ABSTRACT

Background: Matrix metalloproteinases are enzymes involved in cardiovascular (CV) remodeling and hypertension-mediated target organ damage (TOD). Genetic polymorphisms in matrix metalloproteinase 2 (MMP-2) gene [-1575G/A (rs243866); -1306C/T (rs243865); and -735C/T (rs2285053)] are associated with several CV conditions, however the relationship between MMP-2 polymorphisms and resistant hypertension (RH) is unknown. We evaluated whether these genetic single nucleotide polymorphisms (SNPs) in MMP-2 gene are associated with 1) MMP-2 and TIMP-2 levels in RH and mild to moderate hypertensive (HT) subjects, 2) left ventricular hypertrophy (LVH) and arterial stiffness and 3) the presence of RH. Methods: One hundred and nineteen RH and 136 HT subjects were included in this cross-sectional study. Genotypes were determined by real-time PCR using TaqMan probes. Haplotypes were estimated using Bayesian method. Results: The levels of MMP-2 and TIMP-2 were similar among genotypes and haplotypes for the three studied polymorphisms in HT and RH groups. RH showed higher frequency for GCC haplotype (-1575G/A, -1306C/T and -735C/T, respectively) compared to HT (0.77 *vs.* 0.64, respectively), while HT had a higher frequency of GCT (0.17 *vs*. 0.09; p=0.003) and ATC (0.19 *vs*. 0.13; p=0.003, respectively) compared to RH. GCC haplotype was associated to RH apart from potential confounders (odds ratio (OR) =2.09; 95% confidence interval (CI)=1.20-3.64; p=0.01). In addition, CC genotype (OR=2.93; 95% CI= 1.22–7.01; p=0.02) or C allele (OR=2.81; 95% CI=1.26–6.31; p=0.01) for -735C/T polymorphism were independently associated with RH. GCT haplotype was associated with reduced probability of having RH (OR=0.35; 95% CI=0.16–0.79; p=0.01). Finally, no relationship was found between studied MMP-2 SNPs and left ventricular hypertrophy and arterial stiffness in both groups. Conclusion: C allele, CC genotype and GCC

haplotype for -735C/T polymorphism in MMP-2 gene were associated with high probability of having RH, while GCT haplotype showed reduced probability of RH.

Keywords: Refractory Hypertension, tissue inhibitor of metalloproteinases 2, genetic polymorphism, target organ damage.

Highlights:

- MMP-2 polymorphisms [-1575G/A (rs243866); -1306C/T (rs243865); and -735C/T (rs2285053)] were studied to analyze its association with resistant hypertension and target organ damage.
- In -735C/T MMP-2 polymorphism, the C allele and CC genotype were associated with the presence of resistant hypertension.
- Resistant hypertensive group had higher frequency of GCC haplotype, while mild to moderate hypertensive group had higher frequency of GCT and ATC haplotypes.
- GCC haplotype was associated with resistant hypertension, while GCT haplotype was associated with lower probability of having resistant hypertension.

Title: Matrix metalloproteinase-2 -735 C/T polymorphism is associated with resistant hypertension in a specialized outpatient clinic in Brazil.

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1. INTRODUCTION

Resistant hypertension (RH) has been associated with higher cardiovascular (CV) risk [1] and increased prevalence of target organ damage (TOD) compared to controlled hypertension [2, 3]. Several studies have suggested the involvement of metalloproteinases in CV remodeling contributing to TOD development [4, 5].

Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases associated to extracellular matrix (ECM) reorganization. One of the most studied MMPs, the matrix metalloproteinase 2 (MMP-2) is associated with degradation of several vascular and cardiac proteins including type IV collagen, interstitial collagen types I, II and III, troponin I, and myosin light chain and poly(ADP-ribose) polymerase contributing to cardiac dysfunction [6]. In addition, MMP-2 has been associated with hypertension and vascular injury [4, 5]. MMP-2 is regulated at the transcriptional and posttranslational levels. It is activated by cleavage of zymogens and by interaction with endogenous inhibitor, known as tissue inhibitor of metalloproteinases 2 (TIMP-2) [6]. Genetic polymorphisms in the MMP-2 gene [-1575G/A (rs243866); -1306C/T (rs243865); and -735C/T (rs2285053)] have been implicated in the pathogenesis of several CV conditions, such as myocardial infarction [7], stroke [8], left ventricular remodeling in hypertension [9] and coronary disease [10]. However, the relationship between polymorphisms in MMP-2 gene and resistant hypertension and TOD in this population remains unexplored. The aims of this study were to evaluate the association of MMP-2 single nucleotide polymorphisms (SNPs) (-1575G/A, rs243866; -1306C/T, rs243865; and -735C/T, rs2285053) with 1) MMP-2 and TIMP-2 levels; 2) left ventricular hypertrophy (LVH) and arterial stiffness in all hypertensive subjects, and 3) the presence of RH.

2. METHODS

2.1. Study Population

One hundred and nineteen RH patients and 136 mild to moderate hypertensive (HT) patients were included in this cross-sectional study. RH patients were regularly followed at the Outpatient Resistant Hypertension Clinic of the University of Campinas (Campinas, Brazil) and at the Hypertension Clinic of Valinhos (Brazil). The diagnostic of RH was performed according to American Heart Association criteria after 6-months of follow-up. RH was defined as blood pressure (BP) that remains above the BP goal (≥140/90 mmHg) despite the use of 3 or more different classes of antihypertensive drugs at optimal doses, or use of 4 or more antihypertensive drugs with controlled BP [11]. In this period we assessed office BP, ambulatory BP monitoring (ABPM) measurements and biochemical exams in all patients. We excluded secondary hypertension (primary hyperaldosteronism, renal artery stenosis and pheochromocytoma), pseudoresistance (white-coat hypertension) and lack of treatment adherence (determined by Morisky questionnaire and pill counts) to identify true resistance to the treatment [11]. Patients with controlled BP taking at least 3 antihypertensive drugs were considered HT. This study was approved by the Research Ethics Committee at the Faculty of Medical Sciences of University of Campinas (Campinas, Brazil) (approval no 188.161/2013) and conducted in compliance with the guidelines of the Declaration of Helsinki. All participants signed an informed written consent form.

2.2. Inclusion and Exclusion Criteria

The inclusion criteria comprise the diagnosis of non-resistant or resistant hypertension; regular follow-up for at least six months; adherence assessment using questionnaires; volunteer participation and informed consent signature.

Patients with medical history or clinical symptoms of heart failure (ejection fraction < 50%); valvular or pericardial heart diseases; presence of cardiomyopathy; diagnosis of coronary and peripheral vascular disease; autoimmune diseases; nephropathy; liver dysfunction; and pregnancy or declared intention to become pregnant were excluded.

2.3. Blood pressure measurements

Office systolic BP (SBP) and diastolic BP (DBP) were evaluated at least three times using a digital BP monitor (OMRON Healthcare Inc., Bannockburn, IL, USA) with a 3-minute interval between the measurements. Patients were monitored approximately at 8:00 am, in a seated position after a 10-minute rest.

ABPM was performed through twenty-four hour BP monitoring by an automatic oscillometric device (Spacelabs 90207, Spacelabs Inc, Redmon, WA, USA). Patients were instructed to keep their normal daily activities and the BP was measured automatically at a 20-min interval during 24-h period.

2.4. Echocardiography

Left ventricular (LV) dimensions were measured using M-mode echocardiography by a cardiovascular ultrasound machine (Siemens Acuson CV70; Munich, Bavaria, Germany), according to the American Society of Echocardiography (ASE) recommendations [12]. Diastolic and systolic left ventricle diameters, interventricular septal and posterior wall thicknesses were measured according to the QRS wave of electrocardiography at the end of diastole. LV mass index (LVMI) was calculated according to the ASE recommendations, dividing LV mass by the body surface. Left ventricular hypertrophy (LVH) was determined by LVMI $\geq$115 g/m^2 for men and $\geq$95 g/m^2 for women.

2.5. Aortic PWV measurement

Pulse wave velocity (PWV) measurements were obtained using Sphygmocor system (Artcor, Sidney, Australia) to estimate arterial stiffness. Pulse waves were obtained transcutaneously using a tonometer at the right common carotid and femoral arteries. The distance covered by the waves was measured directly between the femoral recording site and the supra sternal notch minus the distance from the supra sternal notch to the carotid recording site. PWV was calculated as distance (meters)/Δt (seconds) [13]. Three consecutive readings were collected to determine the PWV average value that was used in analyses. Arterial stiffness was defined as PWV higher than 10 m/s.

2.6. Biochemical measurements

Blood samples were obtained after overnight fasting period by venipuncture. Heparin-anticoagulated blood samples were centrifuged at 5,300 rpm for 10 minutes to obtain plasma samples. EDTA-anticoagulated total blood samples were collected for DNA extraction. The samples were immediately stored at -80ºC until the measurements. MMP-2 and TIMP-2 were measured by enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Inc., Minneapolis, USA) and plasma aldosterone concentration was measured by radioimmunoassay (Immunotech SAS, Marseille, France) according to the manufacturer's instructions. Creatinine clearance (ml/min/1.73 m2) and urine albumin-to-creatinine ratio (mg/g) were measured in urine sample collected during consecutive 24 hours. Urine albumin-to-creatinine ratio (mg/g) was used to evaluate microalbuminuria (values < 30mg/g were classified as normal and values between 30 and 300mg/g as microalbuminuria).

2.7. Determination of genotypes

Genomic DNA was extracted using the Illustra blood genomic Prep Mini Spin Kit (GE Healthcare, Buckinghamshire, UK) from total blood according to the manufacturer's instructions. MMP-2 genotypes of the SNPs -1575 G/A (rs243866), -1306 C/T (rs243865), and -735 C/T (rs2285053) were determined using the TaqMan allelic discrimination assay (assays ID C_3225942_10, C_3225943_10 and C_26734093_20, respectively; Applied Biosystems, Foster City, CA, USA). DNA quality and quantity was checked by NanoDrop spectrophotometer (ThermoScientific, Wilmington, USA). Polymerase chain reaction (PCR) was conducted with 5 ng of DNA following the steps: (i) 10 minutes at 95°C; (ii) 40 cycles of DNA denaturation at 90°C for 15 seconds; and (iii) annealing/extension at 60°C for 1 minute. Fluorescence signals were detected using StepOnePlus Real Time PCR System (Applied Biosystems, Foster City, CA, USA) and analyzed using the StepOne^{TM} software version 2.1.

2.8. Haplotype inference

Haplotypes combinations for the 3 MMP-2 polymorphisms ordered according to their location in the chromosome (-1575G/A (rs243866); -1306C/T (rs243865); and -735C/T (2258053), respectively) were inferred using the Bayesian statistical-based program PHASE (version 2.1; http://www.stat.washington.edu/stephens/software.html) [14]. Three haplotype combinations were found in these populations including the 3 MMP-2 polymorphisms ordered according to their location in the chromosome (-1575G/A (rs243866); -1306C/T (rs243865); and -735C/T (2258053), respectively. The estimated haplotypes were GCC, GCT and ATC. The haplotypes with observed frequencies <1 % in all groups were excluded from subsequent analyses.

2.9. Statistical analysis

Continuous variables were expressed as mean and standard deviation or median and 1st and 3rd quartiles, according to data distribution. Data distribution was assessed by Shapiro–Wilk test. We performed Student's t-test or Mann–Whitney test to compare two groups, while Kruskal–Wallis or analysis of variance (ANOVA) test were performed to compare three groups, followed by Dunns or Bonferroni multiple comparison test. Categorical variables were presented in frequencies and/or percentages and compared by chi-square or Fisher's test. Hardy–Weinberg equilibrium was evaluated by chi-square test. We used the dominant genetic model to test the association of these polymorphisms with MMP-2 levels and clinical features for all three polymorphisms. AA and AG genotypes of the rs243866 (-1575G/A) polymorphism were gathering as one group, denoted by "A carriers" due to the low frequency of the minor allele. We used the same approach with TT genotype in the rs243865 (-1306C/T) polymorphism and for TT genotype in rs2285053 (-735C/T) polymorphisms. Thus, we analyze the TT and CT as one group - T carriers). Multiple logistic regression analyses were performed by SigmaPlot program version 12.5 (Systat Software, Inc., USA) to evaluate individually the effect of haplotype, genotype and allele on RH presence (adjusted for age, gender, self-declared race, body mass index (BMI) and glycated hemoglobin). The level of statistical significance accepted was 0.05. We considered the alpha error of 0.017 (0.05/3 polymorphisms) as statistically significant for genotype and haplotype analyses.

2.10. Results

General characteristics of HT and RH groups are shown in Table 1. Subjects differ in age, race, diabetes, BMI, waist circumference, fat mass, office SBP, LVMI and PWV. In addition, RH subjects presented higher cholesterol and LDL levels, HbA1c,

glucose, microalbuminuria, aldosterone levels and TIMP-2 levels compared to HT group. Decreased MMP-2/TIMP-2 ratio was found in RH group. Drug distribution is show in table 1. For most of the medications, RH is taking a higher proportion of medications compared to HT, except for angiotensin receptor blocker and statins, which were higher in HT group.

Allele, genotype and haplotype frequencies are shown in Table 2. No deviation from Hardy–Weinberg equilibrium ($p>0.05$) in the studied polymorphisms was found. Allele and genotype frequencies between RH and HT groups were similar for all studied MMP-2 polymorphisms ($p<0.017$). On the other hand, RH had higher frequency for GCC haplotype compared to HT and HT had a higher frequency of GCT and ATC ($p=0.003$) (table 2).

No difference was found between the MMP-2 and TIMP-2 levels according to genotype in each polymorphism for both RH and HT groups (figure 1). Also, we did not find difference in the MMP-2 and TIMP-2 levels comparing haplotypes GCC, GCT and ATC in RH (MMP-2 levels $p=0.64$; TIMP-2 levels $p=0.97$) and HT (MMP-2 levels $p=0.64$; TIMP-2 levels $p=0.32$) groups. Furthermore, no association was found among the studied SNPs (alleles, genotypes and haplotypes) with LVH, arterial stiffness and microalbuminuria in both groups (table 3).

Finally, GCC haplotype was independently associated with RH apart from potential confounders. In addition, CC genotype and C allele of -735C/T polymorphism were associated with the presence of RH. On the other hand, GCT haplotype was associated with lower probability of having RH (table 4).

2.11. Discussion

We found different haplotype frequencies between HT and RH groups (GCC, GCT and ATC, for -1575G/A; -1306C/T; -735C/T). GCC haplotype was associated to

RH phenotype, while GCT haplotype was associated to lower probability of having RH. The CC genotype and the C allele of -735C/T (rs2285053) polymorphism in MMP-2 gene were also associated to RH.

Previous studies have showed the importance of MMP-2 in CV remodeling [15], in hypertension [16] and in TOD development [15]. In addition, patients with increased MMP-2 levels associated with particular genotypes or haplotypes may be exposed at increased risk of CV complications [17]. MMP-2 plays a central role in the degradation of ECM components, both in physiological and in pathophysiological conditions [18, 19], thus there is growing interest to know whether polymorphisms in the MMP-2 gene are associated to altered MMP-2 levels and development of hypertension and TOD.

MMP-2 gene is located on chromosome 16 and variations in the DNA sequence in promoter region might affect transcriptional activity of the adjacent gene [20, 21]. We studied 3 SNPs located in the promoter region of MMP-2 [-1306C/T (rs243865), -1575G/A (rs243866) and -735C/T SNP (rs2258053)], that have been associated to altered MMP-2 expression in CV diseases [7, 9, 22]. The MMP-2 polymorphisms -1306C/T and -735C/T SNPs disrupts a Sp1 promoter site decreasing the promoter activity in the presence of the T allele [20, 21] . In addition, evidences suggest that the G/A transition in the –1575G/A polymorphism located in promoter region, also reduces MMP-2 transactivation activity [23]. Although these studies showed the influence of MMP-2 polymorphisms in MMP-2 levels, we did not find difference in MMP-2 levels and TIMP-2 levels according to genotypes and haplotypes for each polymorphism (-1306C/T, -1575G/A and -735C/T) in both hypertensive groups. In fact, hypertension is a multifactorial and complex pathogenesis and the high number of different medications may influence the levels of MMP-2 [24-26]. In addition, it is likely that these results arise from either interactions of the SNPs with environmental factors or other

polymorphisms in MMP-2 gene, from the studied population, or from the model used in statistical analysis.

The genetic association studies with MMP-2 polymorphisms and hypertension are scarce and still controversial. Some studies have shown that -1306C/T, -735C/T and -1575G/A polymorphisms are not related to hypertension [9, 22, 27-29]. Researchers have also been exploring the association of these polymorphisms with cardiovascular diseases (CVD) and with hypertension-related TOD. The majority of the studies have shown that neither the -1575 polymorphism [30-33] nor the -1306C/T polymorphism are associated with increased risk to develop CVD and TOD [10, 30, 34-37]. Similarly, our results showed no association between these polymorphisms and TOD in resistant hypertension.

Finally, previous studies have showed that the -735TT genotype was associated with increased risk of myocardial infarction [30], while another study suggested TT genotype and T allele as protective factors in subjects with vulnerable plaques [38]. Also, the C allele was associated to chronic heart failure [31] and with ischemic stroke [36]. Conversely, other studies have shown no relationship between the -735C/T polymorphism and other comorbidities including atrial fibrillation [34] and LVMI [9] in hypertensive patients, left ventricular dysfunction in coronary artery disease patients [39] and risk of developing coronary artery disease [37]. Importantly, we found that C allele and CC genotype for -735 polymorphism are both independently associated with resistant hypertension, although this SNP does not modulate MMP-2 levels. In agreement with this, C carriers presented higher probability to have chronic heart failure [31] and ischemic stroke [36], suggesting that C allele is related to similar CV alterations involved in the pathogenesis of RH, apart from its modulatory effects on MMP-2 levels. This polymorphism may also be in linkage disequilibrium with

regulatory sequences modulating transcription of MMP-2 gene, potentially explaining the lack of alteration in MMP-2 levels.

The main limitations of this work includes: i) The small number of patients included; ii) Patients were under pharmacological treatment, which may be a possible confounder for MMP-2 levels; iii) Study power: the lack of association in MMP-2 or TIMP-2 levels may be attributed to low power to detect any difference between genotypes/haplotypes groups. Thus, these findings need to be replicated in independent and larger studies to confirm our findings.

2.11.1 Conclusion

The GCC haplotype was associated to the presence of RH, while the GCT haplotype may act as a protective factor due to its association with lower probability of having RH. Moreover, the association among the former mentioned haplotypes and RH seems to be independent of MMP-2 levels, supporting our hypothesis that this MMP-2 SNP may affect the susceptibility for RH as a MMP-2 levels-independent mechanism. However, we cannot rule out that MMP-2 levels were affected by several antihypertensive drugs taking in RH, which could explain our negative findings. Those haplotype associations with RH may be driven by -735C/T polymorphism or other SNPs in linkage disequilibrium in MMP-2 gene region.

In the present study, we demonstrated for the first time that the GCC and GCT haplotypes, and the C allele and CC genotype of the -735C/T MMP-2 gene polymorphism are associated with the presence of RH.

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Disclosure

The authors report no conflicts of interest to disclose.

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Abbreviation List

Resistant hypertension (RH)

Cardiovascular (CV)

Target organ damage (TOD)

Matrix metalloproteinases (MMPs)

Extracellular matrix (ECM) reorganization

Matrix metalloproteinase 2 (MMP-2)

Tissue inhibitor of metalloproteinases 2 (TIMP-2)

Single nucleotide polymorphisms (SNPs)

Left ventricular hypertrophy (LVH)

Mild to moderate hypertensive (HT)

Blood pressure (BP)

Ambulatory blood pressure monitoring (ABPM)

Systolic blood pressure (SBP)

Diastolic blood pressure (DBP)

Left ventricular (LV)

American Society of Echocardiography (ASE)

Left ventricular mass index (LVMI)

Left ventricular hypertrophy (LVH)

Pulse wave velocity (PWV)

Enzyme-linked immunosorbent assay (ELISA)

Analysis of variance (ANOVA)

Body mass index (BMI)

Low-density lipoprotein (LDL)

Glycated hemoglobin (HbA1c)

Cardiovascular diseases (CVD)

Table(s)

Table 1 – General characteristics of mild-moderate hypertensive (HT) and resistant hypertensive patients (RH)

	HT	**RH**	***P-value***
N	136	119	-
Clinical Parameters			
Age (years)	65 ± 10	60 ± 11	< 0.01
Gender (F %)	62	66	0.51
Race non-W (%)	13	44	<0.01
Diabetes (%)	37	50	0.04
BMI (Kg/m^2)	27.6 (24.6-31.8)	30.8 (26.9-34.9)	< 0.01
Waist circumference (cm)	93.8 (12.8-1.2)	101.1 (14.4-1.6)	<0.01
Fat Mass (Kg)	19.9 (14.6-27.0)	25.9 (19.1-35.1)	<0.01
Office SBP (mmHg)	139 (131-148)	147 (134-159)	< 0.01
Office DBP (mmHg)	81(76-86)	82 (77-91)	0.11
ABPM SBP (mmHg)	126 (118-135)	129 (116-142)	0.22
ABPM DBP (mmHg)	76 (70-81)	76 (70-85)	0.40
LVMI (g/m^2)	100.0 (86.5-119.0)	116.6 (94.7-142.1)	< 0.01
PWV (m/s)	9.8 (8.6-11.6)	9.2 (7.6-11.1)	0.03
Biochemical Parameters			
Total Cholesterol (mg/dL)	165 (140-185)	181 (150-207)	< 0.01
LDL (mg/dL)	86 (67-107)	97 (79-127)	< 0.01
Hb A1c (%)	6.0 (5.8-6.4)	6.3 (5.9-7.8)	< 0.01
Glucose (mg/dL)	97(90-107)	101 (89-132)	0.11
Creatinine (mg/dL)	0.9 (0.8-1.1)	1.0 (0.8-1.2)	0.18
Creatinine Clearance (mL/min/1.73m^2)	65(28-93)	81 (61-98)	0.05
Albumin/Creatinine (mg/g)	(n=39) 6.1 (4.3-13.2)	(n=83) 14 (7.7-80.7)	< 0.01
C-reactive protein (mg/dL)	0.3(0.1-0.6)	0.3 (0.1-0.6)	0.44
Aldosterone (pg/mL)	67.5 (42.5-114.5)	97.9 (60.7-176.9)	< 0.01
MMP-2 (ng/mL)	240.1 (200.6-285.1)	239.1(187.4-308.9)	0.91
TIMP-2 (ng/mL)	70.9 (57.0-89.5)	89.1 (75.3-106.5)	<0.01
MMP-2 / TIMP-2	3.4 (2.6-4.4)	2.6 (2.0-3.5)	<0.01
Medications			
Diuretics	90 (67)	108 (94)	<0.01
Calcium channel blockers	59 (44)	102 (89)	<0.01
β-blockers	19 (14)	83 (72)	<0.01
Angiotensin receptor blockers	101 (75)	67 (58)	<0.01
Spironolactone	2 (1)	46 (40)	<0.01
Angiotensin-converting enzyme inhibitors	22 (16)	44 (38)	<0.01
Sympatholytic drugs	1 (1)	34 (29)	<0.01

Statins	102 (75)	69 (60)	<0.01

Data expressed as mean ± SD or median (1^{st}, 3^{rd} quartiles). HT = mild-moderate hypertensive; RH = resistant hypertensive; N = sample size; F = female; non-W = nonwhite; BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; ABPM = ambulatory blood pressure monitoring; LVMI = left ventricular mass index; PWV = Pulse wave velocity; LDL = low-density lipoprotein; HbA1c = glycated hemoglobin; MMP-2 = matrix metalloproteinase-2; TIMP-2= tissue inhibitor of metalloproteinase 2.

Table 2 – Allele, genotype and haplotype frequencies for MMP-2 polymorphisms in mild-moderate (HT) and resistant hypertensive (RH) subjects

	HT (N=136)	**RH (N=119)**	**P-value**
Allele			
-1575G/A (rs243866)			
G	0.81	0.87	0.09
A	0.19	0.13	
-1306C/T (rs243865)			
C	0.81	0.87	0.12
T	0.19	0.13	
-735C/T rs2285053			
C	0.83	0.90	0.02
T	0.17	0.10	
Genotype			
-1575G/A (rs243866)			
GG	0.64	0.74	
AG	0.34	0.25	0.20
AA	0.02	0.008	
-1306C/T (rs243865)			
CC	0.64	0.74	
CT	0.33	0.25	0.22
TT	0.02	0.008	
-735C/T (rs2285053)			
CC	0.68	0.81	
CT	0.29	0.17	0.04
TT	0.02	0.008	
Haplotype			
GCC	0.64	0.77	
GCT	0.17	0.09	0.003
ATC	0.19	0.13	

HT= mild-moderate hypertensive; RH = resistant hypertensive. Data are expressed as relative frequency (%) and number (n). Chi-square test was used to compare genotype and haplotype distribution between groups and Fisher's test was used to compare allele frequencies between groups. The haplotypes correspond to -1575 G/A (rs243866) -1306 C/T (rs243865) and -735 C/T (rs2285053) polymorphisms, respectively. $p < 0.017$.

Table 3 – Genotypes frequencies of MMP-2 polymorphisms according to the presence of left ventricular hypertrophy, arterial stiffness and renal dysfunction in RH and HT groups

	Left ventricular hypertrophy	Renal dysfunction (Microalb> 30mg/g)	Arterial stiffness (PWV>10m/s)
RH			
-1575G/A (rs243866)			
GG	63 %	33%	36 %
A carriers	71 %	50%	52 %
-1306C/T (rs243865)			
CC	63 %	33%	36 %
T carriers	72 %	50%	50 %
-735C/T (rs2285053)			
CC	66 %	41%	40 %
T carriers	61 %	15%	39 %
HT			
-1575G/A (rs243866)			
GG	50 %	11%	49 %
A carriers	42 %	15%	47 %
-1306C/T (rs243865)			
CC	50 %	11%	49 %
T carriers	43 %	17%	48 %
-735C/T (rs2285053)			
CC	45 %	9%	48 %
T carriers	56 %	20%	52 %

LVH= Left ventricular hypertrophy (≥115 g/m^2 for men and ≥95 g/m^2 for women); Microalb= microalbuminuria; PWV= pulse wave velocity. Fisher's test was used to compare categorical variables between genotypes. * $p<0.05$

Table 4 – Logistic Regression to explore the association of C allele and CC genotype of -735 C/T polymorphism and haplotypes with the presence of resistant hypertension

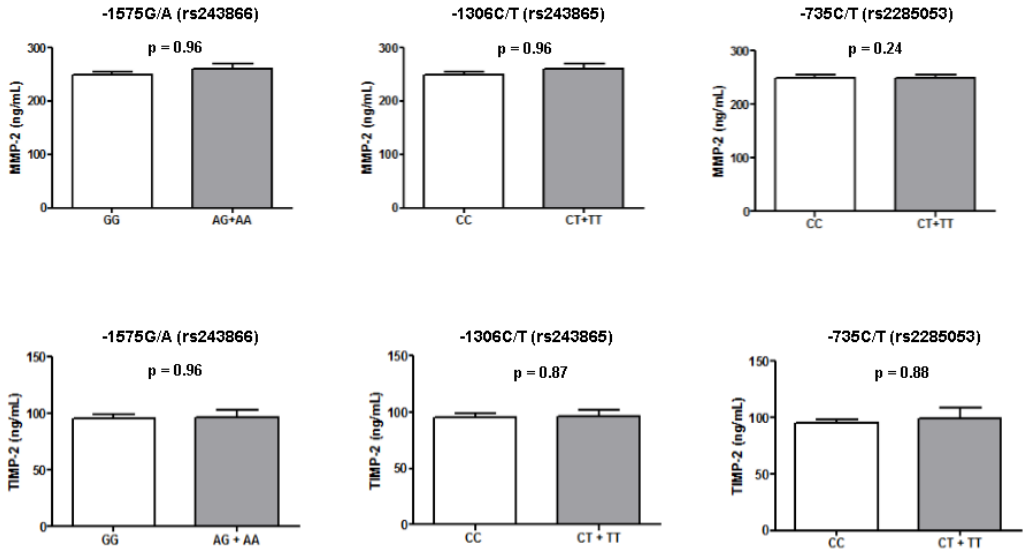
	OR (5%-95%CI)	**p-value**
Haplotype GCC	2.09 (1.20 - 3.64)	0.01
Haplotype GCT	0.35 (0.16 – 0.79)	0.01
Genotype CC -735C/T (rs2285053)	2.93 (1.22 – 7.01)	0.02
Allele C -735C/T (rs2285053)	2.81 (1.26 – 6.31)	0.01

Haplotypes correspond to -1575 G/A (rs243866), -1306 C/T (rs243865) and -735 C/T (rs2285053) polymorphisms. The analyses were individually evaluated for each haplotype, genotype and allele. Adjusted by age, gender, race, BMI and glycated hemoglobin.

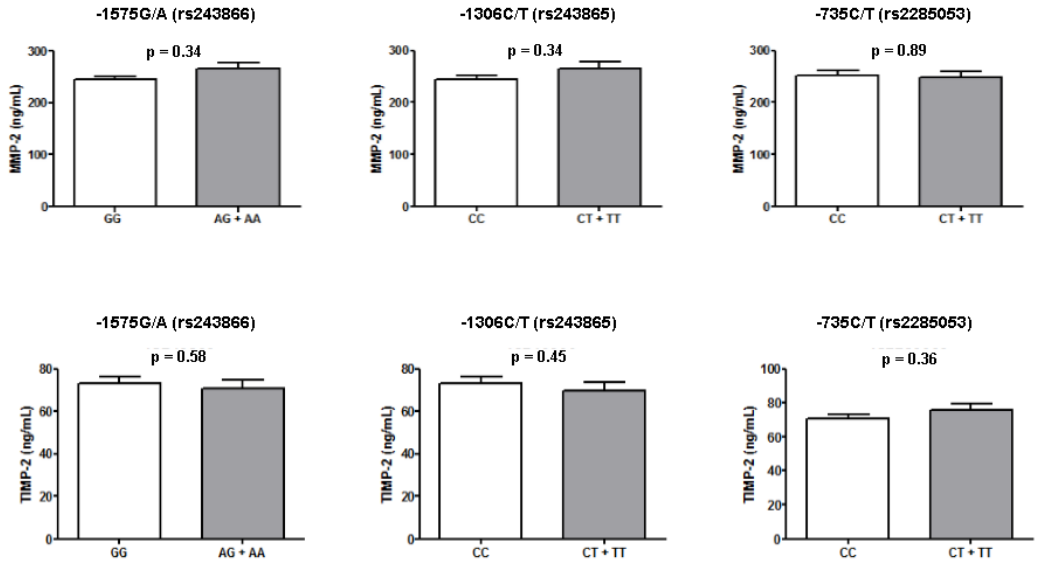
Figure(s)

Figure 1 – MMP-2 and TIMP-2 plasma levels according to genotype in each polymorphism in RH and HT group.

a)



b)



Panel A shows MMP-2 levels and TIMP-2 in RH patients; Panel B shows MMP-2 levels and TIMP-2 levels in HT patients. $p < 0.05$.