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Comprehensive Review of Human Sapoviruses

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SUMMARY

Sapoviruses cause acute gastroenteritis in humans and animals. They belong to the genus *Sapovirus* within the family *Caliciviridae*. They infect and cause disease in humans of all ages, in both sporadic cases and outbreaks. The clinical symptoms of sapovirus gastroenteritis are indistinguishable from those caused by noroviruses, so laboratory diagnosis is essential to identify the pathogen. Sapoviruses are highly diverse genetically and antigenically. Currently, reverse transcription-PCR (RT-PCR) assays are widely used for sapovirus detection from clinical specimens due to their high sensitivity and broad reactivity as well as the lack of sensitive assays for antigen detection or cell culture systems for the detection of infectious viruses. Sapoviruses were first discovered in 1976 by electron microscopy in diarrheic samples of humans. To date, sapoviruses have also been detected from several animals: pigs, mink, dogs, sea lions, and bats. In this review, we focus on genomic and antigenic features, molecular typing/classification, detection methods, and clinical and epidemiological profiles of human sapoviruses.

INTRODUCTION

Sapoviruses cause acute gastroenteritis in humans and animals. They belong to the genus *Sapovirus* within the family *Caliciviridae*. Sapovirus infections are a public health problem because they cause acute gastroenteritis in people of all ages in both outbreaks and sporadic cases worldwide. Outbreaks often occur in semiclosed settings. Outbreaks caused by foodborne transmission of sapovirus have also been reported. In this comprehensive review, we focus mainly on human sapoviruses.

HISTORY

Sapovirus particles are small (about 30 to 38 nm in diameter) and icosahedral and have cup-shaped depressions on the surface, which is a typical calicivirus morphology (Fig. 1) (1). Sapovirus particles were first detected in human diarrheic stool samples in 1976 in the United Kingdom using electron microscopy (EM) (2), and the virus was soon recognized as a new gastroenteritis pathogen (3–11). However, the prototype strain of the *Sapovirus* genus

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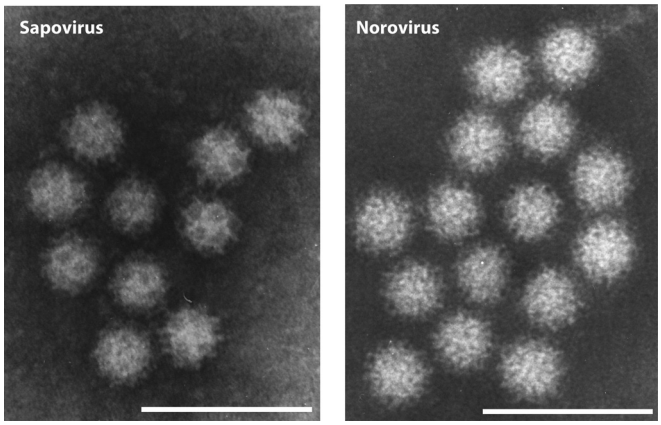

FIG 1 Transmission electron micrographs of sapovirus and norovirus particles from clinical samples. Scale bars indicate 100 nm. (Courtesy of Yasutaka Yamashita, Ehime Prefectural Institute of Public Health and Environmental Science, Japan.)

was from another outbreak in Sapporo, Japan, in 1982 (strain Hu/SaV/Sapporo/1982/JPN), because it has been studied extensively for sapovirus virological and genetic characteristics (10, 12–14).

TAXONOMY

Sapoviruses were previously called "typical human caliciviruses" or "Sapporo-like viruses." In 2002, The International Committee on the Taxonomy of Viruses assigned these viruses to the species *Sapporo virus*, genus *Sapovirus*, in the family *Caliciviridae* (15). Currently, the family *Caliciviridae* consists of five established genera, *Sapovirus*, *Norovirus*, *Lagovirus*, *Vesivirus*, and *Nebovirus* (http://www.ictvonline.org/virusTaxonomy.asp), whereas five new genera (*Bavovirus*, *Nacovirus*, *Recovirus*, *Valovirus*, and *Secalivirus*) have been proposed (16–20).

CELL CULTURE AND ANIMAL INFECTION TRIALS

Attempts to grow human sapoviruses in cell cultures (2, 4, 5, 9, 21–24), have been reported, and two studies describe the propagation of sapoviruses in green monkey kidney cells (23) or primary human embryo kidney cells in the presence of trypsin and actinomycin D (Table 1) (24); however, no confirmed reproduction of these data is available. Currently, only a few porcine sapovirus strains have been grown successfully in primary porcine kidney cells or a porcine kidney cell line (i.e., LLC-PK1) in the presence of porcine intestinal contents or bile acids (Table 1) (25–28). Bile acids likely support porcine sapovirus replication via escape from endosomes during the virus entry step (29). Also, a cellular cyclic AMP (cAMP) signaling pathway induced by intestinal contents or bile acids likely causes downregulation of innate immunity (25, 30). An infection trial of human sapoviruses in mice did not succeed (5). Currently, only the specific porcine sapovirus (Cowden strain) has been studied for its pathogenesis in gnotobiotic pigs (31–33).

PHYSICAL CHARACTERISTICS AND STABILITY

Sapovirus is a nonenveloped virus, and the virus has a buoyant density of 1.36 to 1.41 g/cm^3 (5, 22, 34, 35). The stability of porcine sapovirus under physicochemical treatment is as follows: (i) stable at pH 3.0 to 8.0 at room temperature for 1 h, (ii) sensitive to ethanol treatment (60% and 70%) at room temperature for 30 s, (iii) inactivated by 200 mg/liter (or ppm) sodium hypochlorite at room temperature for 30 min, and (iv) inactivated by heating at 56°C for 2 h (36).

GENOMIC ORGANIZATION

The sapovirus genome has a positive-sense, single-stranded RNA genome, which is approximately 7.1 to 7.7 kb in size and has a 3′-end poly(A) tail. The sapovirus genome contains two open reading frames (ORFs) (Fig. 2). ORF1 encodes a large polyprotein containing the nonstructural proteins followed by the major cap-

TABLE 1 Cell culture trials for human and animal sapoviruses

Origin	Virus growth	Tested cells	Supplement in the culture medium	Reference(s)
Human	No[a]	Human and rhesus monkey amnion, fetal cat cells	None	2
	Yes[a,b]	Green monkey kidney cells	None	23
	No[a]	Human fetal intestinal organ culture, human embryonic kidney cells, monkey kidney cells, human embryonic lung cells, HEp-2 cell line	None	21
	No[a]	Primary rhesus monkey kidney and human embryonic kidney cells	None	4
	No[a]	Primary monkey kidney, primary baboon kidney, primary human embryo intestine, human embryo kidney cells, MRC5, Hep, Vero, and feline lung cells	None	5
	No[a]	HeLa, green monkey kidney cells, and HEL-R66	None	22
	No[a]	Monkey kidney and MRC5 cells	None	9
	Yes[a,b]	primary human embryo kidney cells	Trypsin and actinomycin D	24
Porcine	Yes[c]	Primary porcine kidney cells	Porcine intestinal contents	26
	Yes[c]	LLC-PK1	Porcine intestinal contents	35, 27
	Yes[d]	LLC-PK1	Bile acids	25

[a] Confirmed by electron microscopy.
[b] This result could not be reproduced.
[c] Confirmed by immunofluorescent staining of the virus-infected cells and immune electron microscopy.
[d] Confirmed by immunofluorescent staining of the virus-infected cells and ELISA.

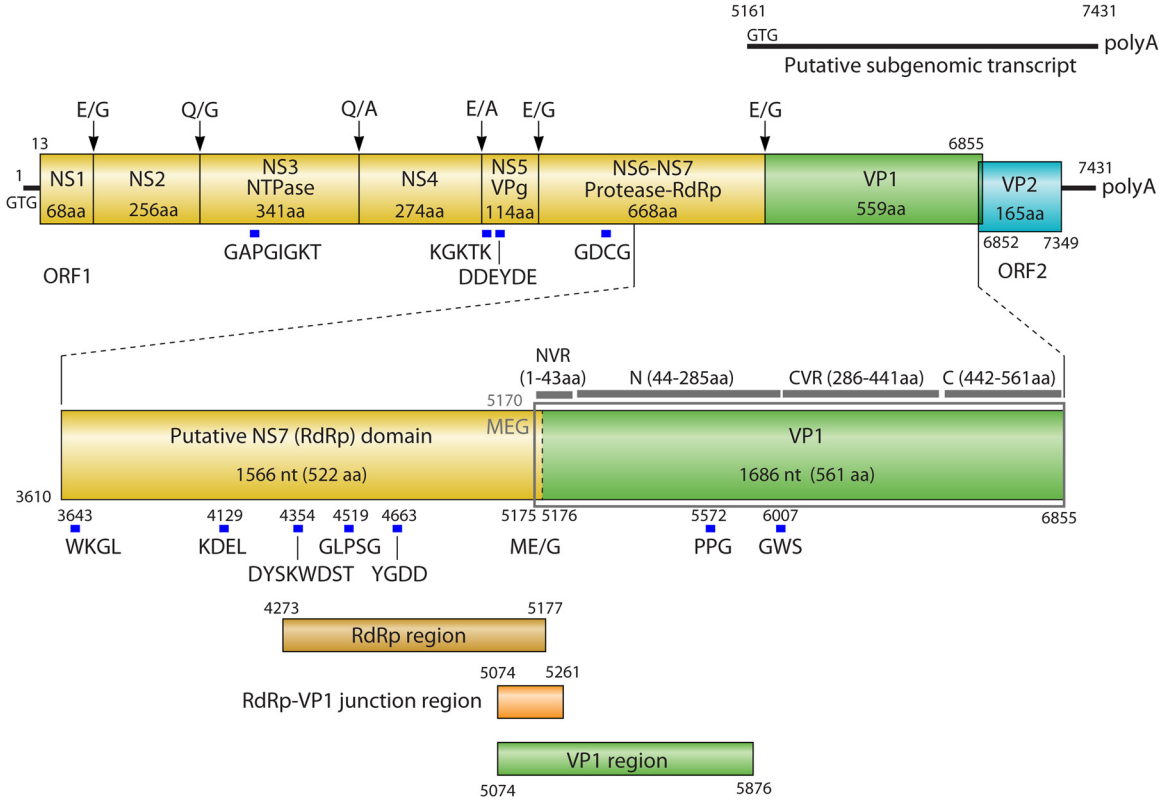

FIG 2 Diagram of sapovirus genomic organization and the RT-PCR target regions for human sapoviruses based on the GI.1 Manchester strain (GenBank accession no. X86560). A schematic diagram of the sapovirus genomic organization, including the putative subgenomic transcript, two common open reading frames (ORF1 and ORF2), the predicted viral nonstructural proteins (NS1, NS2, NS3 [NTPase], NS4, NS5 [VPg], and NS6-NS7 [protease–RNA-dependent RNA polymerase {RdRp}]), and structural proteins VP1 and VP2 is shown. The putative cleavage sites in the ORF polyprotein and their predicted sizes are indicated, according to previous reports (37, 41, 42, 49–51, 55, 272). Typical amino acid motifs for NS3 (GAPGIGKT), NS5 (KGKTK and DDEYDE), NS6 (GDCG), NS7 (WKGL, KDEL, DYSKWDST, GLPSG, and YGDD) and VP1 (PPG and GWS) are also shown. An overview of the RT-PCR target regions (RdRp region, RdRp-VP1 junction region, and VP1 region) is shown, and the detailed primer information is summarized in Tables 2 to 4. The putative first amino acids of VP1 from subgenomic transcript "MEG" and the cleavage site of this motif (ME/G; the slash indicates the cleavage site) from the ORF1 polyprotein are also shown with their nucleotide positions. The putative NS7 (RdRp) region and the cleavage site between RdRp and VP1 are based on previous reports (50, 52, 55, 58). The proposed subdomains in the sapovirus VP1 from the subgenomic transcript (N-terminal variable region [NVR] [1 to 43]), N-terminal region [N] [44 to 285]), central variable region [CVR] [286 to 441]), and C-terminal region [C] [442 to 561]) (47) are also indicated.

sid protein, VP1 (Fig. 2). ORF2 is predicted to encode the minor structural protein VP2 (Fig. 2) (28, 37). A similar genomic organization (i.e., two ORFs, with the first ORF encoding the nonstructural proteins and VP1) is found in other calicivirus genera, such as *Lagovirus*, *Nebovirus*, and the newly proposed genera *Valovirus*, *Bavovirus*, and *Nacovirus* (17, 19, 20, 38, 39). The genomic organization of *Norovirus*, *Vesivirus*, and *Recovirus* differs from that of *Sapovirus*: ORF1 encodes nonstructural proteins, and ORF2 and ORF3 encode structural proteins VP1 and VP2, respectively (18, 37, 40). A third ORF (ORF3) has been predicted in several human (12, 41–47) and bat (48) sapovirus strains; however, its function is unknown.

The ORF1-encoded polyprotein is expressed and processed into at least six nonstructural (NS) proteins (NS1, NS2, NS3, NS4, NS5, and NS6-NS7) and a structural protein (VP1) by virus-encoded protease (Fig. 2) (49–54). *In vitro* studies failed to show cleavage of the NS6-NS7 protein by the viral protease (28, 49, 50, 52, 55, 56), although both the NS6 and NS7 proteins can carry out their respective functions (proteolytic and polymerase) when expressed individually *in vitro* (56–58). The NS6-NS7 protein was also detected in porcine sapovirus-infected cells (28). Similar to the case for sapoviruses, vesivirus also produces the NS6-NS7 protein (fused protease-polymerase) (52, 53, 59–61), whereas noroviruses and lagoviruses produce an individual protease and polymerase, NS6 and NS7, respectively (51, 53, 62–67). The biological functions of the other sapovirus NS proteins have not been experimentally determined; however, NS3 and NS5 have a typical calicivirus NTPase motif (GAPGIGKT) and VPg motifs (KGKTK and DDEYDE), respectively (Fig. 2) (37, 49, 68, 69). VPg is linked to the 5′ end of the viral RNA and is critical for calicivirus genome replication, transcription, and translation (37, 70).

VP1, an approximately 60-kDa protein, is a major component of the complete virus (34, 35). Two mechanisms can be considered in the production of sapovirus VP1. One is that VP1 is cleaved from the ORF1-encoded polyprotein, and the other is that VP1 is translated from a subgenomic RNA (from the 3′-coterminal RNA corresponding to VP1 to the genome end region) (Fig. 2) (71, 72). A subgenomic RNA was confirmed for the sapovirus Cowden strain during replication (25). The VP2 protein has not yet been identified in sapovirus virions; however, the expression of this protein was detected in the *in vitro* translation products of a porcine sapovirus full-length genomic cDNA construct and from

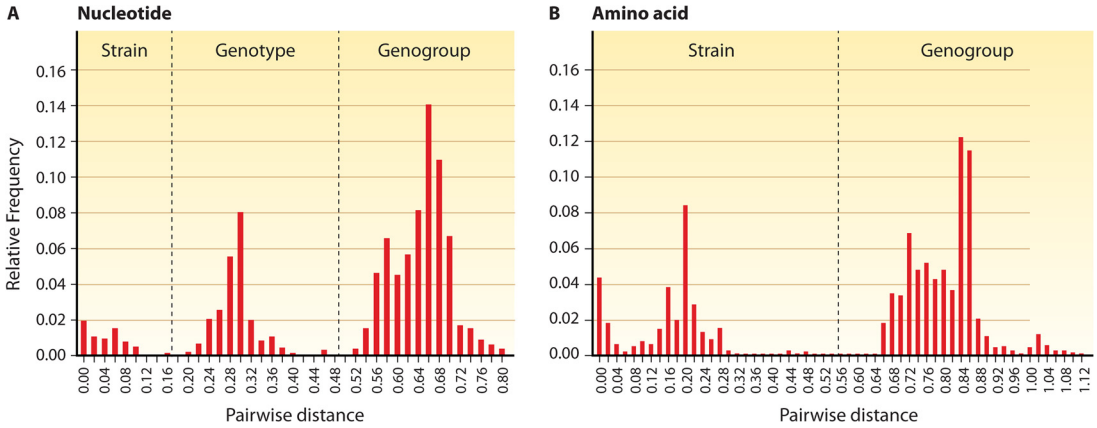

FIG 3 Pairwise distance distribution histograms of 59 representative sapovirus complete VP1 sequences. The vertical dashed lines indicate the cutoff limits for genogrouping and/or genotyping. (A) Limits based on nucleotide sequences, with three peaks (0 to 0.159, 0.198 to 0.471, and 0.522 to 0.807) corresponding to the strain, genotype, and genogroup range, respectively. The mean values ± 3 SD for the pairwise distance peaks were 0 to 0.151, 0.170 to 0.416, and 0.489 to 0.801, and the cutoff values for the genotype and genogroup clusters were designated ≤0.169 and ≤0.488, respectively. (B) Limits based on amino acid sequences, with two major peaks (0 to 0.480 and 0.652 to 1.115) corresponding to the strain and genogroup range, respectively.

porcine sapovirus-infected cells (28). VP2 is predicted to be a strong basic protein and is identified as an interior component of the norovirus particles (73).

The expression of VP1 in insect or mammalian cells resulted in spontaneously assembled virus-like particles (VLPs) (12, 71, 72, 74–81). The sapovirus VLPs are morphologically and antigenically indistinguishable from those of the native sapovirus virions found in clinical specimens (12, 74). Digitized electron cryomicrographs of the human sapovirus VLPs revealed that the icosahedral capsid is formed from 180 molecules of VP1, the same as in norovirus (76). Sapovirus VP1 could be separated into several domains: the N-terminal variable region (NVR), N-terminal region (N), central variable region (CVR), and C-terminal region (C) (Fig. 2) (47). The conserved amino acid motif "GWS" was found in the predicted N and CVR junction (Fig. 2). The "G" in this motif is strictly conserved among caliciviruses (76). Norovirus VP1 has also been separated into several domains, the N-terminal domain, shell domain, and protruding (P) domain, which is further divided into P1 and P2 subdomains (76, 82, 83). The sapovirus VP1 CVR region likely corresponds to the highly variable P2 domain of norovirus VP1 (47, 76).

GENOMIC SEQUENCE AND ANTIGENICITY

The first complete genome of a sapovirus was determined for the Manchester strain detected in the United Kingdom in 1993 (Hu/Manchester/93/UK; GenBank accession no. X86560) (41, 42), which is closely related genetically to the prototype Sapporo strain (14). Thus far, 26 (21 from humans and five from animals [porcine and bat]) complete sapovirus genomes are available in GenBank (as of 1 September 2013). The VP1-encoding region is the most diverse region in the genome (84–86), and sapoviruses are divided into multiple genogroups based on complete VP1 sequences. Five genogroups (GI to GV) are recognized (46, 87), and nine additional genogroups (GVI to GXIV) were recently proposed (88). To date, human sapoviruses have been classified into four genogroups (GI, GII, GIV, and GV).

Distinct antigenicity among sapovirus strains has been demonstrated by using clinical specimens (9, 43, 89–91), recombinant VP1 proteins (77, 92), or virus-like particles (VLPs) (74, 77, 80, 81, 93). Antigenicity differs among GI, GII, GIV, and GV strains (93, 94) and is also distinct among different genotypes within GI and GII (80, 81, 94). These experimental results also support that VP1 determines sapovirus antigenicity. The antigenic differences between human and animal sapoviruses have not yet been determined.

MOLECULAR CHARACTERIZATION

Genogroups and Genotypes

The partial RNA-dependent RNA polymerase (RdRp) or partial VP1 region (Fig. 2) or both of these regions can be used to partially characterize detected sapoviruses, as well as to investigate the similarity of the detected sapovirus for epidemiological surveys. In contrast, the RdRp-VP1 junction region (Fig. 2) is too short for such sequence analysis.

For genetic classification of sapoviruses, VP1 sequences are widely used, because this region is more diverse than the RdRp region (45, 46) and the VP1 sequence correlates with virus phenotype (i.e., antigenicity) (43, 74, 77, 80, 81, 92–95). The International Calicivirus Conference Committee proposed that at least the entire VP1 region sequence is necessary to designate new genogroups or genotypes. We recently established a human sapovirus classification scheme based on the complete VP1 nucleotide sequences (87). In this review, we include newly available sapovirus strains and updated sapovirus genotype numbering along with our previous analytical methods and criteria (87). The frequency histogram with pairwise distance values of 59 representative complete capsid nucleotide sequences of GI, GII, GIII, GIV, and GV sapoviruses resulted in three clearly distinct and nonoverlapping peaks (0 to 0.159, 0.198 to 0.471, and 0.522 to 0.807) (Fig. 3A). These three peaks can be considered to represent the strain, genotype, and genogroup, respectively, as previously described (87). The mean values ± 3 standard deviations (SD) for the pairwise distance peaks were 0 to 0.151, 0.170 to 0.416, and 0.489 to 0.801, respectively (Fig. 3A), and the cutoff values for the genotype and genogroup clusters were designated ≤0.169 and ≤0.488, respectively. Based on the criteria, human sapovirus GI and GII were each subdivided into seven genotypes (GI.1 to GI.7 and GII.1 to

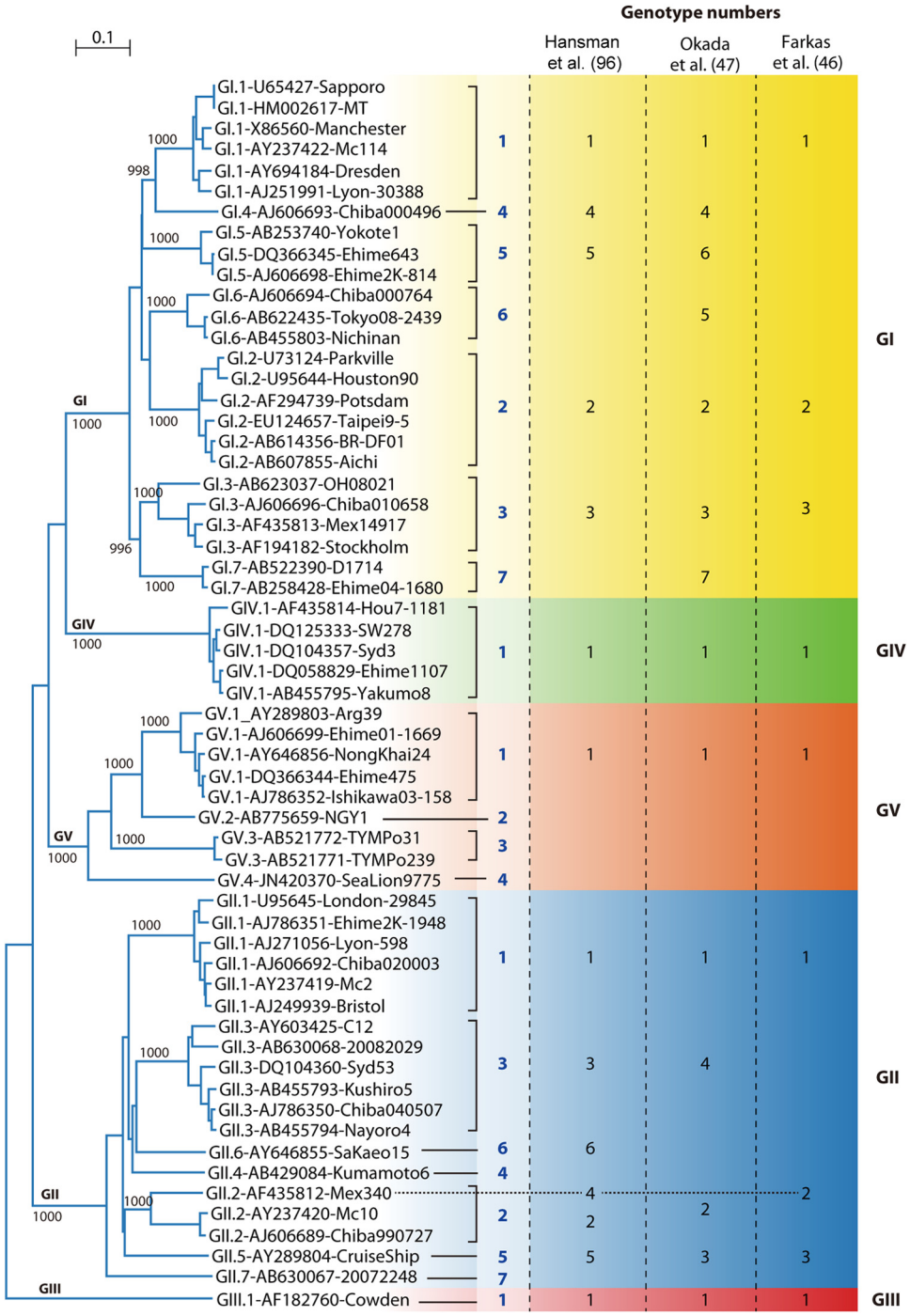

FIG 4 Genogroup and genotypes of GI, GII, GIII, GIV, and GV sapovirus strains based on complete VP1 nucleotide sequences. The phylogenetic tree is based on the complete VP1 nucleotide sequences (approximately 1,690 nt) of a total of 59 sapovirus strains (representing 58 sapovirus strains corresponding to all genotypes within GI, GII, GIV, and GV and one porcine strain representing GIII). The phylogenetic tree was constructed by the neighbor-joining method with 1,000 bootstrap replications using NJPlot software (http://pbil.univ-lyon1.fr/software/njplot.html) (273). The numbers on each branch indicate the bootstrap values of ≥950. The scale represents the nucleotide substitutions per site. Each sapovirus strain is indicated as genogroup/genotype-GenBank accession number-strain name (i.e., GI.1-U65427-Sapporo). Genotyping numbers are updated based on a recent classification scheme (87). Genotyping numbers from three other reports (46, 47, 96) are summarized for comparison.

GII.7). GIV was placed into a single genotype (GIV.1), and GV was subdivided into two genotypes (GV.1 and GV.2) (Fig. 4). GV also includes sapoviruses detected from pigs (GV.3) and sea lions (GV.4). As summarized in Fig. 4, genotype numbering based on the entire VP1 sequences is inconsistent among research groups for GI.5, GI.6, and GII.2 to GII.5 (46, 47, 87, 96). The phylogenetic tree pattern based on the 59 complete VP1 amino acid sequences is similar to that based on the nucleotide sequences (data not

shown); however, the pairwise distance histogram showed only two major peaks (0 to 0.480 and 0.652 to 1.115), and we cannot define the genotype range statistically (Fig. 3B). This differs from the case for noroviruses, because genotypes could be defined statistically by both VP1 nucleotide and amino acid sequences (97).

Evolution and Emergence of Predominant Sapovirus Strains

Genogroup and genotype analysis is important to characterize the currently circulating sapoviruses in the population. Emergence of genetically similar sapoviruses in multiple countries in Europe (98) and dynamic changes of genogroups and genotypes in different years among gastroenteritis patients in the same geographical area in Japan have been reported (99–101). Interestingly, GIV.1 strains were detected predominantly in Japan, Canada, the United States, and Europe around 2007 (98, 101–104). The dynamic change of the detected sapovirus genogroups in 2007 was also identified by national surveillance through regional diagnostic labs network in Japan (http://www.nih.go.jp/niid/en/iasr-table/2784-iasrtve.html; see "IASR Tables Virus" "By Season" "Gastrointestinal Pathogens" "PDF"). This is a distinct trend compared to noroviruses, in which a specific genogroup and genotype (i.e., genogroup II and genotype 4 [GII.4]) have been predominant in the past decade in Japan (http://www.nih.go.jp/niid/en/iasr-table/2784-iasrtve.html) and in multiple other countries (105–108). In the case of norovirus GII.4, time-ordered genetic and antigenic change of VP1 was identified (109, 110). Recent studies from Europe reported similar time-ordered genetic change in the VP1 region of the sapovirus GI.2 strains (98), as reported for norovirus GII.4 strains (111–115).

Due to the inconsistent genotype numbering systems used by different research groups for GI.5, GI.6, and GII.2 to GII.5 (46, 47, 87, 96) (Fig. 4), it is important to indicate which numbering system was used for genotyping, and a harmonized genotype numbering system will facilitate comparison and exchange of information from sapovirus surveillance at national and international levels.

Recombinant Strains

Sapoviruses with inconsistent grouping between the nonstructural protein-encoding region (including the RdRp region) and the VP1 encoding region have been designated "recombinant" or "chimeric" strains. Both intra- and intergenogroup recombinant strains have been reported (Fig. 5). All reported intergenogroup recombinant strains were GIV (based on VP1 sequence), whereas they were clustered together with GII strains in the RdRp region (46, 85, 102, 116, 117). Intragenogroup recombinant strains within GI (118–120), GII (84, 121, 122), and GIII (123) have been identified.

Recently, a norovirus classification scheme has been reported (97). The authors used nucleotide sequences of nearly complete RdRp (1,300 nucleotides [nt]) and both amino acid and nucleotide sequences of VP1 for classification (97). Although sapovirus RdRp (NS7) is fused with protease (NS6) (Fig. 2), we defined the putative complete RdRp-encoding region (1,566 nt) for sapoviruses (Fig. 2) based on our previous *in vitro* studies (52, 58). Among GI, GII, GIV, and GV sapoviruses, the nucleotide sequences spanning the putative complete RdRp- and VP1-encoding regions (Fig. 2) of 26 strains (10 GI, 5 GII, 5 GIV, and 6 GV based on VP1) were available in GenBank as of 1 September 2013. All of these strains and a representative GIII Cowden strain of pig origin were used for phylogenetic analysis based on the RdRp and VP1 regions. We found conserved amino acid motif "WKGL" (Fig. 2) at amino acid positions 12 to 15 in the putative compete RdRp (NS7) region among these sapovirus strains. As shown in Fig. 5, several strains clustered differently on the phylogenetic trees based on RdRp- and VP1-encoding regions. For example, based on the RdRp region, GII and GIV strains are not well separated, as discussed previously (85). The GII.2 Mc10 and GII.3 C12 strains also cluster together in the RdRp region and were previously reported as intragenogroup recombinant strains (84). The GV.4 strain clustered together with other GV strains (GV.1, GV.2, and GV.3) in the VP1 region, but it is separated from other GV strains in the RdRp region. However, the RdRp sequence-based classification is less reliable due to the fewer available sequences compared to the complete VP1 sequences. Further accumulation of sufficient sequence data spanning the complete or sufficient length of the RdRp- to the VP1-encoding regions for all the genogroups and genotypes are critical to provide a better understanding of "recombinant" or "chimeric" strains and to establish a reliable classification scheme for the sapovirus RdRp region in the future, because the putative complete RdRp sequence data for GI.3, GI.4, GI.6, GI.7, GII.4, GII.5, and GII.7 sapoviruses are not yet available (Fig. 5). In addition, it is also critical to amplify a single PCR fragment covering the partial RdRp- and VP1-encoding region for recombination analysis to avoid the possibility of coinfection of different genogroups and/or genotypes of sapovirus strains, as discussed previously (124).

LABORATORY DIAGNOSIS

Virus Particle Detection

Sapoviruses are morphologically distinguishable from other gastroenteritis pathogens (e.g., norovirus, rotavirus, astrovirus, or adenovirus) by their typical "Star of David" surface morphology under the electron microscope (1, 33, 90, 125) (Fig. 1). However, this has low sensitivity compared to nucleic acid detection methods (116, 126–128, 130, 131).

Antigen Detection Methods

Enzyme-linked immunosorbent assays (ELISAs) have been developed for the detection of human sapovirus antigens (91, 93, 132, 190) and have been used for the detection of sapoviruses from clinical samples (43, 91, 132–135, 190). However, these assays are not widely used for diagnosis due to the difficulty in detection of antigenically diverse sapovirus strains, low sensitivity compared to nucleic acid detection methods (43, 91, 93), and current lack of commercial availability. The development of a broadly reactive ELISA or immunochromatography system for the detection of sapovirus antigens depends on the combination of a panel of genogroup/genotype-specific antisera and/or using broadly reactive monoclonal antibodies. These approaches may be feasible, because a common epitope(s) likely exists among GI, GII, GIV, and GV sapovirus strains (94). Broadly reactive norovirus-specific monoclonal antibodies that recognize VP1s of different genogroups of noroviruses were also reported (136–140).

Nucleic Acid Detection Methods

Reverse transcription-PCR (RT-PCR), especially real-time RT-PCR, has become a major and routine method for sapovirus de-

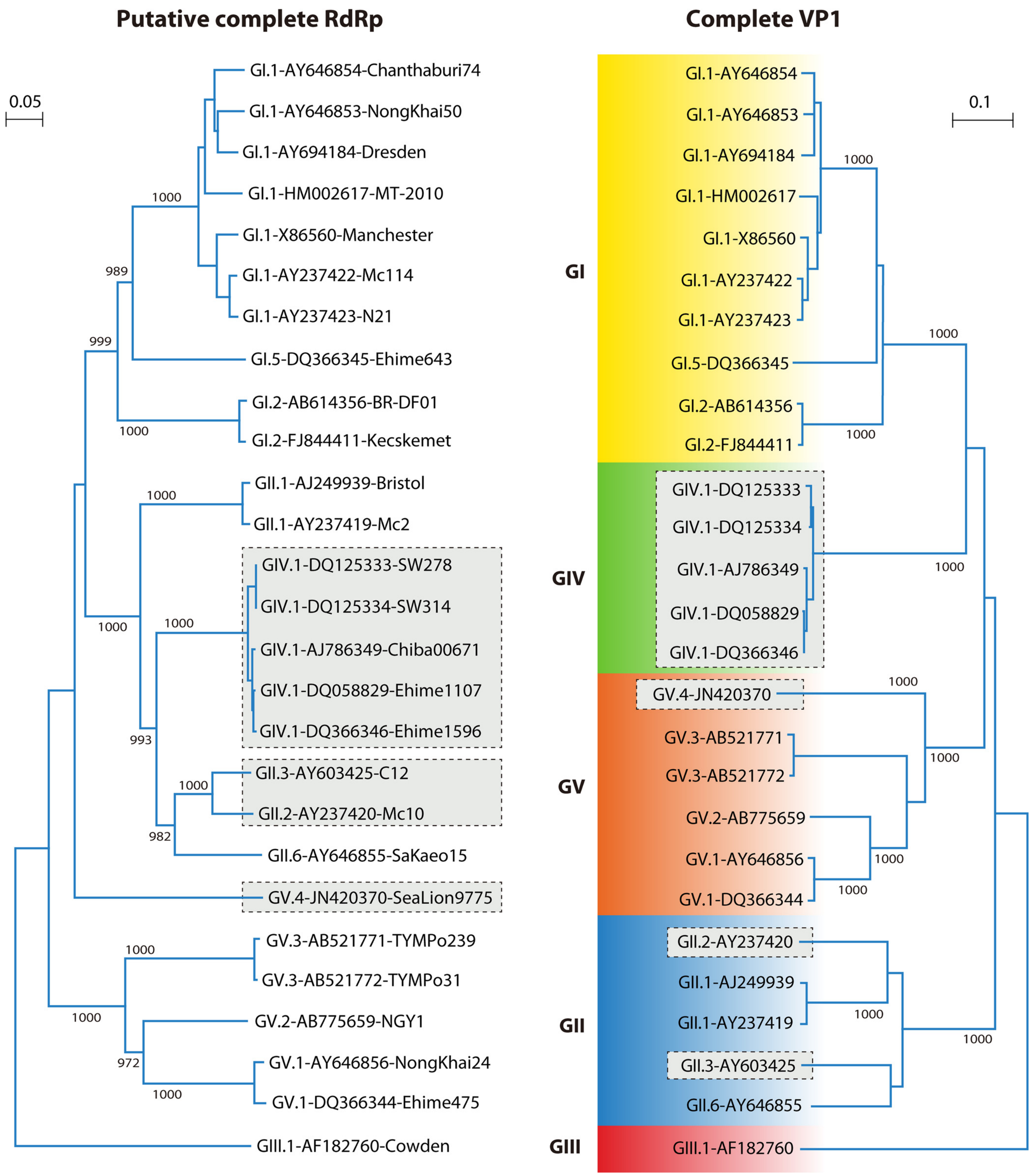

FIG 5 Comparative phylogenetic analysis of sapoviruses based on complete RdRp and VP1 nucleotide sequences. Phylogenetic trees of 27 sapovirus strains (10 GI, 5 GII, 5 GIV, and 6 GV strains and 1 representative GIII strain) whose sequences covering the putative complete RdRp to the end of VP1 had been available at GenBank (www.ncbi.nlm.nih.gov/) by 1 September 2013 are shown. The trees on the left and right are based on the nucleotide sequences of the putative complete RdRp region (approximately 1,570 nt) and the entire VP1 region (approximately 1,690 nt), respectively. The phylogenetic trees were constructed by the neighbor-joining method with 1,000 bootstrap replications using NJPlot software. The numbers on each branch indicate the bootstrap values of ≥950. The scale represents the nucleotide substitutions per site. The sapovirus strains showing inconsistent clustering patterns on the two trees are indicated within dashed boxes.

TABLE 2 Primer combinations targeting the RdRp region for human sapovirus detection

Primer or probe	Sequence (5′ to 3′)	Function[a]	Location in sapovirus genome	Strain	Accession no.	Reference
Sapp36	GTT GCT GTT GGC ATT AAC A	Forward	4273–4291	Manchester	X86560	141
Sapp35	GCA GTG GGT TTG AGA CCA AAG	Reverse	4740–4760	BR-DF01	AB614356	
4490	ACA CGT GGT GGT CTA CCA TCT GG	Forward	4510–4532	Manchester	X86560	143
4485	CAC ACT GTA CAT GCA RTC ATC ACC	Reverse	4666–4689	Manchester	X86560	
p290	GAT TAC TCC AAG TGG GAC TCC AC	Forward	4354–4376	Manchester	X86560	144
p289	TGA CAA TGT AAT CAT CAC CAT A	Reverse	4663–4684	Manchester	X86560	
SR80	TGG GAT TCT ACA CAA AAC CC	Forward	4366–4385	Manchester	X86560	147
JV33	GTG TAN ATG CAR TCA TCA CC	Reverse	4666–4685	Manchester	X86560	
SV-s1	CCC TGG CAA TAT TGG AGA GAT TTG	Forward	4403–4426	Manchester	X86560	148
SV-s2	AAG GCC TAT GAA CCA CAT GGG GC	Forward	4601–4623	Bristol	AJ249939	
SV-s3	GAC ATC AAG TTT GAT ACC AAG GA	Forward	4495–4517	BR-DF01	AB614356	
SV-r-c	GCA TTG TAG GTG GCG AGA GCC	Reverse	5079–5099	Manchester	X86560	
Sapp128	GAT TAC ACC AAA TGG GAT TCC AC	Forward	4354–4376	Manchester	X86560	149
Sapp129	GCA GTC ATC ACC ATA CGT GTG AA	Reverse	4655–4677	Manchester	X86560	
SLV-OFa	ACT SCA AAT GGG ATT CCA CWC AAM ACC	First, forward	4358–4384	Manchester	X86560	150
SLV-OFb	ACW CTA AAT GGG AYT CCA CAC AGA ATC C	First, forward	4358–4385	Manchester	X86560	
SLV-ORa	GTG RAA GAT KGA WGC RGT GGC AGG	First, reverse	4693–4716	Manchester	X86560	
SLV-ORb	TCA AAG ATG GAA GCG GTT GCC G	First, reverse	4694–4715	Manchester	X86560	
SLV-IF	TGG HCT MCC WTC WGG SAT GCC	Second, forward	4518–4538	Manchester	X86560	
SLV-IRa	CAC ACR CTG TAS ATG CAG TCA TCA CC	Second, reverse	4666–4691	Manchester	X86560	
SLV-IRb	CAC AAG GAG TAT ATG CAA TCA TCA CC	Second, reverse	4666–4691	Manchester	X86560	
p290h	GAT TAC TCC AGG TGG GAC TCC AC	Forward	4354–4376	Manchester	X86560	46
p290i	GAT TAC TCC AGG TGG GAC TCA AC	Forward	4354–4376	Manchester	X86560	
p290j	GAT TAC TCC AGG TGG GAT TCA AC	Forward	4354–4376	Manchester	X86560	
p290k	GAT TAC TCC AGG TGG GAT TCC AC	Forward	4354–4376	Manchester	X86560	
p289h	TGA CGA TTT CAT CAT CAC CAT A	Reverse	4663–4684	Manchester	X86560	
p289i	TGA CGA TTT CAT CAT CCC CGT A	Reverse	4663–4684	Manchester	X86560	
SR80	TGG GAT TCT ACA CAA AAC CC	First, forward	4366–4385	Manchester	X86560	152
JV33	GTG TAN ATG CAR TCA TCA CC	First, reverse	4666–4685	Manchester	X86560	
SR80 GI 1–3	CTR KCV GAT ATT GGA RAG ATT T	Second, forward	4404–4425	Manchester	X86560	
SR80 GI 2	AGT CTY TCC ATC TTA GAG AGA	Second, forward	4402–4422	BR-DF01	AB614356	
SR80 GII 1–2	GCT GCR TCY TTG KCA ATC CT	Second, forward	4400–4419	Bristol	AJ249939	
JV33	GTG TAN ATG CAR TCA TCA CC	Second, reverse	4666–4685	Manchester	X86560	
SV36	GTT TCT GTT GGC ATT AAC A	First, forward	4273–4291	Manchester	X86560	153
SRGA	CWG TAB ATG CAR TCA TCA CC	First, reverse	4666–4685	Manchester	X86560	
SVS1	CCC TGG CAA TAT TGG AGA GAT TTG	Second, forward	4403–4426	Manchester	X86560	
SRGE	TAC WGY AAA TGG GAT TCCAC	Second, forward	4357–4376	Manchester	X86560	
SRGA	CWG TAB ATG CAR TCA TCA CC	Second, reverse	4666–4685	Manchester	X86560	
SRGS2A	CAC ATT ACC AGT GTA AGG TGC CC	Second, reverse	4620–4642	Bristol	AJ249939	
Sapp36	GTT GCT GTT GGC ATT AAC A	Forward	4273–4291	Manchester	X86560	100
SV-s1	CCC TGG CAA TAT TGG AGA GAT TTG	Forward	4403–4426	Manchester	X86560	
SV-s2	AAG GCC TAT GAA CCA CAT GGG GC	Forward	4601–4623	Bristol	AJ249939	
SV-s3	GAC ATC AAG TTT GAT ACC AAG GA	Forward	4495–4517	BR-DF01	AB614356	
GV-GLPSGM	GGT CTC CCC TCG GGC ATG	Forward	4560–4577	NongKhai24	AY646856	
SaV1245R	CCC TCC ATY TCA AAC ACT A	Reverse	5159–5177	Manchester	X86560	

[a] Primers used for nested RT-PCR are designated first and second.

tection from clinical specimens (i.e., feces), because of its specificity, sensitivity, and broad reactivity. Numerous primers have been designed for the detection of human sapoviruses (Tables 2 to 4). These primers are designed to amplify the partial RdRp (44, 46, 141–144, 146–153), RdRp-VP1 junction (86, 154–162), or partial VP1 (126, 152, 163–166) region (Fig. 2). Due to the high genetic diversity of sapoviruses, most of the assays include multiple or degenerate primers (Tables 2 to 4). The primers targeting the con-

TABLE 3 Primer and probe combinations targeting the RdRp-VP1 junction region for human sapovirus detection

Primer or probe	Sequence (5′ to 3′)[a]	Function	Location in sapovirus genome	Strain	Accession no.	Reference
CU-SV-F1	GAC CAG GCT CTC GCY ACC TAC	Forward	5074–5094	Manchester	X86560	154
CU-SV-F2	TTG GCC CTC GCC ACC TAC	Forward	5077–5094	BR-DF01	AB614356	
CU-SV-R	CCC TCC ATY TCA AAC ACT AWT TTG	Reverse	5154–5177	Manchester	X86560	
CU-SV-Probe	TGG TTY ATA GGY GGT AC	Probe	5101–5117	Manchester	X86560	
SaV124F	GAY CAS GCT CTC GCY ACC TAC	Forward	5074–5094	Manchester	X86560	86
SaV1F	TTG GCC CTC GCC ACC TAC	Forward	5077–5094	BR-DF01	AB614356	
SaV5F	TTT GAA CAA GCT GTG GCA TGC TAC	Forward	5112–5135	NongKhai24	AY646856	
SaV1245R	CCC TCC ATY TCA AAC ACT A	Reverse	5159–5177	Manchester	X86560	
SaV124TP	CCR CCT ATR AAC CA	Probe	5101–5114	Manchester	X86560	
SaV5TP	TGC CAC CAA TGT ACC A	Probe	5142–5157	NongKhai24	AY646856	
sapo.fwdA	ACC AGG CTC TCG CCA CCT A	Forward	5075–5093	Manchester	X86560	155
sapo.fwdB	ATT TGG CCC TCG CCA CCT A	Forward	5075–5093	BR-DF01	AB614356	
sapo.rev	GCC CTC CAT YTC AAA CAC TAW TTT	Reverse	5155–5178	Manchester	X86560	
sapo.probeA	CTG TAC CAC CTA TGA ACC A	Probe	5101–5119	Manchester	X86560	
sapo.probeB	TTG TAC CAC CTA TGA ACC A	Probe	5101–5119	Manchester	X86560	
sapo.probeC	TGT ACC ACC TAT AAA CCA	Probe	5101–5118	Manchester	X86560	
sapo.probeD	TGC ACC ACC TAT GAA C	Probe	5107–5122	Ehime1107	DQ058829	
F1	CCA GGC TCT CGC CAC CTA C	Forward	5076–5094	Manchester	X86560	248
F2	CCA GGC TCT CGC TAC CTA C	Forward	5076–5094	Manchester	X86560	
F3	TTT GGC CCT CGC CAC CTA C	Forward	5076–5094	BR-DF01	AB614356	
R1	GCC CTC CAT CTC AAA CAC TAT TTT G	Reverse	5154–5178	Manchester	X86560	
R2	GCC CTC CAT TTC AAA CAC TAA TTT G	Reverse	5154–5178	Manchester	X86560	
probe	TGG TTY ATA GGY GGT RCA	Probe	5101–5118	Manchester	X86560	
SLVCF	GAY CWG GCY CTC GCC ACC T	Forward	5074–5092	Manchester	X86560	156
SLVCR	GCC CTC CAT YTC AAA CAC TA	Reverse	5159–5178	Manchester	X86560	
SVLCP	TGY ACC ACC TAT RAA CCA VG	Probe	5099–5118	Manchester	X86560	
SAPOs	CAG GCT CTC GCC ACC TAC	Forward	5077–5094	Manchester	X86560	157
SAPOas	CCC TCC ATY TCA AAC ACT AWT TT	Reverse	5155–5177	Manchester	X86560	
SAPO-XS-QS705	TGG TTC ATA GGT GGT RC	Probe	5101–5117	Manchester	X86560	
F1	TTG GCC CTC GCC ACC TAC	Forward	5077–5094	BR-DF01	AB614356	158
F2	GAY CAS GCT CTC GCY ACC TAC	Forward	5074–5094	Manchester	X86560	
R	CCC TCC ATY TCA AAC ACT A	Reverse	5159–5177	Manchester	X86560	
P	CCR CCT ATR AAC CA	Probe	5101–5114	Manchester	X86560	
F	GAY CAG GCT CTC GCY ACC TAC	Forward	5074–5094	Manchester	X86560	160
F	TTG GCC CTC GCC ACC TAC	Forward	5077–5094	BR-DF01	AB614356	
F	TTT GAA CAA GCT GTG GCA TGC TAC	Forward	5112–5135	NongKhai24	AY646856	
R	CCC TCC ATY TCA AAC ACT A	Reverse	5159–5177	Manchester	X86560	
P	CYT GGT TCA TAG GTG GTR CAG	Probe	5099–5119	Manchester	X86560	
P	CAG CTG GTA CAT TGG TGG CAC	Probe	5138–5158	NongKhai24	AY646856	
SLV5317	CTC GCC ACC TAC RAW GCB TGG TT	Forward	5083–5105	Manchester	X86560	159
SMP-R	CMW WCC CCT CCA TYT CAA ACA C	Reverse	5161–5182	BR-DF01	AB614356	
F	GAY CAG GCT CTC GCY ACC TAC	Forward	5074–5094	Manchester	X86560	161
F	TTT GAA CAA GCT GTG GCA TGC TAC	Forward	5112–5135	NongKhai24	AY646856	
R	CCC TCC ATY TC AAA CAC TA	Reverse	5159–5177	Manchester	X86560	
P	CYT GGT TCA TAG GTG GTR CAG	Probe	5099–5119	Manchester	X86560	
P	CAG CTG GTA CAT TGG TGG CAC	Probe	5138–5158	NongKhai24	AY646856	
GI-F	Tag-CTC GCC ACC TAC AAT GCY TGG TT	Forward	5083–5105	Manchester	X86560	162
GII, GIV, V-F	Tag-ACR GCC AAR GCT GAG GGG	Forward	5036–5053	Ehime1107	DQ058829	
GI-R	Tag-TGG GAT GTG GTC GGV CCA GT	Reverse	5242–5261	Manchester	X86560	
GII, GIV, V-R	Tag-CCC TCC ATT TCA AAC ACT AAT T	Reverse	5160–5181	Ehime1107	DQ058829	
GI probe	CCG AGC CTA GTG TTT GAG ATG GAG GGC AAT GGC TCG G	Probe	5152–5188	Manchester	X86560	
GII, GIV, V-Probe	CCC TGG GCC CCA GTG AAG AGA CCA CCA GGG	Probe	5133–5162	Ehime1107	DQ058829	

[a] Tag, GCAAGCCCTCACGTAGCGAA.

TABLE 4 Primer and probe combinations targeting the VP1 region for human sapovirus detection

Primer or probe	Sequence (5′ to 3′)	Function[a]	Location in sapovirus genome	Strain	Accession no.	Reference
SV-F11	GCY TGG TTY ATA GGT GGT AC	First, forward	5098–5117	Manchester	X86560	126
SV-R1	CWG GTG AMA CMC CAT TKT CCA T	First, reverse	5857–5878	Manchester	X86560	
SV-F21	ANT AGT GTT TGA RAT GGA GGG	Second, forward	5157–5177	Manchester	X86560	
SV-R2	GWG GGR TCA ACM CCW GGT GG	Second, reverse	5572–5591	Manchester	X86560	
SLV5317	CTC GCC ACC TAC RAW GCB TGG TT	Forward	5083–5105	Manchester	X86560	163
SLV5749	CGG RCY TCA AAV STA CCB CCC CA	Reverse	5494–5516	Manchester	X86560	
SV-F13	GAY YWG GCY CTC GCY ACC TAC	First, forward	5074–5094	Manchester	X86560	165
SV-F14	GAA CAA GCT GTG GCA TGC TAC	First, forward	5074–5094	Manchester	X86560	
SV-R13	GGT GAN AYN CCA TTK TCC AT	First, reverse	5857–5876	Manchester	X86560	
SV-R14	GGT GAG MMY CCA TTC TCC AT	First, reverse	5857–5876	Manchester	X86560	
SV-F22	SMW AWT AGT GTT TGA RAT G	Second, forward	5154–5172	Manchester	X86560	
SV-R2	GWG GGR TCA ACM CCW GGT GG	Second, reverse	5572–5591	Manchester	X86560	
SV-F13	GAY YWG GCY CTC GCY ACC TAC	Forward	5074–5094	Manchester	X86560	165
SV-F14	GAA CAA GCT GTG GCA TGC TAC	Forward	5074–5094	Manchester	X86560	
SV-G1-R	CCC BGG TGG KAY GAC AGA AG	Reverse	5561–5580	Manchester	X86560	
SV-G2-R	CCA NCC AGC AAA CAT NGC RCT	Reverse	5483–5503	Mc10	AY237420	
SV-G4-R	GCG TAG CAG ATC CCA GAT AA	Reverse	5413–5432	Ehime1107	DQ058829	
SV-G5-R	TTG GAG GWT GTT GCT CCT GTG	Reverse	5384–5404	NongKhai24	AY646856	
No name	GCT GTT SCY ACT GGT GCA	Forward	5317–5334	Manchester	X86560	164
No name	GGC ATC CTG TCR TTC CAA GCA	Reverse	5391–5411	Manchester	X86560	
No name	CCA ATC SAA TGT CCC TGA GGC AAT ACG SAA	Probe	5337–5366	Manchester	X86560	
SLV5317	CTC GCC ACC TAC RAW GCB TGG TT	First, forward	5083–5105	Manchester	X86560	152
SLV5749	CGG RCY TCA AAV STA CCB CCC CA	First, reverse	5494–5516	Manchester	X86560	
SVpol3′-A	AAG GMR CSY MCA AAA ATA GTG	Second, forward	5144–5164	Manchester	X86560	
SVpol3′-B	GAA GRK RCW MCC AAA TTA GTG	Second, forward	5147–5167	Bristol	AJ249939	
SLV5749	CGG RCY TCA AAV STA CCB CCC CA	Second, reverse	5494–5516	Manchester	X86560	
SaV124F	GAY CAS GCT CTC GCY ACC TAC	First, forward	5074–5094	Manchester	X86560	175
SaV1F	TTG GCC CTC GCC ACC TAC	First, forward	5077–5094	BR-DF01	AB614356	
SaV5F	TTT GAA CAA GCT GTG GCA TGC TAC	First, forward	5112–5135	NongKhai24	AY646856	
SV-R13	GGT GAN AYN CCA TTK TCC AT	First, reverse	5857–5876	Manchester	X86560	
SV-R14	GGT GAG MMY CCA TTC TCC AT	First, reverse	5857–5876	Manchester	X86560	
1245Rfwd	TAG TGT TTG ARA TGG AGG G	Second, forward	5159–5177	Manchester	X86560	
SV-R2	GWG GGR TCA ACM CCW GGT GG	Second, reverse	5572–5591	Manchester	X86560	
SV-F13	GAY YWG GCY CTC GCY ACC TAC	First, forward	5074–5094	Manchester	X86560	180
SV-F14	GAA CAA GCT GTG GCA TGC TAC	First, forward	5074–5094	Manchester	X86560	
SVR-DS3	GGT GAV AVM CCA TTY TCC AT	First, reverse	5849–5868	Ehime1107	DQ058829	
SVR-DS4	GGH GAH ATN CCR TTB TSC AT	First, reverse	5849–5868	Ehime1107	DQ058829	
SaV 1245Rfwd	TAG TGT TTG ARA TGG AGG G	Second, forward	5159–5177	Manchester	X86560	
SVR-DS5	CCC CAC CCK GCC CAC AT	Second, reverse	5482–5498	Manchester	X86560	
SVR-DS6	CCC CAM CCM GCM MAC AT	Second, reverse	5482–5498	Manchester	X86560	
Forward	CAA TCC AAT CCA ATG TCC CT	Forward	5333–5352	Manchester	X86560	166
Reverse	ACY TCA AAV STA CCB CCC CA	Reverse	5494–5513	Manchester	X86560	
probe	ATT AAC CCG TAC ACT TCT CA	Probe	5452–5471	Manchester	X86560	

[a] Primers used for nested RT-PCR are designated first and second.

served motifs of the RdRp region (e.g., p290 and p289 [Table 2]) also amplify other human gastroenteritis viruses (norovirus, rotavirus, and astrovirus) (144, 167, 168). Numerous primers with distinct names are quite similar, especially for RdRp-VP1 junction-targeting primer sets (Table 3).

Multiplex RT-PCR or PCR assays, whose products were differentiated by agarose gel electrophoresis (101, 159, 163, 169), real-time RT-PCR or PCR (156, 158, 162, 170), and a microsphere-based fluorescent PCR product detection assay (e.g., Luminex technology) (160, 166), have been reported for the detection of human sapoviruses together with other gastroenteritis viruses. Although these assays aimed for simultaneous detection of multiple

viruses, it is unclear whether these assays can detect all genogroups of human sapoviruses.

Sapoviruses were also detected by specific primer-independent techniques (i.e., the metagenomic sequence approach) from untreated sewage (16), sewage sludge (171), and feces from California sea lions (172), dogs (173), and humans. These approaches are not widely used for diagnosis but may be applicable for routine clinical diagnosis in the future, when the cost of such assays and data analysis is comparable to that of traditional assays.

Selection of Detection Methods

The nucleic acid detection method is more sensitive than EM (116, 126–128, 130, 131) or ELISA (91). Different detection rates among different PCR assays using the same panel of specimens (clinical specimens, environmental water, and shellfish) were reported (99–101, 174–177). Assays targeting the RdRp-VP1 junction region have the highest detection rate and can be used as the first choice for sapovirus screening from clinical specimens (101, 174). The VP1-targeting RT-PCR is preferred because the products can be sequenced for reliable genotyping (99–101). Similar results were reported for environmental water samples (i.e., river water) (175). RdRp-VP1 junction-targeting real-time RT-PCR was also used for the detection of sapoviruses from shellfish (178, 179): however, the nested RT-PCR targeting the partial VP1 region is superior to the real-time RT-PCR and single-round RT-PCR because of the low level of viral RNA in shellfish compared to clinical specimens (177, 178). Currently, limited primer sets (47, 86, 100, 165, 175, 180) have demonstrated the ability to detect all genogroups of human sapoviruses.

Full-Genome Sequencing Approaches

Full genomic sequence analysis is still not practical for routine diagnosis. A long single-round or nested RT-PCR to amplify a 2- to 2.5-kb PCR fragment to determine the complete VP1 sequences of various sapovirus strains from clinical specimens is feasible by using forward primers targeting the RdRp and/or RdRp-VP1 junction region (Tables 2 to 4) and a reverse primer hybridized to the 3′-end poly(A) tail (Fig. 2) (46, 47, 87, 130, 174, 181, 182). In contrast, the amplification of the 5′-end 5- to 5.5-kb fragment corresponding to the beginning of the genome to the VP1 upstream region is variable because of the lack of universal primers. As a new technology, the specific primer-independent metagenomic sequencing approach (i.e., next-generation sequencing techniques) can be used to determine the nearly complete genome sequences (lacking the 5′ end or both the 5′ and 3′ ends) from fecal specimens (172, 173). 5′ rapid amplification of cDNA ends (RACE) techniques (14, 41, 42) are still necessary to determine 5′ ends to obtain the complete sapovirus genomic sequences.

CLINICAL AND EPIDEMIOLOGICAL OBSERVATIONS

Symptoms and Severity of Disease

Based on the epidemiological data from patients with sapovirus gastroenteritis, the incubation period ranges from less than 1 day to 4 days (5, 8, 44, 130, 135, 178, 183, 184). Major clinical symptoms include diarrhea and vomiting; however, additional constitutional symptoms (i.e., nausea, stomach/abdominal cramps, chills, headache, myalgia, or malaise) are also frequently reported.

TABLE 5 Reported clinical severity scores for sapovirus-, norovirus-, and rotavirus-associated gastroenteritis

Subject age	Clinical severity score (range)			Reference
	Sapovirus	Norovirus	Rotavirus	
<2 yr	6[b]	8[b]	10[b]	185
<2 yr	5.2 (3–10)[b]	7.9 (3–16)[b]	8.4 (1–16)[b]	186
Not specified[a]	6 (0–15)[c]	6 (0–12)[c]	8 (0–14)[c]	191

[a] Described as 0 to 65 and >65 years (191).
[b] Scored with a 0- to 20-point numerical system (192).
[c] Scored with a 0- to 21-point numerical system (191).

Similar to the case for norovirus illness, fever is a rare clinical symptom. Diarrhea usually resolves within 1 week (4, 5, 7–9, 44, 104, 117, 127, 135, 183, 185–189); however, individuals showing symptoms for a longer time (i.e., from over a week to up to 20 days) were also reported (9, 21, 127, 186, 188, 267). In general, the severity of sapovirus gastroenteritis is milder than that for rotavirus and norovirus (Table 5) (185, 186, 191). Gastroenteritis symptoms are usually self-limiting, and patients usually recover within a couple of days; however, the symptoms, severity, and duration of disease are dependent on the individual, and sapovirus infection sometimes leads to hospitalization (22, 152, 167, 193–209). Mortality is rare, but it was reported from outbreaks that occurred in a long-term-care facility for the elderly (104). Human noroviruses are associated with more serious clinical complications in susceptible groups (i.e., premature neonates and immunocompromised patients) (210–212). No such information is available for human sapoviruses, and this requires investigation in the future.

Subclinical (asymptomatic) sapovirus infection was also detected (4, 6, 21, 134, 135, 213–216). Quantitative PCR analysis revealed that asymptomatic individuals also shed sapovirus in the feces at levels comparable to those shed by individuals with gastroenteritis (182, 183).

Shedding Levels and Patterns in Feces

Sapovirus shedding in feces may continue after symptoms disappear (1 to 4 weeks after onset of illness) (6, 22, 174, 191). Sapovirus shedding levels in clinical stool specimens range from 1.32×10^5 to 1.05×10^{11} genomic copies/gram of stool (80, 99, 101, 116, 117, 127–130, 174, 178, 181–183). Sapovirus RNA shedding levels in feces gradually decreased after onset of illness (174). During the prolonged excretion period (i.e., 25 days and 28 days after onset of illness) in some individuals in an outbreak, both synonymous and nonsynonymous nucleotide substitutions in the VP1-encoding region have been identified (174), and this is a possible mechanism for the generation of new variants of sapovirus *in vivo*. Similar to the case for noroviruses, sapoviruses were also detected from an immunocompromised patient who showed prolonged diarrhea (147 days) (217), although further studies with quantitative analysis are necessary.

Sporadic Cases

Sapoviruses are detected worldwide (i.e., in more than 35 countries), and more than 100 papers have described sapovirus detection from clinical specimens. Among them, 13 studies detected more than 30 sapovirus strains from patients with sporadic gastroenteritis (Table 6) (89, 99, 101, 102, 126, 131, 132, 165, 185, 191, 215, 216, 218–226). Although different methods (electron microscopy, ELISA, and PCR assays with different primer sets)

TABLE 6 Sapovirus positive rates in gastroenteritis patients from 13 studies that detected more than 30 strains during the study period

Country	Study period	Age (yr)	Sapovirus positive rate, % (no. positive/total)	Screening method	Genogroup[g]	Positive rate (%) for other viral pathogens	Reference(s)
UK	1979-1981	Unknown	6.6 (39/592)	EM	NA[h]	NA	89
Kenya	1991-1994	≤6	2.2 (32/1431)	ELISA	NA	NA	132
Finland	1993-1995	<2	9.3 (72/775)	Nested RT-PCR[a]	NA	Rotavirus, 23.0; norovirus, 20.2; astrovirus, 8.8; adenovirus, 6.3	185
UK	1993-1996	All	3.8 (92/2422)	RT-PCR[b]	NA	Norovirus, 36.0; rotavirus, 31.3	218
Netherlands	1998-1999	All	6.3 (43/687)	RT-PCR[b]	NA	Norovirus, 16.1; rotavirus, 7.3; adenovirus, 3.8; astrovirus, 2.0	216
Japan	1998-2005	All	9.3 (195/2100)	Nested RT-PCR[c]	GI, GII, GIV, GV	NA	126, 165
Japan	2002-2007	All	12.7 (81/639)	RT-PCR[d]	GI, GII, GIV, GV	Norovirus, 40.7; rotavirus, 7.7; adenovirus, 3.0; enterovirus, 2.0; astrovirus, 1.4	101
Japan	2002-2009	≤15	3.7 (146/3895)	RT-PCR[e]	GI, GII, GIV	NA	102, 220–224
Denmark	2005-2007	≤3	8.8 (97/1104)	qRT-PCR[f]	GI, GII, GV	NA	225
UK	2006-2007	<5	12.7 (74/583)	RT-PCR[b]	NA	Norovirus, 24.5; rotavirus, 19.0	219
USA	2008-2009	<5	5.4 (42/782)	qRT-PCR[f]	NA	Norovirus, 21.4; rotavirus, 18.0; adenovirus, 11.8; astrovirus, 4.9	215
UK	2008-2009	All	8.8 (77/874)	qRT-PCR[f]	NA	Norovirus, 12.4; rotavirus, 7.3; adenovirus, 3.4; astrovirus, 2.5	226
Canada	2008-2009	All	4.2 (107/2486)	qRT-PCR[f]	NA	Norovirus, 17.6; rotavirus, 6.8; astrovirus, 2.0; adenovirus, 1.4	131

[a] First, Sapp36/SLV-r-c; second, S1,S2,S3-Sapp-rc (148).
[b] SR80-JV33 (147).
[c] First, F11/R1 or F13,14,R13,14; second, F21/R2 or F22/R2 (126, 165).
[d] SaV1F, -1,2,4F, -5F, -1245R (86, 101).
[e] SLV5317-5749 (163).
[f] SaV1F, -1,2,4F, -5F, -1245R, and SaV124TP, SaV5TP (86).
[g] Genogroups were determined based on partial VP1 region sequences.
[h] NA, not available.

were used in these studies, the sapovirus positive rates ranged from 2.2% to 12.7%. Eight studies also detected other gastroenteritis pathogens, and sapoviruses ranked second to fourth as the major viral pathogens among patients with sporadic gastroenteritis (Table 6). Similar to the case for noroviruses (101, 131, 185, 198), sapoviruses were detected mainly in the cold season among patients with sporadic gastroenteritis (89, 99–101, 131, 198, 225–227), although different seasonal peaks among years have also been reported (132, 185). Sapovirus illnesses occur more frequently in younger children than in older children and adults (131, 191, 216).

Outbreaks

Although the reported outbreak numbers are less for sapoviruses than for noroviruses (145, 228–230), sapovirus gastroenteritis outbreaks occur throughout the year in all ages of people in various settings, such as child day care centers, kindergartens, schools, colleges, hospitals, nursing homes, restaurants, hotels, wedding halls, and ships (3–7, 9, 80, 98, 103, 104, 116, 117, 127, 129, 130, 145, 174, 181, 182, 186, 188, 189, 228, 231–237). Suspected foodborne sapovirus outbreaks have also been reported (44, 145, 178, 230, 232, 238). The largest foodborne sapovirus outbreak (n = 665) has been reported in Japan in 2010 (183). An epidemiological investigation pointed to contaminated box lunches which were prepared by food handlers who were shedding sapovirus.

Data from four studies suggest that sapovirus caused 1.3 to 8.0% of the gastroenteritis outbreaks (Table 7) (98, 145, 228, 230), and data from the other three studies reported that sapovirus was detected in 5.9 to 22.6% of outbreak samples that tested negative for norovirus or both norovirus and pathogenic bacteria (103, 104, 234) (Table 7).

Coinfections of sapoviruses and multiple enteric viruses (e.g., noroviruses, rotaviruses, astroviruses, adenoviruses, enteroviruses, kobuviruses, etc.) have also been reported among acute gastroenteritis outbreaks (10, 178, 230, 231, 235–237, 239). Coinfections with different sapovirus strains (i.e., different genogroups/genotypes) were also identified from oyster/clam-associated gastroenteritis outbreaks (178, 236).

Sapoviruses in Seafood, Environmental Water, and Animals

Sapoviruses genetically indistinguishable (i.e., similar or identical based on partial virus genome sequences) from those detected in

TABLE 7 Sapovirus positive rates in gastroenteritis outbreaks

Country	Study period	Sapovirus positive rate, % (no. positive/total)	Screening method	Genogroup[g]	Positive rate (%) for other viral pathogens	Reference
Sweden	1994-1998	1.3 (9/676)	EM	NA[h]	Norovirus, 89	145
USA	2000-2004	1.8 (4/226)	RT-PCR[c]	NA	Norovirus, 79.6	228
Netherlands	2007-2009	4.0 (19/478)	qRT-PCR[d]	GI, GIV	NA	98
Germany	2002-2003	5.9 (2/34)[a]	RT-PCR[e]	NA	NA	234
Canada	2004-2007	17.6 (43/244)[b]	qRT-PCR[f]	GI, GII, GIV, GV	NA	103
USA	2002-2009	22.6 (21/93)[b]	qRT-PCR[f]	GI, GII, GIV, GV	NA	104
Japan	2001-2012	8.0 (7/88)	qRT-PCR[f]	GI, GII, GIV	Norovirus, 96.6; kobuvirus, 21.6; astrovirus, 5.7; rotavirus, 1.1	230

[a] Norovirus- and pathogenic bacterium-negative outbreaks.
[b] Norovirus-negative outbreaks.
[c] p290/289 (144).
[d] SLV-CF/SLV-CR, SLV-CP (156).
[e] SR80/JV33 (147).
[f] SaV1F, -1,2,4F, -5F, -1245R, SaV124TP, SaV5TP (86).
[g] Genogroups were determined based on partial VP1 region sequences.
[h] NA, not available.

human clinical specimens have also been detected from shellfish (oysters and clams) (177, 178, 240, 241) and environmental water samples (river water and wastewater) (16, 171, 175, 176, 180, 241–246). These sapoviruses were likely viruses of human fecal origin that were discharged into environmental waters and accumulated in shellfish (i.e., oysters or clams). As evidence, sapoviruses were detected more frequently with higher viral RNA levels from environmental water samples (i.e., sewage and river water) in the cold season (175, 176, 180, 243, 247, 248), when the number of patients with sapovirus-associated sporadic gastroenteritis increased (89, 99–101, 131, 198, 225–227). In addition, similar sapovirus strains were detected from gastroenteritis patients, wastewater, and oysters, which were collected from geographically related areas in the same season (241). In contrast, sapoviruses genetically indistinguishable from those detected in human clinical specimens have not been discovered in other animals (i.e., swine, mink, bats, dogs, and sea lions) (48, 88, 123, 172, 173, 249–253). Based on complete VP1 sequences, GV.3 porcine sapoviruses are closest to human strains; however, they can be clearly separated into a different genotype (Fig. 4). These results suggest the existence of interspecies barriers among human and animal sapoviruses, although further epidemiological studies for other animals and experimental infection studies using human sapoviruses in various animals are necessary. Sapovirus contamination levels were $\sim 1.6 \times 10^4$ copies/g of digestive tissue in various types of shellfish (oyster, cockle, and smooth clam) (179), up to 1.3×10^5 copies/liter in wastewater treatment plant influent (248), and $\sim 1.3 \times 10^9$ copies/liter in untreated wastewater (247).

Transmission Route and Host Susceptibility

Transmission of sapovirus is through the fecal-oral route. Sapoviruses can be transmitted from person to person via contact with sapovirus-positive feces, vomitus, or sapovirus-contaminated materials/surfaces or via contaminated food and drinking water (44, 104, 129, 130, 145, 178, 182, 183, 231, 232, 236–239). These transmission routes are similar to those for norovirus (254), and sapovirus may also have a low infectious dose similar to that of norovirus (i.e., 1,015 to 2,800 genomic copies) (255, 256); however, similar volunteer studies are necessary to confirm this speculation for sapoviruses. No host genetic factors for susceptibility or resistance to human sapovirus infection and disease have been identified. Susceptibility to human sapoviruses is not associated with histo-blood group antigen (HBGA) phenotypes (214). *In vitro* data also support no binding of sapovirus to HBGAs (257, 258). This differs from the case for the prototype norovirus (Norwalk virus): certain HBGA phenotypes (e.g., nonsecretor) of an individual are clearly related to resistance to virus infection (259, 260). Other different genogroups/genotypes of norovirus strains can also bind to HBGAs (113, 261–263) but lack a clear relatedness between the HBGA phenotypes and resistance to infection (264–266). Sialic acids have recently been reported as binding factors for porcine sapovirus (258).

Immunity

The serological responses to sapovirus infection were demonstrated by immune electron microscopy, ELISA, or radioimmunoassay using paired sera (i.e., acute- and convalescent-phase sera) with purified virus from clinical specimens (3, 4, 7, 22, 90, 190, 267, 268). The seroprevalence studies of human sapoviruses using purified virus or recombinant capsid proteins demonstrated a gradually increasing seroprevalence rate with age, and it reached a high level (>90%) in school-age children, and remained high (80 to 100%) in sera or pooled immunoglobulin collected from adults (92, 132, 190, 269–271). These results suggest that sapovirus infection is common during early childhood.

Protective immunity/resistance mechanisms to sapovirus infection at the putative primary infection site (e.g., intestinal lumen) remain to be clarified, but the presence of preexisting serum antibodies to sapoviruses was associated with reduced frequencies of sapovirus infection and illness, at least for antibodies to antigenically homologous sapoviruses (267). A similar phenomenon was also observed in gastroenteritis outbreaks that occurred in mother and baby units (7). Adults who had serum antibodies to antigenically indistinguishable human sapoviruses did not show any clinical symptoms on reinfection (7). Symptomatic reinfections with a different genogroup/genotype of sapovirus were recently reported in a study from Japan (99).

CONCLUSIONS AND FUTURE DIRECTIONS

Recent epidemiological studies with improved diagnostic assays have highlighted the impact of sapovirus-associated gastroenteritis. Genetically highly diverse sapovirus strains were identified through epidemiological surveillance studies. Continuous surveillance with a broadly reactive detection system(s) and molecular characterization will permit the identification of changes in major strains as well as the emergence of new strains and an understanding of the evolution of sapoviruses among humans and animals. However, in contrast to the significant improvement in sapovirus detection methods, the basic understanding of infection/replication sites, pathological changes in infected persons, immunological responses and protective immunity to sapovirus infections in humans, infectious dose, and stability in the environment remain unknown. To date, no vaccines or antiviral drugs are available for the control and prevention of human sapovirus infections. The mechanisms of virus binding and entry into target cells and viral RNA replication and translation are undefined, partially due to the lack of a cell culture system. Extensive studies of human sapoviruses in clinical cases, the use of the cell culture-adapted porcine sapovirus strain as a model, and establishment of a human sapovirus cell culture system will improve our knowledge of sapoviruses and may lead to more targeted control measures for prevention of sapovirus gastroenteritis in the future.

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