Impact of the *PPAR gamma-2* gene polymorphisms on the metabolic state of postmenopausal women

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The relationship Pro12Ala (rs1801282) and C1431T (rs3856806) polymorphisms of *PPAR gamma-2* with glucose and lipid metabolism is not clear after menopause. We investigated the impact of the Pro12Ala and C1431T silent substitution in the 6th exon in *PPAR gamma-2* gene on nutritional and metabolic status in 271 postmenopausal women (122 lean and 149 obese). The general linear model (GLM) approach to the two-way analysis of variance (ANOVA) was used to infer the interactions between the analysed genotypes. The frequency of the Pro-T haplotype was higher in obese than in lean women ($p<0.0349$). In the analysed GLM models according to obesity status, the C1431C genotype was related to a lower glucose concentration ($\beta=-0.2103$) in lean women, and to higher folliculotropic hormone FSH levels ($\beta=0.1985$) and lower waist circumferences ($\beta=-0.1511$) in obese women. The influence of C1431C was present regardless of the occurrence of the Pro12Ala polymorphism. The co-existence of the C1431C and Pro12Pro genotypes was related to lower values for triceps skinfold thickness compared those for the T1241/X and Ala12/X polymorphisms ($\beta=-0.1425$). The presence of C1431C decreased the differences between triceps values that were determined by Pro or Ala allele. In conclusion, C1431T polymorphism seems to have a more essential influence on anthropometric and biochemical parameters than is the case with Pro12Ala polymorphism.

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1. Introduction

The prevalence of obesity increases after menopause and a tendency to visceral fat distribution is observed (WHO 2003; Charles and Laurie 2005; Kelly *et al.* 2008). Moreover, the incidence of impaired glucose tolerance (IGT) increases with age (Chang and Halter 2003) and β-cell dysfunction has been consistently demonstrated with more pronounced defects in older people with IGT (Chang and Halter 2003; Basu *et al.* 2003). Body mass *per se* has an inhibitory effect on the release of gonadotropins, which determine hormonal state after menopause (De Pergola *et al.* 2006).

The metabolism of adipose tissue is complexly regulated by many transcription factors, among which the peroxisome proliferator–activated receptor (*PPAR*) isoform gamma plays a crucial role (Lehrke and Lazarm 2005; Grygiel-Górniak 2014). *PPAR gamma* belongs to the nuclear hormone receptor superfamily and is an essential regulator of lipid and glucose metabolism. It participates in the differentiation of adipocytes, controls the expression of lipoprotein lipase, hormone-sensitive lipase and leptin and, in consequence, regulates insulin sensitivity (Tai *et al.* 2004). Among these variations is Pro12Ala polymorphism, which is located in the exon B *PPAR gamma-2* and which is mainly expressed in adipose tissue (Gurnell *et al.*

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2003) and has been associated with the body mass index (BMI) (Deeb *et al.* 1998), insulin sensitivity (Deeb *et al.* 1998: Koch *et al.* 1999; Altshuler *et al.* 2000), the level of total cholesterol and low density lipoproteins (LDL) fraction (Zietz *et al.* 2002). The Ala variant is protective against type 2 diabetes (Altshuler *et al.* 2000; Lohmueller *et al.* 2003), but the association with adiposity levels is not so consistent (Meirhaeghe *et al.* 2000).

In recent studies we found that blood glucose in heterozygous Pro12Ala subject and energy intake in women with C1431T and T1431T genotypes determined hypertension (Grygiel-Górniak *et al.* 2015). Moreover, Pro12Ala is in linkage disequilibrium with C1431T polymorphism (Valve *et al.* 1999; Meirhaeghe *et al.* 2000), which may modulate the association of Pro12Ala with obesity and the development of diabetes (Doney *et al.* 2002, 2004). The C1431T genotype is also associated with the value of BMI (Tai *et al.* 2004), diabetes, obesity, and metabolic syndrome (Doney *et al.* 2002, 2004; Tai *et al.* 2004; Haseeb *et al.* 2009). However, the relationship of both polymorphisms (Pro12Ala and C1431T) with metabolic parameters is not so clear, especially after menopause. Thus, we aimed to evaluate the association of metabolic risk factors with Pro12Ala and C1431T polymorphism in a group of lean and obese women of postmenopausal age.

2. Subjects and methods

A total of 271 postmenopausal women who underwent health check-ups at a Metabolic Outpatient Clinic were enrolled. They were selected from 1,423 women aged 49–75 year. From this group only women without premenopausal dyslipidemia were recruited to the study (documented proper lipid profile before menopause). To eliminate any influence of pathological factors on glucose and lipid status during the assessments of metabolic state with analysed polymorphisms we used exclusion criteria. Those with diseases such as endocrinological disorders (e.g. diabetes mellitus, thyroid disorders), cardiovascular diseases (with the exception of treated hypertension), renal or liver insufficiency, acute infection and hematologic diseases, previously diagnosed dyslipidemia, using hormonal replacement therapy or taking hypoglycaemic and hypolipidemic medications were excluded from the study. The control group consisted of lean women matched for age who satisfied the same qualification/exclusion criterion. Thus, 122 lean (BMI<25.0 kg/m^2) and 149 obese women (BMI>30.0 kg/m^2) were selected for the subsequent study.

All subjects gave written informed consent on documents approved by the Bioethical Commission of Poznan Medical University nr 792/09.

All procedures performed in studies involving human participants were in accordance with ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

2.1 *Anthropometric measurement*

Weight and height were determined with subjects in underwear using the SECA scale. Weight was measured to the nearest 0.1 kg and height, waist and hip circumferences were measured to the nearest 0.1 cm. Waist circumference was determined at the narrowest point between the costal margin and iliac crest, and the hip circumference was measured at the widest point over the buttocks. In both cases, the subjects were standing and breathing normally. Body mass index (BMI) was calculated as weight/height squared (kg/m^2) and WHR as waist to hip circumference (WHO 2003). Four skinfolds were measured (biceps, triceps, subscapular and suprailiac skinfold) by using the skinfold measurement device – caliper with a 0.2 mm precision (Harpenden company). A bio-impedance analyser (Bodystat 1500, Bodystat Ltd, UK) was used to assess fat content as a proportion of total body mass. The bio-impedance analysis was performed with a single frequency (50 kHz) device.

2.2 *Analytical methods*

Biological samples were collected in the morning (7 a.m.) after a 12 h fast and after a minimum of a 48 h since the last period of physical exercise. Venous blood samples were collected into EDTA containing tubes. The serum samples were taken from clotted (15 min, RT) and centrifuged (15 min, 3000*g*) blood drawn by venous puncture following a 12-hour overnight fast. Glucose and lipid profile [total cholesterol – TC, high density lipoproteins (HDL), triglycerides – TAG] was determined with enzymatic colorimetric assays (Cobas Integra 400 Plus; Roche Diagnostics). Samples were immediately centrifuged and serum was separated and directly used for the assay. The serum level of LDL was calculated using the formula of Friedewald et al. (Friedewald *et al.* 1972). The FSH serum levels were measured via specific chemiluminescence assays from Roche Diagnostic. Plasma insulin levels were determined by means of an enzymatic immuno-assay. The original HOMA model of insulin resistance (HOMA1-IR) was calculated as described by Matthews *et al.* (1985) as

$$\text{HOMA1-IR} = [\text{fasting insulin}(\mu u/\text{mL}) \times \text{fasting glucose}(\text{mM})]/22.5.$$

2.3 *Genotyping*

Genomic DNA was isolated from venous blood samples, which were collected in 5 ml EDTA tubes, according to the manufacturer's protocol (Gentra Puregene Blood Kit,

Qiagen, Germany). Genotypes of the Pro12Ala polymorphism (rs1801282) were determined via a TaqMan genotyping assay (C_1129864_10) (Applied Biosytsem, Foster City, USA) (table 1). The TaqMan mixture contained the forward target-specific polymerase chain reaction primer, the reverse primer, and the TaqMan MGB probes labelled with two special dyes (FAM and VIC) and 15 ng of each sample. The controls for each of the genotypes of the Pro12Ala were run in parallel. An allelic discrimination assay was performed on an ABI7900HT (Applied Biosystems, Foster City, CA, USA). Fluorescence data was obtained after 10 min at 95°C, followed by 40 cycles of 92°C for 15 s and 60°C for 1 min. Automatic allele calling, with the default settings, was carried out via ABI7900HT data collection and analysis software, version 2.2.1 (SDS 2.2.1).

C1431T (rs3856806) genotyping was performed using PCR-restriction fragment length polymorphism (PCR-RFLP) analysis. The 170bp PCR product of exon 6 was digested with *Eco72I* according to the manufacturer's instruction (Fermentas, Vilnius, Lithuania). *Eco72I* cleaves the PCR product from wild type DNA to generate fragments of 127bp and 43bp, but does not cut products containing the variant allele. PCR-digests were analysed on 2.5% agarose gels.

2.4 *Linkage disequilibrium (LD) block determination and haplotype construction*

The genotype data were used to construct the haplotypes between the two polymorphisms by using Haploview 4.2 software to evaluate LD. LD between the SNPs used in haplotype analysis was measured using a pairwise D′ statistic. The structure of the LD block was examined with the method proposed by Gabriel et al. using the 80% confidence bounds of D′ to define sites of historical recombination between SNPs (Gabriel *et al.* 2002). The haplotype frequencies were calculated based on the maximum likelihood method with Haploview 4.2 software. Finally, the associations between haplotypes and the obesity status were checked. Specific haplotype frequencies were compared among lean and obese women (chi-square test).

2.5 *Statistical analysis*

The data analysis was performed using StatSoft, Inc. STATISTICA for Windows, version 10.0. Before the experiment, an initial power analysis of the data was done and the power analysis for 2-way ANOVA with considered amount of analysed subjects was 0.8. The Shapiro-Wilk test was used in order to determine whether the continuous variables were normally distributed. The hypothesis, that the differences between analysed anthropometric and metabolic and factors in the analysed groups were significant, was tested by U Manna–Whitney's test at the significance level of $\alpha=0.05$ or by Student's *t*-test (if data were normally distributed). Because the number of Ala12Ala homozygotes was small (one lean woman and nine obese women), this was collapsed with Pro12Ala heterozygotes, and compared with Pro12Pro homozygotes for all analyses. Allele frequencies were estimated using the gene-counting method, and an exact test was performed to identify departures from Hardy-Weinberg proportions (Court lab – HW calculator.xls). A general linear model(GLM) approach to the 2-way analysis of variance (two-way ANOVA) was used to test for differences between the different genotypes at each polymorphism and the interactions between analysed genotypes. The results were considered significant if $p<0.05$ (SS-test verified the statistical significance of all variables in the models). The values of the Fisher statistic (F) and the corresponding levels of probability (p) were calculated to verify the statistical significance of all variables in the models (three degrees of freedom, df=3). The estimated beta-coefficients of the GLM models showed if the independent variable (or interaction of analysed parameters) in the models had a significant influence on the dependent variable (the anthropometric and biochemical parameters of the analysed populations). This effect was confirmed by the *t*-test.

3. Results

The frequencies of the variants of the Pro12Ala and C1431T polymorphisms were examined as a dichotomous variable, due to the small numbers of the Ala12Ala and T1431T homozygotes (table 2). When the Hardy-Weinberg equilibrium was evaluated, we observed that the Pro12Ala and C1431T polymorphism genotype distributions were in accordance with Hardy-Weinberg expectations: lean women - control group (Pro12Ala $X^2=1.82$, $p=0.18$; C1431T $X^2=1.05$, $p=0.31$) and obese subjects (Pro12Ala $X^2=3.076$, $p=0.08$; C1431T $X^2=1.91$, $p=0.18$). This showed that the study groups were representative. The frequencies of the Pro and Ala allele as well as C1431 and T1431 did not differ significantly between lean and obese women ($p=0.2380$, $p=0.4674$, respectively).

Genotyping data from two polymorphic loci in *PPAR* gene in lean and obese women were used to generate the haplotypes. The construction of haplotypes revealed the presence of Pro-C (freq. 0.833), Pro-T (freq.<0.01), Ala-C (freq 0.108) and Ala-T (freq. 0.051) in lean women and Pro-C (freq. 0.767), Pro-T (freq. 0.131), Ala-C (freq.0.062) and Ala-T (freq. 0.035) in obese women (data not shown in table). The frequency of the Pro-T haplotype was significantly different in obese women from that in lean women ($p<0.0349$). Although the Pro-C haplotype was frequently

Table 1. Polymorphisms of the PPARG gene investigated in the study

Polymorphism	SNP ID	Gene	Location	SNP Type	Codon change	Amino acid change
Pro12Ala	rs1801282	PPARG	Chr.3: 12393125	Transversion Substitution Missense Mutation	CCA,GCA	Pro12Ala
C1431T	rs3856806	PPARG	Chr.3: 12475557	Transition Substitution Silent Mutation	CAC,CAT	H449H

noted in the group of lean women, the result was not statistically significant.

Table 3 shows the results of selected variables, according to BMI values (lean BMI < 25kg/m^2, obese BMI ≥ 30kg/m^2). A state of obesity was characterized by a lower level of the folliculotropic hormone and worse metabolic parameters (higher insulin, glucose, LDL levels and insulin resistance indexes, as well as lower HDL concentrations, $p<0.05$). Because the statistical analysis did not reveal any significant differences in anthropometrical and biochemical data between genotypes Pro12Pro and Ala12/X or between the C1431C and C1431/X ($p>0.05$ for all analysed variables, we used two-way analysis of variance to show the interactions between the genotypes under discussion (GLM models, table 4). We observed some interesting differences regarding the C1431T polymorphism and a few of the interactions between Pro12Ala and C1431T. In the whole group of postmenopausal women, the co-existence of C1431C and Pro12Pro genotypes was associated with lower values for triceps than in the group characterized by T1241/X and Ala12/X polymorphisms ($\beta=-0.1425$, $p=0<0.05$) (figure 1a). A similar phenomenon was observed in the group of obese women ($\beta=-0.2071$, $p=0<0.05$) (figure 1b).

The lean women characterized by C1431C polymorphism had lower blood glucose concentrations ($\beta=-0.2103$,

Table 2. Genotype and allele frequencies of the Pro12Ala (rs1801282) and C1431T (rs3856806) *PPAR gamma-2* polymorphism according to obesity (BMI > 30 kg/m^2) (Data are n (%) for genotypes and n (frequency) for alleles.)

	Postmenopausal women		
Values	Lean n = 122	Obese n = 149	Whole group n=271
Genotype			
Pro12Pro	85 (69.67%)	100 (67.11%)	185 (68.27%)
Pro12Ala	36 (29.51%)	40 (26.85%)	76 (28.04%)
Ala12Ala	1 (0.82%)	9 (6.04%)	10 (3.69%)
Chi-square value (p value)	5.1882 (0.0747)		
Allele frequency			
Pro	206 (0.844)	240 (0.806)	446 (0.823)
Ala	38 (0.156)	58 (0.194)	96 (0.177)
Chi-square value (p-value)	1.3923 (0.2380)		
OR (95% CI)	0.7633 (0.4869 - 1.1964)		
Genotype			
C1431C	85 (69.67%)	113 (75.84%)	198 (73.07%)
C1431T	35 (28.69%)	31 (20.80%)	66 (24.35%)
T1431T	2 (1.64%)	5 (3.36%)	7 (2.58%)
Chi-square value (p value)	2.8257 (0.2434)		
Allele frequency			
C1431	205 (0.840)	257 (0.862)	462 (0.852)
T1431	39 (0.160)	41 (0.138)	80 (0.148)
Chi-square value (p-value)	0.5279 (0.4674)		
OR (95% CI)	1.1925 (0.7414 - 1.9180)		

Table 3. Anthropometric and biochemical characteristics of the analysed postmenopausal women according to BMI values (lean BMI < 25kg/m^2, obese BMI ≥ 30kg/m^2)

Analysed parameters	Analysed group	X ± SD	Median	Lean vs obese p-value
Age [years]	Whole group	59.31 ± 5.46	59.00	0.4755*
	Obesity	59.30 ± 5.10	59.00	
	Lean	59.33 ± 5.90	59.00	
Height [cm]	Whole group	160.94 ± 5.81	161.00	0.5437**
	Obesity	160.11 ± 5.73	160.50	
	Lean	161.12 ± 5.88	161.00	
Body mass [kg]	Whole group	76.37 ± 16.33	75.30	0.00001*
	Obesity	87.92 ± 12.15	86.20	
	Lean	62.25 ± 6.99	62.20	
Triceps skinfold [mm]	Whole group	19.72 ± 6.10	19.00	0.00001**
	Obesity	23.01 ± 5.05	23.00	
	Lean	15.71 ± 4.72	16.00	
Waist circumference [cm]	Whole group	90.38 ± 14.30	91.00	0.00001*
	Obesity	100.57 ± 9.62	100.00	
	Lean	77.94 ± 7.68	78.00	
BMI [kg/m^2]	Whole group	29.37 ± 6.43	30.00	0.00001*
	Obesity	34,30 ± 4,28	33.00	
	Lean	23.35 ± 1.82	24.00	
WHR	Whole group	0.84 ± 0.08	0.84	0.00001*
	Obesity	0.87 ± 0.07	0.87	
	Lean	0.79 ± 0.07	0.78	
FSH [mIU/Ml]	Whole group	70.07 ± 26.34	68.61	0.00001*
	Obesity	60.56 ± 20.77	60.64	
	Lean	81.68 ± 27.81	82.12	
Insulin [mU/ml]	Whole group	9.68 ± 7.50	7.84	0.00001*
	Obesity	12.40 ± 8.96	10.83	
	Lean	6.37 ± 2.68	5.98	
Glucose [mg/dl]	Whole group	96.56 ± 13.55	94.60	0.00002*
	Obesity	99.31 ± 14.91	97.20	
	Lean	93.19 ± 10.82	92.35	
HOMA IR [(mU/ml) × (mmol/l)]	Whole group	2.38 ± 1.99	1.87	0.00001*
	Obesity	3.11 ± 2.36	2.55	
	Lean	1.49 ± 0.75	1.36	
Total cholesterol [mg/dl]	Whole group	230.88 ± 40.95	225.20	0.0975*
	Obesity	236.00 ± 44.62	229.10	
	Lean	224.62 ± 35.16	222.25	
HDL [mg/dl]	Whole group	63.99 ± 14.69	63.10	0.00001
	Obesity	58.71 ± 12.46	57.00	
	Lean	70.44 ± 14.70	69.00	
TAG [mg/dl]	Whole group	117.49 ± 53.88	106.70	0.00001*
	Obesity	134.80 ± 55.12	124.20	
	Lean	96.35 ± 44.05	86.50	
LDL [mg/dl]	Whole group	143.39 ± 36.73	137.40	0.00001*
	Obesity	150.30 ± 39.05	143.90	
	Lean	134.96 ± 31.84	130.30	

BMI – Body Mass Index, WHR –waist to hip ratio, FSH – the folliculotropic hormone, HOMA-IR – homeostasis model assessments of insulin resistance; HDL – high-density lipoproteins, TAG – triglycerides, LDL – low-density lipoproteins.

*U Mann–Whitney's test.

**Student's *t*-test.

Table 4. Anthropometric and biochemical characteristics of the analysed postmenopausal women according to BMI values (lean BMI < 25kg/m^2, obese BMI ≥ 30kg/m^2) and polymorphisms (Pro12Ala; rs1801282 and C1431T; rs3856806) with analysis of interactions between two genotypes (means ± standard deviations in different groups of genotypes and beta-coefficients of the GLM models).

Analysed parameters (the dependent variable of GLM model)	Analysed groups	Analysed polymorphisms (qualitative factors of 2-way Anova)				Joint effect of genotypes interaction (interaction effects of two-way Anova) Pro/Ala x C/T	The GLM model - summary results (two-way Anova with the assess ment of interaction; df=3)	
		Pro12Ala (rs1801282)		C1431T (rs3856806)				
		Pro12Pro n=185	Ala 12/X n=86	C1431C n=194	T1431/X n=77		F-value	p-value
Age [years]		59.36 ± 5.45	59.21 ± 5.52	59.46 ± 5.54	58.91 ± 5.26			
	Whole group	β = −0.0040		β = −0.0108		β = 0.0462	0.1669	0.9186
	Obesity	β = −0.1091		β = 0.1489		β = −0.0488	1.6755	0.1748
	Lean	β = 0.1278		β = −0.1963		β = 0.1762	0.9228	0.1368
Height [cm]		160.74± 5.81	161.39 ±5.83	161.48 ± 5.73	162.14 ± 5.88			
	Whole group	β = −0.0137		β = −0.0519		β = −0.0765	1.0751	0.3600
	Obesity	β = −0.0791		β = −0.0226		β = 0.0061	0.3304	0.8033
	Lean	β = 0.0362		β = −0.0609		β = −0.1673	1.4168	0.2413
Body mass [kg]		77.10 ±15.51	76.03 ± 16.72	76.68 ± 15.52	75.56 ± 18.35			
	Whole group	β = −0.0101		β = 0.0491		β = −0.0708	0.5440	0.6525
	Obesity	β = 0.0233		β = −0.1015		β = −0.0975	1.1004	0.3511
	Lean	β = 0.0333		β = 0.1317		β = −0.0876	0.7536	0.5223
Triceps skinfold [mm]		19.64 ± 6.24	19.90 ±5.82	19.91 ± 5.70	19.22 ± 7.05			
	Whole group	β = 0.0233		β = 0.0826		β = −0.1425*	1.7548	0.1561
	Obesity	β = 0.1093		β = −0.0061		β = −0.2071*	1.7141	0.1666
	Lean	β = −0.0093		β = 0.1071		β = −0.1228	0.7547	0.5217
Waist circumference [cm]		89.55 ± 13.96	92.18 ±14.92	90.79 ± 13.10	89.33 ± 17.09			
	Whole group	β = −0.0919		β = 0.0399		β = −0.0007	0.8017	0.4938
	Obesity	β = −0.1335		β = −0.1511**		β = −0.0394	2.8427	0.0399*
	Lean	β = −0.0585		β = 0.1102		β = 0.1247	1.3312	0.2675
BMI [kg/m2]		29.23 ± 6.52	29.66 ± 0.85	29.67 ± 5.86	28.83 ± 7.41			
	Whole group	β = −0.0209		β = 0.0579		β = −0.0476	0.4008	0.7201
	Obesity	β = 0.0465		β = −0.0984		β = −0.1004	0.9028	0.3785
	Lean	β = −0.0554		β = 0.1748		β = 0.0163	1.1602	0.2845
WHR		0.83 ± 0.07	0.85 ± 0.08	0.84 ± 0.07	0.83 ± 0.09			
	Whole group	β = −0.0890		β = 0.0338		β = 0.0116	0.6856	0.5615
	Obesity	β = −0.1139		β = −0.1011		β = −0.0468	1.7943	0.1508
	Lean	β = −0.0373		β = 0.0859		β = 0.1044	0.8702	0.4586
FSH [mIU/mL]		69.50 ± 26.74	71.28 ±25.57	70.48 ± 25.29	68.99 ± 29.06			
	Whole group	β = −0.0401		β = 0.0755		β = −0.0081	0.5691	0.6357
	Obesity	β = −0.0805		β = 0.1985*		β = 0.0355	2.1927	0.0914
	Lean	β = −0.0474		β = 0.0540		β = −0.0615	0.3074	0.8199
Insulin [mU/mL]		9.39 ± 5.74	10.32 ±10.33	9.54 ± 7.33	10.07 ± 7.96			

Table 4 (continued)

Analysed parameters (the dependent variable of GLM model)	Analysed groups	Analysed polymorphisms (qualitative factors of 2-way Anova)				Joint effect of genotypes interaction (interaction effects of two-way Anova) Pro/Ala x C/T	The GLM model - summary results (two-way Anova with the assess ment of interaction; df=3)	
		Pro12Ala (rs1801282)		C1431T (rs3856806)				
		Pro12Pro n=185	Ala 12/X n=86	C1431C n=194	T1431/X n=77		F-value	p-value
	Whole group	β = −0.0323		β = −0.0160		β = −0.0577	0.6077	0.6104
	Obesity	β = −0.0556		β = −0.0771		β = −0.0367	0.7276	0.5370
	Lean	β = 0.0960		β = −0.0081		β = −0.1333	0.7829	0.5057
Glucose [mg/dL]		97.43 ± 14.13	94.67 ±12.06	96.58 ± 14.04	96.48 ± 12.25			
	Whole group	β = 0.0650		β = −0.0992		β = 0.1155	2.2982	0.0778
	Obesity	β = 0.0763		β = −0.0619		β = 0.1494	1.8539	0.1400
	Lean	β = 0.0623		β = −0.2103*		β = 0.0853	1.7553	0.1595
HOMA IR [(mU/mL) × (mmol/L)]		2.35 ± 1.80	2.44 ±2.35	2.35 ± 1.91	2.48 ± 2.19			
	Whole group	β = −0.0013		β = −0.0308		β = −0.0366	0.2638	0.8513
	Obesity	β = −0.0089		β = −0.0829		β = −0.0147	0.3938	0.7576
	Lean	β = 0.0931		β = −0.0652		β = −0.1004	0.7635	0.5166
Total cholesterol[mg/dL]		231.02 ± 42.21	230.57 ±38.35	232.31 ± 41.45	227.14 ± 39.67			
	Whole group	β = −0.0088		β = 0.0175		β = 0.0281	0.1069	0.9559
	Obesity	β = 0.0008		β = 0.0465		β = 0.1155	0.8931	0.4463
	Lean	β = −0.0374		β = −0.0347		β = −0.0950	0.6552	0.5812
HDL [mg/dl]		63.82 ± 14.31	64.35 ±15.58	63.79 ± 14.57	64.52 ± 15.11			
	Whole group	β = −0.0606		β = −0.0407		β = 0.1269	1.2043	0.3085
	Obesity	β = −0.0815		β = 0.0422		β = 0.1112	0.6686	0.5725
	Lean	β = −0.0716		β = −0.0642		β = 0.1501	0.8374	0.4759
TAG [mg/dL]		115.33 ± 50.36	122.13 ±60.81	118.87 ± 52.23	113.90 ± 58.17			
	Whole group	β = −0.0813		β = 0.0038		β = 0.0553	0.5504	0.6482
	Obesity	β = −0.0864		β = 0.0019		β = 0.1655	1.0721	0.3629
	Lean	β = −0.0888		β = −0.0514		β = −0.0728	0.9622	0.4130
LDL [mg/dL]		144.20 ± 38.44	141.65 ±32.89	144.65 ± 37.49	140.09 ± 34.68			
	Whole group	β = 0.0422		β = 0.0304		β = −0.0372	0.2365	0.8708
	Obesity	β = 0.0509		β = 0.0387		β = 0.0493	0.5041	0.6799
	Lean	β = 0.0267		β = −0.0051		β = −0.1560	0.9237	0.4316

BMI – Body Mass Index, WHR –waist to hip ratio, FSH – the folliculotropic hormone, HOMA-IR – homeostasis model assessments of insulin resistance, HDL – high density lipoproteins, TAG – *triglycerides*, LDL – low density lipoproteins.

*Statistically significant result ($p<0.05$).

** $p=0.07$ – close to statistical significance.

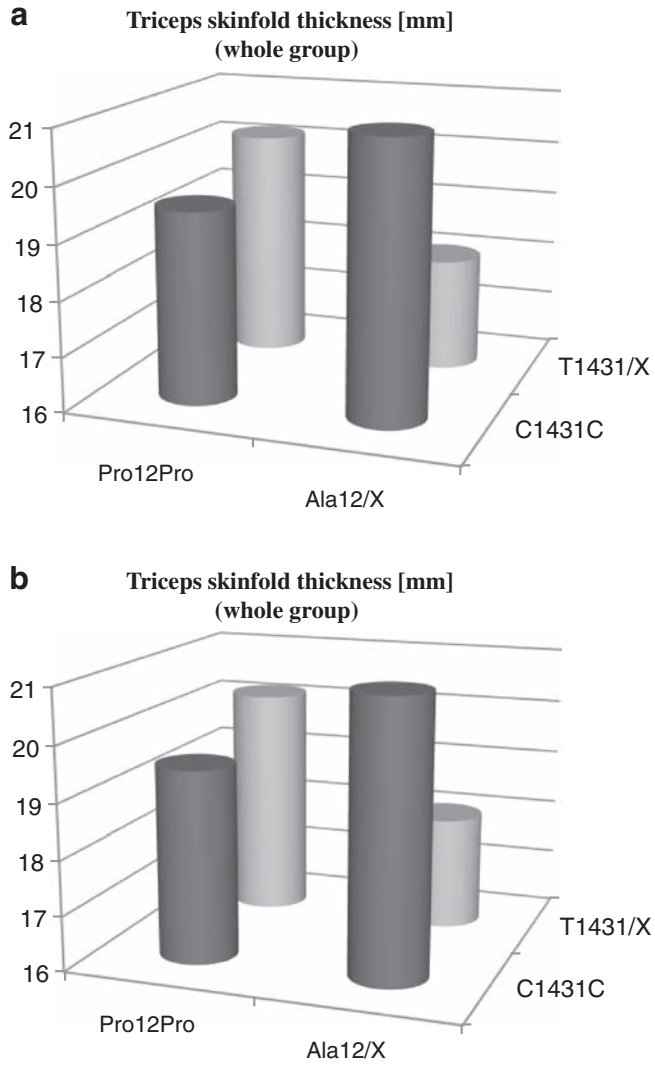

Figure 1. The effect of Pro12Ala (rs1801282) and C1431T (rs3856806) genotypes on triceps skinfold thickness in the whole group of postmenopausal women (**a**) and obese subjects (**b**).

p<0.05) than those with the T1431/X genotype (figure 2a). If C1431C polymorphism and the Ala allele co-existed, the tendencies observed were more evident, but the influence of the Ala allele was not statistically significant.

Obese women with C1431C polymorphism were characterized by lower values for waist circumference. An appropriate GLM model was statistically significant (p=0.0399), and the interactions between analysed variants in this model nearly achieved statistical significance (p=0.07) (figure 2b). In the obese group a higher concentration of FSH (β=0.1985, p<0.05) was also observed (figure 2c) and the influence of the C1431T genotype on waist circumference and FSH concentrations in this group was present regardless of the occurrence of Pro12Ala polymorphism.

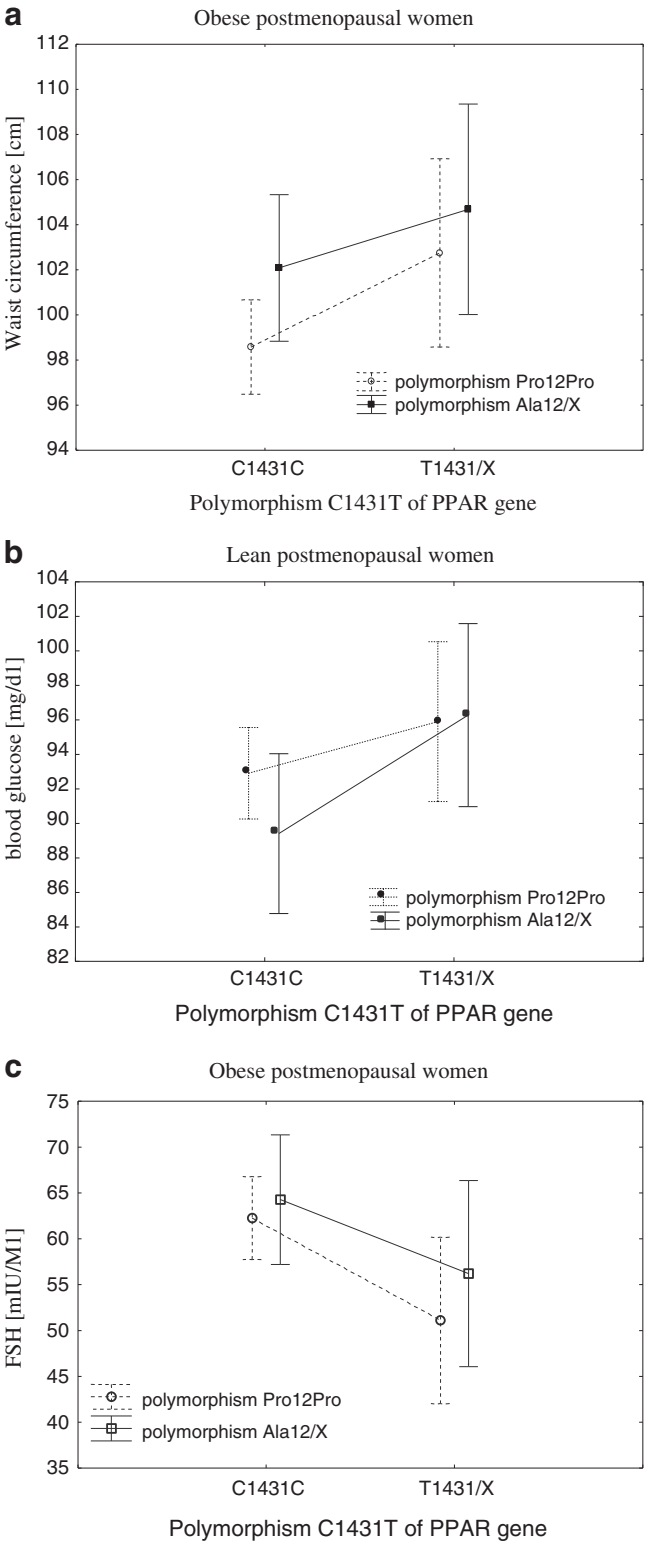

Figure 2. Association between Pro12Ala (rs1801282) and C1431T (rs3856806) genotypes and waist circumference in obese postmenopausal women (**a**), fasting blood glucose in lean postmenopausal subjects (**b**), and serum FSH concentration in obese women (**c**) (means with their confidence intervals CI=0.95).

4. Discussion

The frequency of Ala allele of the Pro12Ala SNP distribution in the whole group of analysed Caucasian Polish women (both lean and obese) was 0.177 (table 1). This value was similar to that found in other studies, in which the frequency varied from 0.11 in the WHO-MONICA population (Meirhaeghe *et al.* 2000; Swarbrick *et al.* 2001), 0.21 in a Czech population (healthy control) and up to 0.23 in healthy Russian subjects (Chistiakov *et al.* 2010. The prevalence of the Pro12Ala genotype in the analysed women was also comparable to other Caucasian populations (González Sánchez *et al.* 2002). The allele frequencies for the C1431T polymorphism also corresponded well with other studies (Haseeb *et al.* 2009; Valve *et al.* 1999; Doney *et al.* 2002, 2004).

Protective (Tai *et al.* 2004; Doney *et al.* 2002, 2004), as well as deleterious effects of Ala allele on metabolic parameters (e.g. glucose and lipid profile) (*Pischon et al.* 2005) have been observed. However, an inconsistent association between Ala allele and hyperglycaemia in postmenopausal age has still been reported. In the literature, the Ala allele has been estimated to reduce the risk of developing type 2 diabetes by 20% (Doney *et al.* 2004; Heude *et al.* 2011). In this study, the anthropometrical and biochemical analyses were similar in the whole group characterized by Ala and Pro or C and T polymorphisms (Table 4). This fact can be explained by the homogeneity of the population in question, which comprised women after menopause, whereas in other studies the range of ages has been much broader and has ranged between 35 and 64 years (González Sánchez *et al.* 2002) or even between 20 and 85 years (Pischon *et al.* 2005). Moreover, in this study, women with diabetes mellitus were excluded from the study to eliminate the influence of the disease on glucose metabolism, while in the literature these genotypes have usually been analysed in diabetic subjects (Altshuler *et al.* 2000; Doney *et al.* 2004; Tai *et al.* 2004).

The construction of haplotypes revealed that the Pro-T haplotype was significantly more prevalent in obese women compared with lean subjects ($p<0.0349$). We suspect that the presence of such genetic constellation can influence metabolic processes. The rare T1431 allele has also been linked inconsistently to increases in weight in contrast to the C allele (Doney *et al.* 2002) and is in linkage disequilibrium with Ala12 allele (Doney *et al.* 2004; Heude *et al.* 2011). In this study we confirmed the positive influence of C1431C polymorphism on the analysed anthropometric parameters. Obese subjects with C1431 allele revealed some tendency to lower values for waist circumference (figure 2b). The influence of C1431C polymorphism on waist circumferences in the analysed groups was observed regardless of the presence of Pro12Ala polymorphism and was statistically significant (table 4).

We also found interactions between the analysed genotypes. The co-existence of the homozygous genotypes C1431C and Pro12Pro was related to lower values for triceps skinfold when compared with the group characterized by T1241/X and Ala12/X genotypes ($\beta=-0.1425$). It was observed both in the whole group (figure 1a) and in obese women (figure 1b); however, the appropriate GLM models were not significant. The presence of C1431C genotype decreases the differences in triceps thickness that were determined by Pro12Pro or Ala12/X genotype. The subcutaneous skinfold of the triceps reflects the value of the total body fat amount, is strictly correlated with body mass, and has been widely used as an index of body fat (Jackson and Pollock 1985). Thus, we suspect that the presence of C1431C may play an important role in postmenopausal obesity and seems to be more essential than the presence of Pro12Ala genotype.

The level of the *folliculotropic hormone* was significantly lower in obese group (table 3). Physiologically, FSH concentrations increase after menopause and is associated with a low level of estrogens. Moreover, altered pulsatile gonadotropin secretion is observed in obese patients and body mass per se seems to have an inhibitory effect on gonadotropin release (De Pergola *et al.* 2006). In consequence, FSH has been recorded as being lower in obese women in comparison to lean subjects (De Pergola *et al.* 2006; Mahabir *et al.* 2006). Interestingly, in this study obese women characterized by C1431T had higher concentrations of FSH, which was independent of the presence of Pro or Ala allele (figure 2c). Thus, this genotype may influence hormonal changes in postmenopausal obesity and its role can be more important than Pro12Ala.

Because multiple risk factors for type 2 diabetes (including obesity and decreased physical activity) are associated with aging and thus predispose postmenopausal women to develop insulin resistance (Chang and Halter 2003; Basu *et al.* 2003), we have identified tissue sensitivity to insulin using glucose and insulin concentrations and HOMA-IR (Matthews *et al.* 1985) (table 3). In the analysed groups, proper tissue insulin sensitivity was indicated only in lean women (the cut off value for HOMA-IR for the Polish population in postmenopausal age is <2.5 mU/ml x mmol/l) (Żyła 2011). Increased HOMA-IR is a risk factor of hypertension, lipid disorders, and the risk of future atherosclerotic heart disease, mainly in obesity state (Chang and Halter 2003; Basu *et al.* 2003). Moreover, in resent studies we determined that blood glucose in subjects with Pro12Ala genotype determined the risk of hypertension (Grygiel-Górniak *et al.* 2015).

Many studies underline the influence of Pro12Ala polymorphism on metabolic disorders (Altshuler *et al.* 2000; Doney *et al.* 2004; Tai *et al.* 2004; Pischon *et al.* 2005), but the data of C1431T variant are limited. In this study, the lean women, which were CC heterozygote had lower blood glucose concentrations when compared with those with the

T1431/X genotype (figure 2a). Correspondingly, in the literature the T1431 allele was reported to be associated with higher glucose levels (Doney *et al.* 2004; Pischon *et al.* 2005). In similar studies, Jaziri et al. confirmed that both polymorphisms (Pro12Ala and C1431T) offer synergistic protection against developing hyperglycaemia (Jaziri *et al.* 2006). In the analysed lean subjects, the presence of both C1431C and Ala allele was associated with the tendency towards a lower glucose level, but the influence of Ala allele was not statistically significant. Thus, the influence of C1431T polymorphism on glucose levels was present regardless of the occurrence of Pro12Ala polymorphism.

An analysis of lipid profiles revealed hypercholesterolemia (LDL levels higher than 130 mg/dL), which was present independently of the analysed polymorphisms (Pro12Ala or C1431T) or body mass (lean and obese group); however, higher LDL levels were observed in obese women ($p=0.0021$) (table 3). Similar data have been reported in studies of Italian (Passaro *et al.* 2011), German (Zietz *et al.* 2002) and Japanese populations (Dongxia *et al.* 2008).

5. Conclusions

Our results show that postmenopausal obesity is associated with higher values for glucose and lipid parameters; however, we did not find any significant differences in anthropometrical and biochemical data between genotypes Pro12Pro and Ala12/X or between the C1431C and C1431/X. This fact can be partially explained by the lack of important metabolic disorders (e.g. diabetes mellitus, metabolic syndrome) in analysed postmenopausal women. We observed the influence of the C1431T genotype on glucose levels in lean groups and waist circumference and FSH concentrations in obese women, which was present regardless of the occurrence of Pro12Ala polymorphism. Thus, we suspect that this C1431T genotype can play a key role in determining the amount and distribution of body fat as well as metabolic state.

6. Study limitations

The main limitation of this study is that it cannot demonstrate causality but only the association between variables. We did not perform adjustment for other potential confounders, such as socioeconomic status, physical activity and family history of obesity. Therefore, these data should be incorporated into future studies.

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