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Insertion/deletion polymorphism of the angiotensin-converting enzyme gene is strongly associated with coronary heart disease in non-insulin-dependent diabetes mellitus

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ABSTRACT **Non-insulin-dependent diabetes mellitus (NIDDM) is considered a model of premature atherosclerosis with a strong genetic component. We have investigated the role of angiotensin-converting enzyme (ACE; EC 3.4.15.1) gene in 316 unrelated NIDDM individuals, 132 who had myocardial infarction or significant coronary stenoses and 184 with no history of coronary heart disease (CHD). A deletion-polymorphism in the *ACE* gene was recently reported to be associated with myocardial infarction especially in people classified as low risk. Here we report that the *D* allele of the *ACE* gene is a strong and independent risk factor for CHD in NIDDM patients. The *D* allele is associated with early-onset CHD in NIDDM, independently of hypertension and lipid values. A progressively increasing relative risk in individuals heterozygous and homozygous for the *D* allele was observed (odds ratios of 1.41 and 2.35, respectively; $P < 0.007$), suggesting a codominant effect on the cardiovascular risk. The percentage of CHD attributable to the *ACE* deletion allele was 24% in this NIDDM population. Identification of NIDDM patients carrying this putative CHD-susceptibility genotype would help early detection and treatment of CHD.**

Non-insulin-dependent diabetes mellitus (NIDDM) is associated with a high risk of coronary heart disease (CHD) and may be considered a model of premature atherosclerosis with a strong genetic component (1). The high incidence of cardiovascular disease in NIDDM (2) is not fully explained by hyperglycemia or by association with other known risk factors such as hypertension and dyslipidemia. Furthermore, genetic factors not only seem to play a role in the development of NIDDM but also might contribute to its vascular complications (3).

A deletion polymorphism in the gene encoding angiotensin-converting enzyme (ACE; EC 3.4.15.1) is strongly related to the level of circulating enzyme (4). It may be a potent risk factor of myocardial infarction especially in people otherwise classified as low risk (nonobese subjects with normal lipid values) (5). This polymorphism was associated with a parental history of myocardial infarction in the same population (6). In addition, this *ACE* gene variant was associated with the pathogenesis of ischemic and idiopathic dilated cardiomyopathies (7). ACE converts angiotensin I to the potent vasoconstrictor angiotensin II and inactivates the vasodilator bradykinin. As these two peptide hormones have opposite effects on vascular tone and on smooth muscle cell proliferation (8), the *ACE* locus may contribute to the pathogenesis of CHD through different mechanisms. Thus, the *ACE* locus may also be a genetic determinant of CHD in populations at high risk for cardiovascular disease, which accounts for 37.4% mortality in France (9). Therefore we have investigated the *ACE* gene polymorphism in a group of French Caucasian NIDDM patients with or without CHD. Our data strongly support the contention that the *D* allele of the *ACE* gene is independently associated with CHD.

MATERIAL AND METHODS

Patients and Controls. We have investigated 316 unrelated Caucasian NIDDM patients (239 men and 77 women). All diabetic patients included in this study fulfilled the World Health Organization criteria for diabetes mellitus (10). Among these individuals, 132 were classified as having CHD (CHD^+); they were patients from Saint Louis Hospital (Paris). Eighty-six were hospitalized for documented transmural myocardial infarction: typical symptoms of myocardial infarction, biological events (significant increase of total creatine phosphokinase levels with specific MB fraction ≥10%), and electrocardiographic evidence of myocardial infarction. The forty-six remaining CHD^+ individuals, who had coronary angiography, presented evidence of heamodynamically significant CHD. Stenoses higher than 70% narrowing in the cross-sectional area of one of the major arteries or higher than 50% on the left main artery were considered significant. A control group (CHD^-) of 184 unrelated NIDDM patients was constituted, presenting neither history of angina pectoris nor abnormal electrocardiography. This control group was obtained from the NIDDM families collected at Centre d'Etude du Polymorphisme Humain, all over France, through a multimedia campaign from 1990 to 1992 (11). Clinical and biological characteristics of the CHD^+ and CHD^- groups are shown in Table 1. Age of patients, duration of diabetes, proportion of smokers, and triglycerides levels were significantly higher in the CHD^+ group. Moreover, HDL cholesterol values were lower in the CHD^+ group. CHD^+ patients had a higher number of cardiovascular risk factors than the NIDDM subjects without CHD (2.2 versus 1.7, respectively, not including diabetes; $P = 0.0001$). Parental history of myocardial infarction, arterial hypertension, smoking habit, total cholesterol ≥6.5 mmol/liter, HDL cholesterol ≤0.9 mmol/liter, triglycerides ≥2.3 mmol/liter, and the presence of a hypolipemic treatment were considered as risk factors for CHD.

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Abbreviations: NIDDM, non-insulin-dependent diabetes mellitus; ACE, angiotensin-converting enzyme; CHD, coronary heart disease; HDL, high density lipoprotein; ECTIM, Etude Cas-Témoin de l'Infarctus du Myocarde; *I*, insertion; *D*, deletion.

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Table 1. Clinical and biological features in CHD+ and CHD− patients

Clinical and biological characteristic	CHD+ (n = 132)	CHD− (n = 184)	P value
Age,* years	62.9 ± 9.4	60.7 ± 9.5	<0.05
Sex, male (%)	107 (81)	132 (72)	NS
Diabetes duration,* years	15.4 ± 10.3	13.2 ± 8.5	<0.05
Body mass index,* kg/m^2	27.2 ± 3.8	27.4 ± 4.6	NS
Hypertension, n (%)	71 (54)	91 (49)	NS
Family history of CHD, n (%)	23 (17)	19 (10)	NS
Smokers, n (%)	86 (65)	94 (51)	<0.02
Total cholesterol,* mmol/liter	6.0 ± 1.2	5.9 ± 1.0	NS
Triglycerides,* mmol/liter	2.0 ± 1.3	1.8 ± 1.1	<0.05
HDL cholesterol,* mmol/liter	1.17 ± 0.35	1.27 ± 0.38	<0.03

NS, not significant; HDL, high density lipoprotein.
*Data are presented as mean ± SD.

DNA Analysis. Lymphocytes were isolated from blood and DNA was prepared by standard techniques (12). Polymerase chain reaction (PCR) was used to detect the two alleles of 490 and 190 bp corresponding, respectively, to the insertion (*I*) and deletion (*D*) fragments. DNAs were amplified on a Techne thermal cycler using primers and PCR cycling conditions as described (13). Amplifications were performed in 3 mM $MgCl_2$ with 100 ng of DNA, 50 ng of each primer and 1.25 units of *Taq* polymerase (Boehringer Mannheim GmbH). PCR products were separated on 2% agarose gels for genotype determinations.

Statistical Analyses. One-way analyses of variance for normal and logarithmically transformed biological data were performed. Qualitative data were compared between groups by the χ^2 test. Allele frequencies were estimated by the gene counting method, and Hardy–Weinberg's equilibrium was checked by a χ^2 test. The relationship between the ACE deletion polymorphism and CHD was assessed by crude odds ratios and by a trend test. Simultaneous adjustments for qualitative and quantitative variables were carried out, using a logistic regression model, in which only variables showing a significant association with CHD in the univariate analyses ($P < 0.05$) were introduced. Statistical analyses were performed with the JMP computer software.

RESULTS

The overall frequencies of the genotypes at the *ACE* locus did not differ significantly between the two subgroups of cases (with myocardial infarction or with significant coronary stenoses), as shown in Table 2. Therefore, the subsequent statistical analyses were performed on all cases. The *ACE* genotype frequencies observed in the subgroup of controls with no history of CHD were in accordance with those reported in the Etude Cas-Témoin de l'Infarctus du Myocarde (ECTIM) study (5) (Table 3). The *D* allele of the *ACE* deletion polymorphism was significantly more frequent in the CHD+ group than in the CHD− group with a relative risk of 1.57 ($P < 0.01$, Table 4). The association was stronger in homozygous than in heterozygous patients (odds ratios *DD/II* and *ID/II* of 2.35 and 1.41, respectively; $P < 0.007$), suggesting a codominant effect of allele *D* and CHD risk. The distribution of the *ACE* genotypes in all groups was in Hardy–Weinberg equilibrium. As the association of the *DD* genotype with myocardial infarction was initially shown in males under 65 years (5), we considered the subgroup of NIDDM male individuals under 65 years. The association between the *DD* genotype at the *ACE* locus and CHD in this population was significant (odds ratio *DD/ID + II* of 2.56, $P < 0.01$). A trend related to the numbers of *D* allele was observed (odds ratios *DD/II* and *ID/II* of 3.88 and 1.76, respectively; $P < 0.005$) in NIDDM males under 65 years. Assuming that the relative risk is well approximated by the odds ratio, the percentage of cardiovascular cases that could be attributable to the *DD* genotype was 24% in the whole NIDDM population.

Table 2. Insertion/deletion polymorphism of *ACE* gene in the three groups studied

Cardiovascular status	Insertion/deletion polymorphism,* n (%)				
	Allele		Genotype		
	I	*D*	*II*	*ID*	*DD*
MI	62	110	12 (14)	38 (45)	36 (41)
Significant CS	43	61	6 (13)	19 (41)	21 (46)
No angina and normal EKG	170	198	40 (22)	90 (49)	54 (29)

MI, myocardial infarction; CS, coronary stenoses; EKG, electrocardiogram.
*Insertion/deletion polymorphism of *ACE* gene.

According to the univariate analyses, the *ACE* deletion polymorphism was found to be a risk factor for CHD in this NIDDM population (Table 4). A multiple logistic regression analysis was performed, introducing the cardiovascular risk factors for CHD that were identified in previous univariate analyses (*P* value under 5% in Table 1) (Table 5). In the whole NIDDM population studied (n = 316), the *DD* genotype appeared to be an independent cardiovascular risk factor (odds ratio of 1.44, $P = 0.031$). When only considering males under the age of 65 years, the relative risk increased significantly to 1.79 ($P = 0.009$), becoming the strongest cardiovascular risk factor associated with CHD. The *ACE* deletion polymorphism was not associated with arterial hypertension (data not shown).

DISCUSSION

A deletion polymorphism in the gene for *ACE* has been suggested to be a potent risk factor for myocardial infarction in male individuals considered to be at low cardiovascular risk (5). Here we report a significant and independent association between the *DD* genotype and CHD in a population of Caucasian NIDDM patients presenting much higher risk for premature atherosclerosis. We also found this association in diabetic males under the age of 65 years. In this subgroup, the association may be partially explained by a slight but not

Table 3. Distribution of *ACE* alleles in the two controls groups compared to the ECTIM study control group

ACE allele	ECTIM study	CHD−	
		All	Males ≤65 years
D, n (%)	790 (54)	198 (54)	89 (48)
I, n (%)	676 (46)	170 (46)	95 (52)
P value*	—	NS	NS

NS, not significant.
*χ^2 ECTIM controls versus the two NIDDM controls.

Table 4. Distribution of *ACE* genotypes and alleles in CHD$^+$ and CHD$^-$ NIDDM patients

	Total (n = 316)				Males ≤65 years (n = 157)			
	CHD$^+$ (n = 132)		CHD$^-$ (n = 184)		CHD$^+$ (n = 65)		CHD$^-$ (n = 92)	
ACE	n	Freq.	n	Freq.	n	Freq.	n	Freq.
Allele								
D	171	0.65	198	0.54	84	0.65	89	0.48
I	93	0.35	170	0.46	46	0.35	95	0.52
Odds ratio (95% CI)		1.57 (1.14–2.19)				1.95 (1.22–3.09)		
P value		<0.01				<0.01		
Genotype								
DD	57	0.43	54	0.29	27	0.42	20	0.22
ID	57	0.43	90	0.49	30	0.46	49	0.53
II	18	0.14	40	0.22	8	0.12	23	0.25
Odds ratio (*DD*/*ID* + *II*) (95% CI)		1.83 (1.15–2.92)				2.56 (1.27–5.15)		
P value		<0.02				<0.01		

CI, confidence interval; Freq., frequency.

significant decrease of the *DD* genotype frequency (Table 4). Our results suggest that the *D* allele has a codominant effect on the cardiovascular risk in NIDDM, as suggested by the trend test considering CHD risk in individuals heterozygous and homozygous for the susceptibility allele.

Case-control studies may suffer from several biases, which may lead to false-positive and to false-negative results (14). We have matched our patient and control groups for the main clinical parameters (type of diabetes, ethnicity, and sex) to avoid this possibility. Moreover, the positive association between the *ACE* deletion polymorphism and CHD in NIDDM is strongly supported by the multivariate logistic regression analyses. Multiple testing for association can show significant association by chance. However, *ACE* is a strong candidate gene that had reasonable prior probability of being involved in vascular complications of diabetes mellitus (15, 16). On the other hand, a survival bias cannot be avoided in a disease association study and prospective studies in diabetic families will be necessary to confirm the role of the *ACE* locus. Moreover, it is possible that an early mortality by CHD of NIDDM patients, due to the *ACE* locus, could lead to an underestimation of the cardiovascular effect of this gene in our study.

The frequency of the *DD* genotype was not significantly different in diabetic patients with history of myocardial infarction and in individuals with severe coronary stenoses. This suggests that the *ACE* deletion polymorphism not only is a risk factor for myocardial infarction but also is associated with coronary atherosclerosis (7). Angiotensin II, which is generated by ACE action, through its effects on contractility and growth of vascular endothelium and vascular smooth muscle cells, might play a key role in the pathologic process leading to coronary atherosclerosis (8, 17, 18). Recent *in vivo* data indicate that infusion of angiotensin II results in rapid and substantial increase in circulating levels of plasminogen activator inhibitor 1 (PAI-1) (19), PAI-1 being the most important physiological inhibitor of tissue-type plasminogen activator. These results suggest a potential relation between the renin–angiotensin system and the trombotic risk. Although the molecular mechanisms of the deleterious effects of the *ACE* deletion polymorphism are unclear, this polymorphism accounts for half of the phenotypic variance of serum ACE (4). This polymorphism might be in linkage disequilibrium with regulatory elements of the *ACE* gene.

The *ACE* deletion polymorphism seems to be a potent cardiovascular risk factor in NIDDM. The association between the *DD* genotype and the risk of cardiovascular disease in NIDDM patients would allow early diagnosis to predict CHD, which would be helpful to modify the incidence of myocardial infarction in this population. Indeed, myocardial infarction or severe coronary stenosis occurred early (56 ± 10 years) in our population. Despite the presence of numerous classical cardiovascular risk factors (2.2 for each patient versus 1.7 in controls, not including diabetes), the *ACE* deletion polymorphism seems to be independent of clinical features, in particular of hypertension and lipid values. It is, to our knowledge, the only genetic factor for CHD described in this population to date whose determination is reliable and independent of glucose control, lipid values, and life-style habits. Early identification of NIDDM patients carrying a genetic susceptibility to CHD should be of particular interest because of the high prevalence of silent myocardial ischemia (20) and sudden deaths in these patients. ACE inhibitors have proven effective in patients after myocardial infarction. They significantly reduced myocardial infarction and unstable angina. Moreover, they reduced morbidity and mortality due to major cardiovascular events in patients with low myocardial ejection fractions (≤40%) (21–23). As the *ACE* deletion polymorphism appeared strongly related to the level of circulating enzyme, these drugs may be more efficient in patients having a genetically determined high ACE concentration. That this polymorphism is of interest in primary

Table 5. Logistic regression analysis of the cardiovascular risk factors associated with CHD in univariate analysis

	NIDDM patients					
	Total (n = 316)			Males ≤65 years (n = 157)		
Cardiovascular risk factor	Odds ratio	*P* value	95% CI	Odds ratio	*P* value	95% CI
Age	1.03	0.020	1.01–1.06	1.04	0.150	0.99–1.09
Diabetes duration	1.02	0.150	0.99–1.05	1.01	0.556	0.97–1.05
Triglycerides	1.14	0.199	0.94–1.38	1.15	0.214	0.92–1.44
HDL cholesterol	0.62	0.204	0.23–1.29	0.49	0.172	0.18–1.36
Smokers	1.51	0.0005	1.20–1.90	1.41	0.022	1.05–1.89
D/*I* polymorphism	1.44	0.031	1.03–2.02	1.79	0.009	1.15–2.78

CI, confidence interval.

prevention and detection of CHD in NIDDM remains to be established, in particular for patients carrying the *DD* genotype.

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