Molecular Epidemiology of G9 Rotaviruses in Taiwan between 2000 and 2002

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Received 7 October 2005/Returned for modification 13 February 2006/Accepted 31 July 2006

Since the mid-1990s, novel G9 rotaviruses have been detected in many countries, suggesting that G9 is a globally important serotype. The molecular epidemiology of G9 rotaviruses in Taiwan from 2000 to 2002 was investigated in this study. G9 rotavirus first appeared in 2000 with 4 cases and constituted 33.8% and 54.8% of the rotavirus-positive samples in 2001 and 2002, respectively. These G9 strains belonged to P[8]G9, subgroup II, and long electropherotype, except one belonged to P[4]G9, subgroup II, and short electropherotype. Nucleotide sequencing and phylogenetic analysis of 52 Taiwanese G9 rotaviruses showed that the VP7 genes shared a high degree of identity to overseas G9 rotaviruses detected after 1993 and that the VP8* portions of the VP4 genes were more closely related to those of local rotaviruses of other G types. The two P[8]G9 strains with high nucleotide identities in the VP7 and the partial VP4 genes, 01TW591 of Taiwan from 2001 and 95H115 of Japan from 1995, varied in four genes, genes 2, 3, 7, and 8, which was revealed by RNA-RNA hybridization. Representative strains for different RNA patterns were also analyzed in the partial VP2 and VP3 genes; the nucleotide identities were high between Taiwanese G9 strains and local G3 or G2 strains. These results suggested that Taiwanese G9 rotaviruses possibly had evolved through reassortment between overseas G9 strains and circulating rotaviruses of other G types.

Group A rotavirus is one of the major pathogens that cause severe gastroenteritis in infants and young children worldwide (19). According to WHO statistical data for the year of 2001, 600,000 infants and young children under 5 years of age died from rotavirus diarrhea, and up to 85% of these deaths occurred in certain developing countries. The infections show seasonal variation, usually occurring in winter in temperate regions (4, 5). Rotavirus belongs to the family Reoviridae, and its virion has a triple-layered icosahedral capsid that surrounds the genome of 11 segments of double-stranded RNA (12). Because of the segmented nature of the genome, the segmented genes will undergo reassortment (15) if two different rotaviruses of the same group coinfect one cell. The genetic heterogeneity of rotaviruses has been characterized by RNA electrophoretic profiles on the gel. RNA electrophoretic analysis is often used for molecular epidemiological studies to monitor rotavirus outbreaks and transmissions (12).

Two outer capsid proteins, VP7 and VP4, define rotavirus serotypes. Both VP7 and VP4 induce neutralizing antibodies and are responsible for the serotype specificity (11, 40). VP7 is a major glycoprotein encoded by gene 7, 8, or 9, and VP7-specific types are abbreviated as G serotypes or G genotypes (11, 12, 27). Group A rotaviruses have been classified into 15 G serotypes and 26 P genotypes (26, 35). The combinations of P[8]G1, P[4]G2, P[8]G3, and P[8]G4 are found to be predominant in human rotaviruses (14, 19). G1 to G4 serotypes are the major targets for vaccine development (9).

G9 rotavirus was first detected in 1983 in the United States (6), and it was also found in Japan, Thailand, Yugoslavia, and India, though only a few cases were identified (10, 29, 38, 42). Since the mid-1990s, G9 rotaviruses have been recognized to be efficiently spreading throughout the world, and G9 may represent the fifth globally important serotype (33, 35). In this study, the molecular epidemiology of G9 rotaviruses between January 2000 and December 2002 was analyzed. G9 rotavirus has been detected in Taipei, Taiwan, since March 2000 (25). G9 became one of the most prevalent G types locally. To explore the origin of these G9 viruses, the viruses were characterized by antigenic and genetic analyses.

MATERIALS AND METHODS

Clinical fecal specimens. Fecal specimens, from children with acute gastroenteritis who either were hospitalized or visited the pediatric clinics of National Taiwan University Hospital (NTUH) in Taipei, a city located in the northern part of Taiwan, were screened by enzyme immunoassay (Rotacclone; Meridian Diagnostic, Cincinnati, OH), and those specimens containing rotaviruses were collected between January 2000 and December 2002. The fecal samples were suspended to approximately 10% (wt/vol) in phosphate-buffered saline, pH 7.2, and clarified by low-speed centrifugation. The supernatant was stored at −70°C.

Viruses cultivation. G9 rotavirus isolate 01TW591 and reference strains, DS-1 (G2), S2 (G2), E210 (G2), and W161 (G9), were grown in a fetal monkey kidney cell line, MA104.
**RESULTS**

**Prevalence of G9 rotavirus.** A total of 648 rotavirus-positive fecal samples were analyzed in this study. They were collected during the period from January 2000 to December 2002 from children with acute gastroenteritis who were either hospitalized (89%) or visited the pediatric clinics (11%) of NTUH. The G and P genotypes of the rotaviruses present in the 648 samples were determined by RT-PCR. The G type distribution varied each year, with G9 being the most common overall (37.7%) (Table 1). In 2000, 4 G9 strains were first recovered in March and constituted 3.3% of the rotavirus-positive samples in that year; G1 and G2 strains constituted 37.5% and 51.7%, respectively. In 2001, the number of G9 strains notably increased to 79, which constituted 33.8% of the rotavirus-positive samples; G1 and G2 strains constituted 37.5% and 15.4%, respectively. In 2002, G9 became the most prevalent G type, 161 G9 strains were found and constituted 51.7% of the rotivirus-positive samples in that year; G1 and G2 strains constituted 37.5% and 1% of the rotavirus-positive samples for each year in this period, respectively. The temporal distribution of G types from 2000 to 2002 is
shown in Fig. 1. The peak of G9 rotavirus was in February 2002. The age distribution of G9 and non-G9 rotavirus from 2001 to 2002 was analyzed. A parallel age distribution was shown between G9 and non-G9 rotavirus infections (Fig. 2). A significant portion of the rotavirus cases, 61.66% for G9 and 61.68% for non-G9, fell within the age range from 6 to 35 months. The portion of the age group younger than 6 months in the G9 rotavirus-infected cases was 9.2%, higher than that in the non-G9 cases, 3.6% (\(P < 0.01\), chi-square test).

**P** genotype, subgroup, and RNA electropherotype of G9 rotaviruses. A total of 244 G9 rotavirus samples were subjected to VP4 genotyping. The VP4 genotypes could be determined for 235 samples. Except for one with P[4], all the other samples contained rotaviruses with P[8]. The subgroup specificity of the G9 rotaviruses in 242 samples was analyzed by ELISA, using subgroup I- and subgroup II-specific MAbs. All of these G9 strains belonged to subgroup II.

Of the 244 G9 samples, the RNA profiles of 226 (92.6%) samples were visible. Six electrophoretic patterns (a to f) could be differentiated (Fig. 3): 5 long electropherotypes and one short electropherotype. The correlations of RNA electrophoretic pattern, subgroup, and VP4 genotype are summarized in Table 2. All the P[8]G9 strains showed a long electropherotype, except for one P[4]G9 strain with a short electropherotype.

**Sequence and phylogenetic analysis of the VP7 genes.** Before 2000, G9 rotaviruses had not been detected in Taiwan for more than 15 years (unpublished data). To investigate the origin of the Taiwanese G9 rotaviruses, nucleotide sequence analysis of the VP8* portion of the VP4 gene and the VP7 gene was conducted. A total of 52 G9 samples were selected, which included all six RNA electrophoretic patterns (Table 2).

For comparative analysis with reference strains, 994 nucleotides of the VP7 genes and the deduced 326-amino-acid sequences were used for comparison. All 52 Taiwanese G9 strains were very similar. The nucleotide sequence and the deduced amino acid sequence identities were 99.1% to 100% and 98.6% to 100%, respectively. Six representative G9 strains with different RNA electrophoretic patterns that were present between 2000 and 2002 were selected for further analysis. These 6 G9 strains included 01TW591 (RNA pattern a), 02TW1532 (b), 02TW498 (c), 02TW569 (d), 01TW1640 (e), and 01TW1288 (f); the first two digits represent the year in which the sample was collected and the last three or four digits indicate the sample number in the corresponding year. The nucleotide sequence identities to most recent G9 strains, 95H115 (Japan, 1995), CMH328 (Thailand, 2000 to 2001), MG9-06 (Australia, 2000), R160 (Brazil, 1997 to 1999), BP785/00 (Hungary, 2000), 0.8-98 (United Kingdom, 1998), MW69 (Malawi, 1997), US1205 (United States, 1996 to 1997), and 480-97

![FIG. 1. Monthly G type distribution of rotavirus strains between 2000 and 2002.](image-url)
The VP7 genes were analyzed phylogenetically. As shown in Fig. 4, the Taiwanese G9 strains, 01TW591, 02TW1532, 02TW498, 02TW569, 01TW1640, and 01TW1288, were closely related to a Japanese strain (95H115) and strains from Thailand (CMH328), Australia (MG9-06), Brazil (R160), United Kingdom (0.8/98 and 480/97), Hungary (BP785/00 and BP1829/01), Malawi (MW69), the United States (US1205 and US1212), India (INL1), and Bangladesh (BD524) isolated after 1994. They were also clustered with Mc345 (Thailand, 1989), 608VN and 684VN (Vietnam, 1999 to 2000), K-1 (Japan, 1999), and T203 (China, 1997) but with greater distance. F45 (Japan, 1986), AU32 (Japan, 1986), WI61 (US, 1983), 116E (India, 1985), and 97SZ37 (China, 1997) were distantly related to most of the recent G9 strains.

Sequence and phylogenetic analysis of the partial VP4 genes. The VP8* portion of the VP4 genes of the 52 G9 strains were analyzed, since great variation has been recognized in this portion of VP4 (12). Excluding the primer sequences, 744 nucleotide sequences and the deduced 248-amino-acid sequences were aligned and compared. Except for the 6 strains with RNA pattern c, the other 45 P[8]G9 strains were similar; the nucleotide sequence identities were from 98.8% to 100%, and the deduced amino acid sequence identities were from 97.2% to 100%. The identities between the strains with RNA pattern c, 02TW498 and 01TW1465, and the other P[8]G9 strains with different RNA patterns were from 89.7% to 89.8% in nucleotide sequences and 91.5% in the deduced amino acid sequences. Compared with the reference strains, 02TW498 and 01TW1465 were more similar to BP785/00 (Hungary, 2000), with identity 99.2% in nucleotide sequences; the other Taiwanese P[8]G9 strains were more closely related to 480-97 (United Kingdom, 1997), with identities of 99.3% to 99.6%, and 95H115 (Japan, 1995), with identities of 98.8% to 99.1%. The identities of P[4]G9 strain 02TW569 to P[4] reference strains, DS-1 and E210, were 94.4% and 98.0%, respectively.

Sequence analyses showed that the VP8* portions of the VP4 genes of Taiwanese G9 strains had three distinct forms. To investigate the origin of these different VP4 genes, 17 local strains of different G types were also included in the phylogenetic analysis. Figure 5 shows that the VP8* portions of the VP4 genes of Taiwanese P[8]G9 strains clustered into two different lineages. One lineage included the 4 Taiwanese P[8]G9 strains with RNA patterns a, b, e, and f. These strains were most closely related to a Taiwanese P[8]G3 strain of 1999, 97TW1832, a Taiwanese P[8]G1 strain of 2001, 01TW1291, and a P[8]G9 strain of United Kingdom, 480-97. Three P[8]G9 reference strains, AU32, F45, and WI61, were distinct from the Taiwanese G9 strains and more closely related to Ku. The other lineage contained two Taiwanese P[8]G9 strains with RNA pattern c, 02TW498 and 01TW1465. These strains were within a cluster with Wa but more closely related to a Hungarian P[8]G9 strain, BP785/00. The Taiwanese P[4]G9 strain, 02TW569, clustered with reference P[4]G2 strains, DS-1 and E210, and were more closely related to local P[4]G2 strains, 98TW762, 99TW967, and 00TW1959.

Sequence alignment of the partial VP4 genes showed that the nucleotide identities of the 4 Taiwanese P[8]G9 strains,
with RNA patterns a, b, e, and f, to 99TW1832 were 99.5% to 100% (Table 3), and the nucleotide identities of the P[4]G9 strain, 02TW569, to P[4]G2 strains, 98TW762 and 99TW967, were 99.3% and 99.6%, respectively.

**Table 2. RNA pattern, VP6 subgroup specificity, and VP4 genotype of the G9 rotaviruses recovered in Taiwan from 2000 to 2002**

<table>
<thead>
<tr>
<th>RNA pattern</th>
<th>RNA e-type&lt;sup&gt;a&lt;/sup&gt;</th>
<th>VP6 subgroup</th>
<th>VP4 genotype</th>
<th>Yr recovered</th>
<th>No. of samples</th>
<th>No. of samples with VP7 and VP4 genes sequenced&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Representative strain&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>d</td>
<td>Short</td>
<td>II</td>
<td>P[4]</td>
<td>2002</td>
<td>1</td>
<td>1</td>
<td>02TW569</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>226</td>
<td>52</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> RNA electropherotype.

<sup>b</sup> The sequences of the full-length VP7 gene and the VP8* portion of the VP4 gene were analyzed.

<sup>c</sup> Representative strain indicates the strain used for most of the comparisons in different genes in this study.

**Sequence and phylogenetic analysis of the partial VP2 and VP3 genes.** The major prevalent P[8]G9 rotaviruses in Taiwan were similar to 95H115, a strain isolated from a geographically close country, Japan, in 1995, in the VP7 and VP4 genes, with identities 98.8% or higher. The genetic relatedness of the 11 RNA segments between 95H115 and the Taiwanese strain 01TW591 was analyzed by RNA-RNA hybridization. RNA extracted from isolate 01TW591 was labeled with peroxidase and used as a probe for RNA hybridization. Hybridization of the RNA extracted from 95H115 to the 01TW591 probe exhibited a hybrid band for each RNA segment (data not shown), though the hybrid bands for RNA segments 2, 3, 7, and 8 showed lower intensity. The genetic background of the 01TW591 strain was examined by incorporating Wa and DS-1 strains in the RNA-RNA hybridization experiments. Except

FIG. 4. Phylogenetic analysis of the nucleotide sequences of the VP7 gene (nt 73 to 942) of G9 strains isolated in Taiwan recently and G9 strains from different parts of the world. A phylogenetic tree was constructed based on the neighbor-joining method within the MEGA package. Percent bootstrap values above 70% are shown at branch nodes. The branch length for a 2% nucleotide difference is indicated at the bottom. For each strain, the P and G genotypes and the country of origin are shown. Taiwanese G9 strains are indicated by boldface italic type, and Taiwanese strains with other G types are indicated by italic type. In addition to one representative G9 strain for each RNA pattern listed in Table 2, a Taiwanese G9 strain, 01TW1465, with RNA pattern c was also included in the analysis.

FIG. 5. Phylogenetic analysis of the nucleotide sequences of the VP8* portions of the VP4 genes (nt 70 to 813). A phylogenetic tree was constructed based on the neighbor-joining method within the MEGA package. Percent bootstrap values above 70% are shown at branch nodes. The branch length for a 2% nucleotide difference is indicated at the bottom. For each strain, the P and G genotypes and the country of origin are shown. Taiwanese G9 strains are indicated by boldface italic type, and Taiwanese strains with other G types are indicated by italic type. In addition to one representative G9 strain for each RNA pattern listed in Table 2, a Taiwanese G9 strain, 01TW1465, with RNA pattern c was also included in the analysis.
the RNA segment 9, the other 10 RNA segments of 01TW591 strain could hybridize with those of the Wa strain, though segment 5 with lower intensity (data not shown).

Because the hybridization intensity between 95H115 and 01TW591 in the VP2 and VP3 segments was low, nucleotide sequences of the VP2 and VP3 genes were further analyzed. The partial sequences of the VP2 (508 bp) and VP3 (605 bp) genes of 95H115, Taiwanese G9 strains, and Taiwanese and reference G1, G2, or G3 strains were analyzed and compared. Phylogenetic analysis of either the VP2 gene (Fig. 6) or the VP3 gene (Fig. 7) showed that the 5 Taiwanese P[8]G9 strains clustered together with reference G1 (KU and Wa) and G9 (WI61 and 95H115) strains, and Taiwanese G1 strains, 97TW1127 and 01TW1291, and G3 strain, 99TW1832; the Taiwanese P[4]G9 strain 02TW569 clustered with reference G2 strains, DS-1, S2, and E210, and Taiwanese G2 strains, 98TW762 and 99TW967. The phylogenetic trees also showed that Taiwanese P[8]G9 strains were more closely related to 99TW1832 and 97TW1127 in VP2, and to 99TW1832, 97TW1127, and 01TW1291 in VP3; the Taiwanese P[4]G9 strain 02TW569 was more closely related to 98TW762 and 99TW967 in both VP2 and VP3.

As shown in Table 3, the nucleotide identities of 01TW591

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>01TW591</th>
<th>95H115°</th>
<th>02TW569</th>
<th>99TW1832</th>
</tr>
</thead>
<tbody>
<tr>
<td>01TW591</td>
<td>P[8]G9</td>
<td>98.8</td>
<td>93.9</td>
<td>92.2</td>
<td>85.5</td>
</tr>
<tr>
<td>02TW1532</td>
<td>P[8]G9</td>
<td>99.5</td>
<td>98.8</td>
<td>97.4</td>
<td>91.1</td>
</tr>
<tr>
<td>02TW948</td>
<td>P[8]G9</td>
<td>89.7</td>
<td>90.0</td>
<td>98.0</td>
<td>91.1</td>
</tr>
<tr>
<td>02TW569</td>
<td>P[4]G9</td>
<td>85.5</td>
<td>82.1</td>
<td>75.4</td>
<td>85.9</td>
</tr>
<tr>
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<td>P[8]G9</td>
<td>99.5</td>
<td>99.0</td>
<td>98.0</td>
<td>98.9</td>
</tr>
<tr>
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<td>P[8]G9</td>
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<td>98.8</td>
<td>97.9</td>
<td>98.9</td>
</tr>
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<td>P[8]G1</td>
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<td>97.8</td>
<td>97.9</td>
<td>98.8</td>
</tr>
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<td>P[8]G1</td>
<td>99.3</td>
<td>95.5</td>
<td>96.7</td>
<td>98.9</td>
</tr>
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<td>98TW762</td>
<td>P[4]G2</td>
<td>85.5</td>
<td>82.5</td>
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<td>85.8</td>
</tr>
<tr>
<td>99TW967</td>
<td>P[4]G2</td>
<td>85.3</td>
<td>82.1</td>
<td>75.4</td>
<td>85.6</td>
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<td>99TW1832</td>
<td>P[8]G3</td>
<td>99.5</td>
<td>99.4</td>
<td>97.7</td>
<td>99.1</td>
</tr>
</tbody>
</table>

*95H115 is a P[8]G9 strain.*

*The comparison was based on 744 bp of the nucleotide sequence of the VP8* portion of the VP4 gene.*

*The comparison was based on 508 bp of the nucleotide sequence of the VP2 gene.*

*The comparison was based on 605 bp of the nucleotide sequence of the VP3 gene.*

![FIG. 6. Phylogenetic analysis of the nucleotide sequences of the partial VP2 genes (nt 490 to 997 of KU strain).](image)

![FIG. 7. Phylogenetic analysis of the nucleotide sequences of the partial VP3 genes (nt 1959 to 2563).](image)
to 95H115 in the partial VP2 and VP3 genes were 93.9% and 92.2%, respectively, lower than the identities of 01TW591 to the other four Taiwanese P[8]G9 strains, 98.8% to 99.0% and 97.4% to 98.0%, respectively. The nucleotide identities of 01TW591 to 99TW1832 were 99.4% and 97.7% in the VP2 and VP3 genes, respectively. The identities among the other P[8]G9 strains, 02TW1532, 02TW498, 01TW1640, and 01TW1288, and 99TW1832 were 99.4% to 99.6% and 99.0% to 99.7% in the VP2 and VP3 genes, respectively. The identities between P[4]G9 strain 02TW569 and P[4]G2 strains, 98TW762 and 99TW967, were 99.6% and 99.2% to 99.7% in the VP2 and VP3 genes, respectively.

**DISCUSSION**

The present study reports on the epidemic of G9 rotavirus that occurred in the northern part of Taiwan from 2000 to 2002. G9 rotaviruses had been detected in children with acute gastroenteritis in the Taipei area since March 2000. G9 rotavirus became dominant and related to more than one half of the cases in 2002. A big outbreak of acute gastroenteritis was noted from 2001 to 2002. Similarly, an outbreak of gastroenteritis associated with G9 rotavirus was also noted in the southern part of Taiwan from 2001 to 2002 (36). In the central part of Taiwan, G9 rotavirus was identified in several samples collected in 2001 to 2002 (22). It appeared that G9 rotaviruses were widespread on the island in 2001 to 2002. In this study, of the 648 human rotavirus samples recovered from January 2000 to December 2002, 244 (37.7%) were of G9 specificity. Most of these Taiwanese G9 rotaviruses belonged to P[8]G9, subgroup II, and long electropherotype, and only one strain belonged to P[4]G9, subgroup II, and short electropherotype. During this period of 3 years, G2 was the major prevalent type and associated with more than one-half of the cases in 2000; G1 became the major prevalent type in 2001 and associated with more than one-fourth of the cases in each of these years. It is interesting that three different serotypes were the dominant types in a 3-year period.

Since the mid-1990s, G9 rotaviruses have emerged around the world. Sequence analysis of the VP7 gene has shown that these novel G9 rotaviruses belong to one phylogenetic lineage and are distinct from the earlier G9 strains (35). In this study, sequence analysis showed that the VP7 genes of Taiwanese G9 rotaviruses shared high identities and clustered closely in the phylogenetic tree. The VP7 genes of these viruses were also very similar to the G9 strains recovered from the central part of Taiwan in 2001 to 2002 (data not shown for sequences with shorter lengths) (22). This indicates that Taiwanese G9 rotaviruses might have the same origin and might have been introduced into Taiwan recently. In addition, based on our unpublished data, this was the first G9 epidemic observed between 1981 and 2005. The VP7 genes of Taiwanese G9 strains were very similar to those of the G9 strains from Japan, Thailand, Australia, Brazil, Hungary, the United Kingdom, Malawi, and the United States; they all belonged to lineage 3, as described previously by Hoshino et al. (16). The VP7 gene appeared to be stable as revealed by the high identity (99.4%) between 01TW591 and a Japanese G9 strain, 95H115, recovered 6 years earlier (31).

In contrast, in the phylogenetic analysis of the VP8* portions of the VP4 genes of these Taiwanese G9 rotaviruses, they could be clearly differentiated into three distinct forms. In addition to a strain with the evident P[4] type, the 51 sequenced G9 strains with P[8] type were clustered into two different lineages. The presence of at least 3 different forms of G9 rotaviruses could be explained by the occurrence of genetic reassortment. 02TW498 and the other 5 strains with RNA pattern c belonged to one P[8] lineage, similar to a Hungarian strain BP785/00, and the other 45 strains with different RNA patterns belonged to the other lineage. The VP8* portions of the VP4 genes of these major P[8]G9 strains were closely related to that of a United Kingdom P[8]G9 strain from 1997, 480-97, and a Japanese P[8]G9 strain from 1995, 95H115, and were even more closely related to a local P[8]G3 strain (99TW1832) from 1999, prevalent before the G9 epidemic, and a local P[8]G1 strain (01TW1291) from 2001. Additional analysis of the partial VP2 and VP3 genes also revealed that the Taiwanese P[8]G9 strains were closely related to 99TW1832. For reference strains with available sequence data for both the VP7 and VP4 genes, 95H115 was quite similar to these Taiwanese P[8]G9 strains. The 95H115 strain belonged to the Wa genogroup (30), the Taiwanese G9 strain 01TW591 characterized in this study also belonged to the Wa genogroup. RNA-RNA hybridization between 01TW591 and 95H115 revealed that 4 of the 11 RNA segments were less closely related. Sequence analysis of the partial VP2 and VP3 genes also showed that these two strains were distinct. It has been suggested that P[8]G9 strains from the United Kingdom have emerged through reassortment in humans between the P[6]G9 strains and the more prevalent cocirculating G1, G3, and G4, which commonly carry VP4 genes of the P[8] type (17). Since genetic reassortment is a common phenomenon that occurs in human rotavirus infection (18, 28), it is reasonable to speculate that the VP4, VP2, and VP3 genes of these major G9 strains of Taiwan were possibly derived from local P[8]G3 or P[8]G1 rotaviruses through genetic reassortment. To further confirm this speculation, sequence analysis of more RNA segments would be helpful. Due to the limited quantities of double-stranded RNA extracted from the fecal samples, it was not feasible to conduct RNA-RNA hybridization analysis.

The P[8]G9 strain 02TW498 with RNA pattern c was closely related to a Hungarian strain, BP785/00 from 2000, in both the VP7 and VP4 genes; however, it was similar to local G3 strain 99TW1832 in both the VP2 and VP3 genes. The possibility of 02TW498 derived from reassortment needs further comparison with BP785/00 in more genes. Only one P[4]G9 strain (02TW569) was recovered in this study. P[4]G9 strains have also been reported in Thailand, Ghana, Brazil, and Australia (1, 2, 20, 41). The rotavirus with the combination of short electropherotype and subgroup II usually reflects a strain resulting from genetic reassortment (32). The result that 02TW569 was closely related to local P[4]G2 strains in the VP4, VP2, and VP3 genes also implied that the strain had possibly evolved by genetic reassortment.

The partial VP3 gene of P[8]G9 strain 01TW591 varied somewhat from that of the other Taiwanese P[8]G9 strains. The variations might be derived from reassortment between different strains or accumulation of point mutations through time. The virus strains showed measurable diversity in different genes, which might imply that G9 rotaviruses had been in the
community for some time before being detected. The 95H115 strain isolated from neighboring Japan in 1995, 5 years before the detection of G9 rotavirus in Taiwan, was closely related to Taiwanese G9 strains in both the VP7 and VP4 genes. In comparison, the United Kingdom strain 480-97 from 1997 was closely related to the major Taiwanese G9 strains in the VP4 gene, but less closely related in the VP7 gene. Therefore, 95H115 appeared to be the possible parental strain of some of the 11 genes. In addition to 95H115, 480-97, and a Hungarian strain, BP785/00, it would be interesting to find other possible parental G9 rotaviruses. The G9 strains, Thailand strain CMH328 and Australian strain MG9-06, detected in 2000 to 2001, Brazilian strain R160 from 1997, United States strain 1205 from 1996 to 1997, and Malawi strain MW69 from 1997, were closely related to Taiwanese strains in the VP7 gene. The relationships with Taiwanese G9 strains were intriguing and need further clarification, but unfortunately, no VP4, VP2, or VP3 gene sequences were available in GenBank for comparison.

The age of 12 to 23 months is considered to be the most susceptible age range for rotavirus infection (4). Our data revealed that approximately 29.6% of the G9 and non-G9 rotavirus infections occurred between 12 to 23 months, which agreed with the usual susceptible age. The age distribution of patients with G9 and non-G9 rotavirus infection appeared to be very similar. However, the portion of the G9 infections that occurred in the age group younger than 6 months was low (9.2%) but was significantly higher than that of non-G9 infections (3.6%) \( P < 0.01 \) (chi-square test). G9 rotavirus was new to the general population in Taiwan. Therefore, this difference between G9 and non-G9 might be explained by the lack of G9-specific neutralizing antibodies in maternal immunity. Compared to the 1995 to 1996 epidemic in the United States, in which a significant portion, about 50%, of the G9 infection occurred in infants younger than 6 months (7), we had much less in Taiwan. In contrast to the P[8]G9 strain as the major epidemic strain in Taiwan, the major epidemic strain was P[6]G9 for the 1995 to 1996 epidemic in the United States. It would be reasonable to suggest that previous existing maternal P[8]-specific antibodies might provide heterotypic protection in our younger population. However, it was different from the observation in the United Kingdom, where G9 infection was as common in children older than 2 years as in younger children (8), and in The Netherlands, where G9 strains caused diarrhea in neonates (39) and the G9 rotaviruses escaped from preexisting immunity (3). There is still a controversy between heterotypic protection and serotype-specific immunity.

This report presents the results of a study on the first wave of G9 rotavirus outbreak, which occurred between 2000 and 2002, in at least the last 25 years in Taiwan. It is noteworthy that between the first appearance of a few cases of G9 rotavirus infection and the start of a big epidemic, there was a period of more than 1 year. If vaccine had been available when G9 rotavirus first appeared, the big epidemic could have been circumvented. According to the results of sequence analysis for the VP7 gene and the partial VP4, VP2, and VP3 genes and RNA-RNA hybridization, we suggested that Taiwanese G9 rotaviruses possibly had evolved through reassortment between overseas G9 strains and circulating rotaviruses of other G types.

ACKNOWLEDGMENTS

We thank Osamu Nakagomi for his kindness in providing the double-stranded RNA of Japanese G9 rotavirus strains AU/32 and 95H115.

This work was supported by grants from the National Science Council (grants NSC-92-2314-B-002-355 and NSC-94-3112-B-002-022) and the Department of Health (grants DOH91-DC-1008 and DOH92-DC-1203) of Taiwan, Republic of China.

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