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Paper

Impact of renin-angiotensin-aldosterone system genes on the treatment response of patients with hypertension and metabolic syndrome

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Key words: essential hypertension, metabolic syndrome, renin-angiotensin-aldosterone system genes, response to treatment

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Abstract

Objective. To evaluate the influence of clinical, biochemical and genetic markers on the response to antihypertensive treatment in patients with essential hypertension and the metabolic syndrome (MetS).

Methods. Measurements of anthropometric indices, blood pressure (BP), and metabolic parameters were obtained from the medical records of 132 (77 women) newly diagnosed, untreated hypertensive patients. Renin-angiotensin-aldosterone system (RAAS) genes polymorphisms (including ACE I/D, angiotensinogen M235T, angiotensin II type 1 receptor [AT_1-receptor] A1166C) were determined. Response to treatment was defined as BP less than 140/90 mmHg.

Results. Patients with MetS (n=60) had higher systolic BP and pulse pressure and a more atherogenic lipid profile than patients without MetS. The frequencies of the ACE and the AT_1-receptor gene polymorphisms were similar between patients with and without MetS. Response to treatment was positively associated with pulse pressure, and the presence of the C allele as well as the AC genotype of the AT_1-receptor gene and inversely with age after adjustment for confounding factors.

Conclusions. RAAS genes distribution does not differ between hypertensive patients with and without the MetS. Higher baseline pulse pressure levels, the presence of the C allele and/or the AC genotype may be in favour of a better response to structured antihypertensive treatment in patients with MetS. However, these findings need to be evaluated in future studies.

Introduction

The metabolic syndrome (MetS) is a constellation of easy-to-measure clinical phenotypes that serve as markers of increased risk for cardiovascular disease (CVD), diabetes mellitus, chronic kidney disease (CKD) and/or albuminuria.[1-4] Although various current definitions of MetS differ in the detail and criteria, there is an agreement on the essential components.[2,4-7] In this syndrome, central obesity and insulin resistance coexist with several micro/macro-vascular and metabolic disorders, such as hypertension, hyperglycaemia, and dyslipidaemia, particularly hyper-triglyceridaemia, low levels of high-density lipoprotein cholesterol (HDL-C) and high fraction of small dense low-density lipoprotein (LDL) particles.[3,4]

In as far as the MetS represents the clustering of numerous independent risk factors for CVD and/or diabetes mellitus, most authorities suggest an aggressive therapeutic approach towards all the MetS individual components.[3-5] One of the major diagnostic features of the MetS is hypertension. Even though antihypertensive therapy is considered a *sine qua non* in the management of hypertension, specific blood pressure (BP) treatment goals have not been yet introduced in this high-risk population.[3-5] It is currently recommended that BP should be reduced to at least achieve a BP of less than 140/90 mmHg (less than 130/80 if diabetes or CKD is present).[4,5] Some investigators believe that angiotensin-converting enzyme inhibitors (ACE) inhibitors or angiotension receptor blockers (ARBs) are better first-line therapy for metabolic syndrome patients, especially when type 2 diabetes or CKD is present, but the issue of the most effective drug has not been entirely resolved.[8] Indeed, inhibition of the renin-angiotensin system (RAS) with ACE-inhibitors or ARBs may lower risk for diabetes itself.[9] There is evidence that the renin-angiotensin-aldosterone system (RAAS) is activated in patients with MetS, while certain RAAS genotypes may influence the response to antihypertensive therapy in patients with essential hypertension.[10-16] Thus, it is intriguing to identify potential predictors of response to BP-lowering treatment in MetS patients.

In the present study, we investigated the associations between clinical, serum biochemical and genetic markers, such as RAAS genes, and the response to BP lowering treatment in patients with essential hypertension and features of the MetS.

Materials and methods

Study population

We reviewed the medical records of new consecutive patients referred to the Outpatient Hypertension Clinic of the University Hospital of Ioannina, Ioannina, Greece (i.e. the referral

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centre for the prefecture of Ioannina) from January 2002 to December 2004. Selection criteria were as follows: (i) subjects aged 20 years or older, (ii) attending the clinic for at least one year, (iii) with no clinical or laboratory evidence of secondary hypertension, (iv) who were followed up four or more times during the study period, and (v) for whom a detailed clinical and laboratory evaluation according to the clinic protocol was recorded.

Patients with (i) a previous history of CVD (angina, myocardial infarction, stroke, peripheral artery disease), (ii) diabetes mellitus (fasting glucose levels greater than 126 mg/dL [7.0 mmol/L] on more than one occasion), (iii) renal function impairment (serum creatinine > 1.2 mg/dL [100.0 µmol/L]), (iv) raised thyroid stimulating hormone levels (greater than 5.0 µU/L), (v) other serious medical conditions, such as neoplasia, congestive heart failure, inflammatory bowel disease, rheumatic disease, and (vi) those receiving antihypertensive and/or lipid lowering agents at baseline were excluded from the study.

The medical records of 132 patients (55 men, 77 women) were finally selected. Collection of data included evaluations at the first visit prior to any therapeutic intervention and at the latest visit. Recordings involved: (i) anthropometric indices (body mass index [BMI], waist circumference), (ii) smoking habits and alcohol consumption, (iii) BP readings as measured in triplicate, following ten minutes rest, at each visit. (Measurements were carried out by trained physicians in the sitting position using a standard mercury sphygmomanometer), (iv) lipid profile total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG), and HDL-C levels, and (v) non-lipid metabolic parameters, including serum glucose, creatinine, urea, electrolytes, and uric acid levels. In addition, RAAS system genes polymorphisms (including ACE I/D, angiotensinogen [AGT] M235T, AT_1-receptor A1166C) were determined in appropriately stored samples in all participants who gave their informed consent.

The diagnosis of MetS was made when two or more of the following risk determinants were present: abdominal obesity (waist circumference [standing position] > 102 cm for men; > 88 cm for women), TG ≥ 150 mg/dL (1.7 mmol/L), low HDL-C (i.e. < 40 mg/dL [1.0 mmol/L] for men; < 50 mg/dL [1.3 mmol/L] for women), and a fasting glucose ≥ 100 mg/dL (5.6 mmol/L).[5]

Prior to treatment initiation, all patients received advice with emphasis on lifestyle changes according to the clinical protocol, including body weight control, increased physical activity, alcohol moderation, sodium reduction, and increased consumption of fresh fruits and vegetables and low-fat dairy products.[17] Individual consultations were offered on every visit.

During the follow-up period, antihypertensive treatment was adjusted according to physician's judgement, in order to achieve the BP treatment goal, defined as systolic blood pressure (SBP) of < 140 mmHg and diastolic blood pressure (DBP) of < 90 mmHg. No specific treatment algorithm was designated. All classes of antihypertensive agents were prescribed (i.e. thiazides, calcium channel antagonists, ACE-inhibitors, ARBs, beta-blockers, alpha-blockers, centrally acting adrenergic agent) and all possible combinations were allowed; upward titration of drug doses for optimal effect was encouraged.

The study protocol conformed to the ethical guidelines of the Declaration of Helsinki. The study was based on all the available eligible consecutive patients that we could assemble over a period of three years. Power calculations *post-hoc* were not performed.

Laboratory determinations

All laboratory measurements were performed at the Laboratory of Biochemistry of the University Hospital of Ioannina, by standardised methods using an Olympus AU 600 clinical chemistry analyser (Olympus Diagnostica, Hamburg, Germany).[18] Blood samples were obtained following a 14-hour overnight fast.

The glutamate dehydrogenase (GLDH) method was used for the determination of urea levels, the uricase/PAP method (an enzymatic colour test) for uric acid, and a modification of the Jaffé-method for creatinine. The creatinine clearance (CrCl) was estimated by the Cockcroft-Gault formula: CrCl = [(140-age) x body weight]/(72 x serum creatinine), where body weight is in kg, age is in years, and serum creatinine is in mg/dL[18] (to convert creatinine from micromole/L to mg/dl sivide by 83.3). For women, the result is multiplied by a factor of 0.85 to compensate for the lower average muscle mass.[19] Glucose was measured by the hexokinase method. The concentrations of TC and TG were determined enzymatically. HDL-C was determined in the supernatant, after precipitation of the ApoB-containing lipoproteins with dextran sulphate–$Mg2^+$ (Sigma Diagnostics, St Louis, MO, USA). LDL-C was calculated using the Friedewald formula provided fasting triglyceride levels were less than 400 mg/dL (4.5 mmol/L).[18]

Genotype determinations

Genomic deoxyribonucleic acid (DNA) from each individual was isolated from peripheral blood leucocytes using a standard NaCl

extraction procedure protocol. The DNA samples were genotyped for the M235T polymorphism of the AGT, the ID polymorphism of the ACE gene and the A1166C polymorphism of the angiotensin II type 1 receptor gene (AT_1-receptor). The AGT M235T genotype was determined by polymerase chain reaction (PCR) amplification followed by digestion with restriction enzyme Tth111I according to the described method.[20] The ID polymorphism in intron 16 of the ACE gene was detected according to Rigat.[21] PCR is known to have a tendency to preferentially amplify short alleles in contrast to larger alleles in a competitive amplification reaction containing two alleles of different sizes as is the case in ACE I versus D alleles. To increase specificity and to avoid preferential amplification of the deletion allele in heterozygotes, DD homozygotes were retyped using insertion-specific primers. To determine the AT_1-receptor A1166C genotype, PCR amplification was also performed under previously described conditions and PCR products were digested by DdeI.[22]

Statistical analysis

Values were expressed as mean±SD. Comparisons of continuous variables were performed by Student's *t*-test for normally distributed variables and Mann-Whitney test for non-normally distributed variables, while chi x^2-tests were used for categorical variables. Relations between variables were explored by determining Pearson's or Spearman's rank correlation coefficients.

The strength of associations of response to treatment with genetic markers and with clinical/biochemical parameters were assessed by means of logistic regression analysis. These associations were first tested by univariate analysis. Multivariate analysis was performed by binary logistic regression analysis, which allows adjustment for confounding factors, such as age, sex, menopause status for women, BMI, smoking habits, alcohol consumption (entered as units per week), length of follow-up, baseline SBP and DBP measurements, serum lipids and other metabolic variables (creatinine, fasting blood glucose, serum uric acid levels) as well as lipid-lowering treatment during follow-up (binary variable) and antihypertensive treatment. Antihypertensive treatment was entered in the model of logistic regression analysis as a categorical variable (four categories: 1 drug, 2, 3, and 4 combined drugs) and also as binary variables to examine the response to treatment by the class of antihypertensive agent used (i.e. diuretic, beta-blocker, calcium antagonist,

Table 1
Baseline demographic, clinical and laboratory characteristics of the study population.

Variable	Patients with essential hypertension			
	All (n=132)	MetS(+) (n=60)	MetS(-) (n=72)	*p
Sex				
Male	55	24	31	0.72
Female	77	36	41	0.72
Age (years)	54±12	56.3±11.1	52.1±12.0	0.12
BMI (kg/m^2)	27.9±4.1	29.6±4.1	26.6±3.5	< 0.001
Waist circumference (cm)				
Men	90.5±11.6	97.8±11.2	91.7±11.9	< 0.01
Women	79.4±9.2	88.6±10.1	76.4±8.8	< 0.01
Blood pressure (BP)				
Systolic BP (mmHg)	152.8±19.9	156.8±21.8	147.5±17.5	0.03
Diastolic BP (mmHg)	94.9±11.6	93.8±13.4	95.9±9.8	0.30
Pulse pressure (mmHg)	57.9±15.7	63.0±15.5	53.6±14.6	< 0.001
Total cholesterol (mmol/L)	6.2±1.2	6.5±1.3	6.0±1.1	0.03
LDL-cholesterol (mmol/L)	3.6±1.8	4.3±1.0	4.1±1.1	0.28
HDL-cholesterol (mmol/L)	1.3±0.3	1.1±0.3	1.4±0.3	< 0.001
Triglycerides (mmol/L)	2.0±0.8	2.3±0.9	1.1±0.5	< 0.001
Glucose (mmol/L)	5.9±1.3	6.3±1.4	5.2±1.3	< 0.001
Creatinine (μmol/L)	80.8±16.7	83.3±16.7	79.1±16.7	0.19
Creatinine clearance (ml/min)	87.4±28.5	86.7±36.2	87.9±26.3	0.81
Uric acid (μmol/L)	339.0±136.8	368.8±130.8	321.2±95.2	0.03

Key: MetS: metabolic syndrome; BMI = body-mass index; * = comparisons between patients with (+) and without (-) the MetS.

Table 2
Prevalence of the diagnostic features of the metabolic syndrome in the study population.

Feature	MetS (+) (n=60)	MetS (-) (n=72)	p
Abdominal obesity	51 (85.0%)	45 (62.5%)	0.004
High triglyceride	40 (66.7%)	2 (2.8%)	< 0.001
Low HDL-C	32 (53.3%)	8 (11.1%)	< 0.001
Fasting glucose (5.6–7.0 mmol/L)	28 (46.7%)	5 (6.9%)	< 0.001

Key: MetS = Metabolic syndrome; HDL-C = high-density lipoprotein-cholesterol.

ACE-inhibitor or ARB). Sequentially, we adjusted for genotype frequencies; each genotype and related alleles entered the model of multivariate analysis.

Significance levels were set at $p<0.05$ in all cases. SPSS 11.0.1 for Windows (SPSS Inc., 1989–2001) was used to perform the statistical analysis.

Results

Baseline characteristics

Demographic, clinical and laboratory characteristics of the study population are shown in table 1. A total of 60 patients (45.4%) with essential hypertension fulfilled the diagnostic criteria of MetS. Of these, 28 patients (46.7%) had two criteria, 26 (43.3%) had three, and six patients (10.0%) had four diagnostic criteria besides the presence of hypertension. The presence of individual diagnostic features was more frequent among patients with essential hypertension and the MetS (table 2). Hypertensive subjects as a whole tended to be overweight (mean BMI 27.9 kg/m^2). Notably, compared to subjects not fulfilling the MetS criteria, abdominal obesity was more evident among patients with the MetS (table 2), who had higher BMIs (29.6±4.1 *vs.* 26.6±3.5 kg/m^2), and waist circumference measurements (both men and women) (table 1). Moreover, patients with the MetS had higher SBP readings and pulse pressure, higher serum concentrations of fasting glucose and uric acid with more pronounced evidence of atherogenic dyslipidemia (i.e. higher levels of TG and lower levels of HDL-C) than patients without MetS (tables 1 and 2).

Genotype distribution

For all polymorphisms determined there was no significant deviation from Hardy-Weinberg equilibrium in this group of subjects. There were no significant differences in the frequencies of the ACE and the AT_1-receptor gene polymorphisms between patients with and

Table 3
Polymorphisms in the study population.

Variable	Patients with essential hypertension			
	All (n=132) *N* (%)	MetS (+) (n=60) *N* (%)	MetS (-) (n=72) *N* (%)	*p
ACE (I/D)				
DD	48 (36.3)	22 (36.7)	26 (36.1)	0.94
II	22 (16.7)	11 (18.3)	11 (15.3)	0.64
DI	62 (47.0)	27 (45.0)	35 (48.6)	0.70
AGT (M235T)				
TT	46 (34.8)	19 (31.7)	27 (37.5)	0.48
CC	26 (19.7)	16 (26.7)	10 (13.9)	0.07
TC	60 (45.5)	25 (41.6)	35 (48.6)	0.42
AT1R (A1166C)				
AA	75 (56.8)	31 (51.7)	44 (61.1)	0.81
CC	11 (8.4)	4 (6.7)	7 (9.7)	0.53
AC	46 (34.8)	25 (41.6)	21 (29.2)	0.13

Key: MetS = metabolic syndrome; * = comparisons (chi^2-test) between patients with (+) and without (-) the MetS; A = adenine; C = cytosine; D = deletion; G = guanine; I = insertion; M = methionine; T = threonine.

Table 4
Pharmacologic treatment in the study population.

Antihypertensive drug class	MetS (+) (n=60)	MetS (-) (n=72)	p
Thiazide diuretic	25 (41.7%)	30 (41.6%)	1.0
Beta-blocker	21 (35.0%)	22 (30.5%)	0.59
Calcium antagonist	26 (43.3%)	29 (40.2%)	0.72
ACE-inhibitor	29 (48.3%)	33 (45.8%)	0.77
Angiotensin receptor antagonist	14 (23.3%)	17 (23.6%)	0.97
Alpha-blocker	3 (5.0%)	4 (5.5%)	0.89
Centrally acting adrenergic agent	6 (10.0%)	7 (9.7%)	0.96

Key: MetS = Metabolic syndrome; ACE = angiotensin-converting-enzyme.

without MetS (table 3). Patients with the MetS showed a trend towards a higher frequency of the M235T CC genotype of the AGT gene (table 3).

Drug treatment

There were no significant differences in the selection of various classes of antihypertensive agents between patients with and without the MetS during the follow-up period (table 4). Twenty patients (33.3%) with MetS *vs.* 23 (31.9%) of patients without MetS received monotherapy, 20 (33.3%) *vs.* 26 (36.1%) received a two-drug combination, 14 (23.4%) *vs.* 17 (23.6%) received three drugs, and six (10.0%) *vs.* six (8.4%) received a combination of four drugs.

Response to treatment

The mean decrease in SBP and DBP levels was greater but non-significant (13.2±3.2 *vs.* 6.2±3.0 mmHg, and 9.0±2.1 *vs.* 5.5±1.5 mmHg, respectively) in patients with the MetS compared with those without MetS. A total of 29 (48.3%) of patients with MetS and 41 (56.9%) of patients without the MetS achieved the treatment goal ('responders') at the end of the follow-up period. In patients with the MetS, the mean decrease in SBP was significantly greater in 'responders' than

Table 5a
Univariate and multivariate logistic regression analyses of the association between baseline clinical, biochemical and genetic markers and the response to treatment in patients with essential hypertension with and without metabolic syndrome.

All patients

	Univariate		Multivariate*	
	Odds ratio (95%CI)	p	Odds ratio (95%CI)	p
Age (yrs)	0.95 (0.92–0.98)	0.003	0.94 (0.89–0.98)	0.02
Pulse pressure (mmHg)	1.15 (1.08–4.99)	0.007	1.13 (1.10–4.58)	0.03
Total cholesterol (mg/dL)	0.99 (0.98–0.99)	0.02	0.99 (0.99–1.00)	0.12
AA genotype	0.45 (0.21–0.96)	0.04	0.42 (0.26–1.22)	0.11
AC genotype	3.06 (1.371–6.81)	0.006	3.39 (1.05–10.94)	0.04
Presence of C allele	2.21 (1.01–4.71)	0.03	2.737 (1.153–6.496)	0.02

Key: Values represent odds ratios per 10 mg/dL changes in total cholesterol; values for pulse pressure represent odds ratios per mmHg. * = Adjustment for age, sex, menopause status for women, body mass index, smoking, alcohol consumption (units/week), length of follow-up, serum lipids, creatinine, fasting glucose, serum uric acid, use of lipid-lowering agents during follow-up (binary variable), and antihypertensive treatment (categorical and binary variables). A = adenine; C = cytosine.

Table 5b
Univariate and multivariate logistic regression analyses of the association between baseline clinical, biochemical and genetic markers and the response to treatment in patients with essential hypertension with and without metabolic syndrome.

Patients with essential hypertension with the metabolic syndrome				
	Univariate		Multivariate*	
	Odds ratio (95%CI)	p	Odds ratio (95%CI)	p
Age	0.93 (0.88–0.98)	0.01	0.94 (0.87–0.98)	0.03
Pulse pressure	1.16 (1.12–3.99)	0.03	1.12 (1.09–4.01)	0.03
AC genotype	4.11 (1.32–12.80)	0.01	3.60 (1.05–12.33)	0.04
Presence of C allele	2.92 (1.06–8.67)	0.04	2.67 (1.04–8.12)	0.04

Key: Values represent odds ratios per 10 mg/dL changes in TC; values for pulse pressure represent odds ratios per mmHg. *Adjustment for age, sex, menopause status for women, body mass index, smoking, alcohol consumption (units/week), length of follow-up, serum lipids, creatinine, fasting glucose, serum uric acid, use of lipid-lowering agents during follow-up (binary variable), and antihypertensive treatment (categorical and binary variables). A = adenine; C = cytosine.

Table 5c
Univariate and multivariate logistic regression analyses of the association between baseline clinical, biochemical and genetic markers and the response to treatment in patients with essential hypertension with and without metabolic syndrome.

Patients with essential hypertension without the metabolic syndrome				
	Univariate		Multivariate*	
	Odds ratio (95%CI)	p	Odds ratio (95%CI)	p
Age	0.96 (0.92–1.01)	0.09	0.96 (0.91–1.02)	0.21
Pulse pressure	0.98 (0.94–1.01)	0.16	1.00 (0.96–1.05)	0.78
AC genotype	2.62 (0.81–8.45)	0.11	2.03 (0.52–7.90)	0.31
Presence of C allele	1.89 (0. 64–5.54)	0.25	1.53 (0.44–5.24)	0.50

Key: Values represent odds ratios per 10 mg/dL changes in TC; values for pulse pressure represent odds ratios per mmHg. *Adjustment for age, sex, menopause status for women, body mass index, smoking, alcohol consumption (units/week), length of follow-up, serum lipids, creatinine, fasting glucose, serum uric acid, use of lipid-lowering agents during follow-up (binary variable), and antihypertensive treatment (categorical and binary variables) A = adenine; C = cytosine.

in 'non-responders' (mean decrease 20.7±4.9 *vs.* 6.2±3.9, p=0.02), while the decrease in DBP was comparable between groups (11.0±3.2 *vs.* 7.2±2.7, p=0.09).

During follow-up, BMI values did not change significantly from baseline in patients with the MetS (29.6±4.1 *vs.* 29.1±4.8 kg/m^2) or without the MetS (26.6±4.5 *vs.* 26.2±4.2 kg/m^2). Furthermore, small but non-significant reductions in BMI were evident both in responders (29.7±4.8 *vs.* 29.2±5.3 kg/m^2) and non-responders (29.5±3.5 *vs.* 28.9±4.4 kg/m^2). The results of the logistic regression analysis indicating the associations of the response to antihypertensive therapy with clinical and biological markers are shown in table 5. In the whole population of patients with essential hypertension, pulse pressure levels, and the presence of C allele as well as AC genotype of the AT_1-receptor gene (with AA as the reference group) were positively associated with response to treatment, whereas age was inversely associated (adjusted odds ratio 0.94 [0.89–0.98] 95% CI (Confidence Interval), p=0.02) (table 5a). These associations were not statistically significant in the subgroup of patients not fulfilling the criteria of the MetS (table 5c). On the contrary, in patients with the MetS, age, pulse pressure, and presence of C allele and the AC genotype of the AT_1-receptor gene were associated with the response to treatment in the same manner (table 5b).

Discussion

The present study indicates that certain biologic parameters could be potential predictors of the response to antihypertensive treatment in subjects with essential hypertension and the

MetS. Among the MetS components, hypertension is the least 'metabolic' and is considered multifactorial in origin.[3-6] There is a limited number of studies linking clinical and genetic markers, such as polymorphisms of RAAS genes, with the response to treatment in subjects with essential hypertension.[12-16] There is evidence that the RAAS is activated in patients with MetS.[10,11] In fact, several components of the RAAS such as angiotensinogen, ACE, and AT_1-receptors are present within human adipose tissue.[23] Experimental studies suggest that the adipose RAAS is regulated by hormonal and nutritional factors and correlates with the degree of obesity and that AII may modulate adipose tissue blood flow, growth and metabolism.[24,25] Thus, an up-regulated adipose RAAS may have deleterious local and systemic effects in obese individuals and contribute to insulin resistance and hypertension. It has also been proposed that both hyperglycaemia and insulin activate the RAAS by increasing the expression of AGT Ang II and the AT_1-receptor which, in concert, may contribute to the development of hypertension in patients with insulin resistance.[26]

It has been reported that several RAAS gene polymorphisms, such as ACE I/D, AGT M235T, AT_1-receptor A1166C, may influence the RAAS activity. However, the exact role of these polymorphisms on the RAAS function remains unclear due to the conflicting results produced by a number of studies.[27-31] The associations of these polymorphisms with particular features of the MetS have also been investigated. Several studies provided evidence supporting an association between increased insulin resistance and the presence of ACE I allele.[32-34] Furthermore, the presence of M235 allele in a Chinese population has been shown to be associated with lower TC and LDL-C levels than in T allele homozygotes.[31] A similar correlation between cholesterol levels and the M235T polymorphism has also been reported in Caucasian populations.[35] In a study of Pima-Indians, Nagi *et al.* showed that plasma ACE concentrations, which were influenced by the ACE I/D polymorphism, were associated with serum TG and TC levels.[36] Furthermore, del Ser T *et al.* demonstrated that serum TG concentrations are elevated in stroke patients in association with the ACE DD genotype.[37]

There is limited data regarding the distribution of RAAS genes in population with MetS and essential hypertension.[38] In our study, there were no statistically significant differences between the MetS+ and the MetS- group, except for a trend of patients with the MetS towards having a higher frequency of the M235T CC polymorphism. Moreover, the presence of the 1166C allele and the AC genotype of the A1166C polymorphism of the AT_1-receptor of the angiotensin II favoured the response to treatment in the whole study population, particularly in subjects with the MetS. Frazier *et al.* demonstrated a diminished BP response to hydroclorothiazide after four weeks of therapy in C allele hypertensive carriers,[39] whereas Kurland *et al.* reported that the A1166C polymorphism does not predict the BP response to a 3-month treatment with irbersartan or atenolol in hypertensive patients.[15] There is also evidence that the presence of the C allele is associated with a series of cardiovascular disease risk factors, including aortic stiffness, increased carotid intima-media thickness, left ventricular hypertrophy, as well as the formation of atheromatous plaques.[40,41] All these considered, we might suggest that patients with essential hypertension with the MetS who have the 1166C allele may constitute a high-risk population that could benefit from structured antihypertensive therapy.

Our data showed an inverse relationship between pulse pressure levels and the response to antihypertensive therapy. Indeed, the determinants of pulse pressure include the cushioning capacity of arteries and the timing and intensity of wave reflections which are influenced by arterial stiffness.[42] Thus, pulse pressure is regarded as a potential clinical marker of arterial wall stiffness, which may be involved in the pathophysiology of hypertension in patients with the MetS.

Study limitations

This was a single centre 'real-life' retrospective study evaluating the effects of genetic polymorphisms on the response to antihypertensive treatment in previously untreated hypertensive patients with the MetS. The application of strict selection criteria, although limiting the sample size, was decided in order to attenuate the heterogeneity of the sample studied and to increase the chances of correctly identifying any associations present. This may represent a study limitation and reduce the generalisability of the results. Although the patients were treated by different regimens according to the physician's judgement, there were no significant differences in the selection of various classes of antihypertensive agents between groups. Finally, given the relatively small size of the homozygote group for the 1166C allele of the A1166C polymorphism of the AT_1-receptor, our findings could be due to an unrecognised bias in sample, not controlled for by our statistical analyses. It would be intriguing if studies with antihypertensive treatment standardised to a particular drug and/or to a particular dosage confirmed our findings.

We conclude that the distribution of the RAAS genes did not differ between patients with essential hypertension with and without the

MetS. Higher baseline pulse pressure levels, the presence of the C allele and/or the AC genotype were in favour of a better response to structured antihypertensive treatment in patients with MetS. Further investigation is needed to evaluate possible implications of these findings in the pathophysiology and treatment of hypertension related to the MetS.

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