Mutual Confounding and Interactive Effects between Hepatitis C and Hepatitis B Viral Infections in Hepatocellular Carcinogenesis: A Population-based Case-Control Study in Taiwan

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Abstract

A population-based case-control study was conducted in Taiwan to determine the hepatitis C virus (HCV)-associated risk of hepatocellular carcinoma (HCC) in a hyperendemic area for hepatitis B virus (HBV) infection. A total of 58 recently diagnosed HCC patients and 225 matched community controls, who participated in a community-based liver cancer screening program, were recruited between March 1991 and March 1994. Control subjects were matched to HCC patients by age (± 3 months), sex, residence, and date of serum sample collection (± 3 months). Serum samples from all study subjects were tested for hepatitis B surface antigen (HBsAg) and antibodies to HCV (anti-HCVs) by enzyme immunoassays, as well as HCV RNA by reverse transcription-PCR assays. Accordingly, patients with HCC were more likely than were controls to be positive for HBsAg (82.8% versus 12.9%, with an odds ratio (OR) of 22.9), anti-HCVs (13.8% versus 4.4%, with an OR of 3.9), and HCV RNA (13.8% versus 5.8%, with an OR of 2.7). After adjustment for anti-HCVs and HBsAg RNA positivities, the matched ORs associated with HBsAg increased to 27.6 and 28.1, respectively, whereas the corresponding adjusted ORs for anti-HCVs and HCV RNA after controlling for HBsAg status were increased to 8.5 and 6.2, respectively. In the meantime, interactive effects between HCV and HBV on risk were also observed. Compared with those who were negative for both anti-HCVs and HBsAg, the matched ORs associated with the sole positivity of anti-HCVs and HBsAg were 4.0 (95% confidence interval = 0.7-24.0) and 24.6 (95% confidence interval = 9.5-64.1), respectively for already 6 HCC cases but none of control subjects were positive for both anti-HCVs and HBsAg. These results indicate that there are mutual confounding and interactive effects between HCV and HBV with respect to their links to HCC in this endemic area of chronic HBV infections.

Introduction

After the cloning of HCV1 genome and the development of an assay for circulating anti-HCV, HCV infection was recognized as a major cause of chronic hepatitis, cirrhosis, and HCC (1-3). On the basis of this generic anti-HCV testing, numerous studies have shown a high prevalence of anti-HCVs in patients with HCC. This prevalence, however, has a wide geographical distribution, with a high positive rate in European and Japanese patients (73-75%; Refs. 4, 5). In contrast, the infection rate was low in South Africa, Taiwan, and China (5.4-29%; Refs. 6-8), where the prevalences of HCC and HBsAg carriage were high. These previous studies, however, were based on false-positive-prone antibody tests, and most of them were conducted based on a series of cases without well-defined comparison groups, making epidemiological interpretations of association more difficult. This population-based case-control study was carried out in Taiwan using both second-generation antibody tests and qualitative/quantitative RNA PCR assays to evaluate HCV infections in patients with HCC and matched community controls to investigate the etiological component played by this hepatotropic virus in an endemic area of chronic HBV infections.

Materials and Methods

In Taiwan, HCC is the leading malignant neoplasm for men and the third highest for women, and a striking geographical variation within the Taiwan area was observed for age-adjusted mortality rates of HCC (9). The highest mortality rate has been found in the Penghu Islets, which are located to the west of Taiwan Island, especially in Paisa and Huhsi townships. Therefore, a community-based liver cancer screening program has been initiated in the Penghu Islets and main Taiwan Island since March 1991 to evaluate the feasibility and cost-effectiveness of the screening program. A total of seven townships were se-
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A total of 13,817 individuals including 4,689 male and 1,795 female residents of the Penghu Islets and 7,333 male residents of main Taiwan Island were recruited for the first-stage screening. The mean ages ± SD were 48.5 ± 10.7 years for male and 49.2 ± 10.2 years for female residents of the Penghu Islets and 47.7 ± 9.8 years for male subjects in main Taiwan Island. There were 2,150 individuals in the Penghu Islets and 2,112 subjects in main Taiwan Island who were positive for at least one of the six first-stage criteria and were subsequently subjected to the second-stage screening by upper abdominal ultrasonography. The ultrasonographic examination identified 60 individuals in the Penghu Islets and 47 subjects in Taiwan Island suspected to be affected with subclinical HCC, and among them, 34 individuals in the Penghu Islets and 24 subjects in Taiwan Island were confirmed as HCC patients according to the following criteria: (a) histological/cytological confirmation (22 cases); or (b) elevated α-fetoprotein levels (400 ng/ml or above) combined with at least one positive image on angiography, sonography, liver scan, computerized tomography, and digital-subtraction angiogram. Pathological examinations were also done for surgically-treated HCC patients.

Of the 58 recently diagnosed HCC patients, 2 were referred to the second-stage screening by upper abdominal ultrasonography. The abdominal ultrasonography was performed with an apparatus with 3.75 MHz real-time linear and sector probes (Models SAL-38B and SSA-240A, Toshiba, Tokyo, Japan) according to a standard procedure. Subjects who had an image compatible with HCC were referred to teaching medical centers for confirmatory examinations, which include aspiration cytology, computerized tomography, and digital-subtraction angiography. Pathological examinations were also done for surgically-treated HCC patients.

With regard to HCV RNA, a single-tube, single-enzyme (tRTh DNA polymerase) combined RT-PCR assay, AMPLICOR HCV (Roche Diagnostic Systems, Branchburg, NJ), was used as described previously (10). Briefly, the combined RT-PCR was performed with a primer pair selected from the highly conserved 5’ UTR of the HCV genome (11, 12). The pair included an upstream primer KY 80, 5’-GCA-GAAAGCGTCTAGCCATGCGGT (nucleotides 56 to 79) and a downstream primer KY 78, 5’-CTGCAAGCACCTATCAGGCAGT (nucleotides 276 to 299). The downstream primer is biotinylated at the 5’ end to facilitate nonradioactive detection of amplification products. The amplification assays were carried out with 100-µl reaction mixtures containing 50 µl of RNA template. Analysis of each serum sample was performed using reagents that provide a Master Mix that contains the tRTh DNA polymerase enzyme, a single primer pair, buffer salts, dATP, dCTP, dGTP, and dUTP. dUTP was incorporated into each amplification product to serve as a substrate for the AmpErase enzyme uracil N-glycosylase to prevent carry-over contamination of previously amplified material. Reaction conditions were optimized for the use of the tRTh DNA polymerase, which, in the absence of manganese, performed both reverse transcription and DNA polymerase functions, obviating the requirement for two enzymes or for two separate reactions. Amplification was carried out in the Perkin-Elmer Cetus GeneAmp PCR system 9600 thermal-cycler with a program that allowed for a 2-min incubation at 50°C (for optimal AmpErase activity to form single-stranded nicks in previously amplified product containing dUTP), followed by a 30-min incubation at 60°C (for the reverse-transcriptase step) and 40 cycles of PCR. Detection of the PCR product was accomplished with an added level of specificity through the use of a solid-phase probe specific for HCV that was coated onto microwell plates. The biotinylated PCR product was hybridized to the microwell plate and detected using an avidin-horseradish peroxidase catalyzed system using conventional microwell plate washer and microwell plate reader (450 nm). The presence of HCV RNA was determined by relating the absorbance of the tested sample to that of the cutoff value. Serum samples of study subjects positive for HCV RNA were further quantitated for HCV RNA levels. For the quantitative PCR assay, HCV RNA was quantified with an RNA QS for 35 PCR cycles. The presence of the QS in each PCR reaction allowed accurate calculation of HCV copy number, normalized for variations in amplification efficiency, and allowed control over potential inhibition of PCR. Detection of amplification products was performed in a microwell format using probes specific for both HCV and the QS. Quantitation was achieved using the ratio of HCV and QS-specific signals and the known copy number of the QS. This assay has a dynamic range from 5 × 10⁴ to 1 × 10⁹/ml.

This single-tube, single-enzyme (tRTh) combined RT-PCR assay, in which HCV RNA can be reverse transcribed into cDNA and then amplified by PCR under a single set of conditions, results in fewer manipulations, and a shorter time required for the assay. In addition to minimizing the possibility of contamination, the use of a thermostable enzyme (tRTh) in the assay permits reverse transcription at elevated temperatures, both improving the specificity of primer extension and improving the efficiency of reverse transcription through destabilization of the secondary RNA structure. The reproducibility of this combined RT-PCR assay, critical to...
The mean ages among patients with HCC according to their HBsAg status were 55.0 ± 5.57, 5.57 ± 0.19, and 6.35 ± 0.13, respectively. The percentage of the coefficient of variation, defined as SD divided by mean of log10-transformed HCV RNA levels, ranged between 2.1 and 5.0%, indicating a low interassay variation. The serum samples of the subjects in this study were analyzed similarly, over the course of multiple runs, without prior knowledge of study group. The PCR primers and the probe used in this study are located in highly conserved regions of the 5' untranslated region of the HCV genome specifically for maximum diagnostic utility. The PCR-based assay successfully amplifies selected samples of known genotype [a, 1b, 2a, 2b, 3, 4, and 6 [classification of Simmonds et al. (13)].

The data were analyzed statistically by several methods. The mean ages among patients with HCC according to their different profiles of HBsAg/anti-HCV test results were compared by ANOVA. The χ² test and Fisher's exact test were used to assess the significances of differences in categorical variables among groups (14). To assess the risk of HCC associated with HBsAg carriage and anti-HCV and HCV RNA positivities, matched and adjusted ORs with their 95% CIs were estimated with the logistic regression model for individually matched data, based on the conditional likelihood function (15). P < 0.05 was used as a criterion for statistical significance, and all calculations were performed using the statistical package of the statistical analysis system (SAS).

Results

Eighty-eight % of 58 cases and 225 controls were male, and 80% of the total subjects had an age between 50 and 64 years old. The mean ages (± SD) of cases and controls were 55.0 (±7.0) and 54.2 (±6.8), respectively. There were no differences in the distribution of age and sex between cases and controls because of the matching scheme.

HBsAg status and anti-HCV and HCV RNA positivities among HCC cases and matched controls are summarized in Table 1. The HBsAg positive rate was statistically significantly higher in HCC cases than in matched controls (82.8% and 12.9%, respectively; P < 0.05). Taking HBsAg negatives as the reference group, the matched OR of the HCC risk was 22.9 (95% CI = 9.7–54.1) for HBsAg positives. After adjustment for anti-HCV and HCV RNA positivities, the matched ORs increased to 27.6 and 28.1, respectively. Anti-HCVs were positive in 8 of 58 (13.8%) HCC cases and 10 of 225 (4.4%) matched controls, with a matched OR of 3.9 (95% CI = 1.4–10.9). After adjustment for HBsAg status, the matched OR increased to 8.8 (95% CI = 1.8–43.0). HCV RNA was detected in 8 of 58 (13.8%) HCC cases and 13 of 225 (5.8%) control subjects, yielding a matched OR of 2.7 (95% CI = 1.1–6.9). After adjustment for HBsAg status, the matched OR associated with HCV RNA positivity increased to 6.2 (95% CI = 1.4–26.6).

To evaluate whether HBsAg status would affect profiles of HCV assays among study subjects, we analyzed the positive rates of anti-HCVs and HCV RNA stratified by the HBsAg status of the subject (data not shown). In the HBsAg-negative stratum, 8 out of 10 (80%) HCC cases were negative for both anti-HCVs and HCV RNA, and the remaining 2 subjects were positive for both markers. Among 196 controls in this stratum, 182 (92.9%) had no evidence of HCV infections. I was positive for anti-HCVs alone, and 4 had sole positivity for HCV RNA, whereas 9 were positive for both tests. In the stratum of HBsAg positivity, anti-HCVs and HCV RNA were both negative for 87.5% (42 of 48) of HCC cases and 100% of 29 controls whereas dual positivity was found in 12.5% (6 of 48) of patients with HCC but none in controls. Overall, there was no statistically significant difference in HCV test results between HBsAg-positive and HBsAg-negative individuals (P = 0.613). In addition, there was also no significant difference in HCV testing profiles between HCC cases and matched controls within each stratum of HBsAg status (P = 0.078 in HBsAg-positive and P = 0.201 in HBsAg-negative strata). We further evaluated the correlation between anti-HCVs and HCV RNA among study subjects and found a high correlation between anti-HCVs and HCV RNA among study subjects; HCV RNA was detectable in all anti-HCV-positive HCC cases and 90% (9 of 10) of anti-HCV-positive control subjects.

We recognized, from the above findings, that assays for anti-HCVs and HCV RNA in the serum were highly correlated and illustrated a similar association with the risk of HCC. In addition, it has been shown that antibody assays are unable to

<table>
<thead>
<tr>
<th>Serological markers</th>
<th>HCC cases</th>
<th>Controls</th>
<th>Matched OR</th>
<th>Adjusted OR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>HBsAg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>10</td>
<td>17.2</td>
<td>196</td>
<td>87.1</td>
</tr>
<tr>
<td>Positive</td>
<td>48</td>
<td>82.8</td>
<td>29</td>
<td>12.9</td>
</tr>
<tr>
<td>Anti-HCVs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>50</td>
<td>86.2</td>
<td>215</td>
<td>95.6</td>
</tr>
<tr>
<td>Positive</td>
<td>8</td>
<td>13.8</td>
<td>10</td>
<td>4.4</td>
</tr>
<tr>
<td>HCV RNA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>50</td>
<td>86.2</td>
<td>212</td>
<td>94.2</td>
</tr>
<tr>
<td>Positive</td>
<td>8</td>
<td>13.8</td>
<td>13</td>
<td>5.8</td>
</tr>
</tbody>
</table>

* Matched by age, sex, residence and date of serum collection.
* In addition to match on age, sex, residence and date of serum collection, the odds ratio associated with HBsAg was also adjusted for anti-HCV, and the odds ratios associated with anti-HCV or HCV RNA were also adjusted for HBsAg status.
* The OR associated with HBsAg was adjusted for HCV RNA.

Table 1 ORs of HCC among 58 HCC cases and 225 matched controls in Taiwan according to serological markers of HBsAg, anti-HCVs, and HCV RNA.
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Table 2  Independent and interactive effects of HCV RNA positivity and HBsAg status on the risk of HCC

<table>
<thead>
<tr>
<th>HBsAg status</th>
<th>HCV RNA positivity</th>
<th>HCC cases</th>
<th>Matched controls</th>
<th>Matched* OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Referent</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>Negative</td>
<td>8 (13.8)</td>
<td>183 (81.3)</td>
<td>1.0</td>
<td>Referent</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>2 (3.5)</td>
<td>13 (5.8)</td>
<td>4.0</td>
<td>0.7-24.0</td>
</tr>
<tr>
<td>Positive</td>
<td>Negative</td>
<td>42 (72.4)</td>
<td>29 (12.9)</td>
<td>24.6</td>
<td>9.5-64.1</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>6 (10.3)</td>
<td>0 (0.0)</td>
<td>×</td>
<td></td>
</tr>
</tbody>
</table>

* Matched by age, sex, residence, and date of serum collection.

Table 3  Association between serum HCV RNA level and HCC among 58 HCC cases and 225 matched controls in Taiwan

<table>
<thead>
<tr>
<th>HCV RNA level (copies/ml)</th>
<th>HCC cases</th>
<th>Matched controls</th>
<th>Matched* OR (95% CI)</th>
<th>HBsAg-adjusted OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undetectable</td>
<td>50 (86.2)</td>
<td>212 (94.2)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Low (≤10⁵)</td>
<td>3 (5.2)</td>
<td>7 (3.1)</td>
<td>1.95 (0.45-8.50)</td>
<td>5.71 (0.60-53.97)</td>
</tr>
<tr>
<td>High (&gt;10⁵)</td>
<td>5 (8.6)</td>
<td>6 (2.7)</td>
<td>3.35 (1.02-11.05)</td>
<td>6.53 (1.12-38.24)</td>
</tr>
</tbody>
</table>

* Matched by age, sex, residence, and date of serum collection.

Discussion

The prevalence of HCV infection in patients with HCC has been extensively investigated worldwide since the development of the first-generation assay for circulating antibodies against HCV (anti-c100-3; Refs. 4, 5, 7, 8, 16). However, the sensitivity and specificity of the first-generation anti-HCV assay, especially for stored sera, has been questioned on several lines of observations (17, 18). It also has been noted that only strongly anti-c100-3 reactive specimens, as defined by absorbance (optical density) 2 or more times higher than the cutoff limit, are specifically associated with the risk of HCC (19). Consequently, the anti-HCV prevalence in patients with HCC may be inflated in these earlier studies. On the basis of the more sensitive and specific second-generation anti-HCV assay, three different global patterns of anti-HCV positivity in HCC patients were observed. HCV infections play a major etiologic role in Japanese and European patients with HCC, with a positive rate ranging from 63 to 66% (20-22); HCV and HBV act in conjunction in HCC patients in Korea, Taiwan, and South Africa, with an anti-HCV positive rate between 17 and 20% (23-25); and HBV has an almost exclusive role in Chinese patients with HCC in eastern and southern China, with the prevalence of anti-HCVs varying from 0 to 9% (6, 26). The prevalence of anti-HCVs among Taiwanese patients with HCC in our current study was 13.8%, which was in accordance with this global pattern. Although the prevalence of anti-HCVs in serum is a reliable marker of HCV infections, its presence does not necessarily indicate active infection with HCV, whereas the presence of HCV RNA in serum, as detected by RNA PCR, provides evidence of active viral replication (27). Accordingly, HCV RNA has been identified in 0 to 62% of patients with HCC worldwide, with the highest prevalence in Japanese and Spanish patients (60% and 62%, respectively; Refs. 20 and 28) and a rate of 0 in Chinese patients in eastern China (26). All these previous studies on HCV RNA among HCC patients, however, were based on case series studies without appropriate comparison groups, rendering the interpretation more difficult. In this current study, HCV RNA positivity was compared between HCC patients and corresponding community controls. Accordingly, HCV RNA was detected in 13.8% of HCC pa-

differentiate between past and present infection, whereas HCV RNA can indicate current active infection. Accordingly, we focused our following analyses on the status of HCV RNA among study subjects.

The independent and combined effects of HBsAg status and HCV RNA positivity on the HCC risk are shown in Table 2. HCV RNA was detected in 20% (2 of 10) of HBsAg-negative HCC patients and 12.5% (6 of 48) of HBsAg-positive HCC patients, whereas 6.6% (13 of 196) of HBsAg-negative controls and none of 29 HBsAg-positive controls had detectable HCV RNA in their serum. Taking subjects who were negative for both HBsAg and HCV RNA as the reference group, the matched OR of developing HCC for HBsAg-negative individuals positive for HCV RNA was 4.0 (95% CI = 0.7-24.0), whereas the corresponding figure for HBsAg-positive subjects negative for HCV RNA was 2.7% (6 of 225) of matched controls had high HCV viremia. Compared with those who had undetectable levels of HCV RNA, the matched ORs of developing HCC for patients with low-viremia and high-viremia HCV infections were 1.95 (95% CI = 0.45-8.50) and 3.35 (95% CI = 1.02-11.05), respectively. After adjustment for HBsAg status, the corresponding figures were 5.71 (95% CI = 0.60-53.97) for those with low-viremia infections and 6.53 (95% CI = 1.12-38.24) for those with high-viremia infections. A statistically significant risk was found in subjects with high viremia levels, whereas there was no significant difference in the HCC risk between high-viremia and low-viremia infections (OR = 1.14; 95% CI = 0.08-17.09).

The prevalence of anti-HCVs in serum is a reliable marker of HCV infections, its presence does not necessarily indicate active infection with HCV, whereas the presence of HCV RNA in serum, as detected by RNA PCR, provides evidence of active viral replication (27). Accordingly, HCV RNA has been identified in 0 to 62% of patients with HCC worldwide, with the highest prevalence in Japanese and Spanish patients (60% and 62%, respectively; Refs. 20 and 28) and a rate of 0 in Chinese patients in eastern China (26). All these previous studies on HCV RNA among HCC patients, however, were based on case series studies without appropriate comparison groups, rendering the interpretation more difficult. In this current study, HCV RNA positivity was compared between HCC patients and corresponding community controls. Accordingly, HCV RNA was detected in 13.8% of HCC pa-
tients and 5.8% of matched controls, yielding a significant matched OR of 2.7. When the effect of HBsAg status was taken into consideration, the matched OR increased to 6.2. This observation implies that in this hyperendemic area of HBV infections, patients with active HCV infections had a 6-fold increased risk of developing HCC compared to noninfected subjects. Recently, Bukh et al. (25) reported a HCV RNA positive rate of 20.3% for 128 southern African blacks with HCC. Their data were consistent with our findings that HCV infections appear to be significantly associated with the HCC risk, whereas HBV was the dominant viral agent responsible for HCC in these endemic areas of chronic HBV infections. In contrast, findings from previous studies in Japan and Europe suggested a more important role for HCV than HBV in the development of HCC in these regions (20–22). These studies with contrasting findings on the role of HBV and HCV in the development of HCC strongly suggest the heterogeneity in the viral etiology of HCC in different geographical areas.

As mentioned earlier, our data demonstrated that individuals with active HCV replication had a 6-fold increased risk of HCC when compared with those without detectable viremia levels, especially among those with high-viremia infections. It has been noted that the severity of liver damage during the course of chronic HCV infections was gradually increased with time (29). Furthermore, serum levels of HCV RNA have been shown to correlate with the clinical stage of liver disease; patients with advanced stages of liver disease including cirrhosis and HCC had higher serum levels of HCV RNA than patients with mild HCV infections (30–32). Taken together, these observations indicate that serum levels of HCV RNA increase with the progression of chronic liver diseases, as demonstrated in previous studies in humans (30–32) and experimentally infected chimpanzees (33), and the infection is still active when the tumor is diagnosed, as observed in the current study. However, solid inferences on the high viremia-associated HCC risk cannot be derived from our analysis, which was based on the case-control study design with a small number of subjects having high viremia levels. The HCC risk associated with high HCV viremia levels might result from the cumulative effect of continuing replication of HCV during the disease progression and/or the loss of immune competence over time in patients with malignant cell transformation. The precise mechanism of replicative HCV-induced liver cell injury and, in turn, hepatocarcinogenesis, needs to be elucidated.

We have shown that HBsAg and HCV positivities are both independent risk factors that significantly confounded one another. This mutual negative confounding (distortion of the estimate of relative risk toward the null value) on the risk of HCC is due to an inverse relationship between HBV and HCV in terms of HBsAg presence in our study population. As shown in Table 2, HCV infection rate was higher in HBsAg-negative individuals than in HBsAg-positive subjects (20 versus 12.5% in HCC patients and 7.1 versus 0% in the control group). This phenomenon has been documented in the literature. That is, on average, anti-HCVs were identified appreciably more often in HBsAg-negative than in HBsAg-positive individuals (59 and 25%, respectively; Ref. 34). These observations underscore the necessity to adjust for this mutual confounding effect between HBV and HCV when studying their individual effects on the risk of HCC. In addition, these findings indicate that HCV plays an important etiological role in HBsAg-negative HCC.

Patients with HCC have been found to have evidence of dual infection with HBV and HCV, with a rate ranging from 4 to 14% in European and Asian patients (20, 23, 24). In addition, the enhanced HCC risk in individuals with dual infection was also observed by several investigators. In a study of Greek patients with HCC, Kaklamani et al. (19) found a 4-fold higher relative risk in anti-HCV-positive patients with HBsAg compared with those negative for HBsAg. Yu et al. (7) conducted a matched case-control study in Taiwan and reported a significant HCC risk associated with dual infection of HBV and HCV. 9 out of 127 HCC cases were positive for both HBsAg and anti-HCVs, compared with none of the 127 matched controls. In accordance with previous studies, 10% of patients with HCC in this study had dual infection, and subjects with such dual infection appear to carry a very high but unmeasurable relative risk when compared with those without dual infection: 6 out of 58 HCC cases and none of the 225 matched controls had dual infection. These study results indicate a conjunctive role of HBV and HCV in the process of hepatocarcinogenesis. On the other hand, a comparison between the ages of HCC patients positive for both HBsAg and anti-HCVs and those possessing only HBsAg does not reveal a significant difference (54.8 ± 3.4 years and 55.5 ± 7.5 years, respectively), as observed in previous studies conducted in Taiwanese HCC patients (35, 36). Furthermore, no clinical differences suggestive of a more aggressive clinical course between patients with dual infection and those without were noted (20). These discrepant observations on the pathogenetic enhancement of dual infection may result from the small numbers of subjects with both HBV and HCV infections included in the studies. Detailed longitudinal studies in patients with dual infection will be required to determine precisely the nature of dual infection in the origin of HCC.

A noteworthy feature of this study was the relatively high frequency (5.8%) of active HCV infections found in community controls, especially for controls from the Penghu Islets (8.5%), where the highest mortality of HCC has been documented (9). In Taiwan, the prevalence of HCV infections in the general population has been estimated to be 1–2% (7, 37). The reasons for such high prevalence of active HCV infections found in our control subjects are unclear. It is possible that control subjects who had participated in the two-stage screening procedures were not a representative sample of the general population. On the other hand, recent studies have identified nondisposable needles for injection and tattooing as major risk factors for the spread of HCV in the community in Taiwan (38, 39). It has been noted that the reuse of needles is still present in some private clinics or illegal medical practices in the study community. Thus, the high prevalence of active HCV infections found in the study area might be attributable to an iatrogenic transmission through the needle or syringe by careless practitioners in the community. Transmission routes for HCV should be clarified for future prevention of HCV propagation in this region.

Before drawing firm conclusions from these data, a number of limitations of the study should be considered. Control subjects in this study were selected from those who were positive for at least 1 of the 6 first-stage screening criteria and were not suspected to be affected with HCC by the second-stage ultrasound examination. As a consequence, individuals in the control group may not be representative of the general population by and large, whereas this selection criterion enhances the comparability between case and control groups in terms of the self-selective participation of the screening program and the intensity of medical surveillance. Thus, the generalizability of the risk estimation may be limited. In addition, as a case-control study of seroprevalent HBV and HCV markers, this study is necessarily limited by our inability to ascribe accurately a temporal relation between viral risk factors and the
risk of developing HCC. The limited number of HCC cases (n = 58) in this study also restrains us from making a precise estimation of the risk (i.e., wide CIs of the relative risk estimate). Nevertheless, this current study incorporating the comparatively epidemiological study design (i.e., cases with a comparable comparison group) with the advances of molecular biological techniques provides the evidence, at population level, that HCV infections do intertwine with chronic HBV infections in HCC and emphasizes the importance of effective intervention on these hepatotropic viral agents to reduce the incidence of HCC in Taiwan.

References
Mutual confounding and interactive effects between hepatitis C and hepatitis B viral infections in hepatocellular carcinogenesis: a population-based case-control study in Taiwan.

C A Sun, H Farzadegan, S L You, et al.