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COMBINED INTERACTION OF HORMONE GENE POLYMORPHISMS REGULATING THE PROCESS OF SPERMATOGENESIS IN MEN WITH MALE INFERTILITY

Irgashev D.S
Head of the hospital , PhD,
andrologist-sexopathologist-reproductologist,
JV OOO Doctor-D-IVF
Uzbekistan, Tashkent
hospitaldoctord@mail.ru
Gasanova Sh.S
Geneticist, Laboratory assistant of the 2nd category,
free applicant
JV LLC " Doctor - D - IVF "
Uzbekistan, Tashkent
sh.gas210@gmail.com
Boboev K.T
MD , PhD , Leading Researcher, Department of Molecular
medicine and cellular technologies at RSSPMCG
Uzbekistan, Tashkent

Abstract: Combinations of unfavorable allelic variants of the GNRHR (Arg262Gln) and LHB (G1502A) genes contribute to the formation of male fertility disorders. The combination of heterozygous genotypes of these genes, which potentiate each other and predispose to disruption of the spermatogenesis process, has the greatest synergistic effect. Conclusion. Molecular analysis and a prognostic model based on a combination of unfavorable genotypic variants of the GNRHR (Arg262Gln) and LHB (G1502A) genes will allow us to assess the risk of reproductive system disorders and infertility in men.

Keywords: male infertility (fertility), GNRHR (Arg262Gln) and LHB (G1502A) polymorphic genes , reproductive system.

Relevance

Currently, about 10-15% of married couples face the problem of impaired fertility in the world, while about 50% of cases are due to a violation or decrease in the reproductive function of a man [1,2]. In most cases, the violation of the reproductive system in men is due to genetic factors [3].

It is known that the process of formation and maturation of spermatozoa is controlled by gonadotropin-releasing hormone (GNRHR) and luteinizing hormone (LHB). At present, both in men and women, key polymorphic variants of these genes associated with reproductive disorders and changes in the hormonal profile have been studied [4].

The gene for the gonadotropin releasing hormone receptor (GNRHR) is located on the long arm of chromosome 4 (4q21.2) and consists of 3 exons [5]. The Arg 262 Gln mutation (c .785 G > A ; rs 104893837) in exon 3 of the GNRHR gene is a single nucleotide substitution of G > A nucleotides on chromosome 4 at position 68606400 [6]. This mutation, attributable to a wide range of different reproductive phenotypes in the form of hypogonadotropic hypogonadism, is also inherited in an autosomal recessive manner and often leads to delayed puberty with the development of infertility in men [7,8] .

luteinizing hormone ß-chain **(LHB)** gene is localized on chromosome 11p13 and contains 3 exons. The gene contains 179 SNPs, among which the most functionally significant are polymorphisms of the coding region of the gene Trp8Arg, Ile15Thr, and Gly102Ser, leading to a decrease in the activity of luteinizing hormone (LH). The G(1502)A polymorphic variant is a substitution in the LH ß-chain gene , leading to the replacement of Gly with Ser at position 102 of the LHB protein (Gly 102 Ser , rs 5030774) [9,10]. Carriers of the LHβ 1052A allele have been found to have lower LH levels, and this polymorphism may be associated with infertility in both men and women [11,12].

Since the literature data on the relationship of single nucleotide substitutions in the genes producing steroid hormones with male fertility are ambiguous, the aim of this work was to study the relationship between the combined interaction of polymorphisms of the GNRHR (Arg262Gln) and LHB (G1502A) genes. in patients with male fertility.

Materials and methods.

The genomic DNA samples of peripheral blood of 140 patients with various clinical manifestations of male infertility served as the material for the molecular genetic study. Of these: 35 (25.0%) were patients with azoospermia , 105 (75.5%) - patients without azoospermia. The control sample was formed from 155 apparently healthy individuals of Uzbek nationality without any violation of the reproductive system.

DNA extraction was carried out using AmpliPrime RIBO-prep DNA extraction kits (NextBio, Russia). The concentration and purity of the isolated genomic DNA was measured using a NanoDrop 2000 instrument (Thermo Fisher Scientific, USA). Genotyping of polymorphic GNRHR (Arg262Gln) and LHB (G1502A) genes using the Rotor system Gene Q (Quagen , Germany) and kits for the determination of single nucleotide substitutions in these genes according to the manufacturer's standard method (NPO Litekh and Sintol, Russia). 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 with calculations of relative risk and confidence interval of the results was carried out using the statistical software package OpenEpi 2009, Version 9.3.

Analysis of the 785 G > A polymorphism of the GNRHR gene

As can be seen from Table 1, the frequency of occurrence of the mutation variant 785 G > A of the GNRHR gene both among patients with MB and among the control group was expectedly low. In both groups, the actual-observed distribution of genotypes of the 785 G > A mutation of the GNRHR gene corresponded to the theoretically expected one ($\chi 2=0.1$; $p=0.7$). The frequencies of the wild 785 G and mutational 785 A alleles, respectively, were 0.98/0.02 in the patient group and 0.99/0.01 in the control group.

Table 1.

Distribution frequency of alleles and genotypes of the Arg262Gln polymorphism of the GNRHR gene in groups of patients and controls

No.	Group	Distribution frequency:									
		alleles				genotypes					
		G		A		G/G		G/A		A/A	
		n	%	n	%	n	%	n	%	n	%
1	Main group (n=140)	273	97.5	7	2.5	133	95.0	7	5.0	0	0
2	With azoospermia (n=35)	70	100	0	0	35	100	0	0	0	0
3	Without azoospermia (n=105)	203	96.7	7	3.33	98	93.3	7	6.7	0	0
4	Control group (n = 155)	308	99.3	2	0.6	153	98.7	2	1.3	0	0

The observed and theoretical frequencies of the G785G, G785A, and A785A genotypes in the general group of patients with male infertility were 0.95/0.95, 0.05/0.05, and 0.0/0.0, respectively. Among conditionally healthy men, the observed and theoretically expected genotype frequencies were 0.99/0.99, 0.01/0.01, and 0.0/0.0. in both groups. Differences in the frequency of genotypes did not differ significantly from the Hardy-Weinberg equilibrium and were at the level of 5.0%, i.e. significance level (χ^2 =0.01 and p>0.05). In both groups, the actual frequency of the homozygous variant A785A of the GNRHR gene , which has a high penetrance to pathology, was equal to H_o=0.

It should be emphasized that homozygous A785A of the GNRHR gene is also extremely rare in the studied populations of the world [13,14,15,16].

Analysis of the G1502A polymorphism of the LHB gene

Table 2 presents the results of the frequency of occurrence, calculations of the deviation of the theoretical and empirical frequencies of the distribution of alleles and genotypes of the missense mutation G1502A of the LHB gene for RHV in groups of patients with MB and population samples.

Table 2

The frequency of distribution of alleles and genotypes of the missense mutation Gly 102 Ser of the LHB gene (G1502A) in groups of patients with male infertility and controls

No.	Group	Distribution frequency:									
		alleles				genotypes					
		G		A		G/G		G/A		A/A	
		n	%	n	%	n	%	n	%	n	%
1	Main group (n=140)	278	99.3	2	0.71	138	98.6	2	1.4	0	0.0
A	WITH azoospermia (n=35)	70	100	0	0	35	100	0	0	0	0.0
b	Without azoospermia (n= 105)	208	99.0	2	0.9	103	98.1	2	1.9	0	0.0
2	Control group, (n=155)	310	100.0	0	0.0	155	100.0	0	0.0	0	0.0

As can be seen from the tables, the occurrence of the mutation variant G1502A of the LHB gene among patients was low. It was found that out of 140 patients with MB, only 1.2% were carriers of the heterozygous variant G1502A (2/140). Both carriers of the missense mutation belonged to a subgroup of patients with infertility, without azoospermia. During the work, the homozygous variant of this mutation was not identified. On the contrary, none of the 155 examined conditionally healthy individuals was a carrier of the missense mutation G1502A of the LHB gene

In the general group of patients, the empirical-actual distribution of alleles and genotypes of the G1502A mutation in the LHB gene corresponded to theoretical-expected at RHV, (χ^2 =0.01; p=0.9, according to Fisher's exact test). The frequencies of the ancestral wild G1502 and minor mutation 1502A alleles, respectively, were 0.99/0.01 in the group of patients. The empirically observed and theoretical frequencies of G/G, G/A, and A/A genotypes of Gly 102 Ser of the LHB gene were 0.99/0.99, 0.01/0.01, and 0.0/0.0, respectively, and the difference level by 5%.

It should be emphasized that among patients with male infertility or in the studied populations of the world, the homozygous variant A1502A of the Gly 102 Ser polymorphism of the LHB gene, which has a high risk of developing a severe form of reproductive system disorders, was also not detected (theoretically, it is extremely rare).

Our data on this mutation indicate the low frequencies of detected actual heterozygotes, and, accordingly, the extremely low level of not only the expected, but also the observed heterozygosity of this locus (H_o = 0.01) in our population.

Results of the analysis of gene-gene interaction

To search for a combination of SNP × SNP loci associated with the risk of developing male infertility, using a bioinformatic approach (OpenEpi software package V 9.2) an analysis of the so-called “gene-gene interaction” of the genes of the spermatogenesis regulators of the GNRHR (Arg262Gln) and LHB (G1502A) genes was carried out in the patient group and in the control group. (table 3)

Our results indicate a trend towards a significant association of SNP×SNP interactions between the genes of the gonadotropin-releasing hormone receptor and luteinizing hormone ß-chain with the development of fertility disorders in men without azoospermia.

As expected, the analysis of combined interactions in a group of patients with male fertility revealed two SNP × SNP locus patterns of DNA marker interactions leading to the risk of developing impaired spermatogenesis . while no such intergenic combination was found in the population sample.

Table 3

Interaction of favorable and unfavorable gene genotypes

GNRHR (Arg262Gln) and LHB (G1502A) among patients with male fertility and control sample

No.	Groups	favorable genes		Unfavorable genotypes			
				1x		2x	
		n	abs	n	abs	n	abs
I	**Main group, n= 140**	133	95.0	5	3.6	2	1.4
II	**Control group, n= 155**	153	98.7	2	1.3	0	0.0

Simultaneous carriage of unfavorable genotypic combinations of 2 SNP loci GNRHR (Arg262Gln) and LHB (G1502A) was found 1.6 times more often among patients with male fertility (without azoospermia) compared with conditionally healthy individuals (1.4% vs. 0.0%, respectively; χ^2=2.3; P=0.1; OR=1.6; 95%CI: 0.247-21.41) (see table 4).

Table 4

Frequency Differences unfavorable genotypes of the GNRHR (Arg262Gln) and LHB (G1502A) genes among patients with male fertility and control sample

unfavorable pleasant genotypes	Number of examined alleles and genotypes				χ^2	p	OR	95% CI
	Main group n= 140		CG n= 155					
	n	%	n	%				
1x	5	3.6	2	1.3	1.6	0.2	2.8	0.540-14.84
2x	2	1.4	0	0.0	2.3	0.1	1.6	0.247-21.41
Blagopr good genotypes	133	95.0	153	98.6	3.4	0.06	0.2	0.050-1.216

Significance analysis of carriage of 1 polymorphic loci of the studied genes showed the most significant contribution and amounted to (χ^2=1.6; P=0.2; OR =2.8; 95% CI : 0.540-14.84).

It should be noted that the combination of 2 SNP×SNPs of favorable genotypes of the GNRHR (Arg262Gln) and LHB (G1502A) genes had a protective efficacy against the formation of male infertility at OR = 0.2 (95.0 versus 98.6%, respectively; χ^2=3.4; P=0.06; 95% CI : 0.050-1.216).

Thus, our study revealed a combined interaction between the gene loci of coding hormones that affect the process of formation and maturation of spermatozoa. The analysis of gene-gene interactions revealed the presence of significant synergy between the studied polymorphic markers and the risk of developing male infertility without azoospermia. At the same time, carriage of combined favorable genotypes of these genes had a significant protective effect. These data are consistent with the theoretical concept of the presence of synergism in the interactions of steroid hormone genes in the mechanism of development of male fertility.

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