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Short communication

Identification of a new clade of hepatitis B virus genotype F

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ABSTRACT

Hepatitis B virus (HBV) is classified into eight main genotypes (A–H) and several subgenotypes. Here, three new genotype F complete genome sequences isolated from patients from Buenos Aires city are reported. The new sequences form a separate monophyletic group from the previously known subgenotype F4 strains. Based on results of phylogenetic, genetic distance and evolutionary analyses, the name F4b is proposed for these isolates and F4a for the formerly known as F4. The identification of new clusters allows deepening the knowledge about the diversification process and evolutionary history of HBV.
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1. Introduction

Hepatitis B virus (HBV) has a compact, circular DNA genome of about 3.2 kb in length with four overlapping open reading frames (ORFs). The overlapping ORFs impose constraints on possible nucleotide substitutions and variable substitution rates for different genomic regions (Yang et al., 1995). The rate at which HBV sequences evolve is, however, uncertain. HBV substitution rates have been estimated in the order of 10^{-4}–10^{-5} substitutions per site per year (s/s/y) depending on the HBeAg status, suggesting that viruses that do not express the e antigen (HBeAg (−)) would evolve faster than viruses that do express it (HBeAg (+)) (Hannoun et al., 2000; Wang et al., 2010).

HBV is classified into eight main genotypes (A–H) (Arauz-Ruiz et al., 2002; Norder et al., 1994; Stuyver et al., 2000) and two additional (I and J) were tentatively proposed (Huy et al., 2008; Kurbanov et al., 2008; Tatematsu et al., 2009). Some genotypes have been further classified into several subgenotypes (designated with numbers), defined by phylogenetic grouping with high support values and intergroup divergences for complete genome sequences ranging from 4.0% to 7.5% (Kramvis et al., 2008; Shi et al., 2013). Subgenotypes have been defined for genotypes A, B, C, D and F (Bowyer et al., 1997; Huy et al., 2004; Kramvis et al., 2002; Sugauchi et al., 2002). Genotype F is one of the most divergent HBV genotypes and its evolutionary history is not yet well understood due to the lack of data (Kramvis et al., 2008; Torres et al., 2011). It has been found in native population of Alaska, Central and South America and four subgenotypes have been described (F1–F4), two of which (F1 and F2) are subsequently divided into two subtypes or clusters (a and b) (Arauz-Ruiz et al., 1997; Campos et al., 2005; Devesa et al., 2008; Huy et al., 2006; Livingston et al., 2007; Piñeiro y Leone et al., 2003).

In the context of an epidemiological screening, the phylogenetic analysis on 44 isolates of HBV genotype F from patients from Buenos Aires city showed that three sequences formed a separate cluster among genotype F sequences available from GenBank, using the S gene and precore-core genetic regions (data not shown). These samples belong to three anti-HBe positive unrelated individuals born in Buenos Aires city, who attended to Hospital Italiano or Hospital F.J. Muñiz. The isolate F4_01 belongs to a 40-year-old man who was diagnosed in screening tests in 1996, F4_02 belongs to a 70-year-old woman who underwent multiple surgeries and was diagnosed in 1997, and F4_03 corresponds to a 65-year-old man; patients F4_01 and F4_03 did not disclose risk factors to assess the source of infection. The study was carried out according to the World Medical Association Declaration of Helsinki and was approved by the Ethical Committee of the School of Pharmacy and Biochemistry, University of Buenos Aires.

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Complete genome sequences were obtained for these three isolates [GenBank ID: KJ676692, KJ676693, KJ676694]. Simplot software was used to exclude a recombinant origin of these sequences (Lole et al., 1999); the window and step sizes were set to 200 bp and 20 bp, respectively.

In order to confirm the subgenotyping of the three isolates, a phylogenetic analysis was performed. Complete genome sequences obtained in this study and reference sequences available at GenBank were aligned with the ClustalX (v2.1) software (Larkin et al., 2007) and edited with the BioEdit (v7.1.3.0) software (Hall, 1999). Phylogenetic trees were constructed using the Maximum Likelihood method performed with the PhyML (v3.0) program (Guindon et al., 2010). Evolutionary models were inferred according to the Akaike Information Criterion (AIC) statistics (Akaike, 1974) obtained with the jModeltest (v2.1) software (Darriba et al., 2012). The robustness of the reconstructed phylogenies was evaluated by bootstrap analysis (1000 replicates). The phylogenetic analysis revealed that all three samples grouped into a separate clade with high bootstrap support associated to subgenotype F4 and that was tentatively named as F4b (Fig. 1).

A further comparative analysis of genetic distances between groups of sequences was assessed in MEGA6 (Tamura et al., 2013). The analysis involved 140 nucleotide sequences (F1a: 6, F1b: 61, F2a: 14, F2b: 4, F3: 22, F4a: 30, F4b: 3). All positions containing gaps and missing data were eliminated. Standard errors (SE) were obtained by a bootstrap procedure (1000 replicates). The new cluster and the other subgenotype F4 strains showed a distance of 3.8% (SE 0.3%) (Table 1). This value is higher than the distance between subtypes F1a and F1b (1.8%) and the distance between F2a and F2b (3.2%), although it is lower than the conventionally accepted 4.0% for considering a new subgenotype (Kramvis et al., 2008; Shi et al., 2013).

Table 1
Estimates of mean divergence (% ± SE) between groups of sequences conducted in MEGA6. The intergroup divergence between F4a and F4b is shown in bold.

	F1a	F1b	F2a	F2b	F3	F4a
F1b	1.8 ± 0.2					
F2a	5.1 ± 0.3	5.8 ± 0.4				
F2b	5.3 ± 0.4	5.7 ± 0.4	3.2 ± 0.3			
F3	5.0 ± 0.3	5.4 ± 0.3	4.3 ± 0.3	4.4 ± 0.4		
F4a	5.4 ± 0.3	5.7 ± 0.3	4.6 ± 0.4	4.6 ± 0.4	4.3 ± 0.3	
F4b	5.6 ± 0.3	6.0 ± 0.3	4.8 ± 0.3	4.8 ± 0.4	4.6 ± 0.3	**3.8 ± 0.3**

By comparison with a subgenotype F4 consensus sequence, isolates F4_01, F4_02 and F4_03 showed 90–102 nucleotide changes along the entire genome (Supplementary Fig. S1) whereas 69 of these changes are shared by the three isolates and would represent the nucleotide markers of the group.

Thus, considering that the new sequences conform a separate monophyletic group from the previously known subgenotype F4 strains, which is supported by high bootstrap values; observing the nucleotidic intergroup divergence; and noting that the new group have a signature pattern of nucleotide and amino acid, it is proposed to name the new group as F4b, and the former F4 strains as F4a, according to the actual recommendation (Kramvis et al., 2008; Shi et al., 2013).

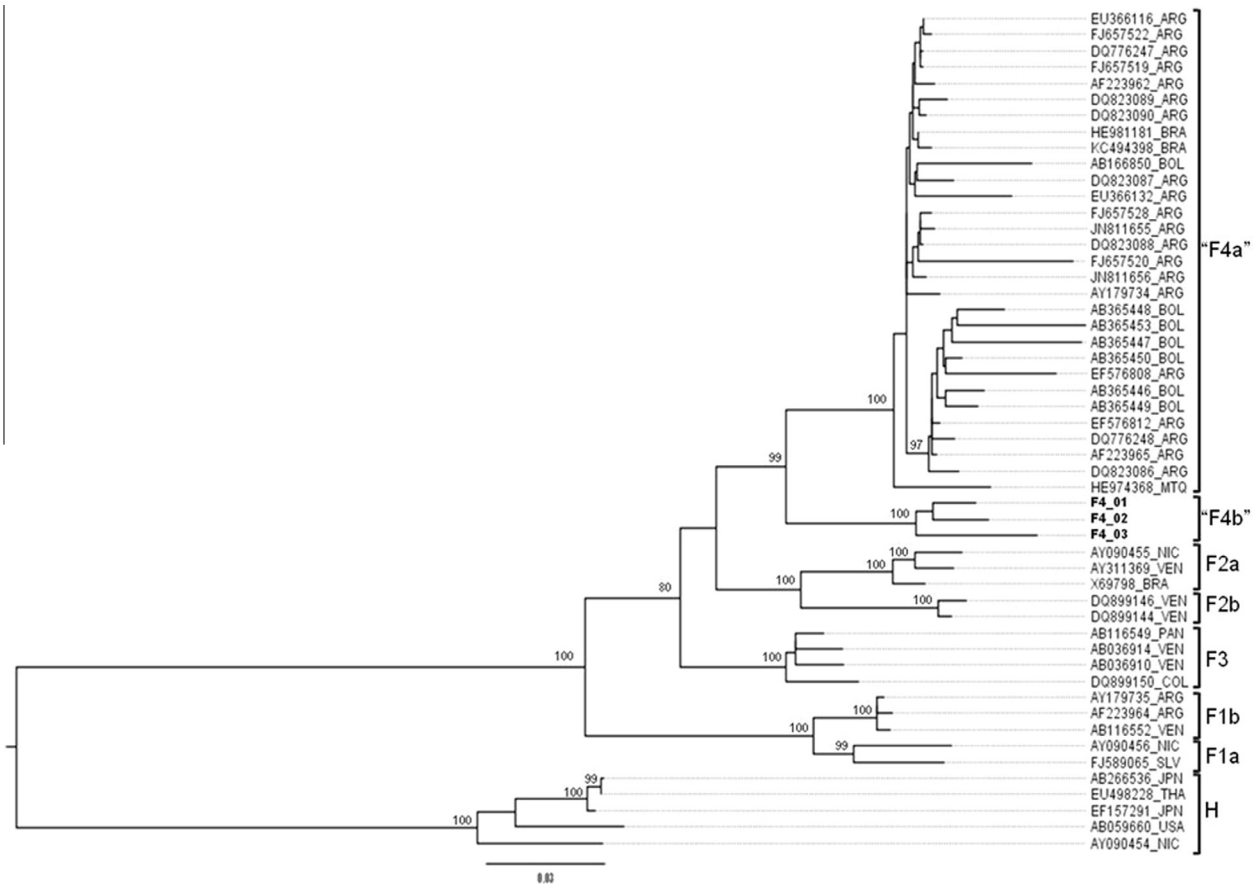

Fig. 1. Maximum-Likelihood phylogenetic tree performed with the PhyML (v3.0) program constructed on the complete genome of F4_01, F4_02, F4_03 and reference sequence from each HBV/F subgenotype available at GenBank. Genotype H was used as outgroup. The numbers at nodes correspond to bootstrap values (1000 replicates) higher than 70%. The scale bar indicates the genetic distances. Sequences reported in this work are shown in bold. Sequences from GenBank are indicated by their corresponding accession numbers and their country of origin is abbreviated with ISO three-letter code.

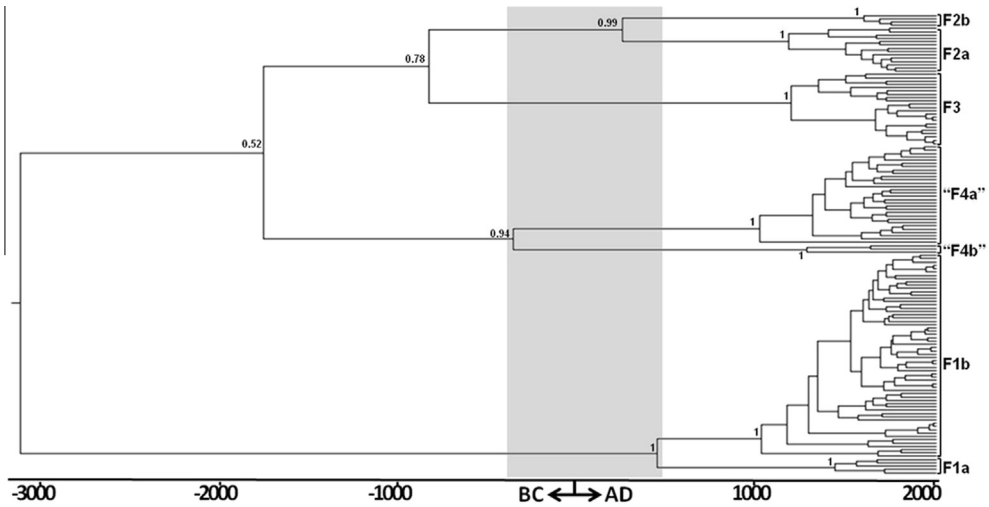

Fig. 2. Time-scaled Maximum clade credibility tree (MCCT) for HBV/F complete genome sequences calibrated with a fixed mean rate of 1×10^{-5} s/s/y. The numbers on the internal nodes represent posterior probabilities and only nodes representing MRCAs for the main groups are shown, for clarity purposes. The scale at the bottom of the tree represents time (years).The shaded area shows the period of cladogenesis (Clades a and b) within the subgenotypes.

Here, a Bayesian coalescent approach was used to estimate the times to the most recent common ancestors (tMRCA) and population dynamics of genotype F and its subgenotypes, calibrating with a fixed mean rate of 1.0×10^{-5} substitutions per site per year (s/s/y) proposed by Torres et al. (2011). For this purpose, once again the dataset of 140 complete genome sequences previously assembled was used. The analyses were carried out using an appropriate substitution model estimated as described above. Different combinations of molecular clocks (strict and uncorrelated lognormal (UCLN)) and demographic models were analyzed in the BEAST v1.8.1 software package (Drummond and Rambaut, 2007). Bayes Factors (BF) were calculated comparing the marginal likelihoods estimated using the Path Sampling (PS) and Stepping Stone (SS) methods (Baele et al., 2012, 2013). The UCLN molecular clock model and the Skyline demographic model were chosen as the best-fit to the data (Supplementary Table S1). Accordingly, the maximum clade credibility tree (MCCT) was obtained using the TreeAnnotator v1.8.1 program and visualized with the FigTree v1.4.2 program (available at http://tree.bio.ed.ac.uk/software/figtree/). This analysis showed that diversification of subgenotype F4 into F4a and F4b would have occurred previously or concomitant to the diversification of subgenotypes F1 and F2 into their "a" and "b" groups. Moreover, the tMRCA of F4b would be dated in the ~1300 AD, similarly to the ancestral times of the rest of currently circulating groups (F1a, F1b, F2a, F2b, F3, F4a) (Fig. 2). It is remarkable that in the MCCT, F4 is shown as the second subgenotype to diverge, after F1, unlike previously published results with the same methodology (Mello et al., 2013; Torres et al., 2011), where it was observed that F3 was the second subgenotype to diverge. These analyses were based on datasets that included all the different subgroups of genotypes F reported until then. Hence, in order to evaluate the impact of the incorporation of the new cluster sequences, datasets of complete genome of genotype F, with and without the F4b strains, were tested under the same analytical conditions. The result indicated that the addition of F4b sequences changed the MCCT topology obtained by Bayesian methods. The datasets were also analyzed by other two methods (Maximum likelihood and Neighbor Joining). In spite of the tree topologies were not congruent to that obtained by Bayesian methods, they showed a significant decrease in the support values of the corresponding nodes (available upon request). The discrepancy in the results may be due to a low phylogenetic signal in the sequences under analysis that not provide enough information to resolve the group formed by subgenotypes F2, F3 and F4.

A further analysis was performed to evaluate which topology could better fit the data in the Bayesian context. Briefly, Bayesian analyses were run in the conditions previously mentioned, constraining the groups (F2,F4) or (F2,F3) as monophyletic. Bayes Factor favored the topology with (F2,F4) as a monophyletic group (Maximum likelihood topology) over the topology with grouping (F2,F3) (log BF = 4.35 for PS and log BF = 3.69 for SS (Supplementary table S2)), suggesting that the former evolutionary hypothesis would have a better phylogenetic and statistical support that the second one. In this regard, it is considered that the incorporation of new sequences or groups in the future could shed light on the phylogenetic relationship between subgroups of genotype F. This scenario highlights the importance of sampling properly the circulating strains and how the inclusion of samples representing new groups or clusters can impact on the evolutionary history of HBV as it is currently accepted.

The identification of this new cluster has different implications. Firstly, some studies have suggested the implication of different genotypes and subgenotypes in clinical outcomes, HBeAg seroconvertion rate, and mutational patterns in precore/core promoter region (Kao, 2002; Yuan et al., 2007). The identification of new subtypes adds a piece of knowledge to the puzzle of the HBV divergence and provides novel opportunities to study the evolutionary behavior of HBV in relation with different clinical status.

Secondly, the geographical distribution of HBV genotypes and subgenotypes is well characterized and some hypotheses about the evolutionary history of HBV have been proposed (Holmes, 2008; Paraskevis et al., 2013; Torres et al., 2011). However, these hypotheses are built based on currently available molecular and epidemiological data. Thus, the finding of sequences belonging to clusters that were not previously sampled allows deepening our understanding about the diversification process and the evolutionary history of viruses.

Therefore, despite the new cluster would currently be a minority population, it could represent the survivors of an ancestral lineage arising at the time of diversification of subgenotypes F. Considering that all sequences of the new cluster are from anti-HBeAg positive patients, it could imply a limited – or even an absent-transmission in recent years. The high nucleotide

divergence observed between these three isolates could be taken as a piece of evidence of a long story of circulation of this strain in the population.

In addition, epidemiological data indicated that all patients were born and raised in Buenos Aires city. However, we speculate that this lineage entered the city from internal migrations occurred since 1940, which conformed part of the current population of big cities in Argentina.

Finally, further research is needed to clarify the origin of new lineages of HBV, their implication in the diversification process and the evolutionary history of genotype F, and their impact on the clinical outcome of HBV infection.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.meegid.2015.06.007.

References

Akaike, H., 1974. A new look at the statistical model identification. IEEE Trans. Autom. Control 19, 716–723. http://dx.doi.org/10.1109/TAC.1974.1100705.

Arauz-Ruiz, P., Norder, H., Visoná, K.A., Magnius, L.O., 1997. Molecular epidemiology of hepatitis B virus in Central America reflected in the genetic variability of the small S gene. J. Infect. Dis. 176, 851–858. http://dx.doi.org/10.1086/516507.

Arauz-Ruiz, P., Norder, H., Robertson, B.H., Magnius, L.O., 2002. Genotype H: a new Amerindian genotype of hepatitis B virus revealed in Central America. J. Gen. Virol. 83, 2059–2073.

Baele, G., Lemey, P., Bedford, T., Rambaut, A., Suchard, M.A., Alekseyenko, A.V., 2012. Improving the accuracy of demographic and molecular clock model comparison while accommodating phylogenetic uncertainty. Mol. Biol. Evol. 29, 2157–2167.

Baele, G., Li, W.L., Drummond, A.J., Suchard, M.A., Lemey, P., 2013. Accurate model selection of relaxed molecular clocks in Bayesian phylogenetics. Mol. Biol. Evol. 30, 239–243.

Bowyer, S.M., van Staden, L., Kew, M.C., Sim, J.G.M., 1997. A unique segment of the hepatitis B virus group A genotype identified in isolates from South Africa. J. Gen. Virol. 78, 1719–1729.

Campos, R.H., Mbayed, V.A., Piñeiro y Leone, F.G., 2005. Molecular epidemiology of hepatitis B virus in Latin America. J. Clin. Virol. 34, S8–S13. http://dx.doi.org/10.1016/S1386-6532(05)80028-9.

Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2012. JModelTest 2: more models, new heuristics and parallel computing. Nat. Methods 9, 772. http://dx.doi.org/10.1038/nmeth.2109.

Devesa, M., Loureiro, C.L., Rivas, Y., Monsalve, F., Cardona, N., Duarte, M.C., Poblete, F., Gutierrez, M.F., Botto, C., Pujol, F.H., 2008. Subgenotype diversity of hepatitis B virus american genotype F in amerindians from Venezuela and the general population of Colombia. J. Med. Virol. 80, 20–26. http://dx.doi.org/10.1002/jmv.

Drummond, A.J., Rambaut, A., 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. 7, 214. http://dx.doi.org/10.1186/1471-2148-7-214.

Guindon, S., Dufayard, J.F., Lefort, V., Anisimova, M., Hordijk, W., Gascuel, O., 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst. Biol. 59, 307–321.

Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 41, 95–98.

Hannoun, C., Horal, P., Lindh, M., 2000. Long-term mutation rates in the hepatitis B virus genome. J. Gen. Virol. 81, 75–83.

Holmes, E.C., 2008. Evolutionary history and phylogeography of human viruses. Annu. Rev. Microbiol. 62, 307–328. http://dx.doi.org/10.1146/annurev.micro.62.081307.162912.

Huy, T.T.T., Ushijima, H., Quang, V.X., Win, K.M., Luengrojanakul, P., Kikuchi, K., Sata, T., Abe, K., 2004. Genotype C of hepatitis B virus can be classified into at least two subgroups. J. Gen. Virol. 85, 283–292. http://dx.doi.org/10.1099/vir.0.19633-0.

Huy, T.T.T., Ushijima, H., Sata, T., Abe, K., 2006. Genomic characterization of HBV genotype F in Bolivia: genotype F subgenotypes correlate with geographic distribution and T (1858) variant. Arch. Virol. 151, 589–597. http://dx.doi.org/10.1007/s00705-005-0671-1.

Huy, T.T.T., Ngoc, T.T., Abe, K., 2008. New complex recombinant genotype of hepatitis B virus identified. J. Virol. 82, 5657–5663. http://dx.doi.org/10.1128/JVI.02556-07.

Kao, J., 2002. Hepatitis B viral genotypes: clinical relevance and molecular characteristics. J. Gastroenterol. Hepatol. 17, 643–650. http://dx.doi.org/10.1046/j.1440-1746.2002.02737.x.

Kramvis, A., Weitzmann, L., Owiredu, W., Kew, M., 2002. Analysis of the complete genome of subgroup A hepatitis B virus isolates from South Africa. J. Gen. Virol. 83, 835–839.

Kramvis, A., Arakawa, K., Yu, M.C., Nogueira, R., Stram, D.O., Kew, M.C., 2008. Relationship of serological subtype, basic core promoter and precore mutations to genotypes/subgenotypes of hepatitis B virus. J. Med. Virol. 80, 27–46. http://dx.doi.org/10.1002/jmv.

Kurbanov, F., Tanaka, Y., Kramvis, A., Simmonds, P., Mizokami, M., 2008. When should "I" consider a new hepatitis B virus genotype? J. Virol. 82, 8241–8242. http://dx.doi.org/10.1128/JVI.00793-08.

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J., Higgins, D.G., 2007. Clustal W and Clustal X version 2.0. Bioinformatics 23, 2947–2948. http://dx.doi.org/10.1093/bioinformatics/btm404.

Livingston, S.E., Simonetti, J.P., McMahon, B.J., Bulkow, L.R., Hurlburt, K.J., Homan, C.E., Snowball, M.M., Cagle, H.H., Williams, J.L., Chulanov, V.P., 2007. Hepatitis B virus genotypes in Alaska native people with hepatocellular carcinoma: preponderance of genotype F. J. Infect. Dis. 195, 5–11. http://dx.doi.org/10.1086/509894.

Lole, K.S., Bollinger, R.C., Paranjape, R.S., Gadkari, D., Kulkarni, S.S., Novak, N.G., Ingersoll, R., Sheppard, H.W., Ray, S.C., 1999. Full-length human immunodeficiency virus type 1 genomes from subtype C-infected seroconverters in India, with evidence of intersubtype recombination. J. Virol. 73, 152–160.

Mello, F.C.A., Araujo, O.C., Lago, B.V., Motta-Castro, A.R., Moraes, M.T.B., Gomes, S.A., Bello, G., Araujo, N.M., 2013. Phylogeography and evolutionary history of hepatitis B virus genotype F in Brazil. Virol. J. 10, 236. http://dx.doi.org/10.1186/1743-422X-10-236.

Norder, H., Couroucé, A.-M., Magnius, L.O., 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.

Paraskevis, D., Magiorkinis, G., Magiorkinis, E., Ho, S.Y.W., Belshaw, R., Allain, J.-P., Hatzakis, A., 2013. Dating the origin and dispersal of hepatitis B virus infection in humans and primates. Hepatology 57, 908–916. http://dx.doi.org/10.1002/hep.26079.

Piñeiro y Leone, F.G., Mbayed, V.A., Campos, R.H., 2003. Evolutionary history of hepatitis B virus genotype F: an in-depth analysis of Argentine isolates. Virus Genes 27, 103–110.

Shi, W., Zhang, Z., Ling, C., Zheng, W., Zhu, C., Carr, M.J., Higgins, D.G., 2013. Hepatitis B virus subgenotyping: history, effects of recombination, misclassifications, and corrections. Infect. Genet. Evol. 16, 355–361. http://dx.doi.org/10.1016/j.meegid.2013.03.021.

Stuyver, L., De Gendt, S., Van Geyt, C., Zoulim, F., Fried, M., Schinazi, R.F., Rossau, R., 2000. A new genotype of hepatitis B virus: complete genome and phylogenetic relatedness. J. Gen. Virol. 81, 67–74.

Sugauchi, F., Orito, E., Ichida, T., Kato, H., Sakugawa, H., Kakumu, S., Ishida, T., Chutaputti, A., Lai, C., Ueda, R., Miyakawa, Y., Mizokami, M., 2002. Hepatitis B virus of genotype B with or without recombination with genotype C over the precore region plus the core gene. J. Virol. 76, 5985–5992. http://dx.doi.org/10.1128/JVI.76.12.5985.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30, 2725–2729. http://dx.doi.org/10.1093/molbev/mst197.

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. Virol. 83, 10538–10547. http://dx.doi.org/10.1128/JVI.00462-09.

Torres, C., Piñeiro y Leone, F.G., Pezzano, S.C., Mbayed, V.A., Campos, R.H., 2011. New perspectives on the evolutionary history of hepatitis B virus genotype F. Mol. Phylogenet. Evol. 59, 114–122. http://dx.doi.org/10.1016/j.ympev.2011.01.010.

Wang, H., Chien, M., Huang, H., Chang, H., Wu, C., Chen, P.-J., Chang, M., Chen, D.-S., 2010. Distinct hepatitis B virus dynamics in the immunotolerant and early immunoclearance phases. J. Virol. 84, 3454–3463. http://dx.doi.org/10.1128/JVI.02164-09.

Yang, Z., Lauder, I., Lin, H.J., 1995. Molecular evolution of the hepatitis B virus genome. J. Mol. Evol. 41, 587–596. http://dx.doi.org/10.1007/BF00175817.

Yuan, J., Zhou, B., Tanaka, Y., Kurbanov, F., Orito, E., Gong, Z., Xu, L., Lu, J., Jiang, X., Lai, W., Mizokami, M., 2007. Hepatitis B virus (HBV) genotypes/subgenotypes in China: mutations in core promoter and precore/core and their clinical implications. J. Clin. Virol. 39, 87–93. http://dx.doi.org/10.1016/j.jcv.2007.03.005.