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THE IMPORTANCE OF GENETIC MARKERS IN THE DIAGNOSIS OF HYPERANDROGENY SYNDROME IN WOMEN OF REPRODUCTIVE AGE

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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. Among all identified polymorphism type mutations, only rs9378252 polymorphism proved to have a statistically reliable positive association between minor allele and heterozygous genotypes and the development of hyperandrogenism.

Keywords: hyperanrogenism, infertility, 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.

The aim of the study. Improving differential diagnosis by identifying CYP21A2 gene polymorphisms in women with hyperandrogenism syndrome.

Object of research. 126 women of reproductive age with hyperandrogenism and 32 healthy women without reproductive disorders were admitted to the Bukhara Regional Center for Reproductive Health.

Research method. Amplification of the SYP21A2 gene sequence was performed during amolecular genetic study. Peripheral blood was obtained from selected women. The product obtained for the study was processed using the GeneAmp® PCR System 9700 device. Polymerase chain reaction products were analyzed on a Complex-3500 automated genetic analyzer (Applied Bio-systems, USA). Amplified genomic DNA sequence was determined using a set of special reagents from Applied Bio-systems

(USA) and Data Collection Software 3.0 special computer program. Using the MEGA special computer program, the image of the spectrum reflecting the DNA sequence of all the studied objects was obtained. Molecular genetic research was conducted in the laboratory of the Scientific and Practical Center of Sports Medicine of the Republic.

Results and their discussion: Based on the results of CYP21A2 gene sequencing performed in our study, single nucleotide polymorphism (SNP) type mutations, such as deletion, translocation, micro- and macro-conversion, strongly affecting the conformation of the expressed enzyme, were identified. not found in the main group. Specific minor allele of polymorphism 683 G>A (K102L) 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% in controls (n=3), rs9378252 (H62L) polymorphism in 19.8% (n=25) of patients and 3.12% (n=1) in controls ($p<0.05$), rs6477 (Leu249=) 10.3% (n=13) patients and 9.3% (n=3) controls, 1389 T>A (M239K) in 7.1% (n=9) patients and 3.3% (n=1) in the control group, non-wild or minor allele ($p>0.05$), 2578 C>T (P453S), 655 A/C>G, 999 A>T (I172N) ($p>0.05$), 2108 C>T The minor allele characteristic of such polymorphisms as (R356W) was detected only in patients and amounted to 3.2% (n=4), 8% (n=10), 5% (n=6) and 0, 8% (n=1), respectively.

The distribution of the results of different polymorphisms in the CYP21A2 gene into alleles and genotype was studied in the main and control groups.

Table 1

Distribution of polymorphisms determined by CYP21A2 gene sequencing in the main and control groups by allele and genotype

	Main group					Control group				
	alleles		genotype			alleles		genotype		
	Wild type (%)	Minor type (%)	Wild homo-zygotes (%)	Hetero zygote (%)	Wild homo-zygotes (%)	Wild type (%)	Minor type (%)	Wild homo-zygotes (%)	Hetero zygote (%)	Wild homo-zygotes (%)
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

According to Table 2, the results of polymorphisms identified in the main and control groups were checked based on the Hardy-Weinberg law in order to check that the distribution of alleles was appropriate at the population level. Accordingly, according to the results obtained in the main and control groups, there was no significant deviation from the expected or observed empirical results or theoretical results for all identified polymorphisms ($\chi 2<3.85$; $P>0.05$) . This shows that the results obtained during the research obey the Hardy-Weinberg law.

Table 2

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

Types of polymorphisms	Main group						χ2	p-value
	Observed			Expected				
	Wild homozygote	Heterozygote	Wild homozygote	Wild homozygote	Heterozygote	Wild homozygote		
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

The results of testing the subjects of the control group for various polymorphisms identified in the CYP21A2 gene according to the Hardy-Weinberg law

Types of poymorphisms	Control group						χ2	p-value
	Observed			Expected				
	Wild homozygote	Heterozygote	Wild homozygote	Wild homozygote	Heterozygote	Wild homozygote		
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

Similarly, the results of CYP21A2 gene sequencing were analyzed and the pathogenetic significance of various polymorphisms in individuals with the mutant allele in the main and control groups was studied. In particular, according to the results of the 683 G>A (K102L) polymorphism of the CYP21A2 gene in the control group with a low percentage ($\chi2<3.85$; $P>0.05$), the minor allele - A prevailed. Accordingly, a non-wild homozygous genotype was found that induces the significance (RR=1.26; 95% CI: 1.16-1.362). Interestingly, this value was also found in the homozygous wild-type genotype (RR=1.07; 95% CI: 0.84-1.37). On the other hand, the heterozygous genotype was found to have a reduced risk of developing the disease (RR = 0.89; 95% CI: 0.687-1.117). But these indicators were not statistically significant, since the difference between the main and control groups in the distribution of genotypes was not statistically significant ($\chi2<3.85$; $P>0.05$).

Table 4

Distribution and pathogenetic significance of the 683 G>A (K102L) polymorphism of the CYP21A2 gene in the main and control groups.

Alleles and genotypes	The amount of alleles and genotypes				χ2	P	RR	95%CI	OR	95%CI
	Main group		Control group							
	N	%	N	%						
G	232	92,0	56	90,3	0,2	0,656	1,05	0,842-1,302	1,24	0,477-3,24
A	20	8,0	6	9,7	0,2	0,656	0,955	0,768-1,188	0,8	0,31-2,1
G/G	108	85,7	26	81,3	0,17	0,675	1,07	0,84-1,37	1,38	0,50-3,83
G/A	16	12,7	6	18,7	0,46	0,49	0,899	0,687-1,176	0,63	0,225-1,77
A/A	2	1,6	0	0	0,51	0,47	1,26	1,16-1,362	-	-

Similarly, when analyzing the results of the rs6468 (L39V) polymorphism of the CYP21A2 gene by allelic parameters, the minor allele was higher in the main group, and the wild allele was higher in the control group, but this difference was not statistically significant. ($\chi2<3.85$; $P>0.05$). In addition, the wild-type or normal allele has a protective effect, reducing the risk of developing hyperandrogenism by 7.5% (95% CI: 0.77-1.12), while the minor or mutant allele increases it by 8% (95% CI : 0.899-1.12). 1.299) was determined to be of inducing significance. According to the analysis of genotyping results, although the wild homozygous genotype has a protective value in the pathogenesis of the disease (RR = 0.938; 95% CI: 0.758-1.16), the influence of the heterozygous genotype increases the risk of developing the disease (RR = 1.06; 95% CI: 0 .86-1.32) revealed a relative risk factor, however, the statistical significance of these indicators was not confirmed $\chi2<3.85$; $P>0.05$).

Table 5

Distribution and pathogenetic significance of the rs6468 (L39V) polymorphism of the CYP21A2 gene in the main and control groups.

Alleles and genotypes	Amount of alleles and genotypes				χ2	P	RR	95%CI	OR	95%CI
	Main group		Control group							
	N	%	N	%						
C	234	82,8	61	95,3	0,5	0,48	0,925	0,77-1,12	0,64	0,18-2,24
G	18	7,2	3	4,7	0,5	0,48	1,08	0,899-1,299	1,56	0,45-5,48
C/C	109	86,5	29	90,6	0,39	0,53	0,938	0,758-1,16	0,63	0,18-2,42
C/G	16	12,7	3	9,4	0,26	0,60	1,06	0,86-1,32	1,4	0,38-5,15
G/G	1	0,8	0	0	0	0	0	0	0	0

On the other hand, when interpreting the result of the rs9378252 (H62L) polymorphism test of the CYP21A2 gene, the wild allele was significantly higher in the control group, and the minor allele was significantly higher in the main group ($\chi2=5$; P=0.026; relative risk factor - RR, respectively), RR=0.8; 95% CI: 0.74-0.894 and RR=1.23; 95% CI: 1.23-1.36). Similarly, according to the results of genotypes, the wild-type homozygous genotype - AA had a protective effect, reducing the progression of the disease by 21% (95% CI: 0.79-1.078; $\chi2>3.85$; P<0.05), heterozygous - AT and wild-type homozygous - T/T genotypes increased the risk of developing the disease by 25% and 26%, respectively (RR=1.25; 95% CI: 1.11-1.42 $\chi2>3.85$; P<0.05, RR=1.26; 95% CI: 1.16–1.36; $\chi2<3.85$;P>0.05) increases .

Table 6

Distribution and pathogenetic significance of the rs9378252 (H62L) polymorphism of the CYP21A2 gene in the initial and control groups.

Alleles and genotypes	The amount of alleles and genotypes				χ2	P	RR	95%CI	OR	95%CI
	Main group		Control group							
	N	%	N	%						
A	226	89,7	63	98,4	5	0,026	0,8	0,74-0,894	0,14	0,018-1,037
T	26	10,3	1	1,6	5	0,026	1,23	1,12-1,36	7,25	0,965-54,46
A/A	101	80,2	31	96,9	5,2	0,02	0,79	0,70-0,899	0,13	0,017-1,00
A/T	24	19	1	3,1	4,8	0,028	1,25	1,11-1,42	7,3	0,948-56,12
T/T	1	0,8	0	0	0,25	0,614	1,26	1,16-1,36	0	0

Similarly, according to the analysis of the rs6477 (Leu=) polymorphism of the CYP21A2 gene, the frequency of the minor allele did not differ between the main and control groups (5.6% and 4.7%, respectively; $\chi2=0.076$; P=0.78). , the frequency of occurrence of the heterozygous genotype was almost the same in the groups of patients and apparently healthy people (9.5% and 9.4%, respectively $\chi2=0.001$; P=0.98). In addition, the homozygous wild-type genotype increased the risk of developing the disease by 26% relative risk factor (RR=1.26; 95% CI: 1.16-1.36), but the chi-square score was robust between wild-type genotype and disease . did not indicate the existence of a link. Based on this, according to the results of our study, it was concluded that there was no correlation between the rs6477 polymorphism and hyperandrogenism ($\chi2>3.85$; P<0.05) .

Table 7

Distribution and pathogenetic significance of the rs6477 (Leu=) polymorphism of the CYP21A2 gene in the main and control groups

Alleles ang genotypes	The amount of alleles and genotypes				χ2	P	RR	95%CI	OR	95%CI
	Main group		Control group							
	N	%	N	%						
C	238	94,4	61	95,3	0,076	0,78	0,97	0,77-1,21	0,84	0,23-3,00
G	14	5,6	3	4,7	0,076	0,78	1,04	0,824-1,3	1,2	0,33-4,29
C/C	113	89,6	29	90,6	0,025	0,875	0,98	0,76-1,257	0,89	0,24-3,36
C/G	12	9,5	3	9,4	0,001	0,98	1,00	0,769-1,3	1,02	0,27-3,8
G/G	1	0,8	0	0	0,25	0,614	1,26	1,!6-1,36	0	0

Finally, in the type of polymorphism 1389 T>A (M239K) of the CYP21A2 gene, although the minor allele A was detected in the main group more often than in the control group, its association with hyperandrogenism was not statistically significant ($\chi2<3.85;P>0.05$). Similarly, the relative risk factor (RR) of homozygous T/T and heterozygous T/A genotypes showed protective and inducing significance in the development of the disease (respectively, RR=0.97; 95% CI: 0.73–1.3 and RR= 1.03; 95). %CI: 0.77–1.37), these pathogenic values were not statistically significant ($\chi2<3.85$; $P>0.05$) .

Table 8

Distribution and pathogenetic significance of polymorphism 1389 T>A (M239K) of the CYP21A2 gene in the main and control groups

Alleles and genotypes	The amount of alleles and genotypes				χ2	P	RR	95%CI	OR	95%CI
	Main group		Control group							
	N	%	N	%						
T	243	95,2	62	96,8	0,03	0,86	0,97	0,733-1,29	0,87	0,18-4,134
A	9	3,57	2	3,13	0,03	0,86	1,03	0,773-1,36	1,15	0,242-5,45
T/T	117	92,9	30	93,7	0,031	0,86	0,97	0,73-1,3	0,87	0,178-4,22
T/A	9	7,1	2	6,3	0,031	0,86	1,03	0,77-1,37	1,15	0,237-5,62
A/A	0	0	0	0	-	-	-	-	-	-

Conclusion:

In conclusion, according to the results of CYP21A2 gene sequencing, among all identified mutations of the polymorphism type, only the rs9378252 polymorphism with a minor allele and heterozygous genotypes has a statistically significant positive association with the development of hyperandrogenism. On the other hand, it was confirmed that polymorphisms 653 G>A, rs6477 and 1389 T>A do not have such an association. Similarly, mutant alleles of polymorphisms 2578 C>T, 655 A/C>G, 999 A>T and 2108 C>T were found only in patients of the main group. In addition, double minor alleles were found in 18.3% and triple minor alleles in 2.4% of patients.

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