CONCISE COMMUNICATION

Genetic Heterogeneity of Hepatitis E Virus Recovered from Japanese Patients with Acute Sporadic Hepatitis

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The recent discovery of a presumably Japan-indigenous hepatitis E virus (HEV) strain (JRA1) spurred analysis of additional isolates from 7 cases of acute sporadic hepatitis E infection. Comparison of a 326-nucleotide region from open-reading frame 1 indicated that 1, 3, and 3 isolates segregated to genotypes I, III, and IV, respectively. Six patients had not traveled abroad recently. One patient had traveled to Hawaii 1 month before becoming ill, and the nucleotide sequence of the HEV isolate infecting her resembled those of US isolates (89%–91% nucleotide identity). However, the isolate was even more homologous to 2 other Japanese isolates (95%–97% nucleotide identity), suggesting that it is more likely a domestic, rather than an imported, strain. Three genotype IV isolates from Japan also had a higher homology to each other (100% amino acid identity) than to 2 Chinese isolates (97%–98% amino acid identity). These findings suggest that HEV strains of at least 3 different genotypes have already made inroads and are spreading in Japan.

Infection with hepatitis E virus (HEV), an enterically transmitted virus, is widespread and often occurs in epidemic fashion in many developing countries but rarely occurs in developed countries. Recent trends worldwide, including in developed countries, however, indicate that HEV infection is occurring in individuals with no history of travel to endemic areas and, of interest, that the nucleotide sequences of HEV isolates from such cases segregate to novel genotypes [1–4]. In Japan, the entire sequence was determined recently for a unique strain of HEV (JRA1) from a patient who had never been abroad [5]. The sequence of this first Japanese HEV isolate was relatively closer among known isolates to those of genotype III from the United States, but it seemed to represent a new genotype. To determine to what extent the other HEV isolates in Japan are genetically related to the prototype JRA1 strain, additional isolates from 7 cases of acute sporadic hepatitis E disease were analyzed in this study for a partial nucleotide sequence of the open-reading frame (ORF) 1.

Materials and Methods

Of the 7 patients with acute hepatitis E disease analyzed in this study, 5 (representing isolates JHA-Sap, JKJ-Sap, JKJ-N, JMY-Haw, and JSY-Sap) were residents of Hokkaido island, and 2 (representing isolates JAK-Sa and JMM-Sai) were from Saitama Prefecture of mainland Honshu, Japan. The onset of disease in each patient was unrelated to onset in other patients in both time and space and was not involved in any outbreak. Six patients had not traveled abroad recently, whereas the patient harboring isolate JMY-Haw had traveled to Hawaii 1 month before the onset of her disease. Serum samples obtained from the patients during the acute phase were stored frozen (at or below −20°C) until virologic analysis.

HEV sequences were analyzed according to methods reported elsewhere [5], with slight modification: nucleic acids were extracted from 25 mL of serum by use of an extraction kit (SMITEST EX-R&D, Genome Science Laboratories) and subjected to first-strand cDNA synthesis at 37°C for 30 min with Moloney murine leukemia virus reverse transcriptase (Stratagene) and a mixture of the antisense primers HE5-4 (5'-CATGCTCTCAGTACATACG-3'; nt 541–560) and HE5-5 (5'-CATGCTCTGCAATCGCTGG-3'; nt 541–560; nucleotide positions correspond to those of the HEV-JRA1 strain.) The cDNA then was subjected to a nested polymerase chain reaction (PCR), using FastStart Taq DNA polymerase (Roche) with the external sense primer HE5-1 (5'-TCGATGCCATGAGGCCCCA-3'; nt 19–37) and a mixture of external antisense primers HE5-4 (5'-GCCYTKGCGAATGCTG-3'; nt 105–123) and a mixture of internal antisense primers HE5-5 (5'-CATGCTCTGCAATCGCTGG-3'; nt 450–469) and HE5-6 (5'-TYAAAACAGTAGGTTCGATC-3') under thermal cycling conditions: 30 cycles at 95°C for 4 min, 95°C for 30 s, 55°C for 30 s, 72°C for 45 s, and then a final cycle at 72°C for 7 min. A 326-nt region thus amplified from ORF1 of the HEV genome

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The nucleotide sequences reported in this article will appear in the DNA Data Bank of Japan, GenBank, and European Molecular Biology Laboratory databases under the accession numbers AB074915–AB074921.

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Figure 1. Phylogenetic tree (neighbor-joining method) for 7 Japanese hepatitis E virus (HEV) isolates (shown within boxes) based on a 326-nt region from open-reading frame 1 (A) and alignment of the amino acid sequence (B). A. The 4 major genotypes of HEV are indicated by Roman numerals I–IV. Bootstrap values >70% are indicated. Accession nos. for the reference sequences are indicated in parentheses.
then was sequenced by use of the Dye Terminator Cycle Sequencing FS Ready Reaction Kit (Perkin-Elmer Applied Biosystems) and the 373A DNA sequencer (Applied Biosystems).

The sequences obtained from these Japanese isolates then were compared with those of isolates from various countries, using computer software (GENETYX-MAC version 10.1; Software Development). The database accession numbers of the reference sequences are shown in figure 1.

**Results**

When the 326-nt ORF1 sequences were compared, the 7 Japanese isolates segregated to 3 major groups: JMM-Sai showed 73.0%–75.7% nucleotide identity to the other 6 isolates; JKN-Sap, JHA-Sap, and JMY-Haw showed 95.4%–98.8% intragroup identity but only 73.0%–79.1% identity to extragroup isolates; and JSY-Sap, JKK-Sap, and JAK-Sai were 89.0%–99.7% identical to each other but only 74.8%–78.8% identical to the other 4 isolates. The prototype full-genome Japanese HEV strain (JRA1) unexpectedly had < 90% nucleotide identity (range, 72.5%–88.3% nucleotide identity) to these new isolates.

Compared with prototype sequences of the 4 major genotypes of HEV, JMM-Sai was most homologous (98.5% identity) to the Burmese strain of genotype I; JKN-Sap, JHA-Sap, and JMY-Haw were most homologous (90.5%–91.4% identity) to the US1 strain of genotype III; and JSY-Sap, JKK-Sap, and JAK-Sai were most homologous (84.3%–85.5% identity) to the Chinese T1 strain of genotype IV. A comparison of these Japanese isolates with other known isolates worldwide is shown in the phylogenetic tree in figure 1A. JMM-Sai was in the midst of the very compact cluster of many Asian isolates and is unequivocally a genotype I isolate. By contrast, JKN-Sap, JHA-Sap, and JMY-Haw formed a mini-cluster with the bootstrap value of 98% within genotype III. JSY-Sap, JKK-Sap, and JAK-Sai, together with a Chinese isolate (S15), similarly formed a mini-cluster having a 90% bootstrap value within genotype IV. The prototype Japanese strain, JRA1, continues to stand as a solitary branch, even after the addition of these 7 new Japanese isolates.

The high degree of relatedness among the 3 genotype III Japanese isolates and among the 3 genotype IV Japanese isolates was further supported by comparing them at the amino acid level: the genotype III isolates (JKN-Sap, JHA-Sap, and JMY-Haw) shared 100% identical amino acid sequences, as did the genotype IV isolates (JSY-Sap, JKK-Sap, and JAK-Sai; figure 1B).

**Discussion**

The phylogenetic analysis in this study was based on a 326-nt region from ORF1. HEV genotyping based on such a short sequence may be misleading; however, our further analysis on the ORF2 gene from 4 of 7 Japanese isolates (JMY-Haw, JKN-Sap, JKK-Sap, and JAK-Sai) showed a segregation pattern similar to that of the 326-nt ORF1 sequence (figure 2).

We did not anticipate that the 7 Japanese HEV isolates analyzed in this study would differ significantly from the prototype Japanese isolate, JRA1, which we found previously and thought to be “possibly a Japan-indigenous strain” [5], and we did not
anticipate the fairly high degree of genetic diversity that existed among them. This small number of isolates spanned 3 major genotypes—I, III, and IV. The origins of these isolates thus are not easily deduced.

The JMY-Haw isolate was from a patient who had returned to Sapporo, Japan, after a 2-week visit to Hawaii 1 month before the onset of acute hepatitis. Since the sequence of JMY-Haw was similar to those of isolates from the United States, it was thought initially that this patient was infected with HEV in Hawaii and that JMY-Haw might represent Hawaiian HEV strains. Although Hawaii has not been recognized as an endemic area for human HEV infection, a very high prevalence of antibodies to HEV was reported recently in wild rats in Hawaii [6]. Considering the potential zoonosis for HEV infection [7], it is not unrealistic to imagine that the patient was infected in Hawaii with a human or rodent HEV. However, the JMY-Haw isolate was more closely related to 2 Japanese isolates recovered from residents in Sapporo than to those from the United States (figures 1 and 2), suggesting that it is more likely that this patient was infected in her home town (Sapporo) or that she was infected in Hawaii with a Japanese strain that had been “exported” to Hawaii some time ago; in fact, there are many Japanese immigrants in Hawaii. Sequence analyses of HEV isolates from Hawaiian patients, in particular of Japanese Hawaiians, would be of great interest in this context.

With respect to the JSY-Sap, JKK-Sap, and JAK-Sai isolates, although they all bear a resemblance to Chinese genotype IV isolates, all were from individuals who had not traveled abroad recently. Nevertheless, it is still possible that these isolates were imported from China, because Japan imports many possible vehicles for HEV transmission, including foods and herbal medicine. Indeed, there was a case report in Japan of hepatitis E that probably had been contracted via a Chinese herbal medicine [8]. However, the fact that JSY-Sap and JKK-Sap were very much homologous to each other (99.7% nucleotide identity) suggests that at least this lineage of HEV has been spreading domestically, even if it was imported from China some time ago.

Isolate JMM-Sai was unambiguously an Asian strain (genotype I). It was surprising that this strain was found in a Japanese person who had never traveled to an HEV-endemic country, and how he became infected remains obscure. But one possible scenario might be that he contracted this Asian strain of HEV in the “mini-Asia” inside Japan. Tokyo and its vicinities (including Saitama, where the patient lives) recently have seen increasing immigration of people from other countries in Asia, providing more chances for Japanese residents there to make close contacts with other Asian people, cultures, and foods (and, perhaps, with Asian HEV, too).

In conclusion, our present study revealed a great range of genetic diversity of HEV in Japan. Reasons for the diversity may come from the difference in the channels through which the ancestral strains of these HEV isolates made inroads in Japan. Irrespective of the origins, however, the existence of pairs of highly homologous isolates (e.g., JKN-Sap and JHA-Sap or JSY-Sap and JKK-Sap; figure 1) implies that these strains of HEV have begun to spread domestically in Japan. Whether the spread is via human-to-human contact or via zoonosis is yet to be determined.

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References