Type 2 Diabetes and Hepatocellular Carcinoma: A Cohort Study in High Prevalence Area of Hepatitis Virus Infection

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This study aimed to elucidate the relationship of type 2 diabetes, other known risk factors, and primary hepatocellular carcinoma (HCC) in countries with a high prevalence of hepatitis infection. We followed a prospective cohort of 54,979 subjects who participated in the Keelung Community-Based Integrated Screening program between 1999 and 2002. A total of 5,732 subjects with type 2 diabetes cases were identified at enrollment on the basis of fasting blood glucose level, and a total of 138 confirmed HCC cases were identified either through two-stage liver cancer screening or linkage with the National Cancer Registry. The independent effect of type 2 diabetes on the incidence of HCC and the interaction between type 2 diabetes and hepatitis infection or lipids profile were assessed using the Cox proportional hazards regression model. After controlling for age, sex, hepatitis B virus (HBV), hepatitis C virus (HCV), smoking, and alcohol consumption, the association between type 2 diabetes and incidence of HCC (excluding 33 prevalent cases identified at enrollment) was modified by HCV status and cholesterol level. The associations were only statistically significant (adjusted hazard ratio [HR] = 2.08 [1.03-4.18]) for being HCV negative and for having hypercholesterolemia (adjusted HR = 2.81 [1.20-6.55]). These statistically significant findings remained even excluding cases of diabetes newly diagnosed at enrollment. In conclusion, in an area with a high prevalence of hepatitis virus infection, type 2 diabetes increases the risk of developing HCC in those who are HCV negative or have a high level of total cholesterol. (HEPATOLOGY 2006;43:1295-1302.)

The association between diabetes mellitus and the incidence of hepatocellular carcinoma (HCC) has been corroborated by epidemiological studies[1-9] and molecular studies related to insulin-like growth factor I (IGF-I) or insulin-like growth factor binding protein-3 (IGFBP-3).[10-12] Despite this, several issues remain unresolved. First, as most of these studies were conducted in Western countries, where the prevalence of hepatitis infection is low, there is some doubt about whether the association between type 2 diabetes and HCC persists in countries with a high prevalence of hepatitis infection. It was speculated that in such countries, type 2 diabetes would play only a minor role in the development of hepatocellular carcinoma. A case-control study in Taiwan found type 2 diabetes not to be associated with the development of HCC.[13] Second, the high prevalence of hepatitis infection also raises the possibility of synergetic interaction between having type 2 diabetes and being hepatitis B positive affecting the risk of HCC. Third, is the question of whether the metabolic changes caused by type 2 diabetes cause steatosis, necrosis (steatohepatitis), and fibrosis, which develop into cryptogenic cirrhosis and further increase the risk of developing HCC.[14,15] As subjects with diabetes are often dyslipidemic, we also investigated whether the effect of type 2 diabetes on the development of HCC is modified by lipid profiles, an issue as yet unaddressed in an epidemiological study. Fourth, the development of HCC may be also affected by lifestyle factors such as smoking or drinking, so it was necessary to take these confounders into account.

Abbreviations: type 2 diabetes: diabetes mellitus; HCC: hepatocellular carcinoma; KCIS, Keelung Community-Based Integrated Screening; LDL: low-density lipoprotein.

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This article is one of a series of studies on Keelung Community-Based Integrated Screening (KCIS 7).

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This study was therefore undertaken to answer two questions:

1. Does diabetes mellitus play an independent role in the development of HCC in Taiwan, as it does in other countries with a low prevalence rate of hepatitis?
2. Is the incidence of type 2 diabetes and liver cancer influenced by the presence of viral hepatitis or modified by lipid profiles or environmental factors, and is there synergy between hepatitis infection and type 2 diabetes or between other factors and type 2 diabetes with regard to the incidence of HCC?

Patients and Methods

Enrollment of Study Cohort. We studied a cohort of participants in the Keelung Community-Based Integrated Screening (KCIS) program in Taiwan in order to elucidate the association between type 2 diabetes, hepatitis infection, and HCC. This multiple screening program, which has been in operation since 1999, was established to offer periodic screening for a variety of cancers and chronic diseases to a target population, the 212,954 residents who were at least 30 years old and were listed in the population registry. Details of the design and implementation of the KCIS program were previously described in full elsewhere.[16,17] In brief, the KCIS program focused on five neoplasms (breast, cervical, colorectal, liver, and oral) and three non-neoplastic chronic conditions (diabetes, hypertension, and high cholesterol). The KCIS program commenced on January 1, 1999, and by the end of 2002, it had recruited a total of 54,979 residents aged 30 years or older, 25% of the entire population. Informed consent was obtained from each participant. The individual components of the multiple screening were phased in at different times. Liver cancer screening, with a two-stage design, was not integrated until April 2000. Because the emphasis of this study was the association between type 2 diabetes and HCC, not the yield of liver cancer screening, and because screening for type 2 diabetes began in September 1999, the enrollment period for HCC cases was from September 1999 to December 2002. We excluded 55 participants who had preexisting primary HCC or other related cancers (ICD-9 codes 155, 156, and 157). We also excluded four individuals for whom the onset of diabetes mellitus occurred before age 18 and four individuals who reportedly died from HCC but for whom no information was available to confirm the diagnosis of HCC (see below). The remaining 54,916 subjects formed the present study cohort.

Study Design. The study design was based on a prospective cohort study, with staggered entry times for participants enrolled between 1999 and 2002. Type 2 diabetes status, including whether newly or previously diagnosed, was assessed at enrollment. HCC cases were identified at enrollment when two-stage liver cancer screening was initiated. The entire study cohort was followed over time to identify incident HCC cases either by subsequent screens or through the linkage of the whole cohort to the Taiwan cancer registry or mortality registry until the end of 2003. After enrollment, each subject had at least 1 year of follow-up. The mean follow-up time was 2.78 ($\pm$ 0.92) years.

Ascertainment of HCC. As liver cancer screening did not begin until April 2000, the HCC cases included 14 cases enrolled prior to initiation of the screening program and 124 cases enrolled after it began. Mass screening for liver cancer was carried out in two stages. In the first stage, high-risk subjects were identified using fasting blood samples tested for HBsAg, anti-HCV antibody, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alpha fetoprotein (AFP). Subjects for whom there was a positive finding, defined as having at least one of these findings— positive for HBsAg, positive for anti-HCV, AST greater than or equal to 60 U/L, ALT greater than or equal to 60 U/L, or AFP greater than or equal to 20 ng/mL—went forward to the second stage of the screening, in which abdominal ultrasound was used to make the assessment. All subjects were offered further monitoring: three monthly abdominal ultrasound examinations for those found to have liver cirrhosis, hemangioma, or pseudotumor, abdominal sonography monthly for 6 months for those with early liver cirrhosis, and annual sonography for those in whom no abnormalities were found. The following criteria were used to confirm HCC in subjects suspected of having the disease based on the findings from ultrasonography (i.e., the surrounding liver parenchyma appearing hypoechoic, isoechoic, or hyperechoic): alpha-fetoprotein level greater than 400 mg/nL, observation of a lesion by enhanced computed tomography scan or angiography, and histological biopsy if an operation or cytological biopsy was indicated. A total of 138 primary hepatocellular carcinomas (ICD-9 code 155) were ascertained: 33 prevalent cases identified at enrollment and 105 incident cases identified at follow-up. Of these 138 newly diagnosed HCC cases, 63 cases were confirmed with an enhanced computed tomography scan or angiography, 34 cases with histological pathology, 2 cases with both imaging and pathological findings, and 39 with a finding of alpha fetoprotein higher than 400 ng/mL in combination with an abnormal finding from ultrasonography. Thirty-three prevalent cases together with 46 of the 105 incident cases were identified though our subsequent liver cancer screening program. The remaining 59 incident cases were ascertained through the

linkage of subjects (including a negative finding at a subsequent ultrasonographic examination and information lacking or absent about any five risk factors at first stage) with the National Taiwan Cancer Registry until the end of 2003 by personal identification number. The cancer registry's criteria for diagnosis of HCC were two imaging findings both showing HCC from different sources (ultrasonography, enhanced CT scan, or angiography), high level of alpha fetoprotein in combination with one positive image finding, or histopathology positive for HCC. We identified 14 HCC cases in 1999, for which there was no information about risk factors prior to the initiation of the screening program, and an additional 49 HCC cases since 2000, after the launch of the two-stage liver cancer screening program. For 8 of the 49 cases, no information about risk factors was available, whereas for the other 41 cases risk factor information was provided. Of the 138 newly diagnosed HCC cases, 57 subsequently died, 43 from HCC and 14 from other causes. This information was ascertained by linking the whole study cohort to the National Taiwan Mortality Registry until the end of 2003. We excluded four subjects whose deaths were attributed to HCC but for whom there was not sufficient information to confirm the diagnosis of HCC. Written informed consent, including on the confidentiality of the data linkage, was provided by each participant.

Ascertainment of Type 2 Diabetes. Subjects who, as a result of screening, were found to have a fasting blood sugar level between 110 and 125 mg/dL were defined as having impaired fasting glucose (IFG) and referred for confirmation of their diabetes status. Those with a fasting blood sugar level of at least 126 mg/dL were defined as having type 2 diabetes and referred for further diabetic care. When participants had their intake into the KCIS program, we also collected data from those with self-reported type 2 diabetes through a questionnaire that requested such information as age at onset, duration of the disease, and medication history. We identified 5,732 subjects with type 2 diabetes, of whom 69% (n = 3,957) had been diagnosed with diabetes prior to entering the study, and 31% (n = 1,775) were newly diagnosed at enrollment. Of the 3,957 previously diagnosed with diabetes, 2,210 (56%) were on medication. The self-reported cases, combined with those subjects whose fasting blood sugar was at least 126 mg/dL at enrollment, were categorized as having type 2 diabetes. Type 1 or type 2 diabetes was determined by age at onset of diabetes mellitus. Four participants with diabetes whose age at onset was younger than 18 years were considered type 1 diabetes and excluded from the analysis.

Data on Confounders. Independent variables that could confound the association between type 2 diabetes and HCC were also collected through a structured closed-form questionnaire and fasting blood samples obtained when participants attended the KCIS program. These variables included sociodemographic factors (such as sex, age, education, and marital status), elevated body mass index (BMI), HBsAg, anti-HCV, hyperlipidemia, and lifestyle factors (smoking, alcohol consumption, betel nut chewing).

BMI was dichotomized into two classes, low (<25 kg/m^2) and high (≥ 25 kg/m^2). HBsAg and anti-HCV status were determined by the ELISA method. A fasting serum total cholesterol of at least 200 mg/dL was defined as hypercholesterolemia. High-density lipoprotein (HDL) level was categorized as low (≤ 35 mg/dL) or high (≥ 35 mg/dL), and low-density lipoprotein (LDL) level was split into two categorie similarly at 130 mg/dL. Note that, to identify those with a high risk of developing cardiovascular disease, the KCIS program has been routinely measuring HDL and LDL levels in participants since 2001. Information on HDL and LDL was therefore available for 28,948 and 28,427 subjects, respectively. There were a total of 1,326 subjects who had self-reported hyperlipidemia, including abnormal levels of cholesterol, triglycerides, HDL, and LDL. Of these 1,326 subjects, 687 were taking cholesterol-lowering medications when they enrolled in the study. Those who had self-reported hyperlipidemia but were found to be normal at enrollment were also treated as having hyperlipidemia in the following analysis.

Smoking, alcohol consumption, and betel nut chewing status were was defined as current, quitting, or never. Information on the duration (in years) and frequency (packs per day for smoking, drinks per week for alcohol consumption, and pieces per day for betel nut chewing) of these three habits also were recorded . Cumulative exposure to the three habits was expressed as duration times frequency, using pack-day for cigarette consumption, glass-week for alcohol consumption, and piece-day for betel nut consumption. Median cumulative exposure to each of the three habits was used in the following associated analysis.

Statistical Analysis. Survival time was measured as the difference between date of entry and date of diagnosis of HCC, date of death attributed to other causes occurring before diagnosis of HCC, or the last date of follow-up (December 31, 2003) for subjects still alive and free of HCC. A Cox proportional hazards regression model was used to assess the univariate and multivariate effects of the various risk factors on time to occurrence of HCC. Hazard ratios (HRs) and their 95% confidence intervals were estimated to show the magnitude and strength of the associations between risk factors and risk

Table 1. Prevalent Cases (n = 33) by Sex and Age Group and Incidence of HCC Cases (n = 105)

	Male				Female			
Age	Prevalent Cases (At Enrollment)	Incident Cases (Follow-up)	Person-Years	Incidence (per 100,000 Person-Years)	Prevalent Cases (At Enrollment)	Incident Cases (Follow-up)	Person-Years	Incidence (per 100,000 Person-Years)
30-39	1	1	10,207.38	9.80	0	0	23,052.22	0.00
40-49	2	5	13,992.36	35.73	0	5	26,739.46	18.70
50-59	3	13	9,718.27	133.77	1	8	18,941.13	42.24
60-69	10	30	10,911.09	274.95	5	18	17,108.80	105.21
70-79	5	13	9,122.20	142.51	5	7	9,088.04	77.02
80+	0	4	1,810.91	220.88	1	1	1,858.51	53.81
Total	21	66	55,762.21	118.36	12	39	96,788.16	40.29

of HCC. The likelihood ratio test, with a significance level of 5%, was used to test for an interaction between type 2 diabetes and other risk factors. The purpose of this was to examine how the association between type 2 diabetes and HCC was modified by relevant covariates, particularly hepatitis B virus (HBV) infection, hepatitis C virus (HCV) infection, and variables related to lipid profiles. We first considered two-way interactions and then three-way interactions. With HCV infection, for example, we tested for a two-way interaction by incorporating a product term (type 2 diabetes × hepatitis C infection) into a model of the main effects plus the confounders age and sex. Where two-way interactions were found to be statistically significant, three-way interactions were also investigated by modifying the product term in the model. Note that because age, sex, HBV infection, and HCV infection were established as risk factors, these variables were always considered in the model. To assess the temporal order of the effect of type 2 diabetes, the main analyses were performed by excluding prevalent HCC cases at enrollment. A subsidiary analysis that excluded those newly diagnosed with type 2 diabetes was conducted to assess whether the association between type 2 diabetes and incidence of HCC still existed. The synergic effect of hepatitis C infection and type 2 diabetes or of lipids and type 2 diabetes was assessed using the synergic index.[18] When the synergy index is larger than 1, a synergistic interaction exists.

Results

Incidence of Hepatocellular Carcinoma. A total of 138 HCC cases, 33 prevalent cases found at enrollment and 105 incident cases found at follow-up, were identified in the study cohort. Table 1 shows prevalent cases by sex and age group and the incidence of HCC (per 100,000 person-years) based on incident cases for participants aged 30 years or older. The incidence of HCC was higher in men than in women by a ratio of approximately 3:1.

Association. Table 2 shows the frequencies by risk factor and the crude hazard ratios for developing HCC. Twenty-five HCC cases occurred in individuals with type 2 diabetes, of which seven were prevalent and 18 were incident. Ten of the 18 incident cases had been diagnosed with type 2 diabetes before entry into the study; the other eight were diagnosed with type 2 diabetes at enrollment but did not develop HCC until later, at follow-up. After excluding 33 prevalent cases, study participants with a history of or a newly diagnosed case of type 2 diabetes had 1.84-fold increase in risk (95% confidence interval [CI] = 1.10-3.07) compared to those who were not diabetic ($P = .02$). Those diagnosed as having IFG were also at increased risk of developing HCC. However, the effect was not statistically significant. Significant factors identified in univariate analysis were age, sex, education, HBV, HCV, total cholesterol, and LDL. It is interesting that those with a high level of LDL had a lower risk of HCC than did those with a low level of LDL (adjusted HR = 0.27, 95% CI = 0.11-0.62).

Effect Modification. Analysis of the models including a term for the two-way interaction between type 2 diabetes and significant variables identified in the univariate analysis (*i.e.*, a series of pairwise interactions) showed only HCV ($P = .04$) and hypercholesterolemia ($P = .01$) to be significantly modifiers of the effect of type 2 diabetes on the risk of developing HCC. This suggests the impact of type 2 diabetes on HCC depends on HCV status and hypercholesterolemia. The test for a three-way interaction between type 2 diabetes, HCV status, and hypercholesterolemia (the type 2 diabetes × HCV × hypercholesterolemia term in the model) was not significant ($P = .83$).

Table 3 shows the association between type 2 diabetes and incident HCC cases (n = 105, excluding the 33 prevalent cases), stratified by hypercholesterolemia, HCV, and HBV status. Within each stratum, adjusted hazard ratios were estimated after controlling for age, sex, HCV, cigarette smoking, and alcohol consumption. This showed only those with type 2 diabetes who were HCV negative had an risk of developing HCC. This subgroup

Table 2. Univariate Analysis of Type 2 Diabetes and Other Relevant Factors Associated With HCC

Variable	HCC Case*	Non-HCC*	Crude	
			Crude HR (95% CI)	*P* Value
Hyperglycemia/type 2 diabetes†				
No	79	46,443	1.00	
IFG (110 ≤ AC ≤ 125 mg/dL)	6	2,221	1.64 (0.72–3.76)	.24
≥126 mg/dL or history	18	5,707	1.84 (1.10–3.07)	.02
Sociodemographic characteristics				
Sex				
Female	51	34,017	1.00	
Male	87	20,761	2.95 (2.09–4.17)	<.001
Age	138	54,778	1.06 (1.05–1.07)	<.001
Education				
High	108	23,965	1.00	
Medium	23	21,155	1.70 (0.65–4.44)	.24
Low	6	9,536	5.96 (2.43–14.63)	<.001
Marriage				
Unmarried	26	8,428	1.00	
Married	6	3,000	1.18 (0.52–2.68)	.70
Divorced/widowed	106	43,349	1.47 (0.60–3.57)	.40
Lifestyle factors				
Cumulative consumption of cigarettes‡				
No	81	41,669	1.00	
Low	18	6,439	1.51 (0.91–2.51)	.11
High	39	6,670	3.11 (2.12–4.56)	<.001
Cumulative consumption of alcohol‡				
No	90	51,143	1.00	
Low	3	1,798	0.93 (0.29–2.93)	.89
High	8	1,837	2.37 (1.15–4.88)	.02
Cumulative consumption of betel nuts‡				
No	129	52,444	1.00	
Low	6	1,164	2.21 (0.97–5.00)	.06
High	3	1,170	1.14 (0.36–3.57)	.83
BMI				
<25 kg/m^2	74	30,897	1.00	
≥25 kg/m^2	59	22,607	1.07 (0.76–1.51)	.69
Hepatitis viral infection				
HBsAg				
Negative	69	44,588	1.00	
Positive	46	6,499	4.85 (3.33–7.07)	<.001
Anti-HCV				
Negative	61	48,999	1.00	
Positive	54	2,087	18.97 (13.06–27.55)	<.001
Biochemical variables				
Total cholesterol				
<200 mg/dL	79	28,904	1.00	
≥200 mg/dL or history	33	21,864	0.58 (0.39–0.87)	.01
Triglycerides				
<200 mg/dL	98	43,171	1.00	
≥200 mg/dL or history	14	7,596	0.87 (0.50–1.52)	.62
HDL				
≥35 mg/dL	54	27,211	1.00	
<35 mg/dL or history	6	1,712	1.77 (0.76–4.11)	.18
LDL				
<130 mg/dL	52	19,470	1.00	
≥130 mg/dL or history	6	8,860	0.27 (0.11–0.62)	<.001

*Number of cases and number of noncases for each risk factor may not equal the total in Table 1 because of missing information for some variables.

†Excluding 33 prevalent HCC cases and two cases without information on blood sugar.

‡The median values were used to split the three lifestyle variables into low and high categories. The median values for cumulative consumption of cigarettes, cumulative consumption of alcohol, and cumulative consumption of betel nuts were 6,940 pack-days, 4,680 glass-weeks, and 37,000 quid-days, respectively.

Table 3. Multivariate Analysis of the Association Between Type 2 Diabetes and HCC by Stratification of HCV, HBV, and Total Cholesterol Level of Either Incident HCC Cases Only or Excluding Newly Diagnosed Cases of Type 2 Diabetes

	Incident HCC Cases (n = 105)		Previously Diagnosed Type 2 Diabetes vs. Normal*	
Stratum	Adjusted HR (95% CI)	*P* Value	Adjusted HR (95% CI)	*P* Value
HCV†				
Positive	0.62 (0.22–1.76)	.37	0.62 (0.22–1.73)	.36
Negative	2.08 (1.03–4.18)	.04	1.98 (0.99–3.96)	.05
HBV‡				
Positive	1.04 (0.36–3.02)	.95	1.04 (0.37–2.93)	.94
Negative	1.46 (0.75–2.85)	.27	1.37 (0.70–2.70)	.36
Cholesterol§				
≥200 mg/dL or history	2.81 (1.20–6.55)	.02	2.79 (1.17–6.64)	.02
<200 mg/dL	0.76 (0.32–1.79)	.53	0.64 (0.26–1.58)	.33

*Excluding 1,765 newly diagnosed type 2 diabetes for non-HCC cases and 10 for HCC cases.

†[a]Adjusted for age, sex, HBV status, cumulative consumption of cigarettes cigarette, and cumulative consumption of alcohol.

‡[b]Adjusted for age, sex, HCV status, cumulative consumption of cigarettes, and cumulative consumption of alcohol.

§[c]Adjusted for age, sex, HBV status, HCV status, cumulative consumption of cigarettes, and cumulative consumption of alcohol.

was twice as likely (95% CI = 1.03-4.18) to develop HCC than were those who did not have type 2 diabetes. In those with a high level of cholesterol, the effect of type 2 diabetes was to increase the relative risk of HCC to 2.81 (95% CI = 1.20-6.55). However, no such association was found in those with a low level of cholesterol (Table 3). The statistically significant findings still remained by only including previously diagnosed diabetes. Both findings suggest a possible temporal sequence for the effect of type 2 diabetes on the occurrence of HCC. Note that education and LDL were not statistically significant after adjusting for other significant variables. So, to keep the model parsimonious, the final model did not include them.

Table 4 shows the results of our evaluation of the synergistic effects of type 2 diabetes and HCV and of type 2 diabetes and hypercholesterolemia on the risk of developing HCC limited to incident cases (n = 105). As a synergy index value is always less than 1, synergistic interactions between type 2 diabetes and status of HCV and between type 2 diabetes and hypercholesterolemia were lacking after controlling for age, sex, HBV status, HCV status, cumulative consumptions of cigarettes, and alcohol.

Discussion

In contrast to previous studies in populations with a low prevalence of HCC, we studied the association between type 2 diabetes and HCC in a country with a high prevalence of hepatitis infection. It is well known that in such high-prevalence areas, the incidence of HCC is heavily influenced by virus-related characteristics that confound other risk factors for HCC, blurring the interaction with hepatitis infection. In our study, the effect of type 2 diabetes on the risk of developing HCC was modified by hepatitis C infection and total cholesterol. With regard to hepatitis C infection, having type 2 diabetes played a more important role in patients who were hepatitis C negative than in those who were hepatitis C positive. Regarding total cholesterol, having type 2 diabetes played a more important role in patients with high cholesterol than in those with low cholesterol. No synergistic

Table 4. Synergistic Interactions Between Type 2 Diabetes and HCV and Between Type 2 Diabetes and Hypercholesterolemia on Risk of HCC

	Crude HR	Synergy Index	Adjusted HR*	Synergy Index
DM(−), HCV(−)	1.00	0.86 (0.38–1.97)	1.00	0.70 (0.30–1.65)
DM(+), HCV(−)	2.92		1.99	
DM(−), HCV(+)	24.75		20.94	
DM(+), HCV(+)	18.37		12.62	
DM(−), hypercholesterolemia(−)	1.00	−7.32 (–†)	1.00	−1.24 (–†)
DM(+), hypercholesterolemia(−)	1.34		0.75	
DM(−), hypercholesterolemia(+)	0.58		0.68	
DM(+), hypercholesterolemia(+)	1.61		1.70	

*Adjusted for age, sex, HBV status, HCVstatus, cumulative consumption of cigarettes, and cumulative consumption of alcohol.

†As synergy index is negative, 95% confidence interval cannot be computed.

effect was found for the interactions of type 2 diabetes with the presence of HCV or type 2 diabetes with high total cholesterol on the risk of HCC after adjusting for age, sex, HBV, and HCV.

Our findings on the effects of the interactions between type 2 diabetes and HCV and between type 2 diabetes and hypercholesterolemia on the occurrence of HCC differed from but did not contradict those of most previous studies, which found evidence of an association between type 2 diabetes and HCC even after controlling for HBV and HCV.[1,2] We believe this disparity results from hepatitis infection being rare in most of the populations previously studied but prevalent in our target population. We found the incidence of HCC to be very strongly related to HBV and HCV status. It seems reasonable, therefore, that the prevalence of hepatitis is a confounder of the effect of type 2 diabetes on the risk for HCC.

The identification of two effect modifiers, hepatitis C infection and total cholesterol, is consistent with current knowledge of the biological mechanism by which HCC develops. The strong effect of type 2 diabetes on HCC in the absence of hepatitis infection suggests that, in addition to the hepatitis C causal pathway, HCC is mediated through the reduction of IGF-1 factors or IGFBP-3 caused by hyperinsulinemia. This, in turn, stimulates the proliferation of live cancer cells, as demonstrated in the Lagiou et al. study.[7] Evidence from the interaction analysis of total cholesterol with type 2 diabetes supports our belief that progress along this pathway also may be accelerated by having high total cholesterol. In the present study, only in patients with high total cholesterol were found to have a high risk of developing HCC. This finding was not observed in previous studies.

Our findings of negative associations between total cholesterol, triglycerides, and LDL-C with HCC (Table 2) are consistent with previous findings.[19] A significant negative association between LDL-C and the risk for HCC was noted. However, the small number of HCC cases precluded us from doing further multivariate analysis. The sparse number of cases may also explain why the interaction between LDL-C and type 2 diabetes on the risk for HCC lacked statistical significance. Future work should be conducted to corroborate this association. Our finding of no association between obesity and incidence of HCC is different from the positive association found in previous studies in the United States.[20-22] Two possibilities account for this disparity. A smaller percentage of people may obese in Taiwan than that in the United States. Or it may be that most HCC cases in Taiwan were a result of virus-related infection rather than alcohol related, as is seen in Western countries. Alcohol-related HCC cases are more likely than virus-related HCC cases to be related to obesity. This is supported by the finding of a nonsignificant association between obesity and two established factors for HCC, HBV infection and HCV infection, in a recent population-based study in Taiwan.[23]

Our study had several advantages over previous ones. First, the study population, covering 25% of the target population, was drawn from the community rather than from a clinical series. We therefore believe this study had minimal selection bias and our results to be externally valid. Despite this, as the KCIS program is an outreach screening service, study subjects enrolled from the KCIS program had a larger percentage of women and a slightly higher proportion of elderly people than is found in the target population (data not shown). To avoid such selection bias, incidence was stratified by sex and age group, as reported in Table 1. Moreover, as age, sex, smoking, alcohol consumption, and other relevant risk factors were controlled in multivariable regression analysis, we believe the influence of selection bias in such a large population-based study would be very small. This is supported by the 10.5% prevalence of type 2 diabetes in our study being very close to the 10.3% prevalence estimated from a previous study in Taiwan.[24] In addition, the incidence of HCC (per 100,000 person-years) by sex (male: 118, female: 40) that we found is comparable to the corresponding nationwide incidence figures (male: 100, female: 36) during a contemporaneous period. Second, unlike other cohort studies, ours adjusted for important confounders. HBV and HCV status are established risk factors for HCC, but few of studies have taken these into account. Hepatitis status has been self-reported in some studies.[25,26] Because hepatitis is often asymptomatic, misclassification is likely. In our study, we also ensured that HBsAg and anti-HCV status were accurately assessed. Third, the synergistic effect between type 2 diabetes and HCV on the risk of HCC was quantitatively assessed. Thus, we investigated whether the combined effect of these two factors was greater than the individual effects.

Fourth, unlike those in some previous studies, our cohort was not comprised solely of diabetics, making it more representative of the baseline population and allowing us to directly compare diabetic and nondiabetic patients.

There is a concern about whether the interaction effects of type 2 diabetes and HCV of type 2 diabetes and high cholesterol on the risk of HCC found in the present study reflect a temporal relationship. A temporal order of type 2 diabetes and occurrence of HCC cases is probable, as we excluded preexisting (previously diagnosed) HCC cases, and the main analysis included only incident, not prevalent, HCC cases. This was strengthened by only including participants with previously diagnosed type 2 diabetes (Table 3). It is also argued that the follow-up

period was too short to reach such definite conclusion. However, HCC cases newly diagnosed more than 1 year after enrollment (36 cases after 1-2 years, 30 cases after 2-3 years, 14 cases after 3-4 years) accounted for 58% of the total number of HCC cases. Given the median survival time of 3 years of the small number of HCC cases reported in a previous study,[27] we believe the mean follow-up time of 2.78 years in the present study was sufficient to assess the effect of type 2 diabetes on HCC.

In conclusion, this prospective cohort study demonstrated that type 2 diabetes increases the risk of developing HCC in those who are HCV negative or have a high level of total cholesterol.

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