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Short communication

Isolation of hepatitis E virus genotype 4 from patients with acute cryptogenic hepatitis in Korea

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ABSTRACT

Background: Autochthonous hepatitis E occurs sporadically in developed countries. The consumption of undercooked pork containing hepatitis E virus genotype 3 (HEV-3) or 4 (HEV-4) is the major risk factor for infection. The serological diagnostic kits currently used in hospitals sometimes produce false-negative or −positive results. Therefore, detection of both HEV RNA and antibodies to the virus is required for confirmative diagnosis of hepatitis E.
Objectives: We aimed to detect HEV in serum samples from patients with cryptogenic hepatitis and to determine the origin of HEV.
Study design: A nested polymerase chain reaction (PCR) method was developed for detection of HEV-3 and HEV-4 in patients with hepatitis. A total of 23 serum samples, deposited in 2006–2012, from patients with acute cryptogenic hepatitis who were serologically negative for hepatitis A, B, C, and E were examined using this method. The amplified PCR products were genetically analyzed.
Results: Four HEV-4 isolates were detected from the 23 serum samples. Phylogenetic analysis indicated that three of the four isolates were closely related to HEV-4 isolates found in pigs in Korea and in patients with hepatitis E in Japan.
Conclusions: The newly developed nested PCR method was useful for detection of HEV in patients with cryptogenic hepatitis. The close relationship between the human HEV-4 isolates identified in this study and swine isolates implied that zoonotic transmission of HEV might be a source of infection in patients with hepatitis.

1. Background

HEV is the major causative agent of acute viral hepatitis worldwide [1]. Although hepatitis E is an endemic disease that occurs primarily in developing countries, sporadic autochthonous cases have recently been reported in industrialized countries [2]. Autochthonous hepatitis E is generally transmitted through consumption of HEV-contaminated foods, particularly pork. In most cases, HEV infection manifests itself as an acute self-limiting hepatitis; however, in certain cases it can also lead to severe acute hepatitis [3]. A recent classification study proposed that HEV isolates infecting humans belong to the genus *Orthohepevirus* in the family *Hepeviridae* [4]. HEV isolates in the genus *Orthohepevirus* are classified into four major genotypes: HEV-1 and HEV-2 have been isolated only from humans, whereas HEV-3 and HEV-4 have been isolated from humans and several animal species, including pigs. HEV-3 and HEV-4 are now recognized as zoonotic agents worldwide [5].

2. Objectives

Detection of anti-HEV antibodies in patient samples is essential for the diagnosis of hepatitis E. However, some serological kits for the detection of anti-HEV antibodies have problems with sensitivity or specificity, resulting in false-negative or – positive results. Therefore, isolation of HEV RNA is recommended as a confirmative diagnostic measure [6]. In this study, we attempted to detect the partial genomic sequences of HEV-3 or HEV-4 in patients diagnosed with acute cryptogenic hepatitis. The genomic sequences of

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Table 1
Primer sequences used for detection of HEV-3 and HEV-4.

Primer	Sequence (nucleotide position, reference strain, GenBank accession no.)	Product (bp)
External Forward	5′-ACG AAT GTK[a] GCK CAR GTC TG-3′ (5003–5022, HE-JA04-1911, AB248520)	
External Reverse	5′-CAG CTG GGG YAG ATC GAC GAC-3′ (5509–5529, HE-JA04-1911, AB248520)	531
Internal Forward	5′-TGG TAC ATA ACC TKA TTG GSA TG-3′ (5064–5087, HE-JA04-1911, AB248520)	
Internal Reverse (G3)[b]	5′-ATT GTG ATA CGA CGT CGG AGG C-3′ (5400–5420, HE-JA04-1911, AB248520)	358
Internal Reverse (G4)[c]	5′-TGG CAT CTC CAT GCA CAG AGC-3′ (5140–5160, JYW-Sap02, AB161719)	152

[a] K for G or T; R for A or G; Y for C or T; S for G or C.
[b] Genotype 3 specific.
[c] Genotype 4 specific.

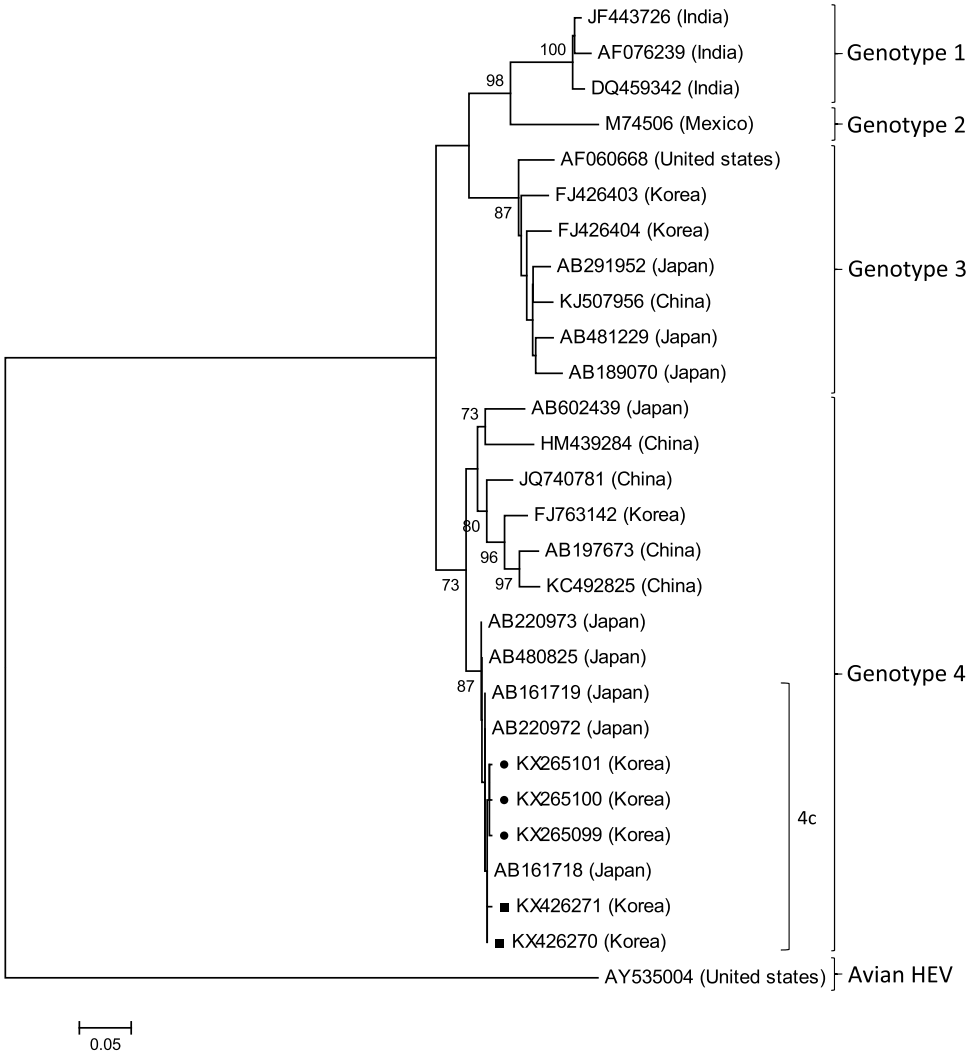

Fig. 1. Phylogenetic analysis of the conserved sequence of the HEV ORF1-ORF2 junction using the neighbor-joining method. The 490-bp nucleotide sequences without primer sequences from three human samples identified in this study and two swine HEV-4 isolates were analyzed with representative strains of HEV-1, HEV-2, HEV-3, and HEV-4. Bootstrap values of more than 70% are shown for the major nodes as a percentage of the data obtained from 1000 repeats. The three human isolates (KX265099, KX265100, and KX265101) and two swine isolates (KX426270 and KX426271) are indicated with closed circles and closed squares, respectively. The scale bar indicates the branch length corresponding to 0.05 substitutions per site.

HEV isolates found in this study were compared with those of other swine and human HEV isolates to determine their genotypes and origins.

3. Study design

Twenty-three serum samples were prospectively collected from patients with acute hepatitis who visited Seoul National University Bundang Hospital in Korea from 2006 to 2012. Written informed consents were obtained from all participants in this study. The study was approved by the Institutional Review Board of Seoul National University Bundang Hospital (B-0611/039-018). All patients enrolled in the study were diagnosed with cryptogenic acute hepatitis because of lack of evidence of viral hepatitis, toxic hepatitis, genetic liver diseases, and autoimmune liver diseases. Viral hepatitis A, B, C, and E and Epstein-Barr virus infection were excluded based on negative serological results. All patients had high aspartate transaminase (AST) and alanine transaminase (ALT) serum levels with compatible clinical features (Supplementary Table S1). Viral RNA was extracted from patient serum samples