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Published in:
Lancet

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Synergistic effects of angiotensin-converting enzyme and angiotensin-II type 1 receptor gene polymorphisms on risk of myocardial infarction

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Summary

We reported from our previous multicentre case-control study that the deletion (D) polymorphism of the gene encoding angiotensin-converting enzyme (ACE) was associated with increased risk of myocardial infarction. The main function of ACE is to convert angiotensin I into angiotensin II, which exerts its known cellular actions through the angiotensin II AT₁ receptor subtype (AGT₁R). We have now investigated the role of a common polymorphism of the AT₁ receptor gene (an A→C transversion at position 1166 of AGT₁R) and looked for an interaction between ACE and AGT₁R gene polymorphisms on the risk of myocardial infarction.

We analysed DNA from 613 patients with myocardial infarction and 723 age-matched population controls. We found a significant interaction between ACE and AGT₁R gene polymorphisms; the odds ratio for myocardial infarction associated with the ACE DD genotype was 1.05 (95% CI 0.75–1.49) for subjects without the AGT₁R C allele, 1.52 (1.06–2.18) in AC heterozygotes, and 3.95 (1.26–12.4) in CC homozygotes (test for trend, $p < 0.02$). Among patients defined as low risk by traditional risk factors (serum apolipoprotein B < 1.25 g/L, body-mass index < 26 kg/m²) the interaction was even stronger (odds ratios 1.64 [0.68–3.92], 7.03 [2.61–19.0], and 13.3 [$p = 0.05$], respectively).

These findings, if confirmed, could have clinical implications for the prevention and treatment of coronary heart disease.

Lancet 1994; **344**: 910–13

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Introduction

The findings of the ECTIM (Etude Cas-Témoin de l'Infarctus du Myocarde) multicentre study suggested that a deletion (D) polymorphism in the gene encoding angiotensin-converting enzyme (ACE) is a risk factor for myocardial infarction.^{1,2} Since those reports, some other studies^{3–8} have confirmed involvement of the ACE I/D polymorphism in the predisposition to coronary heart disease; yet others have not.^{9,10} The frequency of the ACE DD genotype is increased in the presence of left ventricular hypertrophy¹¹ and dilated and hypertrophic cardiomyopathy,^{12,13} whereas high plasma concentrations of ACE are associated with carotid-wall thickening.¹⁴

The ACE DD genotype is associated with raised circulating¹⁵ and cellular¹⁶ concentrations of ACE. The ACE I/D polymorphism is probably a neutral marker in tight linkage to a functional variant yet to be identified.¹⁷ ACE has a key role in the renin-angiotensin system, by converting angiotensin I to angiotensin II; the latter peptide has various effects including vasoconstriction, aldosterone production, and enhanced noradrenergic release from sympathetic nerve endings.¹⁸ Angiotensin II also has hypertrophic, and possibly hyperplastic, effects on vascular smooth muscle cells and cardiomyocytes, and increases extracellular collagen matrix synthesis.^{19,20}

The cellular effects of angiotensin II are mediated by two structurally distinct receptor subtypes, AT₁ and AT₂.²¹ Most of the known actions of angiotensin II are exerted through the AT₁ receptor, which is present in particular in vascular smooth muscle cells and in the myocardium.²² The cDNA encoding human AT₁ receptor, a G-protein-coupled receptor, has been cloned.²³ We have identified a polymorphism in the 3' untranslated region of the AGT₁R gene, corresponding to an A→C transversion at nucleotide position 1166 of the mRNA sequence.²⁴

The physiological role of the AT₁ receptor suggested that the AGT₁R gene could be a candidate gene for myocardial infarction, possibly interacting with the ACE I/D polymorphism. We investigated this hypothesis in the ECTIM study, a case-control study comparing men who survived myocardial infarction with appropriate controls recruited from different European populations.²⁵

Patients and methods

The populations who took part in the ECTIM study have been described.²⁵ Patients with myocardial infarction were recruited between 1989 and 1991 from the registers of the WHO/Multinational Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA) in Belfast (Northern Ireland), Lille (northern France), Strasbourg (eastern France), and Toulouse (southwestern France). Men aged 25–64 years who survived a definite myocardial infarction (MONICA

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	Belfast		Lille		Strasbourg		Toulouse		All subjects	
	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
No of subjects with AGT ₁ R genotype										
AA	98	85	30	64*	86*	104	69	102	283	355
AC	88	81	31	75	103	73	64	90	286	319
CC	15	14	4	9	14	12	11	14	44	49
AGT ₁ R allele frequency	0.71/0.29		0.70/0.30		0.68/0.32†		0.70/0.30		0.69/0.31	
A/C	0.71/0.29		0.70/0.30		0.68/0.32†		0.70/0.30		0.69/0.31	

*Significant deviation from Hardy-Weinberg equilibrium ($p < 0.05$). †Frequency of allele C significantly higher in cases than in controls ($p < 0.05$).

Table 1: Distributions of AGT₁R genotypes in cases and controls in four subpopulations

category D) were recruited 3–9 months after the infarction. Age-matched controls were obtained from the electoral rolls in France and from the lists of general practitioners held by the Central Services Agency in Northern Ireland. To increase ethnic homogeneity, we required that families of patients and controls had been resident in the region for at least two generations and that all four grandparents had been born in Europe or in Ulster. All participants were white Europeans.

Analyses are based on 201 patients with myocardial infarction and 180 controls from Belfast, 65 and 148 from Lille, 203 and 89 from Strasbourg, and 144 and 206 from Toulouse (total 613 cases and 723, respectively). Parental history of myocardial infarction was determined at interview by specially trained staff. In Belfast, data were validated from general practitioners' or hospital records or death certificates,²⁶ and subjects for whom this validation was not possible (15%) were excluded from the analysis. In France, confidentiality legislation does not permit such validation, so we focused the analysis on fatal events, which were judged the most reliable.

High-risk and low-risk groups were defined according to plasma concentration of apolipoprotein B and body-mass index.¹ The low-risk group was made up of subjects not undergoing treatment with hypolipidaemic drugs who had plasma apolipoprotein B below 1.25 g/L and body-mass index lower than 26 kg/m². The high-risk group contained all other subjects.

DNA samples from ECTIM participants were assayed for the ACE *DD* polymorphism by DNA amplification by the polymerase chain reaction (PCR).²⁷ On each assay plate, subjects (cases and controls) from at least two study subpopulations were mixed. All assays were independently replicated and the results were analysed by at least two investigators unaware of clinical details. We developed a new assay to analyse degraded DNA,³ and replication of a subsample of the ECTIM study with this new assay showed no discrepancy. The AGT₁R A¹⁶⁶→C polymorphism was assayed by allele-specific oligonucleotide hybridisations of PCR products.²⁴ The primers used to amplify the AGT₁R region encompassing the A¹⁶⁶→C polymorphism were 5'-AATGCTTGCTAGCCAAAGTCACCT-3' and 5'-GGCTTTGCTTTGTCTTGTTG-3'. The hybridisation procedure²⁴ was slightly modified: each allele was detected by preincubating the amplification products for 2 h with 50 pmoles

of unlabelled oligonucleotide probe specific for the other allele and then incubating for 4 h with 10 pmoles of labelled probe specific for the allele (sequences published previously²⁴); the membranes were then washed and autoradiographed.

Statistical analyses were based on the calculation of odds ratios to provide an estimate of the relative risk of myocardial infarction associated with the ACE *DD* genotype in groups of subjects with different AGT₁R genotypes. The analyses were carried out by means of logistic regression models adjusted for age and population. Heterogeneity of odds ratios between AGT₁R genotypes was tested by introducing corresponding interaction terms in the model. Tests for linear trend were done with the AGT₁R genotype coded as an ordinal variable (0, 1, 2). Fisher's test was used for sets with small numbers.

Results

The AGT₁R genotype distributions were compatible with Hardy-Weinberg expectations, except among cases in Strasbourg and controls in Lille, where there were fewer homozygotes than would be expected ($p < 0.05$, table 1). The genotype and allele frequencies of the AGT₁R polymorphism did not differ between cases and controls (table 1), except in Strasbourg where the frequency of allele C was significantly higher in cases ($p < 0.05$).

We found a significant interaction ($p < 0.05$) between ACE and AGT₁R gene polymorphisms on the risk of myocardial infarction. The association between the ACE *DD* genotype and myocardial infarction was restricted to subjects carrying the AGT₁R C allele, with a pronounced allele dose-effect. In the total population, the odds ratio (which approximates to the relative risk) for myocardial infarction associated with ACE *DD* genotype varied from 1.05 (0.75–1.49, not significant) in subjects without the AGT₁R C allele (*AA* homozygotes) to 1.52 (1.06–2.18, $p = 0.02$) in *AC* heterozygotes and 3.95 (1.26–12.4, $p = 0.02$) in *CC* homozygotes (test for linear trend $p < 0.02$, table 2). Within each AGT₁R genotype, the association was homogeneous across the study subpopulations.

	AGT ₁ R genotype					
	AA		AC		CC	
	Cases	Controls	Cases	Controls	Cases	Controls
All subjects						
ACE <i>DD</i>	86	108	99	86	14	5
ACE <i>DD</i> †	197	247	187	233	30	44
Odds ratio (95% CI)*	1.05 (0.75–1.49)		1.52 (1.06–2.18)†		3.95 (1.26–12.4)†	
Low-risk group						
ACE <i>DD</i>	13	30	21	14	5	3
ACE <i>DD</i> †	22	68	19	62	1	8
Odds ratio (95% CI)*	1.64 (0.68–3.92)		7.03 (2.61–19.0)†		13.3 (0.79–707)§	
High-risk group						
ACE <i>DD</i>	73	78	78	72	9	2
ACE <i>DD</i> †	175	179	168	171	29	36
Odds ratio (95% CI)*	1.01 (0.69–1.49)		1.16 (0.78–1.72)		5.59 (1.02–56)§	

*Groups are defined in the methods section.

†Adjusted for population and age. ‡ $p = 0.02$, §Fisher's test, $p = 0.05$.

Table 2: Distribution of ACE genotypes in cases and controls according to AGT₁R genotype and risk status

	Belfast		Lille		Strasbourg		Toulouse		All subjects	
	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
AGT ₁ R genotype AA										
ACE DD	2	10	1	7	4	5	6	8	13	30
ACE ID+II	8	11	1	14	7	15	6	28	22	68
Odds ratio	0.28		2.00		1.71		3.50		1.64 (0.68-3.92)*	
AGT ₁ R genotype AC+CC										
ACE DD	11	3	5	5	6	2	4	7	26	17
ACE ID+II	8	28	0	13	7	10	5	19	20	70
Odds ratio	12.8†		∞‡		4.29		2.17		7.02 (2.88-17.1)*§	

*Adjusted for population and age. Fisher's test, † $p<0.001$, ‡ $p=0.007$, § $p<0.0001$.

Table 3: Distribution of ACE genotypes in cases and controls of study subpopulations in low-risk group according to AGT₁R genotype

Since the reported effect of the ACE polymorphism is especially strong in subjects at low risk according to traditional risk factors, the analysis was repeated in the two risk groups.¹ In the high-risk group, the association between the ACE DD genotype and myocardial infarction was apparent only in subjects homozygous for the AGT₁R C allele. In the low-risk group, the interaction was stronger than that in the whole population; the odds ratios for myocardial infarction associated with the ACE DD genotype varied from 1.64 (0.68-3.92, not significant) in subjects without the AGT₁R C allele to 7.03 (2.61-19.0, $p<0.0001$) in AC heterozygotes, and 13.3 (Fisher's test, $p=0.05$) in CC homozygotes (test for linear trend $p=0.02$, table 2).

Because the interaction was so strong in the low-risk group, we investigated whether it was present in the low-risk groups in the different subpopulations of the ECTIM study. Because there were small numbers of AGT₁R CC homozygotes in each population, AC heterozygotes and CC homozygotes were pooled for this analysis. In three of the four subpopulations, the effect of the ACE DD genotype on the risk of myocardial infarction was substantially increased in low-risk carriers of the AGT₁R C allele (table 3, risk increase significant in two subpopulations). Despite fluctuations in the size of the odds ratios, no significant heterogeneity was detected across the subpopulations, and the combined odds ratio in carriers of the AGT₁R C allele was estimated at 7.02 (2.88-17.1, $p<0.0001$). The analysis was repeated after exclusion of the Lille sample, because the estimated odds ratio in this population was infinity, but the association remained highly significant (odds ratio 5.26 [2.08-13.3], $p<0.001$). The heterogeneity of odds ratios between AGT₁R genotypes was significant ($p<0.02$).

The association previously found² between the ACE I/D polymorphism and a parental history of myocardial infarction in controls was re-examined in the light of these findings. The odds ratio associated with the ACE DD

genotype was significant in subjects carrying the AGT₁R C allele (3.62 [1.16-11.3], $p<0.03$); it was higher than that in non-carriers of the AGT₁R C allele, although this difference was not significant (table 4). However, we must emphasise that the dilution of genes between generations at the two loci causes a large decrease in power compared with the direct case-control approach.

Discussion

These results suggest that the association between the ACE DD genotype and myocardial infarction is restricted to a subset of individuals, also carriers of the AGT₁R C allele. Moreover, the relative risk associated with the ACE DD genotype seems to increase with the number of AGT₁R C alleles, although we could not test this idea in each subpopulation because of small sample sizes. From the allele frequencies in the control population, we can estimate that 15% of individuals simultaneously carry the ACE DD genotype and the AGT₁R C allele and 2.5% are double homozygotes. Our findings suggest that the ACE DD genotype has a substantial impact, in terms of relative risk for myocardial infarction, in this particular subset of individuals.

Among controls in the high-risk group, there was a significant deficit of subjects simultaneously homozygous for the ACE D and AGT₁R C alleles (2 observed vs 10.7 expected, $p<0.01$) and the deficit was apparent in all four subpopulations. Although purely speculative, one interpretation of this finding is that the combination of the ACE DD and AGT₁R CC genotypes, coupled with other risk factors, confers such a high risk of myocardial infarction that most of these individuals would have already sustained infarctions and possibly died from the disorder. The hypothesis of selection by death has been suggested previously.^{2,3,9}

We have lately reported an excess of the AGT₁R C allele in severely hypertensive compared with normotensive subjects, which suggests involvement of the AGT₁R gene in the predisposition to hypertension.²⁴ In this study, however, we did not find any significant association between the AGT₁R polymorphism and high blood pressure in the whole population.

The biological mechanism by which the ACE and AGT₁R genes interact on the risk of myocardial infarction is unknown, but our findings suggest a possible epistatic effect of the two genes. Whereas the association of the ACE D allele with increased expression of ACE is well known, no intermediate phenotype associated with the AGT₁R C allele has been identified. Since the AGT₁R gene polymorphism does not seem to be independently and directly associated with myocardial infarction, but modulates the relative risk conferred by the ACE D allele, we propose that the AGT₁R C allele is associated with a

ACE genotype	AGT ₁ R genotype AA		AGT ₁ R genotype AC+CC	
	Parental history of MI	No parental history of MI	Parental history of MI	No parental history of MI
DD	16	91	15	73
ID	20	144	24	171
II	6	58	5	63
Odds ratio (95% CI)*				
DD/II	2.22 (0.78-6.30)		3.62 (1.16-11.3)†	
ID/II	1.81 (0.66-4.96)		2.05 (0.71-5.90)	

*Adjusted for age and population. † $p<0.03$.

Table 4: Distribution of ACE genotypes according to parental history of fatal myocardial infarction in controls by AGT₁R genotype

Subjects	Controls
ases	
3	30
2	68
64 (0.68-3.92)	
5	17
7	70
7.02 (2.88-17.1)	

AGT₁R

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modified response of the receptor to angiotensin II. Several G-protein-coupled receptors, including the vascular AT₁ receptor, show desensitisation after exposure to their agonists.²⁸ This phenomenon can result from various mechanisms, including receptor protein modifications and down-regulation of gene expression.²⁸ In our previous study, we were unable to detect any polymorphism modifying the encoded aminoacid sequence of the AGT₁R gene in 50 hypertensive subjects screened by single-strand conformation polymorphism detection.²⁴

We therefore speculate that the AGT₁R C allele is in linkage disequilibrium with a functional variant that could alter the down-regulation of the AGT₁R gene in response to angiotensin II, the latter being present in increased concentrations in the presence of the D allele of the ACE gene. Alternatively, it is conceivable that increased activity of the AT₁ receptor, under genetic control, is associated with over-expression of the ACE gene, since in a rat model AT₁ receptor blockade induces decreased plasma and tissue ACE activities.²⁹ Since the AT₁ receptor and ACE are present in coronary vessel walls and in the myocardium, we also speculate that the gene variants could interact through a local paracrine mechanism, since ACE is a non-limiting step for angiotensin II generation in plasma.³⁰

These results, if confirmed, could have clinical implications for the prevention and treatment of coronary heart disease. Screening for a genetic risk factor becomes worth while if the factor is not uncommon and imparts a high relative risk, and if its detrimental effects can be counteracted pharmacologically or by other means. Since powerful drugs, such as ACE inhibitors and AT₁ receptor antagonists are available, it would be justifiable to consider a clinical trial in patients at high risk of myocardial infarction and carrying the ACE DD genotype and the AGT₁R C allele.

We thank Ms C Souriau, Ms F Defranoux, Ms A Bingham, and Ms V Nicaud for technical assistance. This study was supported by grants from Bristol-Myers Squibb, Merck Sharp & Dohme, Sanofi, Comité Français de Coordination des Recherches sur l'Athérosclérose et le Cholestérol, British Heart Foundation, Institut National de la Santé et de Recherche Médicale, and Institut Pasteur, Lille. AB is the recipient of a Medical Research Council of Canada fellowship award.

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