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Association of ACE gene (I/D) polymorphism and the metabolic syndrome in Moroccan population

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Abstract— **The metabolic syndrome is a cluster of cardiovascular risk factors. Several studies have been riveted on the association of the metabolic syndrome and a number of candidate genes. Our objective was to assess the potential association between angiotensin-converting enzyme (ACE) gene (I/D) polymorphism and metabolic syndrome in an adult Moroccan population using three major proposed definitions.**

We examined 820 subjects from both genders. Metabolic syndrome was defined according to JIS, NCEP-ATPIII and IDF definitions. To determine the ACE genotype of the patients, a genomic DNA fragment on intron 16 of the ACE gene was amplified by polymerase chain reaction using appropriate primers.

The highest rate of metabolic syndrome was obtained in patients diagnosed by JIS (35.73%), succeeded by the IDF (30.24%) and the NCEP-ATPIII (27.93%) criteria respectively. The prevalence was higher in women than in men disregarding of the definition used. Subjects with metabolic syndrome had a low frequency of DD genotypes and high frequency of ID compared to those without. Carriers of the I allele homozygote genotype show a much higher relative risk of developing metabolic syndrome compared to DD homozygous. In all definitions used, a high frequency of D allele was shown in patients with metabolic syndrome.

Our results highlight the role of the ACE (I/D) polymorphism in the etiology of the metabolic syndrome and its components. The deletion of the repetitive sequence of the ACE seems as protective against the development of the metabolic syndrome in an adult Moroccan population.

Index Terms— **Insertion/deletion polymorphism; ACE gene; Metabolic syndrome; Allele; candidate gene.**

I. INTRODUCTION

The metabolic syndrome (MetS) is a constellation of cardiovascular risk factors. It includes abdominal obesity, atherogenic dyslipidemia, and dysglycemia. It is well documented that the presence of MetS is associated with a threefold increased risk of cardiovascular diseases and a three- to fivefold increased risk of cardiovascular death [1], [2]. The pathophysiology of MetS remains unclear. However, increasing evidences suggest that the genetic component is involved in the etiology of the condition. Recently, a large body of studies has been riveted on the association of the MetS and a number of candidate genes [3], [4].

The Angiotensin converting enzyme (ACE) gene has received great attention as a candidate for a variety of medical disorders. The most common polymorphism in ACE gene is a 287 pb Alu insertion-deletion (I/D) in intron 16 of the chromosome 17q23. This polymorphism is a nonsense repetitive DNA domain that leads to three genotypes II, ID and DD [5], [6]. Its association with many MetS components as hypertension [7], [8], diabetes [7], [9], [10], obesity [11], and dyslipidemia [7], [9] has been explored in several studies. Nevertheless, studies on the association of ACE (I/D) polymorphism and the MetS as an entity are scarce and a single MetS definition was used. In this context, our aim is to assess the potential association between ACE (I/D) polymorphism and MetS in an adult Moroccan population using three major MetS definitions: Joint Interim Statement (JIS), National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) and International Diabetes Federation (IDF).

II. MATERIELS AND METHODS

This study was carried out in consultant patients in the diagnostic center of Rabat – Morocco. A total of 820 subjects from both genders, with a minimum age of 19 years old, was included. Collection of the data in this study included anthropometric parameters (age and waist circumference), measurement of blood pressure and the dosage of biochemical parameters (glycemia, triglyceridemia, total cholesterol, and HDL cholesterol).

Informed oral consent was obtained for each patient.

- *Definition of MetS*

MetS was defined according to three major proposed criteria

detailed in Table 1. In JIS and IDF definitions, we applied for the Moroccan population the waist circumference ≥ 80 cm in women and ≥ 94 cm in men to define abdominal obesity.

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TABLE I: THE CRITERIA OF THE THREE USED DEFINITIONS: IDF, ATPIII AND JIS.

	JIS 2009 [12]	IDF 2005 [13]	NCEP-ATP III 2001 [14]
	Three of the five criteria:	Increased waist-circumference, plus more than two of the four criteria:	Three of the five criteria:
Abdominal Obesity	WC ≥ 94 cm in men ≥ 80 cm in women.	WC ≥ 94 cm in men and ≥ 80 cm in women.	WC ≥ 102 cm in men and ≥ 88 cm in women.
Glycemia	≥ 100 mg/dl.	≥ 100 mg/dl or previously diagnosed type 2 diabetes.	≥ 100 mg/dl or specific treatment.
Hypertension	Systolic ≥ 130 mm Hg and/or diastolic ≥ 85 mm Hg.	Systolic ≥ 130 mm Hg and/or diastolic ≥ 85 mm Hg or specific treatment.	Systolic ≥ 130 mm Hg and/or diastolic ≥ 85 mm Hg or specific treatment.
Hyper-Triglyceridemia	≥ 150 mg/dl (1.7 mmol/L).	≥ 150 mg/dl (1.7 mmol/L) or specific treatment.	≥ 150 mg/dl (1.7 mmol/L) or specific treatment.
HypoHDLemia	<1,00 mmol/L (40 mg/dL) in men <1,3 mmol/L (50 mg/dL) in women.	<1,03 mmol/L (40 mg/dL) in men <1,29 mmol/L (50 mg/dL) in women or specific treatment	< 40 mg/dl (0.9 mmol/L) in men or < 50 mg/dl (1.1 mmol/L) in women or specific treatment.

- ***ACE (I/D) polymorphism genotyping***

Genomic DNA was extracted from peripheral blood using the phenol / chloroform method. All genotyping was performed without the knowledge of the case/control status of each individual study.

The primer pairs used were as follows: Forward 5'-CTGGAGACCACTCCCATCCTTTCT-3' and Reverse 5'- GATGTGGCCATCACATTCGTCAGAT-3' which amplify the intron 16 region where the I/D fragment is located. To avoid ID/DD mistyping of heterozygotes as DD homozygotes, all DD genotype samples were confirmed using a pair of primers (Forward 5'-TGGGACCACAGCGCCCGCCACTAC-3' and Reverse 5'-TCGCCAGCCCATGCCCATAA-3') that produce an amplified product only in the presence of the insertion, which was used to verify the polymorphism. All PCR products were visualized after electrophoresis on a 2% agarose gel and ethidium bromide staining. The estimation of the genotypes and allele frequencies was made assuming Hardy-Weinberg equilibrium.

- ***Statistical analysis***

The chi-square test was performed to compare the crude prevalence rate between patients with and without MetS. The analyses reported in this study were performed using the Statistical Analysis System (SAS). P values less than 0.05 were considered statistically significant.

III. RESULTS

A total of 653 women (79.63%) and 167 men (20.37%) were included in this study. The mean age is 52.8 years. Metabolism, clinical and anthropometric characteristics of the studied population are presented in Table 2a. The women show significantly higher levels of total cholesterol, LDL and triglycerides, as well as hypo-HDL compared to men.

Table 2b shows the prevalence of MetS in our study population. The highest rate is obtained in patients diagnosed by JIS (35.73%), succeeded by the IDF (30.24%) and the NCEP-ATPIII (27.93%) criteria respectively. The MetS prevalence is higher in women than in men disregarding of the definition used.

TABLE II: ANTHROPOMETRIC AND METABOLIC CHARACTERISTICS (TABLE 2A), AND THE PREVALENCE OF MetS (TABLE 2B) IN THE STUDY POPULATION.

TABLE IIA

Parameters	Entire Population (N=820), Average ± S.D	Men (N=167), Average ± S.D	Women (N=653), Average ± S.D	P value (sex)
Age (year)	52.84 ± 12.47	53.08 ± 13.05	52.78 ± 12.33	0.79
WC (cm)	87.38 ± 10.24	87.63 ± 6.77	87.31 ± 10.96	0.72
SP (mm Hg)	137.00 ± 18.04	137.64 ± 16.79	136.83 ± 18.36	0.61
DP (mm Hg)	77.03 ± 10.60	76.72 ± 9.46	77.11 ± 10.88	0.67
Gly (g/l)	1.198 ± 0.543	1.189 ± 0.567	1.201 ± 0.537	0.80
ChT (g/l)	1.962 ± 0.424	1.759 ± 0.433	2.014 ± 0.406	**<0.0001*****
HDL (g/l)	0.537 ± 0.188	0.517 ± 0.203	0.543 ± 0.183	0.11
LDL (g/l)	1.166 ± 0.388	1.006 ± 0.377	1.207 ± 0.381	**<0.0001*****
Tg (g/l)	1.293 ± 0.737	1.182 ± 0.811	1.321 ± 0.715	**0.03***

TABLE IIA

Prevalence by Definition	Entire Population (N=820),	Men (N=167), %	Women (N=653), %	P value (sex)

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	%			
JIS	35.73	18.56	40.12	**<0.0001*****
NCEP-ATPIII	27.93	10.78	32.31	**<0.0001*****
IDF	30.24	11.98	34.92	**<0.0001*****

Note: ****P*<0.001; **P*<0.05.
Abbreviations: WC, Waist Circumference; **SP**, Systolic Pressure; **DP**, Diastolic Pressure; **Gly**, Glycemia; **ChT**, Total Cholesterol; **HDL**, High Density Lipoprotein; **LDL**, Low Density Lipoprotein; **Tg**, Triglyceridemia.

Figue 1 indicates a sample of the genotyping on the 2% agarose gel

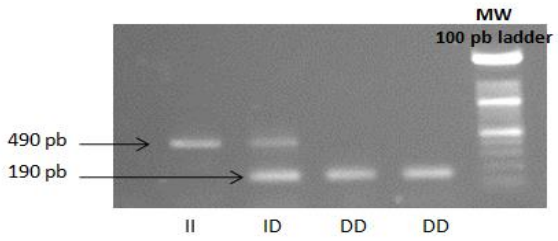

Fig1 : ACE (I/D) polymorphism genotypes

In all used definitions, syndromes had a low frequency of deletion homozygous and high frequency of heterozygosity compared to non-syndromes. The frequency of insertion homozygoty shows no significant difference between syndromes and non-syndromes (Table 3).

Patients with the I allele homozygote show a much higher relative risk of developing MetS compared to DD homozygous regardless of the definition used. One exception is observed in men diagnosed by the IDF definition (Table 4).

In Table 5, the syndromes have a high frequency of D allele compared to patients without MetS in all definitions used.

TABLE III: DISTRIBUTION OF THE GENOTYPIC FREQUENCIES ACE (I/D) IN THE STUDY POPULATION, ACCORDING TO THE SYNDROME STATUS DEFINED BY THE THREE USED DEFINITIONS (JIS, ATPIII AND IDF).

MetS definitions	Polymorphism	Without MetS	With MetS	P value (+/- MetS)
JIS	DD	45.92% (242)	34.81% (102)	**0.002****
	ID	39.09% (206)	48.81% (143)	**0.007****
	II	14.99% (79)	16.38% (48)	0.598
IDF	DD	45.46% (260)	33.87% (84)	**0.002****
	ID	40.38% (231)	47.58% (118)	0.0554
	II	14.16% (81)	18.55% (46)	0.1105
NCEP-ATPIII	DD	44.67% (264)	34.93% (80)	**0.0112***
	ID	40.10% (237)	48.91% (112)	**0.0221***
	II	15.23% (90)	16.16% (37)	0.7412

Note: ***P*<0.01; **P*<0.05

TABLE IV: THE RELATIVE RISK OF DEVELOPING MetS IN PATIENTS COMPARED TO DD GENOTYPE IN THREE USED DEFINITIONS

	MetS definitions	Entire population	Men	Women
Relative risk (odds ratio)	JIS	1.442 (0.941–2.209)	1.190 (0.365-3.881)	1.650 (1.025-2.657)
	IDF	1.758 (1.135-2.723)	1.875 (0.528-6.660)	1.954 (1.203-3.172)
	NCEP-ATPIII	1.357 (0.859-2.143)	0.508 (0.055-4.731)	1.625 (0.993-2.660)

TABLE V: DISTRIBUTION OF THE ALLELIC FREQUENCIES (I/D) IN THE STUDY POPULATION, ACCORDING TO THE SYNDROME STATUS IN THREE USED DEFINITIONS (JIS, ATPIII AND IDF).

MetS definitions	Polymorphism	Without MetS	With MetS	P value (D/I)
JIS	D	65.46% (690)	59.22% (347)	**0.012***
	I	34.54% (364)	40.78% (239)	
NCEP-ATPIII	D	64.72% (765)	59.39% (272)	**0.0446***
	I	35.28% (417)	40.61% (186)	
IDF	D	65.65% (751)	57.66% (286)	**0.0021****
	I	34.35% (393)	42.34% (210)	

Note: ***P*<0.01; **P*<0.05.

IV. DISCUSSION

The application of three major proposed criteria for the MetS shows that the syndrome is common in our population. The female predominance of MetS is inevitable regardless of the definition used. The high prevalence of MetS, especially in women has been reported in several studies [15] - [17]. The stringent thresholds of abdominal obesity proposed for women [12] - [14], as well as the metabolic effect of menopause [17] may partially explain this result. In addition, the genetic background can participate in the emergence of the MetS [4]. In this context, the exploration of the polymorphism of one candidate gene for the genetic basis of MetS was done in this study. Our findings showed that

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syndrome patients carry less DD homozygosity than those without MetS. The DD genotype appears thereby as protective against the apparition of MetS in our population, according to JIS, ATPIII and IDF definitions. This is also supported by the high prevalence of heterozygoty ID in patients with MetS. In fact, the passage from the double (DD) to the simple deletion (ID) of the Alu sequence was responsible for an increase in the prevalence of MetS. In people with insertion homozygoty, the protective effect of the deletion disappears and the prevalence of II genotype shows no difference between patients with and without MetS. Those observations affirm the role that can be played by the genetic factors in the development of MetS. In the study of M. Pacholczyk et al, the relation of MetS with the ACE (I/D) polymorphism in the general population reports a contradictory result [19]. In this work, DD genotype was associated with an increased susceptibility to MetS. A relative small effectif is a limitation of this study. Other studies have investigated the association between MetS and ACE (I/D) polymorphism in specific populations. Studies in Caucasian found that there is no association between ACE (I/D) polymorphism and MetS in type 2 diabetes [10] and hypertensive patients [8]. A similar result was found by Sinorita H, et al in Indonesian type 2 diabetes patients [19]. Reciprocally, a study in Asian patients showed that MetS was associated with the II homozygoty [7] and DD homozygoty genotype [20]. Our results show also that in all definitions used, syndromics presented a predominance of the D allele in contrast to non-syndrome in whom I allele is dominant. A similar result was found by Lee YJ, et al [20]. This finding indicates that in summation to the MetS as an entity, its components may also deliver a common genetic predisposition.

To our knowledge, this is the first study that evaluates the association between ACE (I/D) polymorphism and MetS in three major proposed definitions. Our results show that the risk of developing MetS is much higher in carriers of the II homozygoty than DD hemozygoty regardless of the definition used. The highest exposure is obtained in IDF syndromes. The II genotype is associated with lower circulating levels of ACE (two-time less higher than in those with DD genotype) [21] and consequently with lower levels of angiotensin II. The exception in men diagnosed by the ATP-III definition is partly explained by the threshold values of abdominal obesity who diagnose only patients having a high degree of visceral obesity [14]. This outcome suggests that the used definitions are related to the activation state of the renin angiotensin system for the characterization of MetS in our patient sample. Further studies will be very informative in this regard.

V. Conclusion

The diagnosis of the MetS by three major proposed definitions shows that this entity is common in our study population. The deletion of the repetitive sequence of the ACE seems as protective against the development of the MetS. Carriers of the II homozygosity are more exposed to the MetS compared to patients with DD homozygoty regardless of the definition used. These results emphasize i) the role of the ACE (I/D) polymorphism in the etiology of the MetS and ii) a common genetic predisposition of its components.

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