Genetic variability within the VP1 coding region of echovirus type 30 isolates

Brief Report

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Summary. Genetic relationships of the prototype Bastianni strain of 1958 and of 13 echovirus type 30 (ECV30) isolates associated with meningitis cases in Germany during a period from 1966 to 1997 were investigated using direct sequencing of amplicons derived from a part of the capsid protein VP1 gene. Sequences were aligned both with each other and with known sequences of other type 30 echovirus strains. Phylogenetic analysis indicated that isolates investigated in this work fell into at least three genetic clusters apart from the prototype Bastianni strain. This suggests that genetically distinct groups of ECV30 variants have developed over time.

The genus Enterovirus within the family Picornaviridae consists of positive-stranded RNA viruses, comprising polioviruses, coxsackieviruses A and B, echoviruses, and the numbered enteroviruses 68–71. So far, a total of 64 immunologically distinct serotypes are known to cause infections in humans, ranging from asymptomatic to fatal. Echoviruses (enteric cytopathic human orphan viruses) recently consist of 28 serotypes [4, 5, 8] which have been associated with clinical manifestations ranging from severe meningitis, encephalitis, and myocarditis to mild gastroenteritis and respiratory tract illnesses. Echovirus 30 (ECV30) is one of several serotypes which have often been associated with meningitis.

Non-coding regions (5'NCR and 3'NCR) of the enteroviruses are generally highly conserved, like several regions coding for the nonstructural proteins. The most variable regions of the enterovirus genome are within the genes coding for the capsid proteins VP1, VP2, and VP3 which are partially exposed on the virus surface. VP1 codes for the major antigenic sites and most type-specific neutralisation determinants [5, 14, 15]. Because antigenic variation of capsid proteins may be a survival mechanism for viruses, sequence variation was
expected to be high within VP1. Therefore this genome region was supposed to be the one most suitable for discriminating between enteroviruses based on partial sequence analysis and to be useful with regard to diagnostics and molecular epidemiology of enteroviruses. Furthermore, this genomic region correlates well with the serotype and is therefore useful in molecular typing of enteroviruses [10].

The aim of this study was to analyse the genomic variation of ECV30 isolates over time by direct sequencing of RT-PCR products of a part of the VP1 gene, and to compare the sequences of the isolates with each other in order to reliably discriminate between them and to estimate phylogenetic relationships. Recently Bailly et al. in 1998 (GenBank accession AJ131523) and in 1999 (GenBank accessions AJ133656 to AJ133660) and Oberste et al. in 1999 [9, 10] have published VP1 gene sequences of ECV 30 which were used in part for comparison in this study.

All ECV30 isolates characterised in this study were associated with aseptic meningitis, and were supplied by different Medical Centres. The viruses were isolated from stool, throat swabs, or cerebrospinal fluid by conventional cell culture methods as described previously [1]. Cultures with an enterovirus cytopathic effect were typed by microneutralisation assay using “in-house” rabbit antiserum pools. Procedures for extraction of total RNA from supernatant of infected cell cultures were carried out using QIAamp Viral RNA Kit according to manufacturer’s instructions (Qiagen GmbH, Hilden, Germany). RNA was extracted from 140 µl supernatant of infected cell cultures.

A comparison of previously published sequences of other enterovirus serotypes (deposited in GenBank) revealed some confined, relatively conserved nucleotide sequences within the VP1 genes which were tested for their suitability to be used as primers for RT-PCR within the VP1 region of ECV30 strains. Nucleotide sequences of suitable primers and their location within the VP1 gene are shown in Table 1.

cDNA synthesis at ECV30 RNA was performed with the EC2 primer and MMLV reverse transcriptase at 37 °C for 1 h. First-round PCR amplified a segment of the VP1 region (656 bp) and was performed with primer pair EC2 and EC1. Nested PCR amplification with primer pair EC4 and EC3 resulted in a product

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Polarity</th>
<th>Location&lt;sup&gt;b&lt;/sup&gt; (5'-3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. EC1</td>
<td>5’ CCGGCACTCACAGCTGTYTGAGAC 3’</td>
<td>sense</td>
<td>2545-2567</td>
</tr>
<tr>
<td>2. EC2</td>
<td>5’ CTGATGTGCTTAGCTTTGAAGTA 3’</td>
<td>antisense</td>
<td>3200-3178</td>
</tr>
<tr>
<td>3. EC3</td>
<td>5’ GTGCCAGTGACACTATGCAGAC 3’</td>
<td>sense</td>
<td>2587-2609</td>
</tr>
<tr>
<td>4. EC4</td>
<td>5’ TGGGTATGCWGTTGATTTGTT 3’</td>
<td>antisense</td>
<td>3156-3136</td>
</tr>
</tbody>
</table>

<sup>a</sup>Y = C/T, W = A/T (IUPAC-IUB/GCG code for degenerated bases)

<sup>b</sup>Positions refer to ECV 30 strain Bastianni (GenBank accession AJ131523)