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Short communication

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

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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.

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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).

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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

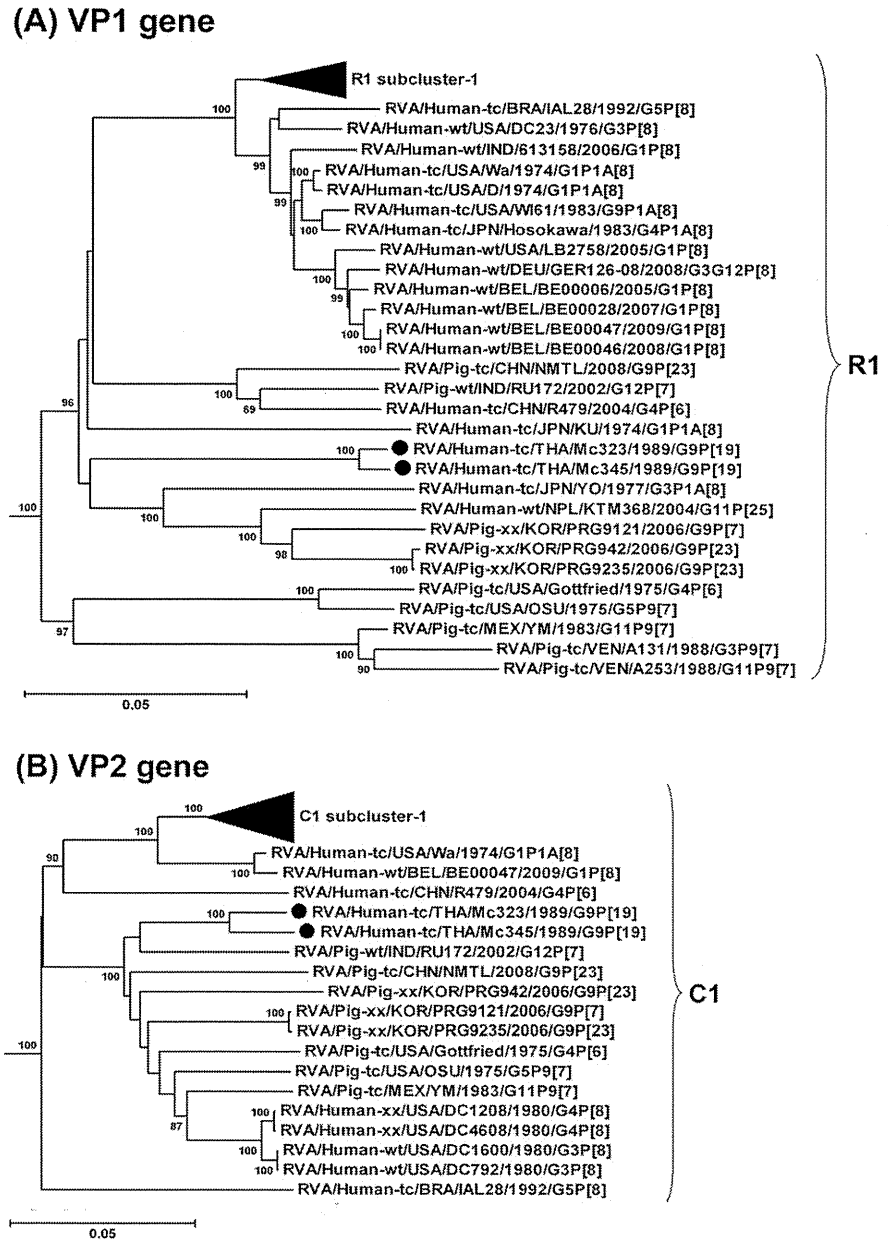

Fig. 1. A–I. Phylogenetic trees constructed from the nucleotide sequences of VP1–3, VP6 and NSP1–5 genes of rotavirus strains RVA/Human-tc/THA/Mc323/1989/G9P[19] and RVA/Human-tc/THA/Mc345/1989/G9P[19] with those of other RVA strains. Although strains representing all the RVA genotypes were included 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.

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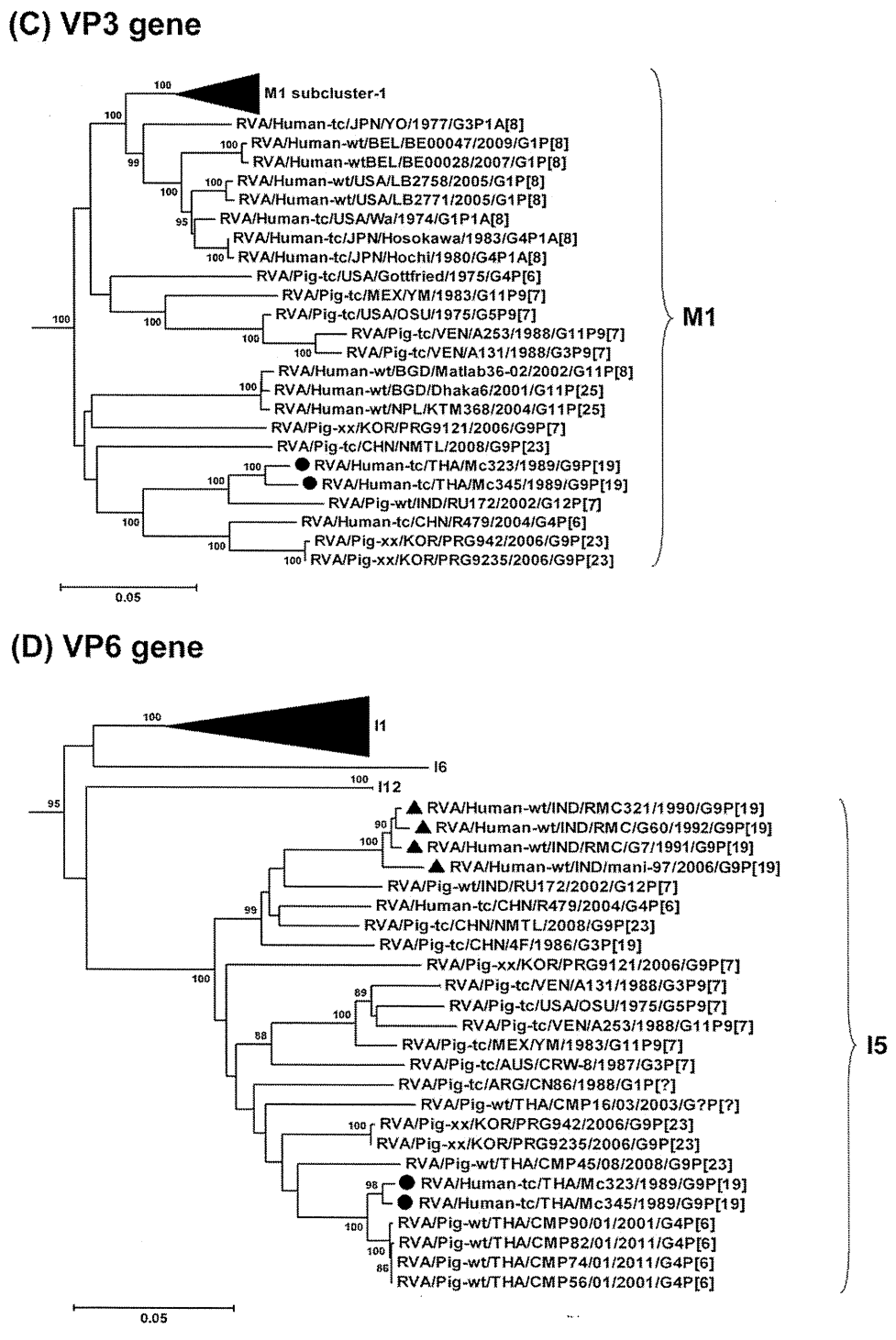

Fig. 1 (*continued*)

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

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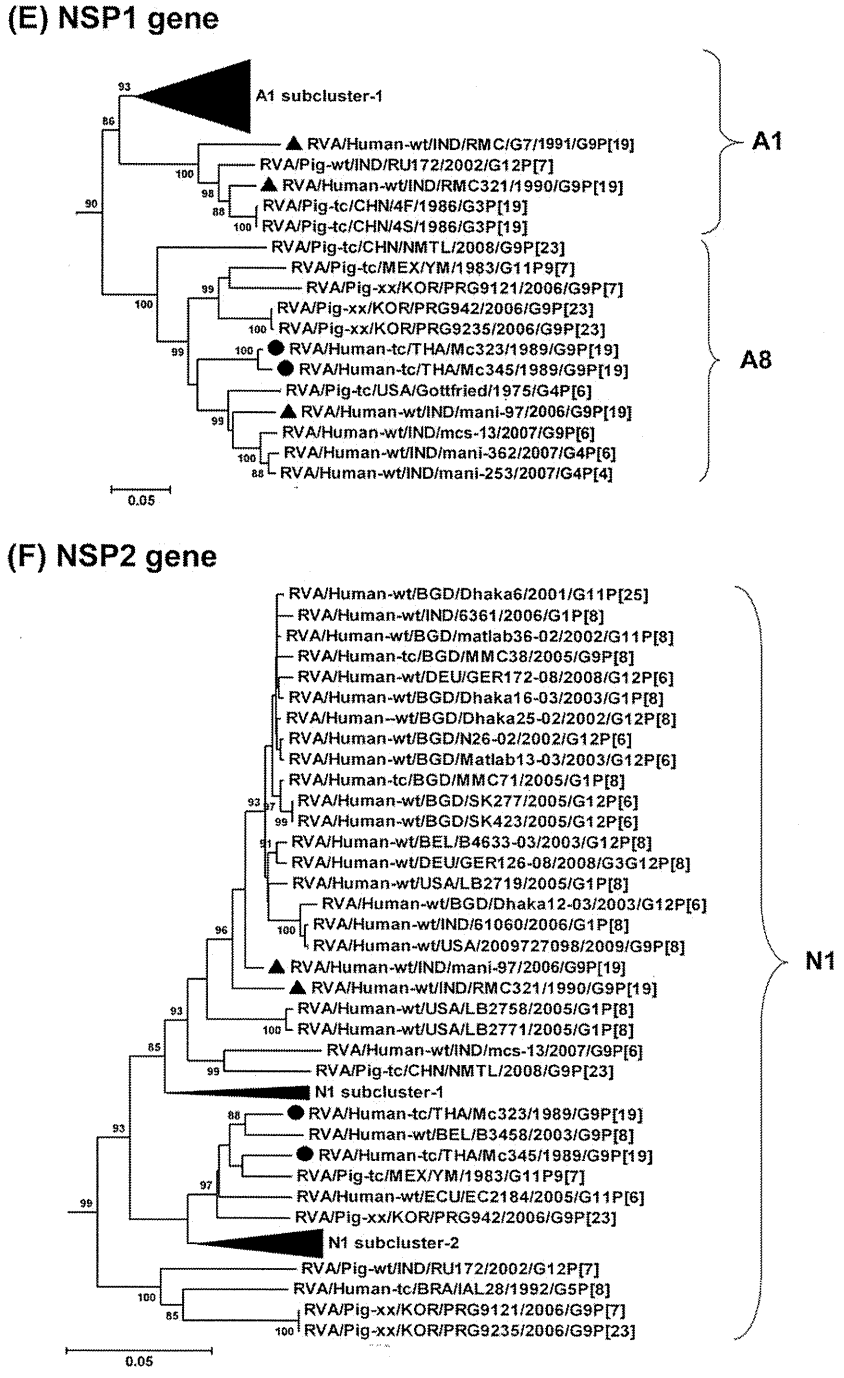

Fig. 1 (*continued*)

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

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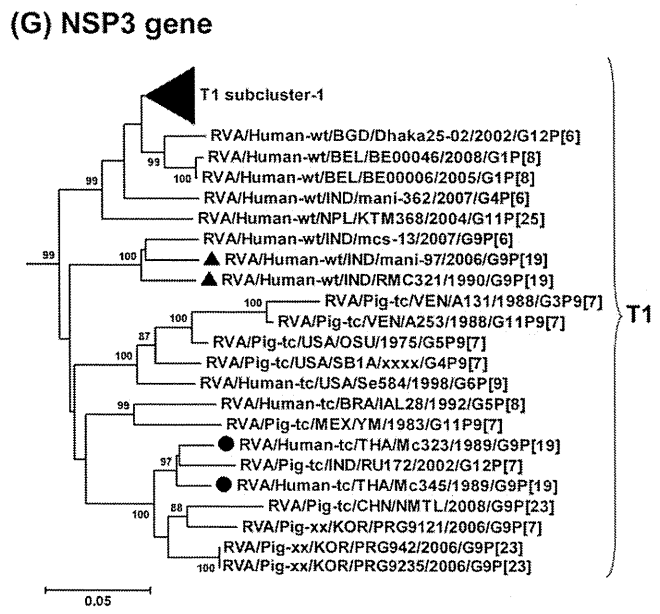

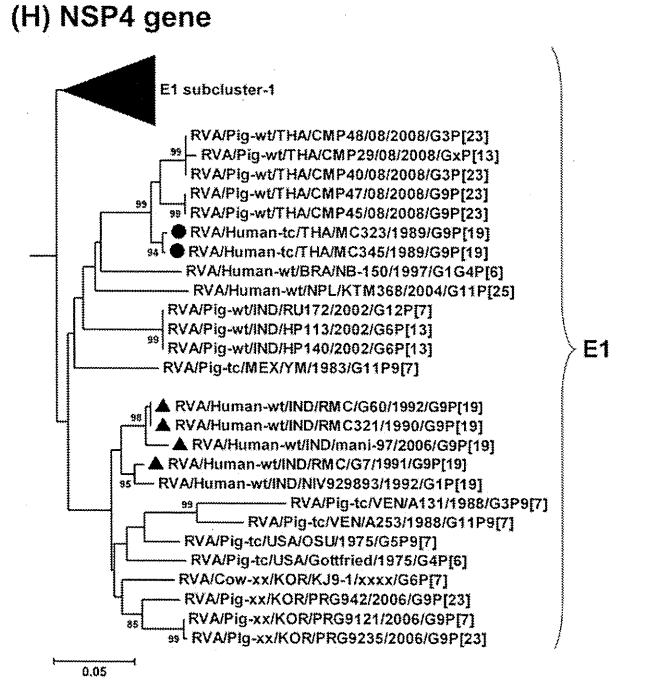

Fig. 1 (*continued*)

Fig. 1 (*continued*)

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.

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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.

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Short Communication

Full genomic analyses of two human G2P[4] rotavirus strains detected in 2005: identification of a caprine-like VP3 gene

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Although G2P[4] rotaviruses are common causes of infantile diarrhoea, to date only the full genomes of the prototype (strain DS-1) and another old strain, TB-Chen, have been analysed. We report here the full genomic analyses of two Bangladeshi G2P[4] strains, MMC6 and MMC88, detected in 2005. Both the strains exhibited a DS-1-like genotype constellation. Excluding the VP4 and VP7 genes, and except for VP3 of MMC88, the MMC strains were genetically more closely related to the contemporary G2P[4] and several non-G2P[4] human strains than the prototype G2P[4] strain. However, by phylogenetic analyses, the VP2, VP3 (except MMC88), NSP1 and NSP3–5 genes of these strains appeared to share a common origin with those of the prototype strain, whilst their VP1, VP6 and NSP2 genes clustered near a caprine strain. The VP3 gene of MMC88 exhibited maximum relatedness to a local caprine strain, representing the first reported human G2P[4] strain with a gene of animal origin.

Group A rotavirus (RV-A) is a major cause of severe diarrhoea in the young of humans and animals (Estes & Kapikian, 2007). The RV-A genome consists of 11 segments of dsRNA that encodes six structural and six non-structural proteins (Estes & Kapikian, 2007). The RV outer capsid proteins, VP7 and VP4, elicit neutralizing antibodies and induce protective immunity. Therefore, monitoring the diversity of the VP7 and VP4 genes are important for vaccine development (Estes & Kapikian, 2007). To date, RV-A strains are classified into at least 25 G and 33 P genotypes on the basis of differences in their VP7 and VP4 gene sequences, respectively (Abe *et al.*, 2009, 2011; Collins *et al.*, 2010; Esona *et al.*, 2010; Estes & Kapikian, 2007; Matthijnssens *et al.*, 2008a, b; Schumann *et al.*, 2009; Solberg *et al.*, 2009; Ursu *et al.*, 2009). Among them, in humans, G1, G2, G3, G4 or G9 in conjunction with P[4], P[6] or P[8] have been reported widely, while, of recent, G12 is emerging as a globally important human genotype (Greenberg & Estes, 2009; Santos & Hoshino, 2005). Although most of the studies on genetic diversity of RV-A are limited to the VP7 and/or VP4 genes, the segmented RV-A genome is vulnerable to frequent reassortment events, and therefore, full genomic analyses of RV-A might be essential to pinpoint the true origin of a strain. Recently, the Rotavirus Classification Working Group (RCWG) proposed a full genome-based classification scheme, providing an ideal platform for deciphering the complex genetic diversity of RV-A strains (Matthijnssens *et al.*, 2008b). However, this scheme has been primarily applied to human RV-A with unusual, or novel G and/or P genotypes (Bányai *et al.*, 2010; Esona *et al.*, 2011; Ghosh *et al.*, 2011; Martella *et al.*, 2010; Steyer *et al.*, 2010), while currently circulating common strains, such as G1P[8] or G2P[4], have been largely ignored.

Although the global prevalence rate of G2P[4] strains does not equal that of G1P[8] strains, G2P[4] RVs are a common cause of viral diarrhoea in humans, with occasional predominance in several countries (Antunes *et al.*, 2009; Gurgel *et al.*, 2007; Kirkwood *et al.*, 2009; Martínez *et al.*, 2010; Paul *et al.*, 2008; Pun *et al.*, 2007; Santos & Hoshino, 2005). However, despite its importance as an enteric pathogen in humans, there are no reports on full genomic analysis of recent human G2P[4] strains, and to date only the full genomes of the prototype G2P[4] strain, DS-1, isolated in 1976, and another Chinese strain, TB-Chen,

The GenBank/EMBL/DDBJ accession numbers for the full-length nucleotide sequences of the VP1–3, VP6 and NSP1–5 genes of strains MMC6 and MMC88 and VP4 gene of MMC88 are HQ641355–HQ641373.

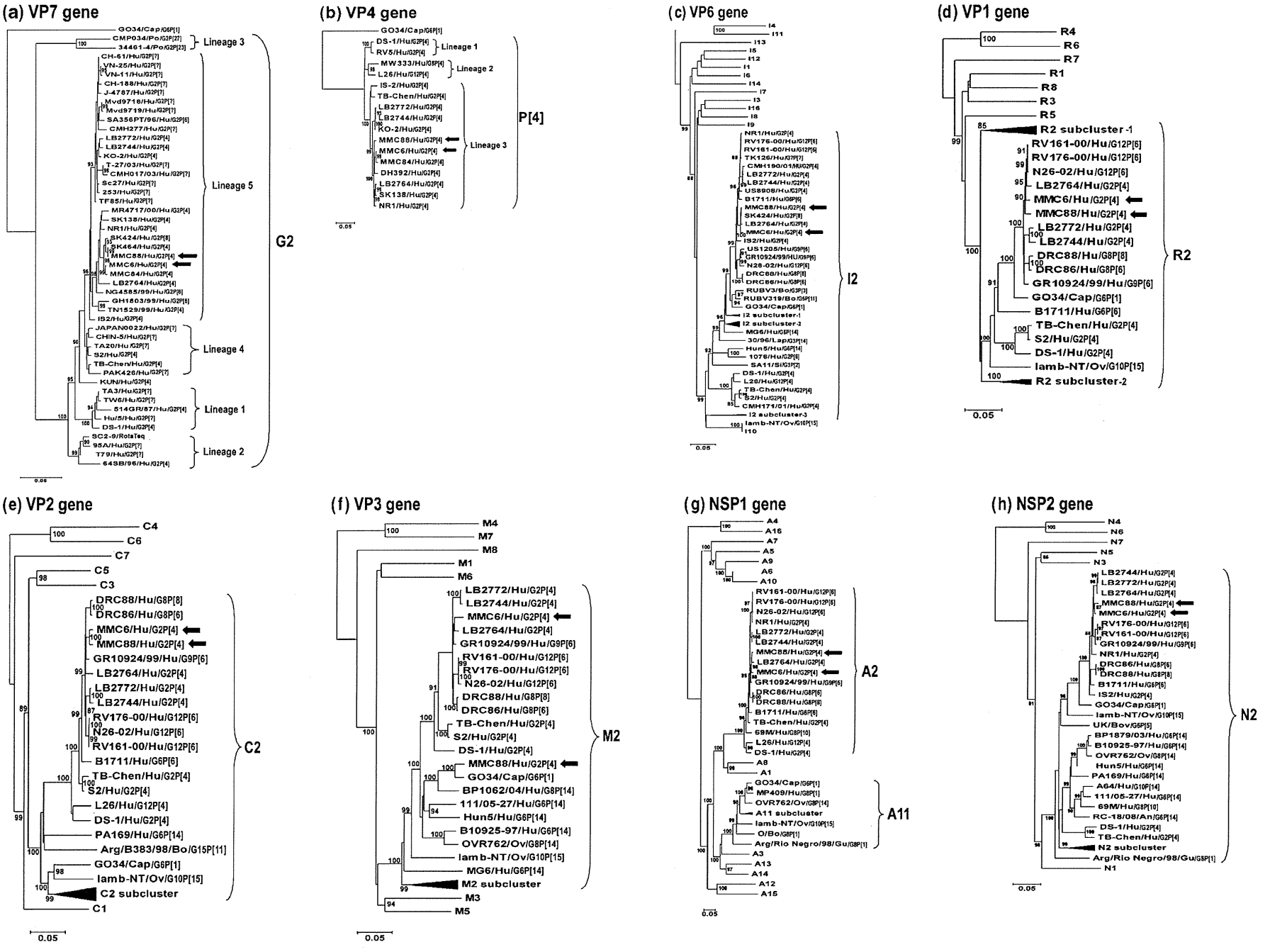
Supplementary material is available with the online version of this paper.

detected in 1996, have been analysed (Chen *et al.*, 2008; Heiman *et al.*, 2008; Matthijnssens *et al.*, 2008a). Comparative analysis of the full genomes of recent and older prototype strains are essential to obtain conclusive data on the evolutionary dynamics of common human strains, as was revealed by recent studies on full genomic analyses of a human G1P[8] strain, Dhaka16-03 (Rahman *et al.*, 2010), and several human G3P[8] strains from the USA (McDonald *et al.*, 2009). Moreover, it would be interesting to investigate as to whether the currently circulating common human strains are indeed true human strains, or are animal–human reassortants. Therefore, in the present study, applying the RCWG classification scheme, the full genomes of two human G2P[4] strains, MMC6 and MMC88, detected in Bangladesh in 2005, were analysed. In addition, a search across the GenBank database revealed the complete or nearly complete coding sequences of the 11 gene segments of three human G2P[4] strains, LB2744, LB2764 and LB2772 (collectively referred to here as the 'LB strains'; GenBank accession numbers are mentioned in Supplementary Table S1, available in JGV Online), detected in USA during 2005–2006. These strains were also included in our analysis.

RV-A G2P[4] strains MMC6 and MMC88 were detected in diarrhoeal stool samples collected from a 10-month-old and a 11-month-old infant, respectively, at the Mymensingh Medical College Hospital, Mymensingh District, Bangladesh, in 2005 (Paul *et al.*, 2008). Both the strains exhibited short RNA migration patterns and had subgroup I specificity, and by RT-PCR-based G- and P- genotyping assays, were assigned to G2 and P[4] genotypes, respectively (Paul *et al.*, 2008). By phylogenetic analyses, the VP7 genes of MMC6 and MMC88 were found to cluster within genotype G2 cluster 5, while the VP4 gene of MMC6 clustered within genotype P[4] cluster 3 (Paul *et al.*, 2008). In the present study, we determined the full-length nucleotide sequences of the VP1–3, VP6 and NSP1–5 genes of strains MMC6 and MMC88 and VP4 gene of strain MMC88. Most of the primers used for obtaining the full-length nucleotide sequences of these gene segments have been reported previously (Gentsch *et al.*, 1992; Taniguchi *et al.*, 1992; Ghosh *et al.*, 2010a, b, 2011; Wang *et al.*, 2010). Additional primers designed in this study are shown in Supplementary Table S2 (available in JGV Online). Nucleotide sequences were determined using the BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems) on an automated sequencer (ABI Prism 3100). Sequence comparisons were carried out as described previously (Ghosh *et al.*, 2010a, b, 2011). Phylogenetic trees were constructed by the neighbour-joining method (Saitou & Nei, 1987) using MEGA (v4.1) software. The trees were statistically supported by bootstrapping with 1000 replicates, and phylogenetic distances were measured by the Kimura two-parameter model.

The complete genomes of human G2P[4] strains MMC6 and MMC88 were 18612 bp in size. By nucleotide sequence identities and phylogenetic analyses, both the strains exhibited a typical DS-1-like genotype constellation (G2-P[4]-I2-R2-C2-M2-A2-N2-T2-E2-H2) (Fig. 1a–k; Supplementary Tables S3 and S4, available in JGV Online). With the exception of the VP3 gene, strains MMC6 and MMC88 exhibited a high degree of genetic relatedness with each other (Fig. 1a–k; Supplementary Tables S3 and S4). With the whole genomes of other RV-A strains, both the strains shared high levels of genetic relatedness with the (i) VP1–2, VP6 and NSP1–5 genes of the G2P[4] LB strains, and human G9P[6] strain GR10924/99 from South Africa, (ii) VP1–2, VP6, NSP1–3 and NSP5 genes of human–animal (bovine and/or porcine) reassortant G8P[6] strain DRC86 and G8P[8] strain DRC88 from Congo, (iii) VP1–2, VP6, NSP1, NSP3 and NSP5 genes of human G12P[6] strains RV161-00, RV176-00 and N26-02 from Bangladesh, (iv) NSP2 genes of strains RV161-00 and RV176-00, and (v) VP2, VP6 and NSP1–5 genes of human–bovine reassortant G6P[6] strain B1711 from Belgium (Fig. 1c–e, g–k; Supplementary Tables S3 and S4). High levels of genetic relatedness were also observed with the partial genomes of several other human strains, such as the VP6 and NSP1–5 genes of G2P[4] strain NR1 and VP6 and NSP2–3 genes of G2P[4] strain IS2 from eastern India, and VP6 and NSP4 genes of G2P[8] strain SK424 from Bangladesh (Fig. 1c, g–k; Supplementary Tables S3 and S4). On the other hand, lower levels of genetic relatedness were observed with the VP1–2, VP6 and NSP1–5 genes of the prototype G2P[4] strain DS-1, the VP1, VP6 and NSP2 genes of strain TB-Chen, and VP1 and VP6 genes of G2P[4] strain S2, detected in 1980 (Fig. 1c–e, g–k; Supplementary Tables S3 and S4). Interestingly, strains MMC6 and MMC88 appeared to be more related to the VP1, VP6 and NSP2 genes of caprine RV-A strain GO34 than those of strains DS-1, TB-Chen and S2 (Fig. 1c, d, h; Supplementary Tables S3 and S4). The nucleotide sequence identities exhibited by the MMC strains to the VP2, NSP1 and NSP3–5 genes of strain TB-Chen, VP2 and NSP3–4 genes of S2, NSP5 gene of IS2, and NSP4–5 genes of G2P[4] strain KUN, isolated in 1982, were comparable to those shared with the G2P[4] LB and above-mentioned non-G2P[4] strains (Supplementary Tables S3 and S4). However, by phylogenetic analyses, MMC6 and MMC88 appeared to be more closely related to the recent G2P[4] LB and the non-G2P[4] human strains than these older G2P[4] strains (Fig. 1e, g, i–k). The VP3 gene of strain MMC6 exhibited high levels of genetic relatedness to those of the G2P[4] LB and DS-1-like non-G2P[4] human strains, whilst that of MMC88 shared maximum nucleotide sequence identities of 97.4 % with that of caprine strain GO34, and by phylogenetic analysis, clustered with the caprine strain, away from the cluster consisting of these human strains (Fig. 1f; Supplementary Table S3).

Taken together, excluding the VP4 and VP7 genes, and with the exception of the VP3 gene of strain MMC88, strains MMC6 and MMC88, the G2P[4] LB strains, and the partial genome of strain NR1, detected in 1999, appeared to be more closely related to different genes of several


(a) VP7 gene
GO34/Cap/G6P[1]
CMP034/Po/G2P[27]
34461-4/Po/G2P[23]
Lineage 3
CH-51/Hu/G2P[7]
VN-25/Hu/G2P[7]
VN-11/Hu/G2P[7]
CH-188/Hu/G2P[7]
J-4787/Hu/G2P[7]
Mvd9716/Hu/G2P[7]
Mvd9719/Hu/G2P[7]
SA356PT/96/Hu/G2P[6]
CMH277/Hu/G2P[7]
LB2772/Hu/G2P[4]
LB2744/Hu/G2P[4]
KO-2/Hu/G2P[4]
T-27/03/Hu/G2P[7]
CMH017/03/Hu/G2P[7]
Sc27/Hu/G2P[7]
253/Hu/G2P[7]
TF85/Hu/G2P[7]
MR4717/00/Hu/G2P[4]
SK138/Hu/G2P[4]
NR1/Hu/G2P[4]
SK424/Hu/G2P[8]
SK464/Hu/G2P[4]
MMC88/Hu/G2P[4]
MMC6/Hu/G2P[4]
MMC84/Hu/G2P[4]
LB2764/Hu/G2P[4]
NG4585/99/Hu/G2P[6]
GH1603/99/Hu/G2P[6]
TN1529/99/Hu/G2P[4]
Lineage 5
IS2/Hu/G2P[4]
JAPAN6022/Hu/G2P[7]
CHN-5/Hu/G1P[7]
TA20/Hu/G2P[7]
S2/Hu/G2P[4]
TB-Chen/Hu/G2P[4]
PAK426/Hu/G2P[7]
Lineage 4
KUN/Hu/G2P[4]
TA3/Hu/G2P[7]
TW6/Hu/G2P[7]
514GR/87/Hu/G2P[4]
Hu/5/Hu/G2P[7]
DS-1/Hu/G2P[4]
Lineage 1
SC2-9/RotaTeq
95A/Hu/G2P[7]
T79/Hu/G2P[7]
64SB/96/Hu/G2P[4]
Lineage 2
G2
0.05
(b) VP4 gene
GO34/Cap/G6P[1]
DS-1/Hu/G2P[4]
RV5/Hu/G2P[4]
Lineage 1
MW333/Hu/G8P[4]
L26/Hu/G12P[4]
Lineage 2
IS-2/Hu/G2P[4]
TB-Chen/Hu/G2P[4]
LB2772/Hu/G2P[4]
LB2744/Hu/G2P[4]
KO-2/Hu/G2P[4]
MMC88/Hu/G2P[4]
MMC6/Hu/G2P[4]
MMC84/Hu/G2P[4]
DH392/Hu/G2P[4]
LB2764/Hu/G2P[4]
SK138/Hu/G2P[4]
NR1/Hu/G2P[4]
Lineage 3
P[4]
0.05
(c) VP6 gene
I4
I11
I13
I5
I12
I1
I6
I14
I7
I3
I16
I8
I9
NR1/Hu/G2P[4]
RV176-00/Hu/G12P[6]
RV161-00/Hu/G12P[6]
TK126/Hu/G3P[7]
CMH190/01/Hu/G2P[4]
LB2772/Hu/G2P[4]
LB2744/Hu/G2P[4]
US8908/Hu/G3P[4]
B1711/Hu/G6P[6]
MMC88/Hu/G2P[4]
SK424/Hu/G2P[8]
LB2764/Hu/G2P[4]
MMC6/Hu/G2P[4]
IS2/Hu/G2P[4]
US1205/Hu/G9P[6]
GR10924/99/Hu/G9P[6]
N26-02/Hu/G12P[6]
DRC88/Hu/G8P[8]
DRC86/Hu/G8P[6]
RUBV3/Bo/G3P[3]
RUBV319/Bo/G5P[11]
GO34/Cap/G6P[1]
I2 subcluster-1
I2 subcluster-2
MG6/Hu/G6P[14]
30/96/Lap/G3P[14]
Hun5/Hu/G6P[14]
1076/Hu/G2P[6]
SA411/Si/G3P[2]
DS-1/Hu/G2P[4]
L26/Hu/G12P[4]
TB-Chen/Hu/G2P[4]
S2/Hu/G2P[4]
CMH171/01/Hu/G2P[4]
I2 subcluster-3
lamb-NT/Ov/G10P[15]
I10
I2
0.05
(d) VP1 gene
R4
R6
R7
R1
R8
R3
R5
R2 subcluster -1
RV161-00/Hu/G12P[6]
RV176-00/Hu/G12P[6]
N26-02/Hu/G12P[6]
LB2764/Hu/G2P[4]
MMC6/Hu/G2P[4]
MMC88/Hu/G2P[4]
LB2772/Hu/G2P[4]
LB2744/Hu/G2P[4]
DRC88/Hu/G8P[8]
DRC86/Hu/G8P[6]
GR10924/99/Hu/G9P[6]
GO34/Cap/G6P[1]
B1711/Hu/G6P[6]
TB-Chen/Hu/G2P[4]
S2/Hu/G2P[4]
DS-1/Hu/G2P[4]
lamb-NT/Ov/G10P[15]
R2 subcluster-2
R2
0.05
(e) VP2 gene
C4
C6
C7
C5
C3
DRC88/Hu/G8P[8]
DRC86/Hu/G8P[6]
MMC6/Hu/G2P[4]
MMC88/Hu/G2P[4]
GR10924/99/Hu/G9P[6]
LB2764/Hu/G2P[4]
LB2772/Hu/G2P[4]
LB2744/Hu/G2P[4]
RV176-00/Hu/G12P[6]
N26-02/Hu/G12P[6]
RV161-00/Hu/G12P[6]
B1711/Hu/G6P[6]
TB-Chen/Hu/G2P[4]
S2/Hu/G2P[4]
L26/Hu/G12P[4]
DS-1/Hu/G2P[4]
PA169/Hu/G6P[14]
Arg/B383/98/Bo/G15P[11]
GO34/Cap/G6P[1]
lamb-NT/Ov/G10P[15]
C2 subcluster
C1
C2
0.05
(f) VP3 gene
M4
M7
M8
M1
M6
LB2772/Hu/G2P[4]
LB2744/Hu/G2P[4]
MMC6/Hu/G2P[4]
LB2764/Hu/G2P[4]
GR10924/99/Hu/G9P[6]
RV161-00/Hu/G12P[6]
RV176-00/Hu/G12P[6]
N26-02/Hu/G12P[6]
DRC88/Hu/G8P[8]
DRC86/Hu/G8P[6]
TB-Chen/Hu/G2P[4]
S2/Hu/G2P[4]
DS-1/Hu/G2P[4]
MMC88/Hu/G2P[4]
GO34/Cap/G6P[1]
BP1062/04/Hu/G8P[14]
111/05-27/Hu/G6P[14]
Hun5/Hu/G6P[14]
B10925-97/Hu/G6P[14]
OVR762/Ov/G8P[14]
lamb-NT/Ov/G10P[15]
MG6/Hu/G6P[14]
M2 subcluster
M2
M3
M5
0.05
(g) NSP1 gene
A4
A16
A7
A5
A9
A6
A10
RV161-00/Hu/G12P[6]
RV176-00/Hu/G12P[6]
N26-02/Hu/G12P[6]
NR1/Hu/G2P[4]
LB2772/Hu/G2P[4]
LB2744/Hu/G2P[4]
MMC88/Hu/G2P[4]
LB2764/Hu/G2P[4]
MMC6/Hu/G2P[4]
GR10924/99/Hu/G9P[6]
DRC86/Hu/G8P[6]
DRC88/Hu/G8P[8]
B1711/Hu/G6P[6]
TB-Chen/Hu/G2P[4]
69M/Hu/G8P[10]
L26/Hu/G12P[4]
DS-1/Hu/G2P[4]
A2
A8
A1
GO34/Cap/G6P[1]
MP409/Hu/G8P[1]
OVR762/Ov/G9P[14]
A11 subcluster
lamb-NT/Ov/G10P[15]
O/BO/G8P[1]
Arg/Rio Negro/98/Gu/G8P[1]
A11
A3
A13
A14
A12
A15
0.05
(h) NSP2 gene
N4
N6
N7
N5
N3
LB2744/Hu/G2P[4]
LB2772/Hu/G2P[4]
LB2764/Hu/G2P[4]
MMC88/Hu/G2P[4]
MMC6/Hu/G2P[4]
RV176-00/Hu/G12P[6]
RV161-00/Hu/G12P[6]
GR10924/99/Hu/G9P[6]
NR1/Hu/G2P[4]
DRC86/Hu/G8P[6]
DRC88/Hu/G8P[8]
B1711/Hu/G6P[6]
IS2/Hu/G2P[4]
GO34/Cap/G6P[1]
lamb-NT/Ov/G10P[15]
UK/Bov/G6P[5]
BP1879/03/Hu/G6P[14]
B10925-97/Hu/G6P[14]
OVR762/Ov/G8P[14]
Hun5/Hu/G6P[14]
PA169/Hu/G6P[14]
A64/Hu/G10P[14]
111/05-27/Hu/G6P[14]
69M/Hu/G8P[10]
RC-18/08/An/G6P[14]
DS-1/Hu/G2P[4]
TB-Chen/Hu/G2P[4]
N2 subcluster
Arg/Rio Negro/98/Gu/G8P[1]
N1
N2
0.05

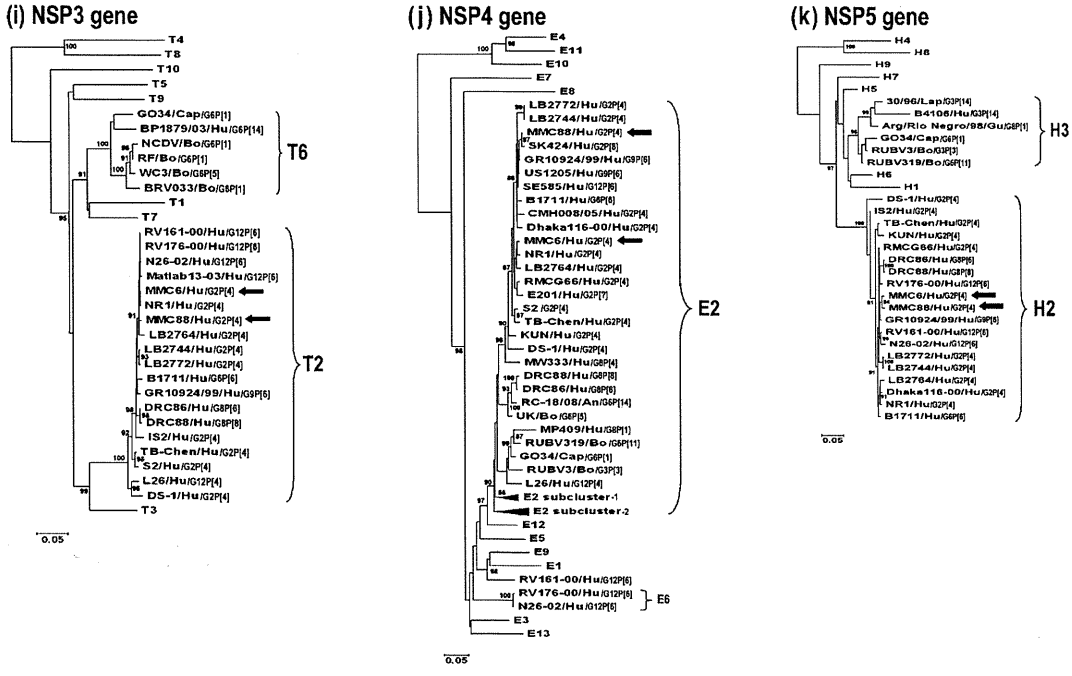


Fig. 1. (a–k) Phylogenetic trees constructed from nucleotide sequences of VP7, VP4, VP6, VP1–3 and NSP1–5 genes of RV strains MMC6 and MMC88 with those of other group A RV strains representing the different G, P, I, R, C, M, A, N, T, E and H genotypes, respectively. In all trees, positions of strains MMC6 and MMC88 are indicated by arrows. Within the I2, R2, C2, M2, N2 and E2 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 represented as subcluster/s. Bootstrap values >85 % are shown. Bar, 0.05 substitutions per nucleotide. An, Antelope; Bo, bovine; Cap, caprine; Gu, guanaco; Hu, human; La, lapine; Ov, ovine; Po, porcine; Si, simian.

contemporary non-G2P[4] human strains (detected in the 2000s) than the older G2P[4] strains, such as DS-1, KUN and S2, detected almost three decades ago, and strain TB-Chen, detected in 1996. However, by phylogenetic analyses, the VP2, VP3, NSP1 and NSP3–5 genes of strains MMC6, MMC88 (except for the VP3 gene), the G2P[4] LB strains and the non-G2P[4] strains (clustering with the MMC strains), and NSP1 and NSP3–5 genes of strain NR1 appeared to evolve from or share the same ancestor as those of the prototype strain DS-1, or other older G2P[4] strains, such as TB-Chen, KUN (NSP4–5 genes) and S2 (VP2–3 and NSP3–4 genes) (Fig. 1e–g, i–k). On the other hand, the VP1, VP6 and NSP2 genes of strains MMC6, MMC88, the G2P[4] LB strains and the related non-G2P[4] strains, and VP6 and NSP2 genes of NR1 clustered near those of the caprine strain GO34 (and VP6 genes of bovine RUBV strains), and appeared to be distantly related to those of strains DS-1, TB-Chen and S2 (VP1 and VP6 genes) (Fig. 1c, d, h). Therefore, not all the genes of these recent G2P[4] strains, or older strains, such as NR1, appear to have evolved from or shared a common ancestor with those of the prototype or other older G2P[4] strains, rather a common origin for the VP1, VP6 and NSP2 genes of these strains and related non-G2P[4] strains with those of the caprine strain was envisaged.

Although data on VP3 gene analysis is limited, this gene was believed to segregate according to species of origin (Cook & McCrae, 2004; Subodh *et al.*, 2006). Moreover, Hoshino *et al.* (1995) had proposed a role of the VP3 in host range restriction. To date, reassortant human RV-A strains which possesses only VP3 gene from other host species has never been reported under natural conditions. However, reassortant strains with the VP3 gene and at least one more gene of animal origin have been reported from cases of human diarrhoea. The VP3 and VP7 genes of human G6P[6] strain B1711 were shown to be derived from ruminant strains (Matthijnssens *et al.*, 2008c), whilst strain 6787/2000/ARN was reported to have porcine-like VP3 and NSP5 genes in the background of Wa genogroup (Esona *et al.*, 2011). Interestingly, the VP3 gene of strain MMC88 exhibited maximum genetic relatedness to that of the caprine RV-A strain, GO34, also detected from the same area in Bangladesh (Ghosh *et al.*, 2010a), whilst its remaining genes were closely related to those of DS-1-like human strains (Fig. 1c–k; Supplementary Tables S3 and S4). Therefore, it is likely that MMC88 might have acquired its VP3 gene from a co-circulating GO34-like caprine strain, possibly through reassortment. The presence of unhygienic conditions and close proximity of humans to livestock at the sampling site might have

facilitated such an event. To our knowledge, the present study is the first report of a reassortant RV-A strain with only the VP3 gene derived from other host species. Moreover, strain MMC88 is the first reported human G2P[4] strain with a gene derived from animal host species.

The VP7 of G2 genotype is a component of the pentavalent RV vaccine, RotaTeq, which has been shown to be both immunogenic and efficacious (Vesikari *et al.*, 2006). Recently, the G2 sequence of RotaTeq was classified as G2 lineage 2, whilst most of the currently circulating strains were found to cluster within G2 lineages 4 and 5 (Matthijnssens *et al.*, 2010). Strains MMC6 and MMC88 (Paul *et al.*, 2008), and the G2P[4] LB strains also belonged to G2 lineage 5 (Fig. 1a). However, comparisons of the deduced VP7 amino acid sequences of these recent G2P[4] strains with those of the older G2P[4] strains and the G2 component of RotaTeq revealed a few amino acid differences in the antigenic regions among these strains, of which, two mismatches at positions 87 and 96 were exclusively between the recent strains and the vaccine component (Supplementary Fig. S1). Although the implication/s of such mismatches on the efficacy of a vaccine remain to be determined, continuous monitoring of such changes may be useful in evaluating and updating the vaccines over time (Rahman *et al.*, 2010).

In conclusion, the present study, though limited to the whole genomic analyses of a few recent strains, highlighted the complex patterns of evolution of G2P[4] RV-A strains, even within the same genogroup. Although intergenogroup reassortants exist, such strains may be less fit and selected against in nature (McDonald *et al.*, 2009). Corroborating this hypothesis, none of the G2P[4] strains analysed in this study exhibited an intergenogroup genomic constellation. However, even within a genogroup, there may be preferences towards maintaining a certain stable genome constellation (McDonald *et al.*, 2009). This might have accounted for the differences observed between the genomes of these recent G2P[4] strains and the prototype strain, and their close relatedness to those of the contemporary non-G2P[4] strains. However, full genomic analyses of several other currently circulating and older G2P[4] strains as well as other common DS-1-like strains are required to validate this hypothesis. Although most studies focus on full genomic analyses of human strains with unconventional G and/or P genotypes, common human strains may also possess genes of animal origin, as was revealed in the present study. Therefore, comparisons of the full genomes of human strains, even those with conventional G–P genotypes, with those of co-circulating animal RV-A strains might be essential to gain a proper understanding of the animal–human reassortment events occurring under natural conditions. Taken together, the present study underscored the importance of full genomic analyses of common circulating strains in obtaining conclusive data on the complex evolutionary dynamics of human RV-A strains.

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Short Communication

Whole-genome analysis reveals the complex evolutionary dynamics of Kenyan G2P[4] human rotavirus strains

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Although G2P[4] rotaviruses are common causes of acute childhood diarrhoea in Africa, to date there are no reports on whole genomic analysis of African G2P[4] strains. In this study, the nearly complete genome sequences of two Kenyan G2P[4] strains, AK26 and D205, detected in 1982 and 1989, respectively, were analysed. Strain D205 exhibited a DS-1-like genotype constellation, whilst strain AK26 appeared to be an intergenogroup reassortant with a Wa-like NSP2 genotype on the DS-1-like genotype constellation. The VP2-4, VP6-7, NSP1, NSP3 and NSP5 genes of strain AK26 and the VP2, VP4, VP7 and NSP1–5 genes of strain D205 were closely related to those of the prototype or other human G2P[4] strains. In contrast, their remaining genes were distantly related, and, except for NSP2 of AK26, appeared to originate from or share a common origin with rotavirus genes of artiodactyl (ruminant and camelid) origin. These observations highlight the complex evolutionary dynamics of African G2P[4] rotaviruses.

Group A rotavirus (RV-A) is a major cause of acute gastroenteritis in African children, accounting for more than 230 000 childhood deaths in sub-Saharan Africa each year (Madhi *et al.*, 2010; Mwenda *et al.*, 2010). The RV-A genome is composed of 11 segments of dsRNA encoding six structural and five or six non-structural proteins (Estes & Kapikian, 2007). The RV-A outer capsid VP7 and VP4 proteins elicit protective antibodies, forming the basis of present-day rotavirus vaccines (Estes & Kapikian, 2007). To date, the RV-A VP7 and VP4 genes have been classified into at least 25 G and 33 P genotypes, respectively (Abe *et al.*, 2009, 2011; Collins *et al.*, 2010; Esona *et al.*, 2010; Estes & Kapikian, 2007; Matthijnssens *et al.*, 2008a, b; Schumann *et al.*, 2009; Solberg *et al.*, 2009; Ursu *et al.*, 2009). Among them, G1, G2, G3, G4 or G9 in combination with P[4], P[6] or P[8] are commonly found in human RV-A strains, while G12 is emerging as an important genotype in human strains (Matthijnssens *et al.*, 2010).

In sub-Saharan Africa during 1990–2009, the most frequent G type detected was G1 (34.9 %), followed by G2 (9.1 %) and G3 (8.6 %), whilst G4, G8 and G9 strains accounted for 1.9, 3.3 and 2.6 % of the infections, respectively (Sanchez-Padilla *et al.*, 2009). Among the P genotypes, P[8] (35.5 %) and P[6] (27.5 %) were predominant, whilst P[4] was reported in 7.3 % of infections (Sanchez-Padilla *et al.*, 2009). The common human G/P combinations, such as G1P[8] and G2P[4] strains, have routinely been detected in most African nations, with varying rates of detection (Cunliffe *et al.*, 1998; Mwenda *et al.*, 2010; Todd *et al.*, 2010). For example, recent studies in countries such as Egypt, Ethiopia, Jordan, Oman, Sierra Leone and Yemen have identified G2P[4] as the most prevalent human RV-A strain (Jere *et al.*, 2011; Khoury *et al.*, 2011; Mwenda *et al.*, 2010), whilst in some African countries, such as Kenya and Uganda, G2P[4] strains have been reported as the second most predominant type in some seasons (Cunliffe *et al.*, 1998; Mwenda *et al.*, 2010; Nyangao *et al.*, 2010). Several unusual human RV-A strains, including animal–human reassortants and zoonotic strains, have been also detected in African countries, sometimes in considerable numbers (Esona *et al.*, 2009a, b, 2011; Ghosh *et al.*, 2011a, b; Jere *et al.*, 2011; Santos & Hoshino, 2005; Todd *et al.*, 2010).

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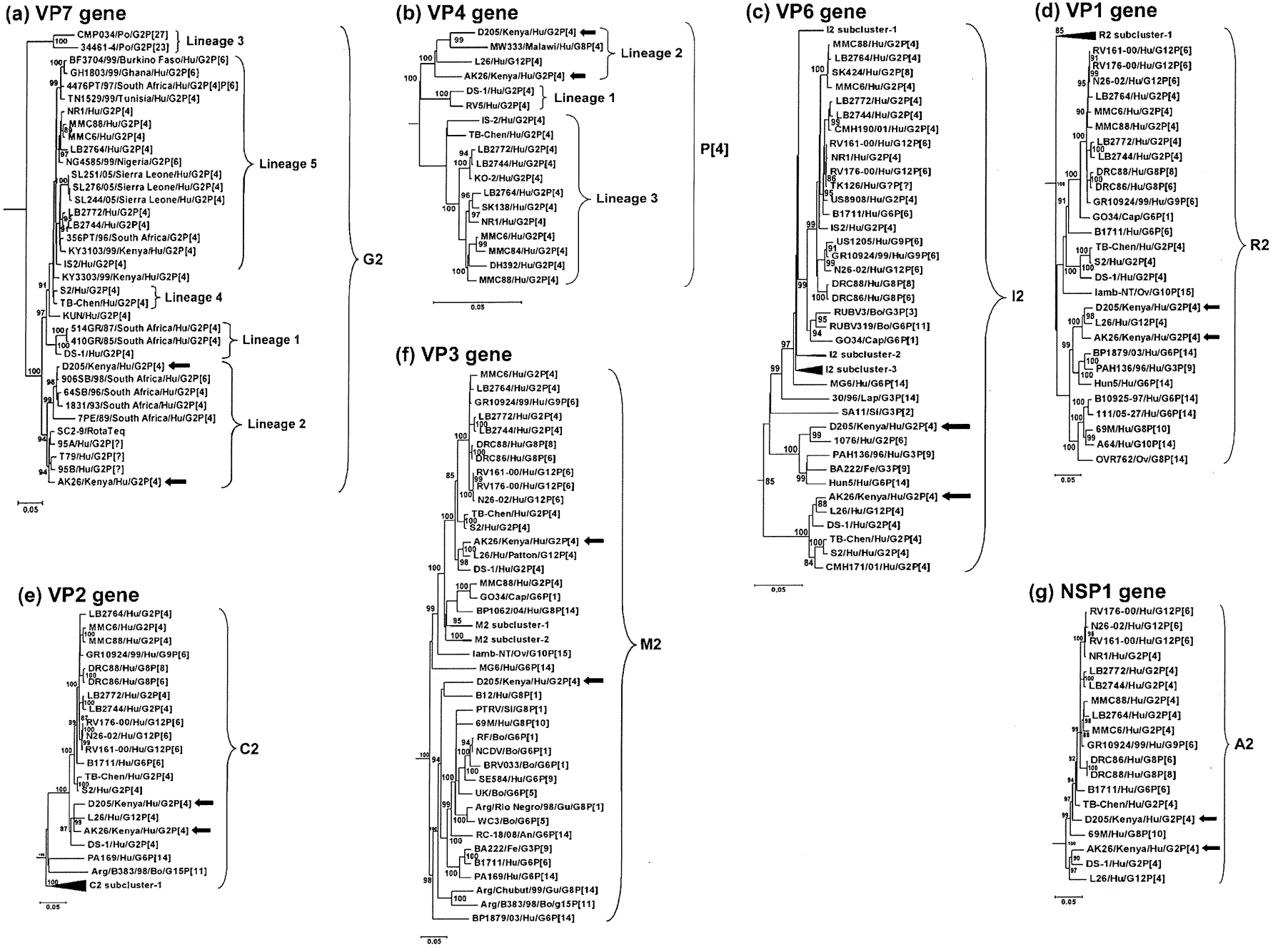
The GenBank accession numbers for the nucleotide sequences of the VP1–4, VP6–7 and NSP1–5 genes of human RV-A G2P[4] strains D205 and AK26 are JF304915–JF304936, respectively.

A supplementary figure comparing the deduced amino acid sequences of the VP7 genes of strains AK26 and D205 with those of other strains and a supplementary table of primers used for amplification of different genes of strains AK26 and D205 are available with the online version of this paper.


(a) VP7 gene
CMP034/Po/G2P[27]
34461-4/Po/G2P[23]
Lineage 3
BF3704/99/Burkino Faso/Hu/G2P[6]
GH1803/99/Ghana/Hu/G2P[6]
4476PT/97/South Africa/Hu/G2P[4]P[6]
TN1529/99/Tunisia/Hu/G2P[4]
NR1/Hu/G2P[4]
MMC88/Hu/G2P[4]
MMC6/Hu/G2P[4]
LB2764/Hu/G2P[4]
NG4585/99/Nigeria/G2P[6]
SL251/05/Sierra Leone/Hu/G2P[4]
SL276/05/Sierra Leone/Hu/G2P[4]
SL244/05/Sierra Leone/Hu/G2P[4]
LB2772/Hu/G2P[4]
LB2744/Hu/G2P[4]
356PT/96/South Africa/G2P[4]
KY3103/99/Kenya/Hu/G2P[4]
IS2/Hu/G2P[4]
Lineage 5
KY3303/99/Kenya/Hu/G2P[4]
S2/Hu/G2P[4]
TB-Chen/Hu/G2P[4]
Lineage 4
KUN/Hu/G2P[4]
514GR/87/South Africa/Hu/G2P[4]
410GR/85/South Africa/Hu/G2P[4]
DS-1/Hu/G2P[4]
Lineage 1
D205/Kenya/Hu/G2P[4]
906SB/98/South Africa/Hu/G2P[6]
64SB/96/South Africa/Hu/G2P[4]
1831/93/South Africa/Hu/G2P[4]
7PE/89/South Africa/Hu/G2P[4]
SC2-9/RotaTeq
95A/Hu/G2P[?]
T79/Hu/G2P[?]
95B/Hu/G2P[?]
AK26/Kenya/Hu/G2P[4]
Lineage 2
G2
0.05
(b) VP4 gene
D205/Kenya/Hu/G2P[4]
MW333/Malawi/Hu/G8P[4]
L26/Hu/G12P[4]
AK26/Kenya/Hu/G2P[4]
Lineage 2
DS-1/Hu/G2P[4]
RV5/Hu/G2P[4]
Lineage 1
IS-2/Hu/G2P[4]
TB-Chen/Hu/G2P[4]
LB2772/Hu/G2P[4]
LB2744/Hu/G2P[4]
KO-2/Hu/G2P[4]
LB2764/Hu/G2P[4]
SK138/Hu/G2P[4]
NR1/Hu/G2P[4]
MMC6/Hu/G2P[4]
MMC84/Hu/G2P[4]
DH392/Hu/G2P[4]
MMC88/Hu/G2P[4]
Lineage 3
P[4]
0.05
(c) VP6 gene
I2 subcluster-1
MMC88/Hu/G2P[4]
LB2764/Hu/G2P[4]
SK424/Hu/G2P[8]
MMC6/Hu/G2P[4]
LB2772/Hu/G2P[4]
LB2744/Hu/G2P[4]
CMH190/01/Hu/G2P[4]
RV161-00/Hu/G12P[6]
NR1/Hu/G2P[4]
RV176-00/Hu/G12P[6]
TK126/Hu/G?P[?]
US8908/Hu/G2P[4]
B1711/Hu/G6P[6]
IS2/Hu/G2P[4]
US1205/Hu/G9P[6]
GR10924/99/Hu/G9P[6]
N26-02/Hu/G12P[6]
DRC88/Hu/G8P[8]
DRC86/Hu/G8P[6]
RUBV3/Bo/G3P[3]
RUBV319/Bo/G6P[11]
GO34/Cap/G6P[1]
I2 subcluster-2
I2 subcluster-3
MG6/Hu/G6P[14]
30/96/Lap/G3P[14]
SA11/Si/G3P[2]
D205/Kenya/Hu/G2P[4]
1076/Hu/G2P[6]
PAH136/96/Hu/G3P[9]
BA222/Fe/G3P[9]
Hun5/Hu/G6P[14]
AK26/Kenya/Hu/G2P[4]
L26/Hu/G12P[4]
DS-1/Hu/G2P[4]
TB-Chen/Hu/G2P[4]
S2/Hu/Hu/G2P[4]
CMH171/01/Hu/G2P[4]
I2
0.05
(d) VP1 gene
R2 subcluster-1
RV161-00/Hu/G12P[6]
RV176-00/Hu/G12P[6]
N26-02/Hu/G12P[6]
LB2764/Hu/G2P[4]
MMC6/Hu/G2P[4]
MMC88/Hu/G2P[4]
LB2772/Hu/G2P[4]
LB2744/Hu/G2P[4]
DRC88/Hu/G8P[8]
DRC86/Hu/G8P[6]
GR10924/99/Hu/G9P[6]
GO34/Cap/G6P[1]
B1711/Hu/G6P[6]
TB-Chen/Hu/G2P[4]
S2/Hu/G2P[4]
DS-1/Hu/G2P[4]
lamb-NT/Ov/G10P[15]
D205/Kenya/Hu/G2P[4]
L26/Hu/G12P[4]
AK26/Kenya/Hu/G2P[4]
BP1879/03/Hu/G6P[14]
PAH136/96/Hu/G3P[9]
Hun5/Hu/G6P[14]
B10925-97/Hu/G6P[14]
111/05-27/Hu/G6P[14]
69M/Hu/G8P[10]
A64/Hu/G10P[14]
OVR762/Ov/G8P[14]
R2
0.05
(e) VP2 gene
LB2764/Hu/G2P[4]
MMC6/Hu/G2P[4]
MMC88/Hu/G2P[4]
GR10924/99/Hu/G9P[6]
DRC88/Hu/G8P[8]
DRC86/Hu/G8P[6]
LB2772/Hu/G2P[4]
LB2744/Hu/G2P[4]
RV176-00/Hu/G12P[6]
N26-02/Hu/G12P[6]
RV161-00/Hu/G12P[6]
B1711/Hu/G6P[6]
TB-Chen/Hu/G2P[4]
S2/Hu/G2P[4]
D205/Kenya/Hu/G2P[4]
L26/Hu/G12P[4]
AK26/Kenya/Hu/G2P[4]
DS-1/Hu/G2P[4]
PA169/Hu/G6P[14]
Arg/B383/98/Bo/G15P[11]
C2 subcluster-1
C2
0.05
(f) VP3 gene
MMC6/Hu/G2P[4]
LB2764/Hu/G2P[4]
GR10924/99/Hu/G9P[6]
LB2772/Hu/G2P[4]
LB2744/Hu/G2P[4]
DRC88/Hu/G8P[8]
DRC86/Hu/G8P[6]
RV161-00/Hu/G12P[6]
RV176-00/Hu/G12P[6]
N26-02/Hu/G12P[6]
TB-Chen/Hu/G2P[4]
S2/Hu/G2P[4]
AK26/Kenya/Hu/G2P[4]
L26/Hu/Patton/G12P[4]
DS-1/Hu/G2P[4]
MMC88/Hu/G2P[4]
GO34/Cap/G6P[1]
BP1062/04/Hu/G8P[14]
M2 subcluster-1
M2 subcluster-2
lamb-NT/Ov/G10P[15]
MG6/Hu/G6P[14]
D205/Kenya/Hu/G2P[4]
B12/Hu/G8P[1]
PTRV/SI/G8P[1]
69M/Hu/G8P[10]
RF/Bo/G6P[1]
NCDV/Bo/G6P[1]
BRV033/Bo/G6P[1]
SE584/Hu/G6P[9]
UK/Bo/G6P[5]
Arg/Rio Negro/98/Gu/G8P[1]
WC3/Bo/G6P[5]
RC-18/08/An/G6P[14]
BA222/Fe/G3P[9]
B1711/Hu/G6P[6]
PA169/Hu/G6P[14]
Arg/Chubut/99/Gu/G8P[14]
Arg/B383/98/Bo/g15P[11]
BP1879/03/Hu/G6P[14]
M2
0.05
(g) NSP1 gene
RV176-00/Hu/G12P[6]
N26-02/Hu/G12P[6]
RV161-00/Hu/G12P[6]
NR1/Hu/G2P[4]
LB2772/Hu/G2P[4]
LB2744/Hu/G2P[4]
MMC88/Hu/G2P[4]
LB2764/Hu/G2P[4]
MMC6/Hu/G2P[4]
GR10924/99/Hu/G9P[6]
DRC86/Hu/G8P[6]
DRC88/Hu/G8P[8]
B1711/Hu/G6P[6]
TB-Chen/Hu/G2P[4]
D205/Kenya/Hu/G2P[4]
69M/Hu/G8P[10]
AK26/Kenya/Hu/G2P[4]
DS-1/Hu/G2P[4]
L26/Hu/G12P[4]
A2
0.05

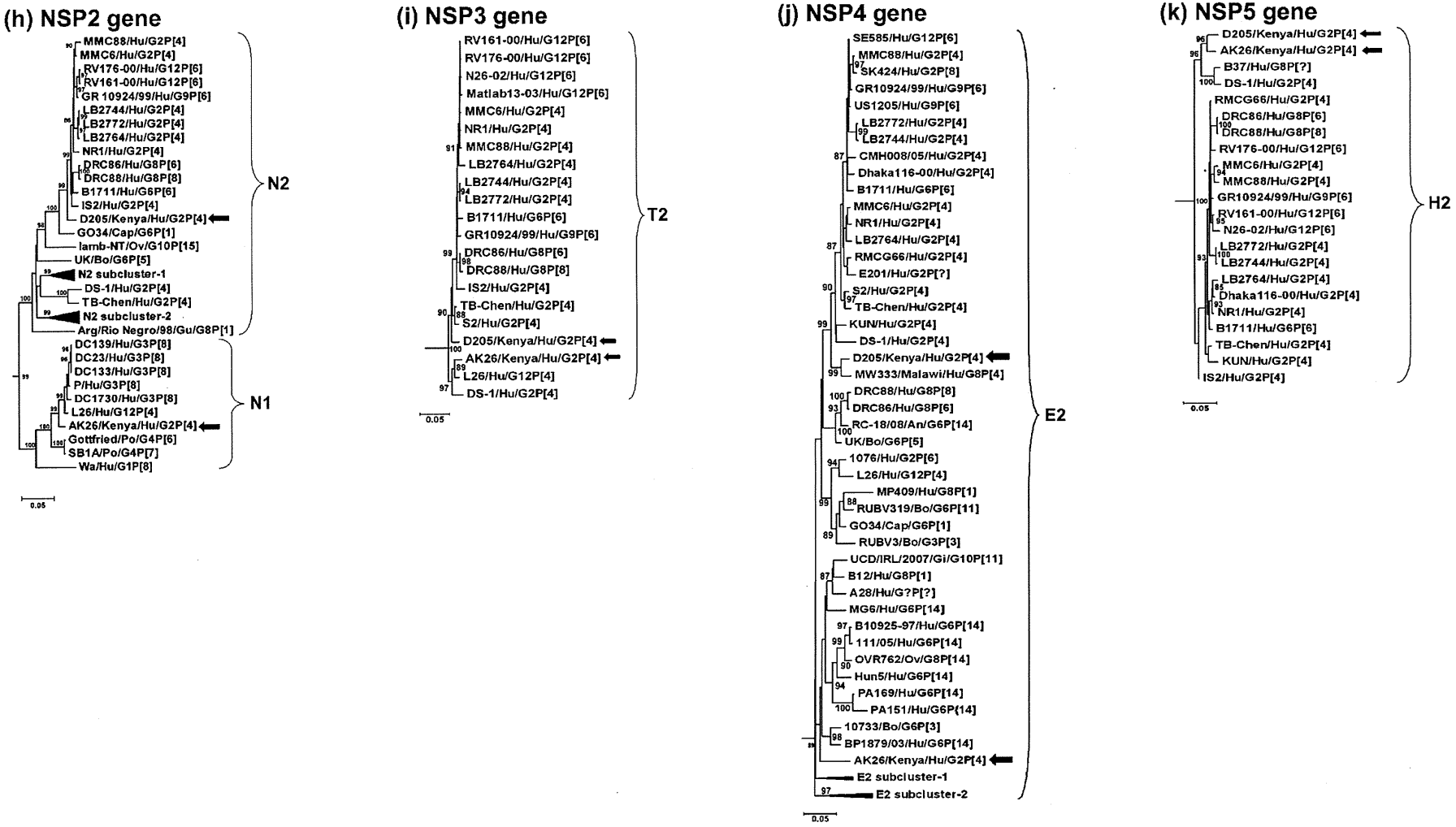


Fig. 1. (a–k) Phylogenetic trees constructed from the nucleotide sequences of VP7, VP4, VP6, VP1–3 and NSP1–5 genes of rotavirus strains AK26 and D205 with those of other group A rotavirus strains representing the G2, P[4], I2, R2, C2, M2, A2, N1 and N2, T2, E2 and H2 genotypes, respectively. Although strains representing the other RV-A genotypes were also included in the phylogenetic analyses to prepare the dendrograms, they are not shown in Fig. 1(a–k). In all trees, the positions of strains AK26 and D205 are indicated by arrows. Within the I2, R2, C2, M2, N2 and E2 genotypes, a clade(s) consisting of strains that is not directly related to the present study, but was included for unbiased analysis, has been compressed and labelled as a subcluster(s). Bootstrap values $\geqslant$85 % are shown. An, Antelope; Bo, bovine; Cap, caprine; Fe, feline; Gi, giraffe; Gu, guanaco; Hu, human; La, lapine; Ov, ovine; Po, porcine; and Si, simian. Bars, 0.05 substitutions per nucleotide.

Studies on genetic diversity of African human RV-A strains have primarily been based on RT-PCR-based G and/or P genotyping assays and/or sequencing of the VP7 and/or VP4 genes (Mwenda *et al.*, 2010; Sanchez-Padilla *et al.*, 2009; Todd *et al.*, 2010). Whole-genome analyses of common human RV-A strains are essential to obtain conclusive data on their evolutionary patterns and genetic relatedness to other strains (Matthijnssens *et al.*, 2008a, b). Moreover, the origin of common human RV-A strains might be more complex than is evident from the sequencing of their VP7 and/or VP4 genes. For example, the VP1, VP6 and NSP2 genes of the recent human G2P[4] strains from Bangladesh (strains MMC6 and MMC88) and USA (strains LB2744, LB2764 and LB2772) were distantly related to the prototype G2P[4] strain and appeared to share a common origin with a caprine strain (Ghosh *et al.*, 2011c). In addition, strain MMC88 had a caprine-like VP3 gene, derived from ruminant–human reassortment events (Ghosh *et al.*, 2011c). Besides, in sub-Saharan African countries, such as Kenya, low socioeconomic status, crowded and unhygienic living conditions, and close proximity of humans to animals offer a favourable environment for complex reassortment and/or interspecies transmission events. However, to date, the whole genomes of only unusual African human RV-A strains, such as G3P[2], G5P[7], G8 and G10, derived from animal–human reassortment events or zoonotic transmission, have been analysed (Esona *et al.*, 2009a, b, 2011; Ghosh *et al.*, 2011a, b; Matthijnssens *et al.*, 2006) In contrast, there are no reports on whole genomic analyses of common human strains, such as G1P[8] or G2P[4] strains, from Africa. Therefore, in the present study, the whole genomes of two Kenyan G2P[4] strains, AK26 and D205, were analysed.

RV-A rotavirus strains AK26 and D205 were detected in diarrhoeal stool samples collected from children (<3 years old) in the districts of Mombasa and Nairobi, Kenya, in July 1982 and August 1989, respectively (Gatheru *et al.*, 1993; Urasawa *et al.*, 1987). Both the strains exhibited short RNA migration patterns in polyacrylamide gels, had subgroup I specificity and were assigned to serotype G2 using serotype-specific mAbs against the important human G (G1–4) serotypes (Gatheru *et al.*, 1993; Urasawa *et al.*, 1987). Following detection, strains AK26 and D205 were successfully isolated by tissue culture in MA-104 cells and stored at −80 °C until further use. For RT-PCR, viral RNA was extracted from the tissue-culture fluid of strains AK26 and D205 by using a QIAamp Viral RNA Mini kit (Qiagen Sciences). Primers that were used for the amplification of different genes of strains AK26 and D205 are shown in Supplementary Table S1 (available in JGV Online). Nucleotide sequences were determined using a BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems) and an automated sequencer (ABI PRISM 3100). Calculation of sequence identities and alignments were carried out as described previously (Ghosh *et al.*, 2010a, b). Phylogenetic trees were constructed by the neighbour-joining method (Saitou & Nei, 1987) using the MEGA (version 4.1) software. The trees were statistically supported by bootstrapping with 1000 replicates, and phylogenetic distances were measured by the Kimura two-parameter model.

Applying the whole genome-based genotyping system (Matthijnssens *et al.*, 2008a, b), the nearly full-length nucleotide sequences (full-length sequences excluding the 5′- and 3′- end primer-binding regions) of the VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5 genes of strains AK26 and D205 were assigned to the G2-P[4]-I2-R2-C2-M2-A2-N1-T2-E2-H2 and G2-P[4]-I2-R2-C2-M2-A2-N2-T2-E2-H2 genotypes, respectively (Fig. 1a–k; Table 1). Therefore, the overall genotype nature of strain D205 was identical to that of the prototype G2P[4] strain, DS-1, whilst strain AK26 appeared to be an intergenogroup reassortant, with a Wa-like NSP2 genotype on the DS-1-like genotype constellation (Table 1).

The VP7 gene of strain AK26 exhibited a maximum nucleotide sequence identity of 99 % against the G2 component [strain SC2-9] of RV-A vaccine RotaTeq, whilst the VP7 gene of strain D205 shared high nucleotide sequence identities of 98, 98 and 99 % with the VP7 gene of South African G2 strains 1831GR/93, 64SB/96 and 906SB/98, respectively. Phylogenetically, both the Kenyan G2P[4] strains belonged to G2 lineage 2 (Fig. 1a). However, within G2 lineage 2, strain AK26 clustered within the same subcluster as the G2 component of RotaTeq, whilst strain D205 formed a separate subcluster with other African G2 strains (Fig. 1a). Comparisons of the deduced amino acid sequences of the VP7 genes of strains AK26 and D205 with those of other G2 strains and the G2 component of RotaTeq did not reveal anything relevant other than a few amino acid differences in the antigenic regions (Supplementary Fig. S1, available in JGV Online). The VP4 genes of strains AK26 and D205 exhibited high levels of genetic relatedness (nucleotide sequence identities of 93.6–96.6 %) with those of human P[4] strains; by phylogenetic analysis they clustered within P[4] lineage 2, with human G12 strain L26, isolated in the Philippines in 1987 (Kobayashi *et al.*, 1989), and G8 strain MW333 from Malawi (Cunliffe *et al.*, 2000) (Fig. 1b).

The VP1–3, VP6 and NSP1–3 genes of strain AK26 and VP1–2 genes of strain D205 were closely related to those of strain L26 (Fig. 1c–i). The VP2–3, VP6, NSP1, NSP3 and NSP5 genes of strain AK26, and the VP2 and NSP5 genes of strain D205 were also closely related to those of the prototype human G2P[4] strain DS-1 (Fig. 1c, e–g, i, k). In contrast, the NSP2 genes of strains AK26 and L26 shared high levels of genetic relatedness with those of human G3P[8] strains from the USA within the Wa-like N1 genotype (Fig. 1h). The VP6 gene of strain D205 exhibited maximum relatedness to that of subgroup I human G2P[6] strain 1076 (Gorziglia *et al.*, 1988) (Fig. 1c). Strain D205 shared high levels of genetic relatedness with the: (i) NSP1 and NSP3 genes of Chinese G2P[4] strain TB-Chen,

Table 1. Genotype nature of the 11 gene segments of group A rotavirus (RV-A) strains AK26 and D205, which were sequenced in this study, with those of selected human and animal RV-A strains with known genomic constellations

The DS-1-like and Wa-like gene segments are indicated by *italic* and boldface type, respectively. Bo, Bovine; Cap, caprine; Fe, feline; Gi, giraffe; Gu, guanaco; Hu, human; Ov, ovine.

Strain/host	Genotypes										
	VP7	VP4	VP6	VP1	VP2	VP3	NSP1	NSP2	NSP3	NSP4	NSP5
AK26/Hu	*G2*	P[4]	*I2*	*R2*	*C2*	*M2*	*A2*	**N1**	*T2*	*E2*	*H2*
D205/Hu	*G2*	P[4]	*I2*	*R2*	*C2*	*M2*	*A2*	*N2*	*T2*	*E2*	*H2*
Wa/Hu	**G1**	**P[8]**	**I1**	**R1**	**C1**	**M1**	**A1**	**N1**	**T1**	**E1**	**H1**
DS-1/Hu	*G2*	P[4]	*I2*	*R2*	*C2*	*M2*	*A2*	*N2*	*T2*	*E2*	*H2*
TB-Chen/Hu	*G2*	P[4]	*I2*	*R2*	*C2*	*M2*	*A2*	*N2*	*T2*	*E2*	*H2*
MMC6/Hu	*G2*	P[4]	*I2*	*R2*	*C2*	*M2*	*A2*	*N2*	*T2*	*E2*	*H2*
MMC88/Hu	*G2*	P[4]	*I2*	*R2*	*C2*	*M2*	*A2*	*N2*	*T2*	*E2*	*H2*
LB2744/Hu	*G2*	P[4]	*I2*	*R2*	*C2*	*M2*	*A2*	*N2*	*T2*	*E2*	*H2*
LB2764/Hu	*G2*	P[4]	*I2*	*R2*	*C2*	*M2*	*A2*	*N2*	*T2*	*E2*	*H2*
LB2772/Hu	*G2*	P[4]	*I2*	*R2*	*C2*	*M2*	*A2*	*N2*	*T2*	*E2*	*H2*
S2/Hu	*G2*	P[4]	*I2*	*R2*	*C2*	*M2*			*T2*	*E2*	
NR1/Hu	*G2*	P[4]	*I2*				*A2*	*N2*	*T2*	*E2*	*H2*
IS2/Hu	*G2*	P[4]*	*I2*					*N2*	*T2*		*H2*
KUN/Hu	*G2*	P[4]								*E2*	*H2*
1076/Hu	*G2*†	P[6]	*I2*							*E2*	
DC133/76/Hu	**G3**	**P[8]**	**I1**	**R1**	**C1**	**M1**	**A1**	**N1**	**T1**	**E1**	**H1**
AU-1/Hu	G3	P[9]	I3	R3	C3	M3	A3	N3	T3	E3	H3
PAH136/96/Hu	G3	P[9]	*I2*	*R2*	*C2*	*M2*	A3	**N1**	T6	*E2*	H3
BA222/Fe	G3	P[9]	*I2*	*R2*	*C2*	*M2*	A3	**N1**	T3	*E2*	H3
GO34/Cap	G6	P[1]	*I2*	*R2*	*C2*	*M2*	A11	*N2*	T6	*E2*	H3
NCDV/Bo	G6	P[1]	*I2*	*R2*	*C2*	*M2*	A3	*N2*	T6	*E2*	H3
UK/Bo	G6	P[5]	*I2*	*R2*	*C2*	*M2*	A3	*N2*	T7	*E2*	H3
BP1879/03/Hu	G6	P[14]	*I2*	*R2*	*C2*	*M2*	A11	*N2*	T6	*E2*	H3
Hun5/Hu	G6	P[14]	*I2*	*R2*	*C2*	*M2*	A11	*N2*	T6	*E2*	H3
B12/Hu	G8	P[1]	*I2*	*R2*	*C2*	*M2*	A3	*N2*	T6	*E2*	H3
Arg/Rio Negro/98/Gu	G8	P[1]	*I2*	R5	*C2*	*M2*	A11	*N2*	T6	E12	H3
Arg/chubut/99/Gu	G8	P[14]	*I2*	R5	*C2*	*M2*	A3	*N2*	T6	E12	H3
OVR762/Ov	G8	P[14]	*I2*	*R2*	*C2*	*M2*	A11	*N2*	T6	*E2*	H3
UCD/IRL/Gi	G10	P[11]	*I2*	*R2*	*C2*		A3	*N2*	T6	*E2*	H3
L26/Hu	G12	P[4]	*I2*	*R2*	*C2*	**M1**/*M2*‡	*A2*	**N1**	*T2*	*E2*	**H1**

*VP4 genotype assigned by RT-PCR-based genotyping by using P-genotype-specific primers.
†VP7 genotype assigned by RT-PCR-based genotyping by using G-genotype-specific primers.
‡Two different nucleotide sequences with accession numbers EF583035 and AY277918 were available for the VP3 gene of strain L26 in the GenBank database.

detected in 1996 (Chen *et al.*, 2008); (ii) NSP1–3 and NSP2–3 genes of Indian G2P[4] strains NR1 and IS2, respectively; (iii) NSP3 gene of G2P[4] strain S2, isolated in Japan in 1980 (Urasawa *et al.*, 1984); and (iv) NSP1–3 genes of recent human G2P[4] strains from Bangladesh (Ghosh *et al.*, 2011c) and the USA (Bányai *et al.*, 2011), non-G2P[4] human RV-A strains, such as G6P[6] strain B1711 (Matthijnssens *et al.*, 2008c), G8 strains DRC86 and DRC88 (Matthijnssens *et al.*, 2006), G9P[6] strain GR10924 (Potgieter *et al.*, 2009) and G12 strains RV161-00 and RV176-00 (Rahman *et al.*, 2007) (Fig. 1 g–i). The NSP4 gene of strain D205 exhibited maximum genetic relatedness to that of strain MW333; by phylogenetic analysis, both strains clustered near the cluster consisting of human G2P[4] strains, including the prototype strain and other human RV-A strains (Fig. 1j).

Interestingly, genes sharing a close relationship with RV-A genes of artiodactyl (ruminant and camelid) origin were identified in both of the Kenyan G2P[4] strains. Phylogenetically, the VP6 genes of strains D205 and 1076 clustered near those of G6P[14] strain Hun5 from Hungary and G3P[9] strains BA222 and PAH136/96 from Italy (Fig. 1c). Strain Hun5 was shown to be a zoonotic strain derived from ruminant host species (Matthijnssens *et al.*, 2009). In contrast, feline strain BA222 and human strain

PAH136/96 were derived from multiple reassortment events involving human, canine/feline and ruminant strains, and both strains possessed VP6 genes of artiodactyl origin (De Grazia *et al.*, 2010; Martella *et al.*, 2011). The VP1 genes of strains AK26, D205 and L26 appeared to cluster near to those of strains Hun5, PAH136/96 and the zoonotic artiodactyl-like G6P[14] strain BP1879/03 from Hungary (Bányai *et al.*, 2009) (Fig. 1d). The VP3 gene of strain D205 exhibited a maximum (but low) nucleotide sequence identity of 89.1 % with G8P[1] strain B12, a zoonotic strain of artiodactyl origin from Kenya (Ghosh *et al.*, 2011b). By phylogenetic analysis, the VP3 gene of strain D205 clustered near strain B12, and, taken together, both strains clustered near the cluster of several artiodactyl and artiodactyl-like human strains (Fig. 1f). The NSP4 gene of strain AK26 exhibited a maximum nucleotide sequence identity of 92.0 % with that of strain BP1879/03, followed by identities of 91.9 and 91.7 % with those of strain UCD/IRL, a bovine-like strain detected in a giraffe (Mulherin *et al.*, 2008), and strain B12, respectively. Phylogenetically, the NSP4 gene of strain AK26 clustered near the cluster of several artiodactyl and artiodactyl-like human strains (Fig. 1j). Therefore, the VP1 and NSP4 genes of strains AK26 and the VP1, VP3 and VP6 genes of strain D205 appeared to originate from, or share a common origin with, RV-A genes that were possibly derived from artiodactyl (ruminant or camelid) strains (Fig. 1c, d, f, j). Moreover, all these genes were distantly related to those of the pure human RV-A strains, such as the prototype strain DS-1 (Fig. 1c, d, f, j).

Taken together, the overall genetic makeup of the Kenyan G2P[4] strains AK26 and D205 appears to be more complex than that of the prototype strain DS-1 or other human G2P[4] strains, for which whole genome sequence is available. Of the 11 gene segments, only the VP2–4, VP6–7, NSP1, NSP3 and NSP5 genes of strain AK26 and the VP2, VP4, VP7 and NSP1–5 genes of strain D205 were closely related to those of the prototype or other human G2P[4] strains. Interestingly, eight of the 11 gene segments of strain AK26 were very closely related to those of the human G12P[4] strain L26, suggesting a common pattern of evolution of these strains. Strain AK26 appeared to be an intergenogroup reassortant, contradicting the common perception that RV-A strains belonging to different genogroups do not readily exchange their genome segments except for the outer capsid coding genes (Bányai *et al.*, 2011). Both the Kenyan G2P[4] strains possessed gene segments which might have originated from, or shared a common origin with, artiodactyl strains. Considering the presence of unhygienic conditions and the close proximity of humans to livestock in countries like Kenya, such close evolutionary relationships between animal and human rotaviruses may not be unlikely, necessitating greater public awareness and adoption of hygienic preventive measures, such as access to clean drinking water and improved sanitary conditions. Overall, our observations emphasized the importance of whole-genome-based studies in obtaining conclusive data on the complex evolutionary dynamics of common African human RV-A strains. Such studies may be useful in developing future vaccine strategies or for evaluating the efficacy of the current RV-A vaccines in Africa. Moreover, information on whole genomes of these old strains, which are rarely available for extensive molecular characterization, will help in better understanding the evolution of recent African G2P[4] strains, the whole genomes of which, however, remain to be determined.

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