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Molecular epidemiology of hepatitis B virus in Afro-Venezuelan populations

Brief Report

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Summary. Hepatitis B virus (HBV) infection among Venezuelan populations of African origin was analyzed. These populations exhibited lower HBV prevalence than the one found in the African continent. Sequence analysis of 6 isolates showed that 3 belonged to genotype F, while the 3 others were HBV genotype A. HBV genotype A was more common in the Afro-Venezuelan groups than in the general Venezuelan population. This might reflect the introduction of genotype A during the slavery period. The absence of the African genotype E among these isolates supports the hypothesis of a recent origin for this HBV genotype. HBV genotype F has already been introduced to these relatively isolated communities.

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In spite of a highly effective vaccine, hepatitis B is still a significant health concern in the world. It is estimated that around 2 billion persons have been infected by hepatitis B virus (HBV) and around 350 millions of them are chronic carriers [28]. The highest HBV prevalence is found in the Western South of Asia and in the Southern and Equatorial Africa. South America exhibits an intermediate prevalence, with HBV endemic clusters among Amerindian populations [27].

Seven genotypes (A–G) of hepatitis B virus (HBV) have been described, HBV genotype F being autochthonous to South America and highly predominant, particularly in Venezuela [3]. Genotypes A and D are predominant in the Old

World but are also widely distributed in all the continents. Genotypes B and C are found mainly in South East Asia and the Far East, while genotype E circulates in sub-Saharan Africa [12]. HBV genotype G has recently been described and its distribution is not yet fully known [23].

Amerindian populations constitute the first human settlers in the Americas and are believed to originate from the South of Siberia and Mongolia, crossing through the Behring Strait, 10,000–30,000 years ago [6, 10, 15]. With the discovery of the New World and during the Colonial period, European and African population groups were introduced in South America. Genetic studies and historical evidence suggest that an important proportion of the slaves who arrived to Venezuela had already a certain degree of admixture in some Islands of the Caribbean [19]. Some Afro-Venezuelan groups have been maintained as small communities in the Venezuelan Coasts or in the mountains, without significant additional admixture since their establishment [4]. Ethnic migrations may have contributed to the circulation of other HBV genotypes in South America. The aim of this study was the molecular characterization of HBV isolates circulating among Venezuelan populations of African origin.

A total of 222 sera from Afro-Venezuelan populations groups were analyzed: Chuao, Aragua State ($n = 90$), Panaquire, Miranda State ($n = 76$), and Macuquita, Falcón State ($n = 56$). A total of 1332 sera from other Venezuelan populations were analyzed in order to compare the prevalence of HBV active infection with the one observed among Afro-Venezuelan groups: rural populations (Caño Delgadito, Portuguesa State, $n = 176$, Caraballeda, Vargas State, $n = 72$, and La Curía, Carabobo State, $n = 66$) and urban populations (pregnant women from Caracas, Federal District, $n = 516$, and Puerto LaCruz, Anzoátegui State, $n = 500$). For Panaquire population, the degree of admixture was previously described [4]. For Macuquita, the systems evaluated were ABO, Duffy and RH (Cc, D, Ee) blood groups, to obtain a quantitative estimation of racial admixture using the gene identity method [5]. HBV surface antigen (HBsAg) was determined by a double sandwich monoclonal immunoassay [20]. Positivity in reactive samples was confirmed by a commercial immunoassay (AUSZYME, Abbott Diagnostics, North Chicago, Ill, US) and/or by the presence of HBV DNA. HBV DNA was determined by nested PCR in the HBsAg region, and PCR amplified products were sequenced, as previously described [21]. Nucleotide and amino acid alignments were performed using DNAMAN 5.2.2. (Lynnon Bio Soft, Canada). Phylogenetic analyses were conducted using MEGA version 2.1 [10]. Nucleotide sequence data have been deposited into the GenBank database under the accession numbers AF479490-AF479495.

From the 222 sera from Afro-Venezuelan populations, 8 (3.6%) were found positive for HBV active infection (Table 1). The HBsAg prevalence of the 3 Afro-Venezuelan communities and of the rural populations were not significantly different (8/222; 3.6% vs. 7/316; 2.2%, $p > 0.05$). In contrast, HBsAg prevalence of all these rural population groups were significantly higher than the one observed in urban groups (6/1016; 0.6% $p < 0.01$). No differences were observed in HBsAg positivity according to sex (data not shown).

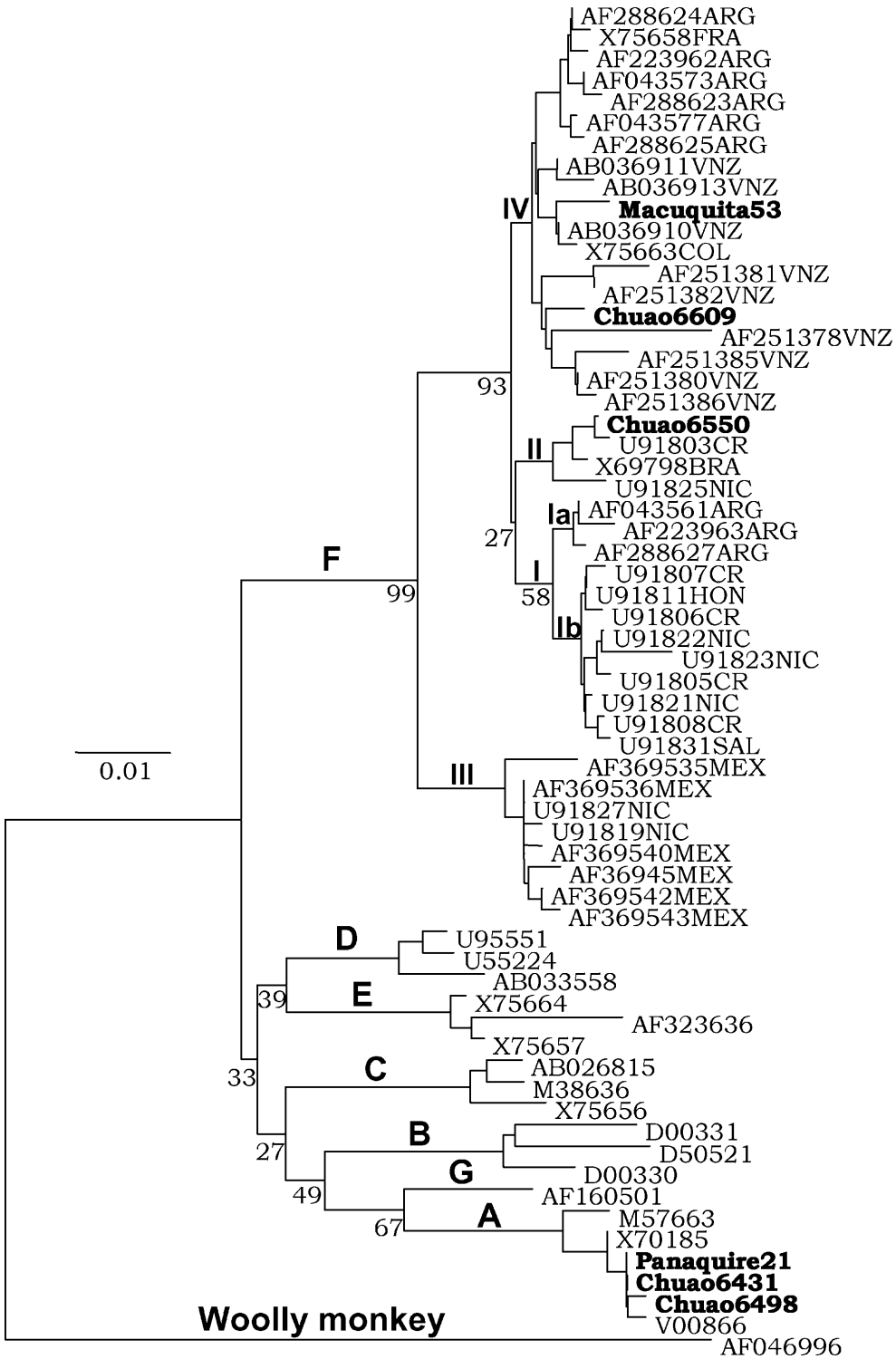
Table 1. HBV active infection in different Venezuelan populations

Population (n)	Genetic admixture	Number of HBsAg positive samples (%)
Afro-Venezuelan:		
Panaquire (76)	59% African, 15% European, 26% Amerindian[a]	3 (3.9)
Chuao (99)	n.d.[c]	4 (4.0)
Macuquita (56)	57% African, 43% European, 0% Amerindian[b]	1 (1.8)
Total Afro-Venezuelan (222)		**8 (3.6)**
Rural:	n.d.[c]	
Caño Delgadito (178)		2 (1.1)
Caraballeda (72)		3 (4.2)
La Curia (66)		2 (3.0)
Total rural (316)		**7 (2.2)**
Urban:	n.d.[c]	
Puerto La Cruz (500)		2 (0.4)
Caracas (516)		4 (0.8)
Total urban (1016)		**6 (0.6)**

[a]Determined in a previous study [4]
[b]Determined in this study ($n = 22$ unrelated individuals)
[c]Not determined

From these 8 HBV isolates, 6 genomic fragments were amplified by PCR in the HBsAg region. Sequence analysis of these 6 isolates showed that 3 belonged to genotype F, and 3 to genotype A (Fig. 1). These Afro-Venezuelan genotype A isolates displayed more than 99.8% identity, while the genotype F isolates were more diverse (up to 1.6% divergence). The analysis of amino acids in the a determinant showed that the 3 genotype A isolates were subtype adw2, 2 genotype F were subtype adw4, as expected, while one isolate F exhibited a less common subtype ayw4 (Fig. 2), which has only been described in few HBV genotype F isolates [1, 3].

In spite of the significant migration of African ethnical groups in the Americas during the slavery trade period, no African genotype E has been found to date in the Americas. However, no study has been conducted in African American groups that have preserved an important proportion of their cultural and genetic origin. This point was addressed in this study, and the HBV isolates circulating among African communities that have been maintained relatively isolated from other ethnic groups, were analyzed. The absence of genotype E in these communities is in agreement with recent phylogenetic studies that suggest a contemporary origin of this genotype in Africa, possibly through a zoonotic introduction from a chimpanzee's hepadnavirus [16, 25]. It is interesting to note that the prevalence of HBV active infection observed among these Afro-Venezuelan communities were not as high as the one found in Africa. This might suggest that the prevalence of HBV infection was lower in Africa during the 19th century, but does not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AF288623ARG
AF043577ARG
AF288625ARG
AB036911VNZ
AB036913VNZ
IV
Macuquita53
AB036910VNZ
X75663COL
AF251381VNZ
AF251382VNZ
Chuao6609
AF251378VNZ
AF251385VNZ
93
AF251380VNZ
AF251386VNZ
Chuao6550
II
U91803CR
X69798BRA
U91825NIC
Ia
AF043561ARG
27
AF223963ARG
F
AF288627ARG
I
U91807CR
99
58
U91811HON
U91806CR
Ib
U91822NIC
U91823NIC
U91805CR
U91821NIC
U91808CR
U91831SAL
0.01
III
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AF369536MEX
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U91819NIC
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AF369542MEX
AF369543MEX
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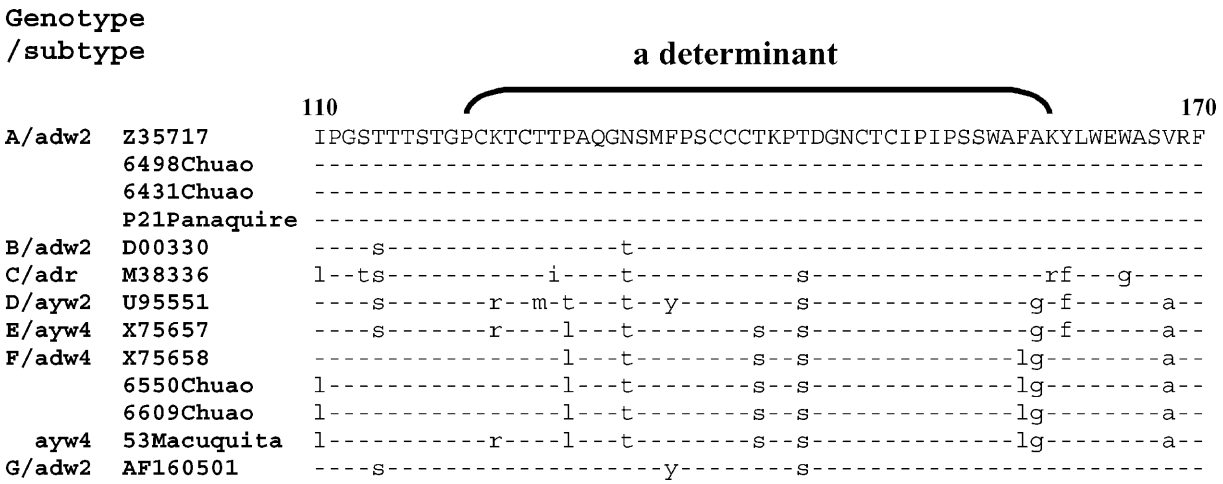

Fig. 2. Amino acid alignments of the sequences of the HBV Afro-Venezuelan isolates (Chuao, Macuquita and Panaquire), showing the a determinant of the HBsAg. Numbers indicate the amino-acid position in the protein. One isolate representative of each genotype is shown: genotype A (Z35717), B (D00330), C (M38336), D (U95551), E (X75657), F (X75658), and G (AF160501). The subtype is shown after the genotype identification

necessarily mean that this continent was free of HBV, as has been hypothesized [16]. Even if the number studied is small, a relatively high frequency of HBV genotype A was found in these African communities, compared to the predominance of HBV genotype F observed in other populations from Venezuela [3]. This result suggests that this genotype was circulating in Africa in the 19th century, and then the slave trade might be one of the routes of introduction of this genotype to the Americas. Further studies are needed in larger Afro-American populations to confirm this hypothesis. Several reports are in agreement with this hypothesis: genotype A is the most frequent genotype in other African populations studied [11, 18, 24]. In addition, the low degree of diversity observed among genotype A isolates in Afro-Venezuelan populations (Fig. 1) supports the hypothesis of a common introduction of these HBV isolates.

Fig. 1. Phylogenetic tree of HBV (surface antigen, partial 556 nt region, nt 205 to 760) isolates from Venezuela, obtained using the Minimum Evolution method with close-neighbor-interchange (100 bootstrap replicas). Genetic distances were evaluated with Kimura 2 parameters corrections [9]. Isolates are designated by their GenBank accession number, except for Afro-Venezuelan isolates (Chuao, Macuquita and Panaquire), which are shown in bold. Bootstrap values for the branching in genotypes and in the clusters of genotype F are shown in the tree. The scale represents the number of substitutions/site/100 bases. Letters indicate HBV genotypes, and roman numbers the clusters within genotype F. All HBV genotype F sequences available in GenBank were included in this tree, except for identical sequences – only one included – and 3 sequences with deletions (in total 45/76 sequences). The country of origin is shown for genotype F sequences after their accession number; *ARG*: Argentine, *BRA*: Brazil, *COL*: Colombia, *CR*: Costa Rica, *FRA*: hemodialysis patient from France, *HON*: Honduras, *MEX*: Mexico, *NIC*: Nicaragua, *SAL*: Salvador, *VNZ*: Venezuela

Even though these African communities have been relatively isolated, the Amerindian HBV genotype F has already been introduced in these ethnical groups. It is interesting to note that this genotype is widely distributed in several countries from Central and South America [2, 3, 13, 26] even in non Amerindian groups. This might be due to a high rate of transmission of this genotype. An alternative explanation for the circulation of the American HBV genotype among Afro-Venezuelan communities is that this introduction might have occurred during the admixture of the slaves with Amerindian groups at their arrival to Venezuela, or even before, in the Caribbean Islands. This last hypothesis might be probable for Panaquire population, which exhibits a significant degree of admixture with Amerindians ethnic groups, but seems less probable for the Macuquita community, where the limited genetic information did not suggested admixture with Amerindians (Table 1).

Four clusters within HBV genotype F have recently been described [13]. Phylogenetic analysis of the S region of all the HBV genotype F sequences available in the GenBank ($n = 76$) showed relatively high bootstrap values for the branching of clusters (Fig. 1). These authors also found signature amino-acid combinations within the clusters, particularly in the overlapping polymerase ORF. The authors proposed 3 amino acids conserved among the polymerases of cluster IV isolates: T401, N466 and Y470. These results were not confirmed by us when sequences from Venezuelan isolates were analyzed. While T401 was conserved, T466 was more frequent than N466 and H470 was more frequent than Y470 in the Venezuelan isolates (data not shown).

Cluster III was found to be the most divergent of the four clusters, and comprised exclusively isolates from Central America. In cluster IV only South American isolates were found, except for two isolates from French hemodialysis patients. Clusters I and II included isolates from both regions. Most of the sequences of the Venezuelan isolates were grouped in cluster IV: isolates circulating in different Amerindian ethnics – Yucpa Amerindians from Western Venezuela and Yanomami Amerindians from Southern Venezuela [14, 21] – and in Afro-Venezuelan communities. Only one Afro-Venezuelan isolate belonged to cluster II. Additional sequences from other Venezuelan isolates were also grouped within cluster II (Pujol, F. H., personal communication), suggesting that isolates belonging to these 2 clusters circulate in Venezuela. More information is needed about which of the HBV genotype F clusters circulate in the Caribbean Islands. Analysis based upon full length genome sequences are also needed to confirm the existence of these clusters of HBV genotype F. However, the limited available phylogenetic information is compatible with the hypothesis that the Afro-Venezuelan genotype F isolates were introduced in these populations after the slaves arrived to Venezuela.

The clinical and biological importance of the genotypic diversity of HBV and the implication in the generation of mutants is not fully understood. There are however some evidences that HBV genetic variability might be associated with different rates of transmission, resistance to interferon or progression to hepatocellular carcinoma [6, 7, 17, 22]. Further studies are needed to evaluate the

contribution of HBV genetic variability in the different epidemiological presentations of this disease.

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