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Genetic associations of bradykinin type 2 receptor, alpha-adrenoceptors and endothelial nitric oxide synthase with blood pressure and left ventricular mass in outpatients without overt heart disease

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ABSTRACT

Background: Physiological pathways such as bradykinin, renin-angiotensin, neurohormones and nitric oxide have been shown to play an important role in the regulation of cardiovascular function. Genetic variants of these pathways may impact blood pressure and left ventricular (LV) mass in different populations. To evaluate associations of genetic polymorphisms of bradykinin B2 receptor (BDKRB2), alpha-adrenergic receptors (ADRA) and endothelial nitric oxide synthase (eNOS) on the modulation of the blood pressure and the left ventricular mass.

Methods: We enrolled 758 individuals without overt heart disease. Blood pressure was estimated by auscultatory method during the clinical examination. Left ventricular (LV) mass was assessed by echocardiography. Genotypes for ADRA1A rs1048101, ADRA2A rs553668, ADRA2B rs28365031, eNOS rs2070744, eNOS rs1799983, and BDKRB2 rs5810761 polymorphisms were assessed by high-resolution melting analysis.

Results: BDKRB2 polymorphism rs5810761 was associated with blood pressure. Carriers of DD genotype had higher levels of SBP and DBP than carrier of II genotype ($p = 0.013$ and $p = 0.007$, respectively). eNOS polymorphism rs1799983 was associated with DBP. Carriers of GT genotype had lower levels of DBP than carriers of GG genotype ($p = 0.018$). eNOS polymorphism rs2070744 was associated with LV mass. Carriers of TC genotype had higher LV mass than carriers of TT genotype ($p = 0.028$).

Conclusions: In a cohort of individuals without overt heart disease, the BDKRB2 rs5810761 polymorphism (DD genotype carriers) were associated higher systolic and diastolic blood pressures, and the eNOS rs1799983 polymorphism (T allele carriers) were associated with lower diastolic blood pressure. The eNOS rs2070744 polymorphism (C allele carriers) was associated with higher left ventricular mass. These data suggest that eNOS and bradykinin receptor genetic variants may be potential markers of common cardiovascular phenotypes.

1. Introduction

Blood pressure and left ventricular mass are phenotypes that may be affected by abnormalities of the cardiovascular system such as hypertension, atherosclerosis, endothelial dysfunction, among others. Both are complex traits mediated by intricate interactions between genetic and environmental factors.

Physiological pathways such as bradykinin, renin-angiotensin, neurohormones and nitric oxide have been shown to play an important role in the regulation of cardiovascular function [1]. Genetic variants of these pathways may influence the subclinical phase of common cardiovascular diseases and genetic differences in the regulation of blood pressure and left ventricular mass may be early markers of subclinical development of cardiovascular disorders in apparently healthy individuals.

Previous studies have suggested associations of genetic variants related to these physiological pathways with blood pressure and left ventricular mass. In relation to left ventricular mass, most studies were conducted in patients with hypertension and evaluated principally genes of renin-angiotensin-aldosterone system. A pivotal study conducted by Schunkert and colleagues demonstrated in a population-based sample of 711 women and 717 men that homozygosity for a deletion polymorphism of the angiotensin-converting-enzyme gene was significantly associated with higher odds for left ventricular hypertrophy (LVH) [2]. In other study with 175 Chinese hypertensive patients,

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a missense variant of angiotensinogen gene at codon 235 (M235T) predicted an increase in left ventricular mass index in subjects with TT genotype [3].

Other works have also shown a possible association between left ventricular mass and genetic variants of bradykinin receptor type 2 (BDKRB2) gene. In 90 Swedish patients with hypertension, the BDKRB2 +9/+9 genotype was associated with lower regression of LVH in response to antihypertensive treatment [4]. In a Japanese cohort of 275 hypertensive and 441 normotensive subjects, in which a BDKRB2 gene polymorphism (58T/C) and an insertion/deletion variant of angiotensin converting enzyme (ACE) gene were genotyped, the prevalence of LVH in patients with hypertension was higher in those with both the *BDKRB2* CC and *ACE* D allele than those with other genotypes [5]. Additionally, genetic variants related to endothelial nitric oxide were also studied regarding the left ventricular mass. In a study with 600 hypertensive patients and 600 healthy controls, a Glu298Asp variant of the endothelial nitric oxide synthase (eNOS) gene was not tied to hypertension, left ventricular mass and carotid artery intima-media thickness [6]. In another study with 127 parents and 167 offspring, analysing the relationship between two variants (Glu298Asp and intron 4) of eNOS gene with ambulatory blood pressure and left ventricular mass, no association was found between the polymorphisms and left ventricular mass index [7].

Works from 90s demonstrated that alpha-adrenergic receptors (ADRA) polymorphisms could be important for blood pressure regulation in specific ethnic populations by mechanisms such as salt excretion, platelet aggregation and baroreceptor sensitivity [8,9]. Freitas and colleagues studied the association between the Arg347Cys polymorphism in the ADRA1A gene and blood pressure phenotypes, in 1568 Brazilians of Vitória City metropolitan area, and they found that Cys/Cys genotype tended to be associated with diastolic blood pressure increase and hypertension in this population [10]. A meta-analysis showed a significant association between the bradykinin B2 receptor polymorphism −58T/C and essential hypertension in 11 studies including 3382 subjects according to different genetic models [11]. Other meta-analysis that evaluated three well-studied eNOS polymorphisms, including 19,284/26,003 cases/controls for G894T (rs1799983), 6890/6858 cases/controls for 4b/a, and 5346/6392 case controls T−786C (rs2070744) revealed that 894T and 4b alleles in Asians and the −786C allele in Caucasians increase the risk of hypertension. The authors suggested that the influence of eNOS variants on the risk of hypertension may be dependent of an ethnic background [12].

The genetic influence on blood pressure and left ventricular mass responses in individuals without cardiovascular disease is not nearly well established as in patients with hypertension. Additionally, there is evidence that in patients without overt heart disease, some subclinical findings such as borderline high-normal blood pressure and left ventricular mass augmentations may precede clinical cardiovascular disease [13]. Our focus was to evaluate common genetic polymorphisms in important pathways related to the regulation of the cardiovascular system which encompassed variants of the bradykinin B2 receptor, alpha-adrenergic receptors and endothelial nitric oxide synthase (eNOS) genes relative to blood pressure and left ventricular mass in a Brazilian cohort of individuals without overt heart disease. We selected the specific genetic polymorphisms based on their importance and functional effects on cardiovascular function as previously described in the literature.

2. Methods

2.1. Study sample

From a cohort of 1015 volunteers aged 18 years old or more, interested in cardiovascular health examination, enrolled between February 2005 and April 2010, we analyzed data from 758 participants, 414 (54.6%) female and 344 (45.3%) male that had blood samples collected for genetic analysis and had undergone a transthoracic 2D echocardiogram. After informed consent, participants underwent clinical examination, 12-lead electrocardiogram and laboratory work up.

2.2. Exclusion criteria

Participants with previous history or evidence of heart disease during the initial clinical evaluation were excluded from the study. Additionally, patients with a history of diabetes mellitus, cerebrovascular disease, cancer, chronic obstructive pulmonary disease, thyroid disease, or other significant systemic diseases were also excluded.

2.3. Blood pressure measurement

Blood pressure was measured during the first appointment in sitting position using an aneroid sphygmomanometer. The patients were instructed to avoid caffeinated beverages, smoking and emptying their bladders at least 30 min before measurement.

2.4. Left ventricular mass measurement

The left ventricular mass was measured by echocardiography following Dereveux formula: left ventricular mass (g) $= 0.8 \times 1.04 \times$ [(LV diastolic diameter + interventricular septum + posterior wall thickness)^3− LV diastolic diameter^3] + 0.6 [14].

2.5. Genotyping

Genomic DNA from subjects was extracted from a peripheral blood following standard salting-out procedure. Genotypes for the polymorphisms ADRA1A rs1048101 (Arg347Cys), ADRA2A rs553668 (1780 C > T), ADRA2B rs28365031 (Del 301-303), eNOS rs2070744 T786C (−786 T > C), eNOS rs1799983 (Glu298Asp), and BDKRB2 rs5810761 (Table 1) were detected by polymerase chain reaction (PCR) followed by high-resolution melting analysis with the Rotor Gene 6000® instrument (Qiagen, Courtaboeuf, France). The QIAgility® (Qiagen, Courtaboeuf, France), an automated instrument, was used according to manufacturer's instructions to optimize the sampling preparation step. One specific disc is able to genotype 96 samples for these polymorphisms.

Amplification of the fragment was performed using the primers for the polymorphisms studied a PCR with 4 cycles. PCR was carried out with the following conditions: denaturation of the template DNA for first cycle of 94 °C for 120 s, denaturation of 94 °C for 20 s, annealing of 53.4 °C for 20 s and extension of 72 °C for 22 s. PCR was performed using a 10 μL reactive solution (10 mM Tris–HCl, 50 mM KCl, pH 9.0; 2.0 mM MgCl2; 200 μM of each dNTP; 0.5 U Taq DNA polymerase; 200 nM of each primer; 10 ng of genomic DNA template) with addition of fluorescent DNA-intercalating SYTO9 ((1.5 μM); Invitrogen, Carlsbad, USA). In the HRM phase, the Rotor Gene 6000 measured the fluorescence at each 0.1 °C temperature increase in the range of 73–85 °C.

Table 1
Study genetic polymorphisms.

Gene	Genetic polymorphism	Genotype	NCBI register	Type
ADRA1A	Arg347Cys	TT, CT, CC	rs1048101	Coding region
ADRA2A	1780 C > T	TT, CT, CC	rs553668	3'UTR
ADRA2B	Del 301/303	II, ID, DD	rs28365031	Coding region
eNOS	−786T > C	TT, TC, CC	rs2070744	Promoter region
eNOS	Glu298asp	GG, GT, TT	rs1799983	Coding region
Type II Bradykinin receptor	BDKRB2	II, ID, DD	rs5810761	Coding region

NCBI, National Center of Biotechnology Information; ADRA1A, alpha-adrenergic receptor 1A; ADRA2A, alpha-adrenergic receptor 2A; ADRA2B, alpha-adrenergic receptor 2B; eNOS, endothelial nitric oxide synthase.