

Received: 2014.10.22  
Accepted: 2014.11.27  
Published: 2015.03.04

# Estrogen Receptor Alpha Polymorphisms, Estradiol Level, and Occurrence of Atherosclerosis Risk Factors in Healthy Postmenopausal Women

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**Source of support:** This study was sponsored by the Institute of Rural Health in Lublin, Poland

**Background:** The objective of the study was to analyze the relationship between interaction of polymorphisms in the estrogen receptor alpha gene ( $ER\alpha$ ) and estradiol (E2), and the occurrence of selected atherosclerosis risk factors in postmenopausal women without the diagnosis of a cardiovascular disease.





**Material/Methods:** The study covered 210 women, a minimum of 2 years after menopause, with FSH >30 mIU/ml, aged 50–60 years, with no chronic diseases diagnosed. In the women examined, the levels of estradiol, total cholesterol, HDL-cholesterol, LDL-cholesterol, and triglycerides were determined, as well as height, waist circumference (W), hip circumference (R), and arterial hypertension. The BMI and W/H ratio were calculated. Genotyping of the  $ER\alpha$  polymorphism was performed using a polymerase chain reaction and restriction enzymes (PCR-RFLP). The alleles of the *XbaI* polymorphism were defined as A and G: heterozygote AG, wild type GG and homozygote AA. The alleles of *PvuII* polymorphism were defined as T and C: heterozygote TC, homozygote TT, and wild type CC.

**Results:** The concentration of endogenous estradiol and  $ER\alpha$  *XbaI* and *PvuII* polymorphisms as independent parameters did not significantly affect the BMI, waist circumference, W/H ratio, levels of CHOL, HDL, LDL, TG, or LDL/HDL, nor the systole and diastole in the postmenopausal women in the study.

**Conclusions:** The presented study suggests that  $ER\alpha$  *XbaI* AA polymorphism may intensify the beneficial effect of estradiol on the distribution of fatty tissue after menopause;  $ER\alpha$  *XbaI* GG and *PvuII* TC genotypes may intensify the beneficial effect of estradiol on HDL level;  $ER\alpha$  *PvuII* TT genotype unfavorably modifies the relation between concentration of estradiol and systolic pressure after menopause.

**MeSH Keywords:** **Atherosclerosis • Estradiol • Estrogen Receptor alpha • Menopause**

**Full-text PDF:** <http://www.medscimonit.com/abstract/index/idArt/892831>

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## Background

Within the last decade, a decrease in mortality has been observed due to diseases developing on the background of atherosclerosis; however, despite this tendency, these diseases are still the main cause of death in many countries [1]. Hence, it is necessary to apply prophylaxis, including, among other things, the control of risk factors. Many studies confirm that the main risk factors of atherosclerosis are obesity and lipid disorders [2]. Based on the results of studies, it was confirmed that the intensification of atherosclerotic changes is positively correlated with obesity and the levels of LDL and VLDL cholesterol, and negatively correlated with the level of HDL cholesterol, irrespective of race, age, and gender [3].

Population studies show that among young women, morbidity due to ischemic heart disease is approximately 6 times lower compared to males in analogous age groups [4]. Nevertheless, this gender-related difference decreases after menopause, when the frequency of occurrence of atherosclerosis and arterial hypertension in females drastically increases [5,6]. These observations suggest that the reduction in the level of endogenous estrogens after menopause may be a critical risk factor in these diseases. Studies of the genetic risk factors of cardiovascular diseases, including atherosclerosis, are still being continued. Although many candidate genes of cardiovascular diseases have been found, the role of genes participating in the impact of sex hormones still remains unclear. Estrogens exert an effect on the endothelium and smooth muscle cells in the vascular wall, where they inhibit cell proliferation and induce vasodilatation [7]. Estrogens also affect the hepatic cells, resulting in decreased LDL cholesterol fraction [8,9] and increase in HDL cholesterol fraction in blood [7,8]. Two estrogen receptors participate in the genome effect of estrogens (ER): alpha receptor (ER $\alpha$ , ESR1) and beta receptor (ER $\beta$ , ESR2) [10]. Both receptors are located on the endothelial cells and in the vascular smooth muscle cells, with the main protective role of the cardiovascular system ascribed to ER $\alpha$  [11]. The gene for this receptor is located on the long arm of chromosome 6, and contains 8 exons encoding receptor protein of 595 amino acids in length and a molecular weight of 66 kDa. Among many polymorphic variants of estrogen receptor gene, the most clinically important are 2 single-nucleotide polymorphisms (SNPs) detected by using restriction enzymes *PvuII* and *XbaI*. The *PvuII* and *XbaI* restriction fragment length polymorphisms of ER $\alpha$  gene are caused by changes in the sequence of DNA of single nucleotides in intron, located within a distance of 397 and 351 alkaline pairs (ap) from exon 2 [12]. Many studies have indicated that there is a relationship between *XbaI* and *PvuII* ER $\alpha$  polymorphism variants, and an increased risk of atherosclerosis in adults [13–16]. It is suggested that *PvuII* polymorphism plays a key role in the regulation of ER $\alpha$  protein expression, and its intensified transcription encoded by allele C

leads to the stronger expression of receptor, stronger estradiol binding of ER $\alpha$  in CC homozygotes, and a stronger effect of estradiol on a cell. The ER $\alpha$  polymorphisms may affect estrogen receptor function, and thus the tissue response to the stimulation with estrogen.

The objective of the study was analysis of an interaction between ER $\alpha$  polymorphism and estradiol, with the occurrence of selected risk factors of atherosclerosis in postmenopausal women without the diagnosis of a cardiovascular disease.

## Material and Methods

The study was conducted during 2011–2013 at the Institute of Rural Health in Lublin. The study group consisted of women from south-eastern Poland. The inclusion criteria were: age 50–65 years, good general health (lack of chronic diseases in medical history, including cardiovascular diseases), and education level at least completed elementary. The women were qualified to the study group also based on clinical symptoms (minimum 2 years from the last menstrual period) and based on the criterion of FSH level (FSH >30 mIU/ml). The exclusion criteria were: active cancerous disease within the period of 5 years after recruitment; addiction to drugs or alcohol; and diagnosed disease entity with the symptoms of dementia. We examined 210 postmenopausal women.

Prior to the collection of blood for laboratory examinations and genetic tests, the women were weighed and their height, waist circumference (W), and hip circumference (R) measured, as well as arterial hypertension. Based on the measurements obtained, the BMI (kg/height in m<sup>2</sup>) and W/H ratio were calculated.

The women under examination had their blood collected to measure the level of estradiol and samples were instantly taken to the laboratory. The determinations were performed by an accredited laboratory; the laboratory standard for estradiol is 0–44.5 pg/ml.

Lipid profile was determined using an automatic biochemistry analyser, Express Plus (Chiron Diagnostics, USA), with reagents by Siemens (Siemens Healthcare Diagnostics, Tarrytown, NY, USA), according to the procedure provided by the manufacturer. The following were determined in blood plasma: triglycerides, and total cholesterol and its fractions HDL cholesterol and LDL cholesterol.

Based on data from the literature, the values of the analyzed parameters that could create risk of cardiovascular diseases were determined: BMI  $\geq 25$  kg/m<sup>2</sup>, waist circumference >88 cm, W/H ratio  $\geq 0.85$ , total cholesterol  $\geq 191$  mg/dl, HDL

cholesterol  $\leq 64$  mg/dl, LDL cholesterol  $\geq 115$  mg/dl, triglycerides  $\geq 150$  mg/dl, LDL/HDL ratio  $>4$ , systole  $>140$  mmHg, and diastole  $>90$  mmHg.

Genomic DNA was isolated using the QIAmp DNA Blood Mini Kit (Qiagen, USA) according to the producer's instructions. Genotyping of the ER- $\alpha$  polymorphism was performed using a polymerase chain reaction and restriction enzymes (PCR-RFLP). PCR reaction was performed in a total amount of 50  $\mu$ l containing: 1 U (1  $\mu$ l) of DNA polymerase (Biotools), 1-PCR buffer (5  $\mu$ l) containing 15 mM MgCl<sub>2</sub> (Biotools), 2.5  $\mu$ l 2 mM dNTPs (final concentration 0.1 mM) (Fermentas, Vilnius, Lithuania), 1  $\mu$ l of 10  $\mu$ M of each primer, 34.5  $\mu$ l nuclease-free water (Applied Biosystems Inc., USA) and 5  $\mu$ l of genomic DNA. The polymerase chain reactions (PCR) were carried out with the following primers [17]:

Forward: 5'- CTG CCA CCC TAT CTG TAT CTT TTC CTA TTC TCC-3'

Reverse: 5'- TCT TTC TCT GCC ACC CTG GCG TCG ATT ATC TGA-3'.

The reactions were performed in a C1000 Thermal Cycler (BioRad) and consisted of the initial denaturation (3 min at 95°C) and 30 cycles, each of which included the proper denaturation (30 s at 95°C), primers annealing (50 s at 62°C), elongation (50 s at 72°C), and the final elongation (7 min at 72°C). Electrophoresis was performed in 2% agarose gel in standard conditions. The products of PCR (1372 bp) were digested overnight at 37°C using 2 separate restriction enzymes for determining the polymorphisms: *PvuII* (c.454-397 T>C) and *XbaI* (c.454-351 A>G). The products of restriction were electrophoresed in 2.5% agarose gel.

The alleles of the *XbaI* polymorphism were defined as A and G: heterozygote AG (fragments: 1372 bp, 936 bp, and 436 bp), wild type GG (fragment: 1372 bp), and homozygote AA (fragments: 936 bp and 436 bp). The alleles of *PvuII* polymorphism were defined as T and C: heterozygote TC (fragments: 1372 bp, 982 bp, and 390 bp), homozygote TT (982 bp and 930 bp), and wild type CC (1372 bp).

Statistical analysis was performed and graphs were created using the software STATISTICA. We estimated numbers (*n*) and frequency distributions (%) for categorical variables and for continuous variables we used means (*M*), reflecting the average level, as well as standard deviations (*SD*), measuring the degree of measurements variation around the arithmetic mean.

The analysis of variance F test was used to investigate whether the age at last menstruation period and lipid parameters depended on *XbaI* and *PvuII* polymorphisms. The  $\chi^2$  test for stochastic independence was applied to examine whether the frequencies of occurrence of cardiovascular risk factors depended on *XbaI* and *PvuII* polymorphisms.

Pearson's correlation coefficient was estimated to check the correlation between the level of estradiol and lipid parameters in the total group examined, and in the groups with *XbaI* and *PvuII* polymorphisms.

Considering the large size of the sample (N=210), border normal distributions of parameters' estimators were assumed. In statistical tests, the level of significance was set at  $p=0.05$ .

Informed consent for participation in the study was obtained from the women. The study was approved by the institute's Ethics Committee.

## Results

The study included 210 postmenopausal women. Table 1 presents their characteristics. Mean age of the women examined was  $56.5 \pm 3.5$  years. Their mean age at last menstruation was  $50.3 \pm 2.7$  years and the mean BMI was  $26.8 \pm 4.04$  kg/m<sup>2</sup>. Nearly a half of the women had secondary school education (47.62%), and 40% had university education, while 9.05% had basic vocational, and 3.33% had primary educational level. In the study group, 71 women (33.81%) had the genotype ER $\alpha$  *XbaI* AA, 104 (49.52%) were carriers of ER $\alpha$  *XbaI* AG, and 35 (16.67%) had the genotype ER $\alpha$  *XbaI* GG. With respect to ER $\alpha$  *PvuII* polymorphism, 57 (27.14%) of women had genotype TT, 97 (46.19%) had genotype TC, and 56 (26.67%) had genotype CC. Age at last menstruation was not significantly dependent on the possessed ER $\alpha$  *XbaI* polymorphism ( $F=0.853$ ,  $p=0.428$ ) or *PvuII* polymorphism ( $F=0.492$ ,  $p=0.612$ ).

Table 2 presents risk factors of cardiovascular diseases and estradiol level in the total examined group and according to ER $\alpha$  polymorphism. The values of analyzed parameters were not significantly dependent on ER $\alpha$  *XbaI* or *PvuII* polymorphisms possessed ( $p>0.05$ ). The mean waist circumference was  $1.54 \pm 12.34$  cm, and the W/H ratio  $0.79 \pm 0.09$ . The mean value of total cholesterol was  $229.09 \pm 42.58$  mg/dl, HDL cholesterol  $53.45 \pm 12.22$  mg/dl, LDL cholesterol  $145.84 \pm 43.70$  mg/dl, and triglycerides  $149.00 \pm 64.13$  mg/dl. The mean LDL/HDL ratio was  $2.91 \pm 1.15$ . The mean value of systolic pressure in the group examined was  $129.11 \pm 18.52$  mmHg, and diastolic pressure  $79.35 \pm 10.46$  mmHg. The mean level of estradiol in the women examined was  $23.53 \pm 20.45$  pg/ml.

Table 3 presents analysis of the occurrence of cardiovascular risk factors according to ER $\alpha$  *XbaI* and *PvuII* polymorphisms in the postmenopausal women in the study. There were 125 women (59.52%) with BMI  $\geq 25$  kg/m<sup>2</sup>, 71 women (33.81%) with waist circumference of more than 88 cm, and 61 (29.05%) women with W/H ratio  $\geq 0.85$ . There were 174 (82.86%) women with CHOL  $\geq 191$  mg/dl, 181 (86.19%) with HDL  $\leq 64$  mg/dl, 166 (79.05%)

**Table 1.** Characteristics of postmenopausal women in the study (N=210).

Variable	Parameter	Estimate
Age (years)	M ±SD*	56.5±3.5
Age at last menstruation (years)	M ±SD	50.3±2.7
BMI (kg/m <sup>2</sup> )	M ±SD	26.38±4.04
Educational level	n (%)	
	Primary	7 (3.33)
	Basic vocational	19 (9.05)
	Secondary	100 (47.62)
<i>Xbal</i>	n (%)	
	AA	71 (33.81)
	AG	104 (49.52)
	GG	35 (16.67)
<i>Pvull</i>	n (%)	
	TT	57 (27.14)
	TC	97 (46.19)
	CC	56 (26.67)

\* Mean ± standard deviation.

**Table 2.** Values of analyzed risk factors of cardiovascular diseases and estradiol in postmenopausal women in general, and according to ERα *Xbal* and *Pvull* polymorphisms. M ±SD\*.

Cardiovascular diseases risk factors	Total (N=210)	<i>Xbal</i>			F**	p***	<i>Pvull</i>			F**	p***
		AA (N=71)	AG (N=104)	GG (N=35)			TT (N=57)	TC (N=97)	CC (N=56)		
BMI (kg/m <sup>2</sup> )	26.38±4.04	26.46±4.33	26.29±3.72	26.48±4.43	0.047	0.954	26.40±4.64	26.71±3.56	25.79±4.17	0.929	0.396
Waist circumference (cm)	81.54±12.34	82.01±13.15	81.46±11.83	80.80±12.43	0.116	0.890	81.86±13.77	82.38±11.71	79.75±11.90	0.833	0.436
W/H ratio	0.79±0.09	0.79±0.09	0.79±0.09	0.80±0.10	0.120	0.887	0.80±0.09	0.79±0.09	0.78±0.09	0.299	0.742
CHOL (mg/dl)	229.09±42.58	225.20±41.43	228.52±41.94	238.69±46.43	1.197	0.304	227.09±41.55	227.35±42.39	234.14±44.27	0.536	0.586
HDL (mg/dl)	53.45±12.22	52.79±13.56	54.08±11.95	52.94±10.27	0.269	0.765	52.74±13.08	53.85±12.94	53.50±10.04	0.147	0.863
LDL (mg/dl)	145.84±43.70	142.77±43.60	145.92±43.32	151.82±45.67	0.501	0.606	144.09±43.93	145.09±42.44	148.90±46.23	0.196	0.822
TG (mg/dl)	149.00±64.13	148.21±62.93	142.62±58.22	169.60±79.33	2.357	0.097	151.30±66.53	142.06±59.22	158.70±69.38	1.247	0.289
LDL/HDL ratio	2.91±1.15	2.93±1.27	2.86±1.08	3.01±1.13	0.226	0.798	2.96±1.30	2.87±1.09	2.92±1.11	0.111	0.895
Systole (mmHg)	129.11±18.52	129.48±19.49	131.04±18.59	122.63±15.01	2.767	0.065	128.16±18.50	131.89±19.40	125.27±16.39	2.402	0.093
Diastole (mmHg)	79.35±10.46	78.77±10.59	80.52±10.24	77.03±10.69	1.627	0.199	79.23±10.07	80.44±11.02	77.57±9.77	1.347	0.262
E2 (pg/ml)	23.53±20.45	22.90±20.76	25.41±21.36	19.20±16.42	1.262	0.285	23.37±21.09	23.70±20.94	23.39±19.27	0.006	0.994

\* Mean ± standard deviation; \*\* F-test; \*\*\* significance level.

**Table 3.** Frequency of occurrence of risk factors of cardiovascular diseases in postmenopausal women in general, and according to ER $\alpha$  XbaI and PvuII polymorphisms n (%).

Cardiovascular diseases risk factors	Total (N=210)	XbaI						PvuII					
		AA (N=71)		AG (N=104)		GG (N=35)		TT (N=57)		TC (N=97)		CC (N=56)	
						$\chi^2*$	p**					$\chi^2*$	p**
BMI $\geq 25$ kg/m <sup>2</sup>	125 (59.52)	40 (56.34)	63 (60.58)	22 (62.86)		0.508	0.776	30 (52.63)	66 (68.04)	29 (51.79)		5.436	0.066
Waistline >88 cm	71 (33.81)	24 (33.80)	38 (36.54)	9 (25.71)		1.371	0.504	18 (31.58)	39 (40.21)	14 (25.00)		3.842	0.146
W/H ratio $\geq 0.85$	61 (29.05)	16 (22.54)	33 (31.73)	12 (34.29)		2.290	0.318	12 (21.05)	32 (32.99)	17 (30.36)		2.546	0.280
CHOL $\geq 191$ mg/dl	181 (86.19)	60 (84.51)	90 (86.54)	31 (88.57)		0.346	0.841	50 (87.72)	81 (83.51)	50 (89.29)		1.150	0.563
HDL $\leq 64$ mg/dl	174 (82.86)	58 (81.69)	84 (80.77)	32 (91.43)		2.198	0.333	45 (78.95)	79 (81.44)	50 (89.29)		2.379	0.304
LDL $\geq 115$ mg/dl	166 (79.05)	54 (76.06)	82 (78.85)	30 (85.71)		1.325	0.515	45 (78.95)	77 (79.38)	44 (78.57)		0.015	0.993
TG $\geq 150$ mg/dl	87 (41.43)	29 (40.85)	41 (39.42)	17 (48.57)		0.918	0.632	25 (43.86)	37 (38.14)	25 (44.64)		0.808	0.668
LDL/HDL ratio >4	34 (16.19)	13 (18.31)	13 (12.50)	8 (22.86)		2.425	0.297	11 (19.30)	13 (13.40)	10 (17.86)		1.076	0.584
Systole >140 mmHg	49 (23.33)	16 (22.54)	27 (25.96)	6 (17.14)		1.177	0.555	13 (22.81)	27 (27.84)	9 (16.07)		2.759	0.252
Diastole >90 mmHg	30 (14.29)	8 (11.27)	17 (16.35)	5 (14.29)		0.889	0.641	5 (8.77)	19 (19.59)	6 (10.71)		4.225	0.121

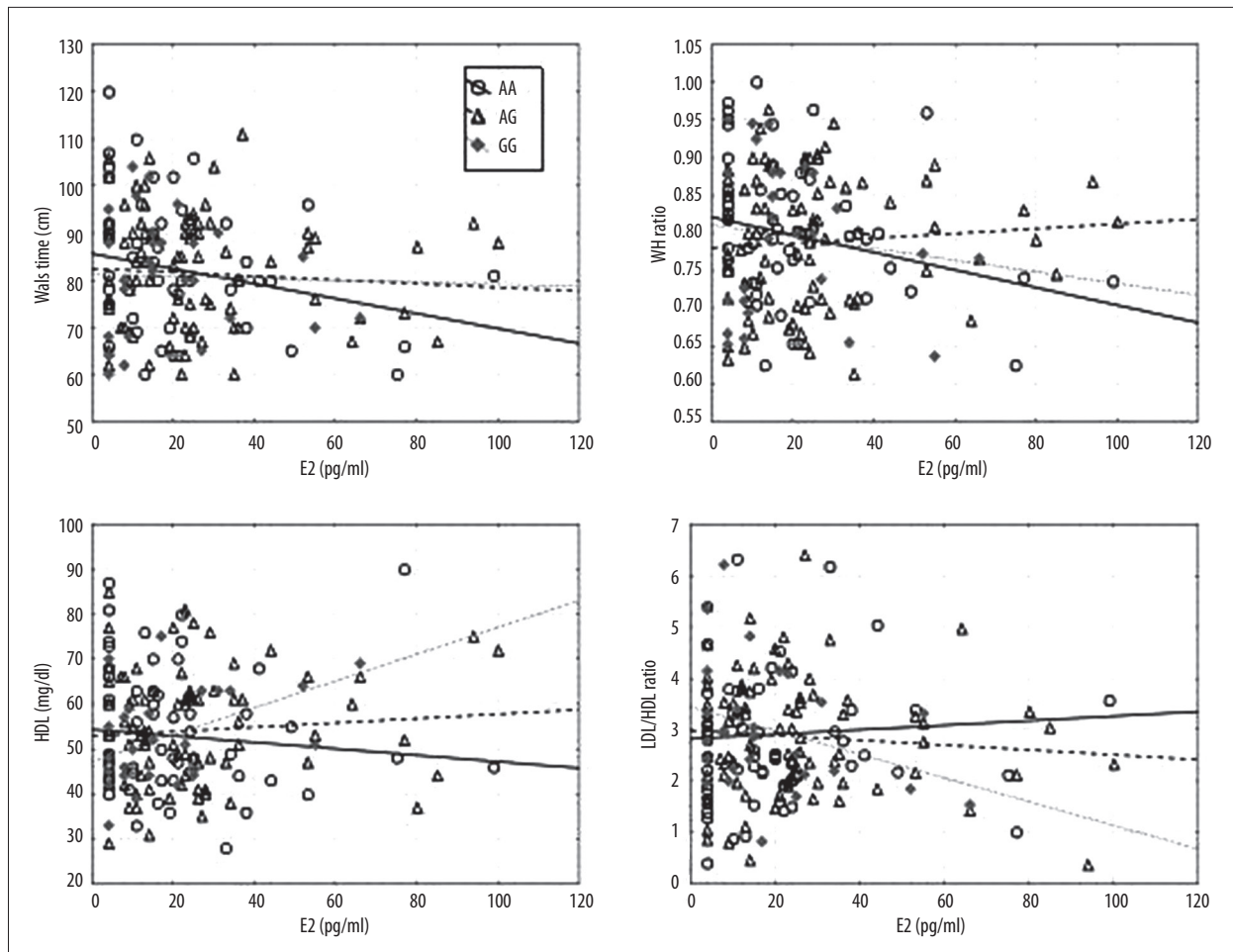
\* Chi-squared test; \*\* significance level.

**Table 4.** Correlation coefficients between estradiol (pg/ml) and risk factors of cardiovascular diseases in general, and according to ER $\alpha$  XbaI and PvuII polymorphisms.

Cardiovascular diseases risk factors	Total (N=210)		XbaI						PvuII					
			AA (N=71)		AG (N=104)		GG (N=35)		TT (N=57)		TC (N=97)		CC (N=56)	
	r*	p**	r	p	r	p	r	p	r	p	r	p	r	p
BMI (kg/m <sup>2</sup> )	-0.066	0.345	-0.051	0.673	-0.117	0.236	0.183	0.292	0.004	0.978	-0.120	0.242	-0.154	0.256
Waist circumference (cm)	-0.128	0.064	<b>-0.251</b>	<b>0.035</b>	-0.071	0.474	0.157	0.369	-0.165	0.220	-0.070	0.497	-0.195	0.150
W/H ratio	-0.077	0.269	<b>-0.283</b>	<b>0.017</b>	0.073	0.459	-0.128	0.465	-0.202	0.131	0.038	0.712	-0.147	0.279
CHOL (mg/dl)	-0.067	0.331	-0.073	0.544	-0.058	0.561	-0.045	0.799	-0.040	0.766	-0.141	0.170	0.037	0.788
HDL (mg/dl)	0.060	0.384	-0.109	0.364	0.089	0.368	<b>0.477</b>	<b>0.004</b>	-0.253	0.058	<b>0.224</b>	<b>0.027</b>	0.114	0.404
LDL (mg/dl)	-0.045	0.521	-0.015	0.905	-0.038	0.704	-0.120	0.492	0.048	0.725	-0.162	0.112	0.063	0.645
TG (mg/dl)	-0.130	0.061	-0.073	0.545	-0.159	0.107	-0.094	0.592	-0.035	0.797	-0.166	0.104	-0.175	0.198
LDL/HDL ratio	-0.066	0.342	0.071	0.556	-0.092	0.351	<b>-0.333</b>	<b>0.050</b>	0.164	0.224	<b>-0.237</b>	<b>0.020</b>	-0.046	0.737
Systole (mmHg)	0.084	0.224	0.130	0.281	-0.002	0.982	0.208	0.230	<b>0.314</b>	<b>0.017</b>	-0.009	0.933	0.004	0.977
Diastole (mmHg)	0.050	0.475	-0.057	0.635	0.059	0.554	0.196	0.259	0.005	0.968	0.127	0.216	-0.067	0.626

\* Correlation coefficient; \*\* significance level.





**Figure 1.** Scatter diagrams comparing estradiol and waist circumference, W/H, HDL, and LDL/HDL ratio, according to *XbaI* polymorphism.

with LDL  $\geq 115$  mg/dl, and 87 (41.43%) with TG  $\geq 150$  mg/dl. The LDL/HDL ratio  $>4$  was observed in 34 (16.19%) women. The values of systolic arterial blood pressure higher than 140 mmHg were found in 49 (23.33%) of the women in the study, and the values of diastolic pressure higher than 90 mmHg were observed in 30 women (14.29%). The occurrence of the analyzed risk factors of cardiovascular diseases was not significantly dependent to the possessed ER $\alpha$  *XbaI* and *PvuII* polymorphisms ( $p > 0.05$ ).

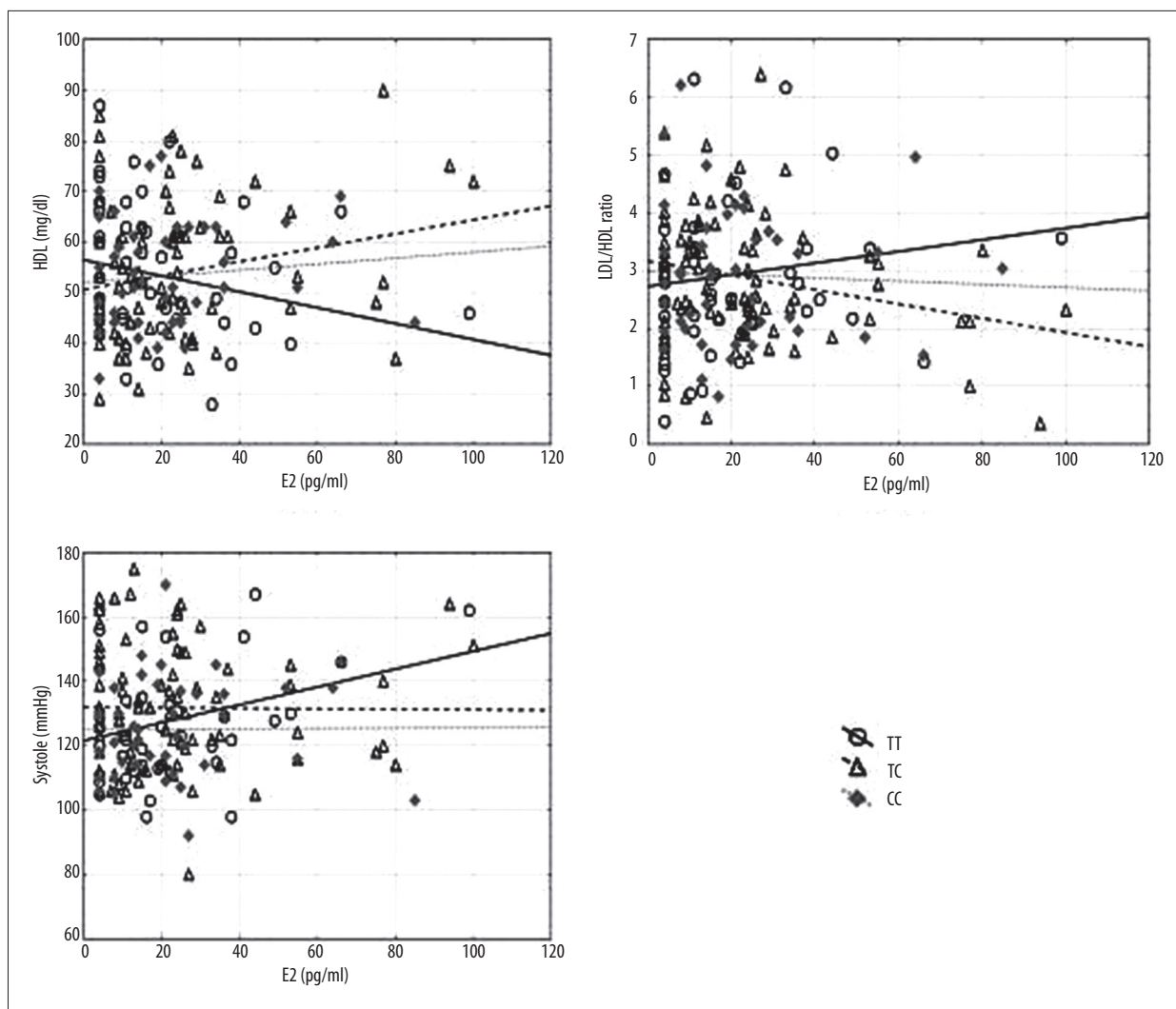
Subsequently, the correlation was investigated between the level of estradiol and the values of analyzed risk factors of cardiovascular diseases in the total examined group, and according to the ER $\alpha$  *XbaI* and *PvuII* polymorphisms possessed by the women in the study (Table 4).

None of the analyzed parameters significantly correlated with the level of estradiol in the total group of examined women. However, the analysis of correlations according genotype ER $\alpha$  polymorphism proved some of them to be significant. The description below concerns only these significant ones.

The level of estradiol correlated significantly negatively to waist circumference ( $r = -0.251$ ;  $p = 0.035$ ) and the W/H ratio ( $r = -0.283$ ;  $p = 0.017$ ) in women with the genotype ER $\alpha$  *XbaI* AA. Women with lower estradiol levels had higher average waist circumference and W/H ratio. Figure 1 shows the regression line between estradiol and waistline goes up, as did the line between estradiol and W/H ratio.

In women with ER $\alpha$  *XbaI* GG genotype, the level of estradiol correlated positively to HDL concentration ( $r = 0.477$ ;  $p = 0.004$ ) and negatively to LDL/HDL ratio ( $r = -0.333$ ;  $p = 0.050$ ). The subgroup of women with higher estradiol levels had higher average HDL level and lower average LDL/HDL ratio. Figure 1 shows that the regression line between estradiol and HDL goes up while the line between estradiol and LDL/HDL goes down among that group of women.

The results obtained also indicated a significant positive correlation between the level of estradiol and systolic blood pressure in women who possessed *PvuII* ER $\alpha$  TT genotype ( $r = 0.314$ ;



**Figure 2.** Scatter diagrams comparing estradiol and HDL, LDL/HDL ratio, and systole, according to *PvuII* polymorphism.

$p=0.017$ ). In this sub-group, an increase in the level of estradiol was accompanied by an increase in the value of systolic blood pressure on average. In women with *PvuII* genotype TC, a positive correlation was noted between the level of estradiol and the level of HDL ( $r=0.224$ ;  $p=0.027$ ), and a negative correlation between the level of estradiol and the LDL/HDL ratio ( $r=-0.237$ ;  $p=0.020$ ). Higher levels of estradiol in this sub-group were associated with higher average HDL level and average LDL/HDL ratio. Figure 2 shows that the regression line between estradiol and systole for TT as well as estradiol and HDL for TC goes up, but goes down for estradiol and LDL/HDL.

The following correlations were calculated: age, age at last menstruation, BMI, risk factors of cardiovascular diseases, and estradiol in the total group of examined women (Table 5). Only the statistically significant correlations are described below.

We found that BMI was positively correlated with waist circumference ( $r=0.607$ ;  $p<0.001$ ) and W/H ratio ( $r=0.635$ ;  $p<0.001$ ) was positively correlated with systolic blood pressure ( $r=0.165$ ;  $p=0.017$ ). Higher levels of BMI were associated with larger waist circumference and higher waist W/H ratio and systolic blood pressure on average.

Age at last menstruation had a positive correlation with LDL/HDL ratio ( $r=0.159$ ;  $p=0.021$ ) and systolic blood pressure ( $r=0.155$ ;  $p=0.025$ ). Women who began menopause later had higher average level of LDL/HDL ratio and systolic blood pressure during menopause.

We found a negative correlation of waist circumference and age of women during a menopause ( $r=-0.157$ ;  $p=0.023$ ). Younger women had larger average waist circumference during menopause.

**Table 5.** Correlation coefficients between age, age at last menstruation, BMI and risk factors of cardiovascular diseases, estradiol in total group of examined women.

Cardiovascular diseases risk factors	Age (years)		Age at last menstruation (years)		BMI (kg/m <sup>2</sup> )	
	r*	p**	r	p	r	p
Waist circumference (cm)	-0.157	0.023	-0.091	0.190	0.607	<0.001
W/H ratio	-0.104	0.134	-0.112	0.105	0.635	<0.001
CHOL (mg/dl)	0.041	0.559	0.094	0.173	-0.004	0.950
HDL (mg/dl)	-0.040	0.566	-0.106	0.124	-0.067	0.334
LDL (mg/dl)	0.019	0.781	0.102	0.141	-0.016	0.817
TG (mg/dl)	0.107	0.123	0.067	0.334	0.104	0.133
LDL/HDL ratio	0.060	0.390	0.159	0.021	0.003	0.963
Systole (mmHg)	0.054	0.440	0.155	0.025	0.165	0.017
Diastole (mmHg)	-0.041	0.552	0.078	0.258	0.119	0.084
E2 (pg/ml)	-0.122	0.078	-0.048	0.490	-0.088	0.207

\* Correlation coefficient; \*\* significance level.

## Discussion

Many studies show that the frequency of occurrence of cardiovascular diseases increases after menopause. It is considered that a decrease in endogenous estrogens is responsible for this unfavorable effect [18,19]. A few studies suggest that there is no reliable evidence for a rapid increase in morbidity due to CVD at postmenopausal age; however, a constant, proportional increase in the occurrence of these diseases is observed with age, caused by the co-existence of risk factors such as obesity, hypertension, lipid disorders, and glucose intolerance [20]. Thus, the question of whether estrogens exert a direct effect on the development of cardiovascular diseases or whether this effect is related to their impact on lipidogram, arterial hypertension, and body weight, remains to be answered. Also, it is not clear in what way the possession of particular ER $\alpha$  genotypes is related to increased risk of these diseases.

In the group of postmenopausal women in the presented study, the values of the parameters analyzed – BMI, WHR, waist circumference, levels of CHOL, LDL, HDL, TG, LDL/HDL ratio, systole, and diastole – were not significantly dependent to the possessed ER $\alpha$  *Xbal* and *PvuII* polymorphisms. Also, the occurrence of cardiovascular risk factors in the group examined, specified in the methodology, was not significantly dependent on the possessed ER $\alpha$  *Xbal* and *PvuII* polymorphisms.

Matsubara et al. also did not confirm any relationships between *Xbal* and *PvuII* polymorphisms, and the levels of total cholesterol, HDL-cholesterol, LDL-cholesterol, and triglycerides [21].

Contrary to the results of the presented study, Kikichi et al. [22] found that *Xbal* polymorphism exerted a significant effect on the level of LDL-cholesterol. Studies by Denga et al. [14] show that ER $\alpha$  gene *PvuII* and *Xbal* polymorphisms are important for the amount of fatty tissue and the BMI in postmenopausal women.

Clinical studies indicate that estrogens greatly affect the amount of fatty tissue. A decrease in their level is related to an increase in the amount of fatty tissue in postmenopausal women [23]. It is noteworthy that estrogens may increase the level of HDL-cholesterol, which explains the lower frequency of occurrence of cardiovascular diseases in pre-menopausal women. This observation has not been confirmed based on the presented material. In the group of women in this study, none of the analyzed parameters were significantly correlated with the level of estradiol.

Studies on the effect of ER $\alpha$  polymorphism and the level of estradiol indirectly showed that women with the ER $\alpha$  *PvuII* CC genotype more effectively respond to the effect of estradiol than those possessing the T allele. In addition, it was discovered that after menopause the level of endogenous estradiol is lowest in women possessing the T allele, whereas this level is the highest in women with the CC genotype, which has not been confirmed in the presented study [24].

Attempts were undertaken to take advantage of the beneficial effect of estrogens on the cardiovascular system in women by applying hormone replacement therapy (HRT). It was observed that the benefits resulting from the implementation of



this therapy may depend on the type of estrogen receptor alpha polymorphism. Herrington et al. [12] published results of studies concerning the level of HDL-cholesterol during the use of HRT, which clearly showed its increase in women with the CC genotype. It is interesting that as early as before the implementation of HRT, the level of HDL cholesterol in homozygotes CC and GG was significantly higher than in women with TT and AA genotypes.

In the presented study, significant negative correlations were found between waist circumference and W/H ratio, and the level of estradiol in women with the ER $\alpha$  *Xbal* AA genotype. In women possessing the ER $\alpha$  *Xbal* GG genotype, a positive correlation was observed between the levels of estradiol and HDL, and a negative correlation between estradiol level and LDL/HDL ratio. This suggests that the AA genotype is related to the strongest effect of estradiol on the distribution of fatty tissue in postmenopausal women, whereas the GG genotype intensifies the beneficial effect of estradiol on the level of HDL. Similarly, a beneficial interaction between the level of estradiol and the level of HDL was discovered in women with the ER $\alpha$  *PvuII* TC genotype. The results of this study concerning the level of endogenous estradiol are partly consistent with the results presented by Herrington et al. [12]; however, in the current study we found that the AA genotype was important with respect to the effect of endogenous estradiol on the distribution of fatty tissue after menopause. In addition, we noted that in the examined women who possessed the ER $\alpha$  *PvuII* TT genotype, the value of systolic blood pressure increased together with the level of estradiol. This suggests a stronger unfavorable effect of estradiol on systolic blood pressure in carriers of the TT allele.

The Rotterdam Study, which covered approximately 4000 women, revealed that those with the TT genotype were more exposed to cardiovascular diseases, which took a more severe and complicated course. The risk of cardiovascular diseases found in the Rotterdam Study occurred irrespective of the classic cardiovascular risk factors, such as BMI, level of lipids,

hypertension, and diabetes [25–27]. A meta-analysis performed by Ding et al. shows interesting results, in which the researchers, after analyzing 21 studies, found that *PvuII* polymorphism plays an important role in the susceptibility to atherosclerosis. They also confirmed that susceptibility related to *PvuII* polymorphism is clearly observed in Asian countries but not significantly in Western countries. The differences in the gene expression and epigenetic effects observed between Eastern and Western populations may lead to differences in the susceptibility to ischemic disease [28,29]. Moreover, the pathomechanism of ischemic disease is related to various factors – both genetic and environmental – with lifestyle, geographic conditions, or climate. These conditionings may affect the gene-environment interactions, and differentiate gene response (e.g., of the ER $\alpha$  genes) related to susceptibility to cardiovascular diseases [30–32].

## Conclusions

1. The level of endogenous estradiol and ER $\alpha$  *Xbal* and *PvuII* polymorphisms as independent parameters did not significantly correlate to BMI, waist circumference, W/H ratio, CHOL, HDL, LDL, TG, LDL/HDL, systole, or diastole of the examined postmenopausal women.
2. The polymorphism of  $\alpha$  estrogen receptor affected correlations between estradiol concentration and the analyzed atherosclerosis risk factors among healthy postmenopausal women:
  - a. A negative correlation of estradiol concentration with waist circumference and W/H ratio was observed in women with genotype AA of ER $\alpha$  *Xbal* polymorphism.
  - b. A positive correlation between estradiol concentration and HDL concentration was observed in women with AA genotype of ER $\alpha$  *Xbal* polymorphism.
  - c. A positive correlation between estradiol concentration and systolic blood pressure after a menopause was observed in women with TT genotype of ER $\alpha$  *PvuII* polymorphism.

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