

Original article

## Influence of GSTP1 gene polymorphism on decreased semen quality

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**Abstract: Background** — Genetic polymorphisms analysis of metabolic and antioxidant systems pathway genes are associated with male infertility is the most perspective and developed field in andrology.

**Purpose** — the aim of the research is to reveal the association of the glutathione S-transferase gene GSTP1 polymorphism C/T (rs1138272) with risk of pathospermia in Russian men in Moscow region.

**Material and Methods** — Case control study was conducted on 68 fertile men and 70 infertile men with various forms of pathospermia. Sperm analysis was performed according to WHO guidelines (WHO, 2010). DNA was extracted from peripheral blood leukocytes. Genotyping of the GSTP1 gene polymorphisms was carried out by generated amplicons from melting curve analysis after real time PCR.

**Results** — statistically significant association of polymorphism GSTP1 C/T (Ala/Val; rs1138272) with asthenozoospermia ( $\chi^2=8.58$ ,  $p=0.003$ ) and teratospermia ( $\chi^2=6.81$ ,  $p=0.009$ ) risk was found. The frequencies of homozygous and heterozygous carries (CT+TT genotypes) for polymorphic locus GSTP1 gene (rs1138272) are 3 times higher for men with disturbance of motility of spermatozoa and 2.5 times higher for men with abnormalities in morphology of spermatozoa, then for men with normozoospermia.

**Conclusion** — The GSTP1 C>T polymorphism (rs1138272) associated with risk of teratospermia and asthenozoospermia in male of reproductive age.

**Keywords:** male infertility, GSTP1 gene, polymorphism, pathospermia, asthenozoospermia, teratospermia, oxidative stress.

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### Introduction

Approximately 17% of couples in Russia are not able to conceive spontaneously within 1 year of unprotected intercourse [1]. It is proven that male infertility factor is about 40-50% of couples' cases [2, 3]. It is known that impaired spermatogenesis is caused by chronic inflammatory diseases of prostate gland, varicocele, genital infections, sperm DNA fragmentation [1, 4-8]. Pathogenic mechanisms of pathospermia do not only include bacterial infection, hypoxia of testis and damages of acrosomal formation, and also are caused by oxidative stress development [7, 9-11]. Oxidative stress (OS) is believed to be an important cause of idiopathic male infertility [5, 11, 12]. As usual, reactive oxygen species (ROS) originate from cellular metabolism of oxygen and normally are neutralized by homeostatic antioxidant systems of humans [9]. The enzymatic antioxidant systems in human seminal plasma are a group of enzymes that include glutathione peroxidase 1 (GXP1) and glutathione S-transferase (GST), manganese superoxide dismutase (SOD2) and catalase (CAT) [13]. Oxidative stress is caused by imbalance of antioxidant enzymes and level of ROS [14]. Besides, morphologically anomalous sperm has an increased ability to generate reactive oxygen species (ROS) and demonstrates low antioxidant activity [12].

Genetic polymorphisms analysis of metabolic and antioxidant systems pathway genes is associated with male infertility is the most perspective and developed field in andrology [10, 15]. There several available studies prove the association of GSTP1 gene polymorphism with male infertility in Asians [15-17]. Some findings are devoted for study the association of GSTP1 polymorphism and infertility in men used cigarette smoke [18, 19].

The aim of the research is to reveal the association of the glutathione S-transferase gene GSTP1 polymorphism C/T (rs1138272) with risk of pathospermia in Russian men in Moscow region.

### Material and Methods

The study includes men living in the Moscow region (n=138): of these, 70 men with impaired reproductive function with various forms of pathospermia of unknown etiology (the 1<sup>st</sup> group of patients) and 68 fertile men of comparable age (2<sup>nd</sup> group of patients). The 1<sup>st</sup> group includes men with abnormal spermiogram, infertility history more than one year, excluded infertility in spouse. The 2<sup>nd</sup> group includes men with normal sperm parameters have one or more children. For both groups we excluded patients under 18 years old, with genital infections, with varicocele, hydrocele and chronic inflammatory diseases of

prostate gland. Informed consent was taken for all participants before study.

All patients underwent a preliminary clinical examination: collection of complaints, anamnesis, examination of objective and urological status, spermogram analysis (WHO, 2010), study of the hormonal profile and karyotype and the presence of deletions in the AZF region of Y-chromosome, mutations in the CFTR gene.

DNA was isolated from leukocyte mass using DNA express-blood kits produced by Synthol (Russia). Genotyping of the GSTP1 gene polymorphism Ala114Val (rs1138272) was performed by generation of amplicons from melting curve analysis after real time PCR (RT-PCR). It was used the amplifier CFX96 (Bio-Rad) with software program CFX Manager TM for genotyping of the GSTP1 gene polymorphism.

Quantitative data are presented as mean and standard deviation –  $M \pm SD$ , binary (such as “yes/no”) variables – as frequencies in absolute values and in percentage – no. (%). The correlation between the carrier of one or another allele and the incidence rate was determined by criterion  $\chi^2$ , 95% CI (confidence interval). The obtained data was processed using the STATISTICA 6.0 software package (StatSoft, USA). The differences were considered significant at  $p < 0.05$ .

### Results

Clinical profile of patients in both groups of participant subjects is shown in Table 1.

Mean age was  $29.5 \pm 3.8$  among all patients. The average body mass index was  $20.4 \pm 4.4 \text{ kg/m}^2$ . The most part of all participant subjects abuses cigarette smoking. No significant differences were observed between the first and second groups of patients with respect to hormonal levels. Consequently, the both groups of patients were concordant with their clinical profile.

Seminal parameters of patients (first group) and fertile men (second group) are shown in Table 2. We found statistically significant differences between the first and second groups of patients with respect to maintenance of morphologically abnormal spermatozoa and quantity of motility active spermatozoa.

All patients had a normal karyotype, no mutations in the CFTR gene, and no AZF micro deletions on the Y chromosome were found. Consequently, the data obtained for men with abnormalities in sperm parameters ( $n=70$ ) were designed as idiopathic infertility.

The frequencies of genotypes GSTP1 polymorphism C>T in groups of participant subjects are shown in Table 3. The frequencies of the CC, CT и TT genotypes GSTP1 (Ala/Val) (C>T, rs1138272) polymorphism were 70.0%, 27.1% и 30.0% for the patients with infertility and 83.8%, 16.2% и 0%, respectively, in the group of fertile men ( $p=0.095$ ). As homozygous genotype TT (rs1138272) was not detected in healthy control and it was in low frequency in infertile men (0.03), heterozygous and homozygous carries of allele T (114Val) were allocated to the separate group (genotype CT+TT; Ala/Val+Val/Val). Carriers of the genotype CT+TT (Ala/Val+Val/Val) in men with pathospermia met with a frequency of 30.0%, in the group of fertile men the frequency of carriers of genotype (CT+TT) was 16.2% ( $p=0.050$ ). The frequencies of the genotypes GSTP1 polymorphism (Ala114Val; C>T) among the men with the pathospermia and healthy control group show no statistically significant differences ( $p > 0.05$ ).

**Table 1. Clinical profile of patients in both groups**

Clinical profile	Groups of patients			
	1 <sup>st</sup> group (n=70)	2 <sup>nd</sup> group (n=68)	p-level	
Age, years	30.0±2.4	27.0±4.9	0.154	
Cigarette smoking	41 (58.5)	38 (55.8)	0.147	
Consumption of alcohol	15 (21.4)	18 (26.4)	0.276	
Body mass index (kg/m <sup>2</sup> )	20.2±4.6	19.7±5.1	0.860	
Hormonal profile	FSH, ME/ml	2.4±1.6	3.1±1.4	0.740
	LH, ME/ml	4.1±0.9	3.9±1.1	0.811
	Testosterone, ng/ml	14.2±1.3	12.9±0.8	0.120
	Prolactin, ME/ml	6.9±2.3	5.6±1.9	0.943

Quantitative data are presented as mean with standard deviation –  $M \pm SD$ , binary (such as “yes/no”) variables – as frequencies in absolute values and in percentage – no. (%).

FSH, follicle stimulating hormone; LH, luteinizing hormone.

**Table 2. Seminal parameters in patients of the first and second groups (mean ± SD)**

Seminal parameters	Groups of patients		
	1 <sup>st</sup> group (n=70)	2 <sup>nd</sup> group (n=68)	p-level
Ejaculate volume, ml	3.8±1.3	4.2±1.0	0.071
pH	7.3±0.1	7.4±0.1	0.083
Sperm concentration, $\times 10^6$ /ml	4.5±2.4	29.7±12.8	<0.001
Motility, %	18.6±6.2	29.6±0.8	0.012
Normal morphology, %	1.2±0.8	5.6±2.5	0.014
Leucocytes, $\times 10^6$ /ml	0.34±0.21	0.52±0.17	0.160

Data are presented as mean with standard deviation –  $M \pm SD$ .

**Table 3. Distribution of GSTP1 genotypes among infertile patients and fertile men (n=138)**

Groups of study	Genotype			
	(Ala/Ala) CC	(Ala/Val) CT	(Val/Val) TT	(Ala/Val + Val/Val) CT+TT
Infertile men (n=70)	49 (70.0)	19 (27.1)	2 (2.9)	21 (30.0)
Fertile men (n=68)	57 (83.8)	11 (16.2)	0 (0)	11 (16.2)
$\chi^2$		4.70		3.70
p-level		0.095		0.050

Binary data are presented as frequencies in absolute values and in percentage – no. (%).

**Table 4. Distribution of genotypes frequencies for GSTP1 polymorphism (C>T; Ala/Val) (rs 1138272) in studied subgroups of patients with different parameters of spermogram**

Parameters of spermograms in studied groups	Genotype				p-level
	(Ala/Ala) CC	(Ala/Val) CT	(Val/Val) TT	(Ala/Val + Val/Val) CT+TT	
Infertile men Asthenozoospermia (n=26)	15 (57.9)	10 (38.4)	1 (3.7)	11 (42.3)	0.014
Teratozoospermia (n=21)	13 (61.9)	7 (33.3)	1 (4.8)	8 (38.0)	0.037
Azoospermia (n=23)	21 (91.3)	2 (8.7)	0 (0)	2 (8.7)	0.734
Healthy men Normozoospermia (n=68)	57 (83.8)	11 (16.2)	0 (0)	11 (16.2)	0.775

Binary data are presented as frequencies in absolute values and in percentage – no. (%).

Further, a comparative analysis of the genotypes frequencies in subgroups of men with different parameters of spermogram was carried out on the studied GSTP1 polymorphism (Ala114Val; rs 1138272); the results are shown in *Table 4*.

Statistically significant differences were found for the genotypes frequencies of GSTP1 polymorphism (C>T; rs1138272) in men with asthenospermia and teratospermia. The frequencies of genotypes CC, CT TT in men with asthenozoospermia was 57.9%, 38.4% and 3.7% ( $\chi^2=8.47$ ,  $p=0.014$ ); and in men with teratospermia – 61.9%, 33.3% и 4.8% ( $\chi^2=6.55$ ,  $p=0.037$ ). The frequencies of homozygous and heterozygous carries of GSTP1 polymorphism (genotypes CT+TT) are three times higher in the case of sperm motility impairment ( $\chi^2=7.16$ ,  $p=0.007$ ), and 2.5 times higher in the case of disrupted morphology of the sperm cells ( $\chi^2=4.6$ ,  $p=0.032$ ).

### Discussion

Male infertility is multifactorial disease associated with individual genetic background [3, 8, 10]. The studies of foreign authors demonstrate that quality of sperm has decreased in the last decades all over the world [2]. This fact manly is believed to be a result of unbalanced diet, adverse environment factors and poor lifestyle, which cause oxidative stress [2, 9]. According to data of many authors oxidative stress is demonstrated to 50% of men with confirmed infertility [1, 5, 11, 12]. Human ejaculate has been shown to contain a significant amount of glutation S-transferases, which can weaken the ROS toxicity to sperm [20]. Weakness of antioxidant glutation S-transferase enzymes may lead to high sensitivity of cells to damages factors of environment and consequently to be involved in many pathogenic mechanisms, including infertility [15]. Free radicals can damage sperm DNA directly by interacting with purine and pyrimidine bases, or cause lipid peroxidation, membrane fluidity and sperm motility reduction and apoptosis [5, 15, 20].

In meta analysis of Safarinjad et al. (2012) it was noted that only two studies were carried out before 2012 to reveal the association GSTP1 polymorphisms with male infertility [15]. In Europe population it was identified an association between GSTP1 polymorphisms and abnormalities in sperm parameters in infertile men in Spain [21]. It is necessary to note that few studies have been carried out to clarify the association of GSTP1 polymorphism with risk of pathospermia but these relationships are still controversial [16, 22]. Nguen et.al. (2018) have reported that the GSTP1 polymorphisms (rs1138272 and rs1695) associated with male infertility in subjects from Vietnam [16]. Early studies have been conducted in Chine did not reveal the association between GSTP1 polymorphisms and pathospermia (odds ratio (OR) 0.86, 95%CI: 0.60–1.25,  $\chi^2=0.197$ ,  $p=0.657$ ) [23]. Later Xiong et al. (2015) have identified the association of GSTP1 polymorphisms with high risk of male infertility (OR 1.53, 95%CI: 1.11–2.11,  $p=0.009$ ) [17]. The data obtained by Feng et al. (2015) have confirmed the association between GSTP1 polymorphisms and infertility in men with azoospermia (OR 1.58, 95%CI: 1.13–2.21,  $\chi^2=13.60$ ,  $p<0.001$ ) [24].

Our findings reveal that the frequencies of GSTP1 genotype did not differ significantly among infertile patients and healthy control ( $p>0.05$ ), but in subgroups in patients with abnormal sperm parameters (asthenozoospermia and teratospermia) the frequencies of GSTP1 genotypes differed significantly ( $p<0.05$ ). That means that patients with sperm motility impairment and

disrupted morphology of the sperm cells are in high risk of oxidative stress. The results of this study concordant with findings of other authors state that oxidative stress is caused by reduction of antioxidant enzymes activity due the polymorphisms of antioxidant systems genes and it affects the spermatozoa worsening their fertility [15, 21].

Detection of point mutation for men with infertility should be applied in preliminary clinical examination for medical correction of spermatogenesis and for successful assistant reproductive technology.

### Conclusion

The GSTP1 polymorphism (Ala/Val) (C>T, rs1138272) associates with male infertility. The frequencies of homozygous and heterozygous carries of GSTP1 polymorphism (Ala/Val; C>T), are three times higher in men with asthenozoospermia, and 2.5 times higher in men with teratozoospermia. These patients are in high risk of oxidative stress and need early medical correction.

### Limitations

We should acknowledge that limitation of this study is the small size of infertile men and healthy control groups. Further studies should be conducted on a larger scale to explore the association of GSTP1 polymorphisms with male infertility with men from different ethnic groups. Second, we have not considered gene-environment interaction in our study.

### Ethical approval

The study was done in accordance with the standards of Good Clinical Practice and principles of Helsinki Declaration. The study was approved by the Bioethics Committee of Peoples' Friendship University of Russia (Moscow, Russia).

### Conflict of interest

The authors declare no conflict of interest.

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