

The Renin-Angiotensin System Genetic Polymorphisms and Rheumatic Mitral Valve Disease

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Background and aim of the study: Angiotensin-converting enzyme (ACE) gene insertion/deletion (I/D) polymorphism, angiotensinogen (AGT) gene polymorphism and angiotensin II type 1 receptor (AT1R) polymorphism in relation to rheumatic mitral valve disease were examined in a case-control study to investigate possible relationships between these gene polymorphisms and rheumatic mitral valve disease in patients undergoing mitral valve replacement (MVR).

Methods: A total of 50 patients with rheumatic mitral valve disease and undergoing MVR was compared with 50 normal, and age- and sex-matched control subjects. ACE I/D, AGT gene M235T and AT1R-adenine/cytosine 1166 (A1166C) genotype polymorphisms were identified by polymerase chain reaction (PCR) -based restriction analysis.

Results: ACE I/D polymorphism differed significant-

ly between the groups. The control group mostly represented the heterozygote ID allele (74%), while the MVR group showed frequencies of 60% for the homozygote DD and II alleles. MM homozygote frequency was significantly greater in controls, but TT homozygote frequency was significantly greater in the MVR group. AT1R-A1166C genotype polymorphism also differed significantly between groups; the MVR group had 73.7% of the AC heterozygote allele, while controls had 64.4% of the AA and 66.7% of the CC homozygote alleles.

Conclusion: These results provided evidence of an association between ACE I/D polymorphism, M235T polymorphism and AT1R-A1166C genotype polymorphism and rheumatic mitral valve disease.

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The renin-angiotensin system (RAS) plays an important role in the pathogenesis of cardiovascular disease (1). Angiotensin is the key peptide of the RAS, and exerts its influence on the heart and blood vessels both through its hemodynamic effects (via an influence on after-load and pre-load and determining coronary vasoconstriction) and through its direct cellular effects (via actions on cell proliferation) (2).

In surgical series, the most common causes of severe mitral regurgitation (MR) are mitral valve prolapse (MVPS) (20-70% of cases), ischemia (13-30%), rheumatic heart disease (3-40%) and endocarditis (10-12%) (3). In Turkey, MR is a common sequela of rheumatic fever, and is typically associated with some degree of mitral stenosis (MS). In the absence of any current data regarding the role of the ACE I/D, AGT-M235T and

AT1R-A1166C polymorphisms in rheumatic mitral valve disease, the present study was designed to investigate, in Turkish patients with rheumatic MS and/or MR and who had undergone mitral valve replacement (MVR), whether genetic polymorphisms of the RAS are associated with rheumatic mitral valve disease.

Clinical material and methods

Patients

Between December 2000 and July 2001, 50 patients (26 women, 24 men; mean age 47.9 ± 11.7 years) with rheumatic mitral valve disease underwent MVR at the authors' institution. Informed consent was obtained from each patient included in the study. Among the patients, 35 were in NYHA functional class III, and 15 were class IV.

Measurements of left ventricular (LV) dimensions were made from two-dimensional echocardiographic images and by M-mode echocardiography. MR, assessed using color-flow Doppler, showed 20 patients (40%) to have grade 2+, 28 (56%) grade 3+, and two

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(4%) grade 4+. The patients' characteristics and echocardiographic findings are detailed in Table I. Four patients had undergone previous heart surgery (two closed mitral valvotomy, one open mitral valvotomy, one mitral ring annuloplasty).

The control group comprised 50 normal subjects (25 men, 25 women; mean age 43.9 ± 0.7 years) in whom cardiac pathology was excluded by echocardiographic assessment.

Surgical technique

Surgery was performed via a median sternotomy on cardiopulmonary bypass (CPB) with moderate hypothermia (28-30°C). Before administration of heparin, 5 ml venous blood was withdrawn from each patient into EDTA tubes for genetic screening. For myocardial protection, one dose of hypothermic crystalloid cardioplegia (St. Thomas' Hospital II) was administered, and terminal warm blood cardioplegia (hot shot) was given prior to removal of the aortic cross-clamp.

A Sorin bileaflet prosthesis (Sorin Biomedica Cardio S.p.A., Saluggia, Italy) was implanted in all patients. All replacements were performed through a standard left atriotomy along the interatrial groove. The Bicarbon bileaflet mechanical heart valve prosthesis was implanted using interrupted everting pledgeted sutures and positioned in anti-anatomical orientation. All efforts were made to preserve the posterior leaflet and the papillary muscles. When the body temperature reached 36.5°C, CPB was discontinued and heparin reversed with protamine sulfate (3.1 mg/kg; Protamine, Roche) following decannulation.

ACE, AGN and AGN receptor polymorphism

Genomic DNA was extracted from peripheral blood lymphocytes according to standard procedures. PCR amplifications of the related regions were carried out in 50 µl volumes of reaction mixtures containing 75 mM Tris-HCl (pH 8.8), 200 mM (NH₄)₂SO₄, 0.1% Tween-20, 2.0 mM MgCl₂, 50 mM of each dNTP, 50 pmol of each set of specific primers, 1.25 unit Taq DNA polymerase (DNamp Ltd., UK) and 0.2-0.5 µg DNA sample. Thirty cycles of 94°C (30 s); 60°C (45 s) (67°C, 35 s for ACE Insertion sequence-specific region) and 68°C (1 min) were performed in an automated thermal cycler (MJR PTC-200, USA). The amplified products were analyzed electrophoretically on 2% agarose gel (4).

ACE, D and I alleles resulted in ~190 bp and ~490 bp amplicons, respectively. DNA samples of DD genotypes were subjected to a second PCR amplification with an insertion-specific primer set (5). In the presence of the I allele a 335-bp product was obtained, with no product in samples homozygous for DD.

The T→C base substitution causes a methionine→

Table I: Patient characteristics and echocardiographic results.

Variable	Study group	Control group
Age (years)*	47.9 ± 11.7	43.9 ± 10.7
Sex (M:F)	24:26	25:25
NYHA functional class		
III	35 (70)	
IV	15 (30)	
Valve area (cm ²)*	1.6 ± 0.4	
Ejection fraction (%)*	56.5 ± 8.0	
PAP (mmHg)*	53.3 ± 14.9	
LAD (cm)*	4.9 ± 0.8	
LVEDD (cm)*	4.7 ± 0.9	
LVESD (cm)*	3.3 ± 0.8	

*Mean ± SD.

Values in parentheses are percentages.

LAD: Left atrial dimension; LVEDD: Left ventricular end-diastolic dimension; LVESD: Left ventricular end-systolic dimension; PAP: Pulmonary artery pressure.

threonine replacement in the protein. To determine the angiotensinogen M235T genotype, 104-bp PCR products were digested with *MspI* restriction enzyme. The homozygote C-type variant digestion products, 73 and 31 bp were analyzed electrophoretically on 4% High Melt-Small Fragment AgarGel (CLP, Inc., USA). The heterozygotes showed three bands, whereas the homozygote T-type variants was not digested and showed only the 104-bp band.

AT1R-A1166C genotypes were analyzed by *DdeI* restriction enzyme digestion, which cuts 850 bp PCR products into 600 bp- and 250 bp-long portions. An additional *DdeI* recognition site is created in the C-type variant, at nucleotide 1166, which is found in the 250-bp fragment. Homozygote CC, homozygote AA and

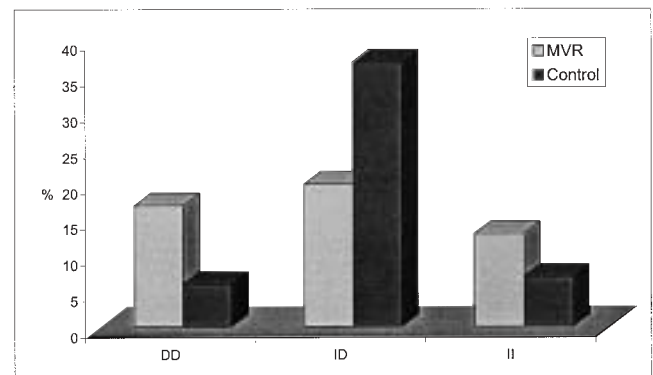


Figure 1: Statistically significant differences between control and study groups according to ACE I/D polymorphism ($p < 0.05$).

heterozygotes produce three, two and four bands (600, 250, 140 and 110 bp long), respectively.

Statistical analysis

Values were presented as mean \pm SD. Differences between groups were determined using the Mann-Whitney *U*-test. Genotypes and allele frequencies were evaluated with the chi-square test. Allele frequencies were estimated by gene counting methods. A *p*-value <0.05 was considered to be statistically significant.

Results

There were no significant differences between the two groups on the basis of patient characteristics (Table I). Neither was there any operative mortality in the MVR group. Measurements of left ventricular dimensions are presented in Table I.

Data of genotype and allele frequency comparison are presented in Figure 1. ACE I/D polymorphism was significantly different between the groups (*p* <0.05). The study (MVR) group was 73.9% homozygous for the DD allele, and 35% homozygous for the II allele, whereas the control group represented 64.9% of the ID allele (Fig. 1).

The results showed the M235T polymorphism to be heterogeneous. The MM homozygote frequency was significantly higher in controls (70.4%), whereas 80% of the TT homozygote frequency was in the MVR group (*p* <0.05). MT allele presence was distributed almost equally between the two groups (Fig. 2).

AT1R-A1166C genotype polymorphism also differed significantly between the two groups. The MVR group incorporated 73.7% of the AC heterozygote allele, whereas the controls had 64.4% of the AA and 66.7% of the CC homozygote alleles (*p* <0.05) (Fig. 3).

Discussion

Cloning of the human genes coding for the angiotensin-converting enzyme (ACE), angiotensinogen (AGT) and angiotensin II type 1 receptor (AT1R) has led to the discovery of several polymorphisms which may play a role as risk factors for cardiovascular diseases (2). In humans, the levels of plasma and cellular ACE are strongly genetically determined (6,7). Following cloning of the ACE gene, an ACE I/D polymorphism was identified which resulted from the presence/absence of a 287-bp fragment in the 16th intron of the ACE gene (8,9). Chou et al. demonstrated that patients with MVPS have a higher frequency of the ACE II genotype, which supports a role for the ACE I/D gene polymorphism in determining the risk of MVPS among the Chinese population (10).

In humans, the AGT gene is located on chromosome 1q42-43 and comprises five exons and four introns spanning 12 kb (11). Several studies have investigated the relationships between cardiovascular disorders and the M235T variant of the AGT gene (11,12). In a recent study, the association of the TT genotype with MVPS was more noteworthy than an overall increase in the frequency of the T allele at the M235T locus. These findings suggest that the M235T polymorphism of the AGT gene is associated with MVPS (13).

During the past few years, several associations between the AT1R-A1166C genotype polymorphisms and certain cardiovascular phenotypes (including blood pressure, coronary vasoconstriction and aortic stiffness) have been reported (14-16). Szombathy et al. demonstrated a significant association between MVPS and the A1166C polymorphism of the AT1R gene in the white population (17). However, in another study performed in Taiwan Chinese, it was concluded that A1166C polymorphism of the AT1R gene was not a

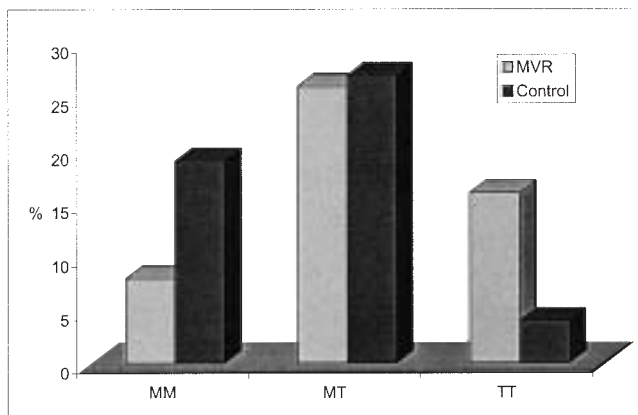


Figure 2: AGT gene polymorphism: Statistically difference between the control and study groups in both homozygote MM and TT frequencies (*p* <0.05).

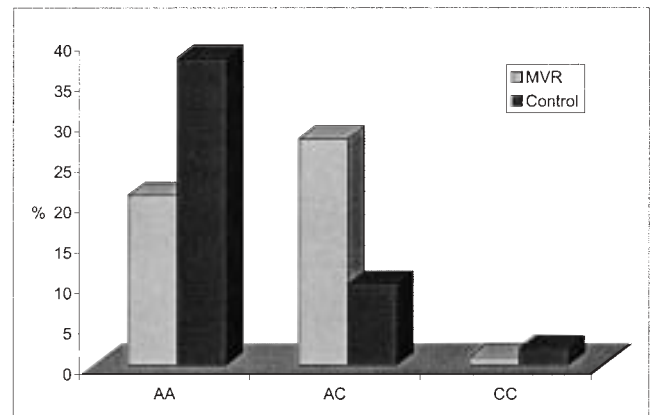


Figure 3: AT1R gene polymorphism: Statistically significant difference between the control and study groups in AA and AC alleles (*p* <0.05).

suitable genetic marker of MVPS (18).

Although MS is almost always of rheumatic origin, on occasion heavy calcification of the mitral apparatus can produce a similar syndrome in the elderly. MR causes chronic LV volume overload, compensatory LV hypertrophy and dilatation, and ultimately progressive LV failure (19). Without surgery, the outlook for patients with severe MR is poor, and the average annual mortality is 5%. Deteriorating LV function and heart failure causes most deaths, but sudden - presumably arrhythmic - death is also common (20). No known medical therapies directly affect the disease process in the valve leaflets in patients with rheumatic valve disease. However, sustained interest has been expressed in the concept of using the ACE inhibitors to reduce the severity of MR and the rate of LV dilatation (21,22).

The present results provide evidence of an association between ACE I/D polymorphism, M235T polymorphism, AT1R-A1166C genotype polymorphism and rheumatic mitral valve disease in patients undergoing MVR. Elsewhere, it has been documented that genotypic differences may play an important role in the etiologies of MVPS. Interestingly, in the present study significant differences were found between the normal population and patients with rheumatic mitral valve disease according to RAS genetic polymorphism, though this finding is difficult to explain. The present results are very similar to those of the related articles, and the present group mostly covers patients who have both MR and MS. Although the etiologies in these patients were known to be of rheumatic origin, the possibility remains that gene polymorphisms might exert an effect which could contribute to the valve regurgitations, as described elsewhere for MVPS (10,13,17,18).

It is believed that further studies with close preoperative and postoperative evaluation of patients with rheumatic mitral valve disease are required to detect whether these polymorphisms have any effect on the progression of the rheumatic mitral valve disease, and which medication should be recommended with regard to the individual ACE polymorphism.

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