ABSTRACT

Genotype E predominates in sub-Saharan Africa (SSA), where hepatitis B virus (HBV) is hyperendemic, with a high incidence of hepatocellular carcinoma. The fact that genotype E is rarely found outside Africa, its low genetic diversity and its presence in Neolithic strains suggest its recent introduction into Africa. In fact, the current phylogeographical analyses showed confined dispersal in Africa with localized transmission and more restricted movement outside Africa. When this study was initiated, genotype E, with unique molecular characteristics and a different natural history to other genotypes, had not been studied *in vitro*. Our major objective was to construct a 1.28-mer genotype E replication-competent clone containing endogenous promoters, for functional characterization and comparison to subgenotypes circulating in SSA. Using the consensus sequence, a clone was constructed and used to transfect Huh7 cells. Detection of cccDNA using real-time polymerase chain reaction and of replicative intermediates using Southern hybridization confirmed the construct was replication competent. Fluorescent microscopy demonstrated HBsAg and HBcAg expression. Although there were no major differences in intracellular and extracellular HBV DNA between genotype E and subgenotypes A1, A2 and D3, genotype E expressed higher levels of HBsAg and HBeAg. Western blotting showed expression of glycosylated and non-glycosylate envelope proteins, with genotype E expressing similar levels to A2 but higher than A1 and D3, with correspondingly high abundance of transcripts as shown by quantitative analysis. These *in vitro* observations correspond with the high viral loads and a high frequency of HBeAg-positivity seen in genotype E-infected individuals. A paediatric case of acute myeloid leukemia, who developed chronic hepatitis B with genotype E after chemotherapy is presented. The patient, treated sequentially with lamivudine and tenofovir, required prolonged treatment following viral breakthroughs caused by lamivudine resistance and immune-escape mutations. Using phylogenetic analysis, a blood transfusion was intimated as the transmission source. The genotype E clone, which completes the panel of African replication-competent clones, can be used for future studies, including infectivity studies and the generation of a cell line stably expressing genotype E. This is an important development considering the paucity of data on this genotype, infecting 17% of chronic carriers globally and having been neglected.