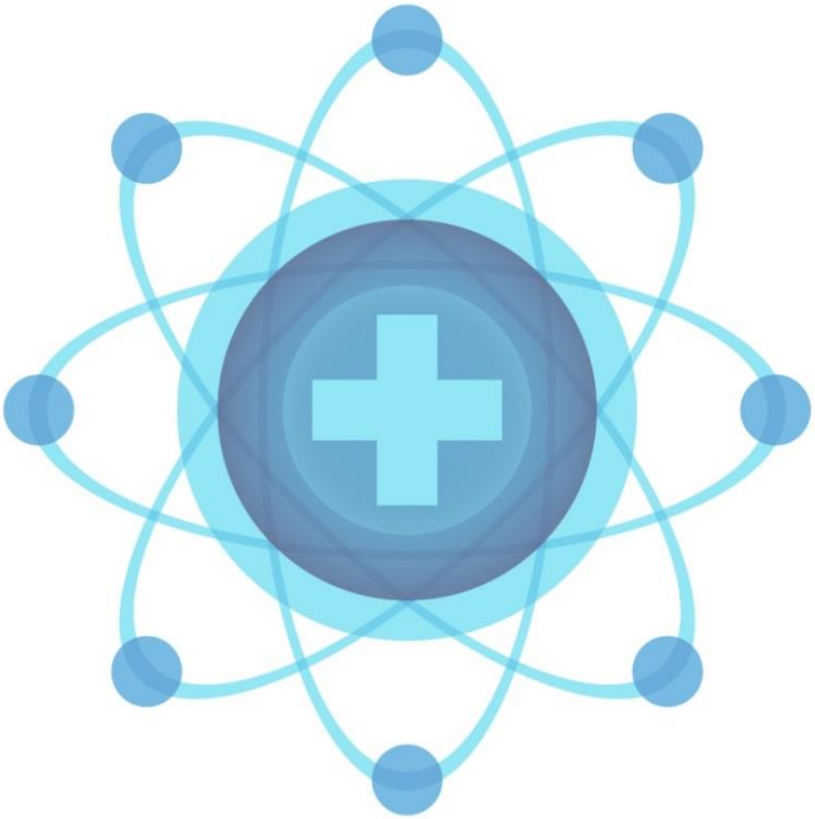

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SIGNIFICANCE OF POLYMORPHISMS OF XENOBIOTS DETOXIFICATION GENES IN THE FORMATION OF INFERTILITY IN MEN

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Abstract. The role of polymorphisms was analyzed Ile462Val of the CYP1A1 gene, C6310T of the CYP1A1 gene, G681A of the CYP2C19 gene, and G636A of the CYP2C19 gene in the formation of male infertility. The work was carried out on samples of 140 patients with various clinical manifestations of male infertility and 155 conditionally healthy fertile Uzbek men. It was found that the proportion of carriage of unfavorable allelic variants of the Ile462Val polymorphisms of the CYP1A1 gene and G636A of the CYP2C19 gene among patients was significantly higher than in individuals without reproductive system disorders, and these loci were associated with an increased risk of developing infertility in men. The results of the analysis of the C6310T polymorphisms of the CYP1A1 gene and G681A of the CYP2C19 gene did not reveal any significant differences in the distribution of allele and genotype frequencies between the studied samples.

Keywords: male infertility, locus, Ile462Val polymorphisms of the CYP1A1 gene, C6310T of the CYP1A1 gene, G681A of the CYP2C19 gene, G636A of the CYP2C19 gene , mutation, reproductive system.

Relevance

Infertility is a common condition affecting about 70 million people worldwide, with male infertility accounting for approximately 50% [1]. In recent years, more and more attention of researchers has been directed to the study of the genetic basis for the development of male infertility [2, 3]. Among reproductive scientists, studies that are devoted to finding a connection between genotypic variants of gene polymorphisms with various reproductive disorders are becoming especially relevant

[4, 5, 6, 7]. Much attention in such studies is focused on the genes of cytochrome P450, which plays an important role in the metabolism of polycyclic aromatic hydrocarbons, which has potential reproductive toxicity in males, exposure to which may have a negative impact on male fertility [8, 9]. Great attention is paid to the study of polymorphic variants of "predisposition" genes, which lead to the appearance of metabolic products with altered physicochemical properties and functional activity parameters [10, 11, 12]. Some authors proved that the body's susceptibility to harmful environmental influences depends on the activity of xenobiotic detoxification enzymes [13, 14].

For the cytochrome P4501A1 (CYP1A1) gene, a key enzyme in the extrahepatic metabolic activation of lipophilic xenobiotics, increased frequencies of two genetically related polymorphisms have been found among infertile men [9] . It can be assumed that the correct regulation of the level of CYP1A1 is essentially important for the optimal functioning of the enzyme. Of the cytochrome genes, the *CYP19A1 gene* encodes the aromatase enzyme, which catalyzes the conversion of androgens to estrogens [15, 16, 17, 18] . Its high expression will disrupt the hormonal balance in the male body, and excessive conversion of androgen to estrogen in the body will further damage testicular spermatogenic function, affect normal sperm development, and cause spermatogenic disorder [19] .

However, despite this, to date, the genetic mechanisms of the formation of a violation of the reproductive system in men, including the role of genetic loci of xenobiotic biotransformation in the development of male infertility, have not been fully elucidated. Particularly little studied are the individual characteristics of the cytochrome P450 genes in the development and clinical course of male infertility, which justifies the relevance of this work.

Materials and methods

The work was carried out on samples of 140 patients with various clinical manifestations of male infertility. Of these: 35 (25.0%) were patients with azoospermia, 105 (75.5%) - patients without azoospermia. The control sample was formed from 155 unrelated healthy individuals of Uzbek nationality without any violation of the reproductive system. Isolation of genomic DNA from peripheral blood was carried out using the standard kit AmpliPrime RIBO-prep (NextBio, Russia). The concentration and purity of the isolated biomaterial was determined using a NanoDrop 2000 (Thermo Fisher Scientific, USA). Genotyping of polymorphisms Ile462Val of the CYP1A1 gene, C6310T of the CYP1A1 gene, G681A of the CYP2C19 gene, and G636A of the CYP2C19 gene was performed by allele-specific PCR on the Rotor instrument Gene Q (Quagen , Germany) using the diagnostic kits of SPF "Litekh" and "Synthol" (Russia) according to the manufacturer's instructions. Estimation of the deviation of the genotype distributions of the studied loci from the Hardy–Weinberg distribution was carried out using the online program "Hardy – Weinberg equilibrium calculator". Statistical analysis of the results was carried out using the statistical software package OpenEpi 2009, Version 9.3.

Results and discussion

Distribution of genotypes of polymorphisms Ile462Val of the CYP1A1 gene, C6310T of the CYP1A1 gene, G681A of the CYP2C19 gene, and G636A of the CYP2C19 gene corresponded to the expected, i.e. in all cases, the Hardy–Weinberg equilibrium is satisfied in both groups.

To clarify the role of the Ile462Val locus of the CYP1A1 gene in the mechanism of infertility formation, we performed a comparative analysis of differences in allele and genotype frequencies for this polymorphism between the studied groups and subgroups. Thus, in a comparative aspect with the control, in the main group of men with infertility, the unfavorable allele G was statistically significantly more frequently recorded (22.1% vs. 13.2%, respectively). The calculated relative chance of detecting this allele in the group of patients compared with the control was 1.9 times ($\chi^2 = 8.1$; $p < 0.05$; O R = 1.9; 95% CI: 1.22-2.96). Moreover, significantly significant differences were also established for the heterozygous genotype A/G, the proportion of which among the subjects of the main group was 1.8 times higher compared to the control (35.7% versus 23.9%; $\chi^2 = 5.0$; $p = 0.03$; O R =1.8 ; 95%CI: 1.08-3.01). For the functionally unfavorable G / G genotype , despite the presence of a difference, it did not differ in statistical significance, but at the same time there was a clear high trend towards its increase among the men of the main group compared to conditionally healthy men (4.3% vs. 1.3%; $\chi^2 = 2.5$; $p = 0.1$; O R = 3.4; 95% CI: 0.75-15.5) (see figures 1 and 2, table 1).

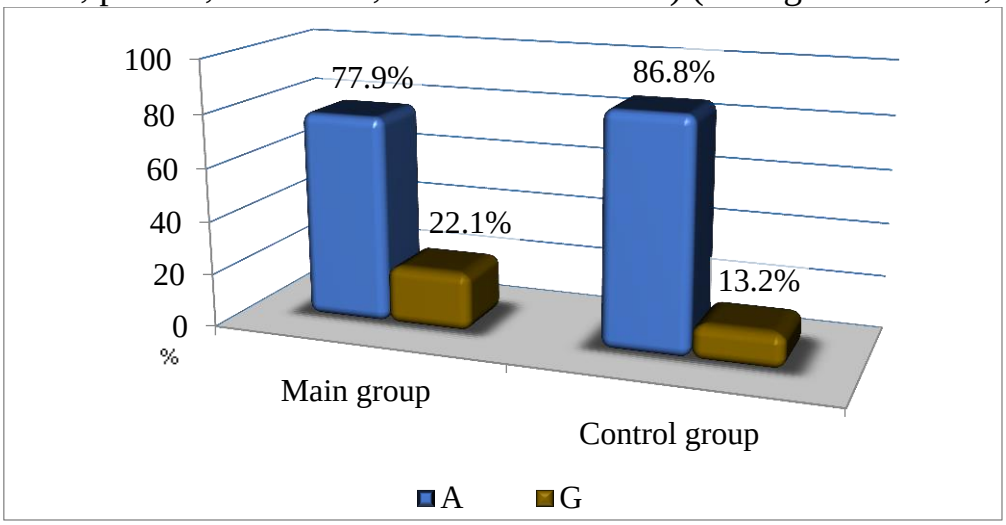

Figure 1. **Differences in the frequency of alleles of the polymorphic locus Ile 462 Val of the CYP 1 A 1 gene in the main groups of male patients with infertility and controls**

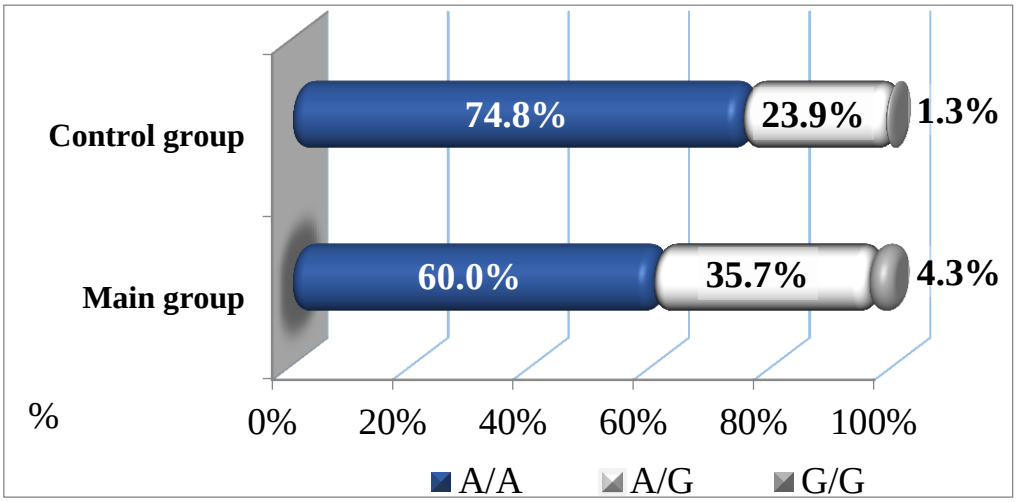

Figure 2 . **Differences in the frequency of genotypic variants of the Ile 462 Val polymorphism of the CYP 1 A 1 gene in the main groups of male patients with infertility and controls**

Table 1

Differences in the frequency of allelic and genotypic variants of the Ile 462 Val polymorphism of the CYP 1 A 1 gene in the main groups of male patients with infertility and controls

Alleles and genotypes	Number of examined alleles and genotypes				χ^2	R	RR	95%CI	OR	95%CI
	Main group		Control group							
	n	%	n	%						
A	218	77.9	269	86.8	8.1	<0.05	0.9	0.63-1.29	0.5	0.31-0.81
G	62	22.1	41	13.2	8.1	<0.05	1.1	0.67-1.8	1.9	1.22-2.96
A/A	84	60.0	116	74.8	7.4	<0.05	0.8	0.51-1.27	0.5	0.3-0.82
A/G	50	35.7	37	23.9	5.0	0.03	1.5	0.94-2.39	1.8	1.08-3.01
G/G	6	4.3	2	1.3	2.5	0.12	3.3	1.45-7.5	3.4	0.75-15.5

A comparative analysis of the results of differences in the distribution of allele and genotype frequencies according to the Ile462Val polymorphism of the CYP1A1 gene , depending on the presence and absence of azoospermia in men with infertility, showed the presence of some features. Compared with the comparative control group, in the group of men with azoospermia, both for the A and G alleles and for the A/A, A/G and G/G genotypes, practically no significant differences were found. On the contrary, compared with the control group, very significant differences were found in the group of men with infertility without azoospermia. So, the proportion of the allele G was statistically significantly higher than that in the subgroup without azoospermia (23.8% versus 13.2%, respectively). The calculated relative chance of detecting this allele in this subgroup of patients compared with the control was 2.1 times (χ^2 =9.7; p< 0.05; O R =2.1; 95%CI:1.32-3.35).

A / G genotype was 2.0 times higher than the control values (38.1% vs. 23.9%; χ^2 =6.1; p =0.01; O R =2.0; 95% CI: 1.15-3.47). Although the frequency of the G / G mutant genotype was 3.8 times higher than in the control group, this genotype

showed only a clear upward trend (4.8% versus 1.3%; χ^2 =2.9; p =0.09; O R =3.8; 95%CI:0.8-17.7) respectively (see figures 3 and 4) .

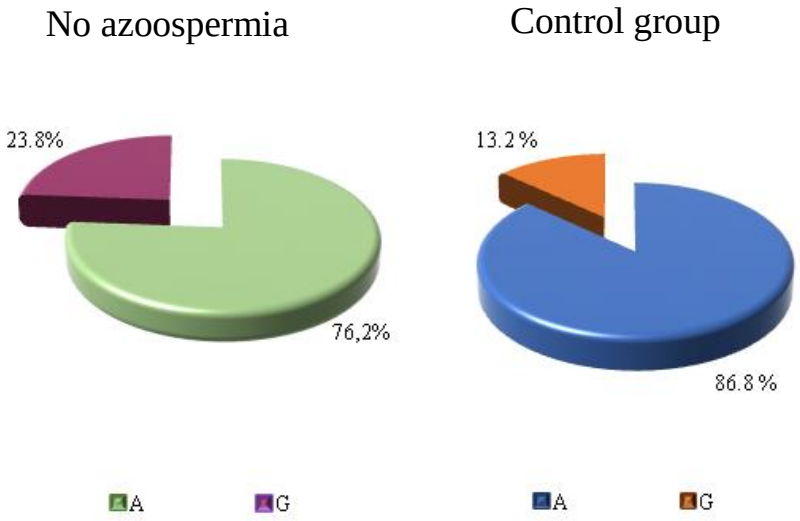

Figure 3. **Differences in the frequency of alleles of the Ile 462 Val polymorphic locus of the CYP 1 A 1 gene in groups of patients without azoospermia and controls**

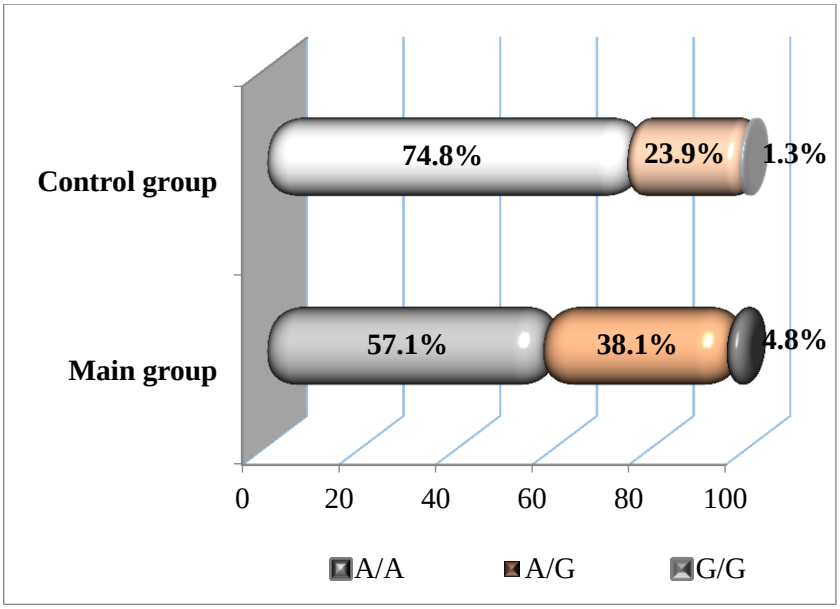

Figure 4. **Differences in the frequency of genotypic variants of the Ile 462 Val polymorphism of the CYP 1 A 1 gene in groups of patients without azoospermia and controls**

Thus, our data allow us to conclude that unfavorable allelic variants of the Ile 462 Val polymorphism of the CYP 1 A 1 gene play an important role in the formation of male infertility without azospermia.

The results of the analysis of the C6310T polymorphisms of the CYP1A1 gene and G681A of the CYP2C19 gene did not reveal significant differences in the distribution of allele and genotype frequencies between the studied samples (Figure 5).

Genotypic frequencies of G/G, G/A, and A/A for the C6310T polymorphism of the CYP1A1 gene in the control were detected in 73.6%, 23.9%, and 2.5% of cases, respectively. In addition, similar data were obtained in the distribution of genotype frequencies in the group of patients - 71.4%, 26.4% and 2.2%, respectively. As can be seen, the proportion of carriers of the unfavorable A/A genotype (associated with low cytochrome production) was slightly lower among patients compared with the control sample (2.5% vs. 2.2%, respectively; $p > 0.05$).

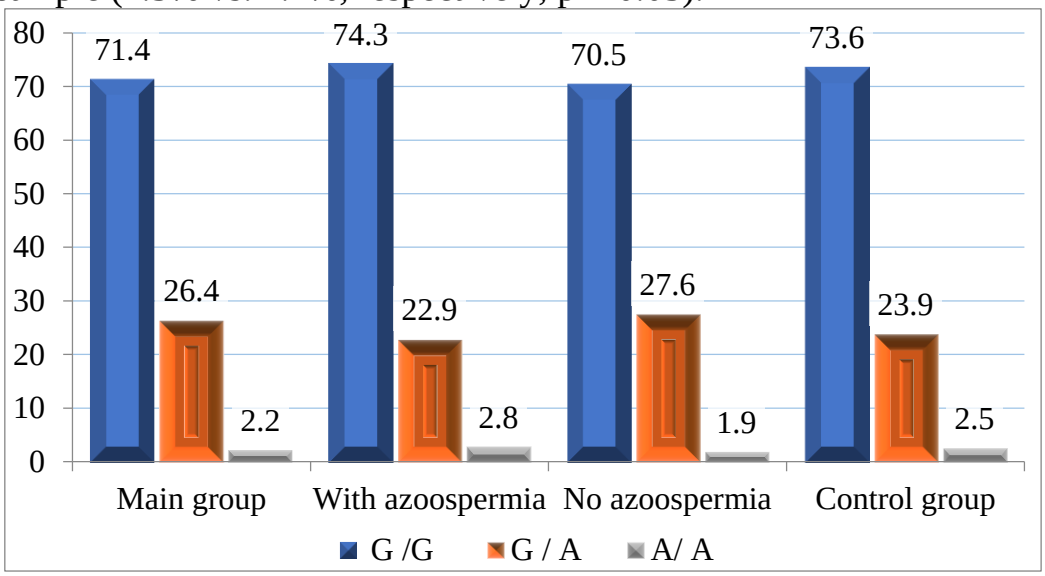

Figure 5. **Distribution of genotypes of polymorphism G 681 A of the CYP 2 C 19 gene in groups of patients and controls**

Significant differences were not found when comparing the distribution of the frequency of genotypes of the C6310T polymorphism of the CYP1A1 gene in the studied groups of patients and controls. The level of reduction of unfavorable C/T and T/T genotypes in the control group compared to the group of patients was insignificant and statistically insignificant (table 2).

Table 2. Differences in the frequency of allelic and genotypic variants of the C 6310 T polymorphism of the CYP 1 A 1 gene in the main group of patients compared with the control

Alleles and genotypes	Number of examined alleles and genotypes				χ^2	R	RR	95%CI	OR	95%CI
	Main group		Control group							
	n	%	n	%						
C	218	77.9	255	82.3	1.8	0.19	0.9	0.6-1.3	0.8	0.6-1.1
T	62	22.1	55	17.7	1.8	0.19	1.1	0.7-1.7	1.3	0.9-1.9
C/C	85	60.7	104	67.1	1.3	0.26	0.9	0.6-1.5	0.8	0.6-1.2
C/T	48	34.3	47	30.3	0.5	0.49	1.1	0.7-1.8	1.2	0.7-2.0
T/T	7	5.0	four	2.6	1.2	0.28	1.9	0.8-4.7	2.0	0.6-6.9

Significant differences were also found when comparing the distribution of the frequency of genotypes of the G636A polymorphism of the CYP2C19 gene in the studied groups of patients and controls (Figure 6).

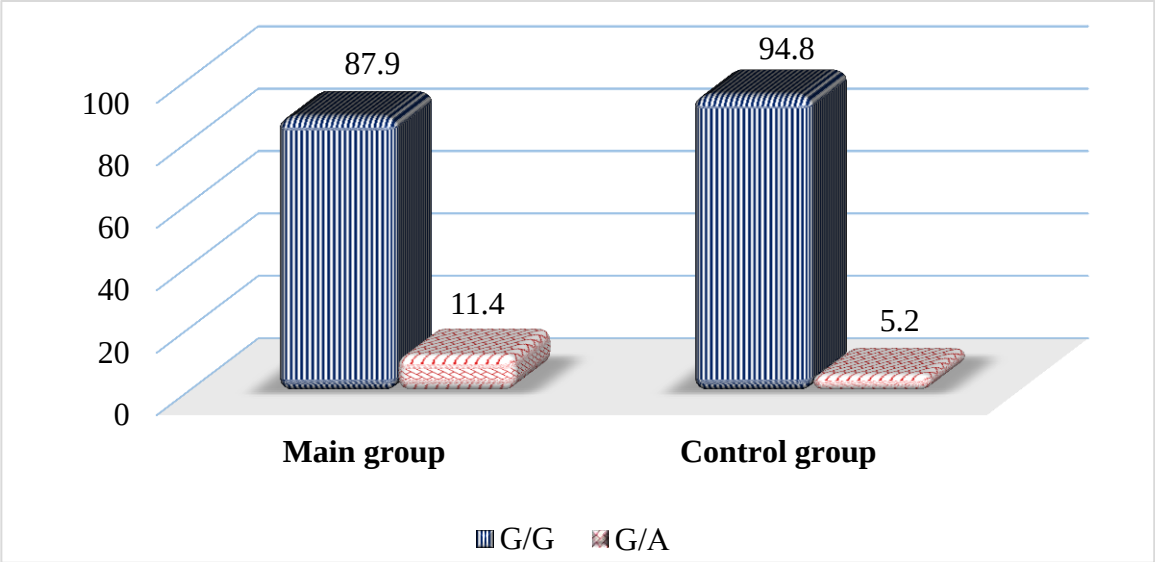

Figure 6. **Distribution of genotypic variants of the G 636 A polymorphism of the CYP 2 C 19 gene in the main and control groups of patients .**

Among the persons of the control group, the carriage of alleles G and A was determined in 97.4 % and 2.6% of cases, while among the persons of the main group they were detected in 93.6% and 6.4% of cases, respectively (χ^2 =5.2; p =0.02). Odds ratio for developing BT in carriers of this genotype was OR =2.6 at 95% CI: 1.14-5.91.

Carriage of the heterozygous G / A genotype among patients also turned out to be significantly higher than in the control sample - 17.1% vs. =3.8; 95%CI: 1.31-11.06).

Meanwhile, the differences in the distribution of allele and genotype frequencies between the groups of men with and without azoospermia were not significant.

These data allow us to conclude that unfavorable allelic variants of the G636A polymorphism of the CYP2C19 gene play an important role in the formation of susceptibility to reproductive system disorders . and is a risk factor for the development of male infertility in people of Uzbek nationality. The risk of developing this pathology in the carriage of allelic variants 636A of the CYP2C19 gene can increase by more than 3.6 times.

Thus, in this study, our results were, in a sense, expected, prior to genotyping of these polymorphic loci . According to our data , unfavorable genotypic variants of the Ile462Val polymorphisms of the CYP1A1 gene and G636A of the CYP2C19 gene among patients were significantly higher than in individuals without reproductive system disorders, and these loci were associated with an increased risk of developing infertility in men.

The frequencies of alleles and genotypes of polymorphisms C6310T of the CYP1A1 gene and G681A of the CYP2C19 gene did not differ significantly in the

studied groups of patients and controls. These data may indicate a possible recessive (less strong) effect of functionally unfavorable genotypic variants in relation to the violation of the reproductive system in men. Since this is one of the few studies in the country on the study of the relationship between the polymorphism of the cytochrome P450 genes and the risk of developing male infertility, in order to finally confirm our findings, we consider it necessary to conduct an extended study of the entire link and synergistically interacting enzyme genes - biotransformation of xenobiotics.

Used literature

1. Abou Alchamat G., Alhlabi M., Issa M. A case report of an XX male with complete masculinization but absence of the SRY gene //Middle East Fertility Society Journal. - 2010. - T . 15. - no. 1. - S. 51-53.

2. Arteaga E., Valenzuela F., Lagos CF, Lagos M., Martinez A., Baudrand R., Carvajal C., Fardella CE Detection of a novel severe mutation affecting the CYP21A2 gene in a Chilean male with salt wasting congenital adrenal hyperplasia. endocrine. 2020 Jan;67(1):258-263.

3. Bieth E., Hamdi SM, Mieusset R. Genetics of the congenital absence of the vas deferens. Hum Genet. 2021 Jan;140(1): 59-76.

4. Nuti F., Krausz C. Gene polymorphisms/mutations relevant to abnormal spermatogenesis. Reprod Biomed Online. 2008 Apr;16(4):504-13.

5. Pan L. et al. Association of the VDAC3 gene polymorphism with sperm count in Han-Chinese population with idiopathic male infertility //Oncotarget. - 2017. - T . 8. - no. 28. - S. 45242

6. Sudhakar D .VS, Shah R., Gajbhiye RK Genetics of Male Infertility - Present and Future: A Narrative Review. J Hum Reprod Sci. 2021 Jul-Sep;14(3):217-227. doi: 10.4103/jhrs.jhrs_115_21. Epub 2021 Sep 28.

7. Yarosh SL, Kokhtenko EV, Starodubova NI, Churnosov MI, Polonikov AV (2013) Smoking Status Modifies the Relationship Between CYP1A1*2C Gene Polymorphism and Idiopathic Male Infertility: The Importance of Gene-Environment Interaction Analysis for Genetic Studies of the Disease. Reprod Sci 20(11) 1302–1307.

8. Amato L .GL, Montenegro LR, Lerario AM, et al. New genetic findings in a large cohort of congenital hypogonadotropic hypogonadism. Eur J Endocrinol. 2019;181(2):103-119.

9. Belloc S ., Hazout A., Zini A., Merviel P., Cabry R., Chahine H., Copin H., Benkhalifa M. How to overcome male infertility after 40: Influence of paternal age on fertility. Maturitas. 2014 May;78(1):22-29.

10. Ji G. et al. Genetic variants in antioxidant genes are associated with sperm DNA damage and risk of male infertility in a Chinese population //Free Radical Biology and Medicine. - 2012. - T . 52. - no. 4. - S. 775-780.

11. Liu SY et al. Strong association of SLC1A1 and DPF3 gene variants with idiopathic male infertility in Han Chinese //Asian journal of andrology. - 2017. - T . 19. - no. 4. - S. 486.

12. Ni W. et al. Lack of association between genetic polymorphisms in three folate-related enzyme genes and male infertility in the Chinese population //Journal of assisted reproduction and genetics. - 2015. - T . 32. - no. 3. - S. 369-374.

13. Nelson D .R., Koymans L., Kamataki T., Stegeman JJ, Feyereisen R., Waxman DJ, Waterman MR, Gotoh O., Coon MJ, Estabrook RW, Gunsalus IC, Nebert DW P450 superfamily: update on new sequences, gene mapping, accession numbers and nomenclature. Pharmacogenetics. 1996 Feb;6(1. - P. 1-42.

14. Josephy PD Genetic variations in human glutathione transferase enzymes: significance for pharmacology and toxicology. Hum Genomics Proteomics. 2010 Jun 13; 2010:876940.

15. Abolins-Abols M., Hanauer RE, Rosvall KA, Peterson MP, Ketterson ED. The effect of chronic and acute stressors, and their interaction, on tests function: an experimental test during testicular recrudescence. J Exp Biol. 2018 Sep 10;221(Pt 17): jeb180869.

16. Aydos SE, Taspinar M., Sunguroglu A., Aydos K. Association of CYP1A1 and glutathione S-transferase polymorphisms with male factor infertility. Fertil Steril. 2009 Aug;92(2):541-7.

17. Aynacioglu AS, Cascorbi I., Mrozikiewicz PM, Roots I. High frequency of CYP1A1 mutations in a Turkish population. Arch Toxicol. 1998 Mar; 72(4):215-8.

18. Boehm U ., Bouloux PM, Dattani MT, de Roux N., Dodé C., Dunkel L, Dwyer AA, Giacobini P., Hardelin JP, Juul A., Maghnie M., Pitteloud N., Prevot V., Raivio T., Tena- Sempere M., Quinton R., Young J. European consensus statement on congenital hypogonadotropic hypogonadism—pathogenesis, diagnosis and treatment. Nat Rev Endocrinol. 2015;11(9): 547–564.

19. Bøllehuus Hansen L., Lorenzen M., Bentin- Ley U., Nielsen JE, Krog H., Berg AH, Håkansson BS, Pedersen AM, Høst T., Juul A., Blomberg Jensen M. Presence of the vitamin D inactivating enzyme CYP24A1 in human sperm and prediction of the success of intrauterine insemination: A prospective study. J Steroid Biochem Mol Biol. 2019 Jul; 191:105353