ドペプチダーゼ処理により感染性の上昇がみられた。一方、KU//rVP4-R247K および KU// rVP4-R231K,R247K は、アルギニルエンドペプチダーゼ処理により感染性の上昇がなく、R247 が重要であることが示された。

ロタウイルス感染性の獲得には R247 における切断が必須であるが、残る２個のアルギニン残基（R231 および R241）における切断は必須ではない可能性が示唆された

今後、リバースジェネティクス系とアルギニルエンドペプチダーゼ/リシルエンペプチダーゼを用いることで、各残基における切断の重要性を詳細に検討したい

ヘルパーウイルスを利用しないリバースジェネティクス系の確立への試みを行った。しかしながら、いまだ成功していない。今後、引き続き、現状の条件下での VP4 の解析を続けるとともに、ヘルパーウイルスを利用しないリバースジェネティクス系の確立への試みを行いたい。

D. 研究発表

論文発表

1. Taniguchi K, Komoto S: Genetics and reverse genetics of rotavirus. Curr Opin Virol 2:399-407, 2012
2. Ghosh S, Shintani T, Urushibara N, Taniguchi K, Kobayashi N: Whole genomic analysis of a human G1P[9] rotavirus strain reveals intergenomic reassortment events. J Gen Virol 98:1700-1705, 2012
3. Rahman S, Higo-Moriguchi K, Htun KW, Taniguchi K, Icatlo FC, Tsuji T, Kodama Y, Nguyen SV, Umeda K, Oo HN, Myint YY, Htut T, Myint SS, Thura K, Thu HM,Fatmawati NND, Oguma K: Randomized placebo-controlled clinical trial of immunoglobulin Y as adjunct to standard supportive therapy for rotavirus-associated diarrhea among pediatric patients. Vaccine 30:4661-4669, 2012
4. Sugata K, Taniguchi K, Yui A, Nakai H, Asano Y, Hashimoto S, Ihira M, Yagasaki H, Takahashi Y, Kojima S, Matsumoto K, Kato K, Yoshikawa T.: Analysis of rotavirus antigenemia in hematopoietic stem cell transplant recipients. Transplant Infect Dis 14(1):49-56, 2012.
5. Kawamura Yoshiki, Sugata Ken, Nakai Hidetaka, Asano Yoshizo, Ohashi Masahiro, Kato Tomochika, Nishimura Naoko, Ozaki Takao, Yui Akiko, Taniguchi Koki, Yoshikawa Tetsushi: Correlation between serum matrix metalloproteinase and antigenemia levels in patients infected with rotavirus. J Med Virol 84:986-991, 2012

学会発表

1. 河本聡志、冨田万祐子、谷口孝喜：ロタウイルスの VP4 トリプシン開裂

部位の解析. 第 60 回日本ウイルス
学会、大阪、2012

E. 知的財産権の出願、登録状況

1. 特許取得: なし。
2. 実用新案登録: なし。
3. その他: なし。

III.　研究成果の刊行に関する一覧表

別紙５

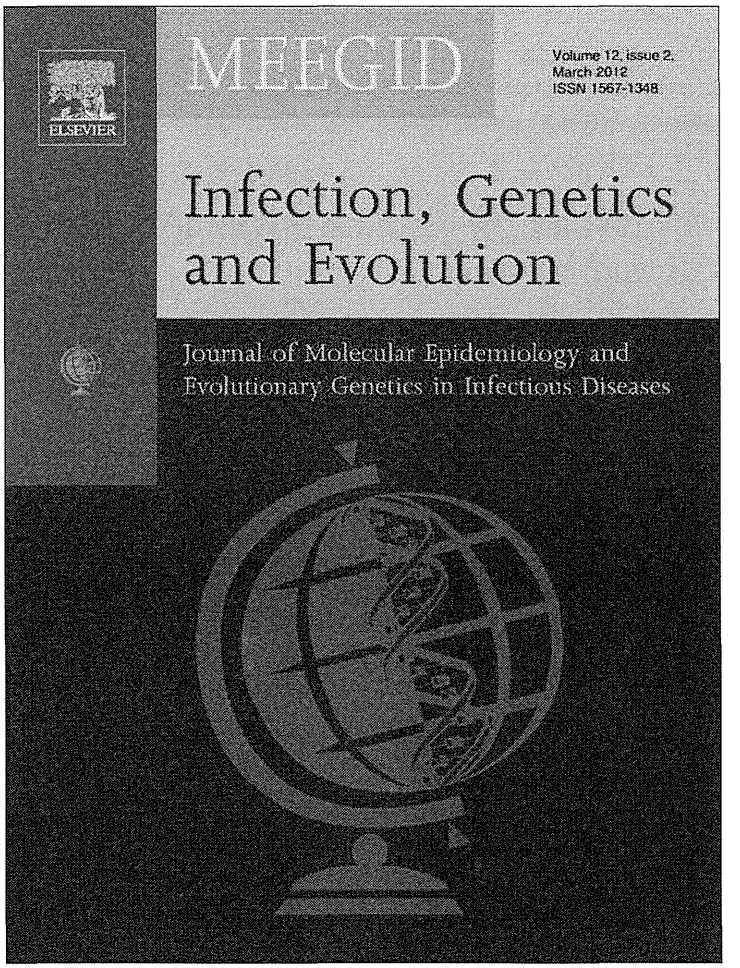
研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Ghosh S, Urushibara N, Taniguchi K, Kobayashi N.	Whole genomic analysis reveals the porcine origin of human G9P[19] rotavirus strains Mc323 and Mc345.	Infect Genet Evol.	12	471-477	2012
Ghosh S, Shintani T, Urushibara N, Taniguchi K, Kobayashi N	Whole genomic analysis of a human G1P[9] rotavirus strain reveals intergenogroup reassortment events.	J Gen Virol	93	1700-1705	2012
Shintani T, Ghosh S, Wang Y-H, Zhou X, Zhou D-J, Kobayashi N	Whole genomic analysis of human G1P[8] rotavirus strains from different age groups in China.	Viruses	4	1289-1304	2012
Ghosh S, Urushibara N, Kawaguchiya M, Shintani T, Kobayashi N	The origin of two rare human P[10] rotavirus strains.	Infect Genet Evol	13	292-300	2013
Taniguchi K, Komoto S	Genetics and reverse genetics of rotavirus.	Curr Opin Virol	2	399-40	2012
Rahman S, Higo-Moriguchi K, Htun KW, Taniguchi K, Icatlo FC, Tsuji T, Kodama Y, Nguyen SV, Umeda K, Oo HN, Myint YY, Htut T, Myint SS, Thura K, Thu HM,Fatmawati NND, Oguma K	Randomized placebo-controlled clinical trial of immunoglobulin Y as adjunct to standard supportive therapy for rotavirus-associated diarrhea among pediatric patients.	Vaccine	30	4661-4669	2012
Sugata K, Taniguchi K, Yui A, Nakai H, Asano Y, Hashimoto S, Ihira M, Yagasaki H, Takahashi Y, Kojima S, Matsumoto K, Kato K, Yoshikawa T.	Analysis of rotavirus antigenemia in hematopoietic stem cell transplant recipients.	Transplant Infect Dis	14	49-56	2012

Kawamura Yoshiki, Sugata Ken, Nakai Hidetaka, Asano Yoshizo, Ohashi Masahiro, Kato Tomochika, Nishimura Naoko, Ozaki Takao, Yui Akiko, Taniguchi Koki, Yoshikawa Tetsushi	Correlation between serum matrix metalloproteinase and antigenemia levels in patients infected with rotavirus.	J Med Virol	84	986-991	2012
Nagaoka Y, Tatsumi M, Tsugawa T, Yoto Y, Tsutsumi H.	Phylogenetic and computational structural analysis of VP7 gene of group a human rotavirus G1P[8] strains obtained in Sapporo, Japan from 1987 to 2000.	Journal of medical virology	84	832-838	2012
Fujii Y, Shimoike T, Takagi H, Murakami K, Todaka-Takai R, Park Y, Katayama K	mplification of all 11 RNA segments of group A rotavirus based on reverse transcription polymerase chain reaction	Microbiol Immunol.	56	630-638	2012
Noguchi A, Nakagomi T, Kimura S, Takahashi Y, Matsuno K, Koizumi H, Watanabe A, Noguchi H, Ito T, Ohtsuka M, Uemura N, Takeda O, Komatsu A, Kikuchi W, Komatsu M, Fukaya H, Miura S, Toda H, Nakagomi O, Takahashi T	Incidence of intussusception as studied from a hospital-based retrospective survey over a 10-year period (2001-2010) in Akita Prefecture	Japan. Jpn J Infect Dis	65	301-305	2012

IV.　研究成果の刊行物・別冊



Infection, Genetics and Evolution 12 (2012) 471–477

Contents lists available at SciVerse ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Short communication

Whole genomic analysis reveals the porcine origin of human G9P[19] rotavirus strains Mc323 and Mc345

Souvik Ghosh [a,*], Noriko Urushibara [a], Koki Taniguchi [b], Nobumichi Kobayashi [a]

[a] Department of Hygiene, Sapporo Medical University School of Medicine, Sapporo, Japan
[b] Department of Virology and Parasitology, School of Medicine, Fujita Health University, Toyoake, Japan

ARTICLE INFO

Article history:
Received 26 October 2011
Received in revised form 25 November 2011
Accepted 13 December 2011
Available online 27 December 2011

Keywords:
Group A rotavirus
Human P[19] strains
Whole genomic analysis
Porcine origin

ABSTRACT

The group A rotavirus (RVA) P[19] is a rare P-genotype of the RVA VP4 gene, reported so far in humans and pigs. Whole genomic analyses of P[19] strains are essential to study their origin and evolutionary patterns. To date, all the 11 genes of only two P[19] strains, RVA/Human-wt/IND/RMC321/1990/G9P[19] and RVA/Human-wt/IND/mani-97/2006/G9P[19], have been analyzed, providing evidence for their porcine origin. In the present study, the whole genomes of the first reported human P[19] strains, RVA/Human-tc/THA/Mc323/1989/G9P[19] and RVA/Human-tc/THA/Mc345/1989/G9P[19], were analyzed. Strains Mc323 and Mc345 exhibited a G9-P[19]-I5-R1-C1-M1-A8-N1-T1-E1-H1 genotype constellation. With the exception of the NSP5 gene, both the strains were closely related to each other. Most of the genes of Mc323 (VP2–4, VP6–7, NSP1–4 genes) and Mc345 (VP2–4, VP6–7 and NSP1–5 genes) appeared to be of porcine origin, whilst the exact origin of VP1 and NSP5 genes of Mc323 and VP1 gene of Mc345 could not be ascertained. Therefore, strains Mc323 and Mc345 were found to have a porcine RVA genetic backbone, and are likely of porcine origin. Taken together, our observations corroborated the hypothesis that P[19] strains might be derived from porcine RVAs, providing important insights into the origin of P[19] strains, and on interspecies transmission of RVAs.

1. Introduction

Group A rotavirus (RVA) is a major cause of acute diarrhea in the young of humans and animals (Estes and Kapikian, 2007). P[19] is a rare P-genotype of the RVA VP4 gene, reported so far in humans and pigs (Burke et al., 1994; Krishnan et al., 1994; Maneekarn et al., 2006; Mukherjee et al., 2010; Nguyen et al., 2008; Urasawa et al., 1992; Varghese et al., 2004; Wu et al., 2011; Zade et al., 2009). P[19] was first identified in a porcine strain, RVA/Pig-tc/CHN/4F/1986/G3P[19] (Burke et al., 1994). In humans, the P[19] genotype was first detected in strains RVA/Human-tc/THA/Mc323/1989/G9P[19] (Mc323) and RVA/Human-tc/THA/Mc345/1989/G9P[19] (Mc345) (Okada et al., 2000). Thereafter, only a few human P[19] strains have been reported in combination with G1, G3, G5 and G9 VP7 genes from India (Krishnan et al., 1994; Mukherjee et al., 2010; Varghese et al., 2004; Zade et al., 2009), Taiwan (Wu et al., 2011) and Vietnam (Nguyen et al., 2008). G3P[19] strains have been also detected in pigs in Thailand (Maneekarn et al., 2006).

Whole genomic analyses of RVA strains are essential to obtain conclusive data on the true origin of a strain, and trace its evolutionary pattern (Ghosh and Kobayashi, 2011; Matthijnssens et al., 2008, 2011). To date, all the 11 gene segments of only two human P[19] strains, RVA/Human-wt/IND/RMC321/1990/G9P[19] (RMC321) and RVA/Human-wt/IND/mani-97/2006/G9P[19] (mani-97) have been analyzed, providing evidence for their porcine origin (Mukherjee et al., 2010, 2011; Varghese et al., 2004, 2006). However, only short-length nucleotide sequences of the VP1–3 genes (653 bp, 651 bp and 662 bp, respectively) of strain RMC321 and VP1–4 genes (651 bp, 622 bp, 623 bp and 839 bp, respectively) of strain mani-97 have been determined, and analysis of strain RMC321 was based on deduced amino acid sequences (Mukherjee et al., 2010, 2011; Varghese et al., 2004, 2006). Partial genomic analyses of the other human P[19] strains have also revealed the presence of porcine-like gene segments (Chitambar et al., 2009; Kojima et al., 1996; Nguyen et al., 2008; Okada et al., 2000; Wu et al., 2011). However, the overall genetic makeup and evolutionary patterns of these strains remain to be elucidated. Moreover, as strains RMC321 and mani-97 were detected in the same geographical region (state of Manipur, India) (Mukherjee et al., 2010; Krishnan et al., 1994; Varghese et al., 2004), whole genomic analyses of P[19] strains from other countries might be useful to gain a proper understanding of the little-known evolutionary

Abbreviations: RVA, group A rotavirus; bp, base pair; ORF, open reading frame.

* Corresponding author. Address: Department of Hygiene, Sapporo Medical University School of Medicine, S 1, W 17, Chuo-Ku, Sapporo, Hokkaido 060-8556, Japan. Tel.: +81 11 611 2111x2733; fax: +81 11 612 1660.
E-mail addresses: souvikrota@gmail.com, souvik8@rediffmail.com (S. Ghosh).

1567-1348/$ - see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.meegid.2011.12.012

Table 1
Genotype nature of the 11 gene segments of group A rotavirus (RVA) G9P[19] strains Mc323 and Mc345 with those of selected human and animal RVA strains with known genomic constellations.

Strain	Genotypes										
	VP7	**VP4**	**VP6**	**VP1**	**VP2**	**VP3**	**NSP1**	**NSP2**	**NSP3**	**NSP4**	**NSP5**
RVA/Human-tc/THA/Mc323/1989/G9P[19]	G9	P[19]	I5	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Human-tc/THA/Mc345/1989/G9P[19]	G9	P[19]	I5	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Human-tc/USA/Wa/1974/G1P1A[8]	G1	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-tc/IND/NIV929893/1992/G1P[19]	G1	P[19]	I1	–	–	–	–	–	–	E1	–
RVA/Human-wt/TWN/03-98s185/xxxx/G3P[19][a]	G3	P[19]	I1	–	–	–	–	–	–	E1	–
RVA/Human-wt/TWN/07-94s126/xxxx/G3P[19][a]	G3	P[19]	I1	–	–	–	–	–	–	E1	–
RVA/Human-wt/TWN/07-97s684/xxxx/G3P[19][a]	G3	P[19]	I1	–	–	–	–	–	–	E1	–
RVA/Pig-tc/CHN/4F/1986/G3P[19]	G3	P[19]	I5	–	–	–	–	–	–	–	–
RVA/Human-wt/IND/mani-253/2007/G4P[4]	G4	P[4]	I1	R1	C1	M2	A8	N1	T1	E1	H1
RVA/Human-wt/IND/mani-362/2007/G4P[6]	G4	P[6]	I1	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Pig-tc/USA/Gottfried/1975/G4P[6]	G4	P[6]	I1	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Pig-wt/THA/CMP90/01/2001/G4P[6]	G4	P[6]	I5	–	–	–	–	–	–	–	–
RVA/Human-wt/TWN/04-97s51/xxxx/G5P[19][a]	G5	P[19]	I1	–	–	–	–	–	–	E1	–
RVA/Pig-wt/IND/HP113/2002/G6P[13]	G6	P[13]	I2	–	–	–	–	–	–	E1	H1
RVA/Pig-wt/IND/HP140/2002/G6P[13]	G6	P[13]	I2	R1	C1	M1	–	–	–	E1	H1
RVA/Human-wt/IND/mcs-13/2007/G9P[6]	G9	P[6]	I1	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Pig-xx/KOR/PRG9121/2006/G9P[7]	G9	P[7]	I5	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Human-wt/BEL/B3458/2003/G9P[8]	G9	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-wt/IND/RMC321/1990/G9P[19]	G9	P[19]	I5	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-wt/IND/RMC/G7/1991/G9P[19]	G9	P[19]	I5	–	–	–	A1	–	–	E1	H1
RVA/Human-wt/IND/RMC/G60/1992/G9P[19]	G9	P[19]	I5	–	–	–	–	–	–	E1	H1
RVA/Human-wt/IND/mani-97/2006/G9P[19]	G9	P[19]	I5	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Human-wt/TWN/07-96s1118/xxxx/G9P[19][a]	G9	P[19]	I12	–	–	–	–	–	–	E1	–
RVA/Human-wt/VNM/VN375/2003/G9P[19]	G9	P[19]	I5	–	–	–	–	–	–	–	–
RVA/Pig-xx/KOR/PRG942/2006/G9P[23]	G9	P[23]	I5	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Pig-xx/KOR/PRG9235/2006/G9P[23]	G9	P[23]	I5	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Pig-wt/THA/CMP45/08/2008/G9P[23]	G9	P[23]	I5	–	–	–	–	–	–	E1	H1
RVA/Human-wt/ECU/EC2184/2005/G11P[6]	G11	P[6]	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Pig-tc/MEX/YM/1983/G11P9[7]	G11	P[7]	I5	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Pig-wt/IND/RU172/2002/G12P[7]	G12	P[7]	I5	R1	C1	M1	A1	N1	T1	E1	H1

Dark gray indicates the gene segments with a genotype identical to that of strain Mc323.
"–" indicates that no sequence data were available in the GenBank database.
[a]Genotype assignment based on those reported by Wu et al. (2011). To our knowledge, to date, the nucleotide sequence accession numbers for the VP4, VP6–7 and NSP4 genes of strains 03-98s185, 07-94s126, 07-97s684, 04-97s51 and 07-96s1118 are not available in the GenBank database.

patterns of P[19] RVAs. Therefore, in the present study, we analyzed the whole genomes of the first reported human P[19] strains, Mc323 and Mc345.

2. Materials and methods

2.1. Virus strains

Strains Mc323 and Mc345 were detected in stool samples collected from patients with acute diarrhea in the city of Chiang Mai, Thailand, in 1989 (Urasawa et al., 1992). Both strains were successfully isolated by tissue culture in MA-104 cells, and stored at −80 °C till further analysis.

2.2. RT-PCR, nucleotide sequencing and sequence analyses

RT-PCR, nucleotide sequencing and sequence analyses were carried out as reported previously (Ghosh et al., 2010a,b, 2011; Wang et al., 2010).

2.3. Nucleotide sequence accession numbers

The GenBank accession numbers for the nucleotide sequences of the VP1–3, VP6 and NSP1–5/6 genes of strains Mc323 and Mc345 are JN104611-JN104618, JN872347, JN104619–JN104626 and JN872348, respectively.

3. Results and discussion

By RNA–RNA hybridization studies and nucleotide sequencing of the VP4, VP7 and NSP5 genes, strains Mc323 and Mc345 were shown to be more related to porcine RVAs than to human strains (Kojima et al., 1996; Maneekarn et al., 2006; Matthijnssens et al., 2010; Urasawa et al., 1992). Although these preliminary observations hinted towards a porcine origin of Mc323 and Mc345, they were not sufficient to ascertain the overall genetic makeup or evolutionary patterns of these RVAs. Therefore, in the present study, the nearly full-length nucleotide sequences (full-length sequences excluding the 5′- and 3′- end primer binding regions) of the remaining gene segments of Mc323 and Mc345 were analyzed. Moreover, the available nucleotide sequence for the NSP5/6 gene of strain Mc323 (GenBank accession No. U54772) was found to lack the putative NSP6 ORF (Supplementary Fig. S1). To confirm this observation, we repeated nucleotide sequencing of the NSP5/6 genes of Mc323 and Mc345.

The VP1–3, VP6 and NSP1–5 genes of strains Mc323 and Mc345 were assigned to the R1, C1, M1, I5, A8, N1, T1, E1 and H1 genotypes, respectively (Table 1, Fig. 1A–I, Supplementary Table S1). Comparisons of the complete genotype constellations of strains Mc323 and Mc345 with those of other P[19] and non-P[19] RVA strains are shown in Table 1. All the 11 gene segments of strain Mc323 exhibited high nucleotide sequence identities to those of Mc345 (Supplementary Table S1) (Okada et al., 2000). By phylogenetic analyses, with the exception of the NSP5 gene, both strains were closely related to each other, (Fig. 1A-I).

Upon comparison with the genomes of other RVAs, the VP2–3 and NSP3 genes of strains Mc323 and Mc345 were closely related to those of porcine strain RVA/Pig-wt/IND/RU172/2002/G12P[7] (Ghosh et al., 2006, 2010b) (Fig. 1B, C and G; Supplementary Table S1). The VP6 and NSP4 genes of Mc323 and Mc345 were closely related to those of several porcine strains (strains with "CMP" in their common names, such as CMP90/01, CMP45/08) from Chiang Mai, Thailand (Fig. 1D and H; Supplementary Table S1). The NSP1 genes of strains Mc323 and Mc345 exhibited maximum nucleotide sequence identities (but low) of 90.5% and 90.2%, respectively, to that of porcine strain RVA/Pig-tc/USA/Gottfried/1975/G4P[6] (Supplementary Table S1), and by phylogenetic analysis, clustered near those of Gottfried and the porcine-like human strains mani-97, RVA/Human-wt/IND/mcs-13/2007/G9P[6], RVA/Human-wt/IND/mani-253/2007/G4P[4] and RVA/Human-wt/IND/mani-362/2007/G4P[6] (Mukherjee et al., 2009, 2011) within the porcine-like A8 genotype (Fig. 1E). The NSP2 gene of strain Mc345 was closely related to that of porcine strain RVA/Pig-tc/MEX/YM/1983/G11P9[7] (Fig. 1F). On the other hand, phylogenetically, the NSP2 gene of strain Mc323 was closely related to that of a human G9P[8] strain, RVA/Human-wt/BEL/B3458/2003/G9P[8] (Fig. 1F). However, the NSP2 genes of Mc323 and B3458 were also closely related to those of porcine strains YM and RVA/Pig-xx/KOR/PRG942/2006/G9P[23] and porcine-human reassortant strain RVA/Human-wt/ECU/EC2184/2005/G11P[6] (Bányai et al., 2009) and clustered separately from the NSP2 genes of the common human

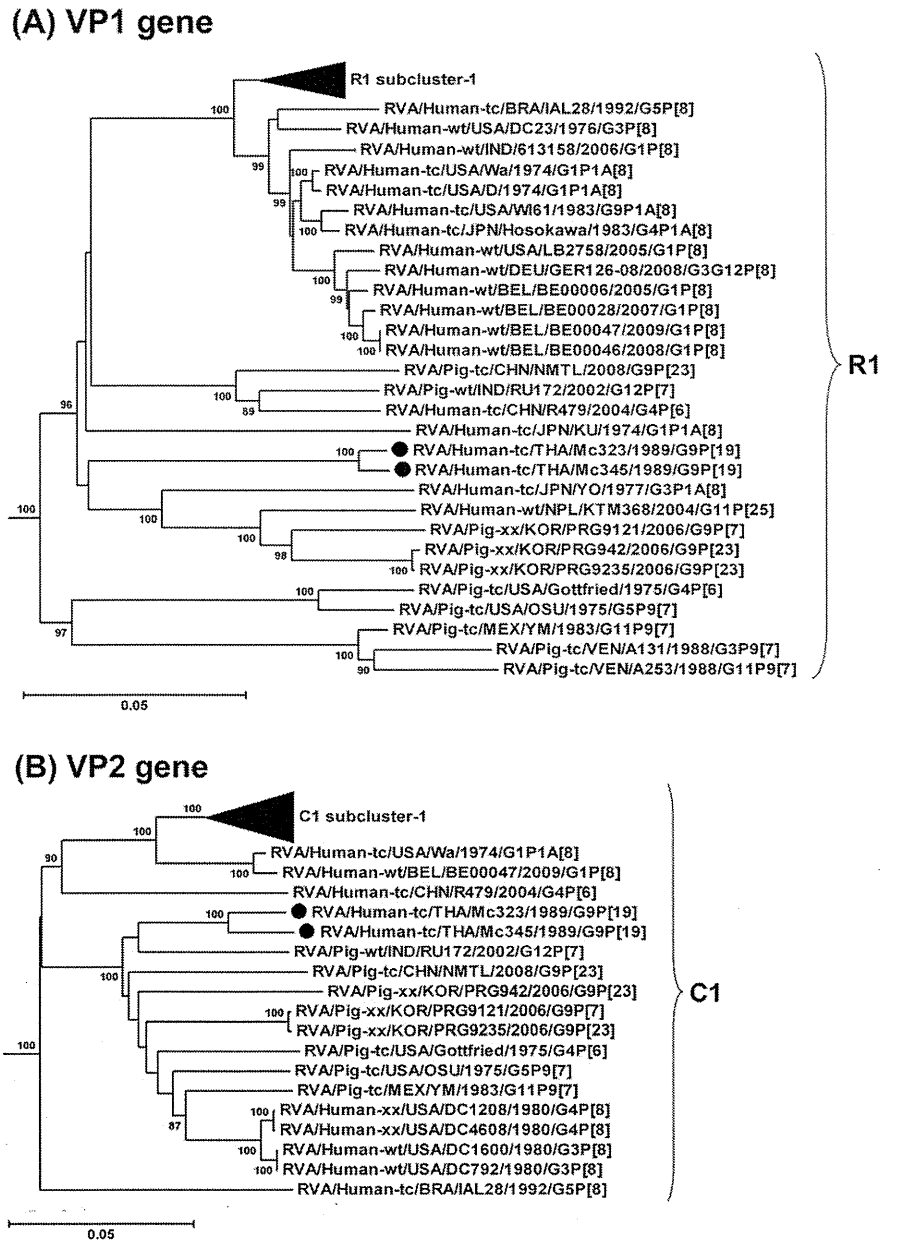


Fig. 1. A–I. Phylogenetic trees constructed from the nucleotide sequences of VP1–3, VP6 and NSP1–5 genes of rotavirus strains RVA/Human-tc/THA/Mc323/1989/G9P[19] and RVA/Human-tc/THA/Mc345/1989/G9P[19] with those of other RVA strains. Although strains representing all the RVA genotypes were included in the phylogenetic analyses to prepare the dendograms, only those relevant to the present analysis are shown in Fig. 1A–I. Within the R1, C1, M1, I1, A1, N1, T1, E1 and H1 genotypes, clade/s consisting of strains that are not directly related to the present study, but were included for unbiased analysis, have been compressed and labeled as subcluster/s. Only short-length nucleotide sequences are available for the VP1–3 genes of strains RVA/Human-wt/IND/RMC321/1990/G9P[19] and RVA/Human-wt/IND/mani-97/2006/G9P[19], and therefore, these were not included in the above analysis. In all trees, positions of strains Mc323 and Mc345 are shown by dark circles, whilst dark triangles indicate those of the other G9P[19] strains. Bootstrap values less than 85% are not shown. Scale bar, 0.05 substitutions per nucleotide.

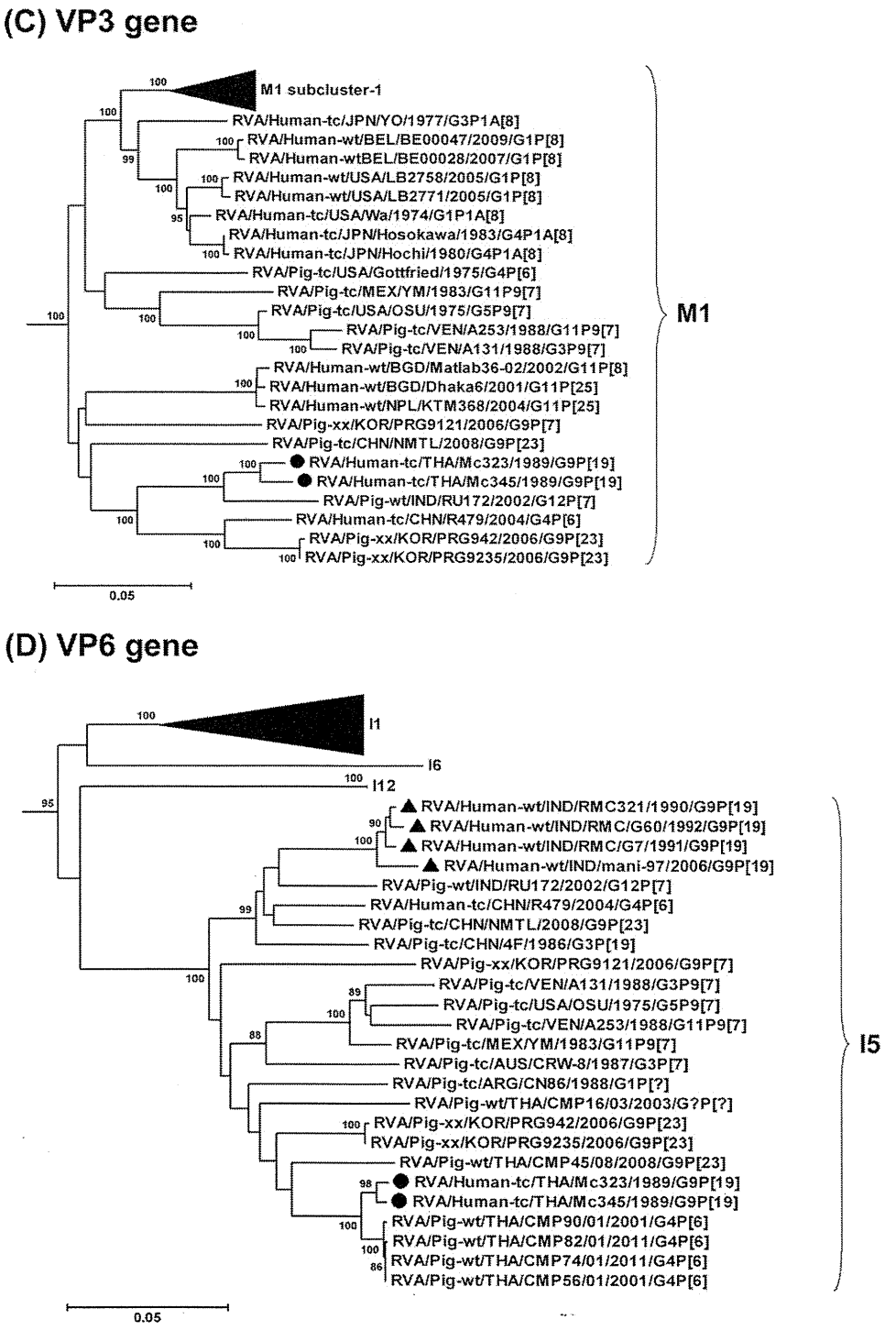


Fig. 1 (*continued*)

strains, such as G1 strains, suggesting a common origin with porcine strains (Fig. 1F). The VP1 genes of Mc323 and Mc345 shared low nucleotide sequence identities (<89%) with other RVAs (Supplementary Table S1), and by phylogenetic analysis, formed a separate cluster within the R1 genotype (Fig. 1A).

The nucleotide sequence of the NSP5/6 gene of strain Mc323 obtained in the present study contained the putative NSP6 ORF (Supplementary Fig. S1). The absence of the putative NSP6 ORF in the previously reported NSP5/6 nucleotide sequence of Mc323 might have resulted from an error in the sequencing process, as the other genes (VP4 and VP7) reported previously exhibited absolute nucleotide sequence identities to those sequenced in the present study (data not shown). Moreover, isolates analyzed in this study were subjected to only five passages in MA-104 cells, and thereafter, stored at −80 °C till the present study. In the previous study, the NSP5 gene of Mc323 was shown to be of porcine origin (Kojima et al., 1996). However, in the present study, the Mc323 NSP5 appeared to cluster between a cluster consisting of distinct human and porcine subclusters within genotype H1 (Fig. 1I) and shared comparable nucleotide sequence identities with those of

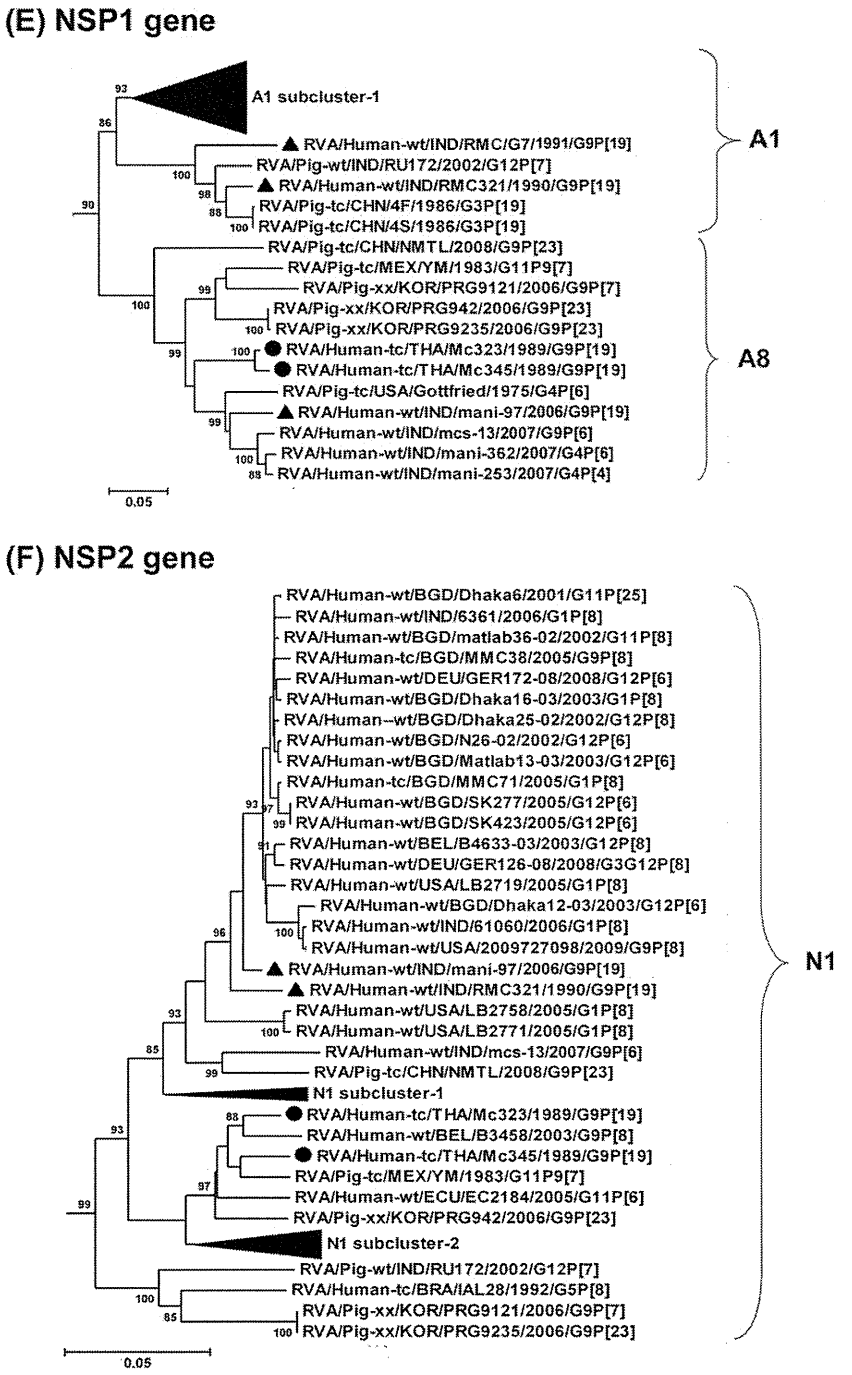
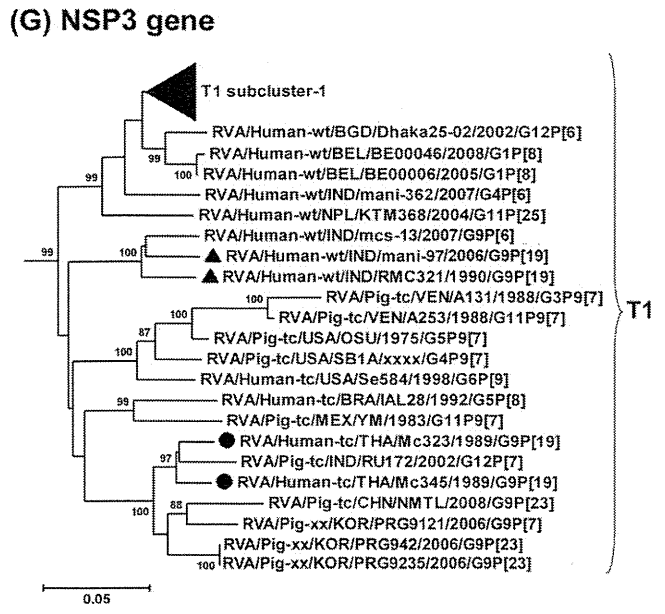


Fig. 1 (*continued*)

several RVA strains from either host species (Supplementary Table S1), making it difficult to establish its exact origin. On the other hand, phylogenetically, the NSP5 gene of strain Mc345 appeared to be closely related to those of porcine strains RVA/Pig-wt/IND/HP113/2002/G6P[13] and RVA/Pig-wt/IND/HP140/2002/G6P[13] (Ghosh et al., 2007) and the porcine-derived NSP5 genes of human G4P[4] and other G9P[19]strains (Mukherjee et al., 2011; Varghese et al., 2004) (Fig. 1I).

Taken together, most of the genes of strains Mc323 (VP2–4, VP6–7, NSP1–4 genes) and Mc345 (VP2–4, VP6–7 and NSP1–5 genes) were found to be closely related to porcine RVA genes. Therefore, both the strains have a porcine genetic backbone, and are likely of porcine origin. This was corroborated by the close relationships observed in the VP4 (VP8*), VP6–7 and NSP4 genes between these strains and the locally circulating porcine strains (Maneekarn et al., 2006; Matthijnssens et al., 2010) (Fig. 1D and H; Supplementary Table S1). On the other hand, the origin of the VP1 and NSP5 genes of Mc323 and VP1 gene of Mc345 could not be ascertained. It may be possible that these genes were acquired through human-porcine reassortment events, following transmission of strains Mc323 and Mc345 from pigs to humans, or they may be of porcine origin.

In conclusion, whole genomic analyses of the first reported human P[19] strains, Mc323 and Mc345, corroborated the hypothesis that P[19] strains might be derived from porcine RVAs. With the exception of the NSP5 gene, both the strains exhibited similar



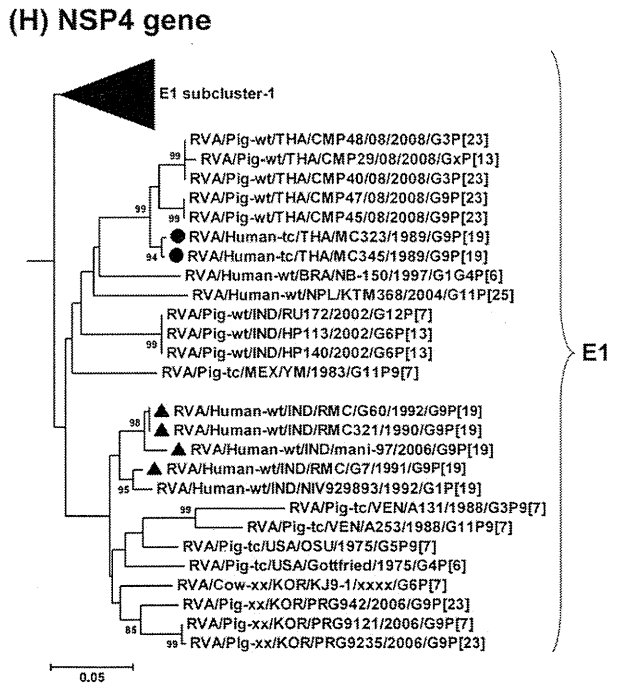


Fig. 1 (*continued*)

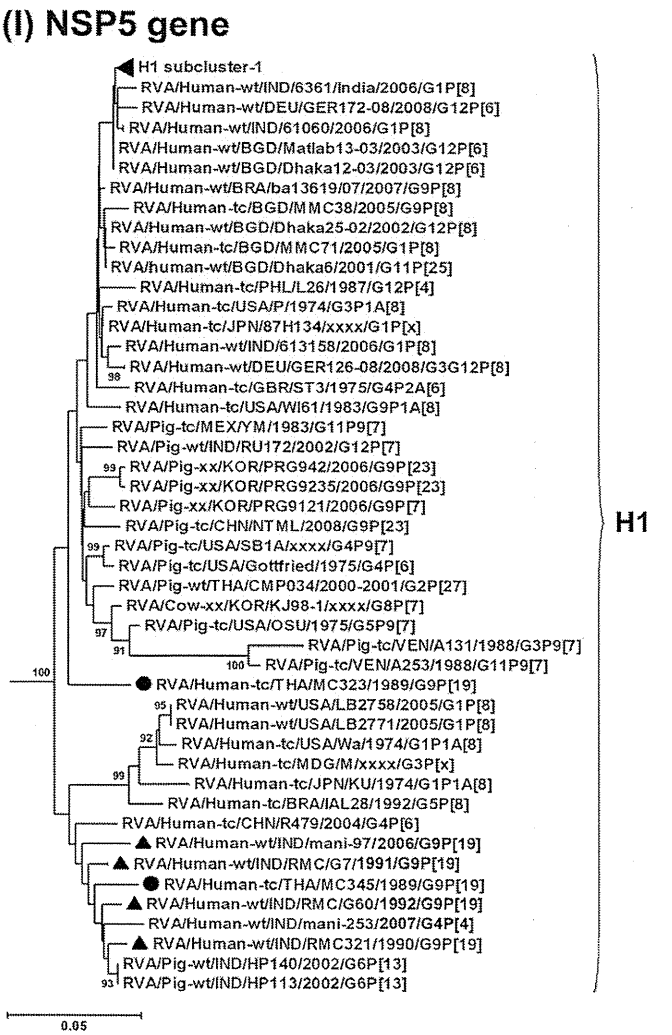


Fig. 1 (*continued*)

Acknowledgements

The study was supported in part by the Grant-in-aid for scientific research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (Grant No. 22406017) and Health and Labour Sciences Research Grants in Research on Emerging and Re-emerging Infectious Disease (H23-shinkou-ippan-005) from the Ministry of Health, Labour and Welfare of Japan.

evolutionary patterns. The porcine origin of strains Mc323 and Mc345 also revealed the increased risk of interspecies transmission events under poor hygienic conditions and close proximity of humans to livestock, especially in developing nations, such as Thailand, necessitating the adoption of hygienic preventive measures in these countries. Although whole genomic analyses of strains Mc323, Mc345, RMC321 and mani-97 provided important insights into the origin of P[19] strains, and on interspecies transmission of RVAs, except for mani-97, these are old strains. On the other hand, to date, only a few porcine P[19] strains have been detected, and none of these have been analyzed for the whole genome. Therefore, detection and analyses of the whole genomes of porcine and recent human P[19] strains might be of significance in context to studies on evolution of P[19] RVAs.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.meegid.2011.12.012.

References

Bányai, K., Esona, M.D., Kerin, T.K., Hull, J.J., Mijatovic, S., Vásconez, N., Torres, C., de Filippis, A.M., Foytich, K.R., Gentsch, J.R., 2009. Molecular characterization of a rare, human-porcine reassortant rotavirus strain, G11P[6], from Ecuador. Arch. Virol. 154, 1823–1829.

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.

Chitambar, S.D., Arora, R., Chhabra, P., 2009. Molecular characterization of a rare G1P[19] rotavirus strain from India: evidence of reassortment between human and porcine rotavirus strains. J. Med. Microbiol. 58, 1611–1615.

Estes, M.K., Kapikian, A.Z., 2007. Rotaviruses and their replication. In: Fields, B.N., Knipe, D.M., Howley, P.M., Griffin, D.E., Lamb, R.A., Martin, M.A., Roizman, B., Straus, S.E. (Eds.), Fields Virology, 5th edn. Lippincott, Williams & Wilkins, Philadelphia, pp. 1917–1974.

Ghosh, S., Kobayashi, N., 2011. Whole-genomic analysis of rotavirus strains: current status and future prospects. Future Microbiol. 6, 1049–1065.

Ghosh, S., Varghese, V., Samajdar, S., Bhattacharya, S.K., Kobayashi, N., Naik, T.N., 2006. Molecular characterization of a porcine Group A rotavirus strain with G12 genotype specificity. Arch. Virol. 151, 1329–1344.

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.

Ghosh, S., Alam, M.M., Ahmed, M.U., Talukdar, R.I., Paul, S.K., Kobayashi, N., 2010a. The complete genome constellation of a caprine group A rotavirus strain reveals common evolution with ruminant and human rotavirus strains. J. Gen. Virol. 91, 2367–2373.

Ghosh, S., Kobayashi, N., Nagashima, S., Chawla-Sarkar, M., Krishnan, T., Ganesh, B., Naik, T.N., 2010b. Full genomic analysis and possible origin of a porcine G12 rotavirus strain RU172. Virus Genes 40, 382–388.

Ghosh, S., Paul, S.K., Hossain, M.A., Alam, M.M., Ahmed, M.U., Kobayashi, N., 2011. Full genomic analyses of two human G2P[4] rotavirus strains detected in 2005: identification of a caprine-like VP3 gene. J. Gen. Virol. 92, 1222–1227.

Kojima, K., Taniguchi, K., Urasawa, T., Urasawa, S., 1996. Sequence analysis of normal and rearranged NSP5 genes from human rotavirus strains isolated in nature: implications for the occurrence of the rearrangement at the step of plus strand synthesis. Virology 224, 446–452.

Krishnan, T., Burke, B., Shen, S., Naik, T.N., Desselberger, U., 1994. Molecular epidemiology of human rotaviruses in Manipur: genome analysis of rotaviruses of long electropherotype and subgroup I. Arch. Virol. 134, 279–292.

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.

Matthijnssens, J., Ciarlet, M., Heiman, E., Arijs, I., Delbeke, T., McDonald, S.M., Palombo, E.A., Go'mara, M.I., Maes, P., Patton, J.T., Rahman, M., Ranst, M.V., 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.

Matthijnssens, J., Ciarlet, M., McDonald, S.M., Attoui, H., Banyai, K., Brister, J.R., Buesa, J., Esona, M.D., Estes, M.K., Gentsch, J.R., Iturriza-Gomara, M., Johne, R., Kirkwood, C.D., Martella, V., Mertens, P.P., Nakagomi, O., Parreno, V., Rahman, M., Ruggeri, F.M., Saif, L.J., Santos, N., Steyer, A., Taniguchi, K., Patton, J.T., Desselberger, U., Van Ranst, M., 2011. Uniformity of rotavirus strain nomenclature proposed by the Rotavirus Classification Working Group (RCWG). Arch. Virol. 156, 1397–1413.

Matthijnssens, J., Heylen, E., Zeller, M., Rahman, M., Lemey, P., Van Ranst, M., 2010. Phylodynamic analyses of rotavirus genotypes G9 and G12 underscore their potential for swift global spread. Mol. Biol. Evol. 27, 2431–2436.

Mukherjee, A., Dutta, D., Ghosh, S., Bagchi, P., Chattopadhyay, S., Nagashima, S., Kobayashi, N., Dutta, P., Krishnan, T., Naik, T.N., Chawla-Sarkar, M., 2009. Full genomic analysis of a human group A rotavirus G9P[6] strain from Eastern India provides evidence for porcine-to-human interspecies transmission. Arch. Virol. 154, 733–746.

Mukherjee, A., Chattopadhyay, S., Bagchi, P., Dutta, D., Singh, N.B., Arora, R., Parashar, U.D., Gentsch, J.R., Chawla-Sarkar, M., 2010. Surveillance and molecular characterization of rotavirus strains circulating in Manipur, north-eastern India: increasing prevalence of emerging G12 strains. Infect. Genet. Evol. 10, 311–320.

Mukherjee, A., Ghosh, S., Bagchi, P., Dutta, D., Chattopadhyay, S., Kobayashi, N., Chawla-Sarkar, M., 2011. Full genomic analyses of human rotavirus G4P[4], G4P[6], G9P[19] and G10P[6] strains from North-eastern India: evidence for interspecies transmission and complex reassortment events. Clin. Microbiol. Infect. 17, 1343–1346.

Nguyen, T.A., Hoang, L.P., Pham, L.D., Hoang, K.T., Okitsu, S., Mizuguchi, M., Ushijima, H., 2008. Use of sequence analysis of the VP4 gene to classify recent Vietnamese rotavirus isolates. Clin. Microbiol. Infect. 14, 235–241.

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.

Urasawa, S., Hasegawa, A., Urasawa, T., Taniguchi, K., Wakasugi, F., Suzuki, H., Inouye, S., Pongprot, B., Supawadee, j., Suprasert, S., Rangsiyanond, P., Tonusin, S., Yamazi, Y., 1992. Antigenic and genetic analyses of human rotaviruses in Chiang Mai, Thailand: evidence for a close relationship between human and animal rotaviruses. 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.

Varghese, V., Ghosh, S., Das, S., Bhattacharya, S.K., Krishnan, T., Karmakar, P., Kobayashi, N., Naik, T.N., 2006. Characterization of VP1, VP2 and VP3 gene segments of a human rotavirus closely related to porcine strains. Virus Genes 32, 241–247.

Wang, Y.H., Kobayashi, N., Nagashima, S., Zhou, X., Ghosh, S., Peng, J.S., Hu, Q., Zhou, D.J., Yang, Z.Q., 2010. Full genomic analysis of a porcine-bovine reassortant G4P[6] rotavirus strain R479 isolated from an infant in China. J. Med. Virol. 82, 1094–1102.

Wu, F.T., Banyai, K., Huang, J.C., Wu, H.S., Chang, F.Y., Yang, J.Y., Hsiung, C.A., Huang, Y.C., Lin, J.S., Hwang, K.P., Jiang, B., Gentsch, J.R., 2011. 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. 83, 1279–1287.

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.

Journal of General Virology (2012), 93, 1700–1705 DOI 10.1099/vir.0.043026-0

Short Communication

Whole-genomic analysis of a human G1P[9] rotavirus strain reveals intergenogroup-reassortment events

Souvik Ghosh,[1] Tsuzumi Shintani,[1] Noriko Urushibara,[1] Koki Taniguchi[2] and Nobumichi Kobayashi[1]

[1]Department of Hygiene, Sapporo Medical University School of Medicine, Sapporo, Japan

[2]Department of Virology and Parasitology, School of Medicine, Fujita Health University, Toyoake, Japan

Correspondence
Souvik Ghosh
souvikrota@gmail.com *or*
souvik8@rediffmail.com

Received 21 March 2012
Accepted 14 May 2012

Group A rotavirus (RVA) strain K8 (RVA/Human-tc/JPN/K8/1977/G1P[9]) was found to have Wa-like VP7 and NSP1 genes and AU-1-like VP4 and NSP5 genes. To determine the exact origin and overall genetic makeup of this unusual RVA strain, the remaining genes (VP1–VP3, VP6 and NSP2–NSP4) of K8 were analysed in this study. Strain K8 exhibited a G1-P[9]-I1-R3-C3-M3-A1-N1-T3-E3-H3 genotype constellation, not reported previously. The VP6 and NSP2 genes of strain K8 were related closely to those of common human Wa-like G1P[8] and/or G3P[8] strains, whilst its VP1–VP3, NSP3 and NSP4 genes were related more closely to those of AU-1-like RVAs and/or AU-1-like genes of multi-reassortant strains than to those of other RVAs. Therefore, strain K8 might have originated from intergenogroup-reassortment events involving acquisition of four Wa-like genes, possibly from G1P[8] RVAs, by an AU-1-like P[9] strain. Whole-genomic analysis of strain K8 has provided important insights into the complex genetic diversity of RVAs.

Group A rotaviruses (RVAs) are a major cause of severe childhood diarrhoea (Cashman *et al.*, 2012; Estes & Kapikian, 2007). To date, RVAs are classified into at least 27 G and 35 P genotypes on the basis of differences in the nucleotide sequences of their outer-capsid VP7- and VP4-encoding genes, respectively (Matthijnssens *et al.*, 2011a). In humans, G1, G2, G3, G4 or G9 strains in conjunction with P[4], P[6] or P[8] have been reported widely, whilst G12 is emerging as an important VP7 genotype (Matthijnssens *et al.*, 2009, 2010a; Santos & Hoshino, 2005).

By RNA–RNA hybridization, human RVAs have previously been classified into at least two major genogroups, represented by reference strains RVA/Human-tc/USA/Wa/1974/G1P1A[8] and RVA/Human-tc/USA/DS-1/1976/G2P1B[4], and one minor genogroup, represented by strain RVA/Human-tc/JPN/AU-1/1982/G3P3[9] (Nakagomi *et al.*, 1989). Recently, a whole genome-based genotyping system has been accepted as the standard method for classification of RVAs by researchers worldwide (Matthijnssens *et al.*, 2008a, b, 2011a). Applying this classification system, the VP1–VP3, VP6 and NSP1–NSP5 genes of most human RVA strains with different G and P genotypes were found to exhibit an RVA strain Wa-like (designated genotypes R1, C1, M1, I1, A1, N1, T1, E1 and H1) or DS-1-like (designated genotypes R2, C2, M2, I2, A2, N2, T2, E2 and H2) genotype, whilst a limited number of strains possessed genes of the AU-1-like (designated genotypes R3, C3, M3, I3, A3, N3, T3, E3 and H3) genotype (Ghosh & Kobayashi, 2011; Heiman *et al.*, 2008; Matthijnssens, *et al.*, 2008a, b, 2011a). Results obtained using this genotyping system concurred with the previous classification of human RVA strains into the three RVA genogroups (Wa, DS-1 and AU-1) (Ghosh & Kobayashi, 2011; Matthijnssens *et al.*, 2008a, b). Human RVA strains possessing mixed genotype constellations have been also reported (Ghosh & Kobayashi, 2011).

RVA G1P[9] is an uncommon VP7–VP4 genotype combination, reported in RVA strains from humans and environmental samples (Matthijnssens *et al.*, 2009; Villena *et al.*, 2003). The first G1P[9] RVA strain, RVA/Human-tc/JPN/K8/1977/G1P[9], was detected in a diarrhoeal stool sample collected from a 14-year-old child in the city of Kitami, Hokkaido prefecture, Japan, in 1977 (Urasawa *et al.*, 1984). Since then, only a few human G1P[9] RVA strains have been reported, from Brazil, Burkina Faso, China, Italy, South Korea and Spain (Bonkoungou *et al.*, 2011; Fang *et al.*, 2002; Grassi *et al.*, 2012; Le *et al.*, 2008; Leite *et al.*, 1996; Santos *et al.*, 2003; Villena *et al.*, 2003).

The GenBank/EMBL/DDBJ accession numbers for the nucleotide sequences of the VP1–VP3, VP6 and NSP2–NSP4 genes of rotavirus strain RVA/Human-tc/JPN/K8/1977/G1P[9] are JQ713645–JQ713651, respectively.

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

Whole-genomic analyses of atypical RVA strains are essential to obtain conclusive data on their true origin and evolution (Ghosh & Kobayashi, 2011; Matthijnssens *et al.*, 2008a, b). However, to date there are no reports on the whole-genomic analysis of the unusual G1P[9] RVA strains. RNA–RNA hybridization studies involving a single G1P[9] strain, K8, pointed towards possible intergenogroup-reassortment events (Nakagomi *et al.*, 1992). By partial genomic analysis, strain K8 was found to possess Wa-like VP7 and NSP1 genes and AU-1-like VP4 and NSP5 genes (Kojima *et al.*, 1996; Matthijnssens *et al.*, 2008b; Taniguchi *et al.*, 1989; Wu *et al.*, 1998). Therefore, to gain insights into the exact origin and overall genetic makeup of a G1P[9] RVA strain, the remaining seven genes (VP1–VP3, VP6 and NSP2–NSP4) of strain K8 were analysed in the present study. Moreover, only a few RVA gene sequences were available during analyses of the NSP1 and NSP5 genes of strain K8 in previous studies (Kojima *et al.*, 1996; Wu *et al.*, 1998), prompting us to repeat phylogenetic analyses of these genes with a larger number of RVA strains.

Human G1P[9] strain K8 was isolated successfully by tissue culture in MA-104 cells in our laboratory (Urasawa *et al.*, 1984) and stored at −80 °C until further analysis. Primers used for the amplification of the VP1–3, VP6 and NSP2–4 genes of strain K8 have been described previously (Ghosh *et al.*, 2010a, b, 2011; Wang *et al.*, 2010). RT-PCR, nucleotide sequencing and sequence analysis were carried out as described previously (Ghosh *et al.*, 2011). Phylogenetic trees were constructed by the neighbour-joining method (Saitou & Nei, 1987) using MEGA (v5.01) software (Tamura *et al.*, 2011). The trees were statistically supported by bootstrapping with 1000 replicates, and phylogenetic distances were measured by the Kimura two-parameter model.

The VP4, VP7, NSP1 and NSP5 genes of RVA strain K8 were shown previously to belong to the P[9], G1, A1 and H3 genotypes, respectively (Matthijnssens *et al.*, 2008b). In the present study, based on nucleotide sequence identities and phylogenetic analyses of the nearly full-length nucleotide sequences (minus the 5′- and 3′-end primer sequences), the VP1–VP3, VP6 and NSP2–NSP4 genes of strain K8 were assigned to the R3, C3, M3, I1, N1, T3 and E3 genotypes, respectively (Table 1; Fig. 1). Therefore, strain K8 exhibited a G1-P[9]-I1-R3-C3-M3-A1-N1-T3-E3-H3 genotype constellation, not reported previously. Four of the 11 genotypes (G1, I1, A1 and N1) of K8 were closely related genomically to those of the Wa-like RVAs, whilst its remaining seven genotypes were AU-1-like, revealing a mixed genotype constellation (Table 1). The Wa-, DS-1- or AU-1-like genogroup is assigned to a human RVA strain if at least seven gene segments belong to the respective Wa-, DS-1-, or AU-1-like genotype (Matthijnssens *et al.*, 2008a). Therefore, strain K8 was assigned to the AU-1 genogroup.

The VP1 gene of strain K8 shared low nucleotide sequence identities (maximum nucleotide sequence identity of 89.9 % with strain RVA/Human-tc/THA/T152/1998/G12P[9], followed by 89.5 % with strain AU-1) with those of other RVAs, and phylogenetically, it clustered separately, near strain AU-1, AU-1-like G12 strain T152 (Matthijnssens *et al.*, 2008a, b; Rahman *et al.*, 2007) and strain RVA/

Table 1. Genotype nature of the 11 gene segments of RVA strain K8 compared with those of selected RVA strains with known genomic constellations

Bold type indicates gene segments with a genotype identical to that of strain K8; – indicates that no sequence data were available in GenBank. Strains K8, Wa and AU-1 are underlined.

Strain	VP7	VP4	VP6	VP1	VP2	VP3	NSP1	NSP2	NSP3	NSP4	NSP5
RVA/Human-tc/JPN/K8/1977/G1P[9]	**G1**	**P[9]**	**I1**	**R3**	**C3**	**M3**	**A1**	**N1**	**T3**	**E3**	**H3**
RVA/Human-tc/USA/Wa/1974/G1P1A[8]	**G1**	P[8]	**I1**	R1	C1	M1	**A1**	**N1**	T1	E1	H1
RVA/Human-wt/BEL/BE00097/2009/G1P[8]	**G1**	P[8]	**I1**	R1	C1	M1	**A1**	**N1**	T1	E1	H1
RVA/Human-wt/USA/DC1505/1976/G3P[8]	G3	P[8]	**I1**	R1	C1	M1	**A1**	**N1**	T1	E1	H1
RVA/Human-tc/JPN/AU-1/1982/G3P3[9]	G3	**P[9]**	I3	**R3**	**C3**	**M3**	A3	N3	**T3**	**E3**	**H3**
RVA/Cat-wt/JPN/FRV1/1985/G3P[9]	G3	**P[9]**	–	–	–	–	–	–	–	**E3**	–
RVA/Human-tc/ITA/PA260-97/1997/G3P[3]	G3	P[3]	I3	**R3**	**C3**	**M3**	A15	N2	**T3**	**E3**	H6
RVA/Human-tc/USA/HCR3A/1984/G3P[3]	G3	P[3]	I3	**R3**	C2	**M3**	A9	N2	**T3**	**E3**	H6
RVA/Cat-tc/AUS/Cat97/1984/G3P[3]	G3	P[3]	I3	**R3**	C2	**M3**	A9	N2	**T3**	**E3**	H6
RVA/Dog-tc/AUS/K9/1981/G3P[3]	G3	P[3]	I3	**R3**	C2	**M3**	A9	N2	**T3**	**E3**	H6
RVA/Simian-tc/USA/RRV/1975/G3P[3]	G3	P[3]	I2	R2	**C3**	**M3**	A9	N2	**T3**	**E3**	H6
RVA/Cat-wt/ITA/BA222/2005/G3P[9]	G3	**P[9]**	I2	R2	C2	M2	A3	**N1**	**T3**	E2	**H3**
RVA/Cat-tc/AUS/Cat2/1984/G3P[9]	G3	**P[9]**	I3	**R3**	C2	**M3**	A3	**N1**	T6	**E3**	**H3**
RVA/Human-wt/THA/CMH120/2004/G3P[9]	G3	**P[9]**	I3	–	–	–	–	–	–	**E3**	–
RVA/Human-wt/THA/CMH134/2004/G3P[9]	G3	**P[9]**	I3	–	–	–	–	–	–	**E3**	–
RVA/Rhesus-tc/USA/TUCH/2002/G3P[24]	G3	P[24]	I9	**R3**	**C3**	**M3**	A9	**N1**	**T3**	**E3**	H6
RVA/Human-wt/JPN/KF17/2010/G6P[9]	G6	**P[9]**	I2	R2	C2	M2	A3	N2	**T3**	**E3**	**H3**
RVA/Human-tc/THA/T152/1998/G12P[9]	G12	**P[9]**	I3	**R3**	**C3**	**M3**	A12	N3	**T3**	**E3**	**H3**

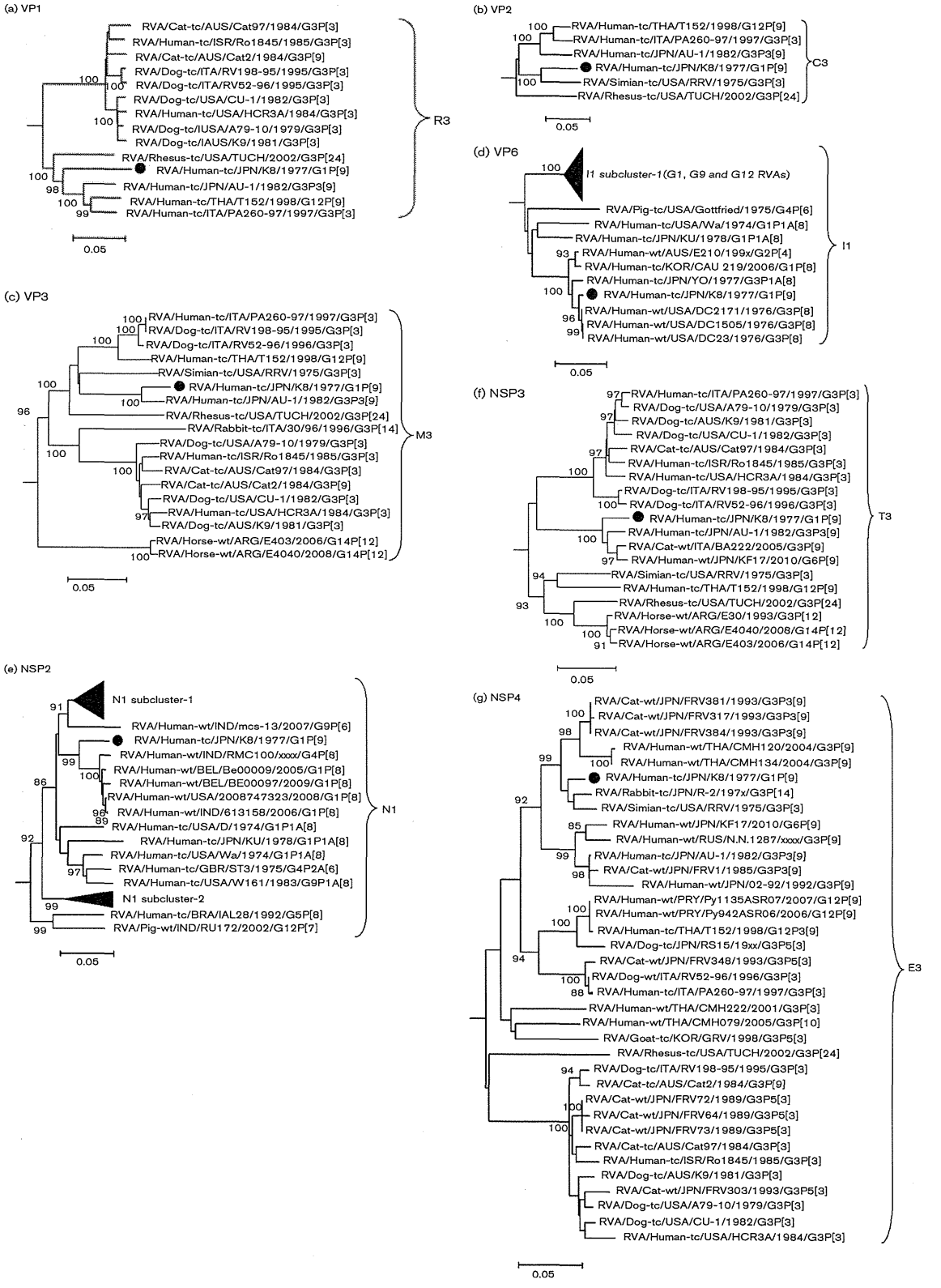


Fig. 1. Phylogenetic analyses of the VP1–VP3, VP6 and NSP2–NSP4 genes (a–g, respectively) of rotavirus strain RVA/Human-tc/JPN/K8/1977/G1P[9]. Although strains representing all RVA genotypes were included in the phylogenetic analyses, only those relevant to the present study are shown. Within the I1 and N1 genotypes, clade(s) consisting of strains that are not directly related to the present study, but were included for unbiased analysis, have been compressed and labelled as subcluster(s). In all trees, the position of strain K8 is highlighted by ●. Bootstrap values >85% are shown. Bar, 0.05 substitutions per nucleotide.

Human-tc/ITA/PA260-97/1997/G3P[3] [a reassortant between RVAs of the AU-1-like and Cat97-like genogroups (Matthijnssens *et al.*, 2011b)] (Fig. 1a). Strain K8 exhibited maximum nucleotide sequence identity of 92.1 % to the AU-1-like VP2 gene of simian strain RVA/Simian-tc/USA/RRV/1975/G3P[3] (Matthijnssens *et al.*, 2010b), followed by identities of 89.0 and 88.9 % to those of strains AU-1 and T152, respectively. Phylogenetically, strain K8 clustered near strain RRV within the VP2-C3 genotype (Fig. 1b). The VP3 gene of strain K8 was related more closely to that of strain AU-1 (nucleotide sequence identity of 95.8 %) than to those of other RVAs (nucleotide sequence identities of <88 %) (Fig. 1c). The VP6 and NSP1 genes of K8 were related closely (nucleotide sequence identities of 99 %) to those of the common human Wa-like G3P[8] RVA strains detected in the USA in 1976 (Fig. 1d; Fig. S1, available in JGV Online). The NSP2 gene of K8 shared nucleotide sequence identities of 94–95 % and clustered phylogenetically with those of the common human Wa-like G1P[8] RVA strains (Fig. 1e).

The NSP3 gene of strain K8 was related more closely (nucleotide sequence identities of 96.0, 95.9 and 95.6 %, respectively) to those of strains AU-1, RVA/Cat-wt/ITA/BA222/2005/G3P[9] (a multi-reassortant strain derived from human, canine/feline, and bovine or bovine-like human RVAs) (Martella *et al.*, 2011) and RVA/Human-wt/JPN/KF17/2010/G6P[9] (a reassortant between bovine-like human and AU-1-like RVAs) (Yamamoto *et al.*, 2011) than those of other RVA strains (nucleotide sequence identities of <88 %) (Fig. 1f). The NSP4 gene of strain K8 exhibited high nucleotide sequence identities of 97.9, 97.5, 96.7, 96.7 and 96.5 % to those of lapine strain RVA/Rabbit-tc/JPN/R-2/197x/G3P[14], strain RRV, feline strains RVA/Cat-wt/JPN/FRV384/1993/G3P3[9], RVA/Cat-wt/JPN/FRV381/1993/G3P3[9] and RVA/Cat-wt/JPN/FRV317/1993/G3P3[9], respectively, and clustered phylogenetically with strains R-2 and RRV, close to strains FRV384, FRV381 and FRV317, within the NSP4-E3 genotype (Fig. 1g). The NSP5 gene of strain K8 formed a separate cluster with strain AU-1 and two other human P[9] RVA strains from Japan within the NSP5-H3 genotype (Fig. S2).

Taken together, the VP6, VP7, NSP1 and NSP2 genes of strain K8 were related closely to those of common human Wa-like G1P[8] and/or G3P[8] strains, whilst its VP1–VP4 and NSP3–NSP5 genes were related more closely to those of AU-1-like RVAs and/or AU-1-like genes of multi-reassortant RVA strains than those of other RVAs. Therefore, human G1P[9] RVA strain K8 might have originated from intergenogroup-reassortment events involving acquisition of four Wa-like gene segments, possibly from G1P[8] RVAs, by an AU-1-like P[9] strain.

Human AU-1-like strains are believed to be derived from feline/canine RVAs, as revealed by RNA–RNA hybridization studies (Nakagomi & Nakagomi, 1989). Among the AU-1-like genes of strain K8, the NSP4 gene was possibly derived from co-circulating feline RVAs (Fig. 1g). Phylogenetically, the VP1, VP3 and NSP3 genes appeared to share a common ancestry with those of typical feline/canine RVAs (Fig. 1a, c, f). The VP4 gene belonged to the same genotype as those of the feline G3P[9] RVAs, such as strains RVA/Cat-wt/JPN/FRV1/1985/G3P3[9], FRV-317, FRV381 and FRV384 from Japan. On the other hand, the VP2 and NSP5 genes appeared to be genetically distinct from those of the typical canine/feline RVAs (Table 1; Fig. 1b; Fig. S2). Moreover, phylogenetically, the NSP5 gene of strain K8 (and AU-1) appeared to share a common ancestry with those of artiodactyl and artiodactyl-like human strains (Fig. S2). Therefore, whole-genomic analyses of more AU-1-like human and typical canine/feline RVAs may be required to obtain conclusive data on the overall genetic relatedness between these RVAs, and with RVAs from other host species.

In conclusion, whole-genomic analysis of human RVA G1P[9] strain K8 provided important insights into the complex genetic diversity and evolutionary patterns of human RVAs. RVAs arising from intergenogroup-reassortment events, such as strain K8, are believed to be selected against in nature (McDonald *et al.*, 2009), as evident from the detection of only a few G1P[9] RVA strains in the last three and a half decades since the isolation of strain K8. However, compared with the high rates of detection of RVAs in humans, to date only a limited number of human RVA strains have been analysed for their whole genomes. Therefore, large-scale whole genome-based surveillance studies may be required to elucidate the actual frequency of RVA intergenogroup-reassortment events occurring under natural conditions, and to monitor the stability of RVA strains arising from such events. To our knowledge, the present study is the first report on the whole-genomic analysis of an intergenogroup-reassortant G1 RVA strain.

Acknowledgements

The study was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (grant no. 22406017) and Health and Labour Sciences Research Grants in Research on Emerging and Re-emerging Infectious Disease from the Ministry of Health, Labour and Welfare of Japan (H23-shinkou-ippan-005).

References

Bonkoungou, I. J., Damanka, S., Sanou, I., Tiendrébéogo, F., Coulibaly, S. O., Bon, F., Haukka, K., Traoré, A. S., Barro, N. & Armah, G. E. (2011). Genotype diversity of group A rotavirus strains in children with acute diarrhea in urban Burkina Faso, 2008–2010. *J Med Virol* **83**, 1485–1490.

Cashman, O., Collins, P. J., Lennon, G., Cryan, B., Martella, V., Fanning, S., Staines, A. & O'Shea, H. (2012). Molecular characterization of group A rotaviruses detected in children with gastroenteritis in Ireland in 2006–2009. *Epidemiol Infect* **140**, 247–259.

Estes, M. K. & Kapikian, A. Z. (2007). Rotaviruses and their replication. In *Fields Virology*, 5th edn, pp. 1917–1974. Edited by B. N. Fields, D. M. Knipe, P. M. Howley, D. E. Griffin, R. A. Lamb,

M. A. Martin, B. Roizman & S. E. Straus. Philadelphia, PA: Lippincott Williams & Wilkins.

Fang, Z. Y., Yang, H., Qi, J., Zhang, J., Sun, L. W., Tang, J. Y., Ma, L., Du, Z. Q., He, A. H. & other authors (2002). Diversity of rotavirus strains among children with acute diarrhea in China: 1998–2000 surveillance study. *J Clin Microbiol* **40**, 1875–1878.

Ghosh, S. & Kobayashi, N. (2011). Whole-genomic analysis of rotavirus strains: current status and future prospects. *Future Microbiol* **6**, 1049–1065.

Ghosh, S., Alam, M. M., Ahmed, M. U., Talukdar, R. I., Paul, S. K. & Kobayashi, N. (2010a). Complete genome constellation of a caprine group A rotavirus strain reveals common evolution with ruminant and human rotavirus strains. *J Gen Virol* **91**, 2367–2373.

Ghosh, S., Kobayashi, N., Nagashima, S., Chawla-Sarkar, M., Krishnan, T., Ganesh, B. & Naik, T. N. (2010b). Full genomic analysis and possible origin of a porcine G12 rotavirus strain RU172. *Virus Genes* **40**, 382–388.

Ghosh, S., Adachi, N., Gatheru, Z., Nyangao, J., Yamamoto, D., Ishino, M., Urushibara, N. & Kobayashi, N. (2011). Whole-genome analysis reveals the complex evolutionary dynamics of Kenyan G2P[4] human rotavirus strains. *J Gen Virol* **92**, 2201–2208.

Grassi, T., Bagordo, F., Cavallaro, A., Guido, M., Malaventura, C., Gabutti, G. & De Donno, A. (2012). Sequence analysis of human rotavirus strains: comparison of clinical isolates from Northern and Southern Italy. *Eur J Clin Microbiol Infect Dis* **31**, 575–582.

Heiman, E. M., McDonald, S. M., Barro, M., Taraporewala, Z. F., Bar-Magen, T. & Patton, J. T. (2008). Group A human rotavirus genomics: evidence that gene constellations are influenced by viral protein interactions. *J Virol* **82**, 11106–11116.

Kojima, K., Taniguchi, K. & Kobayashi, N. (1996). Species-specific and interspecies relatedness of NSP1 sequences in human, porcine, bovine, feline, and equine rotavirus strains. *Arch Virol* **141**, 1–12.

Le, V. P., Kim, J. Y., Cho, S. L., Nam, S. W., Lim, I., Lee, H. J., Kim, K., Chung, S. I., Song, W. & other authors (2008). Detection of unusual rotavirus genotypes G8P[8] and G12P[6] in South Korea. *J Med Virol* **80**, 175–182.

Leite, J. P., Alfieri, A. A., Woods, P. A., Glass, R. I. & Gentsch, J. R. (1996). Rotavirus G and P types circulating in Brazil: characterization by RT-PCR, probe hybridization, and sequence analysis. *Arch Virol* **141**, 2365–2374.

Martella, V., Potgieter, A. C., Lorusso, E., De Grazia, S., Giammanco, G. M., Matthijnssens, J., Bányai, K., Ciarlet, M., Lavazza, A. & other authors (2011). A feline rotavirus G3P[9] carries traces of multiple reassortment events and resembles rare human G3P[9] rotaviruses. *J Gen Virol* **92**, 1214–1221.

Matthijnssens, J., Ciarlet, M., Heiman, E., Arijs, I., Delbeke, T., McDonald, S. M., Palombo, E. A., Iturriza-Gómara, M., Maes, P. & other authors (2008a). 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.

Matthijnssens, J., Ciarlet, M., Rahman, M., Attoui, H., Bányai, K., Estes, M. K., Gentsch, J. R., Iturriza-Gómara, M., Kirkwood, C. D. & other authors (2008b). Recommendations for the classification of group A rotaviruses using all 11 genomic RNA segments. *Arch Virol* **153**, 1621–1629.

Matthijnssens, J., Bilcke, J., Ciarlet, M., Martella, V., Bányai, K., Rahman, M., Zeller, M., Beutels, P., Van Damme, P. & Van Ranst, M. (2009). Rotavirus disease and vaccination: impact on genotype diversity. *Future Microbiol* **4**, 1303–1316.

Matthijnssens, J., Heylen, E., Zeller, M., Rahman, M., Lemey, P. & Van Ranst, M. (2010a). Phylodynamic analyses of rotavirus genotypes G9 and G12 underscore their potential for swift global spread. *Mol Biol Evol* **27**, 2431–2436.

Matthijnssens, J., Taraporewala, Z. F., Yang, H., Rao, S., Yuan, L., Cao, D., Hoshino, Y., Mertens, P. P., Carner, G. R. & other authors (2010b). Simian rotaviruses possess divergent gene constellations that originated from interspecies transmission and reassortment. *J Virol* **84**, 2013–2026.

Matthijnssens, J., Ciarlet, M., McDonald, S. M., Attoui, H., Bányai, K., Brister, J. R., Buesa, J., Esona, M. D., Estes, M. K. & other authors (2011a). Uniformity of rotavirus strain nomenclature proposed by the Rotavirus Classification Working Group (RCWG). *Arch Virol* **156**, 1397–1413.

Matthijnssens, J., De Grazia, S., Piessens, J., Heylen, E., Zeller, M., Giammanco, G. M., Bányai, K., Buonavoglia, C., Ciarlet, M. & other authors (2011b). Multiple reassortment and interspecies transmission events contribute to the diversity of feline, canine and feline/canine-like human group A rotavirus strains. *Infect Genet Evol* **11**, 1396–1406.

McDonald, S. M., Matthijnssens, J., McAllen, J. K., Hine, E., Overton, L., Wang, S., Lemey, P., Zeller, M., Van Ranst, M. & other authors (2009). Evolutionary dynamics of human rotaviruses: balancing reassortment with preferred genome constellations. *PLoS Pathog* **5**, e1000634.

Nakagomi, T. & Nakagomi, O. (1989). RNA–RNA hybridization identifies a human rotavirus that is genetically related to feline rotavirus. *J Virol* **63**, 1431–1434.

Nakagomi, O., Nakagomi, T., Akatani, K. & Ikegami, N. (1989). Identification of rotavirus genogroups by RNA–RNA hybridization. *Mol Cell Probes* **3**, 251–261.

Nakagomi, O., Kaga, E. & Nakagomi, T. (1992). Human rotavirus strain with unique VP4 neutralization epitopes as a result of natural reassortment between members of the AU-1 and Wa genogroups. *Arch Virol* **127**, 365–371.

Rahman, M., Matthijnssens, J., Yang, X., Delbeke, T., Arijs, I., Taniguchi, K., Iturriza-Gómara, M., Iftekharuddin, N., Azim, T. & Van Ranst, M. (2007). Evolutionary history and global spread of the emerging g12 human rotaviruses. *J Virol* **81**, 2382–2390.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

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.

Santos, N., Soares, C. C., Volotão, E. M., Albuquerque, M. C. & Hoshino, Y. (2003). Surveillance of rotavirus strains in Rio de Janeiro, Brazil, from 1997 to 1999. *J Clin Microbiol* **41**, 3399–3402.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* **28**, 2731–2739.

Taniguchi, K., Nishikawa, K., Urasawa, T., Urasawa, S., Midthun, K., Kapikian, A. Z. & Gorziglia, M. (1989). Complete nucleotide sequence of the gene encoding VP4 of a human rotavirus (strain K8) which has unique VP4 neutralization epitopes. *J Virol* **63**, 4101–4106.

Urasawa, S., Urasawa, T., Taniguchi, K. & Chiba, S. (1984). Serotype determination of human rotavirus isolates and antibody prevalence in pediatric population in Hokkaido, Japan. *Arch Virol* **81**, 1–12.

Villena, C., El-Senousy, W. M., Abad, F. X., Pintó, R. M. & Bosch, A. (2003). Group A rotavirus in sewage samples from Barcelona and Cairo:

emergence of unusual genotypes. *Appl Environ Microbiol* **69**, 3919–3923.

Wang, Y. H., Kobayashi, N., Nagashima, S., Zhou, X., Ghosh, S., Peng, J. S., Hu, Q., Zhou, D. J. & Yang, Z. Q. (2010). Full genomic analysis of a porcine-bovine reassortant G4P[6] rotavirus strain R479 isolated from an infant in China. *J Med Virol* **82**, 1094–1102.

Wu, H., Taniguchi, K., Urasawa, T. & Urasawa, S. (1998). Serological and genomic characterization of human rotaviruses detected in China. *J Med Virol* **55**, 168–176.

Yamamoto, D., Kawaguchiya, M., Ghosh, S., Ichikawa, M., Numazaki, K. & Kobayashi, N. (2011). Detection and full genomic analysis of G6P[9] human rotavirus in Japan. *Virus Genes* **43**, 215–223.