

Figure. Phylogenetic analysis of the partial nucleotide sequence encoding the 3D region of bovine kobuviruses isolated in this study and other reference strains recognized to date. The tree was generated on the basis of the neighbor-joining method using the MEGA 3.1 program (7). Scale bar indicates branch length for a 5% nucleotide difference.

identities to each other. In addition, by searching for the closest sequences in the databank, we found that these sequences were closely related to all 13 bovine kobuvirus reference strains available in the GenBank database (U-1, K-35, K-36, K-49, K-38, N-5, K-3, K-4, K-6, K-60, N-2, K-44, and K-55); nucleotide and amino acid sequence identities ranged from 91.0% to 95.0% and 97.4% to 99.4%, respectively. Phylogenetic analysis of partial 3D nucleotide sequences of our bovine kobuvirus strains, together with those of all published bovine kobuvirus reference strains available in the GenBank database, is shown in the Figure. The Aichi virus standard strain was included in the tree as an outlier virus. The phylogenetic tree confirmed that all 6 virus strains belonged to bovine kobuvirus and formed a tight cluster in a monophyletic branch with other published bovine kobuvirus reference strains, but they were distantly related to the Aichi virus standard strain.

Detection and characterization of bovine kobuvirus strains from different geographic areas are important for understanding the worldwide distribu-

tion, heterogeneity, and association of bovine kobuvirus with enteric disease in cattle. Our findings indicate the role of kobuviruses in diarrheal disease in cattle and provide additional information on their relationship to bovine kobuviruses reported previously.

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References

1. Yamashita T, Ito M, Kabashima Y, Tsuzuki H, Fujiura A, Sakae K. Isolation and characterization of a new species of kobuvirus associated with cattle. *J Gen Virol*. 2003;84:3069-77.
2. Racaniello VR. Picornaviridae: the viruses and their replication. In: Knipe DM and Howley PM, editors. *Fields virology*, vol. 1. 5th ed. Philadelphia: Lippincott Williams & Wilkins; 2006. p. 795-838.
3. Oh DY, Silva PA, Hauroeder B, Diedrich S, Cardoso DD, Schreier E. Molecular characterization of the first Aichi viruses isolated in Europe and in South America. *Arch Virol*. 2006;151:1199-206.
4. Pham NT, Khamrin P, Nguyen TA, Kanti DS, Phan TG, Okitsu S, et al. Isolation and molecular characterization of Aichi viruses from fecal specimens collected in Japan, Bangladesh, Thailand, and Vietnam. *J Clin Microbiol*. 2007;45:2287-8.
5. Yamashita T, Sakae K, Kobayashi S, Ishihara Y, Miyake T, Mubina A, et al. Isolation of cytopathic small round virus (Aichi virus) from Pakistani children and Japanese travelers from Southeast Asia. *Microbiol Immunol*. 1995;39:433-5.
6. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. *J Mol Biol*. 1990;215:403-10.
7. Kumar S, Tamura K, Nei M. MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform*. 2004;5:150-63.

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Letters

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4. Kapoor G, Aneja S. Nutritional disorders in adolescent girls. *Ind Pediatr* 1992;29:969-73.
5. Das DK, Biswas R. Nutritional status of adolescent girls in a rural area of North 24 Paraganas district, West Bengal. *Ind J Pub Health* 2005;49:18-21.
6. Das P, Ray SK, Joardar GK, et al. Nutritional profiles of adolescents in rural community of Hooghly district in West Bengal. *Ind J Pub Health* 2007;51:20-1.
7. Lohman TG, Roche AF, Martorell R. *Anthropometric Standardization Reference Manual*. Chicago: Human Kinetics Books, 1988.
8. World Health Organization. *Measuring change in nutritional status*. Geneva: World Health Organization, 1985.

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The Role of Flexible Bronchoscopy in Pediatric Pulmonary Tuberculosis

Tuberculosis (TB) disease in children is complicated by the unique challenges that it poses to the clinician. Despite the availability of effective preventive measures and chemotherapy, the prevalence of TB is increasing in the developing world and in much of the industrialized world as well, the incidence of TB in developed countries has declined over the last several decades. However, in recent years, there has been an increase in reported cases, especially in urban areas. In developing countries, the risk for TB infection and disease is relatively uniform in the population; annual rates of infection often exceed 2%. In pediatric patients, pulmonary tuberculosis (PTB) is a major TB infection, but the diagnosis of PTB presents a challenge. The symptoms of PTB infection are often absent or less specific in the affected pediatric population. Because children aged <8 years old lack sputum production, it is difficult to diagnose by sputum specimen. Children with PTB typically have closed caseous lesions with a relatively small number of mycobacteria. The large cavitory population of tubercle bacilli seen in adults is usually absent in children. This is compounded by the difficulty in collecting sputum in children as they swallow the expectorate coming from the lungs. Furthermore, at present, diagnostic tests are costly, slow and lacking in sensitivity. Flexible bronchoscopy (FB) is a newly available tool in the investigation of pediatric

pulmonary disease. FB has been used mainly to evaluate unresolved chronic respirator symptoms, and in certain circumstances, to obtain cultures from the immune system of compromised hosts. Therefore, the aim of this retrospective survey is to evaluate the role of FB in the diagnosis of pediatric PTB.

In order to evaluate the value of FB for the diagnosis of children with suspected PTB, clinical records were retrospectively reviewed in 206 patients, who were diagnosed with PTB from January 2002 to December 2006 at the Beijing Children's Hospital. All 206 patients were examined by chest X-ray. Purified protein derivative tuberculin skin test (PPD) results were interpreted according to the recommendations of the American Thoracic Society. FB was performed on every patient according to clinical records in our survey. Bronchoalveolar lavage (BAL) was obtained to isolate *Mycobacterium tuberculosis* by FB. The mean age was 4.5 ± 2.7 years. The most frequent symptoms were cough (90%) and fever (85%) in all patients. The PPD results of 150/181 patients were 10 mm or more. Characteristic chest X-ray findings of PTB were 98/206 (47.6%). Thirty-three patients with endobronchial tuberculosis (EBTB) (94.3%) were diagnosed by FB. Bronchoscopic findings of 206 cases were divided into four types by our previous study. The number of indomucous membrane, bronchial lumen, caseating, and blending type was 36, 19, 6 and 145, respectively. FB is a useful tool to diagnose pediatric PTB. FB should be performed especially when chest X-rays are abnormal or when clinical signs suggest PTB.

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Epidemiological and Molecular Analysis of Astrovirus Gastroenteritis in Dhaka City, Bangladesh

Human astrovirus (HAsV) may be the second most common cause of viral gastroenteritis in young children, with rotavirus being the first [1]. HAsVs are members of the *Astroviridae* family. They are non-enveloped viruses possessing a single stranded RNA of positive polarity [2].

A total of 917 fecal specimens were collected from infants and children with acute gastroenteritis in Dhaka City, Bangladesh, during the period of October 2004 to September 2005. The fecal specimens were diluted with distilled water to 10% suspensions, and clarified by centrifugation at 10 000 g for 10 min. The viral genome was extracted from 140 µl of a 10% fecal suspension using a spin-column technique according to the instructions in the QIAamp® Viral RNA Mini Kit Handbook. Briefly, a pair of published primers (PreCAP1 and 82b) for amplifying capsid region of astrovirus was used [3]. The nucleotide sequences of polymerase chain reaction products positive for astrovirus were determined with the Big-Dye terminator cycle sequencing kit and an ABI Prism 310 Genetic Analyzer (Applied Biosystems Inc., CA, USA). Phylogenetic and molecular evolutionary analyses were conducted using the MEGA version 3.2 software package [4]. The following reference strains and accession numbers were used in the phylogenetic analysis: HAstV1 Oxf (L23513), HAstV2 (L13745), HAstV3 (L38505), HAstV4 (L38506), HAstV6 (L38507), HAstV7 (L38508), HAstV-8 (Z66541), PA-COD379-BR (DQ917390), Bcn1.3/97-00 (AF348755), Melb1E/97 (AF175254), JAPAS115 (AB000287), Melb1E/97 (AF175253), Ven835 (AF211956), Arg158 (AY324858), Dresden/04 (AY720892), 5333/JP (EU022556) and 5079/JP (EU022555).

All fecal specimens were tested for the presence of astrovirus by reverse transcription-polymerase chain reaction. The results shown in Table 1 revealed that astrovirus was detected in 4 out of 917 (0.4%) specimens tested. These findings suggested that HAstV is also a causative agent for acute gastroenteritis in infants and children in Dhaka City, Bangladesh. In the present study, astrovirus infection was found in December, January, June and September.

The age of astrovirus infection ranged from 3 to 11 months. It was found that the common clinical symptoms of astrovirus-infected patients were dehydration (100%), abdominal pain (75%) and fever (25%).

TABLE 1

Clinical features of the patients infected with astrovirus

Sign and symptoms	Number of infants and children	(%)
Dehydration	4/4	100
Vomiting	0/4	0
Abdominal pain	3/4	75
Fever	1/4	25
Hospitalization	4/4	100
3-5 times loose stools within 24 h	1/4	25
≥6 times loose stools within 24 h	3/4	75

All the HAstV amplicons were characterized for serotypes and genetic relationship with the reference strains based on their capsid regions. Their partial nucleotide sequences were compared to each other as well as to reference HAstV strains available in the DDBJ DNA/GenBank database by BLAST. This study showed that all Bangladeshi astrovirus sequences belonged only to serotype I (Fig. 1). HAstV detected in this study was closely homologous at the nucleotide and amino acid level.

The nucleotide sequence data of all Bangladeshi HAstV had been submitted to the GenBank (NCBI, Bethesda, MD) and had been assigned accession number EU333900-EU333903, respectively.

This is the first time that data on nucleotide analysis of Bangladeshi astrovirus were reported. Nucleotide sequence analysis indicated that the common serotype I strains were circulating among Bangladeshi children and infants.

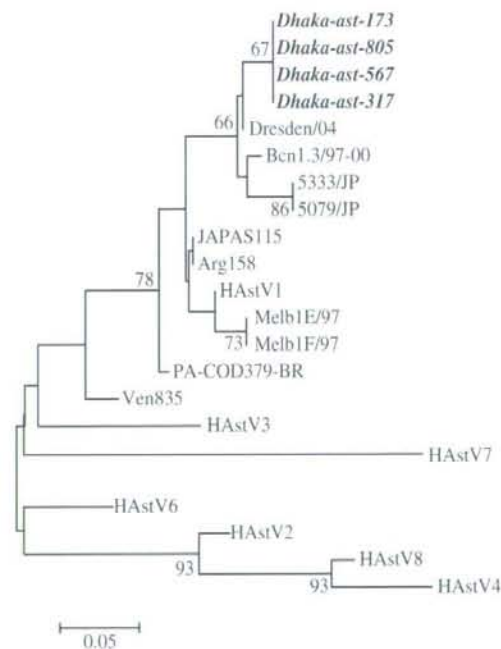


FIG. 1. Phylogenetic tree of the 300 bp region of ORF2 of 4 HAstVs-1 from Dhaka City. The tree was constructed from partial amino acid sequences of capsid region of astrovirus. Reference strains of astrovirus were selected from DDBJ/GenBank under the accession number indicated. Bangladeshi astrovirus was highlighted in italic. The scale indicates nucleotide substitutions per position. The numbers in the branches indicate the bootstrap values. The Genbank accession numbers of this study are: EU333900-EU333903, respectively.

In conclusion, to our knowledge this is the first 1 year molecular epidemiological research of HAsV in Bangladesh.

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References

1. Guix S, Caballero S, Villena C, *et al.* Molecular epidemiology of astrovirus infection in Barcelona, Spain. *J Clin Microbiol* 2002;40:133-9.

2. Clark B, McKendrick M. A review of viral gastroenteritis. *Curr Opin Infect Dis* 2003;24:1-6.
3. Yan H, Yagyu F, Okitsu S, *et al.* Detection of norovirus (GI, GII), sapovirus and astrovirus in fecal samples using reverse transcription single-round multiplex PCR. *J Virol Methods* 2003;14:37-44.
4. Kumar S, Tamura K, Jakobsen IB, *et al.* MEGA2: molecular evolutionary genetics analysis software. *Bioinformatics* 2002;17:1244-5.

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Genetic characterization of group C rotavirus isolated from a child hospitalized with acute gastroenteritis in Chiang Mai, Thailand

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Abstract During an epidemiological survey of human rotavirus infection in Chiang Mai, Thailand, from 2002 to 2004, in which 263 stool specimens tested, one isolate of group C rotavirus was detected from a two-year-old child admitted to hospital with acute gastroenteritis. The human group C rotavirus, named CMH004/03, was characterized further by molecular analyses of its VP4, VP6, and VP7 gene segments as well as determination of RNA pattern by polyacrylamide gel electrophoresis (PAGE). Molecular characterization of VP4, VP6, and VP7 genes by sequence analyses showed high levels of sequence identities with those of human group C rotavirus reference strains isolated worldwide at 95.2% to 99.4% on nucleotide and 97.5% to 100% on amino acid levels. In contrast, the CMH004/03 strain exhibited far lesser nucleotide and amino acid sequence identities at 67.7% to 84.1% and 68.7% to 91.3%, respectively, when compared with those of porcine and bovine group C rotaviruses. Phylogenetic analyses of VP4, VP6, and VP7 genes clearly confirmed that the CMH004/03 strain clustered in a monophyletic branch with other

human group C rotavirus reference strains and distantly related to the clusters of animal group C rotavirus strains. In addition, the RNA electrophoretic migration pattern of CMH004/03 showed a typical pattern (4-3-2-2) of group C rotavirus. To our knowledge, this study is the second report of group C rotavirus infection in pediatric patients in Thailand after it was reported for the first time about two decades ago.

Keywords Rotavirus · Group C · VP4 · VP6 · VP7 · Thailand

Introduction

Rotavirus is the major cause of acute gastroenteritis in infants, young children, and a large variety of young animal species. The virus is a member of the family *Reoviridae* with a double stranded RNA genome of 11 segments, surrounded by a triple-layered capsid protein [1]. The outermost viral capsid primarily comprises of two proteins, VP4 and VP7. The middle layer is formed exclusively by the most abundant protein of the virus, VP6, which defined group and subgroup (SG) specificities. The innermost layer is formed by the VP2 protein, a known RNA binding protein and the transcription enzymes VP1 (viral RNA polymerase) and VP3 (guanylyltransferase) are attached as a heterodimeric complex to the inside of the VP2 innermost surface protein [1]. On the basis of antigenic cross-reactivity and genomic properties of VP6, thus far, rotavirus is classified into seven antigenically distinct groups, A to G. All seven groups have been found to associate with infection in various animal species, but only groups A, B, and C are associated with diarrhea in humans [1–3]. Group A rotavirus is the major cause of childhood

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diarrhea worldwide, and accounts for approximately 454,000–705,000 deaths annually [4]. Human group B rotavirus was first described in China as the cause of nationwide epidemics of diarrhea in adults [5]. Group C rotavirus was first detected from piglets in 1980 [6] and later confirmed as a human pathogen by Bridger et al. [2]. Since then, group C rotavirus has been detected from both sporadic episodes and outbreaks of gastroenteritis throughout the world [7–12]. Host range restriction of group C rotavirus has been documented; only human, porcine, canine, and bovine species were found to be infected with group C rotaviruses [2, 6, 13–15]. All human group C rotaviruses analyzed so far have shown a high degree of sequence conservations, and several evolutionary studies also indicated that human group C rotavirus may have evolved from the same ancestor and possibly belonged to a single genotype [12, 16–18].

In Thailand, only one report of group C rotavirus infection in patients with diarrhea had been documented in 1989 [19]. Since then, it has never been reported as a cause of acute gastroenteritis in Thailand. In contrast to group C rotavirus, which is a less common viral agent causing diarrhea, group A rotavirus is reported as the most predominant viral agent causing diarrhea in humans worldwide [4, 20]. The accumulated data of group A rotavirus surveillance studies in Thailand indicated that group A rotavirus is the leading pathogen that causes diarrhea in children, and it is responsible for about 27% to 58% of diarrhea in hospitalized cases [21–24]. In this study, the group C rotavirus strain was detected from a child hospitalized with acute gastroenteritis. Its VP4, VP6, and VP7 genes were characterized further by nucleotide sequence analyses and the genetic relationship with other group C rotavirus strains circulating worldwide was also investigated.

Materials and methods

Specimen collection

A total of 263 fecal specimens were collected from pediatric patients aged less than 5 years, who were hospitalized with diarrhea at McCormick Hospital, Chiang Mai, Thailand. The study period was from January 2002 to December 2004. Group A rotavirus was first screened in the fecal specimens collected [24]. In addition, the incidence of group C rotavirus infection was also examined by using the same set of fecal samples, with the aim of having an overview of molecular epidemiology of human group C rotavirus in children hospitalized with acute gastroenteritis in Chiang Mai, Thailand.

Detection of group C rotavirus

The RNA genome of rotavirus was first extracted from 10% fecal suspension supernatant using the QIAamp viral RNA Mini Kit (Qiagen, Germany). The presence of group C rotavirus in fecal specimens was detected by RT-PCR using the protocol described previously [25]. A forward primer, G8NS1 (nt 353 to 374) 5'-ATTATGCTCAGACTATCGC-CAC-3', was used in combination with the reverse primer G8NA2 (nt 683 to 704) 5'-GTTTCTGTACTAGCTGGT-GAAC-3', for the amplification of a partial VP7 gene of the group C rotavirus, which specifically generated a PCR amplicon of 352 bp. The group C rotavirus isolated in this study was analyzed further by nucleotide sequence analysis of its VP4, VP6, and VP7 genes.

Polyacrylamide gel electrophoresis of rotavirus RNA

The RNA electrophoretic pattern of group C rotavirus (CMH004/03) was determined based on the method described previously [26]. The dsRNA segments were separated on 10% (w/v) polyacrylamide gel, and the migration pattern was visualized by silver staining [27].

Amplification of the VP4, VP6, and VP7 genes

The partial VP4 gene of group C rotavirus (CMH004/03) was reverse transcribed and amplified using the consensus primers GrC VP4-1F (nt 1 to 20) 5'-GGCTTAAAAAGTAG AGATCG-3' and GrC VP4-1243R (nt 1224 to 1243) 5'-CC AGGATATGATCCTA-3' [18]. The full length of the VP6 gene was amplified using T777 (nt 1 to 21) 5'-GGCTTTAAA AATCTCATTAC-3' and T778 (nt 1334 to 1353) 5'-AGCC ACATAGTTCACATTTC-3' primer pairs. For amplification of the full-length VP7 gene, consensus primers T348 (nt 1 to 22) 5'-GGCTTTAAAAAAGAAGAAGCTG-3' and T349 (nt 1042 to 1063) 5'-AGCCACATGATCTTGTTCAC GC-3' were used [28].

Sequence and phylogenetic analyses of the VP4, VP6, and VP7 genes

The PCR amplicons of the entire VP6 and VP7 genes as well as the partial sequence of the VP4 gene were gel purified with a Wizard SV Gel and PCR Clean-Up System (Promega, Madison, WI), and sequenced in both directions using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) on an automated sequencer (ABI 3100; Applied Biosystems, Foster City, CA). The primers used for amplification of the VP4, VP6, and VP7 genes were also used as sequencing primers. The nucleotide and deduced amino acid sequences were compared with those of group C rotavirus reference strains

available in the NCBI (National Center for Biotechnology Information) GenBank database using the BLAST (Basic Local Alignment Search Tool) server [29]. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 3.1 [30].

Nucleotide sequence accession numbers

The nucleotide sequences of the group C rotavirus strain (CMH004/03) described in this study were deposited in the GenBank database. The accession numbers for the VP4, VP6, and VP7 sequences are EF641109, EF641110, and EF641111, respectively.

Results

Detection of the human group C rotavirus

From a total of 263 fecal specimens included in this study, 98 (37.3%) were positive for group A rotavirus [24], while one isolate (0.4%), named CMH004/03, was identified as group C rotavirus by RT-PCR using primers specific for amplification of VP7 gene of group C rotavirus. The rotavirus strain CMH004/03 was collected in January 2003 from a 2-year-old child admitted with acute gastroenteritis at McCormick Hospital, Chiang Mai province, Thailand. At the time of admission, the patient had fever, nausea, and vomiting without abdominal pain, and had mild dehydration with watery diarrhea for up to 3 to 4 times per day.

Electropherotype of group C rotavirus strain

The RNA electrophoretic migration patterns of the group C rotavirus strain CMH004/03 was characterized by PAGE and compared with RNA patterns of group A rotavirus reference strains available in our laboratory. The CMH004/03 strain exhibited a characteristic of 4-3-2-2 migration pattern, which was typical for group C rotavirus, while group A rotavirus showed a 4-2-3-2 RNA migration pattern (Fig. 1).

Analysis of the VP7 gene sequence

The VP7 gene of the group C rotavirus strain CMH004/03 was 1,063 nucleotides long, which encoded a polypeptide of 332 amino acid residues, similar to other human group C rotaviruses. Comparative analysis of the nucleotide and deduced amino acid sequences of the full-length VP7 gene with those of human and animal group C rotavirus reference sequences indicated that the VP7 gene of CMH004/03 was closely related to those of other human group C rotavirus reference strains (95.2% to 98.8% on nucleotide and 97.5% to 99.3% on amino acid sequence levels)

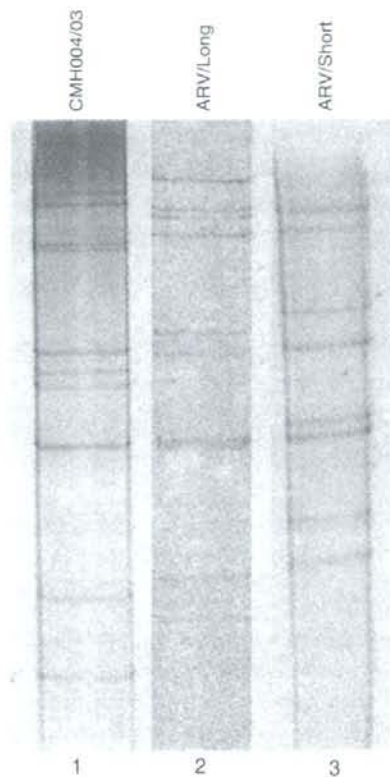


Fig. 1 Electrophoretic migration patterns of rotavirus genomic RNAs. Lane 1 is group C rotavirus detected in this study (CMH004/03), while lanes 2 and 3 are group A rotaviruses representing long and short patterns. The viral RNA were analyzed by electrophoresis in a 10% polyacrylamide gel and visualized by silver nitrate staining

(Table 1). In addition, the VP7 gene of CMH004/03 showed the highest level of nucleotide sequence identity with Japanese group C rotavirus strain OT-99 at 98.8%, whereas the amino acid sequence was most closely related to that of the Columbian group C rotavirus strain Javeriana at 99.3% identity. In contrast, CMH004/03 exhibited sequences distantly related to the porcine and bovine group C rotaviruses at 74.3% to 84.1% nucleotide and 71.0% to 87.0% amino acid sequence identities. A phylogenetic analysis of the VP7 nucleotide sequences of the CMH004/03 strain provided a molecular basis for its genetic relatedness to other human group C rotavirus strains and formed the closest genetic lineage with human group C rotavirus strains Javeriana, OT-99, and KW408 (Fig. 2).

Analysis of the VP4 gene sequence

The partial VP4 nucleotide sequence (nucleotide 1 to 1,241) and deduced amino acid sequence (407 amino acid residues) of CMH004/03 were determined and compared

Table 1 Comparison of nucleotide and amino acid sequence identities of the VP7 gene segment of the CMH004/03 strain with those of the representative group C rotavirus strains

Strain	Host species	Country	Accession No.	Sequence identity (%)	
				Nucleotide	Amino acid
KA4/1018	Human	Sweden	AF225563	97.8	98.4
Uppsala/1004	Human	Sweden	AF225560	98.1	98.7
T97/330	Human	Sweden	AF225558	98.0	98.7
KW290	Human	Japan	AB086967	97.9	97.5
Solano	Human	Argentina	AF120478	97.6	96.9
KU166	Human	Japan	AB086966	97.5	98.1
S-1	Human	Japan	U20995	97.9	97.8
E93	Human	Japan	U20992	98.4	98.7
Javeriana	Human	Columbia	AJ549087	98.6	99.3
OT-99	Human	Japan	AB086969	98.8	99.0
KW408	Human	Japan	AB086968	98.7	98.7
ASP/87	Human	Australia	U20990	97.8	98.1
OK118	Human	Japan	D87543	97.8	97.5
88-220	Human	Japan	M61100	97.4	98.1
A90L	Human	Australia	AY392446	97.2	98.1
Bristol	Human	UK	X77257	97.4	98.1
Preston	Human	UK	X77258	97.2	98.1
BCN21	Human	Spain	AM118023	97.3	97.8
T97/167	Human	Sweden	AF225553	97.5	98.1
Santiago	Human	Chile	U20996	97.8	98.4
Belem	Human	Brazil	X77256	96.9	97.8
ad1015	Human	USA	U20994	97.8	98.4
ad957	Human	USA	U20993	97.8	98.4
Fuan	Human	China	U20987	97.3	98.1
Moduganari	Human	Africa	AF323979	96.3	98.4
V460	Human	India	AY803724	95.3	97.8
Jajeri	Human	Africa	AF323982	95.2	97.8
Cowden	Porcine	USA	M61101	84.1	87.0
WH	Porcine	USA	U31749	82.9	84.6
HF	Porcine	USA	U31748	75.4	71.0
Yamagata	Bovine	Japan	AB108681	74.3	73.7
Shintoku	Bovine	Japan	U31750	75.3	74.0

Bold characters indicate the highest percentage of sequence identity

with those of established VP4 group C rotavirus reference strains available in the GenBank database. The sequence comparison shown in Table 2 indicates that the partial VP4 gene of CMH004/03 was closely related to those of other human group C rotavirus reference strains at 95.5% to 98.3% on nucleotide and 97.5% to 99.5% on amino acid sequence levels. The highest sequence identity was found with the human rotavirus reference strain 208 detected in China at 98.3% and 99.5% of nucleotide and amino acid sequence levels, respectively. The strains detected in animals exhibited much less nucleotide and amino acid sequence identities with the CMH004/03 strain, ranging from 67.7% to 70.4% and 68.7% to 69.5%, respectively. A phylogenetic tree constructed from the nucleotide sequence of CMH004/03 and those of group C rotavirus reference sequences from both human and non-human

origins is shown in Fig. 3. It was again found that the VP4 gene sequence of the Thai group C rotavirus strain formed exclusively in a monophyletic branch with other human group C rotavirus reference strains, and appeared to be most closely related to the Chinese 208 strain. Moreover, based on the VP4 sequences, group C rotavirus strains could be divided into three discrete lineages, one comprised only human rotavirus strains, whereas the other two distinct lineages were formed by porcine and bovine group C rotavirus strains, respectively.

Analysis of the VP6 gene sequence

Analysis of the complete VP6 gene sequence of CMH004/03 revealed that the VP6 gene of this strain was 1,353 nucleotides long, which encoded a polypeptide of 395

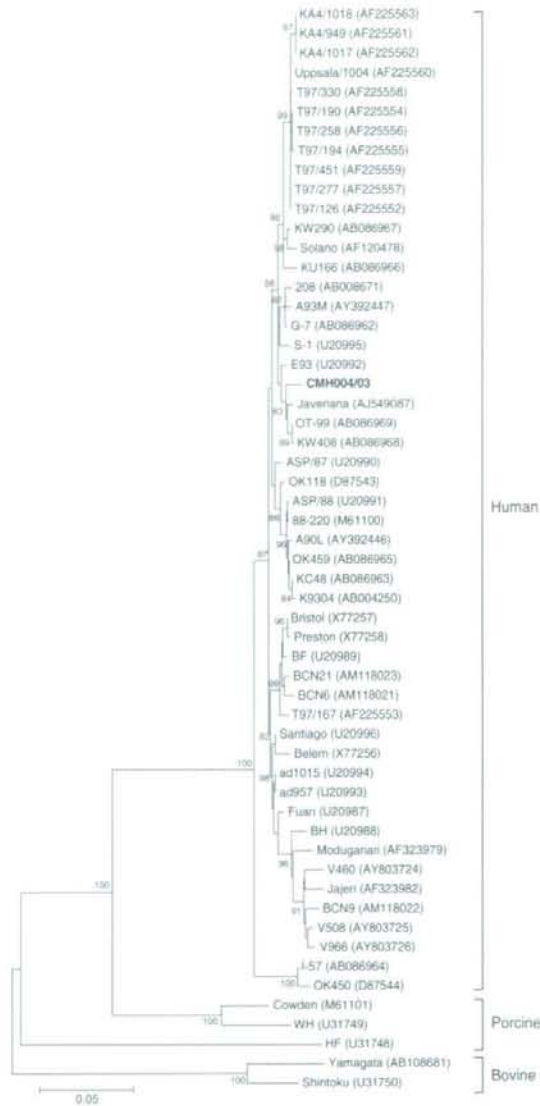


Fig. 2 Phylogenetic analysis of the nucleotide sequences derived from the VP7 genes of the human group C rotavirus strain CMH004/03 and other group C rotavirus reference strains. The tree was constructed based on the neighbor-joining method using the MEGA 3.1 program. The number presented in the branch nodes is the bootstrap value and the scale bar indicates the branch lengths for 5% nucleotide difference

amino acid residues, like other human group C rotavirus strains. Overall comparison of the VP6 nucleotide and deduced amino acid sequences of CMH004/03 with those of the existing group C rotavirus reference strains revealed a high level of sequence identity with human group C rotaviruses circulating all over the world, ranging from 97.2% to 99.4% on nucleotide and 99.2% to 100% on amino acid levels (Table 3). The VP6 sequence of

CMH004/03 was most closely related to the Chinese Wu82 strain at 99.4% nucleotide and 100% amino acid identities. However, the CMH004/03 exhibited far lesser sequence identity to the strains detected in animals (81.6% to 82.9% on nucleotide and 87.5% to 91.3% on amino acid sequence levels). A phylogenetic analysis of the VP6 nucleotide sequences confirmed the finding that the CMH004/03 strain clustered with other human group C rotavirus reference strains in a monophyletic branch and formed the same lineage with the Wu82 strain detected previously in China (Fig. 4). In addition, all human group C rotavirus strains analyzed were distantly related to group C rotaviruses of porcine and bovine origins.

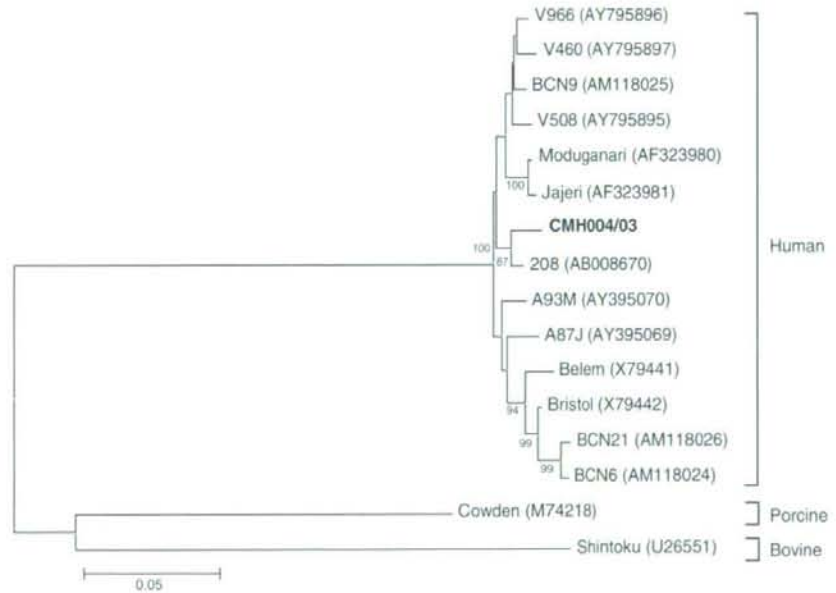
Discussion

From the literature search, the only initial report of group C rotavirus in Thailand was carried out in the central area in 1989 [19]. In that study, four cases were identified as group C rotavirus infections by PAGE, but no genetic information of those group C rotaviruses were described in that report. Since then, the human group C rotavirus has not been reported as a cause of acute gastroenteritis in Thailand until our report in the present study. The intensive epidemiological surveillances of rotaviruses have been conducted consecutively in Chiang Mai, Thailand, since 1984 [21, 26]. Most of those studies focused only on group A rotavirus infections, which is the main causative agent of gastroenteritis that accounted for approximately 17% to 58% of hospitalized children in Chiang Mai area [21, 23, 24], whereas group C rotavirus infections among hospitalized infants and young children have never been investigated in this area before. The present study reports the detection of human group C rotavirus strain (CMH004/03) from a sporadic case of a young girl admitted to hospital with acute gastroenteritis in 2003 in Chiang Mai area, northern Thailand. This is the first report describing the molecular genetic characteristics of group C rotavirus in the country. The low rate of detection (0.4%) in the studied populations suggests that group C rotavirus is an uncommon cause of acute gastroenteritis in hospitalized patients. This observation is in line with a number of publications of group C rotaviruses in sporadic cases of diarrhea with a very low prevalence rate, for instance, 1.2% in Japan [31], 2.3% in Bangladesh [18], 1.8% in Nigeria [28], 1% in Argentina [32], and 0.6% in Hungary [33]. However, several investigators suggested that the patients infected with group C rotaviruses were older (more than 5 years of age) than those infected with human group A rotaviruses [19, 31, 33]. It is possible that very low incidence of group C rotavirus observed in the present study may be because the patients being enrolled in the study were less than

Table 2 Comparison of nucleotide and amino acid sequence identities of the VP4 gene segment of the CMH004/03 strain with those of the representative group C rotavirus strains

Strain	Host species	Country	Accession No.	Sequence identity (%)	
				Nucleotide	Amino acid
V966	Human	India	AY795896	96.9	98.2
V460	Human	India	AY795897	96.7	98.5
BCN9	Human	Spain	AM118025	97.0	98.0
V508	Human	India	AY795895	96.7	98.0
Moduganari	Human	Africa	AF323980	96.9	98.0
Jajeri	Human	Africa	AF323981	96.9	98.0
208	Human	China	AB008670	98.3	99.5
A93 M	Human	Australia	AY395070	97.0	97.5
A87 J	Human	Australia	AY395069	96.5	98.5
Belem	Human	Brazil	X79441	96.2	98.2
Bristol	Human	UK	X79442	96.3	97.7
BCN21	Human	Spain	AM118026	95.5	97.7
BCN6	Human	Spain	AM118024	95.5	97.5
Cowden	Porcine	USA	M74218	70.4	69.5
Shintoku	Bovine	Japan	U26551	67.7	68.7

Bold characters indicate the highest percentage of sequence identity

Fig. 3 Phylogenetic analysis of the nucleotide sequences derived from partial VP4 genes of the human group C rotavirus strain CMH004/03 and other group C rotavirus reference strains. The tree was constructed based on the neighbor-joining method using the MEGA 3.1 program. The number presented in the branch nodes is the bootstrap value and the scale bar indicates the branch lengths for 5% nucleotide difference

5 years old. In addition, Abid et al. reported that 31% of fecal specimens collected in Spain contained the inhibitors of a RT-PCR assay [34]. Since our detection method for group C rotaviruses was RT-PCR, the possibility that a very low detection rate of group C rotaviruses observed in the present study might be due to inhibitors containing in the stool specimens could not be ruled out.

The nucleotide and deduced amino acid sequences of gene encoding for three major structural proteins, VP4, VP6, and VP7, of human group C rotavirus isolated from different regions of the world displayed remarkable

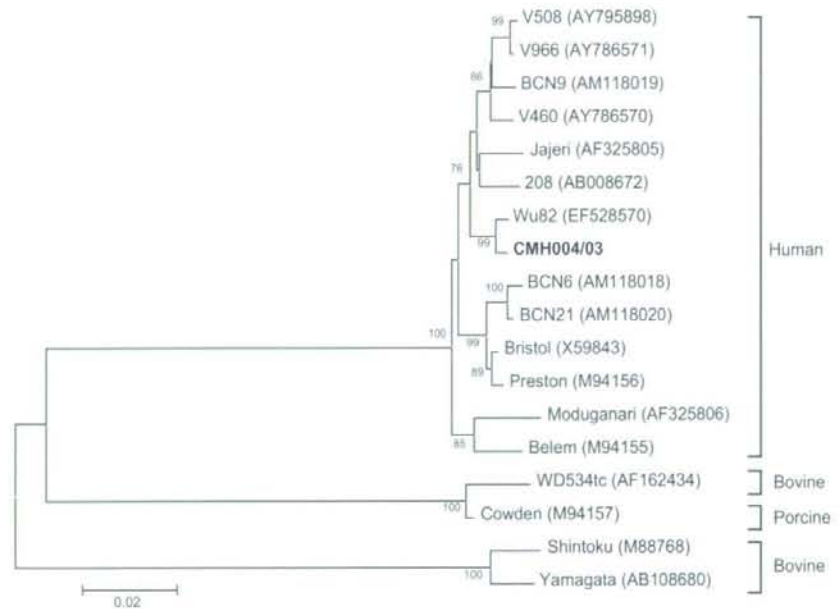
sequence conservations, and greater differences have been noted between human and animal group C rotavirus strains [17, 28, 35]. Up to now, a formal classification system of group C rotaviruses has not yet been well established. Based on nucleotide and deduced amino acid sequence analyses, however, at least four G (VP7), three P (VP4), and three VP6 genetic clusters have been proposed [12, 14, 18]. Phylogenetic analyses of the VP4, VP6, and VP7 genes of our CMH004/03 strain are in good agreement with those of the previous studies [12, 14, 18]. Furthermore, phylogenetic analysis also confirmed that the genome

Table 3 Comparison of nucleotide and amino acid sequence identities of the VP6 gene segment of the CMH004/03 strain with those of the representative group C rotavirus strains

Strain	Host species	Country	Accession No.	Sequence identity (%)	
				Nucleotide	Amino acid
V508	Human	India	AY795898	98.2	99.4
V966	Human	India	AY786571	98.3	99.7
BCN9	Human	Spain	AM118019	98.2	99.7
V460	Human	India	AY786570	98.3	99.7
Jajeri	Human	Africa	AF325805	98.0	98.9
208	Human	China	AB008672	98.3	98.7
Wu82	Human	China	EF528570	99.4	100.0
BCN6	Human	Spain	AM118018	97.6	99.2
BCN21	Human	Spain	AM118020	97.7	99.4
Bristol	Human	UK	X59843	98.1	99.7
Preston	Human	UK	M94156	98.0	99.7
Moduganari	Human	Africa	AF325806	97.2	99.7
Belem	Human	Brazil	M94155	97.4	99.7
WD534tc	Bovine	USA	AF162434	81.9	87.5
Cowden	Porcine	USA	M94157	82.9	91.3
Shintoku	Bovine	Japan	M88768	81.6	88.1
Yamagata	Bovine	Japan	AB108680	81.7	88.6

Bold characters indicate the highest percentage of sequence identity

Fig. 4 Phylogenetic analysis of the nucleotide sequences derived from the VP6 genes of the human group C rotavirus strain CMH004/03 and other group C rotavirus reference strains. The tree was constructed based on the neighbor-joining method using the MEGA 3.1 program. The number presented in the branch nodes is the bootstrap value and the scale bar indicates the branch lengths for 2% nucleotide difference



sequences of VP4, VP6, and VP7 genes formed a monophyletic branch with other human group C rotavirus reference strains. In addition, sequence analyses of the VP4, VP6, and VP7 genes revealed that the sequences are highly conserved (over 95% on nucleotide and 97% on amino acid levels) and the host range is restricted. It is noteworthy to point out that all the genes of our human group C rotavirus strain CMH004/03 were most closely

related to those of human group C rotavirus reference strains reported previously in Asian countries, including Japan (OT-99, KW408) and China (208 and Wu82), than those of other regions of the world, with the exception of one isolate (Javeriana) from Columbia.

In conclusion, the present study reports the first genetic evolutionary analysis of human group C rotavirus detected in Thailand by analyzing of the VP4, VP6, and VP7 genes.

The results demonstrated a close genetic relationship between the VP4, VP6, and VP7 genes of our human group C rotavirus strain CMH004/03 and those of other human group C rotavirus reference strains circulating in other parts of the world. The findings imply that human group C rotaviruses detected worldwide, so far, may have evolved from a common ancestor.

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References

- M.K. Estes, in *Fields virology*, 4th edn., ed. by D.M. Knipe, P.M. Howley, D.E. Griffin (Lippincott, Williams and Wilkins, Philadelphia, PA, 2001), pp. 1747–1785
- J.C. Bridger, S. Pedley, M.A. McCrae, *J. Clin. Microbiol.* **23**, 760 (1986)
- Z.Y. Fang, Q. Ye, M.S. Ho, H. Dong, S. Qing, M.E. Penaranda et al., *J. Infect. Dis.* **160**, 948 (1989)
- U.D. Parashar, C.J. Gibson, J.S. Bresse, R.I. Glass, *Emerg. Infect. Dis.* **12**, 304 (2006)
- L.J. Saif, B. Jiang, *Curr. Top. Microbiol. Immunol.* **185**, 339 (1994)
- L.J. Saif, E.H. Bohl, K.W. Theil, R.F. Cross, J.A. House, *J. Clin. Microbiol.* **12**, 105 (1980)
- N.A. Cunliffe, W. Dove, B. Jiang, B.D. Thinwada Cert, R.L. Broadhead, M.E. Molyneux et al., *Pediatr. Infect. Dis. J.* **20**, 1088 (2001). doi:10.1097/00006454-200111000-00018
- T.G. Phan, S. Nishimura, M. Okame, T.A. Nguyen, P. Khamrin, S. Okitsu et al., *J. Med. Virol.* **74**, 173 (2004). doi:10.1002/jmv.20162
- R.D. Schnagl, K. Boniface, P. Cardwell, D. McCarthy, C. Ondracek, B. Coulson et al., *J. Clin. Microbiol.* **42**, 2127 (2004). doi:10.1128/JCM.42.5.2127-2133.2004
- M. Kuzuya, M. Hamano, M. Nishijima, R. Fujii, H. Ogura, M. Tanaka et al., *Jpn. J. Infect. Dis.* **58**, 255 (2005)
- A. Steyer, M. Poljsak-Prijatelj, T. Bufon, M. Sedmak, L. Vidmar, J.Z. Mijovski et al., *J. Med. Virol.* **78**, 1250 (2006). doi:10.1002/jmv.20687
- I. Abid, S. Guix, M. Aouni, R. Pinto, A. Bosch, *J. Clin. Virol.* **38**, 78 (2007). doi:10.1016/j.jcv.2006.09.012
- E.H. Bohl, L.J. Saif, K.W. Theil, A.G. Agnes, R.F. Cross, *J. Clin. Microbiol.* **15**, 312 (1982)
- B. Jiang, J.R. Gentsch, H. Tsunemitsu, L.J. Saif, R.I. Glass, *Virus Genes* **19**, 85 (1999). doi:10.1023/A:1008196824879
- P. Otto, P. Schulze, W. Herbst, *Arch. Virol.* **144**, 2467 (1999). doi:10.1007/s007050050659
- A.S. Grice, P.R. Lambden, E.O. Caul, I.N. Clarke, *J. Med. Virol.* **44**, 166 (1994). doi:10.1002/jmv.1890440209
- B. Jiang, H. Tsunemitsu, P.H. Dennehy, I. Oishi, D. Brown, R.D. Schnagl et al., *Arch. Virol.* **141**, 381 (1996). doi:10.1007/BF01718407
- M. Rahman, S. Banik, A.S. Faruque, K. Taniguchi, D.A. Sack, M. Van Ranst et al., *J. Clin. Microbiol.* **43**, 4460 (2005). doi:10.1128/JCM.43.9.4460-4465.2005
- M.E. Penaranda, W.D. Cubitt, P. Sinarachatanant, D.N. Taylor, S. Likanonakul, L. Saif et al., *J. Infect. Dis.* **160**, 392 (1989)
- N. Santos, Y. Hoshino, *Rev. Med. Virol.* **15**, 29 (2005). doi:10.1002/rmv.448
- N. Maneekarn, H. Ushijima, *Pediatr. Int.* **42**, 415 (2000). doi:10.1046/j.1442-200x.2000.01251.x
- C. Jiraphongsa, J.S. Bresee, Y. Pongsuwanna, P. Kluabwang, U. Poonawagul, P. Arpornitip et al., *J. Infect. Dis.* **192**, 87 (2005). doi:10.1086/431508
- P. Khamrin, S. Peerakome, L. Wongsawadi, S. Tonusin, P. Sornchai, V. Maneerat et al., *J. Med. Virol.* **78**, 273 (2006). doi:10.1002/jmv.20536
- P. Khamrin, S. Peerakome, S. Tonusin, R. Malasao, S. Okitsu, M. Mizuguchi et al., *J. Med. Virol.* **79**, 1775 (2007). doi:10.1002/jmv.20960
- H. Yan, T.A. Nguyen, T.G. Phan, S. Okitsu, Y. Li, H. Ushijima, *Kansenshogaku Zasshi* **78**, 699 (2004)
- A. Hasegawa, A. Mukoyama, H. Suzuki, S. Inouye, S. Chearskul, P. Thongkrajai et al., *J. Diarrhoeal Dis. Res.* **5**, 165 (1987)
- A.J. Herring, N.F. Inglis, C.K. Ojeh, D.R. Snodgrass, J.D. Menzies, *J. Clin. Microbiol.* **16**, 473 (1982)
- M.I. Adah, A. Wade, M. Oseto, M. Kuzuya, K. Taniguchi, *J. Med. Virol.* **66**, 269 (2002). doi:10.1002/jmv.2141
- S.F. Altschul, W. Gish, W. Miller, E.W. Myers, D.J. Lipman, *J. Mol. Biol.* **215**, 403 (1990)
- S. Kumar, K. Tamura, M. Nei, *Brief. Bioinform.* **5**, 150 (2004). doi:10.1093/bib/5.2.150
- M. Kuzuya, R. Fujii, M. Hamano, M. Nishijima, H. Ogura, *J. Med. Virol.* **79**, 1219 (2007). doi:10.1002/jmv.20910
- A.A. Castello, M.H. Argüelles, G.A. Villegas, A. Olthoff, G. Glikmann, *J. Med. Virol.* **67**, 106 (2002). doi:10.1002/jmv.2198
- K. Bányai, B. Jiang, A. Bogdán, B. Horváth, F. Jakab, E. Meleg et al., *J. Clin. Virol.* **37**, 317 (2006). doi:10.1016/j.jcv.2006.08.017
- I. Abid, S. Guix, M. Aouni, R. Pinto, A. Bosch, *J. Clin. Virol.* **38**, 78 (2006). doi:10.1016/j.jcv.2006.09.012
- H. Tsunemitsu, B. Jiang, L.J. Saif, *Arch. Virol.* **141**, 705 (1996). doi:10.1007/BF01718328

Genetic Diversity of Norovirus, Sapovirus, and Astrovirus Isolated From Children Hospitalized With Acute Gastroenteritis in Chiang Mai, Thailand

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Norovirus (NV), sapovirus (SV), and human astrovirus (HAstV) are important causes of acute gastroenteritis in infants and young children. This study investigated the prevalence of NV, SV, and HAstV infections in children hospitalized with acute gastroenteritis in Chiang Mai, Thailand from May 2000 to March 2002. Fecal specimens were tested for NV, SV, and HAstV by reverse transcription polymerase chain reaction (RT-PCR) using degenerate specific primers. These viruses were characterized further by sequence and phylogenetic analyses of the partial capsid gene. From 296 fecal specimens tested, 13.5% (40 of 296) were positive for NV, SV, and HAstV. Of these, NV most predominant, with a prevalence of 60% (24 of 40), of which 17.5% were NVGI and 42.5% were NVGII. Of note, one specimen was positive for both NVGI and SV. SV was detected in 25%, while HAstV was detected in 17.5%. Analysis of nucleotide and amino acid sequences revealed that NVGI strains comprised GI/3, GI/4, GI/6, GI/7, and GI/13 genotypes. Among NVGII strains, approximately half of them belonged to genotype GII/4 (Lordsdale virus cluster), followed by GII/3, GII/10, GII/1, GII/6, GII/8, and GII/15. Analysis of SV sequences revealed that SVGI (Manchester virus) was more common than SVGII (London virus). The SV genotypes detected in this study belonged to SVGI/1, SVGI/4, SVGI/5, SVGII/1, and SVGII/2, whereas the HAstV belonged to genotypes HAstV-1, HAstV-2, HAstV-3, and HAstV-5. The findings suggest that NV, SV, and HAstV are important enteric viruses cocirculating among hospitalized children in Chiang Mai, Thailand. *J. Med. Virol.* 80:1749–1755, 2008. © 2008 Wiley-Liss, Inc.

KEY WORDS: noroviruses; sapoviruses; astroviruses; acute gastroenteritis; Chiang Mai; Thailand

INTRODUCTION

Acute gastroenteritis is one of the most common diseases in infants, children, and adults worldwide. During the first 5 years of life, every child will contract diarrheal disease, which enhances the risk of dehydration and nutritional deficiency [Jiraphongsa et al., 2005]. In addition to rotavirus (RV), human caliciviruses and astrovirus have emerged as etiologic causes of acute gastroenteritis in this age group. Norovirus (NV) and sapovirus (SV) are classified into the family *Caliciviridae* and are members of nonenveloped, positive-sense, single-stranded RNA viruses. Their genome contain approximately 7,300–8,300 nucleotides long, and a genome-linked protein (VPg) at the 5' terminus and a poly A tail at the 3' terminus [Bertolotti-Ciarlet et al., 2003]. Human astrovirus (HAstV) is a member of the family *Astroviridae*, and has a small (28–30 nm in diameter), round, nonenveloped characteristic. The genome is a positive-sense, single-stranded RNA of approximately 6,800 nucleotides in length [Schnag et al., 2002].

NVs can be divided into five distinct genogroups based on the variation in the capsid gene sequences, in which strains belonging to GI, GII, and GIV are found in humans, whereas GIII and GV are found in cows and mice, respectively [Zheng et al., 2006]. Recently, NVs were classified into 8, 17, 1, 1, and 1 genotypes in GI, GII, GIII, GIV, and GV, respectively [Zheng et al., 2006]. SVs are divided into five genogroups (GI to GV) based on the

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difference of capsid gene sequences, in which GI, GII, GIV, and GV are found in humans, while GIII is found in porcine species [Farkas et al., 2004]. Recently, Akihara et al. [2005] reported that SV strains are classified further into 16 genetic clusters/genotypes (8 in GI, 5 in GII, 1 each in GIII, GIV, and GV) based on the differences of partial capsid amino acid sequences. HAstVs can be divided into eight distinct serotypes or genotypes based on the antigenic difference or variation in the capsid gene sequences: serotype/genotype 1 to 8, in which a high concordance between results of serotyping and genotyping was observed [Sakamoto et al., 2000].

In Thailand, epidemiological studies of NV, SV, and HAstV are less frequent than those of RV. The study conducted in Chiang Mai in 2000 and 2001 [Hansman et al., 2004] reported that NV infection in children hospitalized with diarrhea was 7.6%, while SV infection was 3.8%. One specimen (0.95%) was an NV/SV mixed infection. Later, during 2002 and 2003, Guntapong et al. [2004] reported 11 NV and 9 SV single infections, and 3 were NV/SV mixed infections from a total of 80 stool specimens collected from children hospitalized with acute gastroenteritis in 5 different geographical areas of Thailand (Sa Kaeo, Chanthaburi, Songkhla, Nong Khai, and Tak). For HAstV, the frequency of detection rate ranged from 8.6% to 14% in hospitalized children [Herrmann et al., 1991; Echeverria et al., 1994]. In 2004, a report from Bangkok demonstrated that HAstV was the cause of a neonatal gastroenteritis outbreak, which occurred in the nursery of a maternity ward at Ramathibodi Hospital. HAstV was detected in 4 of 13 (30.7%) diarrheic neonates and 1 member of the nursery staff who had diarrhea [Sirinavin et al., 2006].

This study reports the prevalence and molecular epidemiology of norovirus, sapovirus, and astrovirus infections in sporadic gastroenteritis among hospitalized children in Chiang Mai, Thailand from May 2000 to March 2002.

MATERIALS AND METHODS

Specimen Collection

Two hundred ninety-six fecal specimens were collected from children hospitalized with diarrhea in four different hospitals and one private clinic in Chiang Mai province between May 2000 and March 2002. The ages of the subjects ranged from neonate up to 5 years old.

RNA Extraction and Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

Viral RNA was extracted from 10% fecal supernatant using the QIAamp viral RNA Mini Kit (Qiagen, Hilden, Germany). For RT, the viral RNA was reverse transcribed according to the manufacturer's instruction (Fermentas, Lithuania Glen Burnie, MD). The presence

of NV (GI and GII), SV, and HAstV was detected by RT-PCR using the protocol described previously [Yan et al., 2003]. A forward primer, G1-SKF (nt 5,342–5,361) 5'-CTGCC-CGAATTGTGAATGA-3', was used in combination with the reverse primer, G1-SKR (nt 5,653–5,671) 5'-CCAACCCARCCATRTACA-3', for the amplification of NVGI, which specifically generated a PCR amplicon of 330 bp. For NVGII identification, a forward primer, COG2F (nt 5,003–5,028) 5'-CARGARBC-NATGTT-YAGRTGGATGAG-3', was used in combination with the reverse primer, G2-SKR (nt 5,367–5,389) 5'-CCR-CCNGCATRHCCRTTRTACAT-3', which generated a PCR product size of 387 bp. For SV detection, a 434 bp fragment was generated using the forward primer, SLV5317 (nt 5,083–5,105) 5'-CTCGCCACCTA-CRAW-GCTTGTT-3', and reverse primer, SLV5749 (nt 5,516–5,494) 5'-CGGRCYTCAA AVSTACCBCCC-CA-3'. For amplification of HAstV, a forward primer, PreCAP1 (nt 4,235–4,255) 5'-GGACTGCAAAGCAG-CTTCGTG-3', was used in combination with the reverse primer, 82b (nt 4,934–4,953) 5'-GTGAGCCACCAGC-CATCCCT-3', which generated a PCR product size of 719 bp. All of the positive samples were analyzed further for their genotypes by nucleotide sequence and phylogenetic analyses.

Sequence and Phylogenetic Analyses

The PCR products were purified by the QIAquick Gel Extraction Kit (Qiagen) and sequenced by using the BigDye Terminator Cycle Sequencing Kit (Perkin Elmer-Applied Biosystems, Inc., Foster City, CA) on an automated DNA sequencer (ABI 3100; Perkin Elmer-Applied Biosystems, Inc.). The primers employed for amplification of the partial capsid genes were also used as sequencing primers. The nucleotide sequences obtained were translated into amino acid sequences using the GeneDoc program, and compared to those of NV (GI and GII), SV, and HAstV reference strains available in the GenBank using the BLAST program. The genotypes of NV (GI and GII) and SV were classified based on the recent capsid region classification scheme of Zheng et al. [2006] and Akihara et al. [2005], respectively. The serotypes/genotypes of HAstV were assigned in comparison with those of the reference strains by analysis of their amino acid sequences.

Nucleotide Sequence Accession Numbers

The partial nucleotide sequences of the capsid gene were deposited in GenBank under the accession number EU363852–EU363875 for NV strains, EU363876–EU363885 for SV strains, and EU363886–EU363892 for HAstV strains. The following capsid gene sequences of reference strains published in the GenBank were used in the phylogenetic analysis: NVGI: Boxer/01/US (AF538679), Chiba/00/JP (AB042808), DSV395 (U04469), Hesse (AF093797), Musgrove/89/UK (AJ277614), Norwalk/68 (M87661), Saitama T36GI/01/JP (AB112133), Southampton (L07418),

Winchester/94/UK (AJ277609); NVGII: Amsterdam/98-18/98/NET (AF195848), Bristol (X76716), CS-E1/02/US (AY502009), Erfurt/546/00/DE (AF427118), Fayetteville/98/US (AY113106), Hawaii (U07611), Hillingdon/90/UK (AJ277607), J23/99/US (AY130762), Leeds/90/UK (AJ277608), M7/99/US (AY130761), NongKhai-22/Thai (AY646866), NongKhai-51/Thai (AY646867), SaKao-14/Thai (AY646868), Seacroft/90/UK (AJ277620), Snow Mountain virus (AY134748), Tak-62/Thai (AY646877), Tiffin/99/USA (AY502010), Toronto (U02030), VA97207/97 (AY038599), Wortley/90/UK (AJ277618); SV: 4408/Maizuru/JP (AB180209), 8/DCC/Tokyo/JP/44 (AB236377), Arg39 (AY289803), Chanthaburi-74/Thai (AY646854), Chiba/000671T/99 (AJ412805), Cruise/US (AY289804), Hou7-1181/90 (AF435814), Houston/90 (U95644), Karachi/874/92 (AB181129), Karachi/876/93 (AB181132), Karachi/877/90 (AB181133), Karachi/878/90 (AB181228), Karachi/938/94 (AB181248), Karachi/1021/92 (AB181230), Karachi/1026/92 (AB181134), London/92 (U95645), Lyon/598/97/F (AJ271056), Manchester/93 (X86560), Mex340/90 (AF435812), Moscow/2196/02/RF (AY538722), Moscow/4536/02/RF (AY538716), NongKhai-24/Thai (AY646856), NongKhai-50/Thai (AY646853), PEC (Porcine Enteric Calicivirus) (AF182760), Sapporo/82/JP (U65427), Songkhla-6/Thai (AY646857), Stockholm/318/97/SE (AF194182), Tak-69/Thai (AY646864).

RESULTS

Prevalence and Distribution of Norovirus, Sapovirus, and Human Astrovirus Infections

A total of 296 fecal specimens were collected from children hospitalized with diarrhea, 40 (13.5%) of which were positive for NV, SV, and HAstV. Of these, NV was detected in 24 (8.1%) of the fecal specimens tested. Seven of these (2.4%) were identified as GI genogroup and 17 (5.7%) as GII genogroup. Among the NVs detected, NVGII was more predominant (70.8%) than NVGI (29.2%). SV was detected in 10 (3.4%) specimens and one of these was positive for both NVGI and SV. In addition, HAstV was found in 7 (2.4%) of the specimens tested (Table I). NVGI, NVGII, SV, and HAstV were detected in 27 of 187 (14.4%) specimens in the first period (May 2000–April 2001) and 14 of 109 (12.8%) in the following period (May 2001–March 2002) (Table I). The monthly distribution of NVGI, NVGII, SV, and

HAstV infections in children hospitalized with diarrhea is shown in Figure 1. In the year 2000, NVGII infection was detected at a high peak (~25%) in November. In 2001, infections of almost all NVGI, NVGII, SV, and HAstV tended to occur in the first 7 months of the year, except NVGII, which remained detectable in October. In January 2002, NVGI and GII were detected, while only NVGII was detected in February and HAstV in March. However, SV infection was not detected during this period (January–March 2002). The age of children at infection ranged from 4 months to 5 years old. Among those children, who were infected with NV, SV, or HAstV, 85% were 2 years of age and younger. When the children were grouped into 0–5, 6–11, 12–24, and >24 months of age, no significant difference in the rate of infection with NV, SV, or HAstV was observed among each age group.

Sequence and Phylogenetic Analyses of Noroviruses

All of the NV strains were characterized further for their genotypes by sequencing of the partial capsid genes. The genotypes were classified according to the classification scheme of [Zheng et al., 2006]. Six NVGI strains detected in this study were classified into four distinct GI genotypes as follows: 2 strains of GI/4, 2 strains of GI/6, and 1 strain each of GI/3, and GI/7. In addition, 1 NVGI strain, CMH308/01, could not be classified into any genotype based on the scheme described by Zheng, however, it belonged to GI/13, with a percentage deduced amino acid sequence identity of 100%, according to the classification scheme of [Okada et al., 2005] (Fig. 2).

All 17 NVGII strains were classified further into seven distinct GII genotypes, comprising GII/1, GII/3, GII/4, GII/6, GII/8, GII/10, and GII/15 (Fig. 2). NVGII/4 was found to be the most predominant genotype (9 of 17; 52.9%) of the NVGII strains detected in this study. The GII/3 and GII/10 genotypes were detected at an equally prevalent rate of 11.7% (2 of 17 strains). In addition, a relatively low detection frequency of GII/1, GII/6, GII/8, and GII/15 at 5.8% (1 of 17) was observed.

Sequence and Phylogenetic Analyses of Sapoviruses

A total of 10 SV strains were analyzed and classified further into two distinct genogroups, GI and GII,

TABLE I. The Prevalence of NVGI, NVGII, SV, and HAstV Detected in Children Hospitalized With Acute Gastroenteritis in Chiang Mai, Thailand, From May 2000 to March 2002

Date of specimen collection	No. of specimens tested	No. of specimens positive for virus (%)				Total (%)
		NVGI	NVGII	SV	HAstV	
May 2000–Apr 2001	187	5 (2.7)	11 (5.9)	8 (4.3)	3 (1.6)	27 (14.4)
May 2001–Mar 2002	109	2 (1.8)	6 (5.5)	2 (1.8)	4 (3.7)	14 (12.8)
Total (%)	296	7 (2.4) ^a	17 (5.7)	10 (3.4) ^a	7 (2.4)	40 (13.5) ^a

^aOne specimen was positive for both NVGI and SV.

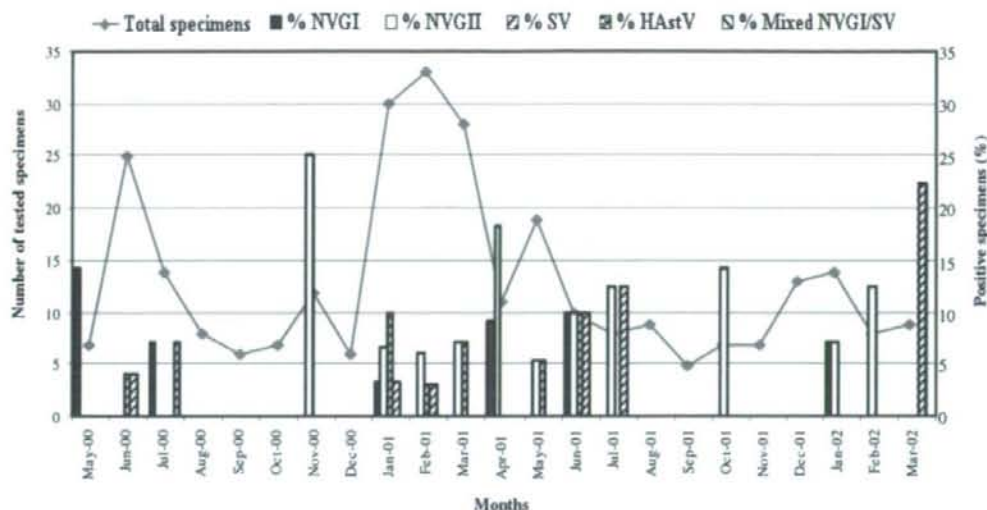


Fig. 1. Monthly distribution of NVGI, NVGII, SV, and HAstV infections among children hospitalized with diarrhea in Chiang Mai, Thailand, from May 2000 to March 2002 [number of tested specimens, \blacklozenge ; positive specimens (%), \square].

according to the recent SV capsid region classification scheme described by Akihara et al. [2005]. It was observed that SVGI was a more common genogroup (80%) than the SVGII (20%) detected in this study. Eight strains of SVGI were classified further into three genotypes, that is, four strains were GI/1, three were GI/4, and one was GI/5. Moreover, two strains of SVGII were also classified into GII/1 and GII/2 genotypes (Fig. 3).

Sequence Analysis of Human Astroviruses

Seven HAstV strains detected in the present study, two of each isolate were HAstV-1, HAstV-2, HAstV-5, and one isolate was HAstV-3. The detection rate of the HAstV in children with acute gastroenteritis was rather low.

DISCUSSION

The present study describes the prevalence of NV and SV infection in children hospitalized with acute gastroenteritis in Chiang Mai city during May 2000–March 2002. The prevalent rates are in good agreement with those reported by Hansman et al. [2004] which also conducted the study in Chiang Mai during July 2000–July 2001. However, the prevalence of NV and SV are somewhat lower when compare to the follow-up study conducted recently in Chiang Mai from March 2002 to December 2004 [Khamrin et al., 2007] as well as to the study conducted in five other regions of Thailand by Guntapong et al. [2004]. The discrepancy of the prevalent rates between our study and others might be due to the difference in the duration and/or geographical area where those studies have been conducted.

Like other studies [Schnagl et al., 2000; Buesa et al., 2002; Oh et al., 2003; Boga et al., 2004; Hansman

et al., 2004], the findings showed that NVGI strains are less common (29.2%) than NVGII (70.8%). It should be noted that in 2002 and 2003, NVGI disappeared completely from five other regions of Thailand [Guntapong et al., 2004]. In addition, NVGI was also undetectable in Chiang Mai area during 2002 and 2004 [Khamrin et al., 2007]. For NVGII, GII/4 has been reported as a major cause of global outbreaks and sporadic cases of gastroenteritis [Foley et al., 2001; White et al., 2002; Lau et al., 2004]. This study in Chiang Mai area found that GII/4 circulated as the most predominant genotype (37.5%), which is similar to those reported by Hansman et al. [2004]. However, a study conducted by Guntapong et al. [2004] in five other regions of Thailand during 2002 and 2003 reported a relatively high GII/4 at the incidence of 64.3%. Interestingly, in the following three consecutive years from 2002 to 2004 [Khamrin et al., 2007], GII/4 increased to 62.8% in Chiang Mai region, which similar to the finding of Guntapong et al. [2004]. For SV infection, SV genogroup I (GI) has been reported worldwide as the most predominant strain [Okada et al., 2002; Phan et al., 2004; Akihara et al., 2005; Phan et al., 2005, 2006]. SVGI/1 strains were previously reported as the most predominant genotype, followed by GII/1 strains and one isolate belonging to an intragenogroup recombinant strain in Chiang Mai during 2000 and 2001 [Hansman et al., 2004; Katayama et al., 2004]. In our study, SVGI was also detected at a very high incidence (80%), with GI/1 as the most predominant strain and followed by GI/4, GI/5, GII/1, and GII/2 strains. The study conducted in the other regions of Thailand by Guntapong et al. [2004] during 2002 and 2003 reported a higher detection rate of SV (15.0%). SVGI/1 was the most prevalent genotype, while the other two strains belonged to SVGV and a novel genotype in the SVGII

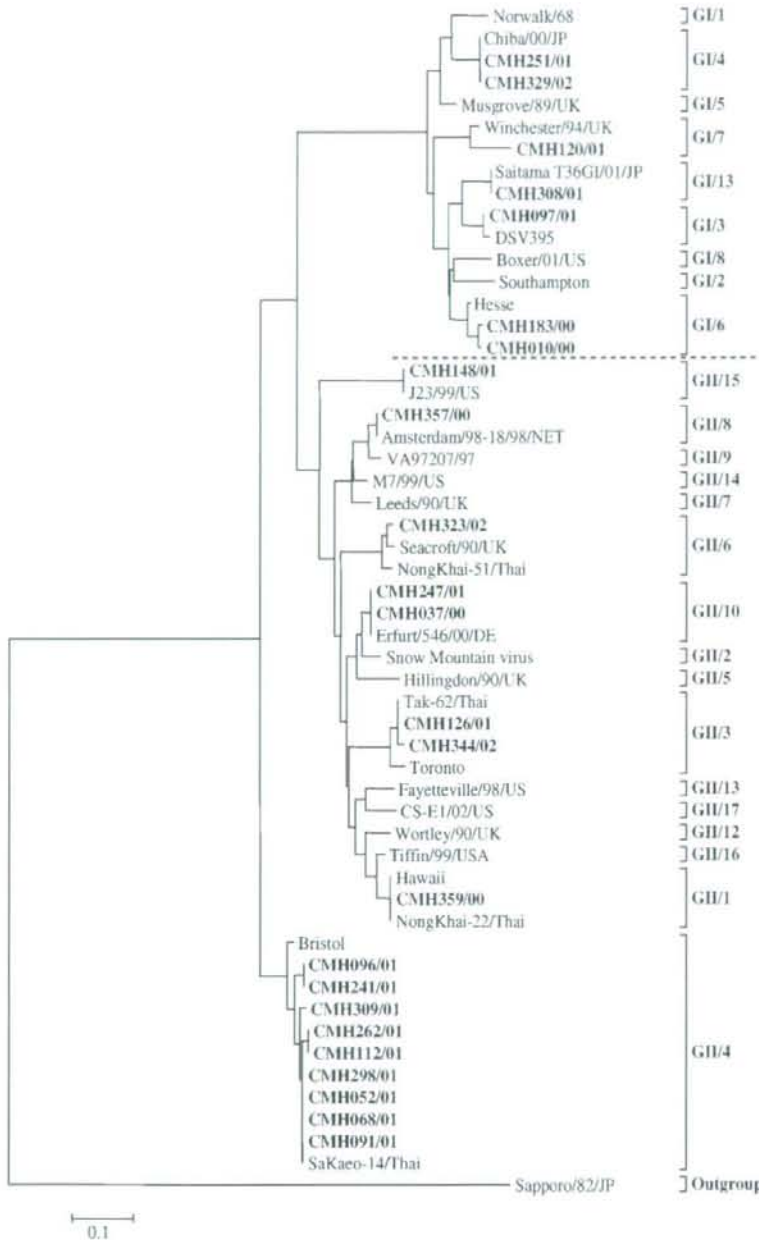


Fig. 2. Phylogenetic analysis of partial capsid deduced amino acid sequences of NVGI and GII strains detected between 2000 and 2002 in Chiang Mai, Thailand. The tree was constructed by multiple alignment of 7 NVGI and 17 NVGII positive sequences (indicated in boldface), 29 reference sequences, and 1 outgroup sequence. In the phylogenetic tree, NVGI strains were classified into eight distinct genotypes from 1

to 8 and NVGII strains were classified into sixteen distinct genotypes from 1 to 10 and 12 to 17 (excepting genotype 11 of porcine NVGII). Sapporo/82/JP was used as an outgroup strain for phylogenetic analysis. Bootstrap values are 1,000 replicates based on neighbor-joining and distance methods. Genotypes or genetic clusters are divided by brackets.

cluster, respectively. Another study in Chiang Mai region from 2002 to 2004 by Khamrin et al. [2007] reported a rather low detection rate of SV (1.2%) in which two strains belonged to SVGI genogroup (SVGI/1, SVGI/2) and other strain belonged to SVGIV genogroup. The data from our study and others [Guntapong et al.,

2004; Khamrin et al., 2007] reveals that the detection rate, genogroup and genotype of NV and SV strains circulating in several regions of Thailand vary from time to time. However, the predominant NVGII/4 and SVGI persist in these regions over a number of years.

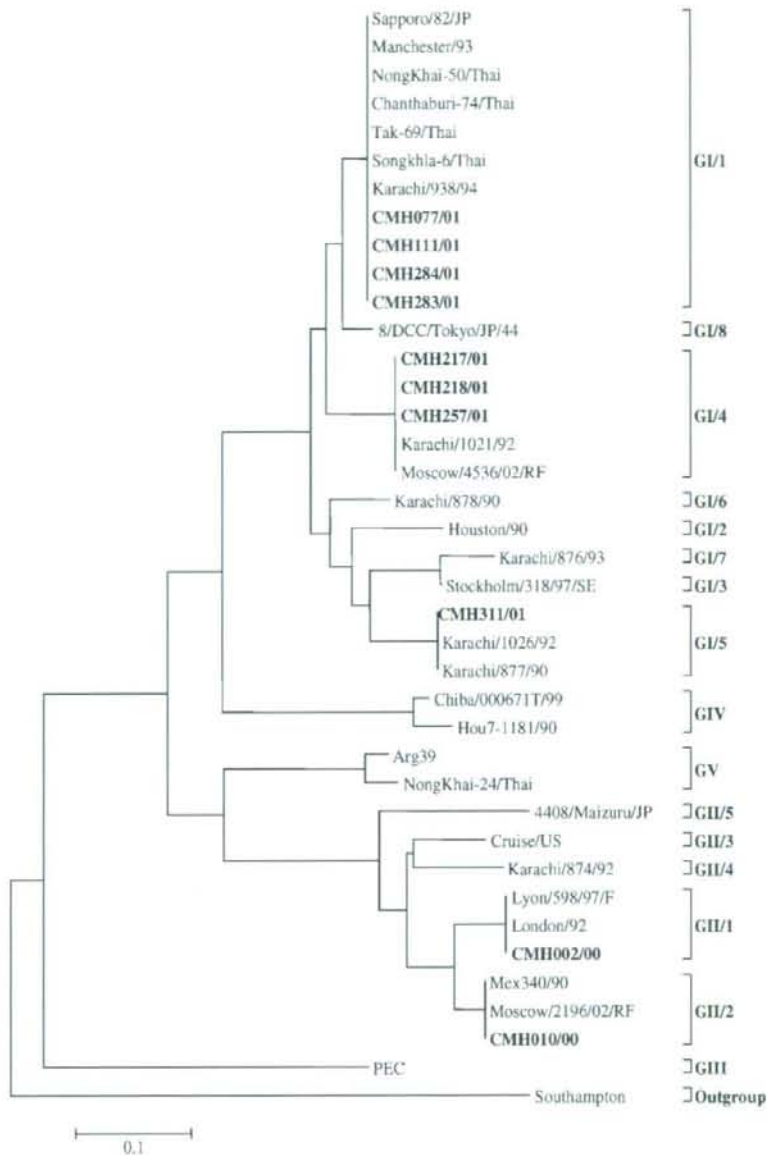


Fig. 3. Phylogenetic analysis of partial capsid deduced amino acid sequences of SV strains detected between 2000 and 2002 in Chiang Mai, Thailand. The tree was constructed by multiple alignment of 10 SV positive sequences (indicated in boldface), 27 reference sequences, and 1 outgroup sequence. In the phylogenetic tree, SV strains were classified into genogroup I, II, IV, and V. SVGI was classified further

into eight genotypes and SVGII into five. SVGIII (Porcine enteric calicivirus, PEC) was also analyzed. Southampton was used as an outgroup strain for phylogenetic analysis. Bootstrap values are 1,000 replicates based on neighbor-joining and distance methods. Tree is unrooted. Genotypes or genetic clusters are divided by brackets.

Epidemiological data of HAsTV as a causative agent of gastroenteritis in Thailand is rather limited. In 1991, Herrmann et al. first detected HAsTV serotype 2 at 8.6% in children hospitalized with diarrhea in Bangkok. Later, Echeverria et al. [1994] reported the detection of HAsTV at 14% in children hospitalized with diarrhea in Ratchaburi province in the central part of Thailand. In 2004, Sirinavin et al. [2006] reported an outbreak of HAsTV with a detection rate of 30.7% in neonates at a

nursery in the maternity ward of Ramathibodi Hospital, Bangkok. However, the detection of HAsTV infection in the previous studies based on serological assays [Herrmann et al., 1991; Echeverria et al., 1994; Sirinavin et al., 2006]. Our study is the first report that describes the distribution of HAsTV genotypes circulating in Chiang Mai city. The data suggest that HAsTV infection is less common in children with acute gastroenteritis compared to rotavirus and norovirus infection

in this area. These percentages are similar to those reported from other regions of the world such as Australia [Mustafa et al., 2000], Germany [Oh and Schreier, 2001], and Spain [Guix et al., 2002].

In conclusion, this study describes the genetic diversity of NV, SV, and HAsV genotypes cocirculating in children hospitalized with diarrhea in Chiang Mai, Thailand.

REFERENCES

- Akihara S, Phan TG, Nguyen TA, Yagyu F, Okitsu S, Müller WE, Ushijima H. 2005. Identification of sapovirus infection among Japanese infants in a day care center. *J Med Virol* 77:595–601.
- Bertolotti-Ciarlet A, Crawford SE, Hutson AM, Estes MK. 2003. The 3' end of Norwalk virus mRNA contains determinants that regulate the expression and stability of the viral capsid protein VP1: A novel function for the VP2 protein. *J Virol* 77:11603–11615.
- Boga JA, Melón S, Nicieza I, De Diego I, Villar M, Parra F, De Oña M. 2004. Etiology of sporadic cases of pediatric acute gastroenteritis in Asturias, Spain, and genotyping and characterization of norovirus strains involved. *J Clin Microbiol* 42:2668–2674.
- Buesa J, Collado B, López-Andújar P, Abu-Mallouh R, Rodríguez Díaz J, García Díaz A, Prat J, Guix S, Llovet T, Prats G, Bosch A. 2002. Molecular epidemiology of caliciviruses causing outbreaks and sporadic cases of acute gastroenteritis in Spain. *J Clin Microbiol* 40:2854–2859.
- Echeverría P, Hoge CW, Bodhidatta L, Tungtaem C, Herrmann J, Imlarp S, Tamura K. 1994. Etiology of diarrhea in a rural community in western Thailand: Importance of enteric viruses and enterovirulent *Escherichia coli*. *J Infect Dis* 169:916–919.
- Farkas T, Zhong WM, Jing Y, Huang PW, Espinosa SM, Martinez N, Morrow AL, Ruiz-Palacios GM, Pickering LK, Jiang X. 2004. Genetic diversity among sapoviruses. *Arch Virol* 149:1309–1323.
- Foley B, O'Mahony J, Hill C, Morgan JG. 2001. Molecular detection and sequencing of "Norwalk-like viruses" in outbreaks and sporadic cases of gastroenteritis in Ireland. *J Med Virol* 65:388–394.
- Guix S, Caballero S, Villena C, Bartolomé R, Latorre C, Rabella N, Simó M, Bosch A, Pintó RM. 2002. Molecular epidemiology of astrovirus infection in Barcelona, Spain. *J Clin Microbiol* 40:133–139.
- Guntapong R, Hansman GS, Oka T, Ogawa S, Kageyama T, Pongsuwanna Y, Katayama K. 2004. Norovirus and sapovirus infection in Thailand. *Jpn J Infect Dis* 57:276–278.
- Hansman GS, Katayama K, Maneekarn N, Peerakome S, Khamrin P, Tonusin S, Okitsu S, Nishio O, Takeda N, Ushijima H. 2004. Genetic diversity of norovirus and sapovirus in hospitalized infants with sporadic cases of acute gastroenteritis in Chiang Mai, Thailand. *J Clin Microbiol* 42:1305–1307.
- Herrmann JE, Taylor DN, Echeverría P, Blacklow NR. 1991. Astroviruses as a cause of gastroenteritis in children. *N Engl J Med* 324:1757–1760.
- Jiraphongsa C, Laosiritaworn Y, Ngowabunpat A. 2005. Diarrhea mortality in children 0–5 years old in Thailand, 2001–2004. *Bull Dep Med Services* 30:43–51.
- Katayama K, Miyoshi T, Uchino K, Oka T, Tanaka T, Takeda N, Hansman GS. 2004. Novel recombinant sapovirus. *Emerg Infect Dis* 10:1874–1876.
- Khamrin P, Maneekarn N, Peerakome S, Tonusin S, Malasao R, Mizuguchi M, Okitsu S, Ushijima H. 2007. Genetic diversity of noroviruses and sapoviruses in children hospitalized with acute gastroenteritis in Chiang Mai, Thailand. *J Med Virol* 79:1921–1926.
- Lau CS, Wong DA, Tong LK, Lo JY, Ma AM, Cheng PK, Lim WW. 2004. High rate and changing molecular epidemiology pattern of norovirus infections in sporadic cases and outbreaks of gastroenteritis in Hong Kong. *J Med Virol* 73:113–117.
- Mustafa H, Palombo E, Bishop R. 2000. Epidemiology of astrovirus infection in young children hospitalized with acute gastroenteritis in Melbourne, Australia, over a period of four consecutive years, 1995 to 1998. *J Clin Microbiol* 38:1058–1062.
- Oh D, Schreier E. 2001. Molecular characterization of human astroviruses in Germany. *Arch Virol* 146:443–455.
- Oh DY, Gaedcke G, Schreier E. 2003. Viral agents of acute gastroenteritis in German children: Prevalence and molecular diversity. *J Med Virol* 71:82–93.
- Okada M, Shinozaki K, Ogawa T, Kaiho I. 2002. Molecular epidemiology and phylogenetic analysis of Sapporo-like viruses. *Arch Virol* 147:1445–1451.
- Okada M, Ogawa T, Kaiho I, Shinozaki K. 2005. Genetic analysis of noroviruses in Chiba prefecture, Japan, between 1999 and 2004. *J Clin Microbiol* 43:4391–4401.
- Phan TG, Okame M, Nguyen TA, Maneekarn N, Nishio O, Okitsu S, Ushijima H. 2004. Human astrovirus, norovirus (GI, GII), and sapovirus infections in Pakistani children with diarrhea. *J Med Virol* 73:256–261.
- Phan TG, Okame M, Nguyen TA, Nishio O, Okitsu S, Ushijima H. 2005. Genetic diversity of sapovirus in fecal specimens from infants and children with acute gastroenteritis in Pakistan. *Arch Virol* 150:371–377.
- Phan TG, Trinh QD, Yagyu F, Sugita K, Okitsu S, Müller WE, Ushijima H. 2006. Outbreak of sapovirus infection among infants and children with acute gastroenteritis in Osaka City, Japan during 2004–2005. *J Med Virol* 78:839–846.
- Sakamoto T, Negishi H, Wang QH, Akihara S, Kim B, Nishimura S, Kaneshi K, Nakaya S, Ueda Y, Sugita K, Motohiro T, Nishimura T, Ushijima H. 2000. Molecular epidemiology of astroviruses in Japan from 1995 to 1998 by reverse transcription-polymerase chain reaction with serotype-specific primers (1 to 8). *J Med Virol* 61:326–331.
- Schnagl RD, Barton N, Patrikis M, Tizzard J, Erlich J, Morey F. 2000. Prevalence and genomic variation of Norwalk-like viruses in central Australia in 1995–1997. *Acta Virol* 44:265–271.
- Schnagl RD, Belfrage K, Farrington R, Hutchinson K, Lewis V, Erlich J, Morey F. 2002. Incidence of human astrovirus in central Australia (1995 to 1998) and comparison of deduced serotypes detected from 1981 to 1998. *J Clin Microbiol* 40:4114–4120.
- Sirinavin S, Techaasensiri C, Okascharoen C, Nuntnarumit P, Tonsuttakul S, Pongsuwan Y. 2006. Neonatal astrovirus gastroenteritis during an inborn nursery outbreak. *J Hosp Infect* 64:196–197.
- White PA, Hansman GS, Li A, Dable J, Isaacs M, Ferson M, McIver CJ, Rawlinson WD. 2002. Norwalk-like virus 95/96-US strain is a major cause of gastroenteritis outbreaks in Australia. *J Med Virol* 68:113–118.
- Yan H, Yagyu F, Okitsu S, Nishio O, Ushijima H. 2003. Detection of norovirus (GI, GII), sapovirus and astrovirus in fecal samples using reverse transcription single-round multiplex PCR. *J Virol Methods* 114:37–44.
- Zheng D, Ando T, Fankhauser RL, Beard RS, Glass RI, Monroe SS. 2006. Norovirus classification and proposed strain nomenclature. *Virology* 346:312–323.

Short
Communication

Genetic linkage among human cytomegalovirus glycoprotein N (gN) and gO genes, with evidence for recombination from congenitally and post-natally infected Japanese infants

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Investigation of sequence polymorphisms in the glycoprotein N (gN; gp4273), gO (gp4274) and gH (gp4275) genes of human cytomegalovirus (HCMV) strains collected from 63 Japanese children revealed that their gO genotype distribution differed slightly from that of Caucasian populations and that there was a significant linkage between the gN and gO genotypes. Linkage of these genotypes in strains obtained from Caucasian populations has been reported, so our similar findings in Japanese infants are consistent with this, and suggest generality of this linkage. Sequence analysis suggests that recombination between two strains of different linkage groups occurred approximately 200 bp upstream of the 3'-end of the gO gene. Further studies are required to elucidate differences in biological characteristics among the linkage groups and the selective constraints that maintain the linkage.

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Human cytomegalovirus (HCMV) infects most people during childhood without clinical symptoms; it is the major viral cause of birth defects and developmental abnormalities. It is also associated with significant morbidity and mortality in immunocompromised individuals. The complete genome of wild-type HCMV strains, such as Merlin, is 236 kb in length, and is predicted to encode 165 genes (Dolan *et al.*, 2004). Genetic characterization of clinical isolates has mainly depended on sequence polymorphisms in the genes that encode viral envelope glycoproteins and cellular homologues (Rasmussen, 1999; Pignatelli *et al.*, 2004). Glycoprotein B (gB), gM, gN, gH, gL and gO are involved in virus entry and egress and are the target molecules recognized by neutralizing antibodies. While extensive sequence variation is found in the gB and gH genes (5–10%), a greater level is found in the gN and gO genes (40–50%); the gL and gM genes are highly conserved among clinical strains. To date, the association of a particular genotype with a particular

clinical outcome has been controversial (Bale *et al.*, 2000; Barbi *et al.*, 2001; Trincado *et al.*, 2000).

We have recently investigated gB, UL144 and UL149 gene polymorphisms and found that the gB3 genotype was more prevalent in congenitally infected individuals with neurological abnormalities (Yan *et al.*, 2008). Recently, a genetic linkage between the gN and gO genes was reported (Mattick *et al.*, 2004). To learn how common this linkage is and whether the linkage group has any correlation with the clinical outcome of congenital infection, we analysed gN, gO and gH gene polymorphisms.

HCMV strains were collected from 45 urine and 24 dried umbilical cord specimens obtained from 63 Japanese children, consisting of 32 congenitally and 31 post-natally infected children. Although samples of both materials were collected from six infants, specimens from each infant were handled as a single entity, as specimens from the same infant yielded the same sequence. Eleven of the congenital cases were identified previously by Ogawa *et al.* (2007). Six were identified by HCMV-specific IgM in maternal or cord blood specimens, a further six were identified by our HCMV screening programme (Inoue & Koyano, 2008) and

The sequence data determined in this study are available under GenBank accession numbers EU348337–EU348364.

Two supplementary tables are available with the online version of this paper.