Association Between Angiotensin I-Converting Enzyme Gene Insertion/Deletion Polymorphism and Risk of Rheumatic Heart Disease

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SUMMARY

Scarring and collagen deposition in the valves and destruction of myocytes may result from the combined effects of a smoldering rheumatic process and a constant trauma to the mitral valve or aortic valve by the turbulent flow in rheumatic heart disease (RHD). It has been suggested that angiotensin I-converting enzyme (ACE) may be responsible for the increased valvular fibrosis and calcification in the pathogenesis of RHD. However, the role of ACE genetic variant in RHD has not been studied among the Chinese population in Taiwan. Hence, a case-controlled study was carried out to investigate the possible relationship between the ACE gene insertion/deletion (I/D) and G2350A polymorphisms and RHD.

A group of 115 patients with RHD documented by echocardiography and 100 age- and sex-matched normal control subjects were studied. ACE gene I/D and G2350A polymorphisms were identified by polymerase chain reaction-based restriction analysis.

There was a significant difference in the distribution of ACE I/D genotypes ($P = 0.02$) and allelic frequencies ($P = 0.04$) between RHD cases and normal controls. An odds ratio for the risk of RHD associated with the ACE I/D II genotype was 2.12 (95% CI, 1.21-3.71). An odds ratio for the risk of RHD associated with the ACE I allele was 1.50 (95% CI, 1.02-2.21). The ACE G2350A polymorphism showed no association with RHD ($P = 0.90$). Further categorization of RHD patients into mitral valve disease and combined valve disease subgroups revealed no statistical difference in these gene polymorphisms when compared between the two subgroups.

This study shows that patients with RHD have a higher frequency of ACE II genotype and I allele, which supports a role for ACE I/D gene polymorphisms in determining the risk of RHD in Taiwan Chinese. (Jpn Heart J 2004; 45: 949-957)

Key words: ACE gene polymorphisms, Rheumatic heart disease, Taiwan

RHEUMATIC fever (RF) is an inflammatory disease that occurs as an autoimmune sequel to group A streptococcal infection of the pharynx.1) Autoimmunity induced by antigenic mimicry between the streptococcal glycoprotein and human

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cardiac myosin and laminin may be responsible for the pathogenesis of rheumatic carditis.\textsuperscript{2,3}) A subgroup of these RF patients has progressive fibrosis of the valves and develops chronic valvular disease, though the factors leading to continued fibrosis and subsequent valve disease remain incompletely defined. The relatively low attack rate of RF after untreated streptococcal pharyngitis (up to 2\% to 3\%), the relatively high concordance rate for RF in monozygotic twins (19\%) in comparison to dizygotic twins (2.5\%), and the high familial incidence of RF suggest the involvement of host genetic factors in susceptibility to RF with consequential progression to rheumatic heart disease (RHD).\textsuperscript{4,5}) Some studies have reported that angiotensin I-converting enzyme (ACE) activities and angiotensin II receptors are present at the sites of high collagen turnover in the heart, including valvular tissue. Dense ACE labeling has been found in all cardiac valves, and this may contribute to collagen synthesis at these sites.\textsuperscript{6-8}) A recent study has reported that in patients with acute rheumatic fever, the ACE DD genotype is associated with an increased risk of subsequent heart valve damage in Turkish patients.\textsuperscript{9})

The human ACE gene has been cloned and localized to chromosome 17q23.\textsuperscript{10}) An insertion/deletion (I/D) polymorphism in intron 16 has been identified for use as a genetic marker.\textsuperscript{11}) There is evidence to suggest the I/D polymorphism is in strong linkage disequilibrium with a major gene effect at the ACE gene locus, which controls up to 44\% of the variability in ACE levels.\textsuperscript{12}) The G2350A polymorphism in intron 17 was reported to have the most significant effect, accounting for 19\% of the total variance in ACE.\textsuperscript{13}) Because there are no data available in the literature regarding the role of ACE gene polymorphisms in the pathogenesis of RHD in Taiwan Chinese, the current study was designed to determine whether the I/D and G2350A polymorphisms of the ACE gene can serve as markers of susceptibility or severity of RHD.

**METHODS**

**Study population:** Between May 2000 and April 2002 a total of 115 unrelated patients (31 men and 84 women, ranging in age from 28 to 80, mean age 51.0 ± 12.2 years) with an echocardiographically documented predominant mitral stenosis (MS) were enrolled in this study. Diagnosis of valve lesions was made by echocardiography and/or catheterization and cineangiography. The patients were further divided into mitral valve disease (MVD; \(n = 53\); 13 men and 40 women; mean age, 51.1 ± 12.4 years) or combined valvular disease (CVD; \(n = 62\); 18 men and 44 women; mean age, 50.9 ± 12.1 years) categories. The MVD category consisted of MS (\(n = 25\)) plus mixed MS + mitral regurgitation (\(n = 28\)). Patients with predominant mitral regurgitation or those with aortic or tricuspid valvular disease
alone were excluded from the study. The control group consisted of 100 age- and sex-matched unrelated healthy volunteers (31 men and 69 women, ranging in age from 26 to 76, mean age, 49.8 ± 16.5 years) who were free of autoimmune diseases and had normal echocardiography and no family history of RHD. All participants were of Chinese Han ethnicity in Taiwan.

The study was approved by the institutional research ethics committee, and informed consent was obtained from each study participant.

Genotyping of the I/D and G2350A polymorphisms of ACE gene: The genomic DNA was prepared from peripheral blood leukocytes using a genomic DNA isolation kit (Blossom, Taipei, Taiwan). The ACE gene I/D polymorphism was determined by polymerase chain reaction using primers flanking the polymorphic region of intron 16 with the following primer sequences: 5’-TGGAGAC-CACTCCCATCCTTTCT-3’ and 5’-CAGGTCTTCATATTTCCGATGTGG-3’. Reactions were carried out in 50-µL volumes containing genomic DNA, 2-6 pmol of each primer, 1X Taq polymerase buffer (1.5 mmol/L MgCl₂), and 0.25 units of AmpliTaq DNA polymerase (Perkin-Elmer, Foster City, Calif., USA). The cycling conditions for I/D were set as follows: one cycle at 94°C for 3 minutes, 30 cycles at 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 60 seconds, and one final cycle of extension at 72°C for 10 minutes. Electrophoresis of the amplified products in 2% agarose gel allowed detection of a 495-base pair (bp) fragment (insertion) and a 208-bp fragment (deletion).

The G2350A polymorphism was typed by the restriction fragment length polymorphism (RFLP) method previously described by Keavney, et al.14)

Statistical analysis: Differences in genotype distribution between patients with RHD and control subjects were tested by the $\chi^2$ test with 2 degrees of freedom (df). For statistical analysis of the allelic frequency distribution in the I/D and G2350A polymorphisms, the two groups were compared using the $\chi^2$ test with 1 df. Allelic frequencies were calculated from genotype frequencies in patients with RHD and control subjects. The ACE DD and ID genotypes were collapsed for calculation of the odds ratio (OR) (DD + ID vs II). The differences among the MVD, CVD, and control groups were estimated using a $\chi^2$ test with 4 df. All statistical analyses were performed with NCSS 2000 Software (Kaysville, Utah, USA). A value of $P < 0.05$ was considered statistically significant.

RESULTS

Table I shows the clinical characteristics of the case and control groups. There were no differences in age, gender, systolic blood pressure, diastolic blood pressure, or body mass index between patients with RHD and control subjects. Of the 115 patients with RHD, 7 (6%) had primary hypertension.
For each ACE polymorphism I/D and G2350A, it was confirmed that the genotype proportions fit the Hardy-Weinberg equilibrium estimated by a $\chi^2$ test. Examination of this distribution showed a significant difference in the ACE I/D polymorphism ($\chi^2 = 7.67, P = 0.02$) among RHD cases and normal controls (Table II). The odds ratio for the risk of RHD associated with ACE II genotype was 2.12 (95% CI, 1.21-3.71). The odds ratio for the risk of RHD associated with the ACE I allele was 1.50 (95% CI, 1.02-2.21).

The analysis of genotype distribution did not show a statistically significant difference for the G2350A polymorphism of the ACE gene between RHD patients and control subjects ($\chi^2 = 0.20, P = 0.90$). The allelic frequencies of the G2350A of the ACE gene were not significantly different between the cases and controls ($\chi^2 = 0.04, P = 0.83$).
As shown in Table III, no statistically significant differences in the G2350A ($\chi^2 = 1.83, P = 0.77$) polymorphisms among the MVD and CVD subgroups and the control group were observed. There was a significant difference in the distribution of I/D polymorphism among the cases and controls. No significant difference in the distribution of I/D polymorphism between the MVD and CVD subgroups was observed ($\chi^2 = 0.15, P = 0.93$).

**DISCUSSION**

RHD is a disorder with autoimmune features, and its pathophysiology is incompletely understood. Although no specific "RF gene" has been identified, several studies have suggested that genetic susceptibility to RF and RHD is linked to HLA class II alleles/haplotypes (HLA-DR2 in American blacks, HLA-DR4 in American Caucasians, HLA-DQA1 *0104 and DQB1 *05031 in Japanese, and HLA-DRB1 *0701 and DQA1 *0201 alleles and DRB1 *0701- DQA1 *0201 haplotype in Egyptians). The most common lesion of acute rheumatic endocarditis is mitral valvulitis. MS usually results from repeated episodes of carditis alternating with healing and is characterized by the deposition of fibrous tissue in the valves and chordae tendineae. Rheumatic involvement is present in 99% of stenotic mitral valves excised at the time of mitral valve replacement.
Guedez reported that MVD patients experienced a significant 5-fold fewer acute recurrent RF episodes compared with the multivalvular lesion patients, with mean rates of RF recurrence of 0.7 and 3.3, respectively. The extent of original inflammation, and recurrence of RF are not the only predictors of the crippling process. Ultimately, the deformed valve is subject to nonspecific fibrosis and calcification. The anatomic changes in severe MS or aortic stenosis may result from the combined effects of a smoldering rheumatic process and a constant trauma to the mitral valve or aortic valve by the turbulent flow. It has been reported that valvular interstitial cells express ACE and collagen I messenger ribonucleic acid (mRNA). In addition, angiotensin II stimulates collagen I gene expression at the mRNA and protein levels. The scarring and collagen deposition in the valves are thought to be the primary changes in RHD. ACE was hypothesized to be possibly involved in the pathogenesis of RHD.

A recent study has reported that in Turkish patients with a history of acute rheumatic fever, the ACE DD genotype is associated with an increased risk of subsequent heart valve damage. They found that patients with valve disease had a higher frequency of ACE DD genotype than that of patients with normal valves. However, they did not compare the difference in the distribution of ACE genotypes between RF patients and the normal healthy controls. In the present case-controlled study, we found an association between ACE II genotype and RHD. The increased risk of RHD associated with ACE I allele was observed. The difference might be caused by ethnic factors. The association of ACE I allele with RHD may be counterintuitive, because Taiwanese individuals with a D allele have higher serum ACE levels. However, I allele has also been shown to be associated with hypertension, insulin resistance, metabolic syndrome, mitral valve prolapse syndrome, and atrial fibrillation with hypertrophic cardiomyopathy. The ACE II genotype can be a genetic risk factor for cardiovascular disease. The activity of renin is thought to be rate-limiting to the production of angiotensin II. Renin, plasma angiotensinogen, and serum and tissue ACE activity are all important determinants for the degree of production of angiotensin II. The angiotensin II receptors have also been shown to mediate the effects of angiotensin II and collagen turnover in cardiac fibroblasts. In human hearts, pressure and volume overload increases cardiac ACE and transforming growth factor-β1 (TGF-β1) in the early stages. This activation of the cardiac renin-angiotensin system may contribute to the observed increase in collagen I and III and fibronectin mRNA expression. A recent study reported that TGF-β1 was an important mediator of the hypertrophic growth response of the heart to angiotensin II. The mechanism of the association of the ACE I allele with RHD in our population should be further clarified. The frequency of the ACE DD genotype in our control Taiwan Chinese population was 18%, which is higher than that
reported in Hong Kong Chinese (13%), but lower than in both Turkish (61.2%) and Caucasian populations (24.8%). The frequency of the D allele in our control Taiwan Chinese population was 44%, which is higher than that reported in Hong Kong Chinese (37.2%), but lower than that in both Turkish (61.2%) and Caucasian populations (54%). The discrepancy in the distribution of ACE gene I/D polymorphism between these studies may be due to ethnic differences. Thus, the findings in a Turkish population can not be extrapolated to a Taiwanese population.

Since the ACE gene polymorphisms were hypothesized to be associated with the severity of RHD, we studied the distribution of the I/D and G2350A polymorphisms in the MVD and CVD subgroups. Our results show no evidence of an association between either ACE I/D or G2350A polymorphism with the severity of RHD. Other factors should be considered to be involved in the pathogenesis of RHD.

**Study limitations:** The current sample of 215 individuals was relatively small in terms of genetic epidemiology studies. Although all people were of Chinese Han ethnicity in Taiwan, they could still derive from a certain ancestry that was prone to develop RHD, and the ACE genotype is a mere marker for such a different population.

It is concluded that the present study demonstrates a significant difference in the frequencies of ACE I/D II genotype and I allele between normal controls and patients with RHD, thereby supporting a role for ACE I/D polymorphism in determining the risk of RHD among the Chinese population in Taiwan. However, no association between the severity of RHD and ACE I/D or G2350A polymorphism was found, indicating that the pathogenesis of RHD is complex and requires further investigation.

**REFERENCES**


