BRIEF REPORT

A quadruple recombination event discovered in hepatitis E virus

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Abstract
Hepatitis E virus (HEV) can infect humans, pigs, and many other animals, but recombination in HEV has rarely been reported. In the present study, phylogenetic and recombination analysis was performed on 557 complete HEV genome sequences from the GenBank database. A potentially significant quadruple recombination event was identified by recombination detection analysis. The recombinant progeny virus, HEV_32_Manchester_301214, was produced by inter-genotype recombination between the major parent HEPAC-44 and the minor parent HE-JA15-1335. HEV_32_Manchester_301214 and HEPAC-44 belong to genotype 3, while HE-JA15-1335 belongs to genotype 1, and these three strains were all isolated 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 recombination regions of the three strains were analyzed, and the analysis results provide valuable information for future research on HEV diversity.

Hepatitis E virus (HEV), a member of the family Hepe-viridae, has a genome comprising a positive-sense, single-stranded RNA molecule of nearly 7.2 kb in length with three partially overlapping open reading frames (ORFs). HEV virions have a diameter of 27–34 nm [4]. Based on pairwise comparisons of complete viral genome sequences, eight genotypes have been discerned within the species Ortho-hepevirus A (HEV-1 to HEV-8), but only HEV genotypes 1 to 4 and 7 have shown 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 offal, 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, recombination has rarely been reported in HEV. In the present study, recombination events between HEV strains were investigated using the 557 complete HEV genome sequences available in the GenBank database, and an interesting recombination phenomenon 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 subsequently 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 (Fig. 1). Although a number of recombinants were found, only one quadruple recombination was identified.
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 occurrence of a quadruple recombination event.

To further confirm the quadruple recombination, the genome sequences were divided into recombinant regions in events 1, 2, 3, and 4, as well as their neighboring non-recombinant regions. Then, the relevant strains were analyzed by the maximum-likelihood (ML) method using MEGA7 software to construct separate phylogenetic trees. In Figure 2, panels A and B show ML trees constructed using the recombinant region (nt 6207-6552) and non-recombinant region (nt 6553-7052) to confirm event 1, while panels C (nt 4242-4526), D (nt 3742-4241), E (nt 5323-5652), F (nt 4823-5322), G (nt 5868-6038), and H (nt 5653-5867) confirm events 2, 3, and 4, respectively.

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 was separate from HE-JA15-1335 and clustered closely with the major parent HEPAC-44 in the non-recombinant region. Thus, the phylogenetic analysis demonstrated the occurrence of event 1. Event 2 was confirmed by a similar phylogenetic analysis, as presented in panels C and D of Figure 2, while panels E-H confirm events 3 and 4 in the same manner. Thus, the occurrence of a quadruple recombination event between HEPAC-44 and HE-JA15-1335 was clearly confirmed by the results of the phylogenetic analysis.

Although viral recombination is a common phenomenon in RNA viruses, quadruple recombination in HEV is very rare. Double recombination events have been reported 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. Although the quadruple recombination event in this study 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 indicating that human infections originate from different host species.
A quadruple recombination event in hepatitis E virus including swine [10] and rabbits [7]. Therefore, the surveillance of HEV should be intensified, and precautions should be taken to prevent the further transmission of the virus among animals.

Recombination can also impact virus-host interactions and affect pathogenesis [12]. The ORF2 region of HEV encodes capsid proteins, which contain antigenic regions, so the recombination events that occur in ORF2 may contribute to escape from the host immune response [1, 2]. Previous studies have shown that recombination in the ORF2 region is likely to increase the virulence of HEV [14]. Specific binding between the viral RdRp 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 origin 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 event is likely to affect viral replication and might be involved in changes in virulence. Moreover, variations in the viral capsid protein also change the viral antigen, which might make vaccines ineffective and make the prevention and control of HEV more difficult. Hence, the phenomenon of multiple recombination is of potential importance.

All available complete HEV genome sequences were analyzed in detail using analytical methods for examining phylogeny and recombination, and the recombinant HEV_32_Manchester_301214 was identified as resulting from a quadruple recombination event occurring in the ORF1 and ORF2 regions. Analysis of quadruple recombination events will lead to a better understanding of virus recombination, and such phenomena are worthy of further research.

Fig. 2 Phylogenetic analysis of the recombinant virus sequences and potential parental sequences. The recombinant, the major parent, and the minor parent are labeled in red, green, and blue, respectively. The phylogenetic trees in panels A-H were constructed using MEGA7 software by the maximum-likelihood method with 1,000 bootstrap replicates.

Author contributions HS designed this research. SL, HS, MD, HG, and HW performed the data analysis. HS, MC, SL, and XB wrote the manuscript.

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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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