

Short Communication

Full-Length Sequence of Hepatitis B Virus Belonging to Genotype H Identified in a Japanese Patient with Chronic Hepatitis

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SUMMARY: We have isolated and cloned the full-length nucleotide sequence of the hepatitis B virus (HBV) genome (denoted HBV-IM806-2) recovered from a Japanese patient with chronic hepatitis. This patient had a history of travel to Bangkok, Thailand, and then suffered the onset of acute hepatitis B 3 months after his return to Japan. The HBV-IM806-2 isolate was composed of 3,215 nucleotides and showed the highest similarity to genotype H of HBV. Interestingly, 24 amino acid residues specific for genotype H were identified throughout the full genome sequence. Furthermore, phylogenetic analysis based on the full genome sequence confirmed that IM806-2 belonged to genotype H and was more closely related to the prototype of the Los Angeles strain than to the Nicaragua strain.

More than 350 million people worldwide are chronically infected with hepatitis B virus (HBV) and are at risk of dying as a result of the occurrence of hepatocellular carcinoma accompanying HBV infection. By characterization of the viral genome, HBV has been classified into genotypes A through G with an inter-genotypic diversity of at least 8% in the full genome sequence (1,2). In addition to this classification, a newly described genotype H has been found in Nicaragua and the U. S. (3). It seems that the distribution of genotype H is restricted to the northern part of Latin America, however, the exact distribution of this genotype remains unclear due to the lack of a rapid and simple method of identification such as genotyping by PCR or PCR-RFLP. Among the sequence records deposited in the database, only 8 isolates of genotype H have been sequenced in the full genome to date. Here we report the identification and entire nucleotide sequence of the HBV belonging to genotype H isolated from a Japanese patient with chronic hepatitis.

A 61-year-old man underwent a medical examination at the International Medical Center of Japan, Tokyo and was diagnosed with chronic hepatitis. Serological findings were positive for HBsAg, HBeAg and anti-HBc, but negative for anti-HCV and HCV RNA. The patient had visited Bangkok, Thailand, 30 years previously and had had sexual contact with a woman there. Three months after his return to Japan, he suffered the onset of acute hepatitis with HBV infection which developed into chronic hepatitis B. We conducted a sequencing analysis of the HBV obtained from this patient. To obtain a full-length sequence, we amplified HBV DNA by PCR using a primer combination of HBV4 (sense; 5'-CCG GAA AGC TTA TGC TCT TCT TTT TCA CCT CTG CCT AAT CAT C-3'; the HindIII site is underlined) and HBV4R (antisense; 5'-CCG GAG AGC TCA TGC TCT TCA AAA AGT TGC ATG GTG CTG GTG-3'; the SacI site is underlined) as reported

previously (4). Viral DNA was extracted from 100 μ l of serum using a DNA/RNA extraction Kit (SepaGene RV-R, Sanko Junyaku Co., Ltd., Tokyo, Japan). The resulting pellet was resuspended in 50 μ l of RNase-free water and maintained at -20°C until use. The PCR conditions included pre-incubation at 94°C, 2-min activation of Blend Taq-Plus DNA polymerase (Toyobo Co., Ltd., Tokyo, Japan) followed by 40 cycles of PCR (94°C for 15 sec, 55°C for 45 sec and 72°C for 3 min 20 sec with a final extension for 7 min at 72°C). The PCR products were separated by 1% agarose gel electrophoresis and purified using a QIA quick gel extraction kit (Qiagen, Inc., Chatsworth, Calif., USA) in preparation for sequence analysis. Purified PCR products were cloned into the HindIII/SacI sites of pUC19 vector. Cloned HBV DNA was subjected to sequencing using an ABI PRISM™ Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, Calif., USA). Sequences of cloned HBV DNA were determined using the automated DNA sequencer, ABI 3100-Avant Genetic Analyzer (Applied Biosystems).

The sequence reported in this paper have been deposited in the DDBJ/GenBank/EMBL under the accession number AB205010.

The HBV genome recovered in this study was compared with the 35 isolates of HBV with a full-length sequence in the database. Nucleotide sequences were multiple aligned using GENETYX for Windows version 7 software (Genetyx, Tokyo, Japan) and were calculated using the Kimura two-parameter method; phylogenetic trees were constructed by the neighbor-joining method (5). To confirm the reliability of the pairwise comparison and phylogenetic tree analysis, bootstrap resampling and reconstruction were carried out 1,000 times.

The full genome sequence of the HBV was obtained from our Japanese patient with chronic hepatitis and was named HBV-IM806-2. IM806-2 was composed of 3,215 bases. When compared with other previously reported HBV isolates with a full genome sequence, IM806-2 showed high overall identity (98.9%) with the prototype of the Los Angeles strain (AY090460) and 97.4% identity with the Nicaragua

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strain (AY090457) belonging to the genotype H group at the nucleotide level. By comparison of the deduced amino acid sequences among the HBV full genome, we identified 24 amino acid residues that were specific for genotype H in each region (Fig. 1). Specifically, 9 strains of genotype H consisting of IM806-2 and 8 isolates from the database showed the consensus sequences of amino acids at 16N, 90Q, 213T, 255S, 270T, 273T, 280E, 306T, 311T, 367S, 461V, 480H, 584A, 743D and 821P in the P gene; 32W, 60A, 102P and 127L in the X gene; 8A, 90P and 219P in the S gene; and 180Q and 184A in the core gene. Furthermore, the HBV-IM806-2 isolate was grouped into genotype H of HBV by phylogenetic analysis based on the full genome sequence (Fig. 2). IM806-2 was found to be more closely related to the Los Angeles strain than to the Nicaragua isolate.

Although extensive studies on HBV have been conducted, the nature of HBV and its true pathogenic role remain controversial. It is possible that HBV genotypes influence the severity of liver diseases and the replication of HBV. Recently, eight different genotypes, A-H, of HBV have been classified. HBV genotypes have been shown to have a distinct geographical distribution and to correlate with the severity of liver diseases (6). It has been reported that genotype A is prevalent in northern and central Europe but is also common in North America and sub-Saharan Africa. Genotypes B and C are confined to Asia. Genotype D is widespread but is the predominant type in the Mediterranean region, while genotype E is found mainly in West Africa. Genotype F shows the highest divergence among the genotypes and is indigenous to aboriginal populations of the Americas (7). The newly described genotype G has been found in the U. S. and France (8). Some of these genotypes have been split into subgroups. Most recently, genotype H has been identified in two Nicaraguans and one American living in Los Angeles (3). Genotype H has been encountered in Nicaragua, Mexico and California, and it seems that its

distribution may be restricted to the northern part of Latin America, including Central America and Mexico. Arauz-Ruiz et al. (3) suggest that the genotype H strain from Los Angeles might be an import from Mexico. Nevertheless, the nature of HBV genotype H throughout the world remains obscure. Regarding the genotypic distribution of HBV in Japan, our data showed that genotype C (74%) was the most prevalent, followed by genotype B (17%) and genotype A (4%) among 100 liver disease patients in the Tokyo area (9). In the present study, we coincidentally found a strain of HBV belonging to the genotype H in a Japanese patient with chronic hepatitis and noted that the isolate recovered was more closely related to the prototype of the Los Angeles strain than to that of the Nicaragua strain. Genotype H is very rare in Japan; in fact, the Japanese Red Cross NAT Screening Research Group recently reported that HBV genotype H was found in only 1 of 328 (0.3%) HBV DNA-positive blood donors in Japan and confirmed that it showed high homology with the strain from Los Angeles (10,11). In addition, Shibayama et al. (12) also reported that genotype H was detected in a Japanese patient co-infected with HIV who had a history of traveling to South America and had had sexual intercourse there. Even though this genotype is very rare in Japan, it is important to survey the infection route of HBV in such patients. In our case, the patient had a history of acute hepatitis B after returning to Japan from Thailand, suggesting that infection had occurred in Thailand. Interestingly, the amino acid changes specific to genotype H were concentrated in the P gene. The P gene product is needed for the encapsidation of viral RNA into core particles and for the conversion of the pregenomic viral RNA molecule into genomic viral RNA. These findings may have an impact on the viral replication, immunological and genetic diagnosis of HBV, as well as on treatment options for the ubiquitous disease it causes. Elucidation of the relationship among genotype H, its pathogenicity in chronic liver diseases and its effects against therapy is awaited with great

Genotype	Amino acid	P gene												C gene		X gene			S gene						
		16	90	213	255	270	273	280	306	311	367	461	480	584	743	821	180	184	32	60	102	127	8	90	219
A	AB076678 Malawi	D	N	C	F	S	S	A	S	S	A	L	D	N	K	S	R	S	G	V	A	I	P	A	S
	M57663 Philippine	D	N	C	F	S	S	A	S	S	A	L	D	N	K	S	R	S	R	V	A	I	P	A	A
	AB126580 Russia	D	N	C	F	N	S	A	C	S	A	L	D	N	K	S	R	S	G	V	A	I	P	T	S
	AF090838 France	D	N	C	F	N	S	A	C	S	A	L	D	N	K	S	R	S	G	V	A	T	P	T	S
B	AB033554 Indonesia	D	D	C	V	N	S	A	H	S	A	L	D	Q	K	S	R	S	G	V	A	I	P	T	T
	AB115551 Cambodia	D	D	C	V	S	S	A	H	S	A	L	D	H	K	S	R	S	G	V	A	I	P	A	T
	AF121249 Vietnam	E	D	C	V	S	S	A	H	S	A	L	N	H	K	S	R	S	G	V	A	V	P	A	T
	AB010291 Japan	D	D	C	V	S	S	A	H	S	A	L	N	H	K	S	R	S	G	V	V	M	P	A	T
C	D00329 Japan	D	D	C	V	S	S	A	H	S	A	L	N	N	N	S	R	S	G	V	A	I	P	A	T
	D50521 Japan	D	D	C	V	S	S	A	H	S	A	L	N	H	K	S	R	S	G	V	A	I	P	A	T
	AB112063 Vietnam	D	N	G	F	S	S	A	H	S	A	L	D	T	K	S	R	S	G	V	A	T	P	A	A
	AB112066 Myanmar	D	N	S	F	S	S	A	H	S	A	L	E	N	K	S	R	S	G	V	A	I	P	A	A
D	AB112471 Thailand	D	N	S	F	S	S	A	H	S	A	L	E	N	K	S	R	S	G	V	A	I	P	A	A
	AB049609 Japan	D	N	G	F	S	S	A	H	S	A	L	D	N	K	S	R	S	G	V	S	I	P	A	A
	AB049610 Japan	D	N	C	F	S	S	A	N	S	A	L	D	N	K	S	R	S	R	V	A	N	P	A	A
	AB115417 Japan	D	N	R	F	S	S	A	N	S	A	L	D	N	N	S	R	T	R	V	A	I	P	A	T
E	AB033559 Papua New Guinea	D	K	S	F	S	A	P	N	S	A	L	D	N	N	S	R	S	G	V	A	I	P	A	T
	AB126581 Russia	D	K	S	F	S	A	P	N	S	A	L	D	N	N	S	R	S	G	V	V	I	P	A	T
	Z35716 Poland	D	K	S	F	S	A	P	N	S	A	L	D	N	N	S	R	S	G	V	V	V	P	A	T
	AB091255 Cote d'Ivoire	E	N	S	F	S	A	A	N	S	A	L	D	N	K	S	R	S	G	V	A	I	P	A	A
F	AB106564 Ghana	E	N	S	F	S	A	A	N	S	A	L	D	N	K	S	R	S	G	V	A	I	P	A	A
	X75657 France	E	N	S	F	S	A	A	N	S	A	L	D	N	K	S	R	S	G	V	A	I	P	A	A
	AB166850 Bolivia	D	S	S	V	S	A	A	P	P	A	L	N	S	N	S	R	S	G	V	G	T	T	A	L
	AY090459 Costa Rica	D	H	S	A	S	A	A	S	S	A	L	N	S	K	S	R	S	G	V	G	I	T	A	T
G	X69798 Brazil	D	S	A	A	S	A	A	P	S	A	L	N	S	T	S	R	S	G	V	G	I	T	A	L
	AB056513 USA	E	T	S	F	S	A	S	S	S	A	L	D	N	K	S	R	S	G	V	V	S	P	A	V
	AB064310 USA	E	T	S	F	S	A	S	S	S	A	L	D	N	K	S	R	S	G	V	V	S	P	A	V
	AF160501 France	E	T	S	F	S	A	S	S	S	A	L	D	N	K	S	R	S	G	V	V	S	P	A	V
H	AY090454 Nicaragua	N	Q	T	S	T	T	E	T	T	S	V	H	A	D	P	Q	A	W	A	P	L	A	P	P
	AY090457 Nicaragua	N	Q	T	S	T	T	E	T	T	S	V	H	A	D	P	Q	A	W	A	P	L	A	P	P
	AY090460 USA	N	Q	T	S	T	T	E	T	T	S	V	H	A	D	P	Q	A	W	A	P	L	A	P	P
	AB179747 Japan	N	Q	T	S	T	T	E	T	T	S	V	H	A	D	P	Q	A	W	A	P	L	A	P	P
IM806-2 Japan	N	Q	T	S	T	T	E	T	T	S	V	H	A	D	P	Q	A	W	A	P	L	A	P	P	

Fig. 1. Comparison of amino acid sequences among different genotypes of HBV. Genotype H-specific 24 amino acid residues can be seen in each region of HBV. Codon numbers indicate the number of position in each region.

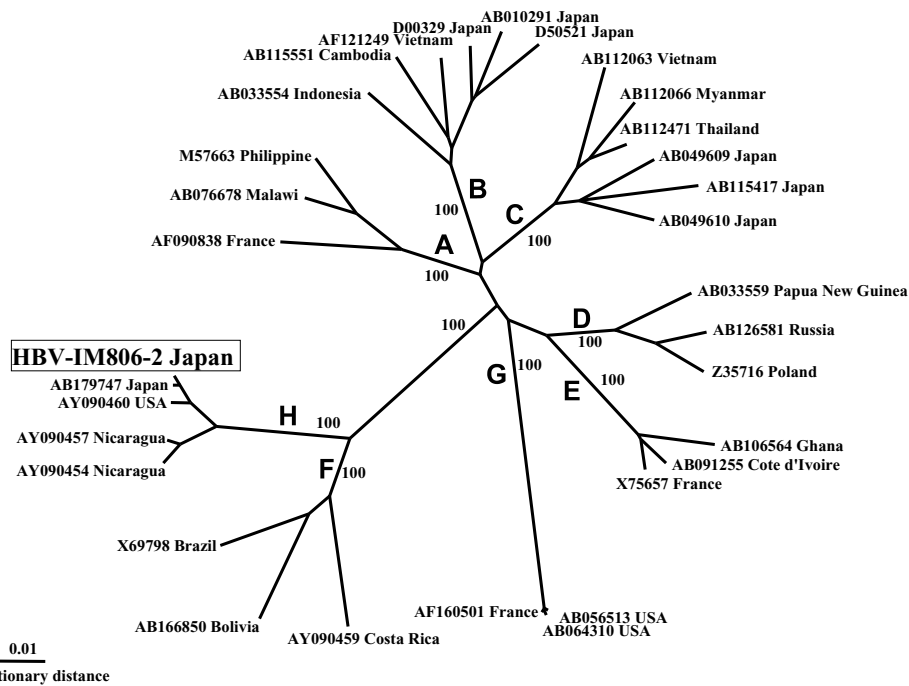


Fig. 2. Phylogram generated by neighbor-joining analysis of genetic distances in the full-length sequence of HBV.

interest. To clarify the virological differences of genotype H, we are now carrying out functional analyses of this genotype by in vitro transfection using the HBV isolate recovered in this study. Recently, it has been reported that HBV shows recombination comprising different genotypes, such as a B/C or A/D recombination. We tested for possibility of recombination using the SimPlot and bootscanning programs, but no recombination was found in the IM806-2 isolate (data not shown).

In conclusion, we identified and cloned the full-length nucleotide sequence of HBV (denoted HBV-IM806-2) recovered from a Japanese patient with chronic hepatitis. Based on phylogenetic analysis of the full genome sequence, it was confirmed that IM806-2 belonged to genotype H and was most closely related to the prototype of the Los Angeles strain.

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