Wnioski: *Częstość występowania polimorfizmu Pro12Ala genu PPARγ2 u kobiet z Dolnego Śląska jest istotnie wyższa niż w innych populacjach europejskich.*

Słowa kluczowe: **zespół jajników policystycznych – PCOS** / **polimorfizm Pro 12Ala** / / **polimorfizm Pro 115Gln** / **gen PPARγ2** /

Introduction

Polycystic ovary syndrome (PCOS) is an endocrine disorder affecting 5-10% of women of reproductive age [1]. It is characterized by excessive androgen production by the ovaries and adrenal glands, abnormalities in gonadotropin secretion by the pituitary gland, chronic anovulation, infertility, as well as insulin resistance [2]. The genetic mechanisms underlying PCOS remain largely unknown. Among others, genetic variations responsible for androgen production, insulin sensitivity, as well as proinflammatory genotypes, may be involved in the genetic predisposition to PCOS [3].

Peroxisome proliferator activated receptors gamma (PPARγ) are nuclear receptors and transcription factors that are expressed in adipocytes. They are involved in adipocyte differentiation, fatty acid metabolism and have been suggested to play a role in the pathogenesis of insulin resistance and atherosclerosis [4,5]. According to many studies, PPARγ Pro12Ala polymorphism carrier status was associated with obesity, however some linked it with protection from obesity and insulin resistance [6, 7, 8, 9, 10, 11, 12, 13, 14, 15].

The frequency of the Pro12Ala polymorphism of the PPARγ2 gene depends on the studied population. In European countries the Ala allele frequency decreases from north to south, from 21% in Baltic countries to 7% in Mediterranean countries [16].

This study was designed to evaluate the frequency of the PPARγ Pro12Ala and Pro115Gln polymorphism in a sample of women from the Lower Silesian population.

Subjects and methods

The study included 54 women with PCOS (mean ± *SD* age: 25.70±6.54 years, BMI: 26.89±7.39 kg/m^2) and 51 healthy women (age: 29.33±7.82 years, BMI: 29.05±7.76 kg/m^2). Each of the studied groups was further divided into two subgroups: non-obese women with BMI <30 kg/m^2 and obese with BMI ≥30 kg/m^2.

PCOS was diagnosed according to the Rotterdam criteria (two of the tree symptoms: 1. oligoovulation, 2. clinical and/or biochemical hyperandrogenism, 3. characteristic ultrasound picture of polycystic ovaries). Subjects with hyperprolactinemia, hypercortisolemia, thyroid disorders, and ovarian or adrenal tumors were excluded from the study.

Patients eligible for the study had not been treated with hormonal or insulin-sensitizing drugs, during the three months prior to the study they had not been on any special diet, consumed alcohol occasionally, and had not suffered from any serious diseases. All subjects received both oral and written information about the study and signed their written informed consent. The study was approved by the Commission of Bioethics at Wroclaw Medical University.

Medical history, physical examination and assessment of antropometric parameters (body mass, height, body mass index (BMI) calculated by the formula: body mass/(height)^2 [kg/m^2], waist circumference, hip circumference, waist-to-hip circumference ratio (WHR)) were carried out in all subjects. Moreover, genetic studies to detect the Pro12Ala polymorphism and the Pro115Gln mutation in the gene of peroxisome proliferator activated receptor γ2 were performed.

Genomic DNA was isolated from peripheral blood lymphocytes using a phenolochloroform extraction. The sequences of the primers used in the polymerase chain reaction (PCR) to detect Pro12Ala polymorphism were: PPAR12-F: 5' – CAA GCC CAG TCT TTC TG TG – 3'; PPAR12-R: 5' – AGT GAA GGA ATC GCT TTC CG – 3'. PCR was carried out in solution with a final volume of 30ml using: 50 mM KCl, 1,5 nM $MgCl_2$, 10 mM TRIS-HCl, 10mmol/each dNTP, 100pmol/each primer, 10ng DNA, and 0.8 U Taq polymerase. PCR was carried out under the following conditions: initial denaturation of 3 min. at 95°C, followed by 40 cycles of denaturation (94°C for 30 sec.), annealing (55°C for 30 sec), extension (72°C for 45 sec), and final extension (72°C for 9 min).

The sequences of the primers used in PCR to detect Pro115Gln polymorphism were: PPAR115-F: 5' – TGC AAT CAA AGT GGA GCC TGC ATG TCT – 3'; PPAR115-R: 5' – AGA AGC TTT ATC TAT CTC CAC AGA – 3'.

PCR was carried out under the following conditions: initial denaturation of 3 min. at 95°C, followed by 35 cycles of denaturation (94°C for 30 sec.), annealing (64°C for 30 sec.), extension (72°C for 45 sec.) and final extension (72°C for 9 min.). The PCR products were incubated overnight with the restrictive enzymes Hpa II (for Pro12Ala) and Hinc II (for Pro115Gln). Then the products were separated by electrophoresis on a 3% agarose gel and visualized using a Vilber-Lourmat detector.

The data were analyzed with Statistica for Windows version 5.1. Statistical analysis was made with Student's *t*, non-parametric Mann-Whitney *U* and the Kruskal-Wallis tests and Pearson correlation analysis. The data are expressed as means ±SE (standard error). Statistical significance was set at $p<0.05$ and categorical variables are expressed as percentages. The chi-squared (χ^2) test with the Yate's correction was used to estimate the statistical significance of allele frequencies.

Results

In the whole studied group (PCOS patients and controls) we did not find the Pro115Gln polymorphism of the PPARγ2 gene.

The frequency of the PPAR γ2 Pro12Ala polymorphism in the studied groups is shown in Table I.

We did not observe statistically significant differences between the frequencies of the Ala allele in the control and the PCOS groups. The differences were examined using the χ^2 test with Yate's correction (χ^2=0.16, p=0.691). Since this was not population research, in the study we examined the results taking the Hardy-Weinberg rule into consideration. The arrangement of

genotype occurance in the control group was in agreement with the Hardy-Weinberg rule (χ^2=3.0474, p<0.05), but not in the PCOS group (χ^2=5.6468, p=0.652).

The frequency of the PPARγ2 Pro12Ala polymorphism in the control and PCOS groups according to BMI is shown in Table II. The frequency of the Ala allele in the women of the control group with BMI <30 was 12.50% and in the PCOS group 18.75%. This difference was not statistically significant (χ^2=0.09, p=0.76).

There was no difference in the frequencies of the Ala allele in women with BMI ≥30 in the control (38.88%) and the PCOS group (38.23%). We observed a statistical difference in the occurrence of the Ala allele in the women in the control group with BMI <30 (12.50%) and BMI ≥30 (38.80%) (χ^2=7.79, p=0.0053). In the PCOS group the difference in Ala allele frequency between women with BMI <30 (18.75%) and with BMI ≥30 (38.23%) was also statistically significant (χ^2=9.09, p=0.0053).

Discussion

Studies of PPARγ2 polymorphism did not reveal the presence of the Pro115Gln polymorphism in the whole studied group. This polymorphism is very rare and found in patients with "morbid" obesity [17].

The frequency of the Ala allele in the total group of 105 women was estimated at 24.76%. This frequency is higher than that observed in the other Caucasian populations and significantly higher than that of non-Caucasians [10, 13, 18, 19, 20, 21, 22, 23]. In our previous report from 2003, in which we investigated 220 unrelated subjects from Lower Silesia, we did not observe differences in the frequency of the Ala allele in obese subjects (28%), patients suffering from diabetes mellitus type 2 (25%), and subjects in the control group (27%). We did not note differences in the frequencies of the Ala allele in females and males. Subjects with the Ala12Ala genotype had significantly higher BMIs than those with "wild-type" Pro12Pro [24].

The frequency of the Pro12Ala polymorphism of the PPARγ2 gene has a high variability and depends on the studied population: 21% in the Balts, 19% in the German, 14% in the Danish, 12% in Finnish, 11% in the French, 7% in the Spanish, 1% in the Samoan and Chinese, 2-3% in the Japanese, and 4% in the Korean populations [10, 13, 19, 20, 21, 22, 23, 25]. In European countries the Ala allele frequency exhibited a clearcut north-to-south gradient, decreasing from 21% in Baltic countries (Estonia) to 7% in Mediterranean countries (Greece, Italy, Portugal, Spain) [16].

In the present study, the frequencies of the Ala allele in the control group (26.47%) and in the PCOS group (23.15%) were similar to that of the whole studied group. We did not note a statistically significant difference in the frequency of the Ala allele in the control and PCOS groups.

In the control group consisting of 51 subjects, the Pro12Ala polymorphism was observed in 6 women with BMI <30 and in 15 with BMI ≥30. In the PCOS group, the Pro12Ala polymorphism was discovered in 11 women with BMI <30 and in 8 with BMI ≥30. The frequency of Ala allele in the obese women in the control group was 38.88% and 12.5% in the non-obese women. This difference was not statistically significant. Similarly, in the PCOS group the frequency of the Ala allele was 38.23% in the obese and 18.75% in the non-obese women (not a statistically significant difference). To conclude, the occurrence of the PPARγ2 Pro12Ala polymorphism in the studied group was related to obesity and not to hyperandrogenism. The distinctly higher frequency of the Ala allele in the obese women (PCOS and control) is related to the occurrence of two Ala alleles, particularly in women with the highest BMI.

Interestingly, the frequency of the Ala allele (about 25%) in our study was significantly higher than those found in studies of other world populations. The most similar frequency of the Ala allele was found in Baltic (21%) and German (19%) in contrast to Asian populations (1-4%) [10, 13, 19, 20, 21, 22, 23, 25]. The opinions of researchers about the Ala allele are divided. Some believe that the Ala allele plays a protective role and prevents the development of obesity (Ala carriers have lower BMI), insulin resistance, and, consequently, type 2 diabetes [6, 9, 10, 11].

The Danish MONICA study revealed that in Danish population (2245 subjects) the frequency of the Ala allele was 14%. In patients with insulin resistance, this frequency was 16.6% and in the group without insulin resistance 14.2%, the difference between these two groups not being statistically significant. On the basis of these results, the researchers concluded that the homozygous variant Ala of 12 codon was a preventive factor against developing insulin resistance [26]. Jaziri et al. showed in a prospective study that the Pro12Ala polymorphism had a protective effect in the development of hyperglycemia and hyperinsulinemia during a six-year period [27].

Radha et al. analyzed the PPARγ2 Pro12Ala polymorphism in South Asians and Caucasians. Although similar frequencies of the Ala allele were found in the two populations, only in the Caucasians did the authors observe a protective role of the Ala allele on diabetes risk. They concluded that there are ethnic differences in the association between the Pro12Ala polymorphism and type 2 diabetes [15]. Ek et al. investigated the Pro12Ala polymorphism in 572 obese and 869 non-obese subjects. The Pro12Pro variant was observed in 540, Pro12Ala in 191, and Ala12Ala in 21 obese patients.

Table I. The frequency of the PPAR γ2 Pro12Ala polymorphism in the studied groups: quantitative and percentage analysis.

	Number of women	Pro12Pro („wild type")		Variant Ala				Frequency of Ala allele
				Pro12Ala		Ala12Ala		
		No	%	No	%	No	%	
All subjects	105	65	61.90	28	26.70	12	11.40	24.76%
Control group	51	30	58.82	15	29.42	6	11.76	26.47%
PCOS group	54	35	64.81	13	24.07	6	11.12	23.15%

Table II. Quantitative and percentage analysis of the frequency of the PPARγ2 Pro12Ala polymorphism in the control and PCOS groups according to BMI.

	Number of women	Pro12Pro („wild type")		Variant Ala				Frequency of Ala allele
				Pro12Ala		Ala12Ala		
		No	%	No	%	No	%	
Control group BMI <30	24	18	75	6	25	0	0	12.5%
Control group BMI ≥30	27	12	44.44	9	33.34	6	22.22	38.88%
PCOS group BMI <30	37	26	70.27	10	27.03	1	2.70	18.75%
PCOS group BMI ≥30	17	9	52.94	3	17.65	5	29.41	38.23%

In the non-obese group, the Pro12Pro variant was noted in 641 Pro12Ala in 214 and Ala12Ala in 14. Similarly to our research, Ala12Ala carriers were extremely obese: mean BMI was 38.9±5.7kg/m^2 [28].

Conclusions

Studies of PPARγ2 polymorphism did not reveal the presence of the Pro115Gln polymorphism in the whole studied group. The frequency of the Pro12Ala polymorphism observed in a sample of women from the Lower Silesian population was significantly higher than in the majority of European populations.

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