Paper

Angiotensin-converting enzyme gene polymorphism in arrhythmogenic right ventricular dysplasia: is DD genotype helpful in predicting syncope risk?

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Key words: angiotensin-converting enzyme gene polymorphism, arrhythmogenic right ventricular dysplasia, sudden cardiac death, syncope, ventricular tachycardia

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Journal of the Renin-Angiotensin-Aldosterone System
(Including other Peptidergic systems)

December 2008
Volume 9
Number 4

Abstract

Introduction. Arrhythmogenic right ventricular dysplasia (ARVD) is a heritable disorder characterised by fibrofatty replacement of right ventricular myocytes and increased risk of ventricular arrhythmias and sudden cardiac death. Angiotensin-converting enzyme (ACE) gene insertion/deletion (I/D) polymorphism affects myocardial ACE levels. DD genotype favours myocardial fibrosis and is associated with malignant ventricular tachycardia. The aim of this study was to explore ACE gene polymorphism in ARVD patients.

Methods. Twenty-nine patients with ARVD and 24 controls were included. All ARVD patients had documented sustained ventricular tachycardia. Thirteen patients had syncopal episodes. Six patients were resuscitated from sudden cardiac death. ACE gene polymorphism was identified by polymerase chain reaction technique.

Results. There was no significant difference in DD genotype frequency between ARVD patients and controls (44.8% *vs.* 45.8%, p=0.94). However, DD genotype frequency was significantly higher in ARVD patients with syncopal episodes compared to those without syncope (69.2% *vs.* 25.0%, p=0.017, odds ratio: 6.750, 95% confidence interval: 1.318–34.565). DD genotype was detected in higher frequency also in patients with a family history of sudden cardiac death (66.7% *vs.* 39.1%, p=0.36).

Conclusion. High prevalence of DD genotype in ARVD patients with syncope suggests that ACE I/D polymorphism might be useful in identifying high-risk patients for syncope.

Introduction

Arrhythmogenic right ventricular cardiomyopathy or dysplasia (ARVD), which was first described in 1977 by Fontaine *et al.*,[1] is an inherited condition characterised by progressive degeneration and replacement of the right ventricular myocardial tissue by fat and fibrosis, constituting the anatomic basis for reentry ventricular arrhythmias.[2-6] It involves mostly the right ventricle leading to right ventricular dysfunction and less commonly the left ventricle.[7] Pathological examinations of patients with ARVD revealed fibrofatty replacement of the myocardium of the right ventricle.[5] The usual clinical presentation of ARVD is that of palpitations, non-sustained and sustained ventricular arrhythmias. Syncope or sudden cardiac death (SCD) may be the first manifestation of the disease. Studies have shown that ARVD is present in up to 20% of individuals who experience SCD and is even more common among athletes who die suddenly.[5,8,9] Most patients with this condition experience the onset of these symptoms between the ages of 20 and 40 years.[5,10] The disease shows a predisposition to occur in men with an incidence of six per 10,000 persons in certain populations.[11]

ARVD appears to have a strong familial association, with more than 30% of patients reporting a family history of the disorder.[4,12,13] The most common pattern of inheritance is autosomal dominant,[11,14] although an autosomal recessive pattern has been reported.[15] Since the identification of the first ARVD locus in 1994,[16] at least 11 loci and five candidate genes have been identified.[17] The majority of the genes (desmoplakin, plakoglobin and plakophilin-2) that cause ARVD code for proteins that are involved in cell-to-cell adhesion. Mutations in these genes result in impairment of cell-to-cell adhesion leading to accelerated apoptosis of myocardial cells, with the repair process consisting of replacement of myocardium by adipose and fibrous tissue.[18] In addition, cardiotropic viruses have been detected in some ARVD cases, and they have been proposed as possible aetiologic agents.[19]

The angiotensin-converting enzyme (ACE-kininase II) is a dipeptidyl carboxypeptidase present on the surface of vascular endothelial cells and in circulating plasma, and plays an important role in blood pressure regulation and electrolyte balance by hydrolysing angiotensin I

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to angiotensin II, a potent vasoconstrictor, and catabolising bradykinin, a potent vasodilator. The ACE gene is located on chromosome 17. After familial studies found that the level of plasma ACE was partly under genetic control,[20] an insertion/deletion (I/D) polymorphism was detected in 1990, which accounts for up to 50% of the variation in circulating and tissue ACE levels.[21] The polymorphism is characterised by the presence (insertion - I) or absence (deletion - D) of a 287-bp *alu* repeat sequence within intron 16, which results in three genotypes (II and DD homozygotes and ID heterozygotes).[22] The frequency of the ACE DD genotype is approximately 0.27 in the general population.[23] Circulating[21] and tissue ACE[24] levels are higher in individuals who are homozygous for the deletion (DD) compared to individuals who are homozygous for the insertion (II) or who are heterozygous (ID). Cardiac renin-angiotensin system is involved in cardiac remodelling and fibrosis. ACE gene polymorphism has been shown to determine the extent of cardiac fibrosis.[25] The D allele is associated with greater myocardial ACE levels,[24] while DD genotype favours myocardial fibrosis and is associated with increased QT dispersion[26] and malignant ventricular arrhythmias.[27]

Since ACE gene polymorphism is involved in cardiac fibrosis and associated with ventricular arrhythmias, we explored whether there was any relation between the ACE gene polymorphism and ARVD.

Methods

The study conformed to the principles outlined in the Declaration of Helsinki. The study was approved by the local ethics committee. All patients gave written informed consent.

The patients who were followed by the Arrhythmia Outpatient Clinics of the Faculty of Medicine, Istanbul University and Marmara University Hospital with a diagnosis of ARVD were invited to the study. Twenty-nine patients (23 male, mean age: 38.0±13.1 years) were included in the study. The diagnosis of ARVD was established on the basis of the criteria set by the Task Force of the Working Group of Myocardial and Pericardial Disease of the European Society of Cardiology and of the Scientific Council on Cardiomyopathies of the International Society and Federation of Cardiology.[28] All the patients had undergone echocardiographic examination which revealed right ventricular dilation and cardiac magnetic resonance imaging which demonstrated myocardial fibrofatty infiltration and replacement together with right ventricular dilation and global or regional wall motion abnormalities of the right ventricle.

The patients' medical histories were obtained both by review of medical records and by patient interview. Information regarding the presenting symptoms as well as major clinical events including syncope, sustained ventricular tachycardia (VT), implantable cardioverter/defibrillator (ICD) implantation and catheter ablation was noted. Patients were also interviewed to determine the family history of SCD.

All ARVD patients had palpitations and documented sustained VT. Thirteen patients (44.8%) had syncope, while six patients (20.7%) were resuscitated from SCD. Twenty-four patients had undergone electrophysiological study; VT was induced in 15 patients (62.5%) and ventricular fibrillation was induced in two patients (8.3%), while no arrhythmia was induced in the remaining seven patients (29.2%). Radiofrequency catheter ablation (RFA) was performed successfully in seven patients (29.2%). However, VT recurred in two patients during the follow-up. ICD was implanted in 15 patients (51.7%) and six patients (40.0%) had received at least one appropriate ICD therapy during one-year follow-up. The medical therapy included beta-blockers in 17 patients, amiodarone in four patients, propafenone in three patients, sotalol in one patient and verapamil in one patient. Six patients (20.7%) had a family history of SCD or undiagnosed syncope.

Twenty-four healthy individuals (19 male, mean age: 40.5±12.4 years), who proved to be in a good state of health and free from any signs of cardiac or other chronic disease after a careful clinical examination and laboratory check-up, were included as the control group. There was no significant difference in age and gender distributions between the patients and controls (p=0.47 for age and p=1.00 for gender). All the healthy controls were non-smokers.

The general characteristics of the ARVD patients and the controls are presented in table 1.

Genotyping

Peripheral blood samples from all patients and controls were collected into EDTA-coated tubes. Genomic DNA was extracted from peripheral blood leukocytes with DNA isolation kit (High Pure DNA Isolation Kit, Roche Diagnostic). The ACE genotypes were assessed by polymerase chain reaction (PCR) technique according to previously published protocols.[29-31] The I/D polymorphism of the ACE gene was assessed by detecting the presence (allele I, insertion) or absence (allele D, deletion) of a 287-bp sequence in intron 16 of the ACE gene in chromosome 17. A set of primers was designed to encompass the polymorphic region in intron 16 of the ACE gene (sense primer 5' CTGGAGACCACTCCCATCCTTTCT

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Table 1
The general characteristics of the ARVD patients and controls.

	ARVD patients (n=28)	Controls (n=24)	p
Age at presentation (mean±standard deviation, year)	38.0±13.1 (age range: 15–65)	40.5±12.4 (age range: 21–65)	0.47
Gender (male) (n-%)	23 (79.3%)	19 (79.2%)	1.00
Body mass index (kg/m^2)	26.04±3.91	24.49±3.95	0.53
Smoking (n-%)	12 (42.9%)	0	
Hypertension (n-%)	2 (7.1%)	0	

Key: ARVD = arrhythmogenic right ventricular dysplasia.

Table 2
Allele and genotype distributions between the groups.

	ARVD patients	Controls	p
D allele (n-%)	38 (65.5%)	29 (60.4%)	0.59
I allele (n-%)	20 (34.5%)	19 (39.6%)	0.59
DD genotype (n-%)	13 (44.8%)	11 (45.8%)	0.94
ID genotype (n-%)	12 (41.4%)	7 (29.2%)	0.36
II genotype (n-%)	4 (13.8%)	6 (25.0%)	0.48

Key: For genotype comparison, p=0.52 (Fisher's Exact test). ARVD = arrhythmogenic right ventricular dysplasia.

3' and antisense primer 5' GATGTGGCCATCACATTCGTCAGAT 3'). DNA was amplified for 35 cycles, each cycle composed of denaturation at 94°C for 30 seconds, annealing at 58°C for 30 seconds, and extension at 72°C for 30 seconds. The PCR products (190-bp D allele and 490-bp I allele) were separated by electrophoresis on 2% agarose gel, stained with ethidium bromide, and viewed with ultraviolet light.

The possibility of mistyping ID heterozygotes as DD homozygotes due to the preferential amplification of the smaller D allele was addressed.[32] To verify the DD allele, each sample found to be DD was subjected to a second, independent PCR with a primer pair (hace5a and hace5c) that permits amplification only in the presence of the I allele, but not the D allele; this was done using the method described by Lindpaintner *et al.*[33]

The operators who performed the I/D genotype determination were unaware of the patients' characteristics.

Statistical analysis

The statistical analysis was performed with a commercially available statistical analysis programme (SPSS 11.0 for Windows, SPSS Inc., Chicago, IL, USA). Categorical variables were expressed as frequency (percentage), while continuous variables were expressed as mean±standard deviation. Pearson chi-square (χ^2) test and Fisher's Exact test were used to compare groups regarding categorical variables. Continuous variables, such as age, were compared with Mann-Whitney *U*-test. p values < 0.05 were interpreted as statistically significant. Genetic variation was distributed according to Hardy-Weinberg equilibrium.

Results

The allele frequencies and genotype distributions between the groups are shown in table 2. There were no significant differences in the allele frequencies (p=0.59) and genotype distributions (p=0.52) between the patients and controls.

To explore any possible relation between ACE polymorphism and clinical presentation of ARVD, ARVD patients were divided into subgroups according to presence of syncope, SCD, inducible (electrophysiologically) arrhythmia and family history of SCD or syncope.

DD genotype was detected in 69.2% of the ARVD patients with syncope, while DD genotype frequency was 25.0% in those without syncope. The difference was statistically significant (p=0.017, odds ratio: 6.750, 95% confidence interval: 1.318–34.565).

DD genotype frequency was higher also in ARVD patients with history of SCD compared to those with no history of cardiac arrest, but the difference was not significant (66.7% *vs.* 39.1%, p=0.36).

Similarly, DD genotype frequency was higher in patients with a family history of SCD or undiagnosed syncope; yet the difference was not significant (66.7% *vs.* 39.1%, p=0.36).

DD genotype was detected in 10 patients (58.8%) with electrophysiologically-inducible arrhythmia (one ventricular fibrillation and nine VT), while only two of the patients (28.6%) with no inducible arrhythmia had DD genotype (p=0.37). Among the seven patients who had undergone RFA, three patients had DD genotype (in one of them, ventricular arrhythmia recurred), three patients had ID genotype (in one of them,

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ventricular arrhythmia recurred) and one patient had II genotype. Among the six patients with appropriate ICD therapy, two patients had DD genotype.

Discussion

ARVD is a rare heart muscle disease characterised by peculiar right ventricle involvement and electrical instability that precipitates ventricular arrhythmias and SCD. It is a familial disease with genetic heterogeneity,[4] and at least 11 loci and five candidate genes have been identified so far.[17] A 2-bp deletion in the plakoglobin has been identified as the first gene responsible for a recessive form of ARVD associated with palmoplantar keratosis and woolly hair (Naxos disease).[18] Mutations of ryanodine receptor type 2 gene and regulatory mutations in transforming growth factor beta3 gene have been found in ARVD2 and ARVD1, respectively.[34,35] Moreover, mutations in desmoplakin (ARVD8) and plakophilin-2 (ARVD9) have been discovered in the absence of skin and hair abnormalities.[15,36,37] The majority of these genes code for proteins involved in cell-to-cell adhesion. Impairment of cell-to-cell adhesion may promote myocyte degeneration and lead to accelerated apoptosis of myocardial cells, with the repair process consisting of replacement of myocardium by adipose and fibrous tissue.[18,38,39]

Despite researches about the genetic aspects of ARVD, ACE gene polymorphism has not yet been studied in ARVD patients. Cardiac renin-angiotensin system is involved in cardiac remodelling and fibrosis. The ACE I/D polymorphism is responsible for ACE activity[29] and the levels of tissue and circulating ACE.[21,29,40,41] Left ventricular ACE activity is higher in individuals with the D allele.[24,42] The DD genotype favours myocardial fibrosis and determines the extent of cardiac fibrosis.[25] As the pathologic hallmark of ARVD is the non-ischaemic death of the myocytes with fibrofatty replacement,[2,5,6] we tried to explore whether DD genotype might have an effect in the fibrotic process and propensity for ventricular arrhythmias and sudden death in ARVD patients.

Our study is the first study exploring the relation between ACE gene polymorphism and ARVD. We did not detect any significant difference in the frequency of DD genotype between the ARVD patients and the controls. Although DD genotype was associated with a higher incidence of idiopathic dilated and ischaemic cardiomyopathy,[43] ACE gene polymorphism did not seem to be related to ARVD development.

Our novel contribution from this study was the demonstration of significantly more DD genotype presence in patients with syncope. DD genotype was detected in 69.2% of the ARVD patients with syncopal episode, while DD genotype frequency was 25.0% in those without syncope (p=0.017, odds ratio: 6.750, 95% confidence interval: 1.318–34.565). Our result was in harmony with previous studies reporting association between DD genotype and malignant arrhythmias. Anvari *et al.*[27] reported that the D allele was associated with increased risk for development of malignant ventricular arrhythmias in patients with coronary artery disease and left ventricular dysfunction. Takezako *et al.*[44] reported that the D allele had additive and dominant effects for the occurrence of significant reperfusion-induced ventricular arrhythmias in patients with acute myocardial infarction.

The natural history of ARVD is determined by the electrical instability of the dystrophic myocardium, which can precipitate arrhythmias at any time during the course of the disease. The D allele was shown to be associated with increased QT dispersion and arrhythmogenic risk in hypertensive patients.[26] We found DD genotype in 58.8% of the patients with electrophysiologically-inducible arrhythmia, while DD frequency was 28.6% in patients with noninducible arrhythmia. Although the difference was not statistically significant, DD genotype was more likely to be associated with inducible arrhythmias. A possible mechanism for increased arrhythmias in DD genotype may be due to calcium overload in cardiac myocytes by angiotensin II-induced stimulation of the calcium channels.[45] Similarly, mutations in the gene encoding the cardiac ryanodine receptor suggest that cytoplasmic calcium overloading may lead to arrhythmias.[11]

Sudden death accounts for the majority of the fatal events in ARVD, but its occurrence is mostly unpredictable. There are no prospective and controlled studies assessing clinical markers that can predict the occurrence of life-threatening ventricular arrhythmias and SCD. At present, only QRS dispersion, history of syncope and right and/or left ventricular abnormalities at radionuclide angiography have been proved to be independent non-invasive predictors of sudden death.[46] As we detected significantly more DD genotype in ARVD patients with syncope, our results suggest that ACE gene polymorphism might be useful in predicting the risk of malignant ventricular arrhythmias and syncope, even in predicting the risk of SCD. Although the difference was not statistically significant, we detected high prevalence of DD genotype in patients with a history of survived cardiac arrest or in patients with a family history of SCD. Similarly, Takezako *et al.*[44] claimed that the ACE-D allele might play a pivotal role in SCD in patients with acute myocardial infarction. We believe that small sample size might be a reason for statistical

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insignificance and further studies with larger populations might yield more accurate interpretations about the relation between ACE gene polymorphism and SCD.

The use of ICD is the treatment of choice for many ARVD patients with ventricular arrhythmias and left ventricular involvement or more affected family number with SCD or undiagnosed syncope.[47] RFA can be useful as adjunctive therapy in management of patients with ARVD with recurrent ventricular arrhythmias despite optimal anti-arrhythmic drug therapy[47] and can achieve a good short-term success rate. Recurrences become increasingly common during long-term follow-up, possibly as a result of the progressive nature of ARVD.[48] Due to our small sample size, we cannot conclude whether ACE gene polymorphism was associated with appropriate ICD therapy or recurrences after RFA.

Study limitations

The major limitation of the study was the small sample size. We know that for searching genetic modifier effects, a much larger patient group is required. However, as ARVD has an incidence of six per 10,000 persons, we do not have a large group of patients. Because the sample size was small, the causative gene polymorphisms were not studied and it was not possible to determine whether ACE gene polymorphism affected the clinical presentation in presence of a certain gene mutation. Although the diagnosis of ARVD was established on the basis of the criteria set by the Task Force, some of the patients lacked endomyocardial biopsy, which is considered the preferred method for diagnosis of ARVD with a specificity of 92%. Further research is needed to identify which genetic factors account for the differing clinical course of ARVD patients. We believe that our study will initiate prospective studies with a larger population and a long follow-up, which will yield better interpretation of ACE gene polymorphism in clinical presentation and prognosis. Nevertheless, this study is the first study exploring the effect of ACE gene polymorphism in ARVD patients, and the results of our study may provide important insights in predicting the risk of syncope and SCD in ARVD patients.

Conclusions

ARVD is an inherited cardiomyopathy characterised by right ventricular dysfunction and ventricular arrhythmias. Identification of the causative genes for ARVD will contribute to the understanding of the pathogenesis and management of this poorly understood condition. Although ACE gene polymorphism is not a causative factor for ARVD, it may be helpful in predicting the high-risk patients for syncope or SCD. Further studies are needed to elucidate the relation between functional and structural changes in different models and populations affected by ARVD, as well as to determine the factors predicting malignant ventricular arrhythmias and SCD in ARVD patients.

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