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ORIGINAL ARTICLE

Molecular characterization of hepatitis C virus genotype 6 subtypes in Thai blood donors

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Abstract *Background:* Hepatitis C virus (HCV) genotype is important for identifying effective antiviral therapy, evaluating pathogenic severity, and tracking transmission routes. In Thailand, HCV genotypes 3 and 1 are the most common. We have previously demonstrated an increasing appearance of genotype 6 in HCV infections in Thailand. However, only limited epidemiological data on genotype 6 in Thailand are available. This study aimed to characterize HCV genotype 6 among apparently healthy Thai blood donors.
Methods: In total, 240 blood samples were collected from Phitsanulok Regional Blood Center, Phitsanulok, Thailand. RNA was reverse transcribed and amplified by the nested polymerase chain reaction. HCV genotyping was performed by direct sequencing and phylogenetic tree analysis of core sequences. Amino acid polymorphism of various subtypes of HCV genotype 6 was investigated.
Results: Of the 240 samples, 192 were successfully sequenced for the core region and 84 were determined to be of HCV genotype 6 by phylogenetic analysis. The most prevalent HCV-6 subtypes were 6f > 6n > 6c > 6i. Amino acid sequences of the partial core region among these four subtypes differed by one to seven residues.
Conclusion: For HCV-6, the subtype 6f was commonly found in Thai blood donors. Comparison of core protein from various HCV-6 subtypes showed substantial polymorphisms, which may form the basis of future studies using samples from patients with clear HCV histories. This feature can be applied to therapies tailored to particular genotype variants.

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Introduction

A common cause of acute and chronic liver diseases arises from hepatitis C virus (HCV), which is classified into six major genotypes (genotypes 1—6) and more than 80 subtypes. HCV genotyping is considered important for predicting a sustained virologic response. Thus, the HCV genotype is an important determinant of effective treatment methods and vaccine development. Antiviral drugs including pegylated interferon-alpha, ribavirin, boceprevir, and telaprevir are current treatments or are under development.[1,2]

The global HCV incidence rate is about 3%; however, the rate varies by region and by genotype: genotypes 1—3 predominate in Europe, North and South America, and Japan [3]; genotype 4 is most frequent in North Africa and the Middle East; genotype 5 is common in South Africa [4,5]; and genotype 6 is largely confined to East/Southeast Asia.[6,7] However, data based on continents conceal local variations, which might provide more accurate insights about the spread of this virus. There have been several studies demonstrating such localization in, for example, Thailand, where HCV genotype frequencies of 3a > 1 > 6 > 2 are found.[8,9] More recently, another region of Thailand demonstrated the prevalence of genotypes 3a > 6 > 1, with genotype 6 in particular being more prevalent in HCV infections.[7] Akkarathamrongsin et al[8] found a prevalence of HCV-6 subtypes in Thai people residing mainly in the central region of the country and subtypes 6f, 6n, 6i, 6j, and 6e were detected using phylogenetic analysis based on the core and NS5B sequences. Another study demonstrated prevalence of infections due to subtypes 6a, 6f, and 6n in four disparate provinces of Thailand.[9] However, failure to detect genotypes 4 or 5 does not preclude their existence in other parts of Thailand.

HCV genotyping commonly uses restriction fragment length polymorphism or polymerase chain reaction (PCR) using type-specific primers and hybridization with specific oligonucleotide probes. However, the genotype specificity of these methods is sometimes questionable and indeed our previous study failed to genotype several samples. By contrast, direct nucleotide sequencing is more reliable for genotyping HCV samples. Although there has been limited epidemiological data on genotype 6 in Thailand, its increasing emergence needs a more thorough assessment of its regional distribution, which might help in limiting its spread and treatment. Therefore, we sought to apply sequence analysis of the core region to understand the molecular epidemiology and genetic diversification of the HCV in order to study the main genotype 6 and also its subtypes among blood donors from Thailand.

Materials and methods

Sample collection

Two hundred and forty serum samples, which were anti-HCV antibody positive, were obtained from blood donors at Phitsanulok Regional Blood Center, Phitsanulok province, Thailand. The blood donors were from Phitsanulok, Phetchabun, Kamphaengphet, Sukhothai, Tak, and Uttaradit provinces in Thailand. Serum samples used in this study were obtained during the years 2006—2007. The study was approved by the Human Ethics Committee of Naresuan University, Phitsanulok, Thailand.

RNA extraction and reverse transcription

RNA was extracted from serum using PureLink Viral RNA/DNA Kits (Invitrogen, USA) according to the manufacturer's instructions. RNA was reverse transcribed into complementary DNA by random primer.

Nested PCR for core gene

The partial core region of HCV was amplified by nested PCR with two sets of oligonucleotide primers using the same optimized condition as described previously.[7] The first-round amplification was carried out with outer sense (Sc2) 5′-GGGAGGTCTCGTAGACCGTGCACCATG-3′ and outer antisense primers (Ac2) 5′-GAGMGGKATRTACCCCATGAGRTCGGC-3′. The second-round PCR was amplified with inner sense (Q2) 5′AGGTCTCGTAGACCGTGCATCATG-3′ and inner antisense primers (AQ2) 5′-CAYGTRAGGGTATCGATGAC-3′. The first-round PCR was conducted for 40 cycles according to the following cycling parameters: preliminary 20 cycles at 94°C for 1 minute, 45°C for 1 minute, and 72°C for 1 minute, followed by 20 cycles at 94°C for 1 minute, 55°C for 1 minute, and 72°C for 1 minute. The-second-round PCR was conducted for 35 cycles according to the following cycling parameters: 94°C for 1 minute, 55°C for 1 minute, and 72°C for 1 minute. The final extension step was carried out at 72°C for 7 minutes.

Direct nucleotide sequencing and phylogenetic analysis by amino acid sequence

HCV genotyping was performed by direct nucleotide sequencing and determined by *Basic Local Alignment Search Tool* analysis [National Center for Biotechnology Information site (NCBI)] and phylogenetic tree analysis. Phylogenetic tree of the core amino acid sequences was constructed using the neighbor-joining method by bootstrap analysis with 1000 replicates in MEGA software (version 6.06) (www.megasoftware.net). External reference sequences for the phylogenetic construction were retrieved from the Los Alamos HCV database (http://hcv.lanl.gov/content/sequence/HCV/ToolsOutline.html) and NCBI.

All sequences obtained from the core region were submitted to GenBank (accession numbers KF577403—KF577411, KF577413—KF577415, KF577417—KF577433, KF577435—KF577475, KF577477—KF577478, and KF577480—KF577493).

Amino acid sequence comparison of genotype 6

To determine the difference of amino acid residues among various HCV-6 subtypes, amino acid sequences of HCV isolates were aligned based on core region with ClustalW2 and BioEdit software. The prototype sequences of HCV genotype 6 subtypes 6f, 6n, 6c, and 6i used for comparison were as follows: D38078 (6f), DQ278894 (6n), D37843 (6c), and D37850 (6i).