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Increased Prevalence of Genotype F Hepatitis B Virus Isolates in Buenos Aires, Argentina

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The genomic coding region of the hepatitis B surface antigen (HBsAg) was partially sequenced from 12 HBsAg-positive sera of carriers residing in Buenos Aires, Argentina. A phylogenetic analysis groups the 12 isolates into genotypes A ($n = 4$), B ($n = 1$), D ($n = 2$), and F ($n = 5$). The occurrence of genotypes A and D is not unexpected, considering the mainly European origin of the studied population. The high prevalence of genotype F and its genetic composition support the suggestion that F strains originated in native populations of the New World.

Hepatitis B virus (HBV) is an etiological agent of acute and chronic liver disease and is distributed throughout the world. The number of chronic carriers exceeds 350 million worldwide.

The serological heterogeneity of hepatitis B surface antigen (HBsAg) has long been established, and the HBV isolates have been classified into nine different subtypes, ayw1, ayw2, ayw3, ayw4, ayr, adw2, adw4, adrq−, and adrq, according to the antigenic determinants and subdeterminants of their HBsAg (3, 4).

The entire nucleotide sequences of HBV genomes of various subtypes have been classified into six genetic groups, named A to F, based on an intergroup divergence of 8% or greater of the complete nucleotide sequence. The relationship of the nine subtypes to genomic groups A to F has been established (15, 16).

The HBV strains within each genomic group show a characteristic geographic distribution, and it has been proposed that HBV diverged into genomic groups according to the distribution of humans among the different continents. However, these studies are still incomplete because the number of isolates analyzed in some parts of the world, including South America, is small (14).

Genetic diversity of HBV and amino acid substitutions may give rise to variations of viral epitopes that are relevant for virus neutralization. Substitution in the primary structure of HBV structural proteins may represent immune-selected mutations on the one hand but could also be unselected naturally occurring variants (9). Therefore, it is important to sequence divergent HBV genomes to describe the extent of natural variation in the encoded genome products (15).

In the present study, HBV isolates from Buenos Aires, Argentina, were characterized by partial sequencing of the viral S gene. Serum samples were provided by the National Reference Center of Viral Hepatitis and Gastroenteritis, INM "C. Malbrán," Buenos Aires, Argentina, and selected from chronically infected adults. All of them were HBsAg positive, and their percutaneous liver biopsies presented anatomical signs of chronic liver hepatitis. Twelve serum samples were used as the source of HBV DNA for sequencing. DNA, extracted from 200 μl of serum by the phenol-chloroform method, was amplified by PCR and sequenced by the dideoxynucleotide chain termination method (17) using the Femtomol sequencing kit (Promega Corp.). The primers used for amplification and sequencing were HBS1 (5′ CAA GGT ATG TTG CCC GTT TG 3′, positions 455 to 474) and HBS2 (5′ AAA GCC CTG CGA ACC ACT GA 3′, positions 713 to 694) (20).

The sequences of nucleotides corresponding to part of the S gene of HBV from 12 HBsAg-positive sera are shown in Fig. 1.

A phylogenetic analysis was carried out using 54 sequences, 42 from GenBank and 12 from the present study. The alignment of the multiple sequences was undertaken by CLUSTALV (10). For DNA phylogeny from distance matrix, the PHYLIP programs DNADIST and KITSCH were consecutively used. The approach made use of output files from the general bootstrap program SEQBOOT (6). The multiple-tree file obtained from KITSCH was used to obtain a majority rule consensus tree (CONSENSE program), and the tree was plotted with DRAWTREE (6).

This phylogenetic analysis allowed the grouping of the amplified viral DNA as genotypes A (samples 5, 8, 12, and 13), B (sample 6), D (samples 1 and 3), and F (samples 2, 4, 7, 14, and 29) (Fig. 2).

The amino acid sequences (residues 110 to 180 of the S antigen) inferred from the nucleotide sequence classify the isolates as subtypes adw2 ($n = 5$), adw4 ($n = 5$), ayw2 ($n = 1$), and ayw3 ($n = 1$) (Fig. 3).

The prevalence of genotypes A and D in Buenos Aires, Argentina, is not unexpected, considering the mainly European descent of its population, while genotype B corresponds to an Asian immigrant carrier.

The prevalence of genotype F is especially noteworthy and constitutes evidence of molecular epidemiological value. Its high occurrence (5 of 12 samples) supports the theory that genotype F is indigenous to the native population of the New World (14). This theory is based on the world distribution of the adw4 subtype (genotype F). It has been previously shown that the adw4 subtype dominates in Córdoba, Argentina (5), and in the northern areas of Brazil (8). This subtype is unique in Venezuela Cuiva Indians and is only rarely found in Europe (5).

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                         500                                                 550
CLONE/isolate       .         .         .         .         .         .         .         .         .         .
pHBV 3200     ATTCCAGGATCAACAACAACCAGTACGGGACCATGCAAAACCTGCACGACTCCTGCTCAAGGCAACTCTATGTTTCCCTCATGTTGCTGTACAAAACCTACGGAT
8             .........................................................................................................
12            .........................................................................................................
13            .........................................................................................................
5             .......................C.................................................................................
6             ............T.G..C.....C..C...................................A.C.......................................C
1             ............T....C.....C............CG........T....A..........A.C........A......C...........C......TT...C
3             ............T....C.....C............CG........................A.C........A......C...........C......T....C
2             ..............G..C.....C.......................A....T.........A.C..................C......T........T....C
4             C..........C..G..C.....C.......................A....T.........A.C...............T..C......T.C.....CT....C
7             C..........T..G..C.....C.......................A....T.........A.C...............C.........T.C.....CT....C
14            C..........T..G..C.....C.......................A....T.........A.C...............C.........T.C.....CT....C
29            ...........T..G..C.....C.......................A....T.........A.C...............C.........T.C.....CT....C

                    600                                                  650
CLONE/isolate .         .         .         .         .         .         .         .         .         .         .
pHBV 3200     GGAAATTGCACCTGTATTCCCATCCCATCGTCCTGGGCTTTCGCAAAATACCTATGGGAGTGGGCCTCAGTCCGTTTCTCTTGGCTCAGTTTACTAGTGCCATTT
8             .........................................................................................................
12            .........................................................................................................
13            .........................................................................................................
5             .........................................................................................................
6             .....C.......................A..T........................................................................
1             .............................A.............G.....T....................C..................................
3             .............................A.............G.....T....................C.........C........................
2             .....C.....T.................A............AG..........................C.........C...................A....
4             .....C.....T.................A............AG..........................C.........C...................A....
7             .....C.......................A..T.........AG..........................C.........C...................A....
14            .....C.......................A..T.........AG..........................C.........C...................A....
29            .....C.......................A..T.........AG..........................C.........C...................A....
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FIG. 1. Nucleotide sequences of the 12 HBV isolates (HBsAg carriers 1 to 8, 12 to 14, and 29) aligned with that of clone pHBV-3200 (19). The nucleotide sequence corresponds to nucleotides 482 to 691 from the *Eco*RI site.

The F genotype was found to be the most divergent HBV genome characterized so far (15). It is also interesting that the five isolates which are grouped as genotype F have a heterogeneous genetic composition. When pairs of the sequences from these five isolates were compared, up to seven synonymous nucleotide differences from a total of 210 sequenced nucleotides were observed (3.3%). In particular, isolate 2 appears as a different subgroup compared to isolates 7, 14, and

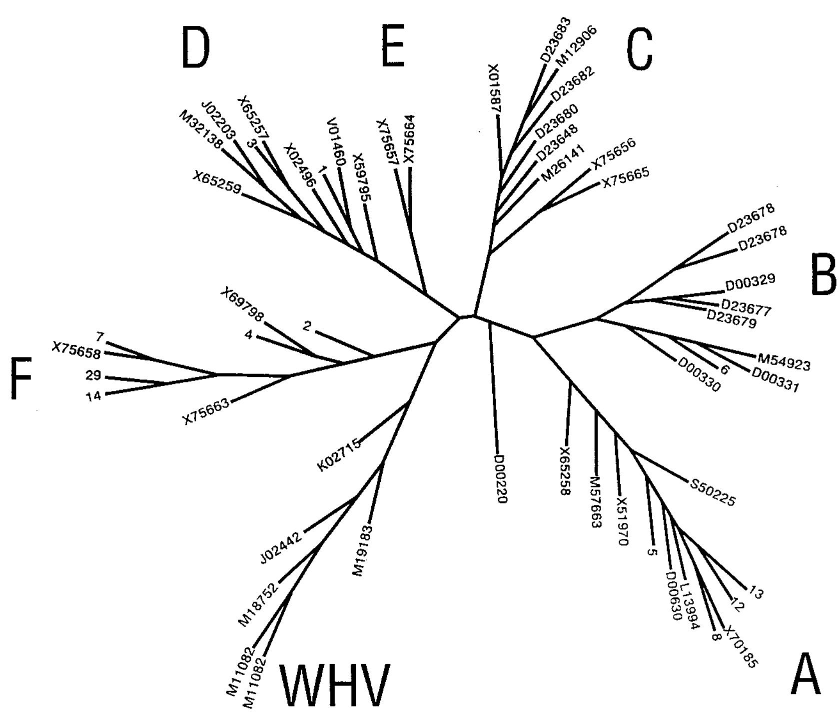

FIG. 2. Unrooted tree showing relationships between 54 partial S gene sequences: 12 from the present study and 42 from GenBank. The woodchuck strains (WHV) were chosen arbitrarily and alternatively as the outgroup, and 100 bootstrap operations were done. A to F indicate subgroup designations (15). The branch lengths are not to scale.

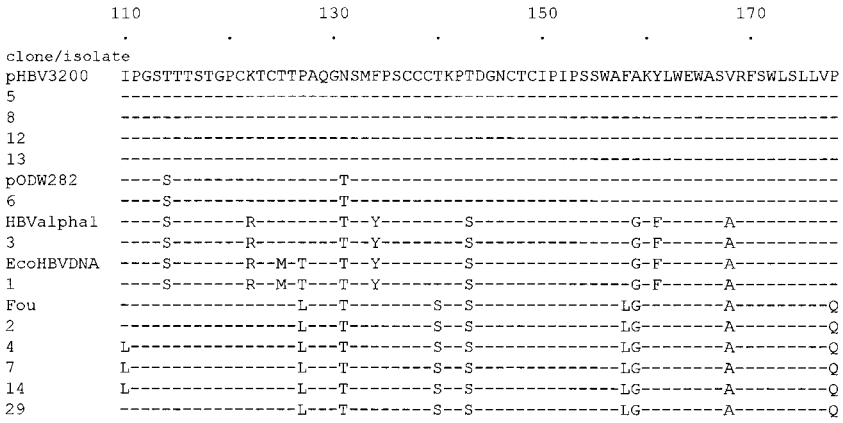

FIG. 3. Amino acid sequences of HBsAg from the 12 HBV isolates compared to those of HBV strains representative of subtype adw2 genotypes A (pHBV-3200) (19) and B (pODW282) (16), subtype ayw2 (18), subtype ayw3 (EcoHBVDNA) (7), and subtype adw4q− (Fou) (15).

29. It should be highlighted that the difference between isolate 2 and isolates 7, 14, and 29 is 3.9%, similar to that observed between genotypes A and B (5.5%) for the region sequenced.

The increased prevalence of genotype F may also have implications in HBV prophylaxis. Changes within the second immunodominant loop of the encoded surface antigen (*a* determinant, residues 139 to 147) may be selected as a consequence of immune pressure. There are reports of an amino acid substitution at residue 145, from glycine to arginine, in several individuals who developed HBV infections despite receiving anti-HBsAg immunoglobulins and vaccine (1, 2, 8) or monoclonal antibodies (13). The E and F genotypes shared a unique substitution at residue 140, from threonine to serine, compared to the vaccine strain, and E strain variants were detected in The Gambia in immunized children with protective anti-hepatitis B surface antibody levels (11). These E strain variants that present a substitution at residue 141 have been reported to occur in conjunction with the residue 140 substitution, and so similar modifications might be selected from F strains (12).

The increased occurrence and the genetic characterization of genotype F in Buenos Aires extend the worldwide molecular epidemiology information on HBV to this geographic area.

Nucleotide sequence accession numbers. The HBsAg coding sequences determined in this study have been submitted to GenBank under accession no. U50169, U50170, U50171, U50172, U50173, U50174, U50175, U50176, U50177, U50178, U50179, and U50180.

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