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Glutathione S-transferase polymorphisms in varicocele patients: a meta-analysis

B. Zhu[1], L. Yin[2,3] and J.Y. Zhang[1]

[1]Biochemistry Department, Luohe Medical College, Luohe, Henan Province, China
[2]Colllege of Life Science, Henan Normal University, Xinxiang, Henan Province, China
[3]Biochemistry Department, Luohe Medical College, Luohe, Henan Province, China

Corresponding author: B. Zhu
E-mail: baoan_zhu@126.com

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ABSTRACT. The glutathione S-transferase (GST) family represents a major group of detoxification and antioxidant enzymes. Studies have shown that high oxidative stress levels are associated with varicocele. The objective of this study was to assess the relationship between *GSTM1* and *GSTT1* null polymorphisms and varicocele using a study group of 497 varicocele patients and 476 control subjects. A systematic literature search (for articles published up to September 2014) utilizing Google Scholar and PubMed was conducted. The chi-square-based *Q* test and I^2 index were used to evaluate data from retrieved studies. The possible publication bias was evaluated by Begg funnel plot and the Egger test. No statistically significant association was found between *GSTM1* or *GSTT1* null genotypes and varicocele in the overall data analysis. In a subgroup analysis, only the null *GSTM1* genotype was observed at a significantly higher frequency in Caucasian varicocele patients. In the Chinese subgroup, no association

was established between the *GSTM1* and *GSTT1* null genotypes and this condition. More attention should be drawn to oxidative stress-related pathological manifestations for Caucasian varicocele patients.

Key words: *GSTM1*; *GSTT1*; Meta-analysis; Polymorphism; Varicocele

INTRODUCTION

Varicoceles (dilations of the pampiniform venous plexus) are found in approximately 15% of the general adult male population, a figure that increases to 35% for men presenting with primary infertility, and 81% for those with secondary infertility (Gorelick and Goldstein, 1993). The exact pathophysiology of varicocele remains unknown, but the most widely accepted concept is that it leads to elevated testicular temperature, which has a deleterious effect on spermatogenesis (Goldstein and Eid, 1989). Several studies have also suggested that this condition is associated with increased oxidative stress (Saleh et al., 2003; Allamaneni et al., 2004; Mancini et al., 2004), and reactive oxygen species (ROS) are found at significantly increased levels in varicocele patients compared to control groups (Hendin et al., 1999).

In testis tissues, GSTA, GSTM, GSTT, and GSTP, which belong to the glutathione S-transferase gene family, act as important protective factors against oxidative stress (Strange et al., 2001). The homozygous deletion (null genotype) of the *GSTM1* or *GSTT1* gene results in the total absence of corresponding enzyme activity. Varicocele patients' susceptibility to ROS may be due to one or more of these GST genetic polymorphisms. As GSTs are important for male reproduction and their malfunctioning is involved in impairment of spermatogenesis, deletion polymorphisms of *GSTM1* or *GSTT1* might be related to male infertility.

Only a small number of studies have focused on the association between GST genotypes and varicocele, and their results are contradictory. We thus carried out the current meta-analysis to assess the association between *GSTM1* and *GSTT1* gene polymorphisms and varicocele, in an attempt to evaluate possible pathophysiologies.

METHODS

Data collection, extraction, and study design

We conducted a systematic literature search (for articles published up to September 2014) of Google Scholar and PubMed databases using the following keywords: "GST-M1", "GST-T1", and "varicocele". The primary reports retrieved were filtered using the following inclusion criteria: 1) studies must consist of a case-control study of *GSTM1* or *GSTT1* polymorphisms and varicocele; and 2) must provide detailed genotype data. Non-case-control studies, reviews, meta-analyses, and duplicate data were excluded. The following information was independently extracted from eligible studies by two investigators (xx and xx), and tabulated: first author and year of publication, country, ethnicity, frequency of each genotype in case and control groups, and genotyping method. All disagreements were resolved by consensus between authors. Subgroup analyses were performed based on ethnicity (Caucasian and Asian) according to the studies involved. A detailed data collection flow chart is shown in Figure 1, and the characteristics of each included study are given in Table 1.

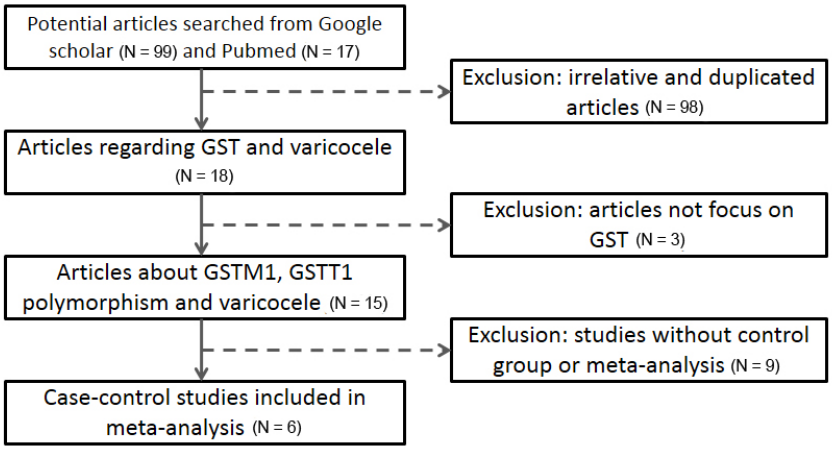

Figure 1. Flow chart describing the identification and retrieval of eligible studies. GST = glutathione S-transferase.

Table 1. Characteristics of each eligible study included in meta-analysis.

First author	Country	Ethnicity	No. of patients for *GSTM1*		No. of patients for *GSTT1*		Genotyping method
			Cases	Controls	Cases	Controls	
Dehghani et al. (2014)	Iran	Caucasian	46	48	46	48	Multiplex PCR
Tang et al. (2012)	China	Asian	65	30	65	30	Multiplex PCR
Acar et al. (2012)	Turkey	Caucasian	109	123	109	123	Multiplex PCR
Ichioka et al. (2009)	Japan	Asian	72	101	72	101	Multiplex PCR
Wu et al. (2009)	China	Asian	-	-	63	54	Multiplex PCR
Chen et al. (2002A)	China	Asian	80	60	-	-	Multiplex PCR
Chen et al. (2002B)	China	Asian	62	60	-	-	Multiplex PCR

PCR = polymerase chain reaction.

Statistical methods

Odds ratios (ORs) with 95% confidence intervals (CIs) were estimated for each study under the null *vs* present genotype genetic model to explore associations between *GSTM1* and *GSTT1* and varicocele. Each eligible study was weighted by sample size. Heterogeneity among studies was evaluated by the chi-square-based *Q* test and I^2 index. When no heterogeneity (I^2 < 50%, P value >0.5) was observed, the fixed-effects model (Mantel-Haenszel method; Mantel and Haenszel, 1959) was applied for OR estimation. Otherwise, the random-effects model was used. Begg's funnel plot and the Egger test were used to evaluate possible publication bias. All analyses were performed using the Stata software (version 12; StataCorp, College Station, TX, USA).

RESULTS

Subject characteristics

Sixeligible case-control studies incorporating 497 cases and 476 controls were included

in our meta-analysis (Chenet al., 2002; Ichioka et al., 2009; Wu et al., 2009; Acar et al., 2012; Tanget al., 2012; Dehghani et al., 2014). The characteristics of these studies are shown in Table 1. For analysis of *GSTM1* polymorphism, the case group comprised 434 patients and the control group 422 individuals; for *GSTT1*, 355 patients were included, along with 356 healthy controls. All polymorphisms were assessed by multiplex polymerase chain reaction genotyping. Five studies were conducted in Asian populations, while two involved Caucasian subjects. Data regarding null, denoted as *GSTM1*(-) and *GSTT1*(-) hereafter, and present genotypes, denoted as *GSTM1*(+) and *GSTT1*(+) hereafter, are shown in Table 2.

Table 2. Genotype distributions in studies included in the meta-analysis.

First author	Cases		Controls		Cases		Controls	
	GSTM1 (-)	*GSTM1* (+)	*GSTM1* (-)	*GSTM1* (+)	*GSTT1* (-)	*GSTT1* (+)	*GSTT1* (-)	*GSTT1* (+)
Dehghani	28	18	20	28	22	24	24	24
Tang	31	34	13	17	29	36	15	15
Acar	50	59	46	77	24	85	28	95
Ichioka	45	27	53	48	34	38	51	50
Wu	-	-	-	-	32	31	23	31
Chen A	35	45	27	33	-	-	-	-
Chen B	26	36	27	33	-	-	-	-

Meta-analysis

Heterogeneity tests of the overall datasets returned I^2 values of 0% for both genes, with P values >0.05 (Table 3). Thus, no statistically significant heterogeneity was observed and we therefore used a fixed-effects model to assess associations within the data. In the overall analysis, no significant association was detected between genotype and varicocele for either *GSTM1* (*GSTM1*(-) *vs GSTM1*(+); OR = 1.29, 95%CI = 0.98-1.70, P = 0.07) or *GSTT1* (*GSTT1*(-) *vs GSTT1*(+); OR = 0.97, 95%CI = 0.71-1.33, P = 0.87; Figure 2A and B). Potential publication bias in the overall dataset was determined by generating Begg's funnel plots and applying Egger's linear regression test. The Begg's funnel plot for both *GSTM1* and *GSTT1* was symmetric (Figure 3), and the Egger's test P values were both greater than 0.05 (Table 3).

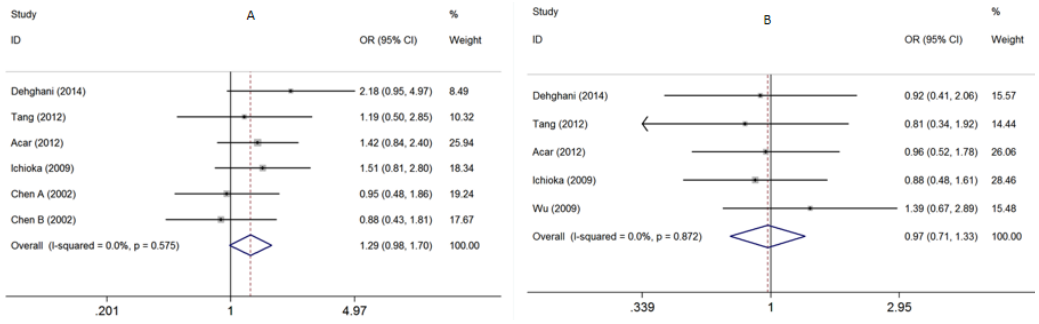

Figure 2. Results of the meta-analysis using the overall dataset. **A.** *GSTM1* null *vs GSTM1* present genotype. **B.** *GSTT1* null *vs GSTT1* present genotype. GST = glutathione S-transferase; OR = odds ratio; CI = confidence interval.

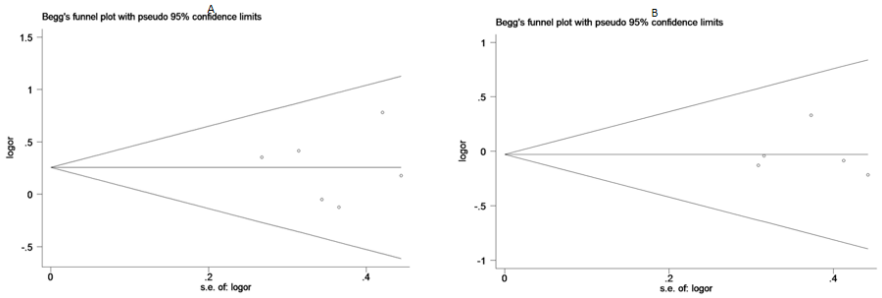

Figure 3. Begg's funnel plots relating to the association between *GSTM1* and *GSTT1* null polymorphisms and varicocele using the overall dataset. **A.** *GSTM1* null *vs GSTM1* present genotype. **B.** *GSTT1* null *vs GSTT1* present genotype. GST = glutathione S-transferase; SE = standard error; OR = odds ratio.

Table 3. Results of the *GSTM1* and *GSTT1* meta-analysis.

Analysis model	Analysis method	Heterogeneity		OR				Publication bias	
		I^2 (%)	P value#	Overall	Lower	Upper	P value*	Begg	Egger
GSTM1(-) *vs GSTM1*(+)	Fixed	0.0	0.575	1.29	0.98	1.70	0.07	1.00	0.96
GSTT1(-) *vs GSTT1*(+)	Fixed	0.0	0.87	0.97	0.71	1.33	0.87	0.81	0.99

OR = odds ratio. #P value from the heterogeneity test. *P value from the OR test.

Heterogeneity tests of the subgroup datasets gave similar results to those above, with I^2 values of 0% and P values >0.05 for both genes, indicating no statistically significant heterogeneity in either Asian or Caucasian subsets (Table 4). Therefore, the fixed-effects model was applied to estimate ORs. Forest plots of the two subgroup analyses are shown in Figure 4. We found that the *GSTM1*(-) genotype was statistically strongly associated with varicocele in the Caucasian dataset (OR = 1.61, 95%CI = 1.02-2.50, P = 0.03; Figure 4A), while in the Asian subgroup, no significant association was detected (OR = 1.13, 95%CI = 0.79-1.60, P = 0.54; Figure 4B). However, analysis of the *GSTT1* gene revealed no significant relationship in either the Caucasian (OR = 0.94, 95%CI = 0.58-1.54, P = 0.81) or Asian datasets (OR = 1.00, 95%CI = 0.66-1.50, P = 0.98; Figure 4C and D). Given the relatively small sample size involved, publication bias was not assessed for the subgroup analysis.

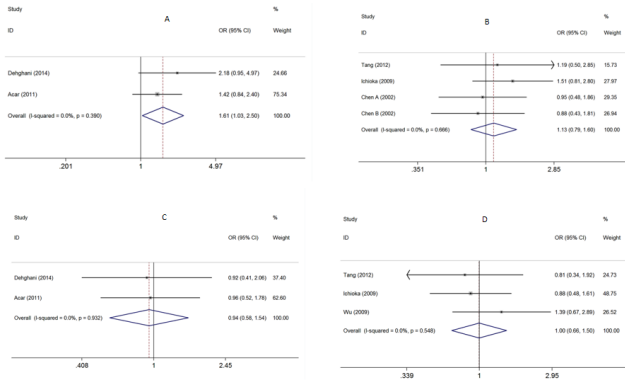

Figure 4. Forest plots from the subgroup analysis of **A.** *GSTM1* in the Caucasian population, **B.** *GSTM1* in the Asian population, **C.** *GSTT1* in the Caucasian population, and **D.** *GSTT1* in the Asian population. GST = glutathione S-transferase.

Table 4. Subgroup meta-analysis for *GSTM1* and *GSTT1* based on ethnicity.

Analysis model	Analysis method	Heterogeneity		OR			
		I^2 (%)	P value#	Overall	Lower	Upper	P value*
Caucasian							
GSTM1(-) *vs GSTM1*(+)	Fixed	0.0	0.39	1.61	1.03	2.50	0.03
GSTT1(-) vs GSTT1(+)	Fixed	0.0	0.93	0.94	0.58	1.54	0.81
Asian							
GSTM1(-) *vs GSTM1*(+)	Fixed	0.0	0.67	1.13	0.79	1.60	0.54
GSTT1(-) *vs GSTT1*(+)	Fixed	0.0	0.55	1.00	0.66	1.50	0.98

OR = odds ratio. #P value from the heterogeneity test. *P value from the OR test.

DISCUSSION

The glutathione S-transferases are a family of isoenzymes that play important roles in protection against oxidative stress. Under aerobic conditions, human spermatozoa generate ROS (Holland et al., 1982) as a normal physiological process (de Lamirande and Gagnon, 1993; Aitken and Fisher, 1994). However, in healthy men's seminal plasma, excessive ROS are neutralized by antioxidants. When the balance between ROS production and antioxidant capacity is shifted, e.g. in pathological conditions where GST activity is reduced, surplus ROS might lead to sperm malfunction (Aitken and Clarkson, 1987; Alvarez et al., 1987; Gopalakrishnan and Shaha, 1998; Aydemir et al., 2007) and a high rate of DNA damage (Lopes et al., 1998).

Here, we presented an up-to-datemeta-analysis including 497 cases and 476 controls, investigating the role of *GSTM1* and *GSTT1* null polymorphisms in varicocele patients, in an attempt to explore possible pathophysiologies of this disease. Our results showed that in an overall population analysis, *GSTM1* and *GSTT1* null polymorphisms were not observed more frequently in varicocele patients than in control subjects. Similarly, Chen et al. (2002) also detected no difference between control and varicocele patient groups regarding the *GSTM1* null genotype. Interestingly, only in the Caucasian subgroup analysis did we find a significantly higher frequency of the *GSTM1* null genotype amongst varicocele patients, with an OR of 1.61 (P = 0.03). In contrast, no such association was discerned in the Asian subgroup. We failed to detect any statistically significant correlation concerning the *GSTT1* null genotype in either Caucasian or Asian populations. As the sperm of varicocele patients with *GSTM1* null genotypes are more vulnerable to oxidative damage (Chen et al., 2002), more attention should be paid to oxidative stress-related pathological manifestations for varicocele sufferers carrying such a null polymorphism. Although the impact of varicocele on male fertility remains unknown (Baazeem et al., 2011), reduced detoxification capacity during oxidative stress seems likely to be a contributory factor for those patients in Caucasian populations with a *GSTM1* null genotype.

It should be noted that there are several limitations to this study. Firstly, only a small number of investigations have focused on the association between *GSTM1* and *GSTT1* polymorphisms and varicocele. Although we included a comprehensive, up-to-date list of publications, it would be preferable to incorporate further data for a more extensive meta-analysis, particularly for ethnicity-based subgroup tests. In addition, other members of the GST family might compensate for the loss of GSTM1 and GSTT1 activity. Due to the limited eligible data, only null genotypes of *GSTM1* and *GSTT1* were assessed in the current meta-analysis. Future studies should include other genetic polymorphisms of varicocele patients.

In conclusion, we investigated *GSTM1* and *GSTT1* polymorphisms in varicocele patients

and found that only amongst those from Caucasian populations was the *GSTM1* null genotype observed at a significantly higher frequency. The performance of epidemiological studies is strongly recommended to validate the role of the *GSTM1* gene in male infertility in Caucasian populations.

Conflicts of interest

The authors declare no conflict of interest.

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