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Full-Genome Sequence of a Hepatitis B Virus Genotype F1b Clone from a Chronically Infected Chilean Patient

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The hepatitis B virus (HBV) is a DNA virus belonging to the *Hepadnaviridae* family. Viral isolates have been classified into 10 genotypes, named from A to J, and several subtypes. We report the full-genome sequence from a single molecular clone of HBV genotype F1b, amplified from a chronically infected Chilean patient.

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Hepatitis B virus (HBV) chronic infections are a major risk for patients with cirrhosis and hepatocellular carcinoma. The HBV genome is a partially double-stranded 3.2-kb DNA that replicates through reverse transcription of an RNA intermediate, globally introducing variability to the viral spread. HBV isolates are classified into 10 genotypes (named A–J) and several subtypes, with a differential geographic distribution (1, 2). In general, genotypes B and C are prevalent within Asia, whereas genotypes A and D circulate in Europe and the United States (1). Genotype G displays a global distribution, and genotype H circulates in Central America (3, 4). Genotypes I and J have been reported in regions in Asia (5, 6). Finally, HBV genotypes E and F are prevalent in Africa and Central and South America (7). Importantly, the genetic diversity, pathology progression, and antiviral therapy response (8, 9) of the European, North American, and Asian genotypes (A–D) (10, 11) have received the greatest attention and more pharmaceutical efforts than those prevalent in other regions (E-H) (12, 13). Similarly, virological and clinical properties of HBV genotypes have been only systematically studied for genotypes A–D. In broad terms, genotype B is more related to mild liver diseases than genotype C, and genotype D has a less positive prognosis than genotype A (14). Other genotypes, including genotype F, have not been thoroughly studied at the clinical or molecular levels.

In previous studies, the HBV genotype F was found to be the most prevalent in Chile (84%) (15), and phylogenetic analyses of complete genome sequences from crude amplified viral DNA were found to correspond to subgenotype F1b (16).

Viral DNA extracted from serum (sample HCUCH4) was amplified as previously described (16, 17), and then ligated with a TOPO XL PCR vector and transformed into TOP10 *E. coli* (Life Technologies). Screening was based on white/blue selection with X-gal. Plasmid DNA was subjected to restriction mapping and fragment size estimation. One clone was completely sequenced from both strands of the viral DNA (Macrogen Inc.), using primers spaced by approximately 500 nucleotides. Consensus sequences were obtained by alignment of both sequenced strands using MegAlign software from the DNAStar package (LaserGene, Inc.). Assembled viral sequences gave 3,215 nucleotides, with canonical HBV overlapping open reading frames for PreC (HBe, 639 nt), C (HBc, 552 nt), X (HBx, 465 nt), PreS1 (LHBs, 1203 nt), PreS2 (MHBs, 846 nt), S (SHBs, 681 nt), and P (Pol, 2532 nt). This isolate was named clone HBV 4.5. Its DNA sequence was compared to that of sample HCUCH4 (GenBank accession number HM585186), resulting in 99% genetic identity, with the changes in clone HBV 4.5 with respect to HCUCH4: A149G, A1149G, and G1698A. Since digestion of the vector release a circularizing HBV genome with no additional sequences, its availability will be important for the initiation of molecular studies on one of the lesser-studied HBV genotypes, such as genotype F, prevalent not only in Chile but also in Central and South America.

Nucleotide sequence accession number. The sequence is available in GenBank under accession number KM233681.

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REFERENCES

1. **Okamoto H, Tsuda F, Sakugawa H, Sastrosoewignjo RI, Imai M, Miyakawa Y, Mayumi M.** 1988. Typing hepatitis B virus by homology in nucleotide sequence: comparison of surface antigen subtypes. J. Gen. Virol. **69:**2575–2583. http://dx.doi.org/10.1099/0022-1317-69-10-2575.
2. **Norder H, Couroucé AM, Coursaget P, Echevarria JM, Lee SD, Mushahwar IK, Robertson BH, Locarnini S, Magnius LO.** 2004. Genetic diversity of hepatitis B virus strains derived worldwide: genotypes, subgenotypes, and HBsAg subtypes. Intervirology **47:**289–309. http://dx.doi.org/10.1159/000080872.
3. **Stuyver L, De Gendt S, Van Geyt C, Zoulim F, Fried M, Schinazi RF, Rossau R.** 2000. A new genotype of hepatitis B virus: complete genome and phylogenetic relatedness. J. Gen. Virol. **81:**67–74.
4. **Arauz-Ruiz P, Norder H, Robertson BH, Magnius LO.** 2002. Genotype H: a new Amerindian genotype of hepatitis B virus revealed in Central America. J. Gen. Virol. **83:**2059–2073.
5. **Yu H, Yuan Q, Ge SX, Wang HY, Zhang YL, Chen QR, Zhang J, Chen PJ, Xia NS.** 2010. Molecular and phylogenetic analyses suggest an additional hepatitis B virus genotype "I." PLoS One **5:**e9297. http://dx.doi.org/10.1371/journal.pone.0009297.

6. **Tatematsu K, Tanaka Y, Kurbanov F, Sugauchi F, Mano S, Maeshiro T, Nakayoshi T, Wakuta M, Miyakawa Y, Mizokami M.** 2009. A genetic variant of hepatitis B virus divergent from known human and ape genotypes isolated from a Japanese patient and provisionally assigned to new genotype J. J. Virol. **83:**10538–10547. http://dx.doi.org/10.1128/JVI.00462-09.
7. **Norder H, Couroucé AM, Magnius LO.** 1994. Complete genomes, phylogenetic relatedness, and structural proteins of six strains of the hepatitis B virus, four of which represent two new genotypes. Virology **198:**489–503. http://dx.doi.org/10.1006/viro.1994.1060.
8. **Pujol FH, Navas MC, Hainaut P, Chemin I.** 2009. Worldwide genetic diversity of HBV genotypes and risk of hepatocellular carcinoma. Cancer Lett. **286:**80–88. http://dx.doi.org/10.1016/j.canlet.2009.07.013.
9. **Tseng TC, Kao JH.** 2008. HBV genotype and clinical outcome of chronic hepatitis B: facts and puzzles. Gastroenterology **134:**1272–1273. http://dx.doi.org/10.1053/j.gastro.2007.12.046.
10. **Erhardt A, Blondin D, Hauck K, Sagir A, Kohnle T, Heintges T, Häussinger D.** 2005. Response to interferon alfa is hepatitis B virus genotype dependent: genotype A is more sensitive to interferon than genotype D. Gut **54:**1009–1013. http://dx.doi.org/10.1136/gut.2004.060327.
11. **Liu WC, Phiet PH, Chiang TY, Sun KT, Hung KH, Young KC, Wu IC, Cheng PN, Chang TT.** 2007. Five subgenotypes of hepatitis B virus genotype B with distinct geographic and virological characteristics. Virus Res. **129:**212–223. http://dx.doi.org/10.1016/j.virusres.2007.07.016.
12. **Erhardt A, Göbel T, Ludwig A, Lau GK, Marcellin P, van Bömmel F, Heinzel-Pleines U, Adams O, Häussinger D.** 2009. Response to antiviral treatment in patients infected with hepatitis B virus genotypes E–H. J. Med. Virol. **81:**1716–1720. http://dx.doi.org/10.1002/jmv.21588.
13. **Kato H, Gish RG, Bzowej N, Newsom M, Sugauchi F, Tanaka Y, Kato T, Orito E, Usuda S, Ueda R, Miyakawa Y, Mizokami M.** 2004. Eight genotypes (A–H) of hepatitis B virus infecting patients from San Francisco and their demographic, clinical, and virological characteristics. J. Med. Virol. **73:**516–521. http://dx.doi.org/10.1002/jmv.20120.
14. **Lin CL, Kao JH.** 2011. The clinical implications of hepatitis B virus genotype: recent advances. J. Gastroenterol. Hepatol. **26:**123–130. http://dx.doi.org/10.1111/j.1440-1746.2010.06541.x.
15. **Venegas M, Muñoz G, Hurtado C, Alvarez L, Velasco M, Villanueva RA, Brahm J.** 2008. Prevalence of hepatitis B virus genotypes in chronic carriers in Santiago, Chile. Arch. Virol. **153:**2129–2132. http://dx.doi.org/10.1007/s00705-008-0231-6.
16. **Venegas M, Alvarado-Mora MV, Villanueva RA, Rebello Pinho JR, Carrilho FJ, Locarnini S, Yuen L, Brahm J.** 2011. Phylogenetic analysis of hepatitis B virus genotype F complete genome sequences from Chilean patients with chronic infection. J. Med. Virol. **83:**1530–1536. http://dx.doi.org/10.1002/jmv.22129.
17. **Günther S, Li B-C, Miska S, Krüger DH, Meisel H, Will H.** 1995. A novel method for efficient amplification of whole hepatitis B virus genomes permits rapid functional analysis and reveals deletion mutants in Immunosuppressed patients. J. Virol. **69:**5437–5444.