Sapovirus (SaV) is a pathogen that causes acute gastroenteritis (1–5). The SaV genome is a positive-sense, single-strand RNA molecule of approximately 7.5 kb, containing two open reading frames (ORFs), and ORF1 is predicted to encode at least six nonstructural (NS) proteins and a structural protein (VP1) in the following order: NS1-NS2-NS3-NS4-NS5-NS6-7-VP1. NS6-7 encodes protease and RNA-dependent RNA polymerase (RdRp) (6). The functions of other NS and ORF2-encoded proteins have not yet been experimentally determined. Based on the VP1 sequences, SaV can be divided into at least five genogroups (GI–GV), and recently, nine additional genogroups (GVI–GXIV) have been proposed (7). Thus far, GI, GII, GIV, and GV SaVs strains have been detected in humans. Human SaVs can be further divided into multiple genotypes (at least GI.1-7, GII.1-7, GIV.1, and GV.1) based on the VP1 sequences (8).

We previously identified 139 SaVs from 1,367 stool samples of outpatients with acute gastroenteritis at three pediatric clinics from June 2002 to March 2011 in Kumamoto Prefecture, Japan, using RT-PCR targeting the human SaV RdRp-VP1 junction region (approximately 105 bp) (1,2). All 139 SaVs were then successfully amplified in combinations with several RT-PCR assays for the partial VP1 region and classified into 4 genogroups and 11 genotypes: GI.1, GI.2, GI.3, GI.5, GI.1, GI.2, GI.3, GI.4, GI.7, GIV.1, and GV.1 (1). The predominant genotypes changed drastically during the study period (i.e., emerging GIV strains in 2007 following conditions: initial denaturation at 94°C for 3 min. The PCR products of expected size were purified and sequenced directly or after cloning as previously described (2,12). The nucleotide sequences corresponding to the partial SaV RdRp region determined in this study have been deposited in GenBank/EMBL/DDJB accession no. AB12825–AB128743.

We amplified the partial RdRp sequences of 119 of the 139 SaV strains (85.6%) corresponding to all 4 genogroups and 11 genotypes (GI.1, GI.2, GI.3, GI.5, GI.1, GI.2, GI.3, GI.4, GI.7, GIV.1, and GV.1) (Fig. 1A). Fourteen of the 20 partial RdRp targeting PCR-negative specimens had relatively low viral copy numbers (data not shown), and this may partly explain...
Fig. 1. Phylogenetic tree of SaV based on (A) partial VP1 or (B) partial RdRp nucleotide sequences. SaV strains were labeled with strain number, detected year and month. The reference SaV strains are indicated with GenBank/EMBL/DDJB accession number in parenthesis. The phylogenetic tree was constructed by the neighbor-joining method with 1,000 bootstrap replications. The numbers on each branch indicate the bootstrap values, where the values of 950 or higher were indicated. The scale represents the nucleotide substitutions per site. The 139 partial VP1 sequences were determined in our previous studies and deposited under the accession numbers AB429079–AB429159 and AB689798–AB689855 (1,2), and the 119 partial RdRp sequences have been determined in this study and deposited under the accession numbers AB812625–AB812743.
the negative result (Fig. 1A). As shown in Fig. 1B, the Sapp36 and SaV-1245R or SV-S1, -S2, -S3, and SaV-1245R primer sets successfully detected the partial RdRp regions of the SaV GI, GII, and GIV strains, whereas GV strains could only be amplified using the SaV GV-GLPSGM and SaV 1245R primer set. A single GI strain was also amplified using this primer combination. On the phylogenetic tree, partial RdRp regions of the 119 SaVs corresponding to the 4 genogroups and 11 genotypes were basically segregated into distinct clusters or branches along with their VP1-based genogroup and genotype except GII.2 and GII.3 strains (Fig. 1B). SaV
GI.2 and GI.3 analyzed in this study formed distinct clusters by the VP1 region (Fig. 1A), whereas they were not segregated well by the RdRp region (Fig. 1B). This is consistent with the previous report that the GI.2 strain (GenBank/EMBL/DDJB accession no. AY237420) detected from an infant hospitalized with acute gastroenteritis in Thailand in 2000 and the GI.3 strain (AY603425) detected from an infant with gastroenteritis in Japan in 2001 were grouped in the same cluster by the RdRp region (13). These genetically similar SaV GI.2 and GI.3 strains likely spread and persist in different countries and areas. Our results suggest that the total clusters based on the RdRp region sequences may not be identical to those based on VP1 region sequences.

In this study, we amplified the partial RdRp region sequence for SaV strains GI.4 and GI.7 for the first time; however, more RdRp sequences corresponding to all VP1-based genogroups and genotypes with a statistically reliably analytical scheme is necessary to establish RdRp sequence-based typing in the future.

Three children (Patients A, B, and C) were positive for SaV twice during the study period as previously described (1). SaVs detected from Patient A consisted of strains GI.1 (29/2007/Jan) and GIV.1 (60/2007/Nov) based on partial VP1 sequences (Fig. 1A). These strains also formed the GI.1 and GIV.1 clusters, respectively, based on the partial RdRp sequences (Fig. 1B). Patient B was positive for SaV strain GI.1 (25/2006/Dec) and then strain GI.3 (101/2009/Mar) (Fig. 1A). The SaV strain isolated from the first sample of this patient also clustered into GI.1 based on the partial RdRp sequence (Fig. 1B); however, we could not amplify the partial RdRp region of the second sample (Figs. 1A and B). The low viral level in the second sample (1.0 × 10^6 copies/g stool) may partly explain this result, although mismatches in the primer target region could also have been possible. Patient C was positive for SaV strain GI.3 (twice within the same month 89/2008/Nov and 90/2008/Nov). The SaV strains from the two stool samples contained sequences with 99.0% (420/424) nucleotide identity within the comparable partial RdRp regions and was consistent with that of partial VP1 region (99.0%), as previously described (1).

In conclusion, our data demonstrated that genetically diverse SaV strains with both nonstructural and structural protein encoding region sequences appeared in succession from 2002 to 2011 in the same geographic area in Kumamoto Prefecture, Japan. The novel primer combinations described in this study can be used as an additional tool to accumulate human SaV partial RdRp region sequences of strains from multiple genogroups and genotypes to further characterize the human SaV genome.

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Conflict of interest None to declare.

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