
Souvik Ghosh,1 Noriaki Adachi,2 Zipporah Gatheru,2† James Nyangao,3 Dai Yamamoto,1 Masaho Ishino,1 Noriko Urushibara1 and Nobumichi Kobayashi1

1Department of Hygiene, Sapporo Medical University School of Medicine, Sapporo, Japan
2Kushiro City General Hospital, Kushiro, Japan
3Centre for Virus Research, Kenya Medical Research Institute, Nairobi, Kenya

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 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).

†Present address: Gynae–Paed Medical Centre, Nairobi, Kenya.

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.
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, 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 $\geq 85\%$ 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). 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.

<table>
<thead>
<tr>
<th>Strain/host</th>
<th>Genotypes</th>
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<tbody>
<tr>
<td>AK26/Hu</td>
<td>G2 P[4] I2 R2 C2 M2 A2 N1 T2 E2 H2</td>
</tr>
<tr>
<td>D205/Hu</td>
<td>G2 P[4] I2 R2 C2 M2 A2 N2 T2 E2 H2</td>
</tr>
<tr>
<td>Wa/Hu</td>
<td>G1 P[8] I1 R1 C1 M1 A1 N1 T1 E1 H1</td>
</tr>
<tr>
<td>TB-Chen/Hu</td>
<td>G2 P[4] I2 R2 C2 M2 A2 N2 T2 E2 H2</td>
</tr>
<tr>
<td>MMC88/Hu</td>
<td>G2 P[4] I2 R2 C2 M2 A2 N2 T2 E2 H2</td>
</tr>
<tr>
<td>S2/Hu</td>
<td>G2 P[4] I2 R2 C2 M2 T2 E2</td>
</tr>
<tr>
<td>IS2/Hu</td>
<td>G2 P[4]* I2 N2 T2 E2 H2</td>
</tr>
<tr>
<td>I076/Hu</td>
<td>G2‡ I2 E2</td>
</tr>
<tr>
<td>DC133/76/Hu</td>
<td>G3 P[8] I1 R1 C1 M1 A1 N1 T1 E1 H1</td>
</tr>
<tr>
<td>AU-1/Hu</td>
<td>G3 P[9] I3 R3 C3 M3 A3 N3 T3 E3 H3</td>
</tr>
<tr>
<td>PAH136/96/Hu</td>
<td>G3 P[9] I2 R2 C2 M2 A3 N1 T6 E2 H3</td>
</tr>
<tr>
<td>BA222/Fe</td>
<td>G3 P[9] I2 R2 C2 M2 A3 N1 T3 E2 H3</td>
</tr>
<tr>
<td>GO34/Cap</td>
<td>G6 P[1] I2 R2 C2 M2 A11 N2 T6 E2 H3</td>
</tr>
<tr>
<td>NCDV/Bo</td>
<td>G6 P[1] I2 R2 C2 M2 A3 N2 T6 E2 H3</td>
</tr>
<tr>
<td>UK/Bo</td>
<td>G6 P[5] I2 R2 C2 M2 A3 N2 T7 E2 H3</td>
</tr>
<tr>
<td>BP1879/03/Hu</td>
<td>G6 P[14] I2 R2 C2 M2 A11 N2 T6 E2 H3</td>
</tr>
<tr>
<td>B12/Hu</td>
<td>G8 P[1] I2 R2 C2 M2 A3 N2 T6 E2 H3</td>
</tr>
<tr>
<td>OVR762/Ov</td>
<td>G8 P[14] I2 R2 C2 M2 A11 N2 T6 E2 H3</td>
</tr>
<tr>
<td>L26/Hu</td>
<td>G12 P[4] I2 R2 C2 M1/M2‡ A2 N1 T2 E2 H1</td>
</tr>
</tbody>
</table>

*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., 2009); 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[4] 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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References


