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# Complex patterns of HCV epidemic in Suzhou: Evidence for dual infection and HCV recombination in East China

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ABSTRACT

*Background:* HCV transmission is closely associated with injection drug use (IDU), and co-circulation of multiple subtypes has been found among injection drug users (IDUs) in China.
*Objectives:* To investigate HCV subtype characterizations among IDUs and general population (GP) in Suzhou, a city at the important "Hu-ning" transportation line.
*Study design:* During January 2010 to May 2011, 123 HCV positive plasma from IDUs and 131 stored HCV positive sera from general individuals were collected in Suzhou. HCV C/E2 and NS5B fragments were amplified using a new multiple RT-nested PCR strategy and subsequent sequenced. Genotypes were characterized by phylogenetic analyses.
*Results:* Eight HCV subtypes (1a, 1b, 2a, 3a, 3b, 6a, 6n, and 6u) were detected among Suzhou IDUs, and six subtypes (1b, 2a, 3a, 3b, 6a and 6n) among GP. HCV subtype distribution is distinct between IDUs and GP. Interestingly, we detected discrepancy of genotyping results between C/E2 and NS5B regions in one general individual, indicating the presence of HCV intersubtype recombinant in China. The recombinant belongs to a 3a/1b recombinant. We also detected dual infections in one general individual and two IDUs. They include dual infections between 1b and 3a, 3a and 6a, and two distinct lineages of 3b.
*Conclusions:* Complex patterns of HCV epidemic among IDUs, as well as GP, in Suzhou, might imply a spread of HCV from IDUs to GP. The finding of one HCV 3a/1b intersubtype recombinant might represent the first report of HCV recombination in China.



## 1. Background

Hepatitis C virus (HCV) is a highly divergent virus that contains a single-stranded positive RNA genome. Currently, 6 genotypes and a large number of subtypes have been identified in HCV based on the genomic sequences.[1] HCV genotypes 1, 2, 3, and 6 are circulating in China.[2] HCV subtype 1b is the most predominant strains among general population (GP), whereas multiple subtypes including 1a, 1b, 3a, 3b, 6a, 6n, and 6u are circulating among injection drug users (IDUs).[3–8]

HCV is transmitted via injection drug use (IDU), blood transfusion, and sexual contact.[9] IDU is the predominant mode of HCV transmission in China.[2,10] Recently, we reported seven HCV subtypes circulating among IDUs in Zhenjiang, Jiangsu, and found that Zhenjiang, as an important transportation station, plays a crucial role in HCV transmission.[6] Suzhou borders with Shanghai and is located at the transportation line linking Shanghai and other regions of China (Fig. 1). Because of higher economic development level, more floating population has been moving to Shanghai and Suzhou. The molecular epidemiological characterization of HCV among IDUs and GP will provide valuable information for understanding HCV transmission in China. Here, we investigated the patterns of HCV epidemic in Suzhou, Jiangsu, by characterizing HCV subtypes, and found that Suzhou has 8 and 6 HCV subtypes circulating among IDUs and GP, respectively. Furthermore, we found one HCV intersubtype recombinant among GP,

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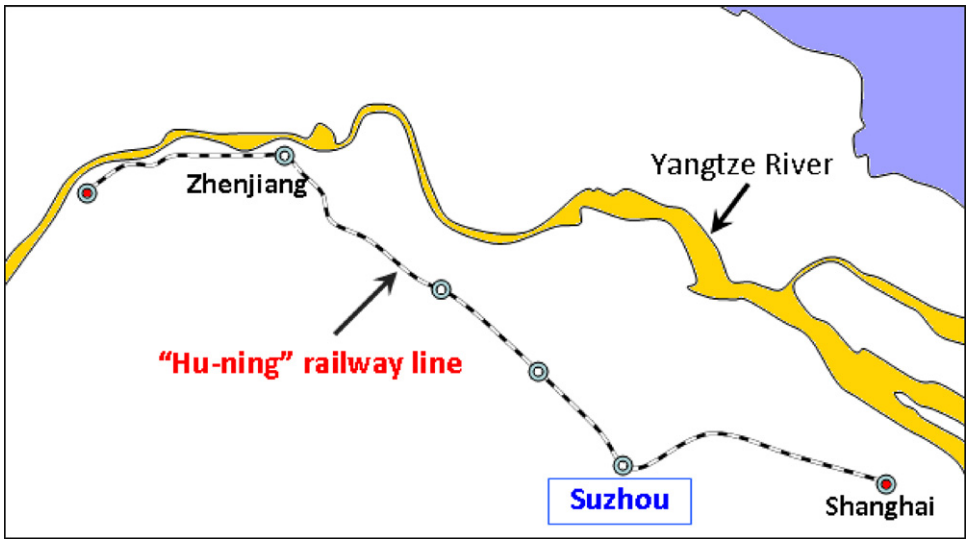


**Fig. 1.** Geographic location of Suzhou city, Jiangsu.

which might represent the first report of HCV recombinant in China.

## 2. Objectives

The purpose of this study was to investigate HCV subtype characterizations among IDUs and GP in Suzhou.

## 3. Study design

### 3.1. Sample collection

The study was performed after approval by the medical ethics committee of Second Affiliated Hospital of Soochow University. Whole blood samples (about 3 mL) were collected from 578 IDUs enrolled in Suzhou drug rehabilitation center using sterile ethylenediaminetetraacetic acid tubes during January 2010 to May 2011. All these IDUs are local residents. Written informed consents were obtained from them. Plasma were separated by centrifugation and anti-HCV antibodies were determined using an enzyme immunoassay method (Yingkexinchuang, Ltd., Xiamen, China). As a result, 123 HCV positive plasma samples were kept. Demographic characterization of HCV infected IDUs in Suzhou is shown in Table S1. In addition, 131 stored HCV positive sera were obtained from 60,323 patients who accepted screening for HCV infection during January 2010 to May 2011 and represent general population. Because this study was not involved in individual privacy, we did not obtain the written informed consents from these patients.

### 3.2. RNA extraction and multiple RT-PCR amplification

The RNA was extracted from 200 μl of HCV-positive plasma or sera with the MiniBEST Viral RNA/DNA Extraction Kit Ver 4.0 (TaKaRa Biotechnology Co. Ltd., Dalian, China). A new multiple RT-nested PCR method was developed to amplify two HCV genomic fragments C/E2 and NS5B (Fig. 2A). In brief, we firstly performed one RT reaction using reverse primers C/E2-R1 and NS5B-R1 to obtain two cDNA fragments. The cDNA products were used as templates for multiple amplifications in the 1st PCR reaction using 2 pairs of outer primers. The 1st PCR product should contain 2 HCV genomic fragments and was used as template for 2 independent 2nd PCR reactions. One reaction uses C/E2-F2 and C/E2-R2 to obtain C/E2 fragment and the other reaction uses NS5B-F2 and NS5B-R2 to obtain NS5B fragment (Fig. 2A). The RT and 1st PCR reactions were performed using the TaKaRa RNA PCR kit (AMV) Ver.3.0 and the 2nd PCR reaction using 2× Power Taq PCR Master Mix (BioTeke, Beijing, China). The primer information was described previously.[6] After the identification using 1% agarose gel, the amplified products were sent to Shanghai Invitrogen Biotechnology Co., Ltd. for sequencing.

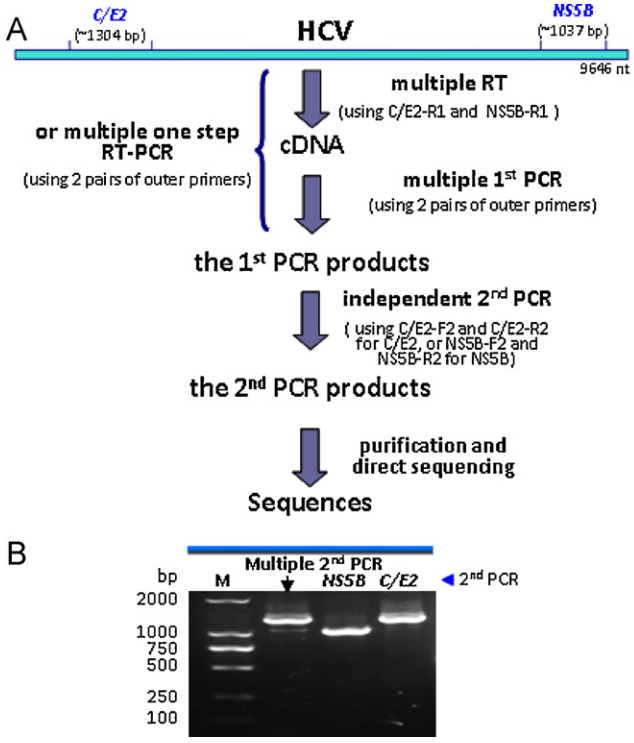


**Fig. 2.** Multiple RT-PCR strategy for amplifying multiple fragments of HCV for subtyping. (A) multiple RT-PCR strategies for HCV amplification, (B) agarose gel electrophoresis of the 2nd PCR products. Relative to the routine way that amplifies two or more viral genomic fragments using 2 or more independent RT-nested-PCR reactions, this multiple RT-PCR strategy can reduce 1 or more RT-PCR reactions, thereby being time-, labor-, reagent- and sample-consuming. The nested primers for C/E2 fragment include outer primers C/E2-F1 and C/E2-R1, and inner primers C/E2-F2 and C/E2-R2. The nested primers for NS5B fragment include outer primers NS5B-F1 and NS5B-R1, and inner primers NS5B-F2 and NS5B-R2. The sequence information and reaction conditions were described in detail in our previous study (Ref. 6). M: DNA marker.