Joint effect of glutathione S-transferase genotypes and cigarette smoking on idiopathic male infertility

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Summary

Inconsistent results of association studies investigated the role of glutathione S–transferase genes in idiopathic male infertility may be explained by ethnical differences in gene–gene and gene–environment interactions. In this study, we investigated a joint contribution of *GSTM1*, *GSTT1* and *GSTP1* gene polymorphisms and cigarette smoking to the risk of idiopathic infertility in Russian men. DNA samples from 203 infertile and 227 fertile men were genotyped by a multiplex polymerase chain reaction (*GSTM1* and *GSTT1* deletions) and PCR-restriction fragment length polymorphism (*GSTP1* I105V) methods. The *GSTP1* genotype 105IV was associated with increased risk of male infertility (OR = 1.50 95% CI 1.02–2.20 $P = 0.04$). Genotype combinations *GSTP1* 105II/*GSTT1* del (G1), *GSTM1* del/*GSTT1* del (G2) and *GSTM1* + /*GSTT1* del (G3) were associated with decreased risk of male infertility ($P \leq 0.003$), whereas a genotype combination *GSTP1* 105IV/*GSTT1* + (G4) was associated with increased disease risk ($P = 0.001$). The genotype combinations G3 and G4 showed a significant association with infertility in smokers; however, nonsmokers carriers did show the disease risk. In conclusion, *GSTM1*, *GSTT1* and *GSTP1* genes are collectively involved in the development of idiopathic male infertility and their phenotypic effects on the disease risk are potentiated by cigarette smoking.

Introduction

The idiopathic form of male infertility is world wide-spread disease found in 40–75% of infertile men (Rowe *et al.*, 2000). The aetiology of idiopathic infertility is thought to be multifactorial (Singh & Jaiswal, 2011) meaning that disease susceptibility is determined by complex interactions between a number of genetic and environmental factors acting in cooperation. There is a concern that low-level xenobiotic exposure of the general population may be responsible for increased prevalence of male infertility and decreased sperm quality registered worldwide over last decades (Kunstmann *et al.*, 1995; Auger *et al.*, 2001; Hansen *et al.*, 2010). It is known that oxidative stress, a pathological condition resulting from the enhanced production of reactive oxygen species and decreased activity of antioxidant defence enzymes, emerges as a key mechanism by which environmental xenobiotics impact on male reproductive functions leading to infertility (Agarwal *et al.*, 2006; Tremellen, 2008; Aitken *et al.*, 2014). This means that polymorphic genes encoding antioxidant and biotransformation enzymes determining inter-individual differences in the ability to activate and detoxify chemical substances of the environment may represent potential modifiers of men's susceptibility to infertility. Pursuing this hypothesis, a number of genetic association studies have been performed to investigate the relationship between polymorphisms in these genes and idiopathic infertility in men (Schuppe *et al.*, 2000; Rubes *et al.*, 2010; Ji *et al.*, 2012; Fang *et al.*, 2014; Sabouhi *et al.*, 2014). Genes for glutathione S-transferases became an object of the most intensive research (Aydemir *et al.*, 2007; Aydos *et al.*, 2009; Finotti *et al.*, 2009; Polonikov *et al.*, 2010; Safarinejad *et al.*, 2010; Tirumala Vani *et al.*, 2010; Jaiswal *et al.*, 2012; Salehi *et al.*, 2012; Lakpour *et al.*, 2013).

Glutathione S-transferases (GST) is an important superfamily of biotransformation and antioxidant defence

enzymes catalysing the conjugation of a large variety of electrophilic and hydrophobic toxic intermediates with reduced glutathione (Hayes *et al.*, 2005). Recent meta-analyses evaluated the associations between *GSTM1*, *GSTT1* and *GSTP1* gene polymorphisms and risk of idiopathic infertility in diverse ethnicities (Chengyong *et al.*, 2012; Safarinejad *et al.*, 2012; Tang *et al.*, 2012; Kan *et al.*, 2013; Li *et al.*, 2013; Song *et al.*, 2013; Wu *et al.*, 2013; Ying *et al.*, 2013). Although several studies provided a strong evidence for the role of *GST* genes in the pathogenesis of male infertility, some studies did not report the association of *GST* gene polymorphisms and disease risk. Although exact reasons of inconsistent results across the genetic association studies in different ethnicities are not yet fully understood, the absence of association between *GST* genes alone and male infertility risk in some populations of the world may be attributed to gene–gene and gene–environment interactions within the framework of common metabolic pathway of xenobiotic biotransformation determined by the enzymes. In particular, our recent studies (Yarosh *et al.*, 2013, 2014) demonstrated an importance of gene–environment interactions analysis for better understanding the role of genes for xenobiotic-metabolising enzymes in the pathogenesis of idiopathic infertility in men. This study was designed to investigate a comprehensive contribution of *GSTM1*, *GSTT1* and *GSTP1* gene polymorphisms and cigarette smoking to the risk of idiopathic infertility in Russian men.

Materials and methods

Study population and diagnosis

All participants of the study provided informed consent before enrolment. The study protocol was approved by the Ethical Review Committee of Kursk State Medical University. A total of 430 unrelated Russian men were recruited from the Family Planning and Reproductive Health Clinic of Kursk Regional Perinatal Centre over a period from 2006 to 2008. The case group included 203 patients with idiopathic male infertility. Criteria for inclusion in the case group were infertility for at least 12 month of regular unprotected intercourse with at least two repeated finding of semen parameter abnormalities and the negative mixed agglutination reaction test. The control group included 227 healthy fertile men (all have normal seminal parameters) who had fathered at least one child. All eligible patients and controls matching the inclusion/exclusion criteria were given the opportunity to be enrolled in the study groups. The mean age of the case group was higher in than in the controls (34.4 years versus 29.2 years; $P < 0.01$).

Semen parameters were evaluated according to World Health Organization guidelines (Rowe *et al.*, 2000). Semen specimens were collected from all study participants through masturbation into a sterile plastic container after at least 2 days of sexual abstinence. Clinical, laboratory and instrumental investigations by experienced andrologist, endocrinologist, genetics and laboratory assistants excluded all possible causes of male infertility such as varicocele, hypogonadotropic hypogonadism, abnormal sexual ejaculatory functions, obstruction of seminal tract, abnormal karyotypes and microdeletions of chromosome Y. The female infertility factor was excluded by experienced gynaecologists. All study participants recorded interviewer administered questionnaire concerning demographic data, life style factors and cigarette smoking habit (ever or never smokers).

Genotyping

Genomic DNA was purified from whole blood samples through phenol–chloroform extraction and ethanol precipitation according to the standard procedure. Genotyping of polymorphism I105V (rs1695) of the *GSTP1* gene was carried out by PCR-RFLP methods, as described in details by Welfare *et al.* (1999). We used sense 5′-GGCTCTATGGGAAGGACCAGCAGG-3′ and antisense 5′-GCACCTCCATCCAGAAACTGGCG-3′ primers for amplification of DNA fragment covering the *GSTP1* gene polymorphism. The PCR product was then digested with endonuclease *BstMAI* (Sibenzyme, Russia). Genotyping *GSTM1* and *GSTT1* null genotypes was performed by a multiplex polymerase chain reaction method in the frame of our previous study (Polonikov *et al.*, 2010). The primer sequences for amplifying the *GSTM1* null polymorphism were sense 5′-GAACTCCCTGAAAAGCTAAAG-C-3′ and antisense 5′-GTTGGGCTCAAATATACGGTGG-3′. The null polymorphism of the *GSTT1* gene was amplified with the following primers: sense 5′-TTCCTT ACTGGTCCTCACATCTC-3′ and antisense 5′-TCACCG-GATCATGGCCAGCA-3′ was used for genotyping. The primer sequences (sense 5′-CAACTTCATCCACGTTCA-CC-3′ and antisense 5′-GAAGAGCCAAGGACAGG TAC-3′) of β-globin gene were used as a control of successful PCR. The PCR products were separated on 2% to 3, 5% ethidium bromide-stained agarose gels and visualised under ultraviolet light on the GDS-8000 Computer Detection System (UVP, Upland, CA, USA). Genotyping was carried out blindly to the case–control status, and the repeatability test was conducted for about 10% of the samples, resulting in a 100% concordance rate.

Statistical analyses

Allele frequencies of polymorphism I105V of the *GSTP1* gene were estimated by gene counting method. The chi-square test was used to identify significant departures from Hardy–Weinberg equilibrium. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to estimate the association between GST genotypes and male infertility risk. Gene–smoking interactions were evaluated by joint categories of GST genotypes and smoking habit with respect to male infertility risk using binary logistic regression analysis. Data on cigarette smoking status were available from 203 infertile men and 164 fertile controls. A two-sided *P*-value ≤0.05 was considered to be statistically significant. Bonferroni correction for *P*-values (P_{adj}) was applied in cases when multiple tests were performed. The statistical calculations were performed using STATISTICA for WINDOWS (v8.0) software package (StatSoft; Tulsa, OK, USA).

Results

Table 1 shows the distribution of alleles and genotypes of polymorphism I105V of the *GSTP1* gene among infertile and fertile men. The *GSTP1* genotype frequencies were in Hardy–Weinberger equilibrium ($P > 0.05$) in the case and control groups. No difference in frequency of allele 105V was seen between the study groups. Meanwhile, the heterozygous genotype 105IV of the *GSTP1* gene was associated with increased risk of idiopathic male infertility (OR = 1.50 95% CI: 1.02–2.20 $P = 0.04$). The distribution of null genotypes for the *GSTM1* and *GSTT1* genes in the case and control groups was reported in our previous study (Polonikov *et al.*, 2010). The frequencies of *GST* genotypes were comparable with those reported previously in our population (Polonikov *et al.*, 2012, 2014). Associations of *GST* genotype combinations with risk of idiopathic male infertility are shown in Table 2. As can be seen from Table 2, two genotype combinations such as *GSTP1* 105II × *GSTT1* del and *GSTM1* del × *GSTT1* del were absent in infertile men. One patient with idiopathic infertility had a genotype combination *GSTM1* + × *GSTT1* del. These genotype combinations were significantly associated with decreased risk of male infertility after adjustment for multiple tests ($P_{adj} \leq 0.003$). Meantime, a genotype combination *GSTP1* 105IV × *GSTT1* + was significantly associated with increased risk of male infertility ($P_{adj} = 0.001$).

As we reported earlier (Yarosh *et al.*, 2013), the case and control groups were comparable with respect to a smoking status. Table 3 shows the results of GST genotypes–smoking interactions analysis. For this analysis, we selected only those combinations of genotypes which were found to be significantly associated with disease risk. As can be seen from Table 3, four genotype combinations showed joint effects with cigarette smoking on male infertility risk, however, only two of them were statistically significant after Bonferroni correction for multiple tests. In particular, a genotype combination *GSTP1* 105IV × *GSTT1* + was associated with increased risk of infertility in smoker men ($P = 0.006$), whereas this genotype combination did show the disease risk in nonsmokers. Absence of genotype combination *GSTM1* + × *GSTT1* del showed a significant association with male infertility in smokers ($P = 0.006$), whereas no association was seen in nonsmoker men.

Discussion

The present study was designed to investigate a joint contribution of *GSTM1*, *GSTT1* and *GSTP1* gene polymorphisms and cigarette smoking to the risk of idiopathic infertility in Russian men. We found only one paper in the literature that investigated the relationship between variation in the *GSTP1* gene and male infertility in which Safarinejad *et al.* (2010) observed a protective effect of this gene polymorphism on the disease risk. This result is inconsistent with our finding that the variant *GSTP1* genotype may be a risk factor for male infertility because the allele 105Val was found to diminish the enzyme

Table 1 Distribution of alleles and genotypes of polymorphism I105V of the *GSTP1* gene among infertile and fertile men

Alleles/genotypes the *GSTP1* gene	Infertile men, *n* = 203 N (%)[a]	Fertile men, *n* = 227 N (%)[a]	Chi-square (*P*-value)
Allele frequencies			
105I	276 (68.0)	319 (70.3)	0.52 (0.47)
105V	130 (32.0)	135 (29.7)	
Genotype frequencies			
105II	90 (44.3)	117 (51.5)	2.23 (0.14)
105IV	96 (47.3)	85 (37.4)	**4.26 (0.04)***
105VV	17 (8.4)	25 (11.0)	0.85 (0.36)

[a]Absolute number and percentage of individuals with particular allele/genotype; *Means a statistically significant association.

Table 2 Associations of GST genotype combinations with risk of idiopathic male infertility

Combinations of genotypes	Infertile men, $N = 203$		Fertile men, $N = 227$		Chi-square (P-value)	OR (95% CI)
	N	%	N	%		
GSTP1 105II × *GSTT1* del	0	0.0	13	5.7	**10.11 (0.001)***	**0.04 (0.00–0.66)**
GSTP1 105IV × *GSTT1* +	95	46.8	72	31.7	**10.26 (0.001)***	**1.89 (1.28–2.80)**
GSTP1 105IV × *GSTT1* del	1	0.5	13	5.7	7.73 (0.01)	0.12 (0.02–0.64)
GSTM1 + × *GSTT1* del	1	0.5	15	6.6	**9.54 (0.002)***	**0.10 (0.02–0.55)**
GSTM1 del × *GSTT1* +	114	56.2	106	46.7	3.84 (0.05)	1.46 (1.00–2.14)
GSTM1 del × *GSTT1* del	0	0.0	14	6.2	**11.06 (0.001)***	**0.04 (0.00–0.64)**

*Means a statistically significant association after adjustment for multiple tests ($P_{adj} \leq 0.003$).

activity towards many xenobiotics, leading to the increased urinary 1-hydroxypyrene excretion (a biomarker of polycyclic aromatic hydrocarbons exposure) (Gaspari *et al.*, 2003; Chen *et al.*, 2007) and increased formation of DNA adducts (Brockstedt *et al.*, 2002). Therefore, we suggest that men with a genotype 105VV of the *GSTP1* gene may be susceptible to environmental toxicants affecting the reproductive system through the induction of chemical and oxidative stress, as it has been demonstrated by a number of studies (Auger *et al.*, 2001; Tremellen, 2008; Hansen *et al.*, 2010; Rubes *et al.*, 2010; Aitken *et al.*, 2014).

As male infertility is a multifactorial disease, the disease phenotype may not accurately be predicted from the knowledge of the effects of individual GST genes because of possible epistatic and additive interactions between the loci determining the common detoxication pathway. This means that the investigation of glutathione-S-transferase genes M1, T1 and P1 in combination may provide a more comprehensive picture of contribution of the genes to the development of male infertility. However, a few genetic association studies (Finotti *et al.*, 2009; Safarinejad *et al.*, 2010; Jaiswal *et al.*, 2012; Salehi *et al.*, 2012) have been designed so far to investigate the role of interactions between GST genes in idiopathic male infertility. In particular, Jaiswal *et al.* (2012) observed that combination of null genotypes of *GSTM1* and *GSTT1* confers protective effect regarding male infertility risk in North Indian population, a finding which is in agreement with our results of the protective effect of genotype combination *GSTM1* del × *GSTT1* del. Meantime, these data are inconsistent with results of other studies in which a combination of *GSTM1* and *GSTT1* null genotypes was found to be associated with increased risk of male infertility in a population of Brazil (Finotti *et al.*, 2009), two Iranian populations (Safarinejad *et al.*, 2010; Salehi *et al.*, 2012) and in recent meta-analysis encompassing 6934 men (Wu *et al.*, 2013). Moreover, Safarinejad *et al.* (2010) observed *GSTP1* wild-type genotype in combination with *GSTM1* null or *GSTT1* null genotype increased probability for male infertility. In contrast, we found that the *GSTP1* 105II × *GSTT1* del genotype combination is associated with decreased risk of male infertility, whereas a genotype combination *GSTP1* 105IV × *GSTT1* + possessed a protective effect on the disease risk. The last relationship can be explained by a strong negative association of the *GSTT1* expressor genotype with infertility risk found in previous study in our Russian population (Polonikov *et al.*, 2010), and then it was confirmed in an independent study in North Indian population (Jaiswal *et al.*, 2012). Such contradictory results across the studies may be explained, on the one hand, by genetic diversity between the studied populations, on the other hand, by heterogeneity of disease pathogenesis in different

Table 3 GST genotype–smoking interactions and susceptibility to idiopathic male infertility

Combinations of genotypes	Infertile men, N (%)		Fertile men, N (%)		OR (95% CI)	
	Smokers ($N = 135$)	Non smokers ($N = 68$)	Smokers ($N = 116$)	Non smokers ($N = 78$)	Smokers	Non smokers
GSTP1 105II × *GSTT1* del	0 (0.0)	0 (0.0)	6 (5.2)	6 (7.7)	0.06 (0.00–1.13)	0.08 (0.00–1.47)
GSTP1 105IV × *GSTT1* +	60 (44.4)	35 (51.5)	32 (27.6)	30 (38.5)	**2.10 (1.24–3.57)***	1.70 (0.88–3.28)
*Non*GSTM1 + × *GSTT1* del	134 (99.3)	68 (100.0)	106 (91.4)	74 (94.9)	**8.84 (1.57–49.9)***	8.28 (0.44–156.6)
GSTM1 del × *GSTT1* del	0 (0.0)	0 (0.0)	5 (4.3)	4 (5.1)	0.07 (0.00–1.37)	0.12 (0.01–2.29)

*The bold values mean a statistically significant association ($P = 0.006$) after adjustment for multiple tests ($P_{adj} \leq 0.013$).

ethnicities and specificity of gene–gene and gene–environment interactions underlying susceptibility to male infertility in distinct human populations.

Over the last years, gene–environment interactions analysis became an emerging branch in genetic research of male factor infertility (Aydos *et al.*, 2009; Rubes *et al.*, 2010; Yarosh *et al.*, 2013). This approach was proposed to overcome a problem of contradictory results between genetic association studies of male infertility and to better understand the mechanisms by which environmental factors provoke the disease in genetically susceptible men. Although such investigations are yet in their infancy, the results of our recent studies clearly demonstrated that oxidant and antioxidant exposures of the environment may potentiate the development of male infertility and their effects mediated by polymorphic genes for antioxidant and xenobiotic-metabolising enzymes (Yarosh *et al.*, 2013, 2014). It is known that smoking status may mask the relationship between polymorphism of GST genes and male infertility risk. In particular, Aydos *et al.* (2009) observed that *GSTM1* null genotype was a significant risk factor for male infertility only after adjustment for smoking status. Although many other studies controlled the observed associations of GST genotypes with male infertility risk for smoking habits and other confounding variables, none of them provided a stratified association analysis separately in smoker and nonsmoker men.

We found for the first time that *GSTM1*, *GSTT1* and *GSTP1* genes are collectively involved in the development of idiopathic male infertility and their phenotypic effects on the disease risk are potentiated by chemicals of cigarette smoke. Although the contribution of GST genes alone to the development of male infertility and abnormal semen parameters has been well documented in the literature, our study results complement these data with a new finding that genetically determined discoordination in the functioning of glutathione S–transferase genes may represent an important part of polygenic background of susceptibility to male infertility, a classic example of multifactorial disease. Taking the study results into account, it is possible to propose that quantity and quality of glutathione S-transferases in male reproductive organs and tissues is likely to be important in an individual's response to environmental chemicals with reproductive toxicities and risk of developing infertility. This means that damaging effects of environmental exposures such as chemicals of cigarette smoke and air population on male reproductive system are potentiated by several glutathione S-transferases possessing diminished detoxication capacity towards xenobiotics-derived intermediates. The study findings justify the need for further investigations which should be focused on a comprehensive evaluation of the pathogenetic role for a number of biotransformation and/or antioxidant genes in combination with different oxidant exposures to identify gene–gene and gene–environment interactions underlying possible toxicogenetic mechanisms of male infertility in the modern world.

References

Agarwal A, Sharma RK, Nallella KP, Thomas AJ Jr, Alvarez JG, Sikka SC (2006) Reactive oxygen species as an independent marker of male factor infertility. *Fertil Steril* 86:878–885.

Aitken RJ, Smith TB, Jobling MS, Baker MA, De Iuliis GN (2014) Oxidative stress and male reproductive health. *Asian J Androl* 16:31–38.

Auger J, Eustache F, Andersen AG, Irvine DS, Jørgensen N, Skakkebaek NE, Suominen J, Toppari J, Vierula M, Jouannet P (2001) Sperm morphological defects related to environment, lifestyle and medical history of 1001 male partners of pregnant women from four European cities. *Hum Reprod* 16:2710–2717.

Aydemir B, Onaran I, Kiziler AR, Alici B, Akyolcu MC (2007) Increased oxidative damage of sperm and seminal plasma in men with idiopathic infertility is higher in patients with glutathione S-transferase Mu-1 null genotype. *Asian J Androl* 1:108–115.

Aydos SE, Taspinar M, Sunguroglu A, Aydos K (2009) Association of CYP1A1 and glutathione S-transferase polymorphisms with male factor infertility. *Fertil Steril* 92:541–547.

Brockstedt U, Krajinovic M, Richer C, Mathonnet G, Sinnett D, Pfau W, Labuda D (2002) Analyses of bulky DNA adduct levels in human breast tissue and genetic polymorphisms of cytochromes P450 (CYPs), myeloperoxidase (MPO), quinone oxidoreductase (NQO1), and glutathione S-transferases (GSTs). *Mutat Res* 516:41–47.

Chen B, Hu Y, Jin T, Lu D, Shao M, Zheng L, Wang Q, Shen Y, Liu H, Liu Y, Zhou Y (2007) The influence of metabolic gene polymorphisms on urinary 1-hydroxypyrene concentrations in Chinese coke oven workers. *Sci Total Environ* 381:38–46.

Chengyong W, Man Y, Mei L, Liping L, Xuezhen W (2012) GSTM1 null genotype contributes to increased risk of male infertility: a meta-analysis. *J Assist Reprod Genet* 29:837–845.

Fang J, Wang S, Wang H, Zhang S, Su S, Song Z, Deng Y, Qian J, Gu J, Liu B, Cao J, Wang Z (2014) The Cytochrome P4501A1 gene polymorphisms and idiopathic male infertility risk: a meta-analysis. *Gene* 535:93–96.

Finotti AC, Costa E, Silva RC, Bordin BM, Silva CT, Moura KK (2009) Glutathione S-transferase M1 and T1 polymorphism in men with idiopathic infertility. *Genet Mol Res* 8:1093–1098.

Gaspari L, Chang SS, Santella RM, Garte S, Pedotti P, Taioli E (2003) Polycyclic aromatic hydrocarbon-DNA adducts in human sperm as a marker of DNA damage and infertility. *Mutat Res* 535:155–160.

Hansen C, Luben TJ, Sacks JD, Olshan A, Jeffay S, Strader L, Perreault SD (2010) The effect of ambient air pollution on sperm quality. *Environ Health Perspect* 118:203–209.

Hayes JD, Flanagan JU, Jowsey IR (2005) Glutathione transferases. *Annu Rev Pharmacol Toxicol* 45:51–88.

Jaiswal D, Sah R, Agrawal NK, Dwivedi US, Trivedi S, Singh K (2012) Combined effect of GSTT1 and GSTM1 polymorphisms on human male infertility in north Indian population. *Reprod Sci* 19:312–316.

Ji G, Gu A, Wang Y, Huang C, Hu F, Zhou Y, Song L, Wang X (2012) Genetic variants in antioxidant genes are associated with sperm DNA damage and risk of male infertility in a Chinese population. *Free Radic Biol Med* 52:775–780.

Kan HP, Wu FL, Guo WB, Wang YZ, Li JP, Huang YQ, Li SG, Liu JP (2013) Null genotypes of GSTM1 and GSTT1 contribute to male factor infertility risk: a meta-analysis. *Fertil Steril* 99:690–696.

Kunstmann JM, Czyglik F, Jouannet P (1995) Decline in semen quality among fertile men in Paris during the past 20 years. *N Engl J Med* 332:281–285.

Lakpour N, Mirfeizollahi A, Farivar S, Akhondi MM, Hashemi SB, Amirjannati N, Heidari-Vala H, Sadeghi MR (2013) The association of seminal plasma antioxidant levels and sperm chromatin status with genetic variants of GSTM1 and GSTP1 (Ile105Val and Ala114Val) in infertile men with oligoasthenoteratozoospermia. *Dis Markers* 34:205–210.

Li X, Pan J, Liu Q, Xiong E, Chen Z, Zhou Z, Su Y, Lu G (2013) Glutathione S-transferases gene polymorphisms and risk of male idiopathic infertility: a systematic review and meta-analysis. *Mol Biol Rep* 40:2431–2438.

Polonikov AV, Yarosh SL, Kokhtenko EV, Starodubova NI, Pakhomov SP, Orlova VS (2010) The functional genotype of glutathione S-transferase T1 gene is strongly associated with increased risk of idiopathic infertility in Russian men. *Fertil Steril* 94:1144–1147.

Polonikov A, Vialykh E, Vasil'eva O, Bulgakova I, Bushueva O, Illig T, Solodilova M (2012) Genetic variation in glutathione S-transferase genes and risk of nonfatal cerebral stroke in patients suffering from essential hypertension. *J Mol Neurosci* 47:511–513.

Polonikov AV, Ivanov VP, Bogomazov AD, Freidin MB, Illig T, Solodilova MA (2014) Antioxidant defense enzyme genes and asthma susceptibility: gender-specific effects and heterogeneity in gene-gene interactions between pathogenetic variants of the disease. *Biomed Res Int*. Article ID 708903. doi: 10.1155/2014/708903.

Rowe PJ, Comhaire FH, Hargreave TB, Mahmoud AMA (2000) WHO Manual for the Standardized Investigation, Diagnosis and Management of the Infertile Male. Cambridge University Press, Cambridge.

Rubes J, Rybar R, Prinosilova P, Veznik Z, Chvatalova I, Solansky I, Sram RJ (2010) Genetic polymorphisms influence the susceptibility of men to sperm DNA damage associated with exposure to air pollution. *Mutat Res* 683:9–15.

Sabouhi S, Salehi Z, Bahadori MH, Mahdavi M (2014) Human catalase gene polymorphism (CAT C-262T) and risk of male infertility. *Andrologia*. doi:10.1111/and.12228.

Safarinejad MR, Shafiei N, Safarinejad S (2010) The association of glutathione-S-transferase gene polymorphisms (GSTM1, GSTT1, GSTP1) with idiopathic male infertility. *J Hum Genet* 55:565–570.

Safarinejad MR, Dadkhah F, Ali Asgari M, Hosseini SY, Kolahi AA, Iran-Pour E (2012) Glutathione S-transferase polymorphisms (GSTM1, GSTT1, GSTP1) and male factor infertility risk: a pooled analysis of studies. *Urol J* 9: 541–548.

Salehi Z, Gholizadeh L, Vaziri H, Madani AH (2012) Analysis of GSTM1, GSTT1, and CYP1A1 in idiopathic male infertility. *Reprod Sci* 19:81–85.

Schuppe HC, Wieneke P, Donat S, Fritsche E, Köhn FM, Abel J (2000) Xenobiotic metabolism, genetic polymorphisms and male infertility. *Andrologia* 32:255–262.

Singh K, Jaiswal D (2011) Human male infertility: a complex multifactorial phenotype. *Reprod Sci* 18:418–425.

Song X, Zhao Y, Cai Q, Zhang Y, Niu YJ (2013) Association of the Glutathione S-transferases M1 and T1 polymorphism with male infertility: a meta-analysis. *J Assist Reprod Genet* 30:131–141.

Tang M, Wang S, Wang W, Cao Q, Qin C, Liu B, Li P, Zhang W (2012) The glutathione-S-transferase gene polymorphisms (GSTM1 and GSTT1) and idiopathic male infertility risk: a meta-analysis. *Gene* 511:218–223.

Tirumala Vani G, Mukesh N, Siva Prasad B, Rama Devi P, Hema Prasad M, Usha Rani P, Pardhanandana Reddy P (2010) Role of glutathione S-transferase Mu-1 (GSTM1) polymorphism in oligospermic infertile males. *Andrologia* 42:213–217.

Tremellen K (2008) Oxidative stress and male infertility-a clinical perspective. *Hum Reprod Update* 14:243–258.

Welfare M, Monesola Adeokun A, Bassendine MF, Daly AK (1999) Polymorphisms in GSTP1, GSTM1, and GSTT1 and susceptibility to colorectal cancer. *Cancer Epidemiol Biomarkers Prev* 8:289–292.

Wu W, Lu J, Tang Q, Zhang S, Yuan B, Li J, Di W, Sun H, Lu C, Xia Y, Chen D, Sha J, Wang X (2013) GSTM1 and GSTT1 null polymorphisms and male infertility risk: an updated meta-analysis encompassing 6934 subjects. *Sci Rep* 3:2258.

Yarosh SL, Kokhtenko EV, Starodubova NI, Churnosov MI, Polonikov AV (2013) Smoking status modifies the relation between CYP1A1*2C gene polymorphism and idiopathic male infertility: the importance of gene-environment interaction analysis for genetic studies of the disease. *Reprod Sci* 20:1302–1307.

Yarosh SL, Kokhtenko EV, Churnosov MI, Ataman AV, Solodilova MA, Polonikov AV (2014) Synergism between the N-acetyltransferase 2 gene and oxidant exposure increases the risk of idiopathic male infertility. *Reprod Biomed Online* 3:362–369. 10.1016/j.rbmo.2014.04.008.

Ying HQ, Qi Y, Pu XY, Liu SR, A ZC (2013) Association of GSTM1 and GSTT1 genes with the susceptibility to male infertility: result from a meta-analysis. *Genet Test Mol Biomarkers* 17:535–542.