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Characterization of Sapoviruses Detected in Hokkaido, Japan

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Sapovirus (SaV) is an important pathogen of acute gastroenteritis that often occurs among young children. The SaV prototype strain was detected for the first time in an outbreak in Sapporo, Hokkaido, Japan, in 1977 (1). The SaV genome is a polyadenylated, single-stranded, positive-sense RNA approximately 7.5 kb long, and it has two or three open reading frames (ORFs). ORF1 encodes nonstructural proteins including protease and polymerase and a major structural (capsid) protein, VP1. ORF2 encodes a putative protein with an unknown function. SaV can be divided into five genogroups (GI to GV) based on the capsid protein gene sequences, among which GI, GII, GIV, and GV are known to infect humans, whereas GIII infects porcine species. An additional ORF, overlapping with the 5’ end of the capsid gene, is found in the strains belonging to GI, GIV, and GV (2,3). Human SaV strains are noncultivable, and the most widely used method to detect SaV is reverse-transcription polymerase chain reaction (RT-PCR), which can be used for genetic analysis (4-8). Real-time RT-PCR for SaV was recently developed (9).

The purposes of this study were: (i) to analyze a gastroenteritis outbreak at a health care center for disabled people in 2007 in Kushiro, Hokkaido, Japan, and (ii) to determine the complete capsid sequences of the 2007 strain in comparison with two previously identified strains detected in outbreaks in 2000 in Yakumo and in 2005 in Nayoro, Hokkaido (10).

The outbreak occurred between May 18 and 22 in 2007 in Kushiro, and 10 people (9 adult students and 1 staff member, ages 17 - 36 years old) developed symptoms of gastroenteritis. During the outbreak, stool specimens were collected from 5 adult students (ages 17 - 28). These specimens were initially screened for norovirus and group A and group C rotaviruses, but were all found to be negative (data not shown). These 5 specimens were then re-examined for SaV using RT-PCR targeting the polymerase gene with the primers Sapp36 and SV-r-c (4); 4 of the 5 specimens were found to be positive for SaV. To confirm the RT-PCR result and determine the copy number of the SaV genome in the stool specimens, real-time RT-PCR targeting the junction region between the polymerase and capsid was performed (9). All 5 specimens were positive, and the number of cDNA copies per gram of stool ranged between 5.21 x 10^6 and 2.94 x 10^9. Sequence analysis revealed that all of the strains belonged to SaV GII, and identical nucleotide sequences were observed in all of the strains (data not shown), indicating that this outbreak was caused by a single GII SaV strain (Kushiro5/2007/JP strain). The specimen that had the highest fecal viral load was subjected to further sequence analysis targeting the partial polymerase, capsid VP1, ORF2, and the 3’ untranslated region of the genome. The 3’ terminus 3.2 kb of the genome was amplified by semi-nested RT-PCR with the forward primer Kushiro PolF1 (5’-GGT TGA GGT GCT CAA TGA ATC-3’) and the reverse primer TX30SXN (5’-GAC TAG TTC TAG ATC GCG AGC GGC CGC CCT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TT-3’) for the first PCR, and the forward primer Kushiro PolF2 (5’-GGT ACT TTA CTG CCT TGA CTA C-3’) and TX30SXN for the second PCR. A final volume of 100 μl of reaction mixture containing 2 μl of the cDNA or the first PCR reaction mixture, 10 μl of 10 X KOD-Plus DNA polymerase buffer, 10 μl of 2.5 mM dNTPs, 4 μl of 25 mM MgSO4, 2.5 μl of dimethylsulphoxide (Sigma, St. Louis, Mo., USA), 2 μl of primers (20 pmol/μl each), and 2 μl of KOD-Plus DNA polymerase (Toyobo, Osaka, Japan), was subjected to PCR at 94°C for 10 min followed by 35 cycles of 94°C for 30 s, 55°C for 30 s, 68°C for 5 min, and a final extension at 72°C for 15 min. The mixture was then held at 4°C. The PCR products were separated with 1% agarose gel electrophoresis, purified with the QIAquick Gel Extraction Kit (Qiagen, Tokyo, Japan), and directly sequenced by a 3130 genetic analyzer (Applied Biosystems, Tokyo, Japan) with the Big Dye Terminator (version 3.1) cycle sequencing kit (Applied Biosystems). Nucleotide sequences were assembled with SEQUENCHER version 4.7 (Gene Codes Corp., Ann Arbor, Mich., USA) and aligned with Clustal
W version 1.83 (http://cluslaw.ddbj.nig.ac.jp/top-j.html). A phylogenetic tree with 1,000 bootstrap replications was constructed by the neighbor-joining method. The distance of the nucleotide substitutions per site was calculated by Kimura’s two-parameter method and illustrated using NJPlot software. The nucleotide and amino acid sequences were analyzed with GENETYX Mac Software, version 12.2.6 (Genetyx Corp., Tokyo, Japan).

We recently reported two SaV strains detected in gastroenteritis outbreaks in 2000 and 2005 in Hokkaido, and determined the partial nucleotide sequences of the polymerase and capsid gene (10). To further characterize these strains, we amplified the 3’ terminus 2.3-kb fragment spanning the capsid start to the genome end using the stool specimens containing capsid gene (10). To further characterize these strains, we mined the partial nucleotide sequences of the polymerase and enteritis outbreaks in 2000 and 2005 in Hokkaido, and determined nucleotide identities when compared with the partial 300 nt of the capsid gene (data not shown). These findings indicate that genetically similar GII SaV likely persisted or circulated between 1999 and 2007 in Japan. GIV Yakumo8/2000/JP was close to Chiba000671/99/JP (AJ786349) detected in 1999 in Chiba (3), to Ehime1596/99/JP (DQ366346) and Ehime1107/02/JP (DQ58829) (12) detected in 1999 and 2002, respectively, in Ehime, and to SW27/04/SE (DQ125333) detected in 2004 in Sweden (12,13), with 99, 98, 99, and 97% nucleotide identities over the 2.3-kb fragment, respectively. In addition, GIV Yakumo8/2000/JP was identical to Tokyo18/DCC/43/00/JP (AB236378) and close to Osaka19-098/07/JP (AB327282), Yokohama16/07/JP (AB305049), and Osaka07-76/07/JP (AB433375) detected in Japan, CMH044/03/THA (EF600796) detected in Thailand, and CUI050202/05/HK (DQ155647) detected in Hong Kong with 97 - 98% nucleotide identities when compared with the partial 300 nt of the capsid gene (data not shown). These findings indicate that genetically similar GIV SaV strains were widely spread and caused gastroenteritis between 1999 and 2008 in Japan and other countries, although the mode of transmission is unknown.

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Fig. 1. Phylogenetic tree of sapovirus (SaV) based on complete capsid nucleotide sequences. GenBank/EMBL/DDJ accession numbers for Kushiro2007/JP (AB455793), Nayoro2005/JP (AB455794), and Yakumo8/2000/JP (AB455795) are shown in bold letters in the tree.

REFERENCES

11. Katayama, K., Miyoshi, T., Uchino, K., et al. (2004): Novel recombinant and ORF2 proteins, respectively (data not shown). These two strains were close to Chiba040507/04/JP (AJ786350), which was detected in a 1-year-old male in 2004 in Chiba (3), with 97 and 98% nucleotide identities, respectively. These strains were also close to Sakai C12/01/JP (AY603425) (11), Chiba990763/99/JP (AJ606690) (3), and Chiba991172/99/JP (AJ606691) (3), with 90% nucleotide identity over the 2.3-kb fragment. In addition, Kushiro5/2007/JP and Nayoro4/2005/JP were close to Ishikawa4-721/04/JP (AM049951), with 96 and 98% nucleotide identities, respectively, when compared with the partial 400-nt capsid gene (data not shown), indicating that a genetically similar GII SaV likely persisted or circulated between 1999 and 2007 in Japan. GIV Yakumo8/2000/JP was close to Chiba000671/99/JP (AJ786349) detected in 1999 in Chiba (3), to Ehime1596/99/JP (DQ366346) and Ehime1107/02/JP (DQ58829) (12) detected in 1999 and 2002, respectively, in Ehime, and to SW27/04/SE (DQ125333) detected in 2004 in Sweden (12,13), with 99, 98, 99, and 97% nucleotide identities over the 2.3-kb fragment, respectively. In addition, GIV Yakumo8/2000/JP was identical to Tokyo18/DCC/43/00/JP (AB236378) and close to Osaka19-098/07/JP (AB327282), Yokohama16/07/JP (AB305049), and Osaka07-76/07/JP (AB433375) detected in Japan, CMH044/03/THA (EF600796) detected in Thailand, and CUI050202/05/HK (DQ155647) detected in Hong Kong with 97 - 98% nucleotide identities when compared with the partial 300 nt of the capsid gene (data not shown). These findings indicate that genetically similar GIV SaV strains were widely spread and caused gastroenteritis between 1999 and 2008 in Japan and other countries, although the mode of transmission is unknown.