Short communication

Detection and characterization of human group C rotavirus in the pediatric population of Barcelona, Spain

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Abstract

Background: The role of group C rotavirus as a cause of childhood diarrhea is not well defined.

Objectives: To determine the prevalence of human group C rotavirus in stools of children in Barcelona, Spain, and to describe the genetic diversity of the rotavirus capsid proteins – VP6, VP7 and VP4 – in these samples.

Study design: Stool specimens were assayed for rotavirus C RNA by an RT-PCR/southern-blot technique that included controls to indicate the presence of inhibitors of RT-PCR in the samples.

Results: Human rotavirus C was detected in 3 of 467 samples. One hundred and forty-five (31%) of these samples showed the presence of inhibitors of the RT-PCR assay. Thus, the corrected estimation for detection of group C rotavirus in Barcelona was of 1%. The entire VP4, VP6 and VP7 sequences were determined for all three isolates, revealing the relatedness of two of them to strains circulating in Europe, while the third was very close to sub-Saharan African strains.

Conclusion: The low rate of detection of group C rotavirus suggests that it is not an emerging pathogen in children in our region.

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Keywords: Human group C rotavirus; Capsid protein sequences; Molecular epidemiology; Internal positive control; Phylogenetic analysis

1. Introduction

Rotaviruses were first identified in 1973 as an important cause of gastroenteritis (Bishop et al., 1973). They are subdivided into seven groups (A–G) on the basis of their dsRNA electropherotypes and their antigenic and genetic properties (Saif, 1990). The members of each group share a common group antigen located on the major inner capsid protein VP6. Group A rotavirus is the most important cause of severe dehydrating diarrheal illness in infants and young children worldwide (Parashar et al., 2003). Group C rotavirus has been associated with sporadic diarrheal illness in many parts of the world (Peñaranda et al., 1989; Qiao et al., 1999; Nilsson et al., 2000; Adah et al., 2002; Castello et al., 2002; Mwenda et al., 2003; Schnagl et al., 2004; Phan et al., 2005; Rahman et al., 2005; Yee et al., 2006) and with limited outbreaks of illness in infants and adults, mainly in Japan and England (Lambden et al., 1992; Oishi et al., 1993; Kuzuya et al., 2005). Group C rotavirus strains are globally distributed and are thought to be an emerging pathogen in humans.

Serotyping of group C rotavirus is hindered because of the difficulties in adapting human group C rotavirus to cell culture propagation (Fujii et al., 2000). Hence, relatedness between human group C rotavirus strains is so far determined by sequence analysis of the VP7 and the VP4 genes. Sequence comparisons suggest that some genetic diversity exists among group C rotaviruses (Tsunemitsu et al., 1992), but this diversity is less than among group A rotavirus. It has been proposed that group C rotaviruses so far analyzed belong to four G serotypes and three P genotypes (Jiang et al., 1996, 1999), with a high degree of conservation between

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them. The human group C rotavirus may have evolved quite recently and possibly constitute a single globally distributed genotype.

2. Material and methods

2.1. Stool samples

Between October 1997 and April 2000, 467 fecal samples were collected from children of different age-groups with gastroenteritis at four hospitals in the Barcelona area (Hospital de la Vall Hebron, Hospital de Terrassa, Hospital de la Santa Creu i Sant Pau and Hospital de Sant Joan de Deu). The groups ranged in age from: 0 to 12 months (n = 184); 12 to 24 months (n = 150); 24 to 36 months (n = 42); 36 to 48 months (n = 17) and >48 months (n = 74). All samples were negative for common bacterial pathogens, several parasites, group A rotavirus and adenovirus. Astrovirus was determined by the method of Guix et al. (2002).

2.2. Detection of human group C rotavirus

For the detection of group C rotavirus, an internal RNA Positive Control (386-bp) was constructed from the baculovirus recombinant “BacVP6C” containing the full-length cDNA of the Cowden strain gene 5. The RNA of stool samples was extracted by guanidine thiocyanate extraction (Boom et al., 1990). RT-PCR was carried out as previously described (Nilsson et al., 2000). PCR products were confirmed by Southern blot hybridization with an internal digoxigenin-labeled probe BMJ 157 (Sánchez-fauquier et al., 2003).

### Table 1
Sequence comparison of the VP6 gene of group C rotavirus

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Sequence comparison (%) of isolates from different locations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vijari</td>
</tr>
<tr>
<td>Vijari</td>
<td>98.1</td>
</tr>
<tr>
<td>Preston</td>
<td>98.0</td>
</tr>
<tr>
<td>Bristol</td>
<td>99.0</td>
</tr>
<tr>
<td>Moduganari</td>
<td>98.0</td>
</tr>
<tr>
<td>Belem</td>
<td>99.0</td>
</tr>
<tr>
<td>BCN6</td>
<td>98.0</td>
</tr>
<tr>
<td>BCN9</td>
<td>99.0</td>
</tr>
<tr>
<td>BCN21</td>
<td>99.0</td>
</tr>
<tr>
<td>Shintoku</td>
<td>87.0</td>
</tr>
<tr>
<td>Yamagata</td>
<td>88.0</td>
</tr>
<tr>
<td>WD534tc</td>
<td>88.3</td>
</tr>
<tr>
<td>Cowden</td>
<td>90.0</td>
</tr>
</tbody>
</table>

Numbers at the top right indicate results of pairwise comparison of the nucleotide sequences and numbers at the bottom left indicate results of pairwise comparison of the deduced amino acid sequences. For the non-Barcelona isolates, the GenBank accession numbers are as follows: Belem, M94155; Bristol, X59843; Preston, M94156; 208, AB008672; Moduganari, AF325806; Vijari, AF325805; Cowden, M94157; WD534tc, AF162434; Shintoku, M88768; Yamagata, AB108680.

### Table 2
Sequence comparison of the VP4 gene of group C rotavirus

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Sequence comparison (%) of isolates from different locations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BCN6</td>
</tr>
<tr>
<td>BCN6</td>
<td>99.5</td>
</tr>
<tr>
<td>BCN21</td>
<td>99.7</td>
</tr>
<tr>
<td>Bristol</td>
<td>99.1</td>
</tr>
<tr>
<td>Belem</td>
<td>98.7</td>
</tr>
<tr>
<td>CHRV/A87J</td>
<td>97.9</td>
</tr>
<tr>
<td>CHRV/A93M</td>
<td>97.5</td>
</tr>
<tr>
<td>Vijari</td>
<td>96.7</td>
</tr>
<tr>
<td>Moduganari</td>
<td>96.6</td>
</tr>
<tr>
<td>BCN9</td>
<td>96.7</td>
</tr>
<tr>
<td>208</td>
<td>97.1</td>
</tr>
<tr>
<td>Cowden</td>
<td>70.9</td>
</tr>
<tr>
<td>Shintoku</td>
<td>69.0</td>
</tr>
</tbody>
</table>

Numbers at the top right indicate results of pairwise comparison of the nucleotide sequences and numbers at the bottom left indicate results of pairwise comparison of the deduced amino acid sequences. For the non-Barcelona isolates the GenBank accession numbers are as follows: Belem, X79441; Bristol, X79442; Preston, M94156; 208, AB008672; CHRV/A87J, AY395069; CHRV/A93M, AY395070; Vijari, AF323981; Moduganari, AF323980; Cowden, U26551; Shintoku, M74218.
2.3. Determination of enzyme inhibition

The internal positive control was used to measure the inhibition of the RT-PCR enzymes. Five microliter of the RNA suspension was heated to 99 °C for 5 min and placed on ice and the RT mix containing 7.5 × 10^5 molecules of the internal control RNA/μl was added. After completion of the RT-PCR assay, 10 μl of the PCR product was analyzed on a 1.5% agarose gel. The DNA bands from the amplification of the viral VP6 gene and the internal control could be differentiated in terms of their size: 596-bp the viral gene and 386-bp the internal control.

2.4. Nucleotide sequencing

The DNA was sequenced using the thermo Sequenase Big Dye Terminator Cycle Sequencing Premix Kit (Amersham Pharmacia Biotech) on an automated sequencer (ABI PRISM 310). The complete sequence of the VP6 gene as well as the VP4 gene was obtained using a previously described set of primers (Adah et al., 2002). For the sequence of the VP7 gene, two primers corresponding to the common 5′ and 3′ ends of the published VP7 gene sequences were used. The nucleotide sequences were submitted to GenBank, under accession numbers AM118018–AM118026.

3. Results and discussion

Group C rotavirus was detected in 3 out of 467 stool sample specimens by RT-PCR and confirmed by Southern blot analysis; one of these samples revealed a mixed infection with astrovirus serotype 4. However, the amplification of the internal control was achieved with only 322 (69%) specimens, indicating that the real incidence of the rotavirus C in Barcelona was about 1%, whereas the incidence of astrovirus in these samples was of 13% (Guix et al., 2002). The use of the internal control increases the accuracy of the incidence of rotavirus C.

The low level of positivity (1%) contrasts with a previous report of a 15% prevalence of rotavirus group C in Madrid (Sánchez-fauquier et al., 2003). The current finding is more in accordance with data from other nearby countries such as France (Chikhi-Brachet et al., 2002) and Tunisia (data not shown) and another Spanish city, Valencia (Javier Buesa, University of Valencia, personal communication), where few or no cases were detected.

The positive specimens belonged to three girls 15, 24 and 36 months old (BCN6, BCN9 and BCN21, respectively) corresponding to the second and the third age-group, confirming that group C rotavirus could infect not only older children and adults, but also young children. Except for the diarrheal symptoms, neither the BCN6 nor BCN9 patients showed any other clinical sign and did not require hospitalization or hydration treatment. The 36-month-old patient (sample...
BCN21) presented a ponderal delay and had a coinfection with an astrovirus serotype 4.

The VP4, VP6 and VP7 complete genes of these three Barcelona strains were sequenced. As expected, they were 2283, 1353 and 1063 nucleotides long, respectively, with ORFs encoding for polypeptides of 744, 395 and 332 amino acids, respectively. The sequences were very similar to each other and to the other sequences so far published at the

![Fig. 1. Phylogenetic tree of VP6, VP7 and VP4 genes of human, bovine and porcine group C rotavirus. (A) Neighbor-joining phylogenetic tree based on the total VP6 gene sequences for BCN6, BCN9, BCN21 and the other established group C rotavirus. (B) Neighbor-joining phylogenetic tree based on the total VP4 gene sequences for the three Barcelona strains and the other published sequences of group C rotavirus. (C) Neighbor-joining phylogenetic tree based on the total VP7 gene sequences for BCN6, BCN9, BCN21 and other group C rotavirus. For the sequences that are not mentioned in Table 3. GenBank database is obtained under the following accession numbers: Moduganari, AF323979; BH, U20988; Preston, X77258; BF, U20989; T97/126-KA4/1018, AF225552–AF225563; OK459, AB086965; A93M, MY392447; OK450, D87544; ad1015, U20994; Fuan, U20987; K9304, AB004250; KC48, AB086963; 88–220, M61100; ASP/87, U20990; ASP/88, U20991; KW408, AB086968; E93, U20992; S-1, U20995; KU166, AB086966; KW290, AB086967. Phylogenetic distances are expressed as the expected number of substitutions per nucleotide site and can be estimated using the scale. The numbers adjacent to the nodes represent the percentage of bootstrap support (of 1000 replicates) for the clusters to the right of the node. Bootstrap values lower than 70% are not shown.]
nucleotide and the amino acid level (≥96%) (Tables 1–3), supporting the hypothesis that all human group C rotaviruses belong to a single globally distributed genotype which is evolving slowly (Jiang et al., 1996).

As seen in the three phylogenetic trees (Fig. 1), the Barcelona strains BCN6 and BCN21 are related to each other and form a separate cluster next to the cluster formed by the English strains Preston and Bristol, whereas strain BCN9 is more closely related to the African strains Moduganari,ajeri and DhakaC13, consistent with the high immigration rates from sub-Saharan Africa into the Barcelona area.

In conclusion, group C rotavirus should not be considered an emerging pathogen in the pediatric population in our area.

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References


