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Molecular evolution of hepatitis B vaccine escape variants in China, during 2000–2016

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ABSTRACT

Hepatitis B vaccine escape variants are the main threat to hepatitis B virus (HBV) infection in vaccination era worldwide. With 215 genotype B HBV and 313 genotype C HBV vaccine escape variants isolated from China during 2000–2016, we reported that genotype B HBV vaccine escape strains diverged in ~1997 (95% HPD; 1987–2005), while genotype C HBV vaccine escape strains diverged in ~1976 (95% HPD; 1955–2003). Additionally, the p-distance of genotype C HBV vaccine escape strains was 0.0291 ± 0.0169, which was significantly higher than that in the genotype B HBV ($t = 131.02$, $p < 0.05$). However, genotype B HBV vaccine escape strains evolved more rapidly than genotype C HBV (2.103×10^{-3} vs 1.083×10^{-3} substitutions/site/year). Bayesian skyline plot analysis showed that the populations of genotype C HBV vaccine escape strains fluctuated more than those in genotype B HBV. Four sites (A5T/S, L21S, T/A126S and T/N131I/A) and 13 sites (N3S, T5A, G10Q/R/E, L21S, T47K/A/V, L98V/P, I/S126N/V/T, Q129H/R/L, T131P/I/N/A, G145A/R, L175S/F, L213I/S, V224A/G) were found to be under positive selection in genotype B and C HBV vaccine escape strains, respectively. More importantly, N3S, L21S, T47K, L98V, I/S126T and L213I mutations were detected in 1 (2.5%), 1 (2.5%), 1 (2.5%), 3 (7.5%), 1 (2.5%), 1 (2.5%) genotype C HBV infected Chinese younger with neonatal HBV vaccination, respectively. Therefore, our results should be valuable in further understanding the molecular evolution of HBV and providing new ideas for the elimination of HBV infection.

1. Introduction

Hepatitis B virus (HBV) is a small enveloped DNA virus that belongs to the family of *Hepadnaviridae*. The virus genome comprises approximately 3200 nucleotides and has four partially overlapping open-reading frames (ORFs): surface (S), pre core (pre-C)/core (C), polymerase (P) and X protein. According to the homogeneity of virus sequences, at least 10 HBV genotypes (A to J) and several subtypes have been defined, respectively, 8% for genotypes and 4–8% for subtypes [1,2]. In China, genotype B and C HBV are both prevalent, as presented in 94% of chronic HBV carriers [3].

Worldwide, it is estimated that one third of the population have been infected by HBV, and every year approximately 500,000 to 700,000 individuals die from HBV-related illnesses, including acute fulminant infection, cirrhosis and hepatocellular carcinoma [4]. Despite improvements in public health and antiviral treatments, vaccination is still the most effective means of preventing HBV infections. In 1991, the World Health Organization (WHO) recommended that all countries with an HBV carrier rate of 8% or more should include the HBV vaccine in their national immunization program. To date, about 200 countries had introduced Childhood vaccination against HBV including birth vaccination [5]. Since the onset of vaccination, significant reductions in morbidity and mortality due to HBV infection have been recorded, most strikingly in countries of high HBV endemic regions [6].

However, HBV has not gone away and always stay in a certain infection rate. What threatens the benefits of vaccination is the emergence of "HBV vaccine escape variants" due to the selection pressure from HBV vaccine immunization or nucleos(t)ide analogue (NA) therapy [7,8]. HBV vaccine escape variants mainly carry

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point nucleotide mutations causing amino acid (aa) substitution in the "a" determinant of the HBsAg (aa 124–147), including I/T126A/N/I/S, Q129H/R, M133L, K141E, P142S, D144A/E and G145R/A [9–13]. While selection of these mutations is rare event, vaccine escape variants have been isolated from the different countries with longstanding hepatitis B vaccination programs, indicating that their possible spread would have considerable consequences in terms of public health [14,15].

HBV has been highly epidemic in China, where epidemiological studies showed about 10% prevalence of HBsAg in general population in 1992. In 2002, China integrated Hepatitis B into the Expanded Program on Immunization, which emphasis on providing a timely birth dose. This strategy is very effective, reducing the rate of HBsAg-positivity from 9.9% to 0.3% in children <15 years [16]. However, in the last decade, more and more HBV vaccine escape variants have been isolated from vaccinated population in China [17–19]. Therefore, in order to better understand the molecular epidemiological characteristics of these variants, we conducted a series of evolution analyses using the S genes of genotype B and C HBV vaccine escape variants isolated from China between 2000 and 2016. Moreover, we further analyzed the mutation characteristics of HBV S gene among 3038 Chinese younger who received neonatal HBV vaccination.

2. Materials and methods

2.1. HBV strains

According to the previous studies, HBV strains with the following mutations in S gene were defined as vaccine escape variants: T116N, P120S/E, I/T126A/N/I/S, Q129H/R, M133L, K141E, P142S, D144A/E and G145R/A [9–13]. In the present study, we firstly collected the full-length nucleotide sequences of HBV S gene from GenBank. Second, all of genotype B and C HBV vaccine escape variants isolated from China during 2000–2016 were selected after alignment of the strains using MEGA 6.06. Third, we omitted strains with 100% nucleotide sequence similarity, the strains with ambiguous sequences and the sequences without information about the sampling dates. Forth, recombination events among the HBV were screened using the RDP 4.39 according to the previous studies [20,21]. The suspected recombinant strains were also excluded. Eventually, 215 genotype B HBV and 313 genotype C HBV vaccine escape variants were included in the current analysis (Table S1).

2.2. Phylogenetic and molecular evolutionary analyses

All included sequences were aligned using CLUSTAL X software (version 2.1), then manually edited with the Bio edit software (version 7.0.5.3). Phylogenetic and molecular evolutionary analyses were performed with Bayesian Markov Chain Monte Carlo (MCMC) method using BEAST v1.6.1 [20–22]. In the current study, GTR+G+I model was used as the nucleotide substitution model and among site rate variation model (selected by JModeltest v2.1.7) [23]. Both strict and relaxed (uncorrelated exponential and uncorrelated lognormal) molecular clocks were used for the present molecular evolutionary analysis. Additionally, constant size and Bayesian skyline coalescent tree prior were used in the study as described before [20–22]. The MCMC chain was run for 30,000,000 steps. The parameter values were sampled at every 3000 steps. The convergence was assessed using Tracer v1.6.1, and the parameters with effective sample sizes (ESS) of 200 or greater after 10% burn-in were accepted [24]. The maximum clade credibility tree was generated by Tree Annotator v1.6.1 (available in BEAST), and the resulting tree file was visualized in the program FigTree 1.4.2.

2.3. Estimation of the pairwise distance (p-distance) frequency distributions

To assess the frequency distribution of HBV vaccine escape strains, the p-distances of genotype B and C HBV vaccine escape strains were calculated using MEGA 6.06 software package as previously described [22,23], respectively. The reliability of the tree was estimated using 1000 bootstrap replications. The HBV strains AB073827 and DQ993693 were used as reference strains for genotype B and C HBV, respectively.

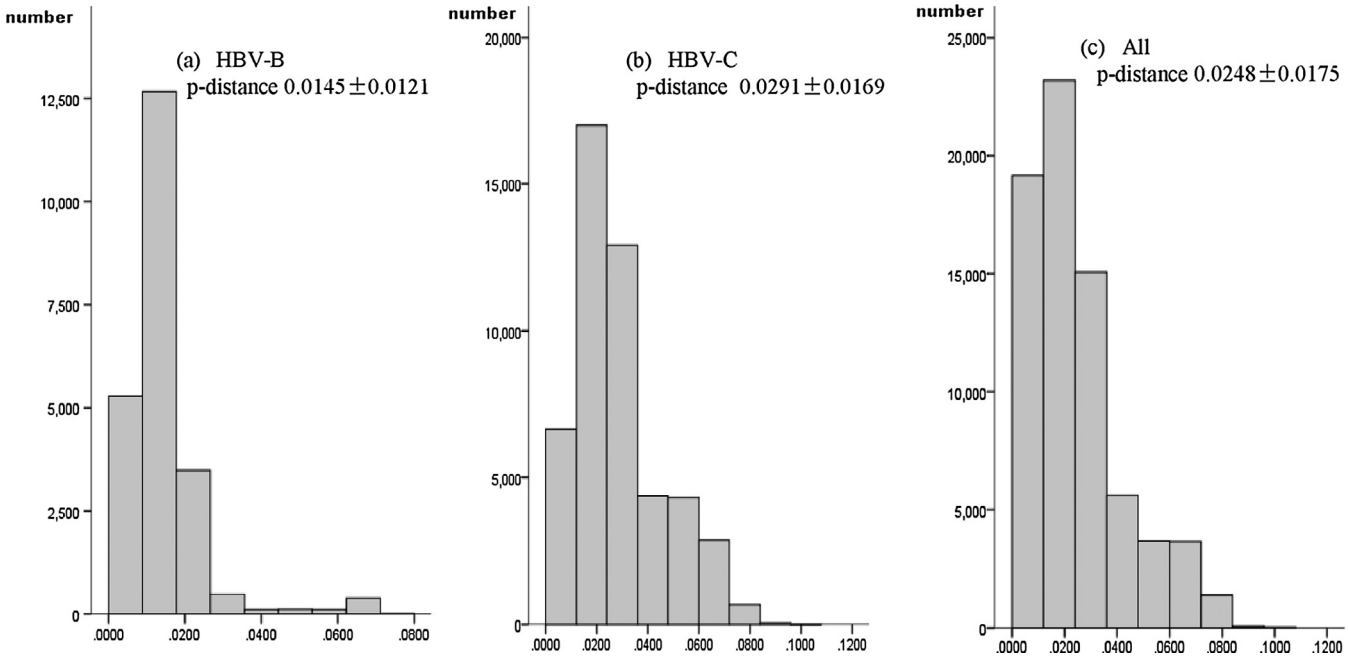

Fig. 1. Distribution of pairwise distances (p-distances) between HBV vaccine escape strains for all viruses (a), genotype B (b), and genotype C (c) based on the S genes.