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Complete Nucleotide Sequence of Hepatitis B Virus Isolated from Two Infants with Fulminant Hepatitis

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Approximately 1% of patients with hepatitis B virus (HBV) infection develop fulminant hepatitis. More than 80% of them require liver transplantation or die of hepatic failure. This is a serious problem, particularly for infants.

The pathogenesis of fulminant hepatitis B is not well understood. Some previous studies have suggested that it is associated with a precore stop codon mutation (1896 G to A) (1,2) or with a double mutation in the basal core promoter region (1762 A to T and 1764 G to A) (3,4). Both variants are expected to be defective in hepatitis B e antigen (HBeAg) expression, which may well modify the immune response of the host. To our best knowledge, a full-length sequence of HBV isolates from newborns and/or infants with fulminant hepatitis or severe hepatic failure has not been reported so far although a few reports have described adults.

We report here the full-length nucleotide sequence of two HBV isolates from two infants, a 3-month-old girl and a 9-year-old 4-month-old girl. They were admitted to the Kyoto University Hospital with diagnoses of fulminant hepatitis and for liver transplantation. Serological findings were positive for HBsAg, anti-HBe, and anti-HBc IgM, but negative for HCV RNA, anti-HAV IgM, and anti-HEV IgM.

Nucleic acid was extracted from serum and liver tissues using a SepaGene RV-R Kit (Sanko-Junyaku, Tokyo), suspended in 50 µl RNase-free water, and stored in -20°C until use. Five microliters of nucleic acid was used for amplification of HBV DNA by PCR. PCR was carried out in the manner as reported (5) using a set of primers to amplify three overlapping fragments that covered the full genome of HBV. Purified amplicons were subjected to direct sequenc-

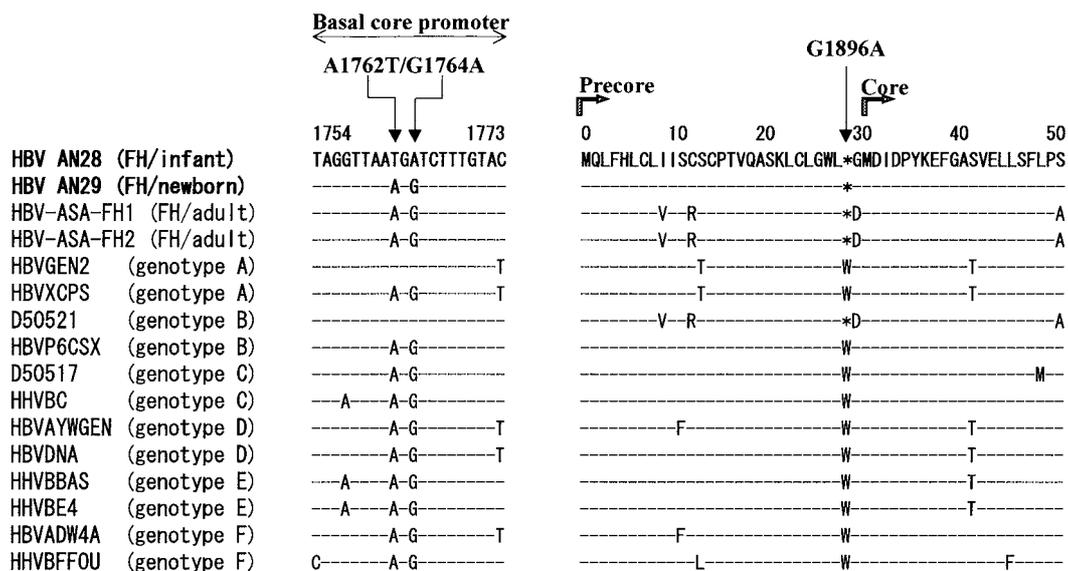


Fig. 1. Comparison of nucleotide and amino acid sequences in precore and core regions of HBV from infants with fulminant hepatitis. FH=fulminant hepatitis, *=stop codon mutation (G1896A).

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ing in 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). Phylogenetic analysis was performed according to the method reported previously (6).

The full-length genomes of the two HBVs were designated as HBV AN28 and HBV AN29, respectively. They were 3215 nucleotides in length and belonged to genotype C as determined by phylogenetic analysis. Both isolates had a stop codon mutation (1896 G to A) in the precore region (Fig. 1). The AN28 had additional mutations in the basal core promoter region (1762 A to T and 1764 G to A), but the AN29 did not.

Though babies born to HBe antigen-positive mothers are prone to become carriers, those borne to HBe antibody-positive mothers are at risk of developing fulminant hepatitis. Friedt et al. (4), studying the genotype D, suggested that those with a combination of basal core promoter mutation (1762-T/1764-A) and precore stop codon mutation (1896 G to A) was a risk of developing fulminant hepatitis in infants. Our two isolates also had the precore stop codon mutation, though only one of them had the basal core promoter mutation (1762 A to T and 1764 G to A). It is interesting that Friedt et al.'s study regarding genotype D and our study regarding genotype C both detected similar, though not identical, mutations in HBVs in fulminant hepatitis in children. The mutation(s) may affect host immune response because the two cases presented here were seropositive for anti-HBe with precore stop codon mutation.

The nucleotide sequence data reported in this paper have

been submitted to the DDBJ/GenBank/EMBL databases under accession no. AB049609 for HBV AN28 and AB049610 for HBV AN29.

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