

The complete nucleotide sequence of Hepatitis B virus, subtype adr (SRADR) and phylogenetic analysis

Motokazu Mukaide, Toshiaki Kumazawa, Akihiko Hoshi, Ryuji Kawaguchi and Kazumasa Hikiji
Genetic Research Laboratory, SRL, Inc., 51 Komiya, Hachioji, Tokyo 192, Japan

Received September 2, 1992; Revised and Accepted October 21, 1992

DDBJ accession no. D12980

We have cloned a genomic DNA of hepatitis B virus (HBV) and the complete DNA sequence was determined. The HBV (1) genomic DNA was purified from serum (2) in a patient with hepatitis who had HBe antigens (3) positive, and the adr subtype of HBV.

The constructed vector with the HBV genome (SRADR) and pUC18 was amplified in *Escherichia coli* strain DH5 alpha, purified and sequenced with dideoxy methods using a T7 Sequencing Kit (Pharmacia LKB Biotechnology, Sweden). The DNA sequence homologies were 99.0% with ADRA (4), 97.7% with ADRC (5), 97.5% with ADRCG (6). Alignment of the region P sequences from SRADR and other HBV defined in Figure 1. To determine the phylogenetic relationships among SRADR, and HPBVCG (Chimpanzee), and other HBV, we constructed evolutionary trees (7) for their pol sequences using both neighbor-joining methods (Figure 2) and UPGMA methods (Figure 3). In both methods, SRADR, ayr and seven previously reported isolates of subtype adr clustered in a closely related group.

ACKNOWLEDGEMENT

We thank Dr Kazuho Ikeo of National Institute of Genetics of Japan.

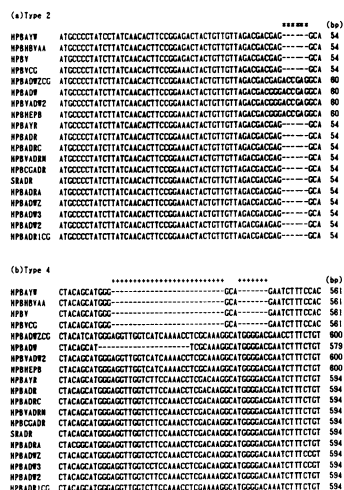


Figure 1. Alignment of the region P sequences from SRADR and other HBV. Identification of specific insertion of type 2 (1) and deletion of type 4 (b). Symbols (*), Positions showing type 2-specific insertion; (+), positions showing type 4-specific deletion.

REFERENCES

1. Blumberg, B.S. and Murray, R. (1965) *J. Am. Med. Assoc.* **191**, 541-546.
2. Dane, D.S., Cameron, C.H. and Briggs, M. (1970) *Lancet* **i**, 695-698.
3. Kaplan, P.N., Greenman, R.L., Gerin, J.L., Purcell, R.H. and Robinson, W.S. (1973) *J. Virol.* **12**, 995-1005.
4. Kobayashi, M. and Koike, K. (1984) *Gene* **30**, 227-232.
5. Takemura, F., Ishii, T., Fujii, N. and Uchida, T. (1991) *Nucleic Acids Res.* **18**, 4587-4587.
6. Fujiyama, A., Miyano, A., Nozaki, C., Yoneyama, T., Ohtomo, N. and Matsubara, K. (1983) *Nucleic Acids Res.* **11**, 4601-4610.
7. Saitou, N. (1990) *Methods Enzymol.* **183**, 584-598.

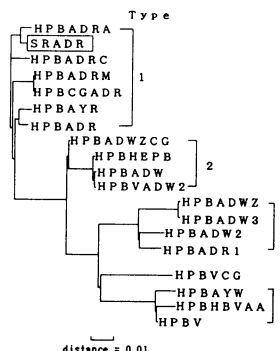


Figure 2. Phylogenetic analysis of the region P sequences from various HBV (GenBank), including the newly isolated SRADR (boxed). The tree was constructed by the neighbor-joining method.

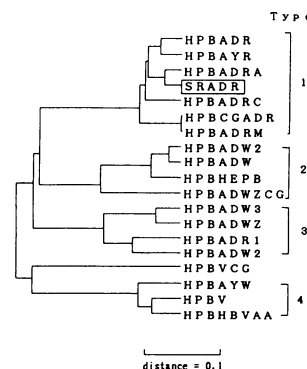


Figure 3. Phylogenetic analysis of the region P sequences from various HBV (GenBank), including the newly isolated SRADR (boxed). The tree was constructed by the UPGMA method.