下咽頭癌とアルコールおよびたばこ代謝関連遺伝子多型についての検討

Hypopharyngeal cancer risk in Japanese: Genetic polymorphisms related to the metabolism of alcohol- and tobacco-associated carcinogens

Abstract

Background Several studies have investigated hypopharyngeal cancer (HC) risk in combination with xenobiotic metabolism-related genetic polymorphisms and the burden of alcohol consumption and smoking in European countries but not in East Asian countries.

Methods This hospital-based case-control study involved 61 male patients with HC and 71 male cancer-free controls. Information on age, body mass index, and alcohol and cigarette consumption was obtained from medical records, a self-completion questionnaire, and a thorough interview by an otolaryngologist. *Alcohol dehydrogenase 1B* (*ADH1B*), *aldehyde dehydrogenase 2* (*ALDH2*), *cytochrome P450 A1* (*CYP1A1*) *Msp*I, *CYP1A1 Ile462Val*, *glutathione S-transferase* (*GST*) *M1*, *GSTT1*, and *GSTP1* gene polymorphisms were determined by polymerase chain reaction-based methods. Univariate and multivariate analyses were performed by adjustment for age by the Mantel–Haenszel method.

Results The burden of alcohol and cigarette consumption significantly increased the risk of HC and showed a synergistic effect. *ADH1B*1/*1* (odds ratio 7.34) and *ALDH2*1/*2* (odds ratio 13.22) were significant risk factors for HC. Individuals with *ADH1B*1/*1* or *ALDH2*1/*2* who consumed alcohol were more susceptible to HC. However, polymorphisms of *CYP1A1* gene and Glutathione S-transferases (GSTs) were not significant cancer risk factors in patients with HC.

*Conclusions ADH1B*1/*1* and *ALDH2*1/*2* were significant risk factors for HC, while polymorphism of *CYP1A1* gene and *GSTs* were not a significant risk factor for HC. These polymorphisms determined the effects of alcohol and cigarette smoke in addition to burden of alcohol and cigarettes intake on the risk of HC.

Key words: cancer risk, polymorphism, alcohol, tobacco smoke, hypopharyngeal cancer

Introduction

Excess consumption of alcoholic beverages and a smoking habit are well-known risk factors for head and neck cancer, particularly hypopharyngeal cancer (HC) and laryngeal cancer (LC). These lifestyle factors are attributed to approximately 80% of head and neck cancers according to the WHO report [1]. Because people who are heavy drinkers and smokers do not always develop HC or LC, individual susceptibility to these cancers may be modulated by genetic polymorphisms in xenobiotic metabolizing enzymes, in addition to the burden of alcohol and smoking themselves.

The appearance rates of these xenobiotic metabolism-related genetic polymorphisms differ among ethnic groups. For example, the genetic polymorphism status of *alcohol dehydrogenase 1B* (*ADH1B*) and *acetaldehyde dehydrogenase 2* (*ALDH2*) are quite different in Japan from central and east European countries [2][3][4][5]. Several studies have investigated the combination of such genetic polymorphisms and alcohol and smoking burden on HC or LC risk in European countries [6][7][8][9], while a few studies have been conducted in East Asian countries, including Japan [10][11][12][13][14].

ADH1B and ALDH2 determine the effective conversion of ethanol to acetaldehyde and its subsequent oxidation to acetate. *ADH1B* (accession number rs1229984, Arg48His) and *ALDH*2 (accession number rs671, Glu487Lys) have genetic polymorphisms that define their enzymatic activity. The homodimer of ADH1B encoded by *ADH1B*1/*1* has only 1/100th and 1/200th of the ethanol-oxidizing capacity of the isozymes encoded by *ADH1B*1/*2* and *ADH1B*2/*2* [15]. The mutant

allele *ALDH2*2* encodes an inactive subunit of ALDH2; its homodimers have null ALDH2 activity and its heterodimers have approximately 6% residual activity [16]. *ALDH2*2* is frequently found in East Asian populations [17], whereas nearly all Europeans are homozygous for the *ALDH2*1* and *ADH1B*1/*1* alleles [18].

Several reports have examined esophageal cancer and alcohol metabolic enzyme polymorphisms [19][20][21]. In one meta-analysis of reports from China, Japan, Thailand, Africa, and Europe, the crude odds ratio (OR) for esophageal cancer was 2.91 for *ADH1B*1/*1* and 1.32 for *ADH1B*1/*2* compared with *ADH1B*2/*2* (21), whereas it was 2.52 for *ALDH2*1/*2* compared with *ALDH2*1/*1* [21]. However, there are few reports on HC and their polymorphisms from East Asia (10, 12). The influence of alcohol on HC risk varies with ethnicity, and gene polymorphisms in *ADH1B* and *ALDH2* may differently affect cancer risk.

Two functional polymorphisms are known in the *CYP1A1* gene: a 3698T>C substitution (*CYP1A1*2A*, rs 4646903) creating an *Msp*I restriction site in the 3'-flanking region, and a 2454A>G substitution (*CYP1A1*2C*, rs 1048943, Ile462Val) in exon 7. *CYP1A1 Msp*I and *CYP1A1 Ile462Val* mutations have been associated with increased risk of head and neck cancers [22]. Glutathione S-transferases (GSTs) are a family of phase II xenobiotic metabolizing enzymes catalyzing conjugation reaction reactive intermediates of electrophilic compounds with cytosolic glutathione. In humans, *GSTs* are mainly coded for at five loci: *GSTA* (a), *GSTT1* (h), *GSTM1* (l), *GSTP1* (p), and *GSTM3* (c) [23].

GSTM1 products catalyze the conjugation of glutathione to epoxide derivatives of polycyclic aromatic hydrocarbons, which are the main carcinogens found in cigarette smoke. *GSTT1* products are important in the detoxification of naturally occurring monohalomethanes, dichloromethanes, and ethylene oxides. *GSTP1* enzyme is widely expressed in the body and detoxifies various potential carcinogens, including cigarette smoke-derived substances such as benzopyrene diol epoxide and acrolein. The polymorphic site in the coding DNA sequence of the *GSTP1* gene is characterized by an adenine (A) to guanine (G) transition at nucleotide 313, translating an isoleucine to valine substitution at codon 105 (Ile^{105} to Val^{105}) in exon 5. GST enzyme activity is significantly lower among individuals with the 105Val allele in the *GSTP1* gene. The *GSTM1* null and *GSTT1* null genotypes are associated with increased risk of head and neck cancer [24][25][26]. Individuals with the Ile/Val and Val/Val genotypes have significantly higher risk of head and neck cancer than those with the Ile/Ile genotype [27]. However, there are inconsistencies among studies [28].

The aim of this study was to clarify the relationship between genetic polymorphisms related to alcohol and tobacco smoke metabolism and cigarette smoking and alcohol intake burden on the risk of HC in Japanese individuals.

Patients and methods

Subjects

This case-control study involved 61 male patients with HC who gave written informed consent for study participation. All HC patients were treated at the university hospital between April 2008 and December 2012. During the same period, 71 cancer-free male control subjects who were treated for inflammatory disease and benign tumors, such as chronic sinusitis, chronic tonsillitis, and benign salivary tumor, were also enrolled. The controls had no cancer-related history.

The study protocol was approved by the Institutional Review Board. This study was conducted in accordance with the principles of the Declaration of Helsinki.

Drinking and smoking habits

Information on age at first visit to our institute was obtained from medical records. Detailed history of alcohol consumption and smoking behavior was obtained from cancer patients and the controls before they started any treatment via a self-completion questionnaire and thorough interview by an otolaryngologist. The burden of cigarette use was reported as daily cigarettes by years. Daily alcohol consumption was expressed in grams per day of ethanol.

Genetic polymorphism analysis

All blood samples were collected into ethylenediaminetetraacetic acid (EDTA)-containing tubes and stored as the buffy coat. Genomic DNA was extracted from the buffy coat using standard protocols with phenol-chloroform extraction and stored at –20°C. *ADH1B* and *ALDH*2 polymorphisms were identified using *Msl*I and *Mbo*II (New England Biolabs Japan, Tokyo, Japan) polymerase chain reaction (PCR)-restriction fragment length polymorphism (PCR-RFLP). The PCR-amplified products of *ADH1B* and *ALDH*2 were digested according to the manufacturer's instructions with the restriction enzymes as described and analyzed following electrophoresis in agarose gel. *CYP1A1 Msp*I and *CYP1A1 Ile462Val* polymorphisms were detected by melting temperature analysis of PCR products amplified with quenching probes using the fully automated genotyping system i-densy TM 5310 (Arkray Inc., Kyoto, Japan); these techniques are named quenching probe methods [29]. *GSTM1* and *GSTT1* polymorphisms were identified by multiplex PCR. *GSTP1* polymorphisms were identified using *BsmAI* (New England Biolabs Japan) PCR-RFLP. The primer sequences used for amplifications and the quenching probe sequences are listed in Table 1.

Statistical analysis

Pearson's chi-square test was used to compare age between cases and controls. Adjustment for age by the Mantel–Haenszel method was used to compare alcohol consumption and smoking behavior between cancer cases and controls. ORs with 95% confidence intervals (CIs) were used to assess the

strength of the association between alcohol consumption, smoking behavior, polymorphisms, and HC risk. Univariate ORs with 95% CIs were calculated after adjustment for age by the Mantel–Haenszel method. Multivariate ORs with 95% CIs were calculated by binominal logistic regression with the stepwise method.

All analyses were performed with SPSS Statistical Package (SPSS for Windows, Version 23.0; SPSS, Inc., Chicago, IL). $P<0.05$ was considered statistically significant.

Results

Subject characteristics

The clinical characteristics of the HC patients and the cancer-free control subjects are shown in Table 2. Patients with smoke consumed only cigarettes, and there was no smoker who consumed cigars, chewing tobacco, or tobacco pipes. The HC patients were significantly older than the controls. The HC patients were more likely to smoke and drink than the controls. Synergistic consumption of alcoholic beverages and cigarettes in the HC patients is shown in Table 3. More than 20% of the HC patients both consumed 50 g/day alcohol and had a Brinkman index ≥800, whereas only 4.2% of the controls did. The OR reached 17.72 in the HC patients compared with patients who consumed small amounts of cigarettes and alcohol.

Univariate ORs due to alcohol consumption and *ADH1B* and *ALDH2* polymorphisms

Alcohol consumption and *ADH1B* and *ALDH2* polymorphisms in cancer patients and controls are shown in Table 4. Those who consumed ≥10 g/day had a higher risk of HC than those who consumed <10 g/day. HC patients who consumed 10–50 g/day of ethanol had an OR of 16.52 (95% CI 2.76–99.06, *P*=0.001), which increased to 36.74 (95% CI 6.50–207.72, *P*=0.000001) for those who consumed ≥50 g/day (Table 4). Compared with *ADH1B*2/*2*, *ADH1B*1/*1* was associated with an increased risk of HC, with an OR of 7.25 (95% CI 2.55–20.83, *P*=0.000243). Compared with *ALDH2*1/*1*, *ALDH2*1/*2* was

associated with an increased risk of HC, with an OR of 6.35 (95% CI 2.68–15.06, *P*=0.000015). These *ADH1B* and *ALDH2* polymorphisms showed a synergistic effect with alcohol on the risk of HC (Table 4).

Table 5 shows the effects of the relationship between alcohol consumption and *ADH1B* and *ALDH2* polymorphisms on the risk of HC. *ADH1B*1/*1* genotype carriers who consumed <50 g/day had a higher risk of HC than *ADH1B*2/*2* genotype carriers (OR 8.20, 95% CI 1.84–37.04, *P*=0.008). *ALDH2*1/*2* carriers who consumed <50 g/day had a higher risk of HC than *ALDH2*1/*1* genotype carriers (OR 6.76, 95% CI 2.27–20.14, *P*=0.001). *ALDH2*1/*2* genotype carriers who consumed ≥50 g/day also had an increased risk of HC compared with *ALDH2*1/*1* genotype carriers who consumed ≥50 g/day (OR 12.97, 95% CI 1.52–110.42, *P*=0.032).

Univariate ORs due to smoking burden and *CYP1A1 MspI, CYP1A1 Ile462Val, GSTM1, GSTT1,* and *GSTP1* polymorphisms

Smokers had an increased risk of HC compared with never smokers (Table 2). A Brinkman index ≥800 was associated with an OR of 7.51 (95% CI 2.56–21.98, 0.00031, Table 6). The effects of smoking burden and genetic polymorphisms (*CYP1A1 Msp*I, *CYP1A1 Ile462Val*, *GSTM1*, *GSTT1*, and *GSTP1*) on HC are shown in Table 6. There were no significant correlations between the polymorphisms examined and cancer risk.

Table 7 and 8 shows the cancer risk of polymorphisms according to smoking burden. In HC,

there were no significant correlations between polymorphisms and cancer risk for any smoking burden.

Multivariate ORs due to risk factors for HC and LC

The multivariate ORs of selected factors for HC are shown in Table 9. In HC, *ADH1B* and *ALDH2* polymorphisms were associated with an increased risk, in addition to alcohol consumption, large smoking burden (Brinkman index ≥800), and age. Compared with *ADH1B*2/*2*, *ADH1B*1/*1* was associated with an increased risk of HC (OR 7.34, 95% CI 1.46–37.04, *P*=0.016). Compared with *ALDH2*1/*1*, *ALDH2*1/*2* was associated with an increased risk of HC (OR 13.22, 95% CI 3.33–52.48, *P*=0.000242).

Discussion

The life style regarding with alcohol intake and cigarette smoking was quite different between male and female in Japan. The incidence of head and neck cancer is also much higher in male than female. For this reason, we employed only male subjects in the present study. The rates of drinking and smoking and the burden of alcohol and cigarette consumption were markedly higher in the HC patients than in the controls. To minimize the sample size effect, the Mantel–Haenszel method was used to control age as influential confounding factors. Although the sample size was limited, our main findings of interest are that *ADH1B*1/*1* and *ALDH2*1/*2* were significant risk factors for HC. Multivariate analysis also revealed alcohol consumption, even low consumption of alcohol beverages, was the dominant risk factor for HC and high burden of cigarette consumption were the dominant risk factor for HC.

Salivary acetaldehyde is at least partly derived from the salivary glands [30]. Salivary acetaldehyde levels of subjects with inactive ALDH2 are higher than those of subjects with active ALDH2 after ethanol ingestion. In addition, acetaldehyde from microbial oxidation of ethanol by the oral microflora is also important for increasing salivary acetaldehyde levels, especially in heavy drinkers and smokers [31]. Asakage et al. [12] reported that inactive *ALDH2*1/*2* and less-active *ADH1B*1/*1* were significant independent risk factors for HC among moderate-to-heavy drinkers but that inactive *ALDH2*1/*2* was not a significant risk factor for oral and oropharyngeal cancers. Because ALDH2 activity in the tongue, gingiva, and esophagus is weak [32][33] and there has been no precise evaluation of

ALDH2 activity in the hypopharynx and larynx, this subsite difference in ALDH2 activity may have influenced their findings. Because previous reports sometimes combined HC with laryngeal, oral, and oropharyngeal cancers as head and neck cancers when assessing cancer risk, the subsite difference should be taken into account when considering the results. In the present study, *ADH1B* and *ALDH2* polymorphisms were prominent risk factors for HC by direct exposure to strong alcoholic beverages.

Mutant alleles encoding an inactive subunit of *ALDH2*2* and a super-active subunit of *ADH1B*2* are highly prevalent among Japanese at 42% and 93%, respectively [2]. Although control subjects in the present study had a relatively high rate of *ALDH2*1/*1* and *ADH1B*1/*1* compared with mainland Japanese, the prevalence of these polymorphisms is similar to those in East Asian countries and markedly different from those in Europe [34]. In the present study, according to *ADH1B* analysis, never to moderate drinkers (0–50 g/day) with *ADH1B*1/*1* showed a significant elevation in HC. Because there was no heavy drinker (>50 g/day) control subject with *ADH1B*1/*1*, the OR of *ADH1B*1/*1* in heavy drinkers could not be calculated. However, 46.9% of HC patients harbored *ADH1B*1/*1*, unlike 0% of control subjects. Thus, in our Japanese population, *ADH1B*1/*1* became a risk factor for HC even in those who consume small amounts of alcohol. According to *ALDH2* analysis, never to moderate drinkers with *ALDH2*1/*2* showed significantly elevated risk of HC. Thus, *ALDH2*1/*2* becomes a risk factor for HC even in those who consume little alcohol. Synergistic effects of *ADH1B* and *ALDH2* polymorphisms on cancer risk were observed for HC, suggesting that these polymorphisms affect

hypopharynx carcinogenesis.

Some studies have investigated smoke metabolism-related polymorphisms and head and neck cancers (25, 26). Ours is the first report to investigate *CYP1A1 MspI*, *CYP1A1 Ile462Val*, *GSTM1*, *GSTT1*, and *GSTP1* polymorphisms in relation to HC in a Japanese population. The data obtained from the control subjects in the present study were quite similar to the previous polymorphism data in Japanese [35]. However, in Japan, polymorphisms in *CYP1A1 Msp*1, *GSTM1*, and *GSTP1* were associated with lung cancer risk [35], in the present study, there was no polymorphism examined that was related to HC risk. A literature search found significant associations of *GSTM1* and *GSTT1* gene polymorphisms with head and neck cancer, particularly oral cavity cancer, although conflicting data including LC were present [11][25][26][36][37]. Stratified according to ethnicity, there was an increased risk of head and neck cancer in Asians with *GSTM1* and *GSTT1* null genotypes compared with Americans and Europeans [26]. Our findings suggest that the influence of enzymatic polymorphisms related to the detoxification of cigarette smoke-derived substances differs among head and neck subsites.

Substantial evidence indicates that smoking and drinking synergistically increase the risk of oral and pharyngeal cancer [38]. The present case-control study clearly revealed that high alcohol and cigarette consumption significantly increased the risk of HC. In addition, alcohol consumption was more prominent risk factor compared with cigarette consumption in HC from multivariate analysis (Table 8). Because the hypopharynx is part of the digestive tract, it may be more easily affected by alcohol than

cigarette smoke.

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