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Angiotensin-converting-enzyme gene polymorphism and heart failure: a case–control study

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Heart failure (HF) is the final outcome of virtually all cardiovascular diseases and is a major and increasingly serious public health problem. The renin–angiotensin system plays an important role in the pathogenesis of cardiovascular disease. Insertion/deletion (I/D) polymorphism of the angiotensin-converting enzyme (ACE) has attracted significant attention; it has been extensively investigated in a spectrum of cardiovascular phenotypes because of its correlation with serum ACE activity. There is controversy regarding the association of ACE I/D polymorphism with cardiovascular disease. The aim of this study was to investigate whether ACE genotype is associated with HF by comparing cases and controls. The study sample consisted of 229 cases with HF due to coronary heart disease or idiopathic dilated cardiomyopathy and 230 controls recruited from the general population. The ACE I/D genotype was identified using a polymerase chain reaction assay. No evidence was found to support an association between ACE genotype and HF.

Keywords: heart failure, angiotensin-converting-enzyme, gene polymorphism.

Introduction

The incidence and prevalence of heart failure (HF) have been increasing steadily over the past few years and have now reached epidemic proportions. The incidence of HF is higher in men than in women (Levy *et al*. 2002) and it increases with age. Data from population studies show that the incidence rate of HF ranges from 2.0 per 1000 person-years at age 45–54 years to about 11.0 per 1000 person-years at age 70–84 years in men, whereas it ranges from 0.7 per 1000 person-years to about 7.0 per 1000 person-years in women at the same ages (Cowie *et al*. 1999, Johansson *et al*. 2001, Wilhelmsen *et al*. 2001). The prevalence of HF in the population ranges from 1.0% at 55–64 years to 10.0% at 75–84 years, with no significant differences between men and women (Mosterd *et al*. 1999, Davies *et al*. 2001). The main causes of this increased prevalence of HF are the progressively ageing population and the increasing number of patients with cardiovascular disease who live long enough to develop HF because of improved medical and surgical treatment (MacIntyre *et al*. 2000, Levy *et al*. 2002). The long-term pharmacological treatment and frequent hospital admissions required by patients with HF have a significant economic impact on health services (Zannad *et al*. 1999).

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HF is the final outcome of virtually all cardiovascular diseases. Coronary heart disease (CHD) and hypertension remain the leading causes (Cowie *et al*. 1997, Zannad *et al*. 1999, Kannel 2000), but other conditions associated with HF include idiopathic dilated cardiomyopathy (IDC), left ventricular hypertrophy (LVH), valvular congenital heart disease, diabetes mellitus, dyslipidaemia, obesity (Cowie *et al*. 1997, Kannel 2000, Wilhelmsen *et al*. 2001), and behavioural and environmental factors (Olivari 2000, Dei Cas *et al*. 2003).

Over the past decade the role of genetic factors (Keavney 2002) in the development of cardiovascular diseases has been actively investigated. The insertion/deletion (I/D) polymorphism of a 287 bp sequence in intron 16 of the gene encoding angiotensin-converting enzyme (ACE) has attracted attention and has been extensively investigated in a spectrum of cardiovascular phenotypes because of its correlation with serum ACE activity (Rigat *et al*. 1990, Danser *et al*. 1995). ACE is a key enzyme in the renin–angiotensin system (RAS) that plays an important role in the regulation of cardiac function and blood pressure. It is associated with the generation of angiotensin II, which stimulates vasoconstriction of the systemic and coronary arteries, aldosterone secretion with renal hydro-saline retention and vascular smooth muscle cell growth, and myocardial hypertrophy and fibrosis. It also inactivates bradykinin, a potent peripheral artery dilator and inhibitor of myocardial cell growth (Carluccio *et al*. 2001). It has been shown that the DD genotype is related to increased local cardiac ACE activity and a higher ACE serum level, and might therefore be implicated in the pathogenesis of cardiovascular abnormalities (Danser *et al*. 1995, Davis *et al*. 2000). ACE I/D polymorphism has been reported to be associated with the incidence of myocardial infarction (MI) (Cambien *et al*. 1992, Keavney *et al*. 2000) and ischaemic heart disease (Lindpaintner *et al*. 1995), with left ventricular systolic performance and cavity size in patients with IDC (Candy *et al*. 1999), and with pulmonary function and exercise capacity in patients with HF (Abraham *et al*. 2002).

The serum level of ACE can be influenced by gender; in particular, it has been shown that oestrogens inhibit circulating renin and ACE and decrease circulating angiotensin II levels (Gallagher *et al*. 1999). As reported in a recent review (Fischer *et al*. 2002), this may explain the findings that genetic polymorphisms of the RAS have more profound effects in men than in women in some studies (Anderson *et al*. 1998, O'Donnell *et al*. 1998, Masoudi *et al*. 2003).

However, since the first report of an association between the D allele and MI in the ECTIM study (Cambien *et al*. 1992), there have been confirmatory, negative and even contradictory (implicating the I allele) findings, as reviewed by Samani *et al*. (1996) and more recently by Keavney *et al*. (2000). Selection bias, the low power of many genetic association studies and the tendency to favour the publication of studies with positive results are the most commonly proposed reasons for the inconsistencies among the various studies (Keavney 2002).

In order to provide further data to clarify this issue, we investigated a possible association between I/D ACE gene polymorphism and HF using a case–control study.

Methods

Study design and subjects

A total of 229 consecutive patients admitted to the Institute of Cardiology of the University and in the main hospital (Spedali Civiu in Brescia) Italy, with a diagnosis of HF between January and December 2002 were enrolled as cases. Only patients born in Italy and with an aetiology of HF due to CHD or IDC were eligible for the study. The diagnosis of HF was based on the presence of the typical clinical signs and symptoms of HF with a left ventricular ejection fraction $\leq 40\%$ on two-dimensional echocardiography (Remme and Swedberg 2002).

In order to estimate the ACE genotype distribution in the general population living in the same area, we included in the study a random sample of subjects from the general population living in an urban area of Brescia. We selected 230 subjects (115 males and 115 females) matched for age (± 5 years) with the HF cases.

The controls underwent a clinical evaluation by a Local Health Unit physician to look for the presence of HF even among those who were unaware they had the disease. Subjects with symptoms or signs that could be related to HF (Framingham criteria; McKee *et al*. 1971) were excluded as controls, and underwent Doppler echocardiography to detect any abnormality in myocardial function. Subjects were considered eligible as controls if they were born in Italy, had no clinical symptoms or signs suggesting the presence of HF, and no history of CHD or IDC.

All HF patients and controls completed a structured questionnaire designed to obtain details of family history of cardiovascular disease, possible causes of HF, the presence of other cardiovascular diseases, current drug therapy for these diseases, previous admissions, and the presence of the main risk factors for HF.

A 20 ml blood sample was taken by venipuncture from all the subjects, and the serum stored at -80°C until analysed.

A local Ethics Committee approved the project and written informed consent was obtained from all subjects.

Determination of ACE genotype

Genomic DNA was extracted from 200 μl of ethylene diamine tetra-acetic acid anticoagulated blood using the Fassst DNA Releaser Kit (Celbio, Pero, Italy). ACE gene I/D polymorphism was detected by polymerase chain reaction (PCR) using oligonucleotide primers flanking the insertion as described elsewhere (Lindpainter *et al*. 1995). The amplification reaction was carried out in a final volume of 50 μl containing 100 μg genomic DNA, 0.5 μM primers, 200 μM deoxynucleotide triphosphates, 1.5 mM magnesium chloride, 1 × PCR buffer (10 mM Tris–HCl, pH 8.8 at 25°C, 50 mM potassium chloride and 0.1% Triton X-100) and 1 U *Taq* DNA polymerase (Finnzymes, Espoo, Finland). The DNA was amplified for 30 cycles with denaturation at 95°C for 1 min and annealed at 64°C for 1 min with extension at 72°C for 1 min using a PCR Express thermal cycler (Celbio, Milano, Italy). The PCR products were analysed using 2% agarose gel electrophoresis (NuSieve 3:1, BMA, Rockland, ME, USA) and visualized by ethidium bromide staining. The PCR products are a 597 bp fragment in the presence of the I allele and a 319 bp fragment in the absence of the I allele (i.e. the D allele).

Statistical methods

Differences in clinical variables and in the distribution of subjects homozygous for the I allele (II genotype), heterozygous subjects (ID genotype) and subjects homozygous for the D allele (DD genotype) between the cases and controls were assessed using contingency tables with χ^2 and exact test analyses. Allele frequencies were estimated by gene counting. To test for Hardy–Weinberg equilibrium, the expected genotype numbers were calculated from the allele frequencies, and deviation from the observed genotype numbers was determined using the χ^2 test. All the statistical tests were performed using an α value of < 0.05 to reject the null hypothesis. All the analyses were conducted using the STATA statistical package (Stata Statistical Software, Release 7.0. College Station, Stata Corporation, Texas, USA).

Results

The mean ages of the cases and controls were 61.9 ± 11.2 years and 62.4 ± 7.8 years, respectively ($p > 0.05$). The distribution of the cases and controls according to sex and risk factors for HF is shown in Table 1; 90.4% of cases and 50.0% of controls were males ($p < 0.05$), and CHD and IDC were present in 46.7% and

Table 1. Distribution of cases and controls according to sex and risk factors for HF.

	Cases ($n = 229$)		Controls ($n = 230$)	
	No.	%	No.	%
Sex*				
Male	207	90.4	115	50.0
Female	22	9.6	115	50.0
Presence of risk factors for HF				
Hypertension	89	48.6	94	51.4
Diabetes*	54	75.0	18	25.0
Dyslipidaemia	99	55.6	79	44.4
CHD	107	46.7		
IDC	122	53.3		

*$p < 0.05$.

53.3%, respectively, of the cases. There were no differences between cases and controls in the presence of other risk factors for HF, except for diabetes, which was present in 75.0% of cases and 25.0% of controls ($p < 0.05$). The association between HF and diabetes was still present when analysed according to sex, whereas association was found between HF and hypertension or dyslipidaemia.

Table 2 shows the distribution of ACE genotypes in the cases and controls. There was no deviation from the Hardy–Weinberg equilibrium for the polymorphism considered when comparing expected and actual genotype frequencies for both cases and controls. No differences were noted in allele frequencies when comparing cases with the control group. The percentage of subjects homozygous for the D allele (DD genotype) was 32.3% for the cases and 37.3% for the controls. When subjects homozygous for the D allele (DD genotype) were compared separately with subjects homozygous for the I allele (II genotype) and heterozygous subjects (ID genotype), no difference in the distribution of genotype frequencies between cases and controls was found. The percentage of 'non-DD' subjects (i.e. II and ID genotypes) was 67.7% for the cases and 62.7% for the controls; again, the difference between cases and controls was not statistically significant.

Table 2. Distribution of I and D polymorphisms of the ACE gene in cases and controls.

	Cases ($n = 229$)		Controls ($n = 230$)	
Genotype	No.	%	No.	%
II	36	15.7	39	17.0
ID	119	52.0	105	45.7
DD*	74	32.3	86	37.3
II+ID	155	67.7	144	62.7
DD*	74	32.3	86	37.3
Allele frequency*				
I		42.0		40.0
D		58.0		60.0

*$p > 0.05$.

Table 3. Distribution of I and D polymorphisms of the ACE gene in males.

	Cases ($n = 207$)		Controls ($n = 115$)	
Genotype	No.	%	No.	%
II	31	15.0	18	15.6
ID	110	53.1	54	47.0
DD*	66	31.9	43	37.4
II+ID	141	68.1	72	62.6
DD*	66	31.9	43	37.4

*$p > 0.05$.

We also investigated the distribution of the ACE genotype according to sex. The distribution of ACE genotypes in males is shown in Table 3. In accordance with our findings in the whole study group, no significant differences were observed between the cases and controls with regard to allele distribution in the male subjects. Similar results were found in females (data not shown).

Further investigations were performed to determine whether I/D polymorphism of the ACE gene is associated with the cause of HF (Table 4). The prevalence of subjects homozygous for the D allele (DD genotype) was 29.0% among cases with CHD and 35.3% among those with IDC. Similarly, the prevalence of 'non-DD' (ID and DD genotypes) patients was 71.0% among cases with CHD and 64.7% among those with IDC. These differences were not statistically significant.

Discussion

The importance of the role of the RAS in the pathogenesis of major cardiovascular diseases is now widely recognized. Over the past decade several studies have suggested that ACE gene polymorphism may be a risk factor for cardiovascular disease. In our study, the role of I/D polymorphism was investigated in patients with HF compared with normal subjects, as HF is the final outcome of all cardiovascular diseases. We found no association between the DD genotype and HF (32.3% in cases versus 37.5% in controls; $p > 0.05$), which is consistent with studies investigating other cardiovascular aspects closely correlated with HF. For example, LVH is an important risk factor for HF (Kannel 2000, Dei Cas *et al*. 2003). Although some studies have reported an association between LVH and ACE

Table 4. Distribution of I and D polymorphisms of the ACE gene in CHD and IDC.

	CHD ($n = 107$)		IDC ($n = 122$)	
Genotype	No.	%	No.	%
II	19	17.7	17	13.9
ID	57	53.3	62	50.8
DD*	31	29.0	43	35.3
II+ID	76	71.0	79	64.7
DD*	31	29.0	43	35.3

*$p > 0.05$.

I/D polymorphism (Schunkert *et al*. 1994, Candy *et al*. 1999), Lindpaintner *et al*. (1996) found no evidence of such an association in the Framingham Heart Study. Furthermore, Kunetzosova *et al*. (2000) conducted a meta-analysis of 23 studies with 5438 participants and concluded that, overall, LVH was not associated with D allele. With regard to MI, Cambien *et al*. (1992) were the first to report a significant association between the ACE DD genotype and an increased risk of MI among 610 cases versus 733 controls. Samani *et al*. (1996) conducted a meta-analysis of 15 published studies on 3394 MI cases and 5479 controls. The overall odds ratio of MI for the DD versus the ID/II genotypes across all the studies was 1.26 (95% confidence interval 1.15–1.39; $p < 0.0001$), which supports a weak association between the D allele and MI risk. However, Lindpaintner *et al*. (1996) could not confirm such an association in 387 patients versus 1475 controls, and another extensive study carried out by Keavney *et al*. (2000) involving 5000 cases and 6000 controls did not confirm the existence of a significant association. IDC is another cardiovascular disorder in which left ventricular dilation and dysfunction lead to HF (Zannad *et al*. 1999, Dei Cas *et al*. 2003). Two studies did not find evidence of any involvement of ACE I/D polymorphism in susceptibility to IDC (Montgomery *et al*. 1995, Tiret *et al*. 2000). In the present study, the cases all had HF due to either IDC or CHD, but the DD genotype was not associated with either of these conditions.

Given that male sex is a risk factor for cardiovascular heart disease and HF (Chen *et al*. 1999) and that the ACE level can be influenced by gender due to inhibition of circulating ACE by oestrogen (Fischer *et al*. 2002), we investigated a possible association of I/D polymorphism with HF according to sex, but again found no difference in DD genotype frequency between cases and controls (31.9% versus 37.4%; $p > 0.05$). A positive association of DD genotype with male sex was found in the study of LVH reported by Schunkert *et al*. (1994). However, Tiret *et al*. (2000) did not find any correlation between I/D polymorphism and IDC or LVH when also controlling for gender.

In conclusion, our data do not suggest a role for ACE I/D genotypes as a risk factor for HF in the population studied. Similar results were also obtained when the patients were divided according to sex or cause of HF.

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