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Angiotensin II type-I receptor and ACE polymorphisms and risk of myocardial infarction in men and women

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Abstract

Background Findings relating an association between an insertion/deletion polymorphism of the angiotensin converting enzyme (ACE) gene and myocardial infarction (MI) have been mixed. While other loci, such as the angiotensin II type-I receptor (AT_1R), may modulate risk, few studies have adequately documented the risk in women. We aimed to study whether the findings in respect of ACE and AT_1R in UK men were borne out in women.

Methods Cases of MI (305 women, 391 men) in Belfast and Glasgow have been compared to controls (291 women, 356 men). These new samples augment the original men (200 cases, 181 controls) included from Belfast in the ECTIM study.

Results Among men, the odds ratio for MI for ACE (DD vs. ID + II) was 1·03 (0·79, 1·34) and among women, 0·69 (0·47, 1·01). This heterogeneity between the risks in men and women was significant in Glasgow ($P = 0·02$). Among men and women the odds ratio for MI for AT_1R (CC vs. AC + AA) was 1·02 (0·71, 1·47). There was a small gradient in risk, such that the odds ratio for DD genotype was 0·86 (0·63, 1·17) among subjects homozygous for the common AT_1R alelle (AA): 0·94 (0·67, 1·30) among heterozygotes and 1·21 (0·53, 2·77) among CC subjects; but this interaction was not statistically significant.

Conclusions Some of the contradictory findings concerning the ACE polymorphism and the risk of MI may be due to heterogeneity in the risk between men and women. The AT_1R^{1196} polymorphism is not an independent risk factor for MI in either sex.

Keywords Genetic risks, myocardial infarction.
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Introduction

In 1996, Samani *et al* [1] reported a meta-analysis of 15 studies investigating the relationship between ACE ins/del polymorphism and coronary risk. With a total sample size of 3394 cases and 5479 controls, the mean odds ratio for myocardial infarction (MI) for DD vs. ID and II was 1·26 (1·15, 1·39). However, there was significant heterogeneity in risk across studies. Some of this may have been attributable to publication bias but might also be related to varying definitions of phenotype and the recruitment of different proportions of men and women. For example, Schuster *et al* [2] in a study not included in the meta-analysis, which recruited 299 men and 88 women undergoing coronary angiography, found an association between DD genotype and history of previous MI that was significant only in women. They went on to propose a 'sexual dimorphism' in the relationship between genotype and phenotype. Anderson *et al* [3] found a significantly elevated risk in women only. The previous study with the largest sample of women (the Copenhagen City Heart Study [4]) found no significant association and the confidence limits around the odds ratio

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estimate (0·67, 1·51) were compatible with a protective effect of DD genotype. Similarly Schunkert *et al* [5] found an association between the D allele at the ACE locus and left ventricular hypertrophy in men but not in women.

It is also possible that discrepancies arise from differing ethnic and genetic backgrounds in the populations studied and the consequent variation in the informativeness of particular marker polymorphisms. Variation in the frequency of other genes and environmental risk factors can be expected to affect the penetrance and impact of specific polymorphisms. We have previously reported an interaction between the ACE ins/del and the angiotensin II type I receptor (AT_1R) polymorphism that might have both public health and therapeutic significance [6].

While there have been fewer studies of this interaction than of the risk conferred by the ACE ins/del polymorphism alone, most studies have recruited small samples of women. Their results have generally not borne out those from the earlier ECTIM report [7]. To clarify whether these discrepant findings might have arisen from what Schuster has described as a 'sexual dimorphism' [2], we have extended the ECTIM study with new samples of both men and women from the Belfast and Glasgow MONICA Project populations in Northern Ireland and Scotland, respectively.

Methods

Study populations and sampling of cases and controls

The sampling frames for both countries were based around the local MONICA (Monitoring Cardiovascular Disease) project coronary event registers. In Northern Ireland, the register covered the greater Belfast and North Down areas, a population of some 500 000. In Glasgow the MONICA register served a population of around 900 000. Male cases were recruited between 3 and 9 months after the index infarction between the ages of 25 and 64 years. Recruitment procedures for female cases in Glasgow were exactly the same as for men but because of the much smaller population base and lower incidence, female subjects in Belfast aged 25–69 years were enrolled up to 2 years after their infarction. Controls of comparable age ($\pm$ 1 year) were recruited from the lists of General Practitioners in the same area. Controls with coronary heart disease were excluded. The recruitment rate based on those who responded to our original postal invitation was 58%.

Each participant completed an interview-administered demographic and lifestyle questionnaire including sections dealing with smoking and alcohol habits and past medical history. This questionnaire and sampling and recruitment procedures closely reflected those used in the original ECTIM study [7].

A blood sample of 20 mL was obtained and placed in tubes containing Na_2EDTA after subjects had fasted for at least 10 h, kept at room temperature and centrifuged (10 min with RCF = 200) within 4 h. After addition of preservative (final concentrations: EDTA, 0·27 mmol L^{-1}; $\in$-amino-*n*-caproic acid, 0·9 mmol L^{-1}; chloramphenicol, 0·6 mmol L^{-1}; and glutathione, 0·3 mmol L^{1}) [8], the plasma was stored at 4 °C for no longer than 6 days and sent at 4 °C to the laboratory in Lille where lipoprotein measurements were performed immediately using the same protocols [9] as in the original ECTIM study.

Genetic Analyses

Genomic DNA was extracted from white blood cells by Miller's salting out method [10]. The polymerase chain reaction (PCR) was used to amplify regions encompassing the ACE ins/del polymorphism and the AT_1-1166 mutation of the angiotensin II Type I receptor. The latter was genotyped as previously described [6] but a description of the primers and amplifiers is given in the Appendix.

Statistical methods

Genotype and allele frequencies were initially compared by x^2 analysis. Odds ratios for MI were estimated by logistic regression adjusted for population, age and gender. Associations with conventional risk factors were tested by analysis of variance adjusted for age, gender and menopausal status in women.

Study approval

The study was approved by the Research Ethical Committee of the Faculty of Medicine, the Queen's University of Belfast and the ethics committees of the West Glasgow Hospitals University NHS Trust, the Royal Infirmary University NHS Trust, the Stobhill Hospital NHS Trust and the West of Scotland General Practice Ethics Committee.

Results

Results I – recruitment and characterisation of ECTIM extension study samples

In total, 201 female cases and 194 female controls were newly recruited in Belfast. An additional 100 male cases and 82 controls were also enrolled to augment the 200 male cases and 181 controls from the original ECTIM study. In Glasgow, 291 male and 104 female cases were recruited along with 274 and 97 controls.

Comparison of original ECTIM and ECTIM-extension subjects

As approximately 6 years elapsed between the original study and recruitment of the additional subjects, some comparison of their characteristics is warranted.

Table 1 Socio-demographic characteristics of controls in Northern Ireland in original ECTIM and ECTIM Extension

	Original ECTIM	ECTIM extension		Test of difference	
		Males	Females	*P**	*P*†
Number of subjects	181	82	194		
Mean Age (years)	54·2	58·6	62·2	< 0·001	< 0·0001
Social Class					
Non-Manual (%)	48·1	53·7	54·2	0·41	0·40
Manual (%)	51·9	46·3	45·8		
Smoking					
Smokers (%)	25·4	19·8	22·0	< 0·0001	0·05
Ex-smokers (%)	29·8	45·7	18·8		
Never-smokers (%)	44·8	34·6	59·2		
Alcohol consumption					
None (%)	40·3	32·9	68·0	< 0·0001	0·002
Light (%)‡	21·5	43·9	32·0		
Moderate (%)§	18·2	12·2	0·0		
Heavy (%)¶	19·9	11·0	0·0		
Subjects on Lipid treatment (%)	7·2	11·0	13·9	0·08	0·43
Mean BMI (kg/m^2)	25·7	26·8	25·9	0·80	0·04
Subjects treated for Hypertension (%)	12·7	17·1	23·2	0·02	0·45

*(Original ECTIM + Males) vs. Females; †Original ECTIM vs. males.
‡Less than 25 g week^{-1}; §between 25 and 50 g wk^{-1}; ¶ more than 50 g wk^{-1}.

Table 1 compares the basic demographic features of the controls in the first and second Belfast studies while Tables 2 and 3 compare the characteristics of cases and controls in both centres.

The male and female controls in the extension are significantly older than those in the original ECTIM sample by roughly four and seven years, respectively. The difference between the male cases, however, was only 1·5 years. Among the male cases the smoking prevalence was 19% lower in the extension sample ($P = 0·006$). Although there was only a small increase in the proportion of cases receiving lipid-lowering medication at the time of recruitment (from 27·5% to 29%), the numbers of controls under treatment had also increased (from 9% to

Table 2 Comparison of socio-demographic characteristics of cases and controls in Northern Ireland

	Male		Female		Test of difference			
	Case	Control	Case	Control	*P**	*P*†	*P*‡	*P*§
Number of subjects	300	263	201	194				
Mean age (years)	54·6	55·5	61·1	61·6	0·14	0·47	< 0·0001	< 0·0001
Social class								
Non-manual	32·0	49·8	32·0	54·2	< 0·0001	< 0·0001	1·00	0·41
Manual	68·0	50·2	68·0	45·8				
Smoking								
Smokers (%)	49·7	23·7	48·8	22·0	< 0·0001	< 0·0001	0·42	< 0·0001
Ex-smokers (%)	24·7	34·7	20·9	18·8				
Non-smokers (%)	25·7	41·6	30·3	59·2				
Alcohol								
None (%)	35·3	38·0	70·6	68·8	0·57	0·32	< 0·0001	< 0·0001
Light (%)¶	33·7	28·5	27·9	32·0				
Moderate (%)**	16·3	16·3	1·0	0·0				
Heavy (%)††	14·7	17·1	0·5	0·0				
Subjects on lipid treatment (%)	28·0	8·4	37·3	13·9	< 0·0001	< 0·0001	0·04	0·08
Mean BMI (kg/m^2)	26·1	26·0	27·1	25·9	0.77	0·02	0·02	0·80
Subjects treated for Hypertension (%)	23·7	14·1	33·5	23·2	0.004	0·02	0·02	0·02

*Case male vs. control male ; †case female vs. control female; ‡case male vs. case female; §control male vs. control female.
¶Less than 25 g week^{-1}; **between 25 and 50 g wk^{-1}; ††more than 50 g wk^{-1}.

Table 3 Comparison of socio-demographic characteristics of cases and controls in Glasgow

	Male		Female		Test of difference			
	Case	Control	Case	Control	*P**	*P*†	*P*‡	*P*§
Number of subjects	291	274	104	97				
Mean age (years)	54·5	54·8	57·3	58·1	0·71	0·40	0·001	<0·0001
Social Class								
Non-Manual	20·6	29·6	27·3	48·5	0·01	0·002	0·22	0·001
Manual	79·4	70·4	72·7	51·5				
Smoking								
Smokers	59·2	40·1	76·5	38·1	< 0·0001	< 0·0001	0·01	0·25
Ex-smokers	26·5	33·2	12·7	26·8				
Non-smokers	14·3	26·6	10·8	35·1				
Alcohol								
% None	28·0	17·9	64·9	34·0	0·02	< 0·0001	< 0·0001	< 0·0001
% Light¶	31·5	35·0	32·0	60·8				
% Moderate**	20·8	28·5	3·1	5·2				
% Heavy††	19·7	18·6	0·0	0·0				
% on Lipid treatment	34·4	6·2	35·6	7·2	< 0·0001	< 0·0001	0·92	0·91
Mean BMI	27·9	27·0	28·2	27·7	0·02	0·49	0·63	0·26
% treated for Hypertension	40·3	17·5	38·5	23·7	< 0·0001	0·02	0·84	0·24

*Case male vs. control male ; †case female vs. control female; ‡case male vs. case female; §control male vs. control female.

12·5%). There was also a material increase in the proportion of cases receiving treatment for hypertension (from 19% to 33%, $P = 0·01$), which was to some extent also evident for controls (from 13% to 19·3%, $P = 0·23$).

In Glasgow, the female cases and controls are roughly three years older than their male counterparts. While cases and controls in Glasgow differ as expected in the proportions from manual social classes, in the proportions who smoke and in the proportions receiving lipid or hypertensive treatment, twice as many female and male controls in Belfast are on lipid medication compared to their Glasgow counterparts (15·3% vs. 7·2% and 10·1% vs. 6·5%, respectively). The frequency of alcohol abstention is also lower in Glasgow than in Belfast.

For the purposes of the remaining analyses the original and extension subjects from Belfast have been aggregated.

Comparison of genotypes

For neither ACE ins/del nor the angiotensin II type-I receptor polymorphism was there any significant departure from Hardy–Weinberg equilibrium proportions ($x^2 = 3·62$; $P = 0·06$, and $x^2 = 0·58$, $P = 0·45$) but there was a modest under-representation of II for the ACE ins/del.

There were no statistically significant differences in the levels of these risk factors between any of the genotypes among control subjects (data not shown). This was true in women irrespective of adjustment for menopausal status.

Table 4 gives the genotype frequencies in cases and controls. Overall there is a small and non-significant under-representation of DD genotypes among cases but

Table 4 ACE I/D and AT_1R genotype distribution in cases and controls

	Men				Women				All			
	Cases		Controls		Cases		Controls		Cases		Controls	
	N	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%
ACE Ins/Del												
II	116	(20·1)	117	(22·6)	68	(23·9)	75	(26·7)	184	(21·4)	193	(24·1)
ID	305	(52·9)	267	(51·5)	155	(54·6)	126	(44·8)	460	(53·4)	393	(49·1)
DD	156	(27·0)	134	(25·9)	61	(21·5)	82	(28·5)	217	(25·2)	215	(26·8)
Odds Ratio*	1·06 (0·81, 1·39)				0·69 (0·47, 1·01)				0·92 (0·74, 1·14)			
AT_1R												
CC	39	(6·8)	35	(6·8)	27	(9·7)	26	(9·8)	66	(7·8)	61	(7·8)
AC	249	(43·7)	212	(41·3)	109	(39·1)	107	(40·2)	358	(42·2)	319	(40·8)
AA	282	(49·5)	266	(51·9)	143	(51·3)	133	(50·0)	425	(50·1)	401	(51·3)
Odds Ratio†	1·00 (0·63, 1·61)				0·99 (0·56, 1·74)				1·00 (0·69, 1·43)			

* DD vs. (ID + II); †CC vs. (AC + AA).

Table 5 Association between genotypes in men and women AT_1R genotype

	AA				AC				CC			
	Cases		Controls		Cases		Controls		Cases		Controls	
	N	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%
All Subjects												
DD	100	24·0	109	27·3	99	27·9	89	28·1	16	24·2	12	19·7
ID + II	317	76·0	290	72·7	256	72·1	228	71·9	50	75·8	49	80·3
Odds ratio*	0·84 (0·61, 1·15)				0·99 (0·71 1·39)				1·31 (0·56, 3·04)			
Men												
DD	73	25·9	68	25·7	75	30·1	59	28·0	8	20·5	6	17·1
ID + II	209	74·1	197	74·3	174	69·9	152	72·0	31	79·5	29	82·9
Odds ratio	1·01 (0·69, 1·49)				1·11 (0·74, 1·66)				1·25 (0·39, 4·03)			
Women												
DD	27	20·0	40	30·3	24	22·6	30	28·3	8	29·6	6	23·1
ID + II	108	80·0	92	69·7	82	77·4	76	71·7	19	70·4	20	76·9
Odds ratio	0·58 (0·33, 1·01)				0·74 (0·40, 1·38)				1·40 (0·41, 4·81)			

*DD vs. (ID + II)

this is more evident for women than men. The overall odds ratio adjusted for age, sex and centre is 0·92 (0·74, 1·14). In Glasgow there was significant heterogeneity between men and women (1·32 vs. 0·55; $P = 0·02$), such that the overall adjusted odds ratio for women, 0·69 (0·47–1·01) was of borderline statistical significance ($P = 0·06$).

There is a small and non-significant over-representation of AT_1R CC genotypes among cases and this is equally evident for both women and men. The overall odds ratio, adjusted for age, sex and centre was 1·02 (0·71, 1·46; $P = 0·93$)

We further categorised the subjects into a low- and high-risk group as was done in the original ECTIM report [7]. Low-risk subjects were those not on hypolipidaemic medication and with a BMI and a plasma apoB level below the median for the sample. Overall, there is no heterogeneity in the ACE (ins/del) or the AT_1R genotype frequency distribution of cases and controls across risk groups. (data not shown; available from authors).

Table 5 shows the association between the genotypes in cases and controls. For both sexes there was an apparent trend in the odds ratios for DD genotype across AGT_1R genotype classes but this did not attain significance.

Discussion

Among the 381 Belfast men included in the original ECTIM report [7], the odds ratio for myocardial infarction was 1·1 ($P > 0·05$). Among this expanded sample of Northern Irish men, the OR ratio for DD genotype is 0·83 (0·58, 1·20) confirming the earlier non-significant finding for Northern Irish men. Although the odds ratio for DD genotype among Glasgow men was 1·32 (0·90, 1·94) this was not significantly different from that in Northern Irish men, yielding a combined odds ratio of 1·03 (0·79, 1·34), for a total sample of over 1100 men. In a combined sample of 604 women – the largest such cohort yet recruited – there was a modest deficit of DD genotypes among cases, of borderline statistical significance, the odds ratio being 0·69 (0·47, 1·01). There was, however, significant heterogeneity in risk conferred by the DD genotype between men and women in Glasgow.

It has been argued that the discrepancies among studies might be attributable to the recruitment of survivors. We recruited women up to two years after their MI and up to nine months after their MI for men. We don't believe that selective mortality biases the findings materially, as nearly 90% of the deaths from MI occur in the first 28 days from onset [11]. Our previous post mortem study offered little support to the notion of survivorship bias [12].

There have been many studies yielding contradictory findings since the publication of the 1996 meta-analysis and the source of this heterogeneity is probably multifaceted. The definition of phenotype is probably most important. While it has been known for some time that individuals with an activated renin-angiotensin system are exposed to a three-fold risk of myocardial infarction [13], the mechanisms whereby the ACE gene may influence the risk of fatal or non-fatal infarction are still largely unknown. The rupture of a plaque leading to MI is not entirely dependent on the atherosclerotic extent of disease and the involvement of the ACE gene may thus occur later in the process, perhaps related to plaque stability, rupture or thrombosis. The findings of Oike *et al.* might support a role in coronary spasm [14].

The diverse findings relating DD genotype with the angiographic severity of disease suggests little involvement with atherosclerotic onset or progression. However the interrelationships will not be easily disentangled in anything other than a true cohort study. Only in such a setting might it be possible to reconcile the apparent association between DD genotype and longevity and the higher risk of MI in *older* (rather than younger) subjects shown by Gardemann *et al* [15].

The rather mixed results may also reflect a real difference in risk according to gender or ethnic group. Schuster *et al* [2] and Anderson *et al* [3], studying samples from Germany and Utah, respectively, found a significantly elevated risk in women only. Our results are thus clearly at variance with these findings and suggest either no relationship or, in Glasgow at least, a protective effect of DD genotype on risk of MI in women. Interestingly, they would seem to accord with the reportedly higher frequency of DD genotype among centenarians [16]. Also, Perola *et al* [17] found that in the general population of Finland, women with a parental history of myocardial infarction were more likely to be II than DD.

Besides the differences in phenotype definition and the proportions of male and female subjects, the frequency of other genes and environmental risk factors might be expected to affect the penetrance and impact of specific polymorphisms. Such was the impetus for studying the angiotensin II type-1 receptor locus. In an earlier report from the ECTIM study [6], there was a gradient in MI risk conferred by DD genotype across AT_1R genotypes which was 1·05 (0·75–1·49) for subjects with AA, 1·52 (1·06, 2·18) for AC and 3·95 (1·26, 12·4) for CC subjects. We have not been able to confirm this relationship and though there was a trend apparent in our data-set, neither this nor any of the point estimates were statistically significant. The allele frequencies, in this extended study, are comparable: $A_{0·71}$:$C_{0·29}$ in cases and $A_{0·717}$:$C_{0·283}$ in controls compared to 0·71/0·29 and 0·70/0·30, respectively [6].

Most previous studies, with only a few exceptions [18,19] have shown little or no association between the C allele and CHD [14,20–23].

A more recent meta-analysis [24] has cast further doubt about the role of these polymorphisms in myocardial infarction. Although no significant differences in odds ratios were noted between men and women, the main source of heterogeneity studied was between large and small studies. It was noted that the odds ratios in the larger studies were smaller than those among the smaller studies and this might have been consistent with publication bias. The larger studies used in that analysis included cases from large randomised controlled trials. The population representativeness of the cases in some of these trials is questionable. Nevertheless our own study may have had limited power if the expected odds ratios were as modest as those suggested by Keavney *et al* [24].

Thus on the basis of the limited evidence available and our own findings, the AT_1R^{A1166C} polymorphism is not a significant risk factor for myocardial infarction. There is reason to believe that the expression of some RAS genes (including the AT_1R gene) might be modified by environmental risk factors, such as salt intake [25], and so there are bound to be interactions between such genes in modulating MI risk that are population specific and that may depend on the expression of other phenotypes that mediate elevated coronary artery disease risk such as hypertension [26] or reduced vascular compliance [27].

In summary, this extension to ECTIM has yielded conflicting results. Its findings might be considered congruent with those from other British populations, in that in MI survivors there is no significant relationship with this polymorphism or with the AT_1R polymorphism. We believe the contribution to MI risk of the ACE I/D polymorphism may be different for men and women and vary in different ethnic groups, independently of other risk factors.

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Appendix

Primers

5′ GGC TTT GCT TTG TCT TGT TG 3′ 5′ AAT GCT TGT AGC CAA AGT CAC CT 3′

ASOs

5′ AAT GAG CCTTAG CTA 3′ (M) 5′ AAT GAG CATTAG CTA 3′ (W)

Primers for ACE ins/del polymorphism

ACE1: 5′ CTG GAG ACC ACT CCC ATC CTT TCT 3′ ACE2: 5′ GATGTGGCCATCACATTCGTCAGAT 3′ ACE3: 5′ TGGGATTACAGGCGTGATACAG 3′

Methods

The products were run on an agarose gel (II revealed by two bands of 479 + 159 bp; ID by three bands of 479 + 191 bp + 159 bp; and DD by one band of 191 bp).

Amplification was carried out using 250 ng of DNA, 25 pmol of ACE1 and ACE2 and 15 pm of ACE3 and 0·2 U of Taq Polymerase. Thirty cycles of amplification were performed on an automated thermocycler with for each cycle: denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, extension at 72 °C for 1 min.