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SHORT REPORT

Identification of hepatitis B virus genotype A/E recombinants in Ghana

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Abstract

Hepatitis B virus (HBV) exhibits a high degree of heterogeneity with at least 10 genotypes (A–J) identified to date. Intergenotypic recombination is relatively common. Previously, we investigated HBV drug resistance in HIV/HBV co-infected individuals in Ghana. After identifying multiple circulating genotypes and a novel D/E recombinant, we sought to determine if additional individuals were also infected with recombinant HBV. Partial genome sequences from three individuals were initially identified as genotype A4. Full-length HBV genomes were obtained using rolling circle amplification followed by PCR and shown to cluster with known A/E recombinant viruses. Similar recombination breakpoints were observed in these three individuals suggesting local spread of this novel recombinant HBV in Ghana.

Keywords Hepatitis B virus · Genotype · Recombinant/recombination · Africa · Ghana

In spite of advances in prevention, diagnosis, and treatment, hepatitis B virus (HBV) infection remains a major global health problem, particularly in sub-Saharan Africa and East Asia where seroprevalence is highest. Globally, an estimated 257 million individuals are chronically infected with HBV, and in 2015, approximately 890,000 individuals died as a result of HBV infection [1]. HBV is the prototype member of the family *Hepadnaviridae* with a partially double-stranded circular DNA genome that is 3.2 kb in length. The genome is organized into four overlapping open reading frames, including the surface (S) or envelope gene, the core (C) gene, the regulatory X gene, and the polymerase (P) gene.

HBV exhibits a high degree of genetic heterogeneity. To date, 10 HBV genotypes (A–J) have been classified by sequence divergence of at least 8% in the entire HBV genome [2, 3]. Genotypes A–D and F have been further classified into subgenotypes with a genetic variation of 4–8% [4]. HBV genotypes and subgenotypes exhibit different clinical and therapeutic outcomes and have distinct global geographic distribution [5]. Intergenotypic recombination occurs in geographic locations where multiple genotypes co-circulate and facilitate diversification within and between individuals. Novel variants generated by recombination between different HBV genotypes have been documented worldwide [6–13].

Approximately 2.7 million HIV-infected individuals are co-infected with HBV worldwide, and HIV/HBV co-infection is a major cause of morbidity and mortality in sub-Saharan Africa [1]. Previously, we investigated the prevalence of HBV-resistant mutations in antiretroviral therapy (ART)-naïve and ART-experienced HIV/HBV co-infected Ghanaian patients on 3TC-containing regimens [14]. 58 of 63 (92.1%) individuals with available sequence data were infected with genotype E, 3 (4.8%) with genotype A4, and 1 (1.6%) each with genotype A1 and genotype D8.

The current analysis investigated the three HBV isolates initially identified by partial genome sequencing as

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belonging to subgenotype A4. HIV-positive adults attending the Fevers (Infectious Diseases) Unit at the Korle-Bu Teaching Hospital in Accra, Ghana between 2012 and 2014 were screened for HBsAg using the Core HBsAg rapid test (Core Diagnostics, Birmingham, UK) following informed consent. Table 1 shows the clinical data for these three patients. Two were male, and two were ART-experienced. HBV DNA levels were 4.56×10^7 to 1.40×10^8 IU/mL. The two treatment-experienced patients had undetectable plasma HIV RNA levels while on therapy with lamivudine/zidovudine/efavirenz, although they had high HBV DNA levels with L180M and M240V mutations. The ART-naïve patient had no HBV-resistant mutations. Based on initial amplification of a 1004 bp fragment of the HBV reverse transcriptase region,these patients clustered with genotype A4 references. Having identified multiple circulating genotypes and a novel D/E recombinant within the parent resistance study [15], we sought to determine if these three A4 viruses were also recombinants.

HBV DNA was extracted from 500 µL of patient serum using the QIAamp UltraSens Virus Kit (Qiagen, Valencia, CA, USA), and the full-length sequence was obtained using rolling circle amplification (RCA) followed by PCR [16]. Next-generation sequencing (NGS) was performed by the Genomics, Epigenomics and Sequencing Core at the University of Cincinnati as previously described [15]. Sequence reads were imported into UGENE for quality control and consensus assembly [17]. Phylogenetic inference was performed using a Bayesian Markov chain Monte Carlo (MCMC) approach as implemented in the BEAST v1.10.1 program [18] under an uncorrelated log-normal relaxed molecular clock and the general time-reversible model with nucleotide site heterogeneity estimated using a gamma distribution. The MCMC analysis was run for a chain length of 200,000,000 with sampling every 20,000th generation. Results were visualized in Tracer v1.7.1 to confirm chain convergence, and the effective sample size (ESS) was calculated for each parameter. All ESS values were > 1000 indicating sufficient sampling. The maximum clade credibility tree was selected from the posterior tree distribution after a 10% burn-in using TreeAnnotator v1.10.1. The jumping profile Hidden Markov Model program was used to investigate potential intergenotypic recombination [19]. The complete genomes of these patients were deposited in GenBank under the accession numbers KU711605–KU711607.

The phylogenetic analysis initially included 84 full-length HBV references, as well as known A/E recombinants. The full-length sequences for these three individuals—006N, 125E, and 218E—clustered together other genotype A references and separate from subgenotypes A1 and A2 (data not shown). Additional genotype A references were downloaded from http://hvdr.bioinf.wits.ac.za/alignments/index.html [20]. As shown in Fig. 1a, the sequences from 006N, 125E, and 218E clustered together with two isolates from Guinea (GQ161753) and France (HF571060). The consensus sequences obtained after RCA and NGS were compared to the previously reported partial sequence amplified via traditional PCR and Sanger sequencing for identification of HBV drug-resistant mutations. The independent amplifications with two different strategies gave identical sequence data, demonstrating the reproducibility of our findings and suggesting that dual infection is unlikely.

Further investigation of the recombination patterns of these viruses indicated similar breakpoints in all three patients (Fig. 1b). A similar recombination pattern was observed in GQ161753 (Fig. 1c). Together, these four viruses are largely genotype A but have breakpoints at nucleotides ~ 2100 and ~ 2400 in the core gene where the viruses contain genotype E sequences. The recombination pattern in HF571060 was distinct. It was also predominantly genotype A; however, it had a larger region of genotype E spanning the entire core gene (nucleotides 1800–2400). Other viruses in the same cluster within the phylogenetic tree—GQ161813, KM606737, AM180623, AY934763, and AY934764—were non-recombinant genotype A viruses (data not shown).

Intergenotypic recombinant HBV genomes have been reported globally (reviewed in [12]). In West Africa, A/B, A/C, A/E, C/E, D/E, and D/E/A recombinants have been identified [6–8, 13, 15, 21–26]. Recombination is an important element of HBV genetic variability, and knowledge of HBV circulating genotypes—and perhaps recombination—may enable clinicians to predict the response to treatment and disease progression. Of note, the recombination breakpoints identified in these three individuals from Ghana align with genomic hotspots found in a systematic

Table 1 Demographic and clinical information for the three individuals included in this study

Patient ID	Gender	Age	ART status	CD4 count	HB e antigen	HB e antibody	HBV DNA (IU/mL)
006N	Female	55	Naïve	382	+	–	45,567,378
061E	Male	43	Experienced	775	+	–	47,794,228
125E	Male	50	Experienced	926	+	–	140,456,193

ART antiretroviral therapy

review of previously reported HBV recombinant viruses [12]. The clustering of these sequences with similar breakpoints suggests that this novel recombinant A/E virus is circulating within Ghana rather than representing a single recombination event. Additionally, the two ART-experienced patients had lamivudine failure despite evidence of good ART adherence. Whether this recombinant

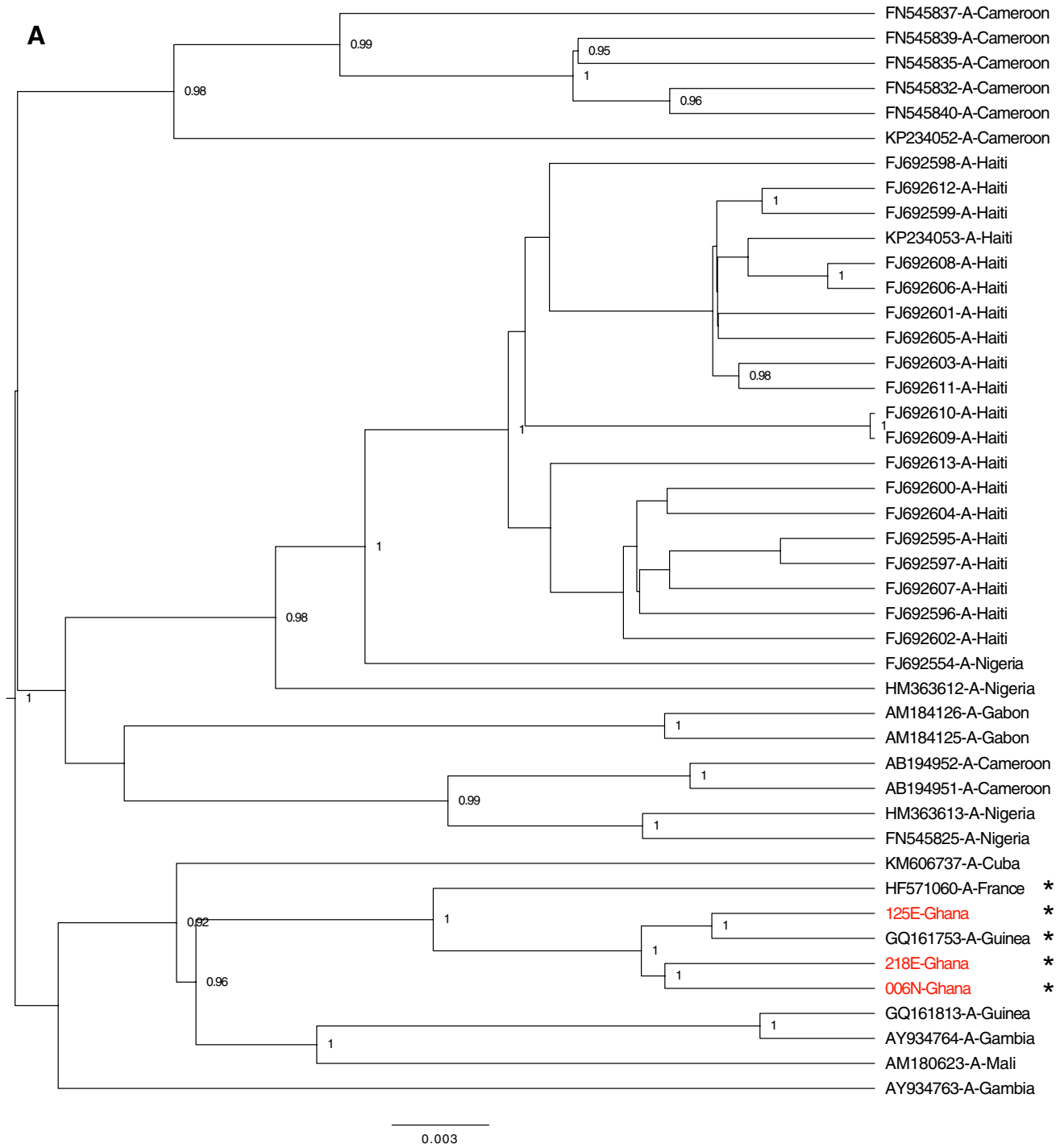

Fig. 1 Phylogenetic and recombination analyses of full-length HBV sequences. **a** The phylogenetic tree was constructed with study sequences 006N, 218E, and 125E highlighted in red and full-length HBV genotype A references. Asterisks denote genotype A/E recombinant viruses. **b** and **c** Jumping profile genome maps were created for the three study sequences, as well as two known A/E recombinant viruses, that clustered near the study sequences to determine the recombination break points

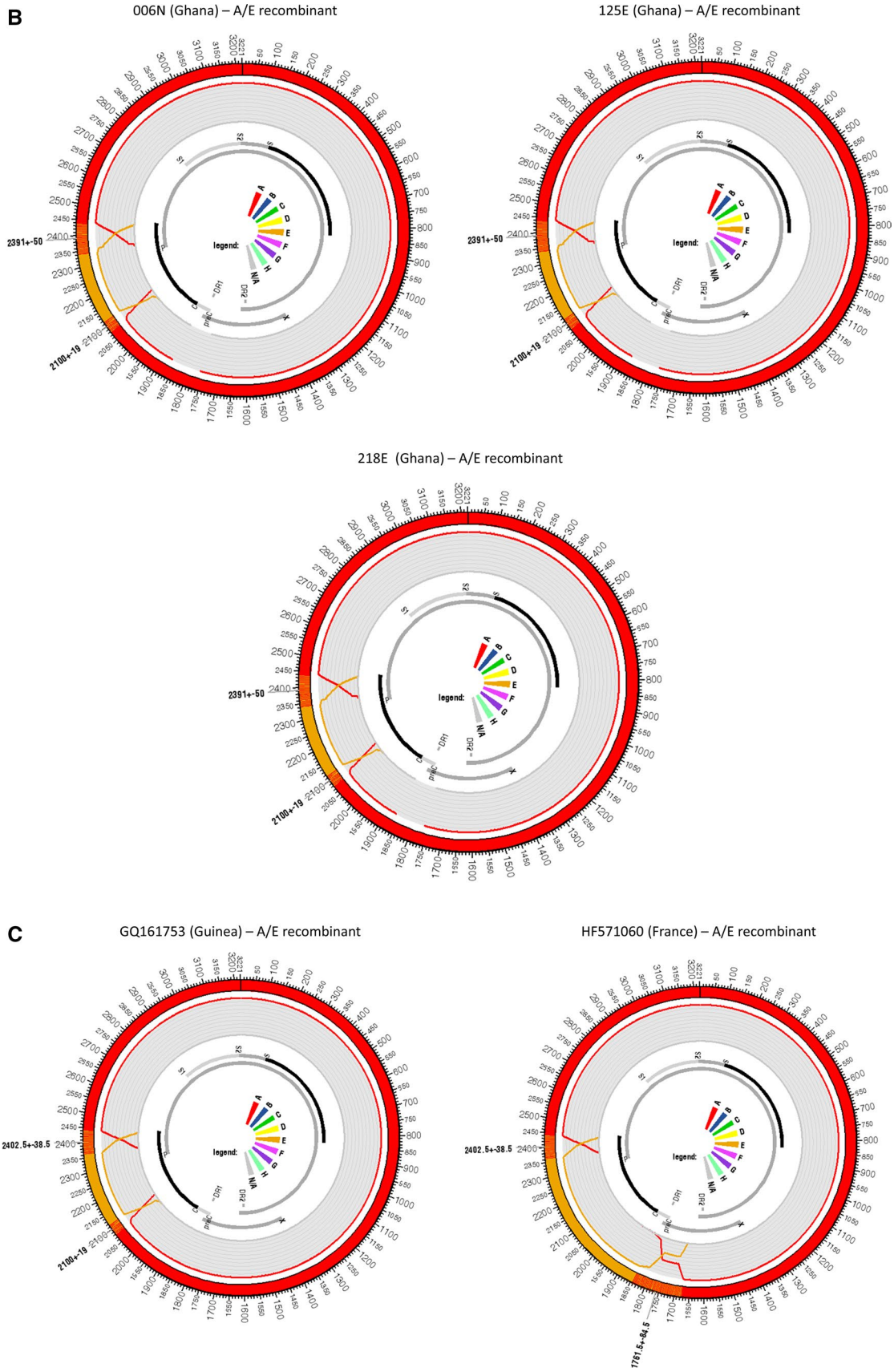

Fig. 1 (continued)

will respond adequately to tenofovir-based ART requires further investigation.

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Compliance with ethical standards

Conflict of interest The authors have no financial disclosures relevant to this article and declare no other conflicts of interest.

Ethical approval The Institutional Review Board (IRB) of the University of Ghana Medical School, Accra, Ghana and Lifespan Hospitals, Providence, Rhode Island reviewed and approved the study. All procedures performed in studies involving human participants were in accordance with the Ethical Standards of the Institutional and/or National Research Committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent All participants provided written informed consent for participation in the parent study of HBV/HIV co-infection.

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