Genomic characterization of HBV genotype F in Bolivia: genotype F subgenotypes correlate with geographic distribution and T^{1858} variant

Brief Report

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Summary. Hepatitis B virus (HBV) strains were classified into eight genotypes from A to H. Genotype F, an indigenous genotype in Central and South America, has been classified into subgenotypes. An in-depth phylogenetic analysis was performed using two full-length Bolivian HBV sequences and other genotype F strains from the database. A novel nomenclature of subgenotypes of genotype F was proposed, in which Bolivia strains belonged to subgenotype F4. This subgenotype had both Leu^{45} and Ile^{110} in the S gene, and linked to the T^{1858} in the precore. This novel nomenclature demonstrated the relation between variability of the HBV genome and the restricted geographical distribution of the virus in some parts of Central and South America.

* Hepatitis B virus (HBV) is an important etiologic agent of acute and chronic hepatitis, with over 350 million chronic infected patients around the world [14]. HBV strains are classified into eight genotypes, from A to H [4, 24, 26, 29]. Genotypes A and D are distributed widely in Europe, genotypes B and C are found in East Asia, and genotype E is confined to the sub-Sahara Africa [24]. Genotype G has been sporadically found in North America, Europe and Japan [10, 28–30]. Genotypes F and H, which are genetically divergent from the other

Nucleotide sequence data reported are available in the DDBJ/EMBL/GenBank databases under the accession numbers: AB166850 (HBV-BL592), AB214516 (HBV-BL597).
HBV genotypes, have been detected indigenously in Central and South America [2, 4, 18, 21, 22]. Genotype F strains have been previously classified as four clusters (I–IV) [6, 19], or two clades (clade 1–2) [25], and recently two subgenotypes F1–F2 [12].

Bolivia, a country in the Southern South America, has a low to intermediate prevalence of HBV infection, ranging from 0.4% to 8% [7, 11]. However, there is no report on HBV sequences, as well as the characteristic of HBV genotype F in this area. In this study, we analyzed the pre-S gene and full genome sequences of HBV genotype F from the sera of six HBV infected patients from the Japanese Hospital, Santa Cruz, Bolivia. These sera were obtained from our previous study on the epidemiology of hepatitis virus infections in Bolivia [11]. Six patients were all positive for HBsAg.

HBV DNA was extracted from 100 µl of sera by using the SepaGene RV-R kit (Sanko Junyaku Co., Ltd., Japan). Polymerase chain reaction (PCR) with type-specific primers was used to confirm genotype F of these HBV isolates [20]. The pre-S gene (522 nucleotides, including pre-S1 and pre-S2) and the full-length 3.2 kb of the HBV genome were amplified by using primers and PCR conditions as described [9]. Due to insufficiency of the sera aliquots, only 2 HBV full-length genomes and 4 other pre-S fragments were amplified. PCR products, after purification with the QIAquick gel extraction kit (Qiagen Inc., USA), were subjected to direct sequencing using the ABI PRISM™ Big Dye Terminator Cycle Sequencing Ready Reaction Kit and automated DNA sequencer ABI 310 (Applied Biosystem, Foster City, USA). Nucleotide sequences and deduced amino acids were multi-aligned [5] with Genetyx for Windows version 6.0 software (Genetyx, Japan). Evolutionary and phylogenetic tree analysis were performed by the MEGA software version 2.1 [13], using the neighbor-joining method, Kimura 2-parameter algorithm with bootstrap 1000 data sets. Genetic distance calculation with standard deviations using bootstrap 1000 data sets were performed by Kimura 2-parameter model (reviewed in Nei and Kumar, 2000 [23]).

Phylogenetic analysis of the pre-S gene of HBV was performed with a total of 51 strains, including 6 Bolivian strains, 25 genotype F and 3 genotype H; together with 17 strains of other reference genotypes from GenBank (Table 1). The woolly monkey HBV strain (Accession no. NC_001896) was used as an outgroup. The division of genotype F into clusters demonstrated previously [6, 19] were supported by this pre-S gene tree (data not shown). Clusters I and II possessed strains reported by Mbayed et al. (2001) [19]. The original cluster III of Mbayed et al. [19] was later renamed genotype H by Arauz-Ruiz et al. [4] due to its high divergence. More recently, the designation of cluster III was adopted for another subclade of F strains from Venezuela [27]. Also, Devesa et al. [6] adopted this new cluster III which comprised all Venezuela strains in this tree. In the present study, all the pre-S genes of Bolivian HBV strains were located in a cluster comprised of reported strains from Mbayed cluster IV [19].

Full-length genome phylogenetic analysis on these two Bolivian HBV strains, together with 25 genotype F strains from the database (Fig. 1A) confirmed the existence of the previously observed four clusters. Recently, Kramvis et al. [12]