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Associations of Polymorphic Variants of the Biotransformation Genes with the Components of the Glutathione System in Men with Infertility

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Abstract

The aim of this research was to investigate the glutathione system components and their association with polymorphisms GST genes in men with infertility.

Materials and Methods: One hundred and sixty Russian men of reproductive age (Caucasians) who came to the public health institution Republican Perinatal Center in Ulan-Ude with an infertility problem of one year and more after marriage were included in the main group. The control group included 104 men with proven fertility. DNA samples were genotyped for polymorphisms in *GSTP1, GSTT1* and *GSTM1*genes and activity of glutathione system enzymes was determined.

Results: The most informative genetic and metabolic indicators in Caucasian males with infertility were combinations of the null genotypes *GSTT1(*0/*0)+GSTM1(*0/*0)* associated with a decrease of GST activity in blood and ejaculate and an increase of GSH and GPO in the blood. Another combination is *GSTP1(Ile105Val)+GSTP1(Ala114Val)*, which is associated with suppression of the blood and ejaculate GPO activity and a decrease in blood concentration of GSH. **(International Journal of Biomedicine. 2017;7(3):226-230.)**

Key words: male infertility • GSTP1 • GSTM1 • GSTT1 • glutathione

Abbreviations

AFO, active forms of oxygen; **DNA**, deoxyribonucleic acid; **GST**, glutathione S-transferase; **GPO**, glutathione peroxidase; **GR**, glutathione reductase; **OS**, oxidative stress.

Introduction

Genetic factors cause 30%–50% of cases of male infertility in different forms.[1-3] The development of molecular biology, biotechnology and gene engineering evoked significant progress in the research mechanisms to control gene expression, which are involved in physiological and pathological processes. Studies aimed at a search for associations between peculiarities of gene polymorphisms and different forms of reproductive function disorder are becoming more relevant.[4-6] Much attention is given to study of the polymorphic variants of the "susceptibility" genes, which, in contrast to mutations, are not evident in the phenotype, but they are not always neutral and often lead to an appearance of metabolic products with modified physical and chemical features and parameters of functional activity.[7] Genetic polymorphisms of biotransformation enzymes determine the intensity of the accumulation of genotoxic metabolites in the cells, participating in DNA damage and control of the enzymes, which detoxify free radicals and enable homeostasis in cells and tissues. Susceptibility of the organism to adverse

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effects of the environment is highly dependent on the activity of the xenobiotic detoxification enzymes.[8,9] Depending on genetically determined peculiarities of xenobiotic biotransformation, its interaction with receptors and enzyme systems, detoxification enzymes define the body's reaction to various toxic substances and pharmaceuticals.[10-14]

The glutathione S-transferase (GST) genes (*GSTM1, GSTT1, and GSTP1*) are involved in the detoxification of a broad range of toxic substances. Polymorphisms in GST genes can affect the expression levels of the GST enzymes. Since GST enzymes play a vital role in cellular defense against environmentally toxic compounds, polymorphisms of GST genes can increase subsceptibility to diseases caused by such xenobiotics. *GSTM1* and *GSTT1* genes show deletion polymorphism.[15] These homozygous gene deletions, called null genotypes, are denoted as *GSTM1*0/*0* and *GSTT1*0/*0*. The percentage of individuals who do not express the GSTM1 enzyme due to a homozygous gene deletion is higher in Caucasians and Asians than in Africans.[16-18] About 60% of Asians, 40% of Africans and 20% of Caucasians do not express the GSTT1 enzyme.[19] The *GSTP1* gene has polymorphism loci within its coding region: *GSTP1 Ile105Val* polymorphism in exon 5 and *GSTP1 Ala114Val* polymorphism in exon 6.[20,21] Polymorphisms within GSTP1 are also associated with alterations in enzyme activity.

The aim of this research was to investigate the glutathione system components and their association with polymorphisms GST genes in men with infertility.

Materials and Methods

One hundred and sixty Russian men of reproductive age (Caucasians) who came to the public health institution Republican Perinatal Center in Ulan-Ude with an infertility problem of one year and more after marriage were included in the main group. The control group included 104 men with proven fertility. All men had a laboratory and clinical examination by an andrologist, including an ultrasonic scan of scrotum and prostate. Macroscopic and microscopic examination of ejaculate was performed in accordance with with the WHO recommendations (2010). The study was conducted in accordance with ethical principles of the Declaration of Helsinki (2000) and approved by the Republican Perinatal Center (Ulan-Ude) Ethics Committee. Written informed consent was obtained from all participants.

Patients with the genetic causes of infertility were excluded from the research (AZF-deletions, CFTR-mutations, mutational changes of the number of CAG repeats, controlled by androgen receptors).

Concentration of the reduced (GSH) and oxidized (GSSG) glutathione was determined by the method of P. Hissin.[22] The activity of GST, GPO and GR was determined using Randox reagents.[23-25] The concentration of conjugates during the reaction was registered spectrophotometrically with a wavelength of 340 nm using a Shimadzu RF-1501 spectro-fluorophotometer.

DNA samples were genotyped for polymorphisms in *GSTP1, GSTT1* and *GSTM1* genes. DNA was isolated from venous blood samples using the sorbent method with the certified reagent kit DNA-Sorb-B (Central Research Institute of Epidemiology, Moscow, Russia). Genetic polymorphism of insertion/deletion (I/D) in the GSTT1 and GSTM1 genes was determined by PCR in the automatic thermocycler Tercyc using the reagent kit of Central Research Institute of Epidemiology(Moscow, Russia).

Deletion status of *GSTM1* and *GSTT1* was simultaneously determined by a multiplex polymerase chain reaction method.[26] To determine the genotypes at codon 105 and 114, respectively, the exon 5 and exon 6 of the *GSTP1* gene were amplified using the relevant primers. Amplification products were detected in 3% agarose gel; the electrophoresis results were registered and documented with the help of the system of computer gel documentation GelDoc. *GSTM1+, GSTT1+* (wild type) and *GSTM1*0* and *GSTT1*0* homozygotes (null genotype) were analyzed.

The statistical analysis was performed using the statistical software STATISTICA 6.1 (StatSoft Inc., USA). Intergenic interaction of the polymorphic variants of the examined genes was estimated with the help of the bioinformatic method of multifactor modelling of genomic interactions – multifactor dimensionality reduction (MDR) in the open access program MDR 3.0.2. The MDR-method allows assessment simultaneously affecting the disease interaction of all examined alleles of the gene polymorphic variant, and decreases the dimension of the number of calculated parameters on the basis of creation of new variables, with assessment of how a combination of genotypes impacts the risk of developing disease.

Results and Discussion

We conducted a comprehensive search using the MDR-method with assessment of all possible combinations of the DNA markers and defined crucial models of intergenic interactions (polymorphic locus combination) for men with infertility defined (Fig. 1).

Fig. 1. *Dendrogram of intergenic interactions of polymorphic locus combination in men with infertility.*

As we can see from the dendrogram and Table 1, the individual marker of development of reproductive disorders for Russian men is carriage of the combination of the polymorphisms *GSTT1(*0/*0)+GSTM1(*0/*0)* and *GSTP1(Ile 105Val)+GSTP1(Ala114Val).*

Established synergic interactions of the polymorphic variants of xenobiotic detoxification genes lead to complete absence of the relevant protein or to appearance of enzymes with modified, usually lower, levels of activity. Taking

into account these data, we performed an analysis of the functionality of the glutathione system in men with infertility.

Table 1.

Entropy value of polymorphic variants of xenobiotics detoxification system genes in various combinations in men with infertility

Polymorphic variants of genes	Caucasians (n=164)
GSTT1(*0/*0)+GSTM1(*0/*0)	0.70 %
GSTP1(Ala114Val)+GSTM1(*0/*0)	0.69%
GSTP1(Ile105Val)+GSTP1(Ala114Val)	1.06%
GSTP1(Ile105Val)+GSTT1(*0/*0)	0.22%

Activities of the antioxidant enzymes are balanced and closely connected to each other.[19] Disproportion in the enzyme components of the antioxidant defense may lead to additional generation of AFO and be one of the causes of OS.[20] Not only do GST enzymes catalyze glutathione accession to the electrophilic center of various chemical compounds, making them less toxic, but also have some peroxidase activity, which plays an important role in intracellular fixation and transportation of a large amount of both endogenous and exogenous compounds.

According to our results, in the main group, those who carried a combination of the null genotypes *GSTT1(*0/*0)+GSTM1(*0/*0)* demonstrated a statistically significant decrease in serum levels of GSH (P=0.003) and GST (P=0.004), an increase in serum level of GPO (P=0.04), and a decrease in GST level (P=0.01)in the ejaculate (Fig. 2).

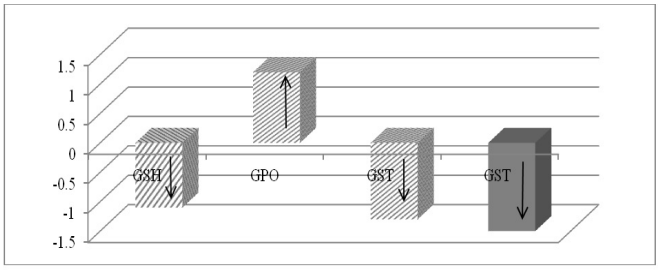

Fig. 2. *Changes in levels of GST enzymes in the main group, those who carried a combination of the null genotypes GSTT1(*0/*0)+GSTM1(*0/*0) (P<0.05).*

Reduction in hydroperoxides content by GPO and GST activity prevents the progression of peroxidation and the appearance of its secondary metabolites. GSTs are an abundant family of dimeric proteins, which have the capacity to conjugate glutathione (GSH) with a variety of electrophilic compounds, primarily produced from exogenous xenobiotics by biotransformation but which can also arise from endogenous substances.

In the main group, those who carried a combination of polymorphisms *GSTP1(Ile105Val)+GSTP1(Ala114Val)* demonstrated a statistically significant decrease in serum levels of GSH and GPO (P=0.04) and a decrease in GPO level (P=0.02) in the ejaculate (Fig. 3).

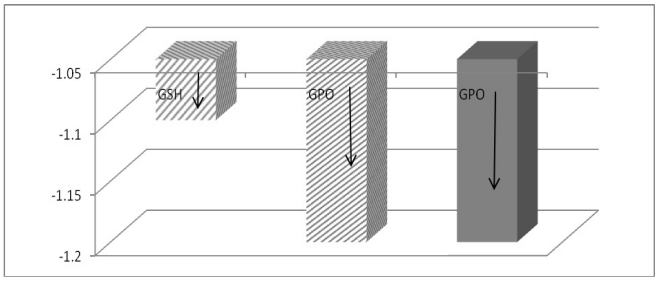

Fig. 3. *Changes in levels of GST enzymes in the main group, those who carried a combination of GSTP1(Ile105Val)+GSTP1 (Ala114Val) (P<0.05).*

The discovered changes of the components of the glutathione system in males with infertility characterize the functional load augmentation in antioxidant systems. GSTs cannot neutralize the toxic impact of various hydrophobic and electrophilic compounds because of their broad substrate specificity and involvement in the metabolism of many endogenous and exogenous electrophilic compounds by conjugation with glutathione.

Two main blood enzymes of the glutathione system associated both with carriage of the combination of the null genotypes *GSTT1(*0/*0)+GSTM1(*0/*0)* and *GSTP1(Ile10 5Val)+GSTP1(Ala114Val* polymorphisms were reduced, but in the ejaculate only one enzyme encoding the mentioned polymorphisms was reduced, which can be considered as a compensatory ejaculate reaction to the impact of damaging factors.

Conclusion

In human testis, reactive oxygen species are involved in the pathogenesis of male reproductive processes by inducing OS that can damage male germ cellular lipids, protein, and DNA.[8,10,11,27,28] Antioxidants, such as *GSTM1* and *GSTT1*, can reduce the toxic effects of OS on male germ lines, suggesting that these two antioxidants genes may play a protective role against OS in spermatogenesis.[12] Thus, the deletion polymorphisms of *GSTM1* and GSTT1 are considered as candidates for genetic susceptibility factors for male infertility. The results showed that the null genotype of *GSTM1* is associated with male infertility, especially in Caucasians and Chinese, indicating that the null genotype of *GSTM1* could increase the risk of male infertility.[11,12]

The interaction between genetic factors (some gene mutations) and the environmental factors (xenobiotics) may play a role in the impaired spermatogenesis and affect the male reproductive function.[29,30] The intake of xenobiotics is different due to diet and pollution conditions, which may result in the different effect of the null genotype of *GSTT1* on male infertility among populations.[12]

Genetically determined imbalance in the system of the glutathione-dependent antioxidant defense determines lipid peroxidation activation and facilitates a significant weakening of metabolic and detoxifying functions of the

organism. As a result, the susceptibility of cells to operations with xenobiotics' harmful influence is significantly increasing, adversely affecting spermatogenesis.

In our study, the most informative genetic and metabolic indicators in Caucasian males with infertility were combinations of the null genotypes *GSTT1(*0/*0)+GSTM1(*0/*0)* associated with a decrease of GST activity in blood and ejaculate and an increase of GSH and GPO in the blood. Another combination is *GSTP1(Ile105Val)+GSTP1(Ala114Val)*, which is associated with suppression of the blood and ejaculate GPO activity and a decrease in blood concentration of GSH.

Genetically determined peculiarities in how the xenobiotic biotransformation system functions make each individual unique with regard to their adaptive capacity - resistance or sensitivity to the damaging exo- and endogenous factors. Identification of carriage of the polymorphic variants of the *GSTT1* and *GSTM1*, as well as determination of the enzymes of the thiol-disulfide system, can be recommended for additional estimation of the risk of developing a disorder of the reproductive functions in males.

Competing interests

The authors declare that they have no competing interests.

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