RESEARCH COMMUNICATION

Esophageal Cancer Risk by *ALDH2* and *ADH2* polymorphisms and Alcohol Consumption: Exploration of Gene-Environment and Gene-Gene Interactions

Chun-Xia Yang[1,5], Keitaro Matsuo[1,6], Hidemi Ito[1], Kaoru Hirose[1], Kenji Wakai[1], Toshiko Saito[1], Masayuki Shinoda[2], Shunzo Hatooka[3], Kazuko Mizutani[4], Kazuo Tajima[1]

Abstract

Alcohol drinking is a major risk factor for esophageal cancer in Japan and its impact may be modulated by levels of ALDH2, ADH2 and CYP2E1, three representative alcohol-metabolizing enzymes which display genetic polymorphisms altering individual alcohol-oxidizing capacity and drinking behavior. To assess the actual influence of *ADH2* Arg47His, *ALDH2* Glu487Lys and *CYP2E1* variant c2 allele polymorphisms on esophageal cancer risk with conjunction with alcoholic consumption, the present 1:3 matched case-control study was conducted. The 165 histologically diagnosed Japanese esophageal cancer cases were here compared with 495 randomly selected controls, matched with respect to sex and age. Conditional logistic regression was used to calculated Odds Ratios (ORs) and 95% confidence intervals (95% CI). Significant gene-environment interactions between alcohol drinking and both ADH2 and ALDH2 were observed regarding esophageal cancer risk. The *ADH2* Arg47His polymorphism showed moderately increased risk (OR for *Arg/His* and *Arg/Arg* relative to *His/His*: 2.01 (1.39-2.90)). In the *ALDH2* case, comparing the *Glu/Lys* with the *Glu/Glu* genotype, ORs were markedly increased to 9.64 (3.23-28.8) and 95.4 (28.7-317) from 1.88 (0.42-8.37) and 4.62 (0.93-23.1) for moderate drinking and heavy drinking, respectively. No significant alteration in risk was observed with the CYP2E1 polymorphism. In conclusion, the present study revealed a significant gene-environment interaction between alcohol drinking and the *ALDH2* polymorphism regarding esophageal cancer risk among a general population in Japan, providing concrete evidence of a role for acetaldehyde in neoplastic development. Interactions between *ALDH2* and *ADH2* need further clarification.

Key Words: esophageal cancer - alcohol - ADH2 - ALDH2 - CYP2E1 - gene-environment interaction - gene-gene interaction

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Introduction

Epidemiological studies have provided convincing evidence that alcohol drinking is a strong risk factor for esophageal cancer development (Castelletto et al., 1994;Kinjo et al., 1998;Brown and Devesa, 2002). Although alcohol itself is not a carcinogen, a primary metabolite, acetaldehyde, has been proven to be carcinogenic in experimental models (Feron et al., 1982;Woutersen et al., 1986;Dellarco, 1988). Basically, alcohol is oxidized to acetaldehyde by the alcohol dehydrogenase enzymes (ADHs), especially by ADH2 (formally ADH1B). Acetaldehyde is further oxidized into acetate by aldehyde dehydrogenase enzymes (ALDHs), and this depends largely on ALDH2. CYP2E1 is also believed to participate in the oxidation of alcohol, resulting in production of reactive free radicals that may initiate lipid peroxidation and consequently impact on carcinogenesis (Albano et al., 1991).

The encoding genes of these three representative alcohol-metabolizing enzymes display polymorphisms which may modulate individual differences in alcohol-oxidizing capacity and drinking behavior (Bosron and Li, 1986;Marchand et al., 1999;Yokoyama et al., 2001). With *ADH2* Arg47His, the 47His allele represents a superactive subunit which has about a 40 times higher V_{max} than the less-active *ADH2 His/His* form (Bosron and Li,

[1]Division of Epidemiology and Prevention, [2]Department of Rehabilitation Service, [3]Department of Thoracic Surgery, and [4]Department of Clinical Laboratory, Aichi Cancer Center; [5]Department of Epidemiology, Huaxi Public Health School, Sichuan University, Chengdu 610041, China. [6]Correspondence to Keitaro Matsuo, M.D., Ph.D, M.Sc. Division of Epidemiology and Prevention, Aichi Cancer Center Research Institute, 1-1 Kanokoden, Chikusa-ku, Nagoya 464-8681, Japan. Fax: +81-52-763-5233. E-mail: kmastuo@aichi-cc.jp

1986;Yoshida et al., 1991). As for the *ALDH2* Glu487Lys polymorphism, the 487Lys allele encodes a catalytically inactive subunit (Bosron and Li, 1986;Yoshida et al., 1991) and individuals with the *ALDH2 Glu/Lys* genotype have only 6.25% of normal *ALDH2 487Glu* activity, indicating a dominant effect of the *ALDH2 487Lys* (Crabb et al., 1989). The *ADH2 47His* and *ALDH2 487Lys* alleles, both leading to high acetaldehyde concentrations, are clustered in east Asian populations such as Japanese (Hamajima et al., 2002;Tamakoshi et al., 2003;Oota et al., 2004), and would be expected to modify the esophageal cancer risk especially in the population whose frequencies for minor alleles are common. Regarding polymorphisms of *CYP2E1*, the variant c2 allele of *CYP2E1* recognized by *RsaI* digestion in the 5'-flanking region of the gene appears to be associated with decreased enzyme activity which might decrease the risk of esophageal cancer(Marchand et al., 1999). Although several studies on *ADH2* or *ALDH2* polymorphisms and esophageal cancer risk have been conducted to clarify their association, the majority were conducted with male alcoholics (Yokoyama et al., 1996;Yokoyama et al., 1998;Yokoyama et al., 1999;Chao et al., 2000) and investigations of non-alcoholic populations have been limited (Hori et al., 1997;Ikehara et al., 2001;Boonyaphiphat et al., 2002). One which focused on both *ADH2* and *ALDH2* polymorphisms suggested a substantial impact on esophageal cancer risk (Yokoyama et al., 2002), however, no confirmation study has been conducted. With regard to the *CYP2E1* results have not been consistent (Hori et al., 1997;Morita et al., 1997;Lin et al., 1998;Tan et al., 2000;Gao et al., 2002).

Therefore, we conducted the present matched case-control study to further assess the actual impact of *ADH2*, *ALDH2* and *CYP2E1* polymorphisms on esophageal cancer risk in a Japanese population in conjunction with alcohol consumption.

Materials and Methods

Subjects

The cases were 165 patients who were histologically diagnosed as having esophageal cancers (159 squamous cell carcinomas and 6 adenocarcinomas) between January 2001 and August 2004 at Aichi Cancer Center Hospital (ACCH). Controls were first visit outpatients who visited ACCH during the same period who were confirmed to have no cancers. They were randomly selected and matched for age and sex to give a 1:3 case-control ratio (n=495). This sample size was defined to detect an odds ratio (OR) 1.8 with a type I error of 0.05 and a type II error of 0.10 (statistical power 0.90) under the assumption that the frequency of individual having an *ALDH2* 487Lys or *ADH2* 47His allele is 40% in the control population. All the subjects, aged 18-80 years, were recruited in the framework of the Hospital-based Epidemiologic Research Program at Aichi Cancer Center (HERPACC), as described elsewhere (Katsuda et al., 2001;Yang et al., 2003). Briefly, in this program all the first visit outpatients are asked to fill out a questionnaire regarding their lifestyle and to provide 7-ml of blood. Approximately 95% of eligible subjects have completed the questionnaire and about 60% have provided blood samples. Our previous study showed lifestyle patterns of first visit outpatients are accordant with those in the general population randomly selected from the Nagoya City electoral roll, supporting external validity of our study results (Inoue et al., 1997). The present study was approved by the ethical committee of Aichi Cancer Center.

Measurement of alcohol exposure

Alcohol consumption of each type of beverage (Japanese sake, beer, shochu, whiskey and wine) was determined by the average number of drinks per day, which was then converted into a Japanese sake (rice wine) equivalent. One drink equates to one ``go'' (180 ml) of Japanese sake which contains 25g of ethanol, one large bottle (720 ml) of beer, two shots (57 ml) of whiskey and two and half-glasses of wine (200 ml). One drink of ``Shochu'' (distilled spirit) which contains 25% ethanol was rated as 108 ml. Total amount of alcohol consumption was estimated as summarized amount of pure alcohol consumption (g/drink) of Japanese sake, beer, shochu, whiskey and wine among current and former regular drinkers.

Genotyping of ALDH and ADH

DNA of each subject was extracted from the buffy coat fraction with BioRobot EZ1 and EZ1 DNA Blood 350μL Kits (Qiagen K.K., Tokyo, Japan). Genotyping was based upon duplex polymerase-chain-reaction with the confronting-two-pair-primer (PCR-CTPP) method (Tamakoshi et al., 2003). Briefly, four primers for the ADH2 polymorphism (F1ADH2: 5'-GGG CTT TAG ACT GAA TAA CCT TGG-3'; R1ADH2: 5'- AAC CAC GTG GTC ATC TGT GC-3'; F2ADH2: 5'- GGT GGC TGT AGG AAT CTG TCA-5'; R2ADH2: 5'- AGG GAA AGA GGA AAC TCC TGA A-3') and four primers for the ALDH2 polymorphism (F1ALDH2: 5'-TGC TAT GAT GTG TTT GGA GCC-3'; R1ALDH2: 5'-CCC ACA CTC ACA GTT TTC ACT TC-3'; F2ALDH2: 5'-GGG CTG CAG GCA TAC ACT A-3'; R2ALDH2: 5'-GGC TCC GAG CCA CCA-3') were mixed in a volume with 25 μL with 0.18mM dNTPs, 0.5 units of AmpliTaq Gold DNA polymerase (Perkin-Elmer Corp., Foster City, CA), and 2.5μL X 10 PCR buffer including 15mM MgCl2. The PCR conditions were as follows: 10 min of initial denaturation at 95 °C, followed by 40 cycles of 1 min at 95 °C, 1 min at 63 °C, and 1 min at 72 °C with 5 min of final extension at 72 °C. The results were confirmed with the PCR-restriction-fragment-length-polymorphism method using *Msl* I (New England BioLab, Inc., MA) for both polymorphisms. The CYP2E1 *Rsa*I genotype was also defined by the PCR-CTPP method using four primers (F1CYP2E1: 5' GCC AGT CGA GTC TAC ATT GTC AGT-3', F2CYP2E: 5' CCC TTC TTG GTT CAG GAG AGG, R1CYP2E1: 5' GTG CTG CAC CTA ACA CTG CAG-3', and R2CYP2E1: 5' CCA GCC AAA TCA CTT GTG GA-3').with 10 min of initial denaturation at 95 °C,

followed by 40 cycles of 1 min at 95 °C, 1 min at 58 °C, and 1 min at 72 °C with 5 min of final extension at 72 °C.

The quality of genotyping was routinely assessed statistically using the Hardy-Weinberg test. When allelic distributions for controls departed from the Hardy-Weinberg equilibrium, genotyping was assessed using another genotyping method. Identification of genotypes was accomplished with a double-blind check, and the results were loaded into the computer by two data researchers independently.

One control each failed to be genotyped for *ALDH2* and *ADH2*, and one case and one control for *CYP2E1*.

Statistical Analysis

Statistical analyses were performed using the STATA statistical package (version 8, STATA, College Station, TX). The ORs and their 95% confidence intervals (95%CIs) generated by conditional logistic regression models were used as measures of association for the risk of esophageal cancer. Three models, a) age-sex, b) age, sex, and smoking adjusted, and c) age, sex, alcohol and smoking adjusted, were examined. Alcohol exposure was categorized into three levels, non-drinker, moderate drinker, and heavy drinker. Heavy drinkers were defined as those drinking alcoholic beverages five or more days per week with an amount of 50g or more ethanol on each occasion and moderate drinkers were defined as all drinkers other than heavy drinkers. Smoking status was also divided into three categories considering cumulative exposure to tobacco: non-smokers, smokers with pack-years (PYs) equal to or less than 40, and smokers with PYs more than 40. Gene-environment interactions were evaluated as interaction terms in the model. Accordance with Hardy-Weinberg equilibrium (HWE) was checked for controls with the chi-square test to assess any discrepancies between genotype and allele frequencies.

Results

The present 165 esophageal cases had an average age of 61.4 years. Males accounted for 89.7%, and current drinkers and heavy drinkers comprised 87.9% and 57.0%, respectively, significantly higher than the 64.2% and 16.4% for the controls. Impact of alcohol drinking according to drinking level was significant. The ORs for esophageal cancer for moderate and heavy drinkers were 5.16 (95%CI: 2.33-11.4) and 27.8 (12.2-63.5), respectively. Smoking habits also differed between cases and controls, with 64.2% of cases being current smokers but only 33.3% of the controls. The ORs for moderate (PY<=40) and heavy (PY>40) smokers were 4.52 (2.32-8.80) and 5.05 (2.58-9.87), respectively (Table 1). Contrary to drinking, impact of heavy exposure to smoking was not remarkable compared with moderate exposure.

The frequencies for *ADH2*, *ALDH2* and *CYP2E1* genotypes and their ORs (95% CIs) for esophageal cancer risk are shown in Table 2. The frequencies of *ALDH2 Glu/Glu*, *Glu/Lys* and *Lys/Lys* were 51.4%, 39.5% and 9.1% among controls, and this was in accordance with the HWE (P=0.38). Harboring the 487Lys allele increased the risk of esophageal cancer. When compared with the *ALDH2 Glu/Glu* genotype, the ORs (95% CI) for *Glu/Lys* and *Lys/Lys* genotypes were 4.13 (2.73-6.24) and 0.17 (0.02-1.26) after adjustment for smoking. Marked difference between ORs with the crude/smoking adjusted models and the drinking and smoking adjusted model indicated the *ALDH2* genotype to define drinking capacity strongly. We therefore decided to exclude the drinking from regression model in further analyses. The ADH2 genotype frequencies were 61.5% for *His/His*, 34.0% for *Arg/His* and 4.5% for *Arg/Arg* among controls, which was also in accordance with the HWE (P=1.00). The *Arg/His* and *Arg/Arg* genotypes were associated with increased risk; smoking adjusted ORs were 2.10 (1.44-3.05) and 1.26 (0.49-3.26), respectively. Individuals harboring the *ADH2* 47His allele showed significantly increased the risk of esophageal cancer (OR=2.01, 1.39-2.90). The *CYP2E1* genotype frequencies were 62.4% for *c1/c1*, 34.8% for *c1/c2* and 4.3% for *c2/c2* among controls, which was also in accordance with the HWE (P=0.09). When compared with c1/c1 genotype, the *c1/c2* genotype showed a non-significantly decreased risk with an OR of 0.78 (0.52-1.15) after adjustment for smoking. This risk reduction was not observed with the c2/c2 genotype.

Data for the combined impact of *ADH2* and *ALDH2* polymorphisms are summarized in Table 3. When compared with commonest genotype, *ADH2 His/His* with ALDH2 Glu/

Table 1. Characteristicd of Cases and Controls

	Cases n=165	Controls n=495	OR (95%CI)
Age			
<50	11 (6.7%)	33 (6.7%)	-
50-64	88 (53.3%)	264 (53.3%)	-
>=65	66 (40.0%)	198 (40.0%)	
Mean age (SD)	61.4 (0.6)	61.4 (0.4)	
Sex			
Male	148 (89.7%)	444 (89.7%)	
Female	17 (10.3%)	51 (10.3%)	
Alcohol drinking ststus			
Never	8 (4.9%)	141 (28.5%)	1.00 (References)
Former	12 (7.3%)	36 (7.3%)	6.20 (2.34-16.4)
Current	145 (87.9%)	318 (64.2%)	9.44 (4.36-20.4)
Alcohol drinking dose			
Non-drinker	8 (4.9%)	141 (28.5%)	1.00 (References)
Moderate	63 (38.2%)	273 (55.2%)	5.16 (2.33-11.4)
Heavy drinker	94 (57.0%)	81 (16.4%)	27.8 (12.2-63.5)
Smoking status			
Never	16 (9.7%)	137 (27.7%)	1.00 (References)
Former	43 (26.1%)	193 (39.0%)	2.41 (1.21-4.83)
Current	106 (64.2%)	165 (33.3%)	6.97 (3.66-13.3)
Smoking dose			
Non-smoker	16 (9.7%)	137 (27.7%)	1.00 (References)
PY<=40	59 (35.8%)	144 (29.1%)	4.52 (2.32-8.80)
PY>40	90 (54.6%)	214 (43.2%)	5.05 (2.58-9.87)

* 1 Heavy drinker means >50g/drink for 5 drinks/wk; PY mean pack-years
* 1 ORs for smoking and drinking were adjusted for age and sex

Table 2. *ADH2, ALDH2* Genotype Frequencies and ORs for Esophageal Cancer

	Cases n=165	Controls n=495*2	OR1*1 (95%CI)	OR2*1 (95% CI)	OR3*1 (95% CI)
ALDH2					
Glu/Glu	38 (23.0 %)	254 (51.4%)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Glu/Lys	126 (76.4%)	195 (39.5%)	4.29 (2.85-6.46)	4.13 (2.73-6.24)	6.43 (4.02-10.3)
Lys/Lys	1 (0.6%)	45 (9.1%)	0.15 (0.02-1.14)	0.17 (0.02-1.26)	1.92 (0.23-15.7)
Glu/Lys+Lys/Lys	127 (77.0%)	240 (48.6%)	3.57 (2.38-5.35)	3.50 (2.32-5.27)	6.43 (4.00-10.3)
ADH2					
His/His	74 (44.9%)	304 (61.5%)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Arg/His	85 (51.5%)	168 (34.0%)	2.09 (1.45-3.02)	2.10 (1.44-3.05)	1.57 (1.04-2.36)
Arg/Arg	6 (3.6%)	22 (4.5%)	1.15 (0.45-2.92)	1.26 (0.49-3.26)	0.62 (0.22-1.72)
Arg/His+Arg/Arg	91 (55.1%)	190 (38.5%)	1.99 (1.39-2.85)	2.01 (1.39-2.90)	1.45 (0.97-2.16)
CYP2E1					
c1/c1	110 (67.1%)	308 (62.4%)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
c1/c2	47 (28.7%)	172 (34.8%)	0.77 (0.52-1.13)	0.78 (0.52-1.15)	0.69 (0.45-1.07)
c2/c2	7 (4.3%)	14 (2.8%)	1.41 (0.55-3.60)	1.37 (0.53-3.59)	1.11 (0.40-3.10)
c1/c2+c2/c2	54 (32.9%)	186 (37.7%)	0.81 (0.56-1.18)	0.82 (0.56-1.20)	0.73 (0.48-1.10)

*1 OR1was age-sex adjusted odds ratio; OR2 adjusted for age, sex and smoking; OR3 was adjusted for age, sex, smoking and drinking
*2 One control could not be genotyped for each polymorphism and was excluded from analysis.

Glu, subjects having the *ADH2* Arg allele with *ALDH2* Glu/Lys showed a markedly elevated risk of esophageal cancer (OR=6.94, 3.91-12.3). In addition, subjects with *ADH2 His/His* and ALDH2 *Glu/Lys*, showed increased risk (OR=3.22, 1.82-5.71). In contrast those with *ALDH2 Lys/Lys* regardless of ADH2 genotypes showed not significant but reduced risk.

Table 4 shows ORs (95% CI) for alcohol drinking according to the *ADH2* and *ALDH2* genotypes. Drinking increased esophageal cancer risk with all genotypes, but particularly among *ALDH2* heterozygotes. The ORs for moderate drinking and heavy drinking were 1.88 (95%CI, 0.42-8.37) and 4.62 (95%CI, 0.93-23.1) , respectively, for the ALDH2 Glu/Glu genotype, and 9.64 (3.23-28.8) and 95.4 (28.7-317) among individuals with ALDH2 Glu/Lys. In contrast, the risk with alcohol drinking was not strongly modified by the ADH2 or CYP2E1 genotypes.

Discussion

The present study demonstrated: 1) the *ALDH2* Glu487Lys polymorphism to be associated with esophageal cancer risk, heterozygotes for 487Lys being at greatest risk,

Table 3. Impact of Combination of *ADH2* and *ALDH2* Polymorphisms on Esophageal Cancer

	Case	Control	OR (95% CI)
ADH2 (*His/His*)+*ALDH2* (*Glu/Glu*)	20 (12.1%)	154 (31.2%)	1.00 (Reference)
ADH2 (*His/His*)+*ALDH2* (*Glu/Lys*)	54 (32.7%)	118 (23.9%)	3.22 (1.82-5.71)
ADH2 (*Arg/His and Arg/Arg*)+*ALDH2* (*Glu/Glu*)	18 (10.9%)	99 (20.1%)	1.35 (0.67-2.70)
ADH2 (*Args/His and Arg/Arg*)+*ALDH2* (*Glu/Lys*)	72 (43.6%)	77 (15.6%)	6.94 (3.91-12.3)
ADH2 (All)+*ALDH* (*Lys/Lys*)	1 (0.6%)	45 (9.1%)	0.19 (0.02-1.46)

* ORs were adjusted for age, sex and smoking
# Two controls were excluded from analyses because genotypes were not defined

Table 4. ORs (95%CI) for Alcohol Drinking According to the *ADH2, ALDH2* and *CYP2E1* Polymorphisms

	Non-drinker	Moderate drinker	Heavy drinker
ALDH2			
Glu/Glu	1.00 (Reference)	1.88 (0.42-8.37)	4.62 (0.93-23.1)
Glu/Lys	1.00 (Reference)	9.64 (3.23-28.8)	95.4 (28.7-317)
P for interaction		0.03	<0.01
ADH2			
His/His	1.00 (Reference)	5.03 (1.67-15.1)	25.8 (8.01-83.3)
His/Arg+Arg/Arg	1.00 (Reference)	8.50 (1.90-38.0)	33.9 (7.34-157)
P for interaction		0.24	0.32
CYP2E1			
c1/c1	1.00 (Reference)	8.17 (2.72-24.5)	37.0 (11.8-117)
c1/c2+c2/c2	1.00 (Reference)	3.26 (0.88-12.2)	18.6 (4.68-74.1)
P for interaction		0.4	0.54

* OR was adjusted for smoking

while homozygotes could be protected; 2) the *ADH2* Arg47His polymorphism might modify esophageal cancer susceptibility, but has an impact only when combined with the *ALDH2* heterozygous state; 3) the *ALDH2* polymorphism modifies the risk due to alcohol drinking, indicating a gene-environment interaction; and 4) *CYP2E1* polymorphism has very limited impact on esophageal cancer risk.

Previous studies showed that the *ALDH2* 487Lys allele encoding an inactive subunit of *ALDH2* is prevalent in Asians (Higuchi et al., 1995), leading to excessive acetaldehyde accumulation on drinking (Enomoto et al., 1991;Mizoi et al., 1994). In individuals with inactive ALDH2 encoded by *ALDH2 Glu/Lys*, the blood acetaldehyde level after drinking is 6-fold the concentration in those with active ALDH2 (Mizoi et al., 1994), increased salivary acetaldehyde levels also being noted (Vakevainen et al., 2000). In light of the consistent evidence for acetaldehyde induced carcinogenesis (Feron et al., 1982;Woutersen et al., 1986;Dellarco, 1988;Morimoto and Takeshita, 1996;Yokoyama et al., 1996;Fang and Vaca, 1997;Homann et al., 1997;Ikehara et al., 2001), our data suggest that this metabolite of alcohol is a strong causative substance for esophageal cancer.

The biological impact of polymorphisms of the *ADH2* gene encoding theenzyme responsible for metabolism of ethanol to acetaldehyde is therefore of great interest (Yokoyama et al., 1999), although there has been one report that it may have little impact on the blood concentration of acetaldehyde after drinking (Mizoi et al., 1994). Our results for the combination of *ALDH2* and *ADH2* polymorphisms in this study might support the hypothesis that the concentration of acetaldehyde after drinking is determined mainly by *ALDH2* enzyme activity. We observed a difference in esophageal risk by the *ADH2* genotype only with those harboring the *ALDH2 Glu/Lys* genotype: the OR for high *ADH2* activity (*His/His*) with reduced *ALDH2* activity (*Glu/Lys*) relative to high *ADH2* acitivity and high ALDH2 acitivity was 3.22 while the OR for low ADH2 activity (*Arg/His* and *Arg/Arg*) with reduced ALDH2 activity (*Glu/Lys*) was 6.94. These data are in line with a former study (Yokoyama et al., 2002). The difference suggests that *ADH2* polymorphism, leading to greater production of acetaldehyde, has an impact on esophageal carcinogenesis when ALDH2 activity is reduced. However, we did not find a statistically significant gene-gene interaction between the *ALDH2* and *ADH2* polymorphisms, so that further studies appear warranted.

CYP2E1 is primarily responsible for the metabolic activation of many low weight carcinogens (Yang et al., 1990), including nitrosamines, which may be involved in carcinogenesis in the esophagus and the enzyme is also believed to participate in the oxidation of alcohol(Albano et al., 1991). The variant c2 allele appears to be associated with decreased enzyme activity and therefore has been hypothesized to decrease risk of cancer (Marchand et al., 1999). Two studies in China in fact showed that harboring the c2 allele significantly decreased esophageal cancer risk (Lin et al., 1998;Tan et al., 2000), but another in China and two previous studies in Japan, as well as the present study, failed to find any association (Hori et al., 1997;Morita et al., 1997;Gao et al., 2002). One possible explanation for the anomalous results might be varying importance of different factors in Chinese and Japanese populations. Where exposure to carcinogens is high, free radicals generated during the oxidation of alcohol may have limited impact on cancer susceptibility. In Japan, alcohol drinking is a critical risk factor for esophageal cancer(Takezaki et al., 2000) and *ALDH2*/*ADH2* gene polymorphisms appear to play more important roles to esophageal cancer risk. The situation is different from that in high risk areas of esophageal cancer in China where environmental carcinogens make a major contribution (Yang, 1980).

Potential limitations of this study should be considered. One methodological issue is selection of the base population for controls. We applied non-cancer patients at the ACCH for this purpose because it is reasonable to assume our cases arise within this population base. The situation is different from precedent case-control studies applying controls from different study bases and excluding all alcoholic cases (Yokoyama et al., 2002). A notable point of our control population is its similarity to the general population in terms of exposures of interest, here smoking and drinking (Inoue et al., 1997). Medical background of controls is another potential source of bias; however, our previous study demonstrated a limited impact in females (Hamajima et al., 1995) and a similar situation would be expected for males. Aichi Cancer Center differs from cancer institutes in other developed countries, where people visit local general clinics first, and are then referred to hospitals which function as secondary and/or specific facilities for further medical treatment. We therefore conclude that it is feasible to use non-cancer outpatients as referents in HERPACC type epidemiological studies. In addition, the present study was free of response information bias to the questionnaire because all data were collected prior to diagnoses.

In conclusion, the present study confirmed a significant gene-environment interaction between alcohol drinking and the *ALDH2* Glu487Lys polymorphism. Gene-gene interactions between *ALDH2* and *ADH2* polymorphisms were also suggested but need further clarification.

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