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ORIGINAL ARTICLE

Male Infertility

Association of polymorphisms in glutathione S-transferase genes (*GSTM1*, *GSTT1*, *GSTP1*) with idiopathic azoospermia or oligospermia in Sichuan, China

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The reported effects of the glutathione S-transferase (GSTs) genes (*GSTM1*, *GSTT1*, and *GSTP1*) on male factor infertility have been inconsistent and even contradictory. Here, we conducted a case-control study to investigate the association between functionally important polymorphisms in GST genes and idiopathic male infertility. The study group consisted of 361 men with idiopathic azoospermia, 118 men with idiopathic oligospermia, and 234 age-matched healthy fertile male controls. Genomic DNA was extracted from the peripheral blood, and analyzed by polymerase chain reaction and restriction fragment length polymorphism analysis. There was a significant association between the *GSTP1* variant genotype (Ile/Val + Val/Val) with idiopathic infertility risk (odds ratio [OR]: 1.53; 95% confidence interval [CI]: 1.11–2.11; *P* = 0.009). Similarly, a higher risk of infertility was noted in individuals carrying a genotype combination of *GSTT1*-null and *GSTP1* (Ile/Val + Val/Val) (OR: 2.17; 95% CI: 1.43–3.31; *P* = 0.0002). These results suggest an increased risk of the *GSTP1* variant genotype (Ile/Val + Val/Val) for developing male factor infertility. Our findings also underrate the significance of the effect of *GSTM1* and/or *GSTT1* (especially the former) in modulating the risk of male infertility in males from Sichuan, southwest China.

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Keywords: genetic polymorphism; glutathione S-transferase; idiopathic infertility; male factor

INTRODUCTION

Infertility is a worldwide reproductive health problem affecting approximately 12%–15% of couples.[1] In about half of the cases, poor semen quality is a major cause.[2,3] Male infertility represents a typical example of complex disease with a substantial genetic basis.[4] The etiology and pathogenesis remain unknown in about 30% of male cases of infertility; this is termed "idiopathic infertility".[5] Over the past two decades, the quality and quantity of human semen have declined,[6] which has raised global concerns about male fertility.

Male germ cells are highly susceptible to stress;[7] therefore, an important way to ensure fertility is protection from oxidative stress. Glutathione S-transferases (GSTs; EC 2.5.1.18) belong to a superfamily of ubiquitous, multifunctional dimeric cytosolic enzymes that play a key role in the Phase II detoxification pathways in humans against various physiological and xenobiotic substances by catalyzing the conjugation of the nucleophilic tripeptide glutathione to a wide range of electrophilic substrates.[8] Hence, GSTs constitute the major defensive antioxidant system against oxidative stress by reducing reactive oxygen species, which are generated by many toxic xenobiotics.[9] In human, GSTs consist of many cytosolic, mitochondrial, and microsomal proteins. The cytosolic family has been categorized into eight separate classes – α (alpha), μ (mu), κ (kappa), ω (omega), π (pi), σ (sigma), θ (theta), and ζ (zeta) – encoded by the *GSTA*, *GSTM*, *GSTK*, *GSTO*, *GSTP*, *GSTS*, *GSTT*, and *GSTZ* genes, respectively.[10] Each class includes several genes and isoenzymes.[10] Functional polymorphisms have mainly been reported in the *GSTM1*, *GSTT1*, and *GSTP1* genes.[11–14]

In India, Tirumala Vani *et al.*[13] identified an association between the *GSTM1* null genotype and idiopathic male infertility. In the United States of America, Olshan *et al.*[15] reported that the *GSTT1* nonnull genotype was associated with reduced sperm concentration and count in semen. In Iran, Safarinejad *et al.*[14] revealed an increased risk of the *GSTM1* and/or *GSTT1* null genotypes for developing infertility with a protective effect conferred by the variant genotypes of *GSTP1*.

In China, Wu *et al.*[16] found that the *GSTT1* null genotype was a predisposing risk factor for sporadic idiopathic azoospermia or oligospermia. Tang *et al.*[17] revealed that *GSTM1* and *GSTT1* null genotypes may predispose sperm to increased oxidative damage in infertile men with varicoceles, while *GSTP1* allelic variation was associated with no difference between cases and controls.

Despite the intriguing results of the two abovementioned studies, there are few reports in China on the association between *GSTs* and idiopathic infertility. Therefore, we designed this case-control study to

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assess the risk of idiopathic male infertility associated with *GSTM1*, *GSTT1*, and *GSTP1* in Sichuan, China.

MATERIALS AND METHODS

Subjects

We carried out a case-control study of 479 male partners of infertile couples who showed an abnormal spermiogram (361 with nonobstructive azoospermia and 118 with oligospermia) and attended the Affiliate Hospital of Sichuan Genitalia Hygiene Research Center (Chengdu, Sichuan, China), from July 2010 to May 2013. All the men had an infertility history of at least 2 years with no indication of hormonal, medical, or surgical causes for their infertility; their spouses had a normal gynecological assessment. Exclusion criteria included a history of epididymo-orchitis, prostatitis, genital trauma and testicular tumors; genital disease such as cryptorchidism, congenital bilateral absence of the vas deferens or varicocele; seminal infections; diabetes; Y chromosome microdeletions, cytogenetic, or karyotype abnormalities; drug, alcohol, or substance abuse; and tobacco use. Semen analysis was performed according to World Health Organization recommendations.[18] Two hundred thirty-four fertile men of a comparable age who had fathered at least one child without assisted reproductive technologies were selected for the control group. All participants were informed about the study according to a protocol that was approved by the Institutional Ethical Review boards of Sichuan University (Chengdu, China), and all gave their written consent.

Genotyping

Genomic DNA was extracted from peripheral venous blood using an Ezup Column Blood Genomic DNA Purification Kit (Sangon Biotech, Shanghai, China) according to the manufacturer's instructions. Spectroscopy was performed to quantify the amount of extracted genomic DNA. DNA samples were stored at − 20°C.

Multiplex polymerase chain reaction (PCR) was performed to detect the *GSTM1* and *GSTT1* genes in a total volume of 25 μl buffered solution containing approximately 100 ng genomic DNA, 0.2 mmol l^{-1} dNTPs (TransGen, Beijing, China), 1.5 mmol l^{-1} Mg^{2+} (Fermentas International Inc., Burlington, ON, Canada), 4 × 10^8 pmol l^{-1} of each primer (Sangon Biotech, Shanghai, China) and 1 U Taq polymerase (Fermentas International Inc., Burlington, ON, Canada). The reaction mixture was heated at 95°C for 5 min, followed by 35 cycles of amplification as follows: a denaturing step at 94°C for 30 s, an annealing step at 60°C for 30 s, and an extension step at 72°C for 45 s. The final extension was at 72°C for 10 min. PCR samples were analyzed on a 2% agarose gel prepared in 1 × TAE buffer containing DuRed nucleic acid gel stain (Abgent Biotechnology Co., Ltd., Suzhou, China) run at 120 V for 35 min at room temperature (**Figure 1a**). To ensure high-quality data, each sample was tested 3 times and the gel photographs were interpreted by two independent readers. No discrepancies were discovered upon replicate testing.

The absence of a 273-bp band for *GSTM1* or a 480-bp band for *GSTT1*, with the presence of a 110-bp β-globin band, was recorded as deleted. This method did not permit detection of heterozygous carriers of *GSTM1* or *GSTT1* deletions, but it identified the null genotypes conclusively.

The *GSTP1* genotype was also determined by PCR-restriction fragment length polymorphism. A 433-bp fragment of the *GSPT1* gene containing an Ile-to-Val substitution in exon 5 (codon 105) was amplified using the primer pair: 5′-GTAGTTTGCCCAAGGTCAAG-3′ and 5′-AGCCACCTGAGGGGTAAG-3′. The reaction mixture and cycling conditions were as described above. The PCR products were then digested with Alw26I (Fermentas International Inc., Burlington, ON, Canada) at 37°C for 3 h in a total volume of 20 μl containing 10 μl PCR mixture, 2 μl buffer, and 1 U Alw26I. The digestion products were analyzed on a 2% agarose gel prepared in 1 × TAE buffer containing DuRed nucleic acid gel stain run at 110 V for 40 min at room temperature (**Figure 1b**).

Homozygous Ile/Ile individuals showed two fragments, of 328 bp and 105 bp. Homozygous Val/Val individuals also showed two bands (three fragments of 222 bp, 106 bp, and 105 bp; the latter two merged into one band). The presence of all three bands corresponded to heterozygous Ile/Val individuals. A random selection of 15% of Ile/Ile and Ile/Val samples, plus all the Val/Val samples, were retested by sequencing by Sangon Biotech (**Figure 2**); no discrepancies were found.

Statistical analysis

The χ^2-test was performed to compare genotype frequencies between groups. Odds ratios (ORs) and 95% confidence intervals (CI) were calculated to measure the risk associated with variant genotypes using the unconditional logistic regression method. Tests for Hardy–Weinberg equilibrium were also conducted by the χ^2-test. $P < 0.05$ was considered statistically significant. The data were analyzed using Statistical Package for Social Sciences software version 20.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Demographic and clinical characteristics of the subjects

Semen parameters in each study group are shown in **Table 1**. Mean age (± s.d.) was 30.8 ± 5.2 years among men with azoospermia (age range 25–40 years), 30.7 ± 5.5 years among men with oligospermia (age range 24–40 years), and 32.8 ± 5.7 years among controls (age range 25–42 years). No significant differences were observed between cases and controls with respect to age.

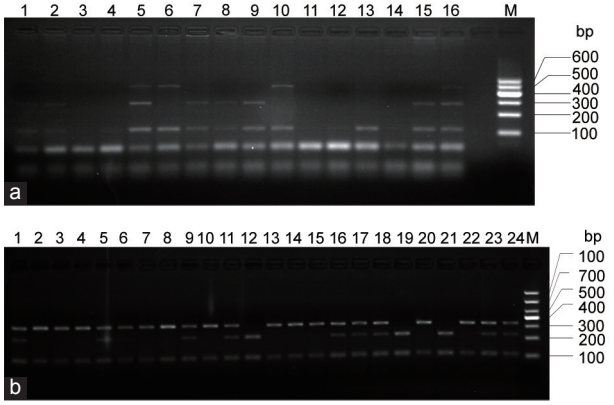

Figure 1: Primers for the *GSTM1* gene were: 5′-CTGCCCTACTTGATTGATGGG-3′ and 5′-CTGGATTGTAGCAGATCATGC-3′; those for *GSTT1* were: 5′-TTCCTTACTGGTCCTCACATCTC-3′ and 5′-TCACCGGATCATGGCCAGCA-3′; primers for human β-globin (110-bp amplicon), used as an internal control, were: 5′-ACACAACTGTGTTCACTAGC-3′ and 5′-CAACTTCATCCACGTTCACC-3′. All primers were manufactured by Sangon Biotech. (**a**) Detection of the *GSM1* and *GSTT1* alleles by multiplex polymerase chain reaction. M: 100 bp DNA ladder (Sangon Biotech, Shanghai, China); lanes 3, 4, 11, 12, 14: Even internal control is missing, these are to be retested; lane 5, 16: *GSTM1* present and *GSTT1* present; lanes 6, 10: *GSTT1* present; lanes 1, 2, 7–9, 15: *GSTM1* present; lane 13: *GSTM1* null and *GSTT1* null. (**b**) Detection of *GSTP1* by restriction fragment length polymorphism. M: DL 1,000 DNA Marker (Takara Biotechnology Co., Ltd., Dalian, China); lanes 1, 5, 9, 11, 16–18, 23, 24: *GSTP1* (Ile/Val); lanes 2–4, 6–8, 10, 13–15, 20, 22: *GSTP1* (Ile/Ile); lanes 12, 19, 21: *GSTP1* (Val/Val).

Association of single gene polymorphisms with male infertility

The frequency of the *GSTM1* null genotype showed almost no difference between the infertile men (51.6%) and the fertile controls (50.9%). Similarly, the *GSTT1* null genotype was not more prevalent in the infertile men (52.0% compared with 47.0% in controls). However, it was found to be more prevalent in people with oligospermia (OR: 1.59; 95% CI: 1.02–2.48; $P = 0.043$). The frequency of the Ile/Ile genotype (the wild-type) of *GSTP1* was found to be higher in controls (62.4%) than in the infertile men (52.0%). Because the frequency of the homozygous mutant (Val/Val) genotype of *GSTP1* was very rare in both the infertile cases (1.9%) and the controls (2.1%), the heterozygous and homozygous mutant genotypes were combined (Ile/Val + Val/Val) and are referred to as variant genotypes of *GSTP1*. The frequency of variant *GSTP1* was higher in cases (48.0%) than in controls (37.6%), showing a significant increased risk for infertility (OR: 1.53; 95% CI: 1.11–2.11; $P = 0.009$) (**Table 2**).

Table 1: Age and semen parameters of the study groups

	Azoospermia (n=361)	*Oligospermia (n=118)*	*Controls (n=234)*
Age (year)	30.8±5.2	30.7±5.5	32.8±5.7
Ejaculate volume (ml)	-	3.4±1.7	3.5±1.6
Total sperm/ejaculate	-	31.3±14.0	135.9±58.7
Sperm density	-	12.1±5.4	52.6±22.7
Motility	-	27.9±13.0	36.6±10.3

The data are expressed as mean±s.d. s.d.: standard deviation

Association of double gene combined polymorphisms with male infertility

When the genotypes of *GSTM1* and *GSTT1* were combined, three genetic combinations were studied: (1) both genotypes present; (2) either *GSTM1*-or *GSTT1*-present; and (3) both genotypes null. There was no significant association between the genotype combinations and infertility.

When combinations of *GSTM1* and *GSTP1* were studied, the frequency of the *GSTM1*-present and *GSTP1*-variant genotype was 114/479 (23.8%), which was higher than that in control subjects (42/234, 17.9%). Similarly, 28.8% of the people with idiopathic oligospermia and 19.7% of controls carried the combination genotype of *GSTM1*-null and *GSTP1*-variant. However, neither reached statistical significance.

Approximately 26% of the infertile people and 14% of the controls carried the combination genotype of *GSTT1*-null and *GSTP1*-variant,

Table 2: Distribution of GSTM1, GSTT1, and GSTP1 genotypes among infertile males and controls

Genotype and allele frequency		*Controls (n=234)*	*Azoospermia (n=361)*	*Oligospermia (n=118)*	*All cases (n=479)*
GSTM1					
Present	*n* (%)	115 (49.1%)	172 (47.6%)	60 (50.8%)	232 (48.4%)
Null	*n* (%)	119 (50.9%)	189 (52.3%)	58 (49.1%)	247 (51.6%)
	Odds ratio (95% CI)		1.06 (0.76–1.48)	0.93 (0.60–1.45)	1.03 (0.75–1.40)
	P		0.721	0.763	0.859
GSTT1					
Present	*n* (%)	124 (53.0%)	181 (50.1%)	49 (41.5%)	230 (48.0%)
Null	*n* (%)	110 (47.0%)	180 (49.9%)	69 (58.5%)	249 (52.0%)
	Odds ratio (95% CI)		1.12 (0.80–1.56)	1.59 (1.02–2.48)	1.22 (0.89–1.67)
	P		0.497	0.043*	0.213
GSTP1					
Ile/Ile	*n* (%)	146 (62.4%)	191 (52.9%)	58 (49.2%)	249 (52.0%)
Ile/Val	*n* (%)	83 (35.5%)	162 (44.9%)	59 (50.0%)	221 (46.1%)
	Odds ratio (95% CI)		1.48 (1.06–2.08)	1.82 (1.16–2.85)	1.56 (1.13–2.15)
	P		0.023*	0.009*	0.007*
Val/Val	*n* (%)	5 (2.1%)	8 (2.2%)	1 (0.8%)	9 (1.9%)
	Odds ratio (95% CI)		1.04 (0.34–3.21)	0.39 (0.04–3.39)	0.88 (0.29–2.65)
	P		0.948	0.378	0.816
Ile/Val and Val/Val	*n* (%)	88 (37.6%)	170 (47.1%)	60 (50.8%)	230 (48.0%)
	Odds ratio (95% CI)		1.48 (1.06–2.07)	1.72 (1.10–2.68)	1.53 (1.11–2.11)
	P		0.023*	0.018*	0.009*

*Statistically significant (P<0.05). CI: confidence interval; GST: glutathione S-transferase

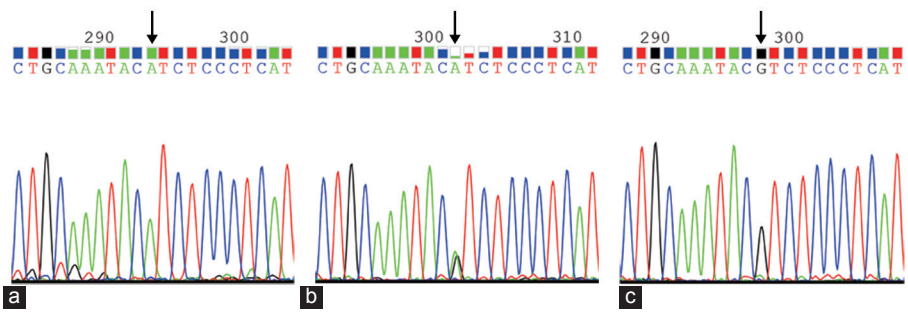

Figure 2: Validation by sequencing. (**a**) *GSTP1* (Ile/Ile); (**b**) *GSTP1* (Ile/Val); (**c**) *GSTP1* (Val/Val).

which conferred a significant increase in infertility risk (OR: 2.17; 95% CI: 1.43–3.31; $P = 0.0002$). Moreover, the risk was found to be further increased (approximately three-fold) in men with idiopathic oligospermia (OR: 3.12; 95% CI: 1.84–5.31; $P = 0.00002$) (**Table 3**).

Association of triple gene combined polymorphisms with male infertility

When the genotypes of three *GST* genes were combined, there were eight possible combinations. Individuals with the null genotypes for *GSTM1* and *GSTT1* and the variant *GSTP1* (Ile/Val + Val/Val) had a greatly increased risk of infertility (OR: 1.79; 95% CI: 1.07–3.00; $P = 0.024$). Individuals with the combination of *GSTM1*-present, *GSTT1*-null, and *GSTP1*-variant were also at higher risk of infertility (OR: 2.35; 95% CI: 1.23–4.49; $P = 0.008$). The other genotype combinations were not associated with significant infertility risk (**Table 4**).

DISCUSSION

In this case-control study, the *GSTP1* variant genotype (Ile/Val + Val/Val) was present at a much higher frequency in every group of infertile men than in the fertile control group. Meanwhile, the distribution of polymorphisms in the *GSTM1/T1* genes did not demonstrate significant differences between the groups. The frequency of the *GSTM1*-null genotype ranges from 42%–60% in Europeans, 42%–54% in Asians, and 16%–36% in Africans, while for the *GSTT1*-null genotype, the reported frequencies are 13%–26% in Caucasians and 35%–52% in Asians.[19] In Asians, the frequencies of the *GSTP1*-Ile/Ile,-Ile/Val, and-Val/Val genotypes are 52%–93%, 24%–44%, and 4%–5%, respectively.[20] In this study, we revealed that, in fertile controls, the frequency of the *GSTM1*-null genotype was 50.9%, the frequency of the *GSTT1*-null genotype was 47.0%, and the frequencies of the *GSTP1*-Ile/Ile,-Ile/Val, and-Val/Val genotypes were 62.4%, 35.5%, and 2.1%, respectively. The distribution of the *GSTP1* genotypes in the controls was in Hardy–Weinberg equilibrium.

Spermatogenesis is an orchestrated process that is regulated by many genes present on both the autosomal and sex chromosomes.[21] Many different mutations and polymorphisms may lead to male infertility.[22,23] Implicated polymorphisms include *CYP1A*,[24] *SOD2*, *NQO1*,[25] *PON1–55*,[26] *ER-α*, *ER-β*,[27] *GSTM1*, *T1*, and *P1* gene polymorphisms.[14] The GST system is one of the most important in protecting cells from oxidative damage; it works to inactivate endogenous unsaturated epoxides, aldehydes, hydroperoxides, and quinines[21,28,29] in conditions of oxidative stress.

It has been suggested that polymorphisms in *GSTM1*, *GSTT1*, and *GSTP1* are associated with male infertility.[13,14,16,17,30,31] Tirumala Vani

Table 3: Distribution of double GST genotypes among infertile males and controls

Double GST genotypes		*Controls (n=234)*	*Azoospermia (n=361)*	*Oligospermia (n=118)*	*All cases (n=479)*
GSTM1 and GSTT1					
Both present	*n* (%)	67 (28.6%)	94 (26.0%)	30 (25.4%)	124 (25.9%)
M1 present and T1 null	*n* (%)	48 (20.5%)	78 (21.6%)	30 (25.4%)	108 (22.5%)
	Odds ratio (95% CI)		1.07 (0.71–1.60)	1.32 (0.78–2.23)	1.27 (0.87–1.85)
	P		0.750	0.296	0.222
M1 null and T1 present	*n* (%)	57 (24.4%)	87 (24.1%)	19 (16.1%)	106 (22.1%)
	Odds ratio (95% CI)		0.99 (0.67–1.45)	0.60 (0.34–1.06)	0.88 (0.61–1.28)
	P		0.943	0.076	0.506
Both null	*n* (%)	62 (26.5%)	102 (28.3%)	39 (33.1%)	141 (26.5%)
	Odds ratio (95% CI)		1.09 (0.76–1.58)	1.37 (0.85–2.22)	1.16 (0.82–1.64)
	P		0.639	0.200	0.414
GSTM1 and GSTP1					
M1 present and P1 (Ile/Ile)	*n* (%)	73 (31.2%)	84 (23.3%)	34 (28.8%)	118 (24.6%)
M1 present and P1 (Ile/Val and Val/Val)	*n* (%)	42 (17.9%)	88 (24.4%)	26 (22.0%)	114 (23.8%)
	Odds ratio (95% CI)		1.47 (0.98–2.22)	1.29 (0.75–2.24)	1.43 (0.96–2.12)
	P		0.064	0.360	0.076
M1 null and P1 (Ile/Ile)	*n* (%)	73 (31.2%)	107 (29.6%)	24 (20.3%)	131 (27.3%)
	Odds ratio (95% CI)		0.93 (0.65–1.33)	0.56 (0.33–0.95)	0.83 (0.59–1.17)
	P		0.687	0.032*	0.286
M1 null and P1 (Ile/Val and Val/Val)	*n* (%)	46 (19.7%)	82 (22.7%)	34 (28.8%)	116 (24.2%)
	Odds ratio (95% CI)		1.20 (0.80–1.80)	1.65 (0.99–2.76)	1.31 (0.89–1.92)
	P		0.376	0.053	0.173
GSTT1 and GSTP1					
T1 present and P1 (Ile/Ile)	*n* (%)	69 (29.5%)	97 (26.9%)	29 (24.6%)	126 (26.3%)
T1 present and P1 (Ile/Val and Val/Val)	*n* (%)	55 (23.5%)	84 (23.3%)	20 (16.9%)	104 (21.7%)
	Odds ratio (95% CI)		0.99 (0.67–1.46)	0.66 (0.38–1.17)	0.90 (0.62–1.31)
	P		0.947	0.157	0.590
T1 null and P1 (Ile/Ile)	*n* (%)	77 (32.9%)	94 (26.0%)	29 (24.6%)	123 (25.7%)
	Odds ratio (95% CI)		0.72 (0.50–1.03)	0.66 (0.40–1.10)	0.70 (0.50–0.99)
	P		0.071	0.108	0.044*
T1 null and P1 (Ile/Val and Val/Val)	*n* (%)	33 (14.1%)	86 (23.8%)	40 (33.9%)	126 (26.3%)
	Odds ratio (95% CI)		1.90 (1.23–2.96)	3.12 (1.84–5.31)	2.17 (1.43–3.31)
	P		0.004*	<0.001*	<0.001*

*Statistically significant (P<0.05). CI: confidence interval; GST: glutathione S-transferase

Table 4: Distribution of triple GST genotypes among infertile males and controls

Triple GST genotypes		*Controls (n=234)*	*Azoospermia (n=361)*	*Oligospermia (n=118)*	*All cases (n=479)*
M1 and T1 present and P1 (Ile/Ile)	*n* (%)	37 (15.8%)	44 (12.2%)	20 (16.9%)	64 (13.4%)
M1 and T1 present and P1 (Ile/Val and Val/Val)	*n* (%)	30 (12.8%)	50 (13.9%)	10 (8.5%)	60 (12.5%)
	Odds ratio (95% CI)		1.09 (0.67–1.78)	0.63 (0.30–1.34)	0.97 (0.61–1.56)
	P		0.719	0.226	0.912
M1 null, T1 present and P1 (Ile/Ile)	*n* (%)	32 (13.7%)	53 (14.7%)	9 (7.6%)	62 (12.9%)
	Odds ratio (95% CI)		1.09 (0.68–1.74)	0.52 (0.24–1.13)	0.94 (0.59–1.48)
	P		0.732	0.095	0.786
M1 null, T1 present and P1 (Ile/Val and Val/Val)	*n* (%)	25 (10.7%)	34 (9.4%)	10 (8.5%)	44 (9.2%)
	Odds ratio (95% CI)		0.87 (0.50–1.50)	0.77 (0.36–1.67)	0.85 (0.50–1.42)
	P		0.614	0.514	0.526
M1 present, T1 null and P1 (Ile/Ile)	*n* (%)	36 (15.4%)	40 (11.1%)	14 (11.9%)	54 (11.3%)
	Odds ratio (95% CI)		0.68 (0.42–1.11)	0.74 (0.38–1.43)	0.70 (0.44–1.10)
	P		0.125	0.372	0.121
M1 present, T1 null and P1 (Ile/Val and Val/Val)	*n* (%)	12 (5.1%)	38 (10.5%)	16 (13.6%)	54 (11.3%)
	Odds ratio (95% CI)		2.18 (1.11–4.26)	2.90 (1.32–6.36)	2.35 (1.23–4.49)
	P		0.021*	0.006*	0.008*
M1 null, T1 null and P1 (Ile/Ile)	*n* (%)	41 (17.5%)	54 (15.0%)	15 (12.7%)	69 (14.4%)
	Odds ratio (95% CI)		0.83 (0.53–1.29)	0.69 (0.36–1.30)	0.79 (0.52–1.21)
	P		0.405	0.245	0.280
M1 null, T1 null and P1 (Ile/Val and Val/Val)	*n* (%)	21 (9.0%)	48 (13.3%)	24 (20.3%)	72 (15.0%)
	Odds ratio (95% CI)		1.56 (0.90–2.67)	2.59 (1.37–4.88)	1.79 (1.07–3.00)
	P		0.108	0.003*	0.024*

*Statistically significant (P<0.05). CI: confidence interval; GST: glutathione S-transferase

et al.[13] concluded that the frequency of the *GSTM1*-null genotype is of great significance to idiopathic male infertility. Wu *et al.*[16] revealed that the *GSTT1*-null genotype predisposes to excess oxidative damage to the spermatocytes of infertile males with varicocele. A similar increase in infertility risk was also reported in Iranian[14] and Brazilian[32] populations. In contrast, one study in the United States reported an association between the *GSTT1*-nonnull genotype and a reduction in sperm concentration and count.[15] Another study, in Russian men with idiopathic infertility, suggested increased infertility with the nondeletion genotype of the *GSTT1* gene.[33] In Iran, Safarinejad *et al.*[14] found that the variant genotypes of *GSTP1* (Ile/Val + Val/Val) resulted in a significant decreased risk of infertility. In China, Tang *et al.*[17] found that *GSPT1* allelic variation showed barely any difference between the infertile and control groups. One study in Iran by Lakpour's group revealed that all individuals in both the oligoasthenoteratozoospermia and normozoospermia groups had the same *GSTP1* (Ile/Ile) genotype, indicating no significant association between *GSTP1* and sperm parameters.[31] The discrepancies among these studies could be explained by factors such as different ethnic backgrounds, study populations, sample collection methods, and study sample sizes.

In a review, Safarinejad *et al.*[34] suggested that the *GSTM1-and GSTT1*-null genotypes are associated with a strong and modest increase in the risk of male infertility, respectively, while the *GSTP1* Ile/Val genotype has a protective effect. In complete contrast, our findings underrated the effect of the null genetic variants of *GSTM1* and *GSTT1* in modulating the risk of male infertility, and suggested, for the first time, that the *GSTP1* variant genotype (Ile/Val + Val/Val) may contribute to the development of infertility. In investigating the combined effect of the GST genes, we found that *GSTT1*-null and *GSTP1* (Ile/Val + Val/Val) have a certain degree of synergism, resulting in a greatly increased risk of infertility; this was not the case for the *GSTM1*-null. Besides, the presence or absence of *GSTM1* had barely any effect when the three GST genes were studied together. The most likely explanation is based on the different biological mechanisms of the three different GST genes. It has been reported that *GSTM1* and *GSTT1* polymorphisms could cause disparities in enzyme activities, and *GSTM1*-null/*GSTT1*-null individuals have a complete absence of activity of these enzymes.[35] However, one study in India revealed that the absence of *GSTM1* activity can be compensated for by the overexpression of *GSTM2*.[36] Another study reported that the induction or repression of *GSTT1* on the sperm surface resulted in decreased sperm motility.[25] *GSTP1*, unlike the former two, did not simply affect GSH metabolism, but interacted with Jun kinase to inhibit apoptosis and promote cellular proliferation.[37,38] By stratification in the infertility group, we demonstrated that individuals with infertility-risk genotypes were more likely to have oligospermia than azoospermia. This is consistent with a study conducted by Tang *et al.*[39] We speculate that protein defects in the GST family alone might be insufficient to cause azoospermia. This is because the major role of the enzymes encoded by *GSTs* is in the mitigation of reactive oxygen species; therefore, these enzymes are more likely to affect the quantity, survival, and/or activity of sperm that have been produced.

We should acknowledge several limitations of this case-control study. First, the relatively small sample size in the oligospermia group and in the controls would substantially decrease the statistical power to explore real associations. Second, this study did not contain complete information about family history of infertility. Third, genetic association studies are prone to population-specific genotype effects. Finally, we did not address gene-gene or gene-environment interactions.

CONCLUSION

In Sichuan, southwest China, males with the variant genotypes of *GSTP1* (Ile/Val or Val/Val) have an increased risk of infertility.

In addition, we have underrated the significance of the effect of *GSTM1* and/or *GSTT1* (especially *GSTM1*) in modulating the risk of male infertility. Further studies should be conducted on a larger scale to confirm our results, and functional studies undertaken to explore the effect of GST variants.

AUTHOR CONTRIBUTIONS

DKX and HHC designed the study, acquired the data, interpreted and analyzed the data, and drafted and revised the manuscript. XPD supervised this study. SHZ and JHZ collected some of the clinical data and acquired some of the data. All authors read and approved the final manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

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