Quantitative assessment of the association between glutathione S-transferase M1 polymorphism and the risk of developing nasopharyngeal cancer

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Abstract. Glutathione S-transferases (GSTs) participate in the detoxification and elimination of electrophilic carcinogens by conjugating them to glutathione. Previous studies have reported a potential association between GSTM1 polymorphism and the risk of developing nasopharyngeal cancer (NPC). However, those findings remain controversial. In the present study, a meta-analysis was conducted by pooling the odds ratios (ORs) with the corresponding 95% confidence intervals (CIs) of all the available case-control studies on NPC. A comprehensive search of PubMed, Embase, Web of Science and China National Knowledge Infrastructure databases up to May 13th, 2014 was performed to identify eligible studies. A total of 12 separate publications, involving 1,593 cases of NPC and 2,868 controls, were included in the meta-analysis. The results demonstrated that the null genotype of GSTM1 was significantly associated with increased risk of developing NPC (OR=1.530, 95% CI=1.348-1.737, $P_{heterogeneity}$=0.370). Subgroup analysis by ethnicity suggested that Asian carriers of the GSTM1 null genotype were more susceptible to NPC than individuals from other ethnic groups (OR=1.516, 95% CI=1.328-1.731, $P_{heterogeneity}$=0.270). Sensitivity analysis confirmed the stability of these observations. In conclusion, the results from the present meta-analysis indicated that the GSTM1 polymorphism may be involved in the development of NPC, particularly in Asians.

Introduction

Nasopharyngeal carcinoma (NPC) is a rare condition worldwide, but it is endemic in certain populations (1). The development of NPC may be attributed to the dynamic interplay of environmental factors and genetic susceptibility. Host factors, including tobacco smoking, consumption of salt-preserved fish, history of chronic respiratory tract diseases and Epstein-Barr virus (EBV) infection, are well-established risk factors for the development of NPC (2,3). However, not all individuals exposed to EBV infection and environmental carcinogenic factors develop NPC, which indicates that genetic susceptibility may also contribute. Glutathione S-transferases (GSTs) are a large family of phase II detoxification enzymes that regulate the conversion of toxic compounds to hydrophilic metabolites (4,5). GSTM1 is one of the main subtypes of GSTs, and is involved in protecting hosts against cancer (4). The GSTM1 gene is located on the short arm of chromosome 1 (1p13.3), and displays several polymorphisms (6). The most common polymorphism in the GSTM1 gene is a null variant, which has been widely investigated as a risk biomarker for various types of cancer (7,8). The GSTM1 null variant may lead to the absence of enzymatic activity, and individuals who carry this variant are thought to be at increased risk of developing cancer (6). Numerous studies have previously evaluated the association of the GSTM1 polymorphism with the susceptibility to NPC, but the results are inconsistent, possibly due to the limited sample size and low power of these studies, which are insufficient to detect the precise effect in a single study (9-23). Thus, in the present study, a meta-analysis was performed in order to quantitatively evaluate the association between the GSTM1 polymorphism and the susceptibility to NPC.

Materials and methods

Search strategy. A comprehensive search of PubMed, Embase, Web of Science and China National Knowledge Infrastructure (CNKI) databases was performed up to May 13th, 2014, in order to identify potentially relevant studies on the association between the GSTM1 polymorphism and the risk of developing NPC. The literature search was conducted independently by 2 investigators, and the disagreements were resolved by consensus. The search terms were as follows: Glutathione S-transferase, GST, GSTs, glutathione S-transferase M1, GSTM, GSTM1, polymorphism, mutation or variation; with nasopharyngeal cancer, nasopharyngeal carcinoma or NPC. A manual search of the references of all the retrieved publications was conducted to identify additional studies. There were no language or sample size limitations.

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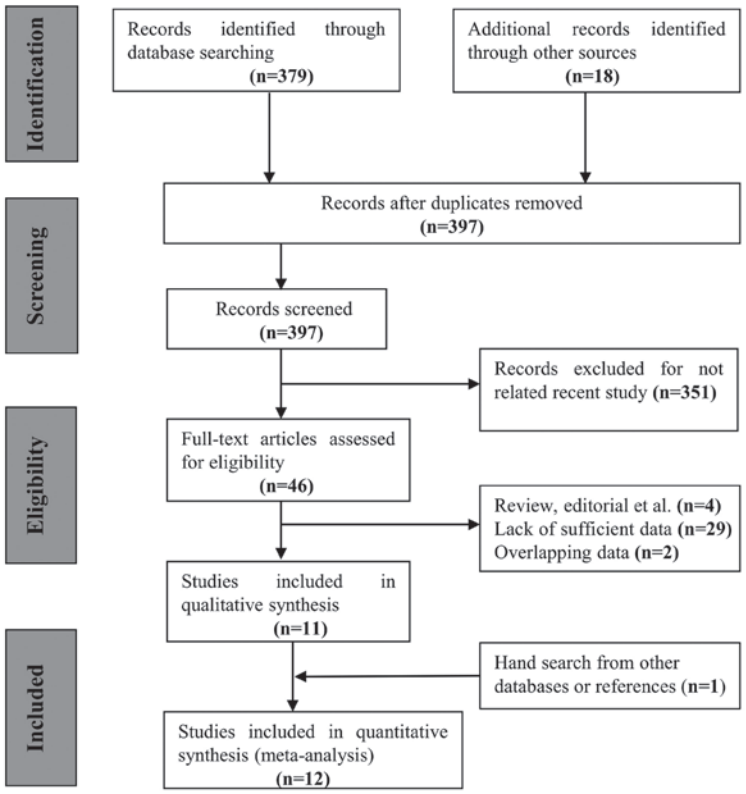

Figure 1. Flow diagram of the selection process followed for identifying and selecting eligible studies for the present meta-analysis.

Inclusion and exclusion criteria. Studies were included in the present meta-analysis according to the following inclusion criteria: i) Studies that evaluated the association between the GSTM1 polymorphism and the risk of developing NPC; ii) those that were case-control studies; iii) those that described the diagnoses of NPC and the sources of cases and controls; iv) those that contained available data for acquiring numbers of the null and present genotypes of GSTM1; and iv) those that presented sufficient information for the calculation of the odds ratio (OR) with its corresponding 95% confidence interval (CI). Single cases and family-based studies were excluded from the meta-analysis. In those cases of overlapping studies, the latest or the most complete one was selected for inclusion in the analysis.

Data extraction. The following data were extracted from the relevant publications: First author's name; year of publication; ethnicity and country of origin of cohort; numbers of cases and controls; genotyping method; source of controls; and genotype distributions in cases and controls. Disagreements were settled through discussion among the 2 investigators mentioned above.

Statistical analysis. The strength of the associations between the GSTM1 polymorphism and the risk of developing NPC was estimated by OR and the corresponding 95% CI, based on the frequencies of the null and present genotypes in cases and controls. The pooled ORs for the null vs. present genotype were calculated using the fixed- or random-effects model (known as the Mantel-Haenszel and DerSimonian and Laird method, respectively), and determined by the Z test (24,25). Q and I^2 tests were adopted to assess the heterogeneity among the studies included in the meta-analysis. $P<0.01$ and $I^2>50\%$ were considered to indicate a statistically significant difference in heterogeneity (26,27). Subgroup analyses were also conducted according to ethnicity: Of the 12 included studies, 10 were conducted in Asians (11-16,18,20-22), 1 in North Africans (17) and 1 in a mixed population (9). Publication bias was evaluated by funnel plot (28) and Egger's linear regression test (29). Sensitivity analysis by omission of each individual study was performed to identify the source of between-study heterogeneity and to confirm the stability and reliability of the pooled results (30). The statistical analyses were performed using STATA12.0 software (StataCorp LP, College Station, TX, USA).

Results

Study characteristics. Relevant studies were retrieved following a systematic literature search of PubMed, Embase, Web of Science and CNKI databases. The selection process for the articles included in the present meta-analysis is depicted in Fig. 1. The initial search yielded 394 study titles and abstracts, which were then subjected to an independent review. Based on the inclusion criteria, 12 individual publications studying the association of the GSTM1 polymorphism and the risk of developing NPC in a total of 1,593 cases and 2,868 individuals were available for analysis (9,11-17,20-22). The clinical characteristics of these studies are listed in Table I. Among the 12 included studies, 10 were conducted in Asians (11-16,18,20-22), 1 in North African (17) and 1 in a mixed population (9).

Table I. Summary of the main characteristics of the studies included in the present meta-analysis.

						GSTM1 polymorphism			
		Cohort		Sample size		Null		Present	
First author	Ref.	Country	Ethnicity	Case	Control	Case	Control	Case	Control
Nazar-Stewart V	9	USA	Mixed	83	142	45	63	38	79
Da SJ	11	China	Asian	80	80	48	36	32	44
Cheng YJ	12	Taiwan	Asian	314	337	173	169	141	168
Liu ZG	13	China	Asian	46	53	28	18	18	35
Deng ZL	14	China	Asian	91	135	56	64	35	71
Deng ZL	15	China	Asian	127	207	78	95	49	112
Tiwawech D	16	Thailand	Asian	78	145	50	74	28	71
Bendjemana K	17	Tunisia	African	45	100	23	33	22	67
Guo X	18	China	Asian	341	590	204	328	137	262
Jiang Y	20	China	Asian	182	366	97	157	85	215
Wei YP	21	China	Asian	126	641	78	305	48	336
Liao ZL	22	China	Asian	80	72	50	32	30	40

GSTM1, glutathione S-transferase M1.

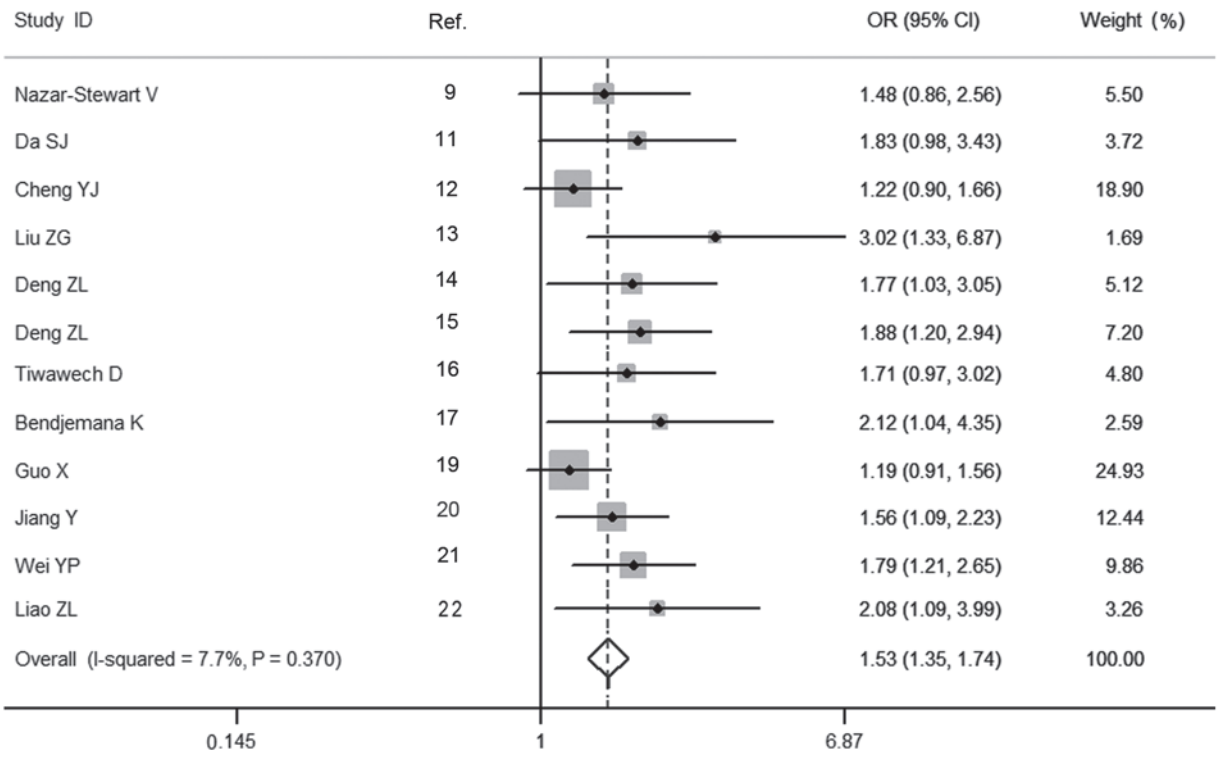

Figure 2. Forest plots describing the associations between the glutathione S-transferase M1 polymorphism and the risk of developing nasopharyngeal cancer. OR, odds ratio; CI, confidence interval.

Pooled analysis results. Estimation of the association between the GSTM1 polymorphism and the susceptibility to NPC was performed, and the pooled OR for the GSTM1 polymorphism suggested a significantly increased risk of developing NPC for carriers of the null genotype, compared with the present genotype (OR=1.530, 95% CI=1.348-1.737, $P_{heterogeneity}$=0.370; Fig. 2). Sensitivity analysis by omission of each individual study further confirmed this significant association

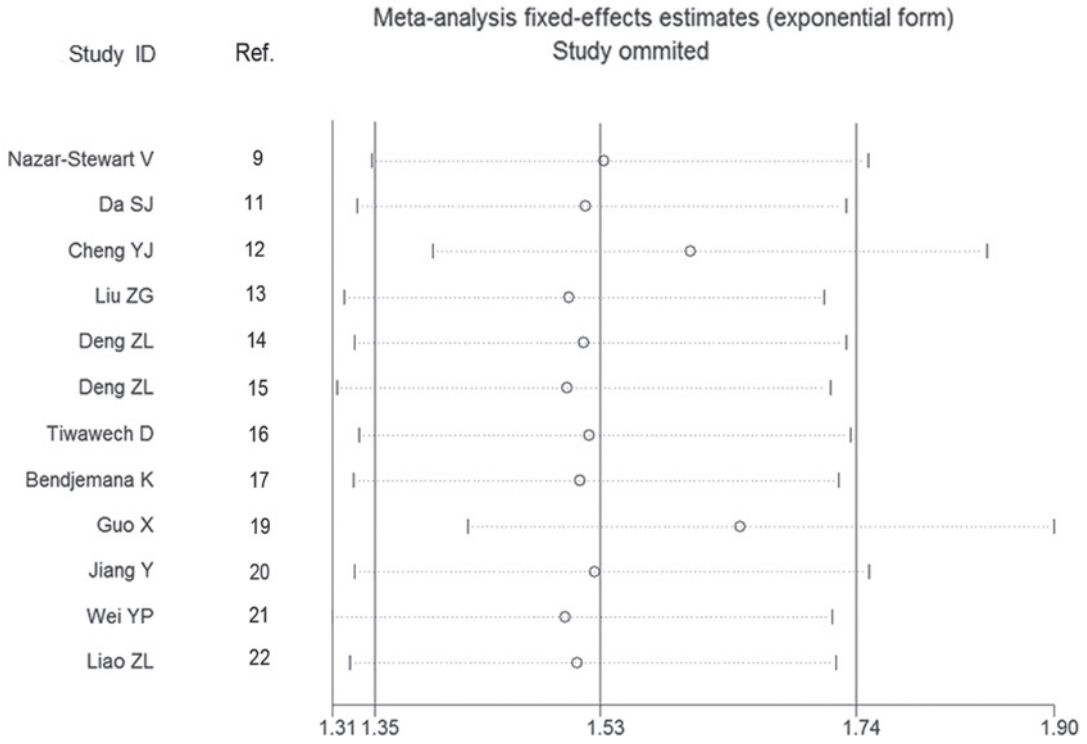

Figure 3. Sensitive analysis of the association between the glutathione S-transferase M1 null genotype and the risk of developing nasopharyngeal cancer.

(Fig. 3). In the subgroup analyses by ethnicity, a significantly increased risk of developing NPC was observed for the polymorphism of GSTM1 in the Asian population (OR=1.516, 95% CI=1.328-1.731, $P_{heterogeneity}$=0.270). Subgroup analyses in the North African and mixed populations were not performed due to insufficient data availability.

Publication bias. Begg's funnel plot and Egger's test were used to estimate the publication bias of all the studies on the associations of the GSTM1 polymorphism and the risk of developing NPC that were included in the present meta-analysis. The funnel plot shapes of the Begg's test did not reveal any evidence of asymmetry (Fig. 4). Furthermore, the P-value of the Egger's test was <0.05, indicating absence of publication bias among the included studies (Fig. 5).

Discussion

GSTs belong to the biotransformation family of enzymes. GSTs are phase II enzymes with catalytic and noncatalytic activities *in vivo*, which are involved in the detoxification of electrophilic compounds by glutathione conjugation, including carcinogens and cytotoxic drugs (31,32). Since the presumed function of GSTs is to protect tissues against toxic and carcinogenic compounds, they are considered to be important determinants in the development of prostate cancer. GSTM1, one of the main subtypes of GSTs, participates in the protection of the host against cancer (4). The GSTM1 gene displays several polymorphisms, among which, the null variant is the most common one, and it has been widely investigated as a risk biomarker for various types of cancer (7,8). The GSTM1 null variant may result in the absence of enzymatic activity, and individuals who carry the null variant are thought to be at increased risk of developing cancer, since the polymorphic deletions of GSTs may affect their ability to detoxify electrophilic carcinogens, which may lead to an increase in the host's susceptibility to environmental toxins and carcinogens (6,33). Mutations in GSTM1 are the most commonly studied polymorphisms of GSTs, regarding genetic susceptibility to cancer (34-36). Numerous individual case-control studies on the GSTM1 polymorphism in association with the risk of developing NPC have been conducted in the past 2 decades (9-23). Certain studies have previously suggested that the GSTM1 null genotype was associated with increased risk of developing NPC (9,20), while other studies did not observe any significant associations between various genetic polymorphisms of GSTM1 and the risk of developing NPC (12).

These discrepancies may arise from the small size of the sample in individual case-control studies, which may result in insufficient statistical power, particularly for weak correlations. In addition, differences in genetic background, study design and source of cases and controls may also contribute to these controversial and inconclusive findings. Therefore, in the present study, a meta-analysis was conducted by pooling the ORs with 95% CIs of all the currently available case-control studies on GSTM1 and NPC, in order to clarify these apparently contradictory findings. A total of 12 eligible studies with 1,593 cases and 2,868 controls were analyzed to quantitatively evaluate the association between the GSTM1 polymorphism and the susceptibility to NPC. The results suggested that the GSTM1 null genotype was significantly associated with the risk of developing NPC.

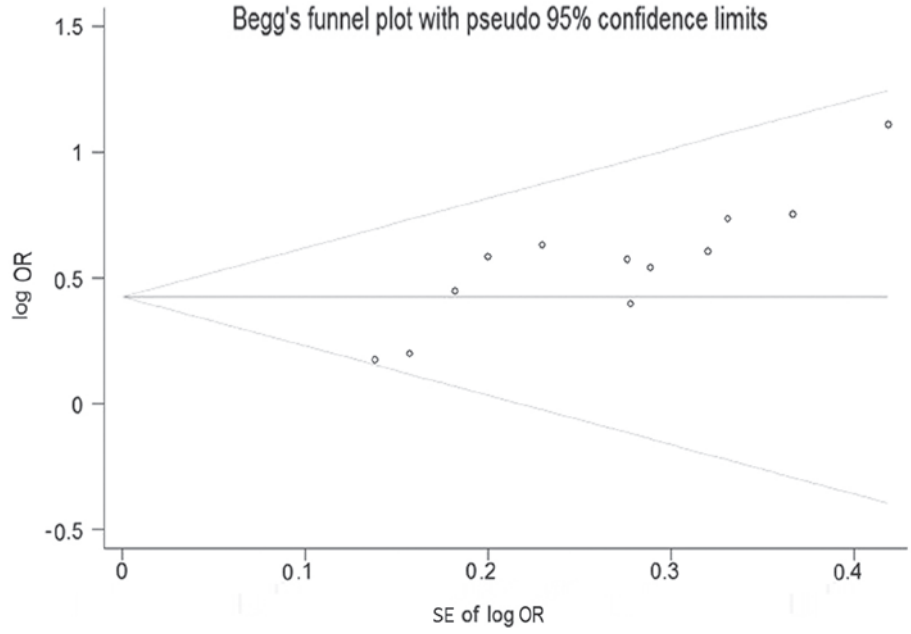

Figure 4. Begg's funnel plot for the risk of publication bias. OR, odds ratio; SE, standard error.

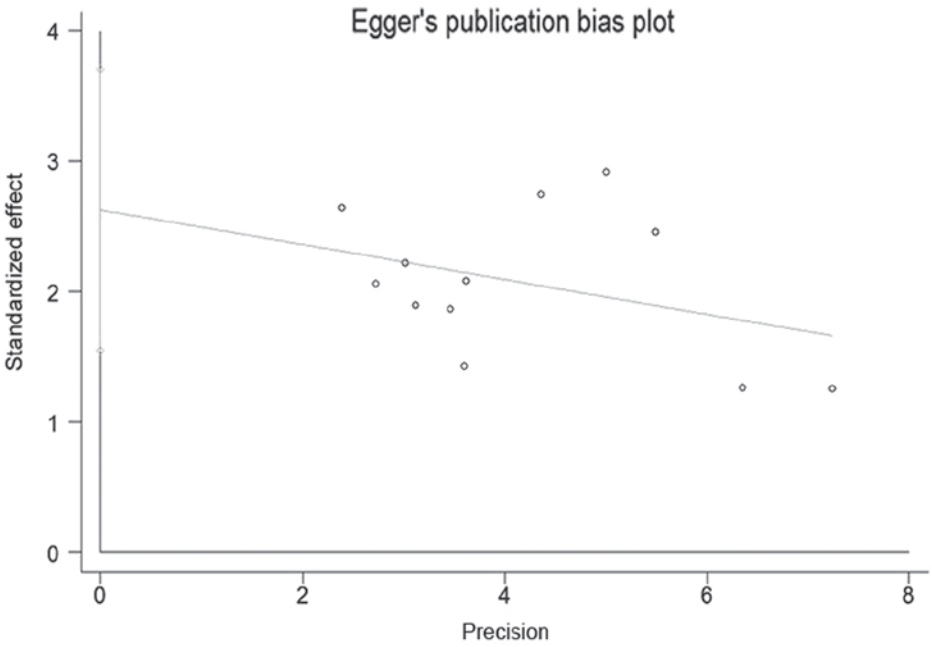

Figure 5. Egger's funnel plot for the risk of publication bias.

However, there were several limitations in the present meta-analysis: i) The adjusted estimation, which is a more precise way of estimating the association, was not pooled; thus, further studies are required to calculate the pooled OR, adjusting for other confounding factors; ii) a potential source of bias in studies of genotypes may be due to the inclusion of individuals from different ethnic backgrounds; and iii) gene-gene and gene-environment interactions were not analyzed in the present meta-analysis due to the unavailability of relevant studies. Therefore, additional case-control studies are required to further analyze these potential interactions and their role in the association between GSTM1 and NCP.

In summary, the results from the present meta-analysis indicate that the GSTM1 polymorphism participates in the development of NPC. However, the significant associations of the GSTM1 polymorphism with the risk of developing NPC must be validated in future studies, which must also account for the influence of gene-gene and gene-environment interactions in this potential association.

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