Association of GSTM1 and GSTT1 Genes with the Susceptibility to Male Infertility: Result from a Meta-Analysis

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The deletion polymorphisms of the glutathione S-transferase M1 (GSTM1) and glutathione S-transferase T1 (GSTT1) genes were considered as candidates for genetic susceptibility factors of male infertility. Previous studies concerning the relationship between the null genotype of the two genes and male infertility have been reported in recent years. However, the results remain elusive. A meta-analysis was performed to estimate the relationship between the deletion polymorphism of the GSTM1 or GSTT1 gene, and male infertility in this study. Sixteen studies concerning the GSTM1 gene, including 2174 cases and 1861 controls, and 13 case-control studies on the GSTT1 gene with a total number of 1992 cases and 1617 controls were processed. The results showed that the null genotype of the GSTM1 gene was associated with male infertility in the overall populations ($P=0.003$, $OR=1.40$, $95\%CI=1.12-1.75$), especially in Caucasian ($P=0.012$, $OR=1.50$, $95\%CI=1.09-2.07$) as well as Chinese ($P=0.001$, $OR=1.55$, $95\%CI=1.19-2.03$). The null genotype of the GSTT1 gene was strongly related to male infertility only in Chinese ($P=0.000$, $OR=1.70$, $95\%CI=1.34-2.14$). These results indicated that the null genotype of the GSTM1 gene might contribute to the susceptibility of male infertility, whereas the null genotype of the GSTT1 gene may be a genetic susceptibility factor of male infertility for the Chinese.

Introduction

MALE INFERTILITY IS A WORLDWIDE polygenic complicated disease, and accounted for approximately half of the infertile couples (Krausz, 2011). Genetic deficiency, infection, varicocele, immunological problems, and environmental factors could affect sperm quality or function (Irvine, 1998; Krausz, 2011). Xenobiotics and endogenous reactive oxygen species (ROS) are the major factors leading to oxidative stress (OS) in the male testis tissue. Since male germ cells are particularly sensitive to OS, which could contribute to testicular and sperm dysfunctions as well as toxicity to spermatogenesis (Aitken and Krausz, 2001; Agarwal and Sushil, 2005; Tremellen, 2008; Turner and Lysiak, 2008), OS could lead to such biological effects as the acceleration of spermatozoa apoptosis (Aitken *et al.*, 2012), abnormality of sperm quality parameters (Badade *et al.*, 2011), decrease of sperm and oocyte fusion capacity (Griveau and Le Lannou, 1997), and damage of DNA integrity in both sperm mitochondrial and nuclear genomes (Aitken *et al.*, 1998). Hence, OS is regarded as a common etiology of male infertility (Tremellen, 2008). OS is caused by disequilibrium of high ROS concentrations and low levels of antioxidants. The antioxidants can remove the excess ROS, and then weaken or prevent the

toxicity of OS in the process of spermatogenesis (Schuppe *et al.*, 2000; Agarwal *et al.*, 2006). In other words, antioxidants play an important protective role on male germ cells against OS.

GSTA, GSTM, GSTT, and GSTP are the glutathione S-transferase (GST) family and act as important antioxidants in testis tissues (Listowsky *et al.*, 1998; Strange *et al.*, 2001). They are extensively present in the testis and seminiferous tubule fluid as well as in the sperm (Hemachand *et al.*, 2002; Mukherjee *et al.*, 1999), and can protect Leydig cells, Sertoli cells, and germ cells against the damage induced by OS (Castellon, 1999), which suggests that GSTs might be important for male reproduction and might be involved in spermatogenesis impairment.

GSTM1 and GSTT1 are two major members of the GST gene family, located on chromosome 1p13.3 and 22q11, respectively. They show deletion polymorphisms in general populations. The homozygous deletion (null genotype) of the GSTM1 or GSTT1 gene results in the total absence of the enzyme activity and increases the level of OS (Seidegard *et al.*, 1988), which suggests that deletion polymorphisms of the two genes might be related to male infertility. In this study, we conducted a meta-analysis to illustrate the possible role of the GSTM1 and GSTT1 gene in male infertility.

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Materials and Methods

The study retrieval was conducted in PUBMED, EMBASE, and CNKI databases using expression of "GSTM1 or GSTT1 and spermatogenesis," "GSTM1 or GSTT1 and infertility," "GSTM1 or GSTT1 and azoospermia or oligozoospermia" dating up to October of 2012. Meanwhile, manual searching of the relative studies was performed to gain the substantial data of GSTM1 or GSTT1 deletion polymorphism and male infertility.

The resulting reports were filtered using the following inclusion criteria: (1) A case-control study of the GSTM1 or GSTT1 polymorphism and male infertility; (2) case and control samples were identified according to WHO guidelines (World Health Organization, 1992, 1999, 2010); (3) studies provided detailed data of genotypes. Non case-control studies, reviews, comments, meta-analysis, and studies with low-level quality scores or duplicate data were excluded. The following data were extracted from the selected studies: the first author and year of publication, country, ethnicity, participants, and frequency of each genotype in case and control groups, genotyping method, *P*-value, odds ratio (OR) and 95% confidence interval (95% CI).

Thirty items related to the STROBE quality score systems (Vandenbroucke *et al.*, 2007) were used to assess the quality score of each eligible study. The scores ranged from 0 to 30 and values of 10, 20, and 30 were considered as low, moderate, and high level of quality, respectively. Two independent investigators searched, identified the studies, extracted the data, and assessed the quality score of articles included. Any assessment result with disagreement was submitted to discussion and a subsequent second assessment by a third investigator.

Meta-analysis was carried out using RevMan5.1.7 (v.5.1.7, Oxford, England) and Stata software (v.12.0; College Station, TX). The overall effect was established by the Z test and the significant difference was considered with a *P*-value lower than 0.05. The OR and 95%CI were used to estimate the strength of the correlation between the null genotype of GSTM1 or GSTT1 and male infertility. Each eligible study was weighted by a sample size. The heterogeneity test was performed using the Q test and estimated I^2 (Higgins *et al.*, 2003), while $P < 0.1$ was considered as a significant difference. The fixed model (Mantel-Haenszel method) was used when the *P*-value of the Q test was more than 0.1 (Mantel and Haenszel, 1959), otherwise, the random model (DerSimonian and Laird, 1986). Sensitivity analysis was performed to assess the stability of meta-analysis by omitting the eligible studies successively and changing the regression model. The Begg's funnel plot and Egger's test were used to evaluate the possible publication bias and $P < 0.05$ was considered as the existence of publication bias (Begg and Mazumdar, 1994; Egger *et al.*, 1997).

Results

Fifty-three articles have been extracted from the databases described above. According to inclusion and exclusion criteria, the following studies were excluded from this study: 28 unrelated to human infertility studies, 1 case group article, 1 review, 1 comment, 2 meta-analysis studies, and 2 duplicate data articles. As a result, 16 and 13 original studies were re-

tained to assess the correlation between the null genotype of GSTM1 or GSTT1 and male infertility, respectively. The detailed flow of retrieval and identification is shown in Figure 1. The baseline characteristics and detailed genotype data of each eligible article are listed in Tables 1 and 2, respectively.

The results of the heterogeneity test and meta-analysis for the overall and subgroups are summarized in Table 3. As shown in Table 3, the results for the Q test of the GSTM1 and GSTT1 gene in the overall population were the same (*P*-value for Q test=0.001); thus, the random model was chosen to evaluate the overall effects. As a result, the null genotype of GSTM1 was associated with male infertility ($P=0.003$, OR=1.40, 95%CI=1.12–1.75), but the null genotype of GSTT1 was not related to male infertility ($P=0.39$, OR=1.16, 95%CI=0.83–1.62) (Fig. 2).

After stratifying the ethnicity into Caucasian, Asian, and Chinese subgroups, the *P*-values of the Q test for the polymorphism of GSTM1 in each subgroup were 0.005, 0.018, and 0.470, respectively. So the fixed model was used for Chinese, and the random model was applied for other populations to evaluate the overall effects. The null genotype of GSTM1 in Caucasians ($P=0.012$, OR=1.50, 95%CI=1.09–2.07) and Chinese ($P=0.001$, OR=1.55, 95%CI=1.19–2.03) were associated with male infertility, but not in Asians ($P=0.110$, OR=1.30, 95%CI=0.95–1.78). The *P*-values of the Q test for the polymorphism of GSTT1 in Caucasians, Asians, and Chinese were 0.001, 0.004, and 0.308, respectively; thus, the fixed model was chosen to estimate the overall effect in Chinese, while the

FIG. 1. The flowchart of retrieval and identification for eligible studies.

TABLE 1. BASELINE CHARACTERISTICS OF EACH ELIGIBLE STUDY

Author and year	Region	Ethnicity	Case group	Control group	Detecting assay	Quality score
Chen <i>et al.</i> (2002)	China	Asian	58 varicocele infertile patients 38 subclinical varicocele infertile patients	106 fertile men	Multiplex PCR	20
Paracchini <i>et al.</i> (2006)	Italy	Caucasian	57 infertile patients	45 fertile men	Multiplex PCR	24
Dhillon <i>et al.</i> (2007)	India	Asian	179 oligoasthenoteratozoospermia infertile patients	200 fertile men	Multiplex PCR	20
Aydemir <i>et al.</i> (2007)	Turkey	Caucasian	52 idiopathic infertile patients	60 fertile men	Multiplex PCR	20
Wu <i>et al.</i> (2008)	China	Asian	78 azoospermia patients 103 oligozoospermia infertile patients	156 fertile men	Multiplex PCR	24
Wu <i>et al.</i> (2009)	China	Asian	63 varicocele infertile patients	54 fertile men	Multiplex PCR	22
Aydos <i>et al.</i> (2009)	Turkey	Caucasian	110 idiopathic infertile patients	105 fertile men	Multiplex PCR	23
Ichioka <i>et al.</i> (2009)	Japan	Asian	72 infertile varicocele patients 202 infertile patients without varicocele	101 fertile men	Multiplex PCR	21
Đorđević <i>et al.</i> (2010)	Serbia	Caucasian	52 infertile patients	56 fertile men	Multiplex PCR	21
Polonikov <i>et al.</i> (2010)	Russia	Caucasian	203 idiopathic infertile patients	227 fertile men	Multiplex PCR	20
Tirumala <i>et al.</i> (2010)	India	Asian	42 infertile patients	43 fertile men	Multiplex PCR	22
Safarinejad <i>et al.</i> (2010)	Iran	Caucasian	166 oligoasthenoteratozoospermia infertile patients	166 fertile men	Multiplex PCR	24
Chen <i>et al.</i> (2010)	China	Asian	75 oligozoospermia patients	36 fertile men	Multiplex PCR	21
Volk <i>et al.</i> (2011)	Slovenia	Caucasian	86 idiopathic azoospermia patients 101 oligoasthenoteratozoospermia infertile patients	194 fertile men	Multiplex PCR	24
Tang <i>et al.</i> (2012)	China	Asian	65 varicocele infertile patients	30 fertile men	Multiplex PCR	22
Jaiswal <i>et al.</i> (2012)	India	Asian	113 idiopathic azoospermia patients	91 fertile men	Multiplex PCR	22
Salehi <i>et al.</i> (2012)	Iran	Caucasian	150 idiopathic infertile patients	200 fertile men	Multiplex PCR	21
Xu <i>et al.</i> (2012)	China	Asian	151 idiopathic azoospermia patients 202 oligozoospermia infertile patients	201 fertile men	Multiplex PCR	23

PCR, polymerase chain reaction.

random model was used in other groups. The null genotype of GSTT1 in Caucasians ($P=0.860$, $OR=1.10$, $95\%CI=0.87-1.40$) and Asians ($P=0.380$, $OR=1.20$, $95\%CI=0.80-1.78$) was not associated with male infertility, while a strong association was detected in Chinese ($P=0.000$, $OR=1.70$, $95\%CI=1.34-2.14$).

Sensitivity analysis was conducted to evaluate the influence of each eligible study and changing the regression model on the pooled OR and the overall effect. The results are shown in Table 3. After omitting any individual study or altering the regression model, the pooled OR and P -value for the overall effect of the null genotype of GSTM1 did not significantly change in the overall, Caucasian and Chinese populations, and similar results for the null genotype of GSTT1 were obtained in the Caucasian and Chinese populations, suggesting that the results of meta-analysis for GSTM1 and GSTT1 were stable in these groups.

The analysis of publication bias shows that the shapes of the Begg's funnel plot were symmetric in each group for GSTM1 and each subgroup for GSTT1 (Fig. 3). However, the asymmetrical shape of a Begg's funnel plot was only found in the overall population for GSTT1 (Fig. 3), and the P -value of the Egger's test (Table 3) was lower than 0.05, suggesting that

the publication bias only existed in the overall population for GSTT1.

Discussion

In human testis, ROS is involved in the pathogenesis of male reproductive processes by inducing OS that can damage male germ cellular lipids, protein, and DNA (Aitken *et al.*, 1998; Irvine, 1998; Tremellen, 2008). Antioxidants, such as GSTM1 and GSTT1, can reduce the toxic effects of OS on male germ lines, suggesting that these two genes may play a protective role against OS in spermatogenesis. The deletion polymorphisms of GSTM1 and GSTT1 are therefore considered as potential factors leading to infertility in male humans. Recently, some case-control studies paid more attention to the association between the polymorphism of GSTT1 or GSTM1 and male infertility, but results from these studies had no cues to confirm it. Thus, whether the polymorphism of GSTT1 or GSTM1 is a genetic factor susceptible to cause male infertility remains unclear.

In this study, the possible association between the deletion polymorphisms of GSTM1 and GSTT1 and male infertility was investigated with a meta-analysis. The results showed

TABLE 2. THE GENOTYPE DISTRIBUTIONS AND RISK GENOTYPE FREQUENCY OF ELIGIBLE STUDIES

Author and year	Case group		Control group		Case group		Control group		P-value*	
	GSTM1(-)	GSTM1(+)	GSTM1(-)	GSTM1(+)	GSTT1(-)	GSTT1(+)	GSTT1(-)	GSTT1(+)	[1]	[2]
Chen <i>et al.</i> (2002)	46	50	42	64	—	—	—	—	0.235	—
Paracchini <i>et al.</i> (2006)	32	25	29	16	—	—	—	—	0.396	—
Dhillon <i>et al.</i> (2007)	59	120	76	124	—	—	—	—	0.306	—
Aydemir <i>et al.</i> (2007)	27	25	28	32	—	—	—	—	0.579	—
Wu <i>et al.</i> (2008)	—	—	—	—	121	60	76	80	—	0.001
Wu <i>et al.</i> (2009)	—	—	—	—	32	31	23	31	—	0.376
Aydos <i>et al.</i> (2009)	59	51	42	63	20	90	20	85	0.045	0.870
Ichioaka <i>et al.</i> (2009)	159	115	53	48	126	148	51	50	0.336	0.438
Đorđević <i>et al.</i> (2010)	29	23	27	29	20	32	15	41	0.432	0.195
Polonikov <i>et al.</i> (2010)	114	89	120	107	1	202	29	198	0.494	0.001
Tirumala <i>et al.</i> (2010)	19	23	9	34	—	—	—	—	0.017	—
Safarinejad <i>et al.</i> (2010)	73	93	46	120	47	119	32	134	0.002	0.053
Chen <i>et al.</i> (2010)	44	31	12	24	48	27	21	15	0.012	0.564
Volk <i>et al.</i> (2011)	97	90	92	102	35	152	46	148	0.385	0.234
Tang <i>et al.</i> (2012)	31	34	13	17	29	36	15	15	0.692	0.625
Jaiswal <i>et al.</i> (2012)	29	84	31	60	6	107	12	79	0.190	0.049
Salehi <i>et al.</i> (2012)	92	58	66	134	51	99	34	166	0.001	0.001
Xu <i>et al.</i> (2012)	238	115	116	85	218	135	94	107	0.022	0.001

*[1]: GSTM1 null versus GSTM1 present genotype by the χ^2 test; [2]: GSTT1 null versus GSTT1 present genotype by the χ^2 test.
GST, glutathione S-transferase.

that the null genotype of GSTM1 is associated with male infertility ($P=0.003$, OR=1.40, 95%CI=1.12–1.75), especially in Caucasian ($P=0.012$, OR=1.50, 95%CI=1.09–2.07) and Chinese ($P=0.001$, OR=1.55, 95%CI=1.19–2.03), indicating that the null genotype of GSTM1 could increase the risk of male infertility. Whereas the association of the null genotype of GSTT1 with male infertility was detected only in Chinese

($P=0.000$, OR=1.70, 95%CI=1.34–2.14), which suggests that the null genotype of GSTT1 might play an important role in male infertility in Chinese.

The result about GSTT1 in the Chinese population in this study is contrary to the earlier results stating that the null genotype of GSTT1 is not associated with male infertility in other populations (Economopoulos *et al.*, 2010; Kan *et al.*,

TABLE 3. THE RESULTS OF META-ANALYSIS FOR GSTM1 OR GSTT1 NULL GENOTYPE AND MALE INFERTILITY

Comparison	Ethnicity	P-value*			OR and 95%CI	
		[1]	[2]	[3]	The fixed model	The random model
GSTM1 null vs. GSTM1 present	Overall	0.000	0.003	0.848	1.39 (1.23–1.59)	1.40 (1.12–1.75)
	Caucasian	0.005	0.012	0.593	1.55 (1.30–1.84)	1.50 (1.09–2.07)
	Asian	0.018	0.110	0.406	1.24 (1.03–1.50)	1.30 (0.95–1.78)
	Chinese	0.470	0.001	0.711	1.55 (1.19–2.03)	1.56 (1.19–2.04)
GSTT1 null vs. GSTT1 present	Overall	0.001	0.39	0.024	1.25 (1.07–2.04)	1.16 (0.83–1.62)
	Caucasian	0.001	0.860	0.190	1.06 (0.56–2.02)	1.10 (0.87–1.40)
	Asian	0.004	0.380	0.124	1.37 (1.12–1.68)	1.20 (0.80–1.78)
	Chinese	0.308	0.000	0.068	1.70 (1.34–2.14)	1.65 (1.26–2.17)

*[1]: P-value for the Q test; [2]: P-value for meta-analysis; [3]: P-value for the Egger's test.
OR, odds ratio; CI, confidence interval.

FIG. 2. The results of meta-analysis in the overall population. (A) GSTM1 null versus GSTM1 present genotype; (B) GSTT1 null versus GSTT1 present genotype. GST, glutathione S-transferase.

2012; Li *et al.*, 2012). This contradiction suggests that the effect of the null genotype of GSTT1 on the susceptibility to male infertility may vary in different populations. One possible cause for this discrepancy could be the difference of the genetic background, in different ethnic populations, which may modify the effect of the null genotype of GSTT1 on male infertility. Moreover, the environmental factors may be another potential reason for the divergence. 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 (Ebisch *et al.*, 2003; Rubes *et al.*, 2007; Axelsson *et al.*, 2010). 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.

This study is more reliable than previous meta-analysis studies because it included the latest published articles and a larger sample size. The results strongly support the findings that the null genotype of GSTM1 could increase the risk of male

infertility reported in previous meta-analysis studies (Economopoulos *et al.*, 2010; Chengyong *et al.*, 2012). In addition, this study is the first one to reveal that the null genotype of GSTT1 is associated with male infertility in Chinese. However, several limitations of this study should be addressed. First, we searched the eligible studies only in PUBMED, EMBASE, and CNKI databases with the English and Chinese language, which might miss some other relevant case-control articles, and might subsequently lead to selection bias. Second, although the sample size in our study was the largest by now, it was not large enough for a comprehensive meta-analysis, especially in ethnic subgroups. Third, GSTM1 and GSTT1 genes strongly interact with environmental factors (Rubes *et al.*, 2007; Vichi *et al.*, 2012), but environmental factors were not taken into account in most of the cases included in this study. Thus, our findings were not based on adjusted OR to estimate. Finally, GSTM1 and GSTT1 were found to interact with other genes, such as MTHFR (Paracchini *et al.*, 2006; Dhillon *et al.*, 2007; Dordevic *et al.*, 2010), CYP1A1 (Aydos *et al.*, 2009), and ABCB1

FIG. 3. The Begg's funnel plot of male infertility risk associated with GSTM1 or GSTT1 deleted polymorphism. (A) GSTM1 null versus GSTM1 present genotype in the overall population; (B) GSTM1 null versus GSTM1 present in Chinese; (C) GSTT1 null versus GSTT1 present in the overall population; (D) GSTT1 null versus GSTT1 present in Chinese.

(Sam *et al.*, 2010), but only a few eligible articles included the gene–gene interaction. As a result, there is no substantial data to estimate the interaction in the meta-analysis study.

In conclusion, the meta-analysis of this study demonstrated that the null genotype of GSTM1 might be a potential factor provoking male infertility in the Caucasian and the Chinese subpopulations, while null genotype of GSTT1 was related to male infertility only in the Chinese population. Considering the limitations of this study, further well-designed epidemiological studies are strongly recommended to validate the role of GSTM1 and GSTT1 genes on male infertility.

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