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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.

Melting curves were generated by the decrease in fluorescence with the increase in the temperature; and in analysis, nucleotide changes resulted in three different curve patterns. Samples of the three observed curves were analysed using bidirectional sequencing as a validation procedure (ABI Terminator Sequencing Kit and ABI 3500XL Sequencer —Applied Biosystems, Foster City, California, USA). The two methods gave identical results in all tests. The wild-type, heterozygous and mutant homozygous genotypes were easily discernible by HRM analysis. In addition, 4% of the samples were randomly selected and reanalysed as quality controls, and gave identical results [15].

3. Demographic and laboratory data

Weight and height were measured, and body mass index (BMI) was calculated. Ethnicity was classified for the Brazilian population according to a set of phenotypic characteristics (such as skin color, hair texture, shape of the nose, and aspect of the lips) and individuals were classified as white, mixed, black and other ethnic groups. The participants were classified as current smokers or non-smokers.

Laboratory workup included fasting plasma glucose, cholesterol and lipoproteins, serum triglycerides, serum creatinine, hemoglobin, leukocyte count, thyroid test and high-sensitivity C-reactive protein.

3.1. Statistical analysis

Continuous data are expressed as mean ± standard deviation. Categorical data are expressed as number and percentage. The Hardy-Weinberg proportions for each polymorphism studied were determined using the chi-square test. The correlation coefficients between continuous variables were performed with Pearson's correlation test. Student's *t*-test and Mann-Whitney *U* test (when appropriate) were performed for comparison of the means between two groups.

Multiple linear regression models were performed to study associations between the study genetic polymorphisms and the response variables systolic blood pressure, diastolic blood pressure and LV mass. The demographic and laboratory covariates included in the models were age, gender, ethnicity, body mass index, smoking status, HDL-cholesterol, total cholesterol and fasting glucose. Analysis of residuals was performed to test whether the requirements of the models were properly met.

3.2. Ethics

The study protocol was approved by the Committee of Ethics on Human Research of the Hospital and all participants were instructed about the study and signed an informed consent.

4. Results

The clinical characteristics of the study population are shown in Table 2. In relation to ethnicity, 502 (66.2%) participants were White, 101 (13.3%) participants were Mixed, 36 (4.8%) participants were Black, 11 (1.4%) participants were from other ethnic groups and in 108 (14.3%) had no report and ethnicity was not retrieved; 145 (19.1%) participants reported to be active smokers.

There were weak correlations between LV mass and systolic blood pressure ($r = 0.22$) and diastolic blood pressure ($r = 0.21$).

In relation to the genetic distributions, all genetic polymorphisms were in Hardy-Weinberg equilibrium, except for the ADRA2B rs28365031 polymorphism ($p < 0.001$).

Table 2
Clinical and laboratory characteristics of the study population.

Variable	Mean (SD)
Age	43.1 (13.2)
Body mass index (Kg/m^2)	26.3 (4.4)
Systolic blood pressure (mm Hg)	124.1 (13.5)
Diastolic blood pressure (mm Hg)	80.9 (9.2)
Creatinine (mg/dL)	0.84 (0.17)
Glucose (mg/dL)	92.4 (8.6)
Total cholesterol (mg/dL)	193.5 (38)
HDL cholesterol (mg/dL)	49.1 (13.5)
LDL Cholesterol (mg/dL)	121.4 (32.6)
Triglyceride (mg/dL)	117.6 (77.4)
Left ventricular mass (g)	158.9 (40.9)

SD, standard deviation.

Table 3
Estimates and significances of the predictor variables included in the final model relative to systolic and diastolic blood pressures.

Variable	Estimate	*p*-Value
Systolic blood pressure		
Blood glucose	−5.24	<0.001
eNOS rs1799983 (GT)*	6.3716	0.06
eNOS rs1799983 (TT)*	9.9154	0.08
BDK2R rs5810761 (Ins/Del)**	4.2216	0.15
BDK2R rs5810761 (Del/Del)**	8.8710	0.013
eNOS rs2070744 (TC)***	0.4523	0.82
eNOS rs2070744 (CC)***	−1.9654	0.53
Diastolic blood pressure		
BMI > 30 kg/m^2	3.80	<0.001
Male gender	1.98	0.01
eNOS rs1799983 (GT)*	−5.2767	0.011291
eNOS rs1799983 (TT)*	−1.4691	0.611497
BDK2R rs5810761 (Ins/Del)**	2.9604	0.164208
BDK2R rs5810761(Del/Del)**	6.7474	0.006561

BMI, body mass index; BDK2R, bradykinin B2-receptor; eNOS, endothelial nitric oxide synthase.
* Considering GG genotype as reference.
** Considering II genotype as reference.
*** Considering TT genotype as reference.

4.1. Blood pressure, LV mass and genetic associations

The predictor variables included in the final regression model relative to systolic and diastolic blood pressures are shown in Table 3. The BDKRB2 rs5810761 polymorphism was associated with blood pressure. Carriers of DD genotype had higher levels of SBP and DBP than carriers of II genotype ($p = 0.013$ and $p = 0.007$, respectively). The eNOS rs1799983 polymorphism was associated with DBP. Carriers of GT genotype had lower levels of DBP than carriers of GG genotype ($p = 0.018$).

The predictor variables included in the final regression model relative to left ventricular mass are shown in Table 4. The eNOS rs2070744 polymorphism was associated with LV mass. Carriers of TC genotype had higher LV mass than carriers of TT genotype ($p = 0.028$).

In the multivariate analysis, genetic polymorphisms of alpha-adrenergic receptors were not associated with blood pressure or LV mass phenotypes.

Table 4
Estimates and significances of the predictor variables included in the final model relative to the left ventricular mass.

Variable	Estimate	p-value
Male gender	41.98	<0.001
BMI > 30 kg/m^2	24.96	<0.001
BMI 25–30 kg/m^2	8.72	<0.001
ADRA1A rs1048101 (Arg/Cys)*	−0.75	0.82
ADRA1Ars1048101 (Cys/Cys)*	−0.10	0.98
eNOS rs2070744 (TC)**	5.31	0.027
eNOS rs2070744 (CC)**	4.10	0.28

BMI, body mass index; eNOS, endothelial nitric oxide synthase.
* Considering Arg/Arg genotype as reference.
** Considering TT genotype as reference.

5. Discussion

In the present study of individuals without overt heart disease, we found significant associations of two functional eNOS genetic polymorphisms with diastolic blood pressure and LV mass as well as a significant association between BDKRB2 rs5810761 polymorphism and both diastolic and systolic blood pressure, even after controlling covariates related to these phenotypes such as age, gender, body mass index, smoking status and metabolic characteristics.

With respect to genetic associations, we observed the BDKRB2 rs5810761 variant significantly influencing the levels of systolic and diastolic blood pressure. It is believed that bradykinin receptors contribute to blood pressure regulation and their inhibition are related to attenuated blood pressure response to angiotensin-converting enzyme inhibition [16,17]. In a British study with 2706 apparently healthy men, 2 functional variants of the bradykinin type I and type II receptors, including the BDK2R deletion polymorphism, were strongly associated with hypertension and with coronary events [18]. Our study is in line with another Brazilian population-based study from Vitória city with 1570 participants that showed the deletion allele of BDKRB2 rs5810761 polymorphism was associated with higher levels of diastolic blood pressure and with the risk of hypertension [19]. In a sample of 166 normotensive white Americans the same polymorphism was associated with systolic blood pressure, but homozygous carriers of deletion allele had lower levels than carriers of the insertion allele [20]. These evidences suggest genetic variants of bradykinin type II receptor gene may participate in blood pressure regulation, but this contribution may suffer influences from geographic differences.

The nitric oxide (NO) molecule is involved with the regulation of blood pressure, principally due its vasodilator effect on the peripheral resistance vasculature, and the eNOS activity is important to this process [21]. Previous studies have evaluated the impact of eNOS polymorphisms on blood pressure in different populations. These data have been controversial and also appear to be influenced by regional characteristics among study populations. Wolff and colleagues observed, in a large cohort of Caucasians, no difference in the distribution of eNOS (Glu298Asp) rs1799983 genotypes between normotensive or hypertensive participants [22]. Conversely, studies in other populations suggested an association between ENOS polymorphisms and blood pressure [23,24]. In our sample, eNOS rs1799983 T allele was associated with lower levels of diastolic blood pressure.

The eNOS rs2070744 C allele was associated with higher left ventricular mass. The eNOS C allele has been related to decreasing in the activity, suppressed eNOS transcription, and reduced NO production [25]. A study with Caucasian hypertensive patients and normotensive controls showed eNOS rs2070744 C allele was associated with blunted endothelium-dependent peripheral vasodilation, which may be a possible mechanism to left ventricular hypertrophy [26]. The relation between eNOS gene and LV mass has been evaluated in some populations with different results. In a multiethnic American population, Zhu and colleagues evaluated the influence of 3 variants of eNOS gene on blood pressure and left ventricular mass [27]. Despite differences in diastolic blood pressure between the analyzed haplotypes, no association was found in relation to LV mass. In a Chinese study with hypertensive patients, there was an association between the left ventricular mass and eNOS rs1799983 but not with eNOS rs2070744 [28], which was the opposite observed in our sample.

Our study is limited by the small number of genotyped polymorphisms. However, the variants which were analyzed in our study have been demonstrated to be functional in vitro and in vivo settings, and to be in linkage disequilibrium with other *loci* in their respective genes, allowing them to be genetic markers of these target genes. The baseline blood pressure assessment were performed only once during the study, which may decrease the accuracy of the measurement. The blood pressure accuracy may also have had decreased by the use of manual measurement instead of automatic one. These facts and the exclusion of hypertensive subjects may explain the small ranges on systolic and diastolic blood pressures found in our population. Nevertheless, we used measurement methods which are performed in our real life day by day settings.

Another limitation to our genetic association study is the complex categorization of the ethnic groups in a multiracial population such as Brazilians. We found a higher predominance of individuals described as white than expected in the entire Brazilian population. In the São Paulo State, there is a high prevalence of descendants of European immigrants, particularly from southern Europe countries such as Portugal and Italy. However, as the degree of miscegenation in Brazil has been very high over the centuries, it is difficult to ascertain accurately the pattern of European, African and Amerindian ancestry in our population, even in individuals with well-defined white or black phenotypes. In a study evaluating ancestry informative SNPs in 200 healthy individuals from 5 regions of Brazil, it was shown a major contribution of European ancestry (0.771), followed by African (0.143) and Amerindian ancestries (0.085) [29].

6. 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.

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Statement of competing interests

The authors report no competing interests.

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