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Molecular Characterization of Sapoviruses Detected in Sporadic Gastroenteritis Cases in 2007 in Ehime Prefecture, Japan

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Sapovirus (SaV) is an important pathogen of acute gastroenteritis in humans (1-7). The SaV genome is a polyadenylated, single-stranded, sense RNA approximately 7.5 kb long, and it has two or three open reading frames (ORFs). ORF1 encodes nonstructural proteins (i.e., protease, polymerase, etc.) and a major structural (capsid) protein, whereas ORF2 and ORF3 encode a putative protein with an unknown function (8). SaV can be divided into at least five genogroups (GI to GV) based on the capsid protein gene sequences, among which GI, GH, GIV, and GV are known to infect humans, whereas GIII infects porcine species (9). Human SaV strains are noncultivable, and electron microscopy (EM), single-round or nested reverse transcription-polymerase chain reaction (RT-PCR) (10-15), and real-time RT-PCR (16) have been the main methods used for SaV detection. At present, characterization of SaV strains is mainly done with short nucleotide sequences of the capsid or polymerase.

The purpose of this study was to determine the nucleotide sequences of the approximately 2.3-kb 3' end of the SaV genome detected in patients with sporadic gastroenteritis in 2007 in Ehime Prefecture, Japan.

During the sporadic gastroenteritis surveillance from June to November of 2007 at the Ehime Prefecture Institute of Public Health and Environmental Science, SaV was detected in 6 cases using nested RT-PCR with primers SV-F11 and SV-R1 for the first PCR and SV-F21 and SV-R2 for the second PCR (14) and real-time RT-PCR (16). All 6 specimens were positive by these two methods, and the number of cDNA copies per gram of feces ranged between 5.52 x 10^7 and 2.74 x 10^8 (Table 1). SaV-like particles were detected in 3 of 6 specimens using EM (Table 1). To better characterize these strains, the approximately 2.3-kb 3' end of the genome including the entire capsid, ORF2, and untranslated region of these 6 strains was amplified with semi-nested RT-PCR followed by direct sequencing analysis as previously described (3). The SaV nucleotide sequences determined in this study were deposited at DDBJ under the accession nos. AB448761-AB448766. Nucleotide sequences were aligned with ClustalW version 1.83 (http://clustalw.ddbj.nig.ac.jp/top-j.html). A phylogenetic tree with 1,000 bootstrap replications was constructed by the neighbor-joining method. The genetic distances were calculated by Kimura’s two-parameter method (17) and illustrated using NJplot software (http://phil.univ-lyon1.fr/software/njplot.html) (18). The nucleotide and amino acid sequences were analyzed with GENETYX MAC Software, version 12.2.6 (Genetyx Corp., Tokyo, Japan). BLAST (Basic Local Alignment Search Tool; http://blast.ddbj.nig.ac.jp/top-j.html) was used to find homologous hits.

Ehime-742S/07/JP (AB448762) was 2261 nt in length from the capsid start codon to the genome end (excluding the polyA tail) and was genetically closest to Lyon/30388/98/Fr (AJ251991), detected in 1998 in France, with 97% nucleotide identity over the approximately 2.3-kb fragment. In addition, Ehime-742S was close to Ehime/2K-927/00/JP (AM049931), Ehime/01-1527/01/JP (AM049930), and Ehime/04-311/04/JP (AM049933) detected in Ehime Prefecture in 2000, 2001, and 2004, respectively, with 99% nucleotide identities when the 390-nt capsid gene parts were compared (data not shown). Furthermore, this strain was close to Chiba/001092F/00/JP (AJ412814), Chiba/010591F/01/JP (AJ412823), and Chiba/040506/04/JP (AM049928), detected in Chiba Prefecture in 2000, 2001, and 2004, respectively, with 99% nucleotide identities when the 390-nt capsid gene parts were compared.

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These strains were genetically close to Ehime1596/99/JP (DQ366346) (19) and Ehime1107/02/JP (DQ058829) (20), detected in Ehime in 1999 and 2002, respectively, and to Yakumo8/00/JP (AB455795) detected in Hokkaido in 2000 (3), with 96% nucleotide identities over the approximately 2.3-kb fragment. In addition, the Ehime-683S, -1097S, -1111S, -1116S, and -1121S strains were identical to Yokohama/16/07/JP (AB305049) (2) and close to Osaka19-086/07/JP (AB327281), Saga8151/07/JP (FJ445097), and Sapporo8411/08/JP (FJ445097), all detected in Japan, with 99% nucleotide identities when the 300- to 400-nt capsid gene parts were compared (data not shown).

Ehime-742S was categorized as GI, whereas Ehime-683S, -1097S, -1111S, -1116S, and -1121S were clustered into GIV based on the complete capsid nucleotide sequences (Fig. 1).

### Table 1. Sporadic gastroenteritis due to sapovirus in Ehime Prefecture in 2007

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Sex</th>
<th>Age</th>
<th>Onset of illness</th>
<th>Specimen collected date</th>
<th>Symptoms at onset of illness</th>
<th>Real-time RT-PCR (copies/g stool)</th>
<th>Accession no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>683S</td>
<td>M</td>
<td>2</td>
<td>June 30</td>
<td>July 4</td>
<td>+ + + + + +</td>
<td>$2.74 \times 10^7$</td>
<td>AB448761</td>
</tr>
<tr>
<td>742S</td>
<td>M</td>
<td>1</td>
<td>July 15</td>
<td>July 18</td>
<td>+ + + + + +</td>
<td>$1.63 \times 10^7$</td>
<td>AB448762</td>
</tr>
<tr>
<td>1097S</td>
<td>M</td>
<td>7</td>
<td>Nov 13</td>
<td>Nov 16</td>
<td>+ + + + + +</td>
<td>$5.52 \times 10^7$</td>
<td>AB448763</td>
</tr>
<tr>
<td>1111S</td>
<td>M</td>
<td>4</td>
<td>Nov 15</td>
<td>Nov 21</td>
<td>+ + + + + +</td>
<td>$2.12 \times 10^7$</td>
<td>AB448764</td>
</tr>
<tr>
<td>1116S</td>
<td>F</td>
<td>5</td>
<td>Aug 24</td>
<td>Aug 28</td>
<td>+ + + + + +</td>
<td>$3.84 \times 10^7$</td>
<td>AB448765</td>
</tr>
<tr>
<td>1121S</td>
<td>M</td>
<td>1</td>
<td>Nov 17</td>
<td>Nov 19</td>
<td>+ + + + + +</td>
<td>$1.32 \times 10^7$</td>
<td>AB448766</td>
</tr>
</tbody>
</table>

*: higher than 37.0 degrees.
EM, electron microscopy.

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**Fig. 1.** Phylogenetic tree of SaV based on complete capsid nucleotide sequences. DDBJ accession numbers for Ehime-683S/07/JP (AB448761), -742S/07/JP (AB448762), -1097S/07/JP (AB448763), -1111S/07/JP (AB448764), -1116S/07/JP (AB448765), and -1121S/07/JP (AB448766) are shown in bold letters in the tree. The number on each branch indicates the bootstrap value, where a value of 950 or higher is considered statistically significant for the grouping. The scale represents genetic distances that means nucleotide substitutions per site.
These results indicated that a genetically similar SaV strain belonging to GI likely persisted or circulated between 1998 and 2007 in Japan and Russia, and that strains belonging to GIV likely persisted between 1999 and 2008 in Japan.

In conclusion, this study demonstrated that SaV strains belonging to GIV were predominant among sporadic gastroenteritis cases due to SaV from June to November in 2007 in Ehime. Similar nucleotide sequences were deposited in DDBJ from other prefectures. In addition, the emergence of SaV strains belonging to GIV was reported in 2007 in Canada (21), although no sequence data are currently available for these strains. These results suggest that the SaV strains belonging to GIV may have spread not only in Japan but also throughout the world in 2007, although the transmission route is unknown. Continuous nationwide and global surveillance for SaV using methods capable of detecting a broad range of genetically diverse human SaV strains, and accumulation of the nucleotide sequences of SaV will be needed to study the geographical distribution of SaV and to better characterize SaV strains.

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REFERENCES