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Hepatitis B virus genotypes from European origin explains the high endemicity found in some areas from southern Brazil

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ABSTRACT

Southern Brazil is considered an area of low Hepatitis B endemicity, but some areas of higher endemicity have been described in the Southwest of Paraná and Santa Catarina states. The aim of this study was to evaluate viral genotypes circulating throughout Paraná state. PCR amplification and partial sequencing of the S gene was carried out in 228 samples from HBsAg positive candidate blood donors. Samples have been collected in seven different counties (Cascavel, Curitiba, Foz do Iguaçu, Francisco Beltrão, Maringá, Londrina and Paranaguá). The most common HBV genotype in Paraná state was D (82.9%; 189/228), followed by A (14.1%; 32/228). Genotypes F (1.3%; 3/228), C (1.3%; 3/228) and H (0.4%; 1/228) were also found. Distribution of genotypes was different in the studied counties, but genotype D was the most frequent in all of them. In Francisco Beltrão, all studied samples belonged to genotype D. The high prevalence of HBV genotype D in South of Brazil is explained by the intense migration of settlers from Europeans countries. Subgenotypes A1 and A2 were identified circulating in all cities where HBV/A was found. As observed in other areas of Brazil, HBV/A1 is more frequent than the HBV/A2 in Paraná state and its presence was significantly larger in black and mulatto individuals. Genotype C was found only in individuals with Asian ancestry from Londrina and Maringá. Most HBV/F sequences identified in this study were classified as subgenotype F2a that was previously described in Brazil. The sole case of subgenotype F4 was from Foz do Iguaçu city, near to Northern Argentina, where F4 is highly prevalent. The single genotype H sample was from Curitiba. This is the first case of this genotype described in Brazil. Further studies should be carried out to determine if more genotype H samples can be found in other populations from Brazil.

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1. Introduction

Hepatitis B virus (HBV) infection is still one of the major causes of chronic liver diseases, including chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC) (Lok and McMahon, 2009). HBV, as a member of the Hepadnaviridae family, is a circular, partially double-stranded DNA virus and is classified into eight genotypes, designated A to H (Kramvis et al., 2008; Norder et al., 2004). Recently, two new additional, HBV genotypes, HBV/I and HBV/J, were proposed, which were isolated in Vietnam (Tran et al., 2008) and Japan (Tatematsu et al., 2009), respectively. However, because these strains are recombinants with other genotypes, their classification as new genotypes is under discussion (Kurbanov et al., 2010).

Currently, HBV genotypes are defined based on an intergroup divergence of 7.5% or more in the complete nucleotide sequence or 4% in the partial sequence of the open reading frame that code for the surface antigens (PreS1/PreS2/S) in the region covering the small protein (HBsAg) (Kramvis et al., 2008; Norder et al., 2004). Furthermore, based on a divergence of >4% and <8% in the complete nucleotide sequence within a genotype, subgenotypes

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have been classified within genotypes A, B, C, D and F (Kramvis et al., 2008; Norder et al., 2004).

The importance of HBV genotypes/subgenotypes in the progression of liver disease and response to interferon therapy have been suggested by several studies, but results are still controversies (Kao and Lin, 2011; Lok and McMahon, 2009; Wiegand et al., 2008).

Most genotypes and some subgenotypes have distinct geographical distribution and are associated with different ethnicities (Kramvis et al., 2008; Kurbanov et al., 2010; Norder et al., 2004). Genotype A is highly prevalent in some regions of Africa, Northern and Middle Europe and North America; genotypes B and C are common in East Asia; genotype D prevails in the Mediterranean basin and in the Middle East; genotype E is indigenous to Africa and predominates throughout its western region; and genotype F is found in South America, especially in populations with Amerindian background (Kurbanov et al., 2010; Norder et al., 2004). The distribution of genotypes G and H is not well known: the first one was found in France, Germany, USA, and Mexico (Sanchez et al., 2007; Stuyver et al., 2000; Vieth et al., 2002); while the latter is frequent in Mexico and has also been found some case in other countries in Central and North America (Alvarado-Esquivel et al., 2006; Arauz-Ruiz et al., 2002; Sanchez et al., 2007). These two genotypes have recently been identified in some regions of South America: genotype G in Colombia (Alvarado Mora et al., 2011) and Brazil (Mendes-Correa et al., 2010); genotype H in Argentina (Flichman et al., 2009).

Brazil is a country with continental dimensions, consisting of five regions (North, Northeast, Central-West, South and Southeast) with distinct environmental and cultural characteristics, and population background. Furthermore, HBV epidemiology is different among these regions, with increasing prevalence from South to North (Souto, 1999). Nevertheless, there are some areas with elevated prevalence inside regions characterized with low or intermediate prevalence. The Southwest of Paraná state, in South of Brazil is an area where the prevalence of HBsAg reached 8.3% up to the 1990's (Carvalho and Dias, 1995; Souto, 1999). In another study carried out in 2006, after the introduction of universal vaccination we identified that HBV infection is still a serious health threat in this area with HBsAg prevalence of 3.8% (Bertolini et al., 2006).

Studies about the distribution of HBV genotypes in areas around the world have showed that it generally reflects the demographic history of this area (Alvarado Mora et al., 2011; Arankalle et al., 2003; Kramvis and Kew, 2007; Thedja et al., 2011). The differences in HBV prevalence among Brazilian regions may result, among other factors, from the origin of the people that live in these different areas nowadays. In this study, trying to elucidate the origins for the relevant prevalence of HBV infection in Southwest of Paraná state, we characterized the distribution of HBV genotypes in seven different regions of Paraná state and identified the demographic data that were associated with the different HBV genotypes identified in this state.

2. Materials and methods

2.1. Samples

Serum samples from 228 volunteers candidate blood donors (177 male and 51 female; age 31.8 ± 9.4 years) positive for HBV surface antigen (HBsAg) were collected in Blood Banks from seven geographically distinct localities at Paraná state, in South of Brazil: Cascavel ($n = 43$), Curitiba ($n = 35$), Foz do Iguaçu ($n = 53$), Francisco Beltrão ($n = 49$), Londrina ($n = 12$), Maringá ($n = 30$) and Paranaguá ($n = 6$).

Each enrolled in this study was submitted to a detailed interview about the following characteristics: sex, age, color (based on self classification according to the Instituto Brasileiro de Geografia e Estatística – IBGE, 2007), education and income levels, ancestry (concerning family immigration from foreign countries as well as moving from other Brazilian regions or other states from southern Brazil – i.e., from Santa Catarina and Rio Grande do Sul states).

Blood samples were collected from May, 1998 to May, 2002 and sera were separated and stored at −20 °C. This study was approved by the local institution ethics committee and all individuals provided written informed consent.

2.2. HBV DNA extraction, amplification and sequencing

HBV nucleic acid was extracted from 100 μL serum samples by the acid guanidinium thiocyanate/phenol/chloroform method (Chomczynski and Sacchi, 1987) and eluted in 50 μL of sterile nuclease-free water.

A fragment of HBV DNA S gene was amplified by nested PCR as previously described (Sitnik et al., 2004). In order to prevent PCR carryover contamination, strict precautions and procedures were strictly followed.

PCR products were purified and submitted to sequencing, using second round primers with fluorescence-labeled dideoxynucleotide chain terminators using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA). The sequences were determined in an automated DNA sequencer (ABI Prism 377, Applied Biosystems, USA).

2.3. Sequence analysis

The quality of each electropherogram was evaluated using the Phred-Phrap software and consensus sequences were obtained by alignment of both sequenced strands (sense and antisense) using CAP3 software available in the web page Electropherogram quality analysis <(http://asparagin.cenargen.embrapa.br/phph/)>.

Sequences were submitted to EMBL/GenBank/DDBJ under accession numbers EF172453–EF172680.

2.4. Phylogenetic analysis

HBV genotypes were determined by phylogenetic analysis of the amplified S gene fragment with sequences from different HBV genotypes and subgenotypes obtained from the GenBank public database <(http://www.ncbi.nlm.nih.gov/)>.

Sequences were aligned and compared using BioEdit (v. 7.0.8) and the integrated CLUSTAL W program (Hall, 1999). Bayesian phylogenetic analyses were conducted using the Markov Chain Monte Carlo (MCMC) simulation implemented in BEAST v.1.6.1 (Drummond and Rambaut, 2007) under relaxed uncorrelated lognormal molecular clock using the model of nucleotide substitution (GTR + G + I) obtained previously by Modeltest v. 3.7 (Posada and Crandall, 1998). The maximum clade credibility (MCC) tree was obtained from summarizing the 10,000 substitution trees and then 10% of burn-in was removed using Tree Annotator v.1.6.1 (Drummond and Rambaut, 2007).

2.5. Statistical analysis

Qualitative variables and genotypes were analyzed using the Chi square test and were described as percentages. Variance analysis was used to compare the mean age of the blood donors infected with different genotypes. Statistical significance level was defined as $P < .05$. SPSS software was utilized for all statistical analyses.

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