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GSTM3 A/B Polymorphism and Risk for Head and Neck Cancer: A Meta-Analysis

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Abstract

Background: Glutathione S-transferase M3 (GSTM3) is an important member of the GSTs that plays a critical role in the development of head and neck cancer (HNC). Several studies have investigated between the GSTM3 A/B polymorphism and risk of HNC, however, the results remain controversial. The aim of this meta-analysis is to evaluate the association between the GSTM3 A/B polymorphism and the risk of HNC.

Methods: All eligible case-control studies published up to July 2013 were identified by searching PubMed and Web of Science. The HNC risk associated with the GSTM3 A/B polymorphism was estimated for each study by odds ratios (OR) together with its 95% confidence interval (CI), respectively.

Results: Fourteen studies from ten publications with 2110 patients and 2259 controls were included. Overall, the GSTM3 A/B polymorphism was associated with a decreased risk of HNC using the dominant model, homozygote comparison model and heterozygote comparison model (OR = 0.82, 95%CI: 0.71–0.94; OR = 0.67, 95%CI: 0.49–0.94; and OR = 0.84, 95%CI: 0.73–0.97, respectively); besides, in stratification analyses by ethnicity, similar results were observed in Caucasian populations. Stratification by tumor site indicated that the GSTM3 polymorphism was associated with a decreased risk of laryngeal cancer under recessive model and homozygote comparison (OR = 0.52, 95%CI: 0.30–0.89; and OR = 0.50, 95%CI: 0.29–0.87, respectively); By stratifying source of control, decreased cancer risk was observed in hospital-based population under all genetic models (OR = 0.67, 95%CI: 0.56–0.81 for the dominant model; OR = 0.66, 95%CI: 0.46–0.95 for the recessive model; OR = 0.55, 95%CI: 0.37–0.83 for the homozygote comparison model, and OR = 0.70, 95%CI: 0.58–0.84 for the heterozygote comparison model).

Conclusions: This meta-analysis suggests that the GSTM3 A/B polymorphism may be an important protective factor for HNC, especially of laryngeal cancer and Caucasian populations.

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Introduction

Head and neck cancer (HNC), including cancers of the oral cavity, pharynx, and larynx, is the sixth most common cancer worldwide [1]. HNC has been associated with high tobacco use and alcohol consumption [2]. There is also evidence that human papillomavirus (HPV) is responsible for HNC [3]. However, not all individuals who are smoking or drinking develop this group of fatal diseases in their normal life span, suggesting that individual genetic make-up may also be involved in disease aetiology.

Glutathione S-transferases (GSTs, Enzyme Commission 2.5.1.18) are a large family of phase II isoenzymes that catalyze the detoxification of reactive electrophilic compounds, including many environmental carcinogens (e.g., benzo[a]-pyrene and other polycyclic aromatic hydrocarbons) [4].

The GST genes are highly polymorphic and frequently inducible. Among the numerous GST genes (comprising types M1 to M5), the GSTM3 gene is located on chromosome 1q13.3 and has 2 alleles identified so far: GSTM3 A and GSTM3 B, of which the latter has a 3 bp deletion in intron 6, known as a recognition motif for the YY1 transcription factor [5]. GSTM3 B allele, having increased transcription potential, enhances detoxification activity of GSTM3-encoded protein [6–7]. This allele has also been linked to decreased risk of laryngeal carcinoma [8].

To date, GSTM3 A/B gene polymorphism has been extensively examined in association with risk of HNC [9–22]. However, the results have been inconclusive or inconsistent. So, we conduct a first meta-analysis to evaluate the association between GSTM3 A/B polymorphism and HNC susceptibility.

Methods

Search strategy

A literature research was conducted using PubMed and Web of Science up to July 2013 without language restrictions. Relevant

studies were identified using the terms: ['Glutathione S-transferase M3 or GSTM3'] AND ['genetic polymorphism or polymorphisms or variant or SNP'] AND ['head and neck cancer or HNC or oral cancer or pharyngeal cancer or laryngeal cancer or hypopharyngeal cancer or oropharyngeal cancer or nasopharyngeal cancer']. The search was restricted to humans. Additional studies were identified by a hand search of references of original or review articles on this topic. If more than one geographic or ethnic heterogeneous group was reported in one report, each was extracted separately.

Inclusion criteria and exclusion criteria

Studies were included if they met the following criteria: (1) studies that evaluated the association between the GSTM3 A/B polymorphism and HNC, (2) in a case-control study design, and (3) had detailed genotype frequency of cases and controls or could be calculated from the article text. While major exclusion criteria were: (1) case-only study, case reports, and review articles, (2) studies without the raw data of the GSTM3 A/B genotype, and (3) studies that compared the GSTM3 A/B variants in precancerous lesions and other cancers.

Data extraction and quality assessment

Two investigators independently extracted data and reached consensus on all of the items. If they generated different results, they would check the data again and have a discussion to come to an agreement. If they could not reach an agreement, an expert was invited to the discussion. Data extracted from the selected articles included the first author's name, year of publication, country of origin, ethnicity, tumor site, genotyping methods, source of control, number of cases and controls. Tumor sites were categorized as oral, pharyngeal, laryngeal, and mixed HNC.

Statistical analysis

Meta-analysis was performed using the Cochrane Collaboration RevMan 5.1 and STATA package version 12.0. The risk of HNC associated with the GSTM3 A/B polymorphism was estimated for each study by odds ratio (OR) and 95% confidence interval (95%CI). Four different ORs were calculated: the dominant model (AB+BB *vs.* AA), the recessive model (BB *vs.* AB+AA), heterozygote comparison (AB *vs.* AA), and homozygote comparison (BB *vs.* AA). A χ^2-test-based Q statistic test was performed to assess the between-study heterogeneity [23]. We also quantified the effect of heterogeneity by I^2 test. When a significant Q test ($P<0.05$) or $I^2>50\%$ indicated heterogeneity across studies, the random effects model was used [24], or else the fixed effects model was used [25]. Before the effect estimation of GSTM3 A/B polymorphism in HNC, we tested whether genotype frequencies of controls were in HWE using χ^2 test. We performed stratification analyses on ethnicity, tumor site and source of control. Analysis of sensitivity was performed to evaluate the stability of the results. Finally, potential publication bias was investigated using Begg' funnel plot and Egger's regression test [26–27]. $P<0.05$ was regarded as statistically significant.

Results

Study characteristics

The search strategy retrieved 51 potentially relevant studies. According to the inclusion criteria, 14 studies [9–22] with full-text were included in this meta-analysis and 37 studies were excluded. The flow chart of study selection in summarized in Figure 1. As shown in Table 1, because the studies [11,13,14] included two tumor types respectively and the study by Park et al [16] included two populations, we treated them separately in this meta-analysis. Furthermore, we removed 4 studies because their genotype distributions among the controls deviated from HWE [19–22]. Therefore, there were 14 case-control studies from 10 publications with 2110 cancer cases and 2259 controls concerning GSTM3 A/B polymorphism. Of the 14 eligible studies, two ethnicities were addressed: 13 studies on Caucasian populations and one study [16] on African-American. Four tumor sites were addressed: 6 studies focused on laryngeal cancer [9,10,12,13,14,18], three

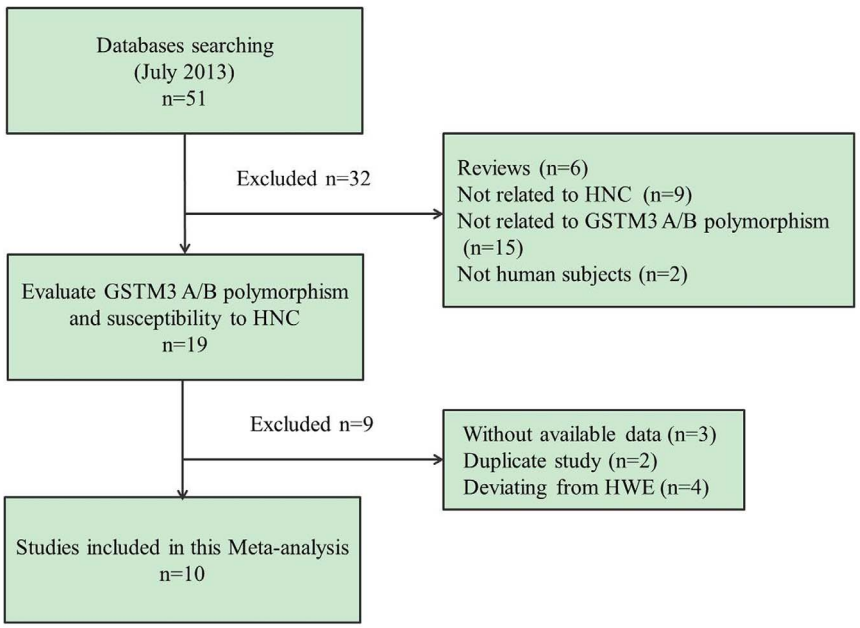

Figure 1. Flow chart showing study selection procedure.
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Table 1. Characteristics of studies included in the meta-analysis.

Study	Year	Country	Ethnicity	Tumor site	Genotyping Methods	Source of control	Age, mean±SD, year		Gender, n (male/female)		Genotype (case/control)				P_{HWE}[3]
							Case	Control	Case	Control	Total	AA	AB	BB	
Chatzimichalis [9]	2010	Greece	Caucasian	Laryngeal	TaqMan	PB[1]	66.5±8.54	62.7±9.62	77/11	76/26	88/102	81/92	7/10	0/0	0.60
Jahnke [10]	1996	England	Caucasian	Laryngeal	PCR	PB	61/58	NR[5]	246/23	NR	100/100	74/66	24/27	2/7	0.09
Jourenkova-Mironova [11]	1999	France	Caucasian	Oral	PCR-RFLP[4]	PB	54.4±10.2	54.9±11.1	113/8	163/9	67/172	49/125	16/42	2/5	0.52
Jourenkova-Mironova [11]	1999a	France	Caucasian	Pharyngeal	PCR-RFLP	PB	54.4±10.2	54.9±11.1	113/8	163/9	50/172	35/125	13/42	2/5	0.52
Jourenkova-Mironova [12]	1999b	France	Caucasian	Laryngeal	PCR-RFLP	PB	55.0	54.9	126/3	163/9	129/172	78/125	47/42	4/5	0.52
Matthias [13]	1998a	German	Caucasian	Pharyngeal	PCR-RFLP	HB[2]	NR	NR	NR	NR	118/173	89/116	22/47	7/10	0.09
Matthias [13]	1998a	German	Caucasian	Laryngeal	PCR-RFLP	HB	NR	NR	NR	NR	256/173	191/116	61/47	4/10	0.09
Matthias [14]	1998b	German	Caucasian	Oral/Pharyngeal	PCR-RFLP	HB	59.3±10	54.1±10.2	93/33	175/44	124/170	95/113	24/50	5/7	0.62
Matthias [14]	1998b	German	Caucasian	Laryngeal	PCR-RFLP	HB	62.0±10.3	54.1±10.2	249/23	175/44	262/170	200/113	58/50	4/7	0.62
Matthias [15]	2003	German	Caucasian	Head and neck	PCR-RFLP	HB	NR	NR	NR	NR	372/170	287/113	76/50	9/7	0.62
Park [16]	2000	USA	Caucasian	Oral	PCR-RFLP	HB	63.6(28–91)	60.4(28–91)	72/29	152/61	99/210	57/122	38/77	4/11	0.45
Park [16]	2000	USA	African-American	Oral	PCR-RFLP	HB	58.6(38–85)	59.6(34–88)	45/18	95/38	63/132	11/15	26/54	26/63	0.32
Rydzanicz [17]	2005	Poland	Caucasian	Head and neck	PCR-RFLP	PB	61.2±9.2	53.1±2.8	178/4	143/0	180/141	124/105	50/33	6/3	0.83
To-Figueras [18]	2002	Spain	Caucasian	Laryngeal	PCR-RFLP	PB	60±7	50±10	202/2	171/32	202/202	142/134	53/62	7/6	0.71

[1]PB: population-based,
[2]HB: hospital-based,
[3]HWE, Hardy-Weinberg equilibrium; P_{HWE} was calculated by goodness-of fit χ^2-test, $P_{HWE}<0.05$ was considered statistically significant,
[4]PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism.
[5]NR, not reported.
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Table 2. Stratified analysis of the GSTM3 polymorphism and HNC risk.

Variables	N[a]	AB+BB *vs.*AA			BB *vs.*AB+AA			BB *vs.*AA			AB *vs.*AA		
		OR[d](95% CI[e])	P[b]	I^2	OR(95% CI)	P[b]	I^2	OR(95% CI)	P[b]	I^2	OR(95% CI)	P[b]	I^2
Total	14	0.82(0.71,0.94)	0.05	42	0.74(0.54,1.00)	0.66	0	0.67(0.49,0.94)	0.54	0	0.84(0.73,0.97)	0.09	35
Ethnicity													
Caucasian	13	0.83(0.72,0.95)	0.04	45	0.73(0.51,1.03)	0.57	0	0.69(0.49,0.99)	0.47	0	0.85(0.73,0.98)	0.07	39
African-American	1	0.61(0.26,1.41)	N/A	N/A	0.77(0.42,1.41)	N/A	N/A	0.56(0.23,1.39)	N/A	N/A	0.66(0.26,1.63)	N/A	N/A
Tumor site													
Laryngeal	6	0.83(0.60,1.15)	0.04[c]	58	0.52(0.30,0.89)	0.24	27	0.50(0.29,0.87)	0.17	37	0.88(0.71,1.08)	0.08	50
Oral	3	0.92(0.65,1.31)	0.56	0	0.79(0.47,1.32)	0.95	0	0.69(0.35,1.34)	0.80	0	0.95(0.66,1.38)	0.67	0
Pharyngeal	2	0.81(0.53,1.22)	0.22	33	1.11(0.47,2.61)	0.76	0	1.02(0.43,2.42)	0.65	0	0.76(0.49,1.20)	0.21	37
Mixed HNC	3	0.77(0.46,1.29)	0.03[c]	72	0.88(0.45,1.72)	0.50	0	0.81(0.42,1.59)	0.39	0	0.76(0.46,1.25)	0.04[c]	68
Source of control													
PB	7	1.06(0.86,1.31)	0.21	29	0.96(0.55,1.69)	0.66	0	0.98(0.56,1.73)	0.60	0	1.07(0.86,1.34)	0.29	19
HB	7	0.67(0.56,0.81)	0.71	0	0.66(0.46,0.95)	0.56	0	0.55(0.37,0.83)	0.62	0	0.70(0.58,0.84)	0.64	0

[a]Number of comparisons.
[b]Test for heterogeneity.
[c]Random-effects model was used when the P-value for heterogeneity test was <0.05, otherwise fixed-effects model was used.
[d]OR, odds ratio.
[e]CI, confidence interval.
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studies on oral cancer [11,16], 2 studies on pharyngeal cancer [11,13], and 3 studies on mixed HNC [14,15,17].

Quantitative data synthesis

As shown in Table 2, overall, the GSTM3 A/B polymorphism was associated with a decreased risk of HNC in three genetic models (OR = 0.82, 95%CI: 0.71–0.94 for the dominant model; OR = 0.67, 95%CI: 0.49–0.94 for the homozygote comparison model; OR = 0.84, 95%CI: 0.73–0.97 for heterozygote comparison model) (Figure 2), but no significant association was observed under the recessive model (OR = 0.74, 95%CI: 0.54–1.00)

In stratified analysis by ethnicity, we found that this polymorphism played different roles in Caucasian and African-American. In the Caucasian population, the GSTM3 A/B polymorphism had significant protective effects on the risk of HNC in three genetic models (OR = 0.83, 95%CI: 0.72–0.95 for the dominant model; OR = 0.69, 95%CI: 0.49–0.99 for the homozygote comparison model; OR = 0.85, 95%CI: 0.73–0.98 for heterozygote comparison model), but no significant association was observed under the recessive model (OR = 0.73, 95%CI: 0.51–1.03); while only one study focused on African-American, found that the GSTM3A/B polymorphism play an important role in risk for oral cancer (Table 2).

In the stratified analysis based on tumor site, significant associations were found in recessive and homozygote comparison model for laryngeal cancer (OR = 0.52, 95%CI: 0.30–0.89; OR = 0.50, 95%CI: 0.29–0.87 respectively); However, no significant association was found for either oral cancer or pharyngeal or mixed HNC (Table 2, Figure 3).

Stratification based on the source of controls showed significant associations between the GSTM3 A/B polymorphism and risk of HNC in the hospital-based subgroup (OR = 0.67, 95%CI: 0.56–0.81 for the dominant model; OR = 0.66, 95%CI: 0.46–0.95 for the recessive model; OR = 0.55, 95%CI: 0.37–0.83 for the homozygote comparison model, and OR = 0.70, 95%CI: 0.58–0.84 for the heterozygote comparison model). However, no significant association was found in the population-based subgroup (OR = 1.06, 95%CI: 0.86–1.31 for the dominant model; OR = 0.96, 95%CI: 0.55–1.69 for the recessive model; OR = 0.98, 95%CI: 0.56–1.73 for the homozygote comparison model; OR = 1.07, 95%CI: 0.86–1.34 for the heterozygote comparison model) (Table 2).

Heterogeneity and sensitivity analysis

There were no inter-study heterogeneity among overall studies of the GSTM3 A/B polymorphism in all four genetic models ($I^2 = 42\%$, $P_{heterogeneity} = 0.05$ for the dominant model; $I^2 = 0\%$, $P_{heterogeneity} = 0.66$ for the recessive model; $I^2 = 0\%$, $P_{heterogeneity} = 0.54$ for the homozygote comparison model; $I^2 = 35\%$, $P_{heterogeneity} = 0.09$ for the heterozygote comparison model). Therefore, we used the fixed-effects model that generated wider CIs. However, it's worth noting that there was moderate heterogeneity among overall studies under dominant model, so we conducted stratified analysis by ethnicity, tumor cites and the source of the controls to find the potential sources of heterogeneity and we found that heterogeneity still exists in Caucasian population ($I^2 = 45\%$, $P_{heterogeneity} = 0.04$), laryngeal cancer ($I^2 = 58\%$, $P_{heterogeneity} = 0.04$) and mixed HNC ($I^2 = 72\%$, $P_{heterogeneity} = 0.03$), however, heterogeneity significantly reduced or removed among oral ($I^2 = 0\%$, $P_{heterogeneity} = 0.56$), pharyngeal cancer ($I^2 = 33\%$, $P_{heterogeneity} = 0.22$), population-based population ($I^2 = 29\%$, $P_{heterogeneity} = 0.21$) and hospital-based populations ($I^2 = 0\%$, $P_{heterogeneity} = 0.71$). Then, sensitivity analysis, after removing one study at a time, was performed to evaluate the stability of the results. We found that the estimated pooled odd ratio changed quite little, indicating that our results were statistically robust.

Publication bias

Begg's funnel plot and Egger's test were performed to assess the potential publication bias in the available literature. The shape of funnel plots did not reveal any evidence of funnel plot asymmetry (Figure 4). Egger's test also showed that there was no statistical significance for the evaluation of publication bias (dominant

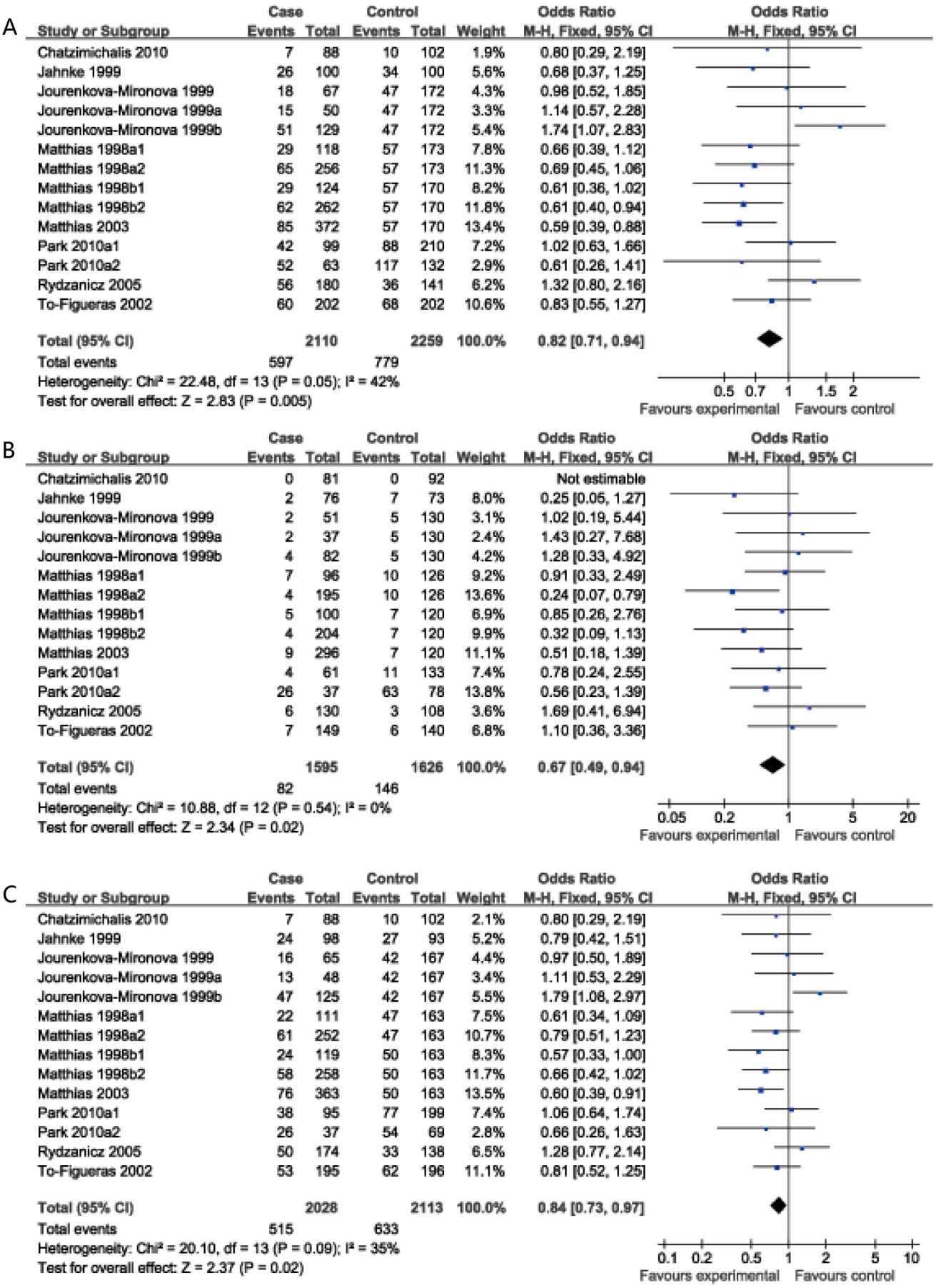

Figure 2. Forest plot of ORs for association between GSTM3 A/B and risk of HNC. (**A** dominant model; **B** BB *vs.* AA; **C** AB *vs.* AA).
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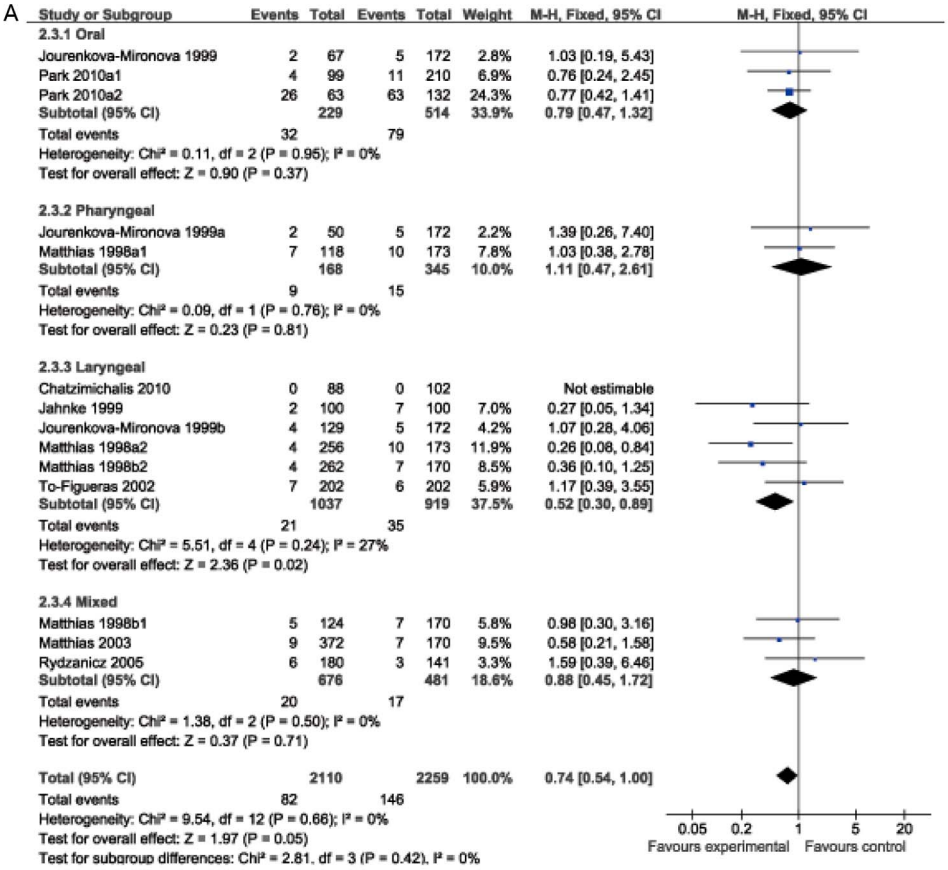
A


Study or Subgroup Events Total Events Total Weight M-H, Fixed, 95% CI
M-H, Fixed, 95% CI
2.3.1 Oral
Jourenkova-Mironova 1999 2 67 5 172 2.8% 1.03 [0.19, 5.43]
Park 2010a1 4 99 11 210 6.9% 0.76 [0.24, 2.45]
Park 2010a2 26 63 63 132 24.3% 0.77 [0.42, 1.41]
Subtotal (95% CI) 229 514 33.9% 0.79 [0.47, 1.32]
Total events 32 79
Heterogeneity: Chi² = 0.11, df = 2 (P = 0.95); I² = 0%
Test for overall effect: Z = 0.90 (P = 0.37)
2.3.2 Pharyngeal
Jourenkova-Mironova 1999a 2 50 5 172 2.2% 1.39 [0.26, 7.40]
Matthias 1998a1 7 118 10 173 7.8% 1.03 [0.38, 2.78]
Subtotal (95% CI) 168 345 10.0% 1.11 [0.47, 2.61]
Total events 9 15
Heterogeneity: Chi² = 0.09, df = 1 (P = 0.76); I² = 0%
Test for overall effect: Z = 0.23 (P = 0.81)
2.3.3 Laryngeal
Chatzimichalis 2010 0 88 0 102 Not estimable
Jahnke 1999 2 100 7 100 7.0% 0.27 [0.05, 1.34]
Jourenkova-Mironova 1999b 4 129 5 172 4.2% 1.07 [0.28, 4.06]
Matthias 1998a2 4 256 10 173 11.9% 0.26 [0.08, 0.84]
Matthias 1998b2 4 262 7 170 8.5% 0.36 [0.10, 1.25]
To-Figueras 2002 7 202 6 202 5.9% 1.17 [0.39, 3.55]
Subtotal (95% CI) 1037 919 37.5% 0.52 [0.30, 0.89]
Total events 21 35
Heterogeneity: Chi² = 5.51, df = 4 (P = 0.24); I² = 27%
Test for overall effect: Z = 2.36 (P = 0.02)
2.3.4 Mixed
Matthias 1998b1 5 124 7 170 5.8% 0.98 [0.30, 3.16]
Matthias 2003 9 372 7 170 9.5% 0.58 [0.21, 1.58]
Rydzanicz 2005 6 180 3 141 3.3% 1.59 [0.39, 6.46]
Subtotal (95% CI) 676 481 18.6% 0.88 [0.45, 1.72]
Total events 20 17
Heterogeneity: Chi² = 1.38, df = 2 (P = 0.50); I² = 0%
Test for overall effect: Z = 0.37 (P = 0.71)
Total (95% CI) 2110 2259 100.0% 0.74 [0.54, 1.00]
Total events 82 146
Heterogeneity: Chi² = 9.54, df = 12 (P = 0.66); I² = 0%
Test for overall effect: Z = 1.97 (P = 0.05)
Test for subgroup differences: Chi² = 2.81, df = 3 (P = 0.42), I² = 0%
0.05 0.2 1 5 20
Favours experimental Favours control

B

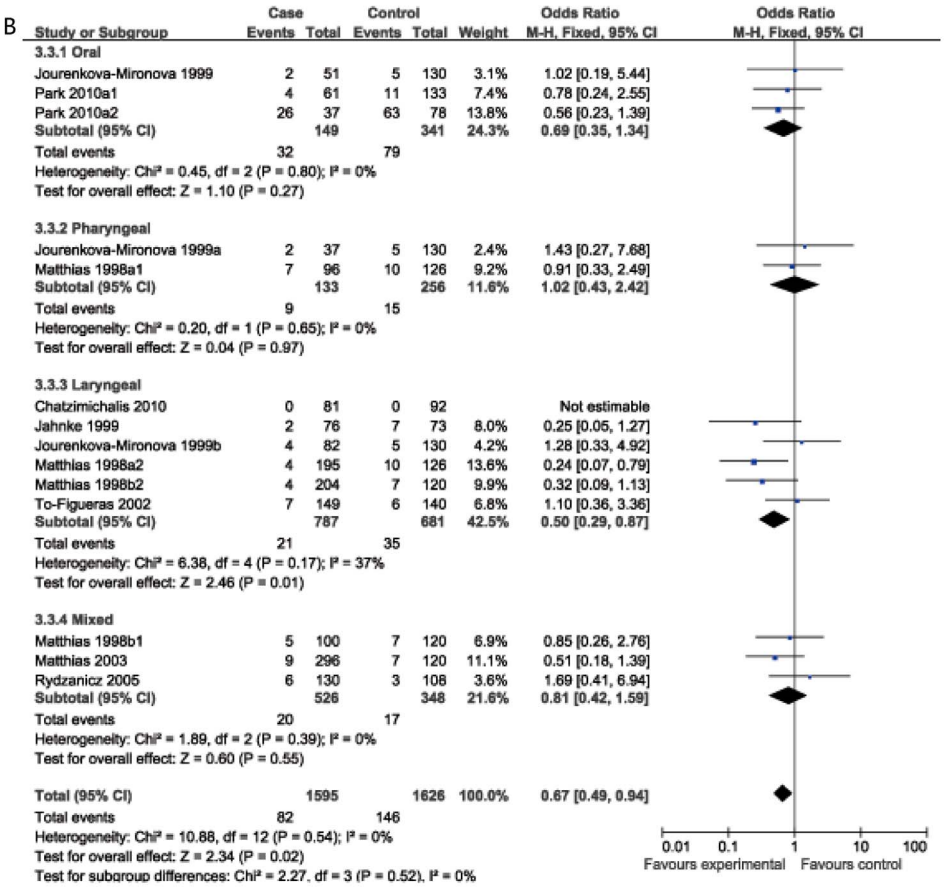

Case Control Odds Ratio Odds Ratio
Study or Subgroup Events Total Events Total Weight M-H, Fixed, 95% CI
M-H, Fixed, 95% CI
3.3.1 Oral
Jourenkova-Mironova 1999 2 51 5 130 3.1% 1.02 [0.19, 5.44]
Park 2010a1 4 61 11 133 7.4% 0.78 [0.24, 2.55]
Park 2010a2 26 37 63 78 13.8% 0.56 [0.23, 1.39]
Subtotal (95% CI) 149 341 24.3% 0.69 [0.35, 1.34]
Total events 32 79
Heterogeneity: Chi² = 0.45, df = 2 (P = 0.80); I² = 0%
Test for overall effect: Z = 1.10 (P = 0.27)
3.3.2 Pharyngeal
Jourenkova-Mironova 1999a 2 37 5 130 2.4% 1.43 [0.27, 7.68]
Matthias 1998a1 7 96 10 126 9.2% 0.91 [0.33, 2.49]
Subtotal (95% CI) 133 256 11.6% 1.02 [0.43, 2.42]
Total events 9 15
Heterogeneity: Chi² = 0.20, df = 1 (P = 0.65); I² = 0%
Test for overall effect: Z = 0.04 (P = 0.97)
3.3.3 Laryngeal
Chatzimichalis 2010 0 81 0 92 Not estimable
Jahnke 1999 2 76 7 73 8.0% 0.25 [0.05, 1.27]
Jourenkova-Mironova 1999b 4 82 5 130 4.2% 1.28 [0.33, 4.92]
Matthias 1998a2 4 195 10 126 13.6% 0.24 [0.07, 0.79]
Matthias 1998b2 4 204 7 120 9.9% 0.32 [0.09, 1.13]
To-Figueras 2002 7 149 6 140 6.8% 1.10 [0.36, 3.36]
Subtotal (95% CI) 787 681 42.5% 0.50 [0.29, 0.87]
Total events 21 35
Heterogeneity: Chi² = 6.38, df = 4 (P = 0.17); I² = 37%
Test for overall effect: Z = 2.46 (P = 0.01)
3.3.4 Mixed
Matthias 1998b1 5 100 7 120 6.9% 0.85 [0.26, 2.76]
Matthias 2003 9 296 7 120 11.1% 0.51 [0.18, 1.39]
Rydzanicz 2005 6 130 3 108 3.6% 1.69 [0.41, 6.94]
Subtotal (95% CI) 526 348 21.6% 0.81 [0.42, 1.59]
Total events 20 17
Heterogeneity: Chi² = 1.89, df = 2 (P = 0.39); I² = 0%
Test for overall effect: Z = 0.60 (P = 0.55)
Total (95% CI) 1595 1626 100.0% 0.67 [0.49, 0.94]
Total events 82 146
Heterogeneity: Chi² = 10.88, df = 12 (P = 0.54); I² = 0%
Test for overall effect: Z = 2.34 (P = 0.02)
Test for subgroup differences: Chi² = 2.27, df = 3 (P = 0.52), I² = 0%
0.01 0.1 1 10 100
Favours experimental Favours control

Figure 3. Subgroup analysis by tumor site of ORs with a fixed-effects model for association between GSTM3 polymorphism and HNC risk. (**A** recessive model; **B** BB *vs.* AA).
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model: $P = 0.574$, recessive model: $P = 0.748$, AB *vs.* AA: $P = 0.718$, BB *vs.* AA: $P = 0.816$).

Discussion

To our knowledge, this is the first meta-analysis which comprehensively assessed the associations between GSTM3 A/B polymorphism and HNC risk. In this study, we found significant associations in the overall comparison using dominant model, homozygote comparison model and heterozygote comparison model. Individuals with the AB/BB genotype could have a decreased risk of HNC. Moreover, in the stratified analyses by several variables, including ethnicity, tumor site, and source of the controls, significant associations were observed in the Caucasian population, laryngeal cancer and hospital-based population.

GSTM3, a major member of the GSTs family, plays a role in metabolism of harmful agents, like polyaromatic hydrocarbons benzo(a)pyrene. The GSTM3 polymorphism could, therefore, confer different efficiencies in the metabolism of carcinogens and has been shown to modulate various cancers risk. Interestingly, it plays different roles in different cancers. Jain et al [28] reported patients who were heterozygous carriers of GSTM3 AB genotype had an enhanced risk for developing esophageal cancer. Loktionov et al [29] found that the GSTM3 B variant presence especially in combination with the GSTM1-null genotype is a risk factor for colorectal carcinogenesis, and Holley et al [30] suggested the GSTM3 AA genotype is associated with improved prognosis especially in those with GSTM1 null. While, some studies found that no significant association between GSTM3 polymorphism and lung [31], gallbladder [32] and adult brain tumor [33] risk was observed. For head and neck cancer, in a recent study, an increase in larynx cancer risk associated with GSTM3 AA genotype was suggested [14], similarly, Chatzimichalis et al [9] found that the presence of the GSTM3 B allele appears to be associated with a reduced risk of laryngeal SCC in a Greek population. Majumder et al [20] reported the GSTM3 AA genotype could increase the risk of oral leukoplakia and cancer among smokers; in contrast, Jourenkova-Mironova et al [11] found the GSTM3 AA genotype was not associated with oropharyngeal cancer risk, and Buch et al [19] reported that no association between the GSTM3 alleles and oral cancer risk was observed. These inconsistent results may be attributed to differences in genetic backgrounds, environmental factors, and other factors, such as small sample size or inadequate adjustment for confounding factors.

In this meta-analysis, we found that individuals with AB+BB genotype had a lower risk of developing HNC under dominant model, besides in the stratified analyses by ethnicity, tumor site, and source of control, we found that B allele carriers had a lower risk of HNC than A allele carriers in Caucasian population, laryngeal cancer and hospital-based population. The results may be explained that the B allele carriers could increase transcription of the GSTM3 gene and expression of GSTM3-related protein, then enhance detoxification activity. In addition, the pathways of carcinogen metabolism are complex, mediated by the activities of multiple genes (such as GSTM1, and CYP1A1) [34,35]. GSTM3 is known to have overlapping substrate specificities with GSTM1 and GSTP1. And GSTM1/GSTP1 effects may be modulated by the GSTM3 genotype [36]. Individuals carrying variant genotypes on these loci might have higher levels of DNA adducts in the exposed tissues [37]. Consequently, there is a possibility that smokers carrying the GSTM1 or GSTP1 or GSTM3 risk genotype will be susceptible to cancer if the DNA adducts remain unrepaired. However, the specific role needs to be tested in future studies.

It would be hard to interpret results, if significant heterogeneity were present, in this meta-analysis, we did not find any obvious heterogeneity and publish bias across studies. While, it's worth noting that there was moderate heterogeneity among overall

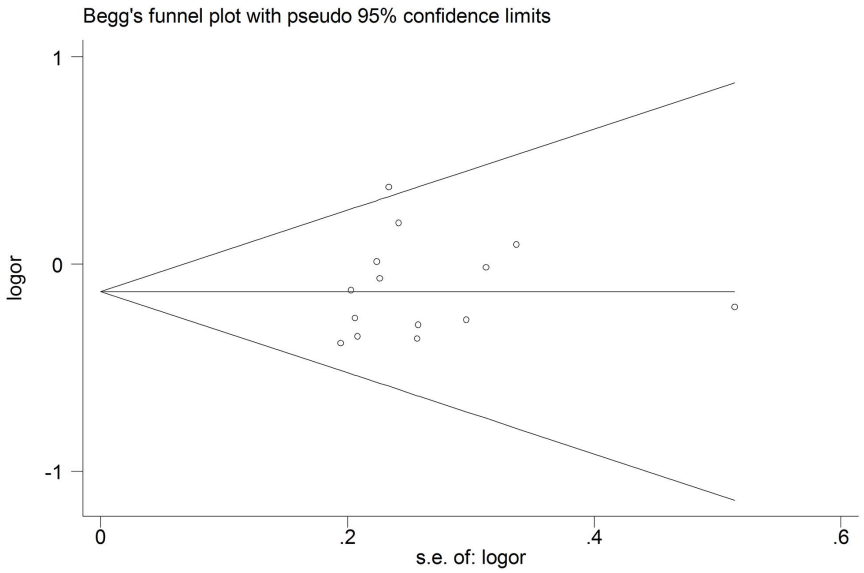

Figure 4. Begg's funnel plot for publication bias test. Each point represents an independent study for the indicated association under the dominant model.
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studies under dominant model, so stratified analysis by ethnicity, tumor cites and the source of the controls was performed and we found that moderate heterogeneity still exists in Caucasian population, laryngeal cancer and mixed HNC, however, heterogeneity significantly reduced or removed among oral, pharyngeal cancer, population-based population and hospital-based populations. The results above suggest that control selection and different tumor types may contribute to the moderate heterogeneity observed in the meta-analysis. Then sensitivity analyses were conducted by excluding one study successively, the estimated pooled OR changed quite little, strengthening the results from this meta-analysis.

Although considerable effort was made to test for the possible association between the GSTM3 A/B polymorphism and risk of HNC, some limitations of this meta-analysis should also be addressed. First, due to limited detailed data presented in the published studies, the potential effect of important risk factors to HNC was not examined, such as smoking, alcohol consumption and HPV status. Second, all studies are focused on Caucasian populations, which may generate selective bias. Third, our results were based on unadjusted estimates, without adjustment for age, gender, family history and other risk factors, while lacking of the information for the date analysis may cause serious confounding bias. Fourth, GSTM3 may influence susceptibility to HNC independently or with other genes such as GSTM1 and GSTP1. However, due to lack of individual data in the present review, we did not perform more detailed analyses, such as analyses of joint effects with other risk factors or gene-gene or gene-environment interactions.

In summary, this meta-analysis indicates that the GSTM3 A/B polymorphism may be an important protective factor for HNC, especially of laryngeal cancer and Caucasian populations. Further studies with standardized unbiased genotyping methods, homogeneous cancer patients, well-matched controls and multiethnic groups would be warranted.

Supporting Information

Checklist S1 PRISMA Checklist for this study.
(DOC)

Checklist S2 MOOSE Checklist for this study.
(DOC)

Author Contributions

Conceived and designed the experiments: W-GD YX. Performed the experiments: YX. Analyzed the data: JW. Contributed reagents/materials/analysis tools: YX JW. Wrote the paper: YX JW.

References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, et al. (2011) Global cancer statistics. CA Cancer J Clin 61:69–90.
2. Sankaranarayanan R, Masuyer E, Swaminathan R, Ferlay J, Whelan S (1998) Head and neck cancer: a global perspective on epidemiology and prognosis. Anticancer Res 18:4779–4786.
3. Kim L, King T, Agulnik M (2010) Head and neck cancer: changing epidemiology and public health implications. Oncology (Williston Park) 24:915–919,924.
4. Ketterer B (1988) Protective role of glutathione and glutathione transferases in mutagenesis and carcinogenesis. Mutat Res 202: 343–361.
5. Shi Y, Lee JS, Galvin KM (1997) Everything you have ever wanted to know about Yin Yang 1…… Biochim Biophys Acta 1332:F49–F66.
6. Yengi L, Inskip A, Gilford J, Alldersea J, Bailey L, et al. (1996) Polymorphism at the glutathione S-transferase locus GSTM3: interactions with cytochrome P450 and glutathione S-transferase genotypes as risk factors for multiple cutaneous basal cell carcinoma. Cancer Res 56:1974–1977.
7. Inskip A, Elexperu-Camiruaga J, Buxton N, Dias PS, MacIntosh J, et al. (1995) Identification of polymorphism at the glutathione S-transferase, GSTM3 locus: evidence for linkage with GSTM1*A. Biochem J 312:713–716.
8. Jahnke V, Strange R, Matthias C, Fryer A (1997) Glutathione S-transferase and cytochrome P450 genotypes as a risk factors for laryngeal carcinoma. Eur Arch Otorhinolaryngol 254:147–149.
9. Chatzimichalis M, Xenellis J, Tzagaroulakis A, Sarof P, Banis K, et al. (2010) GSTT1, GSTM1, GSTM3 and NAT2 polymorphisms in laryngeal squamous cell carcinoma in a Greek population. J Laryngol Otol 124:318–323.
10. Jahnke V, Matthias C, Fryer A, Strange R (1996) Glutathione S-transferase and cytochrome-P-450 polymorphism as risk factors for squamous cell carcinoma of the larynx. Am J Surg 172: 671–673.
11. Jourenkova-Mironova N, Voho A, Bouchardy C, Wikman H, Dayer P, et al. (1999a) Glutathione S-transferase GSTM1, GSTM3, GSTP1 and GSTT1 genotypes and the risk of smoking-related oral and pharyngeal cancers. Int J Cancer 81: 44–48.
12. Jourenkova-Mironova N, Voho A, Bouchardy C, Wikman H, Dayer P, et al. (1999b) Glutathione S-transferase GSTM3 and GSTP1 genotypes and larynx cancer risk. Cancer Epidemiol Biomarkers Prev 8:185–188.
13. Matthias C, Bockmühl U, Jahnke V, Petersen I, Dietel M, et al. (1998a) [Effect of gene polymorphism on detoxifying glutathione-S-transferase enzymes on chromosomal stability of squamous epithelial carcinomas in the area of the head-neck]. Laryngorhinootologie 77: 201–206. (Article in German)
14. Matthias C, Bockmühl U, Jahnke V, Jones PW, Hayes JD, et al. (1998b) Polymorphism in cytochrome P450 CYP2D6, CYP1A1, CYP2E1 and glutathione S-transferase, GSTM1, GSTM3, GSTT1 and susceptibility to tobacco-related cancers: studies in upper aerodigestive tract cancers. Pharmacogenetics 8: 91–100.
15. Matthias C, Jahnke V, Fryer AA, Strange RC (2003) [First results on the influence of polymorphisms at glutathione S-transferase, cytochrome P450, and tumor necrosis factor gene loci on the development of multiple head and neck cancer]. Laryngorhinootologie 82:25–30. (Article in German)
16. Park LY, Muscat JE, Kaur T, Schantz SP, Stern JC, et al. (2000) Comparison of GSTM polymorphisms and risk for oral cancer between African-Americans and Caucasians. Pharmacogenetics 10:123–131.
17. Rydzanicz M, Wierzbicka M, Gajecka M, Szyfter W, Szyfter K (2005) The impact of genetic factors on the incidence of multiple primary tumors (MPT) of the head and neck. Cancer Lett 224: 263–278.
18. To-Figueras J, Gené M, Gómez-Catalán J, Piqué E, Borrego N, et al. (2002) Microsomal epoxide hydrolase and glutathione S-transferase polymorphisms in relation to laryngeal carcinoma risk. Cancer Lett 187: 95–101.
19. Buch SC, Notani PN, Bhisey RA (2002) Polymorphism at GSTM1, GSTM3 and GSTT1 gene loci and susceptibility to oral cancer in an Indian population. Carcinogenesis 23: 803–807.
20. Majumder M, Sikdar N, Paul RR, Roy B (2005) Increased risk of oral leukoplakia and cancer among mixed tobacco users carrying XRCC1 variant haplotypes and cancer among smokers carrying two risk genotypes: one on each of two loci, GSTM3 and XRCC1 (Codon 280). Cancer Epidemiol Biomarkers Prev 14: 2106–2112.
21. Marques CF, Koifman S, Koifman RJ, Boffetta P, Brennan P, et al. (2006) Influence of CYP1A1, CYP2E1, GSTM3 and NAT2 genetic polymorphisms in oral cancer susceptibility: results from a case-control study in Rio de Janeiro. Oral Oncol 42: 632–637.
22. Sikdar N, Paul RR, Roy B (2004) Glutathione S-transferase M3 (A/A) genotype as a risk factor for oral cancer and leukoplakia among Indian tobacco smokers. Int J Cancer 109: 95–101.
23. Lau J, Ioannidis JP, Schmid CH (1997) Quantitative synthesis in systematic reviews. Ann Intern Med 127: 820–826.
24. DerSimonian R, Laird N (1986) Meta-analysis in clinical trials. Control Clin Trials 7: 177–188.
25. Mantel N, Haenszel W (1959) Statistical aspects of the analysis of data from retrospective studies of disease. J Natl Cancer Inst 22: 719–748.
26. Begg CB, Mazumdar M (1994) Operating characteristics of a rank correlation test for publication bias. Biometrics 50:1088–1101.
27. Egger M, Davey Smith G, Schneider M, Minder C (1997) Bias in meta-analysis detected by a simple, graphical test. BMJ 315: 629–634.
28. Jain M, Kumar S, Lal P, Tiwari A, Ghoshal UC, et al. (2007) Role of GSTM3 polymorphism in the risk of developing esophageal cancer. Cancer Epidemiol Biomarkers Prev 16:178–181.
29. Loktionov A, Watson MA, Gunter M, Stebbings WS, Speakman CT, et al. (2001) Glutathione-S-transferase gene polymorphisms in colorectal cancer patients: interaction between GSTM1 and GSTM3 allele variants as a risk-modulating factor. Carcinogenesis 22:1053–1060.
30. Holley SL, Rajagopal R, Hoban PR, Deakin M, Fawole AS, et al. (2006) Polymorphisms in the glutathione S-transferase mu cluster are associated with tumour progression and patient outcome in colorectal cancer. Int J Oncol 28:231–236.
31. Saarikoski ST, Voho A, Reinikainen M, Anttila S, Karjalainen A, et al. (1998) Combined effect of polymorphic GST genes on individual susceptibility to lung cancer. Int J Cancer 77:516–521.

32. Pandey SN, Jain M, Nigam P, Choudhuri G, Mittal B (2006) Genetic polymorphisms in GSTM1, GSTT1, GSTP1, GSTM3 and the susceptibility to gallbladder cancer in North India. Biomarkers 11:250–261.
33. Schwartzbaum JA, Ahlbom A, Lönn S, Warholm M, Rannug A, et al. (2007) An international case-control study of glutathione transferase and functionally related polymorphisms and risk of primary adult brain tumors. Cancer Epidemiol Biomarkers Prev 16:559–565.
34. Hirvonen A (1999) Polymorphisms of xenobiotic-metabolizing enzymes and susceptibility to cancer. Environ Health Perspect 107: 37–47.
35. Wu X, Zhao H, Suk R, Christiani DC (2004) Genetic susceptibility to tobacco-related cancer. Oncogene 23: 6500–6523.
36. Hayes JD, Pulford DJ (1995) The glutathione *S*-transferase supergene family: regulation of GST and contribution of isoenzymes to cancer chemoprotection and drug resistance. Crit Rev Biochem Mol Biol 30: 445–600.
37. Butkiewicz D, Grzybowska E, Phillips DH, Hemminki K, Chorazy M (2000) Polymorphisms of the GSTP1 and GSTM1genes and PAH-DNA adducts in human mononuclear white blood cells. Environ Mol Mutagen 35: 99–105.