Characterization of genotype H hepatitis B virus strain identified for the first time from a Japanese blood donor by nucleic acid amplification test

Hitoshi Ohnuma,1 Akira Yoshikawa,1 Hideaki Mizoguchi1, Hiroaki Okamoto2 and the JRC NAT Screening Research Group

1Saitama Red Cross Blood Center, 1370-12, Takahagi, Hidaka, Saitama-ken 350-1213, Japan
2Division of Virology, Department of Infection and Immunity, Jichi Medical School, Tochigi-ken 329-0498, Japan

The Japanese Red Cross has been conducting a nucleic acid amplification test (NAT) screening for hepatitis B virus (HBV), hepatitis C virus and human immunodeficiency virus 1 among blood donors since July 1 1999. The first case of HBV genotype H was found and reported in Japan. Serological markers of HBV were not detected in this NAT-positive donation. It may be that the positive donation was in the serological window period at the early stage of infection. The complete genome of 3215 nt was sequenced, and the sequence had 99\% homology with the strain from Los Angeles, USA (LSA2523). Here, a leucine zipper motif was found in the region of the HBV surface antigen conserved through genotypes A–H.

Hepatitis B virus (HBV) has been classified into seven genotypes A–G. However, recently genotype H, which is phylogenetically closely related to genotype F, has been reported (Arauz-Ruiz et al., 2002). These genotypes of HBV show a distinctive geographical distribution and a relevance to clinical severity (Mayert et al., 1999; Kobayashi et al., 2002; Locarnini, 2002). They are important in epidemiological studies, analysis of modes of infection and medical treatment.

On July 1 1999, the Japanese Red Cross (JRC) started a nationwide screening by using the nucleic acid amplification test (NAT) of pools of 500 sera. From February 1 2000, the pool size was reduced to 50 (Mine et al., 2003). By March 31 2003, 19 454 693 units in the 500- or 50-sample pools had been tested using NAT screening. During this period, 349 HBV DNA-, 53 hepatitis C virus RNA- and 6 human immunodeficiency virus type 1 RNA-positive donors were found.

The NAT screening system in Japan has been reported previously (Ohtake & Nishioka, 2000; Mine et al., 2003; Minegishi et al., 2003). Samples that are serologically positive and have elevated alanine aminotransferase (>60 IU l\(^{-1}\)) are excluded from NAT screening. All voluntarily donated blood in the JRC is qualified by a questionnaire administered by the JRC blood centres.

HBV DNA loads were calculated from the working curve (10\(^7\), 10\(^6\), 10\(^5\), 10\(^4\), 10\(^3\), 10\(^2\) copies ml\(^{-1}\)) produced by domestic standard samples that were prepared based on the international standard samples (NIBSC). Quantification was carried out using Sequence Detector version 1.7 (PE Applied Biosystems). The data show the mean of quadruplicate tests. The results have already been reported (Minegishi et al., 2003).

The genotypes of HBV are classified based on an intra-group nucleotide divergence of up to 4-2\% of the S-gene sequences or in some cases up to 8-0\% of complete genomes (Norder et al., 1992, 1993, 1994; Okamoto et al., 1988). Precore mutation (from G to A at nt 1896) or core promoter mutations (from A to T at nt 1762 and/or from G to A at nt 1764) were detected and characterized using the methods of Okamoto et al. (1990, 1994). Out of 349 HBV NAT-positive donors, 17 had precore mutants (genotype B, 7 donors; genotype C, 10 donors), 31 had core promoter mutants (genotype A, 2 donors; genotype C, 29 donors) and 13 had mutants with both precore and core promoter mutations (genotype B, 1 donor; genotype C, 12 donors). Sequencing was carried out directly by using a BigDye Terminator Cycle Sequencing kit and ABI Prism 3100 Genetic Analyser (PE Applied Biosystems). To analyse the sequences, Sequencher Mac version 4.1 (Hitachi Software Engineering) or GENETYX-MAC version 9.0 (Software Development) was used.

In Japan, genotypes C and B have been dominant among HBV-viraemic patients. However, recently genotype A has increased in prevalence (Kobayashi et al., 2002; Koibuchi et al., 2001; Orito et al., 2001). Out of 349 HBV NAT-positive donations, there were 40 cases of genotype A
Fig. 1. (a) Dendrogram of hepatitis B virus genotypes A–H, based on 15 complete nucleotide sequences. Fourteen strains (without 02094; indicated in bold) were retrieved from GenBank/EMBL/DDBJ database. The dendrogram was constructed by the UPGMA method (unweighted pair-group method with arithmetic mean) (Nei, 1987). Phylogenetic analysis of the complete genome (3215 nt) of the strain 02094 identified it as a new genotype H, which was separated at the branch of genotype F. Complete genomes of 3215 nt were sequenced, and the sequence was found to have 99-93% homology with the LSA2523 strain. Genetic distance is indicated below the dendrogram. (b) Dendrogram of hepatitis B virus genotypes A–H, based on the S region. Twenty-one strains (without 02094; indicated in bold) were retrieved from GenBank/EMBL/DDBJ database. The dendrogram was constructed by the UPGMA method (Nei, 1987). Genetic distance of genotype H is within 0.0039. Genetic distance is indicated below the dendrogram.
(11.5%), 39 cases of genotype B (11.2%), 264 cases of genotype C (75.6%), 5 cases of genotype D (1.4%) and 1 case of genotype H (0.3%). Genotypes E, F and G were not detected by NAT.

Genotype H was recognized for the first time in Japan by comparing the full sequence (3215 nt) of NAT-positive sample (02094) against LSA2523 (Arauz-Ruiz et al., 2002). To determine the full sequence of HBV DNA genotype H, three overlapping regions were amplified and subjected to sequence analysis: nt 1–668, nt 479–1796, nt 1698–2381, and nt 2332–3215. The nucleotide sequence identity between 02094 and LSA2523 was found to be 99.3%. The dendrogram of the strain 02094 was generated using reference strains of genotypes A–G and seven strains of genotype H made available in GenBank/EMBL/DDBJ through UPGMA methods (Nei, 1987) using GENETYX-MAC version 9.0 (Fig. 1a). Phylogenetic analysis performed on the S gene of genotypes A–H, including the 14 genotype H S-gene sequences available in GenBank/EMBL/DDBJ, is shown in Fig. 1(b).

Genotype H HBV was recovered from a blood sample donated on 9 October 2002 from a 52-year-old Japanese man. We could not obtain any additional information about the source of infection from the donor (02094). However, we found, from the interview sheet, that this subject had not been abroad during the past year, which suggested that he had contracted infection of genotype H HBV in Japan. None of the serological markers of HBV was detected in the sample of 02094. HBV DNA load was \(2 \times 10^6\) copies ml\(^{-1}\). The last of the stocked sample, which was collected 53 days before the NAT-positive donation (02094), proved to be PCR-negative. This may be due to the fact that the NAT-positive donation was in the serological window period at an early stage of infection. From the sequence of precore and core promoter regions, the strain 02094 proved to be a wild-type. The subtype of the strain 02094 was adw, because the codons 122 and 160 in the S region were both lysine (Okamoto et al., 1987a, b).

Between the 02094 strain obtained in the present study and the seven other reported strains of the same genotype (genotype H), there were differences ranging from 24 to 84 nt and from 10 to 47 aa in the entire genome. Of note, the 02094 isolate differed from the 1853NIC, 2928NIC, LAS2523, US2065 and US1122 by only 0–4 aa, but differed from the US10 and US1778 isolates by 13 or 20 aa in the core protein.

Amino acid sequences of the S region (226 aa, 678 nt; 15 strains) were compared between strain 02094 and known strains (Table 1, Fig. 2.). The characteristic amino acids of the S region in genotype F and H were Val\(^{18}\), Leu\(^{61}\), Glu\(^{178}\), Cys\(^{183}\), Leu\(^{193}\), Ile\(^{198}\), Cys\(^{206}\), Cys\(^{220}\) and Ser\(^{225}\). The remarkable amino acids in genotype H were Val\(^{44}\), Pro\(^{45}\), Gly\(^{47}\) and Ala\(^{224}\) (Fig. 2). Specific amino acid in strain 02094 was K\(^{30}\).

There was a conserved region, except for one mutation F85C among genotypes A–H, from codon 69 to 109 reported to be the hydrophobic region, which was the domain of ER-membrane penetration (Mangold & Streeck, 1993). Within this region we found a leucine zipper motif: L-X(6)-L-X(6)-L-X(6)-L (Landschulz et al., 1988) (Fig. 2). This motif may be acting to assemble HBsAg, though the motif has been reported as being nucleic acid-binding and as a eukaryotic transcriptional regulatory motif. On the other hand, the common determinant ‘a’ region from codon 124 to 147 is rather varied, and important in neutralizing antibodies.

**Table 1.** Different number of nucleotides and amino acids between the strain 02094 (not shown) and the seven other reported strains of the same genotype (genotype H)

Parentheses show the percentile identity between 02094 and the other strains.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Nucleic acid</th>
<th>Envelope protein</th>
<th>Amino acid</th>
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<td></td>
<td>Full length 3215 nt</td>
<td>pre-S1</td>
<td>pre-S2</td>
<td>S</td>
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<tr>
<td></td>
<td></td>
<td>119 aa</td>
<td>55 aa</td>
<td>226 aa</td>
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<tr>
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<td>0*</td>
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<tr>
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</table>

*Full-length sequence of US1122 consisted of 3206 nt. The pre-S2 region encodes 52 aa and polymerase gene codes for 840 aa.
Fig. 2. Deduced amino acid sequences of the S gene of HBV genotypes A–H. Fourteen strains (without 02094) were retrieved from GenBank/EMBL/DDBJ. The positions of leucine that formed the leucine zipper motif (Landschulz et al., 1988) are shaded.
Acknowledgements

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References


