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AGT and *ACE* genes influence classic mitral valve prolapse predisposition in Marfan patients

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Abstract

Background: In Marfan syndrome, the mitral valve prolapse, ranging from nonclassic to classic form on the basis of the leaflet thickness, is a common condition characterized by a highly variable structural abnormality. We investigated the role of angiotensinogen (*AGT*) M235T, angiotensin converting enzyme (*ACE*) I/D and angiotensin II type 1 receptor (*AT1R*) A1166C polymorphisms in influencing the susceptibility to classic or non-classic mitral valve prolapse in Marfan patients.
Methods: We studied 135 Marfan patients with mitral valve prolapse, diagnosed by echocardiography. *AGT*, *ACE*, and *AT1R* polymorphisms were identified by polymerase chain reaction-based restriction analysis.
Results: The frequency of the *ACE* D, but not *AGT* 235T and *AT1R* 1166C allele, was significantly higher in patients with classic mitral valve prolapse in comparison to that observed in the non-classic one ($p=0.03$). The percentage of subjects with the contemporaneous presence of *ACE* D and *AGT* 235T alleles was significantly higher in the classic mitral valve prolapse group in comparison to the non-classic one (79% *vs.* 55%, respectively; $p=0.008$). The concomitant presence of these two alleles was associated with increased susceptibility to the classic mitral valve prolapse (OR 3.02, $p=0.016$).
Conclusions: Our findings show a possible role of *ACE* and *AGT* genes as predisposing factors to classic mitral valve prolapse in Marfan patients, thus suggesting a role of renin angiotensin system genes in modulating mitral valve abnormality, and the need for an interventional study with angiotensin II type 1 receptor antagonists, which considers the leaflet thickness progression in Marfan patients with MVP.

Keywords: *AGT*; *AT1R*; *ACE* genes; Mitral valve prolapse; Marfan syndrome

1. Introduction

The mitral valve prolapse, an abnormal displacement into the left atrium of a thickened and redundant mitral valve during systole, represents one of the most common cardiac abnormalities in humans [1]. It is generally sporadic, but is also associated with congenital disorders of connective tissue, including Marfan syndrome. In Marfan syndrome the mitral valve prolapse is characterized by a highly variable structural abnormality, which, in turn, may be associated with clinical complications. Based on leaflet morphology and thickness, patients were classified as having classic and non-classic mitral valve prolapse, according to Freed et al. [2], and by using these criteria data from a population study from the Framingham Heart Study [3], reported a prevalence

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of 1.3% for classic mitral valve prolapse and 1.1% for the non-classic form in the general population.

The renin angiotensin system may play a role in the pathogenesis of mitral valve prolapse. Experimental studies demonstrated that angiotensin II, produced *de novo* in the heart, has various autocrine and paracrine properties on resident cells, and is involved in high-turnover connective tissue formation normally found in heart valve leaflet [4]. Angiotensin II was shown to influence leaflet myofibroblast collagen turnover *via* autocrine-based regulation of TGF-β1 [5], by inducing fibrosis *via* connective tissue growth factor [6], and *via* activation of mitogen-activated protein kinases pathway [7]. Moreover, in human cardiac valve tissue a high angiotensin I-converting enzyme activity has been demonstrated [8]. Polymorphisms in genes encoding for renin angiotensin system components, such as angiotensinogen (AGT), angiotensin converting enzyme (ACE), and angiotensin II type 1 receptor (AT1R), have been investigated in relation to mitral valve prolapse with conflicting results [9–12], but no information is available about the role of these genes in influencing the mitral valve leaflet thickness in Marfan patients with mitral valve prolapse.

In the intron 16 of the gene encoding for the ACE, a polymorphism consisting of an insertion/deletion (I/D) of a 287-bp fragment has been identified, and the *ACE* D allele has been reported to be associated with increased serum levels of circulating enzyme [13]. 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. Data from experimental studies reported both a functional role for the *ACE* I/D polymorphism in modulating angiotensin II levels [14], and an increased mRNA expression in white blood cells from subjects carrying the *ACE* D allele in comparison to subjects carrying the I allele [15].

A functional role for the *AGT* M235T polymorphism has been demonstrated, and data from clinical studies reported that the T235 rare variant accounts for increased AGT levels in circulating blood [16,17]. On the contrary, the functional role of the *AT1R* A1166C polymorphism is still unknown, and a linkage disequilibrium with a mutation that dynamically increases the responsiveness to angiotensin II has been hypothesized. Actually, data from van Geel et al. [18] indicated an increased response to angiotensin II in patients carrying the *AT1R* 1166CC genotype.

We carried out the present study in order to evaluate the influence of these renin angiotensin system polymorphisms on the susceptibility to classic or non-classic mitral valve prolapse in Marfan patients.

2. Materials and methods

2.1. Study population

One hundred and thirty-five Marfan patients with mitral valve prolapse, who strictly fulfilled the diagnostic criteria for Marfan syndrome proposed by De Paepe et al. [19], referred to Center for Marfan Syndrome and Related Disorders of the Azienda Ospedaliero-Universitaria, Careggi, University of Florence, have been investigated. A group of 270 healthy unrelated volunteers recruited from the staff of Hospital and University of Florence and from friends of patients, with no familial history of Marfan syndrome, absence of clinical manifestations or echocardiographic features related to this syndrome, was used as controls. Patients and controls were Caucasian and all subjects signed an informed consent; the study complies with the Declaration of Helsinki and was approved by the local ethic committee.

2.2. Diagnosis of mitral valve prolapse

Colour Doppler echocardiogram was performed in all patients and healthy subjects in order to study mitral valve structure and function. A Hewlett Packard Sonos 2000 (Andover, Massachusetts, USA) with frequency transducer of 2.5 MHz was used. Echocardiographic recordings of the mitral valve were made from parasternal long-axis and apical four-chamber views with subjects in the left decubitus position. Extensive two-dimensional recordings were made from apical four-chamber view to study anatomic features of mitral valve. Colour Doppler was used to evaluate the mitral valve regurgitation, defined as the presence of regurgitant flow jet in the left atrium. Colour jet and left atrial area size were evaluated in the apical chamber. The degree of mitral regurgitation was classified as mild, moderate or severe on the basis of jet area/left atrium ratio <20, 20–40, >40%, respectively [2]. The diagnosis of mitral valve prolapse was made according to recently refined 2-D echocardiographic criteria [2]. Prolapse was defined as the displacement of 1 or both mitral leaflets by more than 2 mm above the high points of the mitral anulus. Prolapse was defined as classic and non-classic form based on leaflet thickness; prolapsing valves with leaflets more than 5 mm thick are considered classic prolapse, while those with leaflets less than 5 mm thick are classified as non-classic prolapse. This echocardiographic evaluation was made from parasternal long-axis view. To minimize the variability of the echo-colour Doppler evaluations, all the echo-colour Doppler evaluations were performed and analyzed by the same physician (C. P.).

2.3. DNA analysis

Genomic DNA extraction was performed from peripheral blood leukocytes using a QUIAmp Blood Kit (QUIAGEN, Hilden, Germany). The *ACE* I/D and *AT1R* A1166C polymorphisms were genotyped as previously described [20]. The M235T polymorphism in exon 2 of *AGT* gene was analyzed as already reported [16].

2.4. Statistical analysis

Statistical analysis was performed with SPSS 10.1 for Windows. The *AGT*, *ACE* and *AT1R* allele frequencies were