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Estrogen receptor α is not a candidate gene for metabolic syndrome in Caucasian elderly subjects [☆]

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ABSTRACT

Objective. Variants of estrogen receptor α (ER α) have been associated with obesity, dyslipidemia, diabetes and blood pressure. The Middle East registers some of the highest rate of metabolic syndrome worldwide. The aim of this study is to investigate the relationship between metabolic syndrome, a clustered combination of these metabolic factors, and polymorphisms *PvuII* and *XbaI* of ER α in Lebanese Caucasian elderly overweight subjects.

Material/Methods. 250 Caucasian Lebanese unrelated elderly men and women, median age 71 years, were studied. ER α intronic polymorphisms variants, *PvuII* and *XbaI* diplotypes and genotypes, were examined. Associations with metabolic syndrome, defined by the American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI), and its components, namely high density lipoprotein (HDL), fasting glucose levels, blood pressure, and waist circumference were evaluated in regression models.

Results. ER α diplotypes and genotypes distributions were similar between participants with and without metabolic syndrome, in the overall group of subjects, and by gender. No consistent associations between the diplotypes and genotypes tested and metabolic syndrome, or its components, could be detected.

Abbreviations: ER α , Estrogen Receptor alpha; AHA/NHLBI, The American Heart Association/National Heart, Lung, and Blood Institute; HDL, High density lipoprotein; NHANES, National Health and Nutrition Examination Survey; MENA, Middle East and North Africa; DNA, Deoxyribonucleic acid; RFLP, Restriction fragment length polymorphism; Bp, base pair (bp); PCR, Polymerase chain reaction; CS_{MS}, Cumulative metabolic syndrome components count; ANOVA, Analysis of Variance; BMI, Body Mass Index; OR [95% CI], Odd ratio [95% Confidence Interval].

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Conclusions. Genetic variants in ER α were not associated with metabolic syndrome or its components, in a group of 250 Lebanese Caucasian elderly participants, a group with a high prevalence of metabolic syndrome.

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1. Introduction

Metabolic syndrome is a cluster of conditions, each of which has been associated with cardiovascular diseases [1]. Elderly subjects have a high prevalence of metabolic syndrome and some studies suggest that the prevalence is higher in women than in men [2]. The prevalence of metabolic syndrome is on the rise and ranged between 17% to 40% in the Middle East and 16.3% in North Africa regions [3]. In Lebanon, the prevalence of metabolic syndrome is quite close (31.2%) to that reported by NHANES 2003–2006 (34%), and this warrants urgent public health measures to prevent morbidity and mortality due to cardiovascular complications in the future [4,5]. Multiple definitions have been proposed to define the metabolic syndrome. These include those by the World Health Organization [6], the European Group for the Study of Insulin Resistance [7], the United States National Cholesterol Education Program Adult Treatment Panel III (ATP III) [8], the American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI) [9], and the International Diabetes Federation [10]. Almost all definitions characterize the metabolic syndrome by the presence of three or more of the following abnormalities: abdominal obesity, elevated triglycerides, low levels of HDL, high blood pressure, and elevated fasting glucose.

Several genome scans have been carried out recently on metabolic syndrome. A genome scan performed in 507 white nuclear families using a 10-cM, (centimorgan), unit for measuring genetic linkage which is the tendency of genes that are located proximal to each other on a chromosome to be inherited together during meiosis, demonstrated a strong link between chromosome band 3q27 and 6 traits, weight, waist circumference, leptin, insulin, insulin/glucose ratio and hip circumference [11]. A recent genome scan using families and sib pairs, analysis for arbitrary pedigrees of the NHLBI Family Heart Study, found significant linkage on chromosome 2 at 240 cM ($p < 0.005$). This same broad region of chromosome 2 has been implicated by at least 13 other studies for phenotypes related to metabolic syndrome [12–24].

Free estrogen levels were significantly higher in subjects with metabolic syndrome than those without metabolic syndrome [25,26]. Furthermore, estrogen levels have been linked to metabolic syndrome components, including glucose tolerance, lipid metabolism and blood pressure [25,27]. Estrogens may modulate these abnormalities through specific receptor, ER α on chromosome 6q25.1, a candidate gene previously implicated in the pathophysiology of cardiovascular diseases. Due to the association between metabolic syndrome and coronary heart diseases, ER α may also predispose its carriers to the development of metabolic syndrome.

Indeed, variants in the ER α genes have been associated with components of the metabolic syndrome, including obesity [28], HDL cholesterol [29] dyslipidemia [30], blood pressure [31,32], and type 2 diabetes [33].

We are aware of only two studies investigating the relationship between ER α polymorphisms and metabolic syndrome by Goulart et al. and Lo et al. [34,35]. This is particularly relevant topic in view of the incidence of metabolic syndrome in the MENA region.

On the basis of genetic background of metabolic syndrome and possible association between ER α and metabolic syndrome, we investigated the relationship between metabolic syndrome and two intronic polymorphisms (*PvuII* and *XbaI*) of ER α in a sample of 250 overweight apparently healthy unrelated elderly.

2. Materials and methods

2.1. Subject selection

The current analyses stem from baseline data of a large randomized vitamin D clinical trial, NCT: 01315366 that enrolled 250 unrelated men and women between January 2011 and July 1, 2013. Briefly, all participants were free of prior history of cancer, chronic disease, autoimmune disease, diabetes, fragility fracture, vitamin D deficiency (<10 ng/ml) or any serious illness that might preclude participation at study entry.

2.2. Protocol description

Subjects enrolled in the above study completed a baseline questionnaire which included questions on demographics (age, marital status and level of education), health characteristics/behaviors (height, weight, waist circumference, alcohol use, smoking status, physical activity), menopausal status for women (age at menarche and age at menopause) and past medical history and actual treatments (history of hypertension, dyslipidemia, etc.). Peripheral venous blood samples were obtained from participants via an ante-cubital vein. This study was approved by the American University of Beirut Medical Center Institutional Review Board, and all subjects gave informed consent.

The primary outcome was metabolic syndrome status, which was defined according to the updated AHA/NHLBI definition. It includes the presence of ≥ 3 of the following: increased waist circumference (≥ 88 cm in women and 102 cm in men), elevated blood pressure ($\geq 130/85$ mmHg or treatment for high blood pressure), abnormal glucose metabolism as identified by a fasting blood glucose level of 100 mg/dl or higher, high triglyceride levels (triglyceride ≥ 150 mg/dl or treatment by fibrates derivatives) and low HDL levels (HDL < 40 mg/dl in men and < 50 mg/dl in women).

Chemistries (HDL, triglyceride, fasting blood glucose) were measured directly using fresh baseline blood samples, using Cobas 602 Roche Diagnostics. Blood pressure was measured using sphygmomanometer by health professionals.

3. Genotype Determination

Genomic DNA was isolated from peripheral leukocytes by standard procedures. The direct molecular haplotyping of the *PvuII* and *Xba I* restriction fragment length polymorphism (RFLPs) was performed. A 346-base pair (bp) polymerase chain reaction (PCR) fragment was generated by a forward primer (5'-GAT-ATC-CAG-GGT-TAT-GTG-GCA-3') and a reverse primer (5'-AGG-TGT-TGC-CTA-TTA-TAT-TAA-CCTGA-3') in a 10- μ l reaction mixture containing 20 ng genomic DNA, 50 mmol/L KCl, 10 mmol/L Tris HCl (pH 8.3), 1.5 mmol/L MgCl₂, 0.2 mmol/L deoxynucleoside triphosphate, 2 pmol/L of each primer, and 0.2 U of Super Taq DNA polymerase (HT Biotechnology, Cambridge, UK). The reactions were performed in a 384-well format on an MJ Tetrad thermocycler (MJ Research, San Francisco, CA) with a cycling protocol of 94 °C, 60 °C, and 72 °C for 45 s each, for 30 cycles. Ten microliters of PCR product was digested by simultaneously adding 5 μ l of digestion mixture containing 5 U of *PvuII*, 7 U of *XbaI* restriction enzyme (both from MBI Fermentas, Hanover, MD), and 1.5 μ l of REact Buffer 2 (Life Technologies, Breda, The Netherlands) and incubating for 90 min at 37 °C. The digestion products were analyzed by electrophoresis in a 3% agarose gel in 0.5 \times TBE (1 \times TBE = 89 mmol/L Tris, 89 mmol/L boric acid, 2 mmol/L Na₂EDTA) for 80 min at 125 V. Separation patterns were documented with a DC120 digital camera (Kodak, Rochester, NY) under ultraviolet illumination (302 nm). To compare ER *PvuII*-*XbaI* haplotype frequencies between our study population and the populations in Oxford, UK and Seta, Japan, inferred haplotype frequencies for those studies were calculated to correspond with those derived from our direct haplotyping method. In this way, the frequencies of identical alleles in the different study populations could be compared. For the Seta study, haplotype frequencies were derived from published genotype frequencies. Haplotype combinations could be unambiguously determined for all genotypes except the *PpXx* genotype. This genotype was assumed to consist of a combination of the haplotypes *px* and *PX*, because subjects homozygous for the alternative haplotype (*px*) were not found in any of the populations studied. The capital P and X denote that *PvuII* and *XbaI* restriction sites are absent, the small p and x denote that *PvuII* and *XbaI* sites are present [36].

4. Statistical analysis

This research investigated the association of the ER α gene polymorphisms with metabolic syndrome and its components traits in overweight elderly subjects. Each component of the metabolic syndrome (triglyceride, HDL, glucose, waist circumference, blood pressure) was coded into a binary variable (present = 1, absent = 0), and a cumulative metabolic syndrome components count [CS_{MS} : 0 (minimum) up to 5 (maximum)] was calculated as the algebraic sum of the five binary variables. Subjects were then accordingly grouped to metabolic syndrome positive ($CS_{MS} \geq 3$) or negative ($CS_{MS} < 3$) which constituted our primary comparison. Demographics, risk factors, genotyping and blood chemistry data were compiled for each of the metabolic syndrome (Yes/No) groups for univariate comparisons. Categorical variables were

expressed as (N, frequency) and were compared using Chi square tests. Continuous variables were expressed as mean \pm SD for normally distributed variables and median [minimum–maximum] if non-normally distributed. Normality of distribution was evaluated using Shapiro–Wilk test. Continuous variables were compared by Analysis of Variance (ANOVA) (normally distributed) or the Kruskal Wallis rank sum test (not normally distributed) as appropriate. Independent t-test was used to compare mean of the continuous variables between groups of including age, blood pressure, HDL, triglyceride, Body Mass Index (BMI) and other continuous variables between genders and between patients with and without metabolic syndrome. Chi square was used to study the relationship between ER α diplotypes and genotypes distribution by gender and absence or presence of metabolic syndrome and metabolic syndrome components count. The relationship of metabolic syndrome or its components and ER α diplotypes and genotypes was further evaluated using binary logistic regression models, where metabolic syndrome or its components were entered as outcome variables, and the predictors were diplotypes or genotypes of the ER α gene. Odd ratio [95% confidence interval] was calculated before and after adjustment for age, smoking, BMI and gender. Separate models were built for metabolic syndrome and for each component. These analyses were stratified by gender. Because there was a little representation of some diplotypes in the study population as follows: N = 48 PXPX, N = 11 PXPx, N = 108 PXpx, N = 6 PpXx, N = 12 Ppxx and N = 65 ppxx, we combined the diplotypes with small N into one category, and diplotypes were therefore categorized into 4 groups as follows: N = 48 PXPX, N = 108 PXpx, N = 65 ppxx and rare haplotypes in our population N = 29. To overcome allele coding discordance between our group and others, the allele coding was converted using the method by Bergink et al., where T allele was converted to C and A allele was converted to G. C, T and G, A were equivalent to P, p and X, x respectively [36]. Statistical analyses were performed using SPSS version 20.0 (IBM, USA). P-values < 0.05 were considered significant and were not corrected for multiple testing.

5. Results

250 Caucasian unrelated study subjects (140 women, 110 men) were studied, median age of 71 years (Table 1). Men were taller, heavier, and had lower BMI than women [Table 1]. Women were more likely to be obese, 54% of women versus 30 % in men, while men were more likely to be overweight (Table 1). There was no difference in systolic or diastolic blood pressure between men and women (Table 1), and medians for triglyceride, HDL, fasting glucose were within the normal ranges in both genders (Table 1). The majority of study subjects had normal fasting glucose, triglyceride and HDL levels (Table 1), but women had higher median HDL than men ($p < 0.001$) (Table 1). Over 70% of subjects were hypertensive and had a waist circumference above upper limit, and a large proportion, that is 45% had metabolic syndrome. Women were more likely than men to have an elevated waist hip circumference and to have metabolic syndrome (Table 1).

The *PvuII* and *XbaI* genotypes frequencies were within the Hardy–Weinberg Equilibrium ($\chi^2 = 1.01$, $p = 0.31$ and $\chi^2 = 0.56$,

Table 1 – Overall and gender demographic and clinical characteristics.^a

	Overall (N = 250)	Women (N = 140)	Men (N = 110)	P value ^b
Age (years)	71 [65–91]	69 [65–81]	72 [65–91]	<0.001
Anthropometric Measurements				
Height (cm)	157.6 ± 8.7	152.7 ± 6.5	164 ± 6.8	<0.001
Weight (kg)	73 [50.5–124.5]	71.75 [50.5–124.5]	75 [52–104]	0.001
BMI (kg/m ²)	29.2 [25–48.4]	30.9 [25–48.4]	27.8 [25–38.6]	<0.001
BMI categories				
Overweight ^c	56.8%	46.4%	70%	<0.001
Obesity ^d	43.2%	53.6%	30%	
Waist circumference (cm)	102.7 [56.3–138]	103 [73–138]	101.3 [56.3–127]	0.17
Biochemical characteristics				
Triglyceride (mg/dl)	118 [48–574]	121[48–419]	110 [49–574]	0.17
HDL (mg/dl)	49 [18–102]	53 [29–102]	43 [18–82]	<0.001
Fasting Glucose (mg/dl)	93.9 ± 9.5	94.4 ± 9.2	93.4 ± 9.9	0.41
Blood Pressure				
Systolic blood pressure (mmHg)	130 [95–186]	130 [100–186]	130 [95–185]	0.63
Diastolic blood pressure (mmHg)	75 [50–108]	75 [50–108]	75 [50–100]	0.57
Metabolic syndrome components count				
HDL				
Normal	160 (64%)	82 (58.6%)	78 (70.9%)	0.04
<40 mg/dl in men and <50 mg/dl in women	90 (36%)	58 (41.4%)	32 (29.1%)	
Blood Pressure				
Normal	71 (28.4%)	38(27.1%)	33 (30%)	0.98
≥130/85 or on antihypertensive treatment	179 (71.6%)	102 (72.9%)	77 (70%)	
Glycaemia				
Normal	182 (72.8%)	102 (72.9%)	80 (72.7%)	0.61
≥100 mg/dl.	68 (27.2%)	38 (27.1%)	30 (27.3%)	
Waist circumference				
Normal	67 (26.8%)	11 (7.9%)	56 (50.9%)	<0.001
≥102 cm in men and ≥88 cm in women	183 (73.2%)	129 (92.1%)	54 (49.1%)	
Triglycerides				
Normal	171 (68.4%)	95(67.9%)	34 (30.9%)	0.83
≥150 mg/dl or on fibrate derivatives	79 (31.6%)	45(32.1%)	76 (69.1%)	
Metabolic syndrome				
No	138 (55.2%)	62 (44.3%)	76 (69.1%)	<0.001
Yes	112 (44.8%)	78 (55.7%)	34 (30.9%)	
Smoking				
Non-smoker	190 (76%)	107(76.4%)	83 (75.5%)	0.85
Smoker	60(24%)	33 (23.6%)	27 (24.5%)	
Anti-hyperlipidemia Treatment				
No	172 (68.8%)	85 (60.7%)	87 (79.1%)	0.007
Yes	78 (31.2%)	55 (39.2%)	23 (20.9%)	
Anti-hypertension Treatment				
No	126 (50.4%)	59 (42.1%)	67 (60.9%)	0.003
Yes	124 (49.6%)	81 (57.9%)	43 (39.1%)	

¶ 29.2% were on statins and 2% on fibrates derivatives.

^a Continuous variables are summarized as median [minimum–maximum] or mean ± Standard Deviation with statistical comparison using Mann–Whitney and T-test respectively. Categorical variables are summarized as count (%) with statistical comparison using Chi-square.

^b P value for difference between genders.

^c Overweight BMI between 25 to 29.9 kg/m².

^d Obesity if BMI ≥30 kg/m².

$p = 0.45$, respectively). ER α diplotypes and genotypes frequencies, in the overall subjects and stratified by gender are shown as follows: the distribution of *PvuII-XbaI* diplotypes was: TATA ($pxpx$) = 26%, CGTA ($PXpx$) = 43.2% and CGCG ($PXPX$) = 19.2%, others (rare diplotypes in our population) = 11.6%. *XbaI* genotypes variants were distributed as follows: AA (xx) = 33.2%, GA (Xx) = 47.6%, GG (XX) = 19.2%. The distribution of *PvuII* genotypes variants was: TT (pp) = 26%, CT (Pp) = 48% and CC (PP) = 26%.

No significant differences were observed in *XbaI*, *PvuII* genotypes or ER α diplotypes in subjects with or without

metabolic syndrome in the overall group (Table 2), or in subgroup analyses by gender [Appendix I].

There were no differences in the mean or median for the various variables used as criteria for defining the metabolic syndrome by ER α diplotypes [Appendix II], *XbaI* genotypes [Appendix III] or *PvuII* genotypes [Appendix IV] in the overall group or in subgroup analyses by gender (Data not shown).

In binary logistic regression analyses, no significant association was found between the ER α gene polymorphisms

Table 2 – Frequency distribution of ER α diplotypes and genotypes using XbaI and PvuII, in subjects with and without metabolic syndrome.^a

	Metabolic Syndrome				P value ^b
	No (N = 138)		Yes (N = 112)		
ER α c diplotypes	N	%	N	%	0.42
pxpx(TATA)	31	(22.5%)	34	(30.4%)	
PXpx(CGTA)	61	(44.2%)	47	(42%)	
PXPX (CGCG)	27	(19.6%)	21	(18.8%)	
Others ^d	19	(13.8%)	10	(8.9%)	
XbaI genotypes					0.74
xx (AA)	43	(31.2%)	40	(35.7%)	
Xx (GA)	68	(49.3%)	51	(45.5%)	
XX (GG)	27	(19.6%)	21	(18.8%)	
PvuII genotypes					0.36
pp(TT)	31	(22.5%)	34	(30.4%)	
Pp(CT)	69	(50%)	51	(45.5%)	
PP (CC)	38	(27.5%)	27	(24.1%)	

Diplotypes and genotypes frequencies did not differ significantly from those expected under Hardy–Weinberg Equilibrium.

^a Categorical variables are summarized as count (%) with statistical comparison using Chi square test.

^b P value for differences between metabolic syndrome (Yes/No).

^c Estrogen Receptor alpha.

^d "Others" represents rare diplotypes in our population.

and the presence or absence of metabolic syndrome in the overall group of subjects, or by gender, both in the unadjusted and adjusted analyses (Tables 3–5). Similarly, there was no association between ER α polymorphisms and each of the individual components of the metabolic syndrome (hypertension, hyperglycemia, high waist circumference, hypertriglyceridemia, low HDL) in the overall group of subjects, or by gender (Tables 3–5).

6. Discussion

6.1. Summary of our findings

Our study explored the impacts of two intronic polymorphisms of the ER α in the metabolic syndrome in the Lebanese Caucasian elderly population. There was no association of XbaI, PvuII diplotypes, and genotypes, with metabolic syndrome, or with any of its components, in the study group of 250 elderly Lebanese with a high prevalence of metabolic syndrome at study entry.

6.2. Estrogen receptor α and metabolic syndrome

The present study supports previous studies that show that there is no association between ER α polymorphisms, mainly PvuII and XbaI, and metabolic syndrome in elderly subjects [34,35]. Goulart et al. did not observe any associations of the tested ER α genotypes (PvuII and XbaI) with metabolic syndrome in 532 Caucasian females with median age of 63 years, in the Women's Health Study (WHS) [34]. This finding is also in the same direction as the Study of Women's Health Across the

Nation (SWAN) genetic study, a community based sample of 1520 pre-menopausal African–American, Caucasian, Chinese and Japanese women aged 42–52 years, who were not using exogenous hormones [35]. Statistically significant relationships between ER β , but not ER α , and metabolic syndrome were observed in Chinese women, but not in Caucasian women or other ethnic groups [35]. In contrast, Gallagher et al., using a family-based approach, found that several polymorphisms of ER α , including XbaI were associated with increased risk of metabolic syndrome, OR = 1.53 (95% CI, 1.05–2.27), but not with individual metabolic traits in younger or middle aged African–American families from the IRAS Family Study. However, the study did not specify the genotype responsible for this association, and only studied it in one ethnic group [37].

6.3. ER α polymorphisms and metabolic syndrome components

6.3.1. Estrogen Receptor α and HDL

It is reported that estrogens increase apoA-I production in hepatic cells by increasing the transcription of the apoA-I gene [38]. This mechanism was clearly demonstrated in a study by Lu et al. on 98 Japanese postmenopausal women, with familial hypercholesterolemia, carrying XX (GG) genotype (wild genotype), where the lack of estrogen leads to a decrease in HDL, apoA-I and apoA-II levels. However, in the same study, women with xx (AA) genotype (mutant genotype) had higher levels of apoA-I, apoA-II and HDL [29]. In the present study, neither XbaI genotypes nor PvuII genotypes were associated with HDL before and after controlling for age, BMI and smoking. Thus, our findings are in accordance with those previously described in the multiethnic groups in the SWAN study [39], in 299 Chinese subjects [40], 397 Iranian subjects [41], 532 American Caucasians in the WHS study [34], 113 Hungarian subjects [42] and 370 Greek subjects [43]. The differing results by Lu et al. may be partially explained by the phenotype of familial hypercholesterolemia of the population studied [29].

6.3.2. Estrogen receptor α and triglycerides

This study shows no association between ER α diplotypes and metabolic syndrome components both before, and after controlling for age, BMI and smoking. This result contradicts the finding of Huang et al., where triglyceride was associated with XbaI genotypes and not diplotypes, in 299 healthy women [40].

6.3.3. Estrogen receptor α and fasting blood sugar

The complex estrogen–estrogen receptor may indirectly affect insulin sensitivity by releasing insulin from granules into the circulation and by regulating β cells proliferation [44]. Hence, the lack of estrogen may lead to insulin resistance and impaired glucose tolerance. In the present study, selected PvuII and XbaI encoding ER α , were not associated with fasting blood sugar both before and after adjusting for age, BMI and smoking. These findings were consistent with Massart et al. [45] who show no association with XbaI either in 99 Italian elderly patients with abdominal aortic aneurysm or in controls, as well as those in 341 Chinese healthy women before and after controlling for sex and age [40].

Other studies reported an association between XbaI and diabetes where X (G) allele was associated with high fasting

Table 3 – Unadjusted and adjusted Odds ratios (OR) for the presence or absence of metabolic syndrome and its components according to PvuII-XbaI diplotypes in the overall group of subjects and by gender.

	Diotypes	N	Metabolic syndrome	Hypertension	Hyperglycemia	High Waist Circumference	Hypertriglyceridemia	Low HDL
Unadjusted								
Overall OR [95% CI]	pxpx (Reference)	65	1.0	1.0	1.0	1.0	1.0	1.0
	PXpx	108	0.70 [0.37-1.30]	0.71 [0.35-1.44]	0.95 [0.48-1.91]	1.08 [0.52-2.22]	0.81 [0.43-1.56]	0.81 [0.43-1.53]
	PXPX	48	0.70 [0.33-1.50]	0.72 [0.31-1.70]	0.97 [0.42-2.24]	0.71 [0.31-1.65]	0.70 [0.31-1.56]	0.75 [0.34-1.63]
	Others	29	0.48 [0.19-1.18]	0.57 [0.21-1.48]	0.99 [0.37-2.64]	0.53 [0.20-1.36]	0.44 [0.15-1.24]	0.78 [0.31-1.56]
Women OR [95% CI]	pxpx (Reference)	65	1.0	1.0	1.0	1.0	1.0	1.0
	PXpx	108	0.58 [0.25-1.31]	0.56 [0.22-1.47]	0.59 [0.23-1.50]	1.20 [0.30-4.77]	0.79 [0.34-1.83]	1.06 [0.47-2.39]
	PXPX	48	0.8 [0.29-2.14]	0.52 [0.17-1.59]	1.10 [0.39-3.13]	1.44 [0.24-8.48]	0.66 [0.23-1.88]	0.75 [0.27-2.03]
	Others	29	0.9 [0.26-3.4]	0.83 [0.18-3.75]	1.45 [0.39-5.38]	-	0.50 [0.11-2.11]	0.84 [0.23-3.04]
Men OR [95% CI]	pxpx (Reference)	65	1.0	1.0	1.0	1.0	1.0	1.0
	PXpx	108	1.03 [0.37-2.81]	0.97 [0.33-2.83]	1.68 [0.56-5]	1.33 [0.50-3.52]	0.86 [0.31-2.37]	0.57 [0.20-63]
	PXPX	48	0.59 [0.16-2.17]	1.16 [0.30-4.44]	0.79 [0.19-3.30]	0.49 [0.14-1.66]	0.76 [0.21-2.68]	0.76 [0.21-2.68]
	Others	29	0.25 [0.04-1.37]	0.50 [0.13-1.86]	0.73 [0.15-3.46]	0.42 [0.11-1.56]	0.41 [0.09-1.83]	0.80 [0.21-3.07]
Adjusted^a								
Overall OR [95% CI]	pxpx (Reference)	65	1.0	1.0	1.0	1.0	1.0	1.0
	PXpx	108	0.72 [0.38-1.36]	0.79 [0.38-1.65]	1.01 [0.50-2.04]	1.46 [0.59-3.59]	0.78 [0.40-1.50]	0.78 [0.41-1.49]
	PXPX	48	0.82 [0.38-1.8]	0.96 [0.40-2.31]	1.11 [0.47-2.63]	1.38 [0.49-3.90]	0.66 [0.29-1.50]	0.76 [0.34-1.70]
	Others	29	0.50 [0.20-1.29]	0.67 [0.25-1.83]	1.06 [0.39-2.87]	0.87 [0.26-2.89]	0.42 [0.15-.21]	0.79 [0.31-1.99]
Women OR [95% CI]	pxpx (Reference)	65	1.0	1.0	1.0	1.0	1.0	1.0
	PXpx	108	0.64 [0.27-1.50]	0.72 [0.26-1.95]	0.65 [0.25-1.71]	0.25 [0.39-16.97]	0.77 [0.32-1.82]	1.2 [0.52-2.76]
	PXPX	48	0.86 [0.31-2.41]	0.76 [0.23-2.46]	1.31 [0.44-3.94]	31.51 [0.31-32.08]	0.64 [0.21-1.86]	0.83 [0.29-2.33]
	Others	29	0.91 [0.24-3.41]	0.80 [0.16-3.86]	1.29 [0.33-4.97]	-	0.52 [0.12-2.24]	0.88 [0.24-3.25]
Men OR [95% CI]	pxpx (Reference)	65	1.0	1.0	1.0	1.0	1.0	1.0
	PXpx	108	0.81 [0.26-2.50]	1.10 [0.35-3.43]	1.60 [0.51-4.99]	1.32 [0.36-4.83]	0.92 [0.31-2.78]	0.41 [0.13-1.26]
	PXPX	48	0.63 [0.14-2.67]	1.72 [0.41-7.13]	0.82 [0.18-3.62]	0.77 [0.14-4.10]	0.88 [0.22-3.38]	0.61 [0.16-2.36]
	Others	29	0.34 [0.05-2.22]	0.81 [0.20-3.29]	0.83 [0.16-4.23]	1.42 [0.26-7.81]	0.49 [0.09-2.44]	0.74 [0.17-3.14]

^a Adjusted for age, BMI and smoking.

Table 4 – Unadjusted and adjusted Odds ratios (OR) for the presence or absence of metabolic syndrome and its components according to XbaI genotypes in the overall group of subjects and by gender.

	XbaI	N	Metabolic syndrome	Hypertension	Hyperglycemia	High Waist Circumference	Hypertriglyceridemia	Low HDL
Unadjusted								
Overall OR [95% CI]	xx (Reference)	83	1.0	1.0	1.0	1.0	1.0	1.0
	Xx	119	0.80 [0.45–1.41]	0.78 [0.41–1.46]	0.96 [0.51–1.79]	1.32 [0.70–2.49]	0.80 [0.44–1.46]	0.86 [0.48–1.55]
	XX	48	0.83 [0.40–1.70]	0.82 [0.37–1.82]	0.96 [0.43–2.15]	0.89 [0.41–1.93]	0.76 [0.35–1.65]	0.79 [0.37–1.67]
Women OR [95% CI]	xx (Reference)	83	1.0	1.0	1.0	1.0	1.0	1.0
	Xx	119	0.58 [0.27–1.26]	0.55 [0.22–1.37]	0.55 [0.23–1.30]	1.16 [0.29–4.58]	0.74 [0.33–1.64]	1.11 [0.52–2.38]
	XX	48	0.78 [0.3–2.03]	0.51 [0.17–1.50]	0.97 [0.35–2.67]	1.23 [0.21–7.24]	0.68 [0.24–1.88]	0.78 [0.29–2.08]
Men OR [95% CI]	xx (Reference)	83	1.0	1.0	1.0	1.0	1.0	1.0
	Xx	119	1.21 [0.49–3]	1.11 [0.44–2.74]	1.86 [0.70–4.90]	1.53 [0.66–3.57]	0.90 [0.36–2.22]	0.60 [0.23–1.50]
	XX	48	0.78 [0.23–2.70]	1.44 [0.42–4.89]	0.90 [0.23–3.48]	0.63 [0.20–1.94]	0.89 [0.27–2.90]	0.79 [0.24–2.55]
Adjusted^a								
Overall OR [95% CI]	xx (Reference)	83	1.0	1.0	1.0	1.0	1.0	1.0
	Xx	119	0.84 [0.47–1.52]	0.85 [0.44–1.64]	1.01 [0.53–1.92]	1.81 [0.81–4.04]	1.81 [0.81–4.04]	0.84 [0.47–1.52]
	XX	48	0.81 [0.38–1.75]	1.05 [0.46–2.4]	1.10 [0.48–2.51]	1.65 [0.62–4.34]	1.65 [0.62–4.34]	0.81 [0.38–1.75]
Women OR [95% CI]	xx (Reference)	83	1.0	1.0	1.0	1.0	1.0	1.0
	Xx	119	1.23 [0.56–2.72]	0.73 [0.28–1.88]	0.63 [0.26–1.54]	2.89 [0.45–18.59]	2.89 [0.45–18.59]	1.23 [0.56–2.72]
	XX	48	0.86 [0.31–2.40]	0.77 [0.24–2.42]	1.21 [0.41–3.55]	3.12 [0.30–31.7]	3.12 [0.30–31.7]	0.86 [0.31–2.40]
Men OR [95% CI]	xx (Reference)	83	1.0	1.0	1.0	1.0	1.0	1.0
	Xx	119	0.45 [0.16–1.21]	1.13 [0.43–2.97]	1.77 [0.65–4.84]	1.26 [0.41–3.83]	1.26 [0.41–3.83]	0.45 [0.16–1.21]
	XX	48	0.66 [0.19–2.26]	1.86 [0.52–6.71]	0.93 [0.23–3.68]	0.71 [0.15–3.27]	0.71 [0.15–3.27]	0.66 [0.19–2.26]

^a Adjusted for age, BMI and smoking.

Table 5 – Unadjusted Odds ratios (OR) for the presence or absence of metabolic syndrome and its components according to PvuII genotypes in the overall group of subjects and by gender.

	PvuII	N	Metabolic syndrome	Hypertension	Hyperglycemia	High Waist Circumference	Hypertriglyceridemia	Low HDL
Unadjusted								
Overall OR [95% CI]	pp (Reference)	65	1.0	1.0	1.0	1.0	1.0	1.0
	Pp	120	0.67 [0.36–1.23]	0.72 [0.36–1.46]	0.99 [0.50–1.94]	0.98 [0.48–1.97]	0.82 [0.43–1.54]	0.80 [0.43–1.50]
	PP	65	0.64 [0.32–1.29]	0.62 [0.28–1.36]	0.92 [0.42–2]	0.68 [0.31–1.47]	0.55 [0.26–1.18]	0.76 [0.37–1.56]
Women OR [95% CI]	pp (Reference)	65	1.0	1.0	1.0	1.0	1.0	1.0
	Pp	120	0.60 [0.26–1.34]	0.59 [0.23–1.51]	0.65 [0.26–1.60]	1.31 [0.33–5.20]	0.81 [0.35–1.85]	1.05 [0.47–2.33]
	PP	65	0.84 [0.33–2.11]	0.56 [0.19–1.62]	1.16 [0.44–3.07]	1.88 [0.32–10.98]	0.55 [0.20–1.49]	0.76 [0.30–1.92]
Men OR [95% CI]	pp (Reference)	65	1.0	1.0	1.0	1.0	1.0	1.0
	Pp	120	0.91 [0.34–2.44]	0.97 [0.34–2.77]	1.62 [0.55–4.74]	1.14 [0.44–2.94]	0.84 [0.31–2.26]	0.59 [0.21–1.63]
	PP	65	0.46 [0.13–1.56]	0.73 [0.23–2.36]	0.66 [0.17–2.49]	0.48 [0.16–1.45]	0.56 [0.17–1.83]	0.80 [0.25–2.48]
Adjusted^a								
Overall OR [95% CI]	pp (Reference)	65	1.0	1.0	1.0	1.0	1.0	1.0
	Pp	120	0.68 [0.36–1.27]	0.81 [0.39–1.67]	1.03 [0.52–2.06]	1.28 [0.53–3.07]	0.78 [0.41–1.48]	0.78 [0.41–1.46]
	PP	65	0.76 [0.37–1.55]	0.81 [0.36–1.82]	1.06 [0.47–2.34]	1.40 [0.53–3.69]	0.52 [0.24–1.14]	0.78 [0.37–1.62]
Women OR [95% CI]	pp (Reference)	65	1.0	1.0	1.0	1.0	1.0	1.0
	Pp	120	0.66 [0.29–1.51]	0.73 [0.27–1.95]	0.70 [0.27–1.78]	2.71 [0.41–17.90]	0.80 [0.34–1.85]	1.18 [0.52–2.70]
	PP	65	0.88 [0.34–2.3]	0.77 [0.25–2.33]	1.33 [0.48–3.65]	3.97 [0.41–38.39]	0.53 [0.19–1.46]	0.82 [0.31–2.12]
Men OR [95% CI]	pp (Reference)	65	1.0	1.0	1.0	1.0	1.0	1.0
	Pp	120	0.75 [0.24–2.3]	1.12 [0.37–3.4]	1.56 [0.51–4.73]	1.27 [0.35–4.49]	0.89 [0.30–2.59]	0.45 [0.15–1.32]
	PP	65	0.55 [0.13–2.17]	1.16 [0.33–4.08]	0.70 [0.17–2.87]	1.07 [0.24–4.82]	0.69 [0.19–2.51]	0.65 [0.19–2.23]

^a Adjusted for age, BMI and smoking.

insulin [46] with low post prandial c-peptide [28], and was an independent risk factor for type II diabetes [33]. Similarly, the p (T) (wild) allele was associated with increased insulin sensitivity [37], whereas the p(C) allele was associated with high insulin level [47], type 2 diabetes, OR = 4.34 (95% CI, 1.61–11.86) [33] higher blood glucose OR = 1.77 (95% CI, 1.09–2.87) and higher post prandial blood glucose, OR = 2.11 (95% CI, 1.29–3.44) [40]. Subjects in the above studies were diabetic or highly predisposed to diabetes, whereas our study subjects were for the most part normo-glycemic.

6.3.4. Estrogen receptor α and waist circumference and measures of adiposity

Estrogen drives deposition of fat into the gynecoid region, while lack of estrogen results in android fat deposition [48]. Although there has been some controversy about the relationship between ER α polymorphisms and measures of obesity (BMI and waist circumference), studies were more likely to be positive [49–55]. In the NILS-LSA study and Huang et al., the X (G) allele was associated with high BMI in 1110 Japanese middle aged women [46] and 108 postmenopausal women [53]. The Framingham Heart Study, investigated the relationship between ER α and BMI as well as waist circumference, and showed that men with XX (GG) had the lowest waist circumference compared to xx (AA) [54]. Possible explanations for our null findings may be that obesity is a complex disease trait, and multiple genes are likely involved. Therefore, it is not surprising that individual genes may not consistently exert large effects.

6.3.5. Estrogen receptor α and blood pressure

There is a wealth of physiological evidence for the expression of the ER α genes in vascular and myocardial cells in humans and animals, and for the role of these receptors in mediating vasomotor tone and protecting against vascular injury [56]. There is evidence to suggest that ER α may be the more important receptor in mediating vasodilatation [57]. In the present study, no evidence for an association between ER α polymorphisms and blood pressure was observed, either in the total group, or when stratifying by gender.

This study has a few limitations. It is not population-based, is of relatively small sample size, and of homogenous ethnicity, factors that may have accounted for the lack of significant findings. However, others studies with a large sample size also did not find any solid association with metabolic syndrome and or its components [34,35]. However, to the best of our knowledge, this is the first study in the Middle East and North Africa, in a group of subjects with a high prevalence of metabolic syndrome and in both genders, reflecting findings in the region, that investigated the association between ER α polymorphisms, and metabolic syndrome and each of its components in overweight elderly unrelated men and women.

7. Conclusion

Metabolic syndrome is a consequence of multiple gene-environment interactions. The worldwide gradual increase in prevalence of obesity as well as obesity related metabolic syndrome, in general and in the Middle East in particular,

renders investigations regarding the underpinnings of the above factors a priority. This increase indicates the importance of environmental influences, such as low levels of physical activity and availability of calorie rich diets that characterize modern societies. However, identification of susceptibility genes for metabolic syndrome and, their functional variants is of utmost importance, as it may enable investigators to design preventive strategies and targeted treatments. The candidate gene approach relies on prior knowledge of biological pathways and association with the phenotype of interest. Unfortunately, to date, few are the large genome-wide association studies that have been conducted in relation to metabolic syndrome. This highlights the need for large scale prospective studies in other ethnic groups.

Author contributions

Dr. Maha Hoteit conceived the research question, protocol design, conducted the literature review, contributed to data acquisition, conducted data analyses and interpretation, drafted and finalized the manuscript. Dr. Asma Arabi contributed to data acquisition, interpretation of results and drafting of the manuscript. Dr. Robert Habib contributed to statistical analyses and interpretation of data. Dr. Rami Mahfouz led the haplotyping of the Estrogen Receptor alpha and contributed to interpretation of analyses. Dr. Rafic Baddoura and Dr. Georges Halaby contributed to revisions of manuscript content. Dr. Ghada El-Hajj Fuleihan has made substantial contributions to conception and design, acquisition of data, analysis and interpretation of data; participated in drafting the manuscript and revising it critically for important intellectual content. All authors approved the final submitted version of the submitted manuscript.

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Conflict of interest

The authors have no conflicts of interests to disclose.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.metabol.2013.08.004>.

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