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Recombination of Genotypes B and C in Hepatitis B Virus Isolated from a Vietnamese Patient with Fulminant Hepatitis

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Hepatitis B virus (HBV) can now be classified into seven major genotypes designated from A to G (1). Accumulated evidence suggests that recombination among different genotypes is relatively frequent (2,3). Within the HBV genome, three recombination hot spots have been identified: in the vicinity of DR1 (direct repeat 1; nucleotide [nt] 1800); at the 3′ end of the core region (nt 2359); and within the 3′ end of the S gene (2,3).

The distribution of HBV genotypes varies geographically, and genotypes B and C are known to be prevalent in Asian countries, including Vietnam (4). Recently, phylogenetic analysis has indicated that B/C recombinants have spread throughout East Asia and A/D recombinants in Italy and South Africa (5,6).

We report here a complete genomic sequence of HBV isolated in Vietnam. The patient was a Vietnamese (male, 30 years old) who was admitted to the Bach Mai Hospital, Hanoi, Vietnam with diagnosis of fulminant hepatitis. At that time, the patient was positive for HBsAg and the IgM class of anti-HBc antibody, but negative for HCV RNA, HDV RNA, anti-HAV IgM, and anti-HEV IgM.

Nucleic acids were extracted from a 100-μl serum sample using a SepaGene RV-R Kit (Sanko-Junyaku, Tokyo). Five microliters of nucleic acids were used for amplification of HBV DNA by PCR. PCR was carried out using a set of primers to amplify five overlapping fragments that cover the full genome of HBV. PCR was performed as reported previously (7). Genotyping of HBV was done by PCR using type-specific primers reported by Naito et al. (8). Purified amplicons were subjected to direct sequencing from both directions using the ABI PRISM™ Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer, Norwalk, Conn., USA). Sequences of amplified DNA were determined using an automated DNA sequencer ABI 377 (Applied Biosystems, Foster City, Calif., USA). A phylogenetic tree was constructed with the MEGA program version 2.1, using the Kimura two-parameter matrix and the neighbor-joining method. To confirm the reliability of the phylogenetic tree analysis, bootstrap resampling and reconstruction were carried out 500 times. Recombination of the HBV genome was determined using the SimPlot program (distributed by Stuart Ray, http://www.welch.jhu.edu/~sray) and bootscanning analysis.

We obtained a full-length HBV genome 3215 nt in length that was designated as HBV-VH133. PCR using type-specific primers designed from pre-S1 through S gene and phylogenetic analysis indicated that the sample belonged to genotype B. However, the genome was clustered with a genotype C-derived sequence in the pre-Core/Core gene (Fig. 1a). Phylogenetic analysis of the HBV full genome indicated that VH133 belongs to subgroup Ba, according to Sugauchi et al. (6) (Fig. 1b). Analysis using SimPlot and bootscanning programs based on six different genotypes revealed that VH133 has a resolution of the breakpoint of genotypes B/C recombination at the position of nt 1880 to 2260 (pre-Core/Core region) (Fig. 2). Thus, VH133 is a recombinant of genotype B with genotype C, which is prevalent in Vietnam.

The nucleotide sequence reported in this paper has been submitted to DDBJ/GenBank/EMBL databases under accession no. AB100695 for HBV-VH133.

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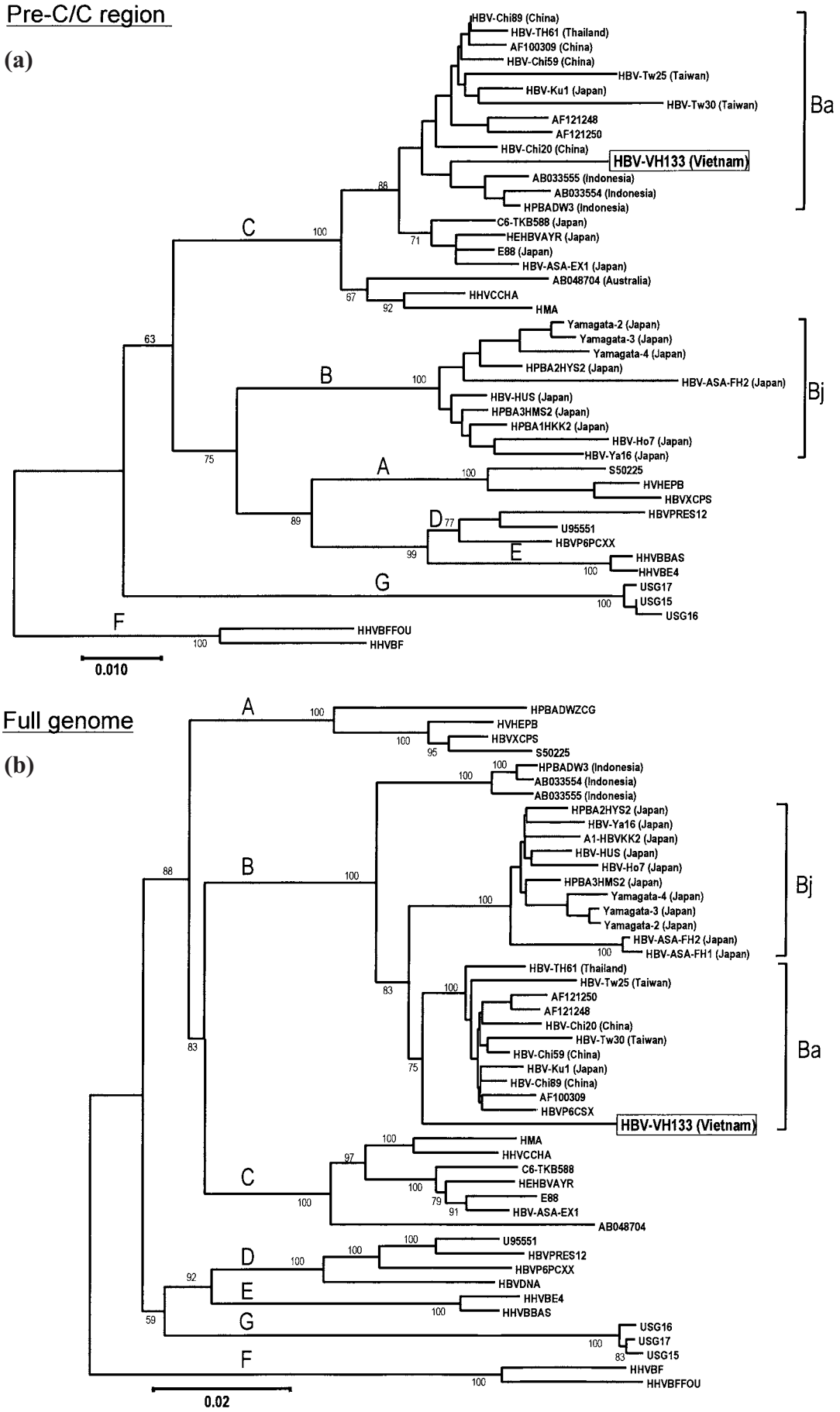

Fig. 1. Phylogram generated by neighbor-joining analysis of genetic distances in the pre-C/C gene (a) and the full-length gene (b) of HBV.

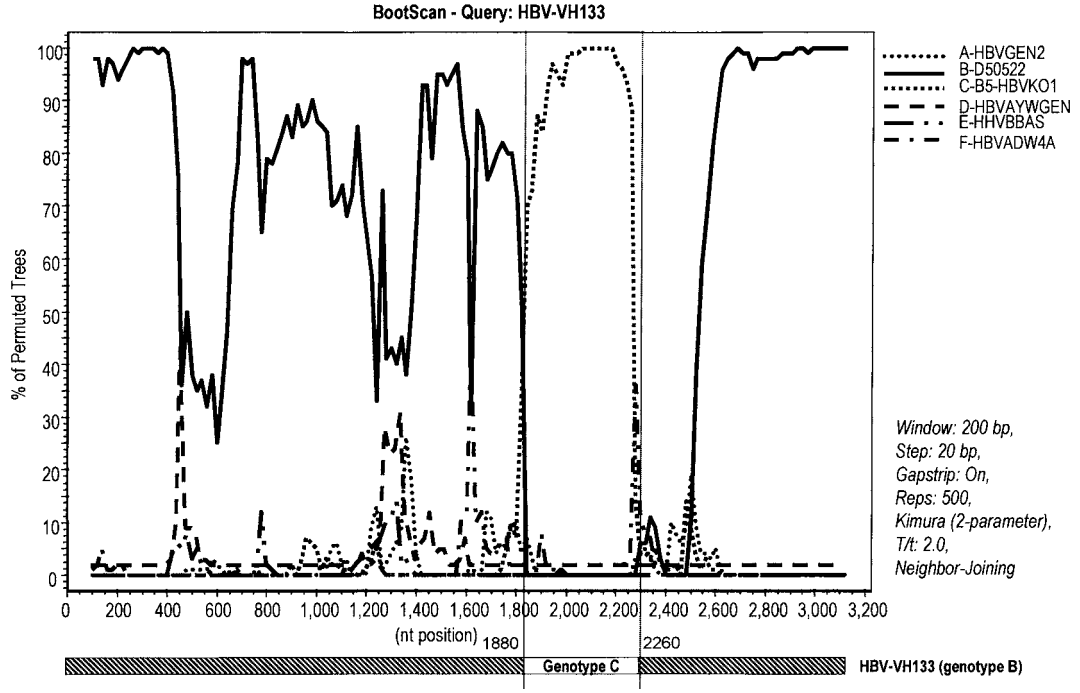

Fig. 2. Resolution of the recombinant event in HBV genomes of distinct genotypes was determined using the SimPlot program and bootscanning analysis.

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