Impact of hepatitis C virus genotype 3 on liver disease progression in a Chinese national cohort

Nan Wu[1], Hui-Ying Rao[1], Wei-Bo Yang[2], Zhi-Liang Gao[3], Rui-Feng Yang[1], Ran Fei[1], Ying-Hui Gao[1], Qian Jin[1], Lai Wei[1,4]

[1]Peking University People's Hospital, Peking University Hepatology Institute, Beijing Key Laboratory of Hepatitis C and Immunotherapy for Liver Disease, Beijing 100044, China;
[2]Department of Infectious Diseases, The First Affiliated Hospital of Kunming Medical College, Kunming, Yunnan 650032, China;
[3]Department of Infectious Diseases, The Third Affiliated Hospital of Sun Yat-Sen University, Guangzhou, Guangdong 510630, China;
[4]Hepatopancreatobiliary Center, Beijing Tsinghua Changgung Hospital, Institute for Precision Medicine, Tsinghua University, Beijing 102218, China.

Abstract

Background: Hepatitis C virus (HCV) genotype 3, particularly subtype 3b, is increasing in prevalence and distribution in China. This study evaluated the prevalence, regional distribution, clinical characteristics, host factors, treatment outcomes, and disease progression of patients with HCV genotype 3 in China.

Methods: A 5-year follow-up was preceded by a cross-sectional study. Treatment choices were at the discretion of treating physicians. Estimated infection time to overall-disease-progression (defined by ≥1 of: newly diagnosed cirrhosis; cirrhosis at baseline, Child-Turcotte-Pugh score increased 2 points or more; progression from compensated cirrhosis to decompensated cirrhosis; hepatocellular carcinoma; liver transplantation; or death) was calculated using the Kaplan-Meier method. Cox regression analyses were conducted to evaluate the risk factors for disease progression.

Results: The cross-sectional study enrolled 997 patients, including 91 with HCV genotype 3 infection. Among them, subtype 3b (57.1%) was more dominant than subtype 3a (38.5%). Five hundred and twelve patients were included into the follow-up phase. Among patients analyzed for estimated infection time to overall-disease-progression, 52/304 (17.1%) patients with HCV genotype 1 and 4/41 (9.8%) with HCV genotype 3 (4/26 with genotype 3b, 0/13 with genotype 3a, and 0/2 with undefined subtype of genotype 3) experienced overall-disease-progression. Patients with HCV genotype 3 were younger than those with genotype 1 (mean age: 39.5 ± 8.7 *vs.* 46.9 ± 13.6 years) and demonstrated more rapid disease progression (mean estimated infection time to overall-disease-progression 27.1 *vs.* 35.6 years).

Conclusions: HCV genotype 3, specifically subtype 3b, is associated with more rapid progression of liver disease. Further analysis to compare HCV subtype 3a and 3b is needed in high prevalence regions.

Trial registration: NCT01293279, https://clinicaltrials.gov/ct2/show/NCT01293279; NCT01594554, https://clinicaltrials.gov/ct2/show/NCT01594554.

Keywords: Hepatitis C virus genotype 3; Chronic hepatitis C; Disease progression

Introduction

Hepatitis C virus (HCV) infection is a major global health concern. According to recent estimates, the global prevalence of HCV infection is about 1.0%, affecting approximately 71 million patients.[1] The prevalence of HCV in China was estimated at around 0.7% of the total population, corresponding to nearly 10 million patients, which is the largest population with HCV infection in the world.[1] The recent development of direct-acting antivirals (DAAs) has revolutionized the therapy of chronic HCV infection, leading to a cure in more than 90% of patients.[2] Due to the extraordinary efficacy of DAAs, the World Health Organization proposes a 70% reduction in HCV incidence by 2030 compared with 2010.[3] However, there are still challenges in achieving this goal, such as the increasing trend from 2001 to 2012 of the large HCV population in China[4] and the difficulties with the elimination of HCV genotype 3.[5]

HCV can be classified into seven major genotypes and 67 confirmed subtypes based on its genetic diversity.[6] Globally, genotype 1 is dominant (44% of all infections), followed by genotype 3 (25% of all infections). While local prevalence of genotypes varies throughout the world, HCV genotype 3 is more prevalent in South (India and Pakistan)

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Correspondence to: Prof. Lai Wei, Hepatopancreatobiliary Center, Beijing Tsinghua Changgung Hospital, Institute for Precision Medicine, Tsinghua University; No. 168, Litang Road, Changping District, Beijing 102218, China
E-Mail: weilai@mail.tsinghua.edu.cn

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and Southeast Asia[1] and can be found in more than 30% of the HCV population in certain European countries, such as Russia, Denmark, and Finland.[1] In China, genotype 1 is the most prevalent overall, while genotype 3 contributes for about 10% of the infected population and is mainly distributed in Southwest provinces and mostly related to intravenous drug use (IVDU).[7,8] In recent years there has been a trend towards increased prevalence and nationwide distribution of HCV genotype 3.[9,10] Unlike South Asian countries, where the majority of genotype 3 infections are subtype 3a, the subtype in China is mainly subtype 3b.[7,8] HCV genotype 3 has been shown to possess a direct steatogenic effect that may be associated with increased fibrosis progression and corresponding increased risk of hepatocellular carcinoma.[5,11,12] Meanwhile, HCV genotype 3 demonstrates a difficult-to-treat character in the era of DAAs. Many currently approved regimens have reduced activity in people with genotype 3 infection.[13,14] Recent results from the Asian and Chinese population demonstrated that only 50% to 73% cirrhotic patients with HCV subtype 3b infection achieved sustained virological response at 12 weeks (SVR12) after treatment with DAA regimen.[15,16] However, the differences in clinical features, clinical outcome, and disease progression between subtype 3a and 3b are still not clear.

CCgenos study (HCV Viral Genotyping Distribution and Genetic Variation in IL28B Distributions among the Han Ethnic Chinese in China, AI452-009/NCT01293279) and the Follow-up Phase of the CCgenos study (AI452-018/NCT01594554) were conducted to evaluate the prevalence, regional distribution, clinical characteristics, host factors, and clinical outcomes in Chinese patients with HCV infection. This study was a subgroup analysis of patients with HCV genotype 3 infection, including subtype 3b, in CCgenos study.

Methods

Ethics approval

The process of completing patients' visit, collecting patients' blood sample, and reviewing patients' data was approved by a central review board and the institutional review boards of each participating center, and by the China National Human Genetic Resource Management Office (2010). All patients provided written informed consent before their enrollment in this study.

Study population

Han ethnic treatment-naïve patients with chronic HCV infection ≥18.0 years old were enrolled at 28 university-affiliated hospitals across China. Provinces in China were grouped into five geographic regions: North, South, East, West, and Central. Enrollment was stratified according to the population demographics of each region. HCV infection was confirmed or reconfirmed (anti-HCV antibody and HCV RNA positive) within 90 days before enrollment. Patients who had received anti-viral or interferon-based therapy for hepatitis C or hepatitis B were excluded. Patients enrolled in the cross-sectional phase of study were invited to enter the 5-year follow-up phase. No other exclusion criteria were applied.

Study design

This study consisted of two phases. The cross-sectional observational phase (conducted between February and June 2011, the CCgenos study, AI452-009/NCT01293279) provided data on the distribution, demographic, clinical characteristics, and socioeconomic status of treatment-naïve patients with HCV infection across the mainland of China.[7] Demographic information, medical histories, physical examinations, blood samples were obtained within 9 days of enrollment. All patients were interviewed to collect information on their lifestyle, HCV transmission risk factors, and socioeconomic status. HCV genotyping, host interleukin 28B (IL-28B), and inosine triphosphate pyrophosphatase (ITPA) genotyping were performed centrally.

The 5-year follow-up phase (AI452-018/NCT01594554) was conducted, between April 2012 and March 2018, to assess the long-term, real-world treatment patterns, and clinical outcomes of HCV infected patients.[17] HCV anti-viral treatment information for each patient between June 2011 and April 2012 was collected retrospectively. Before and during follow-up, choices of treatment options were at the discretion of the treating physicians, and no randomization or protocol-driven treatment was implemented. Patients on active anti-viral treatment visited every 3 ± 1 months to the hospital, patients who remained treatment naïve and those who had completed treatment visited every 6 ± 2 months accordingly. For every visit: (1) clinical information was collected; (2) biochemistry, hematology, blood coagulation functions, and ultrasound were performed at local laboratories; (3) HCV-RNA assessments were performed centrally. If a patient received anti-viral treatment, SVR24 was evaluated by the HCV-RNA results obtained at the closest time point after 24 weeks of treatment cessation.

HCV quantification and genotyping, and host IL-28B, ITPA genotyping

The HCV viral load was quantified using an Abbott RealTime HCV assay (Abbott Laboratories, Des Plaines, IL, USA; lower limit of detection 12 IU/mL). HCV genotype was assessed using a Versant HCV Genotype 2.0 Assay (LiPA) by Siemens (Siemens Healthcare Diagnostics, Tarry-town, NY, USA). Single-nucleotide polymorphisms (SNPs) of IL28B genomic region (rs12979860, rs8099917, rs12980275), and ITPA genomic region (rs1127354) were genotyped. The host genotypes were identified with iPLEX Gold (Sequenom, San Diego, CA, USA) at CapitalBio, a platform that could map SNPs.

Definition of disease progression

Whenever liver biopsy results were available, liver cirrhosis was diagnosed based on biopsy results. If biopsy results were absent, compensated cirrhosis was diagnosed by two of the following criteria: imaging showing nodular liver or splenomegaly, platelet count $<100 \times 10^9$/L in the absence of other explanations, liver stiffness measurement score of more than 13 kPa, or gastro-esophageal varices in

endoscopy. Decompensated cirrhosis was defined as cirrhosis with sequelae such as: ascites, hepatic encephalopathy, variceal hemorrhage, or Child-Turcotte-Pugh (CTP) score ≥7. Overall-disease-progression was defined by any of the following events during follow-up: (1) newly diagnosed cirrhosis; (2) cirrhosis at baseline, CTP score increased 2 points or more; (3) progression from compensated cirrhosis to decompensated cirrhosis; (4) hepatocellular carcinoma; (5) liver transplantation; (6) death. Hepatocellular carcinoma, fatty liver disease, and type 2 diabetes were diagnosed using established guidelines.[18-20] Patients' HCV exposure history was used to estimate the time of their infection.[7]

Statistical analysis

Continuous variables were expressed as mean and standard deviation (SD) for parameters with normal distributions or median and interquartile range for non-normal distributions and compared using the Student's *t* test or Mann-Whitney *U* test; categorical variables were tabulated with counts and percentages and compared using the Chi-squared analysis or Fisher exact test. Survival curves (estimated infection time to disease progression) were calculated using the Kaplan-Meier method and compared using the log-rank test. The association of HCV genotype 3 and other possible risk factors with disease progression was evaluated via univariate and multivariate Cox regression analyses. The risk factors were expressed as a hazard ratio and 95% confidence interval (CI). All analyses were performed with SPSS software 19.0 (SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered to be statistically significant.

Results

Study patients

A total of 1012 patients were enrolled in the cross-sectional study, and 15 of them were excluded for protocol violations (one patient had two violations): two patients were <18.0 years old and/or not ethnic Han; ten patients did not confirm HCV infection within 90 days before enrollment; four patients failed to undergo physical examination and blood sampling within 9 days after enrollment. As a result, 997 patients were included in this analysis, of which 512 patients were included in the follow-up phase from 25 university hospitals across China [Figure 1]. Among them, three patients with HCV genotype 1 infection were diagnosed as cirrhosis based on biopsy results, one patient was diagnosed at baseline, one was diagnosed at the 2nd annual visit, and one was diagnosed at the last visit. There were 41 patients with genotype 3 in the follow-up phase and 24 (58.5%) of them received interferon-based therapy.

Geographical distribution of patients and HCV genotype 3

Overall, genotype 1 was identified in 582 patients (58.4%), genotype 2 in 240 patients (24.1%), genotype 3 in 91 patients (9.1%), and genotype 6 in 63 patients (6.3%). Among the patients with genotype 3, 35 (38.5%) patients were identified as subtype 3a, 52 (57.1%) patients were identified as subtype 3b, and four patients' subtypes failed to be identified. Geographical distribution of patients with HCV genotype 3 is summarized in Supplementary Table 1, http://links.lww.com/CM9/A165. The proportion of genotype 3 infection was highest in the southern region (25.2%,

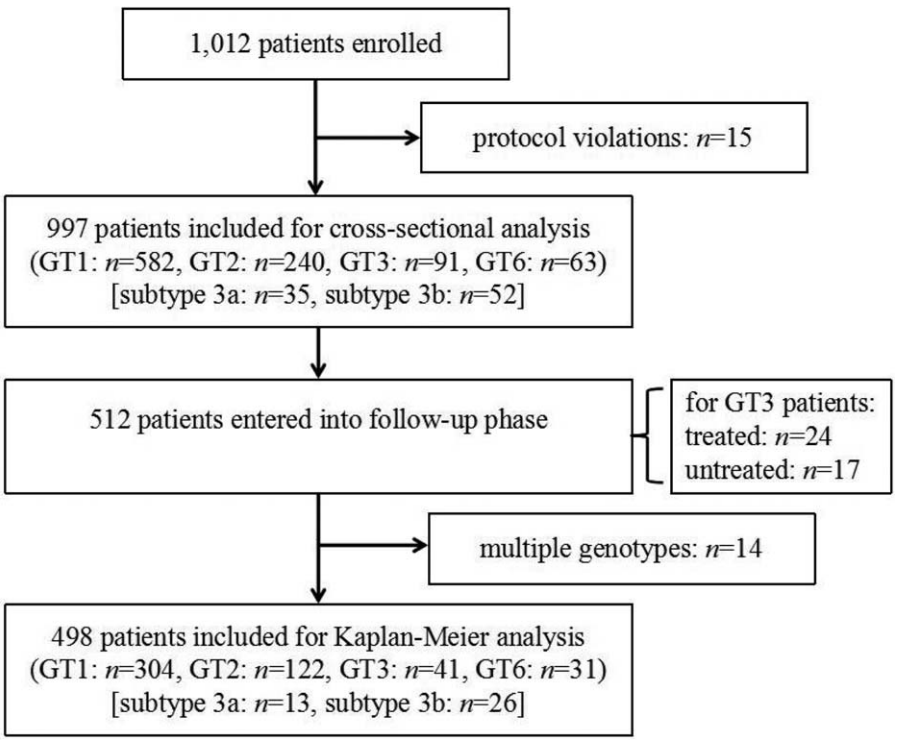

Figure 1: Flow chart of hepatitis C virus patient enrollment and follow-up. GT: Genotype.

39/155), and was 10.5% (22/209) in the western region, 6.9% (15/217) in the eastern region, 6.1% (11/181) in the northern region, and 1.7% (4/235) in the central region. The five provinces with the highest proportions of genotype 3 were: Yunnan (65.0%, 26/40), Ningxia (44.4%, 12/27), Chongqing (25.9%, 7/27), Zhejiang (25.0%, 8/32), and Fujian (18.2%, 2/11).

Baseline characteristics of patients with HCV genotype 3 infection

The baseline characteristics of patients with genotype 3 infection are summarized in Table 1 (baseline characteristic of those entering the follow-up phase can be found in Supplementary Table 2, http://links.lww.com/CM9/A165). The median age of patients infected with subtype 3b was 4.5 years greater than that of patients with subtype 3a ($P = 0.002$). A majority of the patients with genotype 3 infection were male, and the proportion of male patients was comparable in subtype 3a and 3b. The median alanine aminotransferase (ALT) level of patients with subtype 3a was significantly higher than that of patients with subtype 3b ($P = 0.012$). A total of 13.5% of patients infected with subtype 3b had diabetes but no patients with subtype 3a (this difference was significant: $P = 0.038$). Body mass index (BMI), HCV viral load, total bilirubin level, platelet counts, fasting glucose level, and the incidence of fatty liver were similar for patients with subtype 3a and 3b. Baseline cirrhosis status was similar between patients with subtypes 3a and 3b: only one patient was diagnosed with compensated cirrhosis and six patients were diagnosed with decompensated cirrhosis [Table 1]. No patient with genotype 3 was diagnosed with hepatocellular carcinoma at baseline.

Most patients had IL28B genotypes CC (rs12979860), and TT (rs8099917), with little difference between patients with subtypes 3a and 3b [Table 1]. ITPA genotype CC (rs1127354) was numerically higher in patients with subtype 3a than that in patients with subtype 3b [Table 1].

HCV transmission risk factors in patients with HCV genotype 3

The top five transmission risk factors for genotype 3 were IVDU, tattoos or piercings, dental treatment, blood transfusion, and surgery [Table 2]. Exposure to more than one risk factor was reported by 27.5% of patients with genotype 3. The patients with subtype 3a and 3b reported a similar pattern of transmission risk factors, except for blood transfusion which was significantly more frequent among patients with subtype 3a [Table 2].

Transmission risk factors for patients with genotype 3 demonstrated substantial geographic regional variation [Table 3]. Overall, IVDU was the most prevalent transmission risk factor for genotype 3, but it was only reported by patients in the southern and northern regions (in which it was the dominant factor). In the western region, tattoos or piercings and blood transfusion were the most frequently reported transmission risk factors; in the eastern region, dental treatment was most frequently reported; and in the central region there was no trend [Table 3].

Table 1: Comparison of baseline demographic, disease parameters, and host factors in patients infected with genotype 3.

Parameters	Genotype 3 ($n = 91$)	Subtype 3a ($n = 35$)	Subtype 3b ($n = 52$)	Statistics	*P* values
Male, *n* (%)	69 (75.8)	30 (85.7)	37 (71.2)	2.505*	0.287
Age (years), median (Q1, Q3)	38.0 (32.0, 42.0)	35.0 (30.0, 39.0)	39.5 (35.3, 43.5)	772.500†	0.002
Duration of infection (years), median (Q1, Q3)	9.83 (5.46, 15.05)	8.83 (3.56, 14.17)	11.17 (6.08, 17.79)	620.500†	0.152
Body mass index (kg/m^2), mean ± SD	22.3 ± 3.1	22.2 ± 3.1	22.3 ± 3.2	0.140‡	0.886
HCV RNA ($\log_{10}$IU/mL), mean ± SD	5.90 ± 1.00	6.12 ± 0.87	5.85 ± 1.05	1.257‡	0.212
ALT (U/L), median (Q1, Q3)	81.5 (52.0, 140.0)	97.0 (66.0, 151.0)	72.0 (44.0, 117.2)	619.000†	0.012
Total bilirubin (μmol/L), mean ± SD	19.40 ± 11.80	21.73 ± 12.86	18.49 ± 11.17	1.246‡	0.216
Platelet ($\times 10^9$/L), mean ± SD	162.7 ± 59.1	163.1 ± 54.1	159.3 ± 59.1	0.306‡	0.760
Diagnosis at enrollment, *n* (%)				0.826*	0.662
Chronic hepatitis	84 (92.3)	33 (94.3)	47 (90.4)		
Compensated cirrhosis	1 (1.1)	0	1 (1.9)		
Decompensated cirrhosis	6 (6.6)	2 (5.7)	4 (7.7)		
Fatty liver at enrollment, *n* (%)	14 (15.4)	7 (20.0)	6 (11.5)	1.178*	0.278
Fasting glucose (mmol/L), median (Q1, Q3)	4.93 (4.50, 5.44)	4.80 (4.40, 5.15)	4.97 (4.50, 5.59)	772.500†	0.234
Diabetes, *n* (%)	7 (7.7)	0	7 (13.5)	5.124*	0.038
HBsAg positive, *n* (%)	4 (4.4)	3 (8.6)	1 (1.9)	2.108*	0.298
IL28B rs12979860 (CC), *n* (%)	87 (95.6)	34 (97.1)	50 (96.2)	0.000*	1.000.
IL28B rs8099917 (TT), *n* (%)	87 (95.6)	34 (97.1)	50 (96.2)	0.000*	1.000
ITPA rs1127354, *n* (%)				1.383*	0.492
C/C	62 (68.1)	26 (74.3)	34 (65.4)		
C/A	27 (29.7)	9 (25.7)	16 (30.8)		
A/A	2 (2.2)	0	2 (3.8)		

* χ^2 value. † *U* value. ‡ *t* value. HCV: Hepatitis C virus; ALT: Alanine aminotransferase; HBsAg: Hepatitis B surface antigen; IL28B: Interleukin 28B; ITPA: Inosine triphosphate pyrophosphatase.

Table 2: HCV transmission risk factors in patients infected with genotype 3, *n* (%).

Risk factors	Genotype 3 (*n* = 91)	Subtype 3a (*n* = 35)	Subtype 3b (*n* = 52)	χ^2	*P* values
Intravenous drug use	37 (40.7)	12 (34.3)	25 (48.1)	1.628	0.202
Tattoos or piercings	18 (19. 8)	7 (20.0)	10 (19.2)	0.008	0.929
Dental treatment	17 (18.7)	7 (20.0)	10 (19.2)	0.008	0.929
Blood transfusion	14 (15.4)	9 (25.7)	5 (9.6)	4.015	0.045
Surgery	12 (13.2)	6 (17.1)	5 (9.6)	1.073	0.300
Intravenous infusion	4 (4.4)	1 (2.9)	3 (5.8)	0.404	0.646
Long-term exposure	3 (3.3)	2 (5.7)	1 (1.9)	0.903	0.562
Interventional exam and treatment	1 (1.1)	0	1 (1.9)	0.000	1.000
Undefined	10 (11.0)	4 (11.4)	4 (7.7)	0.350	0.709
Multiple risk factors	25 (27.5)	13 (37.1)	12 (23.1)	2.021	0.155

HCV: Hepatitis C virus.

Anti-viral treatment in patients with HCV genotype 3

For patients with genotype 3 in the follow-up phase, 58.5% (24/41) received anti-viral treatment (subtype 3a, $n = 9$; subtype 3b, $n = 13$; unidentified subtype, $n = 2$). The median (Q1, Q3) treatment duration was 47.5 (34.5, 52.5) weeks. Fourteen patients were treated with combination therapy of peg-interferon and ribavirin (median treatment duration: subtype 3a *vs.* 3b: 41.8 [30.0, 53.8] *vs.* 49.8 [33.8, 65.9] weeks), and ten patients were treated with combination therapy of conventional interferon and ribavirin (median treatment duration: subtype 3a *vs.* 3b: 40.6 [27.2, 53.4] *vs.* 54.8 [40.4, 62.8] weeks). Overall SVR24 in patients with genotype 3 was 87.5% (21/24). SVR24 was 66.7% (6/9) in patients with subtype 3a and 100% (13/13) in patients with subtype 3b.

Overall-disease-progression in patients with genotype 3

Kaplan-Meier analysis of disease progression during the follow-up phase was carried out for 41 patients with HCV genotype 3 and 304 patients with genotype 1 [Figures 1 and 2]. The patients with HCV genotype 3 infection were younger than those with genotype 1 (mean age ± SD: 39.5 ± 8.7 *vs.* 46.9 ± 13.6 years), and were infected for a shorter duration than patients with genotype 1 (median [Q1, Q3]: 12.4 [9.0, 17.8] *vs.* 18.6 [15.7, 21.2] years) [Supplementary Table 2, http://links.lww.com/CM9/A165]. The incidence of overall disease progression was comparable between patients with genotype 1 infection and patients with genotype 3b infection: overall-disease-progression was experienced by 17.1% (52/304) of patients with genotype 1 and by 15.4% (4/26) of patients with genotype 3b but by no patients with genotype 3a (0/13) or undefined subtype of genotype 3 (0/2) (making a total of 9.8% (4/41) of all patients with genotype 3). However, median time from estimated infection to overall-disease-progression during follow-up in patients with genotype 3b was nearly one decade shorter than that in patients with genotype 1 (median [95% CI]: 27.1 [24.3–undetermined] *vs.* 35.6 [30.4–53.5] years) [Figure 2]. For genotype 3 patients, incidence of disease progression was comparable between treated and untreated patients [Supplementary Figure 1, http://links.lww.com/CM9/A165].

In univariate Cox regression analyses, disease progression was significantly associated with no treatment, age of being infected ≥40.0 years, age of enrollment ≥40.0 years, abnormal ALT and aspartate aminotransferase (AST), being female, having diabetes, platelet count $\leq 100 \times 10^9$/L, AST to platelet ratio index ≥1.5 and ≥2.0, and not achieving SVR 24 ($P < 0.05$) [Table 4]. Age of enrollment ≥40.0 years, abnormal AST, platelet count $\leq 100 \times 10^9$/L were significantly associated with disease progression in multivariate analyses [Table 4].

Discussion

This analysis expands on previously published results from the CCgenos study,[7] with updated 5-year follow-up data

Table 3: Main transmission risk factors of HCV genotype 3 in different regions, *n* (%).

Risk factors	East (*n* = 15)	West (*n* = 22)	South (*n* = 39)	North (*n* = 11)	Central region (*n* = 4)	χ^2	*P* value
Intravenous drug use	0	0	29 (74)	8 (73)	0	56.873	<0.001
Tattoos or piercings	3 (20)	6 (27)	5 (13)	3 (27)	1 (25)	3.082	0.534
Dental treatment	6 (40)	3 (14)	4 (10)	3 (27)	1 (25)	7.286	0.088
Blood transfusion	3 (20)	6 (27)	3 (8)	1 (9)	1 (25)	5.393	0.196
Surgery	1 (7)	5 (23)	4 (10)	1 (9)	1 (25)	3.376	0.477
Undefined	1 (7)	5 (23)	3 (8)	0	1 (25)	5.408	0.191

HCV: Hepatitis C virus.

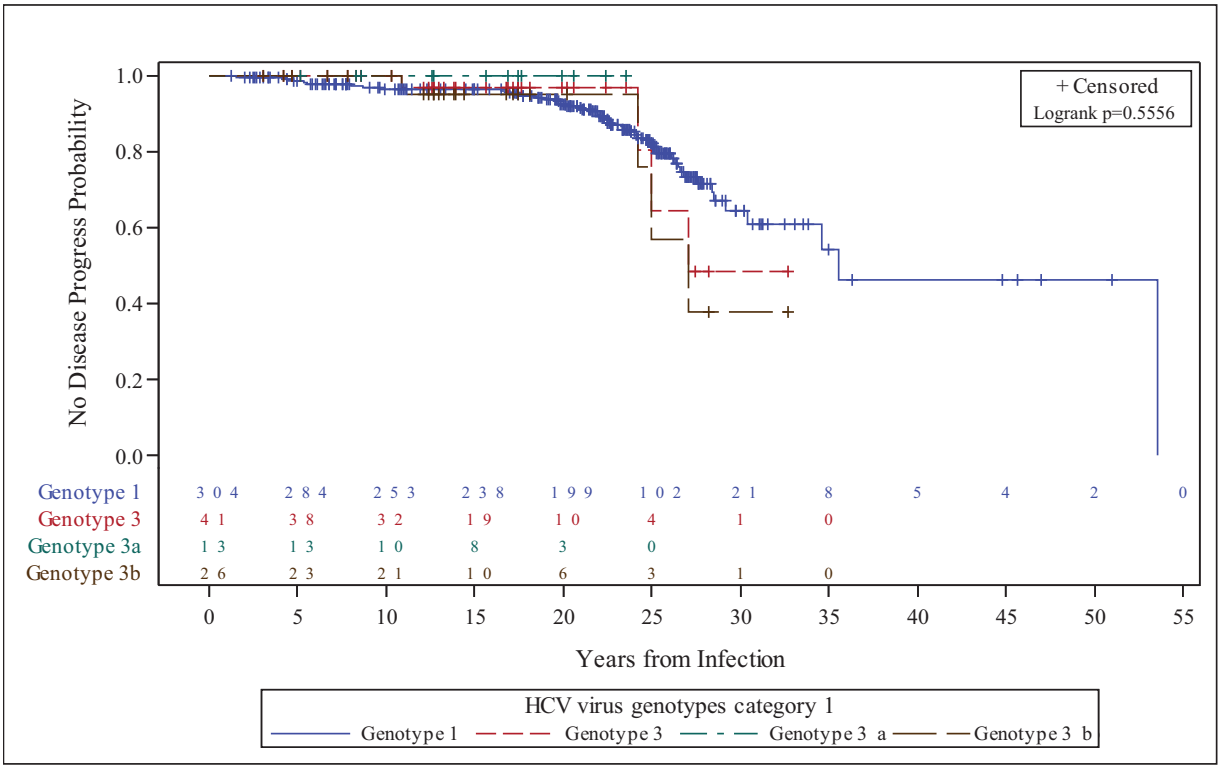

Figure 2: Kaplan-Meier curve for time from estimated infection to overall-disease-progression for HCV genotype 1 and genotype 3 patients. HCV: Hepatitis C virus.

Table 4: Cox regression analyses of the risk factors on estimated infection time to disease progression.

	Univariate analysis			Multivariate analysis		
Risk factors	**Hazard ratio**	**95% CI**	***P* values**	**Hazard ratio**	**95% CI**	***P* values**
Treatment, yes *vs.* no	0.360	0.225, 0.575	<0.001			
Age at time of HCV infection, ≥40 *vs.* <40 years	3.813	1.890, 7.692	<0.001			
Age at time of enrolment, ≥40 *vs.* <40 years	4.935	1.985, 12.272	<0.001	2.955	1.158, 7.543	0.0234
Sex, male *vs.* female	0.561	0.347, 0.906	0.018			
BMI						
24.0–27.9 *vs.* <24.0 kg/m^2	1.410	0.854, 2.327	0.179			
≥ 28.0 *vs.* <24.0 kg/m^2	1.141	0.546, 2.387	0.726			
Genotype						
GT3 *vs.* GT1	1.108	0.399, 3.078	0.844			
GT3a *vs.* GT1	<0.001	–	0.986			
GT3b *vs.* GT1	1.637	0.589, 4.547	0.345			
GT3a *vs.* GT3b	<0.001	–	0.998			
Diabetes history, yes *vs.* no	2.219	1.165, 4.224	0.015			
ALT (U/L)						
Sometimes ≥ ULN *vs.* always < ULN	3.231	1.750, 5.965	<0.001			
Always ≥ ULN *vs.* always < ULN	5.137	2.180, 12.102	<0.001			
AST (U/L)						
Sometimes ≥ ULN *vs.* always < ULN	5.926	2.511, 13.982	<0.001	3.509	1.224, 10.062	0.020
Always ≥ ULN *vs.* always < ULN	18.829	7.680, 46.163	<0.001	7.390	2.413, 22.632	<0.001
Baseline platelet, ≥100 *vs.* <100 ×10^9/L	0.210	0.130, 0.340	<0.001	0.342	0.200, 0.585	<0.001
HBV co-infection, yes *vs.* no	0.329	0.042, 2.554	0.288			
Alcohol, no *vs.* yes	1.362	0.712, 2.602	0.350			
Baseline APRI						
≥ 1.5 *vs.* <1.5	2.236	1.396, 3.582	<0.001			
≥ 2.0 *vs.* < 2.0	1.856	1.147, 3.004	0.012			
SVR24, yes *vs.* no	0.389	0.161, 0.939	0.036			

BMI: Body mass index; ALT: Alanine aminotransferase; ULN: Upper limit of normal; AST: Aspartate aminotransferase; HBV: Hepatitis B virus; APRI: AST to platelet ratio index; SVR: Sustained virologic response; –: Undetermined.

to develop more comprehensive evaluation for HCV genotypes 3 (including subtype 3a and 3b) in China. It is known that China has the largest HCV-infected population in the world, and HCV infection is a major health burden for China.[1] In the era of DAAs, HCV genotype 3 infection has attracted more and more attention, because of poor response to potent anti-viral regimens. Meanwhile, studies focused on genotype dynamic revealed the increasing prevalence of HCV genotype 3 infection in China.[10] The present study analyzed HCV genotype distribution using stratified patient enrollment based on regional population demographics. Therefore, we believe that our study population accurately reflected the HCV genotype distribution in China. In the present cohort, 9.1% patients with HCV infection were identified as genotype 3. Among them, subtype 3b patients (57.1%) were more dominant other than subtype 3a (38.5%). These data are similar to those in a recent report based on more than 30,000 clinical samples from a large, independent laboratory in China, in which the prevalence of subtype 3b infection (7.06%) was higher than subtype 3a (4.62%).[8]

The geographical distribution and genetic diversity of HCV are constantly changing, driven by changes in transmission routes (eg, improvements in blood transfusion safety or increasing IVDU) and increasing global travel.[9] In the present cohort, the distribution of genotype 3 infection showed regional imbalance. HCV genotype 3 was most prevalent in the southern region (25.2%), at the province level it was most prevalent in Yunnan (65.0%). IVDU is a major risk factor for HCV genotype 3 infection in China as well as in European countries.[21,22] It was reported that more than 70% of IVDUs were HCV-positive in the Yunnan Province.[23] Therefore, it is important to control HCV genotype 3 transmission particularly in IVDU and, especially in the southern region. Although IVDU are at the highest risk, it should be noted that, nearly one-third (27.5%) of patients with HCV genotype 3 infection reported exposure to more than one risk factor. HCV transmission through potentially unsafe medical procedures such as dental treatment, which were reported in nearly 20% patients in the present cohort, is also a concern.

In the present study, nearly 60% patients with HCV genotype 3 were treated, and all of them received interferon-based therapy as DAAs were not accessible in China during most of the follow-up period. SVR24 was higher in patients with subtype 3b (13/13, 100%) than in patients with subtype 3a (6/9, 66.7%), and the optimal efficacy might due to the longer treatment duration for patients with subtype 3b in this study. Actually, treatment efficacy was not satisfied for HCV genotype 3 patients in DAAs era.[5,24] HCV subtype 3b showed even more difficult-to-treat character with sofosbuvir-based regimen, especially in cirrhotic patients.[15,16] In a recent clinical trial of sofosbuvir/velpatasvir treatment for HCV infection in an Asian population, patients with subtype 3b infection were primarily enrolled in China and all of whom had baseline resistance-associated substitutions, only 50% (7/14) of the cirrhotic patients achieved SVR12.[16] Latest real-life study in an Asian cohort, in which HCV genotype 3 patients were predominantly infected with subtype 3b, supported that the combination of ribavirin could improve SVR12 significantly.[25] However, in the present cohort, the prevalence of ITPA rs1127354 CC genotype in HCV genotype 3 patients was 68.1%. Considering the association of ITPA rs1127354 CC genotype with a higher risk for hemolysis induced by ribavirin treatment,[26] the possible risk of hemolysis will be relatively high in HCV genotype 3 patients if they receive ribavirin therapy. Therefore, ideal ribavirin-free regimen still warrants further investigation in HCV genotype 3 patients.

In the assessment of disease progression, patients with genotype 3, especially patients with subtype 3b infection, demonstrated a more rapid disease progression than patients with genotype 1. It has been reported in Western populations that HCV genotype 3 may be associated with increased fibrosis progression and risk of hepatocellular carcinoma.[11,12] A recent Korean cohort retrospectively analyzed nearly 1500 patients with chronic HCV infection and found that genotype 3 was an independent risk factor for disease progression.[27] The Korean cohort above was limited by the retrospective nature of its design and an inherent bias in the selection of the cohort (including patients in the same province). The present study prospectively evaluated the impact of HCV genotype 3 on disease progression in a Chinese population. We found that the incidence of overall-disease-progression was not statistically different between patients with genotype 1 and genotype 3 infection. According to the results of multivariate analyses, age at enrollment ≥40.0 years, AST and platelet level might predict the incidence of disease progression. However, the median time from estimated infection to overall-disease-progression during follow-up in patients with genotype 3 was nearly one decade shorter than that in patients with genotype 1. Therefore, HCV genotype 3 infection still needs to be concerned. It was noteworthy that 15.4% (4/26) of patients infected with HCV subtype 3b, comparing with none of patients with subtype 3a infection, experienced disease progression in our cohort. Comparison of clinical characteristics between genotype 3a and genotype 3b has not been well-described in the past. In a previous Chinese cohort,[15] age, male sex, BMI, HCV viral load were similar for patients with subtype 3a and 3b infection. In our cohort, patients infected with subtype 3b were older than patients with subtype 3a at enrollment. Since age of enrollment ≥40.0 years was significantly associated with disease progression in multivariate analyses, the age difference between two groups might partially explain the difference in disease progression. Second, 13.5% of patients with subtype 3b infection, but no subtype 3a patients, had diabetes. For the four patients with subtype 3b infection experienced disease progression during follow-up, two of them had diabetes at the time of enrollment. It was reported that chronic hepatitis C patients who developed diabetes were at an increased risk of liver cirrhosis and its decompensation and hepatocellular carcinoma over time.[28-30] Although the association of having diabetes with disease progression lost statistical significance in multivariate analyses in our cohort, the status of diabetes still need to be concerned for HCV genotype 3 patients. Third, all of the subtype 3a patients

dropped out at the estimated infection time of 25 years. This might cause underestimating of disease progression in this group of patients.

There are a few limitations for our study. Foremost, our findings could potentially be related to limited sample size of HCV genotype 3 patients during follow-up stage. Since patient enrollment and sampling were weighted according to the population density within each region in current study, the relatively low prevalence of HCV genotype 3 at nationwide level and high drop-out rate during follow-up limited sample size. The results need to be interpreted with caution. Further studies analyzing HCV subtype 3b as separate group are warranted in HCV genotype 3 high prevalence regions. Second, very few patients received DAAs treatment during the study due to accessibility limitation under the complex treatment pattern transformation. Therefore, it is necessary to observe the clinical outcomes of HCV genotype 3 patients in DAAs era.

In summary, HCV genotype 3, specifically subtype 3b, is associated with rapid progression of liver disease, and given the trend of increasing prevalence and nationwide distribution of HCV genotype 3, it is a major public health concern in China. Further analysis to compare subtype 3a and subtype 3b is needed in high prevalence region.

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Conflicts of interest

Hui-Ying Rao has received speaker fees from the Bristol-Myers Squibb, Gilead, and Abbvie. Lai Wei has received grants from Abbvie, BMS, and Roche to his institution; has personally provided consulting to Abbvie, Allergan, BMS, Gilead, JNJ, MSD, and Pfizer; received speaker fees from Abbvie, Gilead, and GSK.

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