

## Genetic Diversity of Norovirus and Sapovirus in Hospitalized Infants with Sporadic Cases of Acute Gastroenteritis in Chiang Mai, Thailand

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**Stool specimens from hospitalized infants with sporadic gastroenteritis in Chiang Mai, Thailand, between July 2000 and July 2001 were examined for norovirus and sapovirus by reverse transcription-PCR and sequence analysis. These viruses were identified in 13 of 105 (12%) specimens. One strain was found to be a recombinant norovirus.**

Norovirus and sapovirus are two of the four genera of the family *Caliciviridae* and are well-characterized agents of human acute gastroenteritis (6–9). These viruses can be transmitted by a variety of routes, including food (15) and water (16). Three genogroups (GI, GII, and GIII) for norovirus and four genogroups (GI, GII, GIII, and GIV) for sapovirus are thought to exist, though only norovirus GI and GII and sapovirus GI, GII, and GIV are known to infect humans (11, 18). Numerous molecular epidemiological studies have revealed a global distribution of these viruses (2–4, 17, 19). However, very few molecular epidemiological studies have been conducted in Asian countries other than Japan. In this study, we detected norovirus and sapovirus in stool specimens from hospitalized infants with gastroenteritis in Thailand and partially sequenced the capsid gene to determine genogroups and genotypes.

One hundred five stool specimens collected from hospitalized infants (ranging from 1 month to 5 years of age) with acute sporadic gastroenteritis in Chiang Mai, Thailand, between July 2000 and July 2001 were examined for norovirus and sapovirus by reverse transcription-PCR. This included 52 specimens from McCormic Hospital, 21 specimens from Chiang Mai University Hospital, 23 specimens from Nakornping Hospital, and nine specimens from Sanpatong Hospital. RNA was extracted with the QIAamp viral RNA minivacuum protocol (Qiagen) according to the manufacturer's instructions. Reverse transcription was carried out in a final volume of 20  $\mu$ l with 10  $\mu$ l of RNA in 50 pmol of random hexamer (Takara), 1 $\times$  Superscript II reverse transcription buffer (Invitrogen), 10 mM dithiothreitol (Invitrogen), 0.4 mM each of the four deoxynucleoside triphosphates (Roche), 1 U of RNase inhibitor (Toyobo), and 10 U of Superscript II reverse transcriptase (Invitrogen). Reverse transcription was performed at 42°C for 1 h, followed by deactivation of reverse transcriptase at 72°C for 15 min.

The norovirus PCR primers were selected from three reports that described detection of a broad range of strains (10, 13, 14). For norovirus GI we used primers COG1F (sense) and G1SKR (antisense). For norovirus GII we used primers G2F3 (sense) and G2SKR (antisense). For sapovirus, we used novel capsid gene region primers (corresponding to nucleotides 5083 to 5516 of Manchester virus; GenBank accession number X86560), the SV5317 primer (sense; 5'-CTC GCC ACC TAC RAW GCB TGG TT-3'), and the SV5749 primer (antisense; 5'-CGG RY TCA AAV STA CCB CCC CA-3' [where R is A or G; W is A or T; S is C or G; Y is C or T; V is A, C, or G; and B is C, G, or T]). PCR was carried out with 5  $\mu$ l of cDNA in a PCR mixture containing 33 pmol of each primer, 1 $\times$  *Taq* DNA polymerase buffer B (Promega), 0.2 mM each of the four deoxynucleoside triphosphates, 2.5 U of *Taq* polymerase (Promega), and up to 50  $\mu$ l of distilled water. PCR was performed at 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 60 s, and a final extension at 72°C for 5 min. Reverse transcription-PCR products were sequenced and used for genetic classification. Partial and complete nucleotide sequencing and phylogenetic analysis were performed as previously described (11). The nucleotide sequences determined in this study have been deposited in GenBank under accession numbers AY237410 to AY237423.

Norovirus and sapovirus were detected in 12% (13 of 105) of stool specimens from infants admitted to three of the four hospitals in the Chiang Mai region. The age at infection ranged from 4 months to 5 years. All but one of the infants infected with sapovirus were 12 months of age or younger, the youngest infant being 4 months of age. Norovirus also mostly infected infants 12 months of age or younger. One infant was infected with both norovirus and sapovirus strains. Figure 1a shows the phylogenetic tree of the nine norovirus capsid sequences isolated together with reference sequences. The Thai sequences belonged to three distinct norovirus GI genotypes and three norovirus GII genotypes. One norovirus GII sequence (isolate Mc37) that did not cluster with any of the published genotypes was characterized further by complete genome sequencing.

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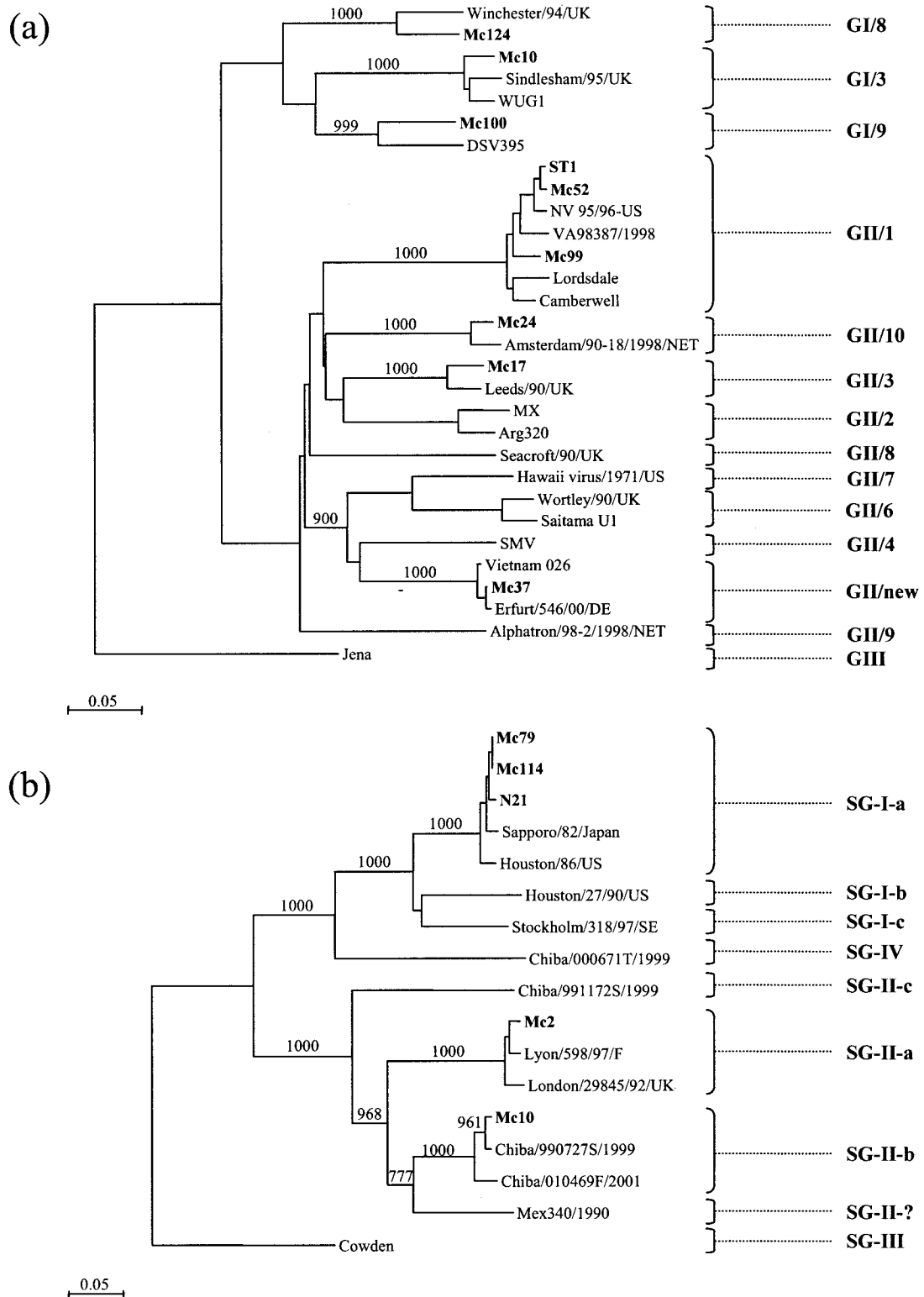


FIG. 1. Phylogenetic analysis of sequences isolated in Thailand. (a) Norovirus capsid sequences (264 bp). (b) Sapovirus capsid sequences (376 bp). The numbers on each branch indicate the bootstrap values for the genotype. Thai sequences are represented in bold. For example, Mc2 is the strain isolated at McCormic Hospital from patient 2. Mc, McCormic Hospital; St, Sanpatong Hospital; N, Nakornping Hospital. Norovirus sequences were classified according to the scheme of Katayama et al. (11), and sapovirus sequences were classified based on the scheme of Okada et al. (18). GenBank accession numbers for the reference strains are as follows: Vietnam 026, AF504671; Alphatron/98-2/1998/NET, AF195847; Amsterdam/98-18/1998/NET, AF195848; Arg320, AF190817; Camberwell, U46500; Chiba/000671T/1999, AJ412805; Chiba/990727S/1999, AJ412795; Chiba/991172S/1999, AJ412797; Chiba/010469F/2001, AJ412820; Cowden, AF182760; DSV395, U04469; Erfurt/546/00/DE, AF427118; Hawaii virus/1971/US, U07611; Houston/86/US, U95643; Houston/27/90/US, U95644; Jena, AJ011099; Leeds/90/UK, AJ277608; London/29845/92/UK, U95645; Lordsdale, X86557; Lyon/598/97/F, AJ271056; Mex340/1990, AF435812; MX, U22498; 408/97003012/1996/FL (NV 95/96-US), AF080558; Saitama U1, AB039775; Sapporo/82/Japan, U65427; Seacroft/90/UK, AJ277620; Sindlesham/95/UK, AJ277615; SMV, AY134748; Stockholm/318/97/SE, AF194182; VA98387/1998, AY038600; Winchester/94/UK, AJ277609; Wortley/90/UK, AJ277618; and WUG1, AB081723.

The genome of Mc37 comprised 7,541 nucleotides, excluding the poly(A) tail, and contained three open reading frames (ORFs). The ORF1 sequence showed 97.2% nucleotide identity to that of Saitama U1 virus (AB039775) but only 71.3 and 67.9% nucleotide identity in ORF2 and ORF3, respectively. Consequently, strain Mc37 likely represents a novel recombinant norovirus.

Figure 1b shows the phylogenetic tree of the five sapovirus sequences isolated together with reference sequences. The sapovirus primers detected both GI and GII sapovirus sequences. Three of the five sapovirus sequences belonged to one sapovirus GI cluster, SG-I-a. The two other sapovirus sequences, sapovirus isolates Mc2 and Mc10, belonged to two distinct sapovirus GII clusters, SG-II-a and SG-II-b, respectively. The sapovirus Mc2 sequence showed 78.5% nucleotide identity to the sapovirus Mc10 sequence. The sequences with the closest matches to the sapovirus Mc10 sequence were from two strains isolated in Japan, Chiba/010469F/2001 virus (AJ412820) and Chiba/990727S/1999 virus (AJ412795), showing 95 and 97% nucleotide identity, respectively. The next closest sequence in the GenBank database (Mex340/1990, AF435812) showed only 82% nucleotide identity.

Our results are consistent with those from similar studies. In a report from Spain, 14.19% of stool specimens were positive for norovirus and sapovirus (1), and the majority of strains belonged to norovirus GII (10.65%), followed by norovirus GI (2.26%) and sapovirus (1.29%). Also, in an Australian report, the overall annual minimum incidence rate in hospitalized children was 8.5% for norovirus and 0.6% for sapovirus infection (12). The majority of norovirus strains detected in this Australian report and another from Ireland (5) were of the Lordsdale virus cluster (GII/1). Our study identified several norovirus sequences in this cluster that closely matched a norovirus 95/96-US strain sequence. Recently, several reports have highlighted the importance of the 95/96-US strain's having global distribution and causing a significant number of outbreaks of gastroenteritis (2, 5, 17, 19). In conclusion, these data have described great genetic diversity among both norovirus and sapovirus strains cocirculating in the Chiang Mai region of Thailand and increased the evidence for the worldwide distribution of these viruses.

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