

# Seroepidemiology and Genotypes of Hepatitis C Virus in Thailand

Srunthron Sunanchaikarn<sup>1</sup>, Apiradee Theamboonlers<sup>1</sup>, Voranush Chongrisawat<sup>1</sup>, Pornsak Yoocharoen<sup>2</sup>, Piyanit Tharmaphornpilas<sup>2</sup>, Porpit Warinsathien<sup>2</sup>, Supakarn Sinlaparatsamee<sup>3</sup>, Siriraj Paupunwatana<sup>4</sup>, Kasemporn Chaiear<sup>5</sup>, Sawan Khwanjaipanich<sup>6</sup> and Yong Poovorawan<sup>1</sup>

**SUMMARY** HCV can be classified into 6 major genotypes based on the phylogenetic analysis of the genomic sequences. The 3 major genotypes found in Thailand are 3, 1 and 6, respectively. In 2004, an epidemiological survey was carried out to evaluate the seroprevalence of HCV infections among populations aged 2-60 years in four provinces of Thailand, representing the North, Northeast, Center and South of the country, respectively. One hundred and twenty five out of 5,825 serum samples (2.15%) were positive for anti-HCV by ELISA. Fifty eight out of 100 anti-HCV positive samples (58.0%) were positive by RT-PCR of the 5'UTR. The core region of 45 representative samples was sequenced allowing classification into genotype variants 1a (6.7%), 1b (26.7%), 2a (2.2%), 2c (2.2%), 3a (51.1%), 3b (2.2%) and 6 (8.9%). This information might be crucial for public health surveillance and prevention of HCV infection.

Viral hepatitis represents a global public health problem with particular prevalence in Asia, Africa, Latin America, and Southern Europe. One hundred and seventy million people (3% of the world population) are already infected with hepatitis C virus (HCV) and 80% of those have developed chronic liver disease.<sup>1</sup> HCV is transmitted parenterally or by direct percutaneous exposure to infectious material, such as contaminated blood products. Hepatocellular carcinoma, with and without cirrhosis has also been associated with HCV infection.<sup>2</sup> In order to effectively prevent HCV infection, identification of chronically infected subjects, screening of blood products and donated organs, using only sterile medical and dental instruments, and avoiding risky parenteral contact, such as skin piercing, high risk sexual practices, etc. are of paramount importance.

HCV is a single stranded RNA virus of positive polarity which belongs to the family *flaviviridae*

and the genus *hepacivirus*. The HCV RNA genome comprises approximately 9,400 nucleotides, which encode a single polyprotein that is post-translationally cleaved into 10 polypeptides. The polyprotein consists of structural and nonstructural (NS) proteins with the structural component including a nucleocapsid core (C) and 2 envelope glycoproteins (E1 and E2). The NS proteins are labeled NS2 through NS5.<sup>3-5</sup> Upon sequence comparison of HCV variants collected from different geographical areas, at least 6 genotypes (1-6) and more than 74 subtypes have been identified and classified.<sup>6-8</sup> Although the

From the <sup>1</sup>Center of Excellence in Viral Hepatitis Research, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand, <sup>2</sup>Bureau of General Communicable Diseases, Department of Disease Control, Ministry of Public Health, Nonthaburi, Thailand, <sup>3</sup>Maharat Nakhon Si Thammarat Hospital, Nakhon Si Thammarat, Thailand, <sup>4</sup>Chiangrai Prachanucroh Hospital, Chiangrai, Thailand, <sup>5</sup>Udon Thani Hospital, Udon Thani, Thailand, <sup>6</sup>Chonburi Hospital, Chonburi, Thailand.  
Correspondence: Yong Poovorawan  
E-mail: Yong.P@chula.ac.th

5' untranslated region (5'UTR) of HCV is highly conserved and specific for each genotype, some genotypes, such as genotypes 1 and 6, have similar sequences in this region. The core gene encodes a nucleocapsid protein that is the most conserved protein in the HCV genome. However, it is more variable than the 5'UTR and contains sufficient sequence information to classify all known subtypes and major genotypes.<sup>9</sup> A patient's sustained response rates to IFN treatment depend on several clinical and virological factors, one of which is the HCV genotype.<sup>10</sup>

In Thailand, there have been many studies on the prevalence of HCV infection, mostly conducted among volunteer blood donors.<sup>11,12</sup> All blood banks try to reduce the risk of transfusion-transmitted HCV through the use of volunteer donors and enhanced donor questioning. Therefore, the seroprevalence in blood donors with a low risk of HCV infection may not exactly reflect the prevalence among the general population. To investigate the prevalence of HCV infection in the Thai population, we tested serum samples of participants in four geographically distinct provinces of the country. Additionally, we also tested anti-HCV positive samples for HCV RNA by PCR amplification of the 5'UTR, followed by sequencing of the core region to determine the genotype of positive samples. Genotypes were classified by phylogenetic analysis of the core region.

## MATERIALS AND METHODS

### Population study

The participants were randomly selected from people in four provinces, Chiangrai, Udon Thani, Nakhon Si Thammarat and Chonburi, representing four geographically distinct areas and thus, populations in the North, North-east, South and Center of the country, respectively. The participants were either healthy children or patients with acute illness who attended the well baby clinic or outpatient clinic of the four main provincial hospitals and two to three district hospitals of each participating province. The study commenced in May 2004 and ended in October 2004. In order to be included into the study, the subjects had to fulfill the following criteria: general good health, no chronic illness, not

subjected to immunosuppressive therapy, no clinical signs or symptoms associated with either HIV infection or any immunodeficiency disorders. This study was part of a wider study aimed at evaluating universal mass vaccination against hepatitis B virus (HBV) infection and determining the seroprevalence of the hepatitis A virus in Thailand. The respective study details have been reported elsewhere.<sup>13,14</sup> The protocol was approved by the Ethics Committee, Ministry of Public Health and Faculty of Medicine, Chulalongkorn University, Bangkok. The subjects or the parents of all participating children were informed about the study objective and their written consent was obtained.

### Laboratory methods

#### *Serological tests*

Sera were collected and kept at -70°C until tested. Each specimen was subjected to enzyme-linked immunosorbent assay for detection of anti-HCV using commercially available third generation ELISA assays and an automated ELISA reader (AxSYM; Abbott Laboratory, North Chicago, IL) according to the manufacturer's instructions.

#### *HCV RNA detection*

Anti-HCV positive samples were selected for detection of HCV RNA by reverse transcription-PCR (RT-PCR) of the virus 5'UTR and core regions. Total RNA was extracted from serum samples applying the guanidinium method and reverse transcribed into cDNA using the primer OC2 (5' CATGGTGC-ACGGTCACGAG 3', position 344-325) targeting the 5'UTR and a down stream primer targeting the core region<sup>8</sup>. RT-PCR was performed as described elsewhere.<sup>15</sup>

For nested amplification, the 5'UTR specific outer primer pair OC1 (5' GCCGACACTCCACCA-TGAAT 3', position 18-37) and OC2 (above), and inner primer pair IC3 (5' GGA ACTACTGTCTTCA-CGCAG 3', position 51-71) and IC4 (5' TCGCAAG-CACCCTATCAGGCA 3', position 310-290), were used (nucleotide position referred from GenBank database accession number M62321). The core region was amplified by nested PCR with both primer pairs

as previously described.<sup>8</sup> The amplification protocol has been described elsewhere<sup>15</sup> except for the annealing temperature, which was changed to 49°C in order to simultaneously amplify both regions. The PCR products (5'UTR; 260 bp, core; 405 bp) were analyzed by electrophoresis in 2% agarose gel stained with ethidium bromide and visualized under UV light.

### Sequencing

The target PCR product of the core region was purified from the agarose gel for sequencing using the Perfectprep Gel Cleanup Kit (Eppendorf, Hamburg, Germany) according to the manufacturer's instructions, and subjected to agarose gel (2%) electrophoresis to ascertain its purity. The concentration of the purified PCR products was determined in a Shimadzu UV 160 A spectrophotometer ( $A_{260} = 1.0$  for 50 mg of double-stranded DNA per ml). For cycle sequencing, 30 to 180 ng of each DNA sample were subjected to double stranded sequencing using the dideoxy-nucleotide chain termination method with the Big Dye Terminator V.3.1 Cycle Sequencing Ready Reaction kit (ABI, Fostercity, CA) and 0.16  $\mu$ M final concentration of sequencing primers in a model 9600 thermocycler (Perkin Elmer Cetus, Norwalk, CT). Cycle sequencing was performed according to the manufacturer's instructions using both sense and anti-sense primers to confirm the sequencing results. We purified the extension products from unincorporated dye terminators by ethanol precipitation and subsequently subjected them to sequence analysis by the ABI Prism 310 Genetic Analyzer according to the manufacturer's instructions (ABI, Fostercity, CA). The sequences were edited using Bioedit 5.0.9 (Ibis Therapeutics, Carlsbad, CA).

### Phylogenetic analysis and genotyping

In order to determine the genotype(s) by phylogenetic analysis, we edited and assembled the sequences using the programs CHROMAS LITE (v.2.0) and SeqMan (DNASTAR, Madison, WI). To establish the relationship between hepatitis C viruses isolated from the patients, genetic and phylogenetic analyses were performed using the PHYLIP package developed by J. Felsenstein (v.3.5C). Bootstrap analysis was performed for values representing 1000 replicates by SEQBOOT. Phylogenetic analysis was

conducted using the Neighbor-joining method. Confidence values were calculated by bootstrap analysis (1,000 replications) and consensus trees were produced by MEGA version 3.1. The core region of different HCV sequences obtained from the GenBank database served as reference for phylogenetic tree analysis. The HCV sequences clustering on the same node were considered to be of identical genotype.

The following reference strains were obtained from GenBank: genotype 1a (AF482730, AF484969, AF484971), 1b (U45461, AY089748, AY089747, AY522238, AF482729), 2a (D10075), 2b (D10077, AY232740), 2c (AY587383), 3a (D14309, AF484989, D14311, AM263149, AF484981), 3b (DQ485279), 3i (AY434137), 4a (AF029298, D45193), 4c (L38338), 5a (D50466, U33434), 6a (AF484980, AF484976), 6b (D37841), 6f (DQ640360) and 6n (AY089763).

## RESULTS

### Serological study of HCV infection

In total, 6,213 subjects aged between 6 months and 60 years from the four provincial hospitals of Chiangrai, Udon Thani, Chonburi and Nakhon Si Thammarat and two to three district hospitals of each participating province were recruited into the study (age stratification was statistically determined). In order to exclude passive immunity conferred by antibody transfer from their mothers only the sera of subjects older than 2 years were studied for anti-HCV seroprevalence. There were 5,825 serum samples left for anti-HCV determination, of which 125 were positive, indicating an overall anti-HCV prevalence of 2.15% (Table 1). The prevalence was high among the 2-4 year olds. In addition, the rate of HCV infection was higher in older patients (Fig. 1.). Among 125 positive samples, only 100 samples were suitable for HCV RNA analysis.

### RT-PCR and sequence analysis results

Fifty eight out of 100 (58%) anti-HCV positive samples were HCV RNA positive by RT-PCR of the 5'UTR. Subsequently, we determined the HCV genotype by sequencing the core region. We selected 45 samples as adequate for sequencing. The data obtained from direct sequencing were aligned

**Table 1** Seroprevalence of hepatitis C virus antibody (anti-HCV) among the study population from four provinces of Thailand

Age (years)	Chiangrai			Nakhon Si Thammarat			Udon Thani			Chonburi			Total		
	No.	No. +	% +	No.	No. +	% +	No.	No. +	% +	No.	No. +	% +	No.	No. +	% +
2-4	153	2	1.31	131	2	1.53	170	2	1.18	120	6	5.00	574	12	2.09
5-6	80	1	1.25	83	2	2.41	111	0	0.00	92	2	2.17	366	5	1.37
7-8	100	0	0.00	86	2	2.33	102	0	0.00	91	1	1.10	379	3	0.79
9-10	83	2	2.41	79	0	0.00	120	1	0.83	103	2	1.94	385	5	1.30
11-12	64	0	0.00	58	2	3.45	62	0	0.00	47	0	0.00	231	2	0.87
13-14	64	1	1.56	50	0	0.00	72	0	0.00	53	0	0.00	239	1	0.42
15-16	46	1	2.17	43	2	4.65	63	2	3.17	32	0	0.00	184	5	2.72
17-18	44	0	0.00	97	2	2.06	65	1	1.54	71	2	2.82	277	5	1.81
19-20	49	2	4.08	47	0	0.00	44	0	0.00	54	1	1.85	194	3	1.55
21-30	199	2	1.01	193	4	2.07	207	4	1.93	189	8	4.23	788	18	2.28
31-40	206	3	1.46	188	4	2.13	203	7	3.45	184	6	3.26	781	20	2.56
41-50	206	2	0.97	188	8	4.26	221	6	2.71	158	8	5.06	773	24	3.10
> 50	173	7	4.05	163	8	4.91	186	2	1.08	132	5	3.79	654	22	3.36
<b>Total</b>	<b>1,467</b>	<b>23</b>	<b>1.57</b>	<b>1,406</b>	<b>36</b>	<b>2.46</b>	<b>1,626</b>	<b>25</b>	<b>1.48</b>	<b>1,326</b>	<b>41</b>	<b>3.12</b>	<b>5,825</b>	<b>125</b>	<b>2.15</b>

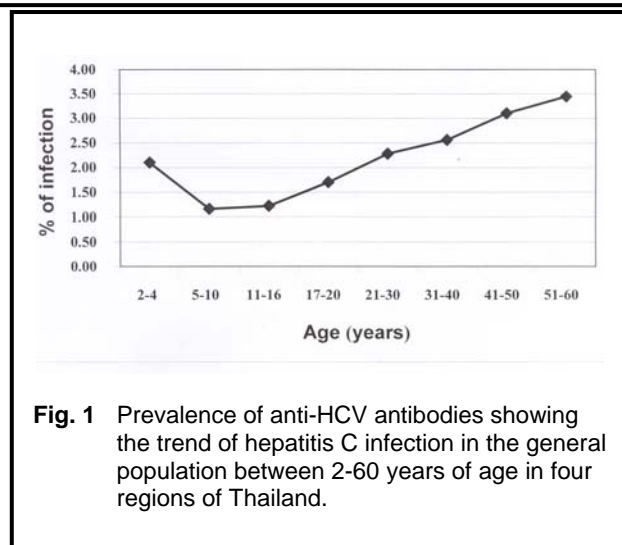
with the known genotype sequences and subjected to phylogenetic analysis (Fig. 2). The genotype distribution emerged as 1a in 3 (6.67%), 1b in 12 (26.67%), 2a in 1 (2.22%), 2c in 1 (2.22%), 3a in 23 (51.11%), 3b in 1 (2.22%) and genotype 6 variants in 4 (8.89%) samples. There was no correlation between genotype distributions and particular geographic areas. Only 2 samples with genotype 2 were found in Chiangrai (northern region of Thailand).

#### Nucleotide sequence accession numbers

New sequences reported in the present study have been submitted to the GenBank database and assigned accession numbers EF543198-543242.

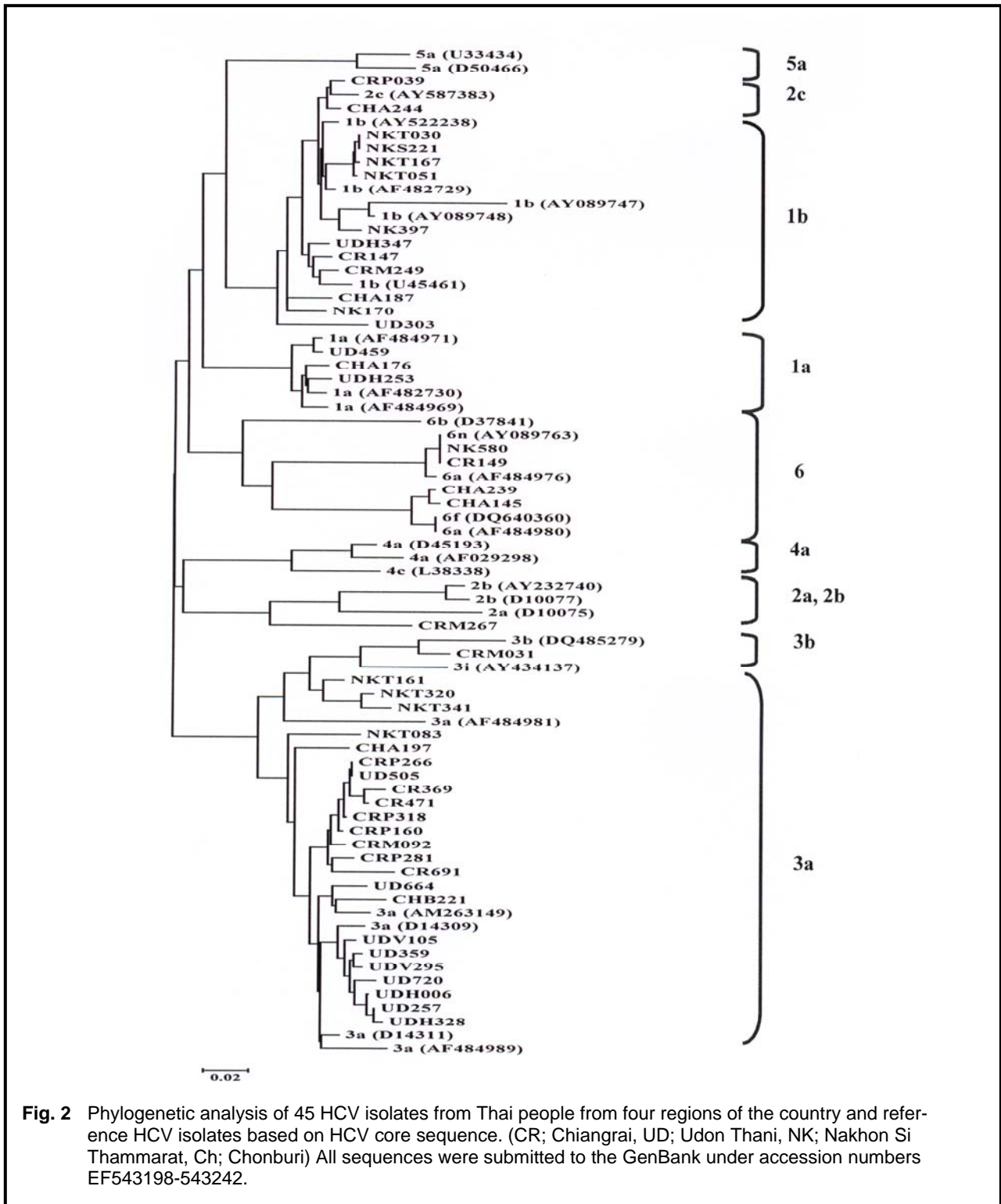
### DISCUSSION

According to previous studies<sup>11,12</sup> conducted on small-sized populations and hence, not representative of the entire nation the prevalence of anti-HCV in Thailand amounted to 0.98-1.5%. This study was carried out on a large scale to reflect the country-wide seroprevalence of HCV infection. Based on four geographically distinct regions of Thailand, our results showed a seroprevalence of 2.15%, exceeding that arrived at in previous studies as well as the



**Fig. 1** Prevalence of anti-HCV antibodies showing the trend of hepatitis C infection in the general population between 2-60 years of age in four regions of Thailand.

0.76% published by the National Blood Centre of the Thai Red Cross Society (National Blood Centre, Thai Red Cross, 2004). According to the present study, the prevalence in children (2-4 years) was relatively high potentially indicating vertical transmission. Perinatal transmission of HCV has been clearly documented. Although early studies indicated that HCV transmission to neonates occurred primarily in association with perinatal human immunodeficiency virus (HIV) transmission, subsequent studies have confirmed that women without HIV infection



**Fig. 2** Phylogenetic analysis of 45 HCV isolates from Thai people from four regions of the country and reference HCV isolates based on HCV core sequence. (CR; Chiangrai, UD; Udon Thani, NK; Nakhon Si Thammarat, Ch; Chonburi) All sequences were submitted to the GenBank under accession numbers EF543198-543242.

can transmit HCV to their offspring. Risk of perinatal acquisition of HCV was found to correlate with the titer of HCV RNA in the maternal serum. Three to 11% percent of infants born to HCV RNA-

positive mothers become infected with HCV.<sup>16,17</sup> On the other hand, the rate of transmission can rise to 36% in mothers with HIV/HCV coinfection.<sup>18</sup> Data on HCV transmission from mother to infant or by

person-to-person contact, either sexually or by non-sexual household contact, are controversial. However, there is almost universal agreement that the presence of concurrent HIV/HCV infection increases the rate of vertical/perinatal HCV transmission as well as transmission through sexual contact. The drawback of the present study has been a lack of HIV and HCV serologies of HCV-infected children's mothers. Nevertheless, we attempted to include the participants without signs and symptoms of either HIV infection or immunodeficiency disease.

The degree to which HCV is transmitted by sexual contact has been debated. Even though sexual transmission of HCV might be inefficient, as demonstrated by low infection rates among the spouses of persons with hepatitis C,<sup>19,20</sup> this study showed that the sexually active age group above 15 years had a higher prevalence of HCV infection (Figure 1). The risk of infection in spouses increased with the duration of marriage and amounted to 60 percent in spouses married for more than 50 years.<sup>21</sup> The mechanisms of sexual and household transmission of HCV have remained unclear since HCV RNA has not been found in saliva, semen, urine, stool, or vaginal secretions of infected patients.<sup>22,23</sup>

According to a seroepidemiological survey in the USA, people aged 30-49 years, who are prone to high risk behavior (e.g. unsafe sex and intravenous drug use), constitute around 65% of infected individuals.<sup>24</sup> Nonetheless, our study demonstrated that people above the age of 50 had the highest prevalence amounting to 17.6% of the entire infected population. This finding might reflect the different modes of HCV transmission in various countries. Several earlier studies have demonstrated that the proportion of cases of HCV infection with no identifiable risk factor had consistently been 35 to 40 percent.<sup>25</sup> However, although high-risk behavior may not be documented within 6 months of HCV diagnosis, a history of more remote risk factors can be obtained for the majority of these cases. Thus, more than 90 per cent of new cases can be attributed to parenteral or sexual transmission.<sup>26</sup> Sporadic HCV infections are those that occur without any known exposure, risk factor, or outbreak. These represent a small minority of cases, and the mode of virus transmission in these instances is not known.

In the present study, we found that 58% of anti-HCV positive samples were viremic while other groups reported approximately 70%.<sup>27</sup> Anti-HCV positive serology without viremia may be due to low viral load, resolved infection or occult infection. Individuals positive for anti-HCV, with normal transaminase levels, and undetectable HCV-RNA levels are generally considered to have cleared the previous hepatitis C infection. Nonetheless, occult HCV infection can be present in two different clinical situations: in anti-HCV negative, serum HCV-RNA negative patients with abnormal liver function tests and in anti-HCV positive subjects with normal values of liver enzymes and without serum HCV-RNA.<sup>28</sup> Persons with occult infection may have detectable HCV RNA in peripheral blood mononuclear cells (PBMC) and in the liver which can lead to HCV-related liver diseases. The detection of HCV-RNA in PBMC in anti-HCV-positive subjects without viremia could reduce false-negative results of HCV-RNA testing by RT-PCR in serum or plasma.<sup>29</sup>

Although the 5' untranslated region (UTR) of HCV is highly conserved between genotypes, it contains insufficient variation to classify HCV on the level of viral subtype and therefore can not be relied on for reliable genotype differentiation. In contrast, a typing method based on the reliable sequence of the core region could identify subtypes as well as major genotypes because its sequence divergence is greater than the divergence of the 5' UTR.<sup>9,30</sup>

In the present study, we confirmed the findings of previous publications that HCV genotype 3a was the most prevalent genotype in Thailand, followed by genotype 1 and genotype 6 variants.<sup>15,27</sup> These three genotypes can be found everywhere whereas genotype 2 is rather rare in the country. Two samples of HCV genotype 2 were found only in Chiangrai located in the north and near the border to Myanmar. Lwin *et al.*, also found HCV genotype 2 in Myanmar.<sup>31</sup> The people from Myanmar and Thailand have frequently crossed the border and closely communicated with each other for a long time. Therefore, virus transmission may have easily occurred. In addition HCV genotype 2 can be found in India, Singapore, Vietnam and the Philippines.<sup>32-35</sup>

In this study, 4 samples had to be classified into genotype 6; 6a (1 sample), 6a or 6f (1 sample)

and 6n (2 samples). The nucleotide sequence of EF543224 appeared to be ambiguous in that it may belong to either genotype 6a or 6f. According to hepatitis C virus sequence databases (<http://hcv.lanl.gov>, <http://www.ncbi.nlm.nih.gov>), BLAST results show that EF543224 belongs to genotype 6f whereas based on phylogenetic analysis it belongs to either genotype 6a or 6f. This may be due to misgenotyping of reference 6a-AF484980 or these 2 reference genotypes largely resemble each other. Therefore, we classified all genotype 6 samples based on phylogenetic analyses as genotype 6 variants.

Even though some studies suggest that HCV recombination is a rare event,<sup>36,37</sup> several studies have demonstrated the occurrence of HCV recombinants in nature.<sup>34,35,38,39</sup> Accordingly, recombination plays a significant role in the evolution of HCV genetic diversity. The genotype of HCV influences the response to interferon treatment and genotype determination based on analysis of a single subgenomic region would limit the use of genotype assays.

The present study has described for the first time the anti-HCV prevalence among the entire population of Thailand. Discovering type 2 variants in Thailand was not surprising because this type has been reported from neighboring countries. Furthermore, this report provides information that might be crucial for health surveillance and prevention.

#### ACKNOWLEDGEMENTS

This research was supported by the National Research Fund from the Ministry of Public Health, the Thailand Research Fund and the Center of Excellence in Viral Hepatitis Research, Chulalongkorn University. We would like to thank the entire staff in the provincial and community hospitals for their assistance in collecting blood samples. We would like to thank Ms. Petra Hirsch and Venerable Dr. Mettando Bhikkhu for reviewing the manuscript.

#### REFERENCES

- World Health Organization. Hepatitis C, Fact sheet (October 2000). <http://www.who.int/dated/1/5/2007>.
- De Mitri MS, Poussin K, Baccharini P, Pontisso P, D'Errico A, Simon N, Grigioni W, Alberti A, Beaugrand M, Pisi E. HCV-associated liver cancer without cirrhosis. *Lancet* 1995; 345: 413-5.
- Choo QL, Kuo G, Weiner AJ, Oveby LR, Bradley DW, Houghton M. Isolate of a cDNA derived from a blood-borne non-A, non-B viral hepatitis genome. *Science* 1989; 244: 359-62.
- Major ME, Rehermann B, Feinstone SM. Hepatitis C virus. In: Knipe DM, Howley PM, Eds. *Fields Virology* 1, 4 Edition, Philadelphia: Lippincott Williams & Wilkins, 2001; pp. 1127-61.
- Major ME, Feinstone SM. The molecular virology of hepatitis C. *Hepatology* 1997; 25: 1527-38.
- Smith DB, Davidson F, Simmonds P. Hepatitis C virus variants and the role of genotyping. *J Hepatol* 1995; 23: 26-31.
- Simmonds P, Holmes EC, Cha TA, Chan SW, McOmish F, Irvine B, Beall E, Yap PL, Kolberg J, Urdea MS. Classification of hepatitis C virus into six major genotypes and a series of subtypes by phylogenetic analysis of the NS-5 region. *J Gen Virol* 1993; 74: 2391-9.
- Mellor J, Holmes EC, Jarvis LM, Yap PL, Simmonds P. Investigation of the pattern of hepatitis C virus sequence diversity in different geographical regions: implications for virus classification. The International HCV Collaborative Study Group. *J Gen Virol* 1995; 76: 2493-2507.
- Mellor J, Walsh EA, Prescott LE, Jarvis LM, Davidson F, Yap PL, Simmonds P. Survey of type 6 group variants of hepatitis C virus in Southeast Asia by using a core-based genotyping assay. *J Clin Microbiol* 1996; 34: 417-23.
- Martinet-Peignoux M, Boyer N, Pouteau M, Castelnaud C, Giuily N, Duchatelle V, Auperin A, Degott C, Benhamou JP, Erlinger S, Marcellin P. Predictors of sustained response to alpha interferon therapy in chronic hepatitis C. *J Hepatology* 1998; 29: 214-23.
- Verachai V, Phutiprawan T, Theamboonlers A, Chinchai T, Tanprasert S, Haagmans B. L., Osterhaus A. and Poovorawan Y. Prevalence and genotypes of Hepatitis C virus infection among drug addicts and blood donors in Thailand. *Southeast Asian J Trop Med Public Health* 2002; 33: 849-51.
- Chainuvati T, Poovorawan Y, Luengrojnanakul P. The prevalence of hepatitis C virus antibody in high risk group of Thai children and adults. *Gastroenterol Jpn* 1991; 26: 176-8.
- Chongsrisawat V, Yoocharoen P, Theamboonlers A, Tharmaphornpilas P, Warinsathien P, Sinlaparatsamee S, Paupunwatana S, Chaiear K, Khwanjaipanich S, Poovorawan Y. Hepatitis B seroprevalence in Thailand: 12 years after hepatitis B vaccine integration into the national expanded programme on immunization. *Trop Med and Inter Health* 2006; 11: 1496-1502.
- Chatproedprai S, Chongsrisawat V, Chatchatee P, Theamboonlers A, Yoocharoen P, Warinsathien P, Tharmaphornpilas P, Warinratwat S, Sinlaparatsamee S, Chaiear K, Khwanjaipanich S, Paupunwatana S, Poovorawan Y. Declining trend in the seroprevalence of infection with hepatitis A virus in Thailand. *Ann Trop Med Parasitol* 2007; 101: 61-8.
- Theamboonlers A, Chinchai T, Bedi K, Jantarasamee P, Sripontong M, Poovorawan Y. Molecular characterization of hepatitis C virus (HCV) core region in HCV-infected Thai blood donor. *Acta Virologica* 2002; 46: 169-73.
- Ohto H, Terazawa S, Sasaki N, Sasaki N, Hino K, Ishiwata C, Kako M, Ujiie N, Endo C, Matsui A. Transmission of hepatitis C virus from mothers to infants. The Vertical Transmission of Hepatitis C Virus Collaborative Study Group. *N Engl J Med* 1994; 330: 744-50.

17. Conte D, Fraquelli M, Prati D, Colucci A, Minola E. Prevalence and clinical course of chronic hepatitis C virus (HCV) infection and rate of HCV vertical transmission in a cohort of 15,250 pregnant women. *Hepatology* 2000; 31: 751-5.
18. Zanetti AR, Tanzi E, Paccagnini S, Principi N, Pizzocolo G, Caccamo ML, D'Amico E, Cambiè G, Vecchi L. Mother-to-infant transmission of hepatitis C virus. Lombardy Study Group on Vertical HCV Transmission. *Lancet* 1995; 345: 289-91.
19. Recommendations for prevention and control of hepatitis C virus (HCV) infection and HCV-related chronic disease. *MMWR Morb Mortal Wkly Rep* 1998; 47 (RR-19): 1-39.
20. Boonyarad V, Chutaputti A, Choeichareon S, Bedi K, Theamboolers A, Chinchai T, Poovorawan Y. Interspousal Transmission of hepatitis C virus in Thailand. *J. Gastroenterol* 2003; 38: 1053-9.
21. Akahane Y, Kojima M, Sugai Y, Sakamoto M, Miyazaki Y, Tanaka T, Tsuda F, Mishiro S, Okamoto H, Miyakawa Y, Mayumi M. Hepatitis C virus infection in spouses of patients with type C chronic liver disease. *Ann Intern Med* 1994; 120: 748-52.
22. Hsu HH, Wright TL, Luba D, Martin M, Feinstone SM, Garcia G, Greenberg HB. Failure to detect hepatitis C virus genome in human secretions with the polymerase chain reaction. *Hepatology* 1991; 14: 763-7.
23. Fried MW, Shindo M, Fong TL, Fox PC, Hoofnagle JH, Di Bisceglie AM. Absence of hepatitis C viral RNA from saliva and semen of patients with chronic hepatitis C. *Gastroenterology* 1992; 102: 1306-8.
24. Alter MJ, Kruszon-Moran D, Nainan OV, McQuillan GM, Gao F, Moyer LA, Kaslow RA, Margolis HS. The prevalence of hepatitis C virus infection in the United States, 1988 through 1994. *N Engl J Med* 1999; 341: 556-62.
25. Alter MJ. Hepatitis C: a sleeping giant? *Am J Med* 1991; 91: 112S-5S.
26. Alter MJ. Epidemiology of hepatitis C. *Hepatology* 1997; 26: 62S-5S.
27. Kanistanon D, Neelamek M, Songsivilai T, Songsivilai S. Genotype distribution of Hepatitis C virus in different region of Thailand. *J Clin Microbiol* 1997; 35: 1772-6.
28. Carreno V. Occult hepatitis C virus infection: a new form of hepatitis C. *World J Gastroenterol* 2006; 12: 6922-5.
29. Caudai C, Padula MG, Bastianoni I, Valensin PE, Shyamala V, Han J, Boggiano CA, Almi P. Antibody testing and RT-PCR results in hepatitis C virus (HCV) infection: HCV-RNA detection in PBMC of plasma viremia-negative HCV-seropositive persons. *Infection* 1998; 26: 151-4.
30. Lole KL, Jha AJ, Shrotri SP, Tandon BN, Prasad VGM, Arankalle VA. Comparison of hepatitis C virus genotyping by 5'noncoding region-and core-based reverse transcriptase PCR assay with sequencing and use of the assay for determining subtype distribution in India. *J Clin Microbiol* 2003; 41: 5240-4.
31. Lewin AA, Shinji T, Khin M, Win N, Obika M, Okada S, Koide N. Hepatitis C virus genotype distribution in Myanmar: Predominance of genotype 6 and existence of new genotype 6 subtype. *Hepatol Res* 2007; 37: 337-45.
32. Das BR, Kundu B, Khandapkar R, Sahni S. Geographical distribution of hepatitis C virus genotypes in India. *Indian J Pathol Microbiol* 2002; 45: 323-8.
33. Lee AS, Ng KY, Chee MK, Vathsala A, Choong HL, Ng HS, Oon CJ. Partial sequence analysis of the hepatitis C viral genome in Singapore patients. *Biochem Biophys Res Commun* 1994; 199: 37-40.
34. Noppornpanth S, Lien TX, Poovorawan Y, Smits SL, Osterhaus AD, Haagmans BL. Identification of a naturally occurring recombinant genotype 2/6 hepatitis C virus. *J virol* 2006; 80: 7569-77.
35. Kageyama S, Agdamag DM, Alesna ET, Leano PS, Heredia AML, Abellanos-Tac-An IP, Jeraza LD, Tanimato T, Yamamura J, Ichimura H. A natural inter-genotypic (2b/1b) recombinant of hepatitis C virus in the Philippines. *J Med Virol* 2006; 78: 1423-8.
36. Simmonds P, Smith DB, Mcomish F, Yap PL, Kolberg J, Urdea MS, Holmes EC. Identification of genotypes of hepatitis C virus by sequence comparisons in the core, E1 and NS-5 regions. *J Gen Virol* 1994; 75: 1053-61.
37. Viazov S, Widell A., Nordenfelt E. Mix infection with two types of hepatitis C virus is probably a rare event. *Infection* 2000; 28: 21-5.
38. Kalinina O, Nordor H, Mukomolov S, Magnius LO. A natural intergenotypic recombinant of hepatitis C virus identified in St. Petersburg. *J Virol* 2002; 76: 4034-43.
39. Legrand-Abravanel F, Claudinon J, Nicot F, Dubois M, Chapuy-Regaud S, Sandres-Saune K, Pasquier C, Izopet J. New natural intergenotypic (2/5) recombinant of hepatitis C virus. *J Virol* 2007; 81: 4357-62.