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The hepatitis C virus core gene yields restriction fragments of variable length and polymorphism

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ABSTRACT: The hepatitis C virus (HCV) contains seven genotypes heterogeneously distributed around the world. HCV displays a high genetic diversity relevant to epidemiology, transmission, and clinical management. To explore the genetic variation of HCV in a local Thai population, we investigated the restriction fragment length polymorphism (RFLP) pattern of the core gene in 31 samples of HCV genotype 3a found in blood donors. The polymorphisms of these HCVs were clustered into five RFLP patterns (IV). Fifteen samples (48%) clustered as the RFLP pattern I and ten samples (32%) as pattern III profiles which have been previously reported. In addition, there were four samples (13%) manifest as pattern II, one sample (3%) as pattern IV, and one sample (3%) as pattern V. This study provides pointers to the molecular epidemiology of HCV genotype 3a distributed in Thailand.

KEYWORDS: molecular epidemiology, genotyping, PCR, transmission

INTRODUCTION

The hepatitis C virus (HCV) is an enveloped single-stranded RNA virus highly associated with acute or chronic hepatitis leading to cirrhosis and hepatocellular carcinoma. The HCV genome encodes a single 3009–3010 amino-acid translational polyprotein precursor flanked by untranslated regions (UTR) at both 5′ and 3′ termini. This polyprotein is composed of structural elements (C, E1, and E2), a small hydrophobic protein (p7) and six different nonstructural proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B). The core gene is relatively well conserved but contains enough sequence information to identify HCV variants[1,2]. HCV can be divided into seven main genotypes[3], and diversification of these can provide clues about geographical spread of the virus in populations. Genotype 3a is common throughout Southeast Asia and this genotype predominates in Thailand[4,5]. More importantly, this genomic region is more amenable to anti-viral therapy and successful clinical outcomes[6–8].

Various combinations of interferon, anti-virals, and protease inhibitors are used to treat HCV infections but these are expensive, the treatments run for several months, and side effects are severe. Successful treatment outcomes depend on several predeterminable factors including the viral genome. While Thailand has an integrated health system, its available resources are relatively small, so knowing the HCV genomic vulnerability can optimize the treatment effectiveness in an infected population. To this end, the present investigation aimed to assess the HCV genotype 3a variability in Thailand, using restriction fragment length polymorphism (RFLP) pattern of the core gene.

MATERIALS AND METHODS

Subjects

In a previous study, 135 samples from blood donors were seropositive for HCV (HIV and hepatitis B negative) and 109 were HCV genopositive[9]. Of these, 40 had been genotyped using type-specific primers to the HCV genome core region using primers Sc2 and Ac2 (first amplification round), and 1a, 3a, 6, S7, 1b, 3b, 2a, 5a, and S2a (second round) (Table 1)[9]. For 31 of these samples, there was enough sample volume remaining for RFLP in the present study. Samples originated from donors resident in Phitsanulok (26 samples) and Phetchabun (5 samples) provinces in Thailand. The present study

Table 1 Oligonucleotide primers for PCR amplification and HCV genotyping.

Name	Primer sequence (5′-3′)
Sc2	GGGAGGTCTCGTAGACCGTGCACCATG
Ac2	GAG[A/C]GG[G/T]AT[A/G]TACCCCATGAG [A/G]TCGGC
1a	GGATAGGCTGACGTCTACCT
3a	GCCCAGGACCGGCCTTCGCT
6	GGTCATTGGGGCCCCAATGT
S7	AGACCGTGCACCATGAGCAC
1b	CCTGCCCTCGGGTTGGCTA[A/G]
3b	CGCTCGGAAGTCTTACGTAC
2a	CACGTGGCTGGGATCGCTCC
5a	GAACCTCGGGGGGAGAGCAA
S2a	AACACTAACCGTCGCCCACAA
Q2	AGGTCTCGTAGACCGTGCATCATG
AQ2	CYAGTRAGGGTATCGATGAC

was approved by the Institutional Review Board of Naresuan University (protocol number 52 02 04 0039).

PCR-RFLP and direct sequencing of PCR products

The first round of PCR was amplified using primers Sc2 and Ac2. The second amplification round was evaluated with primer Q2 and AQ2 (Table 1)[9]. PCR-RFLP followed the method of Sistayanarain et al and Buoro et al[9,10] using the restriction enzymes *Acc*I, *Mbo*I, and *Bst*NI. The amplified fragment from the second round of PCR was incubated with these enzymes for 3 h at 37 °C. All RFLP patterns are shown in Table 2. The PCR products were purified by the DNA Extraction Kit (Fermentas). The amplified fragments were sequenced with primer Q2. Sequences of the core region were assigned by GenBank (accession numbers: HM121987- HM121989). In this study, our two strains (GQ331936 and GQ331938) which had been determined as genotype 3a by direct sequencing were used as reference standards for enzyme digestion[9].

RESULTS

In order to investigate the genetic variation of HCV genotype 3a in Thailand, the core region was selected for PCR-RFLP analysis. DNA banding patterns predicted from *Acc*I, *Mbo*I, and *Bst*NI single digestion of the core fragment of HCV genotype 3a are shown in Table 2. After digestion with *Acc*I, *Mbo*I, or *Bst*NI, one strain (Accession no. GQ331936) showed an RFLP pattern which we term pattern I (Fig. 1) while the other (Accession no. GQ331938)

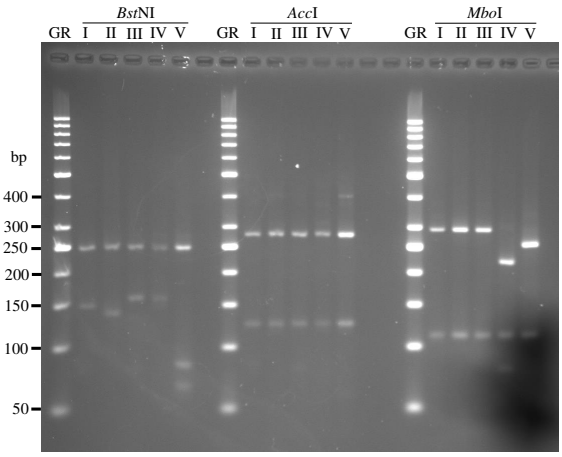

Fig. 1 Agarose gel electrophoresis of five representative samples each showing a different HCV genotype 3a PCR-RFLP patterns. The lanes labelled 'I' are the same sample digested with restriction enzymes *Bst*NI, *Acc*I, or *Mbo*I, and indicate pattern I. Lanes labelled II, III, IV, and V are samples showing the corresponding patterns. The bands reflect the fragment lengths shown in Table 2. GR is a DNA size standard (50 bp gene Ruler).

clustered with our designated pattern III. These two RFLP digestion patterns (Table 2) have been described previously[11–15]. In the present study, RFLP Patterns I and III were discriminated by *Bst*NI digestion (Table 2). After digestion with either *Acc*I, *Mbo*I, or *Bst*NI, all the resultant fragments which yielded RFLP patterns II, IV, and V could be discriminated between each other (Table 2). Of the HCV 3a genotypes, 15 strains (48%) clustered as RFLP pattern I, 10 strains (32%) as pattern III, and 4 strains (13%) clustered as pattern II. The two single isolates showing pattern IV (3%) and pattern V (3%) were remarkably different compared to previous determinations (Fig. 1, Table 2). The RFLP patterns of 3 selected HCV genotype 3a samples (Accession no. HM121988; Accession no. HM121989 and Accession No. HM121987) were confirmed by sequencing.

DISCUSSION

The genotypic diversity of HCV should enable us to identify the transmission route between individuals within a population. The original source of HCV infection can be elucidated using PCR-RFLP analysis[16]. Barusrux et al[17] reported that six major genotypes and subtypes of HCV (3a, 1a, 1b, 6i, 6f, and 6n) were detected from Thai blood samples in northeastern Thailand. In addition, HCV

Table 2 Polymorphism patterns from *Acc*I, *Mbo*I, and *Bst*NI digestion of the HCV genotype 3a sequences within the core fragment.

RFLP pattern	Accession no.	Fragment length (bp) cleaved with:		
		*Acc*I	*Mbo*I	*Bst*NI
I*	D14307, DQ640359, DQ640348, DQ640337	282, 113, 9	297, 107	246, 147, 11
II*	HM121988	268, 136	283, 121	260, 123, 11, 10
III†	DQ430820, D14309, L12355, X76918, AY835224	282, 113, 9	297, 107	246, 158
IV*	HM121989	281, 123	218, 108, 78	247, 157
V*	HM121987	280, 124	257, 109, 38	248, 82, 63, 11

Country of origin: * Thailand; † USA, France, Germany, China.

subtypes 6f, 6n, 6c, and 6i were distributed in five provinces of Thailand[18]. Recently, it was shown that the HCVs isolated from four regions of Thailand (Northeast, South, North, and Central region), genotype 3a was the most prevalent subtype followed by 1a, 1b, 3b, 6f, 6n, 6i, 6j, 6m, 2a, 6c, 6v, 6xa[19]. Thus genotype 3a is the common subtype in Thailand[9,19,20]. In order to assess the variations of HCV genotype 3a, the RFLP of the hepatitis C virus core gene among blood donors from the Phitsanulok regional blood centre were characterized. From our study, we can characterize the RFLPs of core gene of hepatitis C virus genotype 3a into at least five patterns. Patterns I and III were previously reported[11–15]. In the present study, most of HCV genotypes 3a were typically clustered into these two patterns. In previous reports, the RFLP cleavage pattern I had been found in Thailand[11,12] and indeed many of our samples also showed this pattern. From the study of Akkarathamrongsin et al[20], the spread of HCV subtype 3a to Thailand happened during the mid-1970s to early 1980s, and injecting drug use transmission using shared needles probably explains the initial transmission surge. Rigorous blood testing, reduced intravenous drug taking, and national treatment programs have reduced the incidence of HCV infections. However, we also found substantial numbers showing pattern III and some pattern IIs. Pattern III is common in Europe, America, and China[13–15] and such a strain distribution from diverse geographical areas suggests a wider historical movement of viral lineages. Currently, the global spread of HCV has accelerated with the advent of more methods and frequency of transmission routes of populations[21] which probably accounts for the appearance of pattern III HCV. However, we have found three patterns designated as II, IV, and V which may have arisen from mutation by recombination in the local population[22,23]. Mutations of the core region impact on clinical outcomes[24–26]. Thus the emergence of a new HCV variant where the encoded proteins have new properties may change its pathogenesis and susceptibility to treatments. This study describes the molecular epidemiology of local HCV genotype 3a. It demonstrates that HCV genotype 3a isolated clusters as at least 5 RFLP patterns. Such patterns may help to optimize the therapeutic management leading to the ultimate eradication of HCV.

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