Short Communication

Molecular epidemiology of coxsackievirus A16 strains from four sentinel surveillance sites in Peru

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1. Introduction

Coxsackievirus A16 (CVA16) belongs to the genus Enterovirus, family Picornaviridae, and was first isolated in South Africa in 1955. CVA16 infections can be associated with a wide variety of complications including death. The classification of CVA16 is not yet well established. Molecular characterization is carried out based on the VP1 gene, which encodes a protein with immunogenic functionality and is important in antigenic characterization. CVA16 has been classified into three genotypes: A, B, and C.

The molecular epidemiology of CVA16 outside Asia is mostly unknown, particularly in the Americas. This fact has limited the ability of investigators to explore the questions of the origin and divergent patterns of CVA16 genotypes. Prior to May 30, 2016, GenBank searches revealed only partial VP1 sequences from Argentina and the USA. It was thus sought to characterize the genetic diversity and molecular epidemiology of CVA16 in Peru and to determine how this compares to other regions of the world.

2. Methods

CVA16 was isolated from nasopharyngeal swabs. Isolation and identification were performed and reported by the team using cell culture, immunofluorescence, and reverse transcription PCR (RT-PCR). Full-length VP1 and VP4 sequences of the seven CVA16 strains were amplified by RT-PCR using the primers and cycle conditions as described previously. Genetic diversity was analyzed using complete sequences of both the VP1 and VP4 genes. Phylogenetic trees were reconstructed using the Maximum Likelihood algorithm in MEGA software (version 5.2.2). The pairwise distance was calculated with the Kimura 2-parameter model of nucleotide substitution and a bootstrap analysis with 1000 replicates. All analytical procedures were repeated by a blinded operator using the original samples.

3. Results

As a result of previous passive, clinic-based surveillance activities throughout Peru, seven CVA16 isolates reported between 2005 and 2010 were collected and identified. Table 1 presents the demographic and clinical information for these seven CVA16 strains. Four of the isolates were collected in 2009. Isolates were collected in four Peruvian provinces: two coastal region

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provinces (Lima and Piura), one highland region province (Arequipa), and one jungle region province (Iquitos). Six of the seven participants were less than 5 years of age.

Full-length VP1 sequences were obtained for the seven strains; however, due to an insufficient amount of sample, only six full-length VP4 sequences were obtained. All sequences were submitted to the GenBank database under the following accession numbers: VP1 KF956714–KF956720, VP4 KJ010766–KJ010771.

Based on the full-length VP1 sequence, it was found that six isolates clustered in the same lineage and were separate from Asian, European, and Australian strains. All six of these strains grouped together within genotype C (Figure 1a). However, the

Table 1
Clinical and demographic information for Peruvian CVA16 isolates

<table>
<thead>
<tr>
<th>Code</th>
<th>Latitude/longitude</th>
<th>Month and year</th>
<th>Location</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLA1135</td>
<td>–16.41/ –71.53</td>
<td>Feb 2008</td>
<td>Arequipa</td>
<td>&lt;1</td>
<td>F</td>
<td>FV, MA, ST, R</td>
</tr>
<tr>
<td>FLA6784</td>
<td>–12.07/ –77.06</td>
<td>Apr 2009</td>
<td>Lima</td>
<td>1</td>
<td>M</td>
<td>FV, C, ST, H, R</td>
</tr>
</tbody>
</table>

CVA16, coxsackievirus A16; M, male; F, female; FV, fever; MA, malaise; ST, sore throat; R, rhinorrhea; C, cough; A, asthenia; H, headache; E, expectoration; D, diarrhea.

Figure 1. Maximum likelihood trees of coxsackievirus A16 isolates based on: (a) full-length VP1 (891 bp) sequences, and (b) full-length VP4 (207 bp) sequences. Only bootstrap values >70% are shown at the nodes. Representative strains of genogroups A, B, and C were included. Sequences are named as follows: accession number, location, and year. Peruvian coxsackievirus A16 strains are in red. Enterovirus 71 was used to root the tree.
remaining isolate (FLA6916) from Iquitos did not cluster within a known genotype; it actually demonstrated a divergent pattern when compared with genotypes B and C. Phylogenetic analysis using the full-length VP4 gene showed the same relationship as was observed using complete VP1 (Figure 1b).

Recombination screening by RDP3 did not reveal local recombination, although the total Peruvian dataset was small and the whole genome was not sequenced.

4. Discussion

This study presents the first report of the molecular characterization of CVA16 strains in Peru. The findings revealed multiple co-circulating clades in genotype C. The molecular epidemiology of CVA16 in Peru during the years 2006–2009 reflects a pattern of the circulation of genotype C. It was noted that the Peruvian strains from multiple locations (Lima, Piura, and Arequipa) fell into a well-supported Peruvian clade with spatial and temporal clustering. This potentially reflects some localization of CVA16 spread. Interestingly, there was evidence of CVA16 persistence in Peru, with the 2006 taxa forming a well-supported clade with those collected in later years. The single isolate (FLA6916) collected from Iquitos, the largest and most populous city in the Peruvian Amazon, was discovered to be highly divergent from other Peruvian strains and all other genotype B and C strains.

Recombination as the cause of this possible divergence is not likely because enterovirus does not recombine within the VP1 gene (except for homologous recombination); recombination in the P1 (capsid) region is most often deleterious and likely fatal to the virus.6

Two possible inferences can be drawn as to why this divergence may have occurred. First, the molecular epidemiology of CVA16 in the Amazon basin compared with the rest of Peru may be uniquely distinct, allowing for the emergence of novel strains. Second, this isolate may have been introduced independently into Iquitos by outside travelers. Gathering additional isolates will help elucidate the more complete epidemiology of CVA16 in Peru and surrounding countries.

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References