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DOI: 10.1111/j.1745-7262.2008.00347.x

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·*Original Article*·

Genetic polymorphism of glutathione S-transferase T1 gene and susceptibility to idiopathic azoospermia or oligospermia in northwestern China

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Abstract

Aim: To investigate the association of glutathione S-transferase T1 (*GSTT1*) gene polymorphism in patients with idiopathic azoospermia or oligospermia in the northwestern China population. **Methods:** In the case-control study, *GSTT1* genotypes were identified by multiplex polymerase chain reaction (PCR) with peripheral blood DNA samples from 78 patients with idiopathic azoospermia, 103 patients with idiopathic oligospermia and 156 age-matched controls with normal sperm concentration and motility, according to the criteria adapted from World Health Organization guidelines. All of the patients and controls were from northwestern China. **Results:** There is a significant association between *GSTT1* null genotype with idiopathic azoospermia risk (odds ratio [OR]: 2.36, 95% confidence interval [CI]: 1.33–4.20, $P = 0.003$) or idiopathic oligospermia risk (OR: 2.00, 95% CI: 1.17–3.27, $P = 0.010$). **Conclusion:** *GSTT1* null genotype is a predisposing risk factor for sporadic idiopathic azoospermia or oligospermia in northwestern China. *(Asian J Androl 2008 Mar; 10: 266–270)*

Keywords: glutathione S-transferase T1; genetic polymorphism; azoospermia; oligospermia; male infertility

1 Introduction

Idiopathic azoospermia and idiopathic oligospermia are common reasons for male infertility, but little is known about the factors that cause these diseases [1]. Recent evidence suggests that both environmental and occupational exposure affects male fertility [2–4].

Glutathione *S*-transferases (GST) represents an important superfamily of phase II drug metabolizing enzymes that includes at least seven distinct classes; namely, α(A), μ(M), π(P), σ(Sigma), ζ(Zeta), ω(Omega) and θ (T), based on differences in amino acid sequence. GST have the functions of biotransformers, which are to catalyze the conjugation of a large variety of endogenous and exogenous compounds, including toxic or carcinogenic compounds and their metabolites with reduced glutathione [5, 6]. As a result, GST decreases the reactivity of electrophilic substrates, which can affect spermatogenesis and spermatozoa function with cellular macromolecules,

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Received: 2007-05-16 Accepted: 2007-08-23

such as nucleonic acid, lipid and protein.

Human cytosolic GST genes exhibit genetic polymorphisms and the inherited deletion of genes are called null genotypes. Many genetic polymorphisms lead to altered GST activities, which might be partially responsible for individual host susceptibility to xenobiotics. Glutathione S-transferase T1 (*GSTT1*), a member of the GST gene family, is polymorphic in the human population [7]. Total or partial gene deletion of *GSTT1* leads to the absence of enzymatic activity [8]. Previous studies indicate a possible relationship between *GSTT1* null genotype and susceptibility to cancers, diabetes mellitus and coronary artery disease [9–11], but little attention has been paid to the association of *GSTT1* genotype with male infertility.

To achieve a better insight into the relationship between *GSTT1* null genotype and idiopathic azoospermia or idiopathic oligospermia, we determined the association between *GSTT1* null genotype and the potential risk for idiopathic azoospermia or idiopathic oligospermia.

2 Patients and methods

2.1 Study population

Between January 2005 and October 2006, 78 patients with idiopathic azoospermia and 103 patients with idiopathic oligospermia were diagnosed according to World Health Organization (WHO) standards [12] at the First Hospital of Xi'an Jiaotong University (Xi'an, China). The control group comprised 156 healthy volunteers with normal sperm concentration and motility according to the criteria adapted from WHO guidelines [12]. Smoking history was coded by grouping patients into those who smoked more than 10 cigarettes per day for half a year at least or not at all. Drinking history was coded by grouping patients into those who drank more than 50 mL (alcohol) per day for half a year at least or not at all. All of the patients and controls, coming from northwestern China, agreed to participate in the case-control study.

2.2 Blood sampling and DNA extraction

Blood samples (3 mL taken into EDTA by venipuncture) were obtained from all of the subjects. Immediately after collection, whole blood was stored at –80ºC until use. Genomic DNA for polymerase chain reaction (PCR) analysis was isolated from thawed whole blood using Axypre, a whole blood genomic DNA miniprep kit (Axygen biosciences, Union City, CA, USA).

2.3 Genotyping analysis

GSTT1 genotypes were identified by multiplex PCR. The primers for amplifying *GSTT1* gene segment were 5'-TTC CTT ACT GGT CCT CAC ATC TC-3' and 5'-TCA CCG GAT CAT GGC CAG CA-3'. The primes for *β-actin* were 5'-CGT ACT CCT GCT TGC TAA TCC ACA-3' and 5'-CGG GAC CTG ACC GAC TAC CTC A-3'. The PCR was performed in a 25-μL reaction buffer contained 200 μmol/L dNTPs, 1.5 mmol/L $MgCl_2$, 10 pmol of each primer, approximately 200 ng of template DNA, and 2 U of thermostable TaqDNA polymerase (MBI, Lithuania). After a 5 min pretreatment at 94ºC, 35 PCR cycles of 1 min at 94ºC, annealing for 1 min at 63ºC, and extension for 1 min at 72ºC, were performed. The final extension was at 72ºC for 7 min. *β-actin* gene was co-amplified in all samples as an internal standard. The amplification products were separated on 2% agarose gel stained with ethidium bromide. A 480-bp fragment was amplified with *GSTT1* primers; the absence of amplification products was consistent with the null genotype. The fragment of *β-actin* gene was 540 bp. This method can only detect the present (at least one allele present, homozygote or heterozygote) or absent (complete deletion of both alleles, homozygote) genotype. Although this technique does not distinguish between heterozygotes and homozygotes of the positive genotypes, it identifies conclusively the null genotypes. To ensure laboratory quality control, two independent readers interpreted the gel photographs. Samples with ambiguous results, which were generally a result of low PCR yield, were retested and random selections of 15% of all the samples were repeated. No discrepancies were discovered upon replicate testing. The electrophoresis patterns for *GSTT1* null mutation and wild type are shown in Figure 1. A 480 bp band was observed for *GSTT1* (+) and no band for *GSTT1* (–) after PCR amplification.

2.4 Statistical analysis

Data on the age of study subjects were analyzed using one-way analysis of variance. The frequencies of drinking or smoking of study subjects and *GSTT1* genotype between groups were analyzed by χ^2-test. Logistic regression was used to study the effect of *GSTT1* genotype on patients with idiopathic azoospermia or idiopathic oligospermia. The odds ratios (OR) with 95% confidence interval (CI) were reported. A two-tailed $P < 0.05$ was considered statistically significant. All analyses were performed using SPSS version 13.0 statistical software (SPSS, Chicago, IL, USA).

Table 1. Characteristics of the study population. The data are expressed as mean ± SD. $^bP < 0.05$, $^cP < 0.05$, compared with the control group.

Group	*n*	Age (years)	Sperm concentration ($\times 10^6$/mL)	Drinking		Smoking	
				Yes	No	Yes	No
Control	156	29.6 ± 2.3	62.3 ± 22.5	14	142	70	86
Azoospermia	78	27.8 ± 2.7[b]	0	6[b]	72[b]	34[b]	44[b]
Oligospermia	103	28.3 ± 3.8[c]	8.5 ± 3.4 [c]	11[c]	92[c]	45[c]	58[c]

Table 2. Association of glutathione S-transferase T1 (*GSTT1*) polymorphism with idiopathic azoospermia and idiopathic oligospermia. The data are expressed as mean ± SD. $^bP = 0.003$, $^cP = 0.010$, compared with the control group. OR, odds ratio; CI, confidence interval.

Group (*n*)	*GSTT1*		OR (95% CI)
	Null (%)	Present (%)	
Control	76 (48.7)	80 (51.3)	1.00
Azoospermia	54 (69.2)[b]	24 (30.8)[b]	2.36 (1.33–4.20)
Oligospermia	67 (65.0)[c]	36 (35.0)[c]	2.00 (1.17–3.27)

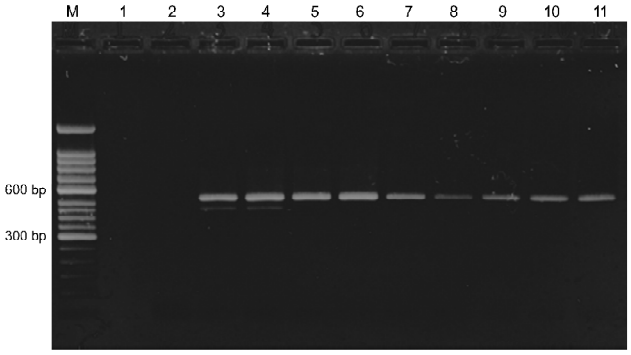

Figure 1. Electrophoresis pattern for null mutation of *GSTT1* by multiplex polymerase chain reaction (PCR). To detect the deletion in *GSTT1*, the gene was amplified using the primers (5'-TTC CTT ACT GGT CCT CAC ATC TC-3' and 5'-TCA CCG GAT CAT GGC CAG CA-3') and a 540-bp fragment of the *β-actin* gene was used as an internal standard using primes (5'-CGT ACT CCT GCT TGC TAA TCC ACA-3' and 5'-CGG GAC CTG ACC GAC TAC CTC A-3'). M: 50 bp DNA ladder (TaKaRa Bio, Japan); Lane 1: water; lane 2: no DNA plate PCR mix buffer; lane 3, 4: *GSTT1* (+) genotype; lane 5-11: *GSTT1* (–) genotype.

3 Results

3.1 The relevant characteristics of the subjects

The relevant characteristics of study subjects are shown in Table 1. No significant differences of age, history of drinking or smoking were observed between cases and controls.

3.2 Association of GSTT1 polymorphism with idiopathic azoospermia or idiopathic oligospermia

The frequencies of *GSTT1* genotype in the control group and the idiopathic azoospermia group or idiopathic oligospermia group are shown in Table 2. The frequencies of *GSTT1* null genotype were 69.2% in the idiopathic azoospermia group, 65.0% in the idiopathic oligospermia group and 48.7% in the control group. The associations between *GSTT1* null genotype with idiopathic azoospermia or idiopathic oligospermia are also shown in Table 2. There were significant associations between *GSTT1* null genotype and idiopathic azoospermia (OR: 2.36, 95% CI: 1.33–4.20, $P = 0.004$) or idiopathic oligospermia (OR 2.00, 95% CI 1.17–3.27, $P = 0.010$).

4 Discussion

With the development of society, more and more chemical substances are used in agriculture, industry and in our daily lives (pesticide, 1,3-butadiene, ethylene oxide, food additives and drugs). Such chemical substances and their metabolites are toxic to the body, especially to the genital system, as they can generate electrophiles. In addition, germinal tissue, especially sperm, has a high demand for respiratory energy, but during the process of oxidative phosphorylation, large quantities of reactive oxygen species (ROS) are generated, which is a main cause of lipid peroxidation, DNA and protein damages and apoptosis [13].

The GST superfamily represents a major group of detoxification and antioxidant enzymes. Their presumptive functions are to protect tissues against toxic compounds and ROS. The gene expressing GST enzymes are polymorphic, and might partially responsible for individual host susceptibility to oxidative stress damage, cancers and other diseases. *GSTT1*, a member of the

theta-class gene family, is polymorphic and the inherited deletion of which causes the phenotypic absence of *GSTT1* activities. Large ethnic differences in the prevalence of the homozygously-deleted genotype of *GSTT1* were observed. Chinese has a frequency of *GSTT1* null genotype of nearly 50% [10, 14] whereas in the Japanese, the frequency is 44.4%–50.0% [15, 16]. However, Indians have a lower frequency (14.5%–20.1%) [9, 17, 18] and the frequency of *GSTT1* null genotype in Caucasian is between 11.0% and 37.9% [19, 20]. Hence, there are significant ethnic differences in *GSTT1* polymorphism.

Previous studies have shown a possible relationship between *GSTT1* null genotype and susceptibility to many diseases [9–11]. However, to our knowledge, there are no studies on the role of *GSTT1* polymorphism in patients with idiopathic azoospermia or idiopathic oligospermia. In the present study, we investigated *GSTT1* gene polymorphism and potential association between *GSTT1* null genotype and idiopathic azoospermia or idiopathic oligospermia. The patients with idiopathic azoospermia or idiopathic oligospermia had a statistically higher prevalence of *GSTT1* null genotype than the controls, but the prevalence in the controls was consistent with that of previous studies [10, 14]. As shown in Table 2, there were significant associations between *GSTT1* null genotype with idiopathic azoospermia or idiopathic oligospermia. Therefore, *GSTT1* null genotype is a risk factor for idiopathic azoospermia or idiopathic oligospermia in the northwestern Chinese population. The reason for this phenomenon might be that people with *GSTT1* null genotype have decreased capabilities in detoxifying some carcinogens and oxygen metabolites. Although some of our data were statistically significant, larger studies are required to confirm the results of the present study.

In conclusion, our results suggest a relationship between *GSTT1* null genotype and increased risks for idiopathic azoospermia and idiopathic oligospermia, which means that *GSTT1* null genotype might play important roles in the pathogenesis of idiopathic azoospermia and idiopathic oligospermia.

References

1 Dohle GR, Colpi GM, Hargreave TB, Papp GK, Jungwirth A, Weidner W. The EAU Working Group on Male In fertility. EAU guidelines on male infertility. Eur Urol 2005; 48: 703–11.

2 Maduro MR, Lamb DJ. Understanding new genetics of male infertility. J Urol 2002; 168: 2197–205.

3 A ZC, Yang Y, Zhang SZ, Li N, Zhang W. Single nucleotide polymorphism C677T in the methylenetetrahydrofolate reductase gene might be a genetic risk factor for infertility for Chinese men with azoospermia or severe oligozoospermia. Asian J Androl 2007; 9: 57–62.

4 Oliva A, Spira A, Multigner L. Contribution of environmental factors to the risk of male infertility. Hum Reprod 2001; 16: 1768–76.

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

6 Strange RC, Jones PW, Fryer AA. Glutathione S-transferase: genetics and role in toxicology. Toxicol Lett 2000; 112: 357–63.

7 Nelson HH, Wiencke JK, Christiani DC, Cheng TJ, Zuo ZF, Schwartz BS, *et al*. Ethnic differences in the prevalence of the homozygous deleted genotype of glutathione S-transferase theta. Carcinogenesis 1995; 16: 1243–5.

8 Bruhn C, Brockmöller J, Kerb R, Roots I, Borchert HH. Concordance between enzyme activity and genotype of glutathione S-transferase theta (GSTT1). Biochem Pharmacol 1998; 56: 1189–93.

9 Sobti RC, Al-Badran AI, Sharma S, Sharma SK, Krishan A, Mohan H. Genetic polymorphisms of CYP2D6, GSTM1, and GSTT1 genes and bladder cancer risk in North India. Cancer Genet Cytogenet 2005; 156: 68–73.

10 Wang G, Zhang L, Li Q. Genetic polymorphisms of GSTT1, GSTM1, and NQO1 genes and diabetes mellitus risk in Chinese population. Bioche and Biophy Res Commu 2006; 341: 310–3.

11 Abu-Amero KK, Al-Boudari OM, Mohamed GH, Dzimiri N. T null and M null genotypes of the glutathione S-transferase gene are risk factor for CAD independent of smoking. BMC Med Genet 2006; 7: 38.

12 World Health Organization. WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction. 3rd edn. Cambridge: Cambridge University Press; 1992.

13 Kowaltowski AJ, Vercesi AE. Mitochondrial damage induced by conditions of oxidative stress. Free Radical Biol Med 1999; 26: 463–71.

14 Zhong SL, Zhou S, Chen X, Huang M. Rapid determination of common mutations in glutathione S-transferase gene by PCR-based methods in healthy Chinese. Clin Chim Acta 2006; 364: 205–8.

15 Kim H, Yang M, Matsumoto T, Kawamoto T, Katoh T, Inatomi H. Effects of glutathione S-transferase (GST) M1 and GSTT1 genotypes on urothelial cancer risk. Cancer Lett 1998; 132: 147–52.

16 Ueda M, Hung YC, Terai Y, Saito J, Nunobiki O, Noda S, *et al*. Glutathione-S-transferase and p53 polymorphisms in cervical carcinogenesis. Gynecol Oncol 2005; 96: 736–40.

17 Mittal RD, Manchanda PK, Bid HK, Ghoshal UC. Analysis of polymorphisms of tumor necrosis factor-alpha and polymorphic xenobiotic metabolizing enzymes in inflammatory

bowel disease: study from northern India. J Gastroenterol Hepatol 2007; 22: 920–4.
18 Srivastava DS, Mandhani A, Mittal B, Mittal RD. Genetic polymorphism of glutathione S-transferase genes (GSTM1, GSTT1 and GSTP1) and susceptibility to prostate cancer in Northern India. BJU Int 2005; 95: 170–3.
19 Steinhoff C, Franke KH, Golka K, Their R, Römer HC, Rötzel C, *et al*. Glutathione transferase isozyme genotypes in patients with prostate and bladder carcinoma. Arch Toxicol 2000; 74: 521–6.
20 Marinho C, Alho I, Arduíno D, Falcão LM, Brás-Nogueira J, Bicho M. GSTM1/T1 and MTHFR polymorphisms as risk factors for hypertension. Biochem Biophys Res Commun 2007; 353: 344–50.