by a polymerase chain reaction (PCR) with confronting two-pair primers (PCR-CTPP) according to Ito et al[22] Genomic DNA (10-150 ng) was amplified in a volume of 25 μL with 0.18mM dNTPs, 12.5 pmol of each primer, 0.5 units of AmpliTaq Gold (Perkin-Elmer Corp., Foster City, CA), and 2.5 μL of 10×PCR buffer including 15 mM $MgCl_2$. The following 4 primers were used for each reaction: F1 (5'-CAGCCCAGACCCAGTG-GACTC-3'), R1 (5'-TGGCTCCTGAGCATGGCGGG-3'), F2 (5'-CAGTGCCGACCTGCGCCAATG-3'), and R2 (5'-GGTAGT-CACAGGGAGGCCCC-3'). PCR was conducted as follows: a 10 minute initial denature at 95℃, 30 cycles for 1 minute at 95℃, 1 minute at 64℃, and 1 minute at 72℃, and a 5 minute final extension at 72℃. PCR products were subjected to electrophoresis in 2% agarose gels and were visualized with ethidium bromide staining. The primer pair F1 and R1 produced the C allele (Ser326) band (252 bp), while F2 and R2 produced the G allele (326Cys) band (194 bp) (Figure 1). To validate the results, 10% were randomly selected for genotyping by using a PCR-restriction fragment length polymorphism analysis,[16] and the results were 100% concordant.

Statistical Analysis

Chi square tests were used for unadjusted comparisons based on frequency. The Wilcoxon's rank sum test was conducted to compare the distribution of age. Unconditional logistic regression models were used to estimate crude and adjusted odds ratios (ORs) of HCC and their 95 percent confidence intervals (CIs) for *hOGG1* genotypes by using dummy variables, with adjustment for potential confounders including sex, age category (40-49, 50-59, 60-69, and 70-79 years), heavy drinking history, smoking status (never, former, and current smokers), and HBsAg and HCVAb status. The gene-environment interaction on HCC risk was evaluated by including in the model the product terms of variables of interest (i.e., *hOGG1* genotype and heavy drinking history/smoking status), as well as main effects and covariates. Likelihood ratio tests were used to examine the overall statistical significance of a set of interaction terms. Tests of linear trend for *hOGG1* genotypes were performed by assigning an ordinal variable to the genotypes in the logistic model. All statistical analyses were performed with the SAS®/PC statistical package (SAS Institute Inc., Cary, NC).

RESULTS

Selected characteristics of study subjects are shown in Table 1. As compared with hospital controls, HCC cases showed significantly higher prevalences of older subjects ($p < 0.01$), HBsAg positives ($p < 0.01$), HCVAb positives ($p < 0.01$), males with heavy drinking history ($p < 0.01$), and male current smokers ($p = 0.03$). As compared to CLD patients, HCC cases revealed significantly greater proportions of males ($p < 0.01$), older subjects ($p < 0.01$), and males with heavy drinking history ($p < 0.01$).

The frequency of *hOGG1* genotype showed no significant difference between HCC cases and either control group ($p = 0.10$ or 0.23) (Table 1). The genotype distributions for hospital controls and CLD patients were in Hardy-Weinberg equilibrium ($p = 0.08$ and 0.14, respectively). After adjustment for sex, age, heavy drinking history, smoking, HBsAg, and HCVAb, the ORs (and 95% CIs) for the Ser/Cys, Cys/Cys, and Ser/Cys+Cys/Cys genotypes relative to the Ser/Ser genotype were estimated at 0.79 (0.35-1.79), 0.48 (0.18-1.27), and 0.68 (0.31-1.46) against hospital controls respectively,, and at 1.51 (0.96-3.37), 0.86 (0.50-1.47), and 1.25 (0.82-1.91),against CLD patients respectively.

Table 2 shows the adjusted ORs of HCC for *hOGG1* genotypes according to heavy drinking history and current smoking status. We could not detect any significant linear trend for the genotypes in any stratum. Among those without heavy drinking history, a significant risk excess for the Ser/Cys vs. Ser/Ser genotype (fully-adjusted OR = 1.82, 95% CI: 1.10-3.01) was observed between HCC cases and CLD patients, yet the risk for the Cys/Cys vs. Ser/Ser genotype was not elevated (OR = 0.90, 95% CI: 0.50-1.64). A similar tendency was observed among those without current smoking. In comparison of HCC cases with hospital controls, additional adjustment for HBsAg and HCVAb substantially altered the OR on some occasions (e.g., OR for Cys/Cys among current smokers), but with a very wide CI. No significant interaction was found between the *hOGG1* genotype and either heavy drinking history or current smoking. Although we conducted corresponding analyses based on daily amount of alcohol drinking and pack-years of smoking, the results were essentially identical (data not shown).

DISCUSSION

In the present study, we could not find any significant association between *hOGG1* Ser326Cys polymorphism and overall HCC risk. In subgroup analyses according to drinking and smoking habits, there was some risk increase for the Ser/Cys vs. Ser/Ser genotype, yet such a finding might be due to chance variation in the light of the absence of risk increase for the Cys/Cys vs. Ser/Ser genotype. In addition, no significant gene-alcohol or gene-smoking interaction was evident.

Chronic inflammation caused by hepatotropic viruses and exposure to alcohol and tobacco stimulate hepatic ROS generation,[7-9] and some reports also have demonstrated that both HCV and HBV infections could induce ROS without inflammation.[23,24] Interestingly, a recent clinical study reported that reducing iron, one of the sources of ROS generation, by phlebotomy and low iron diet decreased hepatic levels of 8-OHdG and eventually the risk of HCC development in patients with chronic hepatitis C after 6 years of follow-up.[25] These reports suggest an important role of oxidative stress in hepatocarcinogenesis.

hOGG1, which acts in the DNA base excision repair pathway, excises 8-OHdG resulting from oxidative stress. The Ser326Cys polymorphism in *hOGG1* may alter glycosylase function, and some studies showed that hOGG1 protein encoded by the 326Cys

Table 1. Selected characteristics of study subjects.

Factor	HCC cases n(%)	Hospital controls n(%)	CLD patients n(%)	P*	P†
Sex					
Male	141 (67.5)	180 (65.5)	205 (53.8)		
Female	68 (32.5)	95 (34.5)	176 (46.2)	0.64	<0.01
Age (year)					
40-49	6 (2.9)	42 (15.3)	73 (19.2)		
50-59	28 (13.4)	85 (30.9)	93 (24.4)		
60-69	76 (36.4)	86 (31.3)	136 (35.7)		
70-79	99 (47.4)	62 (22.6)	79 (9.2)	<0.01	<0.01
Median	69 yr	61 yr	61 yr	<0.01	<0.01
HBsAg					
Negative	190 (90.9)	269 (97.8)	346 (90.8)		
Positive	19 (9.1)	6 (2.2)	35 (9.2)	<0.01	0.97
HCVAb					
Negative	30 (14.4)	254 (92.4)	54 (14.2)		
Positive	179 (85.7)	21 (7.6)	327 (85.8)	<0.01	0.95
Heavy drinking history (male)					
No	95 (67.4)	158 (87.8)	170 (82.9)		
Yes	46 (32.6)	22 (12.2)	35 (17.1)	<0.01	<0.01
Heavy drinking history (female)					
No	65 (95.6)	94 (99.0)	172 (97.7)		
Yes	3 (4.4)	1 (1.1)	4 (2.3)	0.17	0.37
Smoking status (male)					
Never	24 (17.0)	50 (27.8)	54 (26.3)		
Former	51 (36.2)	67 (37.2)	76 (37.1)		
Current	66 (46.8)	63 (35.0)	75 (36.7)	0.03	0.07
Smoking status (female)					
Never	61 (89.7)	88 (92.6)	150 (85.2)		
Former	4 (5.9)	3 (3.2)	15 (8.5)		
Current	3 (4.4)	4 (4.2)	11 (6.3)	0.70	0.66
hOGG1 genotype					
Ser/Ser	56 (26.8)	73 (26.5)	105 (27.6)		
Ser/Cys	110 (52.6)	123 (44.7)	176 (46.2)		
Cys/Cys	43 (20.6)	79 (28.7)	100 (26.2)	0.10	0.23

*: Comparisons were made between HCC cases and hospital controls.

†: Comparisons were made between HCC cases and CLD patients.

HCC: hepatocellular carcinoma

CLD: chronic liver diseases

Table 2. Adjusted odds ratios (ORs) and their 95% confidence intervals (CIs) of HCC for *hOGG1* genotypes according to heavy drinking history and current smoking status.

	HCC cases	Hospital controls	CLD patients	HCC cases vs. hospital controls		HCC cases vs. CLD patients	
	n(%)	n(%)	n(%)	OR* (95% CI)	OR† (95% CI)	OR* (95% CI)	OR† (95% CI)
Without heavy alcohol drinking history‡							
Ser/Ser	40 (25.0)	64 (25.4)	96 (28.1)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Ser/Cys	88 (55.0)	115 (45.6)	154 (45.0)	1.31 (0.77- 2.22)	0.73 (0.28- 1.93)	1.78 (1.07- 1.63)	1.82 (1.10- 3.01)
Cys/Cys	32 (20.0)	73 (29.0)	92 (26.9)	0.80 (0.43- 1.49)	0.42 (0.13- 1.28)	0.90 (0.50- 1.63)	0.90 (0.50- 1.64)
P for trend				0.51	0.13	0.84	0.86
With heavy alcohol drinking history‡							
Ser/Ser	16 (32.7)	9 (39.1)	9 (23.1)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Ser/Cys	22 (44.9)	8 (34.8)	22 (56.4)	1.56 (0.44- 5.59)	0.92 (0.17- 5.06)	0.50 (0.16- 1.50)	0.63 (0.18- 2.19)
Cys/Cys	11 (22.5)	6 (26.1)	8 (20.5)	1.05 (0.24- 4.61)	1.02 (0.10-10.06)	0.70 (0.18- 2.79)	0.84 (0.20- 3.84)
P for trend				0.87	0.99	0.54	0.81
P for interaction§				0.94	0.95	0.13	0.16
Without current smoking							
Ser/Ser	35 (25.0)	56 (26.9)	78 (26.4)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Ser/Cys	77 (55.0)	91 (43.8)	136 (46.1)	1.69 (0.95- 3.00)	1.00 (0.38- 2.59)	1.61 (0.95- 2.72)	1.65 (0.97- 2.81)
Cys/Cys	28 (20.0)	61 (29.3)	81 (27.5)	0.83 (0.42- 1.63)	0.60 (0.20- 1.84)	0.82 (0.44- 1.53)	0.86 (0.46-1.61)
P for trend				0.66	0.39	0.61	0.71
With current smoking							
Ser/Ser	21 (30.4)	17 (25.4)	27 (31.4)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Ser/Cys	33 (47.8)	32 (47.8)	40 (46.5)	0.77 (0.31- 1.92)	0.42 (0.07- 2.42)	1.04 (0.43- 2.52)	1.01 (0.41- 2.52)
Cys/Cys	15 (21.7)	18 (26.9)	19 (22.1)	0.81 (0.29- 2.32)	0.07 (0.003- 1.39)	1.00 (0.35- 2.84)	0.92 (0.32- 2.67)
P for trend				0.69	0.08	0.99	0.89
P for interaction§				0.15	0.70	0.52	0.69

*: Adjusted for sex, age category (40-49, 50-59, 60-69, and 70-79 years), and either smoking status (never, former, and current smokers) or heavy drinking history.

†: Adjusted for the above factors plus HBsAg and HCVAb.

‡: A "heavy alcohol drinking history" was defined as having imbibed ≥69 g ethanol per day for ≥10 years.

§: Calculated by the likelihood ratio test.

HCC : hepatocellular carcinoma

CLD : chronic liver diseases

had substantially lower DNA repair activity than that encoded by the 326Ser allele in an in vitro Escherichia coli complementation activity assay[14] and in human cells in *vivo*[15] whereas others did not find such a difference.[26,27] Thus, there is limited evidence of the genotype-phenotype relation, yet recent epidemiologic studies suggested that the putative low active allele (326Cys) was positively associated with lung, orolaryngeal, esophageal, stomach, and colon cancers,[16-20] but not with breast cancer.[28] To our knowledge, this is the first epidemiologic study on the association between the Ser326Cys polymorphism and HCC risk. Despite its high biological plausibility, however, we could not obtain any significant findings.

In this study, selection bias among controls could be responsible for the lack of association. However, we used two different control groups, and the results based on both control groups showed a similar tendency. Furthermore, the observed frequencies of the 326Ser allele (0.49 among hospital controls and 0.51 among CLD patients) were close to those in two earlier case-control studies among the Japanese (0.53 in both).[19,22] Given the sample size and the genotype frequency of hospital controls, we had an 83% chance of detecting a doubling of the risk for Ser/Cys+Cys/Cys (putative risk genotypes) vs. Ser/Ser (two-sided $p = 0.05$).

The difference of hOGG1 activity potentially caused by the Ser326Cys polymorphism may be compensated by higher induction of other cooperative enzymes (e.g., human MTH homolog 1[29] or human MutY homolog[30]) that prevent 8-OHdG-induced mutagenesis. In addition, regardless of the polymorphism, hOGG1 activity may be functionally inhibited by increased NO production resulting from chronic inflammation, which usually exists as the background of HCC, since NO mediated inhibition of hOGG1 activity has been shown in cholangiocarcinoma cell line.[31] On the other hand, Sugimura et al[32] reported that the risk for lung cancer associated with the *hOGG1* Cys/Cys genotype differed by histological subtypes, being elevated for squamous cell carcinoma but not for adenocarcinoma. HCC might represent a histological type unrelated to this genotype.

In conclusion, our results suggest that the *hOGG1* Ser326Cys polymorphism may not play a major role as an independent factor in hepatocarcinogenesis. Although this case-control study of moderate size is among the largest ones that have been reported on the association between HCC and genetic polymorphisms, we could not exclude the possibility of a weak association (e.g., OR < 2.0) with the *hOGG1* polymorphism and its interaction with environmental factors. Further large studies are needed to address these issues.

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Inverse association between coffee drinking and the risk of hepatocellular carcinoma: a case-control study in Japan

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Coffee use has consistently been associated with lower serum liver enzyme levels and a reduced risk of liver cirrhosis. A limited number of cohort and case-control studies also suggest a decreased risk of hepatocellular carcinoma (HCC) among coffee drinkers, but mostly without consideration of hepatitis virus infection. In the present case-control study, we recruited 209 incident HCC cases and three different controls (1308 community controls, 275 hospital controls, and 381 patients with chronic liver disease [CLD] without HCC), all of whom were aged 40–79 years and residents of Saga Prefecture, Japan. A questionnaire survey elicited information on coffee use during the last 1–2 years and 10 years before, and plasma hepatitis B surface antigen and antibodies to hepatitis C virus were tested for all but community controls. After adjustment for sex, age, heavy alcohol use, smoking status and hepatitis virus markers (except for community controls), coffee use during the last 1–2 years was associated with a decreased risk against any control group. For coffee use 10 years before, comparison between HCC cases and either community controls or CLD patients revealed a decreased risk; adjusted odds ratios for occasional use, 1–2 cups/day and ≥3 cups/day compared with no use were 0.33, 0.27 and 0.22 (P trend < 0.001), respectively, against community controls, and 0.86, 0.62 and 0.53 (P trend = 0.05), respectively, against CLD patients. These results suggest that coffee may protect against the development of HCC, yet further elaborate studies (hopefully, intervention studies) are warranted to corroborate these findings. (*Cancer Sci* 2007; 98: 214–218)

Chronic infections with hepatitis C and B viruses represent established risk factors for hepatocellular carcinoma (HCC), accounting for around 90% of occurrences of this malignancy in Japan.[1–3] However, little is known about the association between the risk of HCC and dietary habits, except for alcohol consumption.[4,5] Recently, coffee drinking has been linked to a decreased risk of HCC; three cohort studies[6–8] and three case-control studies[9–11] demonstrated that coffee drinkers were at a lower risk of HCC than non-coffee drinkers. However, only two case-control studies took into account hepatitis virus infection as a potential confounder.[10,11] For example, if hepatitis virus carriers who have a great risk of developing HCC tend to drink less coffee for any reason (e.g. impaired caffeine clearance due to their liver disease)[12] compared with healthy individuals, it would produce a seemingly protective association between coffee use and HCC.

To further explore the association between coffee drinking and HCC, we conducted a case-control study employing three different controls (community controls, hospital controls, and patients with chronic liver disease [CLD] without HCC). CLD patients were selected as controls because the majority of Japanese patients with HCC have preexisting CLD. Plasma hepatitis virus markers (except for community controls) and other major covariates such as alcohol consumption and cigarette smoking were measured and accommodated in our data analyses.

Materials and Methods

Subjects. The details of the study subjects excluding community controls have been described elsewhere.[13] Briefly, all study subjects were restricted to residents of Saga Prefecture, Japan, who were aged 40–79 years at the time of identification. Incident HCC patients (n = 209, participation rate [PR] = 92%) who were admitted or outpatients of two main hospitals in Saga City (Saga Medical School Hospital and Saga Prefectural Hospital) between April 2001 and March 2004 were recruited as case subjects; 198 cases (95%) had preexisting cirrhosis (n = 167) or chronic hepatitis (n = 31).

Community controls (n = 1308) were residents of Saga City, who were aged 40–79 years as of 31 December 2001. We randomly selected 1000 men and 1000 women from the resident register as follows. Based on computer-generated random numbers for page and line, we first identified corresponding reference lines in the register and then searched for eligible subjects on or after those lines. We asked these subjects to participate in a questionnaire survey by mail in March 2002. Sixty-three people later turned out to be ineligible (31 no delivery, nine erroneous sampling, 10 long absence, 12 inability to respond, one death), and 1338 people successfully responded with a net PR of 69% (1338/1937). Of the 1338 respondents, 30 were excluded due to missing information on current coffee use.

Hospital controls (n = 275, PR = 73%) were first-time visitors at the general outpatient clinic of Saga Medical School Hospital between May 2001 and April 2003, who had no evidence of HCC. From among consecutive visitors, research nurses identified eligible controls based on the following order of priority: (1) men aged 50–79 years; (2) women aged 60–79 years; (3) men aged 40–49 years; and (4) women aged 40–59 years. This order was determined by the sex and age distribution of deaths from liver cancer in Saga Prefecture in 1998. The 275 hospital controls had various, mostly minor, diseases (n = 190), undiagnosed symptoms (n = 49), or no definite abnormality (n = 36).

Patients with CLD (n = 381, 298 patients with chronic hepatitis and 83 patients with cirrhosis, PR = 96%) were out- or inpatients of the two hospitals, the same as the HCC cases, between September 2001 and March 2004. Patients with special types of CLD (primary and secondary biliary cirrhosis, autoimmune hepatitis, and liver disease due to parasitosis, congestive heart failure, or metabolic disorders) were excluded. These CLD patients had no evidence of HCC by radiological findings.

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The study protocol was approved by the ethics committees of the above two hospitals. Written informed consent was obtained from all but community controls. For community controls, having returned the completed questionnaire was regarded as their consent.

Questionnaire survey. Except for community controls, coffee use, alcohol consumption and cigarette smoking were assessed based on an interview survey by research nurses using a uniform questionnaire. For community controls, we carried out a mail survey using a self-administered questionnaire including the same questions on the above lifestyle habits. With regard to coffee use, subjects were asked about their drinking frequency (never, occasional [less than one cup per day], or daily) during the last 1–2 years and 10 years before, with subsequent inquiry to daily users about the number of cups per day; 55 community controls had missing data on coffee use 10 years before. We did not obtain information on the type or brewing method of coffee. A history of heavy alcohol use was defined as having drunk ≥69 g of alcohol per day for ≥10 years. Current smokers were defined as those who currently smoked or stopped smoking less than 1 year before.

Serological tests. Venous blood was drawn from all but community controls, and plasma samples were tested for hepatitis B surface antigen (HBsAg) using a chemiluminescent immunoassay (CLIA; Dainabot, Tokyo, Japan), and for antibodies to hepatitis C virus (HCV) using a second-generation enzyme immunoassay (Abott HCV EIA II; Dainabot) at an external laboratory (SRL, Tokyo, Japan).

Statistical analysis. χ^2-tests and Student's *t*-tests were conducted for univariate analyses. Unconditional logistic regression models were used to estimate odds ratios (OR) and 95% confidence intervals (CI) of HCC according to coffee drinking by using dummy variables, with adjustment for sex, age category (40–49, 50–59, 60–69 and 70–79 years), heavy alcohol use (ever vs never) and smoking status (current vs never/former smokers), as well as HBsAg and anti-HCV status (except for community controls). Linear trends for the association of increasing coffee use with other factors or HCC risk were assessed using the Mantel test (for proportions), linear regression analysis (for means), or logistic regression analysis (for OR) with an ordinal variable assigned for coffee consumption. Tests for interaction between coffee use and alcohol drinking/smoking habits on HCC risk were carried out by including an additional product term of two binary variables (i.e. coffee and either alcohol drinking or smoking) in the logistic model (the Wald test). All statistical analyses were carried out using the Stata statistical package (StataCorp, College Station, TX, USA).

Results

Table 1 shows selected characteristics of study subjects. Compared with at least one of the three control groups, HCC cases showed a significantly higher prevalence of men (against community controls or CLD patients), older subjects (against any control group), HBsAg positivity (against hospital controls), anti-HCV positivity (against hospital controls), men with a history of heavy alcohol use (against any control group), and male current smokers (against hospital controls). HCC cases included significantly less daily coffee users than any control group.

In Table 2, the characteristics of coffee drinkers among community controls and hospital controls are presented. No sex difference in coffee drinking was evident. Heavier coffee drinkers were younger and were more likely to be current smokers (except for female hospital controls). There was some indication of an inverse association between coffee use and either heavy alcohol use (among male community controls) or positivity for HBsAg or anti-HCV (among hospital controls), although it was not statistically significant.

We estimated the adjusted OR of HCC for coffee use during the last 1–2 years by comparing HCC cases with each control group (Table 3). Coffee use during the last 1–2 years was associated with a decreased risk against any control group, although the OR for ≥3 cups/day against hospital controls was unstable and not decreased after additional adjustment for HBsAg and anti-HCV.

Table 1. Selected characteristics of study subjects

Characteristic	HCC cases	Community controls	Hospital controls	CLD patients
Number	209	1308	275	381
Male (%)	67.5	50.2**	65.5	53.8**
Mean age (years)	67.0	56.7**	60.6**	60.4**
HBsAg positive (%)	9.1	ND	2.2**	9.2
Anti-HCV positive (%)	85.6	ND	7.6**	85.8
Heavy alcohol use† (%)				
Male	32.6	12.0**	12.2**	17.1**
Female	4.4	2.9	1.1	2.3
Current smoker (%)				
Male	46.8	48.5	35.0*	36.6
Female	4.4	9.4	4.2	6.3
Daily coffee user (%)				
Male	9.9	51.4**	36.1**	34.6**
Female	10.3	48.2**	27.4**	23.3*

†Having drunk ≥69 g of alcohol per day for ≥10 years. *$P < 0.05$ compared with hepatocellular carcinoma (HCC) cases. **$P < 0.01$ compared with HCC cases. CLD, chronic liver disease; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; ND, not determined.

Because HCC cases could have lately changed their coffee use due to their preexisting liver disease (e.g. impaired caffeine clearance)[12] we also evaluated the association with coffee use 10 years before (Table 4). In this analysis, the comparison between HCC cases and either community controls or CLD patients showed an inverse association between coffee drinking and HCC risk. Such a risk decrease was not observed against hospital controls, and the fully adjusted OR for ≥3 cups/day was rather elevated but very unstable, as illustrated by the wide 95% CI.

Finally, we examined whether the magnitude of the inverse association between coffee and HCC was modified by alcohol drinking and smoking status (Table 5). No significant difference in the adjusted OR for daily versus non-daily coffee users was observed according to daily alcohol use (<23 vs ≥23 g), heavy alcohol use (never vs ever), or smoking status (never/former vs current), as indicated by statistically insignificant *P*-values for interaction. The same was true for coffee use 10 years before (data not shown).

Discussion

The present study provides further evidence that coffee drinking is associated with a decreased risk of HCC. There are now several lines of accumulated evidence for possible beneficial effects of coffee on the liver: (1) a substantial number of cross-sectional and cohort studies have shown a very consistent inverse association between coffee consumption and serum liver enzyme levels;[14–26] (2) several cohort and case-controls studies have demonstrated a consistent protective association between coffee use and the risk of liver cirrhosis;[27–32] and (3) a few animal experiments have revealed that the incidence of liver tumor is lower in rodents given coffee than in those not given coffee.[33,34] Thus, the findings in this study, together with recent observations in several cohort and case-controls studies,[6–11] appear to carry sufficient biological plausibility.

Employing three different controls represents a unique characteristic of the present study. Theoretically, the results based on

Table 2. Characteristics of coffee users

Factor	Daily coffee use during last 1–2 years				*P* trend†
	None	Occasional	1–2 cups	≥3 cups	
Among 1308 community controls					
Number	230	427	387	264	
Male (%)	50.9	47.3	50.6	53.4	0.34
Mean age (years)	64.6	58.2	54.6	50.6	<0.001
Heavy alcohol use‡ (%)					
Male	14.5	12.9	12.8	7.8	0.11
Female	2.7	2.7	2.6	4.1	0.55
Current smoker (%)					
Male	32.5	43.1	49.5	68.1	<0.001
Female	3.5	7.1	9.4	18.7	<0.001
Among 275 hospital controls					
Number	96	88	60	31	
Male (%)	66.7	58.0	75.0	64.5	0.62
Mean age (years)	65.4	60.2	55.8	56.2	<0.001
Heavy alcohol use‡ (%)					
Male	12.5	13.7	15.6	0.0	0.43
Female	3.1	0.0	0.0	0.0	–
Current smoker (%)					
Male	21.9	37.3	48.9	40.0	0.01
Female	6.3	2.7	6.7	0.0	0.53
HBsAg/anti-HCV(+) (%)	12.5	10.2	8.3	3.2	0.13

†Based on the Mantel test or linear regression. ‡Having drunk ≥69 g of alcohol per day for ≥10 years. HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus.

Table 3. Adjusted odds ratios (OR) and 95% confidence intervals (CI) of hepatocellular carcinoma (HCC) for coffee use during the last 1–2 years by comparing 209 HCC cases with 1308 community controls, 275 hospital controls or 381 chronic liver disease (CLD) patients

Factor	Daily coffee use during last 1–2 years				Total	*P* trend
	None	Occasional	1–2 cups	≥3 cups		
Number (%)						
HCC cases	135 (64.6)	53 (25.4)	15 (7.2)	6 (2.9)	209 (100)	–
Community controls	230 (17.6)	427 (32.6)	387 (29.6)	264 (20.2)	1308 (100)	–
Hospital controls	96 (34.9)	88 (32.0)	60 (21.8)	31 (11.3)	275 (100)	–
CLD patients	175 (45.9)	94 (24.7)	66 (17.3)	46 (12.1)	381 (100)	–
Adjusted OR (95% CI) against						
Community controls†	1.00 (reference)	0.31 (0.21–0.46)	0.11 (0.06–0.21)	0.10 (0.04–0.24)	–	<0.001
Hospital controls†	1.00 (reference)	0.54 (0.34–0.87)	0.24 (0.12–0.48)	0.23 (0.08–0.61)	–	<0.001
Hospital controls‡	1.00 (reference)	0.42 (0.19–0.95)	0.23 (0.08–0.68)	1.08 (0.22–5.35)	–	0.03
CLD patients†	1.00 (reference)	0.86 (0.55–1.34)	0.42 (0.21–0.84)	0.28 (0.11–0.72)	–	0.001
CLD patients‡	1.00 (reference)	0.86 (0.55–1.35)	0.42 (0.21–0.84)	0.29 (0.11–0.75)	–	0.001

†Adjusted for sex, age, heavy alcohol use and smoking status. ‡Adjusted for sex, age, heavy alcohol use, smoking status, hepatitis B surface antigen and antibodies to hepatitis C virus.

community and hospital controls, who are recruited in usual settings of case-control studies, would correspond to the results from recent cohort studies in Japan.[6–8] We found a strong inverse association between coffee and HCC risk against community controls, although potential confounding by hepatitis virus infection could not be ruled out. The OR for coffee use against hospital controls were not clearly reduced but were even elevated for the highest intake category after additional adjustment for hepatitis virus markers. These OR were very unstable due to the low prevalence of hepatitis virus infection among hospital controls, and caution must be exercised in interpreting these findings. However, the results based on CLD patients showed a consistent tendency for coffee use during the last 1–2 years and 10 years before, regardless of adjustment for hepatitis virus markers. We speculate that the comparison between HCC cases and CLD patients may convey the most reliable information in terms of both confounding and precision issues. A recent case-control study demonstrated a similar protective association between coffee and HCC among patients with chronic hepatitis C.[11]

Several limitations of this study should be mentioned. First, HCC cases may have reduced coffee consumption because of their preexisting liver disease (e.g. impaired caffeine clearance[12] and gastrointestinal disorders accompanying liver disease). This can lead to a spurious protective association between coffee and HCC. In an attempt to address this issue, we examined the association with coffee intake 10 years before as well as the intake during the last 1–2 years, and found a somewhat weaker protective association against community controls and CLD patients. However, there still exists the possibility that coffee consumption 10 years before could have been affected by worsened liver

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Table 4. Adjusted odds ratios (OR) and 95% confidence intervals (CI) of hepatocellular carcinoma (HCC) for coffee use 10 years before by comparing 209 HCC cases with 1253 community controls, 275 hospital controls or 381 chronic liver disease (CLD) patients

Factor	Daily coffee use 10 years before				Total	*P* trend
	None	Occasional	1–2 cups	≥3 cups		
Number (%) of						
HCC cases	127 (60.8)	53 (25.4)	17 (8.1)	12 (5.7)	209 (100)	–
Community controls	268 (21.4)	496 (39.6)	268 (21.4)	221 (17.6)	1253 (100)	–
Hospital controls	129 (46.9)	73 (26.5)	48 (17.5)	25 (9.1)	275 (100)	–
CLD patients	166 (43.6)	102 (26.8)	59 (15.5)	54 (14.2)	381 (100)	–
Adjusted OR (95% CI) against						
Community controls†	1.00 (reference)	0.33 (0.22–0.48)	0.27 (0.15–0.48)	0.22 (0.11–0.43)	–	<0.001
Hospital controls†	1.00 (reference)	1.10 (0.68–1.78)	0.66 (0.33–1.32)	0.92 (0.40–2.14)	–	0.53
Hospital controls‡	1.00 (reference)	0.99 (0.42–2.32)	0.95 (0.31–2.89)	2.59 (0.58–11.56)	–	0.47
CLD patients†	1.00 (reference)	0.87 (0.56–1.36)	0.64 (0.33–1.23)	0.52 (0.25–1.10)	–	0.05
CLD patients‡	1.00 (reference)	0.86 (0.55–1.34)	0.62 (0.32–1.21)	0.53 (0.25–1.12)	–	0.05

†Adjusted for sex, age, heavy alcohol use and smoking status. ‡Adjusted for sex, age, heavy alcohol use, smoking status, hepatitis B surface antigen and antibodies to hepatitis C virus.

Table 5. Adjusted odds ratios (OR) and 95% confidence intervals (CI) of hepatocellular carcinoma (HCC) for daily versus non-daily coffee users according to alcohol drinking and smoking status

Alcohol drinking or smoking status	Community controls		Hospital controls		CLD patients	
	Ca/Co†	OR‡ (95% CI)	Ca/Co†	OR§ (95% CI)	Ca/Co†	OR§ (95% CI)
Alcohol <23 g/day	157/966	0.19 (0.11–0.35)	157/206	0.79 (0.25–2.46)	157/320	0.48 (0.25–0.95)
Alcohol ≥23 g/day	52/341	0.18 (0.07–0.44)	52/69	0.29 (0.07–1.31)	52/61	0.24 (0.08–0.70)
P interaction	–	0.71	–	0.77	–	0.44
Non-heavy alcohol user	160/1210	0.17 (0.09–0.30)	160/252	0.46 (0.15–1.39)	160/342	0.36 (0.18–0.70)
Heavy alcohol user	49/98	0.30 (0.12–0.75)	49/23	0.26 (0.03–2.30)	49/39	0.57 (0.18–1.87)
P interaction	–	0.27	–	0.43	–	0.29
Never/former smoker	140/929	0.23 (0.12–0.42)	140/208	0.56 (0.18–1.70)	140/295	0.58 (0.29–1.16)
Current smoker	69/379	0.14 (0.06–0.32)	69/67	0.17 (0.02–1.63)	69/86	0.19 (0.07–0.52)
P interaction	–	0.45	–	0.36	–	0.14

†Numbers of cases/controls. ‡Adjusted for sex, age and either smoking status (for OR by alcohol drinking) or heavy alcohol use (for OR by smoking status). §Adjusted for sex, age, hepatitis B surface antigen, antibodies to hepatitis C virus, and either smoking status (for OR by alcohol drinking) or heavy alcohol use (for OR by smoking status).

function at that time among HCC cases. Our comparison between HCC cases and CLD patients may get rid of this issue to some extent, yet intervention studies are needed to address it conclusively.

Second, selection bias could have been introduced in the present study. The catchment area for community controls (Saga City) differed from that for HCC cases (Saga Prefecture), but the results remained unchanged when we excluded the cases that resided outside Saga City in the data analysis (data not shown). We believe that the fairly high PR (69–96%) of study subjects reduced the possibility of self-selection bias. Third, information bias was unlikely because the potential beneficial effect of coffee was perhaps unknown in most study subjects as well as interviewers at the time of investigation. Fourth, misclassification of coffee use, particularly 10 years before, may have been present. Among community and hospital controls, the proportion of more frequent coffee drinkers (1–2 and ≥3 cups/day) during the last 1–2 years (Table 3) was higher than that 10 years before (Table 4), although heavier coffee drinkers were younger, as shown in Table 2. This may be due to underreporting of coffee consumption 10 years before or to generally increasing coffee consumption over the past decade. The possible underreporting, if any, was likely to be non-differential between cases and controls, and thus may have biased relevant OR towards unity. However, this issue would not seriously damage our interpretation.

Although potential confounding effects by known risk factors were taken into account in the data analyses, other possible confounders such as dietary factors (e.g. vegetable consumption)[35] could be relevant. We did not ascertain information on other food items and thus could not address this issue. To our knowledge, however, no specific food has been so strongly associated with HCC risk as to explain the inverse relationship between coffee and HCC by its confounding effect.

Because we previously observed a stronger inverse association between coffee consumption and serum liver enzyme levels (e.g. γ-glutamyltransferase) among male alcohol drinkers than among male non-alcohol drinkers,[21] we evaluated if the inverse association between coffee and HCC was modified by alcohol consumption as well as cigarette smoking. We found no evidence of such an effect modification, which accords with the results from a recent Italian case-control study.[10]

Potential candidates responsible for the inverse association between coffee and HCC may be caffeine,[36] coffee diterpenes (e.g. cafestol and kahweol),[37] antioxidants (e.g. chlorogenic acid)[33] or other unidentified ingredients. We could not evaluate the role of caffeine itself, but previous studies did not find an inverse association between HCC and green tea, another popular source of caffeine in Japan, indirectly suggesting no substantial role of caffeine.[6,7,11,38] Although we did not obtain information on the type or brewing method of coffee from study subjects, instant coffee is most popular in Japan, followed by filtered coffee,[39]

and the use of unfiltered coffee is negligible. As the levels of diterpenes are greatly reduced in instant and filtered coffee,[40] they are unlikely to be responsible for the observed inverse association. Tanaka *et al.* demonstrated that chlorogenic acid exerts an inhibitory effect on chemically induced hyperplastic liver cell foci in hamsters,[33] and some coffee antioxidants including chlorogenic acid may play a crucial protective role in human hepatocarcinogesis. Further studies incorporating genetic polymorphisms of enzymes involved in specific metabolic pathways of the above substances may afford a clue to the above issue.

As already discussed, the major limitation of the present study was a possible decrease of coffee use among HCC cases due to their advanced liver disease. Cohort studies incorporating hepatitis virus markers as well as the severity of liver disease and, hopefully, intervention studies targeting high-risk groups (e.g. patients with chronic hepatitis C) are warranted to establish the protective association between coffee drinking and HCC.

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Alcohol Drinking and Liver Cancer Risk: An Evaluation Based on a Systematic Review of Epidemiologic Evidence among the Japanese Population

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Background: Although alcohol consumption has been recognized as a risk factor for primary liver cancer, it will be informative to summarize relevant epidemiologic data in the Japanese who have characteristic environmental determinants (e.g. hepatitis C virus infection) and genetic traits (e.g. presence of poor acetaldehyde metabolizers).
Methods: We systematically reviewed epidemiologic studies on alcohol drinking and liver cancer among Japanese populations. Original data were obtained through searches of the MEDLINE (PubMed) and *Ichushi* databases, complemented with manual searches. The evaluation was performed in terms of the magnitude of association ('strong', 'moderate', 'weak' or 'no association') in each study and the strength of evidence ('convincing', 'probable', 'possible' or 'insufficient'), together with biological plausibility as previously assessed by the International Agency for Research on Cancer.
Results: Among 22 cohort studies identified, 14 (64%) reported weak to strong positive associations between alcohol and liver cancer risk, 3 (14%) reported no association and five (23%) reported weak to moderate inverse associations; such inverse associations were found mostly in follow-up studies of patients with chronic liver disease (particularly, cirrhotic patients), yet recent studies on patients with chronic hepatitis C presented fairly consistent positive associations. Of 24 case–control studies identified, 19 (79%) showed weak to strong positive associations, whereas the remainder demonstrated no association ($n = 4$) or a moderate inverse association ($n = 1$).
Conclusion: We conclude that there is 'convincing' evidence that alcohol drinking increases the risk of primary liver cancer among the Japanese population.

Keywords: systematic review – epidemiology – alcohol – liver cancer – Japanese

INTRODUCTION

Alcohol has long been viewed as a hepatotoxic agent, and its heavy consumption is known to cause hepatocellular injury that can lead to enhanced fibrosis and eventually to liver cirrhosis through various mechanisms presumed (1). Alcohol drinking has also been implicated in the etiology of primary liver cancer that often develops from cirrhosis (2). In the most recent evaluation by the International Agency for Research on Cancer (IARC), the occurrence of liver cancer has been 'causally' related to the consumption

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of alcoholic beverages (3). In the second report published by the World Cancer Research Fund and the American Institute for Cancer Research, the Panel has judged that alcohol consumption is 'probably' a direct cause of liver cancer (4).

Primary liver cancer is one of the most common cancers in Japan (5). More than 90% of primary liver cancers in this country are hepatocellular carcinomas (HCCs) that are mostly attributable to chronic infections with hepatitis C virus (HCV) and hepatitis B virus (HBV) (6,7); HCV and HBV infections are estimated to account for 70 and 15%, respectively, of the recent occurrences of HCC in Japan (6). This tendency clearly contrasts with the situation in south-east Asia and sub-Saharan Africa where HBV represents a dominant risk factor of HCC, and with that in Western countries where HCV infection plays an increasingly important role (2,8). The role of alcohol in hepatocarcinogenesis might differ between Japan and such areas. Moreover, ~50% of the Japanese are poor metabolizers of acetaldehyde (9), the first metabolite of ethanol, which has been recognized as being possibly carcinogenic to humans (10). Such poor metabolizers have not been found in Africans or Caucasians (9), and thus the Japanese as Mongoloids might be more susceptible to alcohol than other ethnic groups.

The aim of the present study was to review and summarize epidemiologic findings on alcohol drinking and liver cancer among Japanese populations. This work was conducted as part of a project of systematic evaluation of the epidemiologic evidence regarding lifestyles and cancers in Japan (11).

PATIENTS AND METHOD

The details of the evaluation method have been described elsewhere (11). In brief, original data for this review were identified through searches of the MEDLINE (PubMed) and *Ichushi* (*Japana Centra Revuo Medicina*) databases, complemented by manual searches of references from relevant articles where necessary. All epidemiologic studies on the association between alcohol drinking and liver cancer incidence/mortality among the Japanese from 1950 (or 1983 for the *Ichushi* database) to June 2008, including papers in press if available, were identified using the following as keywords: alcohol, liver, hepatocellular, cohort, follow-up, case–control, Japan and Japanese. Papers written in either English or Japanese were reviewed, and only studies on Japanese populations living in Japan were included. The individual results were summarized in the tables separately as cohort or case–control studies.

The evaluation was made based on the magnitudes of association and the strength of evidence. First, the former was assessed by classifying the relative risk (RR) in each study into the following four categories, while considering statistical significance (SS) or no statistical significance (NS): (i) 'strong' (symbol $\downarrow\downarrow\downarrow$ or $\uparrow\uparrow\uparrow$) when RR < 0.5 (SS) or RR > 2.0 (SS); (ii) 'moderate' (symbol $\downarrow\downarrow$ or $\uparrow\uparrow$) when RR < 0.5 (NS), $0.5 \leq$ RR < 0.67 (SS), $1.5 <$ RR ≤ 2.0 (SS) or RR > 2.0 (NS); (iii) 'weak' (symbol $\downarrow$ or $\uparrow$) when $0.5 \leq$ RR < 0.67 (NS), $0.67 \leq$ RR ≤ 1.5 (SS) or $1.5 <$ RR ≤ 2.0 (NS) and (iv) 'no association' (symbol $-$) when $0.67 \leq$ RR ≤ 1.5 (NS); the RR used in this paper denotes ratio measures of effect, including risk ratios, rate ratios, hazard ratios and odds ratios. When RRs for three or more exposure levels were reported, that for the highest level was employed for this classification. In the case of multiple publications of analyses of the same or overlapping data sets, only data from the largest or most updated results were included. Studies that reported RRs for indefinite exposure levels, or did not provide RRs or data necessary for the present authors to calculate relevant RRs, were excluded.

After this process, the strength of evidence was evaluated in a manner similar to that used in the WHO/FAO Expert Consultation Report (12), in which evidence was classified as 'convincing', 'probable', 'possible' and 'insufficient'. We assumed that biological plausibility corresponded to the judgment of the most recent evaluation from the IARC (3). Despite the use of this quantitative assessment rule, an arbitrary assessment cannot be avoided when considerable variation exists in the magnitudes of association among the results of each study. The final judgment, therefore, was made based on a consensus of the research group members, and it was therefore not necessarily objective. When we reach a conclusion that there is 'convincing' or 'probable' evidence of an association, we conduct a meta-analysis to obtain summary estimates for the overall magnitude of association.

MAIN FEATURES AND COMMENTS

We identified a total of 22 cohort (13–34) (Table 1) and 24 case–control studies (35–58) (Table 2). Of those cohort studies, two presented the results by sex (19,31), seven for men only (13–16,26,29,32) and 13 for men and women combined (17,18,20–25,27,28,30,33,34). The respective numbers for the case–control studies are two (45,54), nine (36–38,42,44,48–51) and 13 (35,39–41,43,46,47,52,53,55–58). Several studies showed the results separately according to study areas (16), different age categories (31), the severity of chronic liver disease (CLD) (33) or different control groups (49,54,56).

Study populations in the cohort studies, except for one study based on male alcoholics (26), were classified broadly into two categories: mostly healthy subjects ($n = 7$) such as local residents (14,16,25,31,32), physicians (13) and atomic bomb survivors (19) and patients with CLD (15,17,18,20–24, 27–30,33,34) ($n = 14$) (Table 1). Chronic infections with both HCV and HBV were taken into account in 12 studies, all of which followed patients with CLD (18,20–24, 27–30,33,34). In the case–control studies, excluding one study based on military men exposed to thorotrast (38), a

Table 1. Cohort studies on alcohol drinking and liver cancer among Japanese

Reference	Study period	Study population				Category	Number among cases	Relative risk (95% CI or *P*)	*P* for trend	Confounding variables considered	Comments
		Number of subjects for analysis	Source of subjects	Event followed	Number of incident cases or deaths						
Kono et al. (13)	1965–83	5130 men	Male physicians in western Japan	Death	51 men (primary 9, unspecified 42)	Never/past		1.00		Age, smoking	HBsAg and anti-HCV were not tested.
						Occasional		1.34 (0.61–2.98)			
						<2 go/day		1.80 (0.80–4.02)			
						≥2 go/day		2.36 (1.04–5.35)			
Hirayama (14)	1966–82	122261 men	95% of the census population in 29 health-center-covered areas in six prefectures	Death	788 men (liver cancer) or 123 men (primary liver cancer)	For liver cancer				Age	HBsAg and anti-HCV were not tested
						Not daily		1.00			
						Daily		1.25 ($P < 0.01$)			
						For primary liver cancer					
						Not daily		1.00			
						Daily		1.89 ($P < 0.01$)			
Inaba et al. (15)	1973–88	270 men	Patients with liver cirrhosis at Juntendo University Hospital	Death	46 men	Never		1.00		Age, HBsAg, histories of blood transfusion, hepatitis and surgical operation, smoking	Anti-HCV was not tested
						Current/past		0.41 (0.08–2.20)			
Shibata et al. (16)	1958–86	639 men in a farming area and 677 men in a fishing area	Residents in a farming area or a fishing area in Kyushu	Death	11 men (farming area) and 22 men (fishing area)	Farming area					
						Non-drinker	2	1.0	>0.1	Age	HBsAg and anti-HCV were not tested
						Sake <1 go/day	6	1.1 (0.2–5.5)			
						Sake 1–2 go/day	2	1.6 (0.2–11.6)			
						Sake ≥2 go/day	1	1.1 (0.1–13.5)			
						Fishing area					
						Non-drinker	2	1.0		Age	
						Sake <1 go/day	0	–			
						Sake 1–2 go/day	0	–			
						Sake ≥2 go/day	1	5.5 (0.6–51.1)			

						Fishing area					
						Shochu none	4	1.00	<0.01	Age, smoking	
						Shochu <2 go/day	14	5.85 (1.31–26.18)			
						Shochu ≥2 go/day	4	14.02 (2.34–83.89)			
Kato et al. (17)	1987–90	1784	Patients with decompensated liver cirrhosis or post-transfusion hepatitis	Incidence	122	Never drinker	46	1.00		Sex, age	HBsAg and anti-HCV status was unknown. The total alcohol index was obtained by multiplying the daily ethanol intake (ml) by the number of years of drinking
						Past drinker	19	0.58 (0.32–1.04)			
						Occasional drinker	4	0.43 (0.15–1.24)			
						Current drinker	5	0.41 (0.16–1.06)			
						Total alcohol index					
						0	46	1.00	0.046		
						1–1999	10	0.49 (0.23–1.02)			
						2000+	13	0.53 (0.27–1.04)			
Tsukuma et al. (18)	1987–91	917 (548 men and 369 women)	Patients with chronic hepatitis or compensated cirrhosis at Center for Adult Diseases, Osaka	Incidence	54	Nondrinker		1.00		Age, sex, stage of disease, serum alpha-fetoprotein, HBsAg, anti-HBc, anti-HCV, smoking	HBsAg and anti-HCV status was adjusted for.
						Occasional drinker		0.77 (0.20–2.99)			
						Former drinker					
						<80 g ethanol/day		1.46 (0.56–3.79)			
						≥80 g ethanol/day		1.66 (0.69–3.96)			
						Current drinker					
						<80 g ethanol/day		1.10 (0.39–3.07)			
						≥80 g ethanol/day		1.15 (0.35–3.78)			
Goodman et al. (19)	1980–89	36133	Atomic bomb survivors	Incidence	242 (156 men and 86 women)	For men				Sex, city, age at the time of bombing, age, radiation dose to the liver	HBsAg and anti-HCV were not tested.
						Never-drinker	25	1.00			
						Ever-drinker	126	1.11 (0.72–1.70)			
						Ex-drinker	25	2.33 (1.34–4.07)			
						Quit ≥16 years ago	4	0.96 (0.33–2.77)			

Continued

Table 1. *Continued*

Reference	Study period	Study population				Category	Number among cases	Relative risk (95% CI or *P*)	*P* for trend	Confounding variables considered	Comments
		Number of subjects for analysis	Source of subjects	Event followed	Number of incident cases or deaths						
						Quit 11–15 years ago	8	2.08 (0.93–4.67)			
						Quit ≤10 years ago	12	7.87 (3.89–16.0)			
						Present drinker	100	0.98 (0.63–1.52)			
						<135 ml/week	37	1.09 (0.65–1.81)			
						135–299 ml/week	37	1.11 (0.67–1.86)			
						≥300 ml/week	37	1.12 (0.67–1.87)			
						For women					
						Never/past drinker	56	1.00			
						Present drinker	27	1.25 (0.78–1.98)			
						<27 ml/week	1	0.28 (0.04–2.02)			
						27–69 ml/week	6	1.20 (0.52–2.79)			
						≥70 ml/week	9	2.02 (0.99–4.09)			
Chiba et al. (20)	1977–93	412 (249 men and 163 women)	Patients with HCV-associated chronic hepatitis or compensated cirrhosis at Tsukuba University Hospital	Incidence	63 (54 men and 9 women)	Nondrinker		1.00		Sex, age, stage of disease, serum alpha-fetoprotein, anti-HBs, anti-HBc, histories of blood transfusion, surgical procedure and liver cancer in family, smoking	All subjects were anti-HCV-positive and HBsAg-negative.
						<150 kg ethanol		1.33 (0.60–2.93)			
						150–449 kg ethanol		1.50 (0.71–3.17)			
						≥450 kg ethanol		0.98 (0.43–2.23)			
Ikeda et al. (21)	1980–?	2215 (1544 men and 671 women)	Patients with chronic hepatitis at Toranomon Hospital	Incidence	89	All subjects	($n = 2215$)			Stage of hepatitis, gamma-glutamyl transpeptidase	HBsAg and anti-HCV status was available for all subjects.
						<500 kg ethanol		1.00			
						≥500 kg ethanol		3.04 (1.79–5.14)			
						HBsAg(+) anti-HCV(−) subjects	($n = 610$)			Indocyanine green retention rate	

						<500 kg ethanol		1.00			
						≥500 kg ethanol		8.37 (2.70–25.93)			
						HBsAg(−) anti-HCV(+) subjects	(*n* = 1500)			Stage of hepatitis, γ-glutamyl transpeptidase, history of blood transfusion, albumin	
						<500 kg ethanol		1.00			
						≥500 kg ethanol		1.96 (1.06–3.62)			
Tanaka et al. (22)	1985–95	96 (62 men and 34 women)	Patients with liver cirrhosis at Kyushu University Hospital	Incidence	37 (27 men and 10 women)	Never	16	1.00		Sex, age, years since LC diagnosis, department, hospitalization status, serum albumin, AST, alpha-fetoprotein, HBsAg, anti-HCV, smoking	HBsAg and anti-HCV status was adjusted for. The relative risks were not described in the original paper, and were reestimated by one of the authors (KT). One 'drink' corresponds to 23 ml of ethanol.
						Past	17	0.59 (0.20–1.73)			
						Current					
						<2.4 drinks/day	1	0.06 (0.01–0.57)			
						≥2.4 drinks/day	3	0.17 (0.02–1.42)			
Matsushita et al. (23)	1985–94	267 (165 men and 102 women)	Patients with liver cirrhosis at Kanazawa University Hospital	Incidence	67	Type B or C cirrhosis	(*n* = 202)			Age, anti-HCV	All subjects analyzed were positive for anti-HCV or HBsAg.
						Positive drinking history		1.83 (1.00–3.36)			
						Type C cirrhosis	(*n* = 140)			Age	
						Positive drinking history		2.36 (1.23–4.54)			
Aizawa et al. (24)	1981–98	153 (115 men and 38 women)	Patients with chronic hepatitis or cirrhosis positive for anti-HCV at Jikei University Hospital	Incidence	Not described	Habitual heavy drinking				Sex, age, ALT, interferon therapy, histologic staging, irregular regeneration	All subjects were anti-HCV-positive and HBsAg-negative. Habitual heavy drinking was defined as an average daily consumption of 65 g of ethanol for >5 years.
						No		1.00			
						Yes		3.04 (1.31–7.09)			
Mori et al. (25)	1992–97	3052 (974 men and 2078 women)	Residents in a town in Saga prefecture	Incidence	22 (14 men and 8 women)	History of habitual alcohol consumption				Sex, age	Anti-HCV and HBsAg status was available but not adjusted for.
						No	10	1.00			
						Yes	22	1.27 (0.46–3.47)			
						Never drinker	10	1.00	0.87		One 'drink' corresponds to a glass of sake.
						1–19 drink-years	3	2.05 (0.48–8.79)			
							9				

Continued

Table 1. *Continued*

Reference	Study period	Study population				Category	Number among cases	Relative risk (95% CI or *P*)	*P* for trend	Confounding variables considered	Comments
		Number of subjects for analysis	Source of subjects	Event followed	Number of incident cases or deaths						
						≥20 drink-years		1.14 (0.40–3.26)			
Noda et al. (26)	1972–92	306 men	Alcoholics in Takatsuki city, Osaka, who had been diagnosed at a psychiatric institution	Death	Not described	O/E ratio for hepatocellular carinoma		1.6 (0.3–4.7)		Age, calendar year	Anti-HCV and HBsAg were not tested.
Hamada et al. (27)	1980–2000	469 (227 men and 242 women)	Patients with clinically compensated chronic hepatitis C due to blood transfusion at National Nagasaki Medical Center	Incidence	52	Alcohol consumption				Age, serum bilirubin, platelets, interferon therapy, duration from infection, fibrosis	All subjects were anti-HCV-positive and HBsAg-negative. Excessive alcohol consumption was defined as an alcohol consumption of >50 g/day for 5 years.
						Not excessive		1.00			
						Excessive		2.21 (1.00–3.58)			
Takimoto et al. (28)	1989–?	356	Patients with histologically proven chronic hepatitis C at Niigata University Hospital and one hospital in Niigata, who did not respond to interferon therapy	Incidence	Not described	Alcohol drinking				Age, sex, blood transfusion, viral load, viral subtype, stage of fibrosis, ALT, platelets, interferon dose	All subjects were anti-HCV-positive and HBsAg-negative. Alcohol drinking was defined as having consumed >80 g ethanol daily for >5 years.
						No		1.00			
						Yes		4.30 ($P = 0.048$)			
Uetake et al. (29)	1988–2000	91 men	Patients with HBsAg-negative anti-HCV-negative alcoholic cirrhosis at Jikei University Hospital	Incidence	13 men	Cumulative alcohol intake (kg)				Anti-HBc	All patients were HBsAg-negative, anti-HCV-negative, and alcoholic. The hazard ratio (and 95% confidence interval) was not described in the original paper, and was estimated by one of the authors (KT).
						1200 kg increase		7.7 (1.9–31.5)	0.0047		
Iwasaki et al. (30)	1986–2003	792 (533 men and 259 women)	Hepatitis C patients with or without Child A cirrhosis at Okayama University Hospital and participating institutions, with sustained response to interferon	Incidence	23 (20 men and 3 women)	Alcohol consumption				Fibrosis staging, age	All subjects were anti-HCV-positive and HBsAg-negative.
						<50 g/day		1.00			
						≥50 g/day		3.86 (1.58–9.44)			
Ogimoto et al. (31)	1988–99	66974 (28343 men and 38631 women)	Residents in 45 areas throughout Japan	Death	184 (number by sex and age not described)	Male, 40–59 years	($n = 16715$)			Collaborating institute	HBsAg and anti-HCV were not tested.
						Never drinker		1.00			
						Ex-drinker		8.11 (3.17–20.77)			
						Current drinker		0.65 (0.27–1.52)			

						Male, 60–79 years	($n = 11628$)			
						Never drinker		1.00		
						Ex-drinker		3.48 (1.86–6.54)		
						Current drinker		0.75 (0.43–1.31)		
						Female, 40–59 years	($n = 22528$)			
						Never drinker		1.00		
						Ex-drinker		3.85 (0.48–30.93)		
						Current drinker		0.23 (0.03–1.80)		
						Female, 60–79 years	($n = 16103$)			
						Never drinker		1.00		
						Ex-drinker		4.18 (1.47–11.88)		
						Current drinker		0.59 (0.25–1.43)		
Nakaya et al. (32)	1990–97	21201 men	Residents in 14 municipalities of Miyagi prefecture	Incidence	48 men	Never drinker	3	1.0	0.21	Age, smoking, education, daily consumption of orange and other fruit juice, spinach, carrot or pumpkin, and tomato	HBsAg and anti-HCV were not tested.
						Ex-drinker	10	6.6 (1.8–24.2)			
						Current drinker	35	2.7 (0.8–8.9)			
						<22.8 g alcohol/day	11	2.8 (0.8–10.1)			
						≥22.8 g alcohol/day	24	2.7 (0.8–8.9)			
Ikeda et al. (33)	1995–2005	846 (473 men and 373 women)	Patients with HCV-associated chronic hepatitis or cirrhosis at Kyoto University Hospital and 14 affiliated core hospitals	Incidence	237 (151 men and 86 women)	Patients with chronic hepatitis	($n = 576$)			Sex, age, smoking, alcohol consumption, response to interferon therapy, anti-HBc	All subjects were anti-HCV-positive and HBsAg-negative.
						None	57	1.00 (reference)			
						<30 g/day	14	0.75 (0.39–1.44)			
						≥30 g/day	23	0.65 (0.37–1.12)			
						Patients with cirrhosis	($n = 270$)				
						None	99				

Continued

Table 1. *Continued*

Reference	Study period	Study population				Category	Number among cases	Relative risk (95% CI or *P*)	*P* for trend	Confounding variables considered	Comments
		Number of subjects for analysis	Source of subjects	Event followed	Number of incident cases or deaths						
						<30 g/day	11	1.00 (reference)			
								0.42 (0.22–0.83)			
						≥30 g/day	33	1.03 (0.65–0.83)			
Ohki et al. (34)	1994–2006	1431 (727 men and 704 women)	Patients with positive HCV-RNA at Tokyo University Hospital	Incidence	340	Alcohol consumption				Age, sex, diabetes, body mass index, serum albumin, bilirubin, ALT, prothrombin time, platelets, alpha-fetoprotein	All subjects were anti-HCV-positive and HBsAg-negative.
						≤80 g/day		1.00			
						>80 g/day		1.41 (1.07–1.86)			

CI, confidence interval; HBsAg, hepatitis B surface antigen; anti-HCV, antibody to hepatitis C virus; anti-HBc, antibody to hepatitis B core antigen; HCV, hepatitis C virus; anti-HBs, antibody to hepatitis B surface antige; LC, liver cirrhosis; AST, asparate aminotransferase; ALT, alanine aminotransferase; O/E ratio, ratio of observed to expected number; HCV-RNA, hepatitis C virus RNA.

similar classification was possible based on the type of controls: hospital or community controls (35,37,40–46,48,49,51–56,58) ($n = 18$) vs. patients with CLD (39,47,50,56,57) or HBV carriers (36) ($n = 6$; one study (56) included hospital controls as well) (Table 2). In six case–control studies, both HCV and HBV infections were taken into account or were controlled for (46,47,50,56–58).

A summary of the magnitude of association for the cohort and case–control studies is shown in Tables 3 and 4, respectively. Among all 22 cohort studies identified, nine (13,16,21,23,24,27–30) reported strong positive associations between alcohol drinking and liver cancer, three (14,19,32) reported moderate positive associations and two reported weak positive associations (26,34) (Tables 1 and 3). Of the remaining eight studies, three (18,20,25) observed no association and five (15,17,22,31,33) demonstrated weak to moderate inverse associations; such inverse associations were detected mostly in follow-up studies of patients with CLD (particularly, cirrhotic patients) (15,17,22,33). In some cohort studies targeting mostly healthy subjects, the observed risk was higher in former than current drinkers (19,31,32). Among the seven cohort studies in which mostly healthy subjects were followed, five (13,14,16,19,32) revealed at least weak positive associations, whereas eight (21,23,24,27–30,34) out of the 14 follow-up studies of patients with CLD showed such positive associations.

Among all 24 case–control studies identified, strong positive associations were found in 14 (35,36,40,42–44,47,49–51,54–56,58), moderate positive associations in four (38,41,45,53) and a weak positive association in one (37) (Tables 2 and 4). For the remainder, no association was reported in four (39,46,48,52) and a moderate inverse association was reported in one (57). In the 18 case–control studies employing hospital or community controls, 15 (35,37,40–45,49,51,53–56,58) demonstrated at least weak positive associations, whereas four (36,47,50,56) out of six case–control studies using controls of CLD patients or HBV carriers afforded such positive associations.

Overall, about 60% of the cohort studies identified reported weak to strong positive associations between alcohol drinking and liver cancer risk, although all such studies are done on mostly healthy subjects lacking information on hepatitis virus infection. Since there is no reason to consider that individuals with chronic HCV or HBV infection tend to consume more alcohol than those without, potential confounding by such viral infection is unlikely to explain the positive associations found. Cohort studies of mostly healthy subjects demonstrated fairly consistent positive associations, yet several follow-up studies on CLD patients (particularly, cirrhotic patients) reported no association (18,20) or even inverse associations (15,17,22,33), which may be due to the following reasons.

First, among CLD patients, the severity of liver disease may confound the association with alcohol consumption. If patients with more severe liver disease tend to drink less alcohol at baseline for any reason (e.g. impaired liver

Table 2. Case–control studies on alcohol drinking and liver cancer among Japanese

Reference	Study period	Study subjects				Category	Relative risk (95% CI or *P*)	*P* for trend	Confounding variables considered	Comments
		Type and source	Definition	Number of cases	Number of controls					
Inaba et al. (35)	1977–79	Hospital-based (7 hospitals in Yamanashi)	Cases: 58% were histologically confirmed; Controls: patients without hepatic disease	62 (49 men and 13 women)	62 (49 men and 13 women)	Not daily	1.0		Matched (1:1) for sex, age, and hospital Adjusted for matching factors	HBsAg was tested but not adjusted for. Anti-HCV was not tested.
						Daily	3.2 ($P < 0.05$)			
Oshima et al. (36)	1972–80	Nested case–control (HBsAg-positive blood donors at Osaka Red Cross Blood Center)	Cases: confirmed by record linkage with the Osaka Cancer Registry; Controls: healthy HBV carriers	20 men	40 men	None or <1 go/day	1.0	<0.05	Matched (1:2) for birth year Adjusted for smoking	All subjects were HBsAg-positive. Anti-HCV was not tested.
						1– <3 go/day	5.4			
						≥3 go/day	8.0			
Hiraga et al. (37)	1981–85	Hospital-based (one university hospital)	Cases: 50% were histologically confirmed as HCC; Controls: inpatients or outpatients with various diseases	78 men	78 men	Not daily	1.0		Matched (1:1) for age and residential area Adjusted for matching factors	HBsAg was tested but not adjusted for. Anti-HCV was not tested.
						Daily	1.7 (0.8–4.0)			
Kiyosawa et al. (38)	1980–87	Nested case–control (military men who had undergone angiography with thorotrast between 1943 and 1946)	Cases: confirmed by autopsy and/or serological and imaging examinations; Controls: no liver tumor by biochemical and serological tests and imaging examinations	36 men	67 men	For primary liver cancer			No matching	HBsAg was tested but not adjusted for.
						≥80 g/day	1.0		No adjustment	Anti-HCV was not tested.
						<80 g/day	1.21 (0.54–2.74)			The relative risk was not described in the original paper, and was estimated by one of the authors (KT).
						For HCC				
						≥80 g/day	1.0			
						<80 g/day	2.91 (0.95–8.92)			
Kobayashi et al. (39)	1975–88	Hospital-based (Kanazawa University Hospital)	Cases: cirrhotic patients with HCC at autopsy; Controls: cirrhotic patients without HCC at autopsy	48 (40 men and 8 women)	40 (27 men and 13 women)	Alcohol intake (≥75 g/day, ≥10 years)			No matching	HBsAg was tested but not adjusted for.
						No	1.0		No adjustment	Anti-HCV was not tested.
						Yes	1.4 (0.6–3.4)			The relative risk was not described in the original paper, and was estimated by one of the authors (KT).
Tsukuma et al. (40)	1983–87	Hospital-based (Center for Adult Diseases, Osaka)	Cases: histologically confirmed as HCC; Controls: inpatients with gastrointestinal disease, or examinees for health checkups or gastroendoscopy; no liver	229 (192 men and 37 women)	266 (192 men and 74 women)	Not heavy	1.0		Frequency-matched for sex and age Adjusted for sex, age, HBsAg, history of blood transfusion, smoking, and family history of liver cancer	Anti-HCV was not tested. Heavy drinking was defined as drinking 3 'go's of sake per day for >10 years.
						Heavy	3.2 (2.0–5.1)			
						0–9999 'go's	1.0	0.03		
						10 000–39 999 'go's	1.0 (0.6–1.6)			

Continued