Analysis of the postulated interaction between the angiotensin II sub-type 1 receptor gene A1166C polymorphism and the insertion/deletion polymorphism of the angiotensin converting enzyme gene on risk of myocardial infarction

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Abstract

A synergistic interaction between the insertion/deletion (I/D) polymorphism within the angiotensin-converting enzyme (ACE) gene and an A/C transversion at nucleotide position 1166 within the angiotensin II sub-type 1 receptor (AT1R) gene on risk of myocardial infarction has been reported. The risk associated with the ACE DD genotype increased with the number of AT1R C alleles present. To investigate this further, ACE I/D and AT1R A1166C genotypes were determined in 541 cases recruited at the time of infarction and 507 population-based controls. There was no difference in either the genotype distribution or allele frequencies between cases and controls for either the ACE polymorphism (P = 0.48 and 0.35 respectively) or the AT1R polymorphism (P = 0.35 and 0.21 respectively). Odds ratios for risk of MI associated with the ACE DD and AT1R CC genotypes were 1.09 (95% CI, 0.82–1.45) and 1.06 (0.67–1.68) respectively. 3.1% of cases versus 3.6% of controls were homozygous for both the D and C alleles (P = 0.71). There was no increase in risk associated with the DD genotype in the presence of either one or two AT1R C alleles in the whole cohorts (OR 0.99, 95% CI 0.65–1.51 and 0.76, 95% CI 0.30–1.88, respectively) nor in sub-groups defined by specific risk factors. In conclusion, no evidence was found to support any interaction between the ACE gene I/D polymorphism and the AT1R gene A1166C transversion in determining the risk of myocardial infarction in the population studied. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Gene polymorphism; Angiotensin receptors; Angiotensin converting enzyme; Myocardial infarction

1. Introduction

Epidemiological studies have identified several important risk factors for ischaemic heart disease, although these only partly explain the occurrence of acute coronary syndromes [1]. A positive family history is a strong indicator of risk, part of which accrues from a shared genetic inheritance and part from experiencing a shared early environment [2]. Genetic factors exert their influence on susceptibility to atherosclerosis and acute thrombosis by the modulation of plasma and tissue levels of their biologically active products. Environmental factors may subsequently modify this influence in such a way as to either increase or decrease the associated risk. Acute myocardial infarction is a complex clinical event which, is the culmination of a multiplicity of interacting pathologic processes. In contrast to rare monogenetic diseases, risk of ischaemic heart disease is likely to be carried by several genes, capable of multifaceted interactions with each other and with environmental factors [3].

The best example of a gene–gene interaction influencing the risk of MI reported to date is the purported interaction between the A1166C polymorphism in the angiotensin II sub-type 1 receptor (AT1R) gene and the insertion/deletion (I/D) polymorphism in the an-
giotensin converting enzyme (ACE) gene. In Caucasian male subjects recruited in four European centres 3–9 months following their MI compared with unaffected controls, Tiret et al. [4] found that the risk associated with the ACE DD genotype compared with the ACE II/ID genotypes increased from 1.05 (95% CI 0.75–1.49) in those bearing the AT1R AA genotype, to 3.95 (95% CI 1.26–12.4) in those homozygous for the AT1R CC genotype with an intermediate risk (1.52) (1.06–2.18) in AC heterozygotes. The risk was particularly increased in subjects considered at low risk of MI on the basis of a low apolipoprotein B level and body mass index [4]. If such an interaction could be confirmed it would represent an important advance in our understanding of the genetic basis of acute coronary syndromes and could provide a valuable tool for risk stratification. Therefore, the aim of this study was to further examine the postulated interaction between the ACE I/D and AT1R A/C gene polymorphisms in a cohort of subjects with acute myocardial infarction and population-based controls.

2. Methods

2.1. Subjects

The method of recruitment of subjects has been described previously [5]. In brief, cases were recruited from patients admitted to the coronary care units (CCU) of the Leicester Royal Infirmary, Leicester (July 1993–April 1994) and the Royal Hallamshire Hospital, Sheffield (November 1995–March 1997). Patients were recruited who satisfied the World Health Organisation criteria for acute myocardial infarction in terms of symptoms, elevations in cardiac enzymes or electrocardiographic changes [6]. Recruitment criteria were identical in the two centres and more than 95% of eligible subjects were enlisted. Control subjects were recruited in each hospital from adult visitors to patients with non-cardiovascular disease on general medical, surgical, orthopaedic and obstetric wards to provide subjects likely to be representative of the source population from which the subjects originated. Subjects who reported a history of coronary heart disease were excluded. Again, >95% controls approached agreed to take part. Data on the ACE gene I/D polymorphism from the current Leicester cohort and a previous Sheffield cohort have been reported [7], but a new cohort of cases and controls was recruited for this study in Sheffield to optimise the matching of cases and controls and to improve the data available on classical risk factors for MI.

After informed consent was obtained, cases and controls filled in a standard questionnaire about their personal histories, had height and weight measured, and provided blood samples for genotype analysis and measurement of serum total cholesterol. For the purpose of this study, genotype analysis was restricted to Caucasian cases <75 years of age. The studies in both centres were approved by the respective local clinical research ethics committees.

2.2. Genetic analysis

Deoxyribonucleic acid (DNA) was prepared from a small aliquot of whole blood collected in ethylenediamine tetraacetic acid by using a DNA extraction matrix (Instagene, Biorad, Hemel Hempstead, Hertfordshire, England) and following standard techniques [8]. The extracted DNA was stored at –70°C until analysis. The ACE gene D and I alleles were identified on the basis of polymerase chain reaction (PCR) amplification of the respective fragments from intron 16 of the gene, as previously described [9]. In order to reduce mistyping of ID heterozygotes as DD homozygotes, 5% dimethylsulfoxide was included in the reaction mixture [10]. Amplified fragments were analysed on ethidum-bromide agarose gels as previously described [7].

The AT1R A1166-C polymorphism was also assayed by PCR amplification with a slight modification of the method described by Bonnardeaux et al. [11]. Briefly, 100 ng of DNA was amplified with 100 pmol/l of the sense (5’ GCT TTG TCT TGT TGC AAA AGG 3’) and anti-sense (5’CCC ACT CAA ACC TTT CAA CA 3’) oligonucleotide primers in a final volume of 100 μl. The DNA was amplified for 35 cycles with denaturation at 94°C for 1 min, annealing at 52°C for 1 min, and extension at 72°C for 1 min. 10 μl of PCR product was digested overnight at 37°C with 1 unit of DdeI in a final volume of 20 μl containing 5 mg bovine serum albumin according to the manufacturers’ instructions (New England Biolabs). This reaction yielded fragments of 143, 106 and 47 base pairs in the presence of the C allele, and 249 and 47 base pairs in the presence of the A allele. After the addition of 2 μl of sucrose-based loading buffer, the reaction product was loaded onto a 6% polyacrylamide gel (polyacrylamide to bisacrylamide ratio of 40:1). Electrophoresis was performed at 200 v for 30 min at room temperature. The DNA fragments were visualised by ultraviolet transillumination after staining with ethidium bromide.

Blank controls were routinely included with each set of amplifications to exclude contamination. Genotypes were determined without knowledge of the case or control status, and about 10% were randomly repeated for both polymorphisms. All repeats confirmed the original genotypes.
Table 1
Characteristics of cases and controls according to centre

<table>
<thead>
<tr>
<th></th>
<th>Leicester</th>
<th>Controls</th>
<th>P</th>
<th>Leicester</th>
<th>Controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>62 ± 9</td>
<td>61 ± 9</td>
<td>0.28</td>
<td>62 ± 9</td>
<td>55 ± 12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male (%)</td>
<td>64</td>
<td>62</td>
<td>0.49</td>
<td>72</td>
<td>61</td>
<td>0.01</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>26 ± 4</td>
<td>26 ± 4</td>
<td>0.99</td>
<td>26 ± 4</td>
<td>25 ± 4</td>
<td>0.29</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>6.0 ± 1.2</td>
<td>5.9 ± 1.0</td>
<td>0.22</td>
<td>5.7 ± 1.2</td>
<td>5.6 ± 1.0</td>
<td>0.49</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>29</td>
<td>18</td>
<td>&lt;0.001</td>
<td>34</td>
<td>17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>8</td>
<td>2</td>
<td>&lt;0.001</td>
<td>9</td>
<td>4</td>
<td>0.06</td>
</tr>
<tr>
<td>Current smokers (%)</td>
<td>41</td>
<td>15</td>
<td>&lt;0.001</td>
<td>41</td>
<td>20</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

2.3. Biochemical measurements

Serum total cholesterol was measured using a Kodak Ektachem E700 CXR Automatic Analyser in Leicester and an Olympus AU 5223 machine in Sheffield in national quality-controlled hospital biochemistry laboratories. For cases, the first blood sample taken following admission was used for analysis.

2.4. Statistical analysis

The distribution of the ACE I/D and ATIR A/C genotypes and qualitative risk factors for patients and control subjects were compared using the chi-square test. Quantitative sample means were compared by analysis of variance. Odds ratios and 95% confidence intervals (CI) estimating the relative risk of myocardial infarction associated with the DD/CC genotype were calculated using the Mantel–Haenszel method with stratification, where described, for age, sex and centre.

3. Results

In total, 1048 subjects (541 cases of acute myocardial infarction and 507 control subjects) were analysed from the two centres. Table 1 summarises the characteristics of the cases and controls in each centre. The Sheffield cohorts were well matched for age and sex. In Leicester, the cases were significantly older than controls (average 7 years) and there was a slight male preponderance in the cases. In both centres, approximately one-third of cases had suffered with pre-existing ischaemic heart disease. As would be expected, several classical risk factors (hypertension, diabetes, and smoking prevalence) for acute myocardial infarction were more prevalent in the cases than controls in both centres. However, body mass index and total cholesterol level were similar in cases and controls.

The distributions of the ACE I/D and the ATIR A/C alleles and genotypes in cases and controls are shown in Table 2. For both polymorphisms, all cohorts were in Hardy–Weinberg equilibrium. There was no difference

Table 2
Distribution of ACE I/D and ATIR A/C genotypes in cases and controls

<table>
<thead>
<tr>
<th>ACE</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>II</td>
<td>ID</td>
</tr>
<tr>
<td>Leicester</td>
<td>54 (23)</td>
<td>107 (45)</td>
</tr>
<tr>
<td>Sheffield</td>
<td>50 (16)</td>
<td>155 (51)</td>
</tr>
<tr>
<td>Total</td>
<td>104 (19)</td>
<td>262 (48)</td>
</tr>
<tr>
<td>Allele frequencies</td>
<td>I = 0.434 D = 0.566</td>
<td>I = 0.454 D = 0.546</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ATIR</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA</td>
<td>AC</td>
</tr>
<tr>
<td>Leicester</td>
<td>121 (51)</td>
<td>97 (41)</td>
</tr>
<tr>
<td>Sheffield</td>
<td>143 (47)</td>
<td>128 (42)</td>
</tr>
<tr>
<td>Total</td>
<td>264 (49)</td>
<td>225 (42)</td>
</tr>
<tr>
<td>Allele frequencies</td>
<td>A = 0.696 C = 0.304</td>
<td>A = 0.721 C = 0.279</td>
</tr>
</tbody>
</table>

*a Numbers in parentheses are % of total.
in genotype distributions between the Sheffield and Leicester cohorts. For the ACE I/D polymorphism, there was no significant difference in either the genotype distribution (\(P = 0.48\)) or the allele frequencies (\(P = 0.35\)) between cases and controls. The age, sex and centre-stratified odds ratio (OR) for risk of MI associated with the DD genotype compared with the combined ID/II genotypes was 1.09 (95% CI 0.82–1.45, \(P = 0.57\)). Likewise, there was no significant difference in the genotype distribution (\(P = 0.35\)) or allele frequencies (\(P = 0.21\)) for the AT1R A/C polymorphism (Table 2). The age, sex and centre-stratified OR for risk of MI associated with the CC genotype compared with the combined AA:AC genotypes was 1.15 (95% CI 0.89–1.50, \(P = 0.31\)), and the OR for the AC/CC combined genotypes compared with AA genotype was 1.06 (95% CI 0.67–1.68, \(P = 0.86\)). For both polymorphisms, there was no increase in risk in sub-groups stratified by other risk factors (body mass index, total cholesterol, smoking status, hypertension or diabetes) (data not shown).

Table 3 shows the relative distribution of ACE I/D and AT1R A/C genotypes in cases and controls. There was no increase in the number of DD:CC homozygotes in cases compared with controls (3.1 vs 3.6%, \(P = 0.71\)). No interaction was observed between the two polymorphisms on risk of MI. Specifically, there was no graded increase in risk associated with the DD genotype in the presence of an increasing number of AT1R C alleles. Thus, the OR associated with the DD genotype in subjects with the AA genotype was 1.19 (0.81–1.73, \(P = 0.36\)), in subjects with the AC genotype 0.99 (0.65–1.51, \(P = 0.97\)) and in subjects with the CC genotype 0.76 (0.30–1.88, \(P = 0.51\)). The risk associated with the combined genotypes remained non-significant when subjects were stratified by either age, sex, body mass index or total cholesterol (Table 4).

4. Discussion

Genetic interactions influencing clinical phenotypes are much more plausible when the genes concerned influence the same or related biochemical pathway or cellular process. In this regard, the reported interaction between the ACE gene I/D polymorphism and the AT1R A/C polymorphism is attractive. There is considerable evidence implicating the renin-angiotensin system in vascular biology [12]. Further the ACE I/D polymorphism has a major influence on plasma and tissue ACE levels [13,14] which in turn could affect angiotensin II generation [15], the direct effector molecule for the AT1 receptor. Although, a direct functional effect of the AT1R gene A/C polymorphism, which is located in the 3’ untranslated region of its mRNA, has not been identified, the polymorphism has been associated with increased vascular reactivity [16] and arterial stiffness [17], suggesting a biological effect perhaps through linkage disequilibrium with another polymorphism.

There is an on-going debate about the importance of the ACE I/D polymorphism in influencing the risk of MI [18,19]. Further, most studies that have investigated the AT1R A/C polymorphism have not observed

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Table 3

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA</td>
<td>AC</td>
</tr>
<tr>
<td>II</td>
<td>44 (8.1)</td>
<td>47 (8.7)</td>
</tr>
<tr>
<td>ID</td>
<td>130 (24)</td>
<td>110 (20.3)</td>
</tr>
<tr>
<td>DD</td>
<td>90 (16.6)</td>
<td>68 (12.6)</td>
</tr>
<tr>
<td></td>
<td>44 (8.1)</td>
<td>47 (8.7)</td>
</tr>
<tr>
<td></td>
<td>130 (24)</td>
<td>110 (20.3)</td>
</tr>
<tr>
<td></td>
<td>90 (16.6)</td>
<td>68 (12.6)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses are% of total.

Table 4

<table>
<thead>
<tr>
<th></th>
<th>AA</th>
<th>AC</th>
<th>CC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>1.19 (0.81–1.73)</td>
<td>0.99 (0.65–1.51)</td>
<td>0.76 (0.30–1.88)</td>
</tr>
<tr>
<td>Age &lt; 55</td>
<td>1.66 (0.82–3.36)</td>
<td>0.59 (0.24–1.42)</td>
<td>0.71 (0.16–3.12)</td>
</tr>
<tr>
<td>Male</td>
<td>1.06 (0.67–1.67)</td>
<td>1.15 (0.67–1.98)</td>
<td>0.77 (0.29–2.09)</td>
</tr>
<tr>
<td>BMI &lt; median</td>
<td>1.25 (0.73–2.14)</td>
<td>1.42 (0.77–2.62)</td>
<td>1.40 (0.35–5.63)</td>
</tr>
<tr>
<td>Cholesterol &lt; median</td>
<td>1.19 (0.69–2.06)</td>
<td>1.18 (0.65–2.15)</td>
<td>0.33 (0.08–1.34)</td>
</tr>
<tr>
<td>Non-smokers</td>
<td>1.07 (0.69–1.66)</td>
<td>1.11 (0.67–1.85)</td>
<td>0.58 (0.21–1.54)</td>
</tr>
</tbody>
</table>

* The value next to each odds ratio is the \(P\) value from the comparison within each AT1R genotype between the DD and ID/II genotypes.
* Median for controls in each centre.
an independent effect on MI risk [4,20–22]. Despite
this, an important epistatic interaction between the two
polymorphisms could still exist. However, in a study
involving 541 cases of acute myocardial infarction and
507 control subjects we did not find any evidence to
support this possibility. Our findings are in contrast
with those of Tiret et al. [4] but in accord with more
recent studies [20–23]. Small study size could obscure
any association. However, our study had >99% power
at a significance of 0.01% to detect the 3.95 fold in-
crease in risk observed by Tiret et al. [4] in subjects
carrying the combined DD and CC genotypes.

There are several differences in design between our
study and that of Tiret et al. [4]. Their cases were all
male below the age of 65 and recruited between 3 and
9 months following MI. However, we did not find any
association of the genotypes with risk in younger sub-
jects or when stratified by sex (Table 4). Further re-
cruitment at the time of the incident event as done by
us, should have enhanced the association with MI,
unless the genotypes influenced survival rather than MI
risk. However, no data exists to suggest such a possibil-
ity. It is also noteworthy that in the study by Tiret et al.
[4], the interaction of the two genotypes arose not
because of a relative increase in the number of cases
with the ACE DD genotype in those bearing the AT1R
CC genotype, but instead because of a marked reduc-

tion in the number of cases with the ACE DD genotype
in those bearing the AT1R AA genotype (from 30 to 10%). An
association based on this finding is only possible if one=postulates that controls bearing the combined DD and CC genotypes
die prematurely. However, our findings do
not support this contention, since we found no differ-
ence in the proportion of controls with the ACE DD
genotype bearing the AT1R CC versus AA genotypes
(30 vs 39%).

A further observation in the study of Tiret et al. [4]
was that the effect of the interaction between the ACE
and ATIR polymorphisms was greater in subjects
deemed to be at low risk on the basis of plasma
apolipoprotein B level and body mass index. In this
sub-group the risk in DD/CC subjects was 13.3 (0.79–
707) fold higher. Although we were not able to classify
subjects in precisely the same manner, our sub-group
analyses (Table 4), based on stratification by relevant
individual risk factors, do not provide any evidence to
support this hypothesis. In this regard, our data also
agree with the findings in more recent studies [23].

In summary, in a two centre study of patients with
myocardial infarction recruited at the time of infarction,
no significant interaction was found between the
ACE I/D polymorphism and ATIR A/C polymorphism
in determining risk of myocardial infarction, either in
the whole population or in sub-groups stratified by the
presence or absence of other coronary risk factors.

Taken together with other recent data, our findings
suggest that genotyping for these polymorphisms is
unlikely to be of widespread clinical utility in assessing
the risk of myocardial infarction.

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