Comparison between self-reported facial flushing after alcohol consumption and ALDH2 Glu504Lys polymorphism for risk of upper aerodigestive tract cancer in a Japanese population

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Some Japanese exhibit facial flushing after drinking alcohol. Facial flushing was considered to be caused by acetaldehydemia. The concentration of blood acetaldehyde was concerned with the catalytic activity of acetaldehyde dehydrogenase (ALDH). Acetaldehyde dehydrogenase (ALDH)-2 polymorphism (rs671, Glu504Lys) was known to be associated with upper aerodigestive tract (UAT) cancer due to modulation of ALDH2 enzyme activity. It remains controversial whether facial flushing is useful in predicting UAT cancer risk as a surrogate marker of ALDH2 polymorphism. We conducted a case–control study to assess the risk of UAT cancer and facial flushing and ALDH2 polymorphism. Cases and controls were 585 UAT cancer patients and matched 1170 noncancer outpatients of Aichi Cancer Center Hospital. Information on facial flushing and other lifestyle factors was collected via a self-administered questionnaire. Association between facial flushing, polymorphism, and UAT cancer was assessed by odds ratios and 95% confidence intervals by using conditional logistic regression models. The facial flushing had no significant association with UAT cancer, although ALDH2 Lys allele was significantly associated with UAT cancer. No significant interaction between facial flushing and alcohol consumption was observed in this study, whereas ALDH2 Lys allele had significant association with UAT cancer. The misclassification between facial flushing and ALDH2 genotype was observed in 18% of controls with ALDH2 Glu/Glu genotype and in 16% of controls with ALDH2 Glu/Lys genotype. Facial flushing was less useful to predict UAT cancer risk than genotyping ALDH2 polymorphism. (Cancer Sci 2010; 101: 1875–1880)

A lcohol consumption is one of the most important risk factors for upper aerodigestive tract (UAT) cancer.[1] Acetaldehyde, the first metabolite of ethanol, contributes appreciably to this association.[2]

Alcohol is first oxidized to acetaldehyde, which is then further oxidized to acetate by aldehyde dehydrogenase enzymes (ALDHs), mainly ALDH2. In East Asian populations, the ALDH2 gene displays a polymorphism (rs671, Glu504Lys) that modulates individual differences in acetaldehyde oxidizing capacity.[3–5] Because the Lys504 allele encodes a catalytically inactive subunit, individuals with the ALDH2 Glu/Lys and ALDH2 Lys/Lys genotypes experience a marked elevation in blood acetaldehyde after alcohol ingestion,[4] and many studies have revealed that the ALDH2 Glu/Lys genotype confers higher susceptibility to UAT cancer than the ALDH2 Glu/Glu genotype owing to the decreased elimination of acetaldehyde.[6–12]

Among the adverse reactions some people experience after alcohol consumption, facial flushing is considered to be caused by acetaldehydemia.[13] This response is often exhibited by individuals with the ALDH2 Glu/Lys or ALDH2 Lys/Lys genotype, owing to their low acetaldehyde eliminating capacity,[14–17] but is usually not seen in those with the ALDH2 Glu/Glu genotype.[18,19]

Although some case–control studies have shown an association between facial flushing and UAT cancer,[6,20] no significant association with esophageal cancer was seen in a prospective cohort study.[21] Thus, the association between UAT cancer and facial flushing is controversial. Here, we conducted a case-control study to investigate whether facial flushing was associated with UAT cancer, and then contrasted the association between facial flushing and ALDH2 polymorphism.

Materials and Methods

The subjects were 585 patients with no prior history of cancer who were histologically diagnosed with UAT cancer (oral cavity and pharynx cancer in 264, larynx cancer in 56, esophageal cancer in 265) between January 2001 and December 2005 at Aichi Cancer Center Hospital (ACCH). All of the subjects were recruited within the framework of the Hospital-based Epidemiologic Research Program at Aichi Cancer Center (HERPACC), which is described in detail elsewhere.[22,23] UAT cancer was defined according to the following codes of the International Classification of Diseases and Related Health Problems (ICD10): oral cavity and oropharynx (C00.3–C00.9, C01, C02.0–C02.4, C03, C04, C05.0–C05.2, C06, C09, C10), hypopharynx (C12, C13), oral cavity-oropharynx-hypopharynx not otherwise specified (C02.8, C02.9, C05.8, C05.9, C14), larynx (C32), and esophagus (C15). Malignant neoplasms of the salivary glands (C07, C08), nasopharynx (C11), nasal (C30), and paranasal (C31) were excluded as they have quite distinct etiologies. The controls were 1170 first-visit outpatients at ACCH during the same period who were confirmed to have no cancer and no history of neoplasia. Noncancer status was confirmed by medical examinations, including radiographic examinations. Those who were suspected of having UAT cancer were examined by physical or endoscopic inspection. Radiographic

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examinations were carried out for subjects subsequently suspected of having cancer. Controls were selected randomly and frequency-matched by age category (<40; 40–49; 50–59; 60–69; ≥70 years) and sex (male; female) at a case-control ratio of 1:2. All the subjects provided blood samples.[24] The study was approved by the Institutional Ethical Committee of ACCH.

For each subject, DNA was extracted from the buffy coat fraction with a DNA Blood mini kit (Qiagen, Tokyo, Japan) or BioRobot EZ1 and EZ1 DNA Blood 350 mL Kit (Qiagen). Genotyping for rs671 (*ALDH2* Glu504Lys) was based on Taq-Man Assays (Applied Biosystems, Foster City, CA, USA).

Information on flushing reaction, alcohol consumption, cumulative smoking, fruit and vegetable intake, frequency of hot beverage consumption, and body mass index (BMI) was collected via a self-administered questionnaire. Responses were checked by a trained interviewer. The occurrence of facial flushing after drinking one glass of beer was categorized in the three levels of never, occasional, and usual. Positive facial flushing was defined as the occasional or usual experience of facial flushing. Lifetime 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 ethanol, one large bottle (720 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. In this analysis, we defined one unit of drink as a half *go*. Total alcohol consumption (g per consumption) of Japanese sake, beer, shochu, whiskey, and wine was calculated for current and former regular drinkers, who were then categorized into the four levels of never drinker, moderate drinker, high-moderate drinker, and heavy drinker. Heavy drinking was defined as consumption on 5 days or more per week of four units (46 g ethanol) or more on each occasion; high-moderate drinking as consumption on 5 days or more per week of fewer than four units (46 g ethanol) on each occasion; moderate drinking as consumption on 4 days or fewer per week; and never drinking as never having drunk alcoholic beverages. Cumulative smoking dose was evaluated as pack-years (PY), the product of the number of packs consumed per day and the number of years of smoking. Consumption of fruits and vegetables was determined using a semi-quantitative food frequency questionnaire (SQFFQ), described in detail elsewhere.[25] Briefly, the SQFFQ consisted of 47 single food items with frequencies in eight frequency categories. We estimated average daily intake by multiplying the frequency of intake by the serving size of food (in g). Energy-adjusted intake of fruits and vegetables was calculated by the residual method.[26] The SQFFQ was validated using a 3-day weighed dietary record as standard, which showed that reproducibility and validity were acceptable.[27] Subjects were divided into three groups based on distribution among controls (tertiles). Regarding hot beverage intake, we defined those who consumed coffee or green tea more than three times a day as frequent consumers. Body mass index (BMI) was calculated as the weight in kilograms divided by the height in meters squared. In the analysis, we dichotomized subjects with the threshold of 25 kg/m².

Associations between UAT cancer, facial flushing, and *ALDH2* genotype were assessed by odds ratios (ORs) and 95% confidence intervals (CI) using conditional logistic regression models. The phenotype–environment and gene–environment interactions were assessed using interaction terms. Facial flushing was included as scores (negative, positive). *ALDH2* genotype was included as scores (Glu/Glu; Glu/Lys; Lys/Lys or Glu/Glu; Glu/Lys and Lys/Lys). Potential confounders considered in the multivariate analyses were age as a continuous variable, sex (male; female), smoking (PY<5; 5 ≤ PY<20;

20 ≤ PY<40; 40 ≤ PY), alcohol consumption (never; moderate; high-moderate; heavy), fruit and vegetable intake (tertiles), intake of hot beverages (<three times a day; ≥three times a day) and BMI (BMI<25; BMI≥25). Discrepancies between expected and observed genotype and allele frequencies in the control were assessed by accordance with the Hardy–Weinberg equilibrium using the χ^2-test. Statistical analyses were performed using STATA version 10 (Stata Corporation, College Station, TX, USA). A *P*-value <0.05 was considered statistically significant.

Results

Table 1 shows the characteristics of cases and controls. Alcohol consumption was more prevalent among cases compared with

Table 1. Characteristics of the cases and controls

	Case (%)	Control (%)
Sex		
Male	487 (83.25)	974 (83.25)
Female	98 (16.75)	196 (16.75)
Age at interview (years)		
<40	20 (3.42)	42 (3.59)
40–49	46 (7.86)	101 (8.63)
50–59	186 (31.79)	355 (30.34)
60–69	217 (37.09)	460 (39.32)
≥70	116 (19.83)	212 (18.12)
Cumulative smoking		
PY<5	103 (17.61)	448 (38.29)
5 ≤ PY<20	67 (11.45)	164 (14.02)
20 ≤ PY<40	161 (27.52)	258 (22.05)
40 ≤ PY	249 (42.56)	288 (24.62)
Alcohol consumption†		
Never	94 (16.07)	361 (30.85)
Moderate	89 (15.21)	332 (28.38)
High-moderate	134 (22.91)	287 (24.53)
Heavy	253 (43.25)	170 (14.53)
Frequent intake of hot beverage		
≥3/day	250 (42.74)	472 (40.34)
<3/day	317 (54.19)	671 (57.35)
Body mass index		
<25 kg/m²	493 (84.27)	870 (74.36)
≥25 kg/m²	87 (14.87)	289 (24.70)
Vegetable intake		
Lowest tertile	243 (41.54)	372 (31.79)
Middle tertile	189 (32.31)	370 (31.62)
Highest tertile	138 (23.59)	411 (35.13)
Fruits intake		
Lowest tertile	268 (45.81)	356 (30.43)
Middle tertile	190 (32.48)	392 (33.50)
Highest tertile	113 (19.32)	406 (34.70)
Facial flushing		
Negative	323 (55.21)	556 (47.52)
Positive	243 (41.54)	599 (51.20)
Cancer site		
Oral and pharynx	264 (45.13)	
Larynx	56 (9.57)	
Esophagus	265 (45.30)	
***ALDH2* genotype**		
Glu/Glu	200 (34.19)	583 (49.83)
Glu/Lys	368 (62.91)	480 (41.03)
Lys/Lys	17 (2.91)	107 (9.15)

†Moderate drinking was defined as consumption ≤4 days/week; high-moderate drinking as <46 g ethanol and ≥5 days/week; and heavy drinking as ≥46 g ethanol and ≥5 days/week. ALDH2, aldehyde dehydrogenase 2; PY, pack years.

matched controls. Cumulative exposure to smoking was also common among cases relative to controls. The proportion of subjects who experienced facial flushing was higher in the controls than in the cases. In addition, the distribution of *ALDH2* genotype differed between cases and controls.

Table 2 shows the ORs for UAT cancer and subsites assessed by the facial flushing model and *ALDH2* polymorphism model. Genotype distributions of *ALDH2* among controls were accordant with the Hardy–Weinberg equilibrium. Alcohol consumption and cumulative smoking were confirmed to be significantly associated with UAT cancer and subsites in both models. However, no significant association was observed between facial flushing and UAT cancer. Facial flushing also showed no significant association with head and neck cancer or esophageal cancer. On the contrary, *ALDH2* Glu∕Lys genotype had a significant association with UAT cancer.

Further we investigated the phenotype–environment interaction and gene–environment interaction (Table 3). Facial flushing had no significant interaction with alcohol consumption and cumulative smoking. Similarly, no interactions were observed for head and neck cancer or esophageal cancer. On the other hand, significant interaction was observed between *ALDH2* polymorphism and alcohol consumption for UAT cancer, whereas no significant interaction of this polymorphism was seen with cumulative smoking. We observed the same interactions for esophageal cancer and head and neck cancer.

Table 4 shows the distribution of facial flushing and *ALDH2* genotypes in cases and controls. A correlation between facial

flushing and *ALDH2* genotype was observed. Sensitivity and specificity for identifying *ALDH2* Lys allele carriers among controls were 0.85 and 0.82, respectively. Sensitivity among never, moderate, high-moderate, and heavy drink controls was 0.91, 0.83, 0.77, and 0.68 respectively, and specificity was 0.88, 0.82, 0.81, and 0.80, respectively. Large discordance was observed among heavy drink controls with the *ALDH2* Glu∕Lys genotype. The relationship among cases with *ALDH2* Glu∕Glu and Lys∕Lys genotype was almost similar to that among controls, whereas the discordance among heavy drink cases with *ALDH2* Glu∕Lys genotype was larger than that among heavy drink controls.

Discussion

In this study, we found that facial flushing had no significant association with UAT cancer and subsites. In contrast, *ALDH2* polymorphism was significantly associated with UAT cancer and subsites. In addition, we also found no significant interaction between facial flushing and alcohol consumption on the risk of UAT cancer.

These findings, which are consistent with a previous prospective cohort study in Japan,[21] suggest that facial flushing is not predictive of UAT cancer. Several possibilities may explain the difference in results between facial flushing and *ALDH2* polymorphism in terms of UAT cancer risk. First, facial flushing arises not only as a result of *ALDH2* polymorphism, but also due to other gene polymorphisms and environmental factors.

Table 2. Odds ratios for facial flushing and *ALDH2* genotype

	Upper aerodigestive tract cancer					Esophageal cancer					Oral cavity, pharyngeal, and laryngeal cancer				
	Case	Control	OR	95% CI	*P*-value	Case	Control	OR	95% CI	*P*-value	Case	Control	OR	95% CI	*P*-value
Facial flushing†															
Negative	323	556	1.00	Reference		156	267	1.00	Reference		138	239	1.00	Reference	
Positive	243	599	0.96	0.76–1.21	0.723	104	258	1.22	0.83–1.79	0.306	115	280	0.87	0.62–1.22	0.419
Alcohol consumption†§															
Non-drinker	94	361	1.00	Reference		16	151	1.00	Reference		62	180	1.00	Reference	
Moderate drinker	89	332	1.26	0.88–1.80	0.215	26	151	2.34	1.08–5.08	0.032	55	144	1.27	0.80–2.03	0.309
High-moderate drinker	134	287	2.15	1.49–3.09	<0.001	69	133	7.75	3.64–16.50	<0.001	52	125	1.33	0.80–2.22	0.276
Heavy drinker	253	170	6.87	4.73–9.98	<0.001	149	86	27.12	12.38–59.43	<0.001	86	69	3.78	2.27–6.31	<0.001
Cumulative smoking†															
PY<5	103	448	1.00	Reference		25	191	1.00	Reference		76	223	1.00	Reference	
5 ≤ PY<20	67	164	1.95	1.29–2.95	0.002	27	66	3.38	1.57–7.26	0.002	37	86	1.28	0.76–2.17	0.354
20 ≤ PY<40	161	258	3.07	2.14–4.39	<0.001	82	124	5.10	2.65–9.82	<0.001	62	105	1.90	1.17–3.09	0.009
40 ≤ PY	249	288	4.52	3.18–6.45	<0.001	130	141	7.98	4.22–15.10	<0.001	87	111	2.65	1.63–4.32	<0.001
ALDH2‡															
Glu∕Glu	200	583	1.00	Reference		67	277	1.00	Reference		110	256	1.00	Reference	
Glu∕Lys	268	480	2.51	1.97–3.20	<0.001	197	210	5.55	3.56–8.65	<0.001	142	219	1.51	1.09–2.11	0.015
Lys∕Lys	17	107	1.20	0.66–2.18	0.554	1	43	0.71	0.09–5.61	0.743	12	53	0.90	0.43–1.86	0.772
Alcohol consumption‡§															
Non-drinker	94	361	1.00	Reference		16	151	1.00	Reference		62	180	1.00	Reference	
Moderate drinker	89	332	1.85	1.25–2.74	0.002	26	151	5.95	2.56–13.80	<0.001	55	144	1.45	0.88–2.39	0.145
High-moderate drinker	134	287	3.34	2.23–5.01	<0.001	69	133	24.84	10.29–59.95	<0.001	52	125	1.52	0.88–2.61	0.134
Heavy drinker	253	170	11.03	7.24–16.79	<0.001	149	86	95.98	37.38–246.44	<0.001	86	69	4.43	2.54–7.73	<0.001
Cumulative smoking‡															
PY<5	103	448	1.00	Reference		25	191	1.00	Reference		76	223	1.00	Reference	
5 ≤ PY<20	67	164	1.86	1.22–2.83	0.004	27	66	2.92	1.31–6.50	0.009	37	86	1.27	0.75–2.14	0.377
20 ≤ PY<40	161	258	2.97	2.06–4.28	<0.001	82	124	4.96	2.51–9.81	<0.001	62	105	1.88	1.16–3.06	0.011
40 ≤ PY	249	288	4.15	2.90–5.95	<0.001	130	141	7.02	3.58–13.77	<0.001	87	111	2.58	1.58–4.21	<0.001

†Models included age, sex, alcohol consumption, cumulative smoking, facial flushing, fruit and vegetable intake, frequent intake of hot beverages, and body mass index. ‡Models included age, sex, alcohol consumption, cumulative smoking, ALDH2 genotype, fruit and vegetable intake, frequent intake of hot beverages, and body mass index. §Moderate drinking was defined as consumption ≤4 days∕week; high-moderate drinking as <46 g ethanol and ≥5 days∕week; and heavy drinking as ≥46 g ethanol and ≥5 days∕week. ALDH2, aldehyde dehydrogenase 2; CI, confidence interval; OR, odds ratio; PY, pack years.

Table 3. Interaction between facial flushing/ALDH2 genotype and alcohol consumption/cumulative smoking

| | Facial flushing | | | | | | | | | | | ALDH2 genotype | | | | | | | | | | |
| | Negative | | | | | Positive | | | | | P-interaction | Glu/Glu | | | | | Glu/Lys and Lys/Lys | | | | | P-interaction |
	Case	Control	OR	95% CI	P-value	Case	Control	OR	95% CI	P-value		Case	Control	OR	95% CI	P-value	Case	Control	OR	95% CI	P-value	
Upper aerodigestive tract cancer																						
Alcohol consumption†§ / ‡§																						
Non-drinker	24	75	1.00	Reference		60	276	0.91	0.50–1.64	0.743		28	59	1.00	Reference		66	302	0.52	0.29–0.93	0.027	
Moderate drinker	42	175	1.02	0.55–1.92	0.940	42	156	1.21	0.62–2.36	0.581		37	184	0.52	0.27–0.98	0.042	52	148	0.95	0.50–1.81	0.887	
High-moderate drinker	75	172	1.96	1.05–3.67	0.035	58	113	1.86	0.96–3.58	0.065		46	184	0.76	0.40–1.44	0.405	88	103	2.07	1.10–3.91	0.025	
Heavy drinker	171	122	5.60	3.00–10.45	<0.001	82	47	7.42	3.52–13.76	<0.001	0.796	82	142	1.59	0.84–3.00	0.156	171	28	14.41	7.01–29.61	<0.001	<0.001
Cumulative smoking† / ‡																						
PY<5	51	220	1.00	Reference		43	221	1.22	0.75–1.98	0.432		47	236	1.00	Reference		56	212	1.84	1.15–2.93	0.010	
5 ≤ PY<20	38	75	2.32	1.32–4.10	0.004	27	88	2.05	1.13–3.73	0.019		25	79	1.69	0.93–3.10	0.088	42	85	3.52	2.01–6.16	<0.001	
20 ≤ PY<40	98	128	3.72	2.30–6.02	<0.001	60	127	3.14	1.89–5.22	<0.001		52	144	1.94	1.16–3.24	0.011	109	114	7.14	4.38–11.64	<0.001	
40 ≤ PY	135	128	5.23	3.26–8.37	<0.001	110	158	4.97	3.10–7.99	<0.001	0.803	74	118	3.72	2.23–6.21	<0.001	175	170	7.72	4.88–12.22	<0.001	0.349
Esophageal cancer																						
Alcohol consumption†§ / ‡§																						
Non-drinker	3	23	1.00	Reference		10	125	0.84	0.18–3.97	0.822		5	18	1.00	Reference		11	133	0.40	0.10–1.54	0.181	
Moderate drinker	11	80	1.86	0.42–8.19	0.409	13	71	1.78	0.36–8.74	0.480		10	86	0.73	0.18–2.91	0.654	16	65	1.71	0.42–6.91	0.452	
High-moderate drinker	41	90	5.71	1.31–24.85	0.020	28	41	8.26	1.78–38.22	0.007		17	93	1.46	0.40–5.32	0.567	52	40	11.90	2.94–48.22	0.001	
Heavy drinker	96	68	16.02	3.64–70.49	<0.001	53	18	37.78	7.92–180.15	<0.001	0.152	34	75	3.54	0.90–13.84	0.069	115	11	118.66	23.97–587.55	<0.001	<0.001
Cumulative smoking† / ‡																						
PY<5	13	95	1.00	Reference		8	95	1.30	0.44–3.84	0.640		10	102	1.00	Reference		15	89	3.62	1.30–10.06	0.014	
5 ≤ PY<20	17	34	4.12	1.44–11.78	0.008	10	31	4.10	1.27–13.25	0.018		6	35	1.88	0.46–7.63	0.375	21	31	13.81	4.35–43.78	<0.001	
20 ≤ PY<40	52	66	5.10	2.03–12.82	0.001	29	57	7.72	2.89–20.64	<0.001		21	75	2.83	0.98–8.21	0.055	61	49	29.17	9.93–85.71	<0.001	
40 ≤ PY	74	69	9.22	3.75–22.65	<0.001	56	71	10.66	4.27–26.60	<0.001	0.461	29	62	6.58	2.22–19.51	0.001	101	79	28.21	10.29–77.31	<0.001	0.551
Head and neck cancer																						
Alcohol consumption†§ / ‡§																						
Non-drinker	18	46	1.00	Reference		38	128	0.92	0.46–1.85	0.814		21	39	1.00	Reference		41	141	0.53	0.27–1.04	0.067	
Moderate drinker	28	80	0.96	0.45–2.05	0.923	24	63	1.55	0.68–3.53	0.297		26	82	0.61	0.29–1.28	0.191	29	62	1.03	0.47–2.24	0.945	
High-moderate drinker	26	65	1.43	0.63–3.22	0.388	26	60	1.05	0.46–2.41	0.906		21	73	0.67	0.30–1.49	0.323	31	52	1.03	0.46–2.28	0.945	
Heavy drinker	60	42	4.03	1.82–8.92	0.001	26	26	2.87	1.20–6.88	0.018	0.383	37	54	1.36	0.62–2.99	0.444	49	15	5.34	2.22–12.83	<0.001	<0.001
Cumulative smoking† / ‡																						
PY<5	37	108	1.00	Reference		34	109	1.16	0.66–2.06	0.657		37	118	1.00	Reference		39	105	1.40	0.80–2.44	0.240	
5 ≤ PY<20	19	37	1.42	0.68–2.95	0.352	16	49	1.29	0.62–2.71	0.494		16	39	1.25	0.61–2.59	0.539	21	47	1.72	0.86–3.44	0.127	
20 ≤ PY<40	35	49	2.52	1.32–4.80	0.005	25	55	1.62	0.82–3.18	0.165		24	56	1.52	0.78–2.96	0.222	38	49	3.03	1.61–5.71	0.001	
40 ≤ PY	46	43	3.23	1.69–6.15	0.000	39	67	2.58	1.35–4.93	0.004	0.316	33	41	2.89	1.47–5.67	0.002	54	70	3.14	1.71–5.78	<0.001	0.818

†Models included age, sex, alcohol consumption, cumulative smoking, facial flushing, fruit and vegetable intake, frequent intake of hot beverages, and body mass index. ‡Models included age, sex, alcohol consumption, cumulative smoking, ALDH2 genotype, fruit and vegetable intake, frequent intake of hot beverages, and body mass index. §Moderate drinking was defined as consumption ≤4 days/week; high-moderate drinking as <46 g ethanol and ≥5 days/week; and heavy drinking as ≥46 g ethanol and ≥5 days/week. ALDH2, aldehyde dehydrogenase 2; CI, confidence interval; OR, odds ratio; PY, pack years.

Table 4. The distribution of facial flushing and *ALDH2* genotype

ALDH2	Alcohol consumption‡	Facial flushing		†*P*-value
		Negative (%)	Positive (%)	
Cases				
Glu∕Glu	Overall	183 (85.92)	30 (14.08)	<0.001
	Never	19 (79.17)	5 (20.83)	<0.001
	Moderate	36 (87.80)	5 (12.20)	
	High-moderate	43 (81.13)	10 (18.87)	
	Heavy	79 (88.76)	10 (11.24)	
Glu∕Lys	Overall	163 (42.89)	217 (57.11)	
	Never	5 (9.26)	49 (90.74)	<0.001
	Moderate	15 (25.86)	43 (74.14)	
	High-moderate	39 (43.82)	50 (56.18)	
	Heavy	99 (57.56)	73 (42.44)	
Lys∕Lys	Overall	2 (10.53)	17 (89.47)	
	Never	2 (11.76)	15 (88.24)	0.822
	Moderate	0 (0.00)	2 (100.00)	
	High-moderate	0	0	
	Heavy	0	0	
Controls				
Glu∕Glu	Overall	472 (81.66)	106 (18.34)	<0.001
	Never	50 (87.72)	7 (12.28)	0.653
	Moderate	150 (81.52)	34 (18.48)	
	High-moderate	149 (81.42)	34 (18.58)	
	Heavy	113 (80.14)	28 (19.86)	
Glu∕Lys	Overall	77 (16.28)	396 (83.72)	
	Never	18 (9.14)	179 (90.86)	0.001
	Moderate	25 (17.86)	115 (82.14)	
	High-moderate	23 (22.55)	79 (77.45)	
	Heavy	9 (32.14)	19 (67.86)	
Lys∕Lys	Overall	7 (6.73)	97 (93.27)	
	Never	7 (7.22)	90 (92.78)	0.462
	Moderate	0	7 (100.00)	
	High-moderate	0	0	
	Heavy	0	0	

†*P*-values were calculated using the χ²-test. ‡Moderate drinking was defined as consumption ≤4 days∕week; high-moderate drinking as <46 g ethanol and ≥5 days∕week; and heavy drinking as ≥46 g ethanol and ≥5 days∕week. ALDH2, aldehyde dehydrogenase 2.

Alcohol dehydrogenase (ADH), which metabolizes ethanol to acetaldehyde, may play an important role in the flushing response. Flushing is also influenced by the *ADH1B* Arg48His polymorphism (rs1229984), which modulates alcohol metabolizing capacity.[28–31] In subjects with the *ALDH2* Glu∕Lys genotype, heavy drinkers tended to exhibit facial flushing with less frequency than never drinkers. Reporting bias due to social or cultural pressures is one explanation of the result. A considerable number of individuals with facial flushing in their youth diminished their flushing after long time drinking.[10,20] This suggests that high alcohol consumption may also affect facial flushing.

Second, when used as a surrogate marker of *ALDH2* polymorphism, facial flushing was unable to distinguish between the *ALDH2* Glu∕Lys and *ALDH2* Lys∕Lys genotype. Although the *ALDH2* Glu∕Lys genotype conferred a higher risk of UAT cancer whereas the *ALDH2* Lys∕Lys genotype conferred no significant association, most subjects with either polymorphism exhibit facial flushing, diminishing this characteristic's power to detect *ALDH2* genotype. The relation between facial flushing and *ALDH2* genotype was not particularly close: among controls, 18% with the *ALDH2* Glu∕Glu genotype exhibited facial flushing whereas 16% with the *ALDH2* Glu∕Lys genotype did not. This misclassification was particularly obvious among the high risk group (heavy drinkers with the *ALDH2* Glu∕Lys

genotype). These discrepancies might account for the non-significant association between facial flushing and UAT cancer.

Our present findings were not consistent with those of a previous case–control study.[6,20] This may have been due to several differences between the studies. Among these, the previous study was conducted in men only, whereas our present study included both men and women. Further, the previous study included cases with oral and pharyngeal cancer only[6] or esophageal cancer only,[20] whereas we included not only esophageal cancer but also cancer of the oral cavity, pharynx, and larynx. Finally, the previous study evaluated current and past flushing status, whereas we evaluated flushing status at the time of study enrollment. We saw fewer flushers among heavy drinkers than never drinkers with the *ALDH2* Glu∕Lys genotype, suggesting that flushing status might change with high and long-term alcohol exposure. This possibility should be considered in any future evaluation of flushing status. In the absence of a complete evaluation of flushing status by questionnaire, evaluation by genotyping appears to be efficient in terms of risk evaluation in UAT cancers.

Our study has several methodological strengths. First, it was conducted in a single region in central Japan. Second, potential confounding by age and sex was adjusted for by matching of these factors. In addition, we considered established risk factors as much as possible. Lastly, given that our allele frequencies were comparable to those previously reported in public databases such as HapMap JPT,[32] bias in the distribution of selected polymorphisms appears negligible.

Several potential limitations of our study also warrant mention. One methodological issue is the selection of hospital-based noncancer patients as controls. However, because 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.[33] Drinking habit in controls was equivalent compared with National Health and Nutrition Survey in Japan in 2003. The proportion of facial flushers in HERPACC was comparable to the one in the randomly sampled general population in same area (unpublished data). In addition, to dilute 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 likely underestimate the causal association. Lastly, the moderate number of cases indicates the need for replication of our findings in a larger study in a population with the same ethnicity.

In conclusion, our study showed that facial flushing was not significantly associated with UAT cancer. Facial flushing using a simple questionnaire should not be used as a surrogate marker in predicting UAT cancer risk. Rather, UAT cancer susceptibility should be predicted using genotype *ALDH2* Glu504Lys polymorphism.

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Disclosure Statement

The authors have no conflict of interest.

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