Geographic Variation of Indigenous Hepatitis C Virus Subtypes 6g and 6w in an Endemic Area of Southern Taiwan

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Abstract

Background and Aims: Hepatitis C virus (HCV) genotype (GT) 6 is the most genetically diverse GT and mainly distributed in Southeast Asia and south China but not Taiwan. Earlier studies showed the major GTs in Taiwan were GT 1b and GT 2 with very rare GT6 except in injection drug user and subtype 6a is the main GT 6 subtype among IDUs. Recently we reported a much higher prevalence (18.3%) of GT 6 in Tainan City, southern Taiwan. This study was designed to clarify the subtypes of GT 6 in this endemic area.

Materials and Methods: A total 3022 (1343 men and 1679 women) HCV patients were enrolled. GT 6 subtypes were determined by sequencing of core/E1 and nonstructural protein 5B (NS5B) in 322 of 518 GT 6 patients.

Results: The overall GT6 prevalence rate was 17.1% (518/3022) with higher prevalence districts located in northern Tainan. The major GT6 subtypes in Tainan were 6g (81.0%), followed by 6w (10.8%), 6a (7.5%), and 6n (0.7%).

Conclusions: The high GT 6 prevalence in Tainan was mainly due to subtype 6g and 6w with characteristic geographic distribution, suggesting subtypes 6g and 6w could be indigenous in Tainan for centuries.

Introduction

Genotype (GT) 6 hepatitis C virus (HCV) is the most genetically diverse genotype and mainly distributed in Southeast Asia, southern China.1-4 Up to 31 subtypes from 6a to 6xh have been identified.5 Taiwan is an HCV endemic country with considerably geographic variation of HCV prevalence from 1.7 to 57.9%, the predominant subtypes are 1b and 2a.6-9 Sixty to 70% of HCV genotypes have been reported to be 1b from northern Taiwan, while in southern Taiwan, the prevalence rates of genotypes 1b and 2a are about 50% and 35-41% respectively.8 GT 6 HCV infection was seldom reported from Taiwan except in intravenous drug users (IDUs), especially in IDUs with human immunodeficiency virus (HIV) and the major GT 6 subtypes were 6a, 6k and 6n.10,11 We recently reported unexpected high prevalence rates (17.1~18.3%) of GT 6 in Tainan City with characteristic geographic restriction between two rivers.12,13 To clarify the epidemiology of this unique HCV genotype in southern Taiwan as a local endemic disease and its geographic variation, we further determined the GT 6 subtypes and analyzed their distribution in Tainan City.

Materials And Methods

From 1 Mar. 2016 to 28 Feb. 2019, a total of 3339 patients with HCV viremia who were followed up at Chi Mei medical system, including Yongkang, Liouying, and Chiali (Liuying and Jiali, in Figures) campuses, for reimbursement of direct-acting antivirals (DAAs) treatment were enrolled.

Three hundreds and seventeen patients who are not residents of Tainan city were excluded (Kaohsiung City and Chiayi City/County, at south and north of Tainan). There were 3022(1343 men and 1679 women, mean age, 64.6 ± 12.4 years) patients who live in 37 districts of Tainan City. We excluded the Lonqgi district for only one patient registered in our database. The definition of patient's living district was according to patients' chart records.

The working flow of HCV genotype determination had been reported previously.12 In brief, the Abbott Realtime Genotype II (Des Plaines, IL, USA) was used to determine HCV GTs, and then Abbott Realtime Genotype II PLUS assay (Des Plaines, IL, USA) was used when Realtime GT II revealed ambiguous results. Nonstructural protein 5B (NS5B) and core/E1 sequencing were conducted to check genotypes when PLUS assays still could not determine the genotypes.12 LVN 480-300 labTurbo Virus mini kit (Taigen, Taipei, Taiwan) was used for RNA extraction. Superscript one-step RT-PCR system with platinum Taq DNA polymerase (Invitrogen, Waltham, Massachusetts) and PCR condition using GoTag® Master Mixes (Promega) were followed the manufacturer's instructions. The sequences amplified were 827~1619 for core/E1 and 8230~9266 for NS5B (outer primers) and 847~1325 for core/E1 and 8297~8690 for NS5B (nested PCR).13 Sequences generated were aligned using Basic Local Alignment Search Tool (BLAST) of NCBI database to determine genotypes. MEGA software version 6.0 was used to construct the phylogenetic tree using the neighbor-joining method based on the Kimura 2-parameter distance.
The study was approved by ethical committee of Chi Mei Medical Center and informed consent was obtained from each patient. All experiments were performed in accordance with relevant guidelines and regulations.

The significance of possible associations between discrete variables was compared using Chi-square test. The continuous variables were compared with student t test. The level of statistical significance was set at two-tailed \( P < 0.05 \).

**Results**

The overall numbers and prevalence rates of HCV GT 1a, 1b, 2, 3, 4, 6 and mixed types among chronic hepatitis C (CHC) patients were 119 (3.9%), 956 (31.6%), 1388 (45.9%), 19 (0.6%), 7 (0.2%), 518 (17.1%) and 15 (0.5%) respectively (Table 1). There were no GT 5 and very rare GT 3 and 4 (combined less than 1%). Genotypes 2 and 1b remained two major prevalent HCV genotypes in Tainan as previous studies but with higher GT 2 and much lower GT 1b prevalence. \(^6^\,^9^\) GT 6 prevalence rate 17.1% was significantly higher than previous studies reported but similar to our previous smaller study (18.3%). \(^1^2^\) Numbers and percentages of each GT of 36 districts were showed in Table 1 and Figure 1.

Two major rivers, Jishui River (JR) and Zengwen River (ZR), run through the Tainan city from east mountainous area to west coast (blue lines in Figure 1). We divided into 4 regions according to geographic and HCV prevalence variations (thick black lines in Figure 1). From east to central, the upper stream area between JR and ZR was assigned as Region-1 including 7 districts; the downstream area between JR and ZR was assigned as Region-2 with 6 districts; the southern area of ZR, almost half of Tainan city with 19 districts, we assigned as Region-3; and the northern area of JR was assigned as Region-4 with only 4 districts. Eleven of 13 districts with high GT 6 prevalence > 15% showed cluster between or adjacent to these two major rivers. All districts except one (Xuejia) located between JR and ZR(region 1 and 2) have prevalence of GT 6 more than 10%.

Region-1 has the highest prevalence of GT 6, 31.4% (266/847); 6 of 7 (85.7%) districts with prevalence of GT 6 more than 25% (Figure 1), even the lowest prevalence district (Danei) has 12.5%. Genotype 1a, 1b, 2, 3 and 4 comprise 1.9%, 26.9%, 38.3%, 0.6% and 0.1% respectively (Figure 1, Table 2).

Region-2 has the highest GT 2 prevalence (51.7%). The prevalence rates of GT 1a, 1b, 2, 4 and 6 were 4.1%, 28.2%, 51.7%, 0.4% and 15.3% respectively. All these districts except one (Xuejia 5.8%) had prevalence of GT 6 > 10%.

Region-3 included 19 districts of southern Tainan. The prevalence rates of GT 1a, 1b, 2, 3, 4 and 6 were 4.8%, 31.8%, 50.8%, 1.2%, 0.4%, and 10.7% respectively (Table 2). Two districts, with higher GT6 prevalence of 22.4% and 21.4%, Shanhua and Anding are located close to southern border of ZR. The prevalence rates of the rest districts are all under 20% and only 2 districts with prevalence rates >10% (North and Guanmiao districts)

Region-4 included Houbi, Xinying, Yanshui, and Beimen 4 districts of northern Tainan. The prevalence rates of GT1a, 1b, 2, 3 and 6 were 5.3%, 41.8%, 42.7%, 0.2%, and 9.6% respectively. Xinying with the highest GT 6 prevalence 12.8% is located close to one high GT 6 district in Region-1, Liouying(32.5%).

The GTs distribution of Region-1 showed significantly higher prevalence of GT 6 (Region-1 vs. Region-2, 3, 4; \( P < 0.00001 \)) with lower prevalence of GT 1b (Region-1 vs. Region-3, 4; \( P < 0.05 \)) and. GT 2 (Region-1 vs. Region-2, 3; \( P < 0.00001 \)).

The subtyping results were successfully obtained in 322 of 331(97.3%) of core/E1 and in 320 of 331(96.7%) of NS5B sequences of GT 6 patients (including 7 patients from Chiayi and 7 patients from Kaohsiung, at north and south of Tainan city respectively) with available sera for PCR and sequencing. Subtyping results were concordant in 307 of 331(92.7%) of samples. Genotype 6 subtypes that were determined included 6a, g, n, t, v and w, but only 4 subtypes 6a, g, n, and w with concordant typing results between core/E1 and NS5B. Subtype 6g is the most prevalent subtype in 79.8% (245/307; 81.0%, 239/295 of Tainan samples) of samples, followed by 6w 11.4% (35), 6a 7.5% (24) and 6n 1.0% (3) (Table 3).

The geographic distributions and ratios of 307 GT 6 subtypes from 30 districts of Tainan, Chiayi, and Kaohsiung were summarized in table 3 and figures 2. The distribution of 6w is clearly located in southwestern Tainan, mainly south of ZR, while
the main subtype 6g is distributed at east and north of Tainan, esp. in Region-1, and Chiayi but not Kaohsiung. Subtype 6a was found in downtown area and mountainous area of upstream of ZR. It is relatively rare in hilly southeastern Tainan.

The age distribution of GT 6 subtypes was depicted in figure 3. Subtype 6g and 6w were significantly older than 6a and 6n, suggesting different periods of transmission, cohorts or routes of infection existed in these GT 6 patients.

Discussions

Determination of HCV genotypes and subtypes is crucial in the understanding of viral evolution, transmission, epidemiology, and treatments selection. Even in the era of interferon-free pan-genotypic direct acting antivirals (DAAs), some rare subtypes might harbor intrinsic resistance to DAAs. The efficacy of pan-genotypic DDAs might be suboptimal for these local endemic subtypes as clinical trials covered mainly the global epidemic genotypes/subtypes. Moreover, the geographic differences in distribution of HCV genotypes and subtypes could reflect the epidemiological history of the virus, understanding these local endemic subtypes could help to improve public health strategies to prevent further transmission and spreading.

In this study, more than five hundreds HCV viremic patients were GT 6 in Tainan, southern Taiwan. This is the largest number of GT 6 HCV infection ever reported in Taiwan. In contrast to the peninsular Southeast Asia, GT6 HCV is rarely reported in community- or hospital-based studies from Taiwan. In an earlier study also from southern Taiwan, only 2 of 418 (0.5%) HCV patients were GT 6a. One of the reasons of such low GT 6 prevalence in earlier studies could be the limitation of earlier genotyping assays. Earlier studies using line-probe assay (LiPA) or PCR with type-specific primers aimed for 5' untranslated region (5'UTR) for genotypes determination could not detect GT 6. Highly genetic conservation of 5'UTR is suitable for PCR amplification and RNA detection but lacks sufficient variation to distinguish some GT 1 and 6 and subtypes. Using Abbott Realtime HCV GT II assay for HCV genotyping in our medical systems since 2016, we found a high rate of GT 1 without subtype designation. Among these GT 1 without subtype designation, nearly 80% were confirmed to be GT 6 by Abbott Realtime HCV GT II PLUS and core/NS5B sequencing. Besides subtypes 6a and 6b, Abbott Realtime HCV GT II PLUS assay detects more GT 6 subtypes (6c–6l) and subtypes 1a and 1b in a single reaction. One hundred and sixty-three (including six mixed infection with GT 6) of 210 samples with ambiguous result of Abbott Realtime HCV GT II were identified as GT 6 by GT II PLUS, hence, it is reasonable to assume that such a higher GT6 detection was due to increased detection of subtype 6g but not 6w in this study.

Another reason of low GT 6 prevalence in earlier reports from Taiwan might be the distribution of GT 6 is highly geographic restricted in Tainan only, suggesting a local endemic disease. As most of GT 6g patients resided between two rivers and 6w in the south Tainan. GT 6 prevalence rates in Chiayi and Kaohsiung (north and south of Taiwan) both were much lower than Tainan.

Four GT 6 subtypes 6g, w, a, and n were identified in this study but all were not first reported in Taiwan. Genotype 6, 3 and 1a were more prevalent among IDUs with HIV and subtypes 6a had been reported as the main GT6 subtype (23.5%~37.9%), followed with 6g, 6k, n and w. Extremely high anti-HCV prevalence rates from 96.6~98.7% among IDUs with HIV had been reported in Taiwan. This is most likely because an outbreak of HIV with recombinant circulating form (CRF) 07_BC was spread among IDUs in Taiwan since 2003 and peaked in 2005, long with even greater HCV outbreak since both viruses share similar transmission routes and even higher transmission efficiency. Molecular epidemiological study showed these CRF07_BC HIV sequences from Taiwan resemble the dominant strains circulating among IDUs in China. Evolutionary analyses also revealed that CRF07_BC was introduced into southern Taiwan in 1998–2001 and spread to central–northern Taiwan in 2001–2003, causing the largest HIV/AIDS and also HCV outbreaks in Taiwan. The origins of CRF07_BC HIV and HCV could be traced to Yunnan Province, China and transmitted via heroin trafficking routes. Interestingly 6g and 6w had not been reported in Yunnan and peninsular Southeast Asia but from Jakarta, Indonesia and Hong Kong and Guangzhou, China. In a large study of HCV genotypes and subtypes circulating in China, four GTs and 18 subtypes were identified among 32,030 patients, GT 6 was detected in 2332 samples (7.28%), with the most prevalent subtype being 6a (2045/2332), followed by 6n (226), 6u(36), 6g (4), 6v (2), 6w (2), and 6e, b, j, q, r (each 1). Given the large number of 6g and 6w and less than 10% 6a/6n in our study, it seemed less likely that 6g/w were transmitted from China to Taiwan or disseminated from IDUs. On the contrary, Tainan might be the origin of 6g/w, and those 6g/w detected among IDUs could be “spilled-over” from this area as only 8 and 4 subtypes 6g and 2
6w had been reported among hundreds IDUs/HIV in Taiwan. The phylogenetic analysis of E1 and NS5B from 139 samples of patients older than 70 years old showed 6w strains (D140, D370 and GZ52557) previously defined this subtype were classified closely with 15 GT 6w samples from Tainan (supplemental files), further supported Tainan as the origin of subtype 6w. While GT 6g strains from Jakarta, Indonesia and Hong Kong shared the most recent common ancestor with our 6g samples, the divergent time seemed to be much earlier (supplemental files).

Studies from Hainan island of China, which is close to Vietnam, reported a unique ecosystem of Austronesian descendants (Li tribe) with HCV infection maintained over 600 years with many novel GT6 strains and a new confirmed subtype 6xh that closely related to subtype 6g and 6w. A global collective study of HCV patients treated with different Sofosbuvir-based regimens, including over 14,000 patients, a new GT 6 subtype closely related with 6g and 6w was identified from Tainan. This evidence further supported that subtypes 6g and 6w could have existed in Southern Taiwan for centuries with a new subtype evolved, as these patients with GT6g/w were significantly older than GT6a/n patients (Figure 3). Similarly, new subtypes might exist in those with discordant subtyping results (10514010 and 21004325 in supplemental files). On the other hands, the variants of HCV sequences in Tainan seemed less diverse as in Hainan Island, suggesting a shorter time evolution, maybe less than 600 years. A tempting assumption comes from the colonial history of Taiwan and Indonesia as both Tainan and Jakarta had been colonized by Netherland (Dutch East India Company) in 17th Century, nearly 400 years ago.

In contrast to Hainan Li tribe population reside in a relatively closed environment, Taiwan has been resided by Austronesian populations for 6 thousands years, occupied by Ming-Qing dynasties for hundreds years and colonized by Spain, Netherland and Japan for decades, also with massive populations migration from China to Taiwan after World War II. The epidemiological history of HCV among indigenous Austronesian tribe (Siraya tribe) who has resided in Tainan for centuries seemed more difficult to recover.

In conclusions, we identified GT 6 subtypes 6g and 6w as the indigenous viral subtypes of HCV with characteristic geographic restriction in Tainan, probably for centuries. Tainan could be the origin of subtype 6g/w that were spilled over among IDUs. New emerging strains such as 6a, k, n as well as 1a, GT3 were most likely spread along HIV CRF07_BC outbreak after 21st century. Existence of potentially new subtypes could be anticipated and further complete sequencing data are needed.

**Abbreviations**

HCV: hepatitis C virus; GT: genotype; IDU: injection drug users; RT-PCR: reverse transcription-polymerase chain reaction; NS5B: nonstructural protein 5B; anti-HCV: HCV antibody; HIV: human immunodeficiency virus; JR: Jishui River; ZR: Zengwen River; DAA: direct acting antiviral; LiPA: line probe assay; 5’UTR: 5’ untranslated region; CRF: circulating recombinant form

**Declarations**

**Author note:** We have no known conflict of interest to disclose.

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**Competing interest statement:** None to declare

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**Tables**

Table 1 Distribution of genotypes of 36 districts of Tainan city
| Region | Districts | GT1a | GT1b | GT2 | GT3 | GT4 | GT6 | MT | T(n) |
|--------|-----------|------|------|-----|-----|-----|-----|----|------|
| 1      | Baihe     | 0    | 6    | 5   | 0   | 0   | 5   | 0  | 16   |
|        | Dongshan  | 1    | 21   | 36  | 0   | 0   | 21  | 1  | 80   |
|        | Liouying  | 4    | 53   | 75  | 0   | 0   | 65  | 1  | 198  |
|        | Liujia    | 2    | 30   | 70  | 1   | 0   | 67  | 1  | 171  |
|        | Xiaying   | 3    | 82   | 83  | 2   | 0   | 65  | 3  | 238  |
|        | Guantian  | 5    | 31   | 48  | 1   | 1   | 41  | 1  | 128  |
|        | Danei     | 1    | 5    | 7   | 1   | 0   | 2   | 0  | 16   |
| 2      | Xuejia    | 2    | 27   | 20  | 0   | 0   | 3   | 0  | 52   |
|        | Madou     | 12   | 25   | 66  | 0   | 1   | 23  | 1  | 128  |
|        | Jiangjun  | 2    | 19   | 82  | 0   | 0   | 24  | 1  | 128  |
|        | Chialli   | 6    | 45   | 63  | 0   | 1   | 22  | 0  | 137  |
|        | Xigang    | 1    | 17   | 22  | 0   | 0   | 7   | 0  | 47   |
|        | Qigu      | 0    | 26   | 38  | 0   | 0   | 7   | 0  | 71   |
| 3      | Nanxi     | 0    | 5    | 6   | 0   | 0   | 1   | 0  | 12   |
|        | Yujing    | 1    | 9    | 13  | 0   | 0   | 1   | 0  | 24   |
|        | Nanhua    | 1    | 3    | 3   | 0   | 0   | 0   | 0  | 7    |
|        | Shanshang | 1    | 1    | 5   | 0   | 0   | 0   | 0  | 7    |
|        | Zuozhen   | 0    | 0    | 2   | 0   | 0   | 0   | 0  | 2    |
|        | Shanhua   | 6    | 12   | 20  | 0   | 0   | 11  | 0  | 49   |
|        | Xinshi    | 2    | 16   | 23  | 1   | 0   | 5   | 1  | 48   |
|        | Xinhua    | 0    | 28   | 49  | 0   | 1   | 9   | 1  | 88   |
|        | Guanmiao  | 1    | 5    | 10  | 0   | 0   | 3   | 0  | 19   |
|        | Anding    | 0    | 4    | 7   | 0   | 0   | 3   | 0  | 14   |
|        | Yongkang  | 10   | 75   | 116 | 7   | 0   | 22  | 1  | 231  |
|        | Guiren    | 3    | 11   | 24  | 0   | 0   | 2   | 0  | 40   |
|        | Annan     | 8    | 42   | 69  | 1   | 1   | 20  | 0  | 141  |
|        | Anping    | 2    | 27   | 51  | 0   | 1   | 3   | 0  | 84   |
|        | North     | 6    | 35   | 36  | 1   | 0   | 16  | 0  | 94   |
|        | East      | 2    | 23   | 33  | 2   | 0   | 8   | 0  | 68   |
|        | W. central| 1    | 16   | 21  | 0   | 0   | 4   | 0  | 42   |
|        | South     | 3    | 13   | 35  | 1   | 1   | 2   | 0  | 55   |
|        | Rende     | 3    | 8    | 9   | 0   | 0   | 2   | 1  | 23   |
| 4      | Houbi     | 16   | 44   | 39  | 0   | 0   | 1   | 0  | 100  |
|        | Xinying   | 12   | 146  | 132 | 1   | 0   | 43  | 1  | 335  |
### Table 2 Geographic variation of prevalence of different HCV genotypes

| Region  | Districts | GT1a | GT1b | GT2 | GT3 | GT4 | GT6 | MT | T(n) |
|---------|-----------|------|------|-----|-----|-----|-----|----|------|
| Yanshui |           | 2    | 44   | 57  | 0   | 0   | 10  | 1  | 114  |
| Beimen  |           | 0    | 2    | 13  | 0   | 0   | 0   | 0  | 15   |
| Total   |           | 119  | 956  | 1388| 19  | 7   | 518 | 15 | 3022 |

### Table 3 Distribution of subtypes of 322 genotype 6 HCV patients of 30 districts of Tainan city

| Region-1 | Region-2 | Region-3 | Region-4 | Total |
|----------|----------|----------|----------|-------|
| n        | %        | n        | %        | n      | %     |
| GT 1a     | 16       | 1.9%     | 23       | 4.1%  | 50    | 4.8%  | 30    | 5.3%  | 119   | 3.9%  |
| GT 1b     | 228      | 26.9%    | 159      | 28.2% | 333   | 31.8% | 236   | 41.8% | 956   | 31.6% |
| GT 2      | 324      | 38.3%    | 291      | 51.7% | 532   | 50.8% | 241   | 42.7% | 1388  | 45.9% |
| GT 3      | 5        | 0.6%     | 0        | 0.4%  | 13    | 1.2%  | 1     | 0.2%  | 19    | 0.6%  |
| GT 4      | 1        | 0.1%     | 2        | 0.4%  | 4     | 0.4%  | 0     | 0.2%  | 7     | 0.2%  |
| GT 6      | 266      | 31.4%    | 86       | 15.3% | 112   | 10.7% | 54    | 9.6%  | 518   | 17.1% |
| Mixed GT  | 7        | 0.8%     | 2        | 0.4%  | 4     | 0.4%  | 2     | 0.4%  | 15    | 0.5%  |
| Total     | 847      | 28.0%    | 563      | 18.6% | 1048  | 34.7% | 564   | 18.7% | 3022  |       |
| Region | Districts | GT6a % | GT6g % | GT6n % | GT6w % | ID % | Total |
|--------|-----------|--------|--------|--------|--------|------|-------|
| 1      | Baihe     | 1      | 33.3%  | 2      | 66.7%  | 0    | 0.0%  | 3     |
|        | Dongshan  | 1      | 10.0%  | 4      | 40.0%  | 0    | 0.0%  | 5     |
|        | Liouying  | 0      | 0.0%   | 38     | 90.5%  | 0    | 2.4%  | 42    |
|        | Liujia    | 0      | 0.0%   | 34     | 94.4%  | 0    | 0.0%  | 36    |
|        | Xiaying   | 1      | 2.4%   | 39     | 95.1%  | 0    | 1.8%  | 41    |
|        | Guantian  | 3      | 13.0%  | 19     | 82.6%  | 0    | 1.9%  | 23    |
|        |           | 6      | 3.9%   | 136    | 87.7%  | 0    | 1.9%  | 155   |
| 2      | Xuejia    | 0      | 0.0%   | 2      | 100.0% | 0    | 0.0%  | 2     |
|        | Madou     | 0      | 0.0%   | 18     | 100.0% | 0    | 0.0%  | 18    |
|        | Jiangjun  | 0      | 0.0%   | 9      | 100.0% | 0    | 0.0%  | 9     |
|        | Jiali     | 0      | 0.0%   | 11     | 68.8%  | 1    | 6.3%  | 16    |
|        | Xigang    | 1      | 20.0%  | 2      | 40.0%  | 0    | 2.4%  | 5     |
|        | Qigu      | 0      | 0.0%   | 4      | 80.0%  | 0    | 0.0%  | 5     |
|        |           | 1      | 1.8%   | 46     | 83.6%  | 1    | 1.8%  | 55    |
| 3      | Yujing    | 1      | 100.0% | 0      | 0.0%   | 0    | 0.0%  | 1     |
|        | Shanshang | 1      | 12.5%  | 6      | 75.0%  | 0    | 1.8%  | 8     |
|        | Xinhua    | 0      | 0.0%   | 2      | 50.0%  | 0    | 1.8%  | 4     |
|        | Xinsi     | 1      | 20.0%  | 3      | 60.0%  | 0    | 1.8%  | 5     |
|        | Guanniao  | 1      | 100.0% | 0      | 0.0%   | 0    | 0.0%  | 1     |
|        | Yongkang  | 2      | 16.7%  | 3      | 25.0%  | 1    | 8.3%  | 12    |
|        | Anding    | 1      | 50.0%  | 0      | 0.0%   | 0    | 0.0%  | 2     |
|        | Annan     | 1      | 6.3%   | 5      | 31.3%  | 0    | 9.8%  | 16    |
|        | Anping    | 1      | 50.0%  | 0      | 50.0%  | 0    | 0.0%  | 2     |
|        | North     | 2      | 25.0%  | 5      | 62.5%  | 0    | 12.5% | 8     |
|        | East      | 0      | 0.0%   | 3      | 100.0% | 0    | 0.0%  | 3     |
|        | W. central| 0      | 0.0%   | 0      | 0.0%   | 0    | 0.0%  | 2     |
|        | South     | 0      | 0.0%   | 0      | 0.0%   | 0    | 100.0%| 2     |
|        | Rende     | 0      | 0.0%   | 0      | 0.0%   | 0    | 100.0%| 1     |
|        |           | 11     | 16.7%  | 28     | 42.4%  | 1    | 1.5%  | 66    |
| 4      | Houbi     | 0      | 0.0%   | 1      | 100.0% | 0    | 0.0%  | 1     |
|        | Xinying   | 4      | 12.5%  | 22     | 68.8%  | 0    | 6.3%  | 32    |
|        | Yanshui   | 0      | 0.0%   | 6      | 75.0%  | 0    | 12.5% | 8     |
|        |           | 4      | 9.8%   | 29     | 70.7%  | 0    | 7.3%  | 41    |
|        | Chiayi    | 0      | 0.0%   | 6      | 85.7%  | 0    | 14.3% | 7     |
|        | Kaohsiung | 2      | 28.6%  | 0      | 0.0%   | 1    | 14.3% | 7     |
Excluded districts of Danei, Nanxi, Nanhua, Zuozhen, Shanshang, and Guiren because no sample available for subtype analysis.

**Figures**

![Map of HCV prevalence in Tainan Districts]

**Figure 1**

The HCV prevalence rate of each district of Tainan and two landmark rivers, circle: Yongkang Campus; triangle: Liouying(Liuying) Campus; square: Chiali(Jiali) Campus. Jishui and Zengwen Rivers are showed in blue lines on the north and middle of Tainan. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.
Figure 2

Main genotype 6 subtypes ratios in Tainan and adjacent cities, case numbers are listed in parentheses. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

Figure 3

Age distributions of genotype 6 subtypes in Tainan

Supplementary Files

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