

REVIEW



Human sapoviruses: genetic diversity, recombination, and classification

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SUMMARY

The family *Caliciviridae* contains four genera *Sapovirus*, *Norovirus*, *Lagovirus* and *Vesivirus*, which include *Sapporo virus* (SaV), *Norwalk virus* (NoV), *Rabbit hemorrhagic disease virus* (RHDV) and *Feline calicivirus* (FCV), respectively. SaV is a causative agent of gastroenteritis in children and adults. SaV can be divided into five genogroups (GI–GV), among which GI, GII, GIV and GV are known to infect humans, whereas SaV GIII infects porcine species. Detection methods include ELISA, RT-PCR and real-time RT-PCR. Since few SaV studies have been conducted, it is difficult to draw correlations between or conclusions about rates of incidence, detection and overall prevalence. Nevertheless, most studies agree that SaV infection is more frequent in young children than adults and that infection in children almost always occurs by 5 years of age. In addition, children at day-care centres and institutions are at greatest risk of SaV-associated infection and transmission. Recently, a number of important findings concerning human SaV were discovered. SaV strains were detected in water samples, which included untreated wastewater specimens, treated wastewater samples and river samples. SaV strains were also detected in shellfish samples destined for human consumption, and recombinant SaV strains were identified in a number of different countries. The purpose of this review was to highlight the current knowledge of human SaV, which appears to be an increasingly important virus causing gastroenteritis in humans. Copyright © 2007 John Wiley & Sons, Ltd.

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INTRODUCTION

The family *Caliciviridae* contains four genera *Sapovirus*, *Norovirus*, *Lagovirus* and *Vesivirus*, which include *Sapporo virus* (SaV), *Norwalk virus* (NoV), *Rabbit hemorrhagic disease virus* (RHDV) and *Feline calicivirus* (FCV), respectively. SaV and NoV are etiological agents of human gastroenteritis. SaV is a positive-sense polyadenylated single-stranded RNA virus that infects humans and porcine species [1]. The prototype strain of human SaV, the Sapporo virus, was originally discovered from an outbreak in an orphanage in Sapporo, Japan, in 1977 [2]. In that study, Chiba *et al.* identified viruses with the typical animal calicivirus morphology, the Star-of-David structure, by electron microscopy (EM). Besides having this classical structure, SaV particles are typically 41–46 nm in diameter and have a cup-shaped depression

and/or 10 spikes on the outline (see Figure 3). Because only a limited number of SaV studies have been conducted, it has been difficult to check for correlations between or to draw conclusions about rates of incidence, detection and overall prevalence. SaV can be divided into five genogroups (GI–GV) (Figure 1), among which GI, GII, GIV and GV are known to infect humans, whereas SaV GIII infects porcine species. Phylogenetic studies have also designated SaV clusters or genotypes.

DETECTION OF SaV

SaV was first detected by EM [2]. However, this technique is tedious, since virus particles are difficult to correctly identify and the number of particles is generally low [3]. Several groups have used enzyme immunoassays (EIAs) to screen for SaV antibodies [4–8]. Recently, we designed an SaV ELISA based on hyperimmune rabbit and guinea pig antisera raised against SaV virus-like particles (VLPs) [9,10]. The ELISA had 100% specificity and

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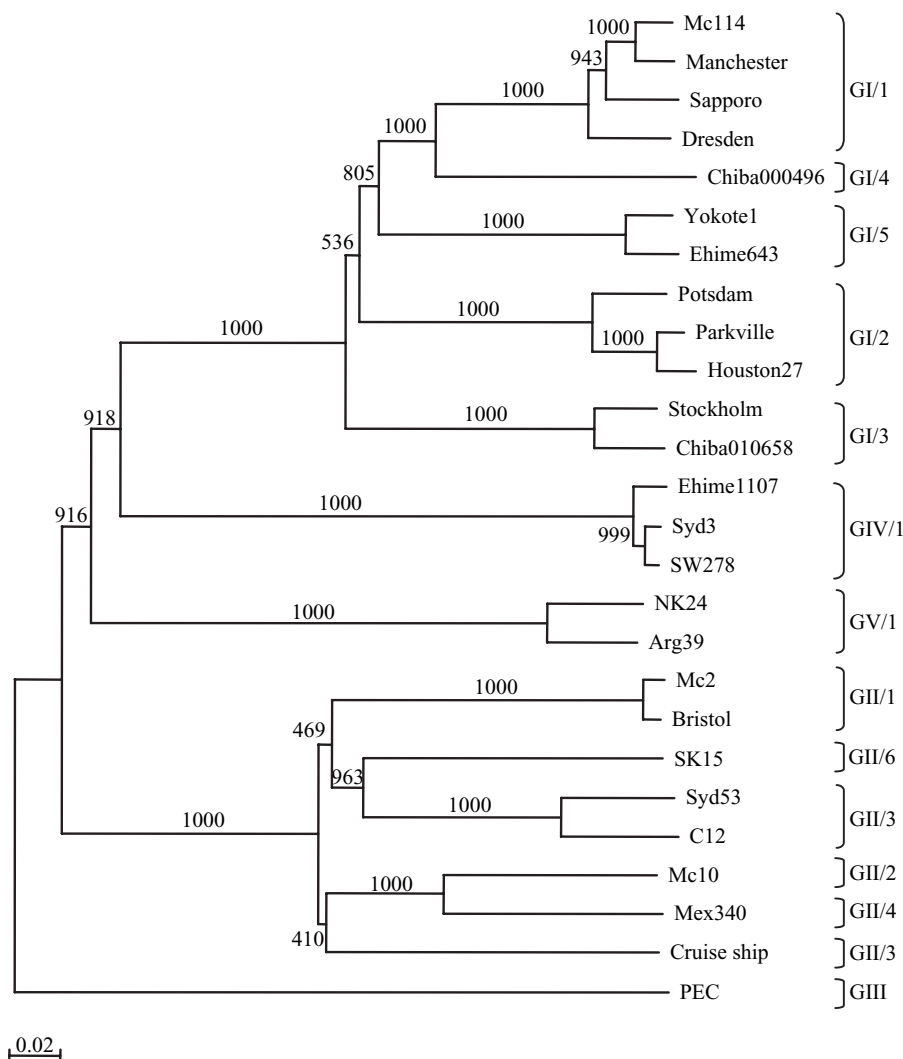


Figure 1. Phylogenetic tree of SaV based upon the entire VP1 nucleotide sequences. Different genogroups and genotypes are indicated. The numbers on each branch indicate the bootstrap values for the genotype. Bootstrap values of 950 or higher were considered statistically significant for the grouping. The scale represents nucleotide substitutions per site. GenBank accession numbers for the reference strains are as follows: Arg39, AY289803; Bristol, HCA249939; C12, AY603425; Chiba000496, AJ412800; Chiba010658, AJ606696; Cruise ship, AY289804; Dresden, AY694184; Ehime643, DQ366345; Ehime1107, DQ058829; Houston27, U95644; Manchester, X86560; Mc2, AY237419; Mc10, AY237420; Mc114, AY237422; Mex340, AF435812; NK24, AY646856; Parkville, U73124; PEC, AF182760; Potsdam, AF294739; Sapporo, U65427; SK15, AY646855; Stockholm, AF194182; SW278, DQ125333; Syd3, DQ104357; Syd53, DQ104360; and Yokote1 AB253740

sensitivities of 60 and 25% when compared to single-round PCR and nested PCR, respectively [9]. Our results showed that ELISA was useful for detecting SaV antigens in clinical stool specimens collected 2 days after the onset of illness. However, the method most widely used these days is reverse transcription-PCR (RT-PCR), which has a high sensitivity and can also be used for

genetic analysis. Several groups have designed RT-PCR primers that can detect a broad range of SaV strains (Table 1 and Figure 2a) [11–15]. More recently, we designed a novel TaqMan-based real-time RT-PCR assay that is sensitive and can detect a broad range of genetically diverse human SaV strains [16]. Analysis using clinical stool specimens revealed that the TaqMan-based real-time

Table 1. Primers for detection of SaV [15]

Name	Location ^a	Sequence
SV-F13	5074–5094	GAYYWGGCYCTCGCYACCTAC
SV-F14	5074–5094	GAACAAGCTGTGGCATGCTAC
SV-R13	5876–5861	GGTGANAYNCCATTKTCCAT
SV-R14	5876–5861	GGTGAGMMYCCATTCTCCAT
SV-F22	5154–5172	SMWAWTAGTGTGTTGARATG
SV-R2	5591–5572	GWGGGRTCAACMCCWGGTGG

^aNucleotide numbers of Manchester virus.

RT-PCR assay could detect SaV GI, II, IV and GV sequences, and no cross-reactivity was observed against other enteric viruses.

SEROLOGICAL STUDIES

In an early study of SaV antibody prevalence in the general community, Sakuma *et al.* [17] indicated that SaV infections were acquired more readily after 2 years of age than before 2 years of age, and especially in infants attending nurseries and children attending kindergarten or primary schools. On the other hand, Matson *et al.* [3] found that SaV-associated diarrhoea was more common

in infants aged 6 months or less and that the SaV detection rate was higher in a day-care centre population than in hospitalised children (in a concurrent study). Grohmann *et al.* [18] also found that infants under 6 months of age had the highest rate of SaV-associated gastroenteritis. However, a direct comparison between these studies is difficult, as the specimens collected by Sakuma *et al.* were from children without gastroenteritis at an outpatient clinic whereas the other two studies collected the specimens from day-care centres. In common, these studies proposed that children in day-care centres and closed institutions were at the greatest risk of SaV infection and that children were likely to acquire SaV antibodies by 5 years of age.

EPIDEMIOLOGICAL AND ENVIRONMENTAL STUDIES

SaV infects both children and adults and have been found to cause outbreaks of gastroenteritis in day-care centres, healthcare facilities and elementary schools. SaV also causes sporadic cases of acute gastroenteritis requiring hospitalisation as well as symptomatic and asymptomatic infections not requiring hospitalisation in the commu-

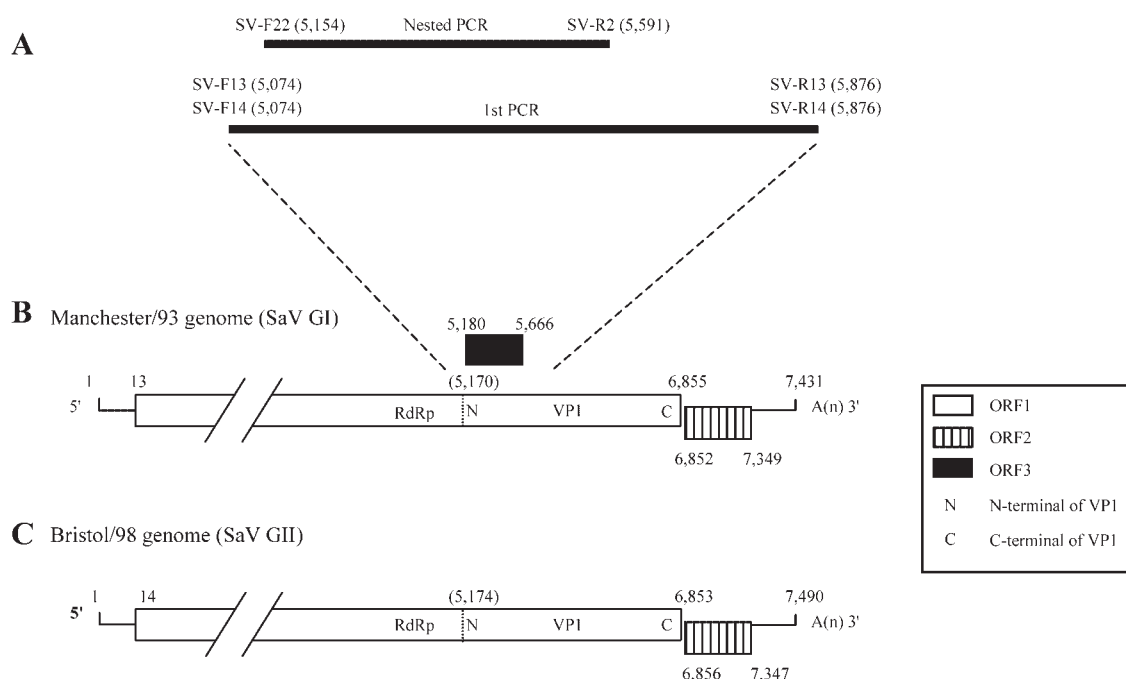


Figure 2. (A) Degenerate primers are directed against a conserved junction sites between the RdRp and the VP1 region and two conserved VP1 sites. (B) The SaV GI, GIV and GV genomes contain three ORFs, whereas (C) the SaV GII genome contains two ORFs

nity [3,5,6,11–14,18–26]. Epidemiological studies have been conducted in many countries, including Australia, Canada, Finland, France, Japan, Mexico, Mongolia, Spain, Sweden, Taiwan, Thailand, UK, US and Vietnam. The rates of incidence, detection and overall prevalence of SaV infections vary in each country and setting and are likely affected by the diagnostic techniques used [27]. A number of reports have noted that SaV detection rates were usually much less frequent than NoV detection rates [24,28–30]. In addition, SaV gastroenteritis appears to produce symptoms milder than those by NoV, so hospitalisation is often unnecessary [28,29,31]. On the other hand, we performed two similar SaV and NoV epidemiological studies among hospitalised infants with gastroenteritis in Thailand and Vietnam, and found SaV in 3.8% (4/105 with single infection) and 0.2% (1/448 with single infection) of stool specimens, respectively [32,33]. This dissimilarity in the SaV detection rates was statistically significant (Fisher's exact $p < 0.005$), whereas the dissimilarity in the NoV detection rates was not statistically significant (in Thailand, 8/105 were NoV-positive with single infection, whereas in Vietnam, 72/448 were NoV-positive with single infection; Fisher's exact $p = 0.1$). The same primers and conditions were used in both studies, thereby suggesting that SaV was an uncommon etiological agent of gastroenteritis in Vietnam. Climatic and environmental conditions as well as cultural differences, including eating habits and hygiene practices, may be important factors in these differences in SaV detection rates between these two countries [34]. NoVs have been detected in oysters, shellfish, drinking fountains, bottled mineral water, ice and community drinking water [35–39]. We recently found SaV in 10% of concentrated water samples, which included untreated wastewater specimens, treated wastewater samples and river samples, but we did not detect SaV in open seawater samples [40]. The SaV samples were isolated in both hot and cold months, that is summer and winter and the sequences matched previously reported SaV sequences. Our results indicated that SaV persists in the natural environment. We also found SaV in shellfish samples destined for human consumption, but we did not detect SaV in oyster samples (manuscript in press). The closely matching SaV sequences detected in the water, shellfish, and patients in Japan suggest that SaV contaminations

in the natural environment may lead to food-borne infections in humans. However, further studies are needed to determine if shellfish contaminated with SaV can cause gastroenteritis in humans.

GENOMIC ORGANISATION

The SaV GI, GIV, and GV genomes contain three open reading frames (ORFs), whereas the SaV GII genome contains two ORFs (Figure 2B,C). ORF1 encodes all the non-structural proteins, including the RNA-dependent RNA polymerase (RdRp), and the major capsid protein (VP1). ORF2 encodes a small protein, believed to be similar to VP2 of NoV [1], and ORF3 encodes a protein of unknown function. The SaV ORF1 is organised in the same way as that of RHDV. Matson *et al.* [41] found that the SaV RdRp gene sequence was closer to RHDV and FCV than to those of other human NoVs. Although human SaV is noncultivable, the expression of the recombinant VP1 (rVP1) in a baculovirus expression system or mammalian expression system results in the self-assembly of VLPs that are morphologically similar to the native virus particles, that is approximately 41–46 nm in diameter with cup-shaped depressions and/or 10 spikes on the outline (Figure 3) [42–45]. In a recent study, we expressed constructs containing SaV N- and C-terminal-deleted rVP1 in a baculovirus expression system, which included two N-terminal-deleted rVP1 constructs that began at 49 and 143 nucleotides from the VP1 start and four C-terminal-deleted rVP1 constructs that had an introduced stop codon at nucleotides 687, 1068, 1260 and 1583 (with respect to the VP1 start) [46]. Our results were similar to those reported in a RHDV N- and C-terminal-deleted rVP1 expression study [47], but were distinct from those reported in a NoV N- and C-terminal-deleted rVP1 expression study [48]. For instance, only SaV and RHDV proteins derived from N-terminal-deleted rVP1 constructs, that is constructs that began at 49 and 90 nucleotides from the VP1 start for SaV and RHDV, respectively, assembled into VLPs, whereas both NoV N- and C-terminal-deleted rVP1 constructs assembled into VLPs. This suggested that SaV and RHDV may have similar expression requirements. Furthermore, the cleavage map of SaV ORF1 appeared to be more closely related to that of RHDV than to those of other caliciviruses [49].

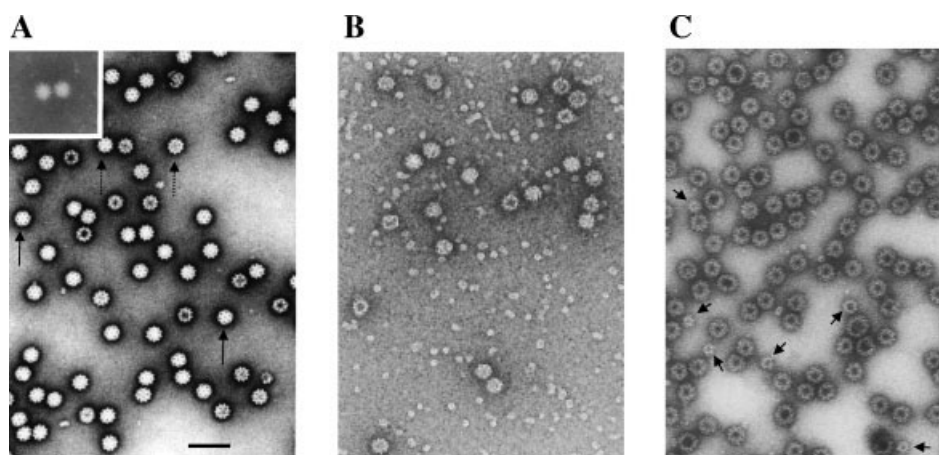


Figure 3. Electron-microscopic images of CsCl purified VLPs negative-stained with 4% uranyl acetate (pH 4) (A) GI Mc114 (Insert, native SaV), (B) GII C12 and (C) GV NK24. The long arrows indicate the Star-of-David structure and the dashed arrows indicate the 10 spikes (A) and the short arrows indicate the small VLPs (C). The bar indicates 100 nm

RECOMBINATION

We recently sequenced the complete genomes of 15 SaV strains (data not shown) and identified the first naturally occurring intragenogroup recombinant SaV strains (Figure 4, i.e. strains Mc10 and C12) [50]. Initially, we grouped Mc10 and C12 into two distinct GII genotypes based on their VP1 sequences (Figure 4B). In addition, the overall genomic nucleotide similarity between Mc10 and C12 was 84.3%, while ORF1 and ORF2 shared 85.5 and 73.3% nucleotide identity, respectively. Our results indicated that they were genetically distinct. However, by comparison of sequence similarity across the length of the genomes, using SimPlot software [51], we discovered a potential recombination site, where the similarity analysis showed a sudden drop in nucleotide identity after the RdRp region (Figure 5). Nucleotide sequence analysis of ORF1 without the downstream VP1 sequence and the VP1 sequence revealed 90.1 and 71.3% nucleotide identity, respectively (Figure 5). These results suggested a single point recombination event occurred at the RdRp–VP1 junction. Interestingly, for Mc10 and C12, there were 44 nucleotides at the RdRp–VP1 junction that matched 100%. This conserved site may represent either the break and rejoin site or the site for copy choice for Mc10 and C12 during viral replication [52], though direct evidence is lacking. More recently, we identified the first intergenogroup recombinant SaV strains (Figure 4, i.e.

strains SW278 and Ehime1107) [53]. Based on the classification scheme of either the partial or complete VP1 sequences in our previous studies, we grouped Manchester and Dresden into GI; Bristol, Mc2, Mc10, C12 and SK15 into GII; PEC into GIII; SW278 and Ehime1107 into GIV; and NK24 into GV [11,45,54]. These genogroups were not maintained when we analysed the non-structural region (i.e. between genome start and VP1 start). We found that SW278 and Ehime1107 clustered into GII for the non-structural region-based-grouping (Figure 4A), but clustered into GIV for the structural region-based-grouping (Figure 4B). Comparisons of the complete genome sequences showed that SW278 and Ehime1107 shared over 97% nucleotide identity and likely represented the same strain, although isolated from different countries (Sweden and Japan, respectively). By comparison of sequence similarity across the length of the genomes, we observed a sudden drop in nucleotide similarity after the RdRp region for SW278 and Ehime1107, indicating that a recombination event occurred at the RdRp–VP1 junction (data not shown). The non-structural region of SW278 and Ehime1107, that is a GIV sequence, has not yet been identified, despite the fact that we have conducted a number of molecular epidemiological studies using broad-range primers. Our studies have found that GIV strains (i.e. VP1 sequences) were detected less often than strains from the other genogroups

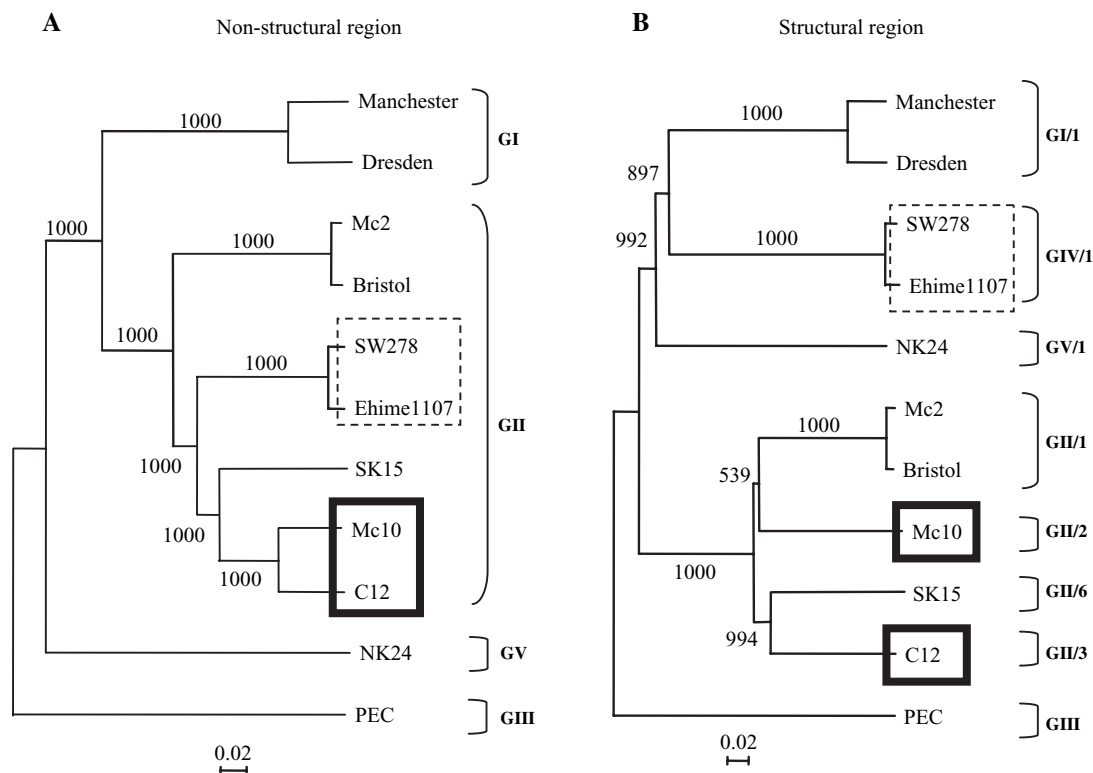


Figure 4. Intra- and intergenogroup recombination among SaV. Phylogenetic tree of (A) non-structural region (i.e. between genome start and VP1 start) and (B) structural region (i.e. between VP1 start and genome end), showing the different genogroups. The numbers on each branch indicate the bootstrap values for the genotype. Bootstrap values of 950 or higher were considered statistically significant for the grouping [62]. The scale represents nucleotide substitutions per site. Mc10 and C12 were the intragenogroup recombinant SaV strains (black box), whereas SW278 and Ehime1107 were intergenogroup recombinant SaV strains (dashed box)

[11,33,40,45,54,55]. Further complete genome analysis of other SaV strains is needed to identify other recombinant strains and determine the extent of recombination in the *Sapovirus* genus.

BINDING TO HUMAN BLOOD GROUP ANTIGENS

In the past several years, increasing evidence has emerged that indicates that human NoVs bind to histo-blood group antigens (HBGAs) [56–60]. These carbohydrate epitopes are present in mucosal secretions and throughout many tissues of the human body, including the small intestine, which certain NoV strains may specifically target. In a recent study, we examined the binding activities of human SaV VLPs to HBGAs present in human saliva and to synthetic carbohydrates [61]. We found that SaV GI and GV VLPs showed no binding activity to HBGAs or synthetic carbohydrates.

However, a number of studies have found that different NoV strains exhibit different binding patterns to HBGAs [58–60]. For example Hawaii virus VLPs had no binding to HBGAs, whereas Norwalk virus VLPs had binding activity to HBGAs. Therefore, further studies are needed to examine the possibility that other human SaV genogroups or genotypes have binding activity to HBGAs.

CONCLUDING REMARKS

SaV-associated infection is becoming increasingly important as a result not only of findings by improved detection techniques but also of greater knowledge of the strains' genetic diversity. Diagnostic techniques are now available to properly screen specimens in order to better understand the overall prevalence of SaV and their epidemiologic characteristics. The recent detection of SaV in

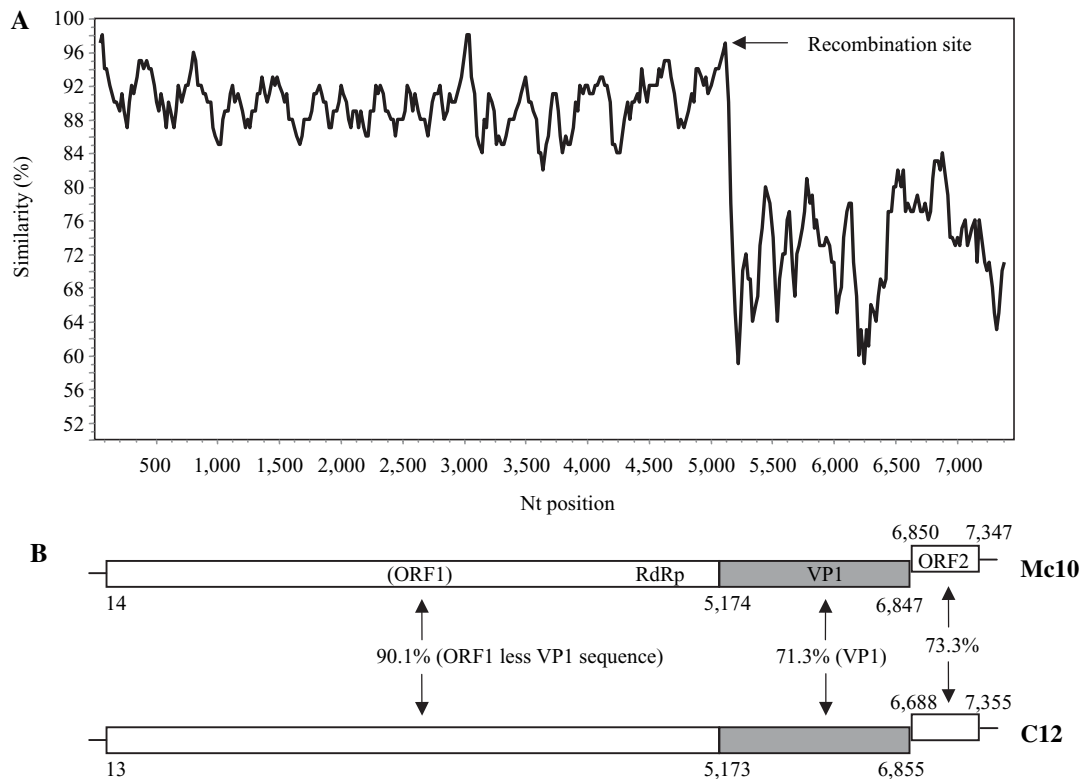


Figure 5. Intragenogroup recombination observed in Mc10 and C12 strains. (A) SimPlot analysis of Mc10 and C12. The Mc10 genome sequence was compared to that of C12 by using a window size of 100 bp with an increment of 20 bp. All gaps were removed. The recombination site is suspected to be located between RdRp and VP1 genes, as the arrow shows. (B) Genome organisation of Mc10 and C12 strains and the nucleotide sequence similarity of different genomic regions

water and shellfish samples creates a greater awareness of these viruses and highlights a possible food-borne transmission route. Recombinant SaV strains are also important and have been identified in a number of countries. And finally, a consensus classification scheme is needed to reduce confusion and properly classify strains while taking recombinant strains and antigenic relatedness into account.

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