

Influence of Angiotensin-converting Enzyme Gene Insertion/Deletion Polymorphism on Rheumatic Valve Involvement, Valve Severity and Subsequent Valve Calcification

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Background and aim of the study: The relationship between the severity of chronic rheumatic heart disease (RHD) and predisposing factors is unknown, and genetic predictors for severe scarring and calcification of the mitral valve are not well defined. A high angiotensin-converting enzyme (ACE) activity has been demonstrated in valve tissue. Thus, a case-control study was conducted to investigate any possible relationship between ACE gene polymorphisms and chronic mitral valve disease severity and calcification.

Methods: This case-control study included 82 patients (24 males, 58 females; mean age 40.3 ± 14.7 years) with chronic rheumatic mitral valve, and 154 control subjects (53 males, 101 females; mean age 43.4 ± 13.4 years). ACE gene insertion/deletion (I/D) polymorphisms were identified using polymerase chain reaction methods.

Results: Among RHD subjects, 31 (30.6%) were D/D, 25 (32.7%) were I/D, and 26 (18.8%) were I/I. Among controls, 57 (57.4%) were D/D, 69 (61.3%) were I/D, and 28 (35.2%) were I/I. The frequency of ACE I/I

genotype was higher in RHD subjects than in controls ($\chi^2 = 7.4$, $df = 2$, $p < 0.030$; D/D versus I/D versus I/I), or ($\chi^2 = 5.5$, $df = 1$, $p < 0.019$; DD + ID versus II). Predisposition to RHD was significantly less frequent in the D/D genotype. There was no statistically significant difference in the genetic analysis of RHD with respect to mitral valve score, severity of mitral regurgitation and left atrial diameter. Mitral valve calcification was significantly associated with a higher frequency of I/I genotype and I/D genotype than D/D genotype alone ($\chi^2 = 6.2$, $df = 2$, $p = 0.043$). The ACE I/I genotype was associated with a predisposition to a greater risk of severe calcific valve disease.

Conclusion: The ACE I/I genotype is more common in patients with rheumatic valve disease than in the normal population. This suggests that the ACE gene polymorphism may be involved in the pathogenesis of rheumatic heart disease.

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Rheumatic heart disease (RHD) remains a major public health problem in developing countries. Beta-hemolytic streptococcal pharyngitis may induce rheumatic fever and RHD in susceptible individuals weeks or years after an acute infection episode, and RHD is a frequent and severe complication. Molecular mimicry between streptococcal antigens (mainly M protein) and heart tissue proteins has been proposed as an important factor leading to the heart lesions found in RHD patients. The CD (cluster of differentiation) T-cells comprise the predominant population at the site of heart lesions (1). Some HLA class II antigens have been linked with a genetic susceptibility to RHD (2).

Genetic determinants of rheumatic valve involvement and any tendency to progressive fibrosis and scarring of the valves or calcification in patients with RHD remain unknown. Aside from rheumatic recurrences alone, the triggering process in the evolution of progressive fibrosis and factors predisposing to severe forms of valves in some patients remain undefined. The results of recent studies have shown that angiotensin-converting enzyme (ACE) receptors and angiotensin II (A2) receptors are present at sites of high collagen turnover in the heart, including valvular tissues (3). ACE activity has been found in all cardiac valves, and this may contribute to collagen synthesis and degradation at these sites (4). Although data derived from different investigations conflict to some extent, the D/D genotype appears to be related to adverse effects with regard to ischemic or idiopathic dilated cardiomyopathy (5), hypertrophic cardiomy-

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opathy (6) and cardiac hypertrophy and remodeling (7). ACE catalyzes the conversion of angiotensin I to angiotensin II, the latter having been implicated in the pathogenesis of atherosclerosis through induction of hyperplasia and hypertrophy of smooth muscle cells and increased expression of platelet-derived growth factor and proto-oncogenes (8,9). One of the most important parameters determining ACE levels is insertion/deletion polymorphism of the ACE gene; indeed, patients with the ACE D/D genotype have been shown to have high circulating and tissue ACE activity (10). ACE gene polymorphism might be an important factor in this respect. Thus, the aim of the present study was to evaluate whether ACE gene polymorphism is a predisposing factor to RHD, and whether it may correlate with the severity of valve scarring or calcification in these patients.

Clinical material and methods

Patient selection

The study group comprised 82 consecutive patients with rheumatic mitral valve disease (24 males, 58 females; mean age 40.3 ± 14.7 years), and the control group 154 age- and gender-matched subjects (53 males, 101 females; mean age 43.4 ± 13.4 years). Patients with coronary artery disease, hypertension, diabetes mellitus and heart failure were excluded. Patients were allocated to the RHD or control (normal valve) groups on the basis of echocardiographic findings. Control subjects had no history of rheumatic fever. All subjects were of Turkish origin and living in the south-east Anatolia region of Turkey.

The study was approved by an institutional committee, and all subjects provided their informed consent before participating in the investigation.

Echocardiography

Transthoracic Doppler echocardiography (TTE) was performed using a commercially available ultrasound machine (Acuson model 128 XP/10). The examination included two-dimensional, M-mode, color, pulsed-wave and continuous-wave Doppler echocardiography. Echocardiography was performed with the patient in the left lateral recumbent position, and standard parasternal and apical long- and short-axis views were recorded. Each valve was assessed for severe stenosis and regurgitation using conventional criteria. The operators were unaware of the results of the ACE genotyping. Rheumatic valve severity was scored on the basis of the following parameters: fusion of the commissures; thickening and calcification of the valve leaflets; fusion and shortening of the chordae tendineae; leaflet motion; and subvalvular involvement. All echocardiographic parameters were scored

separately as either 1 (mild) or 2 (moderate to severe). Valve severity was reflected as the sum of these scores. If none of these parameters was present, the valve morphology was accepted as normal. Visible and measurable calcification (minimum diameter 0.5 cm) was accepted as valve calcification. Immeasurable fine increased echogenicity was not accepted as valve calcification. The acceptable level of inter-observer agreement between the results of the analysis of TTE was given as kappa = 0.62.

Determination of ACE I/D polymorphism

Genomic DNA was extracted from leucocytes using standard methods. ACE genotypes were classified as I/I, I/D and D/D. ACE gene I/D polymorphism was determined by polymerase chain reaction (PCR) using a primer pair flanking the polymorphic region of intron 16 that produces either an amplified 490-bp (I allele) or a 190-bp product (D allele), or both. The reactions were performed according to the method of Rigat et al. (11). The sense nucleotide primer was 5'-CTG-GAGACCACTCCCATCCTTTCT-3', and the antisense primer was 5'-GATGTGGCCATCACATTCGTCA-GAT-3'. PCR reactions were performed in 50 µl reaction volumes with 50 pmol of each primer, 100 ng genomic DNA, 1.5 mmol/l MgCl₂, 50 mmol/l KCl, 10 mmol/l Tris-HCl (pH 8.3), 200 µl/l each dNTP, and 2.5 U Amplitaq DNA polymerase (Fermentas). Amplification was performed as follows: initial denaturation at 95°C for 2 min followed by 30 cycles of denaturation at 95°C for 1 min, annealing at 60°C for 2 min, and extension at 72°C for 3 min. The PCR products were visual-

Table I: Clinical features of the rheumatic valve group.

Parameter	Value/no. of patients
Age (years)*	40.3 ± 14.7 (15-78)
MVA overall (cm ²)*	1.95 ± 0.7 (0.6-3.5)
>1.5 cm ²	44 (54.3)
<1.5 cm ²	37 (45.7)
Mitral valve score*	5.54 ± 2.1 (2-10)
LA diameter (cm)*	4.51 ± 0.82 (3.3-7.2)
Mitral valve calcification	30 (36.6)
Mitral regurgitation	
Mild	23 (28)
Moderate-severe	47 (57.4)
Aortic regurgitation	
Mild	19 (23.2)
Moderate-severe	39 (47.5)
Tricuspid regurgitation	
Mild	14 (17.2)
Moderate-severe	25 (30.5)

*Values are mean ± SD (range).

Values in parentheses are percentages.

MVA: Mitral valve area.

Table II: Distribution of ACE gene polymorphism among rheumatic heart disease (RHD) patients and healthy controls.

Group	Genotype		
	D/D	I/I	I/D
RHD (n)	31 (30.6)	26 (18.8)	25 (32.7)
Controls (n)	57 (57.4)	28 (35.2)	69 (61.3)

Values in parentheses are percentages.
 $\chi^2 = 7.04$, $df = 2$, $p = 0.019$.

ized by electrophoresis in a 2% agarose gel with ethidium bromide and recorded using a gel documentation system (Vilber-Lourmar).

Statistical analysis

The statistical analysis was performed only in patients without any missing values (82 RHD patients, 154 controls). Statistical software SPSS 10.0 was used to analyze the results. Discrete variables were expressed as counts or percentages, and compared using a χ^2 test or Fisher's exact test, as appropriate. Continuous variables were expressed as mean (\pm SD) and compared by means of the unpaired, two-sided *t*-test or ANOVA for two or more groups. A *p*-value <0.05 was considered to be statistically significant. As the inheritance manner of the I allele has not been clarified, its effect was examined separately as either additive (D/D versus I/D versus I/I) or dominant (D/D + I/D versus I/I).

Results

There were no differences with regard to age and gender between RHD patients and control subjects. The main characteristics of the RHD subjects according to echocardiographic findings are listed in Table I.

Distribution of ACE genotypes and allelic frequencies

The distribution of ACE gene polymorphism in RHD patients and healthy controls is indicated in Table II. Among the total cohort of 236 subjects, 88 were homozygous for the deletion D/D, 94 were heterozy-

gous I/D, and 54 were homozygous for the insertion I/I. The frequency of the ACE I/I genotype was higher in RHD subjects than in controls ($\chi^2 = 7.4$, $df = 2$, $p < 0.030$; D/D versus I/D versus I/I), or $\chi^2 = 5.5$, $df = 1$, $p < 0.019$; DD + ID versus II). A predisposition to rheumatic valve disease occurred significantly less often in the D/D genotype; indeed, the D/D genotype appeared to act as a protective factor against rheumatic valve disease.

Rheumatic valve severity and ACE gene polymorphism

There were no statistically significant differences in the genetic analysis of RHD with respect to the mitral valve score ($\chi^2 = 2.2$, $df = 2$, $p = 0.33$), mitral valve regurgitation ($\chi^2 = 2.3$, $df = 3$, $p = 0.40$) and left atrial diameter ($\chi^2 = 2.9$, $df = 2$, $p = 0.22$).

Rheumatic valve calcification and ACE gene polymorphism

Mitral valve calcification was significantly associated with a higher frequency of I/I genotype than the D/D and I/D genotypes alone ($\chi^2 = 6.2$, $df = 2$, $p = 0.043$). On comparing I/I versus D/D + I/D, a stronger relationship was found between mitral valve calcification and the I/I genotype ($\chi^2 = 5.3$, $df = 2$, $p = 0.021$) (Table III).

Discussion

As the precise pathogenetic mechanism of chronic rheumatic heart disease has not yet been defined, the

Table III: Distribution of ACE gene polymorphism among patients with respect of mitral valve calcification.

Calcific	Genotype		
	D/D	I/I	I/D
Positive (n)	20 (64.5)	22 (84.6)	13 (52)
Negative (n)	11 (35.5)	4 (15.4)	12 (48)

Values in parentheses are percentages.
 $\chi^2 = 5.3$, $df = 2$, $p = 0.021$.

present study was initiated to investigate any potential relationship between ACE genotype and rheumatic valve with regard to valve involvement, severity and calcification. The results showed that the genotype frequency of the ACE gene in patients with severe RHD differed from that in the normal subjects, with a low level of ACE activity (I/I genotype) perhaps resulting in negative remodeling and possibly triggering valve scarring and calcification. In contrast, a high level of ACE activity - as reflected by ACE D/D gene polymorphism - was seen to protect RHD subjects from valve scarring or valve calcification by positive remodeling in tissue.

Although most patients exhibit cross-reactivity antibodies, the latter do not appear to contribute to tissue damage. The presence of CD T-cells at lesion sites in the heart has been demonstrated, and this suggests a direct role for these cells in the pathogenesis of RHD (1). Moreover, it is also known that some patients exhibit susceptibility to rheumatic fever due to several different HLA class II specificities (2). Rheumatic carditis may be followed by a healing process or by an ongoing inflammation process (12), and some patients have continued fibrosis, scarring and calcification resulting in a severe form of RHD. The reason why some patients progress to a more severe form of RHD than others remains unclear, however.

Activated endothelium may play a dramatic role in the initial development of rheumatic valvulitis and progression of the disease throughout a patient's lifetime (13). High levels of ACE were found both in endothelial cells and throughout the thickness of the valve matrix in heart valves (14,15). This high ACE activity in valve tissues indicates the presence of active peptide metabolism and local angiotensin II formation. ACE may regulate local concentrations of angiotensin II and bradykinin, which could serve to influence valvular interstitial cell collagen turnover under normal and pathological conditions (e.g. valvular heart disease).

Both plasma and tissue ACE activities were seen to be higher in patients with the ACE D/D genotype (10). In contrast to the present findings, a recent investigation found that in patients with acute rheumatic fever the ACE D/D genotype was associated with an increased risk of subsequent heart valve damage (16), although the patients investigated were not in the chronic phase of the disease. In the present study, the association between ACE genotype and chronic RHD was investigated and, in contrast to the findings of others (16), there was no evidence of any association between ACE D/D gene polymorphism and RHD. However, an unexpectedly higher frequency of I/I genotypes was found, which clearly suggested that the ACE genotype might serve as an important predictor

for the scarring of rheumatic valves. Interesting data were also recently reported by Ozisik et al. (17) in a relatively small series of 50 patients who underwent mitral valve replacement, compared to 50 control subjects. These authors demonstrated a relationship between ACE gene polymorphism and rheumatic mitral valve disease, with the mitral valve replacement group showing higher frequencies of the homozygote D/D and I/I alleles than did the control group. However, Ozisik et al. (17) appear not to have provided adequate information to support the plausibility of an association between ACE D/D and I/I genotypes and a susceptibility to rheumatic fever, as neither D/D nor I/I genotypes could impose opposing biological effects within the same patient population. Although this point awaits clarification, the I/I genotype frequency reported by Ozisik et al. was consistent with that in the present study. Differences in population size, in baseline characteristics and in study design may also serve as reasons for discrepancies between Ozisik et al.'s study and the present investigation with regard to the D/D genotype. For example, mild and moderate to severe mitral valve disease were included in the present study, whereas Ozisik et al. included only severe mitral valve disease subjects; moreover, their control group was also relatively small. Although in the present study no association was found between the severity of rheumatic valve involvement, a higher frequency of the I/I genotype was found in respect of rheumatic valve involvement and the development of rheumatic valve calcification that highlighted the impact of ACE gene polymorphism on RHD.

The present findings provide new evidence suggesting that ACE gene polymorphism plays a detectable role in the structure of the rheumatic valve. To the best of the present authors' knowledge, this study is the first to reveal a possible involvement of the renin-angiotensin system in RHD and subsequent valve calcification. This may suggest that patients with RHD and the ACE D/D genotype have a better prognosis than those with the I/I genotype. By contrast, patients with RHD and the ACE I/I genotype showed more negative tissue remodeling and progressive valve calcification which, in theory, could mean a worse prognosis when compared with patients having D/D genotype involvement. However, it is unclear why lower plasma and tissue levels of ACE (the I/I genotype) would predispose to valve calcification, and this point must be re-emphasized in future studies. An analysis of polymorphisms of the ACE gene may, in the future, play an important role in the risk assessment and medical management of RHD.

Study limitations

ACE gene polymorphism is an important area of

investigation in rheumatic valves. Although the present study utilized a reasonably large cohort for analysis, the observed associations should be replicated in a larger sample size and/or mechanistic studies. The critical defect in this study was that the control group had normal valves and did not necessarily have rheumatic fever; hence, the control and diseased groups may not have been comparable. If there were to be any correlation of pathologic features (rheumatic valve deformity, calcification) with ACE gene polymorphisms from diseased to control valves, it may be the result of different susceptibilities to streptococcal infection, to the risk of progressing to rheumatic fever and/or subsequent valve deformity, or vulnerability to calcification

In conclusion, as the ACE I/I genotype is more common in patients with rheumatic valve disease than in a normal population, it is possible that ACE gene polymorphism might be involved in the pathogenesis of this condition.

Addendum

Our study is strongly supported by the recent published study with strictly similar results (18): Chou et al. found that a significant difference in the frequencies of ACE I/D II genotype and I allele between normal controls and patients with RHD is exist. They demonstrated that patients with rheumatic valve disease have a higher frequency of ACE II genotype and I allele, which strongly supports a role for ACE I/D gene polymorphisms in determining the risk of rheumatic valve disease, thereby supporting results of our study.

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