**Angiotensin-converting enzyme gene polymorphism and premature coronary heart disease**

Frank M. Van Bockxmeer*†, Cyril D. S. Mamotte‡, Valerie Burke§ and Roger R. Taylor§

*Department of Pathology, The University of Western Australia, Perth, Western Australia, Australia, †Department of Biochemistry, Royal Perth Hospital and the West Australian Heart Research Institute, Perth, Western Australia, Australia, ‡Department of Clinical Immunology and Biochemical Genetics, Royal Perth Hospital and the West Australian Heart Research Institute, Perth, Western Australia, Australia, §Department of Medicine, The University of Western Australia, Perth, Western Australia, Australia, and ||Department of Cardiology, Royal Perth Hospital and the West Australian Heart Research Institute, Perth, Western Australia, Australia

**Abstract**

Since the initial report of the association of the deletion/insertion (D/I) polymorphism in the gene for angiotensin-converting enzyme (ACE) with myocardial infarction (MI), there has been considerable controversy. Some have found the D allele to be associated with MI, coronary heart disease (CHD) or other cardiac pathology, while others have not. In the present study 713 consecutive patients, < 50 years of age, documented prospectively with angiographic CHD (> 50% diameter stenosis of at least one coronary artery), with or without MI, were studied, along with 688 community control subjects, also < 50 years of age, selected randomly from the electoral rolls and without a history of CHD or MI. Genotyping was done by standard methods. Most of the subjects in both groups were Anglo–Celtic Caucasians (547 in the CHD group and 642 in the community group), and the report concerns primarily these subjects. ACE genotype distributions were not different between the Caucasian community control group and the CHD or the MI subgroups; the odds ratios and 95% confidence limits for the CHD group were 0.96 (0.73–1.27) for the D allele and 1.02 (0.80–1.31) for D homozygotes; for the MI group these values were 1.00 (0.83–1.20) and 0.99 (0.74–1.32) respectively. This negative result was supported in multivariate analysis accounting for conventional risk factors. There was a significant racial difference in ACE genotypes between Caucasians, Asians and Australian Aborigines in the CHD group (P < 0.001); for example, in this group, 158 of 540 (29%) Caucasians had the DD genotype compared with eight of 84 (10%) Aborigines (P < 0.001) and six of 59 (10%) Asians (P = 0.002). Failure to account for such racial differences would have led to erroneous conclusions. In conclusion, we found no evidence that the D/I ACE gene polymorphism plays a role in the development of CHD or MI at an early age in a Western Australian Caucasian population. While this result refers uniquely to premature CHD and MI, and could be population specific, it is in general agreement with recent meta-analysis of the larger previous studies.

**Key words:** ACE gene, coronary heart disease, myocardial infarction.

**Abbreviations:** ACE, angiotensin-converting enzyme; CHD, coronary heart disease; CI, confidence interval; D/I, deletion/insertion; MI, myocardial infarction.

**Correspondence:** Professor Roger R. Taylor, Department of Cardiology, Royal Perth Hospital, GPO Box X2213, Perth 6847, Western Australia, Australia (e-mail heletoey@rph.health.wa.gov.au).
INTRODUCTION

Controversy surrounds the role of the angiotensin-converting enzyme (ACE) deletion/insertion (D/I) polymorphism in coronary heart disease (CHD) and myocardial infarction (MI). Since the D allele was related to MI in the ECTIM study [1], there have been confirmatory, negative and even contrary (implicating the I allele) reports, as reviewed by Samani et al. [2] and more recently by Keavney et al. [3]. In the present study we have documented prospectively over 6 years patients with angiographic CHD with or without MI. A large, randomly recruited community group of similar age was also studied.

METHODS

Study subjects

A total of 713 subjects of age < 50 years (mean ± S.E.M. 43.6 ± 0.2 years) with CHD and 688 community control subjects, of similar age, were studied. However, this report concentrates on the 547 Caucasian patients in the former group and the 642 Caucasian subjects in the latter. The study was approved by the Ethics Committee of the hospital, and all subjects gave informed consent.

The CHD subjects presenting to the hospital, over approximately 6 years, were documented prospectively for inclusion in the study and for risk factors for CHD and genetic analysis. Patients were required to have at least one obstruction of a major coronary artery (≥ 50% diameter) at angiography, either with (451 of 713; 63%) or without (37%) current or previous MI (based on historical, electrocardiographic and cardiac enzyme documentation).

The community group were subjects between 30 and 50 years of age (40 ± 0.2 years) who were selected at random from the electoral roll and took part in a survey of CHD risk factors in 1994. Each participant completed a questionnaire concerning lifestyle and medical history; their blood pressure, height and weight were recorded, and blood was taken for DNA extraction and genetic analysis. Only those without a history suggestive of CHD were included in the study; three subjects with such a history were excluded from this group.

The patient group, with premature CHD, was comprised predominantly of males (86%), while equal numbers of males and females were recruited in the community group. Racial background was documented accurately in the CHD group: the great majority of patients [547 of 713 (77%)] were Caucasian, 12% were of Australian Aboriginal descent and 8% were Asian. Australian Aboriginals (n = 84) and Asians (n = 59) had a significantly higher II genotype frequency than Caucasians (n = 540) (51% and 37% compared with 22% respectively; P < 0.01 for each) and a significantly lower DD frequency (10% and 10% compared with 29%; P < 0.001 and P = 0.002 respectively). Likewise, in the community group, Caucasians and Asian-born subjects had significantly different genotypes [Caucasian: II, 21%; ID, 49%; DD, 29% (n = 634); Asian: II, 45%; ID, 38%, DD, 17% (n = 29); P = 0.011], while there were no Aboriginals in this group.

Because of these racial differences, and the lack of adequate numbers of controls for other racial groups, the results for Caucasians only with regard to the relationship between ACE genotype and CHD are presented. ACE genotype did not differ between the sexes, and results concerning the influence of ACE genotype on CHD and MI were analysed regardless of gender.

Genotyping

Genomic DNA was extracted from EDTA-anticoagulated blood by a standard Triton X-100 procedure. Genotyping for the 287 bp D/I polymorphism in intron 16 of the ACE gene was carried out, with precautions against mistyping including the use of 63 °C as the annealing temperature and the use of PAGE (12% total; 3.3% cross-linker), rather than agarose-gel electrophoresis, for better resolution of the I and D products, as described previously [4].

Statistical methods

Variables are quoted as means ± S.E.M. Analysis of data was carried out with SPSS for Windows (SPSS Inc., Chicago, IL, U.S.A.). χ² tests were used to examine categorical variables in cross-tabulations. Generalized linear models were used for comparison of means, with adjustment for co-variates as required. Relationships with continuous dependent variables were examined in multiple linear regression with logistic models for dichotomous dependent variables. A P value of < 0.05 was considered significant.

RESULTS

Table 1 presents conventional risk factors for the Caucasian subjects in the CHD and community groups. History of hypertension and of diabetes, current or previous smoking and body mass index all differed significantly between the groups (P < 0.001). The lipid values quoted in Table 1, like the other variables, are for males and females together; however, since the value for each lipid variable in males was more adverse than in females, generalized linear models with adjustment for sex were used to compare groups. Each of the lipid variables was significantly different between groups (P < 0.001), except for low-density lipoprotein cholesterol. The latter was also not significantly different between groups when males and females were analysed separately.
Table 1  Characteristics of Caucasian CHD and community (control) groups

Abbreviations: BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein. Continuous variables are expressed as means ± S.E.M., except for triacylglycerols, which are expressed as the geometric mean and 95% confidence limits. For binary variables, percentages are given in parentheses. Each of the risk factor variables was significantly different between groups (P < 0.001), except for LDL cholesterol. The lipid values quoted are for males and females together, but the values were compared between groups with adjustment for sex.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CHD group (n = 547)</th>
<th>Control group (n = 642)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>43.9 ± 0.2</td>
<td>39.9 ± 0.2</td>
</tr>
<tr>
<td>Males</td>
<td>478 (87%)</td>
<td>329 (51%)</td>
</tr>
<tr>
<td>MI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Previous</td>
<td>167 (31%)</td>
<td>–</td>
</tr>
<tr>
<td>Current</td>
<td>164 (30%)</td>
<td>–</td>
</tr>
<tr>
<td>Previous and current</td>
<td>18 (3%)</td>
<td>–</td>
</tr>
<tr>
<td>Diabetic</td>
<td>76 (14%)</td>
<td>8 (15%)</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>192 (35%)</td>
<td>160 (25%)</td>
</tr>
<tr>
<td>Ex</td>
<td>252 (46%)</td>
<td>186 (29%)</td>
</tr>
<tr>
<td>History of hypertension</td>
<td>181 (33%)</td>
<td>107 (17%)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.4 ± 0.2</td>
<td>25.3 ± 0.2</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.46 ± 0.04</td>
<td>5.29 ± 0.05</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.03 ± 0.01</td>
<td>1.31 ± 0.01</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>3.42 ± 0.04</td>
<td>3.39 ± 0.04</td>
</tr>
<tr>
<td>Triacylglycerols (mmol/l)</td>
<td>1.88 (1.80, 1.97)</td>
<td>1.06 (1.02, 1.11)</td>
</tr>
</tbody>
</table>

Table 2 presents the ACE genotypes of the Caucasian subjects in the total CHD group, the MI subgroup and the community group; there were no significant differences in the distribution of genotypes.

Table 3 presents the odds ratios and 95% confidence interval (CI) when comparing the total CHD group and the MI subgroup with the community group for ACE genotype and conventional risk factors. By univariate logistic regression analysis, the odds ratio for the D allele was 0.96 (95% CI 0.73–1.27) for the total CHD group and 1.00 (0.83–1.20) for the MI subgroup, and for the DD genotype these values were 1.02 (0.80–1.31) for the total CHD group and 0.99 (0.74–1.32) for the MI subgroup. All variables significant in univariate logistic models were used in forward (likelihood ratio) stepwise regression. The final model is shown in the lower section of Table 3. In this analysis high-density lipoprotein cholesterol was the only lipid variable contributing independently to the prediction of CHD or MI; the other lipid variables and ACE genotype functions failed to improve the model with either CHD or MI as the dependent variable. Two subgroups in whom a genetic influence might be expected to play a particular role were analysed separately by logistic regression, namely those who had never smoked and those with a family history of CHD or MI in a first-degree relative occurring at age < 60 years. In neither subgroup was there a significant relationship between

Table 2  ACE genotypes in Caucasian CHD, MI and control groups

Percentages are given in parentheses.

<table>
<thead>
<tr>
<th>ACE genotype</th>
<th>CHD patients (Caucasians)</th>
<th>Community Caucasian group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total group (n = 540)</td>
<td>MI subgroup (n = 349)</td>
</tr>
<tr>
<td>II</td>
<td>120 (22%)</td>
<td>75 (21%)</td>
</tr>
<tr>
<td>ID</td>
<td>262 (49%)</td>
<td>173 (50%)</td>
</tr>
<tr>
<td>DD</td>
<td>158 (29%)</td>
<td>101 (29%)</td>
</tr>
</tbody>
</table>
The frequency of the D allele of the ACE gene in our community Caucasian subjects, who are of U.K. and continental European background, was 54%, which is the same as the overall frequency in the Caucasian-based studies reviewed by Samani et al. [2] and in the CHD cases and controls in the recently reported results from the large ISIS study [3]. We also found very similar D/I genotype frequencies in Caucasians with and without CHD or MI. In the meta-analysis of 15 studies to 1995 by Samani et al. [2], the DD genotype was found to confer an odds ratio for MI of 1.26 (95% CI 1.15–1.39). The largest individual study was published more recently, concerning MI in the ISIS study [3]. The odds ratio for the DD compared with other genotypes was 1.10 (95% CI 1.00–1.21), and that for D homozygotes and heterozygotes compared with I homozygotes was 0.97 (95% CI 0.87–1.08). Keavney et al. [3] also analysed the combined results from 35 smaller studies with less than 200 cases of MI and from the 14 larger studies with more cases. While the relationship between the DD genotype and MI was significant (odds ratio 1.57; 99% CI 1.38–1.78) in the smaller studies, it was not in the larger studies (odds ratio 0.99; 99% CI 0.90–1.08). This evidence of publication bias, suggesting that small negative studies tend not to be published, was similarly documented in the earlier meta-analysis [2]. Between these two meta-analyses [2,3], mixed results continued to be published, with negative results concerning MI from studies in North Karelian Finns [5], Austrians [6], for all patients in a study of Germans [7], and for CHD patients in Welsh subjects [8]. In a subsequent study from the German group [9], based on a large number of patients coming to diagnostic coronary angiography, the D allele was related to the extent of coronary artery disease, although not to MI, in young subjects only (< 61.7 years). Two of the above studies [7,8] reported positive results in selected ‘low-risk’ subgroups, as noted in the original ECTIM report [1], but the variable and rather arbitrary nature of subgrouping could be questioned. Low-risk subgroups were examined in the substantial number of MI cases and controls in the ISIS study [3], with negative results, leading to the conclusion that most of the positive results with ‘low-risk’ subgroups were probably spurious.

Because current or past cigarette smoking was such a dominant risk factor in our study, being present in 80% of CHD patients, we examined only non-smokers as a low-risk group, but found no effect of the ACE genotype. However, there may be a real effect of the ACE genotype in some particular groups. For example, a recent study examined the effect in a high-risk group (213 subjects with heterozygous familial hypercholesterolaemia or familial defective apolipoprotein B100) of average age 57 years, and found an odds ratio for the DD genotype of 2.5 for MI and 2.2 for CHD in males [10].

The role of the D allele in contributing to MI or CHD may also depend upon the frequency of the D allele in the population and upon other racial aspects; the odds ratio for the DD genotype and MI was higher in the Japanese than in the Caucasian studies reviewed by Samani et al. [2]. Race is an important consideration in studies of the ACE genotype, as emphasized by others [2,11] and illustrated in the present study. There was a lower D allele frequency in our Asian compared with our Caucasian subjects (36% and 54% respectively), in line with the values of 39% found in the three Japanese studies reviewed by Samani et al. [2], 36% reported for Han Chinese [12] and 30% for Singaporean Chinese [13] and Thais [14]. In our study, the D allele frequency in Australian Aboriginals was also low, at 29%. If our data had been analysed regardless of race, a fallacious conclusion would have been reached.

Another important consideration is the nature of the recruitment of both cases and controls. Our documentation of cases was carried out prospectively, at the time of hospital presentation, specifically for the purpose of risk factor and genetic studies. Recruitment of controls varies between studies and is particularly contentious; we consider that our use of a randomly selected community group is the optimum. Admittedly, some ‘controls’, even aged under 50 years, might have covert CHD. A control group with ‘no or minimal angiographic coronary obstructive disease at angiography’ has been used in numerous studies. Obviously such patients have usually had coronary angiography because of symptoms, and a substantial number are likely to have a pathological or functionally abnormal coronary circulation.

Despite our negative result and the recent conclusion, from the large ISIS study and meta-analysis of the larger...
earlier studies, that ACE genotype plays little, if any, role in MI [3], the greater frequency of the D allele or the DD genotype reported in children with parents [15] or grandparents [16] with a history of MI or CHD strengthens the concept that there is a real relationship between these factors in some populations. If so, the commonly studied D/I polymorphism may not itself be responsible, but instead one or more of the many polymorphisms in the ACE gene that have been described recently [17].

In conclusion, we did not find a relationship between the ACE genotype and CHD or MI in our Caucasian population. There probably is a relationship in some populations, but the nature of the CHD or MI group studied, the method of recruitment (especially of the control group) and racial differences are important considerations.

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REFERENCES


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