Association of the Glutathione S-transferases M1 and T1 polymorphism with male infertility: a meta-analysis

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Abstract

Background Genes of different pathways regulate spermatogenesis, and the complexity of the spermatogenic process indicates that polymorphisms or mutations in these genes could cause male infertility. Published data on the association between the GSTM1 and GSTT1 polymorphism and male infertility risk are inconclusive. To derive a more precise estimation of the relationship, a meta-analysis was performed. **Methods** A total of 11 studies regarding GSTM1 and 9 studies regarding GSTT1 between 1999 and 2012 were identified through researching MEDLINE, EMBASE and the Chinese Biomedical Database. It was performed to obtain summary estimated odd ratios and 95 % confidence intervals of GSTM1 and GSTT1 for male infertility, with attention to study quality and publication bias.

Results Overall, a significant association was seen between GSTM1 (OR=1.20, 95 % CI=1.02–1.40, $P_{\text{heterogeneity}}=0.000$, $P=0.027$) genotypes and male infertility. Significant associations were also observed in subgroups of Caucasian

populations (OR=1.65, 95 % CI=1.16–2.34, $P_{\text{heterogeneity}}=0.006$, $P=0.005$), but were not observed in Asian populations (OR=1.09, 95 % CI=0.72–1.65, $P_{\text{heterogeneity}}=0.054$, $P=0.697$) when stratified by ethnicity. While there was no significant association was seen between GSTT1 (OR=1.00, 95 % CI=0.74–1.35, $P_{\text{heterogeneity}}=0.000$, $P=0.980$) null genotypes and male infertility. Simultaneously, significant associations were not observed in subgroups of Caucasian populations (OR=0.94, 95 % CI=0.44–2.00, $P_{\text{heterogeneity}}=0.000$, $P=0.867$) and Asian populations (OR=0.93, 95 % CI=0.46–1.87, $P_{\text{heterogeneity}}=0.002$, $P=0.838$) when stratified by ethnicity.

Conclusion Our results suggest the GSTM1 null genotype contributes to male infertility susceptibility, while GSTT1 gene polymorphisms are not associated with male infertility in our study.

Keywords Glutathione S-transferases M1 · Glutathione S-transferases T1 · Polymorphism · Male infertility · Meta-analysis · Molecular epidemiology

Capsule GSTM1 null genotype, rather than GSTT1, contributes to increased risk of male infertility.

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Introduction

Infertility is a global health problem and may result from multifactorial disorder, and in 50 % of infertile couples, the male factor due to deteriorated semen quality is a major cause [1, 2]. In about 30 % of infertile men seeking help for their problem, the etiology and pathogenesis are unknown and their condition is considered idiopathic [3]. Damage to the genetic component of spermatozoa has a crucial role in a majority of cases where current investigations fail to detect the specific cause of male infertility [4]. It has been shown that some genetic polymorphisms are associated with impaired spermatogenesis in infertile men with idiopathic oligoasthenoteratozoospermia [5]. Excessive reactive oxygen species (ROS) have been considered to be one of the major factors leading to an infertile status [6] via

oxidative DNA damages including base damage and DNA strand breaks. Increasing levels of reactive oxygen species (ROS) along with reduced activity of Glutathione S-transferases (GSTs) may cause sperm membrane damage and DNA fragmentation. It belongs to the family of phase II antioxidant enzymes involved in the cellular detoxification of various physiological substances [7]. It constitutes the major defensive antioxidant system against oxidative stress by reducing ROS to less reactive metabolites [8]. Polymorphisms in the GST gene may impair the ability of protection against oxidative stress and lead to the development of a wide range of diseases [9].

GSTs are currently categorized into eight classes, including alpha, mu, kappa, omega, pi, sigma, theta and zeta, that are encoded by GSTA, GSTM, GSTK, GSTO, GSTP, GSTS, GSTT and GSTZ genes, respectively [10, 11]. Three of the GST genes, GSTP1, GSTM1 and GSTT1, have been found to have functional polymorphisms that are frequently present in the general population [12]. The polymorphism in the GSTT1 and GSTM1 gene loci is caused by a gene deletion and results in the virtual absence of enzyme activity, especially in individuals with deletion in both genes (null genotypes) [13]. Published studies are available on the association of genotype in GSTM1, GSTT1 genes and idiopathic male infertility. GSTM1 polymorphism might be an important source of variation in susceptibility of spermatozoa to oxidative damage in patients with idiopathic infertility [14]. *GSTT1* has functional polymorphisms that result in the virtual absence of enzyme activity [13]. Polonikov et al. [15] reported that the nondeletion genotype of the GSTT1 gene is strongly associated with the increased risk of idiopathic male infertility and asthenozoospermia. In a study conducted in Chinese men, Wu et al. [16] reported that there was no statistically significant association between GSTT1 null genotype and infertile patients with varicocele. In another study from China, GSTT1 null genotype was a predisposing risk factor for sporadic idiopathic azoospermia or oligospermia in northwestern China [17].

To date, no complete meta-analysis has been conducted to investigate the association of GSTM1, GSTT1 polymorphisms and male infertility. Hence, a meta-analysis based on a total of 12 independent case–control studies was performed, which may provide the evidence for association of GSTM1 and GSTT1 polymorphisms with male infertility susceptibility.

Materials and methods

Search strategies

We sought to identify all epidemiologic studies that investigated the association of GSTM1, GSTT1 genetic polymorphisms with male infertility. To identify relevant studies, we

conducted a comprehensive systematic bibliographic search through PUBMED, EMBASE, ISI Web of Knowledge, Cochrane Library, and other databases without date and language restrictions for all medical published up to July 2012. The following search strategy was performed by consecutively entering the combined free words “glutathione,” “GSTM1,” “GST,” “GSTT1,” “genotype,” “polymorphism,” “idiopathic,” “male infertility,” “men,” including all alternative locations and combinations of the terms. Moreover, we also supplemented this search by reviewing the reference lists of all retrieved publications and the most recent review articles to ascertain additional undetected published studies. When more than one studies of the same population were included in several publications, only the most recent or complete study was used in this meta-analysis.

Inclusion and exclusion criteria

Two investigators independently reviewed abstracts in duplicate to determine whether they met the general inclusion and exclusion criteria, any discrepancies were resolved by discussion between the investigators.

For the meta-analysis, the following inclusion criteria were considered: (1) only case–control studies that had original data of a quantitative assessment of the relationship of GST variants and male infertility, concentrating upon polymorphisms in GSTM1 and GSTT1; (2) an appropriate description of GSTM1 and GSTT1 polymorphisms in male infertility cases and controls; (3) results expressed as relative risk (RR) or odds ratio (OR); (4) studies with a 95 % CI for RR or OR, or sufficient data to calculate these numbers.

While for the exclusion criteria, we provided as follows: (1) studies without the raw data of genotype of GSTM1 and GSTT1; (2) case-only studies, family-based studies, case reports, editorials, and review articles (including meta-analyses); (3) studies that compare the racial variation of GST variants in healthy population; In studies with overlapping cases/controls, the higher quality score, or the study with more information on origin of cases/controls was included in the meta-analysis.

Quality assessment

Two investigators independently reviewed and scored the quality of the individual study based on the criteria, which employed are shown in the [Materials and Methods](#) section above. Each article was blinded with respect to authors, institutions, countries, and journals. Any disagreements were resolved by consensus and reference to the articles. A quality score was then calculated for the individual study as the percentage of applicable criteria that were met in each study. Items estimating both selection bias and misclassification bias (nine points, items A–I) were given twice the

weight of items evaluating adjustment or matching for confounders and data analysis (nine points, items J–R). Therefore, each quality score could range from 0 % (none of the quality criterion was met) to 100 % (all the quality criteria were met). And the high-quality studies were considered as the ones with at least 60 % of the total score. For each study, the following information was reviewed and abstracted: first author, year of publication, ethnicity of the study population, involved genes, eligible subjects, source of controls, OR, and 95 % confidence intervals (95 % CI).

Data extraction

Information was carefully extracted from all eligible publications independently by two authors according to the inclusion criteria listed above. Disagreement was resolved by discussion between the two authors. The following data were collected from each study: first author's surname, year of publication, country, ethnicity, total numbers of cases and controls, numbers of cases and controls with the GSTM1 and GSTT1 genotypes, and the numbers of normal and null genotype in cases and controls, respectively. Different ethnicity descents were categorized as Caucasian, Asian and Mix (the original studies didn't clarify the race of the subjects or mixed races). We did not limit the number of patients to include a study in our meta-analysis.

Statistical analysis

We used the crude ORs with their corresponding 95 % CI as the metric of choice. Based on the individual ORs, the pooled OR was estimated. To take into account the possibility of heterogeneity across the studies, a statistical test for heterogeneity was performed using the Q statistic [18]. The heterogeneity was considered significantly when P was below 0.10. The heterogeneity was assessed using the I^2 statistic, which takes values between 0 % and 100 % with higher values denoting greater degree of heterogeneity ($I^2=0$ –25 %: no heterogeneity; $I^2=25$ –50 %: moderate heterogeneity; $I^2=50$ –75 %: large heterogeneity; $I^2=75$ –100 %: extreme heterogeneity) [19]. The pooled OR was analyzed jointly using both fixed effects (Mantel–Haenszel) and a random effects model (Der Simonian and Laird) [20]. The fixed effects model was used when there was no heterogeneity of the results of studies. Otherwise, the random-effects model was used.

Subgroup analysis and sensitivity analysis

To explore the reasons of heterogeneity, subgroup analyses were performed by grouping studies that showed similar characteristics, such as ethnicity, control source. The ethnic subgroups were categorized into two ethnic groups (Caucasian, Asian). In addition, sensitivity analyses were

also employed. In sensitivity analysis, each study was excluded one at a time to determine the magnitude of influence on the overall summary estimate [21].

For publication bias assessing, inverted funnel plot, Begg's test [22] and Egger's test [23] were employed. In the funnel plot, the results of the small studies are shown to be more widely scattered than those of the large studies. Where there is absence of publication bias, the plot resembles a symmetrical inverted funnel.

The power of studies was estimated as the probability of finding an association between GSTM1, GSTT1 polymorphisms and male infertility at the 0.05 significant levels, assuming that the genotype risk is 1.2 or 1.5. It was estimated on the basis of the method published by Fleiss et al. [24]. Analyses were performed using Stata (StataCorp, College Station, TX). All the P values were two-sided.

Results

Characteristics of eligible studies

Twenty two studies probing the relationship between GSTM1 and GSTT1 polymorphisms and male infertility were identified. During the extraction of data, 11 articles were excluded, because the contents were lack of control, their contents mainly associated with infertility therapy, or they studied among women other than men. The characteristics of selected studies are summarized in Table 1. A total of 12 eligible studies met the inclusion criteria, including 11 studies for GSTM1 [14, 15, 25–33] and 9 ones for GSTT1 [17, 26, 27, 29–33]. All the included studies were case–control studies. In total, 1,622 cases and 1,573 controls (GSTM1, 1,441 cases and 1,417 controls; GSTT1, 1,349 cases and 1,270 controls) were included in the pooled analyses. Of the 12 studies for polymorphisms, there were 6 with Caucasian ethnicity, 6 with Asian ethnicity, respectively. The controls of all studies mainly came from healthy population and matched for sex and age. All articles used blood samples for genotyping assay.

Meta-analysis results

GSTM1 genotype and male infertility

Aggregated ORs and heterogeneity test results for the association of GSTM1 polymorphism and male infertility were shown in Fig. 1. Overall, there was statistically significant association between GSTM1 genotype (OR=1.20, 95 %CI=1.02–1.40, $P_{\text{heterogeneity}}=0.000$, $P=0.027$) and

Table 1 Association of GSTM1 and GSTT1 polymorphism with male fertility

First author [reference]	Year	Country	Ethnicity	Genotype method	GSTM1		GSTT1	
					Sample size		Sample size	
					Cases/Controls(normal/null)		Cases/Controls(normal/null)	
Dhillon [25]	2007	Indian	Asian	PCR	179(120/59)	200(124/76)	–	–
Aydemir [14]	2007	Turkey	Caucasian	PCR	52(25/27)	60(32/28)	–	–
Wu [17]	2008	China	Asian	PCR	–	–	181(60/121)	156(80/76)
Ichioka [26]	2009	Japan	Asian	PCR	274(115/159)	101(48/53)	274(148/126)	101(50/51)
Aydos [27]	2009	Turkey	Caucasian	PCR	110(51/59)	105(63/42)	110(90/20)	105(85/20)
Vani [28]	2009	Indian	Asian	PCR	42(23/19)	43(34/9)	–	–
Safarinejad [29]	2010	Iran	Caucasian	PCR	166(93/73)	166(120/46)	166(119/47)	166(134/32)
Polonikov [15]	2010	Russian	Caucasian	PCR	203(89/114)	227(107/120)	203(202/1)	227(198/29)
Volk [30]	2011	Slovenia	Caucasian	PCR	187(90/97)	194(102/92)	187(152/35)	194(148/46)
Salehi [31]	2012	Iran	Caucasian	PCR	150(58/92)	200(134/66)	150(99/51)	200(166/34)
Tang [32]	2012	China	Asian	PCR	65(34/31)	30(17/13)	65(36/29)	30(15/15)
Jaiswal [33]	2012	Indian	Asian	PCR	113(84/29)	91(60/31)	113(107/6)	91(79/12)

male infertility. Both Cochran's Q test and the estimate of I^2 revealed significant heterogeneity among the constituent studies. The random effects model was used for large heterogeneity ($I^2=70.3\%$) of studies. To avoid the influence of heterogeneity among studies, we carried out subgroup analyses for ethnic group. In the stratified analysis by ethnicity, significant between-study heterogeneity was detected in all

the comparisons in Asians, but not in Caucasian population. For Caucasian population, there was significant association between GSTM1 polymorphism and increased male infertility risk (OR=1.65, 95 %CI=1.16–2.34, $P_{\text{heterogeneity}}=0.006$, $P=0.005$), but the contrary results were found in Asian population (OR=1.09, 95 % CI=0.72–1.65, $P_{\text{heterogeneity}}=0.054$, $P=0.697$) (Fig. 2).

Fig. 1 Forest plots of the meta-analysis for GSTM1 polymorphism associated with male infertility. Random-effects model was used

Fig. 2 Subgroup analysis by patient ethnicity was held for the association between GSTM1 genotype and male infertility susceptibility

GSTT1 genotype and male infertility

Overall, there was no statistically significant association between GSTT1 genotype (OR=1.00, 95 %CI=0.73–1.35, $P=0.980$) and male infertility (Fig. 3). In the stratified analysis by ethnicity, significant between-study heterogeneity was detected in all the comparisons both in Asians and

Caucasian population. There was no significant association between GSTT1 polymorphism and increased male infertility risk (OR=0.94, 95 %CI=0.44–2.00, $P_{\text{heterogeneity}}=0.000$, $P=0.867$) in Caucasian population, and the same results were also found in Asian population (OR=0.93, 95 % CI=0.46–1.87, $P_{\text{heterogeneity}}=0.002$, $P=0.838$) (Fig. 4). Both Cochran's Q test and the estimate of I^2 revealed significant

Fig. 3 Forest plots of the meta-analysis for GSTT1 polymorphism associated with male infertility. Random-effects model was used

Fig. 4 Subgroup analysis by patient ethnicity was held for the association between GSTT1 genotype and male infertility susceptibility

heterogeneity among the constituent studies. The random effects model was used for large heterogeneity ($I^2=79.9\%$) of studies. To avoid the influence of heterogeneity among studies, we carried out subgroup analyses for ethnic group. In the stratified analysis, there was no significantly increased risks of male infertility. We also failed to detect significant association between the polymorphism and male infertility risk neither in Caucasian nor in Asian populations.

Test of heterogeneity

There was significant heterogeneity for most genetic model comparisons among worldwide populations. After assessing the source of heterogeneity for all genetic model comparison by subgroup analysis on ethnicity, however, there was still significant heterogeneity among different descent population studies.

Sensitivity analysis

One-way sensitivity analyses of the pooled odds ratios and 95 % confidence intervals for GSTM1 and GSTT1 were performed. The pooled ORs were calculated by means of a random effects model. When omitting each dataset in the meta-analysis, the pooled ORs were always persistent. The analysis for carriers of the GSTM1 genotype and carriers of the GSTT1 genotype were shown at Fig. 5a and b, respectively. There is no single study influenced the pooled ORs

qualitatively as indicated by sensitivity analysis, suggesting that the results of this meta-analysis are stable.

Publication bias

The shapes of the funnel plots seemed symmetrical for all analyses. Begg's test was employed and did not suggest publication bias for GSTM1 (Fig. 6a) and GSTT1 (Fig. 7a). Egger's test also conformed the same result about publication bias for GSTM1 (Fig. 6b) and GSTT1 (Fig. 7b). In addition, no evidence of publication bias was found in any subgroup analyses under different ethnic descent models.

Discussion

Mutations and genotype of genes regulating spermatogenesis process may lead to male infertility. Recently, the important influence of oxidative stress in spermatogenesis has been acknowledged. Sperm membrane damage and DNA fragmentation may be a result of reduced action of GST enzyme and increased ROS levels [34]. Increased ROS level causes sperm chromatin damage which results in the production of poor quality of sperm and further lowers the chances of fertilization. GSTs have a protective role during spermatogenesis because its antioxidant properties help in protection from excessive ROS [35]. The GST system is one of the most important systems involved in the metabolism and detoxification of ROS, xenobiotics, and carcinogens

Fig. 5 One-way sensitivity analysis of the pooled ORs and 95 % CI for GSTM1 polymorphism (a) and GSTT1 polymorphism (b), omitting each dataset in the meta-analysis. Random-effects model was used

[36]. Several epidemiological studies have reported that the GSTM1 and T1 null genotypes that result in a lack of functional protein are correlated with an increased susceptibility to diseases associated with oxidative stress [37]. Oxidative stress is a result of the imbalance between ROS and antioxidants in the body. It is a mechanism that can lead to sperm damage, deformity, and eventually male infertility [38]. The possible role in male infertility has been already suggested for GSTM1 and GSTT1 gene variants, but published data are inconsistent.

In order to provide the comprehensive and reliable conclusion, we performed the present meta-analysis of 12 independent case-control studies. Our results regarding the polymorphism of GSTM1 and GSTT1 in the meta-analysis support that GSTM1 polymorphism are associated with an increased risk of male infertility, while there is no significant

relationship between GSTT1 polymorphism and male infertility. Nevertheless, considering that GSTM1 and GSTT1 polymorphism may play different roles in male infertility susceptibility among different ethnic subgroups and frequencies of the two genes polymorphism might be different among different ethnic groups which might contribute to the possible presence of heterogeneity between the studies, we further conducted subgroup analysis by ethnicity in current meta-analysis. In the stratified analysis by ethnicity, our results suggested that GSTM1 genotype was associated with male infertility risk among subjects of Caucasians, but it was not detected in Asians. However, associations of GSTT1 polymorphism with male infertility susceptibility analyzed in overall and subgroup analysis by ethnicity were not found either in Caucasian or Asian populations. The reason for this phenomenon may be caused by the limited

Fig. 6 Begg's (a) and Egger's (b) were held for the detection of publication bias

studies and population numbers of Caucasians and Asians included in the meta-analysis, this may increase the risk of false negative findings, any conclusions at overall population level should be interpreted with caution. Moreover, other ethnic decent studies were absent in our study, for example, Africans and African-Americans. Therefore, we are not sure whether there is a significant association between the GSTT1 polymorphism and increased male infertility risk in the whole population due to low statistical power.

Heterogeneity is a potential problem when interpreting the results of our meta-analysis. In overall analysis, significant between-study heterogeneity was existed among most

of genetic model comparisons. After subgroup analyses by ethnicity, the heterogeneity was removed when we analyzed the association between GSTM1 genotype and male infertility susceptibility in Asians. In this meta-analysis, high levels of heterogeneity were observed in some comparisons. There are some factors that could have contributed to the high heterogeneity. First, there is likely to be considerable genetic heterogeneity between the samples that were drawn from geographically diverse populations. It is known that genotype distributions differ across populations, and genotype-phenotype associations may also depend on population stratification. Second, definition of case group is

Fig. 7 Begg's (a) and Egger's (b) were held for the detection of publication bias

different in different studies, including different semen parameters, such as azoospermia, oligozoospermia, and asthenozoospermia, which could have contributed to the high heterogeneity observed in our meta-analysis. Third, we attempted to determine if the high heterogeneity might also be explained by other variables such as smoking status, and environmental factors included in the different studies, but are unable to provide a reliable answer to this question because we did not have access to individual level data for these variables.

Some limitations of this meta-analysis should be acknowledged. Firstly, as an observational study, there is potential for recall bias from case-control studies. Secondly, a potential source of bias in studies of genotypes might be the inclusion of individuals from different ethnic backgrounds. However, we performed subgroup analysis regarding ethnicity. Thirdly, cases and controls in our

meta-analysis only analyzed Caucasian and Asian population without African and Africa-American population in this study. So it is quite important to have more studies and sample of Africans, and African Americans in the future so that more precise conclusion about the associations between GSTM1 and GSTT1 genotype and male infertility risk could be achieved. Fourthly, our results were based on unadjusted estimates, while a more precise analysis should be conducted adjusted by other factors like daily habits and environmental factors. At last, heterogeneity existed although subgroup analysis was held according to ethnicity, other factors should be considered as the possible sources, for example, the possible specific results (azoospermia, oligozoospermia, and asthenozoospermia). So, further investigations about the association with detailed information between the linkage of GSTM1 and GSTT1 polymorphisms and male infertility susceptibility are needed.

In summary, the results of our meta-analysis indicate GSTM1 null polymorphism contributes to the risk of male infertility, while there is no significant association between GSTT1 genotype and male infertility. The finding provides more information on screening the high risk group of male infertility, and new strategy to prevention it's happen. Large studies with the pooling of individual data should be considered in future association studies to verify results from this meta-analysis and to further evaluate the effect of gene–environment interactions on the GSTM1 and GSTT1 polymorphism-associated male infertility.

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