HCV Genotype 6 Increased the Risk for Hepatocellular Carcinoma Among Asian Patients With Liver Cirrhosis

Mei-Hsuan Lee, PhD[1,8], Tiffany I. Hsiao, MS[2,3,8], Shreenidhi R. Subramaniam[4], An K. Le, BA[2], Vinh D. Vu, BA[2], Huy N. Trinh, MD[5], Jian Zhang, DNP[6], Mingjuan Jin, MD, PhD[7], Vincent Wai-Sun Wong, MD[4], Grace Lai-Hung Wong, MD[4] and Mindie H. Nguyen, MD, MAS[2]

OBJECTIVES: **Hepatitis C virus (HCV) infection is a well-documented risk factor for hepatocellular carcinoma (HCC). Seven HCV genotypes have been classified, and the genotypes show a great variety of geographic distribution. HCV genotype 6 is prevalent in Southeast Asia and has been less studied than the other genotypes.**

METHODS: **This follow-up study was designed to evaluate the natural history of HCV genotype 6. The cohort enrolled 851 Asian patients consisting of 222 with HCV genotype 6 and 629 with other genotypes. The incidence of HCC per 1,000 person-years of various HCV genotypes was estimated by dividing the new HCC cases to the person-years of follow-up. The adjusted hazards ratios (HRs) with 95% confidence intervals (CIs) were estimated by Cox's proportional hazards models.**

RESULTS: **After 4072 person-years of follow-up, there were 96 newly-developed HCC cases, confirming an incidence of 23.6 per 1000 person-years. By stratifying cirrhosis at study entry, the cumulative risk of HCC among HCV genotype 6 vs. non-6 was 2.9 vs. 2.2% for those without cirrhosis (P=0.45) and 76.2% (95% CI: 55.6–96.8%) vs. 36.2% (95% CI: 28.7–39.1%) for those with cirrhosis (P<0.05), respectively. Among patients with cirrhosis, HCV genotype 6 was significantly associated with HCC compared to patients with non-6 genotypes, with the adjusted HR=2.12 (1.33–3.39), P<0.05. In a model treating patients with genotypes other than 1 or 6 as the reference, the adjusted HR for HCC for HCV genotypes 1 and 6 were 1.13 (0.56–2.27) and 2.34 (1.12–4.86), respectively.**

CONCLUSIONS: **Among patients with cirrhosis, those with HCV genotype 6 infection should be given high priority for antiviral therapy to decrease HCC risk and for vigilant adherence to HCC surveillance.**

Am J Gastroenterol 2017; 112:1111–1119; doi:10.1038/ajg.2017.123; published online 25 April 2017

INTRODUCTION

Hepatitis C virus (HCV) infection is a well-established risk factor for hepatocellular carcinoma (HCC), end-stage liver disease, and extrahepatic diseases (1). The most recent estimates of HCV seroprevalence showed an increase over the last 15 years to 2.8%, or more than 185 million infections worldwide (2). The geographic distribution of HCV infection was highly variable, with the seroprevalence in individual countries ranging from lower than 1% to higher than 10% (3,4). More than 50% of people with HCV infection were found to be residing in Asia (4). Out of a global HCV disease burden of 170 million persons, the estimated number of chronic HCV infections was 32.3 million in Southeast Asia and 62.3 million in the western Pacific regions, respectively (5).

HCV exhibits an extraordinarily high genetic diversity. On the basis of phylogenetic and sequence analyses, HCV is classified into 7 genotypes (genotype 1–7) and at least 67 subtypes (6). HCV genotype has a noteworthy geographical distribution. HCV genotype 1 is the most prevalent (46.2%) worldwide, followed by genotype 3 ranked as the second most prevalent type (30.1%) (ref. 7). Genotypes 2, 4, and 6 are responsible for a total of 22.8% HCV infected cases (7). Genotype 6 is found to be regionally located in southern China and Southeast Asia (8,9), including

[1]Institute of Clinical Medicine, National Yang-Ming University, Taipei, Taiwan; [2]Division of Gastroenterology and Hepatology, Stanford University Medical Center, Palo Alto, California, USA; [3]School of Medicine, Virginia Commonwealth University, Richmond, Virginia, USA; [4]Institute of Digestive Disease, The Chinese University of Hong Kong, Hong Kong, China; [5]San Jose Gastroenterology, San Jose, Califonia, USA; [6]Chinese Hospital, San Francisco, Califonia, USA; [7]Department of Epidemiology and Biostatistics, School of Public Health, Zhejiang University, Hangzhou, China; [8]These authors contributed equally to this work.
Correspondence: Mindie H. Nguyen, MD, MAS, Division of Gastroenterology and Hepatology, Stanford University Medical Center, 750 Welch Road, Suite 210, Palo Alto, Califonia 94304, USA or Grace K.-H. Wong, MD, Institute of Digestive Disease, The Chinese University of Hong Kong, 9/F, Lui Che Woo Clinical Sciences Building, Prince of Wales Hospital, Shatin, Hong Kong, China. E-mail: mindiehn@stanford.edu or wonglaihung@cuhk.edu.hk
Received 14 November 2016; accepted 14 March 2017

Vietnam, Thailand, Malaysia, and Cambodia (8–11). In addition, HCV genotype 6 is also prevalent among communities with immigrants coming from Southeast Asia. One study conducted in the San Francisco Bay area found that HCV genotype 6 was as common as genotype 1 among Southeast Asian immigrants (12).

HCV genotype is one of the most important predictors for treatment response, and has been studied most extensively in patients with genotypes 1 to 3, as these genotypes are common in developed Western countries (13,14). In addition to treatment response, HCV genotype 1 has been found to be associated with increased risk for HCC compared to other genotypes (15–17). A recent study also suggested that HCV genotype 3 was a significant determinant for developing either cirrhosis or HCC compared to genotypes 1, 2, and 4 (18). The literature is still sparse in regard to HCV genotype 6 and risks for liver disease progression. Previous studies examining HCV genotype 6 and its impact on advanced liver diseases are limited by small sample size and lack of statistical power (12,19).

In the United States, immigrants from the Asian continent are among the fastest growing ethnic groups, with 15.6 million people and rising (20). There is a considerable number of HCV genotype 6 infected patients who need to receive appropriate care, and evaluating the risk of genotype 6 on liver diseases is important. This larger multicenter patient cohort enrolled 851 Asians to compare the effect of HCV genotype 6 and other genotypes on the risk of HCC.

METHODS

Study population

This retrospective cohort study enrolled 851 consecutive Asian CHC patients at three US centers and one center in Hong Kong between July 1996 and September 2014. Inclusion criteria were adult patients age 18 years or older with CHC and known HCV genotypes who were under clinical monitoring for the development of HCC. Patients who have had solid organ transplantation or known HIV infection were excluded from this study. We retrospectively collected patient clinical seromarkers through clinical chart reviews using a standardized case report form. The patients had periodic health examination approximately every 6–12 months at our clinics. This study was approved by the Institutional Review Board at Stanford University, Stanford, CA, USA and The Chinese University of Hong Kong, Hong Kong, China.

The baseline clinical information including age, gender, body mass index, aspartate transaminase level, alanine transaminase level, total bilirubin, creatinine, albumin, international normalized ratio (INR), platelet count, serum HCV RNA levels, hepatitis B surface antigen status, and the comorbidities of diabetes and chronic kidney diseases were obtained. Almost all treated study patients received interferon/peg-interferon plus ribavirin (97.0%), which was the standard treatment before the approval of direct acting antivirals (DAAs) at the end of 2013 in the United States and is still the standard treatment in Hong Kong to date. Sustained virological response (SVR) was defined as undetectable HCV RNA 6 months or more after the cessation of the treatment. Cirrhosis was determined by biopsy or via imaging, clinical, or laboratory evidence of cirrhosis. Imaging evidence included the presence of nodular/shrunken liver, intra-abdominal varices, ascites, and/or splenomegaly. Clinical evidence included the presence of jaundice, ascites, hepatic encephalopathy, or gastroesophageal varices. Laboratory evidence included signs of portal hypertension, such as thrombocytopenia associated with splenomegaly, or signs of decreased hepatic synthetic function, such as decreased albumin, increased INR, or increased total bilirubin.

Assessment of newly developed HCC

HCC was determined by histological examination or imaging characteristics as per the American Association for the Study of Liver Diseases (AASLD) guidelines (21). All of the patients were diagnosed by (1) a characteristic lesion detected by at least two different imaging techniques (abdominal ultrasonography, angiogram, or computed tomography), or (2) a characteristic lesion detected by one imaging technique and a serum α-fetoprotein level of 400 ng/ml or greater.

Laboratory examinations

The HCV genotype was determined either by core sequencing or INNO-LiPA HCV II (Innogenetics, Ghent, Belgium).

Statistical analysis

Baseline characteristics were reported and compared by patients with various HCV genotype infections. χ^2-tests were used to compare categorical variables, and the Student's *t*-test was used to compare normally distributed continuous variables. The observed person-years were calculated from the study entry date to the incidence of HCC, deaths, or last clinical visit, whichever occurred first. The incidence rates of HCC per 1,000 person-years were calculated by dividing the number of newly developed HCC cases by person-years of follow-up. The differences of cumulative incidence for HCC by HCV genotype 6 and non-6 by follow-up years were detected by Kaplan–Meier methods and compared by log-rank tests. The hazards ratios (HRs) with 95% confidence intervals (CIs) of genotype 6 on the risk of HCC were estimated by Cox's proportional hazards models. The potential risk factors associated with HCC were adjusted in the multivariate models. The two-tailed *P*-value of <0.05 was considered statistically significant. All statistical analyses were performed using statistical analysis software (SAS) version 9.4 (SAS Corporation, Cary, NC).

RESULTS

Baseline characteristics of study participants

A total of 851 consecutive patients were included in the study and all of them were free of HCC at study entry (**Tables 1** and **2**). There were 629 (74%) patients with HCV genotype non-6 whereas 222 (24%) had HCV genotype 6 infection. The mean follow-up was 5.3±4.3 years for patients with HCV genotype 6 and 5.5±4.5 years for patients with genotype non-6. Patients with HCV genotype 6 infection had lower serum levels of alanine transaminase, creatinine and INR, lower model for end-stage liver disease (MELD) scores, higher albumin levels, higher serum levels of HCV RNA, lower HBV co-infection rate, and were more likely to have achieved SVR after antiviral treatment (P<0.05).

Table 1. Baseline characteristics of study participants

	Total population (*N*=851)	Genotype non-6 (*N*=629)	Genotype 6 (*N*=222)	*P* value
Age (mean±s.d.)	53.3±12.3	53.4±12.2	53.1±12.6	0.78
Gender (%)				
Female	359 (42.2)	270 (42.9)	89 (40.1)	0.46
Male	492 (57.8)	359 (57.1)	133 (59.9)	
BMI (kg/m^2)	24.1±3.9	24.1±3.8	23.9±4.0	0.50
Alcohol use (%)	165 (21.8)	118 (20.8)	47 (24.6%)	0.31
AST (U/l)	84.0±99.7	85.9±105.3	78.3±81.1	0.32
ALT (U/l)	101.4±85.4	104.5±92.3	93.6±63.7	0.06
Total bilirubin (mg/dl)	1.3±1.6	1.3±1.8	1.1±1.1	0.15
Creatinine≥1.5 (mg/dl)	55 (11.4)	46 (15.7)	9 (4.7)	**0.0002**
Albumin (g/dl)	4.0±0.6	4.0±0.7	4.2±0.5	**<0.0001**
INR (U/l)	1.2±0.5	1.2±0.6	1.1±0.2	**0.0006**
Platelets (K/ul)	180.0±71.3	180.5±72.0	178.6±69.6	0.75
Log HCV RNA (IU/ml)	5.9±1.1	5.8±1.2	6.0±0.9	**0.01**
HBV co-infection (%)	42 (7.0)	35 (8.8)	7 (3.4)	**0.01**
Diabetes mellitus (%)	189 (22.3)	136 (21.7)	53 (24)	0.47
Chronic kidney disease (%)	60 (7.1)	50 (8)	10 (4.5)	0.09
Follow-up years	5.7±4.5	5.8±4.5	5.5±4.3	0.42
Received treatment (%)	584 (68.6)	431 (68.5)	153 (68.9)	0.91
Achieved SVR (%, among patient received treatment)	316 (54.2)	220 (51.2)	96 (62.8)	**0.01**
Baseline MELD	10.3±4.9	10.9±5.5	9.1±3.4	**0.0002**
Baseline cirrhosis (%)	313 (36.7)	232 (36.8)	81 (36.4)	0.91
HCV genotype (%)				
Genotype 1 (no subtype information)	58 (6.8)			
Genotype 1A	107 (12.6)			
Genotype 1B	305 (35.8)			
Genotype 2	112 (13.2)			
Genotype 3	44 (5.2)			
Genotype 4	3 (0.4)			
Genotype 6	222 (26.1)			

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; HBV, hepatitis B virus; INR, international normalized ratio; MELD, model for end stage liver disease; SVR, sustained virological response. The two-tailed *P*-value of <0.05 was considered statistically significant and indicated in bold.

Overall HCC incidence and presence of cirrhosis

In the total study population, there were 96 newly developed HCC cases after 4,072 person-years of follow-up, creating an incidence of 23.6 per 1000 person-years. The incidence of HCC was 4.1 and 44.0 per 1000 person-years for patients without cirrhosis and with cirrhosis, respectively (P<0.05).

Cumulative risk of HCC by HCV genotypes and presence of cirrhosis

The patients with HCV genotype 6 and non-6 were compared for their cumulative risk of HCC with follow-up. Among the total study population, the cumulative risks of HCC were 14.8 and 21.0 for patients with HCV non-6 and HCV genotype 6, respectively (P=0.07) (**Figure 1a**). After stratifying by liver cirrhosis status at study entry, there was no difference in the cumulative HCC risk by various HCV genotypes among patients without liver cirrhosis, with 2.9% for HCV non-6 and 2.2 for HCV genotype 6, respectively (P=0.45). However, among patients with liver cirrhosis, patients with HCV genotype 6 had increased cumulative risk of HCC compared to non-6, with 76.2% (95% CI: 55.6–96.8%) for HCV-6 vs. 36.2% (95% CI: 28.7–39.1%), respectively (P<0.05).

Table 2. Incidence rate of hepatocellular carcinoma (HCC) by hepatitis C virus (HCV) genotype among study subjects with or without liver cirrhosis at study entry

HCV genotype	Total (*N*=851) *N* (%)	HCC (*N*=96) *N* (%)	Observed period (person-year)	Rate per 1,000 (person-year)
Total population	851	96	4071.95	23.58
Genotype 1 (all)	470 (55.23)	52 (54.17)	2,331.01	22.31
Genotype 1A	107 (12.57)	11 (11.46)	467.15	23.55
Genotype 1B	305 (35.84)	33 (34.38)	1598.12	20.65
Genotype 2	112 (13.16)	9 (9.38)	548.59	16.41
Genotype 3	44 (5.17)	3 (3.13)	164.32	18.26
Genotype 4	3 (0.35)	0 (0.00)	20.81	0.00
Genotype 6	222 (26.09)	32 (33.33)	1007.22	31.77
Non-liver cirrhosis	538	12	2895.07	4.14
Genotype 1(all)	296 (55.02)	8 (66.67)	1600.39	5.00
Genotype 1A	62 (11.52)	2 (16.67)	305.03	6.56
Genotype 1B	199 (36.99)	6 (50)	1117.48	5.37
Genotype 2	76 (14.13)	1 (8.33)	420.84	2.38
Genotype 3	22 (4.09)	1 (8.33)	68.29	14.64
Genotype 4	3 (0.56)	0 (0)	20.81	0.00
Genotype 6	141 (26.21)	2 (16.67)	784.74	2.55
Liver cirrhosis	313	84	1907.48	44.04
Genotype 1(all)	174 (55.59)	44 (52.38)	730.61	60.22
Genotype 1A	45 (14.38)	9 (10.71)	162.11	55.52
Genotype 1B	106 (33.87)	27 (32.14)	480.64	56.18
Genotype 2	36 (11.5)	8 (9.52)	127.75	62.62
Genotype 3	22 (7.03)	2 (2.38)	96.03	20.83
Genotype 4	0 (0.00)	0 (0.00)	0.00	0.00
Genotype 6	81 (25.88)	30 (35.71)	222.48	134.84

HCV genotype 6 and the risk for HCC in univariate analyses by presence of cirrhosis

Table 3 showed the crude HRs and 95% CIs of each determinant and associated risk for HCC. Patients who had increased age, without treatment experience or SVR with treatment, increased levels of creatinine, HBV co-infection, low albumin levels, increased INR and MELD score, and presence of liver cirrhosis were at a higher risk for HCC. HCV genotype 6 showed a borderline significance of risk for HCC with an HR of 1.48 (0.97–2.26). After stratifying by cirrhosis at study entry, HCV genotype 6 was a significant risk factor for HCC development compared to other genotypes. Thus, we further evaluated HCV genotype 6 and its associated risk for HCC in multivariate models in patients with cirrhosis as follows.

HCV genotype 6 and risk for HCC in multivariate analysis in patients with cirrhosis

Table 4 showed the crude HR with 95% CI of baseline predictors. Advanced age, lack of treatment or SVR, HBV co-infection, and increased INR were all significant determinants for newly-developed HCC. Patients with HCV genotype 6 had 2.1-fold risk of developing HCC compared to non-6 ($P<0.05$). In a multivariate model, the risk factors were considered, and the adjusted HR was 2.12 (1.33–3.39) of patients with genotype 6 by using patients with non-6 as a reference group ($P<0.05$; Model 1). We further categorized our study participants and compared the patients with HCV genotype 6, genotype 1, and others (Model 2). We found that patients with HCV genotype 6 had the higher risk for HCC with adjusted HR of 2.34 (1.12–4.86). After excluding the 31 HCC cases that occurred within 6 months after enrollment as they might be prevalent cases, genotype 6 remained significantly associated with higher risk of HCC compared to genotype non-6 in a multivariate model with an adjusted HR of 1.98 (1.06–3.70), $P=0.03$. Compared to patients with genotypes other than 1 or 6, patients with genotype 1 were not at higher risk for HCC. We compared the baseline characteristics among patients with HCV genotype 6 and non-6 among these 313 patients with cirrhosis. However, there were no significant differences in alanine transaminase, HCV RNA, or MELD score, factors which could be relevant for HCC ($P>0.05$).

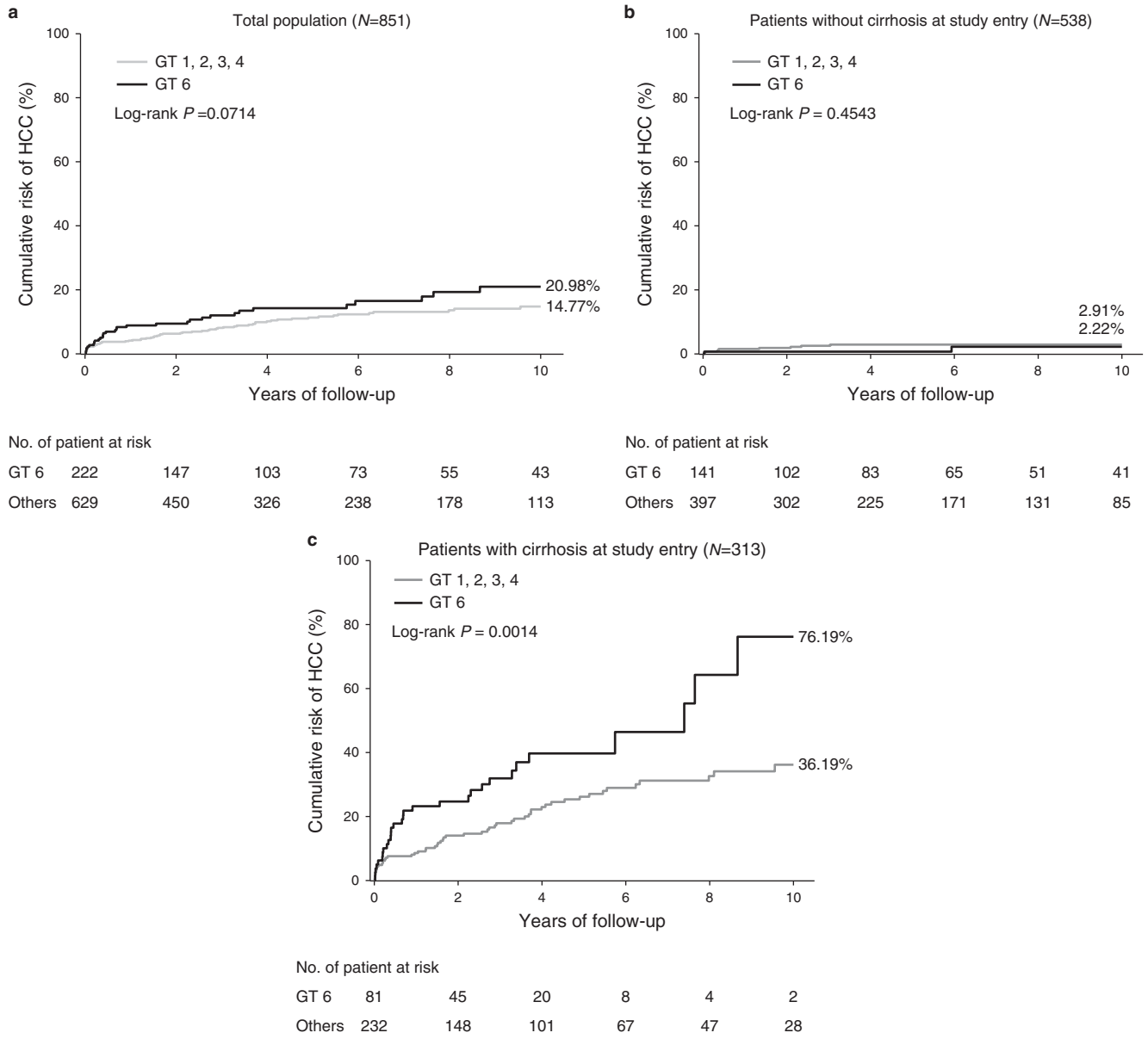

Figure 1. Cumulative risk of hepatocellular carcinoma (HCC) by hepatitis C genotypes (GT) among (**a**) total study population (*N*=851, HCC case=96); (**b**) patients without cirrhosis (*N*=538, HCC case=12); (**c**) patients with cirrhosis (*N*=313, HCC case=84).

DISCUSSION

HCV genotypes 4 through 6 are relatively uncommon in most developed countries. However, these genotypes are widely distributed in many countries of Africa, the Middle East, and Asia. HCV genotype 4 is predominant from Central Africa to the Middle East, while genotype 5 is common in South Africa (7). HCV genotype 6 is dominant in East and Southeast Asia (5,7,10). Most of these regions are also very populous with high HCV seroprevalence and a very large HCV disease burden. To our knowledge, this is the largest study to date that examines the risk of HCV genotype 6 on the development of HCC in an Asian population. We found that especially among patients with liver cirrhosis, HCV genotype 6 was an independent risk factor for HCC. Once patients develop cirrhosis, HCV 6 genotype accelerates disease progression. Since HCC rarely occurs in patients without cirrhosis, this study suggested that early antiviral therapy should be advocated for patients with HCV genotype 6 to prevent development of cirrhosis and subsequent HCC risk.

To date, large epidemiological studies and pivotal trials have generally been conducted in in North America and Western Europe, where large numbers of the HCV genotypes 1, 2, and 3 are prevalent. Thus, data on patients infected with other HCV genotypes, such as genotype 6, are much more limited. The previous standard treatment regimen was based on pegylated interferon plus ribavirin. Patients with HCV genotype 6 showed an SVR rate of 70–80% after 6 months of therapy with pegylated interferon and

Table 3. Crude hazard ratios (HR) relating baseline characteristics at study entry with development of hepatocellular carcinoma in patients with hepatitis C virus (HCV) infection

	Total population (*N*=851)		Without cirrhosis (*N*=538)		Cirrhosis (*N*=313)	
Determinants	**Crude HR (95% CI)**	***P* value**	**Crude HR (95% CI)**	***P* value**	**Crude HR (95%CI)**	***P* value**
Age	1.09 (1.07–1.11)	<0.0001	1.12 (1.06–1.18)	<0.0001	1.04 (1.02–1.06)	0.0004
Gender						
Female	1.00		1.00		1.00	
Male	1.22 (0.81–1.85)	0.34	1.35 (0.41–4.49)	0.62	1.49 (0.96–2.32)	0.07
ALT (U/l)						
<45	1.00		1.00		1.00	
≥45	1.30 (0.71–2.38)	0.40	4.32 (1.12–16.7)	0.03	0.78 (0.42–1.45)	0.42
Creatinine (mg/dl)						
<1.5	1.00		1.00		1.00	
≥1.5	1.89 (1.07–3.35)	0.03	2.73 (0.34–21.99)	0.35	0.82 (0.45–1.49)	0.51
HBV coinfection						
No	1.00		1.00		1.00	
Yes	3.42 (2.02–5.81)	<0.0001	10.14(2.69–38.32)	0.0006	1.82 (1.02–3.26)	0.04
Albumin (g/dl)						
<3.5	1.00		1.00		1.00	
3.5–5.0	0.24 (0.15–0.39)	<0.0001	0.07 (0.02–0.24)	<0.0001	0.80 (0.47–1.37)	0.42
≥5.0	0.11 (0.02–0.82)	0.03			2.39 (0.32–18.07)	0.40
INR (U/l)						
<1.2	1.00		1.00		1.00	
≥1.2	2.94 (1.77–4.89)	<0.0001	1.87 (0.38–9.26)	0.44	1.75 (1.01–3.04)	0.05
MELD						
<8	1.00		1.00		1.00	
8–10	2.21 (1.02–4.77)	0.04	2.93 (0.41–20.84)	0.28	1.47 (0.64–3.41)	0.36
10–12	3.12 (1.25–7.84)	0.02	4.66 (0.42–51.50)	0.21	1.59 (0.59–4.31)	0.36
≥12	4.71 (2.43–9.14)	<0.0001	13.99 (2.32–84.30)	0.0040	1.58 (0.77–3.23)	0.21
Cirrhosis						
No	1.00					
Yes	15.35 (8.37–28.15)	<0.0001				
Treatment						
No	1.00		1.00		1.00	
NSVR	0.85 (0.56–1.28)	0.43	1.20 (0.35–4.14)	0.77	1.02 (0.66–1.58)	0.92
SVR	0.05 (0.02–0.14)	<0.0001	0.09 (0.01–0.83)	0.03	0.12 (0.04–0.39)	0.0004
HCV genotype						
Non-6	1.00		1.00		1.00	
6	1.48 (0.97–2.26)	0.07	0.56 (0.12–2.56)	0.45	2.09 (1.33–3.29)	0.0014

ALT, alanine aminotransferase; CI, confidence interval; HBV, hepatitis B virus; INR, international normalized ratio; MELD, model for end-stage liver disease; SVR, sustained virological response.

ribavirin, which was similar to that for patients with HCV genotypes 2 or 3 infection (22–26). In addition, by comparing patients with HCV genotype 1 and genotype 6 infection in well-matched groups with respect to age, sex, weight, baseline viral load, and liver biochemistry, it was shown that patients with HCV genotype 6 had a better treatment response than patients with genotype 1

Table 4. Adjusted hazard ratios (HR) of hepatitis C virus (HCV) genotype 6 and the risk for hepatocellular carcinoma (HCC) among cirrhosis patients (*N*=313)

	Model 1		Model 2	
Determinants	**Adjusted HR (95% CI)**	***P* value**	**Adjusted HR (95% CI)**	***P* value**
Age	1.03 (1.01–1.05)	0.0073	1.03 (1.01–1.05)	0.0084
Gender				
Female	1.00		1.00	
Male	1.60 (1.02–2.52)	0.0426	1.60 (1.02–2.52)	0.0424
SVR				
No	1.00		1.00	
Yes	0.14 (0.04–0.45)	0.0009	0.14 (0.04–0.45)	0.0010
HBV coinfection				
No	1.00		1.00	
Yes	2.10 (1.17–3.77)	0.0126	2.08 (1.16–3.74)	0.0142
Unknown				
INR (U/l)				
<1.2	1.00		1.00	
≥1.2	2.08 (1.17–3.67)	0.0120	2.08 (1.18–3.68)	0.0116
Unknown				
HCV genotype				
Non-6	1.00			
6	2.12 (1.33–3.39)	0.0016		
HCV genotype				
Others			1.00	
1			1.13 (0.56–2.27)	0.7362
6			2.34 (1.12–4.86)	0.0234

CI, confidence interval; HBV, hepatitis B virus; INR, international normalized ratio; SVR, sustained virological response.

(52% vs. 86%, *P*=0.02) (27). In recent years, new efficacious DAAs have become available for the treatment of HCV (28). Patients with HCV genotype 6 treated with fixed-dose combination therapy with ledipasvir (an inhibitor of the viral protein NS5A) and sofosbuvir (an inhibitor of the viral polymerase NS5B) had SVRs of 96% (all but one of the 25 treated patients) (29). Whether treated with the newer DAAs, which are not yet widely available or affordable for most affected patients in the Asia Pacific region, or with the older pegylated interferon and ribavirin regimen, both regimens produced satisfactory treatment responses. HCV genotype 6 patients should be considered expeditiously for antiviral therapy, given their higher risk for HCC.

HCV genotype was not only a treatment indicator but also a predictor for the end-stage liver diseases. A large-scale study of more than 100,000 veterans in the United States found that HCV genotype 3 was a predictor for liver cirrhosis and HCC (18). However, the veterans' cohort consisted of mainly Caucasian males and their analysis only included patients with HCV genotypes 1 to 4, the most prevalent genotypes in the United States. All participants in our current study were from Asia, thus results on HCV genotype 3 were relatively few. In this study we did not find HCV genotype 1 increased the risk for HCC, as shown in some previous studies (15–17).

Molecular evolutionary analysis suggested that the initial spread time of HCV could predict regional patterns of HCC mortality (30). A previous study has shown that HCV genotype 6 has had a more than 1000-year long development in Southeast Asia (31). It is probable that HCV genotype 6 was introduced and spread across the region earlier than HCV genotype 1. Most Southeast Asian countries, such as Vietnam, Myanmar, and Thailand, experienced long histories of civil conflicts and decades to centuries of colonization and occupation by foreign powers, both neighboring as well as distant powers such as France, Great Britain, and the US HCV likely spread through unsafe medical injections during wartime and other HCV strains were introduced through contacts with persons from other countries during colonization and occupation periods resulting in the very high genetic diversity of HCV genotype 6 in this region (32). Indeed, more than any other HCV genotypes, HCV genotype 6 has almost 30 subtypes (32). To evaluate the seven HCV genotypes and their associated HCC risks, a larger collaborative study including sufficient numbers of the various genotypes will be needed. However, populations with different genetic backgrounds and various dominant HCV genotypes regionally will make such study difficult to carry out. Host and virus interactions on the risk of HCC development should also be considered (33).

A cross-sectional study conducted in the United States found that in patients seen at community gastroenterology clinics for non-liver-related reasons, HCV seroprevalence was 1.7% for non-Asians and 2.9% for Asians, respectively (34). This finding suggested that HCV infection was a considerable issue for immigrants, and thus Asians coming from endemic areas should be screened for HCV. Most of the Asians with HCV infection could not recall any specific risk factors of HCV (12), but these Asians were more likely to have been exposed to iatrogenic risk factors such as blood transfusion, inadequately sterilized medical equipment, and cultural practices, such as acupuncture or cosmetic tattooing, and medical injections (35,36). On the other hand, non-Asian patients with HCV infection were more likely to have typical risk factors such as intranasal cocaine use, intravenous drug injections, and tattoos via contaminated needles (4). In addition to HCV-related risk factors, Asians with HCV-related HCC were found to be more likely to have delayed diagnoses of HCV (37). Among patients with chronic hepatitis C and cirrhosis, Asians had approximately four times the risk of development of HCC compared to Caucasians (38).

However, HCV is often overlooked because HBV is more prevalent in Asia. Our study emphasized the importance of HCV genotype 6, which is one of the prevalent HCV genotypes in the Asian population and its risk for serious liver-related outcomes.

However, as HBV is endemic in Asia and all of our study patients were either immigrant Asians or foreign-born Asian Americans, the majority may have been exposed to HBV, and such prior exposure and occult HBV infection may be associated with higher risk for HCC (39,40). Although we did not test for occult HBV infection in our population, there is currently no evidence to suggest that the distribution of occult HBV infection differs among patients with HCV genotype 6 vs. non-6. Thus, the estimated HRs of HCV genotype 6 on the risk of HCC should not be biased.

The information of SVR in the study was treated as a time-dependent covariate in regression analyses, but as to further stratify for the duration of SVR before the development of HCC, the sample size was unfortunately too small in this subgroup of patients with SVR and with HCC. However, treatment-induced HCV RNA clearance, which may prevent liver disease progressions should decrease the risk for HCC. Future studies with larger sample size are needed to allow for more robust adjustment of background risks, especially in regards to the effect and timing of SVR. In addition, it is also unclear if SVR associated the new direct acting antiviral agents confers similar long-term benefits regarding HCC risk as that seen with IFN-based therapies and needs further investigations.

In summary, in our cohort of 851 Asian patients, patients with HCV genotype 6 and cirrhosis had a significantly higher HCC incidence after long-term follow-up compared to patients with other HCV genotypes. HCV genotype 6 was a significant independent predictor for HCC following adjustment for age, gender, response to antiviral therapy, HBV coinfection, and poor hepatic function. Patients with HCV genotype 6 in particular should be counseled and considered for antiviral therapy to prevent future HCC development.

CONFLICT OF INTEREST

Guarantor of the article: Dr Mindie H. Nguyen, MD, MAS.

Specific author contributions: Study concept and design: Mindie H. Nguyen, Grace Lai-Hung Wong; acquisition of data: Tiffany I. Hsiao, Shreenidhi R. Subramaniam, An K. Le, Vinh D. Vu, Huy N. Trinh, Jian Zhang, Grace Lai-Hung Wong, Mindie H. Nguyen; analysis and interpretation of data and drafting of the manuscript: Mei-Hsuan Lee, Tiffany I. Hsiao, Mindie H. Nguyen; data interpretation and critical review of the manuscript: Shreenidhi R. Subramaniam, An K. Le, Vinh D. Vu, Huy N. Trinh, Jian Zhang, Mingjuan Jin, Vincent Wai-Sun Wong, Grace Lai-Hung Wong; study supervision: Mindie H. Nguyen.

Financial support: None.

Potential competing interests: Mindie H. Nguyen had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Mindie H. Nguyen has received research support from Bristol-Myers Squibb, Gilead Sciences, Janssen Pharmaceuticals and has served as advisory board member or consultant with honoraria for Janssen Pharmaceuticals, Gilead Sciences, Intercept Pharmaceuticals, Alynam Pharmaceuticals, Roche Laboratories, and Dynavax Laboratories. Grace Lai-Hung Wong serves as an advisory board member for Gilead Sciences, and Otsuka, and has received lecture fees from Abbott, Abbvie, Bristol-Myers Squibb, Echosens, Furui, Gilead and Otsuka. Vincent Wai-Sun Wong serves as advisory board member for AbbVie, Gilead Sciences, Otsuka, and Roche, and has received lecture fees from Echosens, Gilead Sciences, Merck; and also was a consultant for Merck, NovoMedica. Mei-Hsuan Lee serves as anadvisory board member for Gilead Sciences, has received lecture fees from Gilead Sciences, Abbvie, and Bristol-Myers Squibb, and hasreceived funding from the Ministry of Science and Technology (MOST 104-2628-B-010-001-MY3 and MOST 105-2628-B-010-003-MY4), Taipei, Taiwan. The remaining authors declare no conflict of interest.

Study Highlights

WHAT IS CURRENT KNOWLEDGE

- ✓ Countries in Southast Asia are populous with high Hepatitis C virus (HCV) seroprevalence, predominantly genotype 6.
- ✓ Genotypes 1–3 infections common in Western countries have been studied most extensively.
- ✓ Prospective study designs have investigated the risk of HCC less in genotype 6 patients.

WHAT IS NEW HERE

- ✓ HCV genotype 6 increased the risk for hepatocellular carcinoma, particular for liver patients with cirrhosis.
- ✓ Patients with HCV genotype 6 need to be prioritized for intensive treatment and surveillance.
- ✓ Our findings contain implications for health policy making.

REFERENCES

1. Lee MH, Yang HI, Lu SN *et al.* Chronic hepatitis C virus infection increases mortality from hepatic and extrahepatic diseases: a community-based long-term prospective study. J Infect Dis 2012;206:469–77.
2. Mohd Hanafiah K, Groeger J, Flaxman AD *et al.* Global epidemiology of hepatitis C virus infection: new estimates of age-specific antibody to HCV seroprevalence. Hepatology 2013;57:1333–42.
3. Lavanchy D. Evolving epidemiology of hepatitis C virus. Clin Microbiol Infect 2011;17:107–15.
4. Hajarizadeh B, Grebely J, Dore GJ. Epidemiology and natural history of HCV infection. Nat Rev Gastroenterol Hepatol 2013;10:553–62.
5. Nguyen LH, Nguyen MH. Systematic review: Asian patients with chronic hepatitis C infection. Aliment Pharmacol Ther 2013;37:921–36.
6. Smith DB, Bukh J, Kuiken C *et al.* Expanded classification of hepatitis C virus into 7 genotypes and 67 subtypes: updated criteria and genotype assignment web resource. Hepatology 2014;59:318–27.
7. Messina JP, Humphreys I, Flaxman A *et al.* Global distribution and prevalence of hepatitis C virus genotypes. Hepatology 2015;61:77–87.
8. Bunchorntavakul C, Chavalitdhamrong D, Tanwandee T. Hepatitis C genotype 6: a concise review and response-guided therapy proposal. World J Hepatol 2013;5:496–504.
9. Chao DT, Abe K, Nguyen MH. Systematic review: epidemiology of hepatitis C genotype 6 and its management. Aliment Pharmacol Ther 2011;34:286–96.
10. Wantuck JM, Ahmed A, Nguyen MH. Review article: the epidemiology and therapy of chronic hepatitis C genotypes 4, 5 and 6. Aliment Pharmacol Ther 2014;39:137–47.

11. Wasitthankasem R, Vongpunsawad S, Siripon N *et al.* Genotypic distribution of hepatitis C virus in Thailand and Southeast Asia. PLoS ONE 2015;10:e0126764.
12. Nguyen NH, Vutien P, Trinh HN *et al.* Risk factors, genotype 6 prevalence, and clinical characteristics of chronic hepatitis C in Southeast Asian Americans. Hepatol Int 2010;4:523–9.
13. Afdhal N, Zeuzem S, Kwo P *et al.* Ledipasvir and Sofosbuvir for untreated HCV genotype 1 infection. N Engl J Med 2014;370:1889–98.
14. Lawitz E, Mangia A, Wyles D *et al.* Sofosbuvir for previously untreated chronic hepatitis C infection. N Engl J Med 2013;368:1878–87.
15. Lee MH, Yang HI, Lu SN *et al.* Hepatitis C virus genotype 1b increases cumulative lifetime risk of hepatocellular carcinoma. Int J Cancer 2014;135:1119–26.
16. Raimondi S, Bruno S, Mondelli MU *et al.* Hepatitis C virus genotype 1b as a risk factor for hepatocellular carcinoma development: a meta-analysis. J Hepatol 2009;50:1142–54.
17. Lee MH, Yang HI, Lu SN *et al.* Hepatitis C virus seromarkers and subsequent risk of hepatocellular carcinoma: long-term predictors from a community-based cohort study. J Clin Oncol 2010;28:4587–93.
18. Kanwal F, Kramer JR, Ilyas J *et al.* HCV genotype 3 is associated with an increased risk of cirrhosis and hepatocellular cancer in a national sample of U.S. Veterans with HCV. Hepatology 2014;60:98–105.
19. Seto WK, Lai CL, Fung J *et al.* Natural history of chronic hepatitis C: genotype 1 versus genotype 6. J Hepatol 2010;53:444–8.
20. U.S. Census Bureau. Asians fastest-growing race or ethnic group in 2012 (No. CB13-112) [Fact sheet]. 2013, June. Retrieved from: https://www.census.gov/newsroom/press-releases/2013/cb13-112.html.
21. Bruix J, Sherman M. American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma: an update. Hepatology 2011;53:1020–2.
22. Nguyen NH, McCormack SA, Vutien P *et al.* Meta-analysis: superior treatment response in Asian patients with hepatitis C virus genotype 6 versus genotype 1 with pegylated interferon and ribavirin. Intervirology 2015;58:27–34.
23. Nguyen NH, Nguyen MH. Current treatment options in patients with hepatitis C virus genotype 6. Gastroenterol Clin North Am 2015;44:871–81.
24. Tsang OTY, Zee JST, Chan JMC *et al.* Chronic hepatitis C genotype 6 responds better to pegylated interferon and ribavirin combination therapy than genotype 1. J Gastroenterol Hepatol 2010;25:766–71.
25. Wong GL, Chan HL, Tse CH *et al.* Impact of IL28B and PNPLA3 polymorphisms on treatment outcomes in patients infected with genotype 6 hepatitis C virus. J Gastroenterol Hepatol 2015;30:1040–8.
26. Lam KD, Trinh HN, Do ST *et al.* Randomized controlled trial of pegylated interferon-alfa 2a and ribavirin in treatment-naive chronic hepatitis C genotype 6. Hepatology 2010;52:1573–80.
27. Fung J, Lai CL, Hung I *et al.* Chronic hepatitis C virus genotype 6 infection: response to pegylated interferon and ribavirin. J Infect Dis 2008;198:808–12.
28. Gerber L, Welzel TM, Zeuzem S. New therapeutic strategies in HCV: polymerase inhibitors. Liver Int 2013;33(Suppl 1):85–92.
29. Gane EHR, An D, Svarovskaia E *et al.* High efficacy of LDV/SOF regimens for 12 weeks for patients with HCV genotype 3 or 6 infection. Hepatology 2014;60:1274A–1275A.
30. Tanaka Y, Kurbanov F, Mano S *et al.* Molecular tracing of the global hepatitis C virus epidemic predicts regional patterns of hepatocellular carcinoma mortality. Gastroenterology 2006;130:703–14.
31. Pybus OG, Barnes E, Taggart R *et al.* Genetic history of hepatitis C virus in East Asia. J Virol 2009;83:1071–82.
32. Lu L, Wu T, Xiong L *et al.* Analysis of HCV-6 isolates among Asian-born immigrants in North America reveals their high genetic diversity and a new subtype. Virology 2016;492:25–31.
33. Lee MH, Yang HI, Lu SN *et al.* Polymorphisms near the IFNL3 gene associated with HCV RNA spontaneous clearance and hepatocellular carcinoma risk. Sci Rep 2015;5:17030.
34. Kin KC, Lin B, Ha NB *et al.* High proportion of hepatitis C virus in community Asian American patients with non-liver-related complaints. J Clin Gastroenterol 2013;47:367–71.
35. Ho EY, Ha NB, Ahmed A *et al.* Prospective study of risk factors for hepatitis C virus acquisition by Caucasian, Hispanic, and Asian American patients. J Viral Hepat 2012;19:e105–e111.
36. Kin KC, Lin B, Chaung KT *et al.* Less-established risk factors are common in Asian Americans with hepatitis C virus: a case-controlled study. Dig Dis Sci 2013;58:3342–7.
37. Yip B, Wantuck JM, Kim LH *et al.* Clinical presentation and survival of Asian and non-Asian patients with HCV-related hepatocellular carcinoma. Dig Dis Sci 2014;59:192–200.
38. Nguyen MH, Whittemore AS, Garcia RT *et al.* Role of ethnicity in risk for hepatocellular carcinoma in patients with chronic hepatitis C and cirrhosis. Clin Gastroenterol Hepatol 2004;2:820–4.
39. Coppola N, Onorato L, Sagnelli C *et al.* Association between anti-HBc positivity and hepatocellular carcinoma in HBsAg-negative subjects with chronic liver disease: a meta-analysis. Medicine 2016;95:e4311.
40. Ikeda K, Marusawa H, Osaki Y *et al.* Antibody to hepatitis b core antigen and risk for hepatitis c-related hepatocellular carcinoma: A prospective study. Ann Intern Med 2007;146:649–56.