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Sequence variants and the risk of head and neck cancer: pooled analysis in the INHANCE consortium

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Previous molecular epidemiological studies on head and neck cancer have examined various single nucleotide polymorphisms (SNPs), but there are very few documented associations. In the International head and neck cancer epidemiology (INHANCE) consortium, we evaluated associations between SNPs in the metabolism, cell cycle, and DNA repair pathways and the risk of head and neck cancer. We analyzed individual-level pooled data from 14 European, North American, Central American, and Asia case–control studies (5,915 head and neck cancer cases and 10,644 controls) participating in the INHANCE consortium. Unconditional logistic regression

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was used to estimate odds ratios (OR) and 95% confidence intervals (CI) for SNP effects, adjusting for age, sex, race, and country. We observed an association between head and neck cancer risk and MGMT Leu84Phe heterozygotes (OR = 0.79, 95% CI = 0.68–0.93), XRCC1 Arg194Trp homozygotes Arg/Arg (OR = 2.3, 95% CI = 1.1–4.7), *ADH1B* Arg48His homozygotes Arg/Arg (OR = 2.7, 95% CI = 1.9–4.0), *ADH1C* Ile350Val homozygotes Ile/Ile (OR = 1.2, 95% CI = 1.1–1.4), and the *GSTM1* null genotype (OR = 1.1, 95% CI = 1.0–1.2). Among these results, *MGMT* Leu84Phe, *ADH1B* Arg48His, *ADH1C* Ile350Arg, and the *GSTM1* null genotype had fairly low false positive report probabilities (<20%). We observed associations between *ADH1B* Arg48His, *ADH1C* Ile350Arg, and *GSTM1* null genotype and head and neck cancer risk. No functional study currently supports the observed association for *MGMT* Leu84Phe, and the association with *XRCC1* Arg194Trp may be a chance finding.

Keywords: SNP, head and neck cancer, INHANCE

INTRODUCTION

Head and neck cancer, including cancers in oral cavity, pharynx (other than nasopharynx), and larynx, is the sixth most common cancer in the world (Parkin et al., 2005). It accounted for about 900,000 of cases and 300,000 deaths in 2008 (Ferlay et al., 2010). The 5-year survival rate was about 61% in the US for all sites (Altekruse et al., 2010) and 26 to 63% in Europe depending on the subsite (Berrino et al., 2007). The major risk factors for head and neck cancer are tobacco smoking and alcohol drinking (Hashibe et al., 2007). The interaction between tobacco smoking and alcohol drinking is greater than the expected multiplicative null with an overall attributable risk of 72% (Hashibe et al., 2009). Other risk factors include human papillomavirus (HPV) infection (IARC Working Group/Human Papillomaviruses, 2007), passive smoking (Lee et al., 2008), low body mass index (BMI; Gaudet et al., 2010), poor diet (World Cancer Research Fund/American Institute for Cancer Research, 2007), and family history of cancer (Negri et al., 2009).

Previous molecular epidemiological studies on head and neck cancer have examined single nucleotide polymorphisms (SNPs), focusing on metabolic and DNA repair genes (Sturgis and Wei, 2002; Hashibe et al., 2003, 2008; Canova et al., 2009); however, very few SNP associations have been consistent for head and neck cancer risk. The heterogeneity may result from study design, e.g., population- or hospital-based controls, study sample size, and study populations, e.g., race/ethnicity (Hashibe et al., 2003; Lohmueller et al., 2003). The International head and neck cancer epidemiology (INHANCE) consortium is a collaboration of research groups leading large molecular epidemiology studies of head and neck cancer. Since larger sample sizes can increase the precision of the effect measure estimates and the ability to detect statistically significant moderate associations, we pooled data on SNPs that were genotyped in common across the INHANCE studies, to evaluate the associations between the SNPs in several pathways and the risk of head and neck cancer.

MATERIALS AND METHODS

The INHANCE consortium (http://inhance.iarc.fr/) was established in 2004. Fourteen studies participating in the consortium contributed SNP data: France (Benhamou et al., 2004), Central Europe (Hashibe et al., 2006), Seattle (Schwartz et al., 2001; Huang et al., 2005), Iowa (Wang et al., 2005), North Carolina (Olshan et al., 2000), Los Angeles (Cui et al., 2006), Houston (Zhang et al., 2005), Puerto Rico (Hayes et al., 1999), Rome (Boccia et al., 2008), Western Europe (Canova et al., 2009), Heidelberg (Risch et al., 2003), Japan (Suzuki et al., 2007), Northeast US (Park et al., 2003), and India (Anantharaman et al., 2007). Descriptions of the individual studies are presented in the **Table A1** in Appendix. To increase power, we included all controls selected for lung and kidney cancers in the Central Europe multicenter case–control study, in addition to the head and neck cancer controls. There were 6,694 head and neck cancer cases and 12,601 controls.

Cases and controls with missing data on age, sex, race/ethnicity, or SNP information, and cases with missing information on the site of origin of their cancer were excluded (779 cases and 1,957 controls). In total, 5,915 cases and 10,644 controls were included in the analysis. Among the cases, 1,901 were oral cancer, 1,751 were pharyngeal cancer, 440 were cancers of the oral cavity or pharynx not otherwise specified, 1,632 were laryngeal cancer and 191 overlapping or subsite missing.

Written informed consent was obtained from all study subjects and the investigations were approved by institutional review boards at each of the institutes involved. Questionnaires were collected from all the individual studies, to assess the comparability of the collected data and of the wording of interview questions among the studies. Each data item was checked for illogical or missing values and inconsistencies were resolved as necessary.

Details on harmonizing questionnaire data have been published previously (Hashibe et al., 2007). Briefly, the definitions for ever smoking and drinking are different across studies. We reclassified ever tobacco smokers as those who have smoked at least 100 cigarettes or 100 cigars or 100 pipes in their lifetime. In our previous analyses, drinking (≥3 alcoholic drinks/day) was associated increased HNC risks (Hashibe et al., 2007), we thus classified heavy drinkers as those who have consumed three or more alcoholic drinks per day.

Single nucleotide polymorphisms reported in more than two studies were included in the current pooled analyses. In total, 28 SNPs in cell cycle (*p21* Ser31Arg rs1801270 and *p53* Pro72Arg rs1042522), DNA repair (*ERCC2* Lys751Gln rs28365048, *MGMT* Leu84Phe rs12917, Ile143Val rs2308321, 171C > T rs1803965, *OGG1* Ser326Cys rs1052133, *XRCC1* Arg194Trp rs1799782, Arg280His rs25489, Arg399Gln rs25487, and *XRCC3* Thr241Met rs861539), folate metabolism (*MTHFR* Ala222Val rs1801133 and Glu429Ala rs1801131), and carcinogen metabolism (*ADH1B* Arg48His rs1229984, *ADH1C* Ile350Val rs698, *CYP1A1* Ile462Val rs1048943, 3801T > C E0322, *CYP2E1* 1054C > T rs2031920,

1143A > T rs6413432, 1293G > C rs3813867, *EPHX1* Try113His rs1051740, His139Arg rs2234922, *GSTM1* null, *GSTM3* Mnl AGG deletion rs1799735, *GSTP1* Ile105Val rs947894, Ala114Val rs1799811, *GSTT1* null, *NQO1* Pro187Ser rs1800566) pathways were included. Hardy–Weinberg equilibrium was tested in the controls by study. SNPs which did not pass the test in a specific study were excluded from the analyses.

The associations between SNPs and head and neck cancer risk were assessed by estimating odds ratios (OR) and 95% confidence intervals (CI) with unconditional logistic regression models for each study. The models included age (categories), sex, race/ethnicity (categories), and country (categories). In the Central Europe study, information on ethnicity was not collected and all subjects were classified as non-Hispanic white, since the majority of these populations were expected to be white. Pooled ORs were estimated with a fixed effects and random effects (DerSimonian and Laird, 1986) logistic regression model. We assessed heterogeneity across studies by the likelihood ratio test. Stratified analyses were conducted by cancer site (oral, pharynx, oral/pharynx not specified, and larynx), age (<45 and ≥45 years), sex, race (White, Black, Hispanic, and Asian) geographic region (Europe, North America, and Latin America), study type (hospital-based and population-based), study size (<300 cases and ≥300 cases), smoking (never and ever), drinking [≤3 and >3 drinks/day (Hashibe et al., 2007)] and fruit and vegetable intake (lower and higher than center-specific median among controls). The analyses were performed using SAS 9 and significant associations were defined as a two-sided *p*-value less than 0.05.

False positive report probability (FPRP; Wacholder et al., 2004) was assessed for all associations in which the two-sided null *p*-value was less than 0.05. FPRP was assessed for OR = 1.5 for probability of true association were 0.25, 0.1, 0.01, and 0.001. For the haplotype analysis, we selected the studies that had information on the multiple SNPs for the gene. We used PHASE v2. (Stephens et al., 2001; Stephens and Donnelly, 2003) to reconstruct the haplotype for genes with multiple SNPs available. Overall, haplotypes for four genes, *CYP2E1*, *EPHX1*, *GSTP1*, and *MTHFR*, could be reconstructed. The most frequent haplotype was treated as the referent group.

RESULTS

A total of 5,915 cases and 10,644 controls were pooled from 14 studies in Europe (2,759 cases and 4,629 controls), North America (2,234 cases and 3,290 controls), Central America (147 cases and 149 controls), and Asia (775 cases and 2,576 controls). Demographic characteristics of the cases and controls are presented in **Table 1**. Among the cell cycle and DNA repair SNPs, we observed associations between head and neck cancer risk and *MGMT* Leu84Phe heterozygotes (OR = 0.79, 95% CI = 0.68–0.93) and *XRCC1* Arg194Trp rare homozygotes (OR = 2.3, 95% CI = 1.1–4.7; **Table 2**).

For the carcinogen metabolism SNPs, associations were observed between head and neck cancer risk and *ADH1B* Arg48His His/His homozygotes (OR = 2.7, 95% CI = 1.9–4.0), *ADH1C* Ile350Val rare homozygotes (OR = 1.2, 95% CI = 1.1–1.4), and the *GSTM1* null type (OR = 1.1, 95% CI = 1.0–1.2; **Table 3**). Further adjusting for cigarette smoking and alcohol consumption (**Table A2** in Appendix) did not change the results greatly. In addition, *XRCC1* Arg280His rare homozygotes showed an association with head and neck cancer risk after adjustment of cigarette smoking and alcohol consumption (OR = 3.3, 95% CI = 1.1–10).

Table 1 | Demographic characteristics of head and neck cancer cases and controls.

		Cases		Controls	
		n	%	*n*	%
All		5915		10644	
SEX					
Female		1251	21.1	2765	26.0
Male		4664	78.9	7879	74.0
AGE					
≤44		655	11.1	1545	14.5
45–49		628	10.6	1117	10.5
50–59		2014	34.0	3333	31.3
60–69		1664	28.1	2991	28.1
70+		954	16.1	1658	15.6
RACE					
White		4676	79.1	7298	68.6
Black		267	4.5	383	3.6
Hispanic		117	2.0	273	2.6
Asian		811	13.7	2647	24.9
Others		44	0.7	43	0.4
STUDY	**COUNTRY**				
France	France	256	4.3	173	1.6
Central Europe	*Romania*	103	1.7	185	1.7
	Poland	189	3.2	814	7.6
	Russia	303	5.1	805	7.6
	Slovakia	40	0.7	196	1.8
Seattle	USA	360	6.1	560	5.3
Iowa	USA	366	6.2	330	3.1
North Carolina	USA	174	2.9	196	1.8
Los Angeles	USA	320	5.4	915	8.6
Houston	USA	828	14.0	865	8.1
Puerto Rico	Puerto Rico	147	2.5	149	1.4
Rome	Italy	277	4.7	293	2.8
Western Europe	Czech Republic	111	1.9	152	1.4
	Germany	175	3.0	179	1.7
	Greece	190	3.2	160	1.5
	Italy	378	6.4	446	4.2
	Ireland	17	0.3	16	0.2
	Norway	110	1.9	137	1.3
	UK	236	4.0	301	2.8
	Spain	76	1.3	81	0.8
	Croatia	52	0.9	46	0.4
Heidelberg	Germany	246	4.2	645	6.1
Japan	Japan	320	5.4	1848	17.4
Northeast US	USA	186	3.1	424	4.0
India	India	455	7.7	728	6.8

Table 2 | Cell cycle and DNA repair SNPs and the risk of head and neck cancer in the INHANCE consortium.

Gene	SNP rs number	Alteration	Referent genotype	No. of cases	No. of controls	Analysis model	Heterozygotes				Rare homozygotes			
							OR	(95%CI)	No. of studies	*p* for heterogeneity	OR	(95%CI)	No. of studies	*p* for heterogeneity
P21	rs1801270	Ser31Arg	Ser/Ser	2301	3920	Fixed effects	1.11	(0.94–1.30)	3	0.01	1.41	(0.75–2.64)	3	0.09
						Random effects	1.24	(0.55–2.77)			1.52	(0.27–8.66)		
P53	rs1042522	Pro72Arg	Arg/Arg	2982	4488	Fixed effects	0.97	(0.87–1.07)	4	0.52	0.97	(0.80–1.17)	4	0.07
						Random effects	0.97	(0.82–1.14)			0.97	(0.71–1.32)		
ERCC2	rs28365048	Lys751Gln	Lys/Lys	2587	4771	Fixed effects	0.97	(0.86–1.09)	5	0.75	1.03	(0.88–1.21)	5	0.48
						Random effects	0.97	(0.82–1.14)			1.03	(0.83–1.29)		
MGMT	rs1803965	171C>T	C/C	2310	3936	Fixed effects	0.98	(0.86–1.11)	3	0.61	1.05	(0.74–1.50)	3	0.21
						Random effects	0.98	(0.73–1.30)			1.06	(0.47–2.38)		
MGMT	rs2308321	Ile143Val	Ile/Ile	2684	4349	Fixed effects	0.90	(0.79–1.02)	6	0.18	1.20	(0.81–1.79)	6	0.68
						Random effects	0.90	(0.76–1.06)			1.20	(0.71–2.02)		
MGMT	rs12917	Leu84Phe	Leu/Leu	1455	3160	Fixed effects	0.79	(0.68–0.93)	5	0.79	1.40	(0.94–2.07)	5	0.69
						Random effects	0.79	(0.64–0.99)			1.40	(0.80–2.44)		
OGG1	rs1052133	Ser326Cys	Ser/Ser	1680	4825	Fixed effects	0.95	(0.83–1.08)	5	0.89	0.98	(0.77–1.24)	5	0.67
						Random effects	0.95	(0.79–1.14)			0.98	(0.69–1.38)		
XRCC1	rs1799782	Arg194Trp	Arg/Arg	1272	2984	Fixed effects	1.02	(0.83–1.26)	4	0.76	2.30	(1.13–4.67)	4	1.00
						Random effects	1.02	(0.72–1.44)			2.30	(0.73–7.26)		
XRCC1	rs25489	Arg280His	Arg/Arg	903	2794	Fixed effects	1.12	(0.88–1.44)	2	0.84	2.87	(0.95–8.67)	2	0.21
						Random effects	1.12	(0.23–5.58)			2.87	(0.00–3719)		
XRCC1	rs25487	Arg399Gln	Arg/Arg	2602	4255	Fixed effects	0.93	(0.83–1.03)	6	0.21	1.00	(0.84–1.19)	6	0.01
						Random effects	0.93	(0.80–1.07)			0.89	(0.58–1.37)		
XRCC3	rs861539	Thr241Met	Thr/Thr	2707	4544	Fixed effects	0.96	(0.86–1.07)	7	0.14	0.90	(0.77–1.06)	7	0.88
						Random effects	0.96	(0.84–1.10)			0.90	(0.74–1.10)		

OR adjusted for age, sex, country, and race.

Table 3 | Carcinogen metabolism SNPs and the risk of head and neck cancer in the INHANCE consortium.

Gene	SNP rs number	Alteration	Referent genotype	No. of cases	No. of controls	Analysis model	Heterozygotes				Rare homozygotes			
							OR	(95%CI)	No. of studies	*p* for heterogeneity	OR	(95%CI)	No. of studies	*p* for heterogeneity
MTHFR	rs1801131	Glu429Ala	Glu/Glu	830	2164	Fixed effects	0.90	(0.75–1.07)	2	0.14	0.95	(0.69–1.31)	2	0.25
						Random effects	0.90	(0.29–2.82)			0.95	(0.12–7.66)		
MTHFR	rs1801133	Ala222Val	Ala/Ala	2605	5444	Fixed effects	0.98	(0.88–1.09)	5	0.87	1.04	(0.88–1.23)	4	0.69
						Random effects	0.98	(0.85–1.14)			1.04	(0.80–1.36)		
ADH1B	rs1229984	Arg48His	His/His	2407	5408	Fixed effects	1.15	(0.92–1.43)	4	0.95	2.73	(1.87–3.98)	3	0.94
						Random effects	1.15	(0.80–1.65)			2.73	(1.19–6.26)		
ADH1C	rs698	Ile350Val	Ile/Ile	3306	6264	Fixed effects	1.06	(0.96–1.18)	9	0.42	1.22	(1.06–1.41)	9	0.09
						Random effects	1.06	(0.93–1.21)			1.19	(0.94–1.50)		
CYP1A1	E0322	3801T > C	T/T	1062	2657	Fixed effects	0.95	(0.80—1.14)	2	0.24	1.41	(0.94–2.12)	2	0.36
						Random effects	0.95	(0.30–2.98)			1.41	(0.10–19.9)		
CYP1A1	rs1048943	Ile462Val	Ile/Ile	2814	4823	Fixed effects	0.96	(0.81–1.14)	7	0.56	0.70	(0.35–1.43)	4	1.00
						Random effects	0.96	(0.77–1.19)			0.70	(0.22–2.23)		
CYP2E1	rs6413432	1143A > T	A/A	722	974	Fixed effects	1.20	(0.85–1.69)	2	0.13	1.39	(0.41–4.74)	2	0.60
						Random effects	1.21	(0.12–11.9)			1.39	(0.00–3889)		
CYP2E1	rs3813867	1293G > C	G/G	1789	3797	Fixed effects	0.86	(0.66–1.13)	5	0.72	1.65	(0.10–26.7)	4	1.00
						Random effects	0.86	(0.59–1.26)			1.65	(0.02–151.9)		
CYP2E1	rs2031920	1054C > T	C/C	981	1414	Fixed effects	1.18	(0.80—1.74)	4	0.66	1.66	(0.10–26.9)	4	1.00
						Random effects	1.18	(0.63–2.22)			1.66	(0.02–152.7)		
EPHX1	rs2234922	His139Arg	His/His	2840	4464	Fixed effects	0.94	(0.84–1.05)	6	0.25	1.24	(0.97–1.58)	6	0.25
						Random effects	0.94	(0.81–1.08)			1.24	(0.89–1.71)		
EPHX1	rs1051740	Tyr113His	Tyr/Tyr	2882	4923	Fixed effects	1.01	(0.91–1.11)	6	<0.01	0.89	(0.75–1.05)	6	0.86
						Random effects	0.94	(0.71–1.26)			0.89	(0.71–1.11)		
GSTM1	NA	Null	present	3857	7232	Fixed effects	NA				1.11	(1.02–1.20)	12	0.33
						Random effects	NA				1.11	(1.00–1.23)		
GSTM3	rs1799735	*Mn*I AGG > del	AGG/AGG	1039	2478	Fixed effects	1.02	(0.85–1.22)	3	0.38	0.90	(0.60–1.36)	3	0.72
						Random effects	1.02	(0.69–1.51)			0.90	(0.37–2.23)		
GSTP1	rs1799811	Ala114Val	Ala/Ala	2447	2757	Fixed effects	1.00	(0.85–1.18)	4	0.46	1.35	(0.77–2.35)	4	0.27
						Random effects	1.00	(0.76–1.31)			1.35	(0.55–3.32)		
GSTP1	rs947894	Ile105Val	Ile/Ile	4086	6461	Fixed effects	1.01	(0.93–1.11)	9	0.23	1.01	(0.88–1.16)	9	0.61
						Random effects	1.01	(0.91–1.13)			1.01	(0.85–1.18)		
GSTT1	NA	Null	present	3704	6919	Fixed effects	NA				0.91	(0.81–1.01)	11	0.13
						Random effects	NA				0.91	(0.78–1.05)		
NQO1	rs1800566	Pro187Ser	Pro/Pro	1896	4038	Fixed effects	1.03	(0.91–1.17)	5	0.73	1.19	(0.88–1.61)	5	0.07
						Random effects	1.03	(0.86–1.23)			1.21	(0.68–2.16)		

OR adjusted for age, sex, country, and race.

Table 4 shows the FPRP for the observed associations for the selected SNPS under a given range of probability of true associations. If the prior probability is greater than 10% and we assume the expected OR = 1.5, the FPRPs for *MGMT* Leu84Phe, *ADH1B* Arg48His, *ADH1C* Ile350Val, and *GSTM1* were lower than 20%.

Odds ratios for selected SNPs by head and neck cancer subsite are shown in **Table 5**. *ADH1B* His48Arg and *ADH1C* Ile350Val were consistently associated with oral, pharyngeal, and oral/pharyngeal NOS cancer, but *ADH1C* Ile350Val was not associated with laryngeal cancer. The association of *GSTM1* null genotype was observed only with oral cancer (OR = 1.2 95% CI = 1.0–1.3).

Figure 1 shows the stratified results for the selected SNPs, comparing the rare homozygotes to the common homozygotes. The effects from *ADH1B* Arg48His tended to be stronger among ever smokers and heavy drinkers than non-smokers and light drinkers. The associations from *ADH1C* Ile350Val were much stronger among Hispanic and Asian; however, these were based on relatively small numbers (case/control in the rare homozygotes: 16/10 for Hispanic and 2/3 for Asian).

Table 6 further evaluated the joint effects among smoking, drinking, and the selected SNPs. There was no evidence for the interaction between drinking and *ADH1B* Arg48His and *ADH1C* Ile350Val, and smoking and *GSTM1* on the risk of head and neck cancer.

Table 4 | False positive report probabilities (FPRP) for observed associations for selected SNPs.

SNPs		Ca	Co	OR	FPRP for OR = 1.5 and for different probabilities of a true association			
					0.250	0.100	0.010	0.001
MGMT L84F	Leu/Phe	307	823	0.79	0.014	0.041	0.319	0.825
	Phe/Phe	43	81	1.40	0.302	0.565	0.935	0.993
XRCC1 R194W	Arg/Trp	155	359	1.02	0.719	0.885	0.988	0.999
	Trp/Trp	16	21	2.30	0.349	0.617	0.947	0.994
ADH1B R48H	Arg/His	467	1194	1.15	0.387	0.655	0.954	0.995
	His/His	1710	3018	2.73	0.001	0.002	0.019	0.161
ADH1C I350V	Ile/Val	1383	2252	1.06	0.463	0.721	0.966	0.997
	Val/Val	579	778	1.22	0.021	0.060	0.413	0.877
GSTM1	null	1906	3386	1.11	0.025	0.073	0.463	0.897

OR adjusted for age, sex, country, race.

Table 5 | Selected SNPs and the risk of head and neck cancer by subsite.

	Oral OR (95% CI)	Pharynx OR (95% CI)	Oral/pharynx. NOS OR (95% CI)	Larynx OR (95% CI)
ADH1B His48Arg (ca/co)	541/5157	593/5157	208/5157	981/5408
His/His	1.00	1.00	1.00	1.00
His/Arg	1.46 (0.86–2.47)	0.98 (0.61–1.27)	1.06 (0.68–1.64)	0.87 (0.56–1.34)
Arg/Arg	2.37 (1.31–4.30)	5.71 (3.22–10.1)	2.27 (1.14–4.52)	1.50 (0.67–3.38)
P_{trend}	<0.01	<0.01	0.03	0.08
His/Arg or Arg/Arg	1.89 (1.29–2.78)	2.80 (2.00–3.92)	2.22 (1.34–3.69)	1.55 (1.16–2.07)
ADH1C I350V (ca/co)	980/6013	1097/6013	240/6013	940/5255
Ile/Ile	1.00	1.00	1.00	1.00
Ile/Val	1.18 (0.99–1.40)	1.10 (0.93–1.30)	1.16 (0.80–1.67)	0.90 (0.75–1.08)
Val/Val	1.40 (1.12–1.76)	1.22 (0.98–1.52)	2.08 (1.19–3.63)	1.11 (0.88–1.41)
P_{trend}	0.01	<0.01	0.22	<0.01
Ile/Val or Val/Val	1.22 (1.04–1.44)	1.13 (0.97–1.32)	1.24 (0.87–1.76)	0.94 (0.80–1.12)
GSTM1 (ca/co)	1236/6637	1068/5911	260/5911	798/3620
Present	1.00	1.00	1.00	1.00
Null	1.15 (1.01–1.32)	0.95 (0.83–1.10)	1.06 (0.82–1.38)	1.06 (0.89–1.26)

OR adjusted for age, sex, country, and race.

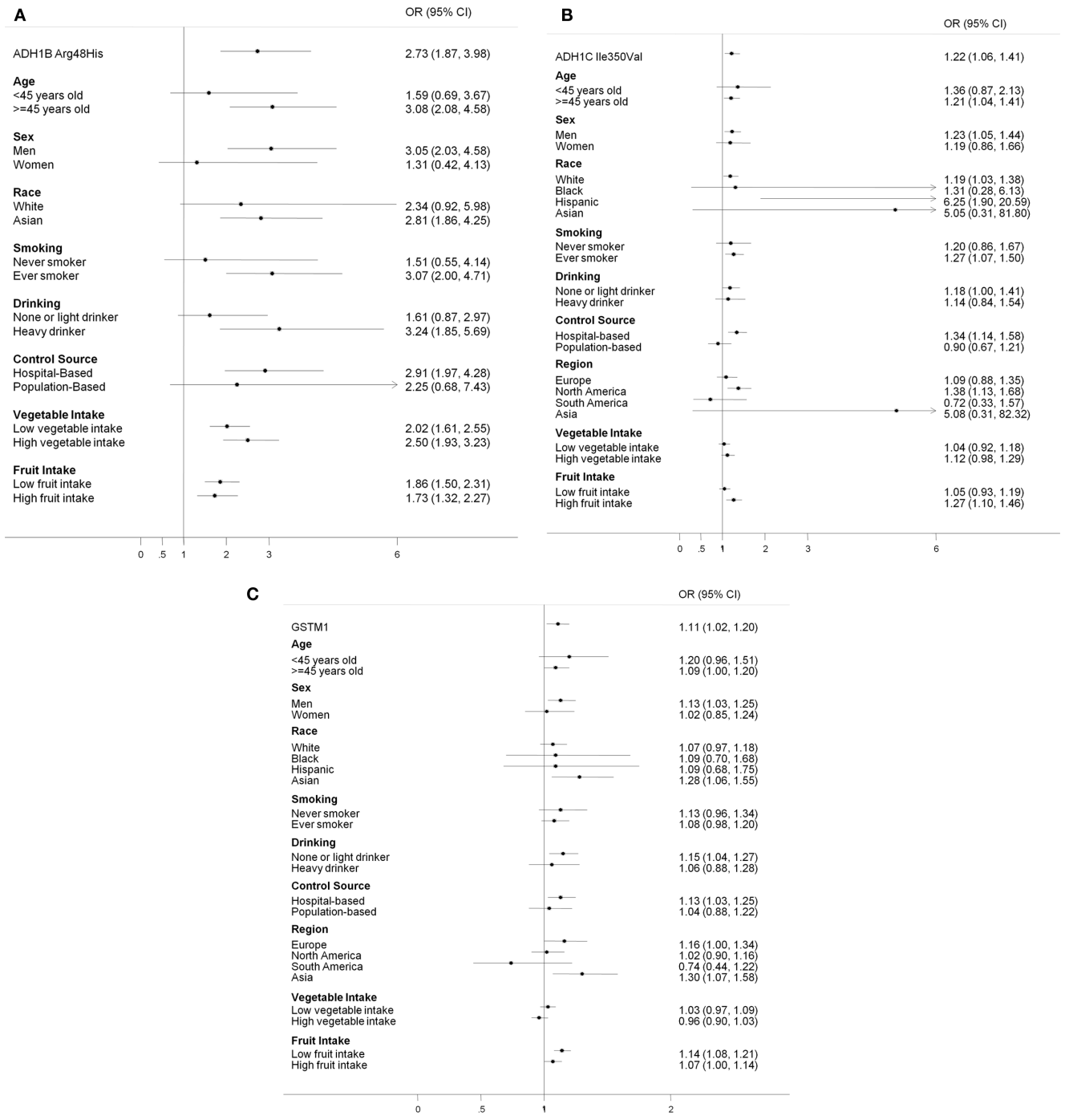

FIGURE 1 | Stratified analysis for selected SNPs: (A) ADH1B Arg48His; His/His vs. Arg/Arg (B) ADH1C Ile350Val: Val/Val vs. Ile/Ile; (C) GSTM1 null genotype.

No obvious associations were observed for the haplotypes of *CYP2E1*, *EPHX1*, *GSTP1*, and *MTHFR* (data not shown).

DISCUSSION

Our results showed associations between head and neck cancer risk and genetic variants in *MGMT* Leu84Phe, *XRCC1* Arg194Trp, *ADH1B* Arg48His, *ADH1C* Ile350Val, and the *GSTM1*. Among them, the results for *XRCC1* Arg194Trp might be a false finding because the FPRP was high even if the prior probability was set to be high (0.25).

Among the four studies reported XRCC1 Arg194Trp, none of them were statistically significant with a total of 16 cases and 21 controls in the Trp/Trp group (**Table 4**). In the International lung cancer consortium, which pooled five studies with 26 cases and 28 controls

Table 6 | Joint effects of drinking and ADH1B Arg48His and ADH1C Ile350Val and smoking and GSTM1 on the risk of head and neck cancer.

Lifestyle	SNP	Case	Control	OR	95% CI
Drinking*	ADH1B Arg48His				
Light	His/His or His/Arg	331	1773	1.00	
Light	Arg/Arg	949	2186	1.73	(0.96–3.14)
Heavy	His/His or His/Arg	292	503	1.96	(1.12–3.43)
Heavy	Arg/Arg	711	552	4.03	(0.95–17.0)
	Interaction			1.21	(0.43–3.41)
Drinking*	ADH1C Ile350Val				
Light	Ile/Ile or Ile/Val	1458	4004	1.00	
Light	Val/Val	327	566	1.18	(0.88–1.59)
Heavy	Ile/Ile or Ile/Val	906	893	2.45	(1.79–3.37)
Heavy	Val/Val	197	117	2.42	(1.64–3.56)
	Interaction			0.97	(0.62–1.52)
Smoking	GSTM1				
No	Present	398	1414	1.00	
No	Null	385	1246	1.16	(0.94–1.42)
Yes	Present	1328	1865	3.03	(1.78–5.17)
Yes	Null	1318	1761	3.11	(1.92–5.06)
	Interaction			0.86	(0.66–1.12)

**Heavy drinkers were defined as those who drank > 40 ml/day.*

in the rare homozygote group, reported a non-statistically significant association with increased lung cancer risk (Hung et al., 2008). In a meta-analysis on DNA damage response genes and head and neck cancer reported an increased oral cancer risk for XRCC1 194Trp in the Asian population; however, no association was observed in the Caucasian population (Flores-Obando et al., 2010). A meta-analysis investigated the associations between DNA repair gene polymorphisms and smoking and found that XRCC1 194Trp, 280His, and 399Gln were associated with smoking behaviors (Hodgson et al., 2010). Nevertheless, further adjusted for smoking and drinking behaviors did not change the results materially, even though the association with 280His becomes statistically significant (**Table A2** in Appendix). Further investigation into the XRCC1 function and its association in tobacco induced gene repair might be helpful to elucidate the associations with head and neck cancer risks.

Association between *MGMT* Leu84Phe and head and neck cancer risk was observed in the present report. The effects were similar regarding the cigarette smoking and alcohol drinking status, age, sex, and race (data not shown). The FPRP was 0.787 if the priority probability was very low (0.001). However, the associations between heterozygotes and rare homozygotes and head and neck cancer risk were in different directions. The results for the rare homozygotes were imprecise across the five studies provided the SNP (OR = 1.60, 0.90, 0.80, 1.55, and 0.66). Excluding the largest study which provided 608 cases and 1925 controls (OR = 1.60) in the analyses yield an OR 0f 0.99 (0.33–3.02). Nevertheless, the function of the *MGMT* Leu84Phe rare allele is not thought to differ from that of common homozygotes (based on the purified protein in the repair of O^6-methylguanine *in vitro*; Pegg et al., 2007). In addition, more than 500 SNPs have been identified in *MGMT* in Caucasians and ~60% of the identified SNPs have minor allele frequency greater than 0.05 (Bugni et al., 2007). The *MGMT* polymorphism might be associated with *MGMT* hypermethylation (Ogino et al., 2007), which could cause mutations in other critical genes (Mukai and Sekiguchi, 2002). The observed association might be a result of the mutated gene or an unidentified causal SNP. More studies would be needed to explore the associations and the mechanisms between *MGMT* polymorphisms and head and neck cancer.

Both *ADH1B* and *ADH1C* gene belongs to the ADH class I family, which plays a major role in ethanol metabolism (Edenberg, 2000). *ADH1C* 350Val and 272Arg result in a faster metabolism of ethanol (Hoog et al., 1986; Carr et al., 1989). Earlier associations on *ADH1B* and head and neck cancer were based on Asian studies (Hori et al., 1997; Yokoyama et al., 1999, 2001; Asakage et al., 2007; Hiraki et al., 2007). Recently, two large case–control studies further confirmed the association in European populations (Hashibe et al., 2006, 2008). The current analysis included the above studies; however, none of the study is influential on the overall pooled estimates. The differences in the published results could be because the frequency of the His allele is extremely low in the European population (International HapMap Project, 2008). In our study, only 1.2% European subjects carried the His/His genotype while 61.3% Asian participants had the polymorphism. Despite the differences in distribution, the effects from the SNP did not differ by populations (**Figure 1**). The effects from *ADH1B* Arg/Arg were stronger among ever smokers or heavy drinkers. People who carry the *ADH1B* Arg/Arg genotype also tended to consume more alcohol than those who carried the His/His or His/Arg genotype (**Table A3** in Appendix).

Earlier studies found no association between *ADH1C* Ile350Val and head and neck cancers (Coutelle et al., 1997; Bouchardy et al., 2000; Olshan et al., 2001; Sturgis et al., 2001; Wang et al., 2005) while some recent studies reported associations as main effects or combined effects with alcohol drinking (Harty et al., 1997; Peters et al., 2005; Hashibe et al., 2006). A large study (Hashibe et al., 2008) combining data from Europe and Latin America further suggested associations of *ADH1B, ADH1C,* and *ADH7* genes with head and neck cancer risk. In the present pooled analysis, which combined data from Europe, North America, Latin America, and Asia, we observed an overall 25% increased risk of head and neck cancer on *ADH1C* Ile350Val, especially among oral and pharyngeal cancer patients. The result from Asia was very unstable possibly due to the low frequency of the homozygotes in the population. Excluding the Asian study from the analysis did not change the results materially.

ADH1B Arg48His and *ADH1C* Ile350Val genes are only 100 kb away and have strong linkage disequilibrium (LD > 0.65; International HapMap Project, 2008). However, after restriction to the *ADH1B* His/His or His/Arg population, *ADH1C* Val/Val still showed strong association on head and neck cancer risk. The frequency was very low in the European population and thus the statistical power was low (data not shown).

GSTM1 belongs to the glutathione *S*-transferases (GST) family producing phase II xenobiotic metabolic enzymes. The GSTs play a role in the metabolism of chemical carcinogens, especially with regard to those present in tobacco smoke (Peters et al., 2006). However, epidemiological studies on the associations between GSTs and head and neck cancer have been inconsistent. The inconsistency could be attributed to study design, for example, population-based controls vs. hospital-based controls, matching criteria, and

incident cases vs. prevalent cases (Geisler and Olshan, 2001). The GST enzymes are also known to be expressed differently by site (Pacifici et al., 1988; Moscow et al., 1989; Howie et al., 1990). In the present study, *GSTM1* null genotype was associated with oral cavity cancer but not the other head and neck cancer sites.

Interestingly, race seems to be a potential effect modifier for the association between *GSTM1* and head and neck cancer, with ORs of 1.1 for Whites, Blacks, and Hispanic and 1.3 for Asians, even though the CI overlapped. The OR for Asians in North America was 1.3 (case/control: 28/64, 95% CI = 0.11–16).

In addition, significant results were only observed in the hospital-based studies for the association between *GSTM1* and head and neck cancer. Hospital-based studies in our study exclude individuals with previous or malignant disease, including respiratory disease (France, Rome, North Carolina, Northeast US, India), tobacco or alcohol related diseases (Central Europe and Western Europe) or from hospital visitors (Houston). The *GSTM1* genotype distribution among the hospital-based controls may not reflect those of the base population in our study. Among controls, the hospital-based studies had lower frequency of the null type *GSTM1* than population-based studies (45 vs. 52%, $p < 0.0001$).

A limitation of our study is that the pooled analysis is based on a heterogeneous population from different geographic regions and ethnicities. However, heterogeneity across studies was not significant for most of our results. In addition, stratified analysis was conducted to assess whether our observations were due to a specific geography and ethnicity. In addition, individual data was available and were harmonized according to standard protocol; thus, we were able to control the potential confounders consistently.

A genome-wide association study (GWAS) was performed independently within the INHANCE consortium using HumanHap300 platform (McKay et al., 2011). The GWAS used the Central Europe and Western Europe studies for the discovery phase and replicated the findings in another subset of the INHANCE consortium: Los Angeles, Houston, Latin America, IARC Multicenter, Boston, and Rome studies, in combination with additional studies not in the current pooled analyses. The *ADH1C* Ile350Val was strongly associated with the head and neck cancer in the discovery phase ($p < 10^{-5}$) and was replicated in the replication phase. The *ADH1B* His48Arg was not tagged on HumanHap300 but was selected as a candidate gene for the replication and showed the strongest association in the replication phase ($p = 3 \times 10^{-12}$). Inconsistency has been observed between results from candidate gene approach and from GWAS in previous studies (Siontis et al., 2010). The consistent findings in the two ADH SNPs in both GWAS (McKay et al., 2011) and a previous analyses (Hashibe et al., 2006) as well as the current pooled analyses based on candidate gene approach further support the role of alcohol metabolism genes on the head and neck cancer etiology. Our observations suggest that, while GWAS may lead to novel hypotheses by elucidating new disease associated loci, traditional hypotheses-driven associations should not be ignored.

In summary, we observed associations between *ADH1B* Arg48His, *ADH1C* Ile350Arg, *GSTM1* null and head and neck cancer risk. An association for *XRCC1* Arg194Trp was not well supported based on the FPRPs. Further investigation is needed for *MGMT* Leu84Phe for the mechanism. *ADH1B* Arg48His and *ADH1C* Ile350Arg could be risk factors or markers of other risk genes for head and neck cancer. Effect of *GSTM1* is slightly associated with increased risk of head and neck cancer but inconsistent across subsites.

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APPENDIX

Table A1 | Summary of individual studies involved in the current analysis.

Study location (reference†)	Recruitment period	Platform/method¥	Case subjects			Control subjects‡	
			Source	Participation rate, %	Age eligibility years	Source	Participation rate, %
EUROPE							
Paris, France (Benhamou et al., 2004)	1987–1992	PCR-RFLP	Hospital	95§	NA	Hospital (unhealthy)	95§
Central Europe (Banska Bystrica, Bucharest, Budapest, Lodz, Moscow)‖ (Hashibe et al., 2006)	1998–2003	TaqMan	Hospital	96	≥15	Hospital (unhealthy)	97
Rome (Boccia et al., 2008)	2002–2007	PCR-RFLP	Hospital	98	NA	Hospital (unhealthy)	94
Western Europe (Canova et al., 2009)	2000–2005	APEX	Hospital	82		Hospital (unhealthy)*	68
Heidelberg, Germany (Risch et al., 2003)	1998–2000	PCR-RFLP	Hospital	96	<80	Population registry	62
NORTH AMERICA							
Seattle, WA Crump et al. (2000), Huang et al. (2005), Schwartz et al. (2001)	1985–1995	PCR-RFLP, multiplex, or matrix-assisted laser desorption/ionization time-of-flight mass spectrometry	Cancer registry	54.4, 63.3¶	18–65	Random digit dialing	63, 61¶
Iowa (Wang et al., 2005)	1993–2006	PCR-RFLP	Hospital	87	>17	Hospital (healthy)	92
North Carolina (Olshan et al., 2000)	1994–1997	Multiplex	Hospital	88	>17	Hospital (unhealthy)	86
Los Angeles, CA (Cui et al., 2006)	1999–2004	PCR-RFLP	Cancer registry	49	18–65	Neighborhood	67.5
Houston, TX (Zhang et al., 2005)	2001–2006	PCR-RFLP	Hospital	95	≥18	Hospital visitors	>80
Northeast, US (Park et al., 2003)	1994–2000	PCR-RFLP	Hospital		NA	Hospital (unhealthy)	
LATIN AMERICA							
Puerto Rico (Hayes et al., 1999)	1992–1995	PCR-RFLP or TaqMan	Cancer registry	71	21–79	Residential records	83
ASIA							
India (Anantharaman et al., 2007)	2001–2004	PCR-RFLP	Hospital		NA	Hospital (unhealthy)	
Japan (Suzuki et al., 2007)	2001–2005	TaqMan	Hospital	61	20–79	Hospital (unhealthy)	41

NA = not applicable/not available.
†Representative publication in which study methods are described.
‡All studies frequency matched control subjects to case subjects on age and sex. Additional frequency matching factors included study center (Central Europe), hospital (France study), ethnicity (Northeast US), neighborhood (Los Angeles study), and tobacco and alcohol habits (India).
§Participation rate was not formally assessed, estimated response rate reported.
‖ Multicenter study.
¶Two response rates are reported because data were collected in two population-based case–control studies, the first from 1985 to 1989 among men and the second from 1990 to 1995 among men and women.
**The three UK centers (Glasgow, Manchester, and Newcastle) from the Western European study were chosen from the same family medical practice (population-based).*
¥The majority of the SNPs were genotyped by the methods.

Table A2 | Single nucleotide polymorphisms and the risk of head and neck cancer, adjusted for smoking and drinking (exclude Northeast US study).

Gene	SNP rs number	Alteration	Referent genotype	Analysis model	Heterozygotes				Rare homozygotes			
					OR	(95%CI)	No. of studies	*p* for heterogeneity	OR	(95%CI)	No. of studies	*p* for heterogeneity
P21	rs1801270	Ser31Arg	Ser/Ser	Fixed effects	1.14	(0.96–1.36)	3	0.01	1.59	(0.81–3.12)	3	0.14
				Random effects	1.24	(0.60–2.56)			1.62	(0.32–8.19)		
P53	rs1042522	Pro72Arg	Arg/Arg	Fixed effects	0.96	(0.86–1.08)	4	0.48	0.96	(0.78–1.18)	4	0.28
				Random effects	0.96	(0.80–1.15)			0.96	(0.69–1.35)		
ERCC2	rs28365048	Lys751Gln	Lys/Lys	Fixed effects	0.95	(0.84–1.09)	5	0.53	1.06	(0.89–1.25)	5	0.63
				Random effects	0.95	(0.79–1.15)			1.06	(0.83–1.35)		
MGMT	rs1803965	171C>T	C/C	Fixed effects	0.97	(0.84–1.11)	3	0.58	1.03	(0.70–1.53)	3	0.08
				Random effects	0.97	(0.71–1.32)			1.08	(0.33–3.58)		
MGMT	rs2308321	Ile143Val	Ile/Ile	Fixed effects	0.90	(0.78–1.03)	6	0.52	1.32	(0.85–2.04)	6	0.66
				Random effects	0.90	(0.75–1.07)			1.32	(0.75–2.34)		
MGMT	rs12917	Leu84Phe	Leu/Leu	Fixed effects	0.80	(0.68–0.95)	5	0.66	1.49	(0.96–2.30)	5	0.83
				Random effects	0.80	(0.63–1.02)			1.49	(0.80–2.76)		
OGG1	rs1052133	Ser326Cys	Ser/Ser	Fixed effects	0.95	(0.82–1.09)	5	0.95	1.00	(0.77–1.30)	5	0.62
				Random effects	0.95	(0.78–1.16)			1.00	(0.70–1.45)		
XRCC1	rs1799782	Arg194Trp	Arg/Arg	Fixed effects	0.94	(0.74–1.18)	4	0.89	2.31	(1.04–5.11)	4	0.98
				Random effects	0.94	(0.64–1.37)			2.31	(0.64–8.40)		
XRCC1	rs25489	Arg280His	Arg/Arg	Fixed effects	1.09	(0.84–1.43)	2	0.87	3.28	(1.06–10.2)	2	0.20
				Random effects	1.09	(0.19–6.24)			3.28	(0.00–5008)		
XRCC1	rs25487	Arg399Gln	Arg/Arg	Fixed effects	0.94	(0.83–1.06)	6	0.48	0.99	(0.82–1.21)	6	0.02
				Random effects	0.94	(0.80–1.10)			0.97	(0.72–1.30)		
XRCC3	rs861539	Thr241Met	Thr/Thr	Fixed effects	0.98	(0.87–1.10)	7	0.06	0.97	(0.81–1.16)	7	0.70
				Random effects	0.98	(0.84–1.14)			0.97	(0.78–1.21)		
MTHFR	rs1801131	Glu429Ala	Glu/Glu	Fixed effects	0.87	(0.71–1.05)	2	0.21	0.83	(0.59–1.19)	2	0.29
				Random effects	0.87	(0.25–3.00)			0.83	(0.08–8.31)		
MTHFR	rs1801133	Ala222Val	Ala/Ala	Fixed effects	1.03	(0.92–1.16)	5	0.92	1.04	(0.87–1.25)	4	0.33
				Random effects	1.03	(0.88–1.22)			1.04	(0.78–1.40)		
ADH1B	rs1229984	Arg48His	Arg/Arg	Fixed effects	1.04	(0.82–1.32)	4	0.95	2.35	(1.57–3.53)	3	0.93
				Random effects	1.04	(0.71–1.53)			2.35	(0.97–5.74)		
ADH1C	rs1042758	Ile350Val	Ile/Ile	Fixed effects	1.01	(0.90–1.14)	9	0.68	1.20	(1.03–1.40)	9	0.22
				Random effects	1.01	(0.89–1.16)			1.17	(0.93–1.46)		
CYP1A1	E0322	3801T > C	T/T	Fixed effects	0.97	(0.80–1.18)	2	0.87	1.52	(0.94–2.47)	2	0.28
				Random effects	0.97	(0.27–3.53)			1.52	(0.07–34.9)		
CYP1A1	rs1048943	Ile462Val	Ile/Ile	Fixed effects	0.97	(0.80–1.18)	7	0.72	0.84	(0.39–1.79)	4	0.99
				Random effects	0.97	(0.76–1.24)			0.84	(0.25–2.86)		
CYP2E1	rs6413432	1143A >T	A/A	Fixed effects	1.32	(0.89–1.96)	2	0.06	1.25	(0.30–5.22)	2	0.97
				Random effects	1.38	(0.05–40.2)			1.25	(0.00–13439)		
CYP2E1	rs3813867	1293G > C	G/G	Fixed effects	0.74	(0.53–1.04)	4	0.88	4.44	(0.27–72.7)	3	1.00

				Random effects	0.74	(0.43–1.27)			4.44	(0.01–2051)		
CYP2E1	rs2031920	1054C > T	C/C	Fixed effects	1.15	(0.68–1.95)	3	0.25	4.45	(0.27–72.7)	3	1.00
				Random effects	1.15	(0.36–3.66)			4.45	(0.01–2052)		
EPHX1	rs2234922	His139Arg	His/His	Fixed effects	0.92	(0.82–1.04)	6	0.82	1.31	(1.00–1.72)	6	0.24
				Random effects	0.92	(0.79–1.08)			1.31	(0.92–1.87)		
EPHX1	rs1051740	Tyr113His	Tyr/Tyr	Fixed effects	0.98	(0.88–1.10)	6	0.01	0.86	(0.71–1.04)	6	0.56
				Random effects	0.92	(0.70–1.22)			0.86	(0.67–1.10)		
GSTM1	NA	null	present	Fixed effects	NA				1.08	(0.98–1.19)	11	0.45
				Random effects	NA				1.08	(0.96–1.22)		
GSTM3	rs1799735	*Mnl* AGG > del	AGG/AGG	Fixed effects	1.03	(0.83–1.27)	2	0.15	0.81	(0.43–1.50)	2	0.44
				Random effects	1.03	(0.25–4.16)			0.81	(0.01–46.1)		
GSTP1	rs1799811	Ala114Val	Ala/Ala	Fixed effects	1.04	(0.87–1.25)	3	0.60	1.21	(0.65–2.24)	3	0.18
				Random effects	1.04	(0.69–1.56)			1.20	(0.27–5.38)		
GSTP1	rs947894	Ile105Val	Ile/Ile	Fixed effects	1.02	(0.92–1.12)	8	0.35	0.95	(0.81–1.11)	8	0.40
				Random effects	1.02	(0.90–1.14)			0.95	(0.79–1.15)		
GSTT1	NA	null	present	Fixed effects	NA				0.92	(0.82–1.04)	11	0.13
				Random effects	NA				0.92	(0.80–1.06)		
NQO1	rs1800566	Pro187Ser	Pro/Pro	Fixed effects	1.02	(0.89–1.17)	5	0.68	1.07	(0.77–1.49)	5	0.01
				Random effects	1.02	(0.84–1.24)			1.11	(0.52–2.37)		

OR adjusted for age, sex, country, race, pack years of smoking, and alcohol drinking. Data from Northeast US were excluded because of missing in alcohol drinking.

Table A3 | Mean and median of alcohol drinking (ml/day) by ADH1B Are48His genotype

Study	Allele	Mean	Median	*p*
Central Europe	His/His or His/Arg	25.28	13.17	0.42
	Arg/Arg	25.01	11.36	
Western Europe	His/His or His/Arg	21.12	9.47	0.01
	Arg/Arg	27.04	15.92	
Heidelberg	His/His or His/Arg	45.96	27.69	
	Arg/Arg	NA	NA	
Japan	His/His or His/Arg	23.58	7.92	<0.01
	Arg/Arg	28.71	23.76	

p-value was determined by Wilcoxon rank sum test.