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 Toshikazu Mizuta,2 Yuichiro Eguchi,2 Tsutomu Yasutake,2 Iwata Ozaki,2 Kyosuke Yamamoto,2 Shingo Onohara,4 Seiji Kawazoe,4 Hirohisa Shigematsu4 and Shunzo Koizumi3

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)

C hronic 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.3,4

Recently, the International Agency for Research on Cancer classified liver cancer as a tobacco-related malignancy.4,5 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).4,5 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).8,9 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.(3)

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,
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. χ² 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 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 control 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) | P²,† | P²,‡ |
|-----------------------------|---------------------|-----------------------------|------------------------|------|------|
| 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 |

²P-value for the difference between hepatocellular carcinoma (HCC) cases and hospital controls.†χ² tests (for continuous variables) 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.
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.

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

|                  | 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)         |
| 1–19 had smoking | 32/31 3.0 (0.9–10.3)              | 32/63 1.3 (0.7–2.5)            |
| 20–39 smoking    | 48/58 0.9 (0.3–2.7)               | 48/62 2.0 (1.1–3.8)            |
| 40+ smoking      | 44/36 0.8 (0.2–2.5)               | 44/52 1.1 (0.6–2.2)            |

The dose–response relationship between cigarette smoking and HCC has been unclear in most epidemiologic studies. The dose–response relationship has also been unclear in most epidemiologic studies, although a part of cohort studies showed a clearer relation.(15–19) 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.

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)           |

During last 20 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 10 years |                                         |                                |
| 0                | 110/151 1.0 (reference)            | 110/239 1.0 (reference)       |
| 1–9              | 56/61 0.6 (0.2–1.6)               | 56/82 1.4 (0.8–2.3)          |
| 10+              | 43/44 1.0 (0.3–2.8)               | 43/60 2.0 (1.1–3.6)          |

During last 5 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 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)          |

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.

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

|                  | 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)         |
| 1–19 had smoking | 32/31 3.0 (0.9–10.3)              | 32/63 1.3 (0.7–2.5)            |
| 20–39 smoking    | 48/58 0.9 (0.3–2.7)               | 48/62 2.0 (1.1–3.8)            |
| 40+ smoking      | 44/36 0.8 (0.2–2.5)               | 44/52 1.1 (0.6–2.2)            |

The dose–response relationship between cigarette smoking and HCC has been unclear in most epidemiologic studies. 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 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.
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 seroergic for HBsAg and/or anti-HCV,27,29,33,34 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, 1320014, 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. Iai K, 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 lb (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, Waki 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, Sparrlos L, Merikas G. Smoking and hepatitis B-negative primary hepatocellular carcinoma. J Natl Cancer Inst 1980; 65: 119–24.
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: 797–801.
12. Chiba T, Matsuzaki Y, Abe 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, Petos 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.
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 Kaper 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.