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Associations of *ESR1* and *ESR2* gene polymorphisms with metabolic syndrome and its components in postmenopausal women

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ABSTRACT

Objectives: Metabolic syndrome (MS) affects a quarter of Polish people and is associated with diabetes mellitus type 2 and ischemic heart disease. The prevalence of MS in postmenopausal women can be increased by the lack of protective effects of oestrogens. In the near future, because of the general increase in life expectancy, the number of postmenopausal women will rise substantially. Therefore, investigating both the environmental and the genetic factors predisposing to MS may have a great impact on women's health. The aim of this study was to determine whether particular oestrogen receptor (ESR) gene polymorphisms can predispose to the development of MS in women after menopause.

Study design: The sample consisted of 147 postmenopausal women. In addition to collecting medical history and analyzing body composition using the TANITA scale, patient's waist size, blood pressure, serum lipids, glucose, insulin, C-reactive protein and adiponectin were measured. The analysis of ESR gene polymorphisms was performed using the Sequenom MassARRAY platform.

Results: Three out of ten analyzed polymorphisms in the ESR1 gene (rs2234693, rs6902771, rs7774230) and one out of eight analyzed polymorphisms in the ESR2 gene (rs3020449) were associated with MS. The ESR1 rs2234693, rs6902771 and rs7774230 polymorphisms were associated with serum concentrations of high-density lipoproteins. The ESR2 rs3020449 polymorphism was associated with serum concentrations of total cholesterol and low-density lipoprotein. Four ESR1 polymorphisms (rs1709183, rs2234693, rs6902771, rs7774230) were associated with total fat tissue content.

Conclusions: Bearing the particular alleles at the ESR gene polymorphisms may impact the development of MS and some of the ESR polymorphisms may influence serum cholesterol concentrations in women after menopause.

1. Introduction

Metabolic syndrome (MS) is a constellation of risk factors, which, when not treated, leads to the development of type 2 diabetes mellitus (T2DM) and coronary heart disease (CHD). It is a prevalent condition, which is associated with the growing epidemic of sedentary lifestyle [1]. MS is diagnosed when at least three out of five following risk factors are present: central obesity, elevated serum triglycerides, low HDL cholesterol concentrations, impaired fasting glucose and elevated systolic (SBP) or/and diastolic blood pressure (DBP) [2]. Women after menopause have a higher risk of MS development due to the lack of oestrogens, which have been shown to possess favourable metabolic effects. They prevent visceral fat accumulation and thus improve insulin sensitivity and its metabolic consequences [3–6]. Nowadays, because of the extension of life expectancy, the number of postmenopausal women constantly increases. Therefore, investigating both the environmental and genetic factors predisposing to the development of MS has a great impact on women's health.

Oestrogens act through two types of nuclear receptors: ERα and ERβ encoded by two different genes (*ESR1* located on the chromosome 7 and *ESR2* located at the chromosome 14, respectively) [7]. Additionally, there are different genetic variants (polymorphisms) of the *ESR1* and *ESR2* genes. Previous research has shown associations between having a particular *ESR* gene polymorphisms and the development of severe diseases in postmenopausal women such as breast, endometrial and ovarian cancer [8–10], T2DM [11], osteoporosis [12] as

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well as depressive disorders [13].

Therefore the aim of this study was to find out whether bearing the particular alleles at the *ESR1* and *ESR2* gene polymorphic sites can be associated with the presence of MS in post-menopausal women.

2. Methods

2.1. Study design

The study was approved by the Ethical Committee of the Medical University of Gdańsk (approval number NKEBN/11/2011), and all the subjects gave a written consent to participate. In order to estimate if the size of the study cohort power calculations were performed using a website based "Genetic Power Calculator" (http://zzz.bwh.harvard.edu/gpc/). According to these calculations a number of at least 138 patients was required to detect a particular association between SNP of the ESR1 or ESR2 and the presence of the MS in postmenopausal women with the power of 80% at the alpha level of 0.05. The study cohort was planned to be 150 women, but few of them resigned during the study for not known reasons. Finally, the study cohort consisted of 136 postmenopausal women aged 47–80 from the Pomeranian region of Poland. All the participants were recruited at the outpatient medical practice by a single physician—a specialist in internal medicine and endocrinology (D.R.) from 2011 until 2014. Postmenopausal status was defined as the absence of menstrual periods for at least the previous 12 months. None of the participants were taking oestrogen/progestin replacement therapy and were relatively healthy. The exclusion criteria were chronic or serious illnesses such as cancer, asthma, rheumatoid arthritis or the presence of cardiovascular disease. Body mass and body composition were evaluated using a TANITA scale (TANITA Corporation, Medconsulting, Poland). According to the manufacture's manual instructions all participants were told to avoid strenuous exercises and excessive food or drink intake 3 h before the measurement. Moreover, the women were asked to urinate before the measurement and check whether their feet are clean. The accuracy of the scale is 0,2 kg. Waist circumference was measured above the anterior superior iliac spines at the end of expiration using the inextensible tape. Blood pressure was measured using the Omron M3 device 3 times after a 5 min rest, and the average was calculated from the last two measurements. MS was defined according to a Joint Scientific Statement (JSS) ofimportant health organizations from 2009 [2], where the presence of at least 3 of the following risk factors is mandatory: central obesity (waist circumference $\geq$80 cm), elevated serum triglycerides concentrations ($\geq$150 mg/dl) or pharmacological treatment for hypertriglyceridemia, lowered HDL cholesterol concentration ($<$ 50 mg/dl) or pharmacological treatment of low HDL-cholesterol levels, elevated blood pressure (systolic [SBP] $>$ 130 mmHg or diastolic [DBP] $>$ 85 mmHg) or pharmacological treatment of hypertension, impaired fasting glucose (IFG $\geq$ 100 mg/dl) or pharmacological treatment of hyperglycaemia.

2.2. Laboratory analyses

Blood samples were drawn in the morning after an overnight fasting period at the medical diagnostic laboratory (Bruss, ALAB Group, Poland), which possesses current ISO certificates and has an accreditation in the field of medical laboratory diagnostics. Serum C-reactive protein (CRP), glucose, insulin, triglycerides (TG), HDL- and total-cholesterol (TCh) concentrations were measured in an electrochemiluminescence immunoassay using Roche Diagnostics-Cobas ECLIA system commercial kits. Serum LDL-cholesterol concentrations were calculated using the Friedewald equation taking into the account its limitations (*i.e.* serum TG $>$ 400 mg/dl). Serum adiponectin concentrations were measured using the immunoenzymaticcommercial kit (ELISA) (R&D Systems) according to manufacturer's instructions. Indices of insulin resistance and sensitivity were calculated using the Homeostasis Model Assesment of Insulin Resistance (HOMA-IR) and Quantitative Insulin Sensitivity Check Index (QUICKI), respectively using the following formulas: HOMA-IR = [serum glucose concentration (mmol/L)] $\times$ [serum insulin concentration (μU/ml)] / 22.5; QUICKI = 1/[log(serum glucose concentration (mmol/L)) + log (serum insulin concentration (μU/ml)].

2.3. DNA isolation

Blood was also drawn into a 5 ml test tubes containing EDTA for the isolation of DNA using a commercially available kit ("Blood Mini", A&A Biotechnology, Gdańsk, Poland) according to the instructions of the manufacturer.

2.4. Evaluation of ESR1 and ESR2 polymorphisms

Ten single nucleotide polymorphisms (SNPs) in the *ESR1* (rs2234693, rs9340799, rs1709181, rs1709183, rs2175898, rs6902771, rs712221, rs7774230, rs827417, rs9322331) and eight SNPs in the *ESR2* gene (rs1256049, rs4986938, rs1255998, rs2987983, rs3020449, rs3020450, rs944050, rs1887944) were evaluated. Genotype analyses were performed on a Sequenom MassARRAY platform (Agena Biosciences, formerlySequenom, San Diego, CA, USA) according to manufacturer's instructions using the iPLEX Gold complete reagents kit.

2.5. Statistical analyses

The Shapiro-Wilk test was used to check the distribution of the acquired data. Normally distributed variables were expressed as arithmetic mean and standard deviation (SD). Non-normally distributed variables are presented as median and interquartile range (Q1–Q3). Genotype frequencies, Hardy–Weinberg equilibrium proportions and the association between the *ESR1* and *ESR2* polymorphisms were evaluated using the chi-squared test. Analysis of variance (ANOVA) was used to evaluate the relationship between the presence of the particular genotypes and the components of the MS, indices of insulin resistance/sensitivity, anthropometric measurements and body composition characteristics. In case of revealing statistically significant differences, the NIR post-hoc test was used. Comparisons between women with the MS and those who did not fulfil the MS criteria were carried out using the student's *t*-test or its non-parametric equivalent, the Mann-Whitney *U* test.

3. Results

The characteristics of all the study participants are shown in Table 1. The prevalence of MS in our cohort was 52% (n = 71). Among women with the MS, 96% had central obesity, 83% had elevated SBP or/and DBP, 76% had elevated serum TG concentrations, 54% had low HDL-Ch concentrations, and 51% had IFG. All genotype frequencies, apart from the *ESR1* rs9322331 polymorphism, were in Hardy-Weinberg equilibrium.

Three out of the ten analysed *ESR1* polymorphisms (rs2234693,

Table 1
Characteristics of the studied women (n = 136).

	Arithmetic mean ± SD	Median (Q1–Q3)
Age (years)	58 ± 6	57 (53–62)
Age at menopause (years)	50 ± 3	50 (48–52)
Time from menopause (years)	8.0 ± 6.6	7.0 (2.0–12.5)
Body weight (kg)	72.0 ± 11.9	69.6 (63.4–78.2)
Waist circumference (cm)	94.8 ± 10.9	93.0 (87.0–100.5)
BMI	27.0 ± 4.3	26.4 (23.9–29.3)

SD, standard deviation, Q1–Q3, interquartile range.