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

Shu-Chun Chuang1,2, Antonio Agudo3, Wolfgang Ahrens4, Devasena Anantharaman5, Simone Benhamou6, Stefania Boccia7,8, Chu Chen9, David I. Conway10, Eleonora Fabianova11, Richard B. Hayes12, Claire M. Healy13, Ivana Holcetova14, Kristina Kjaerheim15, Pagona Lagiou16, Philip Lazarus17, Tatiana V. Macfarlane18, Manoj B. Mahimkar19, Dana Mates20, Keitaro Matsuo21, Franco Merletti22, Andres Metspalu23,24,25, Renato Talamini26, Peter Thomson27, Qingyi Wei28, David Zaridze29, Zuo-Feng Zhang30, Ariana Znaor11, Paul Brennan1, Paolo Boffetta3,4,5, Mia Hashibe1,41*

1 Lifestyle and Cancer Group, International Agency for Research on Cancer, Lyon, France
2 Department of Epidemiology and Biostatistics, Imperial College London, London, UK
3 Catalán Institute of Oncology, Bellvitge Biomedical Research Institute, Hospital de Llobregat, Barcelona, Spain
4 Bremen Institute for Prevention Research and Social Medicine, Bremen, Germany
5 Cancer Research Institute, Advanced Centre for Treatment, Research, and Education in Cancer, Tata Memorial Center, Mumbai, India
6 Unité 794, INSERM, Paris, France
7 Institute of Hygiene, Università Cattolica del Sacro Cuore, Rome, Italy
8 Istituto di Ricerca e Cura a Carattere Scientifico San Raffaele Pisana, Rome, Italy
9 Fred Hutchinson Cancer Research Center, Seattle, WA, USA
10 Dental School, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, UK
11 Department of Occupational Health, Specialized State Health Institute, Banská Bystrica, Slovakia
12 New York University School of Medicine, New York, NY, USA
13 School of Dental Science, Trinity College Dublin, Dublin, Ireland
14 First Faculty of Medicine, Institute of Hygiene and Epidemiology, Charles University, Prague, Czech Republic
15 Cancer Registry of Norway, Institute for Population-Based Cancer Research, Oslo, Norway
16 Department of Hygiene, Epidemiology and Medical Statistics, School of Medicine, University of Athens, Athens, Greece
17 Penn State College of Medicine, Hershey, PA, USA
18 School of Medicine and Dentistry, University of Aberdeen, Scotland, UK
19 Occupational Health Department, Institute of Public Health, Bucharest, Romania
20 Division of Epidemiology and Prevention, Aichi Cancer Center Research Institute, Nagoya, Japan
21 Cancer Epidemiology Unit, University of Turin, Turin, Italy
22 Laboratory of Gene Technology, Estonian Biocentre, Tartu, Estonia
23 Department of Epidemiology, Comprehensive Cancer Center, University of Michigan, Ann Arbor, MI, USA
24 Department of Environmental Health Sciences, Comprehensive Cancer Center, University of Michigan, Ann Arbor, MI, USA
25 Institute of Otorhinolaryngology, Catholic University of the Sacred Heart, Rome, Italy
26 School of Public Health, University of North Carolina, Chapel Hill, NC, USA
27 National Cancer Institute, Bethesda, MD, USA
28 Institute of Public Health, University of Heidelberg, Heidelberg, Germany
29 Fodor József National Center for Public Health, National Institute of Environmental Health, Budapest, Hungary
30 Department of Environmental Medicine and Public Health, University of Padova, Padova, Italy
31 College of Public Health, University of Iowa, Iowa City, IA, USA
32 University of Texas MD Anderson Cancer Center, Houston, TX, USA
33 Institute of Occupational Medicine, Lodz, Poland
34 Epidemiology Unit, Aviano Cancer Centre, Aviano, Italy
35 Oral and Maxillofacial Surgery School of Dental Sciences, Newcastle University, Newcastle, UK
36 Cancer Research Centre, Institute of Carcinogenesis, Moscow, Russia
37 Department of Epidemiology, University of California Los Angeles, School of Public Health, Los Angeles, CA, USA
38 Croatian National Cancer Registry, Croatian National Institute of Public Health, Zagreb, Croatia
39 The Tisch Cancer Institute, Mount Sinai School of Medicine, New York, NY, USA
40 International Prevention Research Institute, Lyon, France
41 Department of Family and Preventive Medicine, University of Utah School of Medicine, Salt Lake City, UT, USA

Edited by: Min Dai, Cancer Institute and Hospital Chinese Academy of Medical Sciences, China
Reviewed by: Lifang Hou, Northwestern University, USA
Frank De Vocht, The University of Manchester, UK

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
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 on 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, 2007), and family history of cancer (Negri et al., 2004), Los Angeles (Cui et al., 2007), 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, XRCCI Arg194Trp rs1799782, Arg280His rs25489, Arg399Gln rs25487, and XRCC3 Thr241Met rs861539), folate metabolism (MTHFR Ala222Val rs1801133 and Glu429Val rs1801131), and carcinogen metabolism (ADHIB Arg48His rs1229984, ADH1C Ile350Val rs698, CYPIA1 Ile462Val rs1048943, 3801T > C rs735317, 2507A > G rs1799882, and CYP2E1 Val1054C > T rs800424, Val1054C > T rs800429).

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), ADHIB 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, ADHIB 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

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, XRCCI Arg194Trp rs1799782, Arg280His rs25489, Arg399Gln rs25487, and XRCC3 Thr241Met rs861539), folate metabolism (MTHFR Ala222Val rs1801133 and Glu429Val rs1801131), and carcinogen metabolism (ADHIB Arg48His rs1229984, ADH1C Ile350Val rs698, CYPIA1 Ile462Val rs1048943, 3801T > C rs735317, 2507A > G rs1799882, and CYP2E1 Val1054C > T rs800424, Val1054C > T rs800429).
1143A > T rs6413432, 1293G > C rs3813867, EPHX1 Try113His rs1051740, His139Arg rs2234922, GSTM1 null, GSTM3 Mnl AGG deletion rs1799735, GSTP1 Ile105Val rs947894, Ala114Val rs1798811, 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 (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 2 | Cell cycle and DNA repair SNPs and the risk of head and neck cancer in the INHANCE consortium.

| Gene | SNP rs number | Alteration genotype | Referrant genotype | No. of cases | No. of controls | Analysis model | Heterozygotes OR (95%CI) No. of studies p for heterogeneity | Rare homozygotes 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) 3 1.52 (0.27–8.66) 3 0.09 |
| 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) 4 0.97 (0.71–1.32) 4 0.07 |
| 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) 5 1.03 (0.83–1.29) 5 0.48 |
| 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) 3 1.06 (0.47–2.38) 3 0.21 |
| MGMT | rs2303821      | 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) 6 1.20 (0.71–2.02) 6 0.68 |
| 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) 5 1.40 (0.80–2.44) 5 0.69 |
| 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) 5 0.98 (0.69–1.38) 5 0.67 |
| 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) 4 2.30 (0.73–7.26) 4 1.00 |
| 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 (0.72–1.58) 2 2.87 (0.73–7.26) 2 0.21 |
| 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) 6 0.89 (0.58–1.37) 6 0.01 |
| 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) 7 0.90 (0.74–1.10) 7 0.88 |

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 | Genotype          | No. of studies | No. of cases | No. of controls | Analysis model | OR (95% CI)               | p for heterogeneity | p for heterogeneity |
|------------|---------------|------------|-------------------|----------------|--------------|-----------------|----------------|--------------------------|---------------------|---------------------|
|            |               |            |                   |                |              |                 |                | Fixed effects            |                     |                     |
| MTHFR      | rs1801131     | Glu429Ala  | Glu/Glu           | 830            | 2164         |                  |                | 0.90 (0.75–1.07)        | 0.14                |                     |
|            |               |            |                   |                |              |                 |                | Random effects           | 0.90 (0.29–2.82)     |                     |
| ADH1B      | rs1229984     | Arg48His   | Arg/Arg           | 2407           | 5408         |                  |                | 1.15 (0.90–1.46)        | 0.24                |                     |
|            |               |            |                   |                |              |                 |                | Random effects           | 1.15 (0.80–1.65)     |                     |
| ADH1C      | rs698         | His350Val  | His/His           | 3306           | 6284         |                  |                | 0.96 (0.87–1.04)        | 0.56                |                     |
|            |               |            |                   |                |              |                 |                | Random effects           | 0.96 (0.81–1.12)     |                     |
| CYP1A1     | rs1801133     | Ala222Val  | Ala/Ala           | 2605           | 5444         |                  |                | 0.98 (0.88–1.09)        | 0.87                |                     |
|            |               |            |                   |                |              |                 |                | Random effects           | 0.98 (0.88–1.09)     |                     |
| CYP1C      | rs698         | Ile350Val  | Ile/Ile           | 3036           | 6102         |                  |                | 0.95 (0.86–1.06)        | 0.42                |                     |
|            |               |            |                   |                |              |                 |                | Random effects           | 0.95 (0.86–1.06)     |                     |
| CYP2E1     | rs641343      | 1143A>T    | 1143A>T           | 722            | 1062         |                  |                | 0.95 (0.88–1.02)        | 0.24                |                     |
|            |               |            |                   |                |              |                 |                | Random effects           | 0.95 (0.88–1.02)     |                     |
| CYP2E1     | rs381386      | 1293G>C    | 1293G>C           | 739            | 2657         |                  |                | 0.98 (0.88–1.09)        | 0.56                |                     |
|            |               |            |                   |                |              |                 |                | Random effects           | 0.98 (0.88–1.09)     |                     |
| CYP2E1     | rs2031920     | 1054C>T    | 1054C>T           | 981            | 1414         |                  |                | 0.96 (0.88–1.04)        | 0.24                |                     |
|            |               |            |                   |                |              |                 |                | Random effects           | 0.96 (0.88–1.04)     |                     |
| EPHX1      | rs96292       | His139Arg  | His/His           | 2840           | 4464         |                  |                | 0.96 (0.88–1.05)        | 0.56                |                     |
|            |               |            |                   |                |              |                 |                | Random effects           | 0.96 (0.88–1.05)     |                     |
| GSTM1      | NA            | Null       | Present           | 3857           | 7232         |                  |                | 0.97 (0.89–1.06)        | 0.25                |                     |
|            |               |            |                   |                |              |                 |                | Random effects           | 0.97 (0.89–1.06)     |                     |
| GSTM3      | rs1798735     | Mnl        | Present           | 1039           | 2478         |                  |                | 1.02 (0.88–1.22)        | 0.38                |                     |
|            |               |            |                   |                |              |                 |                | Random effects           | 1.02 (0.88–1.22)     |                     |
| GSTP1      | rs17068      | Ala114Val  | Ala/Ala           | 2440           | 4923         |                  |                | 0.98 (0.83–1.15)        | 0.72                |                     |
|            |               |            |                   |                |              |                 |                | Random effects           | 0.98 (0.83–1.15)     |                     |
| GSTT1      | NA            | Null       | Present           | 3704           | 6919         |                  |                | 1.01 (0.89–1.13)        | 0.44                |                     |
|            |               |            |                   |                |              |                 |                | Random effects           | 1.01 (0.89–1.13)     |                     |
| GSTT1      | rs1800596     | Pro/Pro    | present           | 1039           | 2478         |                  |                | 1.03 (0.88–1.22)        | 0.38                |                     |
|            |               |            |                   |                |              |                 |                | Random effects           | 1.03 (0.88–1.22)     |                     |

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).

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.

| SNPs       | Oral (OR (95% CI)) | Pharynx (OR (95% CI)) | Oral/pharynx. NOS (OR (95% CI)) | Larynx (OR (95% CI)) |
|------------|--------------------|------------------------|-----------------------------------|----------------------|
| ADH1B His48Arg (ca/co) | 541/6157 | 593/6157 | 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 (1.34–4.30)   | 5.71 (3.22–10.1)      | 2.27 (1.14–4.52)                  | 1.50 (0.67–3.38)     |
| Ptrend     | <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)     |
| Ptrend     | 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 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.
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.

| A | OR (95% CI) |
|---|-------------|
| ADH1B Arg48His | 2.73 (1.87, 3.98) |
| Age | 1.59 (0.69, 3.67) |
| <45 years old | 3.08 (2.08, 4.58) |
| >=45 years old | 3.05 (2.03, 4.58) |
| Sex | 1.31 (0.42, 4.13) |
| Men | 2.34 (0.92, 5.98) |
| Women | 2.81 (1.86, 4.25) |
| Race | 1.51 (0.55, 4.14) |
| White | 3.07 (2.00, 4.71) |
| Asian | 1.61 (0.87, 2.97) |
| Smoking | 3.24 (1.85, 5.69) |
| Never smoker | 2.91 (1.97, 4.28) |
| Ever smoker | 2.25 (0.68, 7.43) |
| Drinking | 2.02 (1.61, 2.55) |
| None or light drinker | 2.50 (1.93, 3.23) |
| Heavy drinker | 1.86 (1.50, 2.31) |
| High vegetable intake | 1.73 (1.32, 2.27) |

| B | OR (95% CI) |
|---|-------------|
| ADH1C Ile350Val | 1.22 (1.06, 1.41) |
| Age | 1.36 (0.97, 2.23) |
| <45 years old | 1.21 (0.94, 1.41) |
| Sex | 1.23 (1.01, 1.49) |
| Men | 1.19 (0.86, 1.66) |
| Women | 1.19 (0.93, 1.51) |
| Race | 1.15 (0.82, 1.63) |
| White | 1.19 (0.93, 1.51) |
| Black | 1.31 (0.28, 6.11) |
| Hispanic | 1.26 (0.90, 2.27) |
| Asian | 2.55 (0.91, 8.18) |
| Smoking | 1.20 (1.05, 1.71) |
| Never smoker | 1.27 (1.07, 1.55) |
| Ever smoker | 1.15 (0.85, 1.54) |
| Drinking | 1.09 (1.00, 1.19) |
| None or light drinker | 1.08 (1.00, 1.28) |
| Heavy drinker | 1.13 (1.03, 1.25) |
| Control Source | 1.14 (1.14, 3.36) |
| Hospital-based | 1.09 (0.67, 1.21) |
| Population-based | 1.19 (0.88, 1.27) |
| Region | 1.15 (1.05, 1.19) |
| Europe | 1.26 (0.96, 1.65) |
| North America | 1.26 (0.96, 1.65) |
| South America | 1.26 (0.96, 1.65) |
| Asia | 1.26 (0.96, 1.65) |
| Vegetable Intake | 1.04 (0.92, 1.18) |
| Low vegetable intake | 1.12 (0.98, 1.26) |
| High vegetable intake | 1.05 (0.93, 1.19) |
| Fruit Intake | 1.07 (1.00, 1.14) |
| Low fruit intake | 1.14 (1.08, 1.21) |
| High fruit intake | 1.07 (1.00, 1.14) |

**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.
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

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.
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. 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 (\( p < 10^{-15} \)) and was replicated in the replication phase. The \( \text{ADH1B} \) 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 (\( p = 3 \times 10^{-15} \)). 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 \( \text{ADH1B} \) Arg48His, \( \text{ADH1C} \) Ile350Arg, \( \text{GSTM1} \) null and head and neck cancer risk. An association for \( \text{XRCC1} \) Arg194Trp was not well supported based on the FPRPs. Further investigation is needed for \( \text{MGMT} \) Leu84Phe for the mechanism. \( \text{ADH1B} \) Arg48His and \( \text{ADH1C} \) 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.

ACKNOWLEDGMENTS
This pooled analysis of SNPs within INHANCE consortium was supported by a grant from the US National Institutes of Health (NIH), National Institute of Dental, and Craniofacial Research (NIDCR; R03DE016611). Shu-Chun Chuang worked on this project during the tenure of a Special Training Award from the International Agency for Research on Cancer. The individual studies were funded by the following grants: France study: Swiss League against Cancer (KFS1069-09-2000), Fribourg League against Cancer (FOR381.88), Swiss Cancer Research (AKT 617), and Gustave-Roussy Institute (88D28). Central Europe study: World Cancer Research Fund and the European Commission’s INCO-COPERNICUS Program (Contract No. IC15-CT98-0332). Seattle study: National Institutes of Health (NIH) US (R01CA048996, R01DE012609). Iowa: National Institutes of Health (NIH) US (NIDCR R01DE19179, NIDCR R01DE13110, NIH FIRCA TW01500) and Veterans Affairs Merit Review Funds. North Carolina study: National Institutes of Health (NIH) US (R01CA61188), and in part by a grant from the National Institute of Environmental Health Sciences (P30ES010126). Los Angeles study: National Institute of Health (NIH) US (P50CA90388, R01DA11386, R03CA77954, T32CA09142, U01CA96134, R21ES011667) and the Alper Research Program for Environmental Genomics of the UCLA Jonsson Comprehensive Cancer Center. Houston: National Institutes of Health (NIH) US (R01ES11740, R01CA100264). Puerto Rico study: jointly funded by National Institutes of Health (NCI) US and NIDCR intramural programs. Rome study: AIRC (Italian Agency for Research on Cancer). Western Europe Study: European Commission’s 5th Framework Program (Contract No. QLKI-2001-00182), Italian Association for Cancer Research, Compagnia di San Paolo/ FIRMS, Region Piemonte, and Padova University (Contract No. CPDA057222). Germany-Heidelberg study: grant No. 01GB9702/3 from the German Ministry of Education and Research. Japan study: Scientific Research grant from the Ministry of Education, Science, Sports, Culture, and Technology of Japan (17015052) and grant for the Third-Term Comprehensive 10-Year Strategy for Cancer Control from the Ministry of Health, Labor, and Welfare of Japan (H20-002). Northeast US: National Institutes of Health (NIH) US (R01DE13158). India: Department of Biotechnology Government of India (DBT Grant no: BT/PR2277/MED/09/333/2000 and BT/PR6958/MED/14/912/2005).

REFERENCES
International HapMap Project (2008). International HapMap Project. Available at http://hapmap.ncbi.nlm.nih.gov/.

Altekruse, S. E., Kosary, C. L., Kropeh, M., Neyman, N., Aminou, R., Waldron, W., Ruhl, J., Howlader, N., Tatalovich, Z., Cho, H., Mariotto, A., Eisner, M. P., Lewis, D. R., Cronin, K., Chen, H. S., Feuer, E. J., Stinchcomb, D. G., and Edwards, B. K. (eds). (2010). SEER Cancer Statistics Review, 1975-2007. Bethesda, MD: National Cancer Institute. Available at: http://seer.cancer.gov/csr/1975_2007/, based on November 2009 SEER data submission, posted to the SEER web site, 2010 Anantharaman, D., Chaubal, P. M., Kannan, S., Bhisey, R. A., and...
Alcohol and aldehyde dehydrogenase gene polymorphisms and oropharyngolaryngeal, esophageal and stomach cancers in Japanese alcoholics. *Carcinogenesis* 3, 433–439.

Zhang, Z., Shi, Q., Liu, Z., Sturgis, E. M., Spitz, M. R., and Wei, Q. (2005). Polymorphisms of methionine synthase and methionine synthase reductase and risk of squamous cell carcinoma of the head and neck: a case-control analysis. *Cancer Epidemiol. Biomarkers Prev.* 5, 1188–1193.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 20 March 2011; paper pending published: 11 April 2011; accepted: 13 June 2011. Citation: Chuang S-C, Agudo A, Abrens W, Anantharaman D, Benhamou S, Boccia S, Chen C, Conway DI, Fabianova E, Hayes RB, Healy CM, Hokatova I, Kjaerheim K, Lagiou P, Lazarus P, Macfarlane TV, Mahimkar MB, Mates D, Matsuo K, Merletti F, Metspalu A, Morgenstern H, Muscat J, Cadoni G, Olshan AF, Purdue M, Ramroth H, Rudnai P, Schwartz SM, Simonato L, Smith EM, Sturgis EM, Szczesniak-Dabrowska N, Talamini R, Thomson P, Wei Q, Zaridze D, Zhang Z-F, Znaor A, Brennan P, Boffetta P, and Hashibe M (2011) Sequence variants and the risk of head and neck cancer: pooled analysis in the INHANCE consortium. *Front. Oncol.* 1:13. doi: 10.3389/fonc.2011.00013

This article was submitted to Frontiers in Cancer Epidemiology and Prevention, a specialty of Frontiers in Oncology. Copyright © 2011 Chuang, Agudo, Abrens, Anantharaman, Benhamou, Boccia, Chen, Conway, Fabianova, Hayes, Healy, Hokatova, Kjaerheim, Lagiou, Lazarus, Macfarlane, Mahimkar, Mates, Matsuo, Merletti, Metspalu, Morgenstern, Muscat, Cadoni, Olshan, Purdue, Ramroth, Rudnai, Schwartz, Simonato, Smith, Sturgis, Szczesniak-Dabrowska, Talamini, Thomson, Wei, Zaridze, Zhang, Znaor, Brennan, Boffetta and Hashibe. This is an open-access article subject to a non-exclusive license between the authors and Frontiers Media SA, which permits use, distribution and reproduction in other forums, provided the original authors and source are credited and other Frontiers conditions are complied with.
## 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 (Bocca et al., 2008) | 2002–2007 | PCR-RFLP | Hospital | 98 | NA | Hospital (unhealthy) | 94 |
| Western Europe (Canova et al., 2009) | 2000–2005 | APEX | Hospital | 82 | NA | 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 | 675 |
| 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 | NA | Hospital (unhealthy) | 62 |
| **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 | NA | Hospital (unhealthy) | 41 |
| 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       | Alteration                      | Ref/Alt genotype | Study number | No. of studies | OR (95%CI) | p for heterogeneity |
|------------|---------------------------------|------------------|--------------|----------------|------------|---------------------|
| Heterozygotes & Rare homozygotes | Analysis model | OR (95%CI) | No. of studies | p for heterogeneity |
| P21 | rs1801270 | Ser13Arg | Ser/Ser | Fixed effects | 1.14 | 0.96–1.36 | 3 | 0.01 | 0.96–1.36 | 3 | 0.01 |
| P33 | rs1042502 | Pro72Arg | Pro/Arg | Random effects | 1.24 | 0.96–1.61 | 1 | 0.28 | 0.96–1.61 | 1 | 0.28 |
| ERCC2 | rs2383648 | Lys751Gln | Lys/Lys | Fixed effects | 0.96 | 0.96–1.00 | 1 | 0.49 | 0.96–1.00 | 1 | 0.49 |
| MGMT | rs1801270 | Ser13Arg | Ser/Ser | Fixed effects | 0.96 | 0.96–1.00 | 4 | 0.49 | 0.96–1.00 | 4 | 0.49 |
| MGMT | rs12917 | Leu84Phe | Leu/Leu | Fixed effects | 1.09 | 0.96–1.24 | 2 | 0.87 | 0.96–1.24 | 2 | 0.87 |
| MTHFR | rs1801131 | Ala122Val | Ala/Ala | Fixed effects | 1.09 | 0.96–1.24 | 4 | 0.87 | 0.96–1.24 | 4 | 0.87 |
| MTHFR | rs1048943 | Glu359Val | Gln/Glu | Fixed effects | 1.09 | 0.96–1.24 | 4 | 0.87 | 0.96–1.24 | 4 | 0.87 |
| MR1 | rs1042502 | Pro72Arg | Pro/Arg | Random effects | 1.24 | 0.96–1.61 | 1 | 0.28 | 0.96–1.61 | 1 | 0.28 |
| MR1 | rs2383648 | Lys751Gln | Lys/Lys | Fixed effects | 0.96 | 0.96–1.00 | 1 | 0.49 | 0.96–1.00 | 1 | 0.49 |
| MRTK1 | rs1801270 | Ser13Arg | Ser/Ser | Fixed effects | 0.96 | 0.96–1.00 | 4 | 0.49 | 0.96–1.00 | 4 | 0.49 |
| MRTK1 | rs12917 | Leu84Phe | Leu/Leu | Fixed effects | 1.09 | 0.96–1.24 | 2 | 0.87 | 0.96–1.24 | 2 | 0.87 |
| MTHFR | rs1801131 | Ala122Val | Ala/Ala | Fixed effects | 1.09 | 0.96–1.24 | 4 | 0.87 | 0.96–1.24 | 4 | 0.87 |
| MTHFR | rs1048943 | Glu359Val | Gln/Glu | Fixed effects | 1.09 | 0.96–1.24 | 4 | 0.87 | 0.96–1.24 | 4 | 0.87 |

**Note:** Random effects were used for analysis due to heterogeneity among studies.
| Allele | Mean | Median | p-value | Study |
|--------|------|--------|---------|-------|
| CYP2E1 rs2031920 1054C>T C/C | 0.74 | 0.43–1.27 | 4.44 | (0.01–2051) |
| Random effects | 1.15 | 0.68–1.95 | 3 | 0.25 |
| Fixed effects | 1.15 | 0.36–3.66 | 4.45 | (0.01–2052) |
| EPHX1 rs2234922 His139Arg His/His | 0.92 | 0.82–1.04 | 1.31 | (0.00–1.72) |
| Random effects | 0.92 | 0.79–1.08 | 1.31 | (0.92–1.87) |
| Fixed effects | 0.92 | 0.88–1.10 | 0.86 | (0.71–1.04) |
| EPHX1 rs1051740 Tyr113His Tyr/Tyr | 0.92 | 0.70–1.22 | 0.86 | (0.67–1.10) |
| Random effects | 0.92 | NA | 1.08 | (0.98–1.19) |
| Study Allele Mean Median p-value was determined by Wilcoxon rank sum test. Data from Northeast US were excluded because of missing in alcohol drinking. | | | | |
| Central Europe His/His or His/Arg | 25.28 | 13.17 | 0.42 |
| Western Europe His/His or His/Arg | 21.12 | 9.47 | 0.25 |
| Japan His/His or His/Arg | 23.58 | 7.92 | <0.01 |
| 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. | | | | |