE) inhibitors in both heart failure [1] and post-myocardial infarction [2, 3] has served to emphasize the pivotal role which the renin–angiotensin–aldosterone system (RAAS) plays in the cardiovascular system. Polymorphisms within the genes controlling this important enzyme system make attractive candidates for elucidating the pathogenesis of cardiovascular disease, and this link is both intriguing and provocative, especially with the advent of such efficacious treatments as ACE inhibitors and, more recently, the angiotensin II receptor blocking agents.

In 1990, Rigat et al. [4] discovered an insertion/deletion polymorphism within the genome of the ACE gene which appeared to account for approximately half the variance of serum ACE concentrations in the normal population. The clinical relevance of this finding was uncertain until 1992, when further evidence emerged. The Etude Cas-Témoin de l’Infarctus du Myocarde (ECTIM) study [5] showed that the DD-ACE genotype was associated with a significantly increased risk of myocardial infarction compared with the II-ACE genotype.

This work was the stimulus for a vast number of clinical studies which have evaluated the impact of the DD-ACE genotype on phenotypic expression in differing populations and diseases. The subsequent reports on the biological effects of the DD-ACE genotype are far from conclusive, as a rapid pool of conflicting data has emerged. Seven years after the
original description it appears timely to review the clinical evidence that the polymorphism of the ACE gene plays a pathophysiological role in cardiovascular disease.

POLYMORPHISM OF ACE

ACE is a zinc-based metallopeptidase; its two primary metabolic roles are the conversion of angiotensin I (ANG I) to angiotensin II (ANG II) and the degradation of bradykinin [6]. ACE occurs as a membrane-bound form and a circulating enzymic form which probably derived from vascular endothelial cells. There appears to be little morphological difference between the two forms of ACE [7] and little intra-individual, but quite marked inter-individual, variation in serum ACE in normal subjects.

The insertion/deletion polymorphism described by Rigat et al. [4] is located within intron 16, is a 287-bp alu repeat sequence and is inherited as either present or absent. The alu repeat is perhaps the commonest family of repeats in the human genome. Homozygosity for the presence of the polymorphic segment is designated II; individuals homozygous for the deleted polymorphism are termed genotype. The polymorphism follows Mendelian characteristics and the Hardy-Weinberg equilibrium accurately predicts the frequency of occurrence of I and D. The I/D polymorphism shows strong linkage disequilibrium with the gene locus involved in the control of serum ACE levels; Rigat et al. [4] showed serum ACE was: 299 ± 49, 393 ± 67 and 494 ± 88 µg/l (mean ± SD) in the II, ID and DD groups respectively, demonstrating a positive relationship with the D-allele.

Linkage equilibrium simply means that alleles (alternative versions of a gene at the same locus) at two linked loci are ‘in equilibrium’ if the frequency of their occurrence together is equal to the product of their individual frequencies. The term ‘disequilibrium’ suggests that some extraneous factor has exerted an influence and there is some selective advantage to the allele selection. The precise cause of this phenomenon is unknown, but common.

ANALYTICAL CONSIDERATIONS

One important consideration is that some of the early work could be subject to misclassification of the D-allele during genotyping. For example, Shanthmugam et al. [8] demonstrated that heterozygotes for the polymorphism may be classified incorrectly with 5% of ID genotypes being misclassified as DD. The current ‘gold standard’ for genotyping uses two restriction enzymes rather than two used previously, and also adds DMSO and raises the temperature of the annealing process. The likely effects of this are uncertain until some of the larger studies are reanalysed using updated techniques. However, Lindpainter et al. [9] specifically addressed this point and found no discrepancy between their analysis and the ECTIM Study [5].

Race may be an important confounding variable when evaluating the clinical impact of the ACE polymorphism in that Rigat’s work has not been replicated in all ethnic groups. For example, a study by Bloem et al. [10] has shown that in contrast to 141 Caucasian children, in whom higher ACE activity was linked to the presence of the DD-ACE genotype, in 62 Afro-Caribbean children there was no difference in ACE activity associated with the carriage of the D-allele. Whether this is relevant to cardiovascular disease remains in question, but it is established that hypertensive Afro-Caribbeans may have low plasma renin activity but still benefit from ACE inhibitors [11]. Another study of African-Caribbean families by Mackenzie et al. [12] demonstrated a relationship between the D-allele and serum ACE levels, but the magnitude was significantly less (9% compared with 52% in the study population of Rigat et al. [4]). In this population multiple loci were responsible for the variance of ACE levels rather than a single polymorphism.

ACE POLYMORPHISM AND MYOCARDIAL INFARCTION

The ECTIM study was the first to describe an association between the ACE polymorphism and phenotypic expression of cardiovascular disease, namely myocardial infarction. Its publication provided the catalyst for a large number of studies. Cambien et al. [5] retrospectively compared data from 610 patients who had suffered an acute myocardial infarction compared with 733 controls from four geographical areas; Northern Ireland and north, east and south-western France. The patients were recruited 6 to 9 months after their index myocardial event. Potential confounding variables of total cholesterol, apolipoprotein B (apoB), fibrinogen, blood pressure, body mass index (BMI) and cigarette smoking were well matched between the two groups. The DD genotype was significantly associated with a myocardial infarction compared with the II genotype [odds ratio (OR) 1.34, P = 0.007]. In a subgroup analysis excluding individuals with conventional risk factors, subjects with a BMI <26 kg/m, not treated with hypolipidaemic drugs and with apoB <125 mg/dl had an even greater risk of myocardial infarction [OR 3.2, 95% confidence interval (CI) 1.7–5.9, P <0.0001], compared with an OR of 1.1 (95% CI 0.9–1.5) for the higher risk group. The numbers within this group were much smaller, with 79 patients in the group with low conventional risk factors compared with 531 patients who had more traditional risk factors. Nonetheless, this study was a landmark and provided a potential insight as to why patients whom we all recognize and were previously felt to be at low risk should suffer a myocardial infarction.
Much work has confirmed this exciting link between the DD-ACE genotype and risk of myocardial infarction. Countries where a strong link has been found are France [5], Wales [13], Northern Ireland [14], Japan [15-18], Germany [19] and Italy [20] (Table 1). However, important differences in the prevalence of the DD-ACE genotype occur in the Japanese studies: the frequency of the D-allele is 0.39 compared with 0.54 in studies based on a Caucasian population [21]. The risk of myocardial infarction with the DD genotype was higher in the Japanese studies than in studies in Caucasians: OR 2.55 (95% CI 1.75-3.70; \( P < 0.0001 \)) compared with 1.18 (1.07-1.30; \( P < 0.0008 \)) respectively. The reasons for this remain uncertain.

On the other hand, the Physicians Health Study by Lindpainter et al. [9] has shown no adverse risk in a large prospective study in the USA. Similarly, no adverse risk of myocardial infarction has been shown in the U.K. [22], Norway [23], Denmark [24, 25] and Austria [26] (Table 2). In particular, Agerholm-Larsen's study [24] looked at 7300 patients in a large case-cohort study. There is no clear geographical or racial pattern which readily explains this differential risk of the ACE polymorphism. It is therefore prudent to consider study design as a possible explanation for the differing results.

Cambien's study [5] recruited hospital patients from France and Northern Ireland, who had suffered a myocardial infarction in the 6 to 9 months before recruitment. This may induce significant recruitment bias in the study because the high mortality associated with a myocardial infarction before hospital admission and in the months after discharge, may result in a significant proportion of patients being excluded. The excess of D-alleles also suggests that these studies are selecting survivors of myocardial infarction and perhaps the DD genotype is actually conferring a survival advantage. Other major flaws in the study were elucidated by Chowdhury et al. [27] and included subgroup analysis without an \( \alpha \)-priori hypothesis, and the designation of low risk of a myocardial infarction based on BMI, apoB and the absence of treatment with hypolipidaemic agents rather than the absence of risk factors such as smoking, diabetes and hypertension.

Evans et al. [14], who also sampled 213 patients from the WHO-MONICA coronary heart disease register in Belfast, tried to remove this selection bias by identifying patients who suffered a fatal myocardial infarction, thus including the patient subgroup who were omitted from Cambien's study. As a result they may have selected a higher risk population with more severe coronary disease or other confounding factors. However, at autopsy, there was an increased frequency of the D-allele \( (P < 0.02) \). The OR for dying from a fatal myocardial infarction or sudden cardiac death compared with death from other causes for DD versus I1 and ID versus I1 was 2.2 and 1.8 respectively \( (P = 0.01) \). Evans also suggested that the D-allele imparted a greater risk of fatal myocardial infarction, rather than myocardial infarction per se. The ECTIM database in Belfast showed that the overall OR for survivors of myocardial infarction carrying the D-allele was 1.1, rather than the much higher level seen in this study.

### Table 1. Summary data for myocardial infarction

<table>
<thead>
<tr>
<th>Author</th>
<th>Reference</th>
<th>OR</th>
<th>P-value</th>
<th>D-allele</th>
<th>n</th>
<th>Major outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cambien et al.</td>
<td>[5]</td>
<td>3.2 (sub)</td>
<td>&lt;0.0001</td>
<td>56</td>
<td>1343</td>
<td>MI</td>
</tr>
<tr>
<td>Mattu et al.</td>
<td>[13]</td>
<td>4.96 (sub)</td>
<td>=0.03</td>
<td>59</td>
<td>200</td>
<td>CAD</td>
</tr>
<tr>
<td>Evans et al.</td>
<td>[14]</td>
<td>2.2</td>
<td>0.01</td>
<td>59</td>
<td>213</td>
<td>MI</td>
</tr>
<tr>
<td>Nakai et al.</td>
<td>[15]</td>
<td>1.38</td>
<td>0.05</td>
<td>43</td>
<td>278</td>
<td>CAD</td>
</tr>
<tr>
<td>Kamitani et al.</td>
<td>[16]</td>
<td>4.43</td>
<td>&lt;0.0001</td>
<td>36</td>
<td>206</td>
<td>MI</td>
</tr>
<tr>
<td>Zhao et al.</td>
<td>[17]</td>
<td>3.83 (ID)</td>
<td>0.024</td>
<td>39</td>
<td>197</td>
<td>MI (in NIDDM)</td>
</tr>
<tr>
<td>Fujisawa et al.</td>
<td>[18]</td>
<td>2.2 (ID)</td>
<td>0.007</td>
<td>63</td>
<td>388</td>
<td>MI</td>
</tr>
<tr>
<td>Gardemann et al.</td>
<td>[19]</td>
<td>sub</td>
<td>0.001</td>
<td>56</td>
<td>52</td>
<td>CAD</td>
</tr>
<tr>
<td>Arbushti et al.</td>
<td>[20]</td>
<td>2.56</td>
<td>0.007</td>
<td>54</td>
<td>270</td>
<td>MI (in NIDDM)</td>
</tr>
<tr>
<td>Beohar et al.</td>
<td>[40]</td>
<td>2.0</td>
<td>0.0001</td>
<td>54</td>
<td>700</td>
<td>MI</td>
</tr>
<tr>
<td>Ruiz et al.</td>
<td>[71]</td>
<td>1.73</td>
<td>0.007</td>
<td>55</td>
<td>920</td>
<td>MI</td>
</tr>
<tr>
<td>Ludwig et al.</td>
<td>[20]</td>
<td>1.59</td>
<td>0.022</td>
<td>55</td>
<td>920</td>
<td>MI</td>
</tr>
</tbody>
</table>

### Table 2. Summary data for myocardial infarction

<table>
<thead>
<tr>
<th>Author</th>
<th>Reference</th>
<th>OR</th>
<th>P-value</th>
<th>D-allele</th>
<th>n</th>
<th>Major outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samani et al.</td>
<td>[22]</td>
<td>1.16</td>
<td>0.44</td>
<td>54</td>
<td>1221</td>
<td>MI</td>
</tr>
<tr>
<td>Lindpainter et al.</td>
<td>[9]</td>
<td>1.05</td>
<td>0.36</td>
<td>55</td>
<td>1862</td>
<td>MI</td>
</tr>
<tr>
<td>Agerholm-Larsen et al.</td>
<td>[25]</td>
<td>1.06</td>
<td>0.54</td>
<td>54</td>
<td>1050</td>
<td>MI, IHD</td>
</tr>
<tr>
<td>Bohn et al.</td>
<td>[23]</td>
<td>0.69</td>
<td>*</td>
<td>56</td>
<td>900</td>
<td>MI</td>
</tr>
</tbody>
</table>
2.2 and 1.8 [14]. The intriguing idea of the D-allele being linked to sudden cardiac death has not been seen in other work to date.

The prospective Caerphilly Heart Study [13] showed that in 1226 subjects in Wales, the DD-ACE genotype conferred an increased risk of coronary artery disease over 5 years. This effect was confined to the same subpopulation which would previously have been considered at low risk from myocardial infarction, i.e. with a low BMI and low apoB level (DD versus II, OR 4.96, P = 0.03) and was not seen as a general population effect. The diagnosis of coronary artery disease was based on the Rose questionnaire and ECGs rather than coronary angiography. Interestingly, when BMI was corrected for in the analysis, the positive relationship between the D-allele and myocardial infarction was lost. Mattu et al. [13] concluded that the D-allele conferred increased risk for coronary artery disease among those previously thought to be at low risk, but that was quantitatively small among the general male population.

The Physicians Health Study (a randomized trial of aspirin and β-carotene and their effects on cardiac events and mortality), conducted by Lindpainter et al. [9], prospectively compared 1250 male physicians with 2340 controls. Essentially painter's study population comprised predominantly American physicians with 2340 controls. Therefore, Lindpainter and Cambien. Cambien's study drew patients from historically stable populations in France and Ulster, whereas Lindpainter's study population comprised predominantly male Caucasian physicians of unknown historical ethnic diversity. One has to make the assumption that Lindpainter's group are likely to be a more heterogeneous genetic group and the possibility remains that linkage disequilibrium has been reduced. Against this argument are; firstly, that the occurrence of the D-allele followed the Hardy-Weinberg equilibrium very closely, and secondly, studies with a positive relationship have been conducted in the U.S.A. [28, 29]. Singer et al. [30] also comment on other design weaknesses; a highly selective study group (physicians) may also create bias and their reported mortality rate is lower than expected. Their incidence of cardiovascular disease is also low.

Katsuya et al. [31] conducted a cross-sectional case-control study and found that although a polymorphism within the angiotensinogen gene was significantly associated with coronary artery disease, there appeared to be no association between the D-allele and coronary artery disease.

Sex differences may also prove to be a confounding variable. Schuster et al. [32] found that in their comparison of 163 acute infarctions, the D-allele was associated with myocardial infarction in women only. However, the control group consisted of 227 patients undergoing coronary angiography for anginal chest pain, so direct comparisons are difficult and this finding has not yet been confirmed.

Thus there are conflicting data regarding the association between the D-allele and myocardial infarction. A recent meta-analysis [21] of 15 studies has analysed data from 3394 patients with myocardial infarction and 5479 controls and concluded that the overall mean OR is 1.26 (95% CI 1.15–1.39, P < 0.0001) for DD versus ID/II genotypes. The ORs were 1.36 (95% CI 1.19–1.55, P < 0.0001) for DD versus II and 1.24 (95% CI 1.11–1.38, P = 0.0001) for DD versus ID. This paper demonstrates that the ACE genotype may be associated with myocardial infarction, but the impact of the D-allele is likely to be small. Specific limitations of meta-analyses including publication bias were addressed by Samani et al. [21], who demonstrated that there was a degree of publication bias with the smaller positive studies and myocardial infarction.

The I/D polymorphism is associated with an increased history of parental [33] and of grandparental myocardial infarction [34]. Tiret et al. [33] questioned individuals about their parents' medical history. Those subjects whose parents had suffered a myocardial infarction showed an increased OR of 2.6 (CI 1.2–5.6) for the carriage of the DD-ACE genotype. Badenhop et al. [34] questioned 404 schoolchildren about their families' cardiac history. Those who had a family history of two grandparents suffering a myocardial infarction had an OR of 2.8 (CI 1.16–6.56) of themselves carrying the DD-ACE genotype. However, the disadvantages of such studies involving family histories, such as unreliability of the data, suggest that they should be treated with some caution. Indeed, in this case, the grandparents were not genotyped themselves to test the validity of the observations drawn. However, the data continue to lend weight to the association between myocardial infarction and the D-allele.

CORONARY ATHEROSCLEROSIS AND LEFT VENTRICULAR DYSFUNCTION

Atherosclerosis

The association between the ACE gene polymorphism and the clinical event rate of myocardial infarction appears to be population specific and quantitatively small as discussed above. There is also little evidence for an association with anatomical abnormalities such as focal coronary artery stenosis. For example, Ludwig et al. [28] examined 679 patients and 203 controls who had been assessed by coronary angiography. He found a significant association between coronary artery stenosis, myocardial infarction and the DD-ACE genotype (OR 1.59, P = 0.002) but no association between the degree of coronary artery stenosis and the DD-ACE genotype alone. This intriguing link suggesting that the
presence of the D-allele increases the risk of myocardial infarction if a stenotic lesion already co-exists, but does not seem to be involved in the actual genesis of a stenotic lesion, is important and certainly needs further evaluation. It may reinforce work done in the stroke arena suggesting that the DD-ACE genotype is not associated with more severe occlusive vascular disease but may increase the risk of an adverse event if a stenotic lesion is already present [35, 36].

Coronary artery spasm

Oike et al. [37] assessed 150 Japanese patients angiographically and grouped them as (i) those with coronary artery spasm on injection of intracoronary ergonovine; (ii) those without coronary artery spasm but with occlusive coronary artery disease, and (iii) those without occlusive coronary artery disease or coronary artery spasm. They found a significant association between the carriage of the D-allele and coronary artery spasm \((P = 0.002)\). However, problems with association studies producing false positive results are present here and the results should be accepted with caution. Kuroki et al. [38] demonstrate in a smaller study \((n = 94)\) that the D-allele did not appear to be associated with coronary artery spasm and found an association with occlusive coronary artery disease, perhaps one of the first to document such a link. A link with coronary spasm would have been important. If there was a link with coronary artery spasm then this could explain the data suggesting that the severity of coronary artery disease is no worse but that the D-allele may increase mortality.

Left ventricular dysfunction

The links between the D-allele and left ventricular size after myocardial infarction are interesting. After an anterior myocardial infarction, the DD-ACE genotype seems to be associated with progressive left ventricular dysfunction. Pinto et al. [39] selected 98 patients from the Captopril and Thrombolysis Study (CATS) who were recruited immediately after their anterior myocardial infarction. Pinto reported a significant increase in echocardiographically determined end-systolic volume and diameter in patients with the DD rather than ID or II-ACE genotype. In addition to this finding, he also demonstrated for the first time that the apparent deleterious effects of the D-allele were abolished by treatment with an ACE inhibitor. If confirmed, then this finding alone has tremendous importance. A Japanese study [40] also showed that in 79 post-infarct patients who underwent coronary angiography (including bi-plane left ventriculography) the ejection fraction was inversely linked to the D-allele; the ejection fraction was significantly different \((P \text{ value} = 0.02)\) with values for DD, ID and II subgroups of 51%, 56% and 62% respectively. There is also a possible arrhythmogenic risk post-myocardial infarction, with evidence emerging that the D-allele correlates to late potentials on signal-averaged ECG recordings [41]. Although both of these studies [39, 40] are relatively small, they address a problem of significant cost to the health service, i.e. the burden of progressive left ventricular impairment after myocardial infarction. If a subgroup who are at particular risk of progressive ventricular dilatation can be identified and successfully treated, a significant morbidity and mortality may be avoided. This problem should therefore be addressed specifically in large prospective well-designed studies.

There appears to be conflicting evidence in the restenosis rate after percutaneous transluminal coronary angioplasty. Samani et al. [42] found no difference after 4 months of follow-up in 233 patients who underwent single-vessel percutaneous transluminal coronary angioplasty as part of the Subcutaneous Heparin and Angioplasty Restenosis trial (SHARP trial). In a similar study, Hamon et al. [43] followed 118 patients for approximately 7.4 months after single-vessel percutaneous transluminal coronary angioplasty and reported that both acutely and at follow-up there was no difference in arterial patency. However, two small studies have suggested that the opposite may be true [44, 45].

Hypertension and left ventricular hypertrophy

Hypertension \textit{per se} does not appear to be associated with the ACE-gene polymorphism. This has been shown in several studies in humans from different regions including the U.K. [46], Belgium [47] and the United Arab Emirates [48]. Other studies have addressed the issue of DD-ACE genotype and left ventricular hypertrophy (LVH). In a German cross-sectional study, Schunkert et al. [49] showed the DD genotype to be more commonly associated with LVH with an OR of 1.76 (95% CI 1.22–2.53; \(P = 0.003)\), which in men increased to 2.63 (95% CI 1.50–4.64; \(P < 0.001)\), and in those who were normotensive the OR increased further to 4.05 (95% CI 1.76–9.28; \(P = 0.001)\). The entire study population of 1428 subjects were Caucasian and of western European descent. There was no apparent link with hypertensive or normotensive women in this study. Electrocardiographic evidence of LVH was found in 290 individuals (149 men and 141 women) but of those only 38% were hypertensive. Criticism of this study may lie in the fact that LVH was not confirmed by any other method, since electrocardiographic LVH is a less sensitive measure than echocardiography [50], although the use of digital acquisition is well validated in clinical outcome studies where electrocardiographic LVH carries an adverse prognostic risk [51]. The finding that normotensive individuals may have LVH is reinforced by results from the Framingham study [52] which showed that 56% of middle-aged men and women had echocardiographic LVH with systolic blood
pressures of less than 140 mmHg. Iwai et al. [53] showed a significant link between left ventricular mass and DD-ACE genotype in a cross-sectional study of patients selected from a hospital outpatient department. They found that there was no association between the D-allele and blood pressure in 268 individuals. Further investigation of 142 subjects with echocardiography identified that the DD-ACE genotype was highly significantly associated with the derived ratio of left ventricular mass/height. In a study based in Scotland, Prasad et al. [54] assessed 83 patients with essential hypertension echocardiographically and found that increased left ventricular mass only correlates with blood pressure when the DD-ACE genotype is present. This finding, although not yet confirmed in other studies of hypertension, implies a need to rethink the principle that haemodynamic overload is the primary determinant of development of left ventricular hypertrophy, and that perhaps those with hypertension and the D-allele are more at risk.

Conflicting data exist however; a small Finnish study [55] reported that in 86 subjects who were considered to be free of cardiovascular disease, there was no correlation between ACE genotype, LVH or left ventricular muscle mass. There was also no correlation between the indices of systolic and diastolic dysfunction and DD-ACE genotype.

**Cardiomyopathies**

The DD-ACE gene polymorphism has an uncertain role in the pathogenesis of cardiomyopathies. Reynolds et al. [29] found that the occurrence of the DD genotype was 48% higher in idiopathic cardiomyopathy and 63% more common in those whose cardiomyopathy was of an ischaemic aetiology (112 and 102 patients respectively). These patients had end-stage cardiac failure and had either undergone or were awaiting cardiac transplantation. Interestingly, controls were the donor heart patients, who had undergone cardiac investigation in the pre-transplant evaluation procedure and were genotyped either from the endomyocardial biopsy at routine follow-up or from stored donor blood samples before implantation. Both donors and recipients/prospective recipients were white Americans. Because of the early nature of this work, the possibility of some mistyping of ID genotype as DDs exists. However, this was disputed when a conflicting study by Montgomery et al. [56] found no relationship. The latter study evaluated 99 patients who had idiopathic dilated cardiomyopathy and compared them with 364 control subjects. The genotype distribution was not different between study groups and, perhaps more interestingly, when followed up for a mean of 28 months, there was no association with the severity of disease progression. A similar prospective study by Andersson and Sylven [57] also found that there was no significant difference in the frequency of the D-allele at the study inception, but that the D-allele was associated with a significant worse prognosis. They studied 193 patients with idiopathic congestive heart failure out of a population of 2711 patients and matched them to 77 controls from the general population. Left ventricular function was assessed using echocardiography. At follow-up after 5 years, four controls (5%) had died; II and ID were considered together of which 37 (29%) had died; of the DD genotypes 34 (51%) had died. This equated with a significant correlation with mortality ($P = 0.006$), and using proportional analysis the DD genotype ($P = 0.04$), NYHA functional class ($P = 0.014$) and left ventricular systolic dimension ($P<0.0001$) were independent predictors of mortality. Methodological problems with this study were that the patients were recruited from a population of patients with a discharge diagnosis of heart failure which was described between 1985 and 1988, therefore a variable amount of time elapsed before patients were enrolled in this prospective study. The authors estimated that concealed coronary artery disease was present in 16% of the study population.

These data suggest that with the D-allele and dilated cardiomyopathy, the role of the D-allele remains unclear, but the work by Andersson and Sylven [57] suggests that there may not be an association between the genesis of dilated cardiomyopathy but rather that the D-allele acts to worsen its prognosis. However, because of the problems with the time since diagnosis with the majority of cardiovascular diseases, if the D-allele both increases the incidence of dilated cardiomyopathy and worsens its prognosis, then the D-allele is likely to be under-represented in the majority of studies at their inception.

There seems to be more consensus with hypertrophic obstructive cardiomyopathy (HOCM). Both American [58] and Japanese [59] groups have shown that the D-allele occurs more frequently in this condition, although the likely clinical impact may be small because of the genetic heterogeneity of this condition. Marian et al. [58], in a short report in *The Lancet*, showed that the D-allele was associated with an increased incidence of HOCM and an increased frequency of sudden cardiac death. The D-allele was found in 69% of patients with HOCM compared with 57% of their unaffected relatives ($P = 0.021$). Some genetic mutations of HOCM are linked to a high incidence of sudden cardiac death. The DD-ACE genotype appeared to be linked to this greater risk of sudden cardiac death; the relative frequency of the D-allele was 0.74 compared with 0.55 ($P = 0.011$) in those who died suddenly. Yoneya et al. [59] examined 80 patients with HOCM and compared them with 88 unaffected siblings. Forty three of the 80 subjects with HOCM were related and likely to have the same genetic origin for the condition, although it was actually found that several were involved. The D-allele was more common in the HOCM group than in the sibling group (42% compared with 35%, $P<0.05$) but, interestingly, spon-
D-allele compared with familial cases which had an OR of 2.97 (95% CI 1.19–7.40; P = 0.035) for the D-allele compared with familial cases which had an OR of 1.46 (not significant). There was no link with any of the described HOCM genotypes in this study.

**Interaction of DD-ACE gene with angiotensin II type 1 receptor (AGT1R) gene**

It is likely that cardiovascular disease, a heterogeneous and multifactorial phenotype, is partly determined by several genes and that the ACE polymorphism is but one candidate gene. Another potential candidate is the AGT1R gene and recently studies have looked at the interaction between these two polymorphisms.

Tiret et al. [60] selected patients from ECTIM study group who had suffered a definite myocardial infarction. A polymorphism of the AGT1R gene (point transversion A→C) was evaluated in 613 patients and 723 control subjects. Those patients who carried the DD genotype and were homozygotes for the A-alleles in the AGT1R gene had an OR of 1.05 (not significant) for a myocardial infarction, but when AC was present the OR was 1.52 (95% CI 1.06–2.18) and for CC, 3.95 (95% CI 1.26–12.4, test for trend P < 0.02). When limited to those subjects with a low BMI and apolipoprotein B, the OR increased even further to DD+AA = 1.64 (95% CI 0.68–3.92), DD+AC = 7.03 (2.61–19.0, P < 0.0001) and DD+CC = 13.39 (0.79–707, P = 0.05).

This paper follows a similar format to the original work by Cambien et al. [5], indeed the same study population was used. Similar criticisms therefore exist; firstly, patients had suffered a myocardial infarction and survived the intervening 6 to 9 months before recruitment. Secondly, we rely on subgroup analysis because the overall results are negative and in this case they look at a low-risk group and those who are homozygous for the C allele. Therefore, these data should be viewed with caution as the numbers in the DD-CC group with low traditional risk factors may mean that a positive link has been missed. Work by Bonithon-Kopp et al. [62] looked at a large sample (n = 1036) of western European subjects and screened for carotid wall thickness. From this they generated a case-control study (96 pairs), matching for the sex, ultrasonographer and degree of atheroma. They found that those subjects who had intimo-medial thickening had a marginally higher plasma ACE activity, but after the exclusion of subjects taking lipid-lowering therapy a significantly higher level of ACE activity was seen in those who had intimo-medial thickening compared with normal carotid dimensions (29.9 ± 7.7 compared with 27.5 ± 8.0 units/l, cases versus controls, P < 0.03). No analysis was performed for the DD-ACE genotype, which may have provided important evidence of the mechanism of the polymorphism's proposed effects. For instance, this may have suggested whether it acts via increased drive on growth factors.

There seems to be little evidence to suggest that there is a small increased risk of ischaemic stroke, perhaps more specifically of lacunar infarcts.

**Possible mechanisms of the interaction of the DD-ACE genotype and cardiovascular disease**

No convincing evidence is yet available which adequately explains the mechanism by which the DD-ACE genotype exerts its protein manifestations. The mere fact that the alu repeat sequence lies within an intron would suggest that it does not
have a role directly in the regulation of ACE, but that it may alter the promoter region activity of the gene. Three plausible explanations are being explored: (i) ACE genotype influencing vascular responsiveness to ANG II, (ii) ACE genotype causing an alteration in endothelial function and (iii) ACE genotype influencing underlying metabolic control mechanisms, i.e. insulin resistance.

As mentioned, ACE has two main physiological functions, firstly the conversion of ANG I to ANG II and secondly the degradation of bradykinin. Both of these alter the balance of endothelial control of arterial blood flow increasing angiotensin II levels (vasoconstriction, increasing blood pressure and total peripheral vascular resistance, exerting trophic cellular changes) and reduced bradykinin (reduced endothelium-dependent vasodilator nitric oxide and prostacyclin).

Vascular responsiveness to ANG II

Two studies have addressed this directly. Lachurie et al. [63] found that in 24 subjects there was no difference in plasma levels of ANG I and II or any potentiation of their vascular effects. They compared 12 subjects with the DD genotype to 12 with the II genotype. Endogenous ANG I and II was effectively suppressed with an infusion of a renin inhibitor (Remikiren). No difference was found in either haemodynamic parameters or the production of ANG II from the infused ANG I between the DD and II groups. These data cannot exclude a significant effect of the D-allele on the conversion of ANG I to ANG II at specific tissue sites. However, other work contradicts this. Ueda et al. [64] found that in 20 subjects (10 with DD and II respectively), those who were homozygous for the D-allele had an enhanced pressor response to ANG I (95% CI 1.3–8.7, \(P = 0.0091\)) and also produced a larger rise in ANG II levels \((P < 0.01)\). In this work endogenous ANG I and II were not suppressed before the infusion of exogenous agents, although the findings were supported by Buikema et al. [65]. These conflicting data need to be resolved, as this mechanism may play a crucial role in any putative adverse cardiovascular risk. Intriguingly, both study groups [63, 64] originate from areas where the D-allele has been seen to have its most consistent effects (U.K. and northern France).

Endothelial function

Bradykinin acts as an endothelium-dependent vasodilator, using nitric oxide to some extent, and ACE determines bradykinin breakdown [66]. Investigating this, Celermajer et al. [67] looked at 184 subjects using high-resolution ultrasound of the brachial artery. In the absence of any cardiovascular disease, there was no link between the D-allele and endothelial dysfunction. However, a study by Zeiher et al. [68] demonstrated that in patients with angina who had mild or minimally affected coronary arteries, a defect exists in endothelium-dependent vasodilatation within the coronary circulation in those with the DD-ACE genotype. This was assessed by changes in the intraluminal diameter of epicardial arteries in response to intracoronary acetylcholine, an endothelium-dependent vasodilator. Further evidence [10] suggests that the DD genotype is associated with an increased basal production of nitric oxide but a decreased level of release to an endothelium-dependent vasoactive agent, in this case methacholine.

Insulin resistance

Insulin resistance is another possible mechanism through which the DD-ACE genotype may act. The evidence relies on two areas; firstly, ACE inhibitor therapy results in improved insulin sensitivity in the majority of studies [69] and secondly, the unproven hypothesis that the D-allele may impart a lower basal level of nitric oxide production and consequently lower basal blood flow. Recent evidence has emerged suggesting skeletal muscle blood flow as a major determinant of peripheral glucose uptake [70]. Some data [71] suggested that the D-allele is strongly associated with coronary artery disease in non-insulin-dependent diabetes mellitus (NIDDM). This led to the hypothesis that the D-allele may be linked to the insulin resistance that characterizes NIDDM. However, two studies have found that there is no link between insulin resistance and the ACE gene polymorphism. Panahloo et al. [72] found if anything, the opposite relationship between genotype and insulin sensitivity in 83 patients with NIDDM when compared with 533 controls. The values for insulin sensitivity in the control group were 57.1 versus 56.5%, DD versus II genotypes; for the NIDDM group these values were 56.4 versus 29.4%, DD versus II genotypes \((P = 0.012\) as \(t\)-test between DD and II genotype), expressed as a percentage of normal controls. In this study insulin sensitivity was assessed using the HOMA method (homoeostasis model assessment) rather than the gold standard, the euglycaemic hyperinsulinaemic clamp, but this method correlates well with clamp techniques [73]. Katsuya et al. [74] found that in 57 patients with NIDDM there was no association between genotype and insulin resistance, glucose intolerance, hyperinsulinaemia or dyslipidaemia when compared with 124 controls. Indeed, the only statistically significant difference was that in the normal control group those with DD genotype were actually more insulin sensitive and had a lower BMI.

CONCLUSION

Many of the observational studies are compromised because of publication bias, post-hoc subgroup analysis and unmeasured confounding factors
such as racial differences. Nevertheless, taken together, the available evidence supports the notion that the DD-ACE genotype exerts a modest influence on cardiovascular diseases (myocardial infarction, LVH, some cardiomyopathies and stroke) but appears to do so mainly in specific geographical areas (northern Europe, parts of the U.S.A. and Japan) and in particular patient subgroups (low BMI, low apoB). It is not yet known whether it does this through an interaction with other genes or by linkage equilibrium with an undiscovered genetic polymorphism or by as yet unexplained biochemical mechanisms. It is unclear why the DD genotype should alter clinical events such as myocardial infarction but not have any demonstrable effect upon the severity of occlusive vascular disease. We should, however, regard the current data with the ACE genotype as an intriguing new clue in the pathogenesis of cardiovascular disease. The way forward would appear to be large prospective studies to further define its clinical consequences allied to detailed mechanistic studies to establish the route by which the DD-ACE genotype exerts its harmful effects. Such knowledge may lead to novel therapeutic opportunities for cardiovascular disease and may enable us to target intervention strategies according to each subject’s ACE genotype. The main factor against this however is that the impact of the DD genotype appears to be small and its clinical manifestations rather heterogeneous.

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