GSTM1 null genotype contributes to increased risk of male infertility: a meta-analysis

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Abstract

Background Many studies have investigated the association between Glutathione S-Transferase M 1 (*GSTM1*) null genotype and risk of male infertility, but the impact of *GSTM1* null genotype on infertility risk is unclear owing to the obvious inconsistency among those studies. This study aimed to quantify the strength of association between *GSTM1* null genotype and risk of male infertility.

Methods We searched the PubMed, Embase and Wangfang databases for studies investigating the association between *GSTM1* null genotype and risk of male infertility. We estimated pooled odds ratio (OR) with its 95 % confidence interval (95 % CI) to assess this possible association.

Results Twelve case–control studies with 1, 589 infertility cases and 1, 537 controls were included. Meta-analysis of total 12 studies showed that *GSTM1* null genotype was associated with increased risk of male infertility (OR=1.34, 95%CI 1.02–1.77, $P=0.036$). In subgroup analysis of Caucasians, there was also an obvious association between *GSTM1* null genotype and increased risk of male infertility (OR=1.51, 95%CI 1.11–2.05, $P=0.006$). Sensitivity analyses by sequential omission of individual studies or omitting studies without high quality did not significantly alter the overall pooled OR. Cumulative meta-analysis further showed a trend of more obvious association as information accumulated. No evidence of publication bias was observed.

Conclusion Meta-analyses of available data suggest that *GSTM1* null genotype contributes to increased risk of male infertility.

Capsule *GSTM1* null genotype contributes to increased risk of male infertility.

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Introduction

Infertility is defined as failure to conceive after 12 months of unprotected intercourse and affects 15 % of couples [1, 2]. Male factors account for the difficulties in 50 % of couples, thus about 8 % of all men of reproductive age may need medical attention for reproductive failure [1, 2]. Despite significant advancements in the diagnostic workup of infertile men, the causes in approximately 50 % of cases remain unknown [3, 4]. Besides, it has been suggested that genetic factors may contribute to increased risk of male infertility [5–7].

The Glutathione S-Transferases (*GSTs*) are the most important family of phase II isoenzymes known to detoxify a variety of electrophilic compounds, including carcinogens, chemotherapeutic drugs, environmental toxins, and DNA products generated by reactive oxygen species, chiefly by conjugating them with glutathione [8]. In addition to this role in phase II detoxification, *GSTs* are able to modulate the induction of other enzymes and proteins important for cellular functions, such as DNA repair [8]. The Glutathione S-Transferase M1 (*GSTM1*) is one of the genes encoding the Mu class of *GSTs*, which is located on chromosome 1p13.3 and contains 10 exons [9]. The most common variant of *GSTM1* gene is the homozygous deletion genotype (null genotype), which has been suggested to be associated with the loss of *GSTs* enzyme activity, and increased vulnerability to cytogenetic damage or oxidative DNA damage [9]. Numerous studies have investigated the association between *GSTM1* null genotype and risk of male infertility, but the available evidence from those genetic studies was weak, owing to sparseness of data or disagreements among studies

[10–14]. Each of these studies typically involved a few cases and controls and failed to confirm a strong and consistent association [10–14]. Small genetic studies have various designs, different methodology and insufficient power, and could inevitably increase the risk that chance could be responsible for their conclusions, while combining data from all eligible studies by meta-analysis has the advantage of reducing random error and obtaining precise estimates for some potential genetic associations [15, 16]. Therefore, we performed a meta-analysis of published data investigating the association between *GSTM1* null genotype and risk of male infertility to shed some light on these contradictory results and to decrease the uncertainty of the effect size of the estimated risk.

Methods

Search strategy

We conducted a comprehensive search of the PubMed, Embase and Wangfang databases from its inception through January 2012. We combined search terms for *GSTM1* null genotype and male infertility. Search terms included *GST*, *GSTM*, *GSTM1*, or glutathione S-transferase M1; gene, polymorphism, mutation, or variant; and male infertility, azoospermia, or oligozoospermia. There was no language limitation. The retrieved studies were manually screened in their entirety to assess their appropriateness for eligibility criteria. All references cited in those studies were also reviewed to identify additional published articles not indexed in the common databases.

Study eligibility

Eligibility criteria included the following: (i) Case–control design of men with and without infertility; (ii) unexplained or idiopathic infertility and infertility with ‘known’ causes were excluded; (iii) provided information on frequency of *GSTM1* genotype. In studies with overlapping cases or controls, the most recent or the largest study with extractable data was included in the meta-analysis. Studies investigating progression, severity, phenotype modification, or response to treatment were all excluded from this meta-analysis. Genome scans investigate linkages and case-only studies were also excluded. In addition, family-based association studies were excluded because they use different study designs.

Data extraction

Two investigators independently extracted data, and disagreements were resolved through consensus. The extracted data included the year of publication, ethnicity of the study

population, definition of infertility, inclusion criteria for infertility patients and normal controls, demographics, matching, genotyping method, and the genotype distribution of cases and controls for the *GSTM1* polymorphism. The frequency of *GSTM1* null genotype was extracted or calculated for cases and controls. All data were extracted from published articles, and we did not contact individual authors for further information. The quality of the included studies was assessed using the following criteria modified from previous reports, and studies with 4 or 5 adequate items were defined as high quality studies [17–19]:

- (1) Description of the case and control groups (adequate, inadequate, not stated).
- (2) Assessment and validation of idiopathic infertility in the patients (adequate, inadequate, not stated). Adequate validation would include confirmation by scan or pathological examination; inadequate validation would include recollection of the patient as the only evidence or a biochemical pregnancy without excluding other ‘known’ causes.
- (3) Description of the laboratory procedures for the genotyping (adequate, inadequate, not stated).
- (4) Elimination of confounding factors in patients (adequate, inadequate, not stated). Adequate elimination refers to the exclusion of the proven causes of infertility (varicocele, Y chromosome deletions, abnormal karyotypes, hypogonadotropic hypogonadism, or seminal tract obstruction).
- (5) Equal assessment for confounding factors in the case and control groups (adequate, inadequate, not stated).

Statistical analysis

We calculated the odds ratio (OR) with its corresponding 95 % confidence interval (95%CI) to assess the strength of the association between *GSTM1* null genotype and risk of male infertility. The significance of the pooled OR was determined by the Z test and a P value of less than 0.05 was considered significant. In our study, two models of meta-analysis for dichotomous outcomes were conducted: the random-effects model and the fixed-effects model [20, 21]. The random-effects model was conducted using the DerSimonian and Laird’s method [21], while the fixed-effects model was conducted using the Mantel-Haenszel’s method [20]. To assess the between-study heterogeneity more precisely, both the chi-square based Q statistic test (Cochran’s Q statistic) to test for heterogeneity and the I^2 statistic to quantify the proportion of the total variation due to heterogeneity were calculated [22, 23]. The I^2 index expressing the percentage of the total variation across studies due to heterogeneity was calculated to assess the between-study heterogeneity. I^2 values of 25 %, 50 %, and

75 % were used as evidence of low, moderate, and high heterogeneity, respectively [22]. If moderate or high heterogeneity existed, the random-effects model was used to pool the ORs; otherwise, the fixed-effects model was used to pool the ORs when I^2 value was less than 50 %. To study the source of between-study heterogeneity, meta-regression was also performed [24]. We also performed a cumulative meta-analysis to provide a framework for updating a genetic effect from all studies and to measure how much the genetic effect changes as evidence accumulated and found the trend in estimated risk effect [25, 26]. In cumulative meta-analysis, studies were chronologically ordered by publication year, and then the pooled ORs were obtained at the end of each year. To validate the credibility of outcomes in this meta-analysis, sensitivity analysis was performed by sequential omission of individual studies or by omitting studies without high quality [27]. For additional analyses, the cases and controls were sub-grouped on the basis of their ethnicity. Racial/ethnic descent was categorized into Caucasians, East Asians, and others according to ethnicity classifications for genetic studies [28, 29]. Publication bias was investigated by funnel plot, in which the standard error of logor of each study was plotted against its logor, and an asymmetric plot suggested possible publication bias [30]. In addition, funnel-plot's asymmetry was further assessed by Egger's linear regression test [31].

All analyses were performed using STATA version 12.0 (StataCorp LP, College Station, Texas). A P value <0.05 was considered statistically significant, except where otherwise specified.

Results

Characteristics of included studies

With our search criterion, 83 individual records were found, and 15 full-text publications were preliminarily identified for further detailed evaluation [10–14, 32–41]. According to the exclusion criteria, two publications were excluded for lack of available data [39, 41] and one was excluded for patients with varicocele [40]. Finally, 12 case–control studies with 1, 589 cases and 1, 537 controls were included into this meta-analysis [10–14, 32–38]. All included studies were English language literature.

Table 1 presented a brief description of those 12 case–control studies, and 8 (66.7 %) were from Caucasian population. The number of cases varied from 42 to 203, with a mean of 132, and the numbers of controls varied from 43 to 227, with a mean of 128 (Table 1). Ten studies excluded known causes for infertility, but the exclusion criteria varied [10, 12–14, 32–34, 36–38]; the other two studies didn't state the exclusion of known causes for infertility [11, 35].

According to the quality criteria, 10 studies had high quality studies [10, 12–14, 32–34, 36–38], and two had low quality studies [11, 35].

Main results

There was obvious heterogeneity among in the meta-analysis of total 12 studies ($I^2=70.2$ %), thus the random-effects model was used. Meta-analysis showed *GSTM1* null genotype was associated increased risk of male infertility (OR=1.34, 95 % CI 1.02–1.77, $P=0.036$) (Fig. 1a). Sensitivity analyses by sequential omission of individual studies or omitting studies without high quality both did not significantly alter the overall combined ORs (Fig. 1b).

In subgroup analysis of Caucasians, there was obvious heterogeneity among those 8 studies ($I^2=65.6$ %), thus the random-effects model was used to pool the ORs. Meta-analysis showed *GSTM1* null genotype was associated increased risk of male infertility in Caucasians (OR=1.51, 95 % CI 1.11–2.05, $P=0.006$) (Fig. 2a). Besides, sensitivity analyses by sequential omission of individual studies or omitting studies without high quality both did not significantly alter the pooled ORs of Caucasians (Fig. 2b). In subgroup analysis of East Asians or India, there was no obvious association between the *GSTM1* null genotype and risk of male infertility.

The cumulative meta-analyses for total 12 studies or 10 studies with high quality both showed a trend of more obvious association as information accumulated (Fig. 3).

Heterogeneity and publication bias

There was obvious heterogeneity in the meta-analysis of total 12 studies ($I^2>50$ %). Subgroup analysis by ethnicity showed the heterogeneity was not eliminated (Fig. 2). The meta-regression showed that the major sources of heterogeneity might be the exclusion of known causes ($P<0.01$) and the quality of studies ($P<0.01$).

Begg's funnel and Egger's test were performed to assess the publication bias. Funnel plots' shape did not reveal obvious evidence of asymmetry, and the P value of Egger's test was 0.845, providing statistical evidence of funnel plots' symmetry (Fig. 4). Thus, the results above suggested that publication bias was not evident in this meta-analysis.

Discussion

This present meta-analysis investigating the association between *GSTM1* null genotype and risk of male infertility is based on the large amount of published data giving greater information to detect significant differences. Sensitivity

Table 1 Characteristics of included studies on *GSTM1* null genotype and male infertility risk

Author (year)	Ethnicity	Cases	Controls	Genotype frequency (Null/Present)	Quality assessment ^a
Jaiswal D 2012 [33]	India	113 nonobstructive azoospermia patients	91 fertile men of comparable age as controls	(case) 29:84 (control) 31:60	1:adequate; 2:adequate; 3:adequate; 4:adequate; 5: not stated;
Salehi Z 2012 [32]	Caucasians	150 men who had unexplained reduced sperm counts	200 age-matched men who had fathered at least 1 child	(case) 92:58 (control) 66:134	1:adequate; 2:adequate; 3:adequate; 4:adequate; 5:adequate;
Volk M 2011 [10]	Caucasians	187 infertile men	194 fertile men with at least one offspring	(case) 97:90 (control) 92:102	1:adequate; 2:adequate; 3:adequate; 4:adequate; 5:adequate;
Tirumala Vani G 2010 [38]	India	42 men with infertility in the age group of 25–45 years	43 healthy individuals who fathered at least one child	(case) 19:23 (control) 9:34	1:adequate; 2:adequate; 3:adequate; 4:adequate; 5: not stated;
Safarinejad MR 2010 [12]	Caucasians	166 patients with idiopathic infertility	166 fertile healthy men	(case) 73:93 (control) 46:120	1:adequate; 2:adequate; 3:adequate; 4:adequate; 5:adequate;
Polonikov AV 2010 [13]	Caucasians	203 patients with idiopathic male infertility	227 fertile volunteers	(case) 114:89 (control) 120:107	1:adequate; 2:adequate; 3:adequate; 4:adequate; 5:adequate;
Finotti AC 2009 [34]	Caucasians	128 men with idiopathic infertility	105 men with normal sperm	(case) 88:40 (control) 64:41	1:adequate; 2:adequate; 3:adequate; 4:adequate; 5:inadequate;
Ichioika K 2009 [36]	East Asians	202 infertile patients without varicocele	101 male controls	(case) 114:88 (control) 53:48	1:adequate; 2:adequate; 3:adequate; 4:adequate; 5:not stated;
Aydos SE 2009 [14]	Caucasians	110 infertile patients with no indication of hormonal, infective, or physical causes	105 healthy fertile men	(case) 59:51 (control) 42:63	1:adequate; 2:adequate; 3:adequate; 4:adequate; 5:adequate;
Dhillon VS 2007 [11]	India	179 infertile patients	200 fertile men	(case) 59:120 (control) 76:124	1:adequate; 2:adequate; 3:adequate; 4:inadequate; 5:not stated;
Aydemir B 2007 [37]	Caucasians	52 men aged 25–49 years with idiopathic infertility	60 male volunteers aged 25–49 years with normal semen analysis	(case) 27:25 (control) 28:32	1:adequate; 2:adequate; 3:adequate; 4:adequate; 5:adequate;
Paracchini V 2006 [35]	Caucasians	57 infertile patients	45 fertile men	(case) 32:25 (control) 29:16	1:adequate; 2:adequate; 3:adequate; 4:inadequate; 5:not stated;

^aQuality assessment codes: 1, description of cases and controls; 2, assessment and validation of the RPL in the patients; 3, description of laboratory methods; 4, Elimination of confounding factors in patients; 5, equal assessment of confounding factors in both groups.)

analysis and the cumulative meta-analysis were also performed. Meta-analysis showed *GSTM1* null genotype was associated with increased risk of male infertility. In subgroup analysis of Caucasians, there was also an obvious association between *GSTM1* null genotype and increased risk of male infertility. Sensitivity analyses by sequential omission of individual studies or omitting studies without high quality both did not materially alter the significance of pooled ORs. Sensitivity analysis and publication bias analysis suggest that it is highly unlikely that the findings may be due to chance (Type 1 error) or bias favoring publication of ‘positive’ studies. Thus, these findings support the concept of *GSTM1* null genotype as a genetic susceptibility factor of male infertility.

Heterogeneity is a potential problem when interpreting the results of all meta-analyses, and finding of the sources of heterogeneity is one of the most important

goals of meta-analysis [42]. The heterogeneity in present meta-analysis may arise from the definition of “male infertility” and “normal controls”. Although 10 studies excluded “known causes” for infertility, the exclusion criteria varied [10, 12–14, 32–34, 36–38]. The criteria for the so-called “normal controls” also varied among those 12 studies. These differences above could result in the heterogeneity in this meta-analysis. Besides, meta-regression analysis showed the major sources of heterogeneity might be the exclusion of known causes and the quality of studies in this meta-analysis. Studies without exclusion of known causes or low quality may have high risk of bias and cause the heterogeneity, as meta-analysis of studies with low quality or high risk of bias can lead to an incorrect estimate of pooled ORs and cause obvious discrepancy from the correct estimate [15, 42].

Fig. 1 Forest plots showed the association between GSTM1 null genotype and risk of male infertility (Results of individual and summary OR estimates, 95 % CI and weights of each study were shown. *Horizontal*

lines represented 95 % CI and *dotted vertical lines* represent the value of the summary OR.)

a **b**

Fig. 2 Forest plots showed the association between GSTM1 null genotype and risk of male infertility in Caucasians (Results of individual and summary OR estimates, 95 % CI and weights of each study

were shown. Horizontal lines represented 95 % CI and dotted vertical lines represent the value of the summary OR.)

Fig. 3 Forest plots showed results of the cumulative meta-analysis (The random effects pooled odds ratio with the corresponding 95 % confidence interval at the end of each information step was shown.)

Fig. 4 Begg's funnel plot for assessing the publication bias risk ($P_{\text{Egger}}=0.845$)

Oxidative stress in the male germ line is thought to affect male fertility and impact upon normal embryonic development, and the decrease in sperm count in infertile males may be due to oxidative DNA damage [43, 44]. Spermatozoa are susceptible to oxidative damage and excessive reactive oxygen species generation may lead to subfertility or infertility [43, 44]. Limited amount of reactive oxygen species production is essential for normal sperm function, whereas excessive reactive oxygen species generation leads to oxidative stress, which may induce sperm DNA damage and adversely influence fertilization and early embryo development [43, 44]. *GSTs* play an important role in the biotransformation and detoxification of many chemical agents, and they reduce reactive oxygen species to less reactive metabolites. Previous studies suggest semen contains *GSTs* and *GSTs* play a role in the protection against oxidative damage of spermatozoa and may play a role in susceptibility to male infertility [44, 45]. *GSTM1* null genotype has been suggested to be associated with the loss of *GSTs* enzyme activity, increased vulnerability to oxidative DNA damage, and excessive reactive oxygen species generation [9]. Therefore, spermatozoa and seminal plasma from male individuals with the *GSTM1* null genotype may exhibit greater susceptibility to oxidative stress and damage than that from *GSTM1* present genotype individuals [14, 40]. Thus, there is obvious biological evidence for that *GSTM1* null genotype contributes to increased risk of male infertility.

As with all meta-analyses, our analysis has limitations that must be considered when interpreting the findings. Firstly, our main analysis was based on unadjusted estimates owing to the lack of adjusted estimates. However, a more precise analysis could be performed if adjusted estimates were available in all studies. To validly examine the influence of confounding on the association between *GSTM1* null genotype and male infertility risk, future analyses can be performed using large pooled data sets of individual

patient data [46]. Secondly, no prospective studies have addressed this association between *GSTM1* null genotype and male infertility risk, and all included studies followed a retrospective case–control design. Thus, owing to the limitations of case–control design, we cannot exclude the possibility of undetected bias. Future prospective studies can investigate whether routine screening for the presence of the *GSTM1* null genotype may improve prediction of male infertility risk. Thirdly, the association between *GSTM1* null genotype and male infertility risk may be affected by population ethnicity. As the subgroup analysis by ethnicity suggest, there is obvious discrepancy in the effect of *GSTM1* null genotype on male infertility in different ethnicities. However, owing to the limitation of the only one study from East Asians, it's difficult for us to obtaining precise estimates for the potential genetic association in East Asians. Thus, the potential genetic association between *GSTM1* null genotype and male infertility risk in Asians need further studies. Finally, gene–gene and gene–environmental factors interactions were not addressed in this meta-analysis for the lack of sufficient data. Previous studies suggest Y chromosome AZFc region gr/gr deletions, androgen receptor CAG-repeat, and MTHFR C677T polymorphism are associated with risk of male infertility, and there may be gene–gene interactions [47–50]. However, we could not perform similar gene–gene and gene–environmental analyses owing to the limited reported information in those included studies. Future studies may further assess the gene–gene and gene–environmental interactions.

In conclusion, this present meta-analysis supports a significant association between *GSTM1* null genotype and risk of male infertility, and *GSTM1* null genotype contributes to increased risk of male infertility especially in Caucasians.

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Conflict of interests None of the authors have any conflict of interests to declare.

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