Folate, alcohol, and aldehyde dehydrogenase 2 polymorphism and the risk of oral and pharyngeal cancer in Japanese

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Folate consumption is inversely associated with the risk of oral and pharyngeal cancer (OPC) and potentially interacts with alcohol drinking in the risk of OPC. Aldehyde dehydrogenase 2 (*ALDH2*) gene polymorphism is known to interact with alcohol consumption. The aim of this study was to investigate potential interaction between folate, alcohol drinking, and *ALDH2* polymorphism in the risk of OPC in a Japanese population. The study group comprised 409 head and neck cancer cases and 1227 age-matched and sex-matched noncancer controls; of these, 251 cases and 759 controls were evaluated for *ALDH* rs671 polymorphism. Associations were assessed by odds ratios and 95% confidence intervals in multiple logistic regression models. We observed an inverse association between folate consumption and OPC risk. The odds ratio for high folate intake was 0.53 (95% confidence interval: 0.36–0.77) relative to low intake (*P* trend = 0.003). This association was consistent across strata of sex, age, smoking, and *ALDH2* genotypes. Interaction between folate consumption, drinking, and *ALDH2* genotype was remarkable (three-way interaction, *P* < 0.001). We observed significant interaction among folate, drinking, and *ALDH2* genotype in the Japanese population. *European Journal of Cancer Prevention* 21:193–198 © 2012 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

A few epidemiologic studies on oral and pharyngeal cancer (OPC) have indicated an inverse association between serum/plasma levels (Frick *et al.*, 2002; Raval *et al.*, 2002; Weinstein *et al.*, 2002; Almadori *et al.*, 2005) or dietary consumption (De Stefani *et al.*, 1999; Pelucchi *et al.*, 2003; Suzuki *et al.*, 2007; Shanmugham *et al.*, 2010; Aune *et al.*, 2011) of folate and OPC risk. Biological functions of folate in so-called 'one-carbon metabolism' are to facilitate de-novo deoxynucleoside triphosphate synthesis and to provide methyl groups required for intracellular methylation reactions. Therefore, folate deficiency is thought to increase the risk of various type of cancer through impaired DNA repair synthesis and disruption of DNA methylation that may lead to protooncogene activation (Duthie, 1999; Choi and Mason, 2000; Wei *et al.*, 2003).

Alcohol consumption is one of the most important risk factors for OPC (World CRFAIfCR, 2007). It has been reported that alcohol interacts with folate consumption to alter the risk of OPC (Pelucchi *et al.*, 2003; Shanmugham *et al.*, 2010) and other alcohol-related cancers such as colorectal and breast cancer (Giovannucci *et al.*, 1995; Zhang *et al.*, 1999; Almadori *et al.*, 2005; Eleftheriadou *et al.*, 2006; Kim *et al.*, 2010). Acetaldehyde, the first metabolite of ethanol, contributes appreciably to the association between alcohol consumption and cancer risk (Seitz *et al.*, 2001). Alcohol is first oxidized to acetaldehyde, which is then further oxidized to acetate by aldehyde dehydrogenase (ALDH) enzymes, mainly by ALDH2. In East Asian populations, the *ALDH2* gene displays a polymorphism (rs671, Glu504Lys) that modulates individual differences in acetaldehyde-oxidizing capacity (Yoshida *et al.*, 1984; Bosron and Li, 1986; Crabb *et al.*, 1989). As the Lys504 allele encodes a catalytically inactive subunit, individuals with the *ALDH2* Lys allele experience a marked elevation in blood acetaldehyde after alcohol ingestion (Yoshida *et al.*, 1984), and many studies have revealed that the *ALDH2* Lys allele confers higher susceptibility to upper aerodigestive tract cancer than the *ALDH2* Glu/Glu genotype due to the decreased elimination of acetaldehyde (Yokoyama *et al.*, 1998, 2006; Matsuo *et al.*, 2001; Yang *et al.*, 2005; Hiraki *et al.*, 2007; Oze *et al.*, 2010). A remarkable gene–environment interaction between *ALDH2* genotype and alcohol drinking has been reported by Oze *et al.* (2010) and Matsuo *et al.* (2001). In

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one study, the odds ratio (OR) for heavy drinking among those with Glu/Glu genotypes was 1.59 [95% confidence interval (CI): 0.84–3.00], whereas that among Lys allele carriers was 14.41 (95% CI: 7.01–29.61; Oze *et al.*, 2010). High consumption of alcohol interferes with folate absorption and increases excretion by the kidney, resulting in a reduced folate level in the body (Romero *et al.*, 1981; Shaw *et al.*, 1989). Furthermore, acetaldehyde is known to be able to cleave folate (Shaw *et al.*, 1989). Therefore, it is reasonable to hypothesize an interaction of folate, alcohol, and *ALDH2* genotype in terms of OPC risk.

We conducted a case–control study to investigate the interaction between folate and alcohol, and further evaluated potential effect modification by *ALDH2* genotype in the risk of OPC in a Japanese population.

Methods

Study population

The study participants were 409 patients, with no prior history of cancer, who were histologically diagnosed with OPC (257 with oral cavity cancer, 72 with oropharyngeal cancer, and 80 with hypopharyngeal cancer) between January 2001 and December 2005 at Aichi Cancer Center Hospital in Japan. All participants were recruited within the framework of the Hospital-based Epidemiologic Research Program at Aichi Cancer Center (HERPACC) (Inoue *et al.*, 1997; Tajima *et al.*, 2000; Hamajima *et al.*, 2001). The study was approved by the Institutional Ethics Committee of Aichi Cancer Center Hospital.

OPC in this study was defined according to the following codes of the International Classification of Diseases and Related Health Problems: oral cavity (C02.0–C02.3, C03, C04, C05.0, and C06), oropharynx (C01, C02.4, C05.1–C05.2, C09, and C10), and hypopharynx (C12 and C13). Malignant neoplasms of the lip (C00.0–C00.9), salivary glands (C07, C08), nasopharynx (C11), nose (C30), paranasal region (C31), and larynx (C32) were excluded. The control participants were 1 227 first-visit outpatients during the same period, who were confirmed to have no cancer and no history of neoplasms. Noncancer status was confirmed by medical examinations, including radiographic examinations, and participants suspected of having upper aerodigestive tract cancer were first examined by physical or endoscopic inspection, and subsequently radiographically, if indicated. Controls were selected randomly and individually matched by age (± 4 years) and sex (male; female) with a case–control ratio of 1 : 3. DNA samples were available for approximately 60% of study participants (251 cases and 759 controls).

Evaluation of exposures

All participants were asked to provide information by a self-administrated questionnaire at the time of the first visit and before any diagnostic procedures. The questionnaire included items on sociodemographic characteristics, family and individual medical history, height and weight, smoking and drinking habits, and vitamin use. Information on dietary habits was collected through a food frequency questionnaire that included 43 single food and beverage items with frequencies in eight categories. The food frequency questionnaire was validated by referring to a 3-day weighted dietary record as a standard, and showed satisfactory validity and reproducibility (Tokudome *et al.*, 2005; Imaeda *et al.*, 2007).

We estimated average daily intake of folates and energy by calculating the sums of their intakes in the single food items as estimated from a food composition table (Resources Council SaTA, Japan, 1982, 1992, 1993) according to the indicated portion size, multiplied by the food frequency. Correlation coefficients for energy and folate were mild to moderate (energy, 0.49 for male and 0.44 female; folate, 0.36 for male and 0.38 for female; Tokudome *et al.*, 2005).

Alcohol consumption of various common beverages (Japanese sake, beer, shochu, whiskey, and wine) was determined in terms of the average number of drinks per day, which was then converted into a Japanese sake (rice wine) equivalent. We asked about the amount consumed in terms of one go (180 ml) of Japanese sake equivalent, which contains 23 g of ethanol, namely one large bottle (633 ml) of beer, two shots (57 ml) of whiskey, or two and a half glasses of wine (200 ml). One drink of 'shochu' (distilled spirit), which contains 25% ethanol, was rated as 108 ml. Drinking status was classified into three categories: never drinker, intermediate drinker [less than four units (one unit = 12.5 g of ethanol equivalent) per day], and high drinker (four or more units per day).

Information on smoking status was obtained in three categories: nonsmoker, former smoker, and current smoker, with former smokers defined as those who had quit smoking at least 1 year before the survey. Cumulative smoking dose was evaluated as pack-years.

DNA was extracted from the buffy-coat fraction using a DNA Blood mini kit (Qiagen, Tokyo, Japan). Genotyping for rs671 (*ALDH2* Glu504Lys) was based on TaqMan Assays (Applied Biosystems, Foster City, California, USA).

Statistical analyses

Associations were assessed by ORs and 95% CIs in multiple logistic regression models. We computed energy-adjusted folate intakes using the residual method (Willett and Stampfer, 1998). The energy-adjusted folate intakes were categorized into quartiles or tertiles based on the control distribution, and the corresponding ORs were estimated using models conditioned on sex and age and adjusted for occupation (blue collar, white collar, and others, categorically), BMI (< 23, < 25, < 27.5, and 27.5 kg/m^2, categorically), nonalcoholic energy intake (tertile 1–3, categorically), smoking (< 5, < 40, and 40 pack-years, categorically), and alcohol drinking (never,

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< 4, and 4 units/day, categorically). Missing values for covariates were treated as dummy variables in the models. Unconditional logistic regression models adjusted for sex and age (continuously) besides the covariates above were applied in stratified analyses.

We evaluated the three-way interaction of folate intake [low–intermediate (tertile 1 and 2): 0 vs. high (tertile 3): 1], alcohol drinking as an ordinal variable (never: 0, < 4 unit/day: 1, ≥ 4 unit/day: 2) and *ALDH2* (Glu/Glu: 0 vs. Lys allele carrier: 1) by including these variables and a three-way interaction term with confounding factors. All analyses were carried out using Stata SE version 11 (Stata Corporation, College Station, Texas, USA).

Results

Table 1 shows characteristics of cases and controls. Age and sex were similar between cases and controls. In terms of occupation, blue-collar workers were more common in cases. Participants with lower BMI or with higher tobacco consumption were significantly prevalent in cases compared with controls. Participants with the *ALDH2* Lys allele, especially of heterozygous genotype, were more common among cases.

Table 2 shows ORs and corresponding 95% CI for OPC according to quartiles of folate intake and three levels of alcohol consumption. We observed a significant inverse association between folate and OPC risk (*P* trend = 0.003). Compared with participants with the lowest quartile of folate (< 243.5 μg/day), ORs for quartile 2 (≥ 243.5 and < 303.1 μg/day), quartile 3 (≥ 303.1 and < 378.4 μg/day), and quartile 4 (≥ 378.4 μg/day) consumers were 0.79 (95% CI: 0.58–1.11), 0.78 (95% CI: 0.55–1.11), and 0.53 (95% CI: 0.36–0.77). Alcohol displayed a significantly positive association with OPC risk. ORs, relative to never drinkers, were 1.21 (0.88–1.65) for intermediate (≤ 4 unit/day) and 2.67 (1.83–3.88) for heavy (≥ 4 unit/day) drinkers. This inverse association by folate consumption was consistently observed across sex and age (< 60 or ≥ 60 years), levels of alcohol drinking, and cumulative exposure to smoking.

To further examine the potential interaction between folate and alcohol consumption on OPC risk, we carried out combined analyses (Table 3). A dose–risk effect of alcohol drinking was modest in the highest folate consumers, whereas a clear association was seen in lower folate consumers (quartile 1–3). The interaction term, however, was not significant.

Table 1 Characteristics of participants

	Cases (%)	Controls (%)	Odds ratios (95% CI)[a]
Total	409	1227	
Age (years)			
<40	39 (9.5)	113 (9.2)	
40–49	39 (9.5)	124 (10.1)	
50–59	123 (30.1)	370 (30.2)	
60–69	127 (31.1)	393 (32.0)	
70–	81 (19.8)	227 (18.5)	
Sex			
Men	296 (72.4)	888 (72.4)	
Women	113 (27.6)	339 (27.6)	
Occupation			
Blue collar	149 (36.4)	333 (27.1)	1 (Ref)
White collar	84 (20.5)	361 (29.4)	0.61 (0.44–0.85)
Others	169 (41.3)	518 (42.2)	0.79 (0.57–1.10)
Unknown	7 (1.7)	15 (1.2)	
BMI (kg/m^2)			
<23	278 (68.0)	652 (53.1)	1 (Ref)
<25	68 (16.6)	286 (23.3)	0.59 (0.43–0.82)
<27.5	41 (10.0)	185 (15.1)	0.53 (0.36–0.78)
27.5–	19 (4.6)	93 (7.6)	0.49 (0.29–0.83)
Unknown	3 (0.7)	11 (0.9)	
Nonalcohol energy (tertiles)			
T1	124 (30.3)	300 (24.4)	1 (Ref)
T2	98 (24.0)	304 (24.8)	0.89 (0.67–1.19)
T3	94 (23.0)	304 (24.8)	0.83 (0.62–1.13)
Unknown	3 (0.7)	5 (0.4)	
Smoking (pack-years)			
<5	126 (30.8)	551 (44.9)	1 (Ref)
<40	153 (37.4)	422 (34.4)	1.21 (0.88–1.65)
40–	126 (30.8)	245 (20.0)	2.67 (1.83–3.88)
Unknown	4 (1.0)	9 (0.7)	
ALDH2 genotype			
Glu/Glu	103 (25.2)	372 (30.3)	1 (Ref)
Lys+	148 (36.2)	387 (31.5)	1.69 (1.18–2.42)
Glu/Lys	136 (33.3)	311 (25.3)	1.73 (1.21–2.48)
Lys/Lys	12 (2.9)	76 (6.2)	1.08 (0.49–2.37)
DNA not available	158 (38.6)	468 (38.1)	

ALDH2, aldehyde dehydrogenase 2; CI, confidence intervals.
[a]Odds ratios from conditional logistic regression models adjusted for age (continuous), occupation (blue collar, white collar, others, and unknown, categorical), BMI (<23, 23–24.9, 25–27.4 and 27.5– and unknown, categorical), nonalcoholic energy intake (tertile 1–3 and unknown, categorical), smoking (pack-years <5, <40, 40–, and unknown, categorical), and drinking [never, <4 units/day (one unit of alcohol indicates 12.5 g ethanol equivalent), 4 or more units/day, and unknown, categorical] other than ALDH2 genotypes. Odds ratios for ALDH2 genotypes were adjusted for the same variables only among those with ALDH2 information (251 cases and 759 controls).

Table 2 Association between dietary folate intake and alcohol consumption and risk of oral and pharyngeal cancer among 409 cases and 1227 matched controls in Japan

	Cases	Controls	Odds ratios[b] (95% CI)
Folate intake (μg/day)[a]			
Mean (μg/day)	105	319.1	
Mean of consumption	105	319.1	
Quartile 1	129	301	1 (Ref)
Quartile 2	99	301	0.79 (0.57–1.11)
Quartile 3	97	301	0.78 (0.55–1.10)
Quartile 4	67	301	0.53 (0.36–0.77)
Unknown	17	23	
P trend[c]			<0.001
Alcohol intake (unit/day)			
Never	113	454	1 (Ref)
<4 units/day	151	560	1.21 (0.88–1.65)
≥ 4 units/day	128	192	2.67 (1.83–3.88)
Unknown	17	21	
P trend[c]			<0.001

CI, confidence intervals
[a]Folate intake was energy adjusted using the residual method and was categorized into four groups by the levels of quartiles among controls; quartile 1 (<243.5 μg/day), 2 (<303.1 μg/day), 3 (<378.4 μg/day), and 4 (≥ 378.4 μg/day).
[b]Odds ratios from conditional logistic regression models adjusted for age (continuous), occupation (blue collar, white collar, others, and unknown, categorical), BMI (<23, 23–24.9, 25–27.4, and 27.5– and unknown, categorical), nonalcoholic energy intake (tertile 1–3 and unknown, categorical), smoking (pack-years <5, <40, 40–, and unknown, categorical), and drinking (never, <4 units/day and ≥ 4 units/day and unknown, categorical).
[c]Trend test excluded those with unknown folate/alcohol driking status from analysis.

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Table 3 Odds ratios[a] and 95% confidence intervals of oral and pharyngeal cancer according to tertile of intake of folate and alcohol in Japan[b]

	Folate intake[c]			
Alcohol consumption	Quartile 4 (highest)	Quartile 3	Quartile 2	Quartile 1 (lowest)
Low (never)	1 (Ref)	1.53 (0.81–2.92)	1.54 (0.82–2.91)	1.80 (0.94–3.45)
Intermediate (<4 units/day)	1.28 (0.67–2.46)	1.62 (0.88–2.99)	2.09 (1.12–3.88)	2.12 (1.14–3.94)
High (≥ 4 units/day)	2.35 (0.97–5.67)	4.15 (2.07–8.35)	2.94 (1.42–6.08)	4.94 (2.53–9.68)

[a]Odds ratios from unconditional logistic regression models adjusted for age (continuous), occupation (blue collar, white collar, others and unknown, categorical), BMI (<23, 23–24.9, 25–27.4 and 27.5– and unknown, categorical), nonalcoholic energy intake (tertile 1–3 and unknown, categorical), and smoking (pack-years <5, 5–19.9, 20–39.9, 40–, and unknown, categorical).
[b]Analysis excluded those with unknown folate and alcohol cosumption.
[c]Folate intake was energy adjusted uisng the residual method and was categorized into four groups by the levels of quartiles among controls: quartile 1 (<243.5 μg/day), 2 (<303.1 μg/day), 3 (<378.4 μg/day), and 4 (≥ 378.4 μg/day).

Table 4 Odds ratios[a] and 95% confidence intevals of oral and pharyngeal cancer according to ALDH2 genotype, folate consumption, and alcohol[b] consumption

	ALDH2 (Glu/Glu)		ALDH2 Lys+		
	Folate intake				
Alcohol consumption	High	Low–intermediate	High	Low–intermediate	*P* interaction[c]
Low (never)					
Cases/controls	7/35	12/34	11/75	29/138	
OR (95% CI)[a]	1 (Ref)	1.95 (0.66–5.70)	0.76 (0.26–2.18)	1.08 (0.42–2.78)	
Intermediate (<4 units/day)					
Cases/controls	9/36	32/148	13/47	41/94	
OR (95% CI)[a]	0.77 (0.25–2.32)	1.18 (0.45–3.11)	1.48 (0.49–4.48)	2.42 (0.90–6.49)	
High (≥ 4 units/day)					
Cases/controls	5/17	28/59	7/7	39/16	
OR (95% CI)[a]	1.63 (0.41–6.43)	2.17 (0.78–6.02)	4.36 (1.04–18.2)	11.9 (3.95–36.1)	<0.001

ALDH2, aldehyde dehydrogenase; CI, confidence interval; OR, odds ratio.
[a]ORs from unconditional logistic regression models adjusted for age (continuous), occupation (blue collar, white collar, others, and unknown, categorical), BMI (<23, 23–24.9, 25–27.4, and 27.5– and unknown, categorical), nonalcoholic energy intake (tertile 1 to 3 and unknown, categorical), and smoking (pack-years <5, 5–19.9, 20–39.9, 40–, and unknown, categorical). Model includes indicator variables for ALDH2 genotype, alcohol consumption, and folate consumption (high, tertile 3 vs. low-intermediate, tertile 1 and 2) combined.
[b]Analysis excluded those with unknown ALDH2 genotype, folate and alcohol cosumption.
[c]*P* interaction was evaluated as a three-way interaction term for ALDH2 Lys genotype, low-intermediate folate consumption, and alcohol consumption (ordinal variable) in the model described in[a].

Table 4 shows the interactive effect of folate, alcohol, and *ALDH2* genotype. The effect of alcohol is stronger in *ALDH2* Lys allele carriers. Among *ALDH2* Lys allele carriers, the effect of alcohol was remarkably strong in low–intermediate folate consumers (OR = 11.9; CI: 3.95–36.1) when compared with high folate consumers (OR = 4.36; CI: 1.04–18.2).

Discussion

In this case–control study, folate intake was inversely associated with the risk of OPC, and alcohol consumption was positively associated. A suggestive interactive effect was observed between folate and alcohol consumption. The most remarkable finding is the significant interaction of folate, alcohol, and *ALDH2* genotype in the risk of OPC.

The association we found between OPC risk and folate consumption is consistent with former findings in different populations (De Stefani *et al.*, 1999; Pelucchi *et al.*, 2003; Shanmugham *et al.*, 2010; Aune *et al.*, 2011) and supports the hypothesis of substantial involvement of folate deficiency in carcinogenesis in the oral cavity and pharynx.

ALDH2 is a key enzyme that catalyzes acetaldehyde into acetate. The polymorphism, rs671, is functionally strong and enough to influence a lot of alcohol-related conditions (Yokoyama *et al.*, 1998; Matsuo *et al.*, 2001, 2006). In this study, we found that the effect of high alcohol consumption was apparently stronger in those with the *ALDH2* Lys allele who consume less folate than those with *ALDH2* Lys allel who consume high folate. Furthermore, the effect of driking and folate was weaker in those with *ALDH2* Glu/Glu. These observations suggest that acetaldehyde plays an important role in the interaction between folate and alcohol consumption. Taking the fact that aldehyde is involved in cleavage of folate in the body (Shaw *et al.*, 1989), our finding is biologically reasonable. Replication of the finding in a population in which the minor allele frequency of rs671 is not rare is warranted. In terms of interaction between folate consumption and alcohol drinking, our finding in the overall analysis is

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consistent with the findings in former studies (Pelucchi *et al.*, 2003; Shanmugham *et al.*, 2010). The absence of significant interaction might be due to a strong influence of *ALDH2* polymorphism in the Japanese population, although confirmation from other Asian data is essential.

Our study has several strengths. First, it was conducted in a single region in Central Japan. Second, potential confounding by age and sex was adjusted by matching these factors. In addition, we considered and adjusted established risk factors. Finally, given that our allele frequencies were comparable with those previously reported in public databases such as HapMap JPT (The IHC, 2005), bias in the distribution of selected polymorphisms seems negligible.

Several potential limitations of our study also warrant mention. One methodological issue is the selection of hospital-based non cancer patients as controls. However, as cases and controls were selected from the same hospital and almost all patients lived in the Tokai area of Central Japan, the internal validity of this case–control study is likely to be acceptable. External validity (generalizability of the results) has been confirmed in our previous study (Inoue *et al.*, 1997). Drinking habit in controls was equivalent to that in the National Health and Nutrition Survey in Japan in 2003. The proportion of facial flushers in HERPACC was comparable with that in a random sample of the general population in the same area (unpublished data). In addition, to reduce any bias that might have resulted from the inclusion of a specific diagnostic group that is related to the exposure, we did not set eligibility criteria for control diseases. A second issue is that the values for self-reported lifestyle factors considered to be potential confounders may be inaccurate. If present, however, any such misclassification would be nondifferential, and would be likely to underestimate the causal association. Finally, the sample size of this study might not be large enough for evaluation of three-way interaction.

In conclusion, our study showed a significant gene–environment interaction between folate/alcohol consumption and *ALDH2* genotype in a Japanese population.

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Conflicts of interest

There are no conflicts of interest.

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