Gene–gene and gene–environment interactions between alcohol drinking habit and polymorphisms in alcohol-metabolizing enzyme genes and the risk of head and neck cancer in Japan

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Alcohol consumption is a strong risk factor for squamous cell carcinoma of the head and neck (SCCHN). The genetic polymorphisms aldehyde dehydrogenase2 (*ALDH2*) Glu487Lys and alcohol dehydrogenase 2 (*ADH2*) His47Arg, which have a strong impact on alcohol metabolism, are common in the Japanese population. To clarify the significance of these polymorphisms in SCCHN carcinogenesis, we conducted a matched case-control study with 239 incident SCCHN subjects and 716 non-cancer controls. Both *ADH2* Arg/Arg and *ALDH2* Glu/Lys were found to be independently associated with increased risk, with odds ratios (OR) of 2.67 (95% confidence interval [CI] 1.51–4.57) and 1.66 (95% CI 1.20–2.31), respectively. Further, compared with subjects having both *ADH2* His/His and *ALDH2* Glu/Glu, the adjusted OR and its 95% CI for those with both *ADH2* Arg/Arg and *ALDH2* Glu/Lys was 5.00 (2.32–10.71) in all subjects. This combination effect was evident in heavy drinkers (OR 11.3, 95% CI 2.97–43.3) but not in moderate or non-drinkers. Statistically significant gene–environment interactions between the two polymorphisms and drinking level were seen (ADH2 $P = 0.035$, ALDH2, $P = 0.013$). Furthermore, we also found a statistically significant gene–gene interaction between the two polymorphisms ($P = 0.042$). In conclusion, this case-control study showed a significantly increased risk of SCCHN in subjects with the *ADH2* Arg/Arg and *ALDH2* Glu/Lys polymorphisms in a Japanese population. In addition, our results also demonstrated that this risk was associated with significant gene–gene interactions between *ADH2* and *ALDH2* polymorphisms, as well as gene–environment interactions between these polymorphisms and alcohol drinking. (*Cancer Sci* 2007; 98: 1087–1091)

Squamous cell carcinoma of the head and neck (SCCHN), which includes cancers of the oral cavity, pharynx and larynx, are the sixth most frequent cancer and the seventh leading cause of cancer-related death worldwide, affecting more than 500 000 individuals each year.[1] It is well known that alcohol drinking is the major risk factor in the etiology of SCCHN.[2]

Alcohol is oxidized to acetaldehyde by the alcohol dehydrogenase enzymes (ADH), particularly ADH2. Acetaldehyde is then further oxidized into acetate by aldehyde dehydrogense enzymes (ALDH), and this oxidation owes much to ALDH2. Genes that encode these two representative alcohol-metabolizing enzymes display polymorphisms that modulate individual differences in alcohol-oxidizing capability and drinking behavior.[3] Regarding *ADH2* Arg47His, the *47His* allele represents a superactive subunit of *ADH2* that confers an approximately 40-times higher V_{max} than the less-active *ADH2* Arg/Arg form,[4,5] whereas for the *ALDH2* Glu487Lys polymorphism, the *487Lys* allele encodes a catalytically inactive subunit.[4,5] Individuals with the *ALDH2* Glu/Lys genotype have only 6.25% of the normal level of ALDH2 487Glu protein. These findings indicate the dominant effect of *ALDH2* 487Lys.[6] The *ADH2* 47His and *ALDH2* 487Lys alleles, which both lead to high acetaldehyde concentrations, are clustered in east Asian populations such as the Japanese population.[7,8] Because these two genetic polymorphisms modify drinking habit,[9] they are expected to affect SCCHN risk, especially in Asian populations in whom the frequency of minor alleles is relatively high. Yokoyama *et al*. showed an increased risk of oropharyngolaryngeal cancer with the *ADH2* Arg/Arg and *ALDH2* Glu/Lys genotypes in Japanese alcoholics.[10] However, little evidence is available on the combined impact of the *ADH2* and *ALDH2* polymorphisms on increased risk for SCCHN by alcohol drinking in non-alcoholics. Further, it has not been determined whether these genetic factors modulate the increased risk of SCCHN caused by environmental factors, including alcohol drinking.

Here, we conducted a case-control study to clarify the impact of both individual and combined *ADH2* and *ALDH2* gene polymorphisms on SCCHN risk. We also investigated gene–gene interactions between these two polymorphisms, as well as gene–environment interactions between polymorphisms and alcohol drinking and exposure.

Materials and Methods

Subjects. The subjects were 239 patients diagnosed histologically with SCCHN (lip and oral cavity cancer in 119, pharynx cancer in 88, and larynx cancer in 32 patients) between January 2001 and December 2004 at Aichi Cancer Center Hospital (ACCH) who had no prior history of cancer, as described elsewhere.[11] Head and neck cancer was defined according to the following codes of the International Classification of Diseases and Related Health Problems (ICD10): lip and oral cavity (C00–C06), pharynx (C10–C14) and larynx (C32). Malignant neoplasms of the salivary glands (C07, C08), tonsil (C09), nasal (C30) and paranasal (C31) were excluded from the present study as they have quite distinct natural histories, and poorly understood etiologies and histological structures. The controls were 716 first-visit outpatients at ACCH during the same period who were confirmed to have no cancer and no history of neoplasia. Controls were selected randomly and matched for age (±3 years) and sex to subjects with a 1:3 case-control ratio to achieve a greater than 80% power to detect an odds ratio (OR) of 2.5 when the proportion of the at-risk genotype among controls was 4%. Mean age was 57.0 years in the subjects and 57.6 years in the controls. Men accounted for 76.9% of the subjects. The controls were selected from the database of the Hospital-based Epidemiologic Research Program at Aichi Cancer Center II (HERPACC-II).

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The framework of the HERPACC-II has been described elsewhere.[12,13] Briefly, all first-visit outpatients aged 18–79 years are asked to fill out a questionnaire regarding their lifestyle as well as to provide 7 mL blood. Approximately 95% of eligible subjects completed the questionnaire and 55% provided blood samples. Approximately 30% of first-visit outpatients were diagnosed as having cancer at ACCH. Under the assumption that the non-cancer population within HERPACC will visit ACCH if they develop cancer in the future, we defined these non-cancer first-visit outpatients as a population in which cases may arise. Our previous study confirmed that the lifestyle patterns of first-visit outpatients were accordant with those in a general population randomly selected from Nagoya City, demonstrating external validity for the study.[14] This investigation was approved by the ethics committee of ACC, and written informed consent to participate was obtained from all subjects.

Genotyping of *ADH2* and *ALDH2*. DNA from each subject was extracted from the buffy coat fraction with a BioRobot EZ1 and EZ1 DNA Blood 350 μL kit (Qiagen K. K., Tokyo, Japan). Genotyping was based on Taqman Assays from Applied Biosystems (Foster City, CA, USA). The principle of the TaqMan Real-Time polymerase chain reaction (PCR) assay system using fluorogenic probes and 5′ nuclease is described by Livak.[15] All of the assays were done in 96-well PCR plates. Amplification reactions (5 μL) were done in duplicate with 30 ng of template DNA, 2× TaqMan Universal Master Mix buffer (Applied Biosystems), 20× primer and probe Mix (Applied Biosystems). Thermal cycling was initiated with a first denaturation step of 20 s at 95°C, and then by 40 cycles of 3 s at 95°C and 30 s at 62°C. After PCR was completed, plates were brought to room temperature, read using a 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA), and the results were analyzed using the 7500 Fast System SDS software. The quality of genotyping was assessed statistically using the Hardy–Weinberg test in our laboratory. When allelic distributions for controls departed from the Hardy–Weinberg frequency, genotyping was assessed using another method.

Assessment of alcohol intake and smoking exposure. Alcohol consumption of various common beverages (Japanese sake, beer, shochu, whiskey and wine) was determined with regard to the average number of drinks per day, which was then converted into a Japanese sake (rice wine) equivalent. One Japanese drink equated to one 'go' (180 mL) of Japanese sake, which contains 25 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. Total alcohol consumption was estimated as the summed amount of pure alcohol consumption (g per consumption) of Japanese sake, beer, shochu, whiskey and wine among current and former regular drinkers. Information on smoking status was obtained in the three categories: nonsmoker, former smoker and current smoker.

Statistical analysis. Statistical analyses were carried out using Stata version 8 (Stata Corporation, College Station, TX, USA). A *P*-value less than 0.05 was considered statistically significant. SCCHN risk was assessed using an unconditional logistic regression models to calculate OR and 95% confidence intervals (CI). Alcohol exposure was categorized into three levels: nondrinker (never drinker), moderate drinker and heavy drinker. Heavy drinkers were defined as those who drank alcoholic beverages 5 days or more per week in an amount of 50 g ethanol or more on each occasion, whereas moderate drinkers were defined as those other than heavy drinkers. Smoking status was also divided into three categories in consideration of cumulative exposure to tobacco: low-level smokers, those with 10 pack-years (PY) or less; moderate smokers, $10 \leq PY < 40$; and heavy smokers, $40 \leq PY$. Potential confounders considered in the multivariate analyses were age, sex and smoking. Accordance with the Hardy–Weinberg equilibrium was checked for controls with the χ^2-test to assess any discrepancies between expected and observed genotype and allele frequencies. Trend of genotype impact was assessed by a score test for each genotype, namely: 0, homozygous for reference allele; 1, heterozygote; and 2, homozygous non-reference allele. Gene–environment and gene–gene interactions were assessed by interaction terms between genes or drinking status with scores of: 0, never; 1, moderate; and 2, heavy.

Results

Table 1 shows the drinking and smoking status among cases and controls. Significant differences between the two groups were seen for both drinking and smoking. Alcohol consumption was significantly increased in the study subjects ($P < 0.001$). The OR for SCCHN in heavy drinkers were increased compared with never drinkers (age–sex smoking adjusted OR, 2.45; 95% CI, 1.62–3.71; trend < 0.001). In addition, the OR for SCCHN in those who drank 75 g ethanol/day or more was increased compared with never drinkers (age–sex smoking adjusted OR, 3.94; 95% CI, 2.10–7.36; trend < 0.001). Current smoking and cumulative exposure to smoking were also significantly increased among subjects (both $P < 0.001$). The OR for SCCHN with high smoking exposure were increased compared with those with low exposure (age–sex drinking adjusted OR, 2.31; 95% CI, 1.47–3.63; trend < 0.001).

Table 2 shows the genotype distributions for *ADH2* and *ALDH2*, and their OR and 95% CI for SCCHN. Genotype frequencies for all polymorphisms were in accordance with the Hardy–Weinberg equilibrium in controls (*ADH2*, $P = 0.273$; *ALDH2*, $P = 0.525$), and allele frequencies were in reasonable accordance with earlier reports in Japan.[16] The frequencies of His/His, His/Arg and Arg/Arg (*ADH2* His47Arg) were 65.8, 30.0 and 4.3% among controls and 57.7, 34.1 and 10.9% among subjects, respectively. A significantly increased risk of SCCHN was observed with Arg/Arg relative to His/His (age, sex, drinking and smoking-adjusted OR, 2.67; 95% CI, 1.51–4.75; trend = 0.004). The frequencies of Glu/Glu, Glu/Lys and Lys/Lys of the *ALDH2* Glu487Lys polymorphism were 49.82, 42.2 and 8.0% among controls and 45.2, 50.2 and 4.6% among subjects. A significantly increased risk of SCCHN was observed with Glu/Lys relative to Glu/Glu (confounder-adjusted OR, 1.66; 95% CI, 1.20–2.31; trend = 0.01).

On analysis of the combination of *ADH2* and *ALDH2* polymorphisms, the adjusted OR for subjects with *ADH2* Arg/Arg and *ALDH2* Glu/Lys compared with those with the *ADH2* His/His and *ALDH2* Glu/Glu was 5.00 (95% CI 2.32–10.71) in all subjects (Table 3) We also found a statistically significant gene–gene interaction between the two polymorphisms ($P = 0.042$). When we stratified the analysis into two categories by drinking level, an increased risk in those with *ADH2* Arg/Arg and *ALDH2* Glu/Lys was observed in heavy drinkers only (OR, 11.3; 95% CI, 2.97–43.3). An increased risk with *ADH2* His/Arg and *ALDH2* Glu/Lys was also observed in heavy drinkers (OR, 2.39; 95% CI, 1.13–5.04).

The impact of *ADH2* and *ALDH2* polymorphisms in combination with drinking are presented in Table 4. For the *ADH2* genotypes, the risk of SCCHN was consistently increased according to drinking level in all *ADH2* genotypes, and was particularly marked with the Arg/Arg genotype. Further, the impact of Arg/Arg in heavy drinkers appeared higher than that in moderate and never drinkers. The OR of heavy, moderate and never drinkers with Arg/Arg were 9.52 (95% CI 3.89–23.3), 2.46 (95% CI 0.89–6.73) and 0.50 (95% CI 0.06–4.11; *P*-trend = 0.008), respectively, when compared with never drinkers with His/His genotypes.

For the *ALDH2* genotypes, the OR of subjects with *ALDH2* Lys+ were increased compared with *ALDH2* Glu/Glu in moderate

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Table 1. Drinking and smoking status among cases and controls

	Cases ($n = 239$)		Controls ($n = 716$)		*P*-value	OR (95% CI)
	n	%	*n*	%		
Alcohol drinking						
Never	56	23.4	236	33.0		1.00 (Reference)†
Ever						
Moderate drinker	74	31.0	292	40.8		1.10 (0.73–1.65)
Heavy drinker	109	45.6	188	26.2	<0.001	2.45 (1.62–3.71)
						P-trend < 0.001
Intake of ethanol (g/day)						
0	57	23.8	236	33.0		1.00 (Reference)†
>0, ≤25	87	36.4	314	43.9	1.24 (0.83–1.84)	
>25, ≤50	47	19.8	107	14.9	1.93 (1.19–3.12)	
<50, ≤75	19	7.9	30	4.2	2.71 (1.38–5.32)	
<75	29	12.1	29	4.0	<0.001	3.94 (2.10–7.36)
						P-trend < 0.001
Smoking						
Smoking status						
Never	56	23.4	256	35.8		
Former	88	36.8	255	35.6		
Current	94	39.4	205	28.6		
Unknown	1	0.4	0	0.0	<0.001	
Smoking exposure						
0 < PY < 10	64	26.8	295	41.2		1.00 (Reference)‡
10 ≤ PY < 40	77	32.2	186	26.0		2.06 (1.33–3.17)
PY > 40	98	41.0	235	32.8	<0.001	2.31 (1.47–3.63)
						P-trend < 0.001

†Odds ratios (OR) adjusted for age, sex and smoking. ‡OR adjusted for age, sex and drinking. CI, confidence interval; PY, pack years.

Table 2. Genotype distributions of *ADH2* and *ALDH2* polymorphisms and their impact on risk of head and neck cancer

	Cases		Controls†		Model 1‡		Model 2‡	
	n	%	*n*	%	OR	95% CI	OR	95% CI
ADH2								
His/His	138	57.7	471	65.8	1.00	Reference	1.00	Reference
His/Arg	75	31.4	213	30.0	1.21	0.87–1.68	1.17	0.83–1.63
Arg/Arg	26	10.9	31	4.3	2.88	1.65–5.01	2.67	1.51–4.75
					P-trend = 0.001		*P*-trend = 0.004	
ALDH2								
Glu/Glu	108	45.2	356	49.8	1.00	Reference	1.00	Reference
Glu/Lys	120	50.2	302	42.2	1.33	0.98–1.80	1.66	1.20–2.31
Lys/Lys	11	4.6	57	8.0	0.64	0.32–1.26	1.43	0.68–3.01
					P-trend = 0.765		*P*-trend = 0.01	

†One control was excluded from the analysis because *ADH2*/*ALDH2* genotypes could not be defined. ‡Model 1 adjusted for age and sex; model 2 adjusted for age, sex, drinking and smoking. CI, confidence interval; OR, odds ratio.

and heavy drinkers (P-trend = 0.037 and 0.003). The impact of the *ALDH2* Glu/Lys genotypes in heavy drinkers (OR, 3.13; 95% CI, 1.46–6.72) appeared higher than that in moderate (OR, 1.05; 95% CI, 0.50–2.20) and never drinkers (OR, 0.75; 95% CI, 0.37–1.53). Statistically significant gene–environment interactions between the two polymorphisms and drinking levels were evident (ADH2, $P = 0.035$; ALDH2, $P = 0.013$).

Discussion

In the present study, we clearly demonstrated that the risk of SCCHN was increased in subjects with the *ADH2* Arg/Arg and *ALDH2* Glu/Lys genotypes. The effect of the *ADH2* Arg+ and *ALDH2* Lys+ genotypes on this increased risk was consistently observed in moderate and heavy drinkers, but not in never drinkers. Further, this risk was also associated with the presence of significant gene–gene interactions between the *ADH2* and *ALDH2* polymorphisms as well as gene–environment interactions between these polymorphisms and alcohol drinking. To our knowledge, this is the first study to examine both *ADH2* and *ALDH2* polymorphisms with reference to SCCHN in non-alcoholic subjects.

Given the role of ADH2 as the predominant determinant of alcohol oxidation, the biological impact of polymorphisms of the *ADH2* gene on the risk of SCCHN is very interesting. Increased risk of head and neck cancers in Japanese alcoholics with *ADH2* Arg/Arg has been observed.[10] Our results that *ADH2* Arg/Arg increases the risk of SCCHN are consistent with this previous study. Further, the significant interaction between *ADH2* polymorphism and drinking level suggests that the impact of drinking differs

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Table 3. Age–sex adjusted odds ratios (OR) for the combination of *ADH2* and *ALDH2* polymorphnisms

	n (case/control)	ALDH2		
	†OR (95% CI)	Glu/Glu	Glu/Lys	Lys/Lys
Overall				
ADH2	His/His	72/238	61/195	5/38
		1.00 (reference)	0.96 (0.65–1.44)	0.45 (0.17–1.19)
	His/Arg	29/103	40/94	6/16
		0.91 (0.56–1.50)	1.39 (0.88–2.21)	1.46 (0.54–3.95)
	Arg/Arg	7/15	19/13	0/3
		1.65 (0.64–4.27)	5.00 (2.32–10.71)	NE
Never and moderate drinker				
ADH2	His/His	36/153	44/164	5/38
		1.00 (reference)	1.13 (0.67–1.89)	0.57 (0.21–1.56)
	His/Arg	12/61	19/74	6/16
		0.84 (0.41–1.73)	1.17 (0.62–2.20)	1.78 (0.64–4.92)
	Arg/Arg	2/9	6/10	0/3
		0.92 (0.189–4.55)	2.57 (0.87–7.61)	NE
Heavy drinker				
ADH2	His/His	36/85	17/31	0/0
		1.00 (reference)	1.37 (0.66–2.86)	NE
	His/Arg	17/42	21/20	0/0
		1.00 (0.49–2.02)	2.39 (1.13–5.04)	NE
	Arg/Arg	5/6	13/3	0/0
		2.19 (0.60–8.08)	11.33 (2.97–43.3)	NE

†OR adjusted for age, sex and smoking. CI, confidence interval; NE, not estimated because of no case in this category.

Table 4. Impact of the *ADH2* and *ALDH2* polymorphisms in combination with drinking

Drinking†	ADH2			*P*-trend	ALDH2			*P*-trend
	His/His	His/Arg	Arg/Arg		Glu/Glu	Glu/Lys	Lys/Lys	
Never	39/163	16/64	1/9	0.942	16/49	31/133	9/54	0.316
	1.00 (Reference)	1.22 (0.63–2.35)	0.50 (0.06–4.11)		1.00 (Reference)	0.75 (0.37–1.53)	0.56 (0.22–1.43)	
Ever: moderate	46/192	21/87	7/13	0.227	34/174	38/115	2/3	0.037
drinker	1.08 (0.66–1.77)	1.06 (0.58–1.94)	2.46 (0.89–6.73)		0.66 (0.32–1.36)	1.05 (0.50–2.20)	3.14 (0.45–21.65)	
Heavy drinker	53/116	38/62	18/9	0.002	58/133	51/54	0/0	0.003
	1.98 (1.20–3.30)	1.66 (1.51–4.71)	9.52 (3.89–23.3)		1.41 (0.69–2.89)	3.13 (1.46–6.72)	NE	
P-trend	0.005	0.056	0.008		0.058	<0.001	0.44	

†Odds ratios (95% confidence intervals) for head and neck cancer stratified by drinking. NE, not estimated because of no case in this category.

with *ADH2* genotype. These results suggest that alcohol metabolism is involved in the carcinogenesis of SCCHN.

The carcinogenic effect of acetaldehyde in various types of cancer is well known.[17] Speculation on the role of the *ADH2* His47Arg polymorphism in acetaldehyde-based carcinogenesis of SCCHN might run as follows: the *ADH2* 47Arg allele results in longer exposure to acetaldehyde, a well-recognized carcinogen, on account of this allele's relatively weak activity in converting alcohol to acetaldehyde. Whether it is alcohol itself rather than acetaldehyde that plays a role in alcohol-induced SCCHN carcinogenesis remains to be elucidated. In any case, it is clear that the *ADH2* polymorphism plays an important role in the carcinogenesis of SCCHN.

Similarly, because ALDH2 is a strong factor in acetaldehyde oxidation, it is of great interest to examine the biological impact of polymorphisms of the *ALDH2* gene. Several studies have reported a strong association between the *ALDH2* Glu/Lys genotype[10,18] and an increased risk of SCCHN, consistent with our present results. In individuals with ALDH2 encoded by *ALDH2* Glu/Lys, the blood acetaldehyde level after drinking is approximately six-fold that in individuals with active ALDH2.[19] On this basis, the impact of acetaldehyde on SCCHN carcinogenesis might be indirectly evaluable by assessing this polymorphism. Thus, the increased risk with the *ALDH2* Glu/Lys genotype is supportive of acetaldehyde-based SCCHN carcinogenesis. The significant gene–environment interaction is also supportive of this carcinogenesis. Moreover, this interaction is similar to the interaction we reported previously for esophageal and colon cancer.[20,21] Considered together with this previous evidence of the important role of acetaldehyde in carcinogenesis in experimental models,[17] our present findings leave little doubt that acetaldehyde plays a pivotal role in the carcinogenesis of SCCHN.

Of note, when the *ADH2* and *ALDH2* polymorphisms were combined, the OR for those with the *ADH2* Arg/Arg and the *ALDH2* Glu/Lys genotypes was higher than that expected from multiplication of the OR for the *ADH2* Arg/Arg and *ALDH2* Glu/Lys genotypes, which is indicative of a strong interaction between these two polymorphisms. As both of these genes are located in the same alcoholic oxidation pathway, either one could play a rate-determining role. These significant gene–gene interactions were enhanced in heavy drinkers, but were not observed in moderate or never drinkers. This suggests the existence of a gene–gene–environment interaction among the *ADH2* and *ALDH2* polymorphisms and drinking.

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Our study has several limitations. One methodological issue is the selection of the control base population. We used non-cancer patients at the ACCH for this purpose on the basis that our subjects arose within this population. We have previously confirmed the similarity of this population to the general population in terms of various exposures of interest, here alcohol drinking.[14] Further, the genotype distribution of the *ALDH2* and *ADH2* polymorphisms in our controls was similar to that in the general population.[16] A second potential source of bias was the medical background of the controls. However, our previous study focusing on women demonstrated that this had only a limited impact: more than 66% of non-cancer outpatients at ACCH had no specific medical condition, and the remaining 34% had specific diseases such as benign tumors, non-neoplastic polyps or both (13.1%), mastitis (7.5%), gastrointestinal disease (4.1%), or benign gynecologic disease (4.1%).[22] A similar situation applies to men. In addition, in contrast to standard hospital-based studies, the HERPACC system is less prone to information bias because all data are collected prior to diagnosis.

In conclusion, our case-control study showed a significantly increased risk of SCCHN in those with the *ADH2* Arg/Arg and *ALDH2* Glu/Lys polymorphisms, especially those with both, in a Japanese population. In addition, our results also demonstrated that this risk was associated with significant gene–gene interactions between *ADH2* and *ALDH2* polymorphisms, as well as gene–environment interactions between these polymorphisms and alcohol drinking.

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