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Gender-related associations of genetic polymorphisms of α-adrenergic receptors, endothelial nitric oxide synthase and bradykinin B2 receptor with treadmill exercise test responses

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ABSTRACT

Background: Treadmill exercise test responses have been associated with cardiovascular prognosis in individuals without overt heart disease. Neurohumoral and nitric oxide responses may influence cardiovascular performance during exercise testing. Therefore, we evaluated associations between functional genetic polymorphisms of α-adrenergic receptors, endothelial nitric oxide synthase, bradykinin receptor B2 and treadmill exercise test responses in men and women without overt heart disease.

Methods: We enrolled 766 (417 women; 349 men) individuals without established heart disease from a check-up programme at the Heart Institute, University of São Paulo Medical School. Exercise capacity, chronotropic reserve, maximum heart-rate achieved, heart-rate recovery, exercise systolic blood pressure (SBP), exercise diastolic blood pressure (DBP) and SBP recovery were assessed during exercise testing. Genotypes for the α-adrenergic receptors ADRA1A Arg347Cys (rs1048101), ADRA2A 1780 C>T (rs553668), ADRA2B Del 301–303 (rs28365031), endothelial nitric synthase (eNOS) 786 T>C (rs2070744), eNOS Glu298Asp (rs1799983) and BK2R (rs5810761) polymorphisms were assessed by PCR and high-resolution melting analysis.

Results: Maximum SBP was associated with ADRA1A rs1048101 (p=0.008) and BK2R rs5810761 (p=0.008) polymorphisms in men and ADRA2A rs553668 (p=0.008) and ADRA2B rs28365031 (p=0.022) in women. Maximum DBP pressure was associated with ADRA2A rs553668 (p=0.002) and eNOS rs1799983 (p=0.015) polymorphisms in women. Exercise capacity was associated with eNOS rs2070744 polymorphisms in women (p=0.01) and with eNOS rs1799983 in men and women (p=0.038 and p=0.024).

Conclusions: The findings suggest that genetic variants of α-adrenergic receptors and bradykinin B2 receptor may be involved with blood pressure responses during exercise tests. Genetic variants of endothelial nitric oxide synthase may be involved with exercise capacity and blood pressure responses during exercise tests. These responses may be gender-related.

KEY QUESTIONS

What is already known about this subject?

- There are few data about genetic associations of exercise test responses. The greater study that addressed this topic, to the best of our knowledge, was conducted by Ingelsson *et al*[9]. Ingelsson studied genetic associations of blood pressure and heart-rate during treadmill exercise tests in a large population of a Framingham study. Of note, exercise capacity was not analysed in Ingelsson's study. Other studies presented more modest samples with heterogeneity in the analysed exercise phenotypes.

What does this study add?

- This study adds new information about genetic associations of important exercise test responses such as exercise capacity and exercise blood pressure.

How might this impact on clinical practice?

- The knowledge about genetic associations of exercise test responses contributes to the understanding of new pathways involved in cardiovascular function and exercise physiology. The genetic variants in this study may represent important markers of cardiovascular regulation during exercise. This information may be utilised in future researches focusing on cardiovascular health.

INTRODUCTION

Cardiovascular responses during and after exercise stress testing, such as heart-rate, blood pressure and exercise capacity, have been reported to predict cardiovascular health in individuals without overt heart disease.[1–4] Several pathways are involved with the regulation of cardiovascular response during exercise.[5] The influence of physiological pathways, including the autonomic

nervous system,[6] renin-angiotensin-aldosterone system and endothelium-derived vasoactive substances,[7 8] on exercise performance have been demonstrated, but the contribution of genetic variations of these systems to inter-individual responses during exercise is not so well documented.

A study of 2982 Framingham Offspring participants evaluated the genetic variants of heart rate and blood pressure responses during and after treadmill exercise testing with the Bruce protocol in 14 genes related to neurohumoral pathways. From 10 associations between the examined variants and exercise phenotypes that reached a nominal significance level, eight included genes encoding α-adrenergic receptors (ADRA), suggesting that this pathway may be an important determinant of exercise haemodynamics in this cohort.[9] The impact of endothelial nitric synthase (eNOS) polymorphism on exercise haemodynamics was also assessed in studies with different methodologies,[10–12] also suggesting a role of eNOS in cardiovascular regulation of exercise.

In this study, we evaluated associations between the common genetic polymorphisms of ADRA (ADRA1A, ADRA 2A, ADRA2B), eNOS and bradykinin B2 receptor with heart rate, blood pressure and exercise capacity responses during treadmill exercise testing in individuals without overt cardiovascular disease. We also hypothesised, due to established sex-related differences in cardiovascular performance during exercise, that these associations may be different between women and men.

METHODS

Study sample

We studied 766 unrelated asymptomatic participants (417 female and 349 male) enrolled in a cohort of patients who underwent a check-up protocol in the General Outpatient Clinics at a university hospital. The study enrolled women and men aged 18 years or older without a medical history of heart disease.

Participants underwent clinical examination (clinical history and physical examination), 12-lead ECG, chest X-ray, echocardiogram and laboratory work up (blood cell count, serum glucose, cholesterol and lipoproteins, triglycerides, creatinine and high-sensitivity C reactive protein) before enrolment.

Exclusion criteria

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

Treadmill exercise test

The participants underwent a symptom-limited treadmill exercise test according to the Ellestad protocol.[13] The criteria for interruption of the exercise were physical exhaustion or exceeding the maximum heart rate predicted for the patient's age. Individuals were encouraged to exercise until they experienced limiting symptoms, even if 85% of maximum predicted heart rate was achieved. Peak exercise capacity was estimated from exercise time and reported as metabolic equivalents.[14]

During exercise and recovery stages of the protocol, we recorded symptoms, blood pressure and heart rate. Predicted peak heart rate was calculated as 220 minus age. Peak heart rate achieved and exercise systolic and diastolic blood pressure achieved were recorded at the end of the exercise stage.

Chronotropic reserve was estimated as follows: (peak heart rate achieved−baseline heart rate)/(predicted peak heart rate−baseline heart rate). After peak exercise, the recovery stage followed, where individuals walked for a 3-min cool-down period at 1.5 mph without an incline. Heart rate recovery was defined as peak heart rate minus heart rate at 1, 2 and 3 min of the recovery stage. Blood pressure recovery was defined as the systolic blood pressure (SBP) values at 1, 2 and 3 min of the recovery stage.

The responses of the exercise testing included in the analysis were chronotropic reserve, heart-rate recovery, exercise SBP, exercise diastolic blood pressure, SBP recovery and exercise capacity.

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 colour, hair texture, shape of the nose and aspect of the lips) and individuals were classified as Caucasian, Intermediate (meaning Brown, *Pardo* in Portuguese), African–American or Asian.[15 16] The participants were classified as current smokers or non-smokers.

Laboratory work up included fasting plasma glucose, cholesterol and lipoproteins, serum triglycerides, serum creatinine, haemoglobin, leucocyte count, thyroid test and high-sensitivity C reactive protein.

Genotyping

Genomic DNA from participants 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 BK2R rs5810761 were detected by PCR followed by high-resolution melting (HRM) analysis with a Rotor Gene 6000 instrument (Qiagen, Courtaboeuf, France).[17] The QIAgility (Qiagen, Courtaboeuf, France), an automated instrument, was used according to manufacturer's instructions to optimise the sample preparation step. One specific disc is able to genotype 96 samples for these polymorphisms.[17]

Amplification of the fragment was performed using the primers for the polymorphisms studied. A 40-cycle 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 $MgCl_2$; 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. 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.

Statistical analysis

Continuous data are expressed as mean±SD. Categorical data are expressed as number and percentage. Differences of means between men and women were estimated by Student's t test. Residual analyses were used to determine whether the data set was well modelled. The treadmill exercise test responses were considered dependent variables, and the genetic polymorphisms were considered independent variables. The Hardy-Weinberg proportions for each polymorphism studied were determined using the χ^2 test.

Multiple linear regression and mixed linear model (when dependent variables were repeated measures) were performed to study the associations between the exercise variables and the genetic polymorphisms in men and women. Interactions between gender and independent variables were included in the models to confirm differences in associations between the genetic polymorphisms and the dependent variables exercise capacity, exercise SBP and exercise diastolic blood pressure. Women were considered as reference and, in addition to this analysis, tests for main effects (for women), interaction effects (difference between women and men) and the sum of these effects (for men) were conducted.[18] The heterozygous genotype was considered as reference. All analyses were performed in the statistical software R (V.2.15.1).

Demographic and laboratory covariates included in the model were age, ethnicity, BMI, smoking status, baseline diastolic and SBP, fasting glucose, total cholesterol, high-density lipoprotein-cholesterol and triglycerides. When interactions p value <0.15, complementary analyses were performed to investigate associations between the polymorphisms and dependent variables for each gender. A p value <0.05 was considered significant.

Ethics

The study protocol was approved by the Ethics Committee on Human Research of the Heart Institute, University of São Paulo Medical School, and all participants were instructed about the study and signed an informed consent.

RESULTS

The characteristics of the study participants are shown in table 1. Mean age was 43 years (age range, 18–79). Participants were more frequently middle-aged participants, with normal levels of arterial blood pressure,

Table 1 Demographic and laboratory characteristics of the study population

Variables	
Gender	Men 349 (45.6%) Women 417 (54.4%)
Smoking*	145 (19.9%)
Ethnicity†	White 502 (77.3%) Black 36 (5.6%) *Pardos* 101 (15.6%) Asian 10 (1.5%)
Age (years)	43 (13.2)
Body mass index (kg/m^2)	26.3 (4.4)
Rest systolic blood pressure (mmHg)	124.1 (13.5)
Rest diastolic blood pressure (mmHg)	80.9 (9.3)
Haemoglobin (g/dL)	14.4 (1.3)
Leucocyte count (n°/ mm^3)	6785 (1857)
Creatinine (mg/dL)	0.85 (0.18)
Plasma 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)
Triglycerides (mg/dL)	117.6 (77.4)
C reactive protein (mg/L)	2.54 (3)
EDLVD (cm)	4.6 (0.3)
ESLVD (cm)	2.9 (0.3)
IV (cm)	0.8 (0.1)
LVPW (cm)	0.8 (0.6)
LVEF (%)	66 (4.8)

Categorical data are expressed as number (percentage) and continuous data as mean (SD).
*Relative to 728 participants with smoking status data.
†Relative to ethnicity data of 649 participants.
EDLVD, end-diastolic left ventricular diameter; ESLVD, end-systolic left ventricular diameter; HDL, high-density lipoprotein; IV, interventricular septum; LDL, low-density lipoprotein; LVEF, left ventricular ejection fraction; LVPW, left ventricular posterior wall.

Table 2 Cardiovascular responses during treadmill exercise test in men and women

Variable	Men		Women		p Value
Exercise capacity	10.5	1.9	8.8	1.9	<0.001
Chronotropic reserve	0.9	0.13	0.85	0.15	<0.001
Maximum SBP (mmHg)	181.1	20.4	161.9	20.3	<0.001
Maximum DBP (mmHg)	87.8	10.7	83.6	10.1	<0.001
SBP 1st minute recovery stage (mmHg)	170.0	21.6	151.9	20.9	<0.001
SBP 2nd minute recovery stage (mmHg)	161.3	21.2	141.1	19.6	<0.001
SBP 3rd recovery stage (mmHg)	148.9	18.7	132.1	16.0	<0.001
SBP recovery 1st minute*	10.8	14.7	8.8	12.1	0.137
SBP recovery 2nd minute*	20.2	17.4	20.0	14.4	0.922
SBP recovery 3rd minute*	31.9	16.3	29.5	16.0	0.114
Peak heart-rate	166.6	15.2	161.8	17.3	<0.001
HR 1st minute recovery stage (bpm)	126.0	18.5	120.8	19.9	0.003
HR 2nd minute recovery stage (bpm)	110.9	17.2	104.2	18.2	<0.001
HR 3rd minute recovery stage (bpm)	104.8	15.5	99.0	15.9	<0.001
HRR 1st minute (bpm)†	40.6	16.8	42.0	14.2	0.328
HRR 2nd minute (bpm)†	55.4	15.1	57.7	13.9	0.087
HRR 3rd minute (bpm)†	61.6	14.4	63.1	14.4	0.246

*Systolic blood pressure recovery was defined as the difference between maximum systolic blood pressure and systolic blood pressure at recovery stage.
†Heart-rate recovery was defined as the difference between peak heart-rate and heart-rate at recovery stage.
DBP, diastolic blood pressure; HR, heart-rate; HRR, heart-rate recovery; SBP, systolic blood pressure.

lipids and fasting glucose, despite the tendency to be overweight (50% of the participants had a BMI >25 mg/kg^2); 145 (19.9%) participants were current smokers.

The responses during the treadmill exercise test are shown in table 2. Rest and exercise blood pressures, exercise capacity and chronotropic reserve were higher in men than in women. There was no significant difference relative to heart rate at the first, second and third minutes of the recovery phase and to SBP at the first, second and third minutes of the recovery phase between men and women. The genotype and allelic distribution of the polymorphisms studied in the sample are depicted in table 3. These polymorphisms, except for the polymorphism ADRA2B rs28365031, are in accordance with the Hardy-Weinberg equilibrium.

Exercise SBP was associated with the polymorphisms ADRA1A rs1048101 (p=0.008) and BK2R rs5810761 (p=0.008) in men, and with ADRA2A rs553668 (p=0.008) and ADRA2B rs28365031 (p=0.022) in women. Interaction effects were statistically significant with p values equal to 0.116, 0.065, 0.008 and 0.128, respectively (table 4). Men with the ADRA1A rs1048101 Cys/Cys genotype had higher exercise SBP than carriers of the Arg/Cys and Arg/Arg genotypes. Men with BK2R rs5810761 DD genotype had higher exercise SBP than carriers of ID and II genotypes. Women with ADRA2A rs553668 CC genotype had lower maximum SBP than women with CT and TT genotypes. Women with ADRA2B rs28365031 DD genotype had lower exercise SBP than women with ID and II genotypes.

Exercise diastolic blood pressure was associated with the polymorphism ADRA2A rs553668 (p=0.002) and eNOS rs1799983 (p=0.015) in women (table 4). In men, the same relationship was observed between exercise diastolic blood pressure and the rs553668, reaching close to statistical significance (p=0.071). Individuals with the ADRA2A rs553668 TT genotype had higher exercise diastolic blood pressure than carriers of the CT and CC

Table 3 Genotypic and allelic frequencies of the study variants

Variant	Genotype n (%)			Allele n (%)	
	CC	CT	TT	C	T
ADRA1A rs1048101	197 (29.9)	328 (49.8)	134 (20.3)	722 (54.8)	596 (45.2)
ADRA2A rs553668	413 (62.6)	215 (32.6)	32 (4.8)	1041 (78.9)	279 (21.1)
eNOS_rs2070744	71 (10.8)	270 (41.3)	313 (47.9)	412 (31.5)	896 (68.5)
	II	ID	DD	I	D
ADRA2B rs28365031	409 (63.4)	153 (23.7)	83 (12.9)	971 (75.3)	319 (24.7)
BKRB2 rs5810761	193 (29.1)	333 (50.2)	137 (20.7)	719 (54.2)	607 (45.8)
	GG	GT	TT	G	T
eNOS rs1799983	329 (50)	272 (41.3)	57 (8.7)	930 (70.7)	386 (29.3)

ADRA, α-adrenergic receptors; eNOS, endothelial nitric synthase.

genotypes. Women with the eNOS rs1799983 GG genotype had lower exercise diastolic blood pressure during exercise than carriers of GT and TT genotypes.

Exercise capacity was associated with the polymorphism eNOS rs2070744 for women (p=0.010), but not for men (interaction p=0.085). Women with TT genotype had higher exercise capacity than carriers of TC and CC genotypes (table 4). In both genders, polymorphism eNOS rs1799983 was associated with exercise capacity. Individuals with eNOS rs1799983 Glu/Glu genotype (p=0.038) and Asp/Asp genotype (p=0.024) had lower exercise capacity than individuals with Glu/Asp genotype. Additional tests showed that there is no difference between Glu/Glu and Asp/Asp genotype (p=0.164).

DISCUSSION

In this study, we found significant associations between treadmill exercise test responses and genetic variants of important physiological pathways associated with circulatory system regulation. We observed that these associations differed among the sexes and were mainly related to blood pressure responses during exercise.

The differences between men and women relative to the impact of genetic variants on exercise responses are multifactorial. The sensitivity and responsiveness of adrenergic receptors have been reported to be distinct between men and women.[19 20] Studies have shown that muscle sympathetic nerve activity is lower in premenopausal women than in men, and sympathetic activity and baroreflex sensitivity may be influenced by female reproductive hormones.[21–24] In addition, women have attenuated vasoconstrictor sensitivity to α_1-adrenergic stimulation but higher vasodilator sensitivity to α_2-receptor stimulation compared to men,[20 25 26] suggesting that the autonomic control of vascular tonus appears to be different between men and women. A sex difference is also noted relative to effects of nitric oxide on vasomotor responses. Women have greater basal nitric oxide biosynthesis than men have, and oestrogen receptors may influence eNOS expression and, consequently, vascular function.[25 27] Our finding suggests that neurohumoral and endothelial influences on exercise performance may have different genetic modulations in men and women.

The associations found in our study were mainly related to blood pressure responses during exercise, with the exception of the association between the eNOS rs2070744 variant and exercise capacity in women. These observations are not unexpected, considering that we have already studied genetic variants of pathways closely related to vasoconstrictor and vasodilatation regulation.[28]

The polymorphism ADRA1A rs1048101 influenced the maximum SBP in men, with greater values in carriers of the Cys/Cys genotype than in carriers of the Arg allele. This polymorphism was associated with autonomic control and with blood pressure responses to antihypertensive drugs.[29–31] In a multiethnic Brazilian study with 1500 participants, the rs1048101 allele Cys was associated with higher levels of blood pressure in a subgroup of physically active participants aged 45 years or lower, but not in sedentary participants,[32] suggesting that this variant may be implicated with the blood pressure adaptations to exercise.

The polymorphism ADRA2A rs553668 was associated with exercise SBP and exercise diastolic blood pressure in women, and with approaching significance in men regarding maximum diastolic blood pressure. The α-adrenergic receptor 2A participates in several physiological responses, such as vascular tonus control, insulin release from pancreatic cells and adipocyte metabolism in humans.[33] The autonomic responses to stress also appear to be exacerbated in carriers of the ADRA2A rs553668 allele T,[34] and in some ethnic groups this allele may be related to hypertension and diabetes mellitus.[33 35] In our study, this polymorphism appears to be a significant marker of blood pressure responses during exercise.

The polymorphism ADRA2B rs28365031 was associated with exercise SBP in women. To the best of our knowledge, there is no study evaluating the impact of this polymorphism on exercise profiles. Previous studies have suggested that the deletion variant is associated with adverse cardiovascular prognosis in select populations.[36 37] In our study, the deletion/deletion genotype was associated with a lower increase in exercise SBP in relation to insertion/insertion and insertion/deletion genotypes, suggesting that in our sample the deletion allele may not be associated with an unfavourable cardiovascular performance during exercise.

The NO produced by endothelium has an important role in vascular tonus control, which may be relevant to balance between muscular vasodilation and vasoconstriction during and after exercise.[8] Genetic variants of endothelial nitric oxide synthase have been reported to affect NO release.[38 39] In our study, the eNOS rs2070744 polymorphism influenced exercise capacity in women and eNOS rs1799983 influenced exercise capacity in both gender. Few data are available on the impact of these polymorphisms on cardiovascular responses during exercise. Two studies with modest samples (49 males and 55 females, respectively) showed divergent results regarding the influence of eNOS rs2070744 polymorphism on blood pressure response to exercise training.[11 12] In a Spanish study, Gómez-Gallego *et al*[40] found a higher prevalence of carriers of genotype TT in elite power athletes than in non–athletic controls. We also found an association between the eNOS rs1799983 polymorphism and exercise diastolic blood pressure in women, with carriers of the GG genotype having lower exercise diastolic blood pressure than carriers of allele T. Despite the lack of an established influence of this variant on eNOS expression, the presence of the T allele has been implicated in some pathological conditions such as hypertension,[41] coronary vasospasms and impaired muscle vasodilation.[10 42] Our finding diverges from the finding in a Korean study with 209 participants, which described an increase in hypertensive response to exercise in

Table 4 Estimates of the associations between treadmill exercise test responses and genetic polymorphism in the study population

	Interaction p Value	Estimate	p Value	Estimate	p Value
Exercise capacity					
ADRA1A rs1048101 (Cys/Cys vs Cys/Arg)	0.220	0.45	0.070	–	–
ADRA1A rs1048101 (Arg/Arg vs Cys/Arg)	0.327	0.18	0.416	–	–
ADRA2A rs553668 (CC vs CT)	0.278	0.01	0.960	–	–
ADRA2A rs553668 (TT vs CT)	0.838	0.21	0.683	–	–
ADRA2B rs28365031 (DD vs ID)	0.709	−0.28	0.436	–	–
ADRA2B rs28365031 (II vs ID)	0.127	−0.23*	0.415*	0.33†	0.302†
eNOS rs2070744 (TT vs TC)	0.085	2.14*	0.010*	0.34†	0.733†
eNOS rs2070744 (CC vs TC)	0.715	0.78	0.390	–	–
eNOS rs1799983 (Glu/Glu vs Glu/Asp)	0.539	−0.47	0.038	–	–
eNOS rs1799983 (Asp/Asp vs Glu/Asp)	0.876	−0.81	0.024	–	–
BK2R rs5810761 (DD vs ID)	0.998	−0.40	0.129	–	–
BK2R rs5810761 (II vs ID)	0.292	−0.15	0.497	–	–
Exercise systolic blood pressure					
ADRA1A rs1048101 (Cys/Cys vs Cys/Arg)	0.116	1.76*	0.538*	8.13†	0.008†
ADRA1A rs1048101 (Arg/Arg vs Cys/Arg)	0.714	−1.31	0.503	–	–
ADRA2A rs553668 (CC vs CT)	0.008	−6.41*	0.008*	3.73†	0.140†
ADRA2A rs553668 (TT vs CT)	0.444	1.97	0.657	–	–
ADRA2B rs28365031 (DD vs ID)	0.128	−8.86*	0.022*	−0.06†	0.990†
ADRA2B rs28365031 (II vs ID)	0.092	−2.90*	0.280*	3.91†	0.220†
eNOS rs2070744 (TT vs TC)	0.562	−2.99	0.121	–	–
eNOS rs2070744 (CC vs TC)	0.621	−5.42	0.072	–	–
eNOS rs1799983 (Glu/Glu vs Glu/Asp)	0.956	−0.29	0.880	–	–
eNOS rs1799983 (Asp/Asp vs Glu/Asp)	0.101	5.21*	0.246*	−6.37†	0.158†
BK2R rs5810761 (DD vs ID)	0.065	6.02*	0.522*	29.06†	0.008†
BK2R rs5810761 (II vs ID)	0.451	8.40	0.200	–	–
Exercise diastolic blood pressure					
ADRA1A rs1048101 (Cys/Cys vs Cys/Arg)	0.946	0.23	0.817	–	–
ADRA1A rs1048101 (Arg/Arg vs Cys/Arg)	0.129	1.22*	0.301*	−0.99†	0.429†
ADRA2A rs553668 (CC vs CT)	0.851	−1.09	0.168	–	–
ADRA2A rs553668 (TT vs CT)	0.032	12.74*	0.002*	6.82†	0.071†
ADRA2B rs28365031 (DD vs ID)	0.599	−2.12	0.114	–	–
ADRA2B rs28365031 (II vs ID)	0.641	−0.79	0.382	–	–
eNOS rs2070744 (TT vs TC)	0.565	1.19	0.172	–	–
eNOS rs2070744 (CC vs TC)	0.786	−0.24	0.861	–	–
eNOS rs1799983 (Glu/Glu vs Glu/Asp)	0.028	−8.35*	0.015*	−2.12†	0.572†
eNOS rs1799983 (Asp/Asp vs Glu/Asp)	0.850	0.28	0.848	–	–
BK2R rs5810761 (DD vs ID)	0.625	0.52	0.608	–	–
BK2R rs5810761 (II vs ID)	0.178	−0.07	0.933	–	–

When interactions p value <0.15, complementary analyses were performed to investigate associations between the polymorphisms and dependent variables for each gender.
*For women.
†For men.
ADRA, α-adrenergic receptors; eNOS, endothelial nitric synthase.

carriers of the GG genotype.[43] This suggests a possible ethnic influence on genetic modulation of exercise responses. Our findings also support the concept that the eNOS gene may play a significant role in exercise performance in individuals without cardiovascular disease.

The bradykinin receptor B2 polymorphism rs5810761 influenced exercise SBP in men in our study. Bradykinin is a polypeptide with important physiological effects on the vascular bed and muscle metabolism.[44 45] Few data exist about the impact of bradykinin receptor genes on blood pressure and exercise performance. In a Brazilian study, the deletion allele was associated with higher diastolic blood pressure in the general population of a metropolitan area.[32] Another BDKRB2 polymorphism has been shown to influence physical performance in marathon runners.[46] These findings suggest that BDKRB2 may participate in the modulation of exercise.

The present study has limitations that must be addressed. In the treadmill exercise test, we used the Ellestad protocol, which is currently used at our institution for individuals without significant functional limitations, instead of the most commonly used Bruce protocol. Despite the more widespread use of the Bruce protocol in specific populations, many studies that evaluated exercise testing variables

used different protocols to study cardiovascular responses to exercise.[2 4] The number of variables included in the model with multiple hypotheses tests may increase family wise error rate and, possibly, the finding of false-positive associations. However, we recognise that our findings are only hypothesis generating, needing validation in other populations. The observed associations may not indicate a direct relationship between the analysed polymorphism and exercise phenotypes, since these variants may be in strong linkage disequilibrium with many other genetic loci not analysed in the present study. Nevertheless, linkage disequilibrium allows that some polymorphisms, despite not being directly associated with the expression of a specific phenotype, become markers of this same phenotype.

CONCLUSIONS

In women, exercise SBP was associated with ADRA2A rs553668 and ADRA2B rs28365031; exercise diastolic blood pressure was influenced by eNOS rs1799983 and ADRA2A rs553668; exercise capacity was associated with eNOS rs2070744 in women and with eNOS rs1799983 in men and women. In men, exercise SBP was associated with ADRA1A rs1048101 and BKRB2 rs5810761.

These findings suggest that genetic variants of ADRA and bradykinin B2 receptor may be involved with blood pressure responses during exercise tests. Endothelial nitric oxide synthase variants may be involved with exercise capacity and blood pressure responses during exercise tests. These responses may be gender-related.

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Competing interests None.

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