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Associations of Estrogen Receptor Alpha and Beta Gene Polymorphisms with Lipid Levels and Insulin Resistance in Men

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ABSTRACT

Objective. The association of four single nucleotide polymorphisms in estrogen receptor alpha (*ESR1*) and beta (*ESR2*) genes with lipid levels and insulin resistance in men.

Design and methods. Lipids, glucose, insulin and HOMA-IR were determined, in a population-based, cross-sectional, cohort of 170 apparently healthy middle-aged Greek men, along with body mass index (BMI), waist circumference (WC) and percentage of body fat content (%fat). Genotyping of *ESR1 for Pvu*II and *Xba*I and *ESR2 for Rsa*I and *Alu*I polymorphisms was performed.

Results. Associations of *Alu*I with LDL-Chol (mean ± SD, aa 4.3 ± 1.1 vs. Aa 3.7 ± 1.0 and AA 4.2 ± 1.1, p = 0.023) and *Rsa*I with HOMA-IR [median (IQR), RR 1.55 (0.88–2.49) vs. Rr/rr 1.69 (0.72–2.29), p = 0.032] were found. Synergistic effects of *Rsa*I and *Alu*I of *ESR2* gene on LDL-Chol levels, %fat and WC, as well as a synergistic effect of both *ESR1* and *ESR2* genes on levels of TChol (p = 0.01) and LDL-Chol (p = 0.027) were also shown. These findings remained significant after adjustment for potential confounders.

Significant independent associations of *Pvu*II with %fat (mean ± SD, pp 24.6 ± 5.3 vs Pp 22.4 ± 5.2 and PP 21.2 ± 6.7, p = 0.044), and RsaI with %fat (RR 22.6 ± 5.5 vs. Rr/rr 25.2 ± 6.3, p = 0.015) and WC (mean ± SD, RR 97.4 ± 10.4 vs. Rr/rr 102.6 ± 12.6, p = 0.013) were found.

Synergistic effects on %fat, between the *ESR1* polymorphisms (p = 0.004), between the ESR2 polymorphisms and among all four ESR polymorphisms studied were also present.

Conclusions. ESR2 is associated with LDL-Chol levels and HOMA-IR in men independently of confounders. Body fat is affected by both genes. Furthermore, a synergistic effect of *ESR1* and *ESR2* on TChol, LDL-Chol and %fat, was shown.

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Abbreviations: %fat, percentage of body fat content; BMI, body mass index; DBP, diastolic blood pressure; *ESR1*, estrogen receptor alpha; *ESR2*, estrogen receptor beta; HDL-Chol, HDL-cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; IQR, interquartile range; LDL-Chol, LDL-cholesterol; RFLP, restriction fragment length polymorphism; SBP, systolic blood pressure; SD, standard deviation; TChol, total cholesterol; Trigs, triglycerides; WC, waist circumference.

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1. Introduction

There is evidence that genetic variation in estrogen receptor alpha (*ESR1*) and beta (*ESR2*) genes is associated with increased cardiovascular risk [1,2], premature ischemic heart disease [3], and unfavorable angiographic changes [4] in both sexes. However, there are data contrasting these observations [5].

Through their receptors, estrogens can modulate cardiovascular risk indirectly via alterations in lipid and glucose metabolism, and directly via their vascular and endothelial effects. Functionally, competent estrogen receptors have been identified in vascular smooth muscle cells, and specific binding sites have been demonstrated in endothelium [6]. Furthermore, apart from the classical genomic effect involving gene expression, non-transcriptional mechanisms for estrogen receptor signaling have also been described [7,8].

On the other hand, genetic association studies have previously shown that *ESR1*[9–11] and *ESR2*[12] gene polymorphisms are associated with lipid levels and interact with hormone replacement therapy [12]. An effect on insulin sensitivity and incidence of type 2 diabetes has also been described [13,14]. However, the effect has not been consistent across different ethnic groups [15,16], across ages [16] and, finally, it was more prominent in females than in males [16,17].

Based on the facts that *ESR2* gene is not included in most association studies, data on males are scarce and inconclusive and there exists significant ethnicity-related variability, we tested the hypothesis that polymorphisms in both genes encoding estrogen receptors (*ESR1* and *ESR2*) might have an impact on somatometric parameters, serum lipid levels and insulin resistance in Caucasian apparently healthy males.

2. Materials and methods

2.1. Sample selection

A total of 170 apparently healthy men, Caucasians, ethnic Greeks, aged 22–59 years (mean age ± SD, 42 ± 8.9), were included in the study. Subjects with diagnosed diabetes mellitus, arterial hypertension, cardiovascular disease, renal or hepatic disease, androgen deficiency and those using any medication were excluded from the study.

The men enrolled in the study were randomly selected from a larger cluster random sample that was drawn from the adult population of the region of Thessaly, Central Greece [18]. The original sample was composed of adults, aged 18–79 years, who had been residents of the region of Thessaly for at least one year. Briefly, the total population of adults was divided into geographical clusters corresponding to the four provinces of the region. The sample of this study was subsequently selected randomly from a) the capital of each province and b) randomly selected smaller towns and villages. The sample size was proportional to the population of the capital and of each village and town according to the 2001 census conducted by the National Statistical Service. Only one adult was selected randomly from each household. An interview in a public health office was arranged for each participant, who was instructed to report in the following morning at 9.00 a.m. after an overnight fast.

2.2. Study protocol

Each participant completed a questionnaire inquiring about health status and health behaviors (smoking and alcohol intake) under a doctor's supervision. Subsequently, each man underwent a thorough physical examination. Anthropometric measurements, such as height, weight and waist circumference (WC), systolic (SBP) and diastolic blood pressure (DBP) were measured in all study participants. Waist circumference was measured using a plastic non-stretchable tailors measuring tape, at a level midway between the lower rib margin and iliac crest to the nearest centimeter. The body mass index (BMI) was calculated according to Quetelet's formula as body weight in kilograms divided by the square of the height in meters.

The body fat content (% fat mass) was assessed by means of bioelectrical impedance analysis, using a commercially bio-impedance analyzer (Omron HBF 302 Body Fat Analyzer, Omron Healthcare, Vernon Hills, IL) with the patient in standing position.

Finally, plasma glucose and serum insulin, total cholesterol, HDL-cholesterol and triglycerides were measured in early morning samples drawn at the fasting state. LDL-cholesterol was estimated with Friedwald method (for triglyceride values not exceeding 400 mg/dl). Homeostasis Model Assessment was used for the calculation of HOMA-IR with the formula: fasting insulin (μU/ml) * fasting glucose (mmol/L)/22.5 [19]. All participants gave written informed consent and the study protocol was reviewed and approved by the Ethical Committee of the University Hospital.

2.3. Assays

All plasma and serum parameters – except insulin – were measured with standard commercially available assays in an Olympus AU600 analyzer (Olympus Optical, Tokyo, Japan). Serum insulin levels were measured using IRMA (Immunotech Beckman Counter Company, Marseille, France). The assay sensitivity for serum insulin measurements was 0.5 IU⁄mL and the interassay coefficient of variation 3.4%.

2.4. Genotyping

All study participants were genotyped for the *Pvu*II (rs2234693) and *Xba*I (rs9340799) polymorphisms of the *ESR1* gene and for the *Alu*I (rs4986938) and *Rsa*I (rs1256049) polymorphisms of the *ESR2* gene. Genomic DNA was extracted from peripheral leucocytes by standard procedures.

For *ESR1* gene, 1.3 kb DNA fragment (which contained intron 1 and a part of exon 2) was amplified using the following primers: (5′-CTGCCACCCTATCTGTATCTTTTCCTATTCTCC-3′ and 5′-TCTTTCTCTGCCACCCTGGGGTCGATTATCTGA-3′).

Polymerase chain reaction (PCR) amplification was performed for 35 cycles at an annealing temperature of 63 °C. Amplified DNA was digested with *Pvu*II or *Xba*I (New England Biolabs, Ipswich, MA) at 37 °C for 3 hours and then electrophoresed in a 1.5% ethidium bromide agarose gel. *Pvu*II digestion reveals genotypes denoted PP (1.3 kb), Pp (1.3 kb, 850 bp, 450 bp) and pp (850 bp, 450 bp), and *Xba*I digestion results in genotypes XX (1.3 kb), Xx (1.3 kb, 910 bp, 390 bp) and xx (910 bp, 390 bp). The restriction fragment length

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