

Polymorphisms in the renin-angiotensin genes

Prevalence, differences in response to treatment and vascular stiffness in hypertensive patients

Rodgerio T¹, Pereira VS¹, Brandão SAB¹, Helfenstein T¹, Monteiro CMC¹, Fischer SCM¹, Santos AO¹, Pova RMS², Manzoli MTB², Fonseca FAH¹, Izar MCO¹

ABSTRACT

Objectives: The renin-angiotensin-aldosterone system (RAAS) has been implicated in many vascular and non-vascular properties. Polymorphisms in genes regulating RAAS can influence blood pressure response to anti-hypertensive drugs and might affect arterial stiffness. The prevalence of ACE I/D, AT1R 1166AC, and CYP11B2 C-344T polymorphisms were evaluated in hypertension stages 1 and 2 (Hy) and compared with healthy normotensive individuals (NT). We also tested whether these variants could affect lipid peroxidation and pulse-wave velocity (PWV), as well as the response to 12-week treatment with ACE inhibitors or diuretics. **Methods:** We evaluated 94 Hy patients, 56±10 y, 51% men (baseline and 12 wk) and 30 NT, 37±9 y, 27% men. Carotid-radial PWV (m/s) was examined at rest and 5 min after arterial occlusion (PH). Casual systolic (SBP) and diastolic blood pressure (DBP), ABPM, TBARS and genotyping (PCR-RFLP) were performed. **Results:** Baseline blood pressure values were higher in Hy, without differences in TBARS or PWV both at rest and PH. Frequencies of hazardous genotypes in Hy and NT were, respectively 83 and 90%, $p=0.05$ for ACE-ID/DD, 44 and 29%, $p=0.0002$ for AT1R 1166AC/CC, and 71 and 90%, $p=0.02$ for CYP11B2-TC/CC. Treatment of hypertension was followed by a reduction in SBP and DBP, as well as in ABPM, with a trend to higher values for SBP by ABPM at sleep in presence of ACE D allele ($p=0.069$), casual SBP in -344C carriers ($p=0.165$) and in DBP in AT1R C carriers ($p=0.122$). TBARS were marginally reduced by treatment ($p=0.054$), with an effect of the ACE D allele on higher levels ($p=0.039$). PWV at rest tended to be reduced ($p=0.103$) after a 12-w treatment and was improved in PH ($p=0.018$), without effect of the studied polymorphisms. The effect of multiple rare alleles (D/C/C) did not affect any of the studied parameters. **Conclusions:** We demonstrated a higher prevalence of polymorphisms in RAS genes in Hy, with small effects of these variants on blood pressure responses, arterial stiffness and oxidative stress. RAAS multiple pathways may account for these effects.

Key words: Renin-angiotensin-aldosterone system; Angiotensin-converting enzyme; Anti-hypertensive treatment; Polymorphisms; Pulse-wave velocity

¹Lipids, Atherosclerosis and Vascular Biology, Cardiology Division, Department of Medicine, Federal University of São Paulo, SP, Brazil.
²Hypertension Section, Cardiology Division, Department of Medicine, Federal University of São Paulo, SP, Brazil

Correspondence: Maria Cristina de Oliveira Izar, Universidade Federal de São Paulo, Setor de Lípidos, Aterosclerose e Biologia Vascular, Disciplina de Cardiologia, Rua Pedro de Toledo 276, 04039-030, São Paulo, SP, Brazil, Phone/Fax: 55-11-5084-8777, E-mail: mcoizar@terra.com.br; mcoizar@cardiol.br

Received: 10.10.2007

Revision accepted: 11.01.2007

Introduction

Hypertension is a common heritable trait resulting of multiple interactions of genetic polymorphisms and environmental factors, with approximately one billion individuals affected worldwide¹. Blood pressure (BP) is maintained by an intricate network of physiological systems, including renal, neuronal, endocrine, and vascular mechanisms, and in fact multiple genes are involved in blood pressure regulation². Identifying and elucidating how each gene plays its role in these complexes and interacting pathways remain to be accomplished. Like most complex diseases, the impact of any individual gene is likely to be small to moderate³. Many candidate genes have been proposed to be associated with hypertension, based on their biological effect on blood pressure and hypertension⁴⁻⁶. Meta-analysis have been published for the most studied genes⁷⁻⁹, and the present work has focused on various renal sodium transport proteins and members of the renin-angiotensin-aldosterone system (RAAS).

The RAAS regulates blood pressure by maintaining vascular tone and the water and sodium balance, being a target for many antihypertensive agents. However, the effects of RAAS blockade can vary in individual hypertensive patients. The relationship between polymorphisms of RAAS genes and hypertension have been previously investigated. Most studies have shown that angiotensinogen (AGT), angiotensin II type 1 receptor (AT1R), angiotensin converting enzyme (ACE), and aldosterone synthase (CYP11B2) gene polymorphisms are associated with high BP levels and risk of hypertension¹⁰⁻¹⁶. In addition, recent studies suggest that genetic variation in these genes may be useful markers for predicting individual differences in response to antihypertensive treatments¹⁷⁻¹⁹.

RAS is implicated in the pathogenesis of atherosclerosis and in the prognosis of coronary artery disease, and is associated with the development of ventricular hypertrophy, myocardial infarction, and remodelling²⁰⁻²⁶. Endothelial dysfunction is a known risk factor for cardiovascular events and can be affected by RAAS activation, also interfering with vascular distensibility^{27,28}. A method that has been used to non-invasively assess vascular compliance is pulse-wave velocity (PWV), also able to provide an indirect measure of endothelial function, when reactive hyperemia is evaluated²⁹.

There are few papers reporting the influence of RAAS gene polymorphisms in the response to different antihypertensive treatment regimens, as well as the effect of these genes in vascular compliance. The aim of our study was to evaluate the prevalence of ACE I/D, AT1R 1166AC and CYP11B2 C-344T polymorphisms in patients with hypertension stages 1 and 2 (Hy) and to compare them with healthy normotensive individuals (NT).

Methods

Study population

For the hypertension group (Hy), 94 individuals with a diagnosis of essential hypertension in stages 1 or 2³⁰, of both sexes (51% men), aging 28-75 years (mean: 56±10 y), in primary prevention of coronary artery disease and naïve of anti-hypertensive treatment were included. These patients were selected among patients from the Hypertension Section, Cardiology Division of the Federal University of Sao Paulo. We also examined 30 healthy controls (NT), 37±9 y, 27% men. Presence of significant liver disease, renal insufficiency, thyroid disorders, cancer or secondary hypertension were the exclusion criteria. Patients with dyslipidemia in use of lipid-lowering drugs, taking antioxidants, and with any known intolerance to the study medications were also excluded. No current smokers were enrolled in both groups.

The protocol was in agreement with the Declaration of Helsinki, and was approved by the local Ethics Committee. All eligible subjects provided written informed consent.

Study design

Subjects were consecutively enrolled in an outpatient setting. After evaluation of eligibility criteria, all participants underwent routine medical examination. Baseline demographics and conventional risk factors for CHD³¹ were documented. Casual systolic (SBP) and diastolic blood pressures (DBP), ambulatory blood pressure monitoring (ABPM), pulse-wave velocity (PWV) in resting conditions and after arterial occlusion were recorded. All participants received a NCEP/ATP III dietary counseling and 12-h fasting blood samples were collected at baseline and 12 wk.

For the control group, a single clinical visit was performed with assessment of PWV. Fasting blood samples were also collected.

Blood pressure measurements

Blood pressures were measured with a standard mercury sphygmomanometer (mm Hg) 5 min after subjects were resting in the sitting position. Systolic and diastolic blood pressures were calculated by averaging two or more measurements differing by no more than 5 mm Hg from each other, obtained 1 to 2 minutes apart³⁰.

Pulse wave velocity

Arterial stiffness was assessed by crPWV with an automated system by using the Complior[®] device as previously described²⁹. This technique is based on the principle that the pressure wave generated by ventricular ejection is propagated into the arterial tree at a speed determined

by the geometric and elastic properties of the arterial wall and the characteristics of the fluid^{28,29}. PWV was determined by a skilled observer who was unaware of the patient's condition. All recordings were obtained from the right side of the patient who was in the supine position and in fasting state, and after a ten-minute period of rest to be sure that the patient was in a steady hemodynamic condition. The carotid and radial arteries were identified by palpation. Surface carotid-radial distance was determined by simple direct measurement of the distance between two probes (m), by using a non-stretching measuring tape. This distance was measured along the right side of the neck, which was slightly extended, and along the course of the brachial and radial arteries, with the arm extended and the shoulder abducted 90°, and informed to the system. Two pressure-sensitive transducers were applied to these arterial sites. Carotid and radial artery pressure waveforms were acquired simultaneously. The time delay between the feet of the two curves was measured directly by the system software, and PWV values were presented in m/s. At least 20 successive pulse waves were recorded for each patient in order to cover a complete respiratory cycle, and 15 of them were chosen to calculate mean crPWV. Post-hyperemic crPWV was also examined, as a noninvasive assessment of the effects of shear stress on vascular distensibility. This method was previously described²⁹ and adapted to our equipment. Briefly, forearm cuff was inflated to suprasystolic pressure for 5 min, and post-hyperemic crPWV was determined 1 min after cuff release, at the expected maximal endothelium-dependent vasodilation (forearm hyperemia). Accuracy and reproducibility of crPWV determinations obtained by using the Complior® were analyzed, with intra-observer repeatability coefficients of 0.84 and 0.90 for crPWV at rest and post-hyperemia, respectively.

Lipid peroxidation

Lipid peroxidation was assessed by measuring thiobarbituric acid-reactive substances (TBARS) as previously described³².

Genotyping

Blood samples for genetic studies were collected from Hy and NT subjects. DNA was extracted of leucocytes from peripheral blood with the GFX™ column (Genomic Blood DNA Purification kit, Amersham Biosciences, USA). Genotypes were determined through the use of polymerase chain reaction amplification of leukocyte DNA, with published primers, restriction enzymes and conditions used for the ACE I/D³³, AT1R 1166AC³⁴, and CYP11B2 C-344T³⁵ polymorphisms. Products were resolved on 1.0

% agarose gel and genotypes were compared with positive and negative controls, with discrepancies resolved by repeat genotyping.

Treatment

After clinical evaluation and collection of baseline procedures, patients in Hy group were randomly assigned to receive perindopril 4-8 mg (ACE-inhibitor group), hydrochlorothiazide 25 mg or indapamide 1.5 mg (diuretic group), or these thiazides plus perindopril 4 mg (combined group) for 12 weeks. For the purpose of this study, genotypes were compared in all treated patients, regardless of treatment assignment, and in controls.

Statistical analysis

All data were analyzed using the statistical software SPSS 11.5 for Windows platform. Unless otherwise stated, data are presented as means \pm SEM. Gene and allele frequencies, deviations from Hardy-Weinberg equilibrium, as well as categorical variables, were tested by the chi-square or Fisher's exact test. The repeatability of crPWV measurements was established by the paired two-tailed Student's t test. Correlation coefficients were used to compare resting and PH-PWV. At baseline, continuous variables were compared by the unpaired Student's t-test between Hy and NT groups.

The primary assessment included changes in parameters of blood pressure, PWV, and oxidative stress, from baseline (within groups analysis) and between genotypes (between groups analysis) using the General Linear Model (GLM) for repeated measures. Because rare genotypes did not allow for individual analysis, they were grouped with heterozygous genotypes for this purpose. All tests were 2-tailed, and a p value < 0.05 was defined for statistical significance.

Results

Baseline characteristics of study population and the effects of treatment are presented in Table I. Patients with Hy were older, predominantly males, who presented higher BMI and waist circumference as compared with NT. As expected, systolic and diastolic blood pressure values were higher in Hy than in NT ($p < 0.0001$). No differences among groups were found between values obtained for TBARS and PWV obtained at rest and after arterial occlusion. There was a reduction in PWV values after arterial occlusion as compared with those obtained at rest both at baseline (9.5 ± 1.5 vs. 9.0 ± 1.6 m/s) and after a 12-week treatment of hypertension (9.2 ± 1.7 vs. 8.5 ± 1.7), suggesting an improvement of vascular distensibility mediated by

shear stress, which can be translated in endothelial-dependent vasodilation. Post-hyperemic PWV was found to be highly correlated with PWV at resting conditions both at baseline ($r=0.605$, $p<0.0001$), after 12 weeks ($r=0.536$, $p<0.0001$), as well as in controls ($r=0.687$, $p<0.0001$) (data not shown).

Twelve weeks after hypertension treatment a consistent reduction was observed in SBP, DBP, and ABPM measures ($p<0.05$ vs. baseline). Treatment of hypertension also promoted a trend to lower TBARS levels and a reduction in PWV after arterial occlusion, reflecting improvement in oxidative stress and vascular compliance (Table 1).

Genotype distribution

Allele frequencies for each polymorphism in the study population were in Hardy-Weinberg equilibrium, except for the AT1R 1166AC polymorphism in the Hy group. Frequencies of hazardous genotypes in Hy and NT were, respectively 83 and 90% ($p=0.05$) for ACE-ID/DD, 44 and 29% ($p=0.0002$) for AT1R-AC/CC, and 71 and 90% ($p=0.02$) for CYP11B2-TC/CC. A higher prevalence of the rare alleles D (ACE/I/D), T (ATR1/1166A-C), and T (CYP11B2/-344C-T) was found in Hy patients as compared with NT. Gene and allele frequencies for the studied polymorphisms are presented in Table 2.

Table 1. Baseline characteristics of study population and effects of 12-week treatment.

Group	Hy (n=94) baseline	Hy (n=94) treatment	p-value vs. baseline	NT (n=30)	p-value vs. Hy
Males (%) ¹	48 (51)	–	–	8(27)	<0.0001
Age (y) ³	56.1±9.9	–	–	37.1±9.3	<0.0001
BMI (kg/m ²) ^{2,3}	28.6±4.6	28.7± 4.8	0.856	25.1±5.1	0.001
WC (cm) ^{2,3}	95.5±12.1	94.3±11.7	0.008	87.6±14.8	0.004
SBP (mm Hg) ^{2,3}	153.7±12.4	136.7±16.1	<0.0001	114.7±8.9	<0.0001
DBP (mm Hg) ^{2,3}	92.1±7.0	84.5±7.6	<0.0001	74.9±12.4	<0.0001
TBARS (nmol/L) ^{2,3}	1.5±0.8	1.3±0.6	0.054	1.75±0.9	0.308
PWV-B (m/s) ^{2,3}	9.5±1.5	9.2±1.7	0.103	9.4±1.4	0.59
PWV-PH(m/s) ^{2,3}	9.0±1.6	8.5±1.7	0.018	8.8±1.4	0.42
ABPMS (mm Hg) ²	131±15	126±14	<0.0001	–	–
ABPMD (mm Hg) ²	81±11	79±11	<0.0001	–	–
ABPMSV (mm Hg) ²	134±16	129±14	<0.0001	–	–
ABPMDV (mm Hg) ²	84±11	82±11	<0.0001	–	–
ABPMSSI (mm Hg) ²	126±16	121±15	<0.0001	–	–
ABPMSDI (mm Hg) ²	77±12	75±12	0.017	–	–

PWV, pulse-wave velocity; B, baseline; PH, post-hyperemia; ABPM, ambulatory blood pressure monitoring; SBP, systolic blood pressure; D, diastolic blood pressure; V, vigil; SI, sleep. $p<0.05$; ¹chi-square, Hy vs. NT; ²baseline vs. 12-weeks, paired Student's t-test; ³Hy vs. NT, unpaired Student's t-test.

Table 2. Distribution of allelic and genotypic frequencies in the study population.

Group	Hy %	Allele frequencies	NT %	Allele frequencies	P for genotypes	P for alleles	
ACE	I/I	17%	10%				
	I/D	49%	I: 0.42	38%	I: 0.29	0.38	0.05
	D/D	34%	D: 0.58	52%	D: 0.71		
AT1R*	AA	56%		71%			
	AC	15%	A: 0.63	29%	A: 0.86	0.06	0.0002
	CC	29%	C: 0.37	0%	C: 0.14		
CYP11B2	–344TT	29%		10%			
	–344TC	63%	T: 0.61	71%	T: 0.45	0.09	0.02
	–344CC	8%	C: 0.39	19%	C: 0.55		

Alleles in Hardy-Weinberg equilibrium, except for AT1R polymorphism in Hy group. $p<0.05$; Hy > NT, chi-square or Fisher's exact test.

Table 3. Effects of ACE I/D, AT1R 1166A/C and CYP11B2 -344T/C polymorphisms on blood pressure control, pulse-wave velocity and TBARS

Variable	ACE I/D polymorphism		AT1R 1166AC polymorphism		CYP11B2 -344TC polymorphism		Effects of 0, 1, 2 or 3 rare alleles	
	p x D allele	p x treatment	p x C allele	p x treatment	p x C allele	p x treatment	p x D/C/C alleles	p x treatment
PWV-B	0.804	0.379	0.689	0.293	0.572	0.130	0.235	0.887
PWV-PH	0.861	0.806	0.529	0.071	0.436	0.167	0.191	0.819
SBP	0.281	<0.0001	0.162	<0.0001	0.165	<0.0001	0.551	<0.0001
DBP	0.284	<0.0001	0.122	<0.0001	0.738	<0.0001	0.531	<0.0001
ABPMS-24h	0.609	<0.0001	0.364	<0.0001	0.604	<0.0001	0.710	0.003
ABPMD-24h	0.852	<0.0001	0.802	<0.0001	0.673	<0.0001	0.782	0.005
ABPMSV	0.554	<0.0001	0.298	<0.0001	0.678	<0.0001	0.565	0.001
ABPMDV	0.919	<0.0001	0.773	<0.0001	0.714	<0.0001	0.728	0.001
ABPMSSI	0.069	0.003	0.694	<0.0001	0.730	0.001	0.439	0.113
ABPMDSI	0.396	0.025	0.947	<0.0001	0.932	0.011	0.795	0.291
TBARS*	0.039	0.249	0.129	0.284	0.672	0.018	0.609	0.164

PWV, pulse-wave velocity; B, baseline; PH, post-hyperemia; ABPM, ambulatory blood pressure monitoring; SBP, systolic blood pressure; D, diastolic blood pressure; V, vigil; Sl, sleep. $p < 0.05$; GLM-repeated measures. *ID + DD > II.

Genotype effects on baseline parameters

For the ACE I/D polymorphism no differences were found regarding age, body weight, BMI, waist circumference, SBP and DBP between II vs. ID + DD individuals. However, higher levels of TBARS were observed in those carrying the D allele ($p=0.043$) (data not shown). For the AT1R 1166AC polymorphism, only heart rate values were higher in those carrying the C allele, with no difference for the CYP11B2 variants, or even when multiple rare alleles were taken into account (data not shown).

Genotype and antihypertensive response

The changes in BP response to antihypertensive treat-

ment in relation to genotypes of RAAS gene polymorphisms are shown in Table 3. The presence of at least one D allele conferred a trend for higher SBP values obtained at sleep by ABPM ($p=0.069$), with equal degree of reduction after treatment. The D allele was also associated with higher TBARS values, which decreased in the same proportion after treatment. The rare C allele of the AT1R 1166 gene polymorphism was not associated with differences in blood pressure, as well as the CYP11B2 C-344T gene polymorphism. The effect of treatment of hypertension was compared in presence of 0, 1, 2, or 3 rare alleles (D/C/C), showing no significant differences in regard of blood pressure parameters, PWV, or TBARS (Table 3 and Figs 1-3).

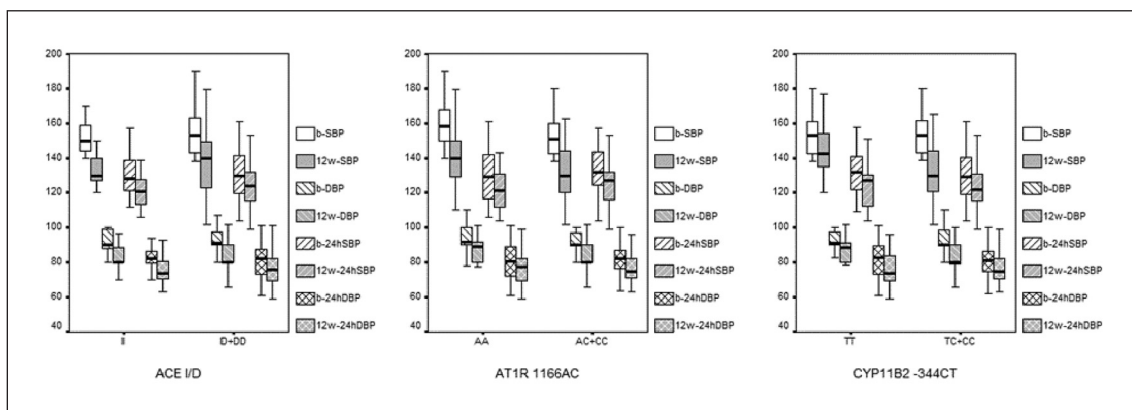


Figure 1. Boxplots showing casual blood pressure measures and 24-h ABPM for ACE I/D, AT1R 1166AC and CYP11B2 -344T/C gene polymorphisms in response to treatment of hypertension. b, baseline; 12w, 12 weeks after treatment; 24h, 24-h blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure.

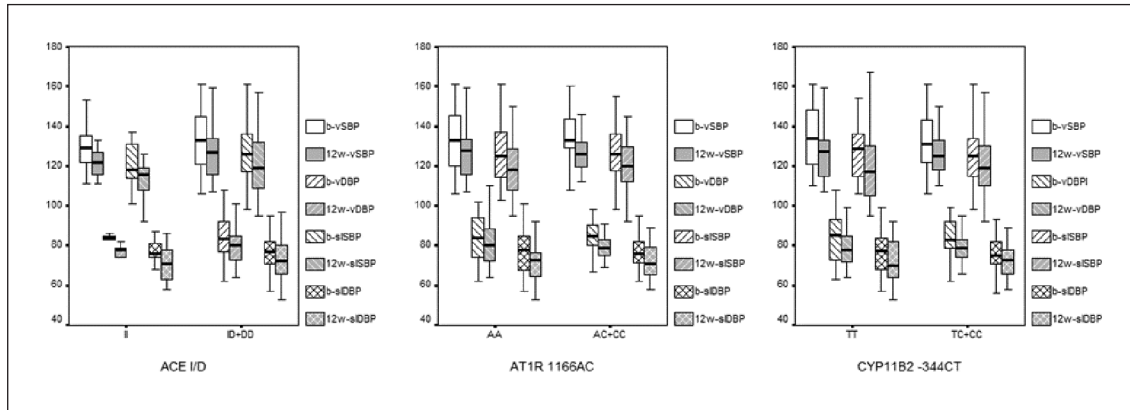


Figure 2. Boxplots showing blood pressure measures obtained by ABPM for ACE I/D, AT1R 1166AC and CYP11B2 -344T/C gene polymorphisms in response to treatment of hypertension. b, baseline; 12w, 12 weeks after treatment; v, vigil; sl, sleep; SBP, systolic blood pressure; DBP, diastolic blood pressure.

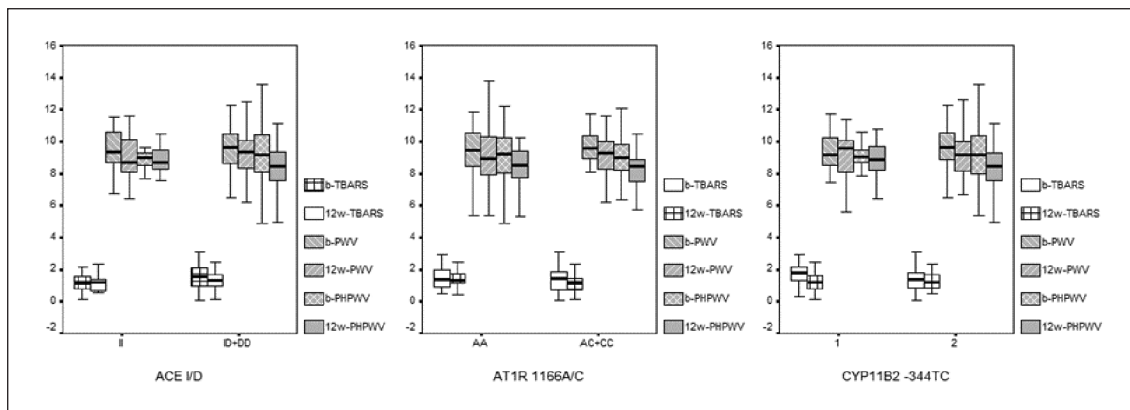


Figure 3. Boxplots showing measures of TBARS and PWV at rest and after arterial occlusion for ACE I/D, AT1R 1166A/C and CYP11B2 -344T/C gene polymorphisms in response to treatment of hypertension. b, baseline; 12w, 12 weeks after treatment, TBARS, thiobarbituric acid-reactive substances; PWV, pulse-wave velocity; PH, post-hyperemia; ID/DD > II; p<0.05, GLM-repeated measures.

Discussion

Our study has demonstrated that polymorphisms in genes that affect the expression of the renin-angiotensin system have distinct prevalences in healthy and hypertensive individuals, with higher prevalence of rare alleles among those with stage 1 or 2 hypertension. However, similar prevalences were observed for the 3 hazardous alleles in both groups.

Treatment of hypertension was accompanied by reduction in casual systolic and diastolic blood pressures, as well as in the measures obtained by ABPM. Pulse-wave velocity after arterial occlusion was ameliorated after treatment, suggesting improvement in vascular distensibility. In our study, a non-invasive and low-cost evaluation of vascular distensibility in resting conditions and after reactive hyperemia in hypertensive and normotensive subjects was also

performed, and a possible correlation of these variables to gene polymorphisms in the RAAS genes was explored.

Genetic polymorphisms did affect blood pressure responses to antihypertensive regimens. Literature information regarding the effects of RAAS polymorphisms and vascular responses is very conflicting. The ACE I/D polymorphism has been evaluated in a meta-analysis and it was not found to contribute to the presence or severity of hypertension³⁶. In another study, an association between premature occurrence of hypertension and ACE I/D variants was found³⁷.

In a study, multiethnic populations were evaluated and no evidence of association between presence of the D allele either in homozygosis or heterozygosis and occurrence of hypertension was found in Americans of Latin or African origin³⁸. In the same study, the AT1R polymorphism

(T variant) increased the risk of hypertension in Americans of African origin, but not in those of Latin origin. Other authors have also demonstrated that ABPM values were associated with the ACE D allele, but not with the AT1R C allele³⁹, with no differences in casual blood pressures depending on the polymorphisms. In our study, the D allele was also associated with higher SBP at sleep by ABPM, with the same degree of response to treatment.

In secondary prevention, the ACE I/D and CYP11B2 polymorphisms were associated with the first event of MI in young patients, but not to disease progression after a long-term follow-up period⁴⁰.

The aldosterone synthase CYP11B2 polymorphism was evaluated in a meta-analysis that included 19 studies presenting heterogeneity, and the authors have shown that homozygous individuals for the -344C allele had a risk of hypertension 17% lower when compared to those for the T allele⁴¹.

In regard of PWV, there are few papers in the literature relating genetic polymorphisms and hypertension. Safar and coworkers⁴² studied the CYP11B2 (-344T variant) in untreated hypertensive subjects and assessed their aortic PWV and have found that the C allele was associated with increased heart rate, reduced stroke volume, and increased PWV with ageing, which modulated the cardiovascular phenotype. In other study, the -344C allele has been implicated in vascular distensibility, and this effect was modulated by salt intake⁴³.

High blood pressure is a major risk factor for coronary artery disease and the alterations in vascular compliance and distensibility, as well as polymorphisms in genes related with RAAS may contribute to cardiovascular risk. In a study carried out in the United Kingdom, the AT1R 1166C allele was associated with a higher cardiovascular risk, regardless of blood pressure levels⁴⁴. However, the AT2R polymorphism, not assessed in our study, has conferred protection. Other recent study showed a linear relationship between the number of rare alleles of many RAAS polymorphisms and increase in carotid IMT⁴⁵.

These data are important for understanding the distinct role of each component of RAAS and their implications in the pathogenesis of arterial hypertension, and the risk of developing coronary artery disease. RAAS may be a modulator in the pathogenesis of cardiovascular disease by a balance of expression of genetic and environmental factors, which could be protective or deleterious.

In summary, this study demonstrated a higher prevalence of polymorphisms in the RAAS genes in hypertensive subjects, with small effects of these variants on blood pressure responses, arterial stiffness, and oxidative stress. A lifetime exposure of subjects to these genetic markers

may have an important role in the risk of hypertension and cardiovascular disease, and may have impact on preventive strategies.

Acknowledgements: This study has been supported by a grant (04/00324-1) from FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo). Tatiane Rodrigo was a recipient of a grant from CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico).

References

1. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL Jr, et al. The seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report. *JAMA* 2003;289(19):2560–72. Erratum in *JAMA* 2003;290(2):197.
2. Doris PA. Hypertension genetics, single nucleotide polymorphisms, and the common disease: common variant hypothesis. *Hypertension* 2002;39(2 pt 2):323–31.
3. Boerwinkle E, Hixson JE, Hanis CL. Peeking under the peaks: following up genome-wide linkage analyses. *Circulation* 2000(16); 102:1877–8.
4. Luft FC. Present status of genetic mechanisms in hypertension. *Med Clin North Am* 2004;88(1):1–18, vii.
5. Turner ST, Boerwinkle E. Genetics of blood pressure, hypertensive complications, and antihypertensive drug responses. *Pharmacogenomics* 2003;4(1):53–65.
6. Angeli F, Verdecchia P, Reboldi GP, Gattobigio R, Bentivoglio M, Staessen JA, Porcellati C. Meta-analysis of effectiveness or lack thereof of angiotensin-converting enzyme inhibitors for prevention of heart failure in patients with systemic hypertension. *Am J Cardiol* 2004;93(2):240–3.
7. Sethi AA, Nordestgaard BG, Tybjaerg-Hansen A. Angiotensinogen gene polymorphism, plasma angiotensinogen, and risk of hypertension and ischemic heart disease: a meta-analysis. *Arterioscler Thromb Vasc Biol* 2003;23(7):1269–75.
8. Province MA, Boerwinkle E, Chakravarti A, Cooper R, Fornage M, Leppert M, et al. Lack of association of the angiotensinogen-6 polymorphism with blood pressure levels in the comprehensive NHLBI Family Blood Pressure Program: National Heart, Lung, and Blood Institute. *J Hypertens*. 2000;18(7):867–76.
9. Kato N, Sugiyama T, Morita H, Kurihara H, Yamori Y, Yazaki Y. Angiotensinogen gene and essential hypertension in the Japanese: extensive association study and meta-analysis on six reported studies. *J Hypertens*. 1999;17(6):757–63.
10. Wu SJ, Chiang FT, Chen WJ, Liu PH, Hsu KL, Hwang JJ, et al. Three single-nucleotide polymorphisms of the angiotensinogen gene and susceptibility to hypertension: single locus genotype vs. haplotype analysis. *Physiol Genomics* 2004;17(2):79–86.
11. Brand-Herrmann SM, Kopke K, Reichenberger F, Schmidt-Petersen K, Reineke T, Paul M, et al. Angiotensinogen promoter haplotypes are associated with blood pressure in untreated hypertensives. *J Hypertens* 2004;22(7):1289–97.
12. Jiang Z, Zhao W, Yu F, Xu G. Association of angiotensin II type 1 receptor gene polymorphism with essential hypertension. *Chin Med J* 2001;114(12):1249–51.
13. Agachan B, Isbir T, Yilmaz H, Akoglu E. Angiotensin converting enzyme I/D, angiotensinogen T174M-M235T and angiotensin II type1 receptor A1166C gene polymorphisms in Turkish hypertensive patients. *Exp Mol Med* 2003;35(6):545–9.

14. Sugimoto K, Katsuya T, Ohkubo T, Hozawa A, Yamamoto K, Matsuo A, et al. Association between angiotensin II type 1 receptor gene polymorphism and essential hypertension: the Ohasama Study. *Hypertens Res* 2004;27(8): 551-6.
15. Xu Q, Wang YH, Tong WJ, Gu ML, Wu G, Buren B, et al. Interaction and relationship between angiotensin converting enzyme gene and environmental factors predisposing to essential hypertension in Mongolian population of China. *Biomed Environ Sci* 2004;17(2):177-86.
16. Brand E, Chatelain N, Mulatero P, Fery I, Curnow K, Jeunemaitre X, et al. Structural analysis and evaluation of the aldosterone synthase gene in hypertension. *Hypertension* 1998;32(2):198-204.
17. Kurland L, Liljedahl U, Karlsson J, Kahan T, Malmqvist K, Melhus H, et al. Angiotensinogen gene polymorphisms: relationship to blood pressure response to antihypertensive treatment. Results from the Swedish Irbesartan Left Ventricular Hypertrophy Investigation vs Atenolol (SILVHIA) trial. *Am J Hypertens* 2004;17(1):8-13.
18. Kurland L, Melhus H, Karlsson J, Kahan T, Malmqvist K, Ohman P, et al. Aldosterone synthase (CYP11B2) -344 C/T polymorphism is related to antihypertensive response: results from the Swedish Irbesartan Left Ventricular Hypertrophy Investigation versus Atenolol (SILVHIA) trial. *Am J Hypertens* 2002;15(5):387-93.
19. Woodiwiss AJ, Nkeh B, Samani NJ, Badenhorst D, Maseko M, Tiago AD, et al. Functional variants of the angiotensinogen gene determine antihypertensive responses to angiotensin-converting enzyme inhibitors in subjects of African origin. *J Hypertens* 2006;24(6):1057-64.
20. Frazier L, Turner ST, Schwartz GL, Chapman AB, Boerwinkle E. Multi-locus effects of the renin-angiotensin-aldosterone system genes on blood pressure response to a thiazide diuretic. *Pharmacogenomics J* 2004; 4(1):17-23.
21. Schuh JR, Blehm DJ, Friedrich GE, McMahon EG, Blaine EH.. Differential effects of renin-angiotensin system blockade on atherogenesis in cholesterol-fed rabbits. *J Clin Invest* 1993;91(4):1453-8.
22. SOLVD Investigators. Effect of enalapril on survival in patients with reduced left ventricular ejection fractions and congestive heart failure. *N Engl J Med* 1991;325(5):293-302.
23. Chobanian AV, Haudenschild CC, Nickerson C, Drago R. Antiatherogenic effect of captopril in the Watanabe heritable hyperlipidemic rabbit. *Hypertension* 1990;15(3):327-31.
24. Pfeffer MA, Braunwald E, Moyé LA, Basta L, Brown EJ Jr, Cuddy TE, et al.. Effects of captopril on mortality and morbidity in patients with left ventricular dysfunction after myocardial infarction. Results of the survival and ventricular enlargement trial. The SAVE investigators. *N Engl J Med* 1992;327(10):669-77.
25. Katsuya T, Koike G, Yee TW, Sharpe N, Jackson R, Norton R, et al. Association of angiotensinogen gene T235 variant with increased risk of coronary heart disease. *Lancet* 1995;345(8965):1600-3.
26. Tiret L, Bonnardeaux A, Poirier O, Ricard S, Marques-Vidal P, Evans A, et al. Synergistic effects of angiotensin-converting enzyme and angiotensin II type 1 receptor DD genotype of the gene polymorphisms on risk of myocardial infarction. *Lancet* 1994;344(8927):910-3.
27. Blacher J, Asmar R, Djane S, London GM, Safar ME. Aortic pulse wave velocity as a marker of cardiovascular risk in hypertensive patients. *Hypertension*. 1999 May;33(5):1111-7.
28. Asmar R, Topouchian J, Pannier B, Benetos A, Safar M. Pulse wave velocity as endpoint in large-scale intervention trial. The Complior study. *J Hypertens*. 2001 Apr;19(4):813-8.
29. Dabela MLG, Izar MCO, Relvas WGM, Santos AO, Helfenstein T, Monteiro CMC, et al. Different impact of coronary risk factors on pulse wave velocity. *Int J Atheroscler* 2007;2(1):75-81.
30. V Brazilian Guidelines for Arterial Hypertension. *Int J Atheroscler* 2006;1(2):71-123.
31. IV Diretriz Brasileira sobre Dislipidemias e Prevenção da Aterosclerose. Departamento de Aterosclerose da Sociedade Brasileira de Cardiologia. *Arq Bras Cardiol* 2007;88 (Suppl 1):1-19.
32. Ohkawa, H; Ohishi, N; Yagi, K. Assay for lipid peroxides in animal tissues by Thiobarbituric Acid Reaction. *Analytical Biochemistry* 1979; 95(1):351-358.
33. Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. *J Clin Invest* 1990; 86 (4):1343-1346.
34. Bonnardeaux A, Davies E, Jeunemaitre X, Féry I, Charru A, Clauser E, et al. Angiotensin II type-1 receptor gene polymorphisms in human essential hypertension. *Hypertension* 1994;24(1):63-9.
35. White PC, Slutsker L. Haplotype analysis of CYP11B2. *Endocrinol Res* 1995;21(1-2):437-42.
36. Mondry A, Loh M, Liu P, Zhu AL, Nagel M. Polymorphisms of the insertion/deletion ACE and M235T AGT genes and hypertension: surprising findings and meta-analysis of data. *BMC Nephrol* 2005;6(1):1.
37. Barbalić M, Skarić-Jurić T, Cambien F, Barbaux S, Poirier O, Turek S, et al. Gene polymorphisms of the renin-angiotensin system and early development of hypertension. *Am J Hypertens* 2006;19(8):837-42.
38. Henderson SO, Haiman CA, Mack W. Multiple polymorphisms in the rennin-angiotensin-aldosterone system (ACE, CYP11B2, AGTR1) and their contribution to hypertension in African Americans and Latinos in the multiethnic cohort. *Am J Med Sci* 2004;328(5):266-73.
39. Spiering W, Zwaan IM, Kroon AA, de Leeuw PW. Genetic influences on 24 h blood pressure profiles in a hypertensive population: role of the angiotensin-converting enzyme insertion/deletion and angiotensin II type 1 receptor A1166C gene polymorphisms. *Blood Press Monit* 2005;10(3):135-41.
40. Franco E, Palumbo L, Crobu F, Anselmino M, Frea S, Matullo G, et al. Renin-angiotensin-aldosterone system polymorphisms: a role or a hole in occurrence and long-term prognosis of acute myocardial infarction at young age. *BMC Med Genet* 2007;8(1):27.
41. Sookoian S, Gianotti TF, González CD, Pirola CJ. Association of the C-344T aldosterone synthase gene variant with essential hypertension: a meta-analysis. *J Hypertens* 2007;25(1):37-9.
42. Safar ME, Cattani V, Lacolley P, Nzietchueng R, Labat C, Lajemi M, et al. Aldosterone synthase gene polymorphism, stroke volume and age-related changes in aortic pulse wave velocity in subjects with hypertension. *J Hypertens* 2005;23(6):1159-66.
43. Wojciechowska W, Staessen JA, Stolarz K, Nawrot T, Filipovsk_ J, Tichá M, et al. European Project on Genes in Hypertension (EP-OGH) Investigators. Association of peripheral and central arterial wave reflections with the CYP11B2 -344C allele and sodium excretion. *J Hypertens* 2004;22(12):2311-9.
44. Jones A, Dhamrait SS, Payne JR, Hawe E, Li P, Toor IS, et al. Genetic variants of angiotensin II receptors and cardiovascular risk in hypertension. *Hypertension* 2003;42(4):500-6.
45. Yazdanpanah M, Aulchenko YS, Hofman A, Janssen JA, Sayed-Tabatabaei FA, van Schaik RH, et al. Effects of the renin-angiotensin system genes and salt sensitivity genes on blood pressure and atherosclerosis in the total population and patients with type 2 diabetes. *Diabetes* 2007(7):1905-12.