A novel B/C inter-genotype recombinant of hepatitis B virus identified in north-west China

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Hepatitis B virus (HBV) has a worldwide distribution. An estimated 350 million individuals suffer from chronic HBV infection, among whom one-third live in China (Liang et al., 2009). HBV has been classified into eight genotypes, designated A–H, based on genomic DNA sequences. The predominant genotypes are B, C and D in China (Chemin et al., 2011). Genetic recombination is an important mechanism for virus evolution, and has imposed challenges on vaccine designation and antiviral therapy strategies (Yang et al., 2006). The characteristics of life-long persistent infection and the prevalence of different genotypes in the same region offer a high probability of co-infection of different genotypes in one host and thus a high risk for virus recombination. In north-west China, HBV inter-genotype recombinants have been reported frequently over the last decade (Wang et al., 2005; Zhou et al., 2011). The present study reports a novel recombinant HBV with a novel genome mosaic structure from Lanzhou, a city in north-west China.

From August 2010 to September 2012, 67 serum samples were collected from asymptomatic HBV carriers with viral loads \( > 10^5 \) in Lanzhou. The serum samples were stored at \(-80 \) °C until use. Approval was obtained from the Fourth Military Medical University institutional ethics committee before the study and written informed consent for participation in this study was obtained from each individual. HBV DNA was extracted from 200 μl serum samples with a viral DNA extraction kit (Tiangen). DNA pellets were dissolved in 20 μl distilled water and 5 μl of the DNA sample was used as a template for HBV DNA amplification by PCR. Two pairs of PCR primers were used to amplify the surface and core genes, respectively, with experimental conditions described previously (Cheng et al., 2009).

Based on the phylogenetic trees acquired with the surface gene and core gene, we found a typical phylogenetic violation in one of the 67 samples, in which the surface gene was clustered into genotype B, while the core gene was clustered into genotype C, indicating a B/C inter-genotype recombination. To further confirm this notion, the complete HBV genome from this sample was amplified using a method described previously (Günther et al., 1995). As reported in previous studies, PCR products were purified with a QIAquick gel extraction kit (Qiagen) and cloned into vector pMD 18-T (Takara) using standard cloning techniques. White colonies were picked and grown in Luria–Bertani medium with ampicillin (100 μg ml\(^{-1}\)). The correct insert size was confirmed using PCR and the restriction enzyme \( SalI \) (Promega). Two clones were selected randomly for sequencing of the complete genome with an ABI 3730 automated DNA sequencer (Applied Biosystems) (Cheng et al., 2009; Wang et al., 2005). The two acquired HBV genomic DNAs were both 3215 bp with a high similarity of 99.9 % and thus only one of them was submitted to GenBank (accession number KC774178).
Full-length genomes from KC774178 and 31 reference strains of HBV were manually edited by visual inspection and multiply aligned with MEGA software (version 5.0). SimPlot and BootScan analyses were employed to determine the possible recombination positions in the KC774178 HBV genome with SimPlot software package (version 3.5.1).

Genetic distances between KC774178 and reference strains are displayed in Fig. 1(a). The distance plots of the complete genome showed regions of higher similarity to genotype B, alternating with regions of higher similarity to genotype C within the core gene, part of the pre-S1/pre-S2 gene and the overlapping polymerase gene. In order to map the breakpoints of possible recombination, BootScan analysis was carried out for the KC774178 sequence. Reference strains of genotypes B and C were utilized for comparison, and genotype F was used as the outgroup control. Fig. 1(b) depicts the results of the BootScan analysis of KC774178, which revealed that the core gene, part of the pre-S1/pre-S2 gene and the overlapping polymerase gene of the sequence were substituted by the corresponding part of genotype C. The locations of the recombination breakpoints were estimated at nt 190 and 1880. In order to identify the precise subgenotypes of the parents of the recombinant fragment, the phylogenetic trees of both recombinant fragments were established and compared with each other (Fig. 2). The fragment between nt 190 and 1880 was clustered into subgenotype B2, while that between nt 1880 and 190 was clustered into subgenotype C3, indicating that the parents of the novel recombinant KC774178 were from subgenotypes B2 and C3, respectively.

Accumulating data suggest that DNA recombination is a significant and relatively frequent event in the evolution of HBV. In the last decade, C/D inter-genotype recombinants of type I (breakpoints at nt 50 and 1450) and type II (breakpoints at nt 10 and 799) have been reported from the Qinghai–Tibet Plateau (Wang et al., 2005). Moreover, in 2011, C/D inter-genotype recombinants were declared with high prevalence among chronic hepatitis B patients in China.

**Fig. 1.** (a) SimPlot result of the novel B/C inter-genotype recombinant KC774178. (b) BootScan result of KC774178. GenBank accession numbers of genotype B reference sequences are AB602818, AF282918, GQ358136, AB368295, GQ924624 and JN792902. GenBank accession numbers of genotype C reference sequences are GQ358154, GQ358158, EU939536, GQ777630 and EU410081. P, C, S and X indicate polymerase, core, surface and X genes.
north-west China, indicating that inter-genotype recombinants may play a much more important role in the spread of HBV than considered previously (Zhou et al., 2011). The existence of B/C inter-genotype recombination has also been reported in Thailand, Vietnam, Indonesia and south China, and the recombination breakpoints were located at nt 1741 and 2443 (Luo et al., 2004; Morozov et al., 2000). However, recombination breakpoints for KC774178 were at nt 190 and 1880, suggesting that a novel B/C inter-genotype recombinant HBV strain has been identified in north-west China. Further investigations are under way to clarify the geographical distribution, prevalence and virological characteristics of the novel B/C inter-genotype recombinant HBV.

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References


