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Research paper

Complicated HCV subtype expansion among drug users in Guangdong province, China

Jin Yan[a,*], Xiao-Bing Fu[a], Ping-Ping Zhou[b], Xiang He[b], Jun Liu[a], Xu-He Huang[b], Guo-Long Yu[a], Xin-Ge Yan[a], Jian-Rong Li[a], Yan Li[a], Peng Lin[a]

[a] Guangdong Provincial Center for Disease Control and Prevention, 160 Qunxian Road, Panyu District, Guangzhou 511430, Guangdong, China
[b] Guangdong Provincial Institute of Public Health, Guangdong Provincial Center for Disease Control and Prevention, 160 Qunxian Road, Panyu District, Guangzhou 511430, Guangdong, China

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ABSTRACT

Guangdong Province is one of the most developed and populous provinces in southern China. The subtype situation of hepatitis C virus (HCV) in Guangdong remains unknown. The aim of this study was to investigate and estimate the HCV subtypes in drug users (DU) using a city-based sampling strategy to better understand the characteristics of HCV transmission in Guangdong. Archived plasma samples ($n = 1074$) from DU who were anti-HCV positive in 2014 were selected randomly from 20 cities in Guangdong Province. Subtypes were determined based on *core* and/or *E1* sequences using phylogenetic analysis. The distributions of HCV subtypes in DU and different regions were analyzed. A total of 8 genotypes were identified. The three main HCV subtypes in DU in Guangdong were 6a (63.0%), 3a (15.2%), and 3b (11.8%). Significant differences were discovered among different registered residency and regions but not among genders, marital status, education level, or drug use patterns. HCV subtype 3b was significantly higher in Guangdong residents than in non-Guangdong residents. In contrast, HCV subtype 6a was significantly lower in Guangdong residents than in non-Guangdong residents. Subtype 1b in eastern Guangdong (eastern) was significantly lower, while 6a was significantly higher when compared with other regions. Subtype 3a in the Pearl River Delta (PRD) region was significantly higher, while 3b was significantly lower when compared with other regions. In western Guangdong, HCV subtype 3a was significantly lower when compared with other regions. Additionally, in northern Guangdong subtypes 1b and 3b were significantly higher, while 6a was significantly lower when compared with other regions. Our study revealed the diversity and distribution of HCV subtypes in DU in nearly all the cities in Guangdong. The results provide essential information that will allow the establishment of specific intervention strategies that may help prevent HCV transmission.

1. Introduction

Guangdong Province, located on the southern coast, is one of the most developed provinces in China and has the largest population and the highest population density. Moreover, the transient population accounts for 17% of the total population. The number of annually reported cases of hepatitis C in Guangdong Province has been increasing for several years since 2005. For this reason Guangdong Province was ranked top for the most number of hepatitis C cases in China in 2017 (Fu et al., 2015) Owing to the lack of epidemiological information for reported hepatitis C cases, it is impossible to understand the hepatitis C virus (HCV) incidence in different groups in Guangdong. However, information regarding drug users (DU) from DU sentinel surveillance sites was available. According to a report in 2016, the average Anti-HCV positive rate in DU in Guangdong Province was 46.51% (Huang et al., 2016), which was much higher than the HCV infection rate in Chinese blood donors (0.34%) (Fu et al., 2010) and in the general population of Guangdong Province (2.25%) (Luo et al., 2005). Due to the lack of large-scale HCV molecular epidemiological studies, the distribution of HCV subtypes is not yet clear. Studies found that the main subtypes of clinical patients in Guangdong Province were 1b, 6a, and 2a (Huang et al., 2018; Yuan et al., 2017) while 1b, 6a, 3b were common

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Abbreviations: HCV, Hepatitis C virus; DU, Drug users; IDU, Injecting drug user; NIDU, Non- Injecting drug user; NA, Not available; χ^2, Chi squared test; PRD region, Pearl River Delta region

[*] Corresponding author at: Institute of HIV/AIDS Control and Prevention, Guangdong Provincial Center for Diseases Control and Prevention, RM. 2530, 160 Qunxian Road, Panyu District, Guangzhou 511430, Guangdong, China.
E-mail addresses: yanjin212@qq.com, iyanjin212@gmail.com (J. Yan).

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in blood donors (Rong et al., 2014). The most common subtypes in DU were found to be 6a, 3a, 3b or 6a, 3a, and 1b (Chen et al., 2011; Kuang et al., 2015). Interestingly, 1b is the main subtype in the general population of Guangdong Province while 6a is the main subtype among DU. However, previous studies had their limitations and they do not reveal any insight into the subtype distribution of non-injecting drug users (NIDU). In this study, we systematically sampled the DU in the DU sentinel surveillance sites across the province. We estimated the distribution of HCV subtypes in the DU in Guangdong using the subtypes of the 1074 archived samples combined with their epidemiological information in the prefecture-level cities in Guangdong. The subtypes and the epidemiology of injecting drug users (IDU) and NIDU were also compared. Our results provide a comprehensive dataset reflecting the characteristics and diversity of HCV in DU in Guangdong.

2. Material and methods

2.1. Study participants and sampling strategy

All the specimens were derived from participants in 38 DU sentinel surveillance sites, including 17 in the PRD, 7 in the eastern, 8 in the western, and 6 in northern Guangdong Province. In 2014, a total of 16,133 DU were monitored in Guangdong Province, of which 7703 cases were anti-HCV positive. A minimum sample size was calculated according to the anti-HCV positive number of each city in 2014 (Table 2). A total of 1074 plasma specimens were randomly selected from the 7703 anti-HCV positive cases from 20 prefecture cities except Heyuan. A questionnaire survey was conducted by all participants and their general demographics, sexual behavior and other relevant information were collected.

2.2. RNA extraction, amplification, and sequencing

Viral RNA was extracted from 200 µl of plasma using the MagPure Viral Nucleic Acid KF Kit (Magen, Guangzhou, China) with a KingFisher Flex system (Thermo Fisher Scientific, Waltham, MA, USA) following the manufacturer's instructions. Both *core* (H77: 337–697 nt) and *E1* (H77: 732–1322 nt) fragments were amplified with PrimeScript One Step RT-PCR Kit and Premix Taq (Takara Bio, Dalian, China). The *core* fragment was amplified using the primers and amplification conditions as previously described (Lu et al., 2005). The *E1* fragment was amplified using the primers as described above first , then the samples that had been failed were amplified with in-house degenerate primers (Table 1) under the following conditions: annealing at 50 °C for 5 cycles then at 54 °C for 25 cycles for the first round and 58 °C for 5 cycles, then 62 °C for 25 cycles for the second round.

2.3. Sequence analysis and subtype determination

The HCV sequences were edited with Sequencher 6 (Gene Codes Corporation), aligned using the HCVAlign tool [1], and then merged with HCV subtyping references from Los Alamos HCV Sequence Database using BioEdit. HCV subtypes were determined based on phylogenetic analysis. The HCV subtype of each patient was determined based on the subtypes of both *core* and *E1* fragments. If only one gene fragment was available, the subtype of that gene was assigned. Neighbor-joining phylogenetic trees were reconstructed to determine the subtypes of *core* and *E1* with the Kimura 2-parameter substitution model and evaluated by the bootstrap method with 1000 replicates using MEGA 6.

2.4. Estimation of HCV subtypes in cities and regions

The basic analysis unit in our study was a specific subtype in a prefecture city. The number of cases for each subtype in a city was estimated by multiplying the proportion of that genotype in that city to the total number of anti-HCV positive cases in that same city. The corrected total numbers and corresponding sampling ratios for each city are listed in Table 2. The estimated number of each subtype in each region across the province was obtained by summing the units across all cities. Since no samples were acquired from the city of Heyuan, only 20 cities were included in calculation.

2.5. Statistical analysis

Statistical analysis was performed using SPSS (Ver. 23.0) and a P value of < 0.05 was considered to be statistically significant. Differences between regions were studied using the corrected number of anti-HCV positive cases and subtypes.

3. Results

3.1. Distribution of HCV subtypes among DU in Guangdong

A total of 1074 samples were selected from the anti-HCV positive cases in this study, accounting for 13.8% of anti-HCV positive cases in all DU sentinel surveillance sites (except Heyuan). A total of 976 cases were determined for the HCV subtypes, of which 939 Core (87.4%) subtypes and 837 (77.9%) E1 subtypes were acquired, and 800 samples (74.5%) were acquired for both subtypes (Table 2).

The subtype distributions are show in Fig. 1 and Table 3. Phylogenetic trees of the *core* (Fig. 2A) and *E1* (Fig. 2B) sequences show that eight HCV subtypes were identified in this study, namely 1a, 1b, 3a, 3b, 6a, 6e, 6u, and 6n. The predominant subtypes circulating in Guangdong are 6a (63.0%), 3a (15.2%), 3b (11.8%) and 1b (6.7%). Minor HCV subtypes included 1a, 6e, 6n, and 6u. These were classified as “other” subtypes.

3.2. Distribution of HCV subtype by geographic region

Guangdong Province can be classified into four regions: the PRD, eastern, western, and northern regions. The PRD region, located in central Guangdong, has a prevalent Cantonese culture and is one of the most densely urbanized areas in the world with a large transient and mosaic population, which includes the nine cities of Guangzhou, Shenzhen, Dongguan, Foshan, Jiangmen, Zhongshan, Huizhou, Zhaoqing, and Zhuhai. Eastern Guangdong is Teochew dialect-based and includes the four cities of Jieyang, Chaozhou, Shantou, and Shanwei. The northern region is mountainous and includes four cities of Meizhou, Heyuan, Shaoguan, and Qingyuan, of which Shaoguan and Qingyuan are inhabited by a largely Cantonese population, while Meizhou and Heyuan are populated by the Hakka people. Western

Table 1
E1 primers.

Primers	Primer sequences	Primer length	Position in H77	Amplification length
E1OF688byrr	CGCGCAATBTGGGYARRGTCATC	23	688–710	932 bp
E1OR1619rdr	GGCCGTGCTRTTDATRTGCCA	21	1599–1619	
E1IF732yr	GCCGAYCTCATGGGRTACAT	20	732–751	590 bp
E1IR1322yydr	GGACCAYTTYADCATCATRTCCCA	24	1299–1322	

Table 2
Anti-HCV positive cases reported in Guangdong in 2014 and sample selection.

Cities	Number of reported cases	Number of selected samples	Sampling Ratio (%)	Number of genotyped samples	Corrected number of represented cases in the study	Representation in the study (%)
Jiangmen	1543	63	4.1	53	1543	3.4
Guangzhou	724	63	8.7	57	725	7.9
Yangjiang	626	53	8.5	40	627	6.4
Qingyuan	549	59	10.7	53	548	9.7
Zhanjiang	482	64	13.3	64	483	13.3
Foshan	427	55	12.9	50	429	11.7
Jieyang	395	81	20.5	70	395	17.7
Yunfu	371	60	16.2	51	371	13.7
Maoming	319	64	20.1	60	320	18.8
Shantou	310	64	20.6	61	310	19.7
Zhaoqing	282	42	14.9	36	282	12.8
Huizhou	249	58	23.3	55	250	22.0
Zhongshan	217	42	19.4	39	217	18.0
Heyuan	208	0	0	0	0	0
Zhuhai	194	47	24.2	46	193	23.8
Shaoguan	161	50	31.1	43	161	26.7
Shenzhen	160	37	23.1	32	160	20.0
Dongguan	138	49	35.5	47	139	33.8
Shanwei	125	49	39.2	48	125	38.4
Chaozhou	114	38	33.3	36	114	31.6
Total	7703	1074	13.8	976	7501	13.0

Guangdong also has a large Cantonese culture base and includes the four cities of Zhanjiang, Maoming, Yangjiang and Yunfu. The corrected HCV subtype distribution is shown in Fig. 1 and Table 3. In all four regions, subtypes 6a, 3a, and 3b predominate. The subtypes between regions were significantly different (Table 3). In the PRD region, subtype 3a (18.2%) is the most common while 3b (8.7%) is the least common in the four regions. In the eastern region, subtype 1b (3.1%) is the least common while subtype 6a (67.2%) is most prevalent. Subtypes 1b (9.8%) and 3b (15.8%) in the northern region are the most common while 6a (56.4%) is the least common. Subtype 3a (9.4%) is the least common in the western region in all four regions.

3.3. HCV genotype distribution by cities

The geographic distributions of the main HCV subtypes in Guangdong are illustrated in Fig. 1 and Table 3. Subtype 6a was

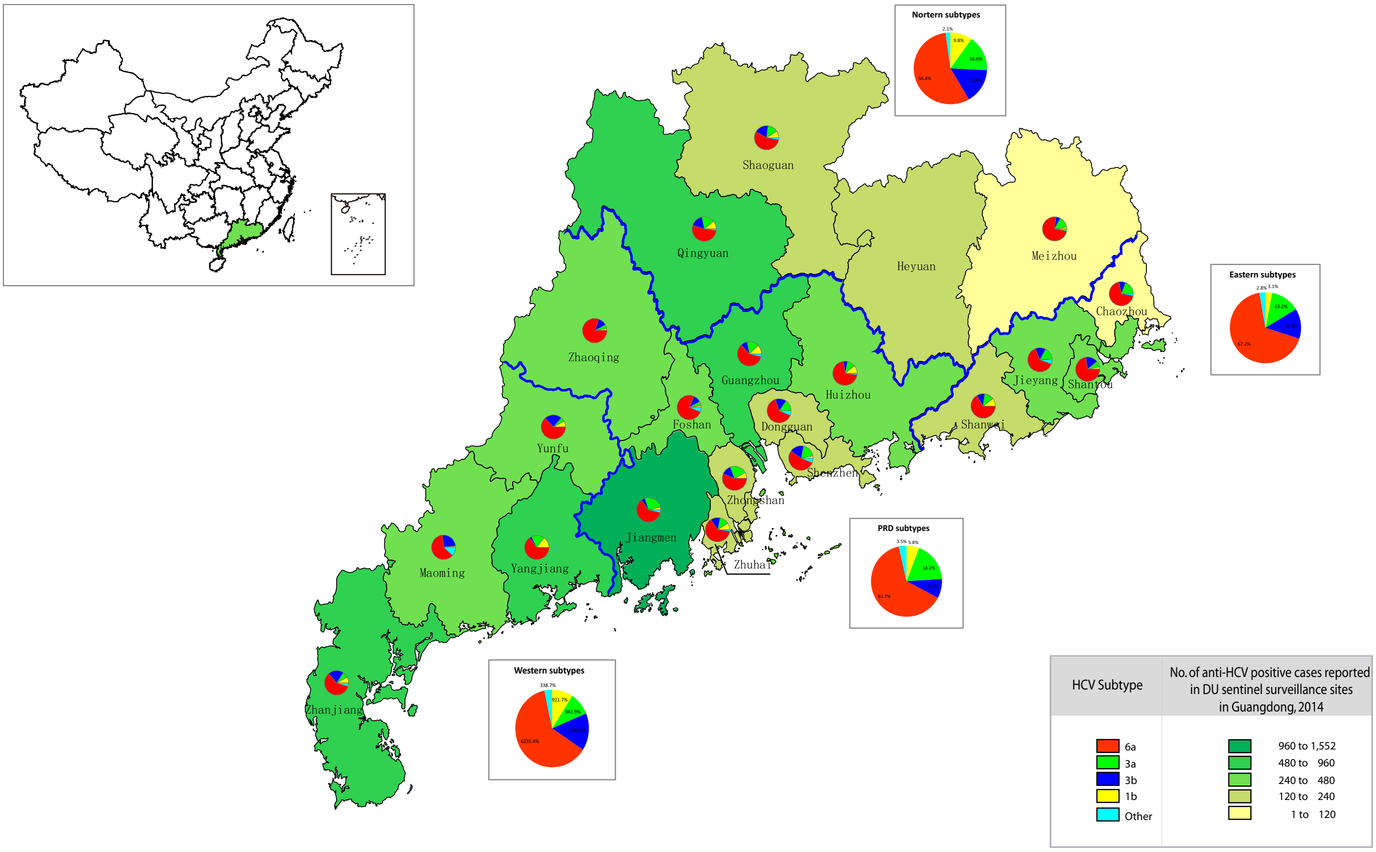

Fig. 1. HCV subtypes distribution in different cities within Guangdong Province. Data is shown on the dataset tabulated in Table 2 and Table 3.

Table 3
Corrected number of subtype distribution among regions and cities.

Regions	Cities	1b	3a	3b	6a	other	Total	x^2	P
PRD		230	717	343	2511	137	3938		
	Jiangmen	58	408	87	933	57	1543		
	Guangzhou	89	114	64	432	26	725		
	Foshan	9	26	43	325	26	429		
	Zhaoqing	8	16	31	227	0	282		
	Huizhou	27	27	14	177	5	250		
	Zhongshan	17	50	28	122	0	217		
	Zhuhai	17	25	25	122	4	193		
	Shenzhen	5	30	30	85	10	160		
	Dongguan	0	21	21	88	9	139		
Eastern		29	125	130	634	26	944		
	Jieyang	6	62	56	248	23	395		
	Shantou	10	25	51	224	0	310		
	Shanwei	13	16	13	83	0	125		
	Chaozhou	0	22	10	79	3	114		
Western		166	170	281	1123	61	1801		
	Yangjiang	94	110	16	407	0	627		
	Yunfu	29	22	87	233	0	371		
	Zhanjiang	38	38	98	286	23	483		
	Maoming	5	0	80	197	38	320		
Northern		80	131	129	461	17	818		
	Qingyuan	62	93	93	290	10	548		
	Shaoguan	15	22	30	90	4	161		
	Meizhou	3	16	6	81	3	109		
Total		505	1143	883	4729	241	7501	198.4	0

detected in all 20 cities in DU in Guangdong in 2014, which accounted for the highest proportion of HCV genotypes in all the cities. Subtype 3a was detected in all the cities included in this study except for Maoming. Subtype 3b was also detected in all the cities in Guangdong while 1b was detected in all the cities in Guangdong except Chaozhou and Dongguan. As for other subtypes, 1a was detected in thirteen cities, except for Huizhou, Shantou, Shanwei, Yangjiang, Yunfu, Zhaoqing and Zhongshan. Subtype 6e was detected in Dongguan, Guangzhou, Huizhou, Maoming, and Shenzhen, while 6n was found in Jieyang and 6u in Dongguan.

3.4. HCV subtypes by demographics and drug using pattern

We found that there were significant differences in the distribution of subtypes between Guangdong residents and non-Guangdong residents (Table 4). For 3b, the number of the non-Guangdong residents (22.8%) was significantly higher than those of Guangdong residents (11.9%), and for 6a, the numbers of non-Guangdong (53.1%) residents was significantly lower than those of Guangdong residents (66.1%). The non-Guangdong residents, mainly from Guangxi (44.8%) and Hunan (22.1%), accounted for 14.9% of total DU. The predominant subtypes in DU from these two provinces were 6a and 3b (53.9% and 23.1% in Guangxi; 46.9% and 28.1% in Hunan, respectively). There were no significant differences in the distribution of subtypes in terms of gender, age, marital status or education level. We divided 976 DU into IDU and NIDU by their drug use patterns, but there was no significant difference in the subtype distribution between them. In terms of sexual behavior, there was no significant difference in the frequency of condom usage between NIDU and IDU who had sex during the past year, either with legal or commercial partners.

4. Discussion

In this paper, an HCV molecular epidemiological study was conducted in DU sentinel surveillance sites in 20 prefecture-level cities across Guangdong Province, and the different subtype distributions between IDU and NIDU were compared. Owing to the lack of representative research on the distribution of HCV subtypes among DU in Guangdong Province in the past, we compared the results with our research in 2015 (Kuang et al., 2015) and found that the main subtypes are basically the same, but we did make four new findings. Firstly, the prevalence of 6a is significantly higher than it was in 2015 (43.6% versus 63.0%). Secondly, the HCV subtypes 1b, 3a, 3b and 6a are significantly different between the four regions. Our third finding is that, HCV subtypes 3b and 6a are significantly different between Guangdong residents and non-Guangdong residents in DU. Lastly, The HCV subtype distribution has no significant difference between NIDU and IDU.

Since 6a had been found to be the second most abundant subtype in three cities within Guangdong in 2005 (Lu et al., 2005), it was estimated that Guangdong had become the second epidemic source of 6a (Fu et al., 2012) and 6a would spread to other provinces of China. Our study confirmed that hypothesis. HCV genotype 6 is mainly prevalent in Southeast Asia, with 22 subtypes of 6a-6v named (Akkarathamrongsin et al., 2010; Wang et al., 2009). Subtype 6a is prevalent in southern China and other northern countries in Southeast Asia. In this study, the proportion of 6a is much higher than that of the 2013 study (Kuang et al., 2015) Subtype 6a was also the main subtype in all 20 cities in Guangdong, thereby confirming the rapid spread of 6a. The recently

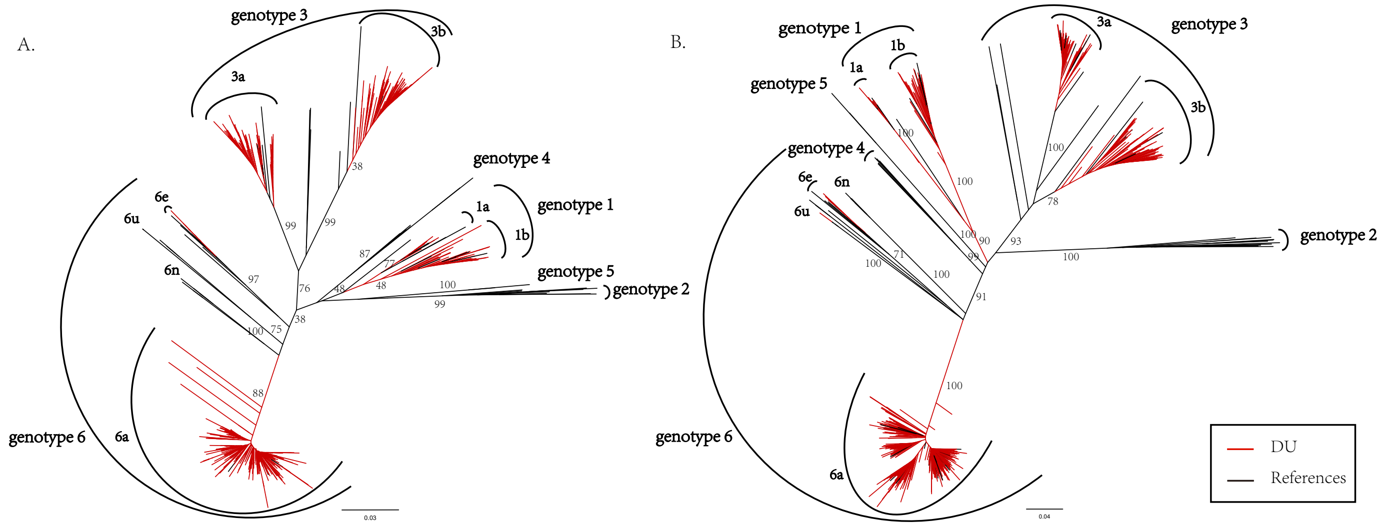

Fig. 2. Phylogenetic trees of the *core* (A) and *E1* (B) sequences. The neighbor-joining trees were reconstructed based on *core* gene (H77: 337–697) (A) and *E1* gene (H77: 752–1298) (B). The red lines represent DU samples in Guangdong, and the black represent reference sequences. The bootstrap values related to subtyping were shown in the phylogenetic trees. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 4
HCV subtype distribution by demographics and drug use pattern.

	1b	3a	3b	6a	others	Total	x^2 Test	
	n(%)	n(%)	n(%)	n(%)	n(%)		x^2	P
Gender						976	4.6	0.31
Male	58(6.3)	119(12.9)	120(13.0)	593(64.4)	31(3.4)	921		
Female	2(3.6)	7(12.7)	12(21.8)	34(61.8)	0(0)	14		
Age(years)						976	15.9	0.39
< 30	6(3.6)	27(16.4)	24(14.5)	99(60.0)	9(5.5)	165		
30-	29(6.8)	49(11.6)	57(13.4)	277(65.3)	12(2.8)	424		
40-	24(6.8)	42(11.9)	49(13.9)	229(65.0)	8(2.4)	352		
≥50	1(2.2)	8(17.4)	2(4.3)	33(71.7)	2(4.3)	46		
Marital status						976	17.9	0.11
Single	14(4.2)	47(14.2)	51(15.4)	213(64.4)	6(1.8)	331		
Married	36(7.4)	57(11.7)	63 (13.0)	312(64.2)	18(3.7)	486		
Live together	4(7.7)	10(19.2)	3(5.8)	31(59.6)	4(7.7)	52		
Divorced	6(5.6)	12(11.2)	15(14.0)	71(66.4)	3(2.8)	107		
Registered residency						976	15.5	0.005
Guangdong	53(6.4)	104(12.5)	99(11.9)	550(66.2)	25(3.0)	831		
Non-Guangdong	7(4.8)	22(15.2)	33(22.8)	77(53.1)	6(4.1)	145		
Education (years)						976	19.7	0.37
< 6	22(5.8)	53(13.9)	54(14.1)	245(64.1)	8(2.1)	382		
< 9	29(5.9)	55(11.2)	63(12.9)	323(65.9)	20(4.1)	490		
< 12	7(8.1)	15(17.4)	14(16.3)	48(55.8)	2(2.3)	86		
> 12	0(0)	1(16.7)	0(0)	4(66.7)	1(16.7)	6		
NA	2(16.7)	2(16.7)	1(8.3)	7(58.3)	0(0)	12		
Drug use pattern						976	8.6	0.37
IDU	40 (6.2)	85(13.3)	90(14.0)	404(63.0)	22(3.5)	641		
NIDU	15 (5.5)	36(13.3)	29(10.7)	185(68.3)	6(2.2)	271		
NA	5(7.8)	5(7.8)	13(20.3)	38(59.4)	3(4.7)	63		

discovered 6e, 6u, and 6n were mainly prevalent in Southeast Asia, and found in the southwestern region of China, and scattered in other province (Cui et al., 2017; Lu et al., 2018). Subtype 6e was the second largest subtype of Vietnam followed by 6a (Li et al., 2014), and the third largest subtype among Guangxi IDU (Tian et al., 2012). Subtype 6n is the most important subtype in northern Myanmar (Lwin et al., 2007) and the second largest subtype among Yunnan IDU (Li et al., 2017). Subtype 6u was first discovered in Myanmar, but was named in Dehong, Yunnan (Wan et al., 2016). In this study, very few cases of 6e, 6u and 6n were found in a few cities of PRD and the western region. Since the first infector of the human immunodeficiency virus (HIV) was detected in DU in 1996 (Lin et al., 2001), drug use had been the main route of HIV transmission in Guangdong until 2009, when the main transmission pattern shifted from drug use to sexual transmission. From then on, the incidence of HIV infection among DU had been maintained at a low level (unpublished data), which reflects the more than decade long intervention efforts in DU in Guangdong Province. HCV has the same transmission route as HIV. In our study, the rapid spread of 6a in DU and the emergence of 6 new subtypes (i.e. 6e, 6u, and 6n) illustrate the fact that the risk factors still exist. Consequently, DU must be educated more around this.

The four regions in Guangdong have their own specificities in dialects and cultures. We found that HCV subtypes are significantly different in all four regions. In the PRD region, 3a is significantly higher compared to other regions. As we know, subtype 3a is common globally, but it is mainly prevalent in India, Pakistan and South Asia (e.g. Thailand) (Esmaeilzadeh et al., 2014). In China, subtype 3a is mainly concentrated in the southwest region (Huang et al., 2018). Located in the central of Guangdong Province, the PRD has the densest and fastest growing population. The mix and flow of these international and interprovincial populations may be why 3a is significantly more common than in other regions. However, we found it strange that subtype 3b in the PRD is significantly less common (8.7%) than in other regions. Subtype 3b is mainly prevalent in Southeast Asia and is one of the major subtypes of IDU in Yunnan and Guangxi in southwestern China (Li et al., 2017; Wan et al., 2016). In recent years, 3b has been reported in DU in Anhui (Cui et al., 2017) and Jiangsu Provinces (Lu et al., 2018). Further research is required to reveal the reason behind this mystery. Since 2010, the prevalence of HCV type 3 has increased rapidly in southwestern and southern China (Du et al., 2019). The monitoring of HCV type 3 should be strengthened because type 3 is not sensitive enough to the most effective drug for hepatitis C Direct-active Antiviral Agent (DAA) (Zanaga et al., 2016) and because it accelerate cirrhosis (Bochud et al., 2009) leading to liver cancer (Nkontchou et al., 2011).

Eastern Guangdong is a Teochew dialect area. We found that subtype 1b (3.1%) in this region was significantly lower than in other areas. Subtype 1b began its spread from the central part of China out into the whole country through illegal blood donation in the mid-1990s (Lu et al., 2013) and is now the main epidemic subtype in the general population of China (Huang et al., 2018; Lu et al., 2005). Subtype 1b is also the main subtype found in unpaid blood donors in Guangdong Province (Rong et al., 2014). Usually the proportion of 1b in DU is very low (Tian et al., 2012; Wan et al., 2016). This pattern of spread from north to south may be the reason that 1b is low in the eastern region. In contrast, the proportion of subtype 1b in Jiangsu (23.8%) (Lu et al., 2018) and Anhui (11.8%) (Cui et al., 2017) DU is not low, suggesting that the same subtype may have different modes of transmission between the eastern and southern regions of China. Although another study (Chen et al., 2011) showed that the percentage of subtype 1b (10.7%) is higher than that in our study (6.7%). The probable reason for this is the differences in the study participants. The study conducted by Chen et al. (Chen et al., 2011) included study participants who were patients from two hospitals in Guangzhou and Shenzhen. We compared the 1b in Guangzhou and Shenzhen in our study with that 2011 study and found that there was a very slight difference between their data (10.7%) and ours (10.6%). Unexpectedly, contrary to the situation of 1b, the prevalence of subtype 6a in the eastern region (67.2%) is the highest, even higher than that in the PRD (63.7%). Subtype 6a is widespread among drug users, suggesting that the eastern region may be the epidemic source of 6a in Guangdong.

In western Guangdong, three of the four cities border Guangxi. We found that in western Guangdong subtype 3a was significantly less common than in other regions. Meanwhile the proportion of subtype 3a in Guangxi was low (Garten et al., 2005; Tian et al., 2012), suggesting that HCV prevalence in the western region was closely related to that in Guangxi. In contrast, in the PRD subtype 3a was the most common suggesting that 3a in PRD probably was originated from different source. Previous studies have shown that 3a was introduced to southwestern China through DU. A study of HIV/HCV co-infections in Guangxi found that 3a was not found in IDU, but was found in a small number of sexually transmitted infectors (5.9%) (Tian et al., 2012), suggesting that the mode of transmission of 3a may have changed, and the sexually transmitted population has also become an important group of people who can spread HCV subtype 3a.

In the northern region, we found that subtype 6a was significantly less common than other regions showing the descent of 6a from southern to northern Guangdong. At the same time, subtype 3b in the north was the most common among the four regions as well as 1b, suggesting a special feature in the north is implicated in the spread of different HCV subtypes from north to south and vice versa in China.

Migrant workers also affect the HCV subtype distribution in Guangdong and other provinces. In this study, the non-Guangdong residents DU accounted for 14.9%, mainly from Guangxi (44.8%) and Hunan (22.1%). Guangxi borders western Guangdong while Hunan borders northern Guangdong and both were the main provinces exporting migrant workers to Guangdong. There were significant differences in the distribution of subtypes between the Guangdong DU and the non-Guangdong DU. Among the infected DU from Guangxi, subtype 6a predominated (53.9%), followed by 3b (23.1%). Additionally, the main subtypes in Guangxi IDU were also 6a (35.6%) and 3b (32.9%) (Tian et al., 2012), suggesting that Guangxi DU have an impact on HCV subtype distribution in Guangdong. The subtypes among DU from Hunan are similar to those in Guangxi, with 6a being the most prevalent (46.9%), followed by 3b (28.1%). Interestingly, 6a is not the main HCV epidemic subtype in Hunan (Huang et al., 2018), suggesting that Hunan DU may get infected in Guangdong and then they bring subtype 6a into Hunan Province with them upon their return home.

In the past, we had no idea of the HCV subtypes of NIDU. In this study, we compared the subtypes between IDU and NIDU and found no significant differences between them, which is both similar (van den Berg et al., 2009), and different to previous data (Tao et al., 2015). There may be two reasons for this. First, some NIDU may not have answered the questionnaire truthfully regarding their drug use patterns. Second, the HCV of NIDU is connected with IDU in some other ways, probably by sexual transmission. It has been reported that sexual risk factors such as multiple partners, commercial partners, and a lack of condom use, were significantly between IDU and NIDU (Xia et al., 2008). Our study shows that there was no significant difference between IDU and NIDU in terms of condom use either with legal or commercial partners.

In summary, our study revealed that subtype 6a has spread quickly across the whole of Guangdong Province. Moreover, the eastern region may be the epidemic source of subtype 6a in Guangdong. We also found that the northern region of Guangdong has a special feature that may be implicated in transmission of HCV. Furthermore, the reason behind the low proportion of 3b in the PRD remains unclear. There may also be some connection between NIDU and IDU in terms of HCV transmission.

We acknowledge that this study has some limitations. First, the DU in this study all came from DU sentinel surveillance sites, which are located in the drug rehabilitation centers. This study did not involve the DU in communities. Second, we didn't obtain samples from Heyuan and there may be some bias in the comparison among regions involving the data in north Guangdong. Third，our study used anti-HCV positive test to identify HCV positive DU for convenience. Although there may be some bias in this assay, comparing to HCV RNA test, the results still reflect the prevalence of HCV subtypes among the DU in Guangdong.

5. Conclusion

Our study clarified the diversity and distribution of HCV subtypes in DU in different cities in Guangdong province. The unique subtype prevalent characteristics of regions and registered residency provide essential information for the establishment of more effective intervention strategies to prevent HCV spreading.

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Availability of data and materials

The data are available upon request to the corresponding authors.

Authors' contributions

JY, XH, PL conceived and designed the study. JY, PPZ, XHH, XGY performed the experiments. XBF, JL, JRL performed the statistical analyses. JY, PPZ, XH analyzed the data and drafted the manuscript. JY, GLY, YL, PL interpreted data and provided critical reviews. All authors reviewed and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Institutional Review Board for Medical Research of Guangdong Provincial Center for Disease Control and Prevention.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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