Sequencing the Whole cDNA of Hepatitis E Virus from Changchun

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Abstract: Through analyzing the whole cDNA sequence of the sporadic Hepatitis E virus gene and comparing it with other genotypes, we can understand its variability. We used RT-nPCR to assay the HEV RNA of a patient who was diagnosed with sporadic hepatitis E virus. We designed the primers according to different segments respectively to amplify the whole gene, and sequenced the product. The whole cDNA of HEV contains 7139bp, which consists of 5′-UTR (nt 1-9), ORF1 (nt 10-5130, 1706aa), ORF2 (nt 5127-7151, 674aa), ORF3 (nt 5155-5499, 114aa), and 3′-UTR (nt 7152-7193). The identity of nucleic acid between it and genotype I, genotype II, genotype III, genotype IV is 73.2 %, 74.1 %, 72.4 %, 73.95 %, 75.2 % and 83.9 %, 85.6 %, respectively. Analyzing the phylogenetic tree showed that it is relevant to HEV genotype IV line. Sporadic hepatitis E virus in Changchun is HEV IV, and it has higher heterogeneity.

Key words: Hepatitis E virus; Isogenesis of nucleic acid; Sequence mensurating.

1.2 PCR

1.4 PCR

Genotype 4 (AJ 272108) HEV JRA1 (AP 003430) 40 80 °C

10d 40 2d 40

25. 9IU/L GGT 110. 0IU/L TBA 36. 4μ mol/L ALT

DBIL 17. 4μ mol/L TBA 26. 4μ mol/L CDH 10. 3

IU/L LP 46. 5IU/L HEV RNA 5. 4μL HEV RNA

(100ng/μL) 0. 4μL dNTP (40mmol/L) 1. 4μL, 4μL,

3 μL HEV RNA 5. 4μL 1. 4μL

65 °C min, 2 °C min, 95 °C min, 20 °C
15 min PCR: 10×PCR buffer (10 mM Tris-HCl, 50 mM KCl, 0.1% Tween-20), 5 μL dNTP (2.5 mM/μL), 5 μL primer mix, 0.5 μL Taq DNA polymerase, 5 μL cDNA, 5 μL MgCl₂ (1.5 mM), 5 μL 0.5 U Taq DNA polymerase, and 5 μL sterilized water were added to the reaction. The reaction was incubated for 5 min at 95 °C to activate the DNA polymerase enzyme, followed by 35 cycles of 95 °C for 30 s (denaturation), 55 °C for 30 s (annealing), and 72 °C for 30 s (extension). After the 35 cycles, the reaction was incubated for 10 min at 72 °C for final extension. The PCR products were analyzed on 1.5% agarose gels.

Table 1 The structure comparison of different HEV genotype

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>ORF1</th>
<th>ORF2</th>
<th>ORF3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype I</td>
<td>1693</td>
<td>660</td>
<td>123</td>
</tr>
<tr>
<td>Genotype II</td>
<td>1691</td>
<td>659</td>
<td>124</td>
</tr>
<tr>
<td>Genotype III</td>
<td>1698-1700</td>
<td>660</td>
<td>122</td>
</tr>
<tr>
<td>Genotype IV</td>
<td>1684-1707</td>
<td>671-674</td>
<td>112-114</td>
</tr>
</tbody>
</table>
2.2 HEV-CCC20 32 HEV IV

ORF1, ORF2, ORF3, HEV-CCC20, HEV IV

HEV IV 日本 JAK-Sai (AB074915) 日本 JKK-Sap (AB074917) 日本 JSN-Sap (AB091395)

China T1 (AJ272108) CCC20

1 HEV ORF1 ORF2 ORF3 HEV-CCC20 HEV IV

Fig. 1 Phylogenetic tree (NJ) on the bases of complete ORF 1, 2, and 3 nucleotide sequence

The bifurcation nodes with a bootstrap score (1000 times) greater than 90% were indicated by asterisk. Accession number for each isolate is in the parenthesis.


