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Circulation of genotype-I hepatitis B virus in the primitive tribes of Arunachal Pradesh in early sixties and molecular evolution of genotype-I

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ABSTRACT

Retrospective serologic screening of 1077 serum samples collected from the primitive tribe from north-eastern India in 1963 revealed high prevalence of HBV (15% HBsAg carrier rate) and HCV (7% anti-HCV positivity) and co-circulation of multiple HBV genotypes-A, C, D and G. Full genome sequencing classified all the G-genotype samples as genotype-I. Comparison of genotype-I-HBV full-genome sequences representing 1963 (n = 5, this study) and 2005 (reported earlier) showed identical recombination break-points of genotypes-A/G/C. Genotype-C and genotype-C-fragment of I-genotype circulating in 1963 were distinctly different. The data demonstrates that the recombination events were not recent. Molecular clock analysis predicted existence of genotype-I in this tribe during 1920s.

1. Introduction

Hepatitis B virus (HBV), a member of the Hepadnaviridae family with partially double stranded, circular DNA genome of approximately 3.2 kb in length is classified into eight genotypes from A to H (Szmaragd and Balloux, 2007). HBV genotypes are known to influence disease progression (Kramvis et al., 2005; Malmström et al., 2012; Sumi et al., 2003).The proposed 9th genotype-I, a unique recombinant of genotypes-A, C, and G has been reported from Asia (Hannoun et al., 2000; Olinger et al., 2008; Tran et al., 2008; Arankalle et al., 2010; Tong et al., 2012; Shen et al., 2014). It is intriguing to note that genotype-I viruses identified in different countries at different time points show almost identical recombination of 3 genotypes. Documentation of genotype-I in the primitive tribe from Arunachal Pradesh, north-east India is of particular interest in terms of generation and transmission of the recombinant from or to the outside world. Availability of stored serum samples collected from the same tribe in 1963 gave us an opportunity to examine if genotype-I, the unique recombinant was prevalent even 5 decades before. The present study reports circulation of genotype-I in the primitive tribe since 1963, distinct differences in the circulating genotype-C and the C-fragment of genotype-I-1963 sequences and evolution of genotype-I.

2. Materials and methods

2.1. Clinical specimens

The study was approved in 2011 by the Institutional Human Ethics Committee. Serum samples (n = 1077) collected from the tribal population of Arunachal Pradesh, north-eastern India in 1963 and stored at −20 °C till tested were used. These samples were collected from apparently healthy male adults aged 19–49 years.

2.2. Serological assays

All the samples were screened for the presence of hepatitis B surface antigen (HBsAg), total antibodies against hepatitis B core antigen (anti-HBc) and antibodies against hepatitis C virus (anti-HCV) using AXSYM system (Abbott, USA).

2.3. Detection of HBV DNA/HCV RNA

150 HBsAg positives and 55 anti-HCV positives were screened for the presence of HBV-DNA/HCV-RNA respectively as described earlier (Arankalle et al., 2010; Jha et al., 1995). Detection of HBV DNA was based on the amplification of pre-core region in nested PCR. For this, HBV DNA was isolated using QIAamp DNA blood mini kit (QIAGEN, Valencia, CA, USA) according to the manufacturer's instructions. 10 µl of the extracted DNA was mixed with the

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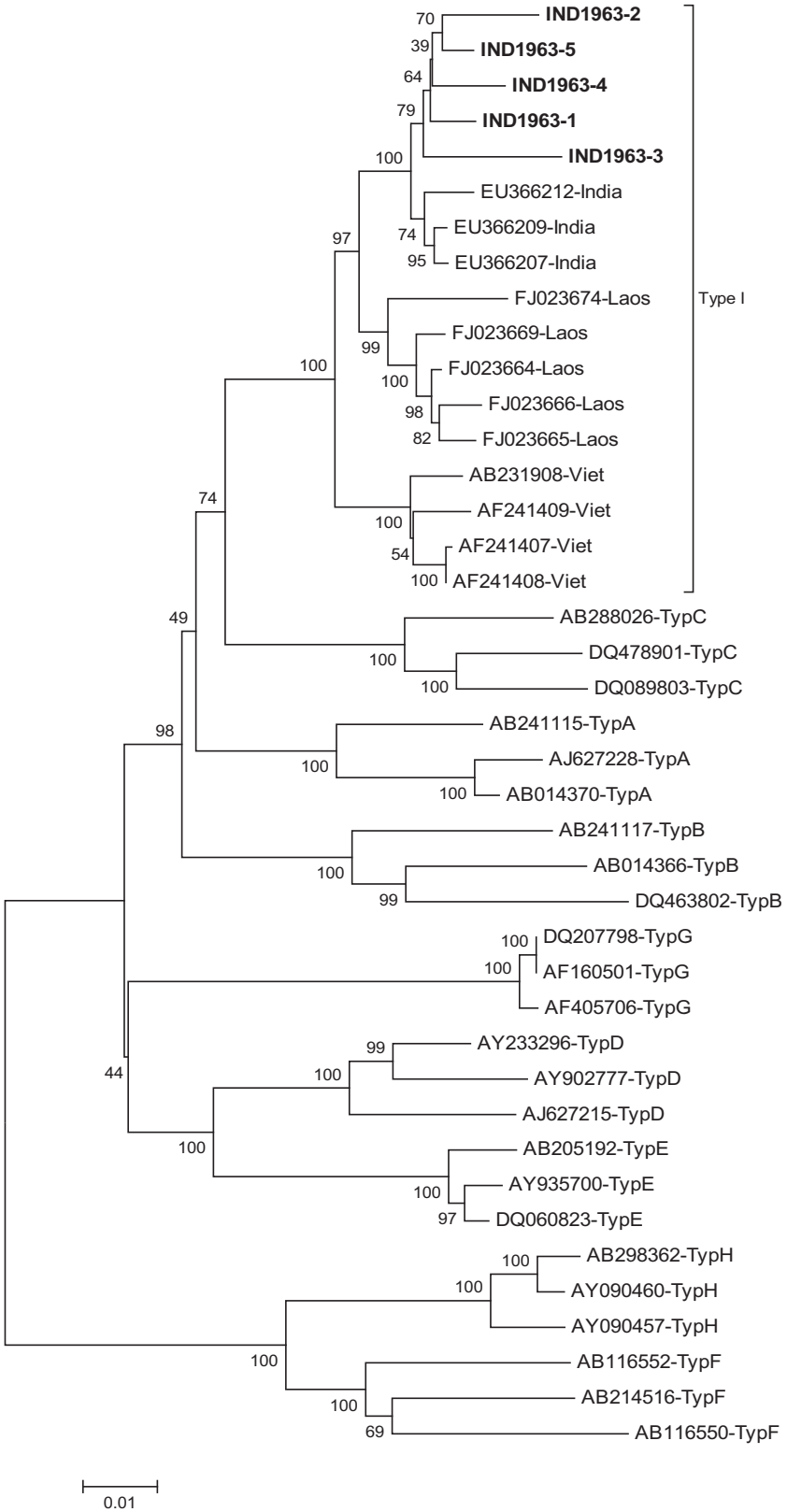

Fig. 1. Phylogenetic analysis of full genome (~3200 nucleotides) sequences of HBV isolates from Arunachal Pradesh, north-eastern India. Accession numbers for the prototype strains representing genotypes A–H, and sequences from Vietnam and Laos are provided in the figure. HBV sequences obtained during this study are denoted as IND1963-1 to 5 (Accession numbers – KF214648, KF214679, KF214680 KF214649 and KF214650). Posterior support is indicated by the values at each node.