Quadruple Recombination Events Discovered in Hepatitis E Virus

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Abstract

Hepatitis E virus (HEV) can infect humans, pigs, and many other animals, but the recombination of HEV has rarely been reported. In the present study, phylogenetic and recombination analyses were performed on 557 complete HEV genomes in GenBank. A potentially significant quadruple recombination event was identified by recombination detection analysis. The recombinant progeny virus HEV_32_Manchester_301214 was produced by recombination between the major parent HEPAC-44 and the minor parent HE-JA15-1335, thereby reflecting inter-genotype recombination. HEV_32_Manchester_301214 and HEPAC-44 belong to genotype 3, while HE-JA15-1335 belongs to genotype 1, and these three strains have all been separated from humans. Three breakpoints of the four recombination events occurred in the ORF2 region, while another occurred in the ORF1 region. This quadruple recombination event was confirmed by phylogenetic analysis. The genotype, host and the recombination regions of the three strains were analyzed. These results of the analyses provide valuable suggestions for future research on HEV diversity.

Introduction

Hepatitis E virus (HEV), a member of the *Hepeviridae* family, has a genome that is positive-sense single-stranded RNA of nearly 7.2 kb in length with three partially overlapped open reading frames (ORFs). HEV virions have a diameter of 27–34 nm[4]. According to the pairwise distances of entire viral genomes, eight genotypes have been discerned within the species Orthohepevirus A (HEV-1 to HEV-8), but only HEV genotypes 1 to 4 and HEV genotype 7 have shown a clinical relevance in humans. HEV-1 and HEV-2, which are mainly transmitted by the fecal-oral route, have been responsible for large HEV outbreaks and epidemics in some countries. HEV-3 spreads worldwide among pigs, and humans most frequently become infected with it by eating undercooked meat or haslet, while HEV-4 is mainly confined to China and has only recently demonstrated a tendency to spread to Europe [3, 13].

Recombination occurs between divergent positive-sense RNA viruses [9, 15, 16], and is a common phenomenon [7, 9, 11, 14-16]. However, the recombination of HEV has rarely been reported. In the present study, recombination events among HEV strains were investigated using the available 557 complete HEV genome sequences in GenBank, interesting recombination phenomenon of HEV was discovered.

The sequences were first screened to exclude artificial and patented mutants. Then, the remaining sequences were aligned in the MAFFT 7.311 program. The aligned sequences were analyzed with Recombination Detection Program 4 (RDP4) [8] using the RDP, GENECONV, BootScan, MaxChi, Chimaera, SiScan, and 3Seq detection methods.

The RDP4 analysis results revealed an interesting phenomenon of virus recombination, namely that an HEV-3 strain from the United Kingdom, HEV_32_Manchester_301214, has undergone four recombination events, which always occurred between the strain HEPAC-44 as the major parent and the strain HE-JA15-1335 as the minor parent (Figure 1). Although a number of recombinants were found, there is only one

quadruple recombination. The major parental strain HEPAC-44 is a French genotype 3 strain that was isolated from the blood of a patient, while the minor parental strain HE-JA15-1335 is a Japanese genotype 1 strain that was isolated from autochthonous human serum. RDP analysis revealed that the recombinant virus HEV_32_Manchester_301214 and the minor parent HE-JA15-1335 shared 99.1% sequence identity over the recombinant region of Event 1, 100% over Event 2, 97.9% over Event 3, and 98.8% over Event 4. Similarly, HEV_32_Manchester_301214 and the major parent HEPAC-44 shared 94.5% sequence identity over Event 1, 95% over Event 2, 95.5% over Event 3, and 95.9% over Event 4. In addition, the SimPlot program was used for analysis to examine the results. The SimPlot analysis suggested that the major parental strain clustered with the minor parental strain, soon after which they separated from each other four times, which indicates the existence of a quadruple recombination event.

To further confirm the existence of quadruple recombination, the genomes were cut into recombinant regions in Events 1, 2, 3, and 4, as well as their neighbored non-recombinant regions. Then, the relevant strains were analyzed by maximum likelihood (ML) trees using MEGA7 software to construct respective phylogenetic trees. Figures 2A and 2B present the ML trees constructed using the recombinant region (6207-6552 nt) and non-recombinant region (6553-7052 nt) to confirm Event 1, while Figs. 2C (4242-4526 nt), 2D (3742-4241 nt), 2E (5323-5652 nt), 2F (4823-5322 nt), 2G (5868-6038 nt) and 2H (5653-5867 nt) respectively confirm Events 2, 3 and 4.

The recombinant virus HEV_32_Manchester_301214 was found to cluster closely with the minor parent HE-JA15-1335 in the recombinant region, whereas it kept away from HE-JA15-1335 and clustered closely with the major parent HEPAC-44 in the non-recombinant region. Thus, the phylogenetic analysis demonstrated the existence of Event 1. Event 2 was confirmed by a similar phylogenetic analysis, as presented in Figs. 2C and 2D, while Figs. 2E-H respectively confirm Events 3 and 4 in the same manner. Thus, the existence of quadruple recombination between HEPAC-44 and HE-JA15-1335 was clearly confirmed by the results of the phylogenetic trees.

Although viral recombination is a common phenomenon in RNA viruses, the quadruple recombination of HEV is very rare. Double recombination events have been reported to exist in HEV [14]; the recombinant swCH31 was produced by both intra- and inter-genotype recombination, which occurred among three potential parental strains belonging to two different genotypes. In this study, although the quadruple recombination event was found to occur between viruses from hosts of the same species, HEV actually has a very broad host range, and there have been several reports that human infections originate from different host species, including swine [10], rabbits [7], etc. Therefore, the detection of HEV should be strengthened and precautions should be taken to prevent the further transmission of the virus between any animals.

Recombination can also impact virus-host interactions and relate to pathogenesis [12]. The ORF2 region of HEV encodes capsid proteins, which contains antigenic regions, so the recombination events that occur in ORF2 may be beneficial to the resistance of the host immune system [1, 2]. Previous studies have shown that the recombination of the ORF2 region is likely to strengthen the virulence of HEV [14].

The specific binding between the RdRp region of the virus and the 3' end of the HEV RNA directs the synthesis of complementary-strand RNA, which may serve as a possible cis-acting element as a potential source of replication [5, 6]. The present analysis demonstrated that Event 2 occurred in the RdRp region of ORF1, while Events 1, 3, and 4 occurred in the ORF2 region of the complete HEV genome. Therefore, this quadruple recombination is likely to lead to replication mutation, and it may be closely related to the evolution of HEV virulence. Moreover, the variation of the viral capsid protein will also change the viral antigen, which will possibly make vaccines ineffective and introduce difficulties to the prevention and control of HEV. Hence, the phenomenon of the multiple recombination of viruses should be seriously considered.

All available HEV complete genomes were analyzed with detailed phylogenetic and recombination analytic methods and we found the recombinant HEV_32_Manchester_301214, which was proved to be produced by a quadruple recombination event. The quadruple recombination events occurred in ORF1 and ORF2 regions and may strengthen the virulence of HEV. The quadruple recombination event will lead to a better understanding of virus recombination and is worthy to do further research.

Declarations

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Author contributions

Shen Hongxing and Wang Hua designed this work. Liu Shuning, Shen Hao, Ding Min and Gu Haixia performed the analyzed the data. Chang Ming, Liu Shuning, Li Yanshuang and Shen Hao wrote the manuscript. Shen Hao and Liu Shuning contributed equally to this work and should be considered co-first authors.

Ethics declarations

Conflict of interest

The authors declare that they have no conflicts of interest.

Ethical approval

The research reported here did not involve experimentation with human participants or animals.

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Figures

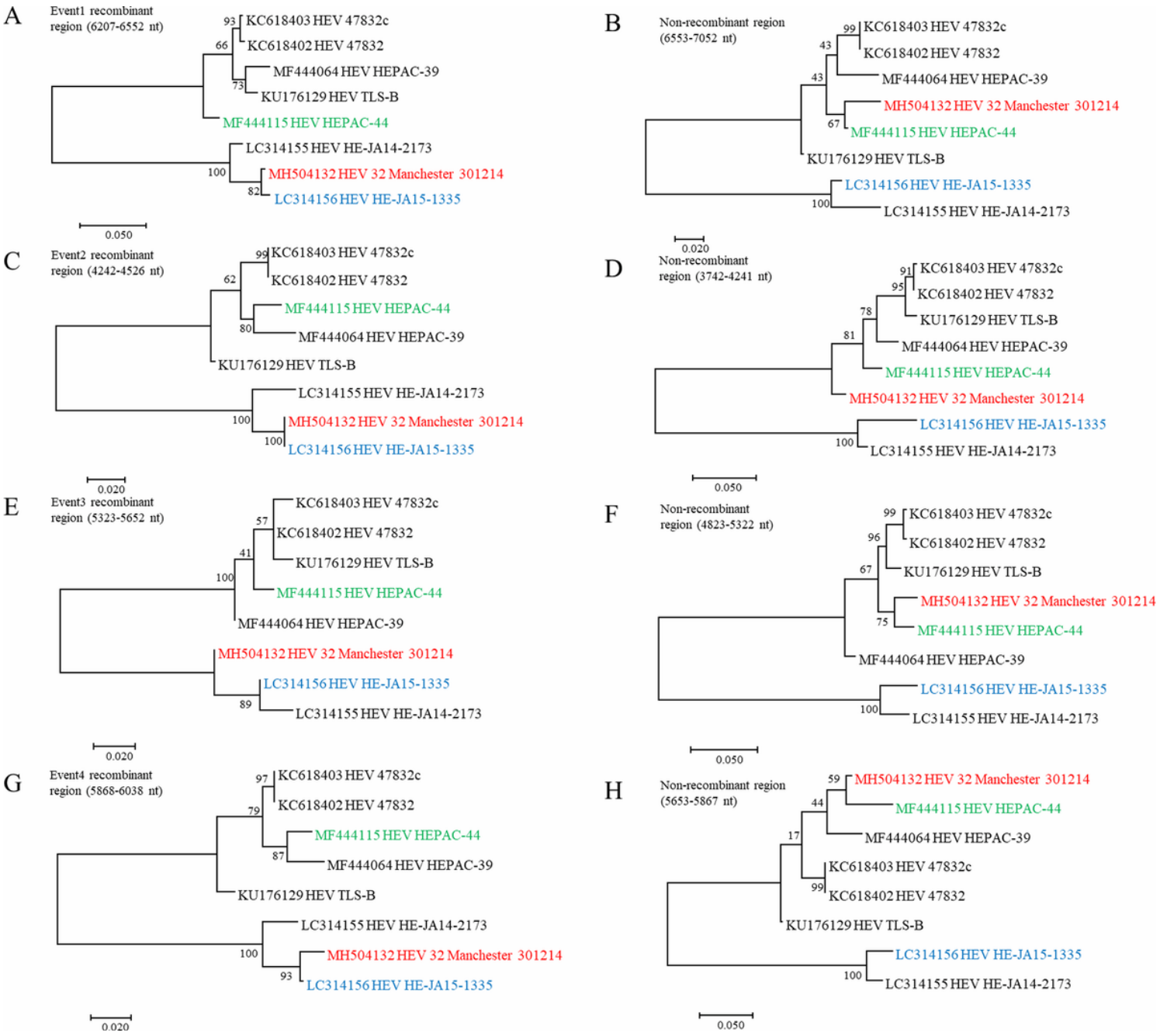

Figure 1

BOOTSCAN evidence for the quadruple recombination origin on the basis of pairwise distance, modeled with a window size 200, step size 20, and 100 Bootstrap replicates.

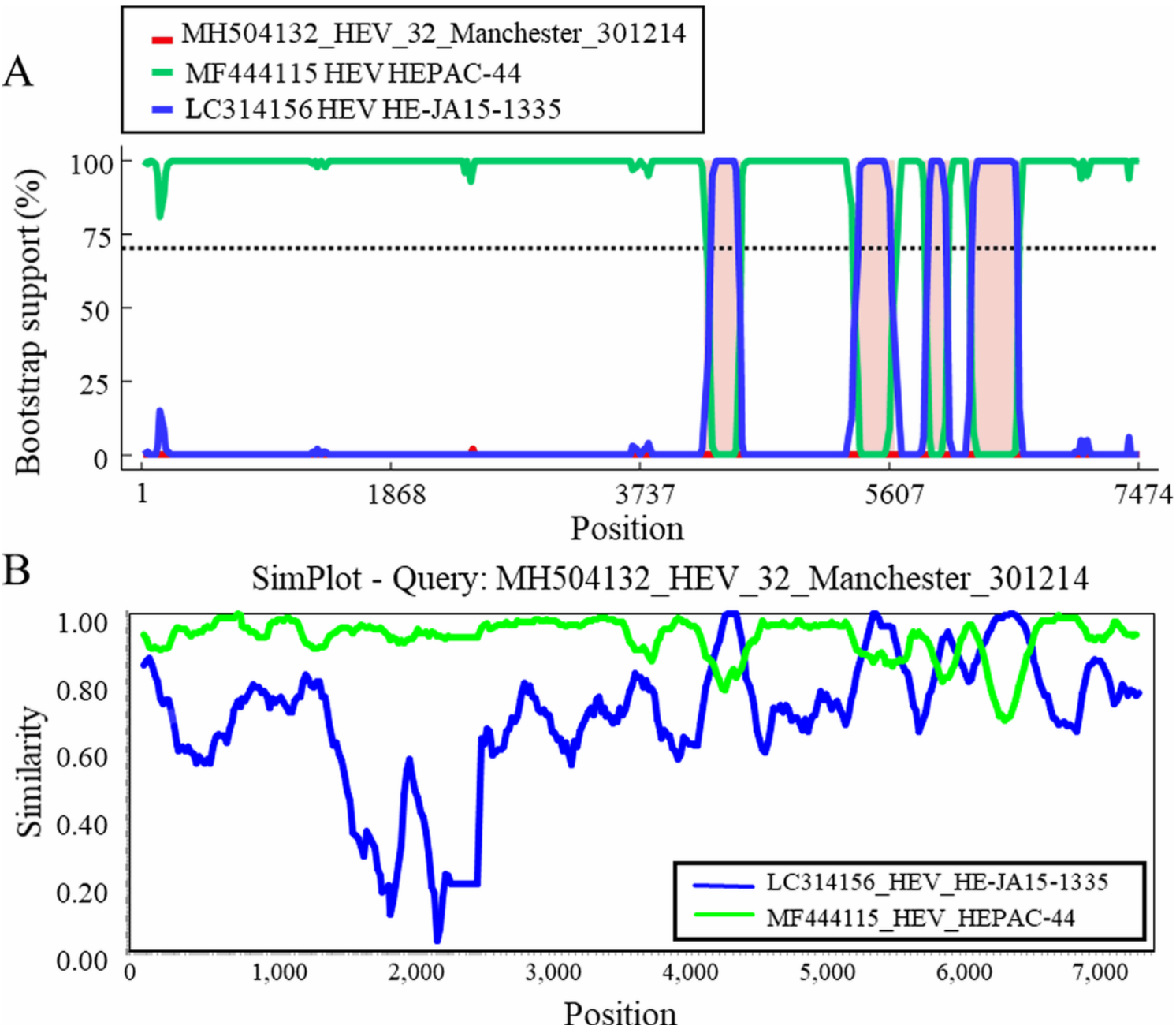

Figure 2

Phylogenetic tree analysis of the recombinant virus sequences and potential parental sequences. The recombinant, the major parent and the minor parent were labeled red, green and blue respectively. The phylogenetic tree A, B, C, D, E, F, G and H were constructed employing the Maximum likelihood method based on 1,000 replicates, using MEGA7 software.