УДК: 616.43:577.175.62

SPECIFIC VARIATIONS IN THE CYP 21A2 GENE IN WOMEN OF REPRODUCTIVE AGE WITH HYPERANDROGENIA

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Abstract. In our study, according to the sequencing analysis of the CYP21A2 gene, only single nucleotide polymorphism mutations were detected in patients with hyperandrogenism, and 3.97% (n=5) of them had a homozygous wild-type genotype, 46.8% (n=59) had a heterozygous genotype (there is one minor allele) was confirmed.

Key words: hyperandrogenism, CYP21A2 gene, molecular genetic analysis, congenital adrenal hyperplasia.

Relevance of the topic. Hyperandrogen syndrome in women of reproductive age remains one of the most pressing problems of endocrine gynecology. The syndrome of hyperandrogenism in women leads from their appearance to the violation of reproductive functions [1]. In 2010, polycystic ovary syndrome (PCOS) occurred in 5-10%, but now this indicator is 15-20%. For this reason, this syndrome causes women to lose their quality of life [2]. The state of hyperandrogenism is directly caused by the violation of androgen metabolism in the body. In women, hyperandrogenism occurs in polycystic ovary syndrome, in the non-classical type of congenital hyperplasia of the cortex of the adrenal gland (non-classical congenital adrenal hyperplasia), when the work of aramatase and 5a-reductase enzymes is disturbed [3,4]. Based on this, since there are many sources of development of hyperandrogenism, there are difficulties in differential diagnosis. Determining the percentage of women of reproductive age with non-classical congenital adrenal hyperplasia and hyperandrogenism will help determine the next stage of treatment. It is known from the literature that in the development of non-classical congenital adrenal hyperplasia, 21-hydroxylase enzyme deficiency is the cause in 90% of cases [5,6,7]. Various changes in the structure of the CYP21A2 gene, which encodes this enzyme, reduce the activity of the enzyme. To confirm the diagnosis of non-classic congenital adrenal hyperplasia, molecular-genetic examination is important.

Research objective. Determination of a differentiated approach to diagnosis by identifying specific changes in the structure of the CYP 21A2 gene in women of reproductive age with hyperandrogenism.

Object and method of research. The study was conducted at the Regional Population Reproductive Health Center. From the women who applied, 126 women of reproductive age with a high level of androgens in the blood were selected. 32 women without reproductive disorders were selected as a control group. Peripheral blood was taken from the selected women for molecular genetic testing. Molecular genetic studies were conducted in the laboratory of the Republican Scientific and Practical Center of Sports Medicine. Sequencing analysis of the CYP21A2 gene was performed.

Results and their discussion. According to the results of the sequencing analysis of the CYP21A2 gene, which was carried out in our study, no mutation types strongly affecting the conformation of the expressed enzyme, such as deletion, translocation, micro- and macroconversion, were detected in the main group. Polymorphism characteristic of mutations of the single nucleotide polymorphism (SNP) type 683 G>A (K102L) minor allele in 14.3% of patients (n=18) and 20% (n=6) of the control group, polymorphism rs6468 (L39V) in 13.5% (n=17) of patients and 9.3% of the control group (n=3), polymorphism rs9378252 (H62L) in 19.8% (n=25) of patients and 3.12% (n=1) of the control group ($p<0.05$), rs6477 (Leu249=) 10.3% (n=13) of patients and 9.3% (n=3) in the control group, 13.89 T>A (M239K) in 7.1% (n=9) of patients and 3.3% (n=1) in the control group, the non-wild or minor allele was detected ($p>0.05$), while the minor allele characteristic of polymorphisms such as 2578 C>T (P453S), 655 A/C>G, 999 A>T (I172N) ($p>0.05$), 2108 C>T (R356W) was detected only in patients, and 3.2% (n=4), 8% (n=10), 5% (n=6) and 0.8% (n=1) respectively.

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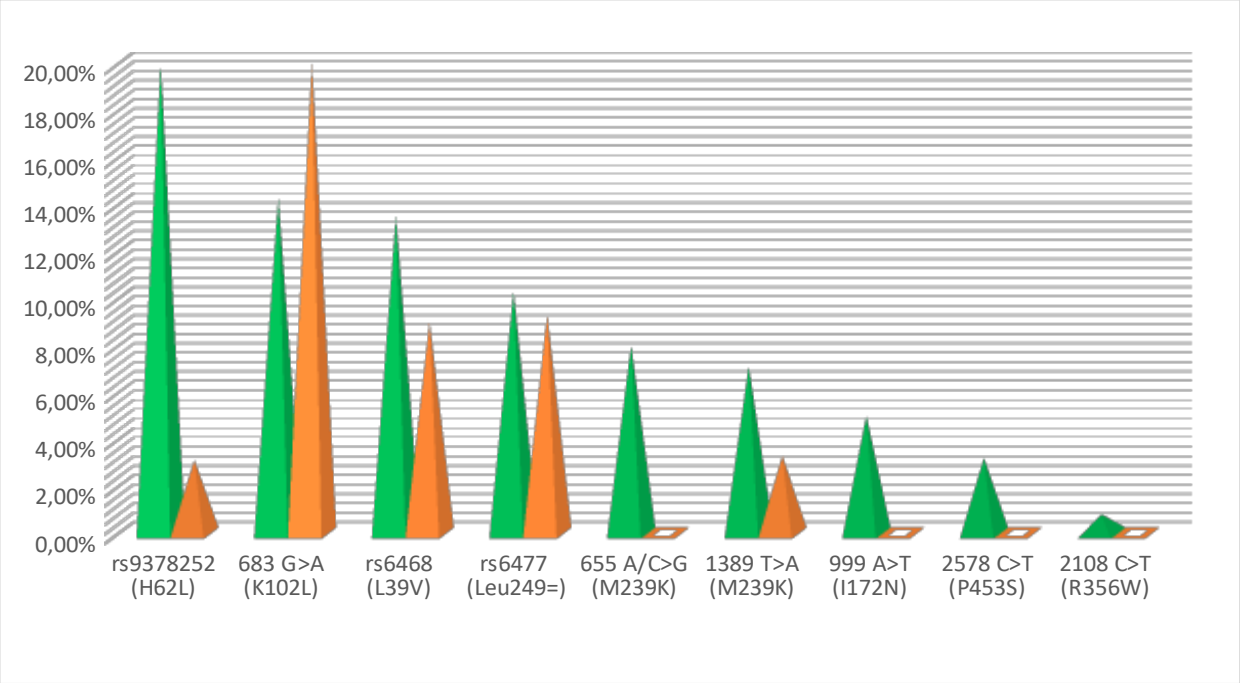

*Figure 1. Results of CYP21A2 gene sequencing in patients of the main and control groups. Indications: * - compared to the control group - p<0.05.*

The results of the distribution of the results of various polymorphisms identified in the CYP21A2 gene by alleles and genotypes in the main and control groups were studied. According to Table 1, the distribution of the result of polymorphisms identified in the main and control groups by alleles was verified at the population level based on the Hardy-Weinberg law. According to the results obtained in the main and control groups, no significant deviation from the observed or theoretical results was found for all identified polymorphisms ($\chi 2<3.85$; $P>0.05$). This indicates that the results obtained during the study violate the Hardy-Weinberg law.

Table 1

Distribution of alleles and genotypes of polymorphisms identified during the sequencing of the CYP21A2 gene in the main and control groups distribution

Polymorphism Type	Main group					Control group				
	Allel		Genotypes			Allel		Genotypes		
	Wild type (%)	Minor type (%)	Homozygous wild (%)	Heterozygous (%)	Homozygous nonwild (%)	Wild type (%)	Minor type (%)	Homozygous wild (%)	Heterozygous (%)	Homozygous nonwild (%)
683 G>A	92,0	8,0	77	12,7	1,6	90,0	10,0	80	20	0
rs9378252	89,7	10,3	80,2	19	0,8	98,4	1,6	96,875	3,125	0
rs6468	82,8	7,2	86,5	12,7	0,8	95,3	4,7	90,625	9,375	0
rs6477	94,4	5,6	89,6	9,5	0,8	95,32	4,68	90,625	9,375	0
655A/C>G	96,03	3,97	92	7,9	0,0	100	0	100	0	0
1389 T>A	96,43	3,57	92,9	7,1	0	96,8	3,2	93,75	6,25	0
2578 C>T	98,4	1,6	96,83	3,17	0	100	0	100	0	0
999 A>T	97,6	2,4	95,2	4,8	0	100	0	100	0	0
2108 C>T	99,6	0,4	99,2	0,8	0	100	0	100	0	0

Table 2

Results of the study of various polymorphisms identified in the CYP21A2 gene in patients of the main group using the Hardy-Weinberg law

Polymorphism Type	Main group						χ2	p-value
	Observed			Expected				
	Homozygous wild	Heterozygous	Homozygous nonwild	Homozygous wild	Heterozygous	Homozygous nonwild		
683 G>A	0,857	0,127	0,016	0,848	0,146	0,006	2,1	0,34
rs6468	0,865	0,127	0,008	0,862	0,132	0,005	0,23	0,89
rs9378252	0,802	0,19	0,008	0,80	0,19	0,01	0,1	0,95
rs6477	0,896	0,095	0,008	0,889	0,11	0,0	1,0	0,58
655 A/C>G	0,92	0,079	0,0	0,922	0,076	0,0	0,21	0,90
1389 T>A	0,929	0,071	0,0	0,93	0,069	0,0	0,17	0,92
2578 C>T	0,968	0,032	0,0	0,968	0,031	0,0	0,03	0,98
999 A>T	0,952	0,048	0,0	0,952	0,048	0,0	0,07	0,96
2108 C>T	0,992	0,08	0,0	0,992	0,08	0,0	0,0	0,99

Instruction: df=1

Table 3

Results of the study of various polymorphisms identified in the CYP21A2 gene in the control group of subjects using the Hardy-Weinberg law

Polymorphism Type	Control group						χ2	p-value
	Observed			Expected				
	Homozygous wild	Heterozygous	Homozygous nonwild	Homozygous wild	Heterozygous	Homozygous nonwild		
683 G>A	0,813	0,187	0,031	0,821	0,17	0,008	0,34	0,84
rs6468	0,9062	0,0937	0,0	0,908	0,089	0,0	0,07	0,96
rs9378252	0,96875	0,0313	0,0	0,969	0,03	0,0	0,008	0,98
1683 G>T	0,96875	0,0313	0,0	0,969	0,03	0,0	0,008	0,98
rs6477	0,9062	0,0937	0,0	0,908	0,089	0,0	0,07	0,96

1389 T>A	93,75	6,25	0	0,9375	0,061	0,0	0,03	0,98

Instruction: df=1

As noted above, the combination of several mutant alleles in the CYP21A2 gene significantly increases the risk of developing hyperandrogenism in women due to the occurrence of adrenal hyperplasia of the non-class type. Therefore, based on the results of sequencing, the frequency of occurrence of combined mutations in the heterozygous form in some patients of the main group was analyzed, and combined mutations were calculated. As a result, it was found that patients with two different mutant alleles were 18.3% (n=23), and patients with three different mutant alleles were 2.4% (n=5).

Table 4

Comparison of double and triple analysis of combined mutations resulting from CYP21A2 gene sequencing

Combined two types of genotypes	**Patients n=126**	**%**	**Combined three types of genotypes**	**Patients n=126**	**%**
683 GA-rs9378252 AT	4	3,2	683 GA-1389 TA-rs6477 CG	1	0,8
683 GA-1389 TA	2	1,6	2108 TA-rs6477 CG-rs9378252 AT	1	0,8
683 GA-rs6468 CG	4	0,8	683 AG-rs9378252 AT-rs6477 CG	2	1,6
999 TA-rs6468 CG	2	1,6	999TA- rs9378252 AT-rs6477 CG	1	0,8
999 TA- rs6477 CG	1	0,8			
999 TA- rs9378252 AT	1	0,8			
rs9378252 AT-rs6468 CT	1	0,8			

rs9378252 AT-rs6477 CG	1	0,8			
rs6468 CT-2578 CT	1	0,8			
rs9378252 AT- 2578 CT	2	1,6			
rs6468 CT- rs6477 CG	4	3,2			
Overall	23	18,3	Overall	5	2,4

Conclusion: According to the results of the sequencing analysis of the CYP21A2 gene, mutations characteristic of only a single nucleotide polymorphism were detected in patients with hyperandrogenism, and it was confirmed that 3.97% (n=5) of them had a non-wild homozygous genotype, and 46.8% (n=59) had a heterozygous genotype (with one minor allele). It was confirmed that all identified polymorphisms correspond to the Hardy-Weinberg law ($\chi 2<3.85$; $P>0.05$). The correlation between the identified polymorphisms and the identified clinical and biochemical changes in patients allows us to identify mutant alleles that cause congenital adrenal hyperplasia.

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