PCOS in women with infertility and role of determinant genes of steroid hormones

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Abstract. We observed 125 women: group 1 - 45 women with primary PCOS and infertility; group 2 - 46 women with infertility and PCOS in preparation for ART; group 3 26 conditionally healthy women. Based on the foregoing, we presented the data of our own studies on the assessment of the state, the genes of steroid hormones (CYP17A1-rs743572, CYP19A1-rs247015) based on the analysis of laboratory data. CY17A1 polymorphism in patients with PCOS, it can be said that the GG mutant genotype was statistically significantly more common in patients compared to the control group. When dividing PCOS patients, they were divided into groups and compared with the control group, MS+ PCOS patients had a lower level of the mutant form (GG) genotype compared to the control group, but in MS-PCOS patients compared to the control group, the mutant gene was determined more.

1 Introduction

Polycystic ovary syndrome (PCOS) is a heterogeneous syndrome characterized by hypersecretion of luteinizing hormone (LH), ovarian hyperandrogenism, polycystic ovaries, hyperinsulinemia due to insulin resistance, and reduced fertility. The variable phenotypic expression of reproductive and metabolic abnormalities in PCOS patients results in differences in oocyte developmental capacity, defined as the ability of oocytes to complete meiosis and undergo fertilization, embryogenesis, and term development. Some women with PCOS who undergo ovarian stimulation for in vitro fertilization (IVF) have normal embryonic development and a normal pregnancy outcome, while others have impaired oocyte development. Women with PCOS who are also overweight are particularly vulnerable and suffer from low egg fertilization and the inability to implant embryos in their own or other women's uterus [1,2,3,4,5].

PCOS is characterized by endocrinological disorders; therefore, polymorphisms in genes encoding sex hormones or regulators of their activity were studied [6,7,8]. The follicle-stimulating hormone receptor (FSHR) gene contains two important single nucleotide polymorphisms (SNPs) in exon 10 that are in linkage disequilibrium and replace two amino acids at positions A307T and N680S. A307T, located in the extracellular domain of FSHR, a site responsible for high-affinity hormone binding [9,10], has been reported to affect hormone transport and signal transduction. Phosphorylation of Ser and Thr residues in

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intracellular regions of FSHR can influence uncoupling from adenylate cyclase. As a result, the amino acid change associated with the respective SNPs can affect post-translational modifications of the FSHR protein, hence receptor function, including FSH efficiency [11,12,13]. Several genetic studies have examined the association between FSHR gene polymorphisms and PCOS.

Several studies have investigated whether polymorphisms of enzymes involved in the biosynthesis and metabolism of sex steroids affect the predisposition to PCOS [14]. A polymorphism was found in the regulatory region of the 17α-hydroxylase (CYP17) gene, which is a replacement of T with C -34 base pairs (bp) from the translation initiation point in the 5'-promoter region of the gene, which creates a new Msp A1 restriction site. The less common "C" allele also results in an additional promoter site like Sp1 (CCACC box), which is expected to increase gene transcription and thus lead to higher androgen levels. Although this base pair substitution is not the primary genetic defect in PCOS, it can exacerbate the clinical picture of hyperandrogenemia, especially in the presence of homozygosity [15,24]. On the other hand, in one of the previous studies, the CYP17 gene was not associated with the synthesis of steroid hormones in PCOS [16]. Several genetic risk factors for PCOS have been studied [17,25]. The CYP1A1 gene, located in the 15q22–q24 region, consists of seven exons and six introns. A polymorphism in the CYP1A1 gene encoding the cytochrome P450 1A1 enzyme has been shown to be associated with PCOS. In addition, studies have shown that the pentanucleotide repeat in the gene is associated with predisposition to PCOS [14,26].

In terms of CYP17A1 T/C polymorphism, proportionally, individuals carrying AG+GG gene variants were more frequently observed in patients than in controls. However, genotype and allele frequencies did not differ between cases and controls ($P > 0.05$). The frequency of the GG CYP17A1 genotype was higher among the main group compared to PCOS with metabolic syndrome. On the other hand, the frequency of the CYP17A1 AG genotype in PCOS patients was higher than in controls. CYP17A1 _ GG genotypes were found in 15% of the main, 11.6% in PCOS women with metabolic syndrome, 19% in PCOS women without metabolic syndrome, 14.4% in the control group. Although there was no statistically significant difference in the distribution of genotypes and alleles between cases and controls in terms of CYP17A1 polymorphism ($P > 0.05$), a high frequency of GG homozygous genotypes was observed in both groups [15].

We then examined the CYP17-34 C/T and CYP1A1 T6235C SNPs. We found that genotype and allele frequencies did not differ between cases and controls in both polymorphisms ($P > 0.05$). Park et al found seven SNPs of the CYP17 gene, which is active in estrogen biosynthesis and located at the 10q24.3 region, and found no significant association between these SNPs and PCOS [16]. For CYP17-34 C/T, we found that the CC genotype was the least common genotype in both cases and controls. The TC genotype by CYP17 was the most frequent in both cases and controls. The genotype distribution of the control group and cases in our study was very similar. We further found that the CC genotype was the least common in both cases and controls. These results are consistent with the results of some previous studies [17]. Diamanti-Kandarakis et al. showed that homozygosity for the polymorphic A2 allele (or CC genotype) was not observed in controls, but a lower percentage (8%) was observed in women with PCOS. Thus, this difference was found to be statistically significant [18]. On the other hand, in our study, we found that the frequency of the "C" allele is 0.44 in cases and 0.36 in controls. The distribution pattern of the two alleles ("C" and "T") was the same in both groups. This result is also consistent with previous studies [19]. Echiburu et al. found that PCOS carriers of the A2 (or C) allele have greater BMI and waist circumference than non-PCOS carriers [20]. However, we found no evidence of an association between clinical and biochemical characteristics and CYP17-34 C/T polymorphism in patients with PCOS ($P > 0.05$). Contrary to previous observations by Babou et al. [21], who suggested that the CYP1A1 polymorphism (T6235C) may represent a risk

factor for PCOS, we did not find any evidence to support an association between CYP1A1 polymorphism and PCOS. In addition, Esinler et al. studied the correlation between CYP1A1 genotypes and women with PCOS [22]. They found that patients with PCOS had a 7.8-fold higher incidence of CYP1A1 of the Ile/Val genotype and a 7.4-fold higher incidence of CYP1A1 of any Val genotype (Ile/Val or Val/Val) in the Turkish population. This discrepancy between the two studies was due to the fact that different regions of the CYP1A1 gene were studied in two studies. We investigated polymorphic variants in the 3'-flanking region (T6235C) of the gene, while Esinler et al. studied Ile462Val (A/G) variants in exon 7 of the gene [23].

Purpose of the study: To achieve prognostic criteria for the outcomes of ART programs in women with infertility in PCOS based on molecular genetic predictors of folliculogenesis disorders.

2 Material and methods

Based on the foregoing, we presented the data of our own studies on the assessment, ultrasound of the pelvic organs (folliculometry with AFA calculation), hormonal studies (AMH, E, FSH, LH, testosterone), steroid hormone genes (CYP191-57A2, rs74) based on the analysis of laboratory parameters.

To solve the tasks set in the work, 125 women were examined: group 1 - 45 women with primary PCOS and infertility; group 2 - 46 women with infertility and PCOS in preparation for ART; group 3 26 conditionally healthy women.

3 Results

In all 106 patients of the observed main group, a genetic study of polymorphism of the CYP17A1(rs743572) and CYP19A1(rs2470152) genes was performed. The control group consisted of 52 healthy volunteers who had no history of predisposition to PCOS. Along with this, 106 patients were also divided into 2 groups. One of them included patients with metabolic syndrome (n=60) (MS+), the second group included patients with PCOS without metabolic syndrome (n=46) (MS-). In patients with PCOS, AA homozygous or wild type of the allelic genotype of the CYP17A1 gene was 36.8%, AG heterozygous genotype in 48.1% of patients, GG homozygous mutant genotype was found in 15.1% of patients.

In our study, polymorphism of the homozygous normal or wild AA genotype of the CYP17A1 gene was observed in 45.0% of patients with MS+, compared with the MS-group where this percentage was 26.1%, in the third observation group this percentage was 40.4%. In addition, among MS+ patients with PCOS, the rate of mutant homozygous GG genotype of the CYP17A1 gene polymorphism was low and amounted to 11.7% in the first group, 19.6% in the second group and 13.5% in the control group. Also, the difference in the occurrence of the heterozygous genotype (AG genotype) in the first and second groups was 9%, and this was dominated by patients of the second group (54.3%), the difference between the indicators of the first and control groups was only 3.2%, where first group.

According to the level of occurrence of allelic variants of the CYP17A1 gene, the percentage of patients with allelic A in the MS + group was 66.7% and 55.3% in patients with the MS group. G allele was determined in 40% of patients of the first group and 43% of patients of the second group (Table 1). When we compared the level of occurrence of CYP17A1 gene polymorphism with the control group, it was found that normal - “wild” AA genotypes with a smaller difference were more common in the control group, while heterozygous AA and mutant GG genotypes slightly prevailed in the group of patients with

PCOS (OR=1.06; 95%CI 0.55-2.10; p<0.8 for heterozygous genotype and OR=1.14; 95%CI 0.44-2.98; P<0.8 for homozygous genotype) (Table 1).

Table 1. Results of the comparison of CYP17A1 gene polymorphism between patients with PCOS and healthy people.

Alleles and genotypes	Number of examined alleles and genotypes				Xi2	P	RR	95%CI	OR	95%CI
	Main group (n=106)		Control group (n=52)							
	N	%	N	%						
A	129	60,8	66	63,0	0,2	p < 0,7	0,96	0,82 - 1,128	0,9	0,551 - 1,45
G	83	39,2	38	37,0	0,2	p < 0,7	1,0	0,88 - 1,213	1,1	0,68 - 1,815
A/ A	39	36,8	21	40,4	0,2	p < 0,7	0,9	0,75 - 1,196	0,86	0,435 - 1,69
A/G	51	48,1	24	46,1	0,05	p < 0,8	1,06	0,85 - 1,328	1,1	0,55 – 2,10
G /G	16	15,1	7	13,5	0,075	p < 0,8	1,04	0,77 – 1,40	1,14	0,44 - 2,98

Interestingly, when we divided patients with PCOS into two groups with the presence of metabolic syndrome disease, we found that in the MC+ group, the wild variant (AA genotype) of the CYP17A1 gene was even higher than in the control group (45.0% and 40.4% respectively), the heterozygous variant (AG genotype) is almost equal (43.3 and 46.1%, respectively), and the mutant variant was more common in the control group. Therefore, we concluded that the importance of the development of PCOS in MS+ patients with the mutant form of the genotype - GG does not matter (chi2=0.08; OR=0.85; 95% CI: 0.27 - 2.60; p=0, 77). (Table 2).

Table 2. The results of genotyping of CYP17A1 gene polymorphism in patients with PCOS MS+, as well as in healthy people are presented.

Alleles and genotypes	Number of examined alleles and genotypes				Xi2	P	RR	95%CI	OR N	95%CI %
	PCOS with metabolic syndrome (n=60)		Control group (n=52)							
	N	%	N	%						
A	80	66,7	66	63,0	0,25	p = 0,61	1,06	0,88 - 1,38	1,15	0,66 - 1,99
G	40	33,3	38	37,0	0,25	p = 0,61	0,9	0,72 - 1,21	0,87	0,50 - 1,50
A/ A	27	45,0	21	40,4	0,24	p = 0,62	1,1	0,77 – 1,54	1,2	0,57 - 2,56
A/G	26	43,3	24	46,1	0,09	p = 0,76	0,95	0,67 – 1,34	0,9	0,42 - 1,88
G /G	7	11,7	7	13,5	0,08	p = 0,77	0,9	0,53 - 1,61	0,85	0,27 - 2,60

However, in the second group of patients (MC-) of the mutant form of the genotype (GG - 19.6%), as well as the heterozygous variant (AG - 54.3%) significantly prevailed in comparison with the control group (in the control group, these figures were 13.5% and 46.1%,

respectively) in the control group, the homozygous AA genotype prevailed with 40.4%. Despite the fact that the value of the mutant form (GG-genotype-) developed PCOS in patients with MS- (OR=1.56) was found, it was considered unreliable (x and 2 = 0.66; 95% CI: 0.53 - 4.60; p < 0.5.) (Table 3).

Table 3. Results of a study of genotyping of CYP17 gene polymorphism in MS-patients with PCOS and healthy people.

Alleles and genotypes	**Number of examined alleles and genotypes**				**Xi2**	**P**	**RR**	**95%CI**	**OR**	**95%CI %**
	PCOS without metabolic syndrome (n=46)		**Control group (n=52)**							
	N	**%**	**N**	**%**						
A	49	53,3	66	63,0	2,1	p < 0,2	0,8	0,59 - 1,07	0,65	0,37 - 1,16
G	43	46,7	38	37,0	2,1	p < 0,2	1,25	0,93 - 1,67	1,5	0,86 - 2,70
A/ A	12	26,1	21	40,4	2,2	p < 0,2	0,69	0,41 - 1,15	0,5	0,22 - 1,23
A/G	25	54,3	24	46,1	0,65	p < 0,45	1,2	0,78 - 1,8	1,39	0,62 - 3,07
G /G	9	19,6	7	13,5	0,66	p < 0,5	1,25	0,76 – 2,04	1,56	0,53 - 4,60

The distribution of genotypes in the studied polymorphic loci was checked for compliance with the Hardy-Weinberg equation. The deviation of genotypes from the Hardy-Weinberg equation in the main and control groups was almost not observed. (D=0.00 and D=-0.01, respectively) (Table 4).

Table 4. Correspondence of the genoptype of the CYP17 gene polymorphism to the Hardy-Weinberg equation in PCOS patients and in healthy patients. (chi-squared test, df=1).

Main group (n=106)					
Alleles	Allele frequency				
A	0,61				
G	0,39				
Genotypes	Genotype frequency		Xi2	p	df
	observed	expected			
A/ A	0,37	0,37	0		
A/G	0,48	0,48	0		
G /G	0,15	0,15	0		
Total	1	1	0,01	0,877	1
Control group					
Alleles	Allele frequency				
A	0,63				
G	0,37				
Genotypes	Genotype frequency		Xi2	p	df
	observed	expected			
A/ A	0,404	0,4	0,01		
A/G	0,461	0,466	0,05		
G /G	0,135	0,136	0,04		
Total	1	1	0,1	0,721	1
Groups	Ho	He	D*		
Main group	0,48	0,48	0,00		

Control group	0,461	0,466	-0,01

Note: D = (Ho - He)/He

As a result of evaluating the efficiency of predicting polymorphism (AUC) of the CYP17A1 rs743572 gene, statistically significant indicators such as sensitivity (SE) and specificity (SP) were determined as independent markers (see Table 5). In patients of the main group, the efficiency of predicting the rs743572 mutant allele A of the CYP17A1 gene was AUC=0.51 (SE=0.63; SP=0.39; OR=1.09; 95% CI=0.75-1.59; p =0.48). Sensitivity, specificity and predictive power of wild alleles are as follows in the group of patients with PCOS who do not suffer from metabolic syndrome: AUC=0.55; SE=0.63; SP=0.47; OR=1.49; 95% CI=0.91-2.44; p=0.64. In the group with metabolic syndrome, respectively, AUC=0.48; SE=0.63; SP=0.33; OR=0.85; 95% CI=0.53-1.37; p=0.66 (AUC is assessed with the following criterion: AUC=0.9-1.0 - excellent quality; AUC=0.8-0.9 - high quality; AUC=0.7-0.8 - good quality ; AUC=0.6-0.7 - average quality; AUC=0.5-0.6 - poor (unsatisfactory) quality) (Table 5).

Table 5. The results of evaluating the effectiveness of predicting the G allele of polymorphism of the CYP17A1 gene rs743572 in the pathogenesis of PCOS.

Factor	Group	SE	SP	AUC	OR	95%CI	P
G	Main group// Control group	0,63	0,39	0,51	1,09	0,75 - 1,59	0,48
	PCOS with metabolic syndrome// Control group	0,63	0,33	0,48	0,85	0,53 - 1,37	0,66
	PCOS without metabolic syndrome// Control group	0,63	0,47	0,55	1,49	0,91 - 2,44	0,64
	PCOS with metabolic syndrome//PCOS without metabolic syndrome	0,53	0,33	0,43	0,57	0,33 - 0,99	0,52

When studying the prognostic efficiency of the CYP17A1 gene in the mutant genotype in the main group, MS+ and MS-groups, AUC=0.51; SE=0.15; SP=0.86; 95% CI - 0.52-2.11; p=0.5 and AUC=0.49; SE=0.12; SP=0.86; 95% CI - 0.29-2.07; p=0.37 also AUC=0.53; SE=0.2; SP=0.86; 95% CI - 0.58-3.55; p=0.29, respectively. (table 6). Predictive performance was not found to be reliable for the mutant genotype and mutant allele. Because in all groups the AUC was less than 0.6.

Table 6. The results of evaluating the effectiveness of predicting the rs743572 polymorphism of the CYP17A1 gene in the pathogenesis of PCOS for the homozygous AA genotype.

Factor	Group	SE	SP	AUC	OR	95%CI	P
G /G	Main group// Control group	0,15	0,86	0,51	1,05	0,52 - 2,11	0,5
	PCOS with metabolic syndrome// Control group	0,12	0,86	0,49	0,78	0,29 - 2,07	0,37
	PCOS without metabolic syndrome// Control group	0,2	0,86	0,53	1,44	0,58 - 3,55	0,29
	PCOS with metabolic syndrome//PCOS without metabolic syndrome	0,12	0,8	0,46	0,54	0,18 - 1,58	0,59

According to the study of CY17A1 polymorphism in patients with PCOS, it can be said that the GG mutant genotype was statistically significantly more common in patients compared to the control group. When dividing PCOS patients, they were divided into groups and compared with the control group, MS+ PCOS patients had a lower level of the mutant form (GG) genotype compared to the control group, but in MS-PCOS patients compared to the control group, the mutant gene was determined more. From this it follows that in the development of PCOS in MS-patients, the CYP17A1 gene mutation (rs743572) plays a

certain role, while in MS+ patients the development of PCOS is due to factors other than the mutation of the CYP17 gene. But this conclusion was not significant when measured through xi2, since our results for chi2 were less than 3.84. The normal wild variant played a protective role in the main group (OR=0.9), especially in MC(-) patients (OR=0.65). When it comes to the Hardy Weinberg equation, we found no significant difference between expected and observed outcomes in the main and control group. Estimates of polymorphism prediction efficiency, as already mentioned, showed only 0.6, which means that the prediction efficiency was not reliable in terms of mutant allele and genotype.

With this we can conclude that the mutant form (AA) of the CYP17A1 rs743572 gene is a risk of PCOS in patients without metabolic syndrome, but this risk is not significant. Our result may be supported by another study: According to Pusalkar M. and colleagues, a CYP17A1 gene polymorphism has been associated with hyperandrogenemia. (Pusalkar, M.; Meherji, P.; Gokral, J.; Chinnaraj, S.; Maitra, A. CYP11A1 and CYP17 promoter polymorphisms associate with hyperandrogenemia in polycystic ovary syndrome. Fertil. Steril. 2009, 92, 653–659.). Rahimi and colleagues also concluded that CYP17A1 polymorphisms are associated with the risk of PCOS.

The mechanism of hyperandrogenemia in terms of the CYP17 polymorphic variant is that the CYp19 mutant variant overexpresses alpha-hydroxylase, which leads to the production of more androgens from hydroxyprogesterone compared to the wild-type variant. Hyperandrogenism in its turn leads to disruption of normal folliculogenesis and, therefore, initiates the appearance of insulin resistance and polycystic ovaries. But in our study, we could not confirm that the polymorphic variant initiated insulin resistance, because in our metabolic syndrome group, the frequency of the CYP17 polymorphic variant was lower compared even with the control group.

Features of the distribution of allelic variants of CYP19A1 gene polymorphisms in patients with polycystic ovary syndrome. In our study, the CYP19A1 gene (rs2470152) was investigated in 106 patients with PCOS. We found that 17% of patients had a homozygous wild-type genotype, 48.1% of patients with a heterozygous GA genotype, and 35.9% of patients with a homozygous mutant AA genotype in patients with PCOS.

When we regrouped the main group into MC (+) and MC (-) according to the CYP19A1 gene polymorphism, we got the following results. The homozygous GG genotype was registered in MS+ PCOS patients homozygous in 25% of cases, while only in MS-PCOS patients in 4.3% and 40.4% in the control group. The heterozygous genotype (GA) was 42.3% in the control group, 38.3% in the first group and the highest rate was in the second group with 61% (MS (-)). It was found that the AA genotype with a homozygous mutant variant was significantly low in the control group - 17.3%, in the other two groups (MC+ and MC-) the result was significantly different from the control group (36.7% and 34.7%) .

According to the percentage of the CYP19A1 gene, depending on the degree of occurrence, the normal G-allele was observed in 44.2% of MS+ PCOS patients. On the other hand, this figure was 34.8% in PCOS MS patients and 61.1% in the control group. The mutant allele was found in 38.9% of patients in the control group, while in the first and second groups it was 55.8% and 65.2%, respectively.

As for the distribution of wild variant genotypes for the CYP19A1 gene in percentage terms for all patients with PCOS and the control group, it was found that the second group prevailed over the first (16% and 40.4%, respectively). The heterozygous genotype showed almost the same result in the two groups of subjects (48.1% and 42.3%, respectively). Although the heterozygous genotype was an inducing factor in terms of influencing the development of PCOS, this variant was not significant. (chi2=0.47; OR=1.2; 95%CI: 0.71 - 2.09 $p < 0.5$). However, when studying the mutant form of AA, it was found that it is detected in most cases in patients, in contrast to the control group, and is confirmed as a significant

risk factor in the development of PCOS. (respectively 35.8% and 17.3%, chi2=5.74; OR=2.7; 95%CI: 1.17–6.06, p<0.02). (table 7).

Table 7. Results of CYP19A1 gene polymorphism genotyping in patients with PCOS and healthy controls.

Alleles and genotypes	**Number of examined alleles and genotypes**				**Xi2**	**P**	**RR**	**95%CI**	**OR**	**95%CI**
	Main group		**Control group**							
	N	**%**	**N**	**%**						
G	85	40,1	64	61,1	12,8	p < 0,001	0,75	0,63 - 0,88	0,4	0,26 - 0,67
A	127	59,9	40	38,9	12,8	p < 0,001	1,33	1,13 - 1,57	2,4	1,48 - 3,86
G/ G	17	16,0	21	40,4	11,3	p < 0,001	0,6	0,41 - 0,87	0,3	0,13 - 0,60
G/A	51	48,1	22	42,3	0,47	p < 0,5	1,1	0,88 - 1,36	1,2	0,65 - 2,5
A /A	38	35,8	9	17,3	5,74	p < 0,02	1,3	1,1 – 1,62	2,7	1,17 - 6,06

During the study, when we divided the polymorphic distribution of the CYP19A1 gene into groups in patients with PCOS, it was found that the mutant form (AA) in patients with metabolic syndrome is an important and reliable factor in the development of PCOS. (chi2=5.2; OR=2.76; 95%CI: 1.1-6.7; p=0.02). In particular, in our study, the homozygous form of the genotype - the "wild" variant (GG) prevailed with a significant difference in the control group (40.4%), this variant was 25% in PCOS patients with MS (+). The mutant form of the homozygous genotype was found in PCOS patients with metabolic syndrome in 36.7%, and the heterozygous genotype in 38.3%. In healthy individuals, this figure was 17.3 and 42.3%, respectively (Table 8).

Table 8. Results of genotyping of CYP19A1 gene polymorphism in PCOS patients with metabolic syndrome and healthy individuals.

Alleles and genotypes	Number of examined alleles and genotypes				Xi2	P	RR	95%CI	OR	95%CI
	PCOS with metabolic syndrome		Control group							
	N	%	N	%						
G	53	44,2	64	61,1	6,7	p = 0,01	0,7	0,56 -0,92	0,5	0,29 - 0,84
A	67	55,8	40	38,9	6,7	p = 0,01	1,38	1,08 -1,77	2,0	1,18 - 3,45
G/ G	15	25,0	21	40,4	3,5	p < 0,1	0,6	0,24 -1,65	0,5	0,25 - 1,03
G/A	23	38,3	22	42,3	0,18	p < 0,7	0,9	0,64 -1,32	0,85	0,39 – 1,80
A /A	22	36,7	9	17,3	5,2	p = 0,02	1,5	1,1 - 2,1	2,76	1,1 - 6,7

Similarly, the mutant homozygous form of the CYP19A1 gene plays a significant role in the development of PCOS in patients without metabolic syndrome (chi2 = 3.9; OR = 2.5; 95% CI 1 - 6.53; p < 0.05). In addition, a mutant variant of the CYP19A1 gene (AA genotype) was registered in patients with MS (+) almost twice as often as in healthy patients (34.8% and 17.3%). Similarly, the heterozygous variant of the genotype was more common in the first group than in the second (60.9% and 42.3%, respectively). As expected, the normal homozygous CYP19A1 gene had a significant advantage in terms of occurrence in healthy

people (4.3% of metabolic syndrome patients with PCOS and 40.4% in healthy people) (Table 9).

Table 9. Results of genotyping of CYP19A1_2 gene polymorphism in patients with PCOS and healthy individuals without metabolic syndrome.

Alleles and genotypes	**Number of examined alleles and genotypes**				**Xi2**	**P**	**RR**	**95%CI**	**OR**	**95%CI**
	PCOS without metabolic syndrome		**Control group**							
	N	**%**	**N**	**%**						
G	32	34,8	64	61,1	13,9	p < 0,001	0,5	0,40 – 0,76	0,33	0,18 - 0,59
A	60	65,2	40	38,9	13,9	p < 0,001	1,8	1,3 - 2,49	3,0	1,67 - 5,37
G/ G	2	4,3	21	40,4	17,6	p < 0,001	0,1	0,04 – 0,56	0,67	0,01 - 0,30
G/A	28	60,9	22	42,3	3,4	p = 0,06	1,5	0,96 -2,32	2,1	0,94 - 4,76
A /A	16	34,8	9	17,3	3,9	p < 0,05	1,6	1,04 -2,33	2,5	1 - 6,53

The distribution of genotypes in the studied polymorphic loci was examined for compliance with the Hardy-Weinberg equilibrium. In the main group of CYP19A1 gene genotypes, no deviations from the level of the expected result were observed (Table 10).

Table 10. Correspondence of the genotype of the CYP19A1_2 gene polymorphism to the Hardy-Weinberg equation in the main group of patients.

Control group					
Alleles	Allele frequency				
G	0,4				
A	0,6				
Genotypes	Genotype frequency		Xi2	p	df
	observed	Expected			
G/ G	0,16	0,16	0		
G/A	0,48	0,48	0		
A /A	0,36	0,36	0		
Total	1	1	0	0,208	1

When examining control groups for the CYP19A1 gene using the Hardy-Weinberg equation, that the result of homozygous (GG and AA) genotypes was higher than expected (empirical-observed for GG and AA showed 0.41 and 0.17, respectively; theoretical-expected 0.37 and 0.15, respectively), and the expected result prevailed over the observed result for the heterozygous genotype (0.42 and 0.48; D=-0.125). (Table 11).

Table 11. Correspondence of the CYP19A1_2 gene polymorphism in healthy people to the Hardy-Weinberg equation.

Control group	
Alleles	Allele frequency
G	0,61
A	0,39

Continuation of Table 11.

Genotypes	Genotype frequency		Xi2	p	df
	observed	expected			
G/ G	0,41	0,37	0,33		
G/A	0,42	0,48	0,72		
A /A	0,17	0,15	0,15		
Total	1	1	1,2	1,98	1
Groups	Ho	He	D*		
Main group	0,48	0,48	0		
Control group	0,42	0,48	-0,125		

Note: D = (Ho - He)/He

In addition, when constructing a predictive model of CYP19A1 gene alleles and various genotype variants, it was found that for mutant alleles (A) in the main group, sensitivity (SE) and specificity (SP) were 0.61 and 0.6, respectively, while patients with MS (+) were 0.61 and 0.56, while patients with MS (-) showed 0.61 and 0.65, respectively. The results of the assessment of the effectiveness of forecasting (AUC) were 0.61, 0.59 and 0.63, respectively. Although the predictive performance was moderate, the simulations appeared to be more reliable in patients without metabolic syndrome than in patients with metabolic syndrome (Table 12).

Table 12. The results of evaluating the effectiveness of predicting the polymorphism of the CYP19A1_2 gene for the allele A in the pathogenesis of PCOS are presented.

Factor	Group	SE	SP	AUC	OR	95%CI	P
A	Main group// Control group	0,61	0,6	0,61	2,34	1,59 - 3,45	0,39
	PCOS with metabolic syndrome// Control group	0,61	0,56	0,59	1,98	1,26 - 3,11	0,55
	PCOS without metabolic syndrome// Control group	0,61	0,65	0,63	2,94	1,78 - 4,86	0,57

However, when calculating the efficiency model of the CYP19A1 gene in the development of PCOS by mutant genotypes (AA), it was found that AUC = 0.6 (SE = 0.36; SP = 0.83; OR = 2.67; 95% CI 1.42–5 .03; P = 0.44) for the main group), and in patients with PCOS with metabolic syndrome AUC = 0.6 (SE = 0.37; SP = 0.83; OR = 2.77; 95% CI 1 .35–5.68; P = 0.31), which indicated the average efficiency of the quality of both groups. For the rest of the groups, the prognostic efficiency was unreliable (Table 13).

Table 13. Evaluation of the efficiency of predicting the polymorphism of the CYP19A1_1 gene in the pathogenesis of PCOS for the homozygous AA genotype.

Group	Factor	SE	SP	AUC	OR	95%CI	P
A /A	Main group// Control group	0,36	0,83	0,6	2,67	1,42 - 5,03	0,44
	PCOS with metabolic syndrome// Control group	0,37	0,83	0,6	2,77	1,35 - 5,68	0,31
	PCOS without metabolic syndrome// Control group	0,35	0,83	0,59	2,55	1,17 - 5,55	0,26

The influence of the CY19A1 rs2470152 gene polymorphism on the development of PCOS led to the fact that mutant alleles were found to be significantly higher in patients than in the control group. When we divided the main group into MC(+) and MC(-) in terms of CYP19A1 and compared with the control group, we found that the homozygous mutant genotype was found to be greater in the MC(+) and MC(-) group compared to the control group. With this, we can conclude that the homozygous mutational form of the CYP19A1 gene plays a convincing inducible role in PCOS and our result was significant (chi2 - 5.74; $p<0.02$ in the main group; chi2=5.2; p=0.02 in patients with metabolic syndrome and

chi2=3.9; p<0.05 in patients without metabolic syndrome). However, the study did not reveal an induced effect on the heterozygous genotype in the development of PCOS (chi2<3.85; p>0.05). At the same time, the wild-type homozygous variant played a protective role in terms of the appearance of PCOS in the main group, as well as in the MC (+) and MC (-) groups (OR≥0.5). When it comes to the Hardy Weinberg equation, we found no significant difference between the expected and observed results in the main group. Estimates of polymorphism prediction efficiency, as already mentioned, showed only 0.6, which means that the prediction efficiency was not reliable in terms of mutant allele and genotype.

4 Conclusion

With this, we can conclude that the mutant form (AA) of the CYP19A1 rs2470152 gene plays a significant inducible role in the pathogenesis of PCOS. Samples that have the wild-type genotype means that they have 2 identical copies of the aromatase gene and can produce sufficient amounts of the aromatase enzyme. Whereas heterozygotes have one normal and another mutant aromatase, a copy of the gene produces insufficient amounts of the aromatase enzyme because both copies of the gene are not equally expressed. As for the homozygous mutant genotype, it produces insufficient amounts of the enzyme, leading to aromatase deficiency. Aromatase deficiency, in turn, can cause hyperandrogenism, as the conversion of testosterone to estradiol is impaired. Hyperandrogenism leads to disruption of normal folliculogenesis and, therefore, stimulates the appearance of insulin resistance and polycystic ovaries.

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