Advances in Medical Sciences 63 (2018) 242–248

Contents lists available at ScienceDirect

Advances in Medical Sciences

journal homepage: www.elsevier.com/locate/advms

Original research article

The preliminary association study of *ADIPOQ*, *RBP4*, and *BCMO1* variants with polycystic ovary syndrome and with biochemical characteristics in a cohort of Polish women

Ewa Czeczuga-Semeniuk[a,*], Marzenna Galar[b], Katarzyna Jarząbek[a], Piotr Kozłowski[c], Nela A. Sarosiek[d], Sławomir Wołczyński[a]

[a] *Department of Reproduction and Gynecological Endocrinology, Medical University of Białystok, Białystok, Poland*
[b] *Department of Hematology, Medical University of Białystok, Białystok, Poland*
[c] *European Centre of Bioinformatics and Genomics (ECBaG), Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Poland*
[d] *Diagnostic Imaging Department, Comprehensive Cancer Center of Białystok, Białystok, Poland*

ARTICLE INFO

Keywords:
PCOS
Polymorphism
ADIPOQ
RBP4
BCMO1

ABSTRACT

Purpose: We aimed to elucidate the frequency of the SNPs in the *ADIPOQ*, *RBP4* and *BCMO1*genes in a population of Caucasian Polish women with polycystic ovary syndrome (PCOS), and to evaluate the possible associations between these variants and the susceptibility to PCOS. Additionally, the relationship of these polymorphisms to a clinical phenotype of this syndrome, and the concentrations of adipokines, were determined.
Materials/methods: Clinical and biochemical profiles, DNA isolation and genotyping, and adipokine assays were performed in 294 PCOS women and 78 controls.
Results: In a cohort of Polish women, for the genotype distribution and allele frequencies (minor allele frequency − MAF) proved that only the SNP rs1501299 in the gene *ADIPOQ* (P = 0.0010, OR = 0.41, 95% C.I.:0.24-0.70) and rs7501331 in the gene *BCMO1* (P = 0.0106, OR = 0.24, 95% C.I.:0.21-0.71), are significantly associated (the latter marginally significant) with the decrease of the risk of the disease. Also for this SNPs there were significant differences in the genotypic frequencies in the study population.

There was a link between rs12934922 of *BCMO1* gen and serum concentration of RBP4 (P = 0.034) and adiponectin (P = 0.038) in the study group but not in the control group. The elevated mean serum concentration of cholesterol (P = 0.020) and LDL cholesterol (P = 0.005) was observed for GG rs1501299 genotype and triglycerides (P = 0.028) for TT rs2241766 genotype.
Conclusions: The results of the present study revealed that the genes variants *RBP4* is not associated with PCO. It seems that rs1501299 of *ADIPOQ* gene influences the occurrence of PCO and lipids profile in those patients.

1. Introduction

Aetiology and pathophysiology of polycystic ovary syndrome (PCOS) has not been elucidated, however many years of observation and clinical studies indicate the role of overproduction of androgens (hyperandrogenism) in ovaries, as well as genetic and environmental factors [1]. This syndrome has been linked with phenotypical and genetic heterogenity, including numerous biochemical and regulatory pathways involved in normal functions of ovaries and adipose tissue, as well as hypothalamic–pituitary–adrenal axis, liver and peripheral tissues [2]. The key factor in the PCOS pathophysiology is deregulated androgen synthesis in the ovary on the very early stage of folliculogenesis [3–5]. Disorders of thecal and granulosa cell functions, as well as distorted folliculogenesis are associated with the activity of gonadotropin, anti-Müllerian hormone (AMH) and insulin [1,2], however, there may be other factors at play. A potentially good candidate is retinoic acid (RA), an analogue of retinol, which is the most active retinoid on the cellular level. A product of carotenoid metabolism, it constitutes the key factor that stimulates meiotic divisions of primary oocytes or oogonia in human ovaries [6]. Moreover, thecal cells of the ovary have been shown to synthetize RA [7], which suggest that these cells are able to convert retinoid precursors to biologically active retinoids [8]. RA influences androgen biosynthesis in normal ovarian thecal cells [8], while increased conversion of retinol to RA contributes

* Corresponding author at: Department of Reproduction and Gynecological Endocrinology, Medical University of Bialystok, Marii Sklodowskiej – Curie 24A, 15 – 276 Bialystok, Poland. Tel: +4885 746 8343.
E-mail address: czeczuga@wp.pl (E. Czeczuga-Semeniuk).

https://doi.org/10.1016/j.advms.2018.01.002
Received 6 July 2017; Accepted 8 January 2018
Available online 08 February 2018

to increased androgen production by thecal cells in PCOS patients [7,8]. More details about abnormal retinoid signalling implicated in PCOS pathogenesis were presented in scientific review published by Jiang et al. in 2017 [9].

In human body, carotenoids from the provitamin A group (e.g. β-carotene), externally supplied with nutrition, are transformed to retinoids in course of an enzymatic reaction. The first step of this reaction requires a specific monooxygenase (beta-carotene 15,15′-monooxygenase 1- BCMO1) [10]. Thus, local concentration and functions of retinol and retinoic acid in particular tissues depend on the regulatory functions of BCMO1. There is a clear positive correlation between cell types sensitive to vitamin A deficiency and cell types expressing BCMO1 [11]. Expression of the BCMO1 mRNA has been found in numerous tissues, such as thecal and granulosa cells in the ovary [10], therefore β-carotene supplied to these cells may be converted in situ to retinoids. Recent research showed a high frequency of single nucleotide polymorphisms (SNPs) in the human *BCMO1* gene, which affect the β-carotene metabolism and its level [12,13]. These SNPs also have a potential effect on stimulating the retinoid-dependent signal transduction. But there is no evidence that *BCMO1* polymorphic variants are implicated in PCOS pathogenesis.

However, research on the secretory activity of the adipose tissue, an element of the endocrine system that produces adipokines and a site where significant amounts of retinol are stored, showed that disorders in the biology of adipocytes, related with distorted synthesis of certain adipokines, may be the key factor in the pathogenesis of metabolic dysfunctions in PCOS, but their role remains elusive. Adiponectin is a protein product of the human gene *ADIPOQ*, which affects metabolic processes in PCOS patients, including glucose level and adipose tissue catabolism [14,15]. This suggests that disruption of adiponectin and/or its receptors plays a key role in the pathogenesis of hyperandrogenism in PCOS. Adiponectin inhibits androgen production in animal [16] and human [15] theca cells. Moreover, the expression of adiponectin receptors in theca cells of PCOS patients is lower compared to normal ovaries [15]. Serum adiponectin test results in PCOS patients are conflicting [17]. Some research findings suggest that adiponectin genes are implicated in PCOS pathogenesis [18–20] with higher prevalence of the rs2241766 45 T/G adiponectin gene variant in women with PCOS [21]. However, only the rs1501299 276 G/T variant of the *ADIPOQ* gene has been linked with the risk of PCOS [19]. In non PCOS women, genetic variants of the *ADPOQ* gene are linked with the level of this adipokine [22,23]. Moreover, the “new adipokine”, or the retinol binding protein 4, RBP4 (coded by the *RBP4* gene on chromosome 10, region 10q23-q24), is responsible for supplying retinol to all tissues of the human body [24]. RBP4 is mainly expressed in the liver and adipose tissue [25], with increased expression in the adipocytes of overweight women with PCOS [26,27]. Research showed that average serum level of RBP in patients with PCOS is significantly higher compared to healthy controls [28–31], regardless of the patient body mass [32,33]. In addition, SNPs of the *RBP4* gene in healthy women were shown to affect the level of this adipokine [34,35] and lipid metabolism [35,36].

Therefore the aim of this study was to determine frequencies of polymorphisms in the following genes: *ADIPOQ* (rs1501299, rs2241766), *RBP4* (rs3758539, rs3758538, rs61461737, rs10882273, rs10882280, rs11187545, rs12265684) and preliminary prospective test in *BCMO1* (rs12934922, rs7501331) in the Polish population of PCOS patients. Another goal was to perform association analysis of these polymorphisms with PCOS incidence in these women and evaluate the association of these polymorphisms with the clinical and biochemical phenotype of PCOS, adiponectin level and RBP4 level.

2. Materials and methods

All subjects were Caucasian ethnicity Polish women and all participated in a previous study [30].

2.1. PCOS women

The patients with PCOS (n = 294) were defined according to the amended Rotterdam Criteria from 2004 [37] after excluding pathology of the adrenal glands, thyroid and hyperprolactinaemia and were hospitalized in the Department of Reproduction and Gynaecological Endocrinology of the Medical University of Białystok. For the previous 6. months, none of the patients had been treated due to the conditions mentioned above or declared taking contraceptives or other medications.

The diagnosis of PCOS was based on the recognition two of the three criteria: disorders in ovulation, such as oligomenorrhoea (≥ 35 days)/amenorrhoea; clinical hyperandrogenism (hirsutism evaluated on the basis of the presence of terminal (coarse) hairs according to the score of the modified Ferriman-Gallwey scale [38,39], score ≥8, acne/seborrhoea or alopecia/hair loss); biochemical hyperandrogenism (evaluation of total testosterone – T or dehydroepiandrosterone sulphate – DHEA-S concentrations); and PCO morphology of the ovary in USG imaging (more than 12 follicles of 2 to 9 mm in diameter in each ovary and/or the volume of each ovary more than 10 ml).

2.2. Controls

The control group (n = 78) were recruited from the healthy female students with regular menorrhea (the length of a cycle being from 28 to 34 days) and without features/symptoms of hirsutism. The control subjects were surveyed according to the questionnaire based on the available literature and related to the medical and gynaecological history, especially taking into consideration the risk of thromboembolic disease. Patients with PCOS (based on the questionnaire [40]) with disorders of ovulation (abnormal menstruation, < 25 or > 35 days), hirsutism, abnormal values of prolactin – PRL (> 25 ng/ml) or androgens (T > 1, 3 ng/ml, DHEA-S > 430 μg/dl), and the morphology of PCO ovaries upon USG imaging were excluded.

2.3. Clinical measurements

Before examination, the patients were informed about the study, and each of them signed the written consent of voluntary participation. Standardized, preliminary screening (physical examination, USG, blood test) was carried out in the first phase of a menstrual cycle.

The Body Mass Index (BMI) was calculated as the weight (kilograms)/height in meters squared.

The Waist – Hip Ratio (WHR) was the ratio of the least circuit measured for the waistline between the iliac crest and the border of the last ribs, to the biggest circuit measured at the level of the buttocks.

2.4. Biochemical assays

Rutine evaluation included blood samples for: total cholesterol, triglycerides, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) of cholesterol, fasting insulin and glucose levels, PRL, total testosterone, DHEA-S, 17-hydroksyprogesterone (17-OHP), thyroid stimulating hormone (TSH) and free thyroxin (fT4). Hormonal examinations were performed in the morning, before meals, between days 2 and 5 of the menstrual cycle. A classic glucose tolerance curve (Oral Glucose Tolerance Test – OGTT) was performed according to the Polish Society of Diabetology.

Insulin resistance was calculated using the homeostasis model assessment-insulin resistance index (HOMA-IR), according to the following formula: fasting serum insulin x fasting plasma glucose (mg/dl)/22.5.

2.5. DNA isolation and genotyping

Genomic DNA was isolated from 2 ml of peripheral EDTA-blood using

Table 1
Characteristics of phenotypical groups of PCOS women and controls subjects.

Phenotypes		Age (years)	Weight (kg)	BMI (kg/m^2)	WHR	RBP4 (ng/ml)	Adiponectin (ng/ml)
A	N	202	202	202	200[a]	197[b]	196[b]
	mean value SD	24.62 ± 4.14	69.30 ± 16.55	25.01 ± 6.11	0.82 ± 0.08	31.17 ± 8.65	118.32 ± 72.19
B	N	34	34	34	34	34	34
	mean value SD	25.00 ± 4.34	69.69 ± 16.50	24.44 ± 5.00	0.80 ± 0.07	34.05 ± 8.53	132.77 ± 69.63
D	N	53	53	53	53	50[b]	50[b]
	mean value SD	25.21 ± 5.06	65.11 ± 13.35	23.68 ± 4.57	0.80 ± 0.08	30.42 ± 8.23	116.70 ± 65.09
controls	N	78	78	78	78	78	78
	mean value SD	23.17 ± 1.54	61.40 ± 10.57	21.61 ± 3.10	0.75 ± 0.07	27.79 ± 11.05	147.21 ± 57.54
controls vs. A		P = 0.033 ES = 0.403	P = 0.001 ES = 0.522	P < 0.001 ES = 0.623	P < 0.001 ES = 0.764	P = 0.002 ES = 0.360	P < 0.001 ES = 0.423
controls vs. B		–	P = 0.013 ES = 0.655	P = 0.005 ES = 0.748	P < 0.001 ES = 0.699	P = 0.001 ES = 0.604	–
controls vs. D		–	–	P = 0.020 ES = 0.548	P < 0.001 ES = 0.576	–	P = 0.013 ES = 0.504

The PCOS patients were divided into the following phenotypical groups: A − Oligomenorrhoea + hyperandrogenism + PCO in the ovarian morphology, B − Oligomenorrhoea + PCO in the ovarian morphology, C − Hyperandrogenism + PCO in the ovarian morphology, D − Hyperandrogenism + oligomenorrhoea.
Due to the small number in the phenotype C group (n = 5), the data obtained were not compared statistically.
The effect size parameter (ES) was calculated because of the differences between groups. ES was divided into the 5 categories:
small effect (> = 0.15 and < 0.40).
medium effect (> = 0.40 and < 0.75).
large effect (> = 0.75 and < 1.10).
very large effect (> = 1.10 and < 1.45).
huge effect > 1.45.
[a] lack of data in two cases.
[b] not all serum samples were available.

the QIAamp Blood Midi Kit (Qiagen), according to the manufacturer's instructions. Briefly, 2 ml of whole blood was mixed with 200 µl QIAGEN Protease and 2.4 ml of lysis buffer, followed by incubation for 10 min at 70 °C. After incubation, 2 ml of 96% ethanol was added and mixed vigorously. For DNA binding, the solution was filtrated using a QIAamp column by centrifugation at 3000 rpm for 3 min. The column was washed with 2 ml of Buffer AW1 by centrifugation at 5000 rpm for 1 min, and then with 2 ml of Buffer AW2 by centrifugation at 5000 rpm for 15 s. The DNA was eluted using 200 µl of distilled water.

Genotyping was performed using the TaqMan SNP Genotyping Assay according to the manufacturer's protocol, on an Applied Biosystems 7500 System by Allelic Discrimination analysis. Eleven SNPs were studied: *ADIPOQ* (rs1501299, rs2241766), *RBP4* (rs3758539, rs3758538, rs61461737, rs10882273, rs10882280, rs11187545, rs12265684), *BCMO1* (rs12934922, rs7501331). To assess the reproducibly of genotyping, 5% of the samples were randomly selected and re-genotyped, and all genotypes matched their initial designated genotypes.

2.6. Adiponectin and RBP4 assay

Details of the adiponectin and RBP4 assay used in the present study have been described in our previous publication [30].

2.7. Statistical analyses

Statistical analyses (for the clinical and biochemical characteristics) were performed using the Student's *t*-test, χ2-test, Mann-Whitney *U* test, Shapiro-Wilk's test and two-way ANOVA, followed by the Newman-Keuls post hoc test or the Kruskal-Wallis test, followed by Dunn's post hoc test, as appropriate. Differences were considered to be significant at $P < 0.05$. All calculations were performed using the statistical block, SPSS.

Association analyses performed on the 11 genotyped SNPs located in the *ADIPOQ*, *RBP4* and *BCMO1* genes also have been described in our previous publication [30].

2.8. Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Bioethics Commission of the Medical University of Bialystok (approval number R-I-002/146/2009).

3. Results

The characteristics of the study population revealed that there were significant higher values of weight, BMI, WHR, concentrations of RBP4 and the lover values of adiponectin in group of PCOS women compared to control subjects (Table 1). Additionally, PCOS group had following T, DHEA-S and 17-OHP values (mean ± SD): 1.01 ± 0.8 ng/ml, 198.63 ± 105.55 µg/dl, and 1.92 ± 2.77 ng/ml respectively (data not presented).

The three SNPs of *RBP4*: rs11187545, rs10882280 and rs1461737 were in linkage disequilibrium. The results obtained in a cohort of Polish women for the genotype distribution and allele frequencies (minor allele frequency – MAF) proved that only the SNP rs1501299 in the gene *ADIPOQ* (P = 0.0010, OR = 0.41, 95% C.I.:0.24-0.70) and rs7501331 in the gene *BCMO1* (P = 0.0106, OR = 0.24, 95% C.I.:0.21-0.71), are significantly associated with the decrease of the risk of the disease. Also for this SNPs there were significant differences in the genotypic frequencies in the study population (Table 2).

There was a link between rs12934922 of *BCMO1* gen and serum concentration of RBP4 (P = 0.034) and adiponectin (P = 0.038) in the study group but not the control group (Table 3). There were no statistically differences in BMI, WHR, and insulin and fasting glucose concentrations between genotypes of analysed genetic variants (data not presented). The elevated mean serum concentration of cholesterol (P = 0.020) and LDL cholesterol (P = 0.005) was observed for GG rs1501299 genotype and triglycerides (P = 0.028) for TT rs2241766 genotype (Table 4). We observed also statistically higher scores in modified Ferriman-Gallwey scale for GG compared to TT rs1501299 genotype (P = 0.032) (data not presented).

Table 2
Genotypic frequencies of *ADIPOQ* (rs1501299, rs2241766), *RBP4* (rs3758539, rs3758538, rs61461737, rs10882273, rs10882280, rs11187545, rs12265684) and *BCMO1* (rs12934922, rs7501331) genes in the study population.

SNP	Chromosome	Genotype	PCOS patients (n = 294)	Controls (n = 78)	P
rs1501299 (G/T)	3q27.3	GG	156 (53.1%)	25 (32.1%)	P < 0.001
		GT	117 (39.8%)	49 (62.8%)	
		TT	21 (7.1%)	4 (5.1%)	
rs2241766 (G/T)	3q27.3a	GT	39 (13.3%)	16 (20.5%)	
		TT	255 (86.7%)	62 (79.5%)	
rs3758539 (C/T)	10q23.33b	CC	199 (67.7%)	55 (70.5%)	
		CT	88 (29.9%)	23 (29.5%)	
		TT	7 (2.4%)	0 (0.0%)	
rs3758538 (C/T)	10q23.33b	GG	6 (2.0%)	2 (2.6%)	
		GT	70 (23.8%)	11 (14.1%)	
		TT	218 (74.1%)	65 (83.3%)	
rs61461737 (A/G)	10q23.33b	AA	253 (86.1%)	66 (84.6%)	
		AG	38 (12.9%)	12 (15.4%)	
		GG	3 (1.0%)	0 (0.0%)	
rs10882273 (C/T)	10q23.33b	CC	38 (12.9%)	8 (10.3%)	
		CT	142 (48.3%)	36 (46.2%)	
		TT	114 (38.8%)	34 (43.6%)	
rs10882280 (A/C)	10q23.33b	AA	3 (1.0%)	0 (0.0%)	
		AC	38 (12.9%)	12 (15.4%)	
		CC	253 (86.1%)	66 (84.6%)	
rs11187545 (A/G)	10q23.33b	AA	252 (85.7%)	66 (84.6%)	
		AG	39 (13.3%)	12 (15.4%)	
		GG	3 (1.0%)	0 (0.0%)	
rs12265684 (C/G)	10q23.33b	CC	195 (66.3%)	53 (67.9%)	
		CG	90 (30.6%)	25 (32.1%)	
		GG	9 (3.1%)	0 (0.0%)	
rs12934922 (A/T)	16q23.2b	AA	75 (25.5%)	19 (24.4%)	
		AT	151 (51.4%)	47 (60.3%)	
		TT	68 (23.1%)	12 (15.4%)	
rs7501331 (C/T)	16q23.2b	CC	185 (62.9%)	47 (60.3%)	P < 0.042
		CT	103 (35.0%)	25 (32.1%)	
		TT	6 (2.0%)	6 (7.7%)	

4. Discussion

Recent years have seen an increased interest in genetic factors associated with PCOS, especially polymorphic variations, such as single nucleotide polymorphism – SNP, which are abundant in human genome and easy to detect. This type of genetic variation is of great importance, because SNPs are highly stabile and can be passed down from generation to generation. Our study is the first evaluation of the link between the rs7501331 variant of the *BCMO1* gene and PCOS. A limitation of our study is the size of compared groups, especially small size of the control group. It causes that statistical power of our analysis for most SNP is low (~30%). However, the power is much higher and exceeds ~70% for the most frequent SNPs (*BCMO1* C/T rs7501331 and *ADIPOQ* G/T rs1501299), assuming the effect of association observed in the study.

Table 3
The adipokines levels for polymorphic variant rs12934922 of *BCMO1* gene.

Level analyzed in control group	*BCMO1* rs12934922
RBP4 level in BB + AB vs AA	
P value	0.8537
Mean BB + AB	27.66 n = 59
Mean AA	28.20 n = 19
Adiponectin level in BB + AB vs AA	
P value	0.5850
Mean BB + AB	145.2 n = 59
Mean AA	153.5 n = 19
Level analyzed in case group	*BCMO1* rs12934922
RBP4 level in BB + AB vs AA	
P value	0.0341
Mean BB + AB	32.00 n = 2
Mean AA	29.53 n = 7
Adiponectin level in BB + AB vs AA	
P value	0.0377
Mean BB + AB	124.8 n = 2
Mean AA	104.9 n = 7

Until present, the polymorphic variants of the *BCMO1* gene were explored as loci connected particularly with the level of circulating carotenoids and retinol. Lindquist et al. and Leung et al. revealed that the inter-individual differences in β-carotene cleavage and absorption may be the consequence of existing genetic variation in the *BCMO1* gene [12,41,]. Leung et al. and Ferruci et al. [12,42] identified SNPs in the *BCMO1* gene related to the concentrations of circulating carotenoids. Furthermore, Leung et al. showed that two out of five studied SNPs, the non-synonymous SNPs rs12934922 and rs7501331, decreased the activity of BCMO1 in the carriers by 32% and 69% respectively [12].

Genetic variants in the enzymes involved in the initial cleavage of carotenoids and their possible role in PCOS remain unknown. Our study is the first that indicated the association between *BCMO1* gene polymorphism and the PCOS risk in Polish women. It has to be noted, however, that due to a small number of the analysed samples, especially low number of rare homozygotes, the association of rs7501331 with PCOS is only marginally significant and should be interpreted carefully. The validation of this association would require replication study in a larger group of samples.

Table 4
The characteristic of clinical and biochemical parameters in genotipic groups rs1501299 and rs2241766 of PCOS women.

		BMI (kg/m^2)	WHR	total cholesterol (mg/dl)	triglycerides (mg/dl)	LDL cholesterol (mg/dl)	HDL cholesterol (mg/dl)	fasting insulin (pmol/l)	fasting glucose (mg/dl)
ADIPOQ G/T rs1501299									
GG	N	156	155	156	156	156	155	156	156
	mean value ± SD	24.83 ± 6.35	0.81 ± 0.08	175.55 ± 30.79	92.39 ± 58.63	102.97 ± 27.18	55.04 ± 16.41	9.33 ± 11.13	84.81 ± 11.43
GT	N	117	116	114	115	114	113	110	116
	mean value ± SD	24.37 ± 4.88	0.81 ± 0.09	165.83 ± 29.56	88.13 ± 59.48	93.87 ± 24.22	56.54 ± 18.43	9.4 ± 8.35	83.47 ± 6.39
TT	N	21	21	21	21	21	21	19	21
	mean value ± SD	25.99 ± 4.93	0.83 ± 0.07	164.48 ± 20.46	94.62 ± 43.98	93.33 ± 20.03	51.10 ± 15.51	7.64 ± 2.57	81.52 ± 8.71
Total	N	294	292	291	292	291	289	283	291
	mean value ± SD	24.73 ± 5.71	0.81 ± 0.08	170.95 ± 30.01	90.87 ± 57.93	98.71 ± 25.93	55.34 ± 17.17	9.24 ± 9.74	84.04 ± 9.55
P		0.332	0.485	0.020 ES = 0.321	0.421	0.005 ES = 0.350	0.449	0.863	0.603
ADIPOQ G/T rs2241766									
GT	N	39	39	37	38	37	37	37	37
	mean value ± SD	23.88 ± 4.80	0.80 ± 0.09	170.41 ± 31.28	75.45 ± 43.97	96.00 ± 22.29	59.29 ± 22.30	7.47 ± 5.48	84.27 ± 6.92
TT	N	255	253	254	254	254	252	246	254
	mean value ± SD	24.86 ± 5.83	0.81 ± 0.08	171.02 ± 29.89	93.18 ± 59.46	99.11 ± 26.43	54.76 ± 16.26	9.51 ± 10.21	84.00 ± 9.89
Total	N	294	292	291	292	291	289	283	291
	mean value ± SD	24.73 ± 5.71	0.81 ± 0.08	170.95 ± 30.01	90.87 ± 57.93	98.71 ± 25.93	55.34 ± 17.17	9.245 ± 9.74	84.04 ± 9.55
P		0.493	0.177	0.724	0.028 ES = 0.307	0.525	0.155	0.110	0.624

(ES – see Table 1).

Analysis of the results obtained in an animal model indicates that genetic variants in the *BCMO1* gene may increase the risk of disorders in lipid metabolism [43]. Our findings did not prove the link between polymorphic variants in the *BCMO1* gene and the lipid profile of the female subjects, but we showed that the genetic variant rs12934922 of the *BCMO1* gene affected the level of both examined adipokines in PCOS patients, which indicates its possible role in the adipose tissue functions. Further studies should focus on the mechanism of rs12934922 action and its influence on the PCOS pathogenesis.

Incidence of metabolic disorders in PCOS patients, apart from the so called Rotterdam Criteria (2004), is known to be high. Many of them show increased BMI, and, as evidenced by our own study, increased WHR. Additionally, obesity seems to affect both clinical and biochemical presentation of PCOS [44]. Metabolic disorders observed in PCOS patients and obese women without PCOS are linked with disorders in adipose tissue functions, such as secreting cytokines, known as adipokines, from adipocytes [45,46,]. In PCOS patients, adiponectin is downregulated [30,47,48], and lower serum levels of this adipokine are observed in both lean and obese women [49], although in some reports the concentrations of adiponectin did not differ between PCOS and control [50]. Many genetic studies supported the role of genetic polymorphisms of the *ADIPOQ* gene in this pathology, but the results are conflicting. Most studies focused on the rs2241766 45 T/G and rs1501299 276 G/T polymorphism since they were associated with the typical disorders connected to PCOS like obesity, insulin resistance and the risk of type 2 diabetes. The first meta-analysis carried out by Wu et al. and published in 2014 indicated a strong association between the *ADIPOQ*-rs2241766 G/T polymorphism and obesity in Chinese studies, but no such relationship in non-Chinese studies [51]. Therefore, rs2241766 G/T in addition to rs1501299 G/T was included into our study in order to find out if this genetic variant is implicated in the pathogenesis of PCOS.

A meta-analysis of the published case-control studies confirmed the presence of rs1501299 G/T variation between the ethnic groups [18]. In Europe populations only, Heinonen et al. found a significant association between the rs1501299 variant and susceptibility to PCOS in Finnish women [21]. In Asian women, such associations were found in two studies on rs1501299 from China and Korea [52,53]. Radavelli-Bagatini et al. reported that the haplotype TGTG of 276 G/T gene variant was linked with susceptibility to PCOS in Brasilian women [20]. In another meta-analyses, the authors suggested that the rs1501299 polymorphism was related to a decreased risk of PCOS [18,19]. The latest updated meta-analysis study (incorporated studies from 2013, 2015 and 2016) indicated that *ADIPOQ* 276 G/T polymorphism was associated with the decreased risk of PCOS, especially in Asian and patients diagnosed according to ESHRE/ASRM (patients diagnosed according to European Society for Human Reproduction and Embryology/American Society for Reproductive Medicine criteria) of PCOS subgroups, suggested the protective role of the T allele for PCOS [54]. Moreover, *ADIPOQ* 276 G/T was associated with the total levels of adiponectin in obese non-PCOS women [22] and may influence production of adiponectin in patients with PCOS [55]. Nevertheless, the findings remain still controversial. No association between rs1501299 and adiponectin levels was found in Indian PCOS women [56]. In the present study on Polish women, a significant association was found between the rs1501299 variant in the *ADIPOQ* gene and the decreased PCOS risk. This genetic variant also affected lipid concentrations, but showed no apparent relationship with adiponectin values. The cumulative results mentioned above are present in Table 5.

Furthermore, we did not demonstrate any association between the *ADIPOQ*-rs2241766 G/T variant and adiponectin levels in control and PCOS subjects. This *ADIPOQ* gene variant was significantly associated with plasma adiponectin concentrations but in non-PCOS patients [23]. The association studies of many genetic variants of *ADIPOQ* gene with adiponectin level demonstrated that there was some ethnic differences in the results [57]. Moreover, the conclusion from the Indian report is that serum adiponectin levels were more dependent on BMI than on adiponectin gene [56]. In humans, another adipokine, RBP4, a known mediator of glucose [58] and lipid [59,60] homeostasis, may play a significant role in PCOS. Many factors (environmental, life-style associated, genetic) may influence the link between RBP4 and PCOS pathophysiology in different geographical regions [31]. Statistically, RBP4 level tends to be higher in the PCOS group compared to control subjects [28,30,31,61] with statistically significant differences between phenotypical groups of PCOS women (control versus oligomenorrhoea + hyperandrogenism + PCOS in the ovarian morphology group, P = 0.002; control versus oligomenorrhoea + PCOS in the ovarian morphology group, P = 0.001; our results, data not presented), and without differences between ovulatory and anovulatory PCOS women [28]. Moreover, RBP4 levels correlated with triglyceride concentrations, partially driven by body fat [62]. The association between RBP4 polymorphic variants and PCOS has not yet been studied. One study on healthy adults from the Chinese Han population demonstrated that RBP4 polymorphic variants were significantly associated with plasma

Table 5
Genotypic frequencies and association with PCOS and adiponectin concentrations of rs1501299 of *ADIPOQ* gene in PCOS women and control subjects of different ethnicity.

Study/Reference No.	Year	Country/Race	Genotype − PCOS patients			Genotype − control			Association with PCOS	Association with adiponectin concentrations
			GG	GT	TT	GG	GT	TT		
Heinonen et al. [21]	2005	Finland/ Caucasian	77 (53.8%)	58 (40.6%)	8 (5.6%)	110 (44.9%)	110 (44.9%)	25 (10.2%)	Yes (susceptibility with PCOS)	–
Xita et al. [55]	2005	Greece/ Caucasian	39 (39%)	49 (49%)	12 (12%)	52 (37.2%)	73 (52.1%)	15 (10.7%)	No	Yes [a]
Zhang et al. [52]	2008	China/Asian	56 (46.7%)	46 (38.3%)	18 (15.0%)	41 (34.2%)	50 (41.6%)	29 (24,2%)	Yes (susceptibility with PCOS)	Yes [a]
Li et al. [53]	2011	Korea/Asian	61 (42.4%)	73 (50.7%)	10 (6.9%)	48 (30.2%)	87 (54.7)	24 (15.1)	Yes	–
Radavelli-Bagatini et al. [20]	2013	Brazil/Mixed	42 (52.5%)	27 (33.7%)	11 (13.8%)	670 (44.7%)	672 (44.8%)	158 (10.5%)	Yes (susceptibility with PCOS)	–
Nambiar et al. [56]	2016	India/Asian	23 (8.2%)	94 (33.3%)	165 (58.5%)	15 (7.5%)	86 (43%)	99 (49.5%)	No	No
Our results		Poland/ Caucasian	156 (53.1%)	117 (39.8%)	21 (7.1%)	25 (32.1%)	49 (62.8%)	4 (5.1%)	Yes (decrease the risk of the PCOS)	No

[a] serum adiponectin concentrations were significantly lower in PCOS carriers of GG + GT than in TT.

RBP4 levels and hypertriglyceridemia risk [35]. Although PCOS patients involved in our study showed statistically significant increase in serum RBP4 levels compared to the control group, which is believed to be a hypertriglyceridemia risk factor in the healthy population [35], the observed triglyceride levels in PCOS patients were comparable to those in the control group, and the studied variants of the *RBP4* gene showed no association with PCOS nor with the levels of particular lipid fractions in the patients (data not presented).

5. Conclusions

Overall, our results showed no link between the studied genetic variants of the *RBP4* gene with the syndrome. It seems that rs1501299 of *ADIPOQ* gene influences the occurrence of PCO and the lipids profile in those patients.

Conflict of interest

The authors declare no conflict of interest.

Financial disclosure

This study was funded by the Medical University of Białystok: Grant no. N/ST/ZB/15/001/1104.

References

[1] Abbott DH, Dumesic DA, Franks S. Developmental origin of polycystic ovary syndrome-a hypothesis. J Endocrinol 2002;174(1):1–5.
[2] van Houten EL, Visser JA. Mouse models to study polycystic ovary syndrome: a possible link between metabolism and ovarian function? Reprod Biol 2014;14(1):32–43.
[3] Gilling-Smith C, Willis DS, Beard RW, Franks S. Hypersecretion of androstenedione by isolated thecal cells from polycystic ovaries. J Clin Endocrinol Metab 1994;79(4):1158–65.
[4] Vendola KA, Zhou J, Adesanya OO, Weil SJ, Bondy CA. Androgens stimulate early stages of follicular growth in the primate ovary. J Clin Invest 1998;101(12):2622–9.
[5] Vendola K, Zhou J, Wang J, Bondy CA. Androgens promote insulin-like growth factor-I and insulin-like growth factor-I receptor gene expression in the primate ovary. Hum Reprod 1999;14(9):2328–32.
[6] Griswold MD, Hogarth CA, Bowles J, Koopman P. Initiating meiosis: the case for retinoic acid. Biol Reprod 2012;86(2):1–7. 35.
[7] Wood JR, Ho CK, Nelson-Degrave VL, McAllister JM, Strauss 3rd JF. The molecular signature of polycystic ovary syndrome (PCOS) theca cells defined by gene expression profiling. J Reprod Immunol 2004;63(1):51–60.
[8] Wickenheisser JK, Nelson-DeGrave VL, Hendricks KL, Legro RS, Strauss 3rd JF, McAllister JM. Retinoids and retinol differentially regulate steroid biosynthesis in ovarian theca cells isolated from normal cycling women and women with polycystic ovary syndrome. J Clin Endocrinol Metab 2005;90(8):4858–65.
[9] Jiang Y, Li C, Chen L, Wang F, Zhou X. Potential role of retinoids in ovarian physiology and pathogenesis of polycystic ovary syndrome. Clin Chim Acta 2017;469:87–93.
[10] Lindqvist A, Andersson S. Biochemical properties of purified recombinant human beta-carotene 15,15'-monooxygenase. J Biol Chem 2002;277(26):23942–8.
[11] Lindqvist A, Andersson S. Cell type-specific expression of beta-carotene 15,15'-mono-oxygenase in human tissues. J Histochem Cytochem 2004;52(4):491–9.
[12] Leung WC, Hessel S, Méplan C, Flint J, Oberhauser V, Tourniaire F, et al. Two common single nucleotide polymorphisms in the gene encoding beta-carotene 15,15'-monoxygenase alter beta-carotene metabolism in female volunteers. FASEB J 2009;23(4):1041–53.
[13] Lietz G, Oxley A, Leung W, Hesketh J. Single nucleotide polymorphisms upstream from the β-carotene 15, 15'-monoxygenase gene influence provitamin A conversion efficiency in female volunteers. J Nutr 2012;142(1):161S–5S.
[14] Michalakis KG, Segars JH. The role of adiponectin in reproduction: from polycystic ovary syndrome to assisted reproduction. Fertil Steril 2010;94(6):1949–57.
[15] Comim FV, Hardy K, Franks S. Adiponectin and its receptors in the ovary: further evidence for a link between obesity and hyperandrogenism in polycystic ovary syndrome. PLoS One 2013;8(11):e80416.
[16] Lagaly DV, Aad PY, Grado-Ahuir JA, Hulsey LB, Spicer LJ. Role of adiponectin in regulating ovarian theca and granulosa cell function. Mol Cell Endocrinol 2008;284(1-2):38–45.
[17] Chen X, Jia X, Qiao J, Guan Y, Kang J. Adipokines in reproductive function: a link between obesity and polycystic ovary syndrome. J Mol Endocrinol 2013;50(2):R21–37.
[18] Xian L, He W, Pang F, Hu Y. ADIPOQ gene polymorphisms and susceptibility to polycystic ovary syndrome: a HuGE survey and meta-analysis. Eur J Obstet Gynecol Reprod Biol 2012;161(2):117–24.
[19] Jia H, Yu L, Guo X, Gao W, Jiang Z. Associations of adiponectin gene polymorphisms with polycystic ovary syndrome: a meta-analysis. Endocrine 2012;42(2):299–306.
[20] Radavelli-Bagatini S, de Oliveira IO, Ramos RB, Santos BR, Wagner MS, Lecke SB, et al. Haplotype TGTG from SNP 45 T/G and 276G/T of the adiponectin gene contributes to risk of polycystic ovary syndrome. J Endocrinol Invest 2013;36(7):497–502.
[21] Heinonen S, Korhonen S, Helisalmi S, Koivunen R, Tapanainen J, Hippeläinen M, et al. Associations between two single nucleotide polymorphisms in the adiponectin gene and polycystic ovary syndrome. Gynecol Endocrinol 2005;21(3):165–9.
[22] de Luis DA, Izaola O, de la Fuente B, Primo D, Fernandez Ovalle H, Romero E. rs1501299 polymorphism in the adiponectin gene and their association with total adiponectin levels, insulin resistance and metabolic syndrome in obese subjects. Ann Nutr Metab 2016;69(3–4):226–31.
[23] Vasseur F, Helbecque N, Dina C, Lobbens S, Delannoy V, Gaget S, et al. Single-nucleotide polymorphism haplotypes in the both proximal promoter and exon 3 of the APM1 gene modulate adipocyte-secreted diponectin hormone levels and contribute to the genetic risk for type 2 diabetes in French Caucasians. Hum Mol Genet 2002;11(21):2607–14.
[24] Kanai M, Raz A, Goodman DS. Retinol-binding protein: the transport protein for vitamin A in human plasma. J Clin Invest 1968;47(9):2025–44.
[25] Muoio DM, Newgard CB. Metabolism: A is for adipokine. Nature 2005;436(7049):337–8.
[26] Tan BK, Chen J, Lehnert H, Kennedy R, Randeva HS. Raised serum, adipocyte, and adipose tissue retinol-binding protein 4 in overweight women with polycystic ovary syndrome: effects of gonadal and adrenal steroids. J Clin Endocrinol Metab 2007;92(7):2764–72.
[27] Yildizhan R, Ilhan GA, Yildizhan B, Kolusari A, Adali E, Bugdayci G. Serum retinol-binding protein 4, leptin, and plasma asymmetric dimethylarginine levels in obese

and nonobese young women with polycystic ovary syndrome. Fertil Steril 2011;96(1):246–50.
[28] Carmina E, Bucchieri S, Mansueto P, Rini G, Ferin M, Lobo RA. Circulating levels of adipose products and differences in fat distribution in the ovulatory and anovulatory phenotypes of polycystic ovary syndrome. Fertil Steril 2009;91(Suppl. (4)):1332–5.
[29] Mahde A, Shaker M, Al-Mashhadani Z. Study of omentin1 and other adipokines and hormones in PCOS patients. Oman Med J 2009;24(2):108–18.
[30] Czeczuga-Semeniuk E, Jarząbek K, Galar M, Kozłowski P, Sarosiek NA, Zapolska G, et al. Assessment of FSHR, AMH, and AMHRII variants in women with polycystic ovary syndrome. Endocrine 2015;48(3):1001–4.
[31] Jia J, Bai J, Liu Y, Yin J, Yang P, Yu S, et al. Association between retinol-binding protein 4 and polycystic ovary syndrome: a meta-analysis. Endocr J 2014;61(10):995–1002.
[32] Chan TF, Tsai YC, Chiu PR, Chen YL, Lee CH, Tsai EM. Serum retinol-binding protein 4 levels in nonobese women with polycystic ovary syndrome. Fertil Steril 2010;93(3):869–73.
[33] Yildiz BO, Bozdag G, Otegen U, Harmanci A, Boynukalin K, Vural Z, et al. Visfatin and retinol-binding protein 4 concentrations in lean, glucose-tolerant women with PCOS. Reprod Biomed Online 2010;20(1):150–5.
[34] Munkhtulga L, Nakayama K, Utsumi N, Yanagisawa Y, Gotoh T, Omi T, et al. Identification of a regulatory SNP in the retinol binding protein 4 gene associated with type 2 diabetes in Mongolia. Hum Genet 2007;120(6):879–88.
[35] Wu Y, Li H, Loos RJ, Qi Q, Hu FB, Liu Y, et al. RBP4 variants are significantly associated with plasma RBP4 levels and hypertriglyceridemia risk in Chinese Hans. J Lipid Res 2009;50(7):1479–86.
[36] Shea JL, Loredo-Osti JC, Sun G. Association of RBP4 gene variants and serum HDL cholesterol levels in the Newfoundland population. Obesity 2010;18(7):1393–7.
Silver Spring.
[37] Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group. Hum Reprod 2004;19(1): 41-47.
[38] Ferriman D, Purdie AW. Association of oligomenorrhoea: hirsuties, and infertility. Br Med J 1965;2(5453):69–72.
[39] Hatch R, Rosenfield RL, Kim MH, Tredway D. Hirsutism: implications, etiology, and management. Am J Obstet Gynecol 1981;140(7):815–30.
[40] Pedersen SD, Brar S, Faris P, Corenblum B. Polycystic ovary syndrome: validate questionnaire for use in diagnosis. Ca Fam Physician 2007;53(6):1041–7.
[41] Lindqvist A, Sharvill J, Sharvill DE, Andersson S. Loss-of-function mutation in carotenoid 15,15'-monooxygenase identified in a patient with hypercarotenemia and hypovitaminosis A. J Nutr 2007;137(11):2346–50.
[42] Ferrucci L, Perry JR, Matteini A, Perola M, Tanaka T, Silander K, et al. Common variation in the beta-carotene 15,15'-monooxygenase 1 gene affects circulating levels of carotenoids: a genome-wide association study. Am J Hum Genet 2009;84(2):123–33.
[43] Lietz G, Oxley A, Boesch-Saadatmandi C, Kobayashi D. Importance of β,β-carotene 15, 15'-monooxygenase 1 (BCMO1) and β,β-carotene 9', 10'-dioxygenase 2 (BCMO2) in nutrition and health. Mol Nutr Food Res 2012;56(2):241–50.
[44] Barber TM, McCarthy MI, Wass JA, Franks S. Obesity and polycystic ovary syndrome. Clin Endocrinol (Oxf) 2006;65(2):137–45.
[45] Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. J Clin Endocrinol Metab 2004;89(6):2548–56.
[46] Reverchon M, Ramé C, Bertoldo M, Dupont J. Adipokines and the female reproductive tract. Int J Endocrinol 2014;2014:232454.
[47] Escobar-Morreale HF. Adiponectin and resistin in PCOS: a clinical, biochemical and molecular genetic study. Hum Reprod 2006;21(9):2257–65.
[48] Shin HY, Lee DC, Lee JW. Adiponectin in women with polycystic ovary syndrome. Korean J Fam Med 2011;32(4):243–8.
[49] Toulis KA, Goulis DG, Farmakiotis D, Georgopoulos NA, Katsikis I, Tarlatzis BC, et al. Adiponectin levels in women with polycystic ovary syndrome: a systematic review and a meta-analysis. Hum Reprod Update 2009;15(3):297–307.
[50] Lecke SB, Mattei F, Morsch DM, Spritzer PM. Abdominal subcutaneous fat gene expression and circulating levels of leptin and adiponectin in polycystic ovary syndrome. Fertil Steril 2011;95(6):2044–9.
[51] Wu J, Liu Z, Meng K, Zhang L. Association of adiponectin gene (ADIPOQ) rs2241766 polymorphism with obesity in adults: a meta-analysis. PLoS One 2014;9(4):e95270.
[52] Zhang N, Shi YH, Hao CF, Gu HF, Li Y, Zhao YR, et al. Association of +45G15G(T/G) and +276(G/T) polymorphisms in the ADIPOQ gene with polycystic ovary syndrome among Han Chinese women. Eur J Endocrinol 2008;158(2):255–60.
[53] Li L, Yun JH, Lee JH, Song S, Choi BC, Baek KH. Association study of +45G15G(T/G) and +276(G/T) polymorphisms in the adiponectin gene in patients with polycystic ovary syndrome. Int J Mol Med 2011;27(2):283–7.
[54] Li X, Wang Y, Tang W, Liu Ch, Chen Y, Zheng Z. Relationship between ADIPOQ polymorphisms and polycystic ovary syndrome: a meta-analysis involving 6735 subjects. Int J Clin Exp Med 2017;10(2):1828–39.
[55] Xita N, Georgiou I, Chatzikyriakidou A, Vounatsou M, Papassotiriou GP, Papassotiriou I, et al. Effect of adiponectin gene polymorphisms on circulating adiponectin and insulin resistance indexes in women with polycystic ovary syndrome. Clin Chem 2005;51(2):416–23.
[56] Nambiar V, Vijesh VV, Lakshmanan P, Sukumaran S, Suganthi R. Association of adiponectin and resistin gene polymorphisms in South Indian women with polycystic ovary syndrome. Eur J Obstet Gynecol Reprod Biol 2016;200:82–8.
[57] Ong KL, Li M, Tso AW, Xu A, Cherny SS, Sham PC, et al. Investigators of the Hong Kong cardiovascular risk factor prevalence study: association of genetic variants in the adiponectin gene with adiponectin level and hypertension in Hong Kong Chinese. Eur J Endocrinol 2010;163(2):251–7.
[58] Graham TE, Yang Q, Blüher M, Hammarstedt A, Ciaraldi TP, Henry RR, et al. Retinol-binding protein 4 and insulin resistance in lean, obese, and diabetic subjects. N Engl J Med 2006;354(24):2552–63.
[59] Promintzer M, Krebs M, Todoric J, Luger A, Bischof MG, Nowotny P, et al. Insulin resistance is unrelated to circulating retinol binding protein and protein C inhibitor. J Clin Endocrinol Metab 2007;92(11):4306–12.
[60] von Eynatten M, Lepper PM, Liu D, Lang K, Baumann M, Nawroth PP, et al. Retinol-binding protein 4 is associated with components of the metabolic syndrome, but not with insulin resistance, in men with type 2 diabetes or coronary artery disease. Diabetologia 2007;50(9):1930–7.
[61] Weiping L, Qingfeng C, Shikun M, Xiurong L, Hua Q, Xiaoshu B, et al. Elevated serum RBP4 is associated with insulin resistance in women with polycystic ovary syndrome. Endocrine 2006;30(3):283–7.
[62] Sopher AB, Gerken AT, Blaner WS, Root JM, McMahon DJ, Oberfield SE. Metabolic manifestations of polycystic ovary syndrome in nonobese adolescents: retinol-binding protein 4 and ectopic fat deposition. Fertil Steril 2012;97(4):1009–15.