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Short communication

The origin of two rare human P[10] rotavirus strains

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ABSTRACT

The Group A rotavirus (RVA) P[10] is a rare genotype of the RVA VP4 gene. To date, the whole genome sequence of only a single P[10] RVA strain, RVA/Human-tc/IDN/69M/1980/G8P4[10], has been determined, revealing a DS-1-like genotype constellation. Whole genomic analyses of P[10] RVA strains with other VP7 genotypes are essential to obtain conclusive data on the origin and genetic diversity of the P10] RVAs. In the present study, the whole genome of a human G4P[10] RVA strain, RVA/Human-tc/IDN/57M/1980/G4P[10], was analyzed. Strain 57M exhibited an unusual G4-P[10]-I1-R1-C1-M1-A1-N1-T2-E1-H2 genotype constellation, and was found to originate from intergenogroup reassortment events involving acquisition of RVA strain 69M-like VP4, NSP3 and NSP5 genes by a co-circulating Wa-like human G4 RVA strain. Although the reference P[10] strain, 69M, exhibits a DS-1-like genotype constellation, the exact origin of this RVA remains to be elucidated. By detailed phylogenetic analyses, we found that the VP1-VP3, VP6, NSP2 and NSP4 genes of 69M originated from artiodactyl and/or artiodactyl-like human P[14] strains, whilst its NSP1, NSP3 and NSP5 genes were more related to those of typical human DS-1-like strains than those of other RVAs. On the other hand, the origin of the VP4 gene of 69M could not be established. Nevertheless, these observations clearly indicated that strain 69M might have originated from reassortment events involving at least the artiodactyl or artiodactyl-like human RVAs and the typical human DS-1-like strains. The present study provided rare evidence for intergenogroup reassortment events involving co-circulating typical human Wa-like RVAs and unusual RVAs of the DS-1-like genogroup, and revealed the presence of artiodactyl-like genes in a human P[10] strain, highlighting the complex evolutionary patterns of the P[10] RVAs.

1. Introduction

Group A rotaviruses (RVA) are a major cause of acute childhood diarrhea (Estes and Kapikian, 2007). The rotavirus P[10] is a rare genotype of the RVA VP4 gene (Matthijnssens et al., 2009a; Santos and Hoshino, 2005). The P[10] genotype was first identified in a human G8 RVA strain, RVA/Human-tc/IDN/69M/1980/G8P4[10] (Qian and Green, 1991), isolated from a child with diarrhea in Indonesia in 1980 (Hasegawa et al., 1984). To date, human P[10] RVAs have been occasionally detected in conjunction with a variety of VP7 genotypes from different countries (Matthijnssens et al., 2009a). In animals, the P[10] genotype has been only reported from a porcine RVA strain with unknown VP7 genotype (Midgley et al., 2012).

The whole genome sequence of a single P[10] RVA strain, 69M, has been determined so far, revealing a DS-1-like genotype constellation (Heiman et al., 2008). Whole genomic analyses of additional P[10] RVA strains, especially those with VP7 genotypes other than G8, are essential to obtain conclusive data on the origin and genetic diversity of these rare RVAs. In the present study, the nearly full-length nucleotide sequences (full-length sequence minus the 5′- and 3′- end primer binding regions) of all the eleven gene segments of a human G4P[10] RVA strain, RVA/Human-tc/IDN/57M/1980/G4P[10], were analyzed. Although the reference P[10] RVA strain, 69M, exhibits a DS-1-like genotype constellation, the genetic relatedness of its VP1-VP3, VP6 and NSP1-NSP5 genes to those of other RVAs within the R2, C2, M2, I2, A2, N2, T2, E2 and H2 genotypes, respectively, remain to be elucidated. Therefore, in the present study, detailed phylogenetic analyses of the VP1-VP3, VP6 and NSP1-NSP5 genes of 69M were also performed to pinpoint the exact origin of this unusual RVA strain.

2. Materials and methods

2.1. Virus strain

Strain 57M was isolated from a diarrheal stool sample collected from a child in the city of Medan, Indonesia, in 1980 (Hasegawa

Abbreviation: RVA, Group A rotavirus; bp, Base pair; UTR, Untranslated region.

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et al., 1984). The source of the tissue culture adapted isolate of strain 57M analyzed in the present study has been described previously (Matsuno et al., 1988).

2.2. RT-PCR, nucleotide sequencing and sequence analyses

Extraction of viral RNA from tissue culture fluid, RT-PCR, nucleotide sequencing and sequence analyses were carried out as reported previously (Ghosh et al., 2012). Primers used for the amplification of different genes of strain 57M are shown in Supplementary Table S1. Phylogenetic trees were constructed by the Neighbor-Joining method (Saitou and Nei, 1987) using MEGA (v5.01) software (Tamura et al., 2011). The trees were statistically supported by bootstrapping with 1000 replicates, and phylogenetic distances measured by the Kimura two-parameter model. The results of phylogenetic analyses were validated using several other genetic distance models, such as Jukes-Cantor, Tamura-Nei, and Tajima-Nei (Tamura et al., 2011).

2.3. Nucleotide sequence accession numbers

The GenBank accession numbers for the nucleotide sequences of the VP1-VP4, VP6, VP7 and NSP1-NSP5 genes of rotavirus strain 57M are JQ863309–JQ863319, respectively.

3. Results and discussion

By nucleotide sequence identities and phylogenetic analyses, strain 57M exhibited an unusual G4-P[10]-I1-R1-C1-M1-A1-N1-T2-E1-H2 genotype constellation (Table 1). Therefore, 57M was found to possess two DS-1-like genotypes (NSP3 and NSP5 genes) and the P[10] VP4 genotype on a Wa-like genotype backbone. The VP7 gene of strain 57M exhibited nucleotide sequence identities of 97–99% to those of several recent and old human G4 RVA strains, including the reference G4 strain RVA/Human-tc/GBR/ST3/1975/G4P2A[6], and phylogenetically, clustered with these strains within VP7-G4 lineage G4a (Fig. 1A). The VP1–VP3, VP6, NSP1, NSP2 and NSP4 genes of strain 57M were found to be closely related (nucleotide sequence identities of 97–99%) to those of RVA strain RVA/Human-tc/USA/Wa/1974/G1P1A[8] and/or other typical human Wa-like G1P[8], G3P[8], G4P[8], and/or G9P[8] strains (Fig. 1C–H, J).

The VP4 and NSP3 genes of 57M were more closely related (nucleotide sequence identities of 99.6%) to those of G8P[10] RVA strain 69M than those of other RVAs (nucleotide sequence identities of ⩽79% and ⩽92%, respectively). Strain 57M shared a nucleotide sequence identity of 92.6% with the only other available P[10]-VP4 gene sequence (partial-length, 879 bp) of that of G3P[10] strain RVA/Human–wt/THA/CMH079/2005/G3P[10] (Khamrin et al., 2009). Phylogenetically, the NSP3 genes of strains 69M and 57M clustered together to form a distinct lineage (shown as T2b) within the DS-1-like T2 genotype (Fig. 1I), whereas their VP4 genes formed the distinct P[10] VP4 genotype cluster (Fig. 1B).

Although the NSP5 gene of strain 57M was assigned to the DS-1-like genotype H2, it was longer (948 bp) than that of strain RVA/Human-tc/USA/DS-1/1976/G2P1B[4] (821 bp), identical to that of strain 69M (Matsui et al., 1990), and one nucleotide longer than that of strain RVA/Human-tc/IDN/B37/197x/G8P[10], a "super short" RVA strain detected in the city of Yogyakarta, Indonesia,

Table 1
Genotype nature of the eleven gene segments of group A rotavirus (RVA) strain 57M with those of strain 69M and other selected RVA strains with known genomic constellations.

Strain	Genotypes										
	VP7	VP4	VP6	VP1	VP2	VP3	NSP1	NSP2	NSP3	NSP4	NSP5
RVA/Human-tc/IDN/57M/1980/G4P[10]	G4	P[10]	I1	R1	C1	M1	A1	N1	T2	E1	H2
RVA/Human-tc/IDN/69M/1980/G8P4[10]	G8	P[10]	*I2*	*R2*	*C2*	*M2*	A2	*N2*	T2	*E2*	H2
RVA/Human-tc/USA/Wa/1974/G1P1A[8]	G1	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-tc/USA/KU/1974/G1P1A[8]	G1	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-tc/USA/D/1974/G1P1A[8]	G1	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-tc/USA/DS-1/1976/G2P1B[4]	G2	P[4]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human-wt/CHN/TB-Chen/1996/G2P[4]	G2	P[4]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human-tc/AUS/RV3/1977/G3P2A[6]	G3	P[6]	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-wt/USA/DC1730/1979/G3P[8]	G3	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-wt/THA/CMH079/2005/G3P[10]	G3	P[10][b]	I8	–	–	–	–	–	–	E3	H6
RVA/Human-tc/GBR/ST3/1975/G4P2A[6]	G4	P[6]	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-wt/USA/DC4996/1977/G4P[8]	G4	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-tc/JPN/Hosokawa/1983/G4P1A[8]	G4 [a]	P[8]	I1	R1	C1	M1	–	–	–	–	–
RVA/Cow-tc/USA/NCDV/1967/G6P6[1]	G6	P[1]	*I2*	*R2*	*C2*	*M2*	A3	*N2*	T6	*E2*	H3
RVA/Cow-tc/GBR/UK/1973/G6P7[5]	G6	P[5]	*I2*	*R2*	*C2*	*M2*	A3	*N2*	T7	*E2*	H3
RVA/Human-wt/HUN/BP1879/2003/G6P[14]	G6	P[14]	*I2*	*R2*	*C2*	*M2*	A11	*N2*	T6	*E2*	H3
RVA/Antelope-wt/ZAF/RC-18-08/G6P[14]	G6	P[14]	*I2*	*R2*	*C2*	*M2*	A11	*N2*	T6	*E2*	H3
RVA/Human-tc/IDN/B37/197x/G8P[10]	G8	P[10][c]	–	–	–	–	–	–	–	–	H2
RVA/Sheep-tc/ESP/OVR762/2002/G8P[14]	G8	P[14]	*I2*	*R2*	*C2*	*M2*	A11	*N2*	T6	*E2*	H3
RVA/Guanoco-wt/ARG/Chubut/1999/G8P[14]	G8	P[14]	*I2*	R5	*C2*	*M2*	A3	*N2*	T6	E12	H3
RVA/Human-tc/USA/WI61/1983/G9P1A[8]	G9	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/human-tc/USA-DC/G2275/1980/G9P[8]	G9	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/human-tc/USA-DC/DC706/1980/G9P[8]	G9	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Giraffe-wt/IRL/UCD/2007/G10P[11]	G10	P[11]	*I2*	*R2*	*C2*	–	A3	*N2*	T6	*E2*	H3
RVA/Human-tc/GBR/A64/1987/G10P11[14]	G10	P[14]	*I2*	*R2*	*C2*	M1	A3	*N2*	T6	*E2*	H3
RVA/Human-wt/BEL/B4633/2003/G12P[8]	G12	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Cow-tc/JPN/Dai-10/2007/G24P[33]	G24	P[33]	*I2*	*R2*	*C2*	*M2*	A13	*N2*	T9	*E2*	H3

Strain 57M is highlighted in bold type, whilst Wa and DS-1, prototype strains of the Wa-like and DS-1-like genogroups, respectively, are underlined.
Gray indicates the RVA genes that are phylogenetically closely related to those of strain 57M.
Italic type indicates the artiodactyl or artiodactyl-like genes with a DS-1-like genotype.
"–" Indicates that no sequence data were available in the GenBank database.
[a]To our knowledge, only partial-length (87 bp) nucleotide sequence of VP7 gene of strain Hosokawa is available in the GenBank database.
[b]Genotype assignment based on analysis of partial-length nucleotide sequence (879 bp) of the VP4 gene.
[c]Genotype assignment based on that reported by Estes and Kapikian (2007).