Table 2. *Continued*

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					
			disease, cancer, or smoking/alcohol-related disease			≥40 000 'go's	2.2 (1.2–4.0)			
Tanaka et al. (41)	1985–89	Hospital-based (Kyushu University Hospital)	Cases: 40% were histologically confirmed as HCC; Controls: health examinees at a public health center	204 (168 men and 36 women)	410 (291 men and 119 women)	Non-drinker	1.0		Frequency-matched for sex and age. Adjusted for sex, age, HBsAg, history of blood transfusion, smoking, and family history of liver disease	Anti-HCV status was available for part of the subjects, but not adjusted for.
						Ever-drinker	1.3 (0.9–2.0)			Heavy drinking was defined as having consumed ≥80 ml of ethanol per day for ≥10 years.
						Not heavy	1.0			The 'drink-years' was calculated by multiplying the daily alcohol use in 'drink' (23 ml of ethanol) by the number of years of consumption.
						Heavy	2.0 (1.2–3.1)			
						Non-drinker	1.0	0.02		
						0.1–33.9 drink-years	1.2 (0.7–2.1)			
						34.0–76.6 drink-years	1.0 (0.5–1.8)			
						≥76.7 drink-years	2.0 (1.2–3.5)			
						Sake				
						<10 drink-years	1.0			
						≥10 drink-years	1.6 (1.1–2.3)			
						Beer				
						<10 drink-years	1.0			
						≥10 drink-years	1.0 (0.7–1.5)			
						Shochu				
						<10 drink-years	1.0			
						≥10 drink-years	1.0 (0.6–1.6)			
						Whisky				

						<10 drink-years	1.0			
						≥10 drink-years	1.8 (1.2–2.9)			
Haratake et al. (42)	1980–90	Hospital-based (University of Occupational and Environmental Health)	Cases: patients with surgically resected HCC; Controls: patients without liver disease	145 (120 men and 25 women)	83 (46 men and 37 women)	Alcohol index, male				HBsAg and anti-HCV status was available for part of the subjects, but not adjusted for.
						<60	1.0		No adjustment	The relative risk was not described in the original paper, and was estimated by one of the authors (KT).
						≥60	2.5 (1.1–5.7)			The alcohol index was calculated by multiplying the daily alcohol use in 'go' of sake (28 ml of ethanol) by the number of years of consumption.
Fukuda et al. (43)	1986–92	Hospital-based (Kurume University Hospital)	Cases: 77% were histologically confirmed as HCC; Controls: inpatients without chronic hepatitis or cirrhosis in 2 general hospitals in Kurume	368 (287 men and 81 women)	485 (287 men and 198 women)	Non-drinker	1.00		Matched (1:1 for males and 1:4 for females) for sex, age, residence, and time of hospitalization Adjusted for matching factors, HBsAg, history of blood transfusion, and parental history of hepatic diseases	Anti-HCV status was available for part of the subjects, but not adjusted for.
						1–29 drink-years	1.75 (1.12–2.74)			The 'drink-years' represented the accumulated amount of alcohol intake by age 40, which was calculated by multiplying the daily alcohol use in 'drink' (23 ml of ethanol) by the number of years of consumption.
						30–59 drink-years	2.08 (1.14–3.79)			
						≥60 drink-years	3.23 (1.61–6.51)			
Yamaguchi (44)	1976–85	Hospital-based (Kurume University Hospital)	Cases: histologically or clinically confirmed as HCC; Controls: patients without chronic hepatic disorders	466 (385 men and 81 women)	466 (385 men and 81 women)	Male, HBsAg-negative			Matched (1:1) for the year of admission, sex, and age	Analysis was done in male HBsAg-negative subjects alone.
						None	1.0	<0.001	No adjustment	'Heavy' was defined as >540 ml of sake/day (approximately 80 ml of alcohol/day) for >10 years, and 'Moderate' as an intermediate volume.
						Moderate	1.3 (0.8–1.9)			
						Heavy	2.7 (1.8–4.0)			

Continued

Table 2. *Continued*

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					
Une et al. (45)	1986–88	Hospital-based (hospitals or clinics located in Iizuka Health Center District)	Cases: identified by death certificates in the district; Controls: patients treated for diseases other than liver diseases in three large hospitals in the district	133 (96 men and 37 women)	132 (92 men and 40 women)	Male			Matched (1:1) for sex and age Adjusted for sex, age, HBsAg, history of blood transfusion, and smoking	Anti-HCV was not tested.
						Positive drinking	1.08 (0.57–2.05)			
						Female				
						Positive drinking	2.87 (0.57–14.40)			
						Both sexes				
						Positive drinking	1.20 (0.66–2.18)			
Tanaka et al. (46)	1992–93	Hospital-based (Center for Adult Diseases, Osaka)	Cases: patients with HCC who responded to questionnaire (no details described); Controls: patients with cancer of stomach, colon, rectum, or breast, or large intestine polyp	137 (116 men and 21 women)	334 (202 men and 132 women)	Nondrinker	1.0		No matching Adjusted for sex, age, education, smoking, HBsAg, and anti-HCV	HBsAg and anti-HCV status was adjusted for.
						Former drinker	8.7 (1.6–46.3)			
						Occasional drinker	0.7 (0.2–2.0)			
						<80 g ethanol/day	0.4 (0.1–1.4)			
						≥80 g ethanol/day	1.4 (0.4–5.5)			
Chiba et al. (47)	1991–93	Hospital-based (Tsukuba University Hospital)	Cases: HCV-associated cirrhotic patients with HCC established by histology or elevated alpha-fetoprotein together with positive imaging study; Controls: HCV-associated cirrhotic patients without HCC	76 (38 men and 38 women)	128 (63 men and 65 women)	Habitual drinking	3.27 (1.46–7.30)		No matching Adjusted for sex, age, and anti-HBV	All subjects were anti-HCV-positive and HBsAg-negative. Habitual drinking was defined as the average daily alcohol consumption of 80 g or more over a period of more than 5 years.
Murata et al. (48)	1984–93	Nested case–control (male participants in a gastric mass screening by Chiba Cancer Association)	Cases: confirmed by record linkage with Chiba Cancer Registry; Controls: participants in the screening without liver cancer	66 men	132 men	Alcohol intake (cups/day)			Matched (1:2) for sex, birth year, and the first digit of the address code No adjustment	Anti-HCV and HBsAg were not tested. One cup corresponds to 180 ml of sake containing 27 ml of ethanol.
						0	1.0	0.3		
						0.1–1.0	0.6			
						1.1–2.0	0.4			
						2.1+	1.5			

Shibata et al. (49)	1992–95	Hospital-based (Kurume University Hospital)	Cases: confirmed as HCC by histological, angiographical, and/or other findings; Hospital controls (HCs): inpatients without chronic hepatitis or cirrhosis in 2 general hospitals in Kurume; Community controls (CCs): randomly sampled citizens of Kurume	115 males	115 male HCs and 115 male CCs	Based on HCs			Matched (1:1) for sex, age, residence (for HCs), and time of hospitalization (for HCs) Adjusted for matching factors	Anti-HCV and HBsAg status was available, but not adjusted for.
						Non-drinker	1.0			The 'drink-years' represented the accumulated amount of alcohol intake by age 40, which was calculated by multiplying the daily alcohol use in 'drink' (23 ml of ethanol) by the number of years of consumption.
						1–29 drink-years	0.9 (0.5–1.8)			
						30–59 drink-years	1.3 (0.6–2.6)			
						≥60 drink-years	1.9 (0.9–4.3)			
						Based on CCs				
						Non-drinker	1.0			
						1–29 drink-years	2.3 (1.1–4.6)			
						30–59 drink-years	2.0 (0.9–4.4)			
						≥60 drink-years	5.0 (2.0–12.7)			
Mukaiya et al. (50)	1991–93	Hospital-based (Sapporo Medical University Hospital)	Cases: histologically and/or clinically confirmed as HCC; Controls: chronic liver disease (hepatitis or cirrhosis) without HCC	104 men	104 men	Not daily	1.00		Matched (1:1) for age Adjusted for age	Additional adjustment for cigarette smoking, and HBV and HCV infections did not materially alter the results.
						Daily	2.31 (1.20–4.42)			
						<1/week	1.00			
						≥1/week	2.17 (1.09–4.29)			
						<20 ml ethanol/day	1.00			
						≥20 ml/day	2.36 (1.26–4.40)			
Takeshita et al. (51)	1993–96	Hospital-based (20 major hospitals in the southern part of Hyogo prefecture)	Cases: 64% were histologically confirmed as HCC; Controls: outpatients or inpatients with various diseases, but without liver disease positive for HBsAg and/or anti-HCV.	102 (85 men and 17 women)	125 (101 men and 24 women)	Men			Frequency-matched for hospital, sex, age, and living area Adjusted for age and smoking	All the controls were HBsAg-negative and anti-HCV-negative by definition.
						0–19 drink-years	1.0	0.007		The 'drink-years' was calculated by multiplying the daily alcohol use in

Continued

Table 2. *Continued*

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					
										'drink' (15 ml of ethanol) by the number of years of consumption.
						20–39 drink-years	1.7 (0.8–3.5)			
						≥40 drink-years	2.7 (1.3–5.5)			
						Women				
						Not described				
Koide et al. (52)	1994	Hospital-based (Nagoya City University Hospital)	QCases: clinically and/or histologically confirmed as HCC; Community controls: selected from the same resident community as cases, with no signs of hepatic diseases or HCC.	84 (64 men and 20 women)	84 (64 men and 20 women)	Never	1.00		Matched (1:1) for sex and age Adjusted for sex and age	Anti-HCV and HBsAg status was available, but not adjusted for.
						Current + former	1.23 (0.59–2.56)			
Iida et al. (53)	1999–2001	Hospital-based (hospitals in Yamanashi prefecture)	Cases: patients with HCC (no details described); Controls: inpatients at the hospitals same as cases (no details described)	495 (363 men and 132 women)	194 (132 men and 62 women)	Non-heavy drinker	1.00		Matched for sex, age, and time of hospitalization Adjusted for sex	Anti-HCV and HBsAg status was available, but not adjusted for.
						Heavy drinker	1.84 (1.13–2.99)			
Matsuo et al. (54)	1995–2000	Hospital-based (Kurume University Hospital)	Cases: confirmed as HCC by histological, angiographical, and/or other findings; Hospital controls (HCs): inpatients without chronic hepatitis or cirrhosis in 2 general hospitals in Kurume; Community controls (CCs): randomly sampled citizens of Kurume	222 (177 men and 45 women)	326 HCs (177 men and 149 women) and 222 CCs (177 men and 45 women)	Male based on HCs			Matched for sex (1:4 for female HCs and 1:1 for other controls), age, residence (for HCs), and time of hospitalization (for HCs)	Anti-HCV and HBsAg status was available except for CCs, but not adjusted for.
						Non-drinker	1.00		Adjusted for matching factors	
						1–29 drink-years	1.31			
						30–59 drink-years	1.65			
						≥60 drink-years	1.95 ($P < 0.05$)			
						Male based on CCs				
						Non-drinker	1.00			
						1–29 drink-years	2.02 ($P < 0.05$)			
						30–59 drink-years	1.53			
						≥60 drink-years				

							3.19 ($P < 0.05$)		
						Female based on HCs			
						Non-drinker	1.00		
						1–29 drink-years	1.25		
						≥30 drink-years	1.15		
						Female based on CCs			
						Non-drinker	1.00		
						1–29 drink-years	0.50		
						≥30 drink-years	1.00		
Munaka et al. (55)	1997–98	Hospital-based (University of Occupational and Environmental Health Hospital)	Cases: no detailed description Controls: no evidence of cancer in any organ	78 (61 men and 17 women)	139 (94 men and 44 women)	Never	1.00	Unmatched Adjusted for sex and age	Anti-HCV and HBsAg status was available, but not adjusted for.
						1 to <200 000 ml	0.31 (0.15–0.62)		
						200 000– <600 000 ml	0.79 (0.40–1.57)		
						≥600 000 ml	4.52 (2.39–8.55)		
Sakamoto et al. (56)	2001–04	Hospital-based (Saga Medical School Hospital and Saga Prefectural Hospital)	Cases: confirmed as HCC by histological, angiographical, or other radiological findings; Hospital controls (HCs): first-time visitors at the general outpatient clinic of Saga Medical School Hospital; Patients with chronic liver disease without HCC (CLDs): patients with chronic hepatitis or cirrhosis not classified as special types (e.g., biliary cirrhosis)	209 (141 men and 68 women)	275 HCs (180 men and 95 women) and 381 CLDs (205 men and 176 women)	Based on HCs		Unmatched	HBsAg and anti-HCV status was adjusted for.
						Never drinker	1.0	Adjusted for sex, age, smoking, HBsAg, and anti-HCV	One 'go' corresponds to 180 ml of sake containing 23 g of ethanol.
						Former drinker	5.3 (1.6–18.6)		
						Current drinker	2.9 (1.2–7.4)		
						Based on CLDs			
						Never drinker	1.0		
						Former drinker	1.3 (0.7–2.2)		
						Current drinker	1.8 (1.0–3.0)		
						Alcohol intake ('go's/day) during last 1–2 years, based on HCs			
						0	1.0		
						0.1–0.9	3.4 (1.1–10.1)		
						1.0–1.9			

Continued

Table 2. *Continued*

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					
							0.8 (0.2–2.9)			
						2.0–2.9	0.6 (0.2–2.4)			
						3.0–3.9	10.2 (1.7–60.5)			
						4.0+	18.0 (3.0–107.9)			
						Alcohol intake ('go's/day) during last 1–2 years, based on CLDs				
						0	1.0			
						0.1–0.9	1.2 (0.7–2.2)			
						1.0–1.9	1.0 (0.5–2.1)			
						2.0–2.9	1.8 (0.8–4.4)			
						3.0–3.9	5.0 (1.3–19.2)			
						4.0+	9.4 (2.5–35.4)			
Fukushima et al. (57)	2001–02	Hospital-based (Osaka City University Hospital)	Cases: HCV-RNA positive patients with HCC confirmed by either histology or radiological findings; Controls: HCV-RNA positive patients without HCC	73 (34 men and 39 women)	253 (131 men and 122 women)	Cumulative ethanol consumption (kg) during lifetime			Matched for sex, age, and the date of first visit Adjusted for matching factors, years since the first identification of liver disease, interferon treatment, ultrasonographic findings, platelet, AST, albumin, and fasting blood sugar	All patients were HCV-RNA-positive and HBsAg-negative.
						Non-drinker	1.00	0.07		
						<260	0.48 (0.18–1.31)			
						≥260	0.37 (0.13–1.07)			
						Cumulative ethanol consumption (kg) after the first identification of liver disease				
						Non-drinker	1.00	0.3		
						<200	0.48 (0.16–1.41)			
						≥200				

							0.55 (0.18–1.66)		
						Cumulative ethanol consumption (kg) after the first identification of liver disease			
						Non-drinker	1.00	0.8	
						<53	1.22 (0.48–3.10)		
						≥53	1.09 (0.35–3.36)		
Ohishi et al. (58)	1970–2002	Nested case–control (atomic bomb survivors in Hiroshima and Nagasaki)	Cases: patients with incident HCC who had stored serum samples available; Controls: survivors without HCC who had stored serum samples available	224 (136 men and 88 women)	644 (387 men and 257 women)	Alcohol consumption (g of ethanol per day)			Matched (1:3) for sex, age, city, time and method of serum storage, and radiation exposure Adjusted for matching factors, hepatitis virus infection, smoking, coffee, body mass index, diabetes, and radiation dose to the liver
						0	1.00	0.004	
						1–19	1.27 (0.56–2.87)		
						20–39	1.02 (0.34–3.05)		
						40+	4.36 (1.48–13.0)		

HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HC, hospital control; CC, community control; CLD, chronic liver disease.

Table 3. Summary of cohort studies on alcohol drinking and liver cancer among Japanese

Reference	Study period	Study population					Magnitude of association
		Sex	Number of subjects	Age range (years)	Event	Number of incident cases or deaths	
Kono et al. (13)	1965–83	Men	5130	Not specified	Death	51	↑↑↑
Hirayama (14)	1966–82	Men	122261	≥40	Death	788	↑↑
Inaba et al. (15)	1973–88	Men	270 (liver cirrhosis)	Not specified	Death	46	↓↓
Shibata et al. (16)	1958–86	Men	639 (farming area)	40–69	Death	11	–
			677 (fishing area)	40–69	Death	22	↑↑↑
Kato et al. (17)	1987–90	Men and women	1784 (cirrhosis and posttransfusion hepatitis)	≥16	Incidence	122	↓↓
Tsukuma et al. (18)	1987–91	Men and women	917 (chronic liver disease)	40–69	Incidence	54	–
Goodman et al. (19)	1980–89	Men	36 133 (men and women)	Not specified	Incidence	156	–
		Women		Not specified	Incidence	86	↑↑
Chiba et al. (20)	1977–93	Men and women	412 (HCV-associated chronic liver disease)	40–72	Incidence	63	–
Ikeda et al. (21)	1980–?	Men and women	2215 (chronic hepatitis)	13–75	Incidence	89	↑↑↑
Tanaka et al. (22)	1985–95	Men and women	96 (liver cirrhosis)	40–69	Incidence	37	↓↓
Matsushita et al. (23)	1985–94	Men and women	267 (liver cirrhosis)	Not specified	Incidence	67	↑↑ (type B or C) ↑↑↑ (type C)
Aizawa et al. (24)	1981–98	Men and women	153 (HCV-associated chronic liver disease)	20–65	Incidence	Not described	↑↑↑
Mori et al. (25)	1992–97	Men and women	3052	≥30	Incidence	22	–
Noda et al. (26)	1972–92	Men	306 (alcoholics)	21–77	Death	Not described	↑
Hamada et al. (27)	1980–2000	Men and women	469 (HCV-associated chronic liver disease)	Not specified	Incidence	52	↑↑↑
Takimoto et al. (28)	1989–?	Men and women	356 (HCV-associated chronic hepatitis)	Not specified	Incidence	Not described	↑↑↑
Uetake et al. (29)	1988–2000	Men	91 (alcoholic cirrhosis)	34–72	Incidence	13	↑↑↑
Iwasaki et al. (30)	1986–2003	Men and women	792 (HCV-associated chronic liver disease with sustained response to interferon)	Not specified	Incidence	23	↑↑↑
Ogimoto et al. (31)	1988–99	Men	16 715	40–59	Death	184 (number by sex and age not described)	↓
		Men	11 628	60–79	Death		–
		Women	22 528	40–59	Death		↓↓
		Women	16 103	60–79	Death		↓
Nakaya et al. (32)	1990–97	Men	21 201	40–64	Incidence	48	↑↑
Ikeda et al. (33)	1995–2005	Men and women	576 (HCV-associated chronic hepatitis)	Not specified	Incidence	94	↓
			270 (HCV-associated cirrhosis)	Not specified	Incidence	143	–
Ohki et al. (34)	1994–2006	Men and women	1431 (HCV-associated chronic liver disease)	Not specified	Incidence	340	↑

↑↑↑, strongly positive; ↑↑, moderately positive; ↑, weakly positive; –, no association; ↓, weakly inverse; ↓↓, moderately inverse.

Table 4. Summary of case–control studies on alcohol drinking and liver cancer among Japanese

Reference	Study period	Study subjects				Magnitude of association
		Sex	Age range (years)	Number of cases	Number of controls	
Inaba et al. (35)	1977–79	Men and women	Not specified	62	62	↑↑↑
Oshima et al. (36)	1972–80	Men	Not specified	20	40	↑↑↑
Hiraga et al. (37)	1981–85	Men	Not specified	78	78	↑
Kiyosawa et al. (38)	1980–87	Men	Not specified	36 (primary liver cancer)	67 (exposed to thorotrast)	–
				20 (hepatocellular carcinoma)	67 (exposed to thorotrast)	↑↑
Kobayashi et al. (39)	1975–88	Men and women	Not specified	48	40 (cirrhotic patients)	–
Tsukuma et al. (40)	1983–87	Men and women	≤74	229	266	↑↑↑
Tanaka et al. (41)	1985–89	Men and women	40–69	204	410	↑↑
Haratake et al. (42)	1980–90	Men	Not specified	145	83	↑↑↑
Fukuda et al. (43)	1986–92	Men and women	40–69	368	485	↑↑↑
Yamaguchi (44)	1976–85	Men	Not specified	466	466	↑↑↑ (HBsAg-negative)
Une et al. (45)	1986–88	Men	Not specified	96	92	–
		Women	Not specified	37	40	↑↑
Tanaka et al. (46)	1992–93	Men and women	40–79	137	334	–
Chiba et al. (47)	1991–93	Men and women	Not specified	76	128 (HCV-associated cirrhosis)	↑↑↑
Murata et al. (48)	1984–93	Men	Not specified	66	132	–
Shibata et al. (49)	1992–95	Men	40–69	115	115 hospital controls	↑
					115 community controls	↑↑↑
Mukaiya et al. (50)	1991–93	Men	Not specified	104	104 (chronic liver disease)	↑↑↑
Takeshita et al. (51)	1993–96	Men	Not specified	85	101	↑↑↑
Koide et al. (52)	1994	Men and women	46–79	84	84	–
Iida et al. (53)	1999–2001	Men and women	Not specified	495	194	↑↑
Matsuo et al. (54)	1995–2000	Men	40–75	177	177 hospital controls	↑↑
					177 community controls	↑↑↑
		Women	40–75	45	149 hospital controls	–
					149 community controls	–
Munaka et al. (55)	1997–98	Men and women	34–92	78	138	↑↑↑
Sakamoto et al. (56)	2001–2004	Men and women	40–79	209	275 hospital controls	↑↑↑
					381 patients with chronic liver disease	↑↑↑
Fukushima et al. (57)	2001–2002	Men and women	17–85	73	253 (HCV-RNA-positive)	↓↓
Ohishi et al. (58)	1970–2002	Men and women	Not specified	224	644	↑↑↑

↑↑↑, strongly positive; ↑↑, moderately positive; ↑, weakly positive; –, no association; ↓↓, moderately inverse.

function or physicians' advice), even in those with a similar diagnosis (e.g. chronic hepatitis or cirrhosis), alcohol drinking may seem to play no, or even protective, role. Second, among cirrhotic patients, competing risks (i.e. deaths from causes other than liver cancer) may be responsible. For example, if cirrhotic patients with alcoholism continue to drink heavily, they may die of hepatic failure or variceal bleeding before the development of liver cancer. Third, drinking habits at baseline among CLD patients may have changed substantially during follow-up, and the resultant misclassification may have distorted a true association. Fourth, alcohol consumption may actually play no important role in the development of liver cancer from cirrhosis. However, it appears difficult to differentiate these possibilities by observational studies.

In some cohort studies based on mostly healthy subjects, former drinkers experienced a higher risk of liver cancer than never drinkers (19,31,32); in all such studies,

information on hepatitis virus infection and the presence or absence of CLD was missing. In this regard, a plausible explanation is that former drinkers may have included high-risk individuals such as hepatitis virus carriers and CLD patients who had abstained from alcohol because of illness.

In the case–control studies identified, alcohol consumption was almost consistently associated with increased liver cancer risk. This was the case regardless of the type of controls (mostly healthy subjects vs. CLD patients or hepatitis virus carriers), and only one study on patients with chronic hepatitis C reported an inverse association (57), which somewhat differs from the situation in the cohort studies. A possible change in recent drinking habits among CLD patients can be taken into account in case–control studies, but not usually in cohort studies, and this matter might partly account for the above difference, although the exact reason remains unknown.

Since about 90% of patients with HCC in Japan are known to be chronically infected with HCV or HBV (6), the postulation that heavy alcohol consumption causes alcoholic cirrhosis and thereby leads to the development of HCC does not appear to play a major role. Instead, the potential modifying effect of alcohol on HCC risk among HCV- or HBV-infected individuals is likely to be more important. In this connection, most follow-up studies of patients with chronic hepatitis C over the past decade showed fairly consistent positive associations between alcohol drinking and HCC risk (21,24,27,28,30,34), with few exceptions (33). It remains unclear to what extent alcohol consumption increases the HCC risk among the Japanese general population who are not infected with HCV or HBV because no study exists on this issue.

Potential mechanisms linking the use of alcohol with the development of liver cancer are discussed elsewhere (3). As for the role of alcohol among those with HCV infection, which is the most important risk factor of HCC in Japan, several mechanisms including increased viral replication, enhanced HCV quasispecies complexity, increased liver-cell death, suppression of immune responses, iron overload and increased oxidative stress have been suggested (59,60).

The Japanese may be more susceptible than other ethnic groups, to potential carcinogenic effects of alcohol because about half of them represent heterozygous or homozygous carriers of the inactive aldehyde dehydrogenase (ALDH) 2 allele (*ALDH2*2*) (9), who have an excessive accumulation of acetaldehyde after alcohol intake; acetaldehyde has been classified as being possibly carcinogenic to humans (10). Epidemiologic data on the role of the *ALDH2* genotype in hepatocarcinogenesis has been conflicting (49,51,52,55,56,61). Overall, no material differences have been observed in the *ALDH2* genotype distribution between liver cancer patients and control subjects, although two studies of relatively small size reported a significantly increased risk among heterozygous or homozygous carriers of *ALDH2*2* (55,61). Two studies suggested a significantly elevated risk of HCC for *ALDH2*2* carriers vs. non-carriers among drinkers, but not among non-drinkers (55,56).

The IARC has concluded that there is sufficient evidence for the carcinogenicity of ethanol in experimental animals (3). Taken together, this systematic review confirms a biologically plausible positive association between alcohol drinking and liver cancer risk among the Japanese, and a meta-analysis should be conducted to obtain summary estimates for the overall magnitude of association. However, the studies included in this review employed very different categories of alcohol consumption (particularly in reference categories), which has made a meaningful meta-analysis unfeasible. A meta-analysis of several large-cohort studies using common alcohol consumption categories is now underway, and we hope it will address the above issue.

EVALUATION OF EVIDENCE ON ALCOHOL DRINKING AND LIVER CANCER RISK AMONG JAPANESE

From these results and based on assumed biological plausibility as previously evaluated by the IARC (3), we conclude that there is 'convincing' evidence that alcohol drinking increases the risk of primary liver cancer among the Japanese population. High-risk individuals such as patients with CLD and hepatitis virus carriers are strongly recommended to abstain from alcohol use.

Funding

This work was supported by a Grant-in-Aid for the Third Term Comprehensive Control Research for Cancer from the Ministry of Health, Labor and Welfare, Japan.

Conflict of interest statement

None declared.

References

1. Lieber CS. Alcohol and the liver: 1994 update. *Gastroenterology* 1994;106:1085–105.
2. Fattovich G, Stroffolini T, Zagni I, Donato F. Hepatocellular carcinoma in cirrhosis: incidence and risk factors. *Gastroenterology* 2004;127: S35–50.
3. International Agency for Research on Cancer. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans Volume 96. Consumption of Alcoholic Beverages and Ethyl Carbamate (Urethane). Lyon, France: IARC (in press).
4. World Cancer Research Fund, American Institute for Cancer Research. Food, Nutrition, Physical Activity, and the Prevention of Cancer: a Global Perspective. Washington, DC: AICR 2007.
5. Gann Monograph on Cancer Research No. 51. Cancer Mortality and Morbidity Statistics: Japan and the World-2004. Tokyo, Japan: Scientific Societies Press 2004.
6. Ikai I, Arii S, Okazaki M, Okita K, Omata M, Kojiro M, et al. Report of the 17th Nationwide Follow-up Survey of Primary Liver Cancer in Japan. *Hepatol Res* 2007;37:676–91.

7. Tanaka K, Ikematsu H, Hirohata T, Kashiwagi S. Hepatitis C virus infection and risk of hepatocellular carcinoma among Japanese: possible role of type 1b (II) infection. *J Natl Cancer Inst* 1996;88:742–6.
8. Llovet JM, Burroughs A, Bruix J. Hepatocellular carcinoma. *Lancet* 2003;362:1907–17.
9. Goedde HW, Agarwal DP, Fritze G, Meier-Tackmann D, Singh S, Beckmann G, et al. Distribution of ADH2 and ALDH2 genotypes in different populations. *Hum Genet* 1992;88:344–6.
10. International Agency for Research on Cancer. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans Volume 71. Re-evaluation of Some Organic Chemicals, Hydrazine and Hydrogen Peroxide. Lyon, France: IARC 1999.
11. Inoue M, Tsuji I, Wakai K, Nagata C, Mizoue T, Tanaka K, et al. Evaluation based on systematic review of epidemiological evidence among Japanese populations: tobacco smoking and total cancer risk. *Jpn J Clin Oncol* 2005;35:404–11.
12. World Health Organization. WHO Technical Reports Series 916. Diet, Nutrition and the Prevention of Chronic Diseases: Report of a Joint WHO/FAO Expert Consultation. Geneva, WHO 2003.
13. Kono S, Ikeda M, Tokudome S, Nishizumi M, Kuratsune M. Cigarette smoking, alcohol and cancer mortality: a cohort study of male Japanese physicians. *Jpn J Cancer Res* 1987;78:1323–8.
14. Hirayama T. A large-scale cohort study on risk factors for primary liver cancer, with special reference to the role of cigarette smoking. *Cancer Chemother Pharmacol* 1989;23(Suppl.):S114–7.
15. Inaba Y, Kikuchi S, Namihisa T, Ichikawa S. The effect of smoking and drinking habit on the process from liver cirrhosis to liver cancer. *Gan No Rinsho* 1990;36:299–304 (in Japanese).
16. Shibata A, Fukuda K, Toshima H, Tashiro H, Hirohata T. The role of cigarette smoking and drinking in the development of liver cancer: 28 years of observations on male cohort members in a farming and fishing area. *Cancer Detect Prev* 1990;14:617–23.
17. Kato I, Tominaga S, Ikari A. The risk and predictive factors for developing liver cancer among patients with decompensated liver cirrhosis. *Jpn J Clin Oncol* 1992;22:278–85.
18. Tsukuma H, Hiyama T, Tanaka S, Nakao M, Yabuuchi T, Kitamura T, et al. Risk factors for hepatocellular carcinoma among patients with chronic liver disease. *N Engl J Med* 1993;328:1797–801.
19. Goodman MT, Moriwaki H, Vaeth M, Akiba S, Hayabuchi H, Mabuchi K. Prospective cohort study of risk factors for primary liver cancer in Hiroshima and Nagasaki, Japan. *Epidemiology* 1995;6:36–41.
20. Chiba T, Matsuzaki Y, Abei M, Shoda J, Tanaka N, Osuga T, et al. The role of previous hepatitis B virus infection and heavy smoking in hepatitis C virus-related hepatocellular carcinoma. *Am J Gastroenterol* 1996;91:1195–203.
21. Ikeda K, Saitoh S, Suzuki Y, Kobayashi M, Tsubota A, Koida I, et al. Disease progression and hepatocellular carcinogenesis in patients with chronic viral hepatitis: a prospective observation of 2215 patients. *J Hepatol* 1998;28:930–8.
22. Tanaka K, Sakai H, Hashizume M, Hirohata T. A long-term follow-up study on risk factors for hepatocellular carcinoma among Japanese patients with liver cirrhosis. *Jpn J Cancer Res* 1998;89:1241–50.
23. Matsushita E, Kaneko S, Kobayashi K. Evaluation of background factors related to carcinogenesis in cirrhosis. *Proceedings of 20th Inuyama Symposium*. Tokyo: Chugai-igakusha 1998,159–64 (in Japanese).
24. Aizawa Y, Shibamoto Y, Takagi I, Zeniya M, Toda G. Analysis of factors affecting the appearance of hepatocellular carcinoma in patients with chronic hepatitis C. A long term follow-up study after histologic diagnosis. *Cancer* 2000;89:53–9.
25. Mori M, Hara M, Wada I, Hara T, Yamamoto K, Honda M, et al. Prospective study of hepatitis B and C viral infections, cigarette smoking, alcohol consumption, and other factors associated with hepatocellular carcinoma risk in Japan. *Am J Epidemiol* 2000;151:131–9.
26. Noda T, Imamichi H, Tanaka H, Kawata A, Hirano K, Ando T, et al. Cause-specific mortality risk among male alcoholics residing in the Osaka metropolitan area. *Psychiatry Clin Neurosci* 2001;55: 465–72.
27. Hamada H, Yatsuhashi H, Yano K, Daikoku M, Arisawa K, Inoue O, et al. Impact of aging on the development of hepatocellular carcinoma in patients with posttransfusion chronic hepatitis C. *Cancer* 2002;95: 331–9.
28. Takimoto M, Ohkoshi S, Ichida T, Takeda Y, Nomoto M, Asakura H, et al. Interferon inhibits progression of liver fibrosis and reduces the risk of hepatocarcinogenesis in patients with chronic hepatitis C: a retrospective multicenter analysis of 652 patients. *Dig Dis Sci* 2002;47:170–6.
29. Uetake S, Yamauchi M, Itoh S, Kawashima O, Takeda K, Ohata M. Analysis of risk factors for hepatocellular carcinoma in patients with HBs antigen- and anti-HCV antibody-negative alcoholic cirrhosis: clinical significance of prior hepatitis B virus infection. *Alcohol Clin Exp Res* 2003;27:47S–51S.
30. Iwasaki Y, Takaguchi K, Ikeda H, Makino Y, Araki Y, Ando M, et al. Risk factors for hepatocellular carcinoma in hepatitis C patients with sustained virologic response to interferon therapy. *Liver Int* 2004;24:603–10.
31. Ogimoto I, Shibata A, Kurozawa Y, Nose T, Yoshimura T, Suzuki H, et al. Risk of death due to hepatocellular carcinoma among drinkers and ex-drinkers. Univariate analysis of JACC study data. *Kurume Med J* 2004;51:59–70.
32. Nakaya N, Tsubono Y, Kuriyama S, Hozawa A, Shimazu T, Kurashima K, et al. Alcohol consumption and the risk of cancer in Japanese men: the Miyagi cohort study. *Eur J Cancer Prev* 2005;14:169–74.
33. Ikeda K, Marusawa H, Osaki Y, Nakamura T, Kitajima N, Yamashita Y, et al. Antibody to hepatitis B core antigen and risk for hepatitis C-related hepatocellular carcinoma: a prospective study. *Ann Intern Med* 2007;146:649–56.
34. Ohki T, Tateishi R, Sato T, Masuzaki R, Imamura J, Goto T, et al. Obesity is an independent risk factor for hepatocellular carcinoma development in chronic hepatitis C patients. *Clin Gastroenterol Hepatol* 2008;6:459–64.
35. Inaba Y, Maruchi N, Matsuda M, Yoshihara N, Yamamoto S. A case-control study on liver cancer with special emphasis on the possible aetiological role of schistosomiasis. *Int J Epidemiol* 1984;13:408–12.
36. Oshima A, Tsukuma H, Hiyama T, Fujimoto I, Yamano H, Tanaka M. Follow-up study of HBs Ag-positive blood donors with special reference to effect of drinking and smoking on development of liver cancer. *Int J Cancer* 1984;34:775–9.
37. Hiraga M, Araki S, Murata K, Yokoyama K, Terao H. Assessment of the interaction of hepatitis B antigen and alcohol in primary hepatocellular carcinoma: a case-control study. *Jpn J Public Health* 1986;33:636–9 (in Japanese).
38. Kiyosawa K, Imai H, Sodeyama T, Franca ST, Yousuf M, Furuta S, et al. Comparison of anamnestic history, alcohol intake and smoking, nutritional status, and liver dysfunction between thorotrast patients who developed primary liver cancer and those who did not. *Environ Res* 1989;49:166–72.
39. Kobayashi K, Unoura M, Tanaka N, Hattori N. A comparison between hepatocellular carcinoma-developing and non-carcinoma-developing patients with cirrhosis over a long follow-up period. *Hepatogastroenterology* 1990;37:445–8.
40. Tsukuma H, Hiyama T, Oshima A, Sobue T, Fujimoto I, Kasugai H, et al. A case-control study of hepatocellular carcinoma in Osaka, Japan. *Int J Cancer* 1990;45:231–6.
41. Tanaka K, Hirohata T, Takeshita S, Hirohata I, Koga S, Sugimachi K, et al. Hepatitis B virus, cigarette smoking and alcohol consumption in the development of hepatocellular carcinoma: a case-control study in Fukuoka, Japan. *Int J Cancer* 1992;51:509–14.
42. Haratake J, Kasai T, Takeda S. An epidemiologic comparative study of hepatocellular carcinoma caused by various etiologic factors. *Gan No Rinsho* 1992;38:297–301 (in Japanese).
43. Fukuda K, Shibata A, Hirohata I, Tanikawa K, Yamaguchi G, Ishii M. A hospital-based case-control study on hepatocellular carcinoma in Fukuoka and Saga Prefectures, northern Kyushu, Japan. *Jpn J Cancer Res* 1993;84:708–14.
44. Yamaguchi G. Hepatocellular carcinoma and its risk factors–their annual changes and effects on the age at onset. *Kurume Med J* 1993;40:33–40.
45. Une H, Osajima K, Momose Y, Esaki H. A case-control study on liver cancer in the Chikuho area, Fukuoka prefecture. *Minzoku Eisei* 1993;59:241–7.
46. Tanaka H, Hiyama T, Tsukuma H, Imaoka S, Morisada K, Iwanaga T. Association of HBV, HCV, drinking and smoking with the development of hepatocellular carcinoma: a case-control study using hospitalized patients. *Shokaki Gan* 1995;5:117–22 (in Japanese).
47. Chiba T, Matsuzaki Y, Abei M, Shoda J, Aikawa T, Tanaka N, et al. Multivariate analysis of risk factors for hepatocellular carcinoma in

patients with hepatitis C virus-related liver cirrhosis. *J Gastroenterol* 1996;31:552–8.
48. Murata M, Takayama K, Choi BC, Pak AW. A nested case-control study on alcohol drinking, tobacco smoking, and cancer. *Cancer Detect Prev* 1996;20:557–65.
49. Shibata A, Fukuda K, Nishiyori A, Ogimoto I, Sakata R, Tanikawa K. A case-control study on male hepatocellular carcinoma based on hospital and community controls. *J Epidemiol* 1998;8:1–5.
50. Mukaiya M, Nishi M, Miyake H, Hirata K. Chronic liver diseases for the risk of hepatocellular carcinoma: a case-control study in Japan. Etiologic association of alcohol consumption, cigarette smoking and the development of chronic liver diseases. *Hepatogastroenterology* 1998;45:2328–32.
51. Takeshita T, Yang X, Inoue Y, Sato S, Morimoto K. Relationship between alcohol drinking, ADH2 and ALDH2 genotypes, and risk for hepatocellular carcinoma in Japanese. *Cancer Lett* 2000;149: 69–76.
52. Koide T, Ohno T, Huang XE, Iijima Y, Sugihara K, Mizokami M, et al. HBV/HCV infection, alcohol, tobacco and genetic polymorphisms for hepatocellular carcinoma in Nagoya, Japan. *Asian Pac J Cancer Prev* 2000;1:237–43.
53. Iida F, Yamagata Z, Iida R, Hosoda K, Okada S, Matsuda M, et al. Hepatocellular carcinoma in Yamanashi prefecture – a trial of case-control study (third report). *Yamanashi Igaku* 2002;30:1–7 (in Japanese).
54. Matsuo M. Association between diabetes mellitus and hepatocellular carcinoma: results of a hospital- and community-based case-control study. *Kurume Med J* 2003;50:91–8.
55. Munaka M, Kohshi K, Kawamoto T, Takasawa S, Nagata N, Itoh H, et al. Genetic polymorphisms of tobacco- and alcohol-related metabolizing enzymes and the risk of hepatocellular carcinoma. *J Cancer Res Clin Oncol* 2003;129:355–60.
56. Sakamoto T, Hara M, Higaki Y, Ichiba M, Horita M, Mizuta T, et al. Influence of alcohol consumption and gene polymorphisms of ADH2 and ALDH2 on hepatocellular carcinoma in a Japanese population. *Int J Cancer* 2006;118:1501–7.
57. Fukushima W, Tanaka T, Ohfuji S, Habu D, Tamori A, Kawada N, et al. Does alcohol increase the risk of hepatocellular carcinoma among patients with hepatitis C virus infection? *Hepatol Res* 2006;34: 141–9.
58. Ohishi W, Fujiwara S, Cologne JB, Suzuki G, Akahoshi M, Nishi N, et al. Risk factors for hepatocellular carcinoma in a Japanese population: a nested case-control study. *Cancer Epidemiol Biomarkers Prev* 2008;17:846–54.
59. Vento S, Cainelli F. Does hepatitis C virus cause severe liver disease only in people who drink alcohol? *Lancet Infect Dis* 2002;2303–9.
60. Koike K, Tsutsumi T, Miyoshi H, Shinzawa S, Shintani Y, Fujie H, et al. Molecular basis for the synergy between alcohol and hepatitis C virus in hepatocarcinogenesis. *J Gastroenterol Hepatol* 2008;23(Suppl. 1):S87–91.
61. Kato S, Tajiri T, Matsukura N, Matsuda N, Taniai N, Mamada H, et al. Genetic polymorphisms of aldehyde dehydrogenase 2, cytochrome p450 2E1 for liver cancer risk in HCV antibody-positive japanese patients and the variations of CYP2E1 mRNA expression levels in the liver due to its polymorphism. *Scand J Gastroenterol* 2003;38: 886–93.

Appendix

Research group members: Shoichiro Tsugane [principal investigator], Manami Inoue, Shizuka Sasazuki, Motoki Iwasaki, Tetsuya Otani [until 2006], Norie Kurahashi [since 2007], Taichi Shimazu [since 2007] (National Cancer Center, Tokyo); Ichiro Tsuji [since 2004], Yoshitaka Tsubono [in 2003] (Tohoku University, Sendai); Yoshikazu Nishino (Miyagi Cancer Research Institute, Natori, Miyagi); Kenji Wakai (Nagoya University, Nagoya); Keitaro Matsuo [since 2006] (Aichi Cancer Center, Nagoya); Chisato Nagata (Gifu University, Gifu); Tetsuya Mizoue (International Medical Center of Japan, Tokyo); Keitaro Tanaka (Saga University, Saga).

Case–control study on cigarette smoking and the risk of hepatocellular carcinoma among Japanese

Megumi Hara,[1] Keitaro Tanaka,[1,5] Tatsuhiko Sakamoto,[1] Yasuki Higaki,[1] Toshihiko Mizuta,[2] Yuichiro Eguchi,[2] Tsutomu Yasutake,[2] Iwata Ozaki,[2] Kyosuke Yamamoto,[2] Shingo Onohara,[4] Seiji Kawazoe,[4] Hirohisa Shigematsu[4] and Shunzo Koizumi[3]

Departments of [1]Preventive Medicine, [2]Internal Medicine and [3]General Medicine, Faculty of Medicine, Saga University, 5-1-1 Nabeshima, Saga 849-8501; [4]Department of Internal Medicine, Saga Prefectural Hospital Koseikan, 1-12-9 Mizugae, Saga 840-8571, Japan

(Received August 27, 2007/Revised September 13, 2007/Accepted September 15, 2007/Online publication October 23, 2007)

Emerging epidemiologic data suggest that cigarette smoking may increase the risk of hepatocellular carcinoma (HCC), yet considerable controversies (e.g. inconsistent dose–response relationships) still exist with this association. We examined whether smoking was associated with HCC risk in a case–control study including 209 incident HCC cases and two different control groups (256 hospital controls and 381 patients with chronic liver disease [CLD] without HCC). Comparison of HCC cases with CLD patients, but not with hospital controls, demonstrated a significantly increased risk of HCC for current smokers. After adjustment for sex, age, heavy drinking history and hepatitis virus markers, odds ratios (and 95% confidence intervals) for former and current smokers relative to never smokers were 1.0 (0.6–1.7) and 2.5 (1.4–4.6), respectively, against CLD patients, as compared with 0.8 (0.3–2.3) and 1.8 (0.6–5.1), respectively, against hospital controls. In terms of pack-years during lifetime, dose–response relationship was not evident against either control group (*P* trend = 0.43), but it became clearer for more recent cigarette use among CLD patients. For example, regarding cumulative cigarette consumption during the last 5 years, adjusted odds ratios (and 95% confidence intervals) for 1–4 and 5+ pack-years relative to no use were 1.9 (1.1–3.6) and 2.8 (1.5–5.2) (*P* trend = 0.003), respectively. These results suggest that cigarette smoking may play a crucial role in the late stage of HCC development and that CLD patients may benefit from their earliest smoking cessation. (*Cancer Sci* 2008; 99: 93–97)

Chronic infections with hepatitis C and B viruses are two major causative factors for hepatocellular carcinoma (HCC) in Japan, where more than 90% of HCC occurrences are attributable to at least either infection.[1,2] However, there is considerable experimental and epidemiologic evidence indicating that HCC development is a multistage process and is influenced by other environmental and genetic factors, and tobacco use has been suspected as one such candidate.[3]

Recently, the International Agency for Research on Cancer classified liver cancer as a tobacco-related malignancy.[4] However, the following issues remain to be resolved. First, the dose–response relationship between smoking and HCC risk has been unclear in most epidemiologic studies (particularly, case–control studies).[5] Second, possible confounding by hepatitis virus infection has not been considered in most studies (especially, cohort studies). Third, potential virus and smoking interactions have not fully been explored. For example, initial case–control studies suggested an increased risk of HCC among smokers that are seronegative for hepatitis B surface antigen (HBsAg),[6,7] whereas a recent nested case–control study revealed an elevated risk among smokers seropositive for antibody to hepatitis C virus (anti-HCV).[8] In addition, study populations have substantially been heterogeneous (e.g. almost healthy individuals, hepatitis virus carriers, or patients with chronic liver disease [CLD]), making the target population of possible smoking intervention indefinite.

In an attempt to address the above issues, we conducted a case–control study of HCC including two different control groups (hospital controls and patients with CLD without HCC); the former represents a conventional control group when the natural history of disease is unknown, and the latter was selected based on the clinically established finding that the majority of HCC patients, at least in Japan, have pre-existing CLD.[1]

Materials and Methods

Subjects. The details of the study subjects and methods have been described elsewhere.[9] In brief, all study subjects were restricted to being residents of Saga prefecture, Japan, who were 40–79 years old at the time of identification. During a 3-year period between April 2001 and March 2004, we identified 226 incident cases with HCC from among in- or outpatients of two main hospitals in Saga City (Saga Medical School Hospital and Saga Prefectural Hospital), of whom 209 (92%) agreed to participate. Confirmation of their HCC diagnosis was based on biopsy ($n = 59$), angiography ($n = 123$), or other imaging methods ($n = 27$). Of the 209 cases, 198 (95%) had pre-existing CLD (cirrhosis 167, chronic hepatitis 31).

Hospital controls 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: (i) men aged 50–79 years; (ii) women aged 60–79 years; (iii) men aged 40–49 years; and (iv) 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. Of 379 eligible outpatients contacted, 275 (73%) agreed to participate. These controls had various, mostly minor, diseases ($n = 190$), undiagnosed symptoms ($n = 49$), and no definite abnormality ($n = 36$). We excluded 19 controls whose final diagnoses for their current visits were smoking-related diseases,[10] (cancer seven, coronary heart disease two, chronic obstructive pulmonary disease five, chronic pharyngitis/laryngitis two, gastric ulcer three), leaving 256 controls for data analysis.

Patients with CLD without HCC were out- or inpatients of the two hospitals 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. Of 397 eligible patients contacted, 381 (96%) agreed to participate. These CLD patients had chronic hepatitis ($n = 298$; 266 with hepatitis C alone, 20 with hepatitis B alone,

[5]To whom correspondence should be addressed.
E-mail: tanakake@post.saga-med.ac.jp

doi: 10.1111/j.1349-7006.2007.00645.x

three with both, and nine with other origins) or cirrhosis ($n = 83$; 56 with hepatitis C alone, 10 with hepatitis B alone, two with both, and 15 with other origins) and had no evidence of HCC by radiological findings.

The study protocol was approved by the ethics committees of the two hospitals, and written informed consent to the use of blood and clinical information for this study was obtained from all subjects.

Interviews. Research nurses interviewed the study subjects using a structured questionnaire on demographic and lifestyle factors. The questionnaire elicited information on whether they had a 'heavy drinking history', which was defined as having drunk 69 g or more of ethanol per day for 10 or more years. A closed-end question queried about current smoking habit (never, former, or current smokers), with subsequent inquiries to former and current smokers about the number of cigarettes smoked per day and the duration of smoking in years, as well as the time of quitting smoking for former smokers. We defined 'never smokers' as individuals who had never smoked or had smoked for less than one year, 'former smokers' as those who had stopped smoking one or more years before, and 'current smokers' as those who currently smoked or had stopped smoking less than one year before. The cumulative amount of smoking was calculated as pack-years (packs [1 pack = 20 cigarettes]/day × years of smoking) during lifetime or different time periods of life (e.g. last 10 years).

Hepatitis virus markers. Venous blood was collected from each subject, and plasma HBsAg and anti-HCV were assayed using a chemiluminescent immunoassay (CLIA; Dinabot, Tokyo) and a second-generation enzyme immunoassay (Abbott HCV EIA II; Dinabot, Tokyo), respectively, at an external laboratory (SRL, Tokyo).

Statistical analysis. χ^2 tests (for numbers and proportions) and Mann–Whitney tests (for continuous variables) were used for univariate analyses. The odds ratios (OR) and 95% confidence intervals (CI) of HCC for smoking habits were estimated by using unconditional logistic regression analysis, with adjustment for sex, age category (40–49, 50–59, 60–69, and 70–79), heavy drinking history (never and ever), and HBsAg and anti-HCV status. To assess linear trends in HCC risk associated with pack-years, a continuous variable of pack-years as well as covariates was included in the logistic model. Since female smokers were very few, we made analyses for men and women combined with adjustment for sex. All reported P-values are two-sided, and P-values less than 0.05 were considered statistically significant. All statistical analyses were carried out with the STATA statistical package (StataCorp, College Station, TX, USA).

Results

Table 1 shows basic characteristics of study subjects. As compared with at least either control group, HCC cases presented higher proportions of males ($P < 0.01$ against CLD patients), older subjects ($P < 0.01$ against both control groups), HBsAg positives ($P < 0.01$ against hospital controls), anti-HCV positives ($P < 0.01$ against hospital controls), males with a heavy drinking history ($P < 0.01$ against both control groups), and male current smokers ($P = 0.03$ against hospital controls, $P = 0.07$ against CLD patients). The median years since smoking cessation among former smokers ranged from 10 to 22 and did not significantly differ between HCC cases and either control group in either sex.

The relationship between smoking histories and HCC is shown in Table 2. After adjustment for sex, age, heavy drinking history, HBsAg and anti-HCV, the HCC risk was elevated for current versus never smokers (OR 1.8, 95% CI 0.6–5.1 against hospital controls; OR 2.5, 95% CI 1.4–4.6 against CLD patients) but not for former versus never smokers (OR 0.8 and 1.0, respectively). In terms of pack-years during lifetime, the dose–response relationship with HCC risk was not evident against either control group (P trend = 0.43), although some risk excess was observed for light to moderate consumption categories.

Since the comparison between HCC cases and CLD patients demonstrated a significantly increased risk for current smoking but not for pack-years during lifetime, we speculated that more recent cigarette use might be associated with higher HCC risk. To examine this possibility quantitatively, we calculated pack-years during different time periods (last 40, 20, 10 and 5 years) and associated OR (Table 3). Although no significant association was detected against hospital controls, significant dose–response relationships with pack-years during the last 10 or 5 years were observed against CLD patients. For example, the adjusted OR (and 95% CI) for 1–4 and 5+ pack-years during the

Table 1. Basic characteristics of study subjects

Factor	HCC cases (n = 209)	Hospital controls (n = 256)	CLD patients (n = 381)	$P^{a,\dagger}$	$P^{b,\dagger}$
Male : female (number)	141:68	167:89	205:176	0.61	<0.01
Age (years, median)	69	61	61	<0.01	<0.01
HBsAg-positive (%)	9.1	2.3	9.2	<0.01	0.97
Anti-HCV-positive (%)	85.6	7.8	85.8	<0.01	0.95
Heavy drinking history, male (%)	32.6	12.6	17.1	<0.01	<0.01
Heavy drinking history, female (%)	4.4	1.1	2.3	0.20	0.37
Smoking habit, male (%)					
Never smoker	17.0	28.1	26.3	0.03	0.07
Former smoker	36.2	37.7	37.1		
Current smoker	46.8	34.1	36.6		
Smoking habit, female (%)					
Never smoker	89.7	94.4	85.2	0.47	0.66
Former smoker	5.9	2.2	8.5		
Current smoker	4.4	3.4	6.3		
Years since smoking cessation (median)					
Male former smoker	18.0	15.0	15.5	0.09	0.22
Female former smoker	14.0	21.5	10.0	1.00	0.58

†P-value for the difference between hepatocellular carcinoma (HCC) cases and hospital controls.‡χ^2 tests (for numbers and proportions) or Mann–Whitney tests (for continuous variables).§P for the difference between HCC cases and chronic liver disease (CLD) patients. Anti-HCV, antibody to hepatitis C virus; HBsAg, hepatitis B surface antigen.

doi: 10.1111/j.1349-7006.2007.00645.x

Table 2. Adjusted odds ratios (OR) (and 95% confidence intervals [CI]) of hepatocellular carcinoma (HCC) according to smoking habits

Smoking habits	HCC cases versus hospital controls		HCC cases versus CLD patients	
	Number of cases/controls	OR† (95% CI)	Number of cases/controls	OR† (95% CI)
Never smoker	85/131	1.0 (reference)	85/204	1.0 (reference)
Former smoker	55/65	0.8 (0.3–2.3)	55/91	1.0 (0.6–1.7)
Current smoker	69/60	1.8 (0.6–5.1)	69/86	2.5 (1.4–4.6)
1–19 cigarettes/day	30/20	2.5 (0.7–9.2)	30/37	2.3 (1.1–4.7)
20+ cigarettes/day	39/40	1.4 (0.4–4.6)	39/49	2.7 (1.4–5.6)
Pack-years during lifetime				
0	85/131	1.0 (reference)	85/204	1.0 (reference)
1–19	32/31	3.0 (0.9–10.3)	32/63	1.3 (0.7–2.5)
20–39	48/58	0.9 (0.3–2.7)	48/62	2.0 (1.1–3.8)
40+	44/36	0.8 (0.2–2.5)	44/52	1.1 (0.6–2.2)
		P trend = 0.43		*P* trend = 0.43

†Adjusted for sex, age, heavy drinking history, hepatitis B surface antigen, and antibody to hepatitis C virus. CLD, chronic liver disease.

Table 3. Adjusted odds ratios (OR) (and 95% confidence intervals [CI]) of hepatocellular carcinoma (HCC) according to pack-years during different time periods

Pack-years	HCC cases versus hospital controls		HCC cases versus CLD patients	
	Number of cases/controls	OR† (95% CI)	Number of cases/controls	OR† (95% CI)
During last 40 years				
0	90/136	1.0 (reference)	90/207	1.0 (reference)
1–39	81/95	1.1 (0.4–2.9)	81/135	1.4 (0.8–2.2)
40+	38/25	1.4 (0.4–4.8)	38/39	1.6 (0.8–3.1)
		P trend = 0.58		*P* trend = 0.18
During last 20 years				
0	110/151	1.0 (reference)	110/239	1.0 (reference)
1–19	56/61	0.6 (0.2–1.6)	56/82	1.4 (0.8–2.3)
20+	43/44	1.0 (0.3–2.8)	43/60	2.0 (1.1–3.6)
		P trend = 0.99		*P* trend = 0.06
During last 10 years				
0	129/176	1.0 (reference)	129/264	1.0 (reference)
1–9	40/39	1.4 (0.5–3.7)	40/64	1.4 (0.8–2.3)
10+	40/41	1.4 (0.5–3.9)	40/53	2.3 (1.3–4.3)
		P trend = 0.49		*P* trend = 0.01
During last 5 years				
0	135/187	1.0 (reference)	135/285	1.0 (reference)
1–4	34/28	2.2 (0.8–6.4)	34/47	1.9 (1.1–3.6)
5+	40/41	1.6 (0.5–4.4)	40/49	2.8 (1.5–5.2)
		P trend = 0.37		*P* trend = 0.003

†Adjusted for sex, age, heavy drinking history, hepatitis B surface antigen, and antibody to hepatitis C virus. CLD, chronic liver disease.

last 5 years compared with no use were estimated at 1.9 (1.1–3.6) and 2.8 (1.5–5.2), respectively, with a *P* trend of 0.003.

Discussion

Our findings from the comparison between HCC cases and CLD patients lend further support to the positive association between cigarette smoking and HCC risk. Several epidemiologic studies on patients with CLD[11–14] demonstrated a clearer association between smoking and HCC, as seen in this study. On the other hand, our comparison between HCC cases and hospital controls did not show any significant association with cigarette smoking, although some risk elevation was noted for current smokers and more recent cigarette use. This finding also accords with the results from most Japanese case–control studies using hospital or community controls.[5] The above discrepancy was partly because only 2% and 8% of our hospital controls tested positive for HBsAg and anti-HCV, respectively, and adjustment for both markers made the relevant OR very unstable.

The dose–response relationship between cigarette smoking and HCC has been unclear in most epidemiologic studies,[15–19] although a part of cohort studies showed a clearer relation.[11,12,20–22] Based on our comparison of HCC cases with CLD patients, no dose–response relation was evident for pack-years during lifetime, yet more recent cigarette consumption such as pack-years during the last 5 years was significantly associated with HCC risk in a dose-dependent manner. Similarly, Tanaka *et al.* reported that current, but not former, heavy smoking (Brinkman index ≥800) was an independent risk factor for HCC (RR = 4.9) in a case–control study using hospitalized patients.[23] This suggests the possibility that a change in recent smoking habit may have a large effect on smoking-HCC relations, thereby distorting dose–response relationships with pack-years during lifetime or cigarette consumption measured in the remote past.

Based on the results from large cohort studies, Hirayama,[20] and Tsukuma *et al.*[11] suggested that cigarette smoking may be involved in end-stage development of liver cancer, such

as cirrhosis to HCC. Our results supported their hypothesis, since most smokers among HCC cases had lately suffered from advanced CLD such as cirrhosis. For information, the comparison between CLD patients and hospital controls without CLD in this study did not show increased risk for the development of CLD among smokers (data not shown). In light of these findings, cigarette smoking may facilitate tumor promotion or progression, rather than initiation, in multistage hepatocarcinogenesis. Experimental data suggest that rodents exposed to tobacco constituents demonstrate a higher incidence of liver tumor than control animals,[24,25] and that tobacco smoke enhances chemically induced rat hepatocarcinogenesis.[26]

In Japan, only a few epidemiologic studies have considered serologic markers for both hepatitis B and C viruses as potential confounders for the smoking-HCC relation,[11,12,23] although several studies in foreign countries took this consideration.[27–35] Among these, three cohort studies,[11,12,34] and three case–control studies,[23,28,33] reported an overall significant risk increase for smoking although some insignificant risk increase was observed in other studies.[27,29,30] Except for studies on hepatitis virus carriers or CLD patients,[11,12] statistical adjustment for both viral markers generally renders relevant risk estimates imprecise as a result of a relatively low seropositivity among study populations or control groups. Such was the case in our comparison of HCC cases with hospital controls, and much more hospital controls would be required to overcome this problem. However, studying defined high risk populations such as hepatitis virus carriers or CLD patients, rather than making a strenuous effort to recruit a large number of hospital controls, would provide more practical information if one considers that the majority of HCC patients develop from such high risk individuals.

Several studies reported that the positive association with smoking was restricted to or stronger in subjects seronegative for HBsAg and/or anti-HCV,[6,7,27,33,34] as compared with seropositive subjects although other studies demonstrated almost opposite findings.[8,35,36] In the present study, only 17 HCC cases (8.1%) tested negative for both HBsAg and anti-HCV, and thus it was difficult to examine the above virus–smoking interaction. However, our results revealed that cigarette smoking was associated with an increased risk of HCC among CLD patients (predominantly of hepatitis C origin), who can be regarded as a target population for possible smoking intervention. CLD patients may benefit from their earliest smoking cessation, which has not yet been commonly recommended by clinicians or the general public in Japan.

Acknowledgments

We express our deep appreciation to Emeritus Professor J. Tadano of the Department of Laboratory Medicine, Saga Medical School, and the staff of all of the relevant departments for their kind cooperation. This study was supported by Grants-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology (Grant nos. 11670344, 13220014, 14031216, and 15390188) and Grants-in-Aid for the Research on Hepatitis and for the Third Term Comprehensive Control Research for Cancer from the Ministry of Health, Labor and Welfare, Japan.

References

1 Ikai I, Arii S, Ichida T *et al.* Report of the 16th follow-up survey of primary liver cancer. *Hepatol Res* 2005; **32**: 163–72.
2 Tanaka K, Ikematsu H, Hirohata T, Kashiwagi S. Hepatitis C virus infection and risk of hepatocellular carcinoma among Japanese: possible role of type 1b (II) infection. *J Natl Cancer Inst* 1996; **88**: 742–6.
3 Chen CJ, Yu MW, Liaw YF. Epidemiological characteristics and risk factors of hepatocellular carcinoma. *J Gastroenterol Hepatol* 1997; **12**: S294–308.
4 International Agency for Research on Cancer. *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Vol 83. Tobacco Smoke and Involuntary Smoking. Lyon, France: IARC, 2004.
5 Tanaka K, Tsuji I, Wakai K *et al.* Cigarette smoking and liver cancer risk: an evaluation based on a systematic review of epidemiologic evidence among Japanese. *Jpn J Clin Oncol* 2006; **36**: 445–56.
6 Trichopoulos D, MacMahon B, Sparros L, Merikas G. Smoking and hepatitis B-negative primary hepatocellular carcinoma. *J Natl Cancer Inst* 1980; **65**: 111–14.
7 Trichopoulos D, Day NE, Kaklamani E *et al.* Hepatitis B virus, tobacco smoking and ethanol consumption in the etiology of hepatocellular carcinoma. *Int J Cancer* 1987; **39**: 45–9.
8 Fujita Y, Shibata A, Ogimoto I *et al.* The effect of interaction between hepatitis C virus and cigarette smoking on the risk of hepatocellular carcinoma. *Br J Cancer* 2006; **94**: 737–9.
9 Sakamoto T, Hara M, Higaki Y *et al.* Influence of alcohol consumption and gene polymorphisms of ADH2 and ALDH2 on hepatocellular carcinoma in a Japanese population. *Int J Cancer* 2006; **118**: 1501–7.
10 Rothman KJ, Greenland S. Case-control studies. In: Rothman KJ Greenland S, eds. *Modern Epidemiology*, 2nd edn. Philadelphia: Lippincott, Williams & Wilkins, 1998: 93–114.
11 Tsukuma H, Hiyama T, Tanaka S *et al.* Risk factors for hepatocellular carcinoma among patients with chronic liver disease. *N Engl J Med* 1993; **328**: 1797–801.
12 Chiba T, Matsuzaki Y, Abei M *et al.* The role of previous hepatitis B virus infection and heavy smoking in hepatitis C virus-related hepatocellular carcinoma. *Am J Gastroenterol* 1996; **91**: 1195–203.
13 Mukaiya M, Nishi M, Miyake H, Hirata K. Chronic liver diseases for the risk of hepatocellular carcinoma: a case-control study in Japan. Etiologic association of alcohol consumption, cigarette smoking and the development of chronic liver diseases. *Hepatogastroenterology* 1998; **45**: 2328–32.
14 Chen ZM, Liu BQ, Boreham J, Wu YP, Chen JS, Peto R. Smoking and liver cancer in China: case-control comparison of 36 000 liver cancer deaths vs. 17 000 cirrhosis deaths. *Int J Cancer* 2003; **107**: 106–12.
15 Tsukuma H, Hiyama T, Oshima A *et al.* A case-control study of hepatocellular carcinoma in Osaka, Japan. *Int J Cancer* 1990; **45**: 231–6.
16 Tanaka K, Hirohata T, Takeshita S *et al.* Hepatitis B virus, cigarette smoking and alcohol consumption in the development of hepatocellular carcinoma: a case-control study in Fukuoka, Japan. *Int J Cancer* 1992; **51**: 509–14.
17 Goodman MT, Moriwaki H, Vaeth M, Akiba S, Hayabuchi H, Mabuchi K. Prospective cohort study of risk factors for primary liver cancer in Hiroshima and Nagasaki, Japan. *Epidemiology* 1995; **6**: 36–41.
18 Tanaka K, Hirohata T, Fukuda K, Shibata A, Tsukuma H, Hiyama T. Risk factors for hepatocellular carcinoma among Japanese women. *Cancer Causes Control* 1995; **6**: 91–8.
19 Mizoue T, Tokui N, Nishisaka K *et al.* Prospective study on the relation of cigarette smoking with cancer of the liver and stomach in an endemic region. *Int J Epidemiol* 2000; **29**: 232–7.
20 Hirayama T. A large-scale cohort study on risk factors for primary liver cancer, with special reference to the role of cigarette smoking. *Cancer Chemother Pharmacol* 1989; **23** (Suppl): S114–17.
21 Hsing AW, McLaughlin JK, Hrubec Z, Blot WJ, Fraumeni JF Jr. Cigarette smoking and liver cancer among US veterans. *Cancer Causes Control* 1990; **1**: 217–21.
22 Liaw KM, Chen CJ. Mortality attributable to cigarette smoking in Taiwan: a 12-year follow-up study. *Tob Control* 1998; **7**: 141–8.
23 Tanaka H, Hiyama T, Tsukuma H, Imaoka S, Morisada K, Iwanaga T. Association of HBV, HCV, drinking and smoking with the development of hepatocellular carcinoma: a case-control study using hospitalized patients (in Japanese). *Shokaki Gan* 1995; **5**: 117–22.
24 Hecht SS, Chen CB, Ohmori T, Hoffmann D. Comparative carcinogenicity in F344 rats of the tobacco-specific nitrosamines, N′-nitrosonornicotine and 4-(N-methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone. *Cancer Res* 1980; **40**: 298–302.
25 Rivenson A, Hoffmann D, Prokopczyk B, Amin S, Hecht SS. Induction of lung and exocrine pancreas tumors in F344 rats by tobacco-specific and Areca-derived N-nitrosamines. *Cancer Res* 1988; **48**: 6912–17.
26 Nishikawa A, Furukawa F, Miyauchi M *et al.* Enhancement by cigarette smoke exposure of 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline-induced rat hepatocarcinogenesis in close association with elevation of hepatic CYP1A2. *Jpn J Cancer Res* 2002; **93**: 24–31.
27 Yu MC, Tong MJ, Govindarajan S, Henderson BE. Nonviral risk factors for hepatocellular carcinoma in a low-risk population, the non-Asians of Los Angeles County, California. *J Natl Cancer Inst* 1991; **83**: 1820–6.
28 Tzonou A, Trichopoulos D, Kaklamani E, Zavitsanos X, Koumantaki Y, Hsieh CC. Epidemiologic assessment of interactions of hepatitis-C virus

doi: 10.1111/j.1349-7006.2007.00645.x

with seromarkers of hepatitis-B and -D viruses, cirrhosis and tobacco smoking in hepatocellular carcinoma. *Int J Cancer* 1991; **49**: 377–80.
29 Yu MW, You SL, Chang AS, Lu SN, Liaw YF, Chen CJ. Association between hepatitis C virus antibodies and hepatocellular carcinoma in Taiwan. *Cancer Res* 1991; **51**: 5621–5.
30 Yu MW, Chen CJ. Elevated serum testosterone levels and risk of hepatocellular carcinoma. *Cancer Res* 1993; **53**: 790–4.
31 Pyong SJ, Tsukuma H, Hiyama T. Case-control study of hepatocellular carcinoma among Koreans living in Osaka, Japan. *Jpn J Cancer Res* 1994; **85**: 674–9.
32 Shin HR, Lee CU, Park HJ *et al*. Hepatitis B and C virus, Clonorchis sinensis for the risk of liver cancer: a case-control study in Pusan, Korea. *Int J Epidemiol* 1996; **25**: 933–40.
33 Kuper H, Tzonou A, Kaklamani E *et al*. Tobacco smoking, alcohol consumption and their interaction in the causation of hepatocellular carcinoma. *Int J Cancer* 2000; **85**: 498–502.
34 Wang LY, You SL, Lu SN *et al*. Risk of hepatocellular carcinoma and habits of alcohol drinking, betel quid chewing and cigarette smoking: a cohort of 2416 HBsAg-seropositive and 9421 HBsAg-seronegative male residents in Taiwan. *Cancer Causes Control* 2003; **14**: 241–50.
35 Franceschi S, Montella M, Polesel J *et al*. Hepatitis viruses, alcohol, and tobacco in the etiology of hepatocellular carcinoma in Italy. *Cancer Epidemiol Biomarkers Prev* 2006; **15**: 683–9.
36 Jee SH, Ohrr H, Sull JW, Samet JM. Cigarette smoking, alcohol drinking, hepatitis B, and risk for hepatocellular carcinoma in Korea. *J Natl Cancer Inst* 2004; **96**: 1851–6.

Available online at www.sciencedirect.com
ScienceDirect
Cancer Letters 271 (2008) 98–104
CANCER Letters
www.elsevier.com/locate/canlet

Interaction between interleukin-1β −31T/C gene polymorphism and drinking and smoking habits on the risk of hepatocellular carcinoma among Japanese

Tatsuhiko Sakamoto [a], Yasuki Higaki [a], Megumi Hara [a], Masayoshi Ichiba [b], Mikako Horita [a,b], Toshihiko Mizuta [c], Yuichiro Eguchi [c], Tsutomu Yasutake [c], Iwata Ozaki [c], Kyosuke Yamamoto [c], Shingo Onohara [d], Seiji Kawazoe [d], Hirohisa Shigematsu [d], Shunzo Koizumi [e], Keitaro Tanaka [a,*]

[a] *Department of Preventive Medicine, Faculty of Medicine, Saga University, 5-1-1 Nabeshima, Saga, Japan*
[b] *Department of Social and Environmental Medicine, Faculty of Medicine, Saga University, Saga, Japan*
[c] *Department of Internal Medicine, Faculty of Medicine, Saga University, Saga, Japan*
[d] *Department of Internal Medicine, Saga Prefectural Hospital Koseikan, Japan*
[e] *Department of General Medicine, Faculty of Medicine, Saga University, Saga, Japan*

Received 7 April 2008; accepted 26 May 2008

Abstract

The risk of hepatocellular carcinoma (HCC) increases with the severity of hepatic inflammation. Interleukin (IL)-1β and tumor necrosis factor (TNF)-α are proinflammatory cytokines with multiple biological effects and may play essential roles in inflammation-linked tumor development. We conducted a case-control study including 209 incident HCC cases and two control groups (275 hospital controls and 381 patients with chronic liver disease [CLD] without HCC) to investigate whether *IL-1B* and *TNF-A* gene polymorphisms influence HCC susceptibility with any interaction with alcohol and tobacco. By comparing HCC cases with CLD patients, we found that *IL-1B* −31T/C polymorphism was associated with HCC risk among never drinkers and current smokers; adjusted odds ratios (and 95% confidence intervals) for C/T and T/T genotypes compared with C/C genotype were 1.70 (0.76–3.77) and 2.46 (1.05–5.76) (P trend = 0.03), respectively, among never drinkers, and 1.53 (0.60–3.99) and 2.54 (0.81–7.95) (P trend = 0.11), respectively, among current smokers. Similarly, HCC risk associated with heavy alcohol intake and current smoking differed by this polymorphism among CLD patients. *IL-1B* −31T/C polymorphism may modify HCC risk in relation to alcohol intake or smoking.

Keywords: Hepatocellular carcinoma; Interleukin-1β; Tumor necrosis factor-α; Alcohol; Smoking; Case-control study

1. Introduction

Hepatocellular carcinoma (HCC) is one of the leading causes of cancer mortality in Japan, where most HCC patients have chronic infection with

* Corresponding author. Tel.: +81 952 34 2280; fax: +81 952 34 2065.
E-mail address: tanakake@post.saga-med.ac.jp (K. Tanaka).

0304-3835/$ - see front matter © 2008 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.canlet.2008.05.036

hepatitis C virus or hepatitis B virus [1,2]. Epidemiological data also demonstrate that other environmental factors such as alcohol and tobacco enhance the risk of HCC [3,4].

Although the exact mechanism of hepatocarcinogenesis is still incompletely understood, HCC risk increases with the severity of hepatic inflammation [5,6]. Chronic inflammation developing through the action of various inflammatory mediators is known as a cofactor in carcinogenesis [7]. Among inflammatory mediators, proinflammatory cytokines such as interleukin (IL)-1β and tumor necrosis factor (TNF)-α play essential roles and have been implicated in inflammation-associated tumors [8].

IL-1B gene polymorphisms in the promoter region at positions −511C/T and −31T/C, which are in tight linkage disequilibrium [9], have been associated with susceptibility to HCC [10–13] although the direction of this association has been conflicting. A *TNF-A* polymorphism in the promoter region at −308G/A, which has been related to various diseases [14,15] but is known to be rare in the Japanese [16], has also been linked to HCC risk [17]. Another *TNF-A* polymorphism in the promoter region at −1031T/C, which has been related to several diseases [18,19] and is observed in a relatively large proportion of the Japanese [16], has not been examined in causation of HCC.

We conducted a case-control study to investigate whether the above gene polymorphisms of *IL-1B* and *TNF-A* affect HCC risk with any interaction with alcohol and tobacco, both of which have been implicated as not only risk factors of HCC but also correlates with the production of inflammatory cytokines [20,21]. Two different control groups (hospital controls and patients with chronic liver disease [CLD] without HCC) were employed in this study; the former represents a conventional control group, and the latter was selected based on the clinically established finding that the majority of HCC patients in Japan have preexisting CLD [22].

2. Methods

2.1. Subjects

The details of this study have been described elsewhere [23]. Briefly, all study subjects were restricted to residents of Saga Prefecture, Japan, who were aged 40–79 years at the time of identification. We recruited 209 incident HCC cases (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; 198 cases (95%) had preexisting cirrhosis ($n = 167$) or chronic hepatitis ($n = 31$).

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. We selected these controls based on the following order of priority: (i) men aged 50–79 years; (ii) women aged 60–79 years; (iii) men aged 40–49 years; and (iv) women aged 40–59 years. This order was determined by the sex and age distribution of mortality from liver cancer in Saga Prefecture in 1998. The controls had various, mostly minor, diseases ($n = 190$), undiagnosed symptoms ($n = 49$), and no definite abnormality ($n = 36$). Patients with CLD without HCC ($n = 381$, 298 patients with chronic hepatitis and 83 cirrhotic patients, PR = 96%) were out- or in-patients of the two hospitals 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.

The study protocol was approved by the Ethics Committees of the two hospitals, and written informed consent to the use of their blood and clinical information for this study was obtained from all subjects.

2.2. Interview

Research nurses interviewed study subjects on drinking and smoking habits using a uniform questionnaire. We defined "never drinkers" as those who had never drunk or had drunk less than once per week and/or for less than one year, "former drinkers" as those who had quit alcohol one or more years before, and "current drinkers" as those who currently drank or had quit alcohol less than one year before. A "history of heavy drinking" was regarded as present if subjects had imbibed 69 g or more of ethanol per day for 10 or more years. "Never smokers" were defined as individuals who had never smoked or had smoked for less than one year, "former smokers" as those who had stopped smoking one or more years before, and "current smokers" as those who currently smoked or had stopped smoking less than one year before.