Molecular characterization of a rare G1P[19] rotavirus strain from India: evidence of reassortment between human and porcine rotavirus strains

Shobha D. Chitambar, Ritu Arora and Preeti Chhabra

Enteric Viruses Department, National Institute of Virology, Pune 411001, India

Correspondence

Shobha D. Chitambar
chitambar@hotmail.com

Received 14 May 2009
Accepted 9 August 2009

This study pertains to the characterization of a human rotavirus strain (NIV929893) with a rare specificity of G1P[19]. Three structural genes (VP4, VP6 and VP7) and one non-structural gene (NSP4) of strain NIV929893 were subjected to RT-PCR for amplification of entire coding regions. All of the amplicons were sequenced to carry out phylogenetic analysis. The complete amino acid sequences of the VP7 and VP4 gene products showed clustering of the VP7 gene with G1 strains of human origin and the VP4 gene with P[19] strains of porcine origin. The two viral proteins VP6 and NSP4, described previously as genetically linked proteins, were shown to be subgroup II and genotype B of human and porcine origins, respectively. The findings of this study provide evidence of reassortment between VP7/VP6 genes of humans and VP4/NSP4 genes of porcine species and an independent segregation of VP6 and NSP4 genes in a group A human rotavirus strain with G1P[19] specificity.

INTRODUCTION

Group A rotaviruses of the family *Reoviridae* are recognized as the single most common cause of non-bacterial acute gastroenteritis. The viral genome consists of 11 segments of dsRNA ranging from 0.6 to 3.3 kb, which encode six structural (VP1–4, VP6 and VP7) and six non-structural (NSP1–6) proteins (Estes, 2001). A dual classification system based on two outer-layer proteins, VP7 and VP4, has been established for rotaviruses. In recent years, the application of molecular methods has allowed the identification of 20 G and 28 P types in human and animal rotavirus infections (Solberg *et al.*, 2009). Ten G and 11 P types have been detected in human infections, although five G (G1–4 and G9) and two P (P[4] and P[8]) types are found most commonly (Santos & Hoshino, 2005; Grimwood & Kirkwood, 2008; Matthijssens *et al.*, 2008).

The middle-layer protein, VP6, determines the rotavirus group and subgroup (SG) specificities. More recently, on the basis of the NSP4 gene, rotaviruses have been classified into six genotypes: A (prototype strain KUN), B (Wa), C (AU-1), D (EW), E (avian-like) and F (Ch-1) (Horie *et al.*, 1997; Ciarlet *et al.*, 2000; Ito *et al.*, 2001; Mori *et al.*, 2002; Matthijssens *et al.*, 2008).

Surveillance of rotavirus strains in the human population has revealed variation in the distribution of G and P

genotypes, together with the emergence of unusual G/P combinations in different geographical locations (Gentsch *et al.*, 2005; Santos & Hoshino, 2005). Many such genotypes have been found in regions where animals live in close proximity to humans (Urasawa *et al.*, 1992). Human group A rotavirus strains, which carry genes commonly found in animal rotaviruses, have been isolated from infected children in both developed and developing countries. There is increasing evidence to demonstrate a significant contribution of animal rotaviruses in giving rise to reassortment and genetic diversity among human rotavirus strains (Varghese *et al.*, 2004; Maneekarn *et al.*, 2006). The possibility of the reassortant strains upholding their virulence in both species has been also described on account of genetic similarity between animal and human rotavirus strains (Nakagomi *et al.*, 1990; Urasawa *et al.*, 1992). In this context, porcine rotavirus genes VP4, VP6, NSP1, NSP3 and NSP4 have been reported to have a close relationship with their counterparts in human rotaviruses, and hence porcine rotaviruses are considered a potential source of interspecies rotavirus infection (Martella *et al.*, 2005).

The present study reports the characterization of a rotavirus strain with an unusual specificity of human VP7 (G1) and porcine VP4 (P[19]) genes identified in a rotavirus surveillance study conducted among children with diarrhoea (Zade *et al.*, 2009).

METHODS

Specimen. The rotavirus strain NIV929893 was isolated from a faecal specimen collected from an 11-month-old male patient

Abbreviation: SG, subgroup.

The GenBank/EMBL/DDBJ accession numbers for the sequences determined in this study are DQ886957 (VP7), DQ887060 (VP4), FJ685614 (VP6) and FJ685615 (NSP4).

hospitalized for acute gastroenteritis in Pune, western India, in July 1992. The specimen was collected within 24 h of hospitalization with prior informed consent from the parents. A 10% faecal suspension was prepared in 0.01 M PBS (pH 7.4) and rotavirus positivity was identified by antigen-capture ELISA (IDEIA Rotavirus; Dako). The specimen was stored in aliquots at -20°C until analysed.

RNA extraction and RT-PCR. Viral RNA was extracted from a 30% faecal suspension using Trizol LS Reagent (Invitrogen) according to the manufacturer's instructions. RT-PCR was carried out for full-length VP7 (1062 bp), VP4 (2359 bp), VP6 (1356 bp) and NSP4 (752 bp) genes using a One-step RT-PCR kit (Qiagen) and primers specific for each of the genes (Table 1). The PCR conditions involved an initial reverse transcription step of 30 min at 45°C , followed by PCR activation at 95°C for 15 min, 40 cycles of amplification (1 min at 94°C , 1 min at 45°C and 2.5 min at 70°C) and a final extension of 7 min at 70°C . All of the PCR products were electrophoresed in 2% agarose gels containing ethidium bromide ($0.5\text{ }\mu\text{g ml}^{-1}$) and visualized under a UV transilluminator.

Nucleotide sequencing and phylogenetic analyses. The PCR amplicons were excised from the gel for purification (QIAquick Gel Extraction kit; Qiagen). Sequencing was carried out using a BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems) and the sequence data were collected using an ABI 3130XL automated sequencer (Applied Biosystems). Multiple alignments were carried out using CLUSTAL W (Thompson *et al.*, 1994). Phylogenetic analyses were conducted using the MEGA v3.1 software program (Kumar *et al.*, 2004). The statistical significance of the relationships was estimated by bootstrap resampling analysis (1000 replications).

RESULTS

Sequence analysis of the VP7 gene

Phylogenetically, the VP7 gene of rotavirus strain NIV929893 segregated with the G1 genetic cluster, which contained the prototype strains KU and Wa, as well as other G1 strains from Pune, India, detected in human infections (Fig. 1a). Nucleotide and amino acid sequence identities were 92.0–99.9% and 95.7–100%, respectively. The strain showed only 73.0–76.2% nucleotide and 75.8–79.8% amino acid identity with selected strains of other G types (Table 2).

Sequence analysis of the VP4 gene

The VP4 gene of strain NIV929893, when analysed phylogenetically, clustered with P[19] rotavirus strains

indicating a porcine origin (Fig. 1b). The nucleotide and deduced amino acid sequences showed 96.2–97.4% and 97–98.8% identities, respectively, with Mc323, Mc345, RMC/G60, RMC321 and RMC/G7 strains of P[19] specificity and only 71.8–77.2% and 59.7–73.6% nucleotide and amino acid identities with selected strains of other P genotypes (Table 2).

Sequence analysis of the VP6 gene

Comparative analysis of the sequences of the VP6 gene of strain NIV929893 showed 92.3–92.8% nucleotide and 98.2–99% amino acid identities with human rotavirus strains Dhaka12-03, Matlab13-03, RMC100 and RMC437, and 81.2–82.3% and 93.5–93.7% nucleotide and amino acid identities, respectively, with porcine rotavirus strains OSU and YM (Fig. 2a, Table 2). The strain was placed in SGII on the basis of amino acid residues at positions 248 (Phe), 305 (Asn), 306 (Ala), 310 (Gln) and 315 (Gln) of VP6, which are all conserved in SGII rotaviruses (Greig *et al.*, 2006).

Sequence analysis of the NSP4 gene

The nucleotide and corresponding amino acid sequences of the NSP4 gene of strain NIV929893 were compared with those of rotavirus strains representing genotypes A–F. Phylogenetically, the NSP4 gene of strain NIV929893 grouped with genotype B and was closely related to the NSP4 gene of rotavirus strain RMC/G7 with 98.4 and 99.4% nucleotide and amino acid identities, respectively, indicating porcine origin (Fig. 2b, Table 2). However, nucleotide and amino acid identities with genotype B strains of human origin were 88.8–89.4% and 90.9–93.7%. With the other genotypes – A, C, D, E and F – the nucleotide and amino acid identities were 78.9–82.6% and 81.7–85.7%, 77.3–80.9% and 82.3–82.9%, 66.7–67.2% and 62.9–64%, 49.8–51.9% and 29–31.4%, and 50.5% and 31.5%, respectively.

DISCUSSION

Group A rotaviruses are the most common cause of diarrhoea in humans and animals. Rotaviruses are

Table 1. List of primers used in this study

Gene	Primer name	Primer sequence (5'→3')	Reference
VP7	C2	GGCTTTAAAAGAGAGAATTTCCGTCTGG	Gouvea <i>et al.</i> (1990)
	C1	GGTCACATCATACAATTCTAATCTAAG	Gouvea <i>et al.</i> (1990)
VP4	VP4, Gen F	GGCTATAAAATGGCTTCGCTCA	Matthijnssens <i>et al.</i> (2006)
	VP4, Gen R	GGTCACATCCTCAATAGCGTTCT	Matthijnssens <i>et al.</i> (2006)
VP6	VP6-R1F	GGTCACATCCTCAATAGCGTTCT	This study
	VP6-1366R	GGCTTTTAAACGAAGTCTTC	This study
NSP4	NSP4F	TAAAGATTCTGTCCGAGAGAG	This study
	NSP4 722R	TTAAGACCGTTCCTCCATTAAC	This study

Fig. 1. Phylogenetic analysis of complete nucleotide sequences of VP7 (a) and VP4 (b) genes of rotavirus strain NIV929893. Clusters containing the NIV929893 strain are boxed, and for each strain the following data are given: GenBank accession number/strain name/genotype.

generally species-specific; however, cross-species transmission has been described in several studies (Okada *et al.*, 2000; Varghese *et al.*, 2004). The segmented nature of the rotavirus genome and a close genetic relationship between human and animal rotavirus strains favours generation of reassortants during mixed infection resulting from two different strains. The present study reports characterization of one such reassortant, G1P[19], which caused infection in a human.

Table 2. Comparative nucleotide and amino acid sequence identities of strain NIV929893 with other rotavirus strains

NA, Sequence information not available. High nucleotide and amino acid identities are indicated in bold.

Strain	Nucleotide/amino acid identities (%)			
	VP7	VP4	VP6	NSP4
G1P[8]/KU	92.5/96.4	77.2/73.6	91.1/98.2	90.1/93.1
G2P[4]/TB-Chen	73.1/76.3	76.4/72.1	80.8/92.4	82.3/83.4
G5P[7]/OSU	75.4/77.0	NA	81.2/93.7	94.4/96.6
G11P[7]/YM	73.7/79.1	71.8/59.7	82.3/93.5	91.8/94.9
G9P[19]/RMC321	76.2/79.8	97.3/98.2	82.0/93.5	97.0/97.7
G9P[19]/RMC/G7	75.8/78.9	97.4/98.8	82.3/93.2	98.4/99.4
G3P[19]/Mc345	75.5/75.8	96.7/98.2	NA	NA

Globally, rotaviruses of the G1–G4, G9, P[4] and P[8] genotypes have been found to be a common cause of rotavirus diarrhoea in humans. G1 is the most commonly found genotype in human rotavirus infections. Although the combination of G1 with P[8] is well known worldwide, the combination of G1 with other P types has also been reported (Santos & Hoshino, 2005).

P[19], a rare genotype, was first described for a porcine rotavirus strain, 4F, with G3 specificity (Burke *et al.*, 1994). Subsequently, G3P[19] was isolated from faecal specimens of piglets with diarrhoea in Thailand (Maneeakarn *et al.*, 2006; Theamboonlers *et al.*, 2008). However, in human infection, P[19] was identified with G9 strains from Thailand and India (Okada *et al.*, 2000; Varghese *et al.*, 2004). The study presented here documents for the first time, to our knowledge, the presence of P[19] specificity in association with G1.

In addition to the binary classification based on VP7 and VP4 genes, which display multiple combinations of G and P types, rotaviruses are also classified on the basis of the less diverse VP6 and NSP4 genes (Iturriza-Gómara *et al.*, 2003). VP6 is a major structural protein that interacts with the outer structural proteins VP4 and VP7 and the core protein VP2, whilst NSP4 is a non-structural protein that has been described as a receptor for VP6 during viral morphogenesis (Iturriza-Gómara *et al.*, 2003; Varghese *et al.*, 2004). Among human rotaviruses, VP6 associates mainly with two subgroup specificities, SGI and SGII,

Fig. 2. Phylogenetic analysis of complete nucleotide sequences of the VP6 (a) and NSP4 (b) genes of rotavirus strain NIV929893. Clusters containing the NIV929893 strain are boxed, and for each strain the following data are given: GenBank accession number/strain name/genotype.

whilst NSP4 is restricted to two (A and B) of the six genotypes A–F (Iturriza-Gómara *et al.*, 2002). In a study conducted on rotavirus strains isolated from humans, it was suggested that both VP6 and NSP4 genes were linked to each other in common and reassortant rotavirus strains with 100 % linkage (Iturriza-Gómara *et al.*, 2003). Accordingly, SGI of VP6 occurs in association with NSP4 genotype A, whilst SGII of VP6 associates with NSP4 genotype B. However, evidence of a VP6 gene related to bovine and human SGI strains, and a porcine NSP4 gene of genotype B has been documented for porcine group A rotavirus strains HP113 and HP140 (Ghosh *et al.*, 2007).

In the present study, a VP6 gene with SGII specificity and an NSP4 gene of genotype B were identified in strain NIV929893. Interestingly, phylogenetic analysis of the deduced amino acid sequences of these genes showed a human origin for the VP6 gene and a porcine origin for the NSP4 gene. Thus, the findings of this study suggest that the linkage between the NSP4 genotype and VP6 subgroup appears to be vital in group A rotaviruses that cause infections in humans. Although the reassortment resulted from independently segregated genes, a combination of VP6

SGII and NSP4 genotype B was maintained in the recovered human strain, NIV929893. Independent segregation of VP6 and NSP4 genes has been reported previously in asymptomatic rotavirus infections (Banerjee *et al.*, 2007).

To conclude, studies on the diversity of human rotavirus VP7, VP4, VP6 and NSP4 genes are of ecological importance and can provide insight into the mechanism involved in rotavirus evolution, interspecies transmission and exchange of genetic material during reassortment. The present study provides novel information on the occurrence of the unusual human rotavirus strain G1P[19] and direct evidence to support the interspecies transmission and reassortment of human and porcine rotaviruses in nature. Identification of such novel rotavirus reassortant strains is of epidemiological importance and can lead to serious implications regarding rotavirus vaccine development and implementation.

ACKNOWLEDGEMENTS

We thank Dr A. C. Mishra, Director, National Institute of Virology, Pune, India, for his constant support.

REFERENCES

- Banerjee, I., Iturriza-Gomara, M., Rajendran, P., Primrose, B., Ramani, S., Gary, J. J., Brown, D. W. & Kang, G. (2007). Molecular characterization of G11P[25] and G3P[3] human rotavirus strains associated with asymptomatic infections in south India. *J Med Virol* 79, 1768–1774.
- Burke, B., McCrae, M. A. & Desselberger, U. (1994). Sequence analysis of two porcine rotaviruses differing in growth *in vitro* and in pathogenicity: distinct VP4 sequences and conservation of NS53, VP6 and VP7 genes. *J Gen Virol* 75, 2205–2212.
- Ciarlet, M., Liprandi, F., Conner, M. E. & Estes, M. K. (2000). Species specificity and interspecies relatedness of NSP4 genetic groups by comparative NSP4 analysis of animal rotaviruses. *Arch Virol* 145, 371–383.
- Estes, M. K. (2001). Rotaviruses and their replication. In *Fields Virology*, 4th edn, pp. 1747–1785. Edited by D. M. Knipe & P. M. Howley. Philadelphia: Lippincott Williams & Wilkins.
- Gentsch, J. R., Laird, A. R., Bielfelt, B., Griffin, D. D., Banyai, K., Ramachandran, M., Urasawa, S., Hasegawa, A., Urasawa, T. & other authors (2005). Serotype diversity and reassortment between human and animal rotavirus strains: implications for rotavirus vaccine programs. *J Infect Dis* 192, S146–S159.
- Ghosh, S., Varghese, V., Samajdar, S., Bhattacharya, S. K., Kobayashi, N. & Naik, T. N. (2007). Evidence for independent segregation of the VP6- and NSP4-encoding genes in porcine group A rotavirus G6P[13] strains. *Arch Virol* 152, 423–429.
- Gouvea, V., Glass, R. I., Woods, P., Taniguchi, K., Clark, H. F., Forrester, B. & Fang, Z. Y. (1990). Polymerase chain reaction amplification and typing of rotavirus nucleic acid from stool specimens. *J Clin Microbiol* 28, 276–282.
- Greig, S. L., Berriman, J. A., O'Brien, J. A., Taylor, J. A., Bellamy, A. R., Yeager, M. J. & Mitra, A. K. (2006). Structural determinants of rotavirus subgroup specificity mapped by cryo-electron microscopy. *J Mol Biol* 356, 209–221.
- Grimwood, K. & Kirkwood, C. D. (2008). Human rotavirus vaccines: too early for the strain to tell. *Lancet* 371, 1144–1145.
- Horie, Y., Masamune, O. & Nakagomi, O. (1997). Three major alleles of rotavirus NSP4 proteins identified by sequence analysis. *J Gen Virol* 78, 2341–2346.
- Ito, H., Sugiyama, M., Masubuchi, Y., Mori, Y. & Minamoto, N. (2001). Complete nucleotide sequence of a group A avian rotavirus genome and a comparison with counterparts of mammalian rotaviruses. *Virus Res* 75, 123–138.
- Iturriza-Gomara, M., Wong, C., Blome, S., Desselberger, U. & Gray, J. (2002). Molecular characterization of VP6 genes of human rotavirus isolates: correlation of genogroups with subgroups and evidence of independent segregation. *J Virol* 76, 6596–6601.
- Iturriza-Gomara, M., Anderton, E., Kang, G. & Gallimore, C. (2003). Evidence for genetic linkage between the gene segments encoding NSP4 and VP6 proteins in common and reassortant human rotavirus strains. *J Clin Microbiol* 41, 3566–3573.
- Kumar, S., Tamura, K., Jacobsen, I. B. & Nei, M. (2004). MEGA3: integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. *Brief Bioinform* 5, 150–163.
- Maneekarn, N., Khamrin, P., Chan-it, W., Peerakome, S., Sukchai, S., Pringprao, K. & Ushijima, H. (2006). Detection of rare G3P[19] porcine rotavirus strains in Chiang Mai, Thailand, provides evidence for origin of the VP4 genes of Mc323 and Mc345 human rotaviruses. *J Clin Microbiol* 44, 4113–4119.
- Martella, V., Ciarlet, M., Baselga, R., Arista, S., Elia, G., Lorusso, E., Banyai, K., Terio, V., Madio, A. & other authors (2005). Sequence analysis of the VP7 and VP4 genes identifies a novel VP7 gene allele of porcine rotaviruses, sharing a common evolutionary origin with human G2 rotaviruses. *Virology* 337, 111–123.
- Matthijnssens, J., Rahman, M., Martella, V., Xuelei, Y., De Vos, S., De Leener, K., Ciarlet, M., Buonavoglia, C. & Van Ranst, M. (2006). Full genomic analysis of human rotavirus strain B4106 and lapine rotavirus strain 30/96 provides evidence for interspecies transmission. *J Virol* 80, 3801–3810.
- Matthijnssens, J., Ciarlet, M., Heiman, E., Arijs, I., Delbeke, T., McDonald, S. M., Palombo, E. A., Iturriza-Gomara, M. I., Maes, P. & other authors (2008). Full genome-based classification of rotaviruses reveals a common origin between human Wa-like and porcine rotavirus strains and human DS-1-like and bovine rotavirus strains. *J Virol* 82, 3204–3219.
- Mori, Y., Borgan, M. A., Ito, N., Sugiyama, M. & Minamoto, N. (2002). Diarrhoea inducing activity of avian rotavirus NSP4 glycoproteins, which differ greatly from mammalian rotavirus NSP4 glycoproteins in deduced amino acid sequence, in suckling mice. *J Virol* 76, 5829–5834.
- Nakagomi, O., Ohshima, A., Aboudy, Y., Shif, I., Mochizuki, M., Nakagomi, T. & Gotlieb-Stematsky, T. (1990). Molecular identification by RNA-RNA hybridization of a human rotavirus that is closely related to rotaviruses of feline and canine origin. *J Clin Microbiol* 28, 1198–1203.
- Okada, J., Urasawa, T., Kobayashi, N., Taniguchi, K., Hasegawa, A., Mise, K. & Urasawa, S. (2000). New P serotype of group A human rotavirus closely related to that of a porcine rotavirus. *J Med Virol* 60, 63–69.
- Santos, N. & Hoshino, Y. (2005). Global distribution of rotavirus serotypes/genotypes and its implication for the development and implementation of an effective rotavirus vaccine. *Rev Med Virol* 15, 29–56.
- Solberg, O. D., Hasing, M. E., Trueba, G. & Eisenberg, J. N. (2009). Characterization of novel VP7, VP4, and VP6 genotypes of a previously untypeable group A rotavirus. *Virology* 385, 58–67.
- Theamboonlers, A., Bhattarakosol, P., Chongsrisawat, V., Sungkapalee, T., Wuthirattanakowit, N. & Poovorawan, Y. (2008). Molecular characterization of group A human rotaviruses in Bangkok and Buriram, Thailand during 2004–2006 reveals the predominance of G1P[8], G9P[8] and a rare G3P[19] strain. *Virus Genes* 36, 289–298.
- Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 22, 4673–4680.
- Urasawa, S., Hasegawa, A., Urasawa, T., Taniguchi, K., Wakasugi, F., Suzuki, H., Inouye, S., Pongprot, B., Supawadee, J. & other authors (1992). Antigenic and genetic analyses of human rotaviruses in Chiang Mai, Thailand: evidence for a close relationship between human and animal rotaviruses. *J Infect Dis* 166, 227–234.
- Varghese, V., Das, S., Singh, N. B., Kojima, K., Bhattacharya, S. K., Krishnan, T., Kobayashi, N. & Naik, T. N. (2004). Molecular characterization of a human rotavirus reveals porcine characteristics in most of the genes including VP6 and NSP4. *Arch Virol* 149, 155–172.
- Zade, J. K., Chhabra, P. & Chitambar, S. D. (2009). Characterization of VP7 and VP4 genes of rotavirus strains: 1990–1994 and 2000–2002. *Epidemiol Infect* 137, 936–942.