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Lone and secondary nonvalvular atrial fibrillation: Role of a genetic susceptibility

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Abstract

Background: An involvement of the renin angiotensin system in atrial fibrillation (AF) has been hypothesized, and *ACE* DD genotype has been suggested to influence the predisposition to AF. The aim of this study was to investigate the role of the *ACE* I/D polymorphism in relation to the different clinical forms of AF, lone and secondary nonvalvular atrial fibrillation (NVAF).

Methods: 510 consecutive patients with documented NVAF (106 patients had lone, and 404 secondary NVAF), and 520 controls with a negative history of cardiovascular disease have been studied.

Results: A significant difference in allele frequency between lone and secondary NVAF ($p=0.002$) has been found. The *ACE* D allele was associated with the predisposition to lone NVAF under a dominant, recessive and additive model, both at univariate and multivariate analysis, after adjustment for age and gender (multivariate analysis: dominant OR=2.87, $p=0.02$; recessive OR=2.01, $p=0.003$; additive OR=4.47, $p<0.0001$). *ACE* D allele was significantly associated with secondary NVAF at both univariate and multivariate analysis under a recessive and additive, but not dominant, model (multivariate analysis: recessive OR=1.89, $p=0.001$; additive OR=2.50, $p<0.0001$).

Conclusions: This study highlights the role of *ACE* gene in predisposing to both lone and secondary NVAF, further contributing to penetrate the genetic mechanisms responsible for this complex disease. The clinical relevance of our results may be related to the possible characterization of subjects predisposed to NVAF in the absence of traditional risk factors, and to the use of ACE-inhibitors therapy able to improve the arrhythmogenic substrate.

Keywords: ACE I/D polymorphism; Predisposition to disease; Nonvalvular atrial fibrillation

1. Introduction

Nonvalvular atrial fibrillation (NVAF) is the commonest cardiac arrhythmia in clinical practice and is associated with substantial morbidity and mortality. Its incidence and prevalence are increasing, and it represents a potential cause of thromboembolic events [1]. Independent risk factors include male sex, increasing age, hypertension, thyroid disease, diabetes, smoking habit, valvular heart disease, and myocardial infarction [2], besides left atrial dilation and left ventricular hyperthrophy [3]. Nevertheless, the molecular basis for the development of structural remodeling of human atria is still a matter of investigation. There are increasing information on the relevance of investigating polymorphisms in candidate genes for atrial fibrillation (AF), in order to evaluate their influence on susceptibility to this complex condition. Genetic case-

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Table 1
Demographic and clinical characteristics of the study population

Variable	Patients (n=510)	Healthy subjects (n=520)	P-value
Age (years)[a]	71.3±10.2	70.1±13.0	0.07
Males, n (%)	312 (61.2)	326 (62.7)	0.6
Females, n (%)	198 (38.8)	194 (37.3)	0.6
Hypertension, n (%)	308 (60.4)	119 (22.9)	<0.0001
Dyslipidemia, n (%)	113 (22.2)	86 (16.5)	0.04
Diabetes, n (%)	85 (16.7)	22 (4.2)	<0.0001
Smoking habit, n (%)	128 (25.1)	87 (16.7)	0.001
CAD (%)	114 (22.4)	–	
Left atrial dimension >38 mm (%)	427 (83.7)	–	
LVEF<50% (%)	169 (33.1)	–	
ACE-Inhibitor (%)	109 (21.4)	12 (2)	
β-blocker (%)	20 (4)	2 (0.4)	
Angiotensin receptor antagonist (%)	30 (6)	2 (0.4)	
Calcium antagonist (%)	49 (10)	1 (0.2)	
Diuretic (%)	90 (18)	1 (0.2)	
Digoxin (%)	104 (20)	–	
Statin (%)	23 (5)	–	
Aspirin (%)	118 (23)	–	
Oral anticoagulant (%)	25 (5)	–	
Antiarrhythmic (%)	58 (11)	–	
Oral antidiabetes (%)	31 (6.1)		

[a] Continuous data are presented as mean±SD; CAD = coronary artery disease; LVEF = left ventricular ejection fraction.

control association studies look for correlations between phenotype and genotype and represent the most common studies used to evaluate the genetic basis of disease predisposition. Some polymorphisms have functionally significant effects on the gene product and they represent the most useful type of polymorphisms in disease association studies.

In the intron 16 of the gene encoding for the angiotensin converting enzyme (ACE), a polymorphism consisting of an insertion/deletion (I/D) of a 287-bp fragment has been identified [4], and the ACE D allele has been reported to be associated with increased serum levels of circulating enzyme [5]. The ACE DD genotype is associated with higher plasma levels of the enzyme, the II genotype with lower ACE levels, and the ID genotype with intermediate levels [5]. Data from experimental studies reported both a functional role for the *ACE* I/D polymorphism in modulating angiotensin II levels [6], and an increased mRNA expression in white blood cells from subjects carrying the ACE D allele in comparison to subjects carrying the I allele [7].

The ACE I/D is an appealing polymorphism in relation to susceptibility to cardiovascular diseases, including hypertensive heart disease [8], congestive heart failure [9], coronary artery disease [10], and cardiomyopathy [11]. An involvement of the renin angiotensin system (RAS) in AF has been suggested, due to the finding of increased *ACE* expression in the atrial tissue of patients with AF [12]. Angiotensin II can induce via activation of mitogen activated protein kinases, such as Erk1/Erk2, the proliferation of fibroblasts as well as extracellular matrix protein accumulation [13], and it has been hypothesized that the demonstrated activation of the Erk pathway within the atrial interstitium may be one molecular mechanism explaining fibrotic changes of the atrial tissue in patients with AF.

We previously showed a role for the *ACE* DD genotype as a predisposing factor to AF in 148 patients with persistent AF compared with 210 healthy controls [14].

The aim of this report was to investigate the influence of ACE I/D polymorphism in relation to the clinical forms of lone and secondary NVAF, in a larger population.

2. Materials and methods

2.1. Study population

The study population consisted of 510 consecutive hemodynamically stable patients with documented NVAF lasting >24 h without periods of sinus rhythm (mean duration 5±0.4 weeks, as documented by electrocardiography) referred to the Division of Cardiology, University of Florence, Careggi Hospital, Florence, and 520 control subjects, comparable for age and gender, invited to participate in the study, and consisted of partners or friends of patients, and healthy subjects from a population study "Progetto Nutrizione per la Salute e la Prevenzione di Malattia" conducted between 2002 and 2004 in Florence, Italy. Exclusion criteria for the controls were: personal and family history of cardiovascular disease. A detailed interview addressed to personal and familial history was performed in the frame of a physical examination by expert physicians, in order to identify symptom-free subjects and to exclude those who were suspected of having any form of vascular disease. Moreover, we accurately interviewed our control subjects, in order to identify the existence of symptoms related to arrhythmias.

For all subjects a 12-lead ECG, 24-h ECG recordings, stress-test, chest roentgenogram, routine laboratory screening, thyroid function test, and echocardiography were obtained. Transthoracic echocardiography was performed to measure left atrial dimension. In particular, in patients showing symptoms related to the presence of cardiovascular disease, a more accurate clinical and instrumental evaluation was performed (e.g. echo-stress testing, myocardial scintigraphy, and/or coronarography).

For the inclusion in the current study, all subjects needed to have performed one or more complete echocardiographic examinations. All of the original echocardiographic data

Table 2
ACE I/D polymorphism in subjects receiving ACE-inhibitors therapy

ACE I/D polymorphism	Patients (n=109)	Healthy subjects (n=12)
DD	44 (40.4)	5 (41.7)
ID	51 (46.8)	5 (41.7)
II	14 (12.8)	2 (16.6)

Table 3
Genotype distribution and allele frequencies of *ACE* I/D polymorphism

Genotype	Allele	All patients	Lone AF patients	Secondary AF patients	Healthy subjects
Distribution	Frequency	(n=510) (%)	(n=106) (%)	(n=404) (%)	(n=520) (%)
ACE II		70 (13.7)	9 (8.5)	61 (15.1)	126 (24.2)
ACE ID		218 (42.7)	49 (46.2)	169 (41.8)	251 (48.3)
ACE DD		222 (43.5)	48 (45.3)	174 (43.1)	143 (27.5)
	ACE D	0.65 [a]	0.68 [b]	0.64	0.52

[a] *ACE* D frequency in all NVAF patients vs. healthy subjects, $p<0.0001$; genotype distribution $p<0.0001$.
[b] *ACE* D frequency in lone NVAF patients vs. secondary NVAF patients, $p=0.002$; genotype distribution $p=0.2$.

were available for the offline measurements of left atrial dilation and ejection fraction.

Exclusion criteria for patients included one of the following: familial AF, hyperthyroidism, valvular heart disease, including mitral valve prolapse, symptomatic heart failure, cardiomyopathy, chronic obstructive pulmonary disease, cardiomegaly apparent on the chest radiography, AF due to trauma, surgery, or acute medical illness. The number of patients initially assessed for inclusion into the study was 632. One-hundred and twelve patients were ineligible for inclusion after initial assessment as 28 had thyroid disease, 24 valvular heart disease, 26 symptomatic heart failure, 11 cardiomyopathy, 15 pulmonary disease, and 8 a familial history of AF. Moreover, 10 AF patients refused to assent with the genetic analysis.

Patients who were younger than 65 (years) and had no identifiable cause of AF were classified as lone NVAF patients.

The presence of traditional cardiovascular risk factors was assessed on the basis of patients' interview, echocardiography data and hospital records. Current smoking status was determined at the time of blood collection. The subjects were considered to have hypertension according to the guidelines of the European Society of Hypertension/European Society of Cardiology [15] or if they were taking antihypertensive drugs. Dyslipidemia was defined according to the Third report of the National Cholesterol Education Program (NCEP) [16], and diabetes in agreement with the American Diabetes Association [17].

Coronary heart disease was defined on the basis of a history of myocardial infarction or stable and unstable angina. Left atrial dilation was defined as a diameter >38 mm at echocardiography [18], and left ventricular dysfunction was defined as ejection fraction <50%.

All subjects in the patient and control group were Caucasian, unrelated to each other, and resident in the same geographic area. All subjects gave informed consent and the study complies with the Declaration of Helsinki and was approved by the local ethic committee.

2.2. Molecular analysis

Genomic DNA extraction was performed from peripheral blood leucocytes by using a QIAmp Blood Kit (QIAGEN, Hilden, Germany).

The *ACE* I/D polymorphism was genotyped according to previously reported methods [19].

ACE I/D polymorphism was evaluated through PCR reaction, carried out in a PCR thermocycler (MJ Research) at an annealing temperature of 58 °C, according to Rigat et al. [20]. The reaction was performed in a final volume of 15 μl with 0.2 mM of each dNTP, 5% DMSO, $MgCl_2$ 3 mM, 4 pmol of forward primer (5′-CTGGAGACCACTCCCATCCTTTCT-3′), 4 pmol of reverse primer (5′-GATGTGGCCATCA-CATTCGTCAGAT-3′, 0.5 U of Taq polymerase (GoTaq, Promega Italia, Milano, Italy) in PCR 1X buffer. ACE DD genotype samples were subjected to a second PCR reaction at an annealing temperature of 67 °C in order to specifically amplify the I allele [19].

2.3. Statistical analysis

Statistical analysis was performed by using the SPSS (Statistical Package for Social Sciences, Chicago, USA) software for Windows (Version 11.5). Continuous variables

Table 4
Univariate analysis for ACE I/D polymorphism according to dominant, recessive and additive genetic models

Variable	Odds ratios (95% CI)	P
Age	1.007 (0.99–1.02)	0.4
Gender	0.95 (0.71–1.26)	0.7
Hypertension	10.54 (7.72–14.31)	<0.0001
Diabetes	5.72 (3.48–9.32)	<0.0001
Smoking habit	2.31 (1.70–3.21)	<0.0001
Dyslipidemia	1.53 (1.21–2.14)	0.007

ACE I/D polymorphism	Dominant model		Recessive model		Additive model	
	Odds ratios (95% CI)	P	Odds ratios (95% CI)	P	Odds ratios (95% CI)	P
Lone NVAF	2.89 (1.21–6.04)	0.02	2.18 (1.40–3.51)	<0.0001	4.69 (2.22–9.96)	<0.0001
Secondary NVAF	1.44 (0.95–2.19)	0.08	1.99 (1.51–2.60)	<0.0001	2.51 (1.72–3.67)	<0.0001

Table 5
Multivariate analysis for ACE I/D polymorphism according to dominant, recessive and additive genetic models

ACE I/D	Dominant model		Recessive model		Additive model	
Polymorphism	Odds ratios (95% CI)	*P*	Odds ratios (95% CI)	*P*	Odds ratios (95% CI)	*P*
Lone NVAF [a]	2.87 (1.19–6.89)	0.02	2.01 (1.31–3.09)	0.003	4.47 (2.01–9.92)	<0.0001
Variables						
Age	0.99 (0.97–1.02)	0.8	1.01 (0.98–1.02)	0.8	1.003 (0.97–1.03)	0.8
Gender	1.42 (80.83–2.46)	0.2	1.15 (0.73–1.89)	0.5	1.27 (0.66–2.46)	0.5
Secondary NVAF [b]	1.56 (0.95–2.56)	0.07	1.89 (1.32–2.78)	0.001	2.50 (1.57–3.99)	<0.0001
Variables						
Age	1.02 (0.98–1.03)	0.2	0.99 (0.89–1.01)	0.7	1.003 (0.98–1.02)	0.9
Gender	1.11 (0.74–1.67)	0.6	1.20 (0.78–1.84)	0.3	0.96 (0.61–1.53)	0.9
Hypertension	8.92 (5.97–13.31)	<0.0001	11.72 (7.9–17.2)	<0.0001	9.22 (5.88–14.44)	<0.0001
Diabetes	3.83 (2.02–7.26)	<0.0001	4.16 (2.2–7.8)	<0.0001	4.02 (1.94–8.33)	<0.0001
Smoking habit	2.38 (1.50–3.78)	<0.0001	1.84 (1.2–2.9)	0.007	1.81 (1.08–3.02)	0.02
Dyslipidemia	1.96 (81.25–3.07)	0.003	1.19 (0.8–1.7)	0.3	1.25 (0.88–1.78)	0.2

[a] Adjusted for age, gender.
[b] Adjusted for age, gender, hypertension, smoking habit and diabetes, ACE I/D polymorphisms, and presence of CAD.

are expressed as mean±Standard Deviation (SD), and the nonparametric Mann–Whitney test for unpaired data was used for comparison between single groups. The χ^2-test was used to test for deviation of genotype distribution from Hardy–Weinberg equilibrium. The association between ACE I/D polymorphism and NVAF was assessed using logistic regression analysis under a dominant, recessive and additive genetic model. The dominant genetic model compares individuals with one or more polymorphic alleles with a baseline group with no polymorphic alleles (e.g. *ACE* DD+ID vs. II genotype). The recessive genetic model compares the DD genotype with the combined ID+II genotypes, which form the baseline group. The additive genetic model assumes that there is a linear gradient in risk between the DD, ID and II genotypes (II genotype baseline). This is equivalent to a comparison of the D allele vs. the I allele (baseline). All variables that resulted with a *p*-value <0.2 in the univariate analysis (hypertension, diabetes, dyslipidemia, and smoking habit) were introduced into a multivariable model. Variables as age and gender were also included into the multivariable model. Odds ratio (OR) with 95% confidence interval was determined. Statistical significance was accepted at *p*-value <0.05. A sample size of at least 300 subjects/group was deemed sufficient to prove/exclude an association between ACE I/D polymorphism and atrial fibrillation with a statistical power (β) of 90%, and significance value of 0.05 (α). A sample size of about 300 subjects in each group would limit the β-error of 0.10 (i.e. the power of the study would be 90%).

Table 6
ACE ID Polymorphisms and left atrial dimension in lone and secondary NVAF

Genotype distribution	Allele frequency	Lone NVAF(a)	Lone NVAF(b)	Secondary NVAF(c)	Secondary NVAF(d)
		>38 mm	<38 mm	>38 mm	<38 mm
		(*n*=75) (%)	(*n*=31) (%)	(*n*=352) (%)	(*n*=52) (%)
ACE II		8 (10.7)	1 (0.30)	57 (16.2)	6 (11.5)
ACE ID		35 (46.6)	14 (45.0)	152 (43.2)	18 (34.6)
ACE DD		32 (42.7)	16 (52.0)	143 (40.6)	28 (53.8)
	ACE D	0.63	0.74	0.62	0.71

a vs. b allele frequency $p=0.19$, genotype distribution $p=0.4$.
c vs. d allele frequency $p=0.07$, genotype distribution $p=0.19$.

3. Results

The demographic and clinical characteristics of the study population are reported in Tables 1 and 2.

The genotype distribution and allele frequency of the *ACE* I/D polymorphism were in agreement with those predicted by Hardy–Weinberg equilibrium in both patients and controls. The genotype distribution and allele frequency of *ACE* I/D polymorphism are shown in Table 3. The prevalence of the *ACE* D allele was 0.65 in NVAF patients, and 0.52 in controls. The analysis was carried out on the basis of the different clinical forms of the disease (lone, $n=106$ and secondary, $n=404$), and according to a dominant, recessive and additive genetic model. We observed a significant difference in allele frequency, and in genotype distribution, between NVAF patients and controls (Table 3); as concerning lone and secondary clinical form, we found a significant difference in ACE D allele frequency, but not in genotype distribution (Table 3). The *ACE* D allele was associated to the predisposition to lone NVAF under a dominant, recessive and additive model (Table 4), which remained significant under the three genetic models after adjustment for age and gender (Table 5).

As regards to the secondary NVAF, at univariate analysis we observed a significant association between ACE D allele and the susceptibility to the disease, under a recessive and additive, but not dominant, model (Table 4). After

adjustment for age, gender, traditional cardiovascular risk factors and presence of CAD, *ACE* D allele still remained a predisposing factor to the disease under the recessive and additive genetic model (Table 5).

As left atrial dimension is considered, we observed a higher prevalence of *ACE* D allele in both lone and secondary NVAF patients with left atrial dimension <38 mm, in comparison to that observed in those with left atrial dimension >38 mm (Table 6).

4. Discussion

This report strengthens previous indications about the role of *ACE* I/D polymorphism as a predisposing factor for NVAF. In the present study *ACE* I/D polymorphism was found to be associated with both lone and secondary NVAF, providing support for its investigation in evaluating the susceptibility to the disease.

These findings are consistent with the observation of the involvement of RAS in NVAF. An increased ACE activity, and consequently increased angiotensin II levels, may cause electrophysiological alterations via different mechanisms. In particular, extensive interstitial fibrosis is an important pathophysiological substrate of AF. Angiotensin II can induce the proliferation and differentiation of fibroblasts through the involvement of extracellular signal-regulated kinases proteins [21]. Moreover, angiotensin II can contribute to cell differentiation and fibroblast activation by the involvement of other protein kinases like cJun-N-terminal kinase or p38 mitogen activated protein kinase [22]. Angiotensin II may also induce the development of interstitial fibrosis by stimulating collagen synthesis and inhibiting collagenase activity [23]. Furthermore, the ability of angiotensin II to stimulate fibroblasts and alter the metabolism of fibrillar collagen may be mediated by transforming growth factor-β_1, endothelin-1, and plasminogen activator inhibitor-1 [24], and experimental studies provided evidence that osteopontin represented a critical mediator of the proinflammatory and profibrotic effects [25,26]. Data from experimental and clinical studies [27,28] reported a role for ACE I/D polymorphism in influencing interstitial fibrosis. Really, it has been demonstrated, in a genetic rat model, a different development of cardiac fibrosis in relation to different ACE I/D polymorphism genotypes, in particular rats with high ACE plasma levels with ACE DD genotype, have more accumulation of collagen I and fibronectin in comparison to animals with low plasma ACE activity, and the other two genotypes [25]. Accordingly, data from patients with untreated essential hypertension, have demonstrated the association between ACE DD genotype and elevated serum carboxy-terminal propeptide of procollagen type I (PICP), a marker of myocardial fibrosis, concentration, thus proposing a link between the ACE DD genotype and myocardial fibrosis in humans [26].

The observation that *ACE* D allele does not influence the left atrial dimension in both lone and secondary NVAF, leads us to hypothesize that the real mechanism by which this gene influences the predisposition to the disease, lies in the profibrotic effect of angiotensin II on cardiac tissue. In particular, the datum of a higher, but not significant, prevalence of *ACE* D allele in NVAF patients with left atrial dimension <38 mm, in comparison to that observed in those with left atrial dilation, supports this hypothesis.

The results from the current study are in keeping with our previous report [14], and underline the role of the *ACE* gene in modulating the susceptibility to both lone and secondary NVAF in a larger population. Actually, in our study the number of subjects investigated was sufficient to detect absolute differences in allele frequency, with a statistical power of 90% (β=0.9) and a significance value of 0.05 (α). Recently a role for multiple RAS genes in the development of nonfamilial structural AF has been suggested [29]. Our findings are at variance with those of Tsai et al. [29], who found an association between AF and the ACE I/D polymorphism only in a subclass of patients with specific angiotensinogen gene variants, and with valvular disease. These conflicting results may be due to the different prevalence of the *ACE* D allele, probably related to the different genetic background of the study populations. Moreover, our patients had no valvular disease, and we analyzed patients with both lone and secondary AF. Control subjects in the study of Tsai et al. did not consist of healthy individuals, but rather of patients with diabetes, hypertension, or CAD and the only difference was the presence or absence of AF.

Recently, a prospective study by Zaman et al. [30] explored the role of ACE-inhibitors in facilitating cardioversion of persistent AF, and reported that the use of long-term therapy facilitated electrical defibrillation in persistent AF patients. Our findings suggest that ACE I/D polymorphism determination may be used as a guide to identify AF subjects who are most likely to benefit from pharmacological treatment with ACE-inhibitors. There is evidence that responses to drugs are, at least partially, under genetic control [31]; therefore the growing need for better identifying people who have the highest benefit from pharmacological interventions and the lowest risk of developing side-effects, assumes that pharmacogenetic, which explores the individual response to a particular drug, may significantly assist in choosing the adequate drug.

The clinical implication of our results is mainly related to the physiopathological meaning of these findings; really, the ACE D allele modulates angiotensin II levels, so contributing to the cardiac remodeling and to the development of atrial fibrillation. Pharmacological inhibition of the ACE appears to reduce atrial structural remodeling, and prevent the loss of atrial microcapillaries [32]. Moreover, data from a recent meta-analysis [33] reported that the use of ACE-inhibitors and ARBs following cardioversion, appears promising in the prevention of atrial fibrillation.

This study has two limitations. First, the control group was composed of apparently healthy subjects very seldom

affected by diabetes or hypertension, which make this group quite different from the patient group. One could hypothesize that the *ACE* D allele influences susceptibility to either diabetes or hypertension, or both; nevertheless, the observation that the *ACE* D allele is associated with lone NVAF strongly supports the genetic relevance in the pathogenesis of this condition. The second limitation lies in lack of information about serum ACE levels. The *ACE* D allele has been reported to be associated with increased serum levels of circulating enzyme in both healthy subjects [5], and in coronary artery disease [34], thus modulating angiotensin II availability. Actually, this study provides evidence of the association between *ACE* D allele and NVAF, but it does not demonstrate the direct mechanism by which *ACE* I/D polymorphism causes the disease.

In summary, this study highlights the role of *ACE* gene in predisposing to both lone and secondary NVAF, thus contributing to investigate the molecular mechanisms responsible for this complex condition. Therefore, the clinical relevance of our findings lies on the one hand in the possible characterization of subjects predisposed to NVAF in the absence of traditional risk factors, and on the other hand in the use of ACE-inhibitors therapy, thus improving the arrhythmogenic substrate. It will be intriguing to verify the hypothesis of a role of the ACE I/D polymorphism as a genetic determinant of response to ACE in AF.

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