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Research paper

ESR2 gene G1730A variant is associated with triglycerides level and myocardial infarction in young men but not in women

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ABSTRACT

Objectives: The aim of the study was to investigate the role of estrogen receptor type 2 gene (*ESR2*) variant G1730A in myocardial infarction (MI) in young age.
Methods: Genotyping was performed with restriction fragments length polymorphism method in 158 patients (79.1% men) with MI aged < 50 years (studied group) and in control groups: 150 healthy individuals aged < 50 years (63.3% men) and 202 patients (64.3% men) with MI aged ≥ 50 years.
Results: The AA genotype of *ESR2* G1730A variant was significantly more frequent in men with MI aged < 50 comparing to men with MI aged ≥ 50 (21.6% vs. 8.4%, $P = 0.004$) and to healthy young men (21.6% vs. 11.6%, $P = 0.048$). There was statistically significant difference between AA genotype and GA + GG genotypes male carriers with MI aged < 50 in median triglyceride (TG) level (2.0 vs. 1.7 mmol/l respectively, $p = 0.023$).
Conclusions: Our findings suggest a possible role of *ESR2* G1730A variant as the risk factor of MI in a young age not as an independent but a potential risk factor associated with TG level in men but not in women.

1. Introduction

Distinct incidence of myocardial infarction (MI) in pre-menopausal women and men at the same age is thought to be associated with the influence of estrogens on the development of atherosclerosis. The biological effect of estrogens depends mostly on the expression of their two nuclear receptors – ERα, encoded by *ESR1* gene and ERβ encoded by *ESR2* – both acting through genomic as well as non-genomic mechanisms, in a different manner in men and in women (Traupe et al., 2007). Action of estrogens receptors modulates lipid metabolism, endothelial function, atherothrombotic plaque status, mediates acute vasodilation of epicardial coronary arteries, affects coronary artery vasoreactivity and expresses the cardioprotective effect in ischemia reperfusion injury (Deschamps et al., 2010; Kim et al., 2008).

Estrogen β receptor seems to be particularly important for vascular effect of estrogens since heat shock protein 27 (HSP27), a biomarker of atherosclerosis secreted at reduced level in affected arteries, was identified as an ERβ-associated protein modulating estrogen signaling (Miller et al., 2005). The ablation of the ERβ gene decreases post ischemic functional recovery in female, but not in male hearts (Wang et al., 2008). Moreover, chronic estrogen action and activation of the ERβ could lead to the increased NO/SNO signaling and thus play an essential role in cardioprotection which seems to be particularly important in women (Lin et al., 2009).

The expression of ERβ might be associated with a *ESR2* gene variant leading to the modulation of coronary artery disease (CAD) risk factors, in a different manner, regarding gender, race, population or ethnicity. There have been several *ESR2* gene variants reported to be involved in this process. For instance in the North American population women, but not men with history of MI were more likely to have the T allele of the rs1271572 variant and less likely to have the allele A of the rs1256049 variant (Rexrode et al., 2007). On the other hand, rs1271572 variant T allele was associated with increased risk of MI in Spanish men but not in women (Domingues-Montanari et al., 2008). G1730A (rs4986938) variant of the *ESR2* was associated with cardiovascular risk factors as obesity and cholesterol level in postmenopausal women undergoing coronary angiography (Saltiki et al., 2009). Although the genetic background seems to be particularly important in young patients, there

Abbreviations: ACS, acute coronary syndrome; BMI, body mass index; CAD, coronary artery disease; ESR1, estrogen receptor type 1; ESR2, estrogen receptor type 2; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MI, myocardial infarction; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; TG, triglycerides

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is a scarce data regarding the association of estrogen receptors genes variants with premature CAD in association with classical risk factors and gender.

The aim of this study was to investigate the prevalence of the *ESR2* G1730A (rs4986938, *Alu*I) variants in young Caucasian patients with MI and thus its possible role in the pathogenesis of premature CAD in association with lipids level. We sought to determine particularly whether the studied genetic variants could account for the differences in susceptibility to CAD between young men and women. Moreover, we analyzed clinical risk factors of premature MI and the possible association between the genetic variants of the *ESR2* and components of the metabolic syndrome as a condition predisposing to the development of CAD in young age.

2. Study population and methods

2.1. Study population

The examined population included 510 subjects, participants of the recently published study (Ambroziak et al., 2018). The studied group consisted of 158 patients (125 men and 33 women, 79.1% vs. 20.9%) aged under 50 (from 26 to 49, mean 44 years), hospitalized in the Department of Cardiology Medical Centre of Postgraduate Education, Grochowski Hospital in Warsaw, Poland due to the first episode of acute coronary syndrome (ACS). The ACS was defined on the basis of clinical symptoms (stenocardial pain), ecg (non-ST elevation and ST elevation myocardial infarction) and troponin level.

There were two control groups in the study. The first one – the healthy control group – consisted of 150 healthy people aged 30–49, mean 42 years, 95 men (63.3%) and 55 women (36.7%) without history of CAD, randomly recruited from the healthy blood donors in co-operation with the Regional Blood Centre. The second control group consisted of 202 patients aged over 50 (from 50 to 92, mean 65 years), 130 men (64.3%) and 72 women (35.7%), hospitalized due to the first episode of ACS (fulfilling the criteria mentioned above).

The data regarding co-morbidities, including ongoing treatment were collected based on the patient's questionnaire and physical examination on admission. Hypertension was assessed based on the medical history and treatment or based on mean value of two measurements of systolic (SBP) and diastolic (DBP) blood pressure performed after at least 5 min sitting, made in 5 min interval. Hypertension was defined as values ≥140 mmHg SBP and/or ≥90 mmHg DBP accordingly to ESH/ESC (European Society of Hypertension, European Society of Cardiology) guidelines (Mancia et al., 2013). Diabetes mellitus was assessed based on the medical history and treatment or based on fasting plasma glucose ≥126 mg/dl (7.0 mmol/l) or ≥ 200 mg/dl (11.1 mmol/l) in oral glucose tolerance test according to WHO and ADA (American Diabetes Association) guidelines (American Diabetes Association, 2010). Depression and smoking status, including duration and intensity (cigarettes number per day) of smoking, were assessed based on history of patient. BMI was calculated as weight (kg)/height (m^2).

Blood samples were collected on admission (for glucose at admission) and next morning (for other parameters). Biochemical analyses, including glucose, total, HDL and LDL cholesterol as well as triglycerides (TG) plasma concentrations were performed in fasting blood samples (except but glucose at admission) by standard enzymatic methods using COBAS INTEGRA 800 regents and equipment (Roche Diagnostics Gmbh).

The participants gave a written informed consent for participation in the study. The investigation conforms to the principles outlined in the Declaration of Helsinki and the study protocol was approved by the Ethical Committee of the Medical Centre of Postgraduate Education.

2.2. Genotyping

Genomic DNA was extracted from peripheral blood mononuclear cells by the salting-out method (Miller et al., 1988). Genotyping of the selected polymorphism in the studied gene was performed by PCR amplification followed by digestion with a restriction enzyme (restriction fragment length polymorphism method – RFLP) as described previously (Roszkowska-Gancarz et al., 2010). Shortly, a 307 bp fragment of the *ESR2* gene was amplified using the following primers: forward 5′ TTTTTGTCCCCATAGTAACA 3′ and reverse 5′AATGAGGGACCACAGCA 3′. PCR conditions were as follows: initial denaturation 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, annealing at 57 °C for 30 s, then extension at 72 °C for 30 s, and a final step at 72 °C for 5 min. Each 12.5 µl reaction contained 50 ng of DNA, 2.0 mM $MgCl_2$, 10 pmol of each primer, 0.25 mM of each deoxynucleoside triphosphate and 1 unit of *Taq* polymerase (Invitrogen Carlsbad, USA) in a corresponding buffer. 2.5 µl of the PCR product was digested with 1 unit of the *Alu*I restriction enzyme (Fermentas, Lithuania) in 65 °C for 3 h. The obtained restriction fragments were visualized on a 3% agarose gel. Presence of the *Alu*I restriction site (the A allele) resulted in two fragments: 243 bp and 64 bp, whereas in case of the G allele – the PCR 307 bp PCR product remained undigested (Fig. 1).

2.3. Statistical analysis

All analyses were performed using GraphPadPrism Version 6.07 software (GraphPad Software, Inc., La Jolla, USA) and Statistica Version 12 software package (StatSoft Inc., Tulsa, USA). For all tests, the level of significance was established at 0.05. The comparison of the studied groups including all clinical data was performed using *t*-test (for age) and Mann-Whitney *U* test for others clinical and biochemical data.

The genotype distribution was analyzed using the Web-Assotest program (available at: http://www.ekstroem.com/assotest/assotest.html) assuming dominant and recessive models of inheritance. Under each model, the odds ratio (OR) with a 95% confidence interval (CI) and the *p* value for an association were calculated. Based on the observed alleles frequency, the expected frequency of genotypes was calculated and compared by the χ^2 test with 3 × 2 table to assess the Hardy-Weinberg equilibrium. The observed genotypes distribution in all studied groups and subgroups was in Hardy-Weinberg equilibrium. Power analysis was performed using the DSS software available online http://www.dssresearch.com.

The multivariate logistic regression model was performed to investigate the association between independent covariates and binary endpoints. Gender, BMI, smoking status, co-morbidities (hypertension,

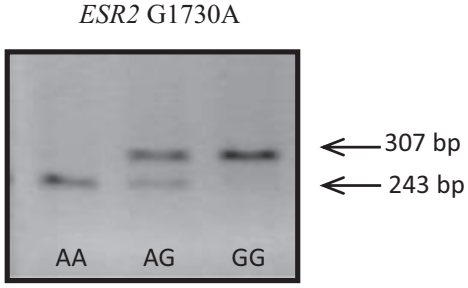

Fig. 1. Restriction fragments of studied gene *ESR2* G1730A variant in agarose gel. Presence of the *Alu*I restriction site (the A allele) resulted in two fragments: 243 bp and 64 bp, whereas in case of the G allele – the PCR 307 bp PCR product remained undigested. The AA genotype was recognized when the PCR product (307 bp) was completely digested into two fragments 243 bp and 64 bp (the shorter fragment was not visualized), the GG genotype when the PCR product remained undigested. Heterozygotic patients were identified were two bands (307 bp and 243 bp) were present (bp - base pairs, *ESR2* - estrogen receptor type 2 gene).