VP6 genotyping was carried out by multiplex RT-PCR with subgroup-I- and II-specific primers (GAVP6-SG1 and GAVP6-SG2, respectively) as described previously [38]. NSP4 genotypes A, B, or C were determined by multiplex RT-PCR with NSP4 genotype-specific primers as described previously [38].

Propagation of rotaviruses in cell culture

Among all the rotavirus specimens genotyped, 24 specimens from children and adults representing individual G-types were selected for propagation of rotavirus strains in MA104 cells. Briefly, stool suspension filtered with sterile syringe filter (pore size 0.45 μm, Corning) was activated with 10 μg/ml acetylated trypsin (Type V–S from bovine pancreas, Sigma) and inoculated onto MA104 cells in glass tubes with Eagle's minimum essential medium containing 1 μg/ml acetylated trypsin, followed by incubation in rolling culture using a rotator. Culture fluid of rotavirus was harvested 3–14 days after infection and was subsequently inoculated onto MA104 cells serially, until the fourth passage. The culture fluid of the final passage was examined for rotavirus RNA segments by PAGE.

Sequence and phylogenetic analysis

Sequences of rotavirus genes encoding VP7, VP4, VP6, NSP1-NSP5 were determined directly with RT-PCR products from these genes. PCR products were purified using a Wizard® SV Gel and PCR Clean-Up System (Promega Inc.). Sequencing reactions were performed with fluorescent dideoxy chain termination chemistry using the BigDye Terminator version 1.3 cycle sequencing kit (Applied Biosystems). DNA sequences were determined by the use of an ABI Prism 3100 genetic analyzer (Applied Biosystems). Accession numbers of the sequences determined in this study are given in Table 2.

Genetyx-WIN Version 5.1 (Software Development, Tokyo, Japan) was used to perform pairwise alignment and calculate sequence identity of VP7 and VP4 genes from different strains. Phylogenetic analysis was performed with MEGA software version 4.1 based on the neighbor-joining method. Phylogenetic distances were measured by the Kimura two-parameter model, and phylogenetic trees were statistically supported by bootstrapping with 1,000 replicates. For drawing of phylogenetic dendrograms of VP7 and VP4 genes, an outgroup sequence was chosen from a human rotavirus strain having a genotype which was different from the analyzed type: the Wa (G1)-VP7 gene for VP7 gene dendrograms of G2, G9, and G12 rotaviruses; the DS-1 (G2)-VP7 gene for VP7 gene dendrograms of G1 rotaviruses; the Wa (P[8])-VP4 gene for VP4 gene dendrograms of P[4] and P[6] rotaviruses; and the DS-1 (P[4])-VP4 gene for VP4 gene dendrograms of P[8] rotaviruses. For the phylogenetic trees of VP6 and NSP1-NSP5, sequences of the avian rotavirus strain PO-13 or murine rotavirus strain EW were used as outgroups.

Results

Prevalence of rotaviruses

In total, group A rotavirus was detected in 26.4% of stool specimens from children (430/1,627) and 10.1% of specimens from adults (92/913). Among adults, the rotavirus-positive rate was highest in the 31–40-year-old

Table 1 Frequency of G-types and P-types in rotaviruses from children and adults in Mymensingh, Bangladesh

Genotype		Frequency (%)	
G-type	P-type	Children	Adults
G1	P[6]	9 (8.0)	
G1	P[8]	10 (8.8)	6 (14.3)
G1	P[4] + [8]	1 (0.9)	
G1	P[4] + [6] + [8]	3 (2.7)	5 (11.9)
G1	ND	1 (0.9)	
G2	P[4]	52 (46.0)	18 (42.9)
G2	P[6]	3 (2.7)	
G2	P[8]		1 (2.4)
G2	P[4] + [6]	6 (5.3)	
G2	P[4] + [6] + [8]		1 (2.4)
G4	P[4]	1 (0.9)	
G4	P[6]		1 (2.4)
G4	P[8]		1 (2.4)
G9	P[4]	1 (0.9)	
G9	P[6]	5 (4.4)	
G9	P[8]	5 (4.4)	4 (9.5)
G9	P[4] + [6]	4 (3.5)	
G9	P[4] + [8]	2 (1.8)	
G9	ND	1 (0.9)	
G12	P[4]	1 (0.9)	
G12	P[6]	3 (2.7)	1 (2.4)
G1 + G2	P[4]	1 (0.9)	
G1 + G2	P[6]	1 (0.9)	
G1 + G2	P[4] + [6] + [8]		3 (7.1)
G1 + G2 + G4	P[6]		1 (2.4)
G2 + G4 + G9	P[4] + [6] + [8]	1 (0.9)	
ND	P[6]	1 (0.9)	
ND	P[8]	1 (0.9)	
Total		113 (100)	42 (100)

ND not determined

group (14.3%), followed by the 51–60-year-old group (12.6%). During the study period, high incidence of rotavirus was found in March to April in both children and adults, while a high detection rate in August was found only in children.

Frequency of G and P types

Among the rotavirus-positive specimens, a total of 155 specimens (113 and 42 from children and adults, respectively) were selected to analyze genetically. These specimens were chosen from all three hospitals, and the numbers of these specimens were determined generally in proportion to the total number of specimens in each month to reduce the possibility of the occurrence of selection bias. Thus, the selected rotavirus specimens were considered to generally represent the rotaviruses prevalent in the whole study period. Among the selected 113 specimens from children, 81 specimens (71.7%) were derived from male patients, and 61 specimens (54.0%) were from infants 7–12 months of age.

Table 1 shows the frequencies of G and P types identified for the rotaviruses detected in this study. In both children and adults, G2 was the most predominant (54.0 and 47.6%, respectively), followed by G1 (21.2 and 26.2%, respectively) and G9 (15.9 and 9.5%, respectively). G4 was detected in three specimens from a child and adults, while G3 was not detected. G12 was identified in four specimens from children and one specimen from an adult by sequence analysis of the VP7 gene. Among the G2 rotaviruses, the G2P[4] genotype was the most frequent in both children and adults, while a few G2 viruses were associated with P[6] or P[8]. G1 rotaviruses were mostly combined with P[8] or P[6]. Although only the G9P[8] genotype was detected among G9 rotaviruses from adults, genotypes combined with P[4], P[6], and P[8] were found in G9 viruses from children. Four G12 rotaviruses, including one strain from an adult, had P[6], whereas P[4] was associated with one virus from a child. The detection rate of P[6] alone was significantly higher in children (19.5%) than in adults (7.7%) ($P < 0.05$), although there was no significant difference in incidence of other P-types between children and adults. Among all the specimens examined, mixed G-types were detected in 7 specimens (4.5%), and mixed P-types were found in 26 specimens (16.8%).

RNA profiles of isolated strains

Figure 1 shows representative RNA patterns of G1, G2, G9, and G12 rotaviruses isolated from adults and children. RNA patterns from children and adults were identical in individual G1 and G12 types. Although G9 or G2 rotaviruses from adults and children showed similar RNA patterns, minor differences in migration were found in RNA segments 2, 3, 7, and 8. All of the G2 rotaviruses exhibited short RNA patterns, while G12 viruses showed long patterns.

Phylogenetic analysis of VP7 genes

A total of 39 rotavirus strains, listed in Table 2, were analyzed phylogenetically for their genes encoding VP7,

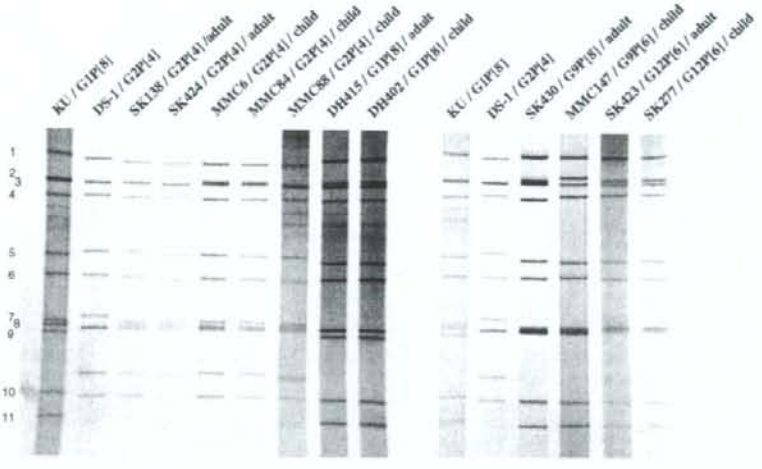


Fig. 1 RNA patterns of representative rotavirus strains isolated in this study. The strain name, G and P type, and derivation (adult or child) are indicated above lanes. Strains KU and DS-1 are references for long and short RNA patterns. RNA segment numbers for the strain KU are indicated on the *left*. RNA segments 1–11 encode VP1, VP2, VP3, VP4, VP6, NSP1, NSP3, NSP2, VP7, NSP4, and NSP5, respectively [10]

Table 2 Profiles of rotaviruses analyzed genetically and their gene sequence information

Rotavirus (specimens)[a]	Genotype				Specimen/patient			GenBank accession number	
	G-type	P-type	NSP4	VP6	Date of collection	Age	Sex	VP7 gene	VP4 gene
DH415	G1	P[8]	B	II	Dec. 2005	48 years	M	EU839906	EU839955
SK417	G1	P[8]	B	I + II	Apr. 2005	25 years	M	EU839907	
SK438	G1	P[8]	B	II	Apr. 2005	22 years	F	EU839908	EU839956
SK420	G1	P[8]	B	II	Dec. 2005	10 years	M	EU839909	
SK469	G1	P[8]	B	I + II	Jan. 2006	34 years	F	EU839910	
MMC56	G1	P[8]	B	I + II	Mar. 2005	8 months	M	EU839911	
MMC71	G1	ND[e]	B	II	Nov. 2005	10 months	M	EU839912	
MMC82	G1	P[8]	B	I + II	Jan. 2006	9 months	M	EU839913	
SK322	G1	P[8]	B	II	Jan. 2006	1 year	M	EU839914	EU839957
DH378	G1	P[8]	B	II	Nov. 2004	1 year	M	EU839915	
DH402	G1	P[8]	B	II	Jan. 2005	9 months	M	EU839916	EU839958
SK138	G2	P[4]	B	I	Aug. 2004	40 years	F	EU839917	EU839945
DH392	G2	P[4]	B	I	Dec. 2004	37 years	F	EU839918	EU839949
DH397	G2	P[4]	B	I	Nov. 2004	42 years	F	EU839919	
SK424[b]	G2	P[8]	B	I	Nov. 2005	35 years	F	EU839920	EU839954
SK464	G2	P[4]	A	I	Jun. 2006	20 years	M	EU839921	
SK468	G2	P[4]	A	I	Jan. 2006	28 years	M	EU839922	
MMC6	G2	P[4]	B	I	Sep. 2005	10 months	M	EU839923	EU839950
MMC84	G2	P[4]	B	I	Jan. 2005	5 months	F	EU839924	EU839951
MMC88	G2	P[4]	A	I	Jan. 2005	11 months	F	EU839925	
SK299	G2	P[4]	A	I	Apr. 2005	9 months	F	EU839926	
DH404	G2	P[4]	A	I	Feb. 2005	3 years	F	EU839927	
DH408	G2	P[4]	A	I	Dec. 2005	3 years	F	EU839928	
DH396	G9	P[8]	B	II	Nov. 2004	34 years	F	EU839929	EU839959
SK427	G9	P[8]	B	I + II	Nov. 2005	35 years	M	EU839930	
SK430	G9	P[8]	B	II	Nov. 2005	23 years	M	EU839931	EU839960
SK432	G9	P[8]	B	II	Dec. 2005	12 years	M	EU839932	
SK441	G9	P[8]	B	I + II	Jun. 2005	26 years	F	EU839933	
MMC24	G9	P[6]	B	I + II	Mar. 2005	4 months	F	EU839936	EU839952
MMC38	G9	ND[e]	B	II	Apr. 2005	1 year	F	EU839937	
MMC147	G9	P[6]	B	II	Mar. 2005	8 months	M	EU839938	EU839953
MMC172	G9	P[6]	B	II	Apr. 2005	4 months	M	EU839939	
DH373	G9	P[8]	B	II	Oct. 2004	3 years	M	EU839940	EU839961
DH375	G9	P[8]	B	II	Oct. 2004	7 months	F	EU839941	EU839962
SK423[c]	G12	P[6]	B	II	Dec. 2005	59 years	F	EU839934	EU839946
MMC29	G12	P[6]	B	I	May 2005	13 months	F	EU839935	EU839947
MMC206	G12	P[4]	B	I + II	Jun. 2005	8 months	M	EU839942	
SK277[d]	G12	P[6]	B	II	Dec. 2005	18 months	M	EU839943	EU839948
SK327	G12	P[6]	B	II	Jan. 2006	5 years	F	EU839944	

[a] "MMC", "SK", "DH" in the specimen name indicate hospitals of the sample collection, Mymensingh Medical College Hospital, SK hospital, and Dharmapasha Thana Health Complex, respectively

[b] GenBank accession numbers of VP6 and NSP4 genes are EU839963 and EU839964, respectively

[c] GenBank accession numbers of genes encoding VP6 and NSP1-NSP5 are EU839965-EU839970, respectively

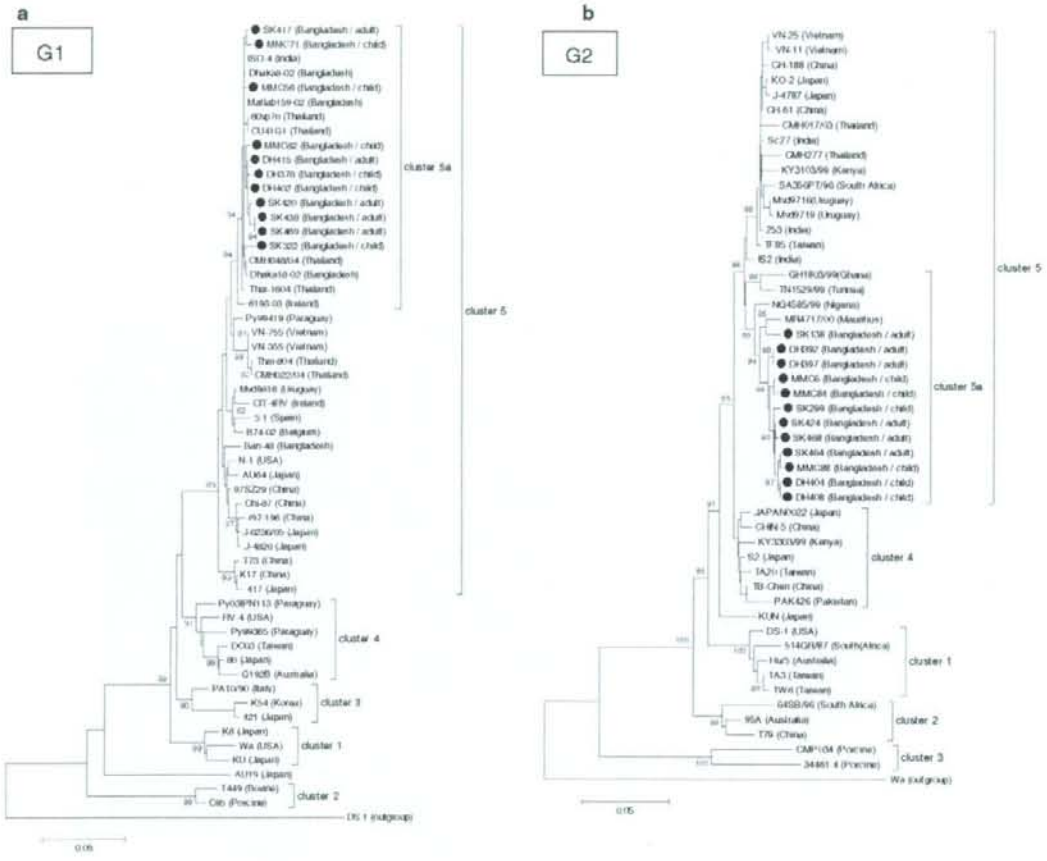
[d] GenBank accession numbers of genes encoding VP6 and NSP1-NSP5 are EU839971-EU839976, respectively

[e] Not determined

Fig. 2 Phylogenetic dendrograms based on the complete VP7 genes from G1 rotaviruses (**a**), G2 rotaviruses (**b**), G9 rotaviruses (**c**), and G12 rotaviruses (**d**), constructed by the neighbor-joining method using the MEGA program. Dendrograms are rooted with human rotavirus strain KU or DS-1. The variation scale (substitutions per site) is indicated at the *bottom*. Percent bootstrap support is indicated by the values at each node, and the values <80 are omitted. Rotaviruses analyzed in the present study are indicated by *closed circles* with their derivation (adult or child). The name of each rotavirus strain is shown with its country of origin. The clusters are indicated at the *right* side. Reference sequences used in the analysis were obtained from the GenBank database. Accession numbers of the sequences are listed as follows: **a** AU19 (AB018697), T449 (M92651), C95(L24165), K8 (D16344), Wa (K02033), KU (AB222788), PA10/90 (DQ377587), K54 (U26377), 421 (D16326), Py03IPN113 (EF179194), RV-4 (M64666), Py99365 (DQ15682), DC03 (AF183859), 80 (D16325), G192B (AF043678), T73 (AF450291), K17 (D16320), 417 (D16328), 97'SZ29 (AF260952), Chi-87 (DQ512998), J-6236/05 (EF088838), J-4826 (DQ512990), r97-196 (AF453426), N-1 (AB081792), AU64 (AB081801), Ban-48 (U26364), CIT-4RV (AF254138), Mvd9816 (AF480293), 5.1 (DQ672628), B74-02 (AY635048), Py99419 (DQ15683), VN-755 (DQ512974),VN-355 (DQ512968), Thai-804 (DQ512979), CMH022/04 (EF199717), 6193-03 (DQ207389), Thai-1604 (DQ512981), CMH048/04 (EF199711), Dhaka18-02 (AY631051), 60vp7n (DQ674881), CU41G1 (DQ236055), Matlab159-02 (AY631055), ISO-4 (AY098670), Dhaka8-02 (AY631049). **b** CMP034 (DQ534015), 34461-4 (AY766085), 64SB/96 (AY261341), 95A (U73955), T79 (AF450292), DS-1 (AB118023), 514GR/87 (AY261338), Hu/5 (A01028), TA3 (AF106280), TW6 (AF044338), KUN (D50124), JAPAN0022 (D50117), CHIN-5 (D50114), KY3303/99 (AY261350), S2 (M11164), TA20 (AF106281), TB-Chen (AY787646), PAK426 (D50125), GH1803/99 (AY261353), TN1529/99 (AY261357), NG4585/99 (AY261351), MR4717/00 (AY261358), IS2 (X95273), TF85 (AF106299), 253 (X95370), Mvd9716 (AF480275), Mvd9719 (AF480276), CMH277 (AY707784), SA356PT/96 (AY261342), KY3103/99 (AY261349), Sc27 (AJ293722), CMH017/03 (EF199724), CH-61 (DQ904516), CH-188 (DQ904519), KO-2 (AF401754), J-4787 (DQ904511), VN-25 (DQ904515), VN-11(DQ904512). **c** 97'SZ37 (AF260959), WI61 (AB180969), AU-32 (AB045372), F45 (AB180970), 116E (L14072), 97'SZ37 (AF260959), Hokkaido-14 (AB176677), t203 (AY003871), JP3-6 (AB176678), JP32-4 (AB176682), K-1 (AB045374), 99-SP1904VP7 (AB091754), Mc345 (D38055), Perth-G9.1 (AY307094), Syd.G9.1 (AY307093), Bulumkutu (AF359358), R160 (AF274971), R136 (AF438228), MR4730/00 (AY262749), 95H115 (AB045373), 84/02-1 (AY184813), CMH319 (AY699301), 97CM108 (AY866504), N23 (AJ491177), MW69 (AJ250545), 6222LP/99 (AF529871), INL1 (AJ250277), BD524 (AJ250543), BD431 (AJ250542), PH301 (AJ491184), US321 (AJ250275), RMC321 (AF501578), 684VN (AB091778), 608VN (AB091777), DV38 (AJ491168), US1205 (AF060487), 3710CM (AY816184), 3298CM (DQ647423), MD28 (AB297791), KY6923/02 (DQ822604), ISO-3 (AF501580), 00-SP2737VP7 (AB091752), GH3574 (AY211068), BS1414/02 (DQ822599), L169 (DQ873674), 17025-03 (DQ207390), SE121 (AJ491192), CAU202 (EF059922), GUH13 (AB364384), 05SLC057 (AB306267), BA201 (AY695811), SI1791 (AY630924), R77 (AY196129), Melb-G9.10 (AY307087), 6.9 (DQ672630), AHP66 (AB364375), 14-9 (EF159957), 01TW591 (DQ490173), CMH028/04 (EF199735), R2 (AY196111), KNIH-13 (DQ990319), 02TW498 (DQ096292), KUMS04-102 (DQ056300). **d** L26 (M58290), RU172 (DQ204743), T152 (AB071404), CP727 (AB125852), K12 (AB186120), CP1030 (V), GJ0612314 (EU259895), DQ111868 (DQ111868), ISO5 (AF508734), 36B2 (DQ099749), Matlab13-03 (DQ146676), Dhaka12-03 (DQ146665), 05K066 (AB275301), ISO29 (DQ062129), 04N338 (AB264006), ISO16 (DQ099751), Se585 (AJ311741), ISO-1 (AY206861), 05N065 (AB275297), ISO25 (DQ062126), Dhaka 25-02 (DQ146654), N26-02 (DQ146687), RV176-00 (DQ490556), RV161-00 (DQ490550), B4633-03 (DQ146643), 13B2 (DQ099746), CAU214 (EF059917), 05N054 (AB275291), MD844 (AB269689)

VP4, and other viral proteins. Among these rotaviruses, NSP4 genotype B was assigned to all of the G1, G9 and G12 viruses, while both genotypes A and B were detected in G2 rotaviruses. While VP6 genotype I was assigned to all of the G2 rotaviruses and one G12 virus, VP6 genotype II was observed in G1, G9 and G12 rotaviruses, with mixed type I + II in some viruses.

Figure 2 shows phylogenetic dendrograms of VP7 genes for G1, G2, G9, and G12 rotaviruses analyzed in this study and representative strains belonging to individual G-types previously reported in the world. G1 rotaviruses from six children and five adults (Table 2) exhibited extremely high sequence identities in the VP7 gene (98.9–99.9%) and were found in cluster 5 (cluster 5a), which includes many strains from Asian countries (Fig. 2a). The eleven Bangladeshi G1 strains were the closest to those from India and Thailand within the cluster 5a, while they showed 92.1–92.7% identities to the prototype G1 strain Wa in cluster 1. Twelve G2 rotaviruses from six children and six adults (Table 2) showed more than 97% identities of VP7 gene sequence to each other. In the phylogenetic tree, these G2 strains were clustered with some strains from African countries (cluster 5a) (Fig. 2b). The G2 strains showed highest VP7 sequence identity to the strain MR4717/00 from Mauritius (97.5–98.3%) and 93.1–93.6% identity to the prototype strain DS-1 in the cluster 1. G9 rotaviruses from six children and five adults showed more than 98% VP7 gene sequence identity to each other and were clustered in a single branch within cluster 3, which contains globally spreading G9 strains in the phylogenetic tree (Fig. 2c). Among the G9 viruses of cluster 3, the 11 strains in Mymensingh in the present study were more closely related to some strains isolated outside Bangladesh, e.g., CMH319 (Thailand) and Perth-G9.1 (Australia) (sequence identities of 98.4–99.2% and 98.2–98.8%, respectively) than Bangladeshi strains BD431 and BD524 reported previously (96.7–97.6% identity). G12 rotaviruses from four children and one adult had 97.8–100% identities to each other in the VP7 gene sequence. In the phylogenetic tree, they were located in cluster 3, which contained G12 rotaviruses isolated from many countries in the world after 2000 (Fig. 2d). The five G12 rotaviruses showed extremely high VP7 sequence identity (97.5–98.2%) to the G12 rotavirus strains recently detected in Bangladesh (e.g., Dhaka25-02), India (e.g., ISO25), and Nepal (e.g., 05N065) and lower identities (90.0–90.5%) to the G12 prototype strain L26 in the cluster 1.



Phylogenetic analysis of VP4 genes

Complete VP4 gene sequences of P[4], P[6], and P[8] strains were phylogenetically analyzed with those of representative strains from the world deposited in GenBank database (Fig. 3). Four G2P[4] Bangladeshi strains (two children and two adults) exhibited 98.1–98.6% VP4 gene sequence identity to each other and were located in a single branch in cluster 3 (Fig. 3a). These P[4] viruses showed higher VP4 sequence identities (95.8–98.1%) to strains IS-2 (India) and KO-2 (Japan) in cluster 3 than strains DS-1 (cluster 1; 93.6–94.1%) and L26 (cluster 2; 93.1–93.7%). Five P[6] strains, one from an adult (SK423, G12) and four from children (G9 strains MMC24 and MMC147, G12 strains MMC29 and SK277), showed more than 98% VP4 sequence identity to each other. Particularly, strains SK423 and SK277 had identical VP4 and VP7 gene sequences. In the phylogenetic tree, the P[6] strains analyzed were located in cluster 2, which is distinct from cluster 1 containing P[6] prototype strain M37 (Fig. 3b). Nine P[8] rotaviruses with G1, G2, or G9 from four children and five adults exhibited more than 98% VP4 gene sequence identity to each other, and all of them were found in cluster 3 (Fig. 3c). VP4 genes of these P[8] viruses showed lower identity to those of strains Wa (cluster 1; 90.2–90.5%) and KU (cluster 2; 93.0–93.6%).

Phylogenetic analysis of a G2P[8] strain and G12P[6] strains

A rotavirus strain, SK424, with the unusual genotype combination G2P[8], and two G12P[6] strains from a child (SK277) and an adult (SK423) were further analyzed genetically to find out the derivation of the other viral gene

Fig. 2 continued

segments. VP6 and NSP4 sequences of strain SK424 were classified into clusters of DS-1-like rotaviruses (Fig. 4a, e). In contrast, VP6 and NSP4 of the two G12 strains were clustered in the Wa-like group, i.e., subgroup II and NSP4 genotype B, respectively. It was remarkable that the nucleotide sequences of the VP6 and NSP1-NSP5 genes of the G12 strains SK277 and SK423 were identical. These two G12 strains were genetically classified into the clusters of Wa-like rotaviruses in the phylogenetic analysis of NSP1-NSP3 and NSP5 (Fig. 4b–f). In any phylogenetic trees of VP6, NSP1, NSP2, NSP4 and NSP5, G12 rotaviruses SK277 and SK423 were located close to G12 rotavirus strains isolated recently in Bangladesh (Dhaka25-02, Dhaka12-03, Matlab13-03, RV161-00, and RV176-00).

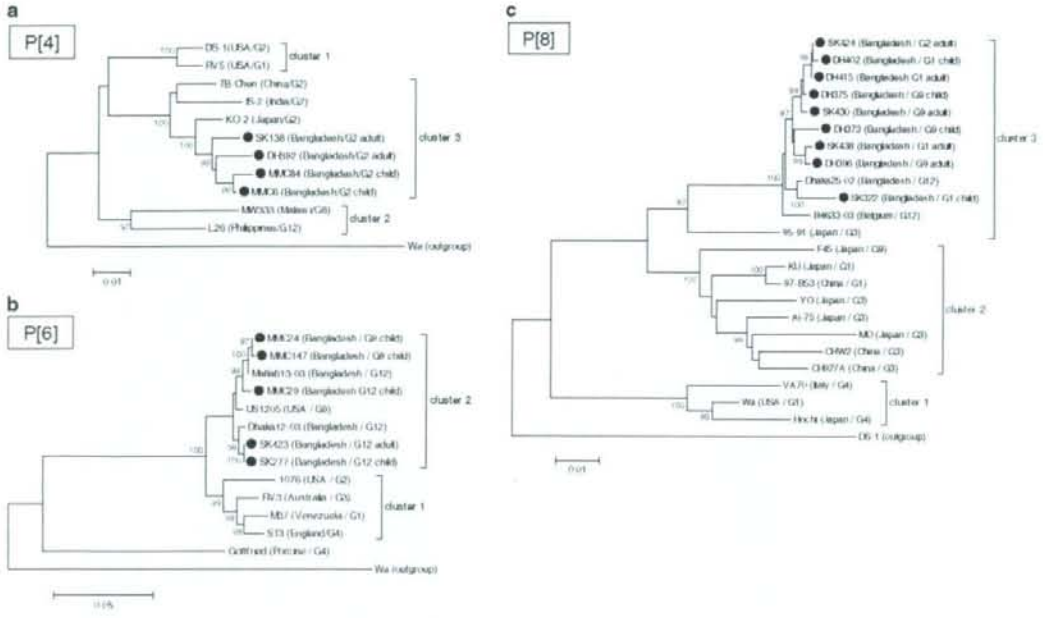


Fig. 3 Phylogenetic dendrograms based on the complete VP4 genes from P[4] rotaviruses (**a**), P[6] rotaviruses (**b**), and P[8] rotaviruses (**c**) constructed by the neighbor-joining method using the MEGA program. Dendrograms are rooted with human rotavirus strain Wa or DS-1. The variation scale (substitutions per site) is indicated at the *bottom*. Percent bootstrap support is indicated by the values at each node, and the values <80 are omitted. Rotaviruses analyzed in the present study are indicated by *closed circles* with their G-types and derivation (adult or child). The name of each rotavirus strain is shown with its country of origin. The clusters are indicated at the *right* side. Reference sequences used in the analysis were obtained from the GenBank database. Accession numbers of the sequences are listed as follows: **a** DS-1 (AB118025), RV5 (M32559), MW333 (AJ278256), L26 (EF672591), IS-2 (X82323), TB-Chen (AY787644), KO-2 (AF401755). **b** Gotfried (M33516), 1076 (M88480), RV3 (U16299), M37(L20877), ST3 (EF672612), Dhaka12-03 (DQ146663), US1205 (AF079356), Matlab13-03 (DQ146674). (c) VA70 (AJ540229), Wa (L34161), Hochi (AB039943), F45 (U30716), 97-B53 (AF260932), KU (AB222784), YO (AB008279), Ai-75 (AB008285), MO (AB008278), CHW2 (AB008277), CH927A (AB008272), 95-91 (AB008291), B4633-03 (DQ146641)

Particularly, all of the viral proteins of SK277 and SK423 analyzed in this study were closely related to those of the strain Dhaka12-03, while some viral proteins of SK277 and SK423 were genetically distinct from those of other Bangladeshi G12 strains.

Discussion

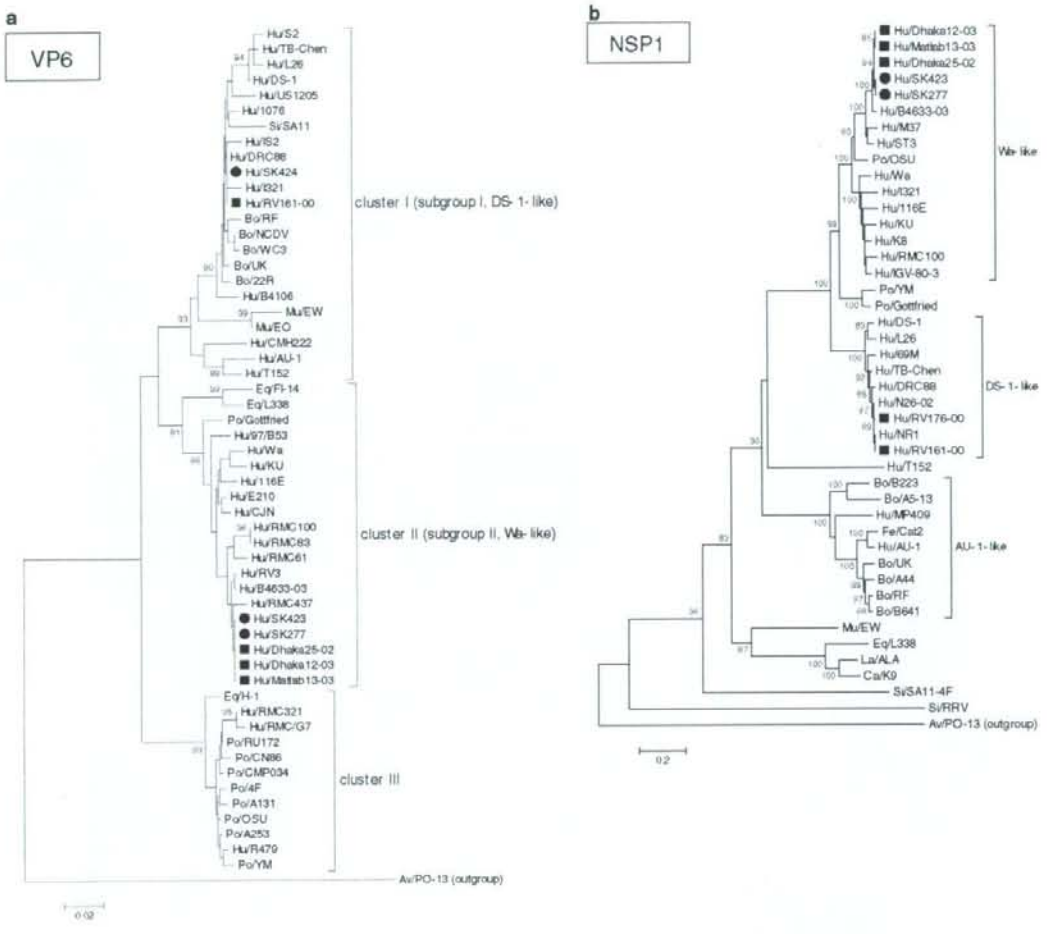
Rotavirus diarrhea in adult populations has been noted mostly as sporadic cases and occasionally as outbreaks. The detection rate of rotavirus in sporadic cases of diarrhea in adults has been described as 2–15% in most reports from many countries in the world [4, 20, 33, 36]. Although incidence of rotavirus diarrhea is highest in the age group less than 5 years of age, detection rates ranging from 7 to 17% were reported throughout age groups of adults in recent studies in China and Brazil [7, 38]. In the present study, the rotavirus-positive rate in adult specimens was 10.1% in total, which is comparable to that in the epidemiologic study of rotavirus in Wuhan, China, describing a detection rate of 9.0% in the adult population [38]. Therefore, it is suggested that rotavirus is prevalent as a minor pathogen of diarrheal diseases in adults of mostly all age groups at similar rates in countries of both temperate and tropical regions.

To date, few studies have examined the phylogenetic relatedness of rotaviruses distributed to children and adults. Only the hospital-based epidemiologic study in China reported predominance of G3 rotaviruses in both children and adults (15 years or older), and revealed a close phylogenetic relatedness of the G3 viruses from these two groups [38]. In the present study in Mymensingh, Bangladesh, the same G/P type (G2P[4]) was predominant

Fig. 4 Phylogenetic dendrograms based on the complete amino acid sequences of VP6 (**a**), NSP1 (**b**), NSP2 (**c**), NSP3 (**d**), NSP4 (**e**), and NSP5 (**f**) constructed by the neighbor-joining method using the MEGA program. Dendrograms are rooted with the avian rotavirus strain PO-13 or the murine rotavirus strain MW. The variation scale (substitutions per site) is indicated at the *bottom*. Percent bootstrap support is indicated by the values at each node, and the values <80 are omitted. *Closed circles* indicate rotaviruses analyzed in the present study, and closed squares represent recently characterized G12 rotavirus strains in Bangladesh [27]. The host species of reference rotavirus strains are indicated with individual strain names : *Hu* human, *Po* porcine, *Eq* equine, *Bo* bovine, *Si* simian, *Ca* canine, *Mu* murine, *La* lapine, *Fe* feline, *Ov* ovine, *Av* avian. The clusters of rotaviruses represented as Wa-like, DS-1-like, and AU-1-like viruses are indicated at the *right* side. Reference sequences used in the analysis were obtained from the GenBank database. Accession numbers of the sequences are listed as follows: **a** PO-13 (D16329), CMP034 (accession no. DQ534018), CN86 (U10031), 4F (L29184), RU172 (DQ204741), RMC/G7 (AY601551), RMC321 (AF531913), YM (X69487), A253 (AF317122), A131 (AF317124), H-1 (AF242394), OSU (AF317123), Gottfried (D00326), Wa (K02086), 116E (U85998), KU (AB022768), CJN (AF461757), 97/B53 (AF260931), E210 (U36240), RV3 (U04741), RMC61 (AY601549), RMC437 (AY601554), RMC100 (AF531912), RMC83 (AY601550), FI-14 (D00323), L338 (D82974), EW (U36474), EO (AY947543), CMH222 (DQ288659), R479 (DQ873675), S2 (Y00437), TB-Chen (AY787645), 1076 (D00325), SA11 (L33365), B4106 (AY740737), UK (X53667), RF (K02254), NCDV (AF317127), WC3 (AF411322), US1205 (AF079357), DRC88 (DQ005110), IS2 (X94617), 22R (AB040055), I321 (X94618), Dhaka12-03 (DQ146664), Dhaka25-02 (DQ146653), AU-1 (DQ490538), T152 (DQ146702), DS-1 (DQ870507), L26 (DQ146695), B4633-03 (DQ146642), Matlab13-03 (DQ146675), RV161-00 (DQ490549). **b** PO-13 (AB009633), T152 (AB097459), DS-1 (L18945), L26 (D38150), 69M (D38151), TB-Chen (AY787647), DRC88 (DQ005108), RV-176-00 (DQ490557), N26-02 (DQ146688), NR1 (AF506017), RV161-00 (DQ490540), EW (U08428), SA11-4F (AF290884), L338 (D38158), K9 (AF111946), RRV (Z32535), ALA (AF084549), B223 (Z12105), A5-13 (D38148), MP409 (AF141916), Cat2 (U23727), AU-1 (D45244), UK (L11575), A44 (U23726), RF (M22308), B641 (AF458087), YM (D38154), Gotfried (U08431), M37 (U11491), ST3 (U11492), B4633-03 (DQ146644), Dhaka25-02 (DQ146655), Dhaka12-03 (DQ146666), Matlab13-03 (DQ146677), OSU (U08432), Wa (L18943), I321 (U08418), 116E (U85999), KU (AB022769), K8 (D38152), RMC100 (AY601546), IGV-80-3 (X59297). **c** PO-13 (AB009625), AU-1 (DQ490534), T152 (DQ146703), KU (AB022770), L26 (DQ146696), KJ44 (DQ494401), K291 (EU542713), RMC100 (AF506014), Wa (L04534), RMC321 (AF506293), Dhaka12-03 (DQ146667), Dhaka25-02 (DQ146656), B4633-03 (DQ146645), MG6 (EF554100), SA11 (J02353), WC3 (EF990700), NCDV (L04530), RF (Z21640), BRV033 (EF990704), 30/96 (DQ205227), B4106 (AY740734), UK (J02420), DS-1 (L04529), TB-Chen (AY787648), PA169 (EF554133), Hun5 (EF554111), RV161-00 (DQ490541), I321 (Z47975), IS2 (X94562), NR1 (AF506018), DRC88 (DQ005107), DRC86 (DQ005118), Matlab13-03 (DQ146678). **d** PO-13 (AB009626), SA11 (EF460843), 69M (X81425), RV161-00 (DQ490542), NR1 (AF506019), DRC86 (DQ005117), DRC88 (DQ005106), IS2 (X76645), L26 (DQ146697), DS-1 (EF136660), TB-Chen (AY787649), S2 (X81428), AU-1 (DQ490535), T152 (DQ146704), RRV (X81426), UK (K02170), Hun5 (EF554112), MG6 (EF554101), PA169 (EF554134), WC3 (EF990701), RF (Z21639), NCDV (X81429), BRV033 (EF990705), 30/96 (DQ205228), B4106 (AY740733), RMC321 (AF541920), KJ44 (DQ494403), OSU (X81431), K291 (EU542719), GO (X81430), MA/68 (EU169870), WI61 (X81437), I321 (X81433), KU (AB022771), ST3 (X81436), Wa (X81434), IGV-P (AF190170), RMC100 (AF506015), Dhaka25-02 (DQ146657), Dhaka12-03 (DQ146668), B4633-03 (DQ146646), Matlab13-03 (DQ146679). **e** EW (U96335), FRV303 (AB048199), RRV (L41247), AU-1 (D89873), FRV-1 (D89874), T152 (DQ146705), FRV348 (AB048201), RV176-00 (DQ490560), N26-02 (DQ146691), RMC439 (AB196491), Dhaka12-03 (DQ146669), RV161-00 (DQ490543), B4633-03 (DQ146647), RMC132 (AB201459), R1 (DQ339147), Dhaka25-02 (DQ146658), R26 (DQ339150), M37 (U59109), Wa (AF200224), YO (AB008236), KU (AB022772), RV-3 (U42628), 116E (U78558), BD524 (AJ400634), RU172 (DQ204740), YM (X69485), OSU (D88831), RMC321 (AF541921), H-1 (AF144800), FI-14 (AF144803), R-2 (AF144794), FI-23 (AF144802), WC3 (AY050273), RF (AY116593), MF66 (AY527227), 1076 (U59105), Hg18 (AY527226), RUBV3 (EF200572), L26 (AJ311732), SA11 (AF087678), RV5 (U59103), DS-1 (AAG09190), DRC88 (DQ005105), DRC86 (DQ005116), TB-Chen (AY787650), S2 (U59104), BD426 (AJ400636), NR1 (AF506291), Se585 (AJ311731), RMC/G66 (AY601545), US1111 (AJ400637), US1205 (AF079358), MW69 (AJ400643), E210 (U59107), Matlab13-03 (DQ146680). **f** PO-13 (AB009628), UK (K03385), VMRI (M33606), 69 M (M33607), NR1 (AF508732), RMC/G66 (AY769694), RV176-00 (DQ490561), RV161-00 (DQ490544), N26-02 (DQ146692), DRC88 (DQ005104), DRC86 (DQ005115), KUN (AB091727), TB-Chen (AY787651), DS-1 (EF672583), B37 (M28378), RV-5 (P18037), Hun5 (EF554114), LLR (AY622998), K8 (AB008655), AU-1 (AB008656), 30/96 (DQ205231), B4106 (AY740731), ALABAMA (P17467), RRV (AAK15265), SA11 (AF306493), Mc323 (U54772), CC86 (X80537), C60 (D00474), OSU (X15519), CN86 (X80538), Mc345 (U54773), Wa (AF306494), KU (AB008661), YM (X69486), RU172 (DQ204739), L26 (DQ146698), B4633-03 (DQ146648), Dhaka25-02 (DQ146659), RMC100 (AF373605), Matlab13-03 (DQ146681), Dhaka12-03 (DQ146670)

in both children and adults. Moreover, the predominant G2 viruses from children and adults had, genetically, almost identical VP7 genes and were included in a single subcluster by phylogenetic analysis with many rotavirus strains from the world. Similarly, VP7 genes of less prevalent G1, G9, and G12 rotaviruses were phylogenetically closely related between children and adults. In the present study, VP4 sequences of P[4], P[6], and P[8] were also analyzed, and their close relatedness between children and adults was demonstrated. These findings imply that genetically identical or similar rotaviruses, irrespective of the predominant type or less prevalent types, might have been distributed to both child and adult populations endemically.

Numerous studies of epidemiologic trends of human rotavirus genotypes have reported the predominance of G1P[8] worldwide, among the common types G1-G4, and G9, and P[4], P[6], and P[8] [30]. While G2P[4] tends to be less prevalent than other genotypes, occasional predominance have been observed in various countries, including England, China, Taiwan, South Africa, and Brazil [13, 21, 23, 32, 40, 41]. In Bangladesh, G1 and G2 were most frequently detected in Mymensingh in 1988–1989 [1, 2], and a transient peak of G2 was reported in the same period in Matlab [6]. However, G2P[4] became a predominant type in Mymensingh, as revealed in the present study (2004–2006), and also in Dhaka and Matlab (2005–2006) after predominance of G1 from 2002–2004 [26],



suggesting that G2 rotaviruses have been prevailing in a wide area of Bangladesh since 2004 or 2005.

It was notable that G2 rotaviruses detected in Mymensingh were phylogenetically closely related to those isolated in African countries (Ghana, Tunisia, Mauritius, and Nigeria) [22] from 1997 to 2000 in the same cluster (5a), while different from those of G2 viruses from Taiwan, Thailand, and Japan [18]. Accordingly, it is suggested that the predominant G2 rotaviruses in Bangladesh might not have originated from the eastern Asian countries but have a relationship to strains from African countries. Because the genotype G2 has been most frequently reported for rotaviruses from adult diarrheal cases [12, 16], it may be suggested that G2 rotaviruses might have been transmitted by adults during travel and introduced to less prevalent regions. Although transmission of rotaviruses with common G-types over the continent has not yet been well known, more phylogenetic analysis of rotaviruses correlated with the changing pattern of genotypes will disclose global migration of rotaviruses.

Global spread and increased detection rates of G9 and G12 have been noted since 1995 and 2000, respectively [27, 30]. In Bangladesh, G9 emerged in 1995 and became predominant in 2000 and 2001 [37], while G12 was first detected in 2000–2001 and increased to 13.6% in the 2005–2006 season in studies in Dhaka and Matlab [26]. In Kolkata, in eastern India, located near Bangladesh, G12 has become one of the dominant types of rotaviruses [29]. In

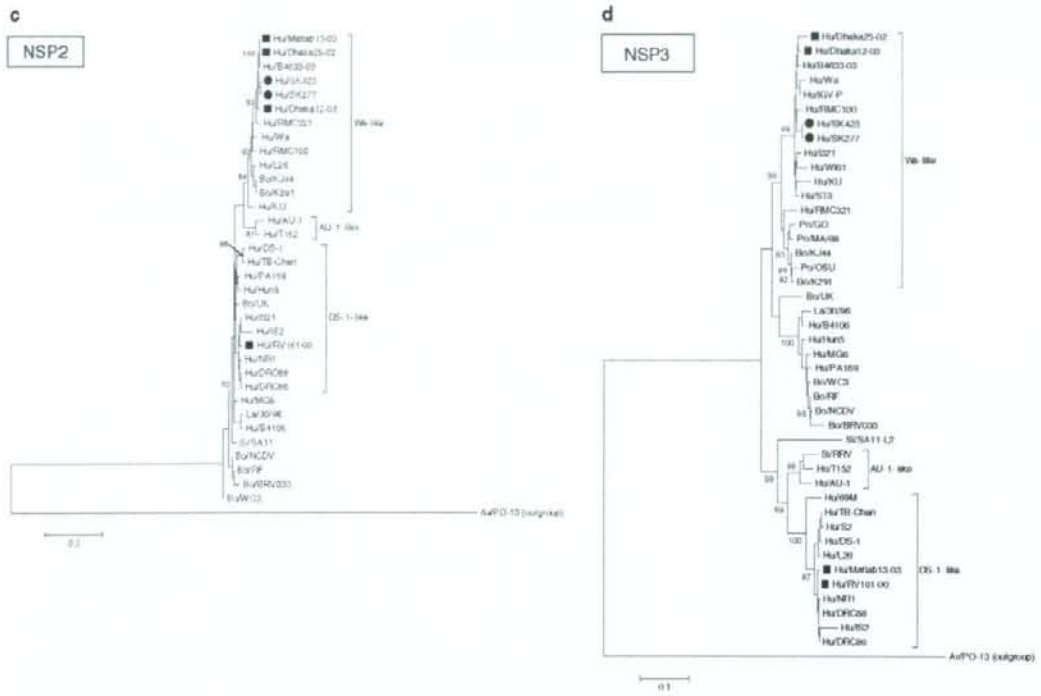


Fig. 4 continued

the present study, G9 was detected as the third predominant genotype, and G12 was first identified in Mymensingh, suggesting that these types have been widespread in Bangladesh. G12 rotaviruses, as well as G9 rotaviruses, have been reported to carry RNA segments with considerable genetic diversity, suggesting the frequent occurrence of reassortment events [27–31]. In the present study, G12P[6] strains SK277 and SK432 in Mymensingh were found to be genetically closely related to those isolated in Dhaka and Matlab in 2000–2003 [27] and had a genetic constellation identical to that of the strain Dhaka12–03, among the six reassortant strains representing different constellations of RNA segments reported previously in Bangladesh [27]. Furthermore, VP4 gene sequences of G2P[8] and G9P[8] viruses showed extremely high identities to those of G1P[8] viruses, and some G12P[6] viruses had VP4 gene sequences almost identical to those of G9P[6] viruses. These findings suggest that reassortment of the VP4 gene segment might have occurred among G1, G2, and G9 rotaviruses, and also between G9 and G12 rotaviruses.

The present study in Bangladesh, together with the recent study in China [38], suggest that rotaviruses might have been circulating among children and adults in a locality. In a recent study in Equador, asymptomatic carriage of rotavirus in adults has been also documented despite its low frequency [9]. Moreover, it has been hypothesized that rotaviruses excreted from asymptomatic adults may be a source of infection for susceptible children in a hospital environment [5]. Therefore, for prevention of rotavirus diarrhea in children, control of rotavirus transmission from adults should also be considered.

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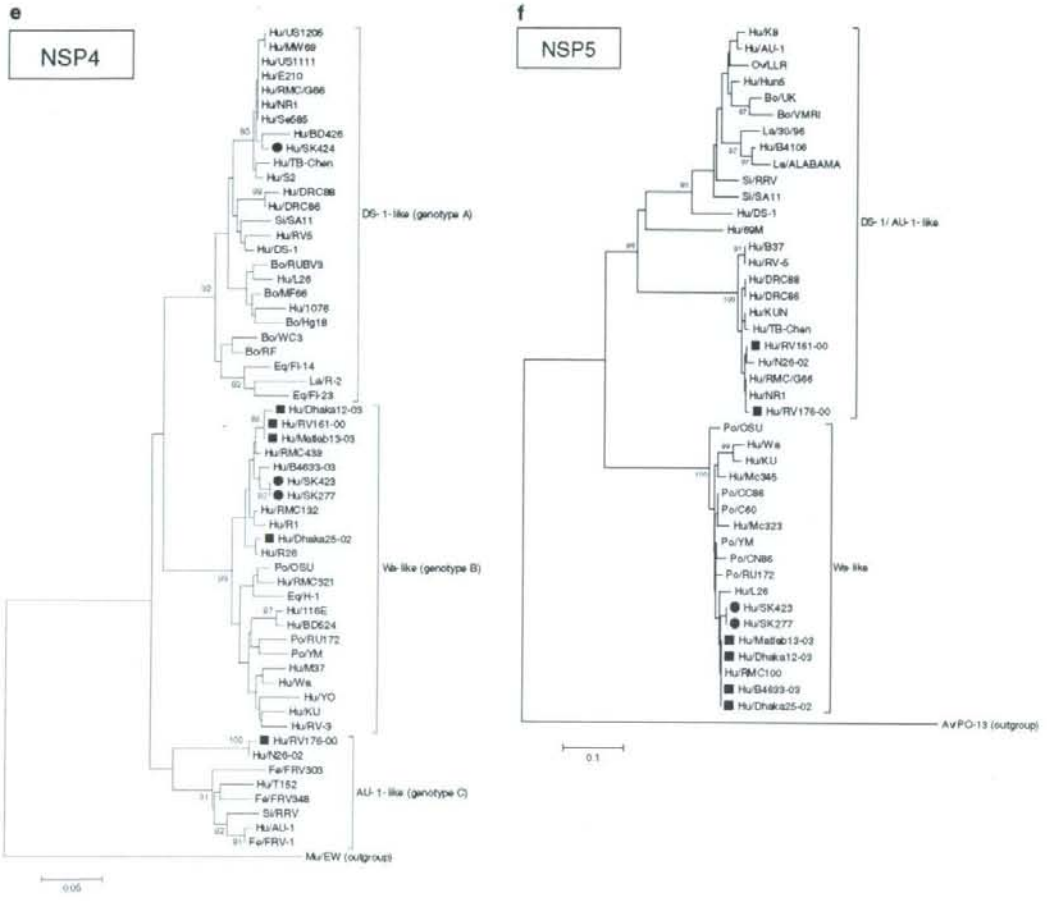

Fig. 4 continued

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Phylogenetic Analysis of Rotaviruses With Predominant G3 and Emerging G9 Genotypes From Adults and Children in Wuhan, China

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Prevalence and phylogenetic relatedness of rotaviruses causing diarrheal diseases in children and adults were analyzed in Wuhan, China. During a period between June 2006 and February 2008, group A rotavirus was identified in 24.9% (280/1126) and 7.6% (83/1088) of specimens taken from children and adults, respectively. G3P[8] was the most frequent genotype in both children (66.3%) and adults (62.7%), followed by G1P[8] (20.3% and 26.2%, respectively). G9 was detected in specimens from six children (2.0%) and seven adults (5.6%). The VP7 genes of G3P[8] rotaviruses from children and adults showed extremely high sequence identities to each other (98.9–100%) and also to those of G3 viruses isolated in Wuhan in 2003–2004. In the phylogenetic analysis of the VP7 gene, the G3P[8] rotaviruses in Wuhan were clustered into a single lineage with some G3 viruses, which had been referred to as "the new variant G3" rotaviruses, reported recently in East Asia and Southeast Asia. Similar to G3P[8] rotaviruses, extremely high sequence identities between children and adults were observed for VP7 genes of G1 and G9 rotaviruses. The G9 viruses were clustered in the lineage of globally spreading strains, while G1 viruses were genetically close to those reported previously in China and Japan. These findings indicated the persistence of the variant G3 rotaviruses and spread of G9 rotaviruses derived from the global G9 lineage in Wuhan, and suggested that the rotaviruses were circulating among children and adults, irrelevant to the G types. ***J. Med. Virol. 81:382–389, 2009.*** © 2008 Wiley-Liss, Inc.

KEY WORDS: rotavirus; genotype; lineage; G3; G9

INTRODUCTION

Group A rotaviruses are the leading cause of acute severe gastroenteritis in children in both developing and developed countries. Rotavirus infection is the single greatest cause of deaths associated with diarrhea among children [Bresee et al., 2005]. It is estimated that rotavirus diarrhea causes the deaths of 611,000 infants and young children each year worldwide [Parashar et al., 2006]. Virtually every child experiences rotavirus gastroenteritis at least once during the first few years of life, although most deaths due to rotavirus diarrhea occur in poor developing countries [Velázquez et al., 1996]. To reduce the death and disease burden associated with rotavirus infections, at least two practical rotavirus vaccines have been developed and employed for voluntary or routine administrations for children in many countries [Dennehy, 2008]. In adults, rotavirus gastroenteritis occurs at low frequency, but rotavirus infections have been present in epidemic outbreaks, travel-related gastroenteritis, and endemic diarrheal cases [Hrdy, 1987; Anderson and Weber, 2004]. However, epidemiologic and genetic information of rotaviruses causing sporadic gastroenteritis cases in adults is limited [Iturriza-Gómara et al., 2000; Nakajima et al., 2001; Pietruchinski et al., 2006], and therefore the significance of rotavirus diarrhea in adults and its possible influence on rotavirus infection in children are not well elucidated.

The genus rotavirus is a member of the family *Reoviridae* and is classified into at least seven established groups (A–G) or a newly proposed group [Alam et al., 2007; Estes and Kapikian, 2007]. Among these

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groups, group A rotavirus is the most prevalent worldwide as an important diarrheal pathogen in children. The genome of rotavirus consists of 11 segments of double-stranded RNA (dsRNA) encoding six structural viral proteins (VP) and six non-structural proteins (NSP). Neutralization antigens of rotavirus exist on the two structural proteins VP7 and VP4, which constitute the outermost layer of the rotavirus particle. Based on the antigenicity of VP7 and VP4, group A rotaviruses have been serologically classified into G serotypes and P serotypes, respectively. Recently, genotypes of group A rotavirus, G type and P type defined by VP7 gene and VP4 gene, respectively, have been commonly utilized to characterize rotaviruses because these types generally represent the G and P serotypes [Estes and Kapikian, 2007]. At least 15 G types and 28 P types have been described so far in rotaviruses from humans and various animal species. In human rotaviruses, the major genotypes are G1, G2, G3, G4, and G9, which are combined with P[4], P[6], and P[8] [Santos and Hoshino, 2005]. However, predominant genotypes vary depending on countries or regions, and seasons or years. It is considered important to survey genotypes of rotavirus and to detect presently prevalent strains in individual countries in order to know the global trend of predominant rotaviruses and their characteristics, which provide significant information relevant to the estimation of vaccine efficacy. Numerous recent epidemiologic studies revealed the global expansion of G9 rotaviruses since the 1990s as well as the emergence and widespread development of rare G12 rotaviruses to many countries since 2000 [Santos and Hoshino, 2005; Rahman et al., 2007].

In China, prevalent antigenic types have been changing according to epidemiologic studies reported since the 1980s [Zheng et al., 1989; Woods et al., 1992]. In Wuhan, G2 and G1 were predominant in 1994 and 1995, respectively [Wu et al., 1998]. However, more recently, G3 rotaviruses predominated from 2000 to 2006, and a G9 rotavirus was detected in 2004 for the first time in this city [Wang et al., 2007]. It was of note that the predominant G3 rotaviruses from children and adults were genetically closely related, suggesting that identical rotaviruses were circulating among children and adults. The present study was conducted from 2006 to 2008 in Wuhan to survey rotavirus genotypes continuously after the preceding study. Rotaviruses with predominant and minor genotypes were analyzed phylogenetically to ascertain their relatedness to rotaviruses distributed among other countries, and to further determine genetic relatedness of rotaviruses between children and adults.

MATERIALS AND METHODS

Specimens

The present study was conducted as a hospital-based survey of sporadic rotavirus diarrhea. Fecal specimens were collected from inpatients and outpatients in four hospitals (Renming Hospital of Wuhan University, Wuhan Commercial Staff Hospital, Wuhan the Sixth Hospital, Wuhan Children's Hospital) during a period between June 2006 and February 2008. The specimens were stored at −84°C until analyzed. In addition to the above specimens, rotavirus-positive specimens detected in the same hospitals from October 2005 to May 2006 which had not been genotyped in the previous study [Wang et al., 2007] were determined for their G and P types, and a number of them were analyzed phylogenetically.

Detection of Rotavirus

The presence of rotavirus in stool specimens was determined by detection of dsRNA segments of rotavirus in polyacrylamide gel electrophoresis (PAGE). Viral dsRNA was extracted from 400 μl of 10% stool suspension with sodium dodecyl sulfate (SDS) and phenol, and precipitated with ethanol as described previously [Kobayashi et al., 1989]. RNA segments of rotavirus were separated by PAGE and stained with silver nitrate as described previously [Herring et al., 1982]. Rotavirus groups were discriminated by the electrophoretic migration patterns (electropherotypes) of the rotavirus RNA segments.

Genotyping of Rotavirus

Rotavirus G-type and P-type were determined by reverse transcription-polymerase chain reaction (RT-PCR) as described previously [Gouvea et al., 1990; Gentsch et al., 1992]. The viral dsRNA was extracted from stool suspension using guanidine isothiocyanate and an RNaid kit (BIO 101, Inc., La Jolla, CA). For G genotype-specific amplification in the multiplex PCR, primer set 1 (RVG9, aAT8, aBT1, aCT2, aDT4, aET3, and aFT9) [Gouvea et al., 1990] or primer set 2 (G1, G2, G3, G4, G8, G9, G10, and VP7-R) [Iturriza-Gómara et al., 2004] was used. For P type-specific amplification, primer set 1 (con3, 1T-1, 2T-1, 3T-1, 4T-1, and 5T-1) [Gentsch et al., 1992] or primer set 2 (A, C, D, E, and F) [Wu et al., 1994] was used.

Sequence and Phylogenetic Analysis

Full-length nucleotide sequences of rotavirus VP7 genes and VP4 genes were determined directly with RT-PCR products from these genes. PCR products were purified by Wizard^{R} SV Gel and PCR Clean-Up System (Promega, Inc.). Sequencing reaction was performed with fluorescent dideoxy chain termination chemistry using the BigDye Terminator version 3.1 cycle sequencing kit (Applied Biosystems, Carlsbad, CA). DNA sequence was determined by the use of the ABI Prism 3700 genetic analyzer (Applied Biosystems). Multiple alignments and sequence identity of VP7 and VP4 genes from different strains were analyzed with Genetyx-WIN Version 5.1 (Software Development, Tokyo, Japan) and phylogenetic relationships among sequences were analyzed by the neighbor-joining method using the CLUSTAL W program. Phylogenetic dendrogram was visualized with Treeview.

Accession Numbers of Rotavirus Genes

The nucleotide sequences of VP7 gene for G1, G3, G9, and G4 rotavirus strains analyzed in this study were deposited in the GenBank database under accession numbers EU708570–EU708573 (4 strains), EU708575–EU708590 (16 strains), EU708591–EU708601 (11 strains), and EU708602 (1 strain), respectively. The VP4 gene sequence of a strain L621 was deposited in the GenBank database under accession number EU708574.

RESULTS

During a period between June 2006 and February 2008, a total of 2,214 fecal specimens from 1,126 children (under 15 years old) and 1,088 adults (15 years old or older) were collected. Group A rotavirus was detected in 24.9% (280/1,126) and 7.6% (83/1,088) of the specimens from children and adults, respectively. Group C rotavirus was identified in eight specimens, while group B rotavirus was not detected. Monthly detection rates of group A rotavirus varied from 12.4% to 62.4% and 0% to 22.3% in children and adults, respectively, with a peak in November. The highest incidence of rotavirus was detected in an age group of 7–24 months in children. In adults, the detection rate was highest in an age group of 50–59 years (10.3%), followed by 20–29 years (8.5%).

G and P types were determined for all 426 rotavirus-positive specimens, including 63 specimens obtained from Oct. 2005 to May 2006 (Table I). The most frequent genotype was G3P[8] present in 65.3% of the specimens, followed by G1P[8] (22.1%) and G2P[4] (4.0%). In both children and adults, the G3P[8] was the most prevalent (66.3% and 62.7%, respectively). G3P[9] was detected in two specimens from adults. Twelve G9P[8] rotavirus specimens (2.8%; six children and six adults) were identified, and G9P[6] was detected in an adult specimen.

The VP7 gene sequence was determined for seventeen G3P[8] rotavirus strains from seven children and ten adults detected in different years, and a G3P[9] rotavirus strain L621 from an adult. The VP7 gene sequences of G3P[8] rotaviruses from children and adults in 2005–2007 showed extremely high identities to each other (98.9–100%) and also to those of G3 rotaviruses in Wuhan in 2003–2004 (e.g., Y111) (more than 99%). In the phylogenetic dendrogram of G3 rotavirus VP7 genes, the G3P[8] rotaviruses in Wuhan were clustered in the sublineage 4 of the lineage 3 together with some strains from Asian countries including India, Malaysia, Thailand, Viet Nam, Taiwan, and Japan (Fig. 1a). Strains MaCH09004 in Malaysia and CMH054 in Thailand showed more than 99% VP7 gene sequence identities to the Wuhan G3P[8] rotaviruses. In contrast, G3 prototype human rotavirus strain YO (sublineage 1) and simian rotavirus strain SA11 showed lower identities (95.5–95.9% and 81.5–81.6%, respectively) to the Wuhan strains.

The VP7 gene of G3P[9] strain L621 was clustered in the sublineage 3 of the lineage 3, and exhibited 98.9–99.0% identity to the Thai strains with G3P[9] (CMH120/04 and CMH134/04), and lower identities to those of G3P[8] Wuhan strains (94.5–95.1%), strains YO (91.2%), and SA11 (81.6%). The entire VP4 gene sequence of the strain L621 was determined and analyzed. In the phylogenetic tree of P[9] VP4 gene, L621 was clustered in the lineage 3 which includes most human P[9] rotaviruses (Fig. 2). L621 VP4 gene had higher sequence identities to those present in human rotavirus strain AU-1 (97.3%), feline rotavirus FRV-1 (97.1%), and the G3P[9] human rotaviruses from Thailand (96.2–96.4%, VP8-coding region). In contrast, L621 VP4 showed lower identities to those in strains K8 (G1, lineage 1) (95.2%) and T152 (G12, lineage 2) (89.4%).

Figure 1b indicates alignment of VP7 amino acid sequences of representative G3 rotaviruses of sublineages 1–4 in the lineage 3. Wuhan strain E887 (2007), as well as the strain L174 (2004), had identical VP7 sequences to strains MaCH09004 (Malaysia), CMH054 (Thailand), VN–330 (Viet Nam), and 5091 (Japan) belonging to the sublineage 4. VP7 sequences of other sublineage 4 viruses, that is, 107E1B, Y111, and R1469

TABLE I. Frequency of G Types and P Types in Rotaviruses From Children and Adults in Wuhan, China

G type/P type	Number of specimens (%)		
	Children	Adults	Total
G1/P[8]	61 (20.3)	33 (26.2)	94 (22.1)
G2/P[4]	14 (4.7)	3 (2.4)	17 (4.0)
G3/P[8]	199 (66.3)	79 (62.7)	278 (65.3)
G3/P[6]	7 (2.3)	1 (0.8)	8 (1.9)
G3/P[9]	0 (0)	2 (1.6)	2 (0.5)
G3/ND[a]	10 (3.3)	1 (0.8)	11 (2.6)
G4/P[6]	1 (0.3)	0 (0)	1 (0.2)
G9/P[8]	6 (2.0)	6 (4.8)	12 (2.8)
G9/P[6]	0 (0)	1 (0.8)	1 (0.2)
G1 + G3/P[8]	1 (0.3)	0 (0)	1 (0.2)
G1 + G9/P[8]	1 (0.3)	0 (0)	1 (0.2)
Total	300 (100)	126 (100)	426 (100)

[a]Not determined.

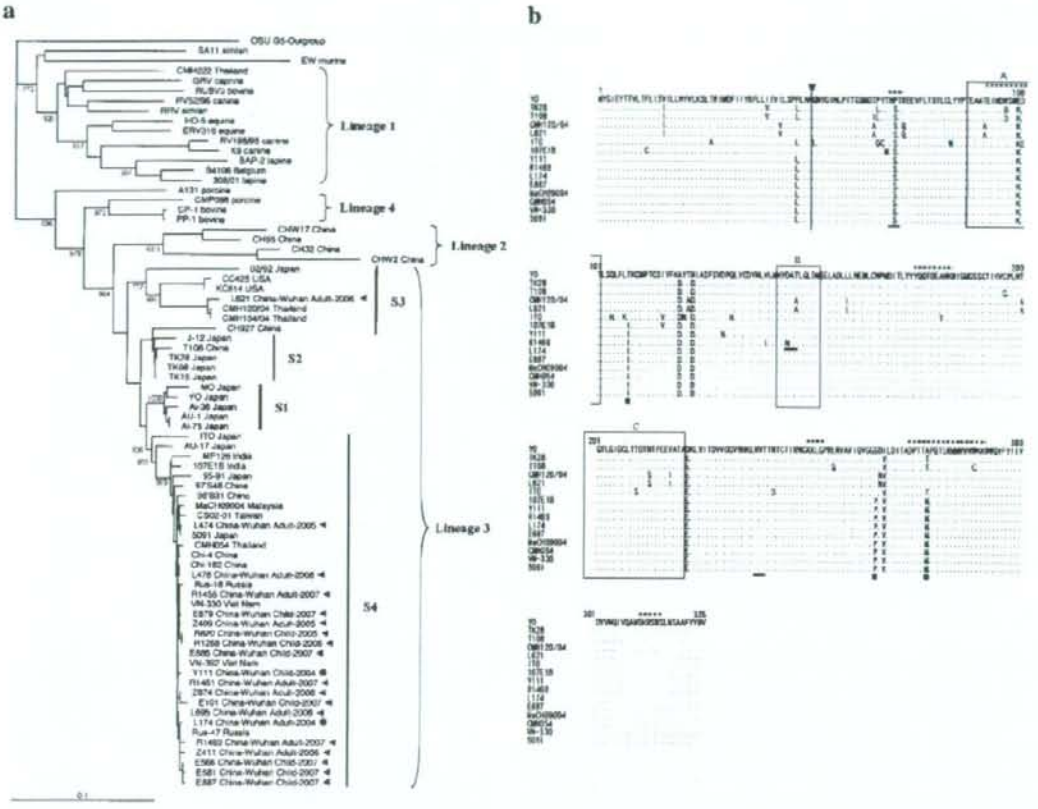

Fig. 1. a: Phylogenetic dendrogram based on the complete VP7 genes from G3 rotaviruses constructed by neighbor-joining method with CLUSTAL W program. Dendrogram is rooted with the G5 porcine rotavirus strain OSU (GenBank accession no. X04613), and the scale bar represents a genetic distance that is equivalent to 0.1 substitutions per site. Bootstrap values of 1,000 trials are indicated at the branch nodes (values <750 not shown). Rotaviruses analyzed in the present study are indicated by arrowheads with their derivation (adult or child) and year of detection. Solid circles indicate the representative rotavirus strains analyzed in the previous study in Wuhan [Wang et al., 2007]. Name of each rotavirus strain is described with country or animal of its origin. The lineages are indicated at the right side. Reference sequences used in the analysis were obtained from the GenBank database. b: Alignment of the deduced amino acid sequences of the VP7 of G3 rotaviruses belonging to the lineage 3 (a), including strains detected in Wuhan in the present study (E887, L621, R1469) and the previous study (Y111, L174) [Wang et al., 2007]. Sublineages (S1–S4) and country of individual reference strains are as follows; YO (S1, Japan), TK28 (S2, Japan), T108 (S2, China), CMH120/04 (S3, Thailand), ITO (S4, Japan), 107E1B (S4, India), MaCH09004 (S4, Malaysia), CMH054 (S4, Thailand), VN-330 (S4, Viet Nam), and 5091 (S4, Japan). Dot indicates identical amino acid to that of strain YO. Antigenic regions A–C are shown by squares. Amino acid 1–50 is a signal peptide marked with a line with an arrowhead. Putative *N*-glycosylation sites are indicated by underlines, and hydrophilic regions are shown by dotted lines above the sequence. Amino acids, which are distinctive between S4 and other sublineages are shown with solid squares under the sequences.

were almost identical to that of E887 with only a few amino acid differences. In contrast, VP7 sequence of E887 was different from that of human rotavirus strain YO (sublineage 1) isolated in Japan (1977) by 10 amino acids, and strains TK28 (Japan) and T108 (China) (sublineage 2) detected in the 1990s by 7 and 11 amino acids, respectively. Amino acid differences in the antigenic region of VP7 between the sublineage 4 viruses and other sublineages were found at amino acid position 99 (sublineage 1), 96 (sublineage 2), and 91, 147, 213, and 218 (sublineage 3). Three amino acids of the sublineage 4 rotaviruses at positions 108 (isoleucine), 266 (proline), and 278 (methionine) in the non-antigenic regions were distinct from those of other lineages.

G9 rotaviruses from four children and seven adults showed 97.9–99.9% VP7 gene sequence identities to each other and were clustered in the lineage 3 which contains globally spreading G9 strains in the phylogenetic tree (Fig. 3). These G9 viruses detected in Wuhan from 2005 to 2007 exhibited extremely high VP7 sequence identity to G9 strain L169 isolated in Wuhan in 2004 (97.9–98.8%), and also to other strains in the

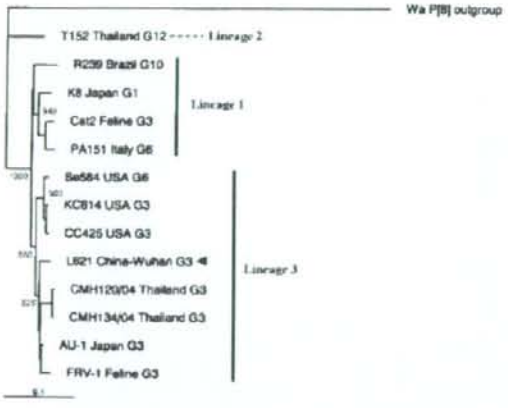

Fig. 2. Phylogenetic dendrogram based on the complete VP4 genes from P[9] rotaviruses constructed by neighbor-joining method with CLUSTAL W program. Dendrogram is rooted with the P[8] human rotavirus strain Wa (GenBank accession no. L34161), and the scale bar represents a genetic distance that is equivalent to 0.1 substitutions per site. Bootstrap values of 1,000 trials are indicated at the branch nodes (values <750 not shown). Rotavirus strain L621 analyzed in the present study is indicated by an arrowhead. Name of each rotavirus strain is described with country or animal of its origin and G type. The lineages are indicated at the right side. Reference sequences used in the analysis were obtained from the GenBank database.

Fig. 3. Phylogenetic dendrogram based on the complete VP7 genes from G9 rotaviruses. For explanation of the dendrogram, see figure legends of Figure 1. Reference sequences used in the analysis were obtained from the GenBank database.

lineage 3 represented by PerthG9.1 (more than 98%), but exhibited lower identities to a G9 prototype strain WI61 (89.3–89.7%).

VP7 genes of the four G1 rotaviruses, one from an adult and three from children, were closely related to each other (97.6–99.4% identities) and were clustered in the sublineage 5 of the lineage 5 in the dendrogram of G1 VP7 genes (Fig. 4), together with some strains from Japan and China showing more than 98% identities. Wuhan G1 strains showed 96.6–97.8% identities to the strains belonging to other sublineages (S1–S4) in the lineage 5, which includes strains from many Asian countries. In contrast, G1 strains in Wuhan exhibited lower VP7 gene sequence identities (85–95%) to the viruses in the lineages 1 through 4.

In the present study, only one G4P[6] rotavirus strain E931 was detected in an 8-month-old infant in February 2008. Phylogenetic analysis of the VP7 gene of G4 rotaviruses indicated that E931 was closely related to a strain R479 isolated in Wuhan in 2004 [Wang et al., 2007] showing 96.0% identity (data not shown), and these strains were clustered in a G4 lineage with a porcine rotavirus strain Gottfried (data not shown). However, the strain E931 had lower identities (82–88%) to those in the major G4 lineage containing most of G4 human rotaviruses.

DISCUSSION

The incidence of rotavirus in sporadic cases of diarrhea in adults has been described as 2–17% in most reports from many countries in the world [Svenungsson et al., 2000; Nakajima et al., 2001; Anderson and Weber, 2004]. In the present study, the rotavirus-positive rate in adult specimens was 7.6% in total, which was similar to that (9.0%) in the preceding epidemiologic study in Wuhan, China, from 2000 to 2006 [Wang et al., 2007]. The findings in the present and preceding studies indicated that rotavirus has been constantly distributed to adult populations at a low frequency. Although in the present study, rotaviruses were detected by the identification of RNA patterns in PAGE in order to detect any groups of rotaviruses, the detection rate of group A rotavirus would be slightly higher if more sensitive detection methods such as EIA were used.

Phylogenetic relatedness of rotaviruses distributed to children and adults was first analyzed in the preceding study in Wuhan (2000–2006) and close phylogenetic

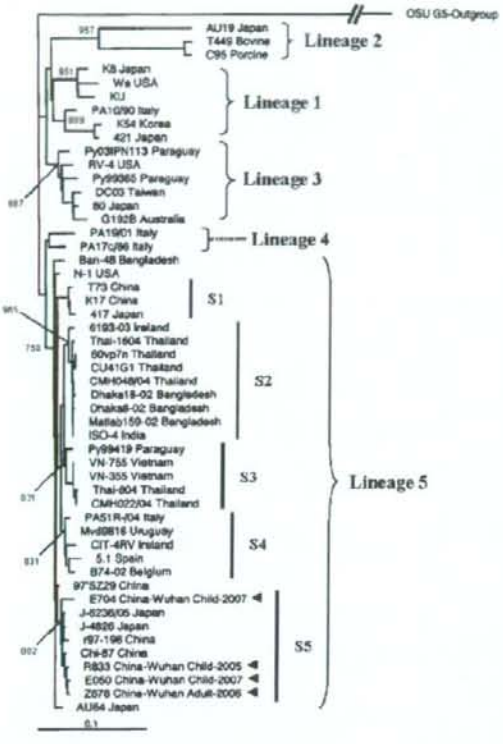

Fig. 4. Phylogenetic dendrogram based on the complete VP7 genes from G1 rotaviruses. For explanation of the dendrogram, see figure legends of Figure 1. Reference sequences used in the analysis were obtained from the GenBank database.

relatedness in children and adults was demonstrated for rotaviruses with G3 which was the most predominant in both groups [Wang et al., 2007]. In the present study, in addition to the predominant G3P[8] viruses, G1 and G9 rotaviruses from children and adults were revealed to be genetically highly related and clustered in a same lineage (sublineage) phylogenetically. These findings suggested that rotaviruses with less prevalent types, as well as predominant types, were also circulating among children and adults.

In China, G1 was described as a predominant type of rotavirus in many studies that had been done before 2001 in different areas of the country [Fang et al., 2002, 2005; Orenstein et al., 2007]. In Wuhan, predominance of G2 was noted in 1994, before the predominance of G1 in 1995 [Wu et al., 1998]. However, epidemiologic change of predominant type, from G1 to G3 occurred since the year 2000 by unknown reasons in China [Fang et al., 2005; Lo et al., 2005], and G3 was highly prevalent until 2006 in Wuhan [Wang et al., 2007]. In the present study, persistence of G3 until early 2008 was confirmed. Sequence analysis of VP7 indicated that the G3 rotaviruses prevailing in Wuhan from 2005 to 2007 were virtually identical to those from 2003 to 2004, and also to strains from Malaysia, Thailand, Viet Nam, and Japan reported recently [Phan et al., 2007a]. In contrast, VP7 of G3 viruses in Wuhan was genetically distinct from G3 strains in China from the late 1980s to the 1990s, that is, strains in the lineage 2 and lineage 4-sublineage 2 [Wen et al., 1997]. Trinh et al. [2007] characterized the difference in the VP7 amino acid sequence between current Chinese G3 rotaviruses and those isolated in 1986–1992, and noted distinct amino acids at positions 96 and 213 in the antigenic regions. At these sites, the current Chinese G3 rotavirus strains, as well as those analyzed in the present study in Wuhan, have identical amino acids (asparagine), which are different from those (aspartic acid) in the old G3 viruses. The long persistence of the current G3 rotaviruses in Wuhan appears to be unusual because in Japan, the predominance of the G3 rotavirus, which is genetically similar to those in Wuhan, was observed for a short duration from 2003 to 2004, but since 2004 G1 has replaced it as a predominant type [Phan et al., 2007b]. In Thailand and Malaysia, G3 has been a minor genotype according to the recent epidemiologic studies [Hung et al., 2006; Khamrin et al., 2007a; Theamboonlers et al., 2008]. Thus, virological factors related to rotavirus protein(s) other than VP7, or host and population factors peculiar to Chinese inhabitants are suggested to be ascribable to the G3 predominance.

Phan et al. [2007a] described the Japanese G3 rotaviruses detected in 2003–2004, represented by the strain 5091, as having significant amino acid sequence differences compared with G3 rotaviruses in Japan in 1990–1995, and called the latest G3 virus a "new variant" rotavirus. They noted some amino acid substitutions in the antigenic regions in the VP7 of the new variant viruses. The present study indicated that the Wuhan G3 rotavirus E887 had identical VP7 sequence to the new variant strain 5091, and some amino acid differences were detected in the antigenic regions compared with G3-lineage 3, sublineages 1–3 rotaviruses. However, the strain E887, together with the strain 5091, was found to have only one different amino acid at position 99 in the antigenic regions, compared with G3 prototype strain YO which was isolated in Japan in 1977 [Urasawa et al., 1984]. Amino acids at position 96, 213 in the antigenic regions were the same between the new variant G3 rotaviruses (E887 and 5091) and old prototype strain YO. This means that amino acid divergence in the antigenic regions may not be specific traits of the recently prevailing new variant G3 viruses. In contrast, three amino acids located at the constant region (positions 108, 266, 278) were commonly found in the sublineage 4 rotaviruses containing the new variant G3 viruses, but were distinct from other sublineages. Therefore, amino acids at these positions are considered to be a feature of the new variant G3 viruses, although the influence of these amino acids to the infectivity or virulence of rotavirus has yet to be elucidated.

The genotype G9, as a first recognized emerging type of rotavirus, has been widespread globally since the mid-1990s [Santos and Hoshino, 2005; Santos et al., 2005]. In China, G9 has been detected since 1997 but detection rate has been approximately 5% or less [Fang et al., 2002, 2005]. In Wuhan, G9 was first detected in 2004, among the surveillance of the rotaviruses since 2000 [Wang et al., 2007]. The isolated G9 strain L169 was clustered in the lineage (lineage 3) of the globally spreading G9 viruses, while genetically distinct from the G9 strains in China, which have been reported so far. In the present study, G9 rotaviruses were detected in both children and adults, albeit in low frequency, and they were grouped into the lineage 3. Other than Wuhan, G9 strains were isolated in the northwest areas of China and they were also clustered in the lineage 3 [Yang et al., 2008]. It is suggested by these findings that the G9 rotavirus from the global lineage may become an important genotype in China in the near future, considering that G9 rotavirus scarcely affected Chinese people previously. In addition, it should be also noted that the G9 rotavirus was reported to be associated with more severe diseases in children in Latin American countries where G9 became prevalent [Linhares et al., 2006]. Thus, further surveillance of the rotavirus genotyping and cautions against an increase of G9 will be necessary in China.

A rotavirus strain L621 with a rare genotype G3P[9] had the VP7 gene which was phylogenetically distinct from those present in G3P[8] viruses in Wuhan. In contrast, both VP7 and VP4 genes of L621 were genetically close to those in G3P[9] strains CMH120/04 which was detected in Thailand [Khamrin et al., 2007b], and the VP4 gene was related to that of the feline rotavirus strain FRV-1, as well as the feline-like human rotavirus AU-1 [Nakagomi et al., 1993]. Another rare type, G4, was detected in a single strain E931 which was genetically close to the strain R479 detected in the preceding study in Wuhan, and the R479 was found to have a porcine rotavirus-like VP6 gene [Wang et al., 2007]. As indicated recently by an exhaustive genetic analysis of rotaviruses [Matthijnssens et al., 2008], origins of animal species for individual RNA segments of rotavirus can be estimated by their sequence analysis. Hence, sequence determination of remaining gene segments of the strains with rare genotypes will elucidate the possible origin of animal species for individual RNA segments, and may support the occurrence of interspecies transmission and reassortment event in humans.

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