Human infection with novel G3P[25] rotavirus strain in Taiwan

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Abstract

Genotype P[25] rotaviruses are rare and to date have been reported to occur only in a few countries of mainland Asia. Here we report the molecular characterization of a novel human rotavirus genotype combination, G3P[25], detected in a 17-month-old child hospitalized due to severe gastroenteritis during 2009 in central Taiwan. Sequencing and phylogenetic analysis of the VP4 gene demonstrated a distinct origin from other strains bearing the P[25] VP4 gene, whereas the VP7, VP6 and NSP4 gene phylogenies identified common origins with cognate genes of other, presumed human-porcine reassortment Taiwanese strains. These results suggest that interactions between human and animal strains appear to contribute to the generation of genetic and antigenic diversity of rotavirus strains, with potential public health importance in Taiwan.

Keywords: Genotype, phylogenetic analysis, reassortment, Taiwan, zoonosis

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Group A rotaviruses, a highly diverse group of pathogens within the family *Reoviridae*, are the main cause of acute dehydrating diarrhoea in childhood. Traditional classification of group A rotaviruses is based on a dual nomenclature to assign G and P serotype specificities for the neutralization antigens, VP7 and VP4, respectively [1]. Nucleotide sequencing has demonstrated a strong correlation between genotypes of the VP4 and VP7 genes and serotypes determined by neutralization. To date, at least 25 rotavirus G genotypes and 33 P genotypes have been identified in various species by nucleotide sequence-based classification [2,3], of which, 12 G types (G1-G6, G8-G12 and G20) and 15 P types (P[1]-P[11], P[14], P[19], P[25] and P[28]) have been identified in human strains. The globally common human G and P types include only five strains, G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8], but at least 60 additional G-P combinations have been found to occur in humans, often from geographically and temporally separated cases [1]. During the last 5 years numerous newly identified human rotavirus neutralization antigen specificities (P[7], P[25], P[28], G11 and G20) have been described [4–6], and close genetic relatedness to animal rotaviruses is commonly implicated in the origin and evolution of these new human antigen specificities. However, in several cases no closely related genes have been identified in any animal hosts investigated. Nonetheless, available evidence suggests that animal rotaviruses may cross the host species barriers through direct interspecies transmission on rare occasions, or, more frequently, by reassortment with human rotavirus strains [7]. The emergence of G9 and G12 VP7 specificities during the 1990s and 2000s demonstrates how certain antigens that are new to humans become epidemiologically important globally [8–17], presumably through reassortment of novel animal rotavirus genes into the genetic backbone of human Wa-like or DS1-like strains [18,19]. These emergences draw increasing attention to newly identified antigen specificities and reaffirm the importance of strain monitoring in defining the occurrence and spread of strains bearing novel neutralization antigens.

The genotype P[25] VP4 is among the numerous newly described human rotavirus antigen specificities; the first case was described only in 2005 [5]. Interestingly, the few genotype P[25] strains that have been detected to date in human infections were all in combination with G11 VP7 specificity.

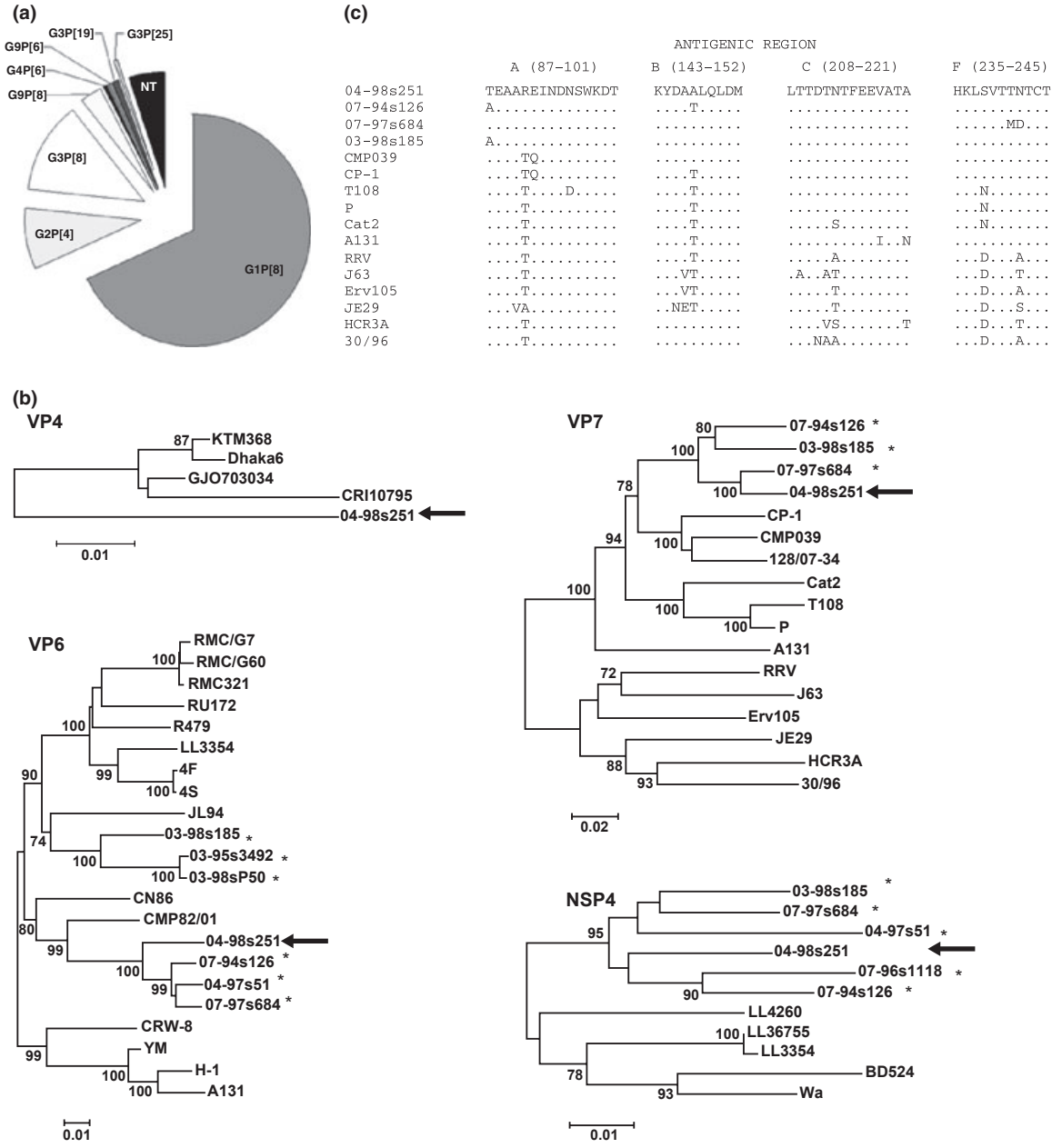

FIG. 1. Detection and molecular analysis of a Taiwanese G3P[25] strain, 04-98s251. (a) Prevalence data of human rotavirus strains detected during 2009 indicate low r G3P[25] (1 of 159, 0.6%). NT, partially non-typeable strains. (b) Neighbour-joining trees of rotavirus VP7, VP4, VP6 and NSP4 genes. Arrows indicate the novel G3P[25] strain, 04-98s251, identified in Taiwan, while asterisks show other gentically related Taiwanese strains. Each tree is prepared using single genotype specificities. Bootstrap values >60 are indicated. The scale bar is proportional to the genetic distance. The genotype combinations of other Taiwanese strains included in this analysis are: G3P[19] (03-98s185, 07-94s126, 07-97s684); G5P[19] (04-97s51); G9P[19] (07-96s1118); G4P[6] (03-95s3492); G5P[6] (03-98sP50). (c) Primary structure of the predicted VP7 antigenic sites A, B, C and F of Taiwanese and reference G3 rotavirus strains. Dots indicate amino acid residue identities in the alignment.

Independent detections of G11P[25] strains in Bangladesh [5], India [20], Nepal [21] and South Korea [unpublished, GenBank entry] suggest that this particular antigen combination may be more transmissible than a variety of other uncommon surface antigen combinations. Recent molecular characterizations of the Nepalese and the Bangladeshi G11P[25] strains identified a common evolutionary origin of the neutralization antigens, and revealed that acquisition of the backbone genes from the epidemiologically major human Wa-like rotavirus strains through reassortment may have been important in their subsequent

transmission in children. The complete genome configuration of these G11P[25] strains provided indirect evidence that the P[25] VP4 could have originated from an animal host, perhaps pigs, although additional sequence information about potential parental strains from the suspect animal hosts is needed to validate this hypothesis [1, 22]. In this report, we describe an unusual Taiwanese rotavirus genotype combination, G3P[25], that is novel in humans, and partially characterize its genome to help understand its evolutionary roots.

Among 159 strains genotyped during the 2009 surveillance season in Taiwan, one (0.6%) rotavirus strain, designated 04-98s251, was found to bear the G3P[25] genotype combination by using a nucleotide sequencing-based genotyping assay (Fig. 1a). This strain was identified in a 17-month-old boy with acute dehydrating diarrhoea treated at a hospital in Changhua, mid-Taiwan, during December 2009. The disease manifestation was severe, with main symptoms of fever (2 days with a maximum temperature of 38.5°C), vomiting (1 day duration, maximum two episodes/day) and diarrhoea (6 days duration, maximum seven episodes/day). Neither enteric bacterial pathogens nor other enteric viruses were detected during routine microbiological examination.

In an attempt to understand the evolutionary history of this strain, genes encoding surface antigens (VP7 and VP8*, the variable region of VP4), the inner capsid antigen (VP6) and the enterotoxin (NSP4) were further analysed. In brief, consensus primers spanning fragments (1062 bp, VP7; 1356 bp, VP6; 794 bp, VP4; 738 bp, NSP4) of each gene were used in gene-specific two-step RT-PCR assays and purified amplicons were sequenced using the same PCR primers [23]. Relevant gene sequences for phylogenetic analysis were retrieved from GenBank [24] and from our own sequence collection. To obtain phylogenetic trees for each gene we used the MEGA4 software (neighbour-joining method, Kimura two-parameter nucleotide substitution model, 500 bootstrap replicates) [25].

When the G3 VP7 gene in the strain 04-98s251 was analysed, no closely related strains were found in GenBank; the best match was seen with porcine and bovine-porcine reassortment rotavirus strains (e.g. CMP039 and PP-1; 89% nt identity). In contrast, three Taiwanese G3P[19] strains detected during 2005 (07–94s126, 92%), 2008 (07–97s684, 96%) and 2009 (03–98s185, 93%) were more closely related and clustered on a single common branch of the phylogenetic tree (Fig 1b). In the major antigenic regions this strain shared the highest similarities with three Taiwanese G3P[19] strains; of note, the arginine residue in position 91 (region A) seems to be conserved in all of these unusual Taiwanese strains (Fig 1c). The P[25] VP4 gene was moderately related to cognate VP4 gene sequences deposited in GenBank and seemed to represent an independent genetic lineage in the P[25] VP4 gene phylogeny (92–94% inter-lineage nt identity between 04-98s251 and CRI 10795, Dhaka6, GJ0703034 and KTM368; 96–99% intra-lineage nt similarity among CRI 10795, Dhaka6, GJ0703034 and KTM368). Similarly to the VP7 gene, only limited genetic relatedness was seen in the VP6 and NSP4 genes of the 04-98s251 strain to other strains in GenBank (VP6, 92% maximum nt identity to CMP82/01 and related genotype I5 porcine strains; NSP4, 92–93% maximum nt identity to LL4260 and related Wa-like, genotype E1 human strains), while most closely related strains were identified with a handful of Taiwanese P[19] rotavirus strains (VP6, 95% nt identity; NSP4 gene, 95% nt identity).

In conclusion, the molecular analysis of the unusual Taiwanese genotype P[25] strain demonstrated a lack of common evolutionary roots with other P[25] strains that were identified in mainland Asia during the last 5 years. The molecular analysis of the VP7, VP6 and NSP4 genes also confirmed that this and other unusual Taiwanese strains (e.g. P[19] strains [23]) had a distinct evolutionary pathway compared with the globally common strains, implying that animal, perhaps porcine, rotaviruses may contribute to the local strain diversity on this island. The perception that P[25] strains are repeatedly identified in parts of Asia is noteworthy and requires continuous monitoring to see if a more suitable genomic constellation generated by reassortment with the backbone human strains will facilitate the epidemiological spread of these newly described human rotavirus strains.

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

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Transparency Declaration

Conflicts of interest: nothing to declare.

References

1. Matthijnssens J, Bilcke J, Ciarlet M *et al.* Rotavirus disease and vaccination: impact on genotype diversity. *Future Microbiol* 2009; 4: 1303–1316.
2. Esona MD, Mijatovic-Rustempasic S, Conrardy C *et al.* Reassortant group A rotavirus from straw-colored fruit bat (*Eidolon helvum*). *Emerg Infect Dis* 2010; 16: 1844–1852.
3. Abe M, Ito N, Masatani T *et al.* Whole genome characterization of new bovine rotavirus G21P[29] and G24P[33] strains provides evidence for interspecies transmission. *J Gen Virol* 2011; 92: 952–960.
4. Esona MD, Geyer A, Bányai K *et al.* Novel human rotavirus genotype G5P[7] from child with diarrhea, Cameroon. *Emerg Infect Dis* 2009; 15: 83–86.
5. Rahman M, Matthijnssens J, Nahar S *et al.* Characterization of a novel P[25],G11 human group a rotavirus. *J Clin Microbiol* 2005; 43: 3208–3212.
6. Solberg OD, Hasing ME, Trueba G, Eisenberg JN. Characterization of novel VP7, VP4, and VP6 genotypes of a previously untypeable group A rotavirus. *Virology* 2009; 385: 58–67.
7. Martella V, Bányai K, Matthijnssens J, Buonavoglia C, Ciarlet M. Zoonotic aspects of rotaviruses. *Vet Microbiol* 2010; 140: 246–255.
8. Bányai K, Bogdán Á, Domonkos G *et al.* Genetic diversity and zoonotic potential of human rotavirus strains, 2003–2006, Hungary. *J Med Virol* 2009; 81: 362–370.
9. Carmona RC, Timenetsky Mdo C, Morillo SG, Richtzenhain LJ. Human rotavirus serotype G9, São Paulo, Brazil, 1996–2003. *Emerg Infect Dis* 2006; 12: 963–968.
10. Khamrin P, Peerakome S, Wongsawasdi L *et al.* Emergence of human G9 rotavirus with an exceptionally high frequency in children admitted to hospital with diarrhea in Chiang Mai, Thailand. *J Med Virol* 2006; 78: 273–280.
11. Steyer A, Poljsak-Prijatelj M, Barlic-Maganja D, Bufon T, Marin J. The emergence of rotavirus genotype G9 in hospitalised children in Slovenia. *J Clin Virol* 2005; 33: 7–11.
12. Bányai K, Bogdán A, Kisfali P *et al.* Emergence of serotype G12 rotaviruses, Hungary. *Emerg Infect Dis* 2007; 13: 916–919.
13. Mukherjee A, Chattopadhyay S, Bagchi P *et al.* Surveillance and molecular characterization of rotavirus strains circulating in Manipur, north-eastern India: increasing prevalence of emerging G12 strains. *Infect Genet Evol* 2010; 10: 311–320.
14. Cunliffe NA, Ngwira BM, Dove W *et al.* Serotype G12 rotaviruses, Lilongwe, Malawi. *Emerg Infect Dis* 2009; 15: 87–90.
15. Pun SB, Nakagomi T, Sherchand JB *et al.* Detection of G12 human rotaviruses in Nepal. *Emerg Infect Dis* 2007; 13: 482–484.
16. Iturriza-Gómara M, Dallman T, Bányai K *et al.* Rotavirus surveillance in Europe, 2005–2008: web-enabled reporting and real-time analysis of genotyping and epidemiological data. *J Infect Dis* 2009; 200 (Suppl 1): S215–S221.
17. Castello AA, Argüelles MH, Rota RP *et al.* Molecular epidemiology of group A rotavirus diarrhea among children in Buenos Aires, Argentina, from 1999 to 2003 and emergence of the infrequent genotype G12. *J Clin Microbiol* 2006; 44: 2046–2050.
18. Rahman M, Matthijnssens J, Yang X *et al.* Evolutionary history and global spread of the emerging G12 human rotaviruses. *J Virol* 2007; 81: 2382–2390.
19. Laird AR, Gentsch JR, Nakagomi T, Nakagomi O, Glass RI. Characterization of serotype G9 rotavirus strains isolated in the United States and India from 1993 to 2001. *J Clin Microbiol* 2003; 41: 3100–3111.
20. Banerjee I, Iturriza-Gomara M, Rajendran P *et al.* Molecular characterization of G11P[25] and G3P[3] human rotavirus strains associated with asymptomatic infection in South India. *J Med Virol* 2007; 79: 1768–1774.
21. Uchida R, Pandey BD, Sherchand JB *et al.* Molecular epidemiology of rotavirus diarrhea among children and adults in Nepal: detection of G12 strains with P[6] or P[8] and a G11P[25] strain. *J Clin Microbiol* 2006; 44: 3499–3505.
22. Matthijnssens J, Rahman M, Ciarlet M *et al.* Reassortment of human rotavirus gene segments into G11 rotavirus strains. *Emerg Infect Dis* 2010; 16: 625–630.
23. Wu FT, Bányai K, Huang JC *et al.* Diverse origin of P[19] rotaviruses in children with acute diarrhea in Taiwan: detection of novel lineages of the G3, G5, and G9 VP7 genes. *J Med Virol* 2011; 83: 1279–1287.
24. Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J, Sayers EW. GenBank. *Nucleic Acids Res* 2010; 38: D46–D51.
25. Tamura K, Dudley J, Nei M, Kumar S. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* 2007; 24: 1596–1599.