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In response to identification of a quadruple mutation that confers tenofovir resistance in chronic hepatitis B patients

To the Editor:

Park *et al.* recently reported a quadruple mutation that conferred resistance to tenofovir in South Korean patients with chronic hepatitis B virus (HBV) infection.[1] In 2 patients who experienced viral breakthrough during treatment, the CYEI mutation within the HBV reverse transcriptase (RT) gene resulted in resistance to tenofovir with IC_{50} and IC_{90} values of 15.3- and 26.3-fold higher than those of wild-type virus, respectively. A triple mutation (CYE) also reduced tenofovir susceptibility by 3.7-fold. Because tenofovir resistance is rarely reported in clinical practice, we evaluated the presence of this mutation utilizing a large database of publicly available HBV sequences.

Full-length HBV genome references were downloaded from http://hvdr.bioinf.wits.ac.za/alignments/index.html.[2] This included 4,244 unique sequences including 508 from genotype A, 1,000 from genotype B, 1,543 from genotype C, 727 from genotype D, 230 from genotype E, 170 from genotype F, 20 from genotype G, 17 from genotype H, and 29 from genotype I. Babylon Translator was then used to trim genotype A to I amino acid sequences corresponding to the RT only.[3] Sequences were aligned using Clustal X 2.1, and the relevant amino acid positions were visualized in AliView 1.18.1 including rtS106C, rtH126Y, rtD134E, and rtL269I (with the quadruple mutation having the combined amino acid sequence CYEI).

As shown in Fig. 1, there was considerable amino acid variability at these 4 positions across the published sequences. At position 106, the most common amino acid was S (n = 4,154; 97.9%). However, other amino acids were also present including C (n = 79), P (n = 5), A (n = 2), X (n = 2), T (n = 1), and Y (n = 1). At position 126, H was the most common amino acid (n = 3,206; 75.5%), although other amino acids included Y (n = 850), R (n = 179), Q (n = 3), C (n = 2), L (n = 1), N (n = 1), P (n = 1), and X (n = 1). At position 134, the most common amino acid was D (n = 3,276; 77.2%) with other amino acids including N (n = 805), E (n = 58), S (n = 45), H (n = 21), B (n = 10), I (n = 9), G (n = 5), X (n = 5), Y (n = 5), A (n = 1), C (n = 1), Q (n = 1), T (n = 1), and V (n = 1). At position 269, I (n = 3,452; 81.3%) was the most common amino acid; others included L (n = 776), deletion (n = 8), F (n = 2), V (n = 2), P (n = 1), S (n = 1), T (n = 1), and X (n = 1).

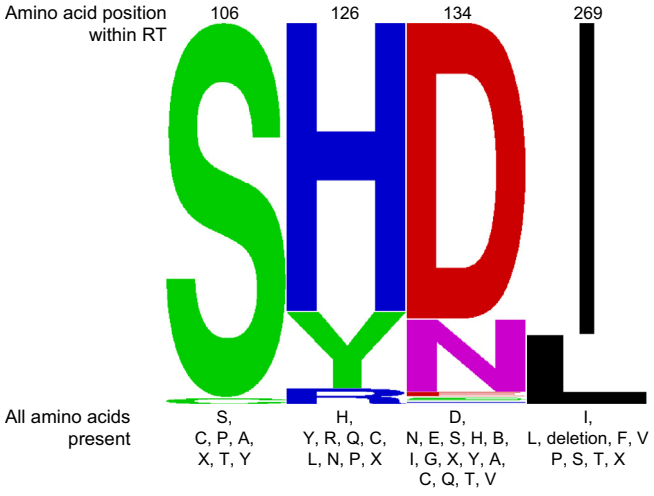

Fig. 1. Amino acid variation witin the reverse transcriptase protein. (This figure appears in colour on the web.)

Among the 4,244 HBV sequences evaluated, there were no sequences with the triple mutation CYE. However, one sequence (accession number DQ089802) had the quadruple mutation CYEI. This isolate (HK1371) was first reported by Chan *et al.* from a Chinese patient chronically infected with HBV genotype C prospectively followed in a Hepatitis Clinic in Hong Kong.[4] Individuals in the cohort were recruited between 1997 and 2000 and were excluded if they were treated with antiviral agents or had evidence of hepatitis C virus infection or hepatocellular carcinoma.[5] The HIV status of individual HK1371 and the risk factor(s) for infection were not reported.

Tenofovir disoproxil fumarate (TDF) – trade name Viread® – was approved by the United States Food and Drug Administration (FDA) on October 26, 2001 for treatment of HIV. The fixed-dose combination of tenofovir with emtricitabine was approved on February 8, 2004. TDF was approved by the Chinese FDA for the treatment of chronic HBV in adults and pediatric patients >12 years of age in October 2013.[6] Thus, the presence of the CYEI mutation in HK1371 arose spontaneously prior to the use of TDF in China. The quadruple CYEI mutation reported recently by Park *et al.*[1] is quite rare (0.024%). However, because this mutation can arise spontaneously in the absence of TDF, its presence should be evaluated in individuals with a suboptimal response to TDF, as well as in those with viral breakthrough despite good adherence. Polymorphisms at the rt106, rt126, and rt134 positions have been reported but not functionally characterized in nucleos(t)ide-treatment-naïve patients with chronic HBV infection.[7]

This re-analysis of existing sequence data suggests that the CYEI mutation can occur in people with no exposure to tenofovir. Multiple factors can shape viral diversity, including human genetic variability, co-infections, race, gender, and immunologic selection pressures such as antibody responses, cell-mediated responses, and vaccine-induced immune pressures. Given the overlap between HBV open reading frames (ORFs), mutations with the S ORF may lead to the development of drug resistance mutations within the P ORF. While a combination of 4 mutations is statistically unlikely in a treatment naïve individual, it is clearly possible in rare instances.

Conflicts of interest

The authors declare no conflicts of interest that pertain to this work. Please refer to the accompanying ICMJE disclosure forms for further details.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2019.07.010.

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Reply to: "In response to identification of a quadruple mutation that confers tenofovir resistance in chronic hepatitis B patients."

To the Editor:

We thank Drs. Blackard, Kwara, and Sherman for their interest in our article.[1] The error rate of hepatitis B virus (HBV) polymerase is high (approximately 1 per 10^5 to 10^7 base syntheses).[2] Due to the highly error-prone nature of HBV reverse transcriptase (RT), numerous combinations of amino acid substitutions are generated during viral replication. Among them, some quasispecies with the highest viral fitness are selected and become predominant clones under selective pressure, such as antiviral treatment or host immunity.

In the Letter, Blackard *et al.* emphasized that among 4,244 evaluated HBV sequences, one sequence, which was derived from an antiviral-naïve Hong Kong patient, showed a quadruple substitution (rtS106C [C], rtH126Y [Y], rtD134E [E], and rtL269I [I]) that was previously proven to confer tenofovir resistance in 2 heavily treated patients.[1] Considering the fidelity of HBV RT is sufficiently low to produce large pools of HBV diversity and the replication capacity of a CYEI mutant is even higher than wild-type HBV under no selective pressure (as shown in our study[1]), detecting a CYEI mutant from antiviral-naïve patients may be possible. However, before establishing a complete CYEI mutation, other intermediate mutations (*e.g.*, CYE mutants) that have a very low replication capacity compared with wild-type HBV might have difficulty overcoming selective pressure. Thus, finding a CYEI mutation may be more difficult than wild-type HBV in antiviral-naïve patients. Collectively, the Letter confirmed that in viral evolution, drug-resistant mutants are only selected from numerous preexisting pools of HBV diversity, and not generated by specific selection pressure. In this regard, we agree that the presence of a CYEI mutation is highly suspicious when a patient shows either suboptimal response or non-response to tenofovir treatment, even in patients who did not receive prior antiviral treatment. In addition, the substitution of polymorphic as well as conserved sites in RT should be suspected of causing antiviral resistance, when considering that all 4 codons of CYEI mutation are polymorphic sites according to the previous definition (>1% variation).[3] In the same context, several patients who showed viral breakthrough or persistent viremia during 96 weeks of tenofovir alafenamide treatment had 1 or more substitution at these 4 sites.[4]

Another notable finding stated in the Letter was that the amino acid I at codon rt269 was the most common amino acid (81.3%) in HBV of various genotypes. However, according to our previous study,[5] most of the Korean antiviral-naïve patients (95.5%, 21 of 22) harbored the amino acid L at that codon at baseline. The rtL269I substitution was observed along with a YMDD mutation following phenotypical antiviral resistance. These clinical data indicate that YMDD + rtL269I was selected due to antiviral pressure. The dominant HBV in Korea is genotype C2. Therefore, the rtL269I substitution is predominant in other genotypes except genotype C. Further investigation of the population with rtL269I substitutions, using a large cohort of genotype C patients, would be informative. Since the YMDD mutant is replication defective, we showed that the rtL269I substitution is associated with markedly restored replication capacity in the multidrug-resistant mutant.[5] This substitution resulted in approximately 7-fold higher replication ability on a drug-resistant HBV backbone, however, drug resistance was not conferred. Molecular modeling indicated that the rtL269I substitution may increase polymerase activity *via* structural change.[5] Based on the results from our 2 studies[1,5], rtL269I is a compensatory mutation for low replicative HBV with antiviral resistance at least in genotype C HBV. Viral fitness is apparently acquired by the rtL269I substitution and sufficiently important to be selected as a dominant clone under TDF pressure. Our results indicate the resistance barrier to TDF may be lower in other genotypes than in genotype C.