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# Estrogen receptor alpha XbaI polymorphism is associated with higher relaxin-3 levels in women with metabolic syndrome

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## Abstract

Relaxin-3 is known to play a role in appetite regulation, increasing food intake and body weight. This study aimed to assess the relation of relaxin-3 with ERS1 *Xba*I and *Pvu*II polymorphisms. One hundred female patients with metabolic syndrome were investigated. The genotypes of ESR1 *Xba*I and *Pvu*II polymorphisms were assessed with PCR-RFLP. Serum relaxin-3 level was measured for all participants using ELISA. Higher relaxin-3 levels showed association with the minor GG genotype of ESR-1 *Xba*I polymorphism but not *Pvu*II polymorphism.

## Keywords

*Plicosepalus acacia*
Loranthaceae
Antimicrobial activity

## 1. Introduction

Relaxin-3 (also known as INSL7) is an insulin/relaxin superfamily neuropeptide that was first discovered in 2002 (Bathgate et al. 2002). It is involved in the regulation of food intake, stress response, and arousal and exploratory behaviors (Hu et al., 2016).

Metabolic syndrome is a combination of several physiological, biochemical, clinical, and metabolic factors that increase the risk of cardiovascular disease, type 2 diabetes mellitus. Insulin resistance, visceral adiposity, atherogenic dyslipidemia, endothelial dysfunction, genetic susceptibility, elevated blood pressure, prothrombotic state, and chronic stress are the several factors that constitute the syndrome (Kaur, 2014). The prevalence of metabolic syndrome in women appears to be increasing, especially in those of childbearing age (Ramos and Olden, 2008).

Levels of estrogen or its receptors were linked to metabolic syndrome in both humans and experimental animals (Matic et al., 2013). Estrogen signaling is mediated by multiple receptors. Most of

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the known estrogenic effects are mediated via direct interaction of estrogen with the DNA-binding transcription factors, estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ) (Dahlman-Wrigh et al., 2006). Estrogen receptor alpha (ESR1) gene codes for ERα. The human ESR1 gene is located on the long arm of chromosome 6 (6q25.1) and contains 8 exons separated by 7 intronic regions (Dunbier et al., 2011). Several single nucleotide polymorphisms (SNPs) of the ESR1 gene have been identified. The most extensively investigated SNPs of the ESR1 gene have been, *Xba*I (rs9340799) and *Pvu*II (rs2234693), which are both in the first intron (Molvarec et al., 2007).

This study aimed to investigate the possible relation of ESR1 *Pvu*II and *Xba*I polymorphisms with the relaxin-3 serum level in Egyptian women with metabolic syndrome in Suez Canal Area.

## 2. Materials and methods

### *2.1. Study population*

The study was conducted on 100 female metabolic syndrome patients. They were selected from the Outpatient Clinic of the Suez Canal University Hospital. metabolic syndrome was diagnosed according to the Third Report of the National Cholesterol Education Program's Adult Treatment Panel (ATPIII); where metabolic syndrome is diagnosed by the presence of any three or more of the following factors: waist circumference ≥88 cm in women; fasting blood glucose (FBG) ≥ 100 mg/dL or known diabetes; serum TG ≥ 150 mg/dL; HDL-C < 50 mg/dL in women; and blood pressure ≥ 130/85 mmHg or treated hypertension (NCEP, 2002).

No smokers were included in the study. Subjects suffering from heart failure, myocardial infarction, diabetes mellitus type I, any type of cancer, renal failure or chronic liver disease were excluded. Pregnant or lactating women and those receiving hormone replacement therapy or contraceptive medications were also excluded. All participants had regular menstruation.

The present study was conducted according to the principles of the Declaration of Helsinki, and all the participants provided written informed consent following a protocol approved by the Faculty of Pharmacy, Suez Canal University Research Ethics Committee.

### *2.2. Relaxin-3 measurement*

Peripheral blood was drawn after a 12 h fast and a portion was collected with EDTA and used for DNA extraction. The serum was separated from the remaining portion of the blood samples and used for measurement of relaxin-3 level using EIAab human relaxin-3 ELISA kit (Wuhan EIAab Science Co., Ltd., China).

### *2.3. Genomic DNA extraction and genotyping*

Genomic DNA was isolated from 300 μL of whole blood collected in EDTA anticoagulated tubes using the Wizard genomic DNA purification kit (Promega, Madison) according to the manufacturer's instructions. The *Xba*I and *Pvu*II polymorphisms within the ESR1 gene were detected using polymerase chain reaction-based restriction fragment length polymorphism (PCR-RFLP). A fragment of 1300 base pairs (bp) was amplified by a forward primer (5′-

CTGCCACCCTATCTGTATCTTTTCCTATTCTC C-3′) and a reverse primer (5′-TCTTTCTCTGCCACCCTGGCGTCGATTATCTG A-3′). PCR amplifications were performed in a total volume of 50 µL containing 250 ng genomic DNA, 25 µL Go Taq® Green Master Mix, 2× (Promega, Madison) and 0.2 µmol/L of each primer (Ghattas et al., 2013a).

Thermal cycling conditions were as follows: an initial denaturation step at 95 °C for 5 min; followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 53 °C for 40 s and extension at 72 °C for 90 s; with a final extension at 72 °C for 7 min. Thermal cycling was performed in an Eppendorf Mastercycler® machine (Eppendorf, Hamburg, Germany). The PCR products were digested with *Xba*I and *Pvu*II restriction enzymes (Promega, Madison) at 37 °C for 4 h (Ghattas et al., 2013a).

Digestion with *Xba*I, produced two fragments of 900 + 400 bp (A allele) while undigested product produced a single 1300 bp fragment (G allele) **(Figure 1)**, and digestion with *Pvu*II produced two fragments 850 + 450 bp (T allele) while undigested product produced a single 1300 bp fragment (C allele) **(Figure 2)**. The cleavage products were electrophoresed on a 1% agarose gel.

### *2.4. Statistical analysis*

Hardy-Weinberg's equilibrium was evaluated using Chi-square test. Associations of genotypes with the metabolic syndrome component traits and relaxin-3 were analyzed by one way analysis of variance (ANOVA) test followed by Tukey's test for multiple comparisons. Statistical analyses were performed with the SPSS ver. 20 software package. A value of p

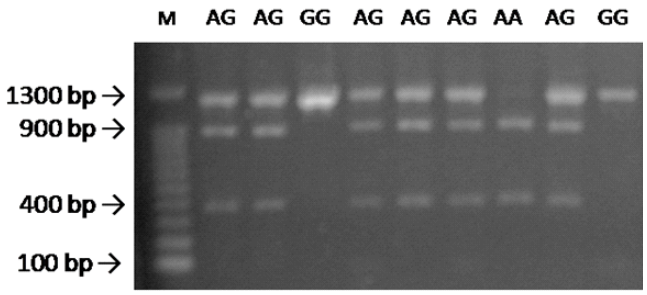


**Figure 1: Detection of ESR1 *Xba*I genotypes by agarose gel electrophoresis. Samples containing two types of fragments (900bp and 400 bp) have genotype AA; samples with only one type of fragment (1300 bp) represent genotype GG; and samples with all three types of fragments (1300 bp, 900 bp and 400 bp) are of genotype AG.**

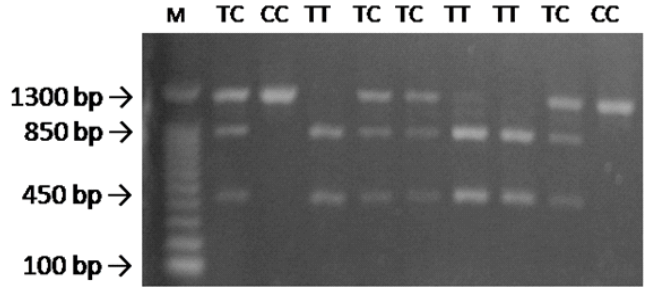


**Figure 2: Detection of ESR1 *Pvu*II genotypes by agarose gel electrophoresis. Samples containing two types of fragments (850pb and 450 bp) have genotype TT; samples with only one type of fragment (1300 bp) represent genotype CC; and samples with all three types of fragments (1300 bp, 850 bp and 450 bp) are of genotype TC.**

< 0.05 was considered statistically significant. Data are expressed as mean ± standard deviation (SD).

## 3. Results and discussion

ERα has been found to play a role in glucose and lipid homeostasis in several tissues including the brain, skeletal muscle, adipose tissue, pancreas, liver, and heart; that may have important implications to risk factors associated with the metabolic syndrome (Barros and Gustafsson, 2011).

The genotype distribution of ESR1 *Xba*I and *Pvu*II polymorphisms in the study sample is presented in

**Table 1: Observed and expected allele frequency and genotype distribution of ESR1 *Xba*I and *Pvu*II polymorphisms in 100 metabolic syndrome patients.**

| ***Xba*I polymorphism** | | | | ***Pvu*II polymorphism** | | | |
|---|---|---|---|---|---|---|---|
| **Genotypes** | **Observed** | **Expected** | ***p*** | **Genotypes** | **Observed** | **Expected** | ***p*** |
| **AA** | 15 | 18.06 | 0.210 | **TT** | 30 | 27.04 | 0.235 |
| **AG** | 55 | 48.87 | | **TC** | 44 | 49.92 | |
| **GG** | 30 | 33.06 | | **CC** | 26 | 23.04 | |
| **Allele** | | | | **Allele** | | | |
| **A** | 85 (42.5%) | | | **T** | 104 (52%) | | |
| **G** | 115 (57.5%) | | | **C** | 96 (48%) | | |

**Table 1**. The percentage of the minor G allele of *Xba*I polymorphism is 57.5%, whereas the percentage of the minor C allele of *Pvu*II polymorphism is The frequency of the genotypes is in accordance with Hardy-Weinberg's equilibrium.

As shown in **Table 2**; serum relaxin-3 was significantly higher in carriers of the minor GG genotype of ESR-1 *Xba*I polymorphism compared to carriers of the major AA genotype ($p < 0.014$). There was no significant difference between the serum relaxin-3 levels in the three different genotypes of ESR-1 *Pvu*II polymorphism.

These results match with previous findings; where the minor G allele of *Xba*I polymorphism of the ESR1 gene, was found to be associated with metabolic syndrome, whereas *Pvu*II polymorphism showed no association with metabolic syndrome (Ghattas et al., 2013a). Relaxin-3 was considered to be a potential marker of metabolic syndrome where serum levels of relaxin-3 were higher among metabolic syndrome female patients compared to normal subjects (Ghattas et al., 2013b).

This study assumes a possible role of estrogen in regulation of relaxin-3 secretion. This assumption can be supported by the suggested role of relxin-3 in regulation of the hypothalamic-pituitary-gonadal axis (McGowan et al., 2008). The interaction between the hypothalamic-pituitary-gonadal axis and estrogen is well known (Chrousos et al., 1998). Relaxin is concerned with follicle growth, ovulation, implantation and placental establishment (Anand-Ivell and Ivell, 2014). Additionally, relaxin-3 was found to be associated with lipid accumulation in adipocytes (Yamamoto et al., 2014).

## 4. Conclusion

The GG genotype of ESR1 *Xba*I polymorphism was significantly associated with increased serum relaxin-3 levels in women with metabolic syndrome. In contrast, ESR1 *Pvu*II polymorphism genotypes showed no association with serum relaxin-3 levels.

**Table 2: Serum relaxin-3 level in carriers of the different genotypes of ESR-1 gene *Xba*I and *Pvu*II polymorphisms.**

| ***Xba*I genotypes** | **Serum relaxin-3 level (ng/mL)** | ***Pvu*II genotypes** | **Serum relaxin-3 level (ng/mL)** |
|---|---|---|---|
| **AA** | 130.03 ± 27.40 | **TT** | 134.08 ± 28.32 |
| **AG** | 134.83 ± 28.02 | **TC** | 134.27 ± 27.50 |
| **GG** | 143.60 ± 27.01* | **CC** | 142.14 ± 27.94 |

Data are presented as mean ± SD. Comparisons were performed by with way ANOVA test followed by the Tukey's test for multiple comparison. * indicates significant difference from carriers of AA.

## 5. Conflict of interest

The authors report no declaration of conflict of interest.

## 6. Acknowledgements

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