Meta- and Pooled Analyses of *GSTM1*, *GSTT1*, *GSTP1*, and *CYP1A1* Genotypes and Risk of Head and Neck Cancer

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Abstract

Sequence variation in the *GSTM1*, *GSTT1*, *GSTP1*, and *CYP1A1* genes may potentially alter susceptibility to head and neck cancers, although evidence from previous studies has not been consistent. To explore these associations, we conducted a meta-analysis of 31 published case–control studies (4635 cases and 5770 controls) and a pooled analysis of original data from nine published and two unpublished case–control studies (2334 cases and 2766 controls). In the meta-analysis, the summary odds ratios (ORs) for head and neck cancer were 1.23 [95% confidence interval (95% CI), 1.06–1.42] for the *GSTM1* null genotype, 1.17 (95% CI, 0.98–1.40) for the *GSTT1* null genotype, 1.10 (95% CI, 0.92–1.31) for carrying the *GSTP1* Val105 allele, and 1.35 (95% CI, 0.95–1.82) for carrying the *CYP1A1* Val462 allele. The pooled analysis ORs were 1.32 (95% CI, 1.07–1.62) for the *GSTM1* null genotype, 1.25 (95% CI, 1.00–1.57) for the *GSTT1* null genotype, 1.15 (95% CI, 0.86–1.53) for carrying the *GSTP1* Val105 allele, and 0.98 (95% CI, 0.75–1.29) for carrying the *CYP1A1* Val462 allele. Increasing risk of head and neck cancer was observed with inheritance of increasing numbers of modest risk genotypes at the three *GST* loci (P for trend = 0.04), with the combination of carrying the *GSTM1* null, *GSTT1* null, and *GSTP1* Val105 alleles conferring an OR of 2.06 (95% CI, 1.11–3.81). In conclusion, both the meta- and pooled analysis support modest associations of *GSTM1* and *GSTT1* genotypes with head and neck cancer risk, and our pooled analysis supports the notion of greater risk when genotypes at multiple *GST* loci are considered in a multigenic model.

Introduction

Sequence variation in genes coding for phase I and phase II enzymes, such as members of the cytochrome P450 (CYP) and glutathione *S*-transferase (GST) families may potentially alter individual susceptibility to cancer. Polymorphisms that confer a modest disease risk (relative risk <2) can be a substantial public health burden if they are common (1). A review on *GSTM1* and *GSTT1* deletion genotypes and head and neck cancer summarized the results of case–control studies as inconclusive (2). Of the 21 studies reviewed for the *GSTM1* deletion genotype, 13 studies reported odds ratios (ORs) between 0.9 and 1.3, whereas 8 studies reported ORs between 1.4 and 3.9 (2). For the *GSTT1* deletion genotype, eight studies reported ORs from 0.5 to 1.2, whereas six reported ORs from 1.4 to 2.6 (2). A meta-analysis that identified 25 studies on the *GSTM1* null genotype and the risk of head and neck cancer reported a summary OR of 1.20 [95% confidence interval (95% CI), 1.08–1.33 (3)]. Because these carcinogen-metabolizing enzymes may be among numerous genes involved in the multistage pathway of cancer, they are expected to be modest to moderate risk factors that may be difficult to detect. However, even modest single gene effects on cancer risk are of biological and medical importance because of the possibility of identifying, under multigenic models, high-risk individuals for target prevention activities.

Tobacco smoke contains a range of different carcinogens, including polycyclic aromatic hydrocarbons, aromatic amines, and nitrosamines (4). The extent of exposure of the upper aerodigestive tract to carcinogens may depend on whether the carcinogen is activated by phase I enzymes and whether it is detoxified by phase II enzymes. An individual's exposure to tobacco carcinogens may therefore be altered by sequence variation in genes coding for these enzymes.

The *CYP1A1* gene codes for a phase I enzyme that activates tobacco procarcinogens, such as benzo[α]pyrene and aromatic amines, into their carcinogenic forms (5). An A→G base substitution at nucleotide 2455, which is strongly linked to 3801T>C in the 3′-flanking region, encodes for an amino acid replacement of isoleucine by valine at codon 462 and has been reported to be associated with increased enzyme activity (6, 7). The variant genotype is suggested to be harmful, possibly by increasing carcinogen activation and generating reactive oxygen species (8). Moreover, smokers with the *CYP1A1* variant genotype may have elevated DNA adduct levels (9).

The GST family includes phase II enzymes that detoxify carcinogens and reactive oxygen species (10). Individuals who have homozygous deletions for the *GSTM1* and *GSTT1* gene

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Table 1 Summary of case–control studies on *GSTM1*, *GSTT1*, *GSTP1* (ILE105VAL), and *CYP1A1* (ILE462VAL) genotypes and head and neck cancers

Ref.	First author	Year of publication	Country	Control source	Matching	Site		
						Oral	Pharynx	Larynx
(13)	Buch	2002	India	Hospital-healthy	Individual	X		
(14)	Cheng	1999	United States	Hospital	Individual		Head & neck; site NS	
(15)	Coutelle	1997	France	Alcoholism clinic	None		X	X
(16)	Deakin	1996	United Kingdom	Hospital	None	X		
(17)	Gonzalez	1998	Spain	Hospital-healthy	None	X	X	X
(18)	Gronau	2003	Germany	Hospital-healthy	Individual	X	X	X
(19)	Hahn	2002	Germany	Hospital-healthy	Individual	X		
(20)	Hamel	2000	Canada	Mixed	Individual	X	X	X
(21)	Hanna	2001	United States	Hospital	Individual			X
(22)	Hong	2000	Korea	Hospital	None			X
(23)	Hung	1997	Taiwan	Population based	Frequency	X		
(24)	Jaskula-Sztul	1998	Poland	NS, healthy	None			X
(25, 26)	Jourenkova	1999	France	Hospital	Frequency	X	X	X
(26, 27)	Jourenkova	1999	France	Hospital	Frequency	X	X	X
(28)	Kao	2002	Taiwan	Hospital	None	X		
(29)	Katoh	1999	Japan	Hospital-healthy	None	X		
(30)	Katoh	1999	Japan	Hospital-healthy	None	X		
(31)	Kietthubthew	2001	Thailand	Population based	Individual	X		
(32)	Kihara	1997	Japan	Hospital-healthy	None	X	X	X
(33)	Ko	2001	Germany	Hospital-healthy	None		Head & neck; site NS	
(34)	Matthias	1998	Germany	Hospital	None	X	X	X
(35)	Matthias	1999	Germany	Hospital	None	X	X	X
(36)	McWilliams	2000	United States	Hospital	None	X	X	X
(37)	Morita	1999	Japan	Hospital-healthy	None	X	X	X
(38)	Nomura	2000	Japan	Hospital	None	X	X	
(39)	Olshan	2000	United States	Hospital	Frequency	X	X	X
(40)	Oude Ophuis	1998	Netherlands	Hospital-healthy	None	X	X	X
(41)	Oude Ophuis	2003	Netherlands	Hospital-healthy	None	X	X	X
(42)	Park	1997	United States	Hospital	Frequency	X	X	X
(43)	Park	1999	United States	Hospital	Frequency	X	X	X
(44)	Park	2000	United States	Hospital	Frequency	X	X	X
(45)	Risch	2003	Germany	Population based	Frequency			X
(46)	Sato	2000	Japan	Hospital-healthy	None	X		
(47)	Sreelekha	2001	India	Hospital-healthy	Individual	X		
(48)	Tanimoto	1999	Japan	Hospital	Individual	X		
(49)	To-Figueras	2002	Spain	Hospital-healthy	None			X
(50)	Trizna	1995	United States	Hospital-healthy	None	X	X	X
No. of studies in meta-analysis								
Cases								
Controls								
Summary OR (95% CI)								
Test for heterogeneity								
Publication bias (Egger's test)								

have no GSTM1 and -T1 enzyme activity. Lack of these enzymes may potentially increase cancer susceptibility because of a decreased ability to detoxify carcinogens such as benzo[α]pyrene-7,8-diol epoxide, the activated form of benzo[α]pyrene. The missense substitution Ile105Val results from an A→G base substitution at nucleotide 313. The Val105 form of the GSTP1 enzyme may be 2–3 times less stable than the canonical Ile105 form (11) and may be associated with a higher level of DNA adducts (12).

A previous review on *GSTM1*, *GSTT1*, and head and neck cancer included journal articles written in English and published between 1993 and 2000 (2), whereas a published meta-analysis on *GSTM1* included publications up to May 2001 (3). Because published reports on additional study populations not included in the review and meta-analysis are available, we conducted an updated meta-analysis of case–control studies evaluating the relationship between head and neck cancer and *GSTM1*, as well as *GSTT1*, *GSTP1*, and *CYP1A1*, to assess whether the available evidence supports these associations and to determine the sources of heterogeneity among the study results. In addition, we pooled the raw datasets from 11 case–control studies on the relationship between these genes, which encode carcinogen-metabolizing enzymes, and head and neck cancer to explore the main effect of the genes as well as gene–gene and gene–environment interactions.

Materials and Methods

Meta-analysis

A MEDLINE search was conducted for case–control studies reported up to August 2003 on *GSTM1*, *GSTT1*, *GSTP1*, and *CYP1A1* and the risk of head and neck cancer, including oral, pharyngeal, and laryngeal cancers. We focused on the null alleles of the *GSTM1* and *GSTT1* genes, the Val105 allele of the *GSTP1* gene, and the Val462 allele of the *CYP1A1* gene. The

Table 1 Continued

Cases (*n*)	Controls (*n*)	*GSTM1* null, crude OR[a] (95% CI)	*GSTT1* null, crude OR (95% CI)	*GSTP1* (any Val), crude OR (95% CI)	*CYP1A1* (any Val), crude OR (95% CI)
297[b,c]	450[c]	2.95 (2.16–4.04)[d]	1.60 (1.06–2.41)[d]		
162	315	1.51 (1.03–2.21)	2.30 (1.48–3.56)		
39[c,e,f]	37[c,e,f]	2.38 (0.93–6.06)[d,g]			
40[i]	577[i]	1.01 (0.53–1.92)[d]	0.59 (0.20–1.71)[d]		
75[f]	200[f]	1.34 (0.78–2.29)[d]			
187	139	0.78 (0.50–1.21)[d]	1.07 (0.59–1.97)[d]		1.30 (0.70–2.41)[d]
94	92	1.29 (0.72–2.31)[d]			0.64 (0.17–2.34)[d]
90[b,h]	90	0.96 (0.53–1.73)[d]	2.57 (1.12–5.90)		
20	20	4.00 (0.98–16.27)[d,g]	0.71 (0.14–3.66)[d]		
82[c,f]	63[c,f]	1.96 (0.99–3.86)[d]	2.34 (1.19–4.58)[d]		
41[f]	123[f]	1.03 (0.50–2.12)[d]	1.26 (0.61–2.58)[d]		
171	180	0.71 (0.46–1.08)	0.77 (0.45–1.31)		
250[c]	172[c]	1.09 (0.74–1.60)[d]	1.38 (0.82–2.30)[d]		
250[c]	172[c]			1.23 (0.86–1.82)[d]	
106	146				5.42 (2.83–10.38)[d,g]
92	147	1.65 (0.98–2.80)	0.88 (0.52–1.48)		1.29 (0.76–2.18)
83	122			1.91 (1.04–3.52)[d]	
53	53	3.02 (1.36–6.71)[d]	0.58 (0.26–1.26)		
156[h]	472	1.29 (0.90–1.86)[d]			
312	300	1.22 (0.88–1.67)[d]	1.01 (0.68–1.50)[d]		
380	193				1.04 (0.64–1.70)[d]
398[h]	219[h]	1.18 (0.82–1.68)[d]	0.99 (0.66–1.49)[d]	1.39 (0.98–1.96)[d]	
160[h,i]	149[i]	0.99 (0.62–1.59)	0.91 (0.47–1.74)	1.26 (0.78–2.04)[d]	0.42 (0.18–0.99)
145	164	0.94 (0.60–1.46)[d]		0.73 (0.44–1.21)[d]	0.88 (0.55–1.41)[d]
109[h]	33	2.43 (1.10–5.38)[d,g]			
182[i]	202[i]	0.96 (0.64–1.46)[d,g]	1.47 (0.84–2.58)[d]	1.25 (0.81–1.92)[d]	1.33 (0.58–3.06)[d]
185[b,h]	207	0.97 (0.65–1.44)[d]	0.95 (0.58–1.56)[d]		1.15 (0.68–1.93)[d]
235	285			0.80 (0.57–1.13)[d]	
131	131				2.58 (1.17–5.66)[d]
154	246			0.91 (0.60–1.38)[d]	
164	344	1.34 (0.92–1.95)[d]			
245	251	0.92 (0.65–1.32)[d]	1.13 (0.69–1.86)[d]		
142	142	2.24 (1.40–3.61)			1.88 (1.17–3.03)[d]
98	60	1.92 (0.99–3.74)[d]	2.48 (0.87–7.06)[d]		5.21 (2.37–11.43)[d]
100	100	1.04 (0.59–1.83)[d]			
204[i]	203[c,i]	0.92 (0.62–1.35)[d]	0.67 (0.41–1.09)[d]	1.01 (0.68–1.49)[d]	
186[i]	42[i]	2.37 (1.20–4.67)	1.47 (0.71–3.02)		
		26	21	9	11
		4224	3346	1768	1764
		5333	3829	1699	1585
		1.23 (1.06–1.42)	1.17 (0.98–1.40)	1.10 (0.92–1.31)	1.32 (0.95–1.82)
		0.00	0.01	0.13	0.00
		0.99	0.97	0.52	0.89

[a] OR, odds ratio; CI, confidence interval; NS, not specified.
[b] Prevalent and incident cases.
[c] All ever tobacco smokers and/or chewers.
[d] Odds ratio calculated.
[e] All drinkers.
[f] All males.
[g] Study excluded.
[h] Includes nasopharyngeal, maxillary sinus, and/or salivary gland cancers.
[i] Number of cases and controls genotyped varied for each gene, thus overall numbers are presented.

following keywords were used in the Medline search: "Glutathione," "GSTM1," "GSTT1," "GSTP1," and "CYP1A1." In addition, we reviewed the literature cited by each of the journal articles that we identified. When several articles were identified for the same population, we referred to the most updated information source. A total of 37 publications were identified, with 4635 cases and 5770 controls from 31 different populations (13–50).

We focused on studies that genotyped individuals by PCR and excluded ~30 studies that assessed gene expression by measurement of protein levels. Positive controls for *GSTM1* and *GSTT1* genotyping were mentioned for the majority of studies. *GSTP1* was genotyped in all studies by PCR combined with restriction fragment length polymorphism analysis. For *CYP1A1* genotyping, three studies used allele-specific PCR (19, 28, 46), one study used PCR combined with single-strand conformational polymorphism analysis (42), and the other studies used PCR combined with restriction fragment length polymorphism analysis.

For each study, we abstracted the publication date, country

Table 2 Distribution of *GSTM1*, *GSTT1*, *GSTP1* (ILE105VAL), and *CYP1A1* (ILE462VAL) genotypes among head and neck cancer cases and controls

Ref.	Country	% *GSTM1* null		% *GSTT1* null		% *GSTP1* any Val		% *CYP1A1* any Val	
		Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
Asia									
(13)	India	49.2	24.7	18.2	12.2				
(47)	India	49.0	33.3	18.4	8.3			51.0	16.7
(29, 30)	Japan	58.7	46.3	47.8	51.0	37.3	23.8[a]	45.7	39.5
(32)	Japan	55.1	48.7						
(37)	Japan	49.0	50.6			24.8	31.1	33.8	36.6
(38)	Japan	67.0	45.5						
(46)	Japan	64.8	45.1					52.1	36.6
(48)	Japan	43.0	42.0						
(22)	Korea	68.3	52.4	57.3	36.5				
(23)	Taiwan	58.5	57.7	58.5	52.8				
(28)	Taiwan							86.8	54.8[a]
(31)	Thailand	56.6	30.2	34.0	47.2				
Overall in Asia		55.0	41.7	30.9	27.7	29.3	28.0	52.7	39.5
Europe									
(15)	France	69.2	48.6						
(25–27)	France	54.4	52.3	20.4	15.7	55.2	50.0		
(18)	Germany	42.8	48.9	16.0	15.1			17.1	13.7
(19)	Germany	59.6	53.3					4.3	6.7
(33)	Germany	53.2	48.3	20.5	20.3				
(34, 35)	Germany	57.4	53.4	22.0	22.2	55.0	46.9[a]	15.0	14.5
(45)	Germany	51.8	53.8	15.5	13.9				
(40, 41)	Netherlands	50.8	51.7	19.5	20.3	50.6	56.1	18.4	16.4[a]
(24)	Poland	49.1	57.8	17.5	21.7				
(17)	Spain	58.7	51.5						
(49)	Spain	47.1	49.3	17.2	23.6	50.7	50.2		
(16)	United Kingdom	55.0	54.8	11.8	18.5				
Overall in Europe		52.7	52.4	19.3	19.6	53.3	51.4	15.0	13.8
North America									
(20)	Canada	56.7	57.8	22.2	10.0				
(14)	United States	53.1	42.9	32.7	17.5				
(21)	United States	80.0	50.0	15.0	20.0				
(36)	United States	46.3	46.5	16.9	18.3	58.9	53.2	6.5	14.0
(39)	United States	43.6	44.6	18.6	13.5	66.3	61.1	7.6	5.8
(42–44)	United States	43.3	36.3			62.3	64.6	17.6	7.6[a]
(50)	United States	68.3	47.6	44.9	35.7				
Overall in North America		52.5	43.1	26.5	16.8	62.7	60.9	9.4	7.8
Overall		53.3	47.0	22.9	20.6	52.7	50.6	23.9	21.1

[a] Departure from Hardy–Weinberg equilibrium detected ($P < 0.05$). Hardy–Weinberg equilibrium was assessed among the controls for *GSTP1* and *CYP1A1*.

where the study was conducted, site within the head and neck cancer studied, control source, numbers of cases and controls, and whether controls were matched to cases. Healthy subjects recruited from hospitals as controls were categorized as "hospital-healthy."

Statistical Analysis. We calculated for each study crude odds ratios (ORs) and 95% confidence intervals (95% CIs) for head and neck cancer when possible. For a study from France, we combined the data for cancers of the oral cavity, pharynx, and larynx that had been presented in separate publications (25–27). We abstracted crude ORs from the publications when they were available. When both the data and crude ORs were presented and there was a discrepancy between the two estimates (47), we retained the calculated crude OR because the OR presented in the original publication may have been adjusted for some factors. We did not perform a meta-analysis of the adjusted ORs because adjustment was not comparable among the studies. For *GSTP1* and *CYP1A1*, we combined the heterozygous and homozygous genotypes because of the limited number of subjects who were homozygous mutant.

When possible we estimated or abstracted study-specific ORs separately by site within the head and neck (oral cavity, larynx) and by smoking status (never, ever smokers). For several studies, we were unable to separate cancer cases of the nasopharynx, nasal cavity, sinus, or salivary gland from other head and neck cancers (20, 32, 36, 40). The histology of head and neck cancer cases was squamous cell carcinoma in most studies, but two studies (23, 47) did not specify the histology, and one study (32) included 20 of 156 cases with "other miscellaneous histologies."

Summary ORs were estimated with the statistical program STATA, version 8.0, by inverse-variance weighting, using a random-effects model that included a term for heterogeneity among studies (51). We estimated summary ORs when there were at least three risk estimates available. Thus, for some strata, summary ORs could not be estimated because of the small number of studies. Tests for heterogeneity among the studies were conducted for each analysis. Publication bias was assessed with the funnel plot of Begg and Mazumdar (52) and regression asymmetry test of Egger *et al.* (53).

We conducted influence analyses, in which each study was excluded one at a time to determine the magnitude of influence on the overall summary estimate. The influence analyses showed that the inferences for *GSTM1*, *GSTT1*, and *GSTP1* did not change as

Table 3 Meta-analysis of case–control studies for *GSTM1*, *GSTT1*, and head and neck cancer

	GSTM1				*GSTT1*			
	No. of studies	OR[a] for null genotype (95% CI)	Test for heterogeneity	Egger's test	No. of studies	OR[a] for null genotype (95% CI)	Test for heterogeneity	Egger's test
Overall	30	1.30 (1.12–1.50)	0.00	0.02	21	1.17 (0.98–1.40)	0.01	0.97
Excluding studies	26[b]	1.23 (1.06–1.42)	0.00	0.99				
Cancer site								
Oral	10[c]	1.45 (1.05–2.00)	0.00	0.31	7[d]	1.15 (0.82–1.63)	0.10	0.44
Larynx	9[e]	1.10 (0.86–1.41)	0.02	0.65	7[f]	1.00 (0.74–1.36)	0.09	0.77
Smoking status								
Never smokers	4[g]	0.98 (0.58–1.65)	0.85	0.78				
Ever smokers[h]	8[i]	1.37 (0.97–1.94)	0.00	0.59	5[j]	1.24 (0.92–1.66)	0.24	0.90
Region								
Asia	9	1.58 (1.16–2.14)	0.00	0.29	6	1.31 (0.88–1.96)	0.04	0.99
North America	6	1.24 (0.98–1.57)	0.17	0.76	6	1.59 (1.12–2.26)	0.17	0.34
Europe	11	1.02 (0.90–1.15)	0.57	0.91	9	0.96 (0.81–1.14)	0.63	0.43
Year of publication								
1995–1999	14	1.22 (1.03–1.43)	0.04	0.45	9	1.14 (0.87–1.48)	0.04	0.50
2000–2003	12	1.24 (0.96–1.59)	0.00	0.65	12	1.20 (0.93–1.55)	0.02	0.69
No. of cases and controls								
<100 cases or <100 controls	9	1.42 (1.16–1.74)	0.45	0.69	9	1.26 (0.86–1.85)	0.04	0.02
≥100 cases & 100≥controls	17	1.16 (0.97–1.39)	0.00	0.16	12	1.14 (0.93–1.39)	0.02	0.47
Control source								
Hospital-healthy or population	16	1.27 (1.02–1.59)	0.00	0.02	12	1.03 (0.85–1.25)	0.17	0.77
Hospital	10	1.17 (1.02–1.34)	0.66	0.96	9	1.41 (1.04–1.93)	0.03	0.64
Matching								
Individual matching	7	1.38 (0.92–2.08)	0.00	0.16	7	1.50 (1.01–2.22)	0.03	0.50
Frequency matching	5	1.07 (0.89–1.27)	0.69	0.93	4	1.30 (0.98–1.71)	0.91	0.83
No matching	14	1.21 (1.02–1.43)	0.02	0.15	10	0.97 (0.79–1.18)	0.22	0.70

[a] OR, odds ratio (adjusted for study center); CI, confidence interval.
[b] Excluded (15, 21, 31, 38).
[c] Included (13, 16, 19, 23, 25, 26, 29, 31, 37, 38, 46–48).
[d] Included (13, 16, 23, 26, 29, 31, 47).
[e] Included (15, 18, 21, 22, 22, 24–26, 32, 35, 37, 45, 49).
[f] Included (21, 22, 24, 25, 35, 45, 49).
[g] Included (32, 39, 43, 45).
[h] Ever smoking was categorized by different criteria in the studies: smoked for at least 5 years (23); smoked at least 5 cigarettes/day for 4 years (33); smoked at least 5 cigarettes/day for 5 years (25–27); smoked at least 100 cigarettes in a lifetime (39); smoked >0 pack-years (44, 45); and not specifically defined (30, 32).
[i] Included (13, 15, 22, 25, 26, 32, 33, 39, 43, 45).
[j] Included (22, 25, 26, 33, 39, 45).

a result of the exclusion of any one study. However, the summary estimate for *CYP1A1* was statistically significant only when we included one specific study that reported a high OR relative to the other studies (28). We considered this study to be a possible outlier and thus excluded the study. Further, for *GSTM1*, we observed four studies of small sample size that had identified strong positive associations and led to asymmetry in the Begg funnel plot. We excluded these four studies in an attempt to minimize publication bias. Both summary estimates including all studies identified and excluding several studies are presented.

Pooled Analysis

The data on *GSTM1*, *GSTT1*, *GSTP1*, *CYP1A1*, and head and neck cancers for the pooled analysis were extracted from the International Collaborative Study on Genetic Susceptibility to Environmental Carcinogens database (54, 55), which contains individual level data from case–control studies on genes that metabolize environmental carcinogens. Investigators who had published their results from case–control studies on genetic polymorphisms and cancers were identified through a MEDLINE search and requested to provide published and unpublished original data from their studies. Our data included 11 case–control studies, of which 9 had been published and included in the meta-analysis (13, 15, 16, 20, 25–27, 29, 30, 34, 35, 40, 42–44). Cases of cancer of the nasopharynx, maxillary sinus, and salivary glands were excluded from the analysis. Of the 2334 head and neck cancer cases included in the analysis, there were 840 oral cavity cancers, 501 pharyngeal cancers, 904 laryngeal cancers, and 79 cases with unspecified cancer within the head and neck, whereas the control group included 2766 subjects.

Statistical Analysis. To assess the association of the genotypes with head and neck cancer, the logistic regression model was used to estimate study-specific ORs and 95% CIs. We estimated a crude OR and an OR adjusted for age, sex, and race for each study. ORs estimated for individual studies and numbers of cases and controls may not precisely match those reported in the publications. Heterogeneity among studies was assessed with the test for heterogeneity, whereas publication bias was assessed with the funnel plot of Begg and Mazumdar (52) and regression asymmetry test of Egger *et al.* (53). A summary OR was estimated by inverse-variance weighting with the random-effects model (51) because of the heterogeneity detected among studies. In the pooled analysis, we did not assess the effect of study characteristics because of the small number of studies available.

Results

Study-specific crude ORs and overall summary ORs from the meta-analysis of the *GSTM1*, *GSTT1*, *GSTP1*, and *CYP1A1* genotypes are shown in Table 1. The distribution of genotypes at these four loci among the head and neck cancer cases and controls from

Table 4 Meta-analysis of case–control studies for *GSTP1* (ILE105VAL), *CYP1A1* (ILE462VAL), and head and neck cancer

	GSTP1				*CYP1A1*			
	No. of studies	OR[a] for any Val (95% CI)	Test for heterogeneity	Egger's test	No. of studies	OR[a] for any Val (95% CI)	Test for heterogeneity	Egger's test
Overall	9	1.10 (0.92–1.31)	0.13	0.52	12	1.48 (1.01–2.16)	0.00	0.76
Excluding studies					11[b]	1.32 (0.95–1.82)	0.00	0.89
Cancer site								
Oral	3[c]	1.52 (1.05–2.20)	0.63	0.22	5[d]	1.48 (0.77–2.83)	0.00	0.80
Larynx	5[e]	0.94 (0.73–1.20)	0.20	0.42	3[f]	1.03 (0.65–1.62)	0.29	0.07
Smoking status								
Never smokers								
Ever smokers[g]	3[h]	1.15 (0.87–1.51)	0.49	0.86				
Region								
Asia					4	1.73 (0.93–3.23)	0.00	0.18
North America	3	1.11 (0.86–1.43)	0.48	0.56	3	1.14 (0.41–3.21)	0.01	0.26
Europe	4	1.08 (0.85–1.38)	0.14	0.94	4	1.10 (0.82–1.49)	0.79	0.35
Year of publication								
1995–1999	5	1.15 (0.87–1.53)	0.08	0.97	5	1.18 (0.89–1.57)	0.23	0.01
2000–2003	4	1.02 (0.82–1.27)	0.33	0.07	6	1.37 (0.73–2.56)	0.00	0.48
No. of cases and controls								
<100 cases or <100 controls					3	1.74 (0.57–5.28)	0.00	0.99
≥100 cases & 100≥controls	8	1.06 (0.90–1.24)	0.24	0.68	8	1.20 (0.88–1.63)	0.04	0.88
Control source								
Hospital-healthy or population	4	0.98 (0.70–1.17)	0.07	0.36	7	1.43 (0.96–2.12)	0.01	0.74
Hospital	5	1.21 (1.01–1.45)	0.65	0.56	4	1.12 (0.58–2.13)	0.02	0.99
Matching								
Individual/frequency matching	3	1.12 (0.89–1.42)	0.47	0.60	5	1.84 (0.98–3.46)	0.02	0.72
No matching	4	1.18 (0.90–1.55)	0.12	0.90	6	1.09 (0.79–1.50)	0.05	0.14

[a] OR, odds ratio (adjusted for study center); CI, confidence interval.
[b] Excluded (28).
[c] Included (26, 30, 37).
[d] Included (19, 29, 37, 46, 47).
[e] Included (27, 35, 37, 41, 49).
[f] Included (34, 37, 43).
[g] Ever smoking was categorized by different criteria in the studies: smoked at least 5 cigarettes/day for 5 years (26, 27), smoked at least 100 cigarettes in a lifetime (39), and not specifically defined (30).
[h] Included (26, 27, 30, 39).

the 31 populations are presented in Table 2. The frequencies of the genotypes varied among controls: 24.7–57.8% for the *GSTM1* null genotype, 8.3–52.8% for *GSTT1* null genotype, 23.8–64.6% for the *GSTP1* valine genotype, and 5.8–39.5% for the *CYP1A1* valine genotype. The percentage of Caucasians in the United States studies were as follows: 88.9% of cases and 87.9% of controls (14), 95.6% of cases and 93.3% of controls (36), 62% of cases and 86% of controls (39), 100% of cases and controls (42), 66.2% of cases and 67.3% of controls (43), 61.5% of cases and controls (44), or not specified (21, 50).

Of the 30 identified studies on *GSTM1*, 4 were excluded to minimize publication bias (15, 21, 31, 38). For the remaining 26 studies, the summary OR for the *GSTM1* null genotype was modestly elevated (OR, 1.23; 95% CI, 1.06–1.42; Table 3). There appeared to be heterogeneity among the studies according to the test for heterogeneity. The ORs for the *GSTM1* null genotype were higher for studies with smaller sample size than for larger studies ($P = 0.07$) and for studies from Asia relative to studies from Europe ($P = 0.08$). The summary OR for the *GSTT1* null genotype and risk of head and neck cancer was 1.17 (95% CI, 0.98–1.40; Table 3) for 21 studies. None of the studies was excluded because there were no strong indications of excessive influence or publication bias. Geographic region may again be a source of heterogeneity in the *GSTT1* studies ($P = 0.03$). For *GSTP1*, we estimated a summary OR of 1.10 (95% CI, 0.92–1.31) for carrying the Val105 allele, including nine case-control studies (Table 4). The risk of oral cancer may be higher than the risk of laryngeal cancer for the *GSTP1* any valine genotype ($P = 0.04$). Twelve case–control studies were identified for the association between the *CYP1A1* genotype and head and neck cancers. After the exclusion of one study suspected to be an outlier (28), the summary OR for carriage of the Val462 allele was 1.35 (95% CI, 0.95–1.82; Table 4). Differences between ORs for the *CYP1A1* genotype in the stratified analysis were not identified.

Results of the pooled analysis of *GSTM1*, *GSTT1*, *GSTP1*, *CYP1A1*, and the risk of head and neck cancer are shown in Table 5. The race/ethnicity distribution for the Lazarus study was 64.3% Caucasian and 35.7% African American among cases, and 61.8% Caucasian and 38.2% African American among controls. For the Romkes study, the cases were 97.4% Caucasian and 2.6% African American, whereas the controls were 78.9% Caucasian, 15.5% African American, 4.2% Hispanic, and 1.4% Asian. Including 11 studies, the OR adjusted for study center was 1.32 (95% CI, 1.07–1.62) for the *GSTM1* null genotype. When further adjusted for age, sex, and race, the OR was 1.18 (95% CI, 0.97–1.44; not presented). The risk of head and neck cancer was also elevated by the *GSTT1* null genotype (OR adjusted for study center, age, sex, and race was 1.41; 95% CI, 1.00–1.57; not presented), but not by the *GSTP1* and *CYP1A1* missense substitutions, according to the pooled analysis. Differences among ORs in the stratified analysis were not observed in the pooled analysis.

Gene–gene interactions were assessed for all combinations of *GSTM1*, *GSTT1*, *GSTP1*, and *CYP1A1* on the risk of head and neck cancer in the pooled analysis, but strong inter-

Table 5 Pooled analysis of case–control studies on the *GSTM1*, *GSTT1*, *GSTP1* (ILE105VAL), and *CYP1A1* (ILE462VAL) genotypes and head and neck cancer

Principal investigator	First author (Refs.)	Country	Control source	Site			Cases (*n*)	Controls (*n*)	*GSTM1* (null), OR[a] (95% CI)	*GSTT1* (null), OR (95% CI)	*GSTP1* (any Val), OR (95% CI)	*CYP1A1* (any Val), OR (95% CI)
				Oral	Pharynx	Larynx						
Benhamou	Jourenkova (25–27)	France	Hospital	X	X	X	250	172	1.09 (0.74–1.60)	1.38 (0.82–2.30)	1.23 (0.83–1.82)	
Bhisey	Buch (13)	India	Hospital-healthy	X			300[b–d]	678[c,d]	1.73 (1.49–1.99)	1.61 (1.11–2.33)		1.06 (0.76–1.48)
Cascorbi	Unpublished	Germany	Hospital-healthy		X	X	505[f]	223[f]	2.01 (1.34–3.01)	1.26 (0.65–2.43)		1.02 (0.51–2.04)
Coutelle	Coutelle (15)	France	Alcoholism clinic		X	X	39[c,d,e]	76[c,d,e]	2.37 (1.05–5.36)			
Foulkes	Hamel (20)	Canada	Mixed	X	X	X	196[b,f]	199[f]	1.09 (0.74–1.62)	1.15 (0.67–1.98)		
Katoh	Katoh (29, 30)	Japan	Healthy	X			45	91	1.59 (0.78–3.27)			
Lazarus	Park (42–44)	United States	Hospital	X		X	185[f]	367[f]	1.40 (0.96–2.03)		0.87 (0.57–1.33)	
Manni	Oude Ophuis (40)	Netherlands	Hospital-healthy	X	X	X	245[b,f]	159[f]	1.33 (0.77–2.29)	0.56 (0.30–1.05)	0.77 (0.52–1.14)	0.63 (0.33–1.19)
Romkes	Unpublished	United States	Healthy	Head and neck			40[f]	70[f]	0.79 (0.36–1.73)	0.84 (0.25–2.84)		
Strange	Deakin (16)	United Kingdom	Hospital	X			107[f]	493[f]	0.69 (0.45–1.05)	1.38 (0.84–2.27)	1.53 (0.94–2.48)	2.11 (0.85–5.24)
Strange	Matthias (34, 35)	Germany	Hospital	X	X	X	422[f]	238[f]	1.31 (0.94–1.83)	1.54 (0.91–2.63)	1.56 (1.11–2.19)	0.87 (0.55–1.38)
No. of studies (cases/controls) in data									11 (2224/2517)	8 (1929/1830)	5 (1164/982)	5 (1558/1467)
Summary OR[a]									1.32 (1.07–1.62)	1.25 (1.00–1.57)	1.15 (0.86–1.53)	0.98 (0.75–1.29)
Test for heterogeneity									0.00	0.22	0.04	0.28
Publication bias (Egger's test)									0.15	0.14	0.75	0.79
Oral									1.20 (0.89–1.63)	1.34 (0.99–1.82)	1.37 (0.88–2.14)	0.97 (0.56–1.69)
Pharynx									1.25 (0.98–1.61)	1.11 (0.66–1.87)	1.10 (0.58–2.05)	0.77 (0.47–1.25)
Larynx									1.53 (1.17–2.00)	1.10 (0.81–1.49)	1.08 (0.81–1.44)	0.93 (0.64–1.34)
Never smokers									1.58 (1.11–2.23)	1.29 (0.83–1.99)	1.38 (0.46–4.12)	0.95 (0.62–1.45)
Ever smokers									1.33 (1.01–1.74)	1.23 (0.77–1.94)	1.01 (0.76–1.33)	0.87 (0.50–1.51)
Caucasians									1.19 (0.93–1.51)	1.17 (0.91–1.50)	1.15 (0.86–1.54)	0.95 (0.64–1.43)
SCC									1.24 (0.99–1.54)	1.17 (0.88–1.55)	1.13 (0.83–1.54)	

[a] OR, odds ratio (all ORs from the pooled analysis are adjusted for study center); CI, confidence interval; SCC, squamous cell carcinoma.
[b] Prevalent and incident cases.
[c] All ever tobacco smokers and/or chewers.
[d] All males.
[e] All drinkers.
[f] Number of cases and controls genotyped varied for each gene; thus, overall numbers are presented.

actions were not identified. We also analyzed the data for possible gene–environment interactions between each genotype and smoking, but again interactions were not obvious. However, from the subset of studies that had genotype data on all three *GST* loci (906 cases and 543 controls; Refs. 26, 30–32, 37, 38, 42), we observed an increasing risk of head and neck cancer with inheritance of modest risk genotypes at increasing numbers of the *GST* loci that we studied. The null genotypes for *GSTM1* and *GSTT1* and carrying the Val105 allele of *GSTP1* were considered likely to confer modestly increased risk. Taking the subjects with the genotype of *GSTM1* present, *GSTT1* present, and Ile/Ile for *GSTP1* as the reference, the OR was 1.13 (95% CI, 0.83–1.53) for subjects who inherited a modest risk genotype at one *GST* locus, 1.19 (95% CI, 0.87–1.63) for subjects who inherited modest risk genotypes at two *GST* loci, and 1.69 (95% CI, 0.99–2.88) for subjects who carried likely modest risk genotypes at all three *GST* loci when adjusted for study center (test for trend, $P = 0.08$). When further adjusted for age, sex, and race, the OR was 1.16 (95% CI, 0.83–1.63) for one modest risk *GST* genotype, 1.23 (95% CI, 0.86–1.75) for two modest risk *GST* genotypes, and 2.06 (95% CI, 1.11- 3.81) for carrying three modest risk *GST* genotypes (test for trend, $P = 0.04$). When stratified by smoking, the results were not statistically significant.

Discussion

The results from the meta-analysis supported the hypothesis that specific genotypes at the *GSTM1*, *GSTT1*, and *CYP1A1* loci modestly increase the risk of head and neck cancer. Potential sources of heterogeneity included sample size and geographic region. The pooled analysis confirmed the association of head and neck cancer with *GSTM1* and *GSTT1*, but the associations with *GSTP1* or *CYP1A1* missense substitutions were not clear. The pooled analysis was based on a subset of published studies from the meta-analysis that tended to report no associations or weak associations. Although pooling of the data provided increased statistical power to detect gene–environment interactions, we did not observe any strong interactions. One possible explanation for the lack of interaction may be that these gene–environment interactions are heterogeneous by ethnicity, in which case pooling data across different ethnicities may have diluted the interaction. A

relationship was suggested, however, between genotypes at multiple *GST* loci and head and neck cancer risk.

Case–control studies with small sample size (<100 cases or 100 controls) may be reporting inflated ORs. These results suggest caution in the interpretation of small case–control studies. The summary ORs for *GSTM1* and *GSTT1* may also differ by geographic region. The prevalences of these genotypes in controls varied widely among and within regions. In the Indian population, the prevalence of the *GSTM1* and *GSTT1* null genotypes seemed to be particularly low. It will be of interest to further explore whether these genotypes are more relevant in specific ethnic groups, with respect to the risk of head and neck cancer.

Because we were unable to control for matching factors in the meta-analysis, we may have bias in our study-specific effect estimates. However, matching did not seem to be a source of heterogeneity among the studies, and individually matched studies did not have ORs that were biased toward the null, as might be expected, when compared with unmatched studies. Therefore, not controlling for matching factors may not be a strong limitation.

The modest association we observed between the risk of head and neck cancer and the *CYP1A1* Val462 allele (OR, 1.35; 95% CI, 0.95–1.82) could reflect a possible association with the *MspI* variant allele because the *CYP1A1* Val462 allele has been reported to be in strong linkage disequilibrium with the *CYP1A1 MspI* variant allele in Japanese (56) and Finnish populations (57). The *CYP1A1* Ile462Val studies in our meta-analysis that had also examined the *CYP1A1 MspI* sequence variation in most cases showed similar association results for either marker (18, 34, 40, 46). One study that had presented data for a combination of these sequence variants (40) did not show any associations for either sequence variant alone or together, possibly because of the limited number of subjects who carried the sequence variants. Further studies with adequate sample size that examine combinations of *CYP1A1* sequence variants will be helpful in clarifying their role on the risk of head and neck cancer.

Carcinogen metabolism is complex, involving the interaction of numerous carcinogens and enzymes. The GSTs have a variety of substrates, including environmental carcinogens, pesticides, drugs, and endogenous molecules of lipid peroxidation as well as inducing agents, some of which double as substrates, including polycyclic aromatic hydrocarbons, phenolic antioxidants, isothiocyanates, and reactive oxygen species (58). The metabolic action of GST enzymes may differ by cancer site; the highest concentrations of GSTP1 have been observed in oral and pharyngeal tissues, and the highest concentrations of GSTM1 have been observed in laryngeal tissue, relative to the other GSTs (2). GST enzyme expression may also differ according to the general controls of gene expression, such as the rates of transcription, translation, and degradation as well as possible posttranslational modifications.

Individually, sequence variants in carcinogen-metabolizing genes may be modest to moderate risk factors, explaining the inconsistent results seen in epidemiological studies. This meta-analysis supports the hypothesis that genotypes at the *GSTM1*, *GSTT1*, and *CYP1A1* loci are modest risk factors for head and neck cancer. On the other hand, combinations of genotypes that each confer a small relative risk may add up to a relative risk large enough to be observed in epidemiological studies. Our pooled analysis supported the idea that inheritance of multiple modest risk *GST* genotypes may confer a greater risk of head and neck cancer. Future epidemiological studies focusing on complex genotypes within the same gene family or other related gene families may be helpful in identifying individuals at high risk for head and neck cancers and in elucidating gene–gene interactions.

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