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Association between GSTM1 and GSTT1 Allelic Variants and Head and Neck Squamous Cell Cancinoma

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Abstract

Backgrounds: GSTM1 and GSTT1 are involved in the detoxification of carcinogens such as smoking by-products, and polymorphisms in these two genes with a result of loss of enzyme activity may increase risk of carcinogenesis. Although many epidemiological studies have investigated the association between GSTM1 or GSTT1 null genotype and head and neck squamous cell carcinoma (HNSCC), the results remain conflicting. To elucidate the overall association of GSTM1, GSTT1 and HNSCC, we included all available studies and performed this meta-analysis.

Methodology/Principal Findings: A dataset including 42 articles for GSTM1, 32 articles for GSTT1, and 15 articles for GSTM1 and GSTT1 in combination were identified by a search in PubMed. Associations beween HNSCC and polymorphisms of GSTM1 and GSTT1 alone and in combination were analysed by software RevMan 5.1. Stratification analysis on ethnicity and smoking status, sensitivity analysis, heterogeneity among studies and their publication bias were also tested. Association was found in overall analysis between HNSCC and GSTM1 and GSTT1 null genotype. Stratified by ethnicity, we found increased risks of HNSCC in carriers with GSTM1 null genotype in Asian, GSTT1 null genotype in South American, and dual null genotype in European and Asian. When stratified by smoking, a more significant association of GSTM1 null genotype with HNSCC risk was observed in smokers.

Conclusions/Significance: This meta-analysis presented additional evidence of the association between GSTM1 and GSTT1 polymorphisms and HNSCC risk.

Introduction

Head and neck neoplasms are the sixth leading cause of death by cancer [1]. The most common histological type is the squamous cell carcinoma, accounting for about 90% of all cases [2,3]. Being a multifactorial disease, the etiology of head and neck squamous cell carcinoma (HNSCC) is still a much debated question. Smoking of cigarettes, consumption of alcohol and genetic causes are some of the foci of former etiological studies.

Enzymes of the glutathione S-transferase (GST) family are present in eukaryotes and in prokaryotes, which are composed of many cytosolic, mitochondrial, and microsomal proteins. They catalyze various reactions and participate in the phase II biotransformation of xenobiotics. GSTs contribute to the detoxification of by-products of smoking and alcohol and other exogenous chemical carcinogens which may induce HNSCC, so they have been considered as potential candidates for HNSCC susceptibility. Classes *ı* and *μ* of the GST superfamily have been paid lots of attention, which are encoded by GSTT1 and GSTM1 genes. The GSTM1 and GSTT1 gene have been localized to chromosome 1p13.3 and 22q11.2. Both of the genes are polymorphic and frequent homozygous deletions of the genes

presenting null genotype are associated with loss of the corresponding enzyme activity. Therefore, carriers with null genotype will increase the risk of the development of HNSCC due to the decreased ability to detoxify carcinogens theoretically.

In 2003, a meta-analysis conducted by Hashibe *et al.* indicated modest associations of GSTM1 and GSTT1 genotypes with head and neck cancer risk [4]. However, more than twenty independent studies from various populations have further examined the relationships between these two genes and HNSCC risk, and still reported conflicting results. Some studies in HNSCC have indicated that the null genotype of GSTM1 or GSTT1 is a risk factor of HNSCC development [5–7]. However, such an association was not observed in some other groups [8–10]. Therefore, it is necessary to reevaluate the association of GSTM1 or GSTT1 null genotype with the risk of HNSCC by pooling the new published studies using meta-analysis. The present study included all eligible published case-control studies to establish a relatively comprehensive picture of the relationship between these two genes and HNSCC.

Materials and Methods

Selection criteria and identification of eligible studies

Candidate studies were identified through computer-aided literature searches in PubMed for relevant articles in English and Chinese (1995 to May 2012). To identify all articles that studied the association of *GSTT1* and *GSTM1* polymorphisms with HNSCC, we conducted the search using the following keywords and subject terms: 'GSTT1' or 'GSTM1', and 'squamous'. We also searched the references cited in the articles and included published works. Abstracts, case-only articles, editorials, review articles and repeated literatures were excluded. Of the articles with the overlapping data, we only included the publication with the most extensive information. The inclusion criteria in the current meta-analysis were as follows: (a) they are unrelated studies; (b) identification of squamous cell carcinoma was histologically confirmed; and (c) they have original data of genotype frequency and provided sufficient information to calculate the odds ratio (OR) or P-value.

Data extraction

Two reviewers (Zhang Y and Ni Y) independently examined the studies for inclusion in the meta-analysis and collected data on the genotype of *GSTT1* and *GSTM1*. We extracted the following information from each study: first author, year of publication, country, ethnicity, numbers of case and control, smoking status and genotyping information. Disagreements between two reviewers were discussed and resolved with consensus. When essential information was not found in articles, we made effort to get the data from the authors (Figure 1).

Statistical analysis

The meta-analysis for *GSTM1* or *GSTT1* null genotype or dual null genotype compared HNSCC vs. controls. Odds ratio (OR) and its 95% confidence interval (CI) were assessed for each study. The Cochran's Q-statistic was used to test heterogeneity, and the heterogeneity was considered statistically significant when $P<0.1$ [11]. The Mantel-Haenszel method was used to calculate the OR for the included data in a fixed effects model in the absence of between-study heterogeneity, while random effects model was used for those with heterogeneity. P-value<0.05 was considered statistically significant, and $0.05\leq$P-value<0.10 was indicated suggestive. In addition, we also performed stratification analyses on ethnicity, smoking and combined analyses of *GSTM1* and *GSTT1* on HNSCC risk. The sensitivity analysis was carried out to test the stability of the pooled effect after excluding individual studies. Begg's funnel plot was used to evaluate publication bias.
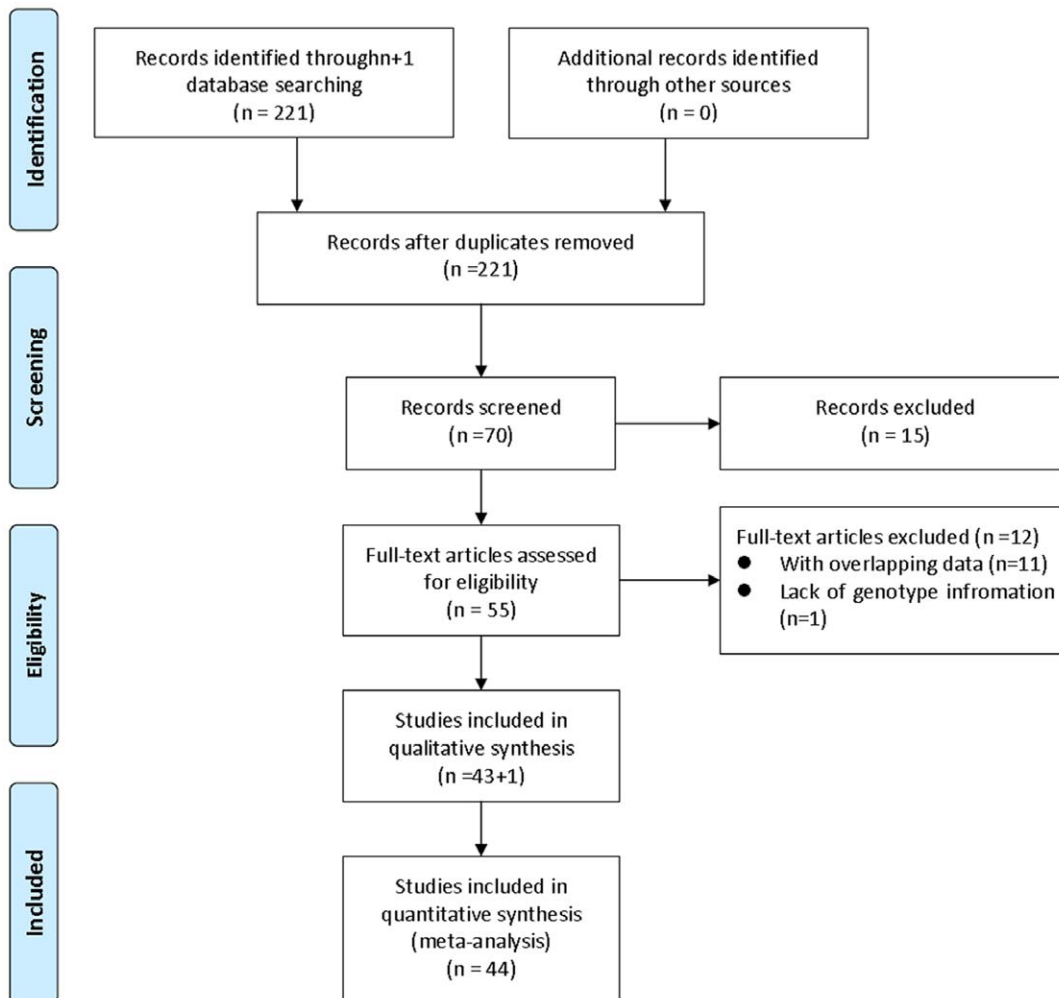

Figure 1. Flow diagram of study identification.
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Table 1. Characteristics of studies included in meta-analysis.

Author (Ref)	Year	Country	Ethnicity	Case	Control	Whether has genotype distribution information			
						GSTM1	GSTT1	Dual genes	Tobacco consumption
Jahnke et al. (23)	1996	Germany	European	269	216	Yes	Yes	No	No
Park et al. (24)	1997	USA	European	133	133	Yes	No	No	No
González et al. (25)	1998	Spain	European	75	200	Yes	No	No	No
Oude Ophuis et al. (13)	1998	Netherlands	European	185	207	Discarded	Discarded	Yes	No
Cheng et al. (26)	1999	USA	European	162	315	Yes	Yes	Yes	No
Katoh et al. (27)	1999	Japan	Asian	92	147	Yes	Yes	No	No
Morita et al. (28)	1999	Japan	Asian	145	164	Yes	No	No	No
Nazar-Stewart et al. (29)	1999	USA	European	48	144	Yes	No	No	Yes
Sato et al. (30)	1999	Japan	Asian	142	142	Yes	No	No	No
Tanimoto et al. (31)	1999	Japan	Asian	100	100	Yes	No	No	No
Hamel et al. (32)	2000	Canada	European	90	90	Yes	Yes	No	No
Olshan et al. (33)	2000	USA	European	182	202	Yes	Yes	No	Yes
Hahn et al. (34)	2002	Germany	European	94	92	Yes	No	No	No
To-Figueras et al. (35)	2002	Spain	European	204	203	Yes	Yes	No	No
Gronau et al. (36)	2003	Germany	European	187	139	Yes	Yes	Yes	No
Drummond et al. (37)	2004	Brazil	South American	70	82	Yes	No	No	No
Evans et al. (38)	2004	USA	European	283	208	Yes	Yes	No	Yes
Li et al. (39)	2004	China	Asian	89	164	Yes	No	No	No
Drummond et al. (40)	2005	Brazil	South American	87	81	No	Yes	No	No
Gajecka et al. (41)	2005	Poland	European	292	321	Yes	Yes	No	No
Acar et al. (42)	2006	Turkey	Asian	110	197	Yes	Yes	No	No
Biselli et al. (10)	2006	Brazil	South American	60	60	Yes	Yes	Yes	No
Gatta's et al. (43)	2006	Brazil	South American	103	102	Yes	Yes	Yes	No
Oude Ophuis et al. (44)	2006	Netherlands	European	185	285	Yes	Yes	No	No
Peters et al. (45)	2006	USA	European	692	753	Yes	Yes	No	Yes
Sharma et al. (46)	2006	India	Asian	40	87	Yes	Yes	No	Yes
Sugimura et al. (47)	2006	Japan	Asian	122	241	Yes	Yes	No	Yes
Anatharaman et al. (48)	2007	India	Asian	451	727	Yes	Yes	No	Yes
Cha et al. (49)	2007	Korea	Asian	72	209	Yes	No	No	Yes
Suzen et al. (8)	2007	Turkey	Asian	98	120	Yes	Yes	Yes	Yes
Boccia et al. (9)	2008	Italy	European	210	245	Yes	Yes	No	No
Buch et al. (50)	2008	USA	European	196	414	Yes	Yes	No	No
Harth et al. (51)	2008	Germany	European	312	300	Yes	Yes	No	No
Hatagima et al. (52)	2008	Brazil	South American	231	212	Yes	Yes	No	No
Losi-Guembarovski et al. (53)	2008	Brazil	South American	91	81	Yes	Yes	No	No
Amtha et al. (54)	2009	Indonesia	Asian	81	162	Yes	Yes	No	No
Li et al. (55)	2009	China	Asian	76	76	Yes	Yes	No	Yes
Chatzimichalis et al. (56)	2010	Greek	European	88	102	Yes	Yes	No	No
Leme et al. (57)	2010	Brazil	South American	100	100	Yes	Yes	Yes	No
Sam et al. (58)	2010	India	Asian	408	220	Yes	Yes	Yes	No
Soucek et al. (59)	2010	Czech	European	122	179	Yes	Yes	No	No
Lourenço et al. (6)	2011	Brazil	South American	142	142	Yes	Yes	No	No
Ruwali et al. (5)	2011	India	Asian	500	500	Yes	Yes	Yes	Yes
Shukla et al. (60)	2012	India	Asian	150	141	Yes	No	No	No

Study or Subgroup	Case Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Random, 95% CI	Year
Jahnke et al. 1996	151	269	112	216	2.8%	1.19 [0.83, 1.70]	1996
Park et al. 1997	68	133	68	133	2.4%	1.00 [0.62, 1.62]	1997
González et al. 1998	44	75	103	200	2.2%	1.34 [0.78, 2.29]	1998
Morita et al. 1999	71	145	83	164	2.5%	0.94 [0.60, 1.46]	1999
Tanimoto et al. 1999	43	100	42	100	2.1%	1.04 [0.59, 1.83]	1999
Katoh et al. 1999	54	92	68	147	2.2%	1.65 [0.98, 2.80]	1999
Cheng et al. 1999	86	162	135	315	2.7%	1.51 [1.03, 2.21]	1999
Sato et al. 1999	92	142	64	142	2.4%	2.24 [1.39, 3.61]	1999
Nazar-Stewart et al. 1999	26	48	63	142	1.8%	1.48 [0.77, 2.86]	1999
Hamel et al. 2000	51	90	52	90	2.0%	0.96 [0.53, 1.72]	2000
Olshan et al. 2000	76	176	88	195	2.6%	0.92 [0.61, 1.39]	2000
Hahn et al. 2002	56	94	49	92	2.1%	1.29 [0.72, 2.31]	2002
To-Figureras et al. 2002	96	204	100	203	2.7%	0.92 [0.62, 1.35]	2002
Gronau et al. 2003	80	187	68	139	2.5%	0.78 [0.50, 1.21]	2003
Evans et al. 2004	136	282	102	208	2.8%	0.97 [0.68, 1.39]	2004
Drummond et al. 2004	46	70	40	82	1.8%	2.01 [1.04, 3.88]	2004
Li et al. 2004	50	89	69	164	2.2%	1.77 [1.05, 2.97]	2004
Gajecka et al. 2005	140	292	164	321	2.9%	0.88 [0.64, 1.21]	2005
Acar et al. 2006	57	110	74	197	2.4%	1.79 [1.11, 2.87]	2006
Sharma et al. 2006	21	40	29	87	1.6%	2.21 [1.03, 4.75]	2006
Gatta´s et al. 2006	59	103	39	102	2.1%	2.17 [1.24, 3.79]	2006
Peters et al. 2006	403	692	404	753	3.3%	1.20 [0.98, 1.48]	2006
Biselli et al. 2006	25	60	29	60	1.7%	0.76 [0.37, 1.57]	2006
Sugimura et al. 2006	59	122	126	241	2.5%	0.85 [0.55, 1.32]	2006
Oude Ophuis et al. 2006	94	185	163	285	2.7%	0.77 [0.53, 1.12]	2006
Cha et al. 2007	37	72	123	209	2.2%	0.74 [0.43, 1.27]	2007
Anatharaman et al. 2007	198	451	269	727	3.2%	1.33 [1.05, 1.69]	2007
Suzen et al. 2007	67	98	57	120	2.1%	2.39 [1.37, 4.17]	2007
Hatagima et al. 2008	95	231	93	212	2.7%	0.89 [0.61, 1.30]	2008
Harth et al. 2008	166	612	145	300	3.0%	0.40 [0.30, 0.53]	2008
Losi-Guembaro et al. 2008	36	91	42	81	2.0%	0.61 [0.33, 1.11]	2008
Boccia et al. 2008	112	210	128	245	2.7%	1.04 [0.72, 1.51]	2008
Buch et al. 2008	126	196	276	414	2.8%	0.90 [0.63, 1.29]	2008
Li et al. 2009	45	76	32	76	1.9%	2.00 [1.05, 3.81]	2009
Amtha et al. 2009	49	81	90	162	2.2%	1.23 [0.71, 2.11]	2009
Leme et al. 2010	66	100	75	100	2.0%	0.65 [0.35, 1.19]	2010
Soucek et al. 2010	64	116	70	122	2.3%	0.91 [0.55, 1.53]	2010
Chatzimichal et al. 2010	74	88	88	102	1.5%	0.84 [0.38, 1.88]	2010
Sam et al. 2010	109	408	48	220	2.7%	1.31 [0.89, 1.93]	2010
Ruwali et al. 2011	236	500	160	500	3.1%	1.90 [1.47, 2.46]	2011
Lourenço et al. 2011	58	142	66	142	2.4%	0.80 [0.50, 1.27]	2011
Shukla et al. 2012	51	150	27	141	2.2%	2.18 [1.27, 3.73]	2012
Total (95% CI)		**7584**		**8651**	**100.0%**	**1.14 [1.00, 1.29]**	
Total events	3673		4123				

Heterogeneity: Tau² = 0.12; Chi² = 147.35, df = 41 (P < 0.00001); I² = 72%
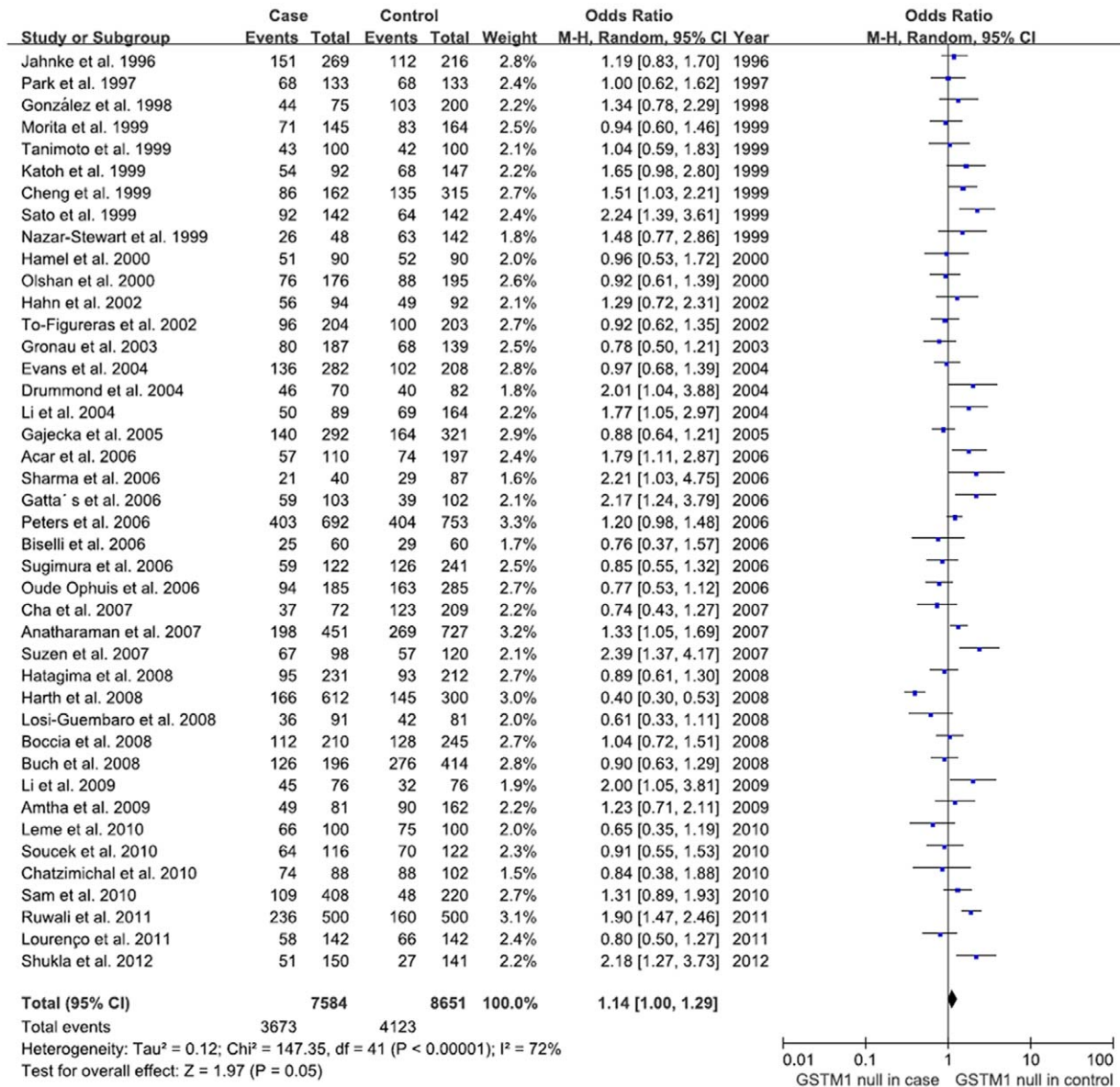Test for overall effect: Z = 1.97 (P = 0.05)

Figure 2. Forest plot of *GSTM1* associated with HNSCC under random-effects model. Each study is shown by point estimate of OR and 95% CI by a horizontal line. The diamond shows the overall risk and the line represent the 95% CI for each meta-analysis. Events: null genotype.
doi:10.1371/journal.pone.0047579.g002

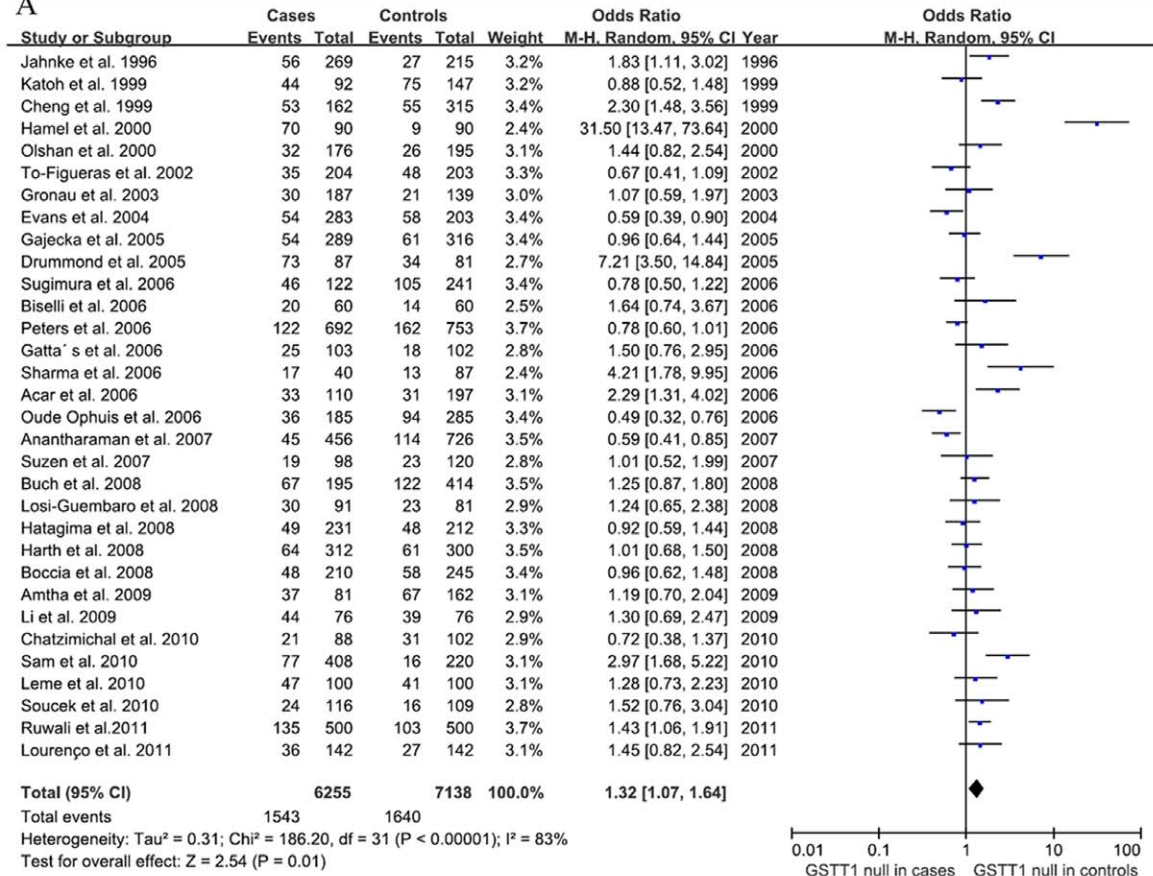**Table 2** Genotype distribution of *GSTM1* and *GSTT1* in different Ethnicities.

	GSTM1			GSTT1			GSTM1+GSTT1		
	European	Asian	South Am	European	Asian	South Am	European	Asian	South Am
Cases (n/N[a])	2049/4111	1239/2676	385/797	766/3458	497/1983	280/814	83/534	200/1769	48/354
Controls (n/N)	2378/4475	1361/3397	384/779	849/3884	586/2476	205/778	57/661	185/2088	48/343
OR[b]	0.96	1.48	1.05	1.21	1.32	1.63	2.01	1.56	0.96
95% CI[c]	0.82–1.13	1.24–1.75	0.71–1.57	0.87–1.69	0.93–1.88	1.03–2.58	1.15–3.53	1.05–2.33	0.62–1.48
P[d]	0.64	<0.00001**	0.80	0.26	0.12	0.04*	0.01*	0.03*	0.85

Abbreviations: [a], number of carriers with null genotype/ total number; [b], odds ratio; [c], confidence interval; [d], value for heterogeneity; OR, odds ratio; 95% CI, 95% confidence interval.
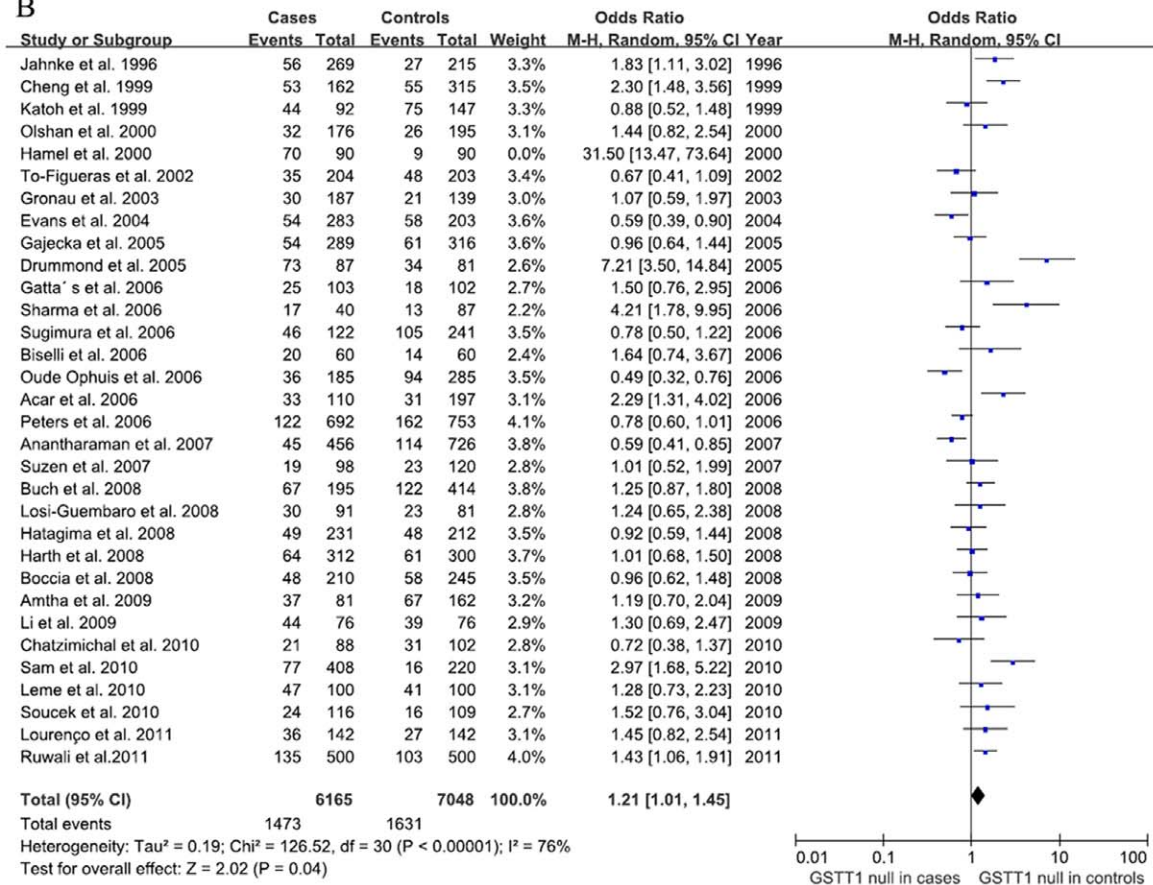**P <0.01; * 0.01≤P<0.05
doi:10.1371/journal.pone.0047579.t002

A

Study or Subgroup	Cases Events	Total	Controls Events	Total	Weight	Odds Ratio M-H, Random, 95% CI	Year
Jahnke et al. 1996	56	269	27	215	3.2%	1.83 [1.11, 3.02]	1996
Katoh 1999	44	92	75	147	3.2%	0.88 [0.52, 1.48]	1999
Cheng et al. 1999	53	162	55	315	3.4%	2.30 [1.48, 3.56]	1999
Hamel et al. 2000	70	90	9	90	2.4%	31.50 [13.47, 73.64]	2000
Olshan et al. 2000	32	176	26	195	3.1%	1.44 [0.82, 2.54]	2000
To-Figueras et al. 2002	35	204	48	203	3.3%	0.67 [0.41, 1.09]	2002
Gronau et al. 2003	30	187	21	139	3.0%	1.07 [0.59, 1.97]	2003
Evans et al. 2004	54	283	58	203	3.4%	0.59 [0.39, 0.90]	2004
Gajecka et al. 2005	54	289	61	316	3.4%	0.96 [0.64, 1.44]	2005
Drummond et al. 2005	73	87	34	81	2.7%	7.21 [3.50, 14.84]	2005
Sugimura et al. 2006	46	122	105	241	3.4%	0.78 [0.50, 1.22]	2006
Biselli et al. 2006	20	60	14	60	2.5%	1.64 [0.74, 3.67]	2006
Peters et al. 2006	122	692	162	753	3.7%	0.78 [0.60, 1.01]	2006
Gatta´s et al. 2006	25	103	18	102	2.8%	1.50 [0.76, 2.95]	2006
Sharma et al. 2006	17	40	13	87	2.4%	4.21 [1.78, 9.95]	2006
Acar et al. 2006	33	110	31	197	3.1%	2.29 [1.31, 4.02]	2006
Oude Ophuis et al. 2006	36	185	94	285	3.4%	0.49 [0.32, 0.76]	2006
Anantharaman et al. 2007	45	456	114	726	3.5%	0.59 [0.41, 0.85]	2007
Suzen et al. 2007	19	98	23	120	2.8%	1.01 [0.52, 1.99]	2007
Buch et al. 2008	67	195	122	414	3.5%	1.25 [0.87, 1.80]	2008
Losi-Guembaro et al. 2008	30	91	23	81	2.9%	1.24 [0.65, 2.38]	2008
Hatagima et al. 2008	49	231	48	212	3.3%	0.92 [0.59, 1.44]	2008
Harth et al. 2008	64	312	61	300	3.5%	1.01 [0.68, 1.50]	2008
Boccia et al. 2008	48	210	58	245	3.4%	0.96 [0.62, 1.48]	2008
Amtha et al. 2009	37	81	67	162	3.1%	1.19 [0.70, 2.04]	2009
Li et al. 2009	44	76	39	76	2.9%	1.30 [0.69, 2.47]	2009
Chatzimichal et al. 2010	21	88	31	102	2.9%	0.72 [0.38, 1.37]	2010
Sam et al. 2010	77	408	16	220	3.1%	2.97 [1.68, 5.22]	2010
Leme et al. 2010	47	100	41	100	3.1%	1.28 [0.73, 2.23]	2010
Soucek et al. 2010	24	116	16	109	2.8%	1.52 [0.76, 3.04]	2010
Ruwali et al.2011	135	500	103	500	3.7%	1.43 [1.06, 1.91]	2011
Lourenço et al. 2011	36	142	27	142	3.1%	1.45 [0.82, 2.54]	2011
Total (95% CI)		6255		7138	100.0%	1.32 [1.07, 1.64]	
Total events	1543		1640				

Heterogeneity: Tau² = 0.31; Chi² = 186.20, df = 31 (P < 0.00001); I² = 83%
Test for overall effect: Z = 2.54 (P = 0.01)

Odds Ratio M-H, Random, 95% CI
0.01 0.1 1 10 100
GSTT1 null in cases   GSTT1 null in controls

B

Study or Subgroup	Cases Events	Total	Controls Events	Total	Weight	Odds Ratio M-H, Random, 95% CI	Year
Jahnke et al. 1996	56	269	27	215	3.3%	1.83 [1.11, 3.02]	1996
Cheng et al. 1999	53	162	55	315	3.5%	2.30 [1.48, 3.56]	1999
Katoh et al. 1999	44	92	75	147	3.3%	0.88 [0.52, 1.48]	1999
Olshan et al. 2000	32	176	26	195	3.1%	1.44 [0.82, 2.54]	2000
Hamel et al. 2000	70	90	9	90	0.0%	31.50 [13.47, 73.64]	2000
To-Figueras et al. 2002	35	204	48	203	3.4%	0.67 [0.41, 1.09]	2002
Gronau et al. 2003	30	187	21	139	3.0%	1.07 [0.59, 1.97]	2003
Evans et al. 2004	54	283	58	203	3.6%	0.59 [0.39, 0.90]	2004
Gajecka et al. 2005	54	289	61	316	3.6%	0.96 [0.64, 1.44]	2005
Drummond et al. 2005	73	87	34	81	2.6%	7.21 [3.50, 14.84]	2005
Gatta´s et al. 2006	25	103	18	102	2.7%	1.50 [0.76, 2.95]	2006
Sharma et al. 2006	17	40	13	87	2.2%	4.21 [1.78, 9.95]	2006
Sugimura et al. 2006	46	122	105	241	3.5%	0.78 [0.50, 1.22]	2006
Biselli et al. 2006	20	60	14	60	2.4%	1.64 [0.74, 3.67]	2006
Oude Ophuis et al. 2006	36	185	94	285	3.5%	0.49 [0.32, 0.76]	2006
Acar et al. 2006	33	110	31	197	3.1%	2.29 [1.31, 4.02]	2006
Peters et al. 2006	122	692	162	753	4.1%	0.78 [0.60, 1.01]	2006
Anantharaman et al. 2007	45	456	114	726	3.8%	0.59 [0.41, 0.85]	2007
Suzen et al. 2007	19	98	23	120	2.8%	1.01 [0.52, 1.99]	2007
Buch et al. 2008	67	195	122	414	3.8%	1.25 [0.87, 1.80]	2008
Losi-Guembaro et al. 2008	30	91	23	81	2.8%	1.24 [0.65, 2.38]	2008
Hatagima et al. 2008	49	231	48	212	3.5%	0.92 [0.59, 1.44]	2008
Harth et al. 2008	64	312	61	300	3.7%	1.01 [0.68, 1.50]	2008
Boccia et al. 2008	48	210	58	245	3.5%	0.96 [0.62, 1.48]	2008
Amtha et al. 2009	37	81	67	162	3.2%	1.19 [0.70, 2.04]	2009
Li et al. 2009	44	76	39	76	2.9%	1.30 [0.69, 2.47]	2009
Chatzimichal et al. 2010	21	88	31	102	2.8%	0.72 [0.38, 1.37]	2010
Sam et al. 2010	77	408	16	220	3.1%	2.97 [1.68, 5.22]	2010
Leme et al. 2010	47	100	41	100	3.1%	1.28 [0.73, 2.23]	2010
Soucek et al. 2010	24	116	16	109	2.7%	1.52 [0.76, 3.04]	2010
Lourenço et al. 2011	36	142	27	142	3.1%	1.45 [0.82, 2.54]	2011
Ruwali et al.2011	135	500	103	500	4.0%	1.43 [1.06, 1.91]	2011
Total (95% CI)		6165		7048	100.0%	1.21 [1.01, 1.45]	
Total events	1473		1631				

Heterogeneity: Tau² = 0.19; Chi² = 126.52, df = 30 (P < 0.00001); I² = 76%
Test for overall effect: Z = 2.02 (P = 0.04)

Odds Ratio M-H, Random, 95% CI
0.01 0.1 1 10 100
GSTT1 null in cases   GSTT1 null in controls

Figure 3. Forest plot of *GSTT1* associated with HNSCC under random-effects model. A: Overall analysis. B: Sensitivity analysis with exclusion of the study by Hamel et al. 2000. The diamond shows the overall risk and the line represent the 95% CI for each meta-analysis. Events: null genotype.
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Results

Eligible studies and meta-analysis databases

We identified 221 articles through the initial computerized search of published work. After reading titles, abstracts, 55 articles were retained. For the analysis of *GSTM1* or *GSTT1*, after discarding 11 articles [7,12–21] due to the overlapping data and 1 article [22] due to lack of essential genotype information, 44 case-control studies [5,6,8–10,23–60] finally met our criteria for inclusion. Among them, 42 studies described the association between *GSTM1* null genotype and HNSCC, and 32 between *GSTT1* null genotype and HNSCC. For the association between dual null genotype and HNSCC, 1 discarded article [13] containing the distribution information of dual null genotype was reincorporated, and 15 studies were included (Table 1). For the analyses stratified by smoking, eight studies [5,8,29,33,45,47,48,55] for *GSTM1*, and seven studies [5,33,38,45–48] for *GSTT1* were included.

Heterogeneity result

Cochran's Q tests indicated heterogeneity exist in different studies in the analysis except studies of dual genes in South American ($P = 0.51$, $I^2 = 0\%$) and *GSTM1* in non-smokers ($P = 0.65$, $I^2 = 0\%$). The random or fixed effect model was selected for comparisons with or without heterogeneity, respectively.

Meta-analysis results

A total of 7584 HNSCC cases and 8576 controls for *GSTM1*, 6255 cases and 7138 controls for *GSTT1*, 2657 cases and 3092 controls for dual genes were investigated.

For *GSTM1* polymorphism, the overall meta-analysis showed a suggestively increased risk in null genotype as compared to wild

genotype (OR = 1.145, 95% CI: 1.00–1.29, $P = 0.05$) (Figure 2). In sensitivity analysis by temporarily excluding individual studies, no single study substantially affected the pooled OR, indicating that the results of these meta-analyses are stable. Analysis after stratification by ethnicity indicated *GSTM1* null genotype tended to be associated with HNSCC in Asian (OR = 1.48, 95% CI: 1.24–1.75, $P < 0.01$), while no significant association was found in European or South American (Table 2).

For *GSTT1* polymorphism, null genotype was associated with an increased risk of HNSCC (OR = 1.32, 95% CI: 1.07–1.64, $P = 0.01$) (Figure 3A). Sensitivity analysis showed that the association still exist even with exclusion of the study of Hamel et al. which was obviously deviating from others (OR = 1.21, 95% CI: 1.01–1.45, $P = 0.04$) [32] (Figure 3B). Analysis stratified by ethnicity indicated that *GSTT1* null genotype increased the HNSCC risk in South American (OR = 1.63, 95% CI: 1.03–2.58, $P = 0.04$) (Table 2).

Combined analysis of *GSTM1* and *GSTT1* on HNSCC risk showed that OR of individuals with dual null genotype was elevated (OR = 1.48, 95% CI: 1.12–1.96, $P = 0.006$) compared to *GSTM1* or *GSTT1* individual null genotype (Figure 4). After stratification for ethnicity, we observed a significant association for HNSCC in European (OR = 2.01, 95% CI: 1.15–3.53, $P = 0.01$) and Asian (OR = 1.56, 95% CI: 1.05–2.33, $P = 0.03$) populations among *GSTM1* and *GSTT1* dual null individuals (Table 2). The exclusion of individual studies did not change these results qualitatively.

We further performed stratification analysis by smoking status. As shown in Table 3, significant association of *GSTM1* deletion with risk of HNSCC was observed in smoking group (OR = 1.51, 95% CI: 1.05–2.17, $P = 0.03$) but not in non-smoking group (OR = 1.14, 95% CI: 0.90–1.43, $P = 0.28$). However, we did not found any significant associations for *GSTT1* in either smokers (OR = 1.01, 95% CI: 0.64–1.60, $P = 0.96$) or non-smokers
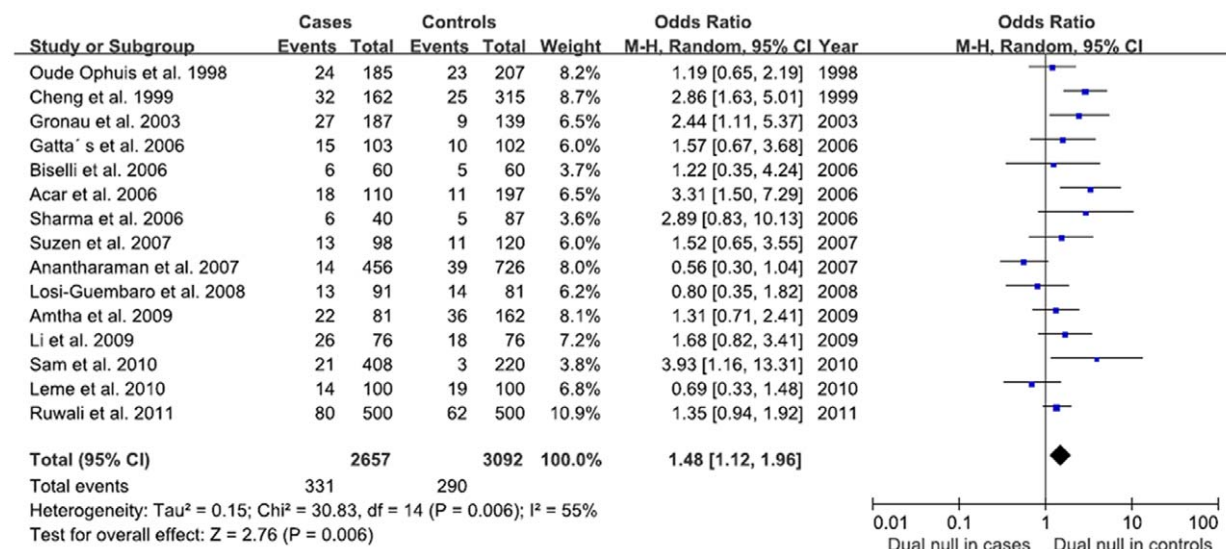
Figure 4. Forest plot of *GSTM1* and *GSTT1* associated with HNSCC under random-effects models. The diamond shows the overall risk and the line represent the 95% CI for each meta-analysis. Events: null genotype.
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Table 3. Genotype distribution of GSTM1 and GSTT1 in different smoking status.

	GSTM1		GSTT1	
	Non-smoker	Smoker	Non-smoker	Smoker
Cases (n/N[a])	255/455	862/1638	94/462	347/1671
Controls (n/N)	473/1031	658/1519	222/981	325/1527
OR[b]	1.14	1.51	1.13	1.01
95% CI[c]	0.9–1.43	1.05–2.17	0.68–1.86	0.64–1.6
P[d]	0.28	0.03[*]	0.64	0.96

Abbreviations: [a], number of carriers with null genotype/total number; [b], odds ratio; [c], confidence interval; [d], value for heterogeneity; OR, odds ratio; 95% CI, 95% confidence interval
*$0.01 \leq P < 0.05$
doi:10.1371/journal.pone.0047579.t003

(OR = 1.13, 95% CI: 0.68–1.86, $P = 0.64$) (Table 3), which may be due to the limited number of study with smoking information.

Publication bias

Funnel plots were performed to assess the publication bias, and these shapes did not suggest any obvious evidence of asymmetry in the analyses of GSTM1, gene-gene interaction, and GSTT1 analysis stratified by smoking status. When one study [32] for GSTT1 analysis and two studies [46,49] for GSTM1 analysis stratified by smoking status were omitted, funnel plots illustrated symmetric shape.

Discussion

Genetic factors play an important role in the etiology of tumors. For HNSCC, genes encoding xenobiotic-metabolizing enzymes (XMEs) are some of the most likely candidates that could affect individual's susceptibility to the disease, due to their involvement of the metabolic activation and detoxification of the environmental carcinogens [61]. Conjugation is one of the most common pathways of xenobiotic metabolism and is considered phase II metabolism which is catalyzed by multiple enzyme superfamilies including Glutathione S-transferases (GSTs). GSTs mediate the reactions of glutathione with electrophiles, resulting in the elimination of potentially carcinogenic chemicals [62]. GSTM1 and GSTT1 genes belonging to GSTs have been studied extensively due to their important detoxification function and high-frequency polymorphisms. GSTM1 and GSTT1 homozygous deletions (null genotype) may lead to deficient enzyme activity [63]. In the present study, the overall frequency of GSTM1 and GSTT1 null genotype in controls were 47.65% and 23.77% respectively in accordance with other studies [64–67]. After stratification for ethnicity, the frequency of GSTM1 and GSTT1 null genotype in controls in European, Asian and South American were 53.14%, 40.06%, 49.29% and 21.86%, 23.67%, 26.34% respectively, which indicated ethnic differences.

The importance of GSTM1 and GSTT1 polymorphisms effects on HNSCC has been a concern in recent years, but the data of existing studies are contradictory. An increase in the risk of HNSCCC was observed in cases with null genotypes of GSTM1 or GSTT1 in some studies [5–8]. However the risk was not found in other studies. For example, Boccia [9] and Biselli [10] did not find the association between GSTM1 or GSTT1 and HNSCC. Although the confused effect of these polymorphisms may be a result of various reasons such as demographic features of subjects and different life styles, comparatively small sample size in individual study might lead to lower statistical power and bias. The present meta-analyses of 42 studies including 7584 cases and 8651 controls for analysis of GSTM1, 32 studies including 6255 cases and 7138 controls for analysis of GSTT1, and 15 studies including 2657 cases and 3092 controls provide more comprehensive information on the relationships between two genes and HNSCC.

This meta-analysis showed that both GSTM1 and GSTT1 null genotype confers susceptibility to HNSCC in the overall analysis. GSTM1 can deals with large hydrophobic electrophiles including polycyclic aromatic hydrocarbons derived epoxides (PAH) [68,69], while GSTT1 targets a more restricted kind of compounds, like monohalomethane and ethylene oxide [70]. Different GST isoforms exhibit overlapping substrate specificity, combinations of GSTM1 and GSTT1 null genotype may theoretically confer a higher risk to HNSCC. Comparing to homozygous deletion of GSTM1 and GSTT1 alone, deletion of two genes in combination significantly increases the risk of HNSCC as showed in our combined analysis, indicating a synergenic role of GSTT1 and GSTM1 in cancergenesis.

Analyses after stratification by ethnicity revealed ethnicity-specific associations between two genes and HNSCC. Our findings indicate that GSTM1 may be an important factor in Asians in the development of HNSCC, which is similar to the results reported by Hashibe et al. [4]. However, GSTT1 but not GSTM1 may be important in South Americans, while GSTM1 and GSTT1 in combination play a vital role in Europeans and Asians. This result may be attributed to the different habits of smoking, alcohol consumption, intake of food and different genetic backgrounds in different ethnic groups.

Both GSTT1 and GSTM1 can prevent the accumulation of tobacco smoke carcinogens, and compared with non-smokers, mutations of these two genes theoretically increase the risk of HNSCC in smokers. To investigate potential gene-environment interaction, we stratified the data by smoking status. A significant association was observed in smokers with GSTM1, whereas no difference was observed between smokers and non-smokers for GSTT1. Previous studies showed that GSTT1 and GSTM1 are involved in the detoxification of carcinogens such as smoking by-products, and polymorphisms in these two genes with a result of loss of enzyme activity may increase risk of carcinogenesis and have different role in detoxification. [68–70]. Although we found higher risk of GSTM1 null genotype in smokers (OR = 1.51) than non-smokers (OR = 1.14), further individual large study are required to evaluate the interaction of GSTM1 and smoking on HNSCC risk.

Although our result of this meta-analysis is constructive, its limitations and some potential bias should be addressed. First, despite that a well-designed search strategy was used to identify eligible studies, it was possible that some relevant studies were not included. This study only focused on full-text papers published in English and Chinese in PubMed, so some eligible studies in other languages or in other databases might be missed. Second, adjustments over age, gender and other environmental factors such as alcohol drinking might help better detect the association between GSTM1, GSTT1 and HNSCC. If available detailed individual data are enough for an adjusted estimate in the future, a more precise analysis should be conducted. Third, ethnicity was determined roughly by subject's country due to inadequate available data, and this classification can help us have a regional concept of these genes functions. Fourth, the controls in the included studies were recruited in different ways and not uniformly defined, which may have distorted the meta-analysis. Finally,

because all the studies were designed with retrospective studies, we cannot clearly determine the causal relationship between the risk factor and HNSCC. Given the limitations and biases above, the conclusions or interpretations made from the results of this meta-analysis should be explained with caution.

Conclusions

The results of this meta-analysis suggest that *GSTM1* and *GSTT1* null genotypes may be associated with an increased risk of HNSCC. Further large well-designed studies are warranted to confirm these findings.

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