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Original Article

Hypertension is associated with a variant in the *RARRES2* gene in populations of Ouro Preto, Minas Gerais, Brazil: a cross-sectional study

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Abstract: Background: Arterial hypertension (AH) is implicated in vascular health and contributes significantly to cardiovascular morbidity and mortality. In addition to the contribution of usual risk factors for AH, elucidating the influence of genetic factors is a promising area of investigation. Therefore, we evaluated the association between AH and cardiovascular risk factors (CVRFs) and genetic polymorphisms in communities in Southeast Brazil. Methods: A total of 515 adults aged 18-91 years, who were cross-sectionally assessed between 2015-2016, were included. Demographic, clinical, behavioral, anthropometric characteristics, and laboratory parameters and 12 single nucleotide polymorphisms in seven candidate genes involved in cardiovascular risk (*RARRES2, AGT, NOS3, GNB3, APOE, APOB, APOC3, LDLR*, and *PPARG*) were evaluated, with AH as the outcome. Sex, age, and laboratory parameters were considered the main confounding factors. Results: There was a significant association between age >60 years (odds ratio [OR] =6.74), alcohol dependence (OR=3.84), smoking (OR=1.74), overweight (OR=1.74), high plasma triglyceride (TG) levels (OR=1.98) and low high-density lipoprotein (HDL-c) (OR=6.22), diabetes (OR=3.68), and insulin resistance (OR=2.40) and AH. A significant association was observed between rs4721 in *RARRES2* and AH. The T allele in homozygosis was a potent chance modifier for AH. The highest chance gradients for AH were characterized by the presence of the TT genotype and DMT2 (OR=9.70), high TG (OR=6.26), low HDL-c (OR=8.20), and age more than 60 years (OR=9.96). Conclusion: The interaction of the T allele of the rs4721 polymorphism in *RARRES2* with CVRFs may predispose carriers to a higher cardiovascular risk.

Keywords: Cardiovascular risk, hypertension, dyslipidemia, diabetes, obesity, molecular epidemiology, *RARRES2* gene

Introduction

Arterial hypertension (AH) is an important risk factor for cardiovascular disease (CVD) and is characterized by a pathological multifactorial clinical condition in which there is a sustained increase in systolic blood pressure (SBP) greater than or equal to 140 mmHg and/or diastolic blood pressure (DBP) greater than or equal to 90 mmHg [1, 2]. This condition negatively impacts vascular health and is an essential predictor of cardiovascular morbidity and mortality.

In 2010, AH caused 9.4 million premature deaths worldwide, contributing to a global burden of 7% of disability-adjusted life years (DALY) [3]. Globally, the prevalence of AH is highest in Africa and the Americas, reaching 46% and 35%, respectively [4]. The number of adults with AH increased from 594 million in 1975 to 1.13 billion in 2015. This increase was observed to mainly occur in low- and middle-income countries [4]. In Brazil, AH affects 32.5% (36 million) of adults and more than 60% of elderly people, contributing directly or indirectly to 50% of deaths from CVD [2].

In addition to the classical physiological mechanisms, genetic factors may also be responsible for regulating blood pressure and predisposition to AH and have been a promising area of investigation [5-7]. Notably, AH is influenced by age, sex, ethnicity, overweight, physical inactiv-

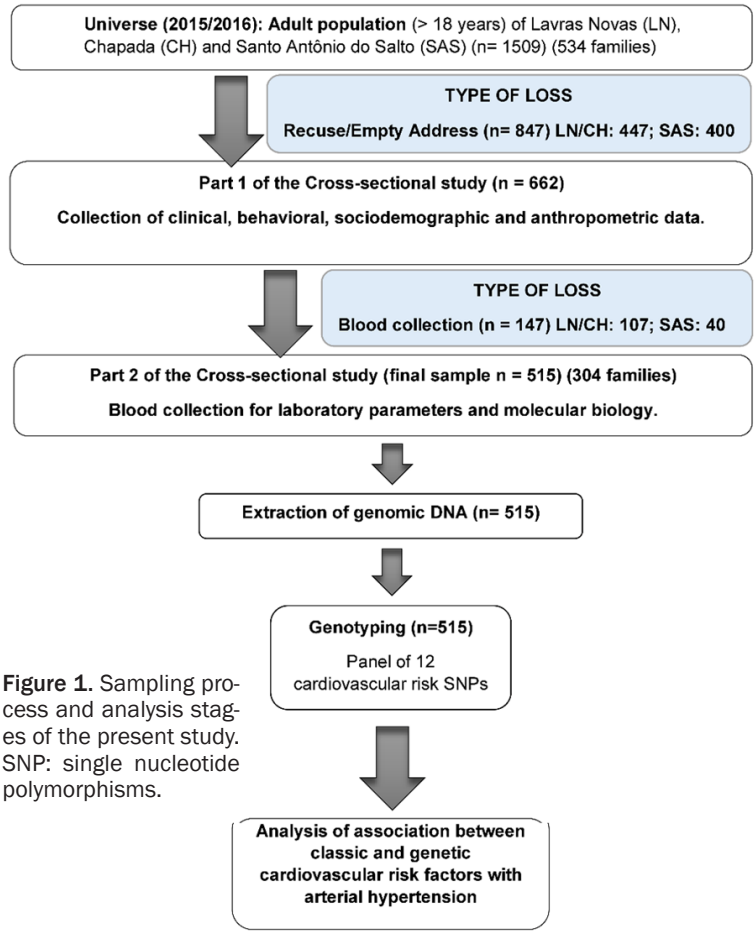

Figure 1. Sampling process and analysis stages of the present study. SNP: single nucleotide polymorphisms.

ity, excessive salt, alcohol intake, and socioeconomic factors [2].

Ouro Preto is located in the southeastern region of Brazil, in the Iron Quadrangle region; the population is formed by the miscegenation of Caucasians, blacks, and indigenous people around the gold mining activity (dating XVIII century). The first studies based on the urban population indicated a high prevalence of AH (37.7%), as well as a high prevalence of overweight and obesity in adults (30.0% and 11.9%, respectively) [8] and children and adolescents (8.7% and 6.2%, respectively) [9], indicating that this population requires attention.

Recently, the adult population of Ouro Preto was the focus of epidemiological studies that pointed to the persistently high prevalence of cardiovascular risk factors (CVRFs) [10, 11], such as AH, overweight, obesity, dyslipidemia, and the influence of new biochemical and genetic factors on the development of these risk factors [11]. Given that AH is a major cardiovascular risk factor, the present study explored a possible association between AH and the usual CVRFs and genetic polymorphisms in two rural populations of Ouro Preto/Minas Gerais, Brazil.

Materials and methods

Study design and study area

The present work adopted a cross-sectional design (April 2015 to January 2016) to evaluate two rural populations of Ouro Preto, Brazil. The areas included were the districts of Lavras Novas, its subdistrict Chapada, and Santo Antônio do Salto.

Inclusion/exclusion criteria

The study included adults (>18 years old) of both sexes who had lived for at least five years in these regions. Participants, who did not complete the interview, did not attend the blood draw or refused/withdrew, and did not sign the consent form, were excluded from the study.

Sampling process, final sample, and homogeneity in relation to losses

The sample was defined based on the following criteria: universe of 1509 subjects, confidence level and expected error (95% and 5%, respectively), expected frequency of the outcome (AH) in Ouro Preto (38%), and expected losses (10%). The calculated sample size comprehend 600 individuals. The factors used to balance the sampling were: sex and age group. These factors were stratified in each household, and all eligible residents were invited to participate in the study.

Figure 1 presents the sampling and analysis stages of the present study. The losses of the sample showed no difference between sexes but were higher in young men (18-39 years).

Blood pressure was measured in the refusers and compared with the participants to control for possible selection bias. There was no significant difference between the refusal group [SBP: 133.1 mmHg (±16.0) and DBP: 83.8

mmHg (±12.6)] and the participants [SBP: 130.8 mmHg (±22.7) and DBP: 79.6 mmHg (±14.6)] (P=0.558).

Exposure, outcome, and confounding factors

Demographic, clinical, behavioral, anthropometric characteristics, and laboratory parameters and 12 polymorphisms (single nucleotide polymorphisms-SNPs) linked to cardiovascular risk phenotypes were evaluated as exposures. The AH was the outcome assessed. Sex, age, and laboratory parameters were considered as the main confounding factors.

Data collection

The participants included in the study answered a semi-structured questionnaire to obtain sociodemographic data, such as sex, age, income, marital status, education, self-reported skin color, behavior (smoking and alcohol consumption), and medication use.

Smoking: Smoking was categorized into three groups: never smokers, smokers, and ex-smokers. Non-smokers were characterized by never having smoked or had smoked less than 100 cigarettes during their lifetime. Ex-smokers were those who smoked at least 100 cigarettes during their lifetime but quit smoking more than six months ago. Smoker was defined as the person who had smoked 100 or more cigarettes in her lifetime and still smoked at the time of the study [12]. For the bivariate analysis, the ex-smoker and non-smoker groups were grouped.

Alcohol consumption: Alcohol dependency was defined using the CAGE questionnaire (Cut-down, Annoyed, Guilty, Eye-opener) [13].

Blood pressure: Arterial blood pressure was measured following instructions described in the Brazilian Society of Cardiology protocol [2]. For measurement, the participant was at rest in a sitting position with the arm supported. Three measurements were carried out using an automatic digital blood pressure monitor, HEM-705CP® (Omron Healthcare, USA). The interval between measurements was 1-2 min. The lowest of three measures was recorded [2]. Hypertensive individuals were defined as follows: individuals with a casual blood pressure measurement of ≥140/90 mmHg and were already taking antihypertensive medication [2]. In situations where SBP and DBP were in different groups, the higher value was adopted to classify blood pressure.

Anthropometric measurements: The anthropometric measurements included weight, height, and waist circumference (WC). Body weight was measured on a TANITA® portable scale (maximum capacity of 150 kg and accuracy of 0.1 kg). Participants were instructed to remain barefoot and wear as little clothing as possible. Height was measured using a portable stadiometer (Charder, Taiwan). The participant was instructed to stand barefoot with her or his back to the vertical rod, feet together, arms bending along the body, head erect, eyes fixed forward, and back of the head touching the rod. The body mass index (BMI) was defined as the weight in kilograms divided by the height square in meters, and was classified as normal (BMI <25 kg/m^2), overweight (BMI 25-29.9 kg/m^2), and obese (BMI ≥30 kg/m^2) [14]. WC was measured using a simple inelastic tape. The participants were in an upright position with a relaxed abdomen, arms extended laterally along the body, feet slightly apart, and weight equally distributed between the two lower limbs. The cut-off point was established by the World Health Organization (WHO); 94 cm for men and 80 cm for women [15].

Laboratory parameters

Lipid profile: Total cholesterol, high-density lipoprotein (HDL-c) and serum triglyceride (TG) alevels were determined by the enzymatic-colorimetric method (Invitro diagnostic/Human, Brazil), in the Chemwell R6® Automatic Analyzer (Awareness Technology, USA). The low-density lipoprotein (LDL-C) and very-low-density lipoprotein (VLDL-c) fractions were calculated using the Friedewald (1972) formula [16] (considering a TG levels of ≤400 mg/dL). Dyslipidemias were classified according to reference values for ten-hour fasting: low HDL-c (men <40 mg/dL and women <50 mg/dL) alone or in association with an increase in LDL-c or TG, isolated hypercholesterolemia (LDL-c ≥160 mg/dL), isolated hypertriglyceridemia (TG ≥150 mg/dL), mixed hyperlipidemia (LDL-c ≥160 mg/dL and TG ≥150 mg/dL) [17]. The classification of dyslipidemia also considered cholesterol levels ≥190 mg/dL.

Fasting blood glucose: Fasting blood glucose was measured by a colorimetric enzymatic (Invitro diagnosis/Human, Brazil) in a Chemwell R6® Automatic Analyzer (Awareness Technology, USA). Serum glucose levels <100 mg/dL were considered normal; 100-125 mg/dL, altered; and ≥126 mg/dL, elevated [18]. The use of oral hypoglycemic medication or insulin with or without fasting blood glucose ≥126 mg/dL was defined as diabetes. Pre-diabetic individuals (100-125.9 mg/dL) who were not using hypoglycemic drugs were not considered diabetic.

Fasting insulinemia: Fasting insulin levels were determined by indirect chemiluminescence using an automated Access 2 Immunoassay System® (Beckman Coulter, USA). Insulin resistance (IR) was defined according to the homeostatic insulin resistance index assessment model (HOMA-IR): fasting insulin (μIU/mL) × fasting glucose (mmol/L)/22.5 [19]. The cutoff for adults was 2.71 [20].

Chemerin adipokine: The adipokine chemerin was evaluated by enzyme-linked immunosorbent assay (ELISA) (Abcam Kit, Chemwell R6® Automated Analyzer, Awareness Technology, USA). Serum chemerin levels were categorized into quartiles for the bivariate analyses, and the median (160 ng/mL) was used as the cutoff point.

Molecular assays

Extraction of genomic DNA: Genomic DNA was extracted from whole blood collected in ethylenediaminetetraacetic acid (EDTA) using the Wizard® genomic DNA purification kit (Promega®, USA).

Genetic markers of cardiovascular risk: The selection criteria for a panel of 12 polymorphisms involved a literature review to survey the SNPs with the highest frequency of positive association with the CVRFs addressed in the present study [AH, obesity, dyslipidemia, type 2 diabetes mellitus (T2DM), and IR]. Those that were presented in at least five previous studies were selected, at least one of which was a genome-wide association study (GWAS) and in ethnic groups similar to the study population, and the possibility of the presence of some rare alleles in studies of African and European populations (main ancestors in the formation of the Brazilian population) was checked.

The SNPs selected were (1) associated with AH: rs699 in the *AGT* gene [21], rs1799983 in the *NOS3* gene [21], and rs5443 in the *GNB3* gene [21]; (2) associated with dyslipidemia: rs429358 and rs7412 in the *APOE* gene [22], rs693 in the *APOB* gene [23], rs4520 [24], and rs5128 [25] in the *APOC3* gene, rs5925 in the *LDLR* gene [26]; and (3) associated with obesity, IR, and T2DM: rs1801282 in the *PPARG* gene [27], rs4721, and rs17173608 in the *RARRES2* gene [28].

Two SNPs (rs429358 and rs7412) in the *APOE* gene characterized a haplotype. The method of SNPs evaluation was described elsewhere [11].

The technique for allelic discrimination real-time PCR (qPCR) using specific primers and probes for each SNP (TaqMan® Minor Groove Binder-MGB, TaqMan® System; 7500 Fast Real-Time PCR Systems, Applied Biosystems, USA). Amplifications proceeded as described: initial denaturation at 95°C for 10 min, 40 cycles at 95°C for 15 s, and final extension at 60°C for 1 min. Reagents and DNA sample volumes were 10 μL mix (TaqMan Universal PCR Master Mix, 20x Primer Mix, and water) plus 1 μL DNA (±1-20 ng/μL).

Statistical analysis

The data were processed and analyzed using the SPSS software version 22 (IBM, USA). The genotype and allele frequencies of 12 SNPs were evaluated and tested for Hardy-Weinberg equilibrium, and those in disequilibrium were excluded. Proportion comparisons of categorical variables between the hypertensive and normotensive group, such as the distribution of the 12 SNP panel genotypes, were performed using Pearson's chi-square test. One-way analysis of variance (ANOVA) was performed for continuous variables after testing for normal distribution using the Shapiro-Wilk test. Binary logistic regression analysis was performed to adjust the estimates of association by confounders. The odds ratio (OR) was calculated to identify the genetic markers and other risk factors significantly ($P<0.05$) and independently associated with AH.

Ethical aspects

This study was approved by the local Human Research Ethics Committee (Federal University of Ouro Preto, Nos. 125017/2015 and 516-

66115.5.0000.5150). All participants signed an informed consent form after being informed about the research objectives and risks.

Results

Demographic characteristics

329 participants were women (63.9%, mean age 49.2±17.3) and 186, men (36.1%, mean age 49.8±16.4). The age range was 18-91 years. Twenty participants (3.9%) lived in Chapada, 189 (36.7%) in Lavras Novas, and 306 (59.4%) in Santo Antônio do Salto.

Comparisons of the sociodemographic and behavioral characteristics of the study population between the hypertensive and normotensive groups are shown in **Table 1**. As expected, AH was more likely to develop with advancing age and was more likely to affect those who were illiterate, married or divorced/widowers, and smokers. An average income was more predisposing to AH than an income below the minimum wage. Sex and self-reported skin color showed no differences between groups.

Anthropometric, laboratory parameters, and clinical characteristics

Anthropometric and clinical characteristics, and laboratory parameters distribution by normotensive and hypertensive patients are shown in **Table 2**. In the study population, we observed that CVRFs, such as overweight, increased waist circumference, dyslipidemia, DMT2, and IR, were also associated with AH.

Genetics characteristics

The 12 SNPs were tested for Hardy-Weinberg equilibrium; three were out of balance (*LDLR*/rs5925 P=0.002; *APOC3*/rs5128 P=0.002; *APOC3*/rs4520 P=0.037) and were removed from the analyses. **Table 3** presents the analyses of the allelic and genotypic frequencies of the nine genetic markers of the SNPs in Hardy-Weinberg equilibrium in the AH group (hypertensive and normotensive individuals). There was a difference only between the ancestral homozygous genotype, GG, and the TT variant of rs4721 polymorphism in relation to AH, where the presence of the TT allele increased the chance of having AH by 92%.

Factors associated with AH after adjustment for confounders

Anthropometric and body composition data were adjusted by the multivariate analysis for laboratory parameters, sociodemographic, behavioral, and genetic variables. **Table 4** presents the variables that remained significantly associated with AH after adjustment.

Based on the laboratory parameters and DMT2 variable that were obtained in the adjusted model presented above (**Table 4**). An analysis of chance modification for AH was performed by taking the absence or the presence of changes in these variables, together with each genotype of the rs4721 polymorphism, like strata of exposure, where the homozygous GG was considered a reference group (**Table 5**). In general, we observed that the variables evaluated presented a chance gradient for AH, with the most excellent chances of having AH, indicated by the concomitant presence of the homozygous TT genotype and the altered variable.

In **Table 5**, it is observed that individuals with variables that changed in the presence of the TT genotype had a significant chance of having AH; among them, there was a concomitant presence of T2DM and the TT genotype, where individuals with this trait had a 9.7 times greater chance of AH than non-diabetic individuals with the GG genotype. Another remarkable finding was that, in relation to the reference group, the chance of AH was more than 8- and 6-fold higher for the TT homozygotes in those with altered HDL-c and TG, respectively.

Discussion

The present study evaluated the association of AH with sociodemographic variables and the presence of CVRFs and genetic polymorphisms in populations from rural areas of Ouro Preto, Minas Gerais, in Southeast Brazil. After adjustment, there was a significant association between age over 60 years, alcohol dependence, smoking, overweight, high plasma TG levels, low HDL-c, DMT2, and IR with AH, which aligns with the findings presented in the literature [4, 29].

Interestingly, after adjustment, a significant association was observed among the genetic polymorphisms evaluated between the rs4721 polymorphism in the *RARRES2* gene and AH. Further, the presence of the T allele in homozygosis was an important chance modifier for AH. The highest chance gradient for AH was indicated by the concomitant presence of DMT2

Table 1. Sociodemographic and behavioral characteristics, by groups of hypertensive and normotensive individuals, in the study population

Variables	Hypertension			OR (95% CI)	P*
	No (n=294)	Yes (n=221)	Total (n=515)		
Age group					
18-34 years	133 (45.2)	19 (8.6)	152 (29.5)	1.0	-
35-59 years	124 (42.2)	99 (44.8)	223 (43.3)	5.58 (3.22-9.67)	<0.0001
≥60 years	37 (12.6)	103 (46.6)	140 (27.2)	19.49 (10.6-35.7)	<0.0001
Sex					
Female	186 (63.3)	143 (64.7)	329 (63.9)	1.0	
Male	108 (36.7)	78 (35.3)	186 (36.1)	0.93 (0.65-1.35)	0.736
Schooling					
Literate	280 (95.6)	192 (86.9)	472 (91.8)	1.0	
Illiterate	13 (4.4)	29 (13.1)	42 (8.2)	3.25 (1.64-6.41)	<0.0001
Missing data**	1	-	1		
Marital status					
Not married	96 (32.8)	40 (18.1)	136 (26.5)	1.0	
Married or SU	177 (60.4)	137 (62.0)	314 (61.1)	1.85 (1.21-2.85)	0.0045
Others†	20 (6.8)	44 (19.9)	64 (12.5)	5.28 (2.77-10.1)	<0.0001
Missing data**	1	-	1		
Income					
≥4 MS	31 (11.1)	36 (17.1)	67 (13.6)	1.0	
2-3 MS	166 (59.3)	108 (51.2)	274 (55.8)	0.56 (0.32-0.96)	0.033
≤1 MS	83 (29.6)	67 (31.8)	150 (30.5)	0.69 (0.38-1.23)	0.217
Missing data**	14	10	24		
Self-reported skin color					
White + yellow	36 (12.3)	26 (11.9)	62 (12.2)	1.0	
Black + brown	256 (87.7)	192 (88.1)	448 (87.8)	1.03 (0.60-1.77)	0.891
Missing data**	2	3	5		
Smoking‡					
No	207 (71.1)	118 (54.1)	325 (63.9)	1.0	
Yes	84 (28.9)	100 (45.9)	184 (36.1)	2.08 (1.44-3.02)	<0.0001
Missing data**	3	3	6		
Alcohol drinking§					
Low risk/none Alcohol drinking	268 (93.4)	190 (88.4)	458 (91.2)	1.0	
Alcohol dependence	19 (6.6)	25 (11.6)	44 (8.8)	1.85 (0.99-3.46)	0.050
Missing data**	7	6	13		

Data are reported as numbers and percentages. *P<0.05, Pearson's chi-square test. **Missing data were excluded from the analysis; SU: stable union; MS: minimum salary; CI: confidence interval; OR: odds ratio; †separated/divorced or widowed; ‡self-reported lifetime and/or current smoking; §according to the CAGE (Cut-down, Annoyed, Guilty, Eye-opener) questionnaire.

Table 2. Anthropometric and laboratory parameters, by groups of hypertensive and normotensive individuals, in the study population

Variables	Hypertension			OR (95% CI)	P*
	No (n=294)	Yes (n=221)	Total (n=515)		
BMI (kg/m^2)					
<25	145 (49.5)	82 (37.4)	227 (44.3)	1.0	0.007
≥25	148 (50.5)	137 (62.6)	285 (55.7)	1.63 (1.14-2.34)	
Missing data**	1	2	3		

WC‡ (cm)					
Normal	146 (50.2)	77 (35.5)	223 (43.9)	1.0	0.001
CR	145 (49.8)	140 (64.5)	285 (56.1)	1.83 (1.27-2.62)	
Missing data**	3	4	7		
TG (mg/dL)					
<150	243 (82.7)	141 (63.8)	384 (74.6)	1.0	<0.0001
≥150	51 (17.3)	80 (36.2)	131 (25.4)	2.70 (1.80-4.10)	
TC (mg/dL)					
<190	161 (54.8)	104 (47.1)	265 (51.5)	1.0	0.083
≥190	133 (45.2)	117 (52.9)	250 (48.5)	1.36 (0.96-1.93)	
LDL-c (mg/dL)					
<130	243 (82.7)	171 (77.4)	414 (80.4)	1.0	0.135
≥130	51 (17.3)	50 (22.6)	101 (19.6)	1.39 (0.90-2.15)	
HDL-c (mg/dL)					
≥40 (M)	277 (94.2)	186 (84.2)	463 (89.9)	1.0	<0.0001
≥50 (F)					
<40 (M)	17 (5.8)	35 (15.8)	52 (10.1)	3.06 (1.66-5.63)	
<50 (F)					
nHDL-c (mg/dL)					
<160	249 (84.7)	168 (76.0)	417 (81.0)	1.0	0.013
≥160	45 (15.3)	53 (24.0)	98 (19.0)	1.74 (1.12-2.71)	
Dyslipidemia					
No	137 (46.6)	52 (23.5)	189 (36.7)	1.0	<0.0001
Yes	157 (53.4)	169 (76.5)	326 (63.3)	2.83 (1.92-4.17)	
FG (mg/dL)					
<100	164 (55.8)	80 (36.2)	244 (47.4)	1.0	
100-125	121 (41.2)	110 (49.8)	231 (44.9)	1.86 (1.28-2.70)	0.0009
>125	9 (3.0)	31 (14.0)	40 (7.7)	7.06 (3.20-15.5)	<0.0001
FI (uLU/mL)					
<p75	237 (80.6)	150 (67.9)	387 (75.1)	1.0	0.001
≥p75	57 (19.4)	71 (32.1)	128 (24.9)	1.96 (1.31-2.95)	
HOMA-IR					
<2.71	269 (91.5)	171 (77.4)	440 (85.4)	1.0	<0.0001
≥2.71	25 (8.5)	50 (22.6)	75 (14.6)	3.14 (1.87-5.27)	
DMT2					
No	284 (96.6)	182 (82.4)	466 (90.5)	1.0	<0.0001
Yes	10 (3.4)	39 (17.6)	49 (9.5)	6.46 (3.16-13.2)	
QM (ng/mL)					
≤160	151 (52.0)	104 (47.9)	255 (50.2)	1.0	0.377
>160	140 (48.0)	113 (52.1)	253 (49.8)	1.17 (0.82-1.66)	
Missing data**	3	4	7		

Data are reported as numbers and percentages. *P<0.05, Pearson's chi-squared test. **Missing data were excluded from the analysis; BMI: body mass index; WC: waist circumference; CR: cardiovascular risk; TG: triglyceride; TC: total cholesterol; LDL-c: low-density lipoprotein; HDL-c: high-density lipoprotein; FG: fasting glycemia; FI: fasting insulinemia; HOMA-IR: homeostatic model assessment of insulin resistance; DMT2: type 2 diabetes mellitus; QM: chemerin; F: female; M: male; ‡according to WHO, 2008; CI: Confidence interval; OR: Odds ratio.

and the TT genotype. TT homozygous individuals presented two other high-chance gradients of note that attracted attention with altered TG and HDL-c levels.

Table 3. Genotype frequency of polymorphisms and allele frequency of the APOE haplotype (rs429358+rs7412), by groups of hypertensive and normotensive individuals, in the study population

Gene/rs ID/Genotypes†	Hypertension			OR (95% CI)	P*
	No (n=294)	Yes (n=221)	Total (n=515)		
PPARG/rs1801282					
CC	289 (98.3)	213 (96.4)	502 (97.5)	1.0	
CG	5 (1.7)	8 (3.6)	13 (2.5)	2.17 (0.70-6.72)	0.169
GG	-	-	-	-	
RARRES2/rs4721					
GG	71 (24.1)	40 (18.1)	111 (21.6)	1.0	
GT	151 (51.4)	103 (46.6)	254 (49.3)	1.21 (0.76-1.92)	0.416
TT	72 (24.5)	78 (35.3)	150 (29.1)	1.92 (1.20-3.20)	0.010
RARRES2/rs17173608					
TT	208 (70.7)	161 (72.9)	369 (71.7)	1.0	
GT	75 (25.6)	52 (23.5)	127 (24.7)	0.89 (0.59-1.34)	0.597
GG	11 (3.7)	8 (3.6)	19 (3.6)	0.93 (0.36-2.39)	0.895
NOS3/rs1799983					
GG	157 (53.4)	110 (49.8)	267 (51.8)	1.0	
GT	114 (38.8)	97 (43.9)	211 (41.0)	1.21 (0.84-1.74)	0.297
TT	23 (7.8)	14 (6.3)	37 (7.2)	0.86 (0.42-1.76)	0.696
GNB3/rs5443					
CC	35 (11.9)	28 (12.7)	63 (12.2)	1.0	
CT	137 (46.6)	118 (53.4)	255 (49.5)	1.07 (0.61-1.87)	0.794
TT	122 (41.5)	75 (33.9)	197 (38.3)	0.76 (0.43-1.36)	0.368
APOB/rs693					
GG	170 (57.8)	136 (61.5)	306 (59.4)	1.0	
AG	97 (33.0)	75 (33.9)	172 (33.4)	0.96 (0.66-1.40)	0.859
AA	27 (9.2)	10 (4.5)	37 (7.2)	0.46 (0.21-0.98)	0.042
Alleles *APOE*/rs429358+rs7412					
ε2	3 (1.0)	1 (0.5)	4 (0.8)	1.0	
ε3	226 (76.9)	166 (75.1)	392 (76.1)	1.07 (0.58-1.96)	0.813
ε4	65 (22.1)	54 (24.4)	119 (23.1)	0.44 (0.04-4.29)	0.471
Genotype *APOE*/rs429358+rs7412					
E2/2	3 (1.0)	1 (0.5)	4 (0.8)	1.0	
E2/3	17 (5.8)	20 (9.0)	37 (7.2)	3.52 (0.33-37.14)	0.270
E2/4	209 (71.1)	146 (66.1)	355 (68.9)	2.09 (0.21-20.34)	0.514
E3/3	9 (3.1)	1 (0.5)	10 (1.9)	0.33 (0.01-7.13)	0.468
E3/4	51 (17.3)	50 (22.6)	101 (19.6)	2.94 (0.29-29.2)	0.336
E4/4	5 (1.7)	3 (1.4)	8 (1.6)	1.8 (0.12-26.19)	0.665
AGT/rs699					
GG	128 (43.5)	104 (47.1)	232 (45.0)	1.0	
AG	139 (47.3)	91 (41.2)	230 (44.7)	0.80 (0.55-1.16)	0.253
AA	27 (9.2)	26 (11.8)	53 (10.3)	1.18 (0.65-2.15)	0.577

Data are reported as numbers and percentages; *P<0.05, Pearson's chi-squared test; †The ancestral allele in homozygosis was taken as the reference group with the exception of the *APOE* haplotype rs429358 and rs7412, where the reference was the ε2 allele; CI: confidence interval; OR: odds ratio.

Regarding age, elderly individuals and TT homozygotes also had one of the highest chances of having AH (OR =9.96; 95% CI: 3.83-25.9; P<0.0001, data not shown in the table), which

Table 4. Cardiovascular risk factors independently associated with arterial hypertension in the study population

Variable	Crude OR (95% CI)	P	Adjusted OR* (95% CI)	P
Age group				
≥60 years	6.06 (3.92-9.36)	<0.0001	6.74 (4.06-11.20)	<0.0001
Alcohol consumption				
Dependence	1.85 (0.99-3.46)	0.050	3.84 (1.86-7.92)	<0.0001
Smoking				
Smoker	2.08 (1.44-3.02)	<0.0001	1.74 (1.11-2.72)	0.014
BMI				
≥25 kg/m^2	1.63 (1.14-2.34)	0.007	1.74 (1.10-2.75)	0.017
HDL-c				
<40 mg/dL (M)	5.65 (1.86-17.2)	0.001	6.22 (1.54-25.2)	0.010
<50 mg/dL (F)				
TG				
≥150 mg/dL	2.70 (1.80-4.10)	<0.0001	1.98 (1.21-3.26)	0.007
DMT2				
Yes	6.46 (3.16-13.2)	<0.0001	3.68 (1.63-8.30)	0.002
HOMA-IR				
≥2.71	3.14 (1.87-5.27)	<0.0001	2.40 (1.24-4.65)	0.009
rs4721†				
GT	1.21 (0.76-1.92)	0.416	1.20 (0.70-2.00)	0.531
TT	1.92 (1.20-3.20)	0.010	2.00 (1.11-3.70)	0.022

*P<0.05, adjusted by sex, age, HOMA-IR, TG, and HDL-c by binary logistic regression; †homozygous genotype for the ancestral allele (GG): reference group; CI: confidence interval; OR: odds ratio; BMI: body mass index; HDL-c: high-density lipoprotein; F: female; M: male; TG: triglyceride; DMT2: type 2 diabetes mellitus; HOMA-IR: homeostatic model for assessing insulin resistance.

Table 5. Adjusted bivariate risk association for rs4721 polymorphism genotypes, laboratory parameters, and diabetes with arterial hypertension in the study population

GN	HDL-c†	OR (95% CI)	P*	TG‡	OR (95% CI)	P*	DMT2§	OR (95% CI)	P*
GG	Normal	1.0		Normal	1.0		Normal	1.0	
GT	Normal	1.16 (0.66-2.04)	0.599	Normal	1.46 (0.78-2.75)	0.235	Normal	1.21 (0.68-2.14)	0.499
TT	Normal	1.86 (0.99-3.49)	0.052	Normal	1.94 (0.96-3.91)	0.063	Normal	1.98 (1.06-3.71)	0.032
GG	Altered	2.66 (0.50-14.0)	0.247	Altered	3.07 (1.05-8.92)	0.039	Altered	4.92 (1.05-22.8)	0.042
GT	Altered	2.92 (0.93-9.21)	0.066	Altered	1.79 (0.81-3.96)	0.147	Altered	3.31 (0.99-11.1)	0.052
TT	Altered	8.20 (2.19-30.6)	0.002	Altered	6.26 (2.31-16.9)	<0.0001	Altered	9.70 (1.73-54.2)	0.010

*P<0.05, binary logistic regression adjusted for age group, alcohol consumption, smoking, BMI, TG, DMT2, and HOMA-IR; GN: genotype; CI: confidence interval; OR: odds ratio; HDL-c: high-density lipoprotein; TG: triglyceride; DMT2: type 2 diabetes mellitus. †reference group (Normal) ≥40 mg/dL in men and ≥50 mg/dL in women; risk group (Altered) <40 mg/dL in men and <50 mg/dL; ‡reference group (Normal) <150 mg/dL; risk group (Altered) ≥150 mg/dL; §nondiabetic reference group (Normal); diabetic (Altered) risk group.

may indicate that the detection of children and young people with a TT genetic profile may prove to be an important tool for detecting groups that are susceptible to AH and require more attention.

As rs4721 polymorphism has an allele frequency that varies between populations, the T variant allele (62%) of this polymorphism is more frequent than the ancestral G allele in Europeans, which is in agreement with a study conducted in Germany (63%) [30]. However, for African populations, the frequency of the variant T allele was slightly higher at 76% (https://www.ncbi.nlm.nih.gov/snp/rs4721, accessed May 2021). In the present study, the frequency of the T allele was higher (53.8%), which was lower than that in the European and African populations. Among the hypertensives in the present study, the GT (46.6%) and TT (35.3%)

genotypes showed high frequencies, which is in agreement with data from another survey in southeastern Brazil, where the same genotypes (GT: 54.5% and TT: 25.7%) showed high frequencies in patients with hypertension [31]. In the same study, the T allele was also the most frequent (53%), which draws attention to the higher risk that it can confer to the development of AH.

The rs4721 polymorphism (formerly known as rs10278590) is a common variant located on chromosome 7 in the 3' untranslated region (3' UTR) of the *RARRES2* gene. This genetic variation is an attractive target, as it is still poorly understood, and is proposed to affect only gene expression and not the function of the gene product, as it is located within non-coding regions [30]. To date, few studies on cardiovascular risk have evaluated this polymorphism as a target; however, its association with the development of visceral fat in non-obese individuals is consistent [28, 30]. Despite little evidence, it is plausible to consider that it may be involved in the genesis of other cardiovascular outcomes through mechanisms linked to visceral adiposities, such as DMT2 and dyslipidemia, which justifies the need to conduct more studies in the future.

Few studies have reported a relationship between AH and rs4721 polymorphism. However, it has already been associated with SBP in a population in Brazil [31]. Further, when the sample was stratified by BMI, only obese individuals continued to show this relationship, in which carriers of the T allele showed the highest blood pressure levels. Müssing et al. (2009) [30] did not observe an association with systolic and diastolic blood pressure, even after adequate adjustment, in either the additive or the dominant models. In the present study, this association persisted even after adjusting BMI, which encouraged further investigation to understand whether obesity is a confounding factor in this relationship.

It is relevant to suggest a direct path that joins the rs4721 polymorphism with AH, taking into account the association mentioned above of this genetic polymorphism with the development of visceral fat in non-obese individuals [30]. The relationship between increased visceral adipose tissue and AH has been well documented since the Framingham study [32] and supported by data showing that a simple 10% reduction in body weight, without other types of intervention, can reduce or even normalize blood pressure in obese people [33]. In a recent review, Leggio et al. (2017) [29] identified the main mechanisms accepted over the years that explain the relationship between adipose tissue and AH: (1) activation of the sympathetic nervous system; (2) hyperinsulinemia; (3) altered adipokine secretion, inflammatory and endocannabinoid markers; (4) changes in renal function, sodium excretion, pressure related to natriuresis, and salt sensitivity; and (5) super stimulation of the renin-angiotensin-aldosterone system.

Given the aspects presented, the influence of the rs4721 polymorphism on visceral adiposity can be an important predisposing factor for cardiovascular risk, and the interaction of its T allele with other risk phenotypes may be an example of a genetic factor modifying the effect of usual risk factors for AH and deserves to be the focus of future studies to deepen the knowledge about the functionality of this polymorphism, mainly in mixed populations. In addition, it is crucial that future studies also consider the interaction of environmental and behavioral aspects, such as physical activity and diet, with this genetic factor.

The strength of the present study is in the evaluation of classical and emerging CVRFs in a population in the southeast region of Brazil, never previously studied, while obtaining a representative sample. However, this study has limitations. One, is the small sample size, which may have limited some results concerning genetic polymorphisms, requiring more studies with larger samples to confirm these possible associations. Other, is the low adherence of the young adult male population (18 to 39 years old) to the study, which may have limited the external validity (inference capacity) for this specific group.

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Disclosure of conflict of interest

None.

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