RESEARCH COMMUNICATION

Alcohol Dehydrogenase-2 and Aldehyde Dehydrogenase-2 Genotypes, Alcohol Drinking and the Risk of Primary Hepatocellular Carcinoma in a Chinese Population

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Abstract

Objective: To investigate the relationship of alcohol dehydrogenase-2 (ADH2) and aldehyde dehydrogenase-2 (ALDH2) genotypes as well as alcohol drinking to the susceptibility of primary hepatocellular carcinoma (HCC). Methods: A case-control study including 208 cases of HCC and 208 controls matched with sex, age and residential area was carried out in Taixing city of Jiangsu province, China. Blood samples were collected and tested for ADH2 and ALDH2 genotypes by PCR-RFLP method. Results: There were no significant differences in the frequency of either ADH2 or ALDH2 genotypes between cases and controls. Compared with no-drinkers possessing ALDH21*1 genotypes, drinkers with ALDH21*2 or ALDH22*2 genotypes and cumulative amount of alcohol consumption >3 (Kg * years) were at a significantly higher risk of developing HCC (OR=3.30, 95%CI: 1.24-8.83). In contrast, there was no significant difference in cancer risk between no-drinkers with ADH21*1 and drinkers with ADH2 1*2 or ADH22*2 genotypes. A dose-dependent positive result was found (P=0.044) between cumulative amount of alcohol consumption and the risk of HCC in individuals carrying ALDH21*2 or ALDH22*2 genotypes. Drinkers with cumulative amount of alcohol consumption >3 (Kg * years) who possessed both inactive ALDH2 (ALDH21*2 or ALDH22*2) and inactive ADH (ADH21*2 or ADH22*2) genotypes were not at a significantly higher risk of HCC (adjusted OR=4.26, 95%CI: 0.63-29.08) compared to no-drinkers possessing ADH21*1 and ALDH21*1 genotypes. Compared with individuals possessing ALDH21*1, with negative HBsAg and cumulative amount of alcohol consumption ≤3 (Kg * years), those with ALDH21*2 or ALDH22*2, positive HBsAg, and cumulative amount of alcohol consumption >3 (Kg * years) had a significantly higher risk of HCC (OR=49.71, 95%CI: 5.51–448.96). Conclusion: These results revealed that it was not ADH2 but ALDH2 polymorphisms that had a significant interaction with heavy alcohol consumption in the development of HCC. This result suggests that to help lower their risk for HCC , persons with ALDH21*2 or ALDH22*2 genotypes should be encouraged to reduce their consumption of alcoholic beverages.

Key Words: Alcohol - consumption - metabolism - HCC - China

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Introduction

Many epidemiological studies have consistently shown heavy alcohol drinking as a risk factor for hepatocellular carcinoma (HCC) (Munaka et al., 2003). The fact that only a small number of heavy drinkers develop HCC raises concerns about genetic susceptibility. Since cancer results from interactions between environmental and genetic factors, study of genetic variation in alcohol metabolic enzymes is importnat. Ethanol is eliminated from the body by oxidation to acetaldehyde and then to acetate, catalyzed by alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH), respectively (Bosron and Li, 1986). The oxidative metabolite of ethanol, acetaldehyde, is recognized to be carcinogenic for animals and is suspected to have similar effects in man (Matsuo et al., 2001). Aldehydes have the potential to bind to cellular proteins and DNA, thus leading to carcinogenic effects (Seitz and Oneta, 1998). The level of *in vivo* aldehyde is not only related to the quantity and frequency of alcohol consumption, but also to the activity of ADH and ALDH in the body. Both ADH and ALDH have multiple subtypes, among with ADH2 and ALDH2, which are polymorphic. Aldehyde dehydrogrnase-2 (ALDH2) generates acetic acid from acetaldehyde (Bosron and Li, 1986). People homozygous for the ALDH2*2 allele (ALDH2*2/2) do not have any ALDH activity. Even those heterozygous for the normal and variant alleles (ALDH2*1/2) show only 1/16 of the activity in homozygotes of ALDH2*1 (Enomoto et al., 1991). Like ALDH2, ADH2 is

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polymorphic, and its variant ADH2*2 allele is highly prevalent among East Asians. The β2β2 isozyme encoded by ADH2*2 has a 40-fold higher Vmax than that of the β1β1 isozyme, which is encoded by ADH2*1 (Bosron and Li, 1986). Under predicted physiologic conditions, β2β2 enzymes oxidize ethanol 20-fold faster than doβ1β1 enzymes (Bosron et al., 1988). Therefore, shortly after alcohol drinking, individuals carrying both variant ADH2 and ALDH2 would accumulate a large amount of aldehyde that cannot be efficiently oxidized to the non-toxic acetic acid. The different combinations of genotypes of ADH2 and ALDH2 are possible to influence the individual susceptibility to organ damage and/or cancer.

At present, studies of various drinking populations have only associated ADH2 and ALDH2 polymorphisms with oropharyngolaryngeal and esophageal cancers, but not with HCC (Chao et al., 2000; Takeshita et al., 2000; Yokoyama et al., 2001; 2002). Taixing City, located in the middle part of Jiangsu Province, has relatively high incidence and mortality rates for HCC, not only compared with other regions in the province, but also in China as a whole. In the 1990s, the age-adjusted mortality rates (per 100,000) for HCC in Taixing City, in Jiangsu Province and in China were 67.9, 36.8, and 20.4, respectively. Oyb previous study has shown that more than 40% of adult residents in Taixing drink wines and drinking is a risk factor for HCC in this area (Ding et al., 2001). To define the individual and combined roles of ADH2, ALDH2 polymorphisms and drinking habits in the risk for HCC development, we conducted a case-control study in this high-incidence area.

Table 1. Background Information for HCC cases and Controls

	Males			Female		
	No. cases	No. controls	P value	No. cases	No. controls	P value
Age						
20-39	24	27		9	9	
40-49	51	48		5	6	
50-59	51	48		11	10	
60-69	27	30		8	8	
≥70	8	9		5	5	
Total	170	170	0.961	38	38	0.100
Occupation[a]						
Farmer	118	116		32	34	
Worker	22	18		3	1	
Others	29	34		3	3	
Total	169	168	0.667	38	38	0.588
Education[b]						
None	16	11		16	14	
Primary	61	49		11	10	
Junior#	68	82		7	13	
Senior#	19	25		4	1	
College	5	2		0	0	
Total	169	169	0.227	38	38	0.588
Income/month[c]						
≤120 yuan	90	76		25	24	
>120 yuan	75	90		13	13	
Total	165	166	0.11	38	37	0.931

a.Information was not obtained for two male controls, and one male case with HCC b.Information was not obtained for one male control, and one male case with HCC #Middle school c.Information was not obtained for four male and one female controls, and five male cases with HCC

Materials and Methods

Study subjects

We recruited 208 patients aged 22-79 years who were first diagnosed as having hepatocellular carcinoma from September 1998 to August 2002. Population-based controls were recruited from healthy residents in the villages or towns where cases resided. Doctors of the local public health center visited the local governmental office and randomly selected one control for each case, using the lists of residents after matching for sex, ethnicity, and age within 2 years of each case. All study subjects have completed a questionnaire administrated by a trained interviewer, covering residential, occupational, social, lifestyle, psychological and economic factors. The interviewer then collected the blood samples of subjects from a peripheral vein after obtaining their oral informed consents. The collected blood samples were shipped to the public health center within a day. Buffy coat was then separated and stored at -30˚C.

The items on drinking habits include frequency, types of alcohol (beers, hard liquors, medium liquors, and fruit liquors) and total amount of alcohol consumed. We defined a drinker as a person who drinks hard liquors at least once per week and continuously drinks for at least half a year. Cumulative amounts of alcohol consumption were expressed as “kilogram (Kg)*year”. One “kg*year” was defined as drinking hard liquor one kg per day and continuously drinking for one year. No patients refused to participate, and the response rates were 100% for cases and >90% for controls.

DNA extraction and genotyping of ADH2 and ALDH2

Whole blood was collected into EDTA- coated tubes and centrifuged for 15min at 3,000 rec/min. The buffy layer was isolated and shipped to the Cancer Research Institute of Jiangsu Province. Genomic DNA was extracted from 200μL of buffy coat using Qiagen QIAamp DNA Blood Mini kit (Qiagen Inc., Valencia, CA). Genotyping of ADH2 (Groppi et al., 1990) and ALDH2 (Chao et al., 1997) was performed by PCR-RFLP. Briefly, primers 5’- ATTCTGTAGATGG-TGGCTGT-3’ and 5’-GAAGGGGGG-TCACCAGGTTG-3’ were used for amplification of the ADH2 genes. Primers 5’-CCCTTTGGTGGCTAGAA-GATG-3’ and 5’-CCACACTCACAG-TTTTCTCTT-3’ were used for amplification of the ALDH2 genes. In a total volume of 25μL PCR mixture containing 100-200ng genomic DNA, 1μmol/L each primer, 0.25mmol/L of each dNTP, 0.25 mmol/L $MgCl_2$, and 1U of Taq DNA polymerase (Takara). Thirty-five cycles of PCR were performed in a thermocycler. For ADH2 each cycle consisted of 30s at 95˚C, 30s at 62˚C, 30s at 72˚C, and for ALDH2 each cycle consisted of 1 min at 95˚C, 2 min at 58˚C, 1 min at 72˚C.

The PCR products were digested with MaeIII(Roche)

Table 2. Odds Ratios (ORs) and 95% Confidence Intervals (CIs) for HCCs according to ADH2 and ALDH2 Polymorphisms

Genotype	Cases (%) (n=208)	Controls (%) (n=207)	OR (95%CI)
ALDH21*1	120 (57.7)	133 (64.3)	1.00
ALDH21*2	64 (30.8)	59 (28.5)	1.20 (0.76-1.89)*
ALDH22*2	24 (11.5)	15 (7.25)	1.77 (0.85-3.74)**
ALDH21*2+			
ALDH22*2	88 (42.3)	74 (35.8)	1.32 (0.87-2.00)***
ADH21*1	21 (10.1)	26 (12.6)	1.00
ADH21*2	132 (63.8)	97 (46.9)	1.68 (0.86-3.32)#
ADH22*2	54 (26.1)	84 (40.6)	0.80 (0.39-1.64)##
ADH21*2+			
ADH22*2	186 (89.9)	181 (87.4)	1.27 (0.66-2.45)###

*x^2=0.7, P=0.403; **x^2M-H=2.68, P=0.102; ***x^2M-H=1.61, P=0.204 #x^2M-H=2.64, P=0.10; ##x^2M-H=0.45, P=0.50; ###x^2M-H=0.60, P=0.44

or Mbo II (Takara) and then separated on 15% polyacrylamide gel. The gels were stained with ethidium bromide and visualized under UV light. Genotypes were determined without knowledge of the subjects' statuses. Among 416 examined samples, visualized PCR products could not be obtained for 1 control and 1 case for ADH2 and 1 control for ALDH2.

Statistical Analysis

All analyses were performed with the SAS (version 6.02) and Epi-info (version 6.04) statistical package. Odds ratios and 95% confidence intervals were adjusted by multiple logistic regression analysis. The Mantel-Haenszel x^2 method was used to test for significant associations between the ADH2 or ALDH2 genotype and cancer risk.

Results

The age distributions of controls and cases were similar, with mean values of 52 years. Most of study subjects were farmers. Occupation, education, and income status were also similarly distributed in cases and controls (Table 1).

The positive rates of serum HBsAg in cases (72.1%) were higher than those in controls (22.2%) (OR=9.05, 95%CI:5.66-14.5, P<0.001) (data not shown).The frequencies of ALDH2 and ADH2 genotypes demonstrated no significant differences between cases and controls (Table 2). The allelic distribution of ADH2 and ALDH2 polymorphism was in Hardy-Weinberg equilibrium (P>0.05).

Table 3 shows that drinkers with ALDH21*2 or ALDH22*2 genotypes and cumulative amount of alcohol consumption >3 (Kg * year) were at a significantly higher risk of developing PHC compared with no-drinkers carrying ALDH21*1 genotype (OR=3.30, 95%CI: 1.24-8.83). When compared with those with different ADH2 genotypes, no significant difference was found (OR=1.46, 95%CI: 0.39-5.47).

Furthermore, we found a dose-dependent positive result (P=0.044) between cumulative amounts of alcohol consumption and the risk of HCC in individuals with ALDH21*2 or ALDH22*2 genotypes (Table 4).

Compared with those no-drinkers with ADH21*1 and ALDH21*1 genotypes, drinkers with ALDH21*2 or

Table 3. Odds Ratios (ORs) and 95% Confidence Intervals (CIs) for HCCs According to the Combination of Selected Drinking Status, and ADH2 and ALDH2 Polymorphisms

Drinking	Genotypes	Cases	Controls	OR# (95%CI)
never	ALDH21*1	47	59	1.00
	ALDH21*2+			
	ALDH22*2	51	47	1.44 (0.74-2.81)
drinking	ALDH21*1	73	74	1.12 (0.62-2.02)
	ALDH21*2+			
	ALDH22*2	37	27	2.02 (0.97-4.20)
cumulative amounts of alcohol consumption (Kg*year)				
never	ALDH21*1	47	59	1.00
≤3	ALDH21*1	35	42	0.89 (0.45-1.76)
>3	ALDH21*1	38	32	1.20 (0.85-1.71)
never	ALDH21*2+			
	ALDH22*2	51	47	1.44 (0.74-2.81)
≤3	ALDH21*2+			
	ALDH22*2	16	18	1.37 (0.56-3.36)
>3	ALDH21*2+			
	ALDH22*2	21	9	3.30 (1.24-8.83)
never	ADH21*1	7	9	1.00
	ADH21*2+			
	ADH22*2	91	97	0.85 (0.25-2.87)
drinking	ADH21*1	14	17	0.80 (0.17-3.78)
	ADH21*2+			
	ADH22*2	95	84	0.99 (0.30-3.35)
cumulative amounts of alcohol consumption (Kg * years)				
never	ADH21*1	7	9	1.00
≤3	ADH21*1	7	10	0.54 (0.09-3.51)
>3	ADH21*1	7	7	1.10 (0.38-3.19)
never	ADH21*2+			
	ADH22*2	91	97	0.85 (0.25-2.87)
≤3	ADH21*2+			
	ADH22*2	43	48	0.65 (0.18-2.45)
>3	ADH21*2+			
	ADH22*2	52	36	1.46 (0.39-5.47)

#adjusted for age, sex, HBsAg, hepatocirrhosis, and schistosomiasis.

Table 4. Odds ratios (ORs) for HCC According to Combination of ALDH2 Genotypes and Cumulative Amount of Alcohol Drinking (Kg * years)

Amount of alcohol drinking (Kg * years)	ALDH/ALDH (ALDH21*2+ALDH22*2)			ALDH (ALDH21*1)		
	cases	controls	OR(95%CI)	cases	controls	OR(95%CI)
≤ 1	57	57	1.00	62	75	1.00
≤ 3	10	8	1.25(0.42-3.79)	20	26	0.93(0.45-1.92)
≤ 5	7	4	1.75(0.42-8.57)	17	9	2.28(0.88-6.01)
> 5	14	5	2.80(0.87-10.53)*	21	23	1.10(0.53-2.30)#

*χ2 trend=4.056; P=0.044; # χ2 trend=0.742, P=0.389

Table 5. Odds ratios (ORs) for HCC According to Combination of ADH2, ALDH2 Genotypes and Cumulative Amount of Alcohol Drinking (Kg * years) in Alcohol Drinkers

ALDH2*	ADH2**	drinking***	cases	controls	OR# (95%CI)
-	-	-	5	6	1.00
-	-	+	6	6	1.41 (0.20-9.70)
+	+	-	30	35	0.60 (0.12-3.00)
+	-	-	2	4	0.32 (0.02-6.11)
-	+	+	32	27	1.11 (0.24-5.20)
+	+	-	13	13	1.49 (0.28-8.02)
-	-	+	1	1	0.55 (0.02-19.8)
+	+	+	20	9	4.26 (0.63-29.1)

*-: ALDH21*1, +: ALDH21*2+ALDH22*2, **-: ADH21*1, +: ADH21*2+ADH22*2, *** drinking: - ≤3 Kg * years, + >3Kg * years.#adjusted for age, sex, HBsAg, hepatocirrhosis and schistosomiasis

Table 6. Odds ratios (ORs) for HCC According to Combination of ALDH2 Genotypes, HBsAg and Cumulative Amount of Alcohol drinking (Kg * years) in Alcohol Drinkers

ALDH2*	HBsAg	drinking**	case	control	OR# (95%CI)
-	-	-	9	29	1.00
-	-	+	9	25	1.15 (0.38-3.51)
+	-	-	4	14	1.18 (0.30-4.73)
+	-	+	7	8	2.86 (0.79-10.4)
-	+	-	26	13	7.33 (2.52-21.4)
+	+	-	12	4	12.43 (3.06-50.4)
-	+	+	29	7	15.39 (4.76-49.8)
+	+	+	14	1	49.71 (5.51-449.)

* -: ALDH21*1, +: ALDH21*2+ALDH22*2, ** drinking: - ≤3 Kg * years, + >3Kg * years.x^2 trend= 52.78, P=0.000 #adjusted for age, sex, HBsAg , hepatocirrhosis and schistosomiasis.

ALDH22*2 genotypes and ADH21*2 or ADH22*2 genotypes and cumulative amount of alcohol consumption >3 (Kg * years) were at an increased risk of developing HCC (adjusted OR=4.26, 95%CI: 0.63-29.08), although this observation did not reach statistical significance (Table 5).

Finally, we examined the relationship among the ALDH2 genotype, HBsAg and HCC according to cumulative amount of alcohol drinking (Kg * years) in alcohol drinkers. As shown in table 6, the positiveness of HBsAg independently increases the risk of HCC significantly in alcohol drinkers, regardless of their ALDH2 genotypes. On the contrary, alcohol consumption showed some but not enough statistically significant relationship with HCC when considered as an independent factor. However, cooperative relationships between the two factors were found. In alcohol drinkers with ALDH21*1 genotype, the positiveness of HBsAg and cumulative amounts of alcohol consumption >3Kg * years were at a significantly higher risk than HBsAg negatives whose cumulative amounts of alcohol consumption ≤3 Kg * years (OR=15.39, 95%CI: 4.76-49.77). Furthermore, Compared with individuals possessing ALDH21*1, with negative HBsAg and cumulative amount of alcohol consumption ≤3 (Kg * years), those with ALDH21*2 or ALDH22*2, positive HBsAg and cumulative amount of alcohol consumption >3 (Kg * years) had a significantly higher risk of HCC (OR=49.71, 95%CI: 5.51–448.96).

Discussion

The correlation between habitual alcohol drinking and the risk of HCC has been observed in previous studies. However, many of these studies did not support a contribution of acetaldehyde, an active metabolite of ethanol, to HCC development, but indicated a direct involvement of ethanol in hepatocancinogenesis (Yokoyama et al., 1998; Takeshita et al., 2000).

It has been generally accepted that both environmental exposures/lifestyle and genetic makeup influence the genetic susceptibility to cancer. In studies of cancer risk from alcohol exposure, it is necessary to investigate alcohol metabolism, which is mainly decided by the genetic variations of ADH and ALDH. Up to this date there are only two papers addressing the relationship between ADH2 and ALDH2 genetic polymorphisms and HCC susceptibility (Takeshita et al., 2000; Zhang et al., 2002). In both studies, no association of ADH2/ALDH2 genotypes with HCC development was found. However, neither of the studies reported the combined effects of alcohol metabolic enzyme polymorphisms and drinking habits.

In addition, cancer risks related to ADH2 and ALDH2 genotypes should be adjusted by alcohol consumption (Yokoyama et al., 2001). In this study, no association between ADH2 genotype and HCC was found after adjusted by drinking habits. We also found that the risk of HCC from alcohol consumption could be modified by both ALDH2 genotype and the amount of alcohol consumption, but neither of these factors alone. As shown in our results, for drinkers with ALDH21*2 or ALDH22*2 genotypes, only those with >3 (Kg*years) cumulative alcohol consumption had a dose-dependent elevated risk for PHC, compared to no-drinkers carrying ALDH21*1 genotypes. Previous studies have shown that it is not the ADH2 genotypes but the ALDH2 genotypes that determined an individual's peak blood acetaldehyde concentration (Crabb et al., 1989, Yoshihara et al., 2000). ALDH2 generates acetic acid from acetaldehyde metabolism and the ALDH2 variant genotypes lack this enzyme activity. Therefore, long durations and large amounts of alcohol consumption will increase the concentration of acetaldehyde in the body. Some studies found that the plasma concentration of acetaldehyde in drinkers with ALDH2 variant genotype is six folds higher than in those with wild genotype (Mizoi et al., 1994). The accumulation of acetaldehyde in the body could increase the risk of hepatocarcinogenesis when the concentration reaches a certain level.

Hepatitis B virus (HBV) plays an important role in the etiology of HCC in China. Lin and Cheng observed that there was a sound geographical correlation between the prevalence of the mutant ALDH2*2 alleles and HBV infections (2002). Populations with a high ALDH2*2 prevalence were from HBV-endemic areas. Furthermore, they found that HBV and alcohol drinking exhibited a synergistic effect upon liver cirrhosis and cancer. In the

present study, HBsAg significantly increased the risk for HCC in drinkers, regardless of their genotypes. Also, compared with HBsAg negative individuals with ALDH21*1 genotype and a few amounts of alcohol consumption, HBsAg positive individuals possessing ALDH2 variant genotypes and large amounts of alcohol consumption were at a tremendously higher risk of HCC (OR=49.7). The results of this study may help to screen the high-risk group for developing HCC. Education and intervention targeting high risk individuals who are HBsAg positive and possess ALDH2 variant alleles is vitally important in a new strategic approach aimed at preventing HCC.

It is generally thought that acetaldehyde accumulation in the blood causes uncomfortable symptoms including facial flushing, palpitation and head-ache after small amounts of alcohol being consumed; therefore further alcohol consumption is limited. These symptoms might be related to certain combinations of ADH2 and ALDH2 genotypes. Those with both variant ADH2 and ALDH2 alleles could generate a greater amount of acetyaldehyde but lack the ability to further oxidize the aldehyde to the non-toxic acetic acid. However, alcohol drinkers in China tend to drink more, even with uncomfortable symptoms, on some special occasions. Our results show that drinkers with ALDH21*2 or ALDH22*2 genotypes and ADH21*2 or ADH22*2 genotypes as well as cumulative amount of alcohol consumption >3 (Kg * years) were at an increased risk of developing PHC compared with those no-drinkers with ADH21*1 and ALDH21*1 genotypes. However, this observation did not reach statistical significance, and it needs to be further investigated.

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