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Complex evolutionary patterns of two rare human G3P[9] rotavirus strains possessing a feline/canine-like H6 genotype on an AU-1-like genotype constellation

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ABSTRACT

The group A rotavirus (RVA) G3P[9] is a rare VP7–VP4 genotype combination, detected occasionally in humans and cats. Other than the prototype G3P[9] strain, RVA/Human- tc/JPN/AU-l/1982/G3P3[9], the whole genomes of only two human G3P[9] RVA strains and two feline G3P[9] RVA strains have been analyzed so far, revealing complex evolutionary patterns, distinct from that of AU-1. We report here the whole genomic analyses of two human G3P[9] RVA strains, RVA/Human-tc/CHN/L621/2006/G3P[9] and RVA/Human-wt/CHN/E2451/2011/G3P[9], detected in patients with diarrhea in China. Strains L621 and E2451 possessed a H6 NSP5 genotype on an AU-1-like genotype constellation, not reported previously. However, not all the genes of L621 and E2451 were closely related to those of AU-1, or to each other, revealing different evolutionary patterns among the AU-1-like RVAs. The VP7, VP4, VP6 and NSP4 genes of E2451 and L621 were found to cluster together with human G3P[9] RVA strains believed to be of possible feline/canine origin, and feline or raccoon dog RVA strains. The VP1, VP3, NSP2 and NSP5 genes of E2451 and L621 formed distinct clusters in genotypes typically found in feline/canine RVA strains or RVA strains from other host species which are believed to be of feline/canine RVA origin. The VP2 genes of E2451 and L621, and NSP3 gene of L621 clustered among RVA strains from different host species which are believed to have a complete or partial feline/canine RVA origin. The NSP1 genes of E2451 and L621, and NSP3 gene of E2451 clustered with AU-1 and several other strains possessing a complete or partial feline RVA strain BA222-05-like genotype constellation. Taken together, these observations suggest that nearly all the eleven gene segments of G3P[9] RVA strains L621 and E2451 might have originated from feline/canine RVAs, and that reassortments may have occurred among these feline/canine RVA strains, before being transmitted to humans.

1. Introduction

Group A rotaviruses (RVA) are a major cause of severe childhood gastroenteritis (Estes and Kapikian, 2007). By RNA–RNA hybridization, human RVAs have been previously 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). By whole genome sequencing, most human RVA strains have been found to exhibit a Wa-like (I1-R1-C1-M1-A1-N1-T1-E1-H1) or DS-1-like (I2-R2-C2-M2-A2-N2-T2-E2-H2) genotype constellation, whilst, to date, only a few strains have been found to possess gene segments exhibiting G3P[9] RVA strain AU-1-like genotypes (I3, R3, C3, M3, A3, N3, T3, E3 and H3) (Matthijnssens and Van Ranst, 2012).

The G3P[9] is a rare RVA VP7-VP4 genotype combination, found in strain RVA/Human-tc/JPN/AU-l/1982/G3P3[9], the representative strain of the human RVA AU-1-like genogroup (Matthijnssens et al., 2008; Nakagomi et al., 1989). To date, G3P[9] RVA strains have been detected occasionally in humans (De Grazia et al., 2010; Grant et al., 2011; Hwang et al., 2011; Khananurak et al., 2010; Matthijnssens et al., 2009; Santos and Hoshino, 2005), and have also been recovered from cats with diarrhea (Birch et al., 1985; Martella et al., 2010, 2011; Oka et al., 2001). By RNA-RNA hybridization and whole genome sequencing, the prototype

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Abbreviation: RVA, Group A rotavirus.

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G3P[9] strain, AU-1, was shown to display feline RVA characteristics (Matthijnssens et al., 2008; Nakagomi and Nakagomi, 1989). Other than AU-1, the whole genomes of only two human G3P[9] RVA strains, RVA/Human-wt/ITA/PAH136/1996/G3P[9] and RVA/Human-wt/ITA/PAI58/1996/G3P[9], and two feline G3P[9] RVA strains, RVA/Cat-wt/ITA/BA222/2005/G3P[9] and RVA/Cat-tc/AUS/Cat2/1984/G3P[9], have been analyzed so far, revealing unusual genotype constellations, possibly derived from multiple reassortment events involving feline, human and bovine RVAs (De Grazia et al., 2010; Martella et al., 2011; Matthijnssens et al., 2011; Tsugawa and Hoshino, 2008).

Therefore, based on limited studies, the evolutionary patterns of the human G3P[9] RVAs appear to be complex, raising curiosity on the overall genetic makeup of additional G3P[9] RVA strains, especially those detected in other countries. In the present study, we report the analyses of 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 two human G3P[9] RVA strains, RVA/Human-tc/CHN/L621/2006/G3P[9] and RVA/Human-wt/CHN/E2451/2011/G3P[9], detected in patients with diarrhea in China.

2. Material and methods

2.1. Virus strains

Strains L621 and E2451 were detected in diarrheal stool samples collected from a 46 year old man and a 2 year 6 month old male child in the city of Wuhan, Hubei province, China, in 2006 and 2011, respectively. Only L621 was successfully isolated by tissue culture in MA-104 cells.

2.2. RT-PCR, nucleotide sequencing and sequence analyses

The nucleotide sequences of the VP4 and VP7 genes of strain L621 have been determined previously (Wang et al., 2009). Therefore, the nucleotide sequences of the remaining genes of L621 and whole genome of strain E2451 were obtained in this study. Viral RNA was extracted from the tissue culture fluid of L621 and stool sample of E2451 using the QIAamp Viral RNA Mini Kit (Qiagen GmbH, Germany). Primers used for the amplification of different RVA genes are shown in Supplementary Table S1. RT-PCRs were

Table 1
Genotype nature of the eleven gene segments of group A rotavirus (RVA) G3P[9] strains L621 and E2451with those of selected RVA strains with known genomic constellations.

	Genotypes										
Strain	**VP7**	**VP4**	**VP6**	**VP1**	**VP2**	**VP3**	**NSP1**	**NSP2**	**NSP3**	**NSP4**	**NSP5**
RVA/Human-tc/CHN/**L621**/2006/G3P[9]	G3a	P[9]a	I3a	R3c	C3b	M3a	A3a	N3c	T3c	E3d	H6
RVA/Human-wt/CHN/**E2451**/2011/G3P[9]	G3a	P[9]a	I3a	R3c	C3a	M3a	A3a	N3c	T3a	E3a	H6
RVA/Human-tc/JPN/**AU-l**/1982/G3P3[9]	G3a	P[9]a	I3a	R3a	C3a	M3a	A3a	N3a	T3a	E3a	H3
RVA/Human-tc/THA/**T152**/1998/G12P[9]	G12	P[9]e	I3d	R3a	C3a	M3a	A12	N3b	T3c	E3c	H6
RVA/Human-tc/JPN/**K8**/1977/G1P[9]	G1	P[9]b	I1	R3a	C3b	M3a	A1	N1	T3a	E3d	H3
RVA/Human-tc/ITA/**PA260-97**/1997/G3P[3]	G3b	P[3]	I3c	R3a	C3a	M3a	A15	N2	T3b	E3c	H6
RVA/Cat-tc/AUS/**Cat97**/1984/G3P[3]	G3b	P[3]	I3b	R3b	C2	M3b	A9	N2	T3b	E3b	H6
RVA/Dog-tc/ITA/**RV198-95**/1995/G3P[3]	G3b	P[3]	I3c	R3b	C2	M3a	A9	N2	T3b	E3b	H6
RVA/Dog-tc/ITA/**RV52-96**/1996/G3P[3]	G3b	P[3]	I3c	R3b	C2	M3a	A9	N2	T3b	E3c	H6
RVA/Dog-tc/USA/**CU-1**/1982/G3P[3]	G3b	P[3]	I3b	R3b	C2	M3b	A9	N2	T3b	E3b	H6
RVA/Dog-ct/AUS/**K9/1981**/G3P[3]	G3b	P[3]	I3b	R3b	C2	M3b	A9	N2	T3b	E3b	H6
RVA/Dog-tc/USA/**A79-10**/1979/G3P[3]	G3b	P[3]	I3b	R3b	C2	M3b	A9	N2	T3b	E3b	H6
RVA/Human-tc/ISR/**Ro1845**/1985/G3P[3]	G3b	P[3]	I3b	R3b	C2	M3b	A9	N2	T3b	E3b	H6
RVA/Human-tc/USA/**HCR3A**/1984/G3P[3]	G3b	P[3]	I3b	R3b	C2	M3b	A9	N2	T3b	E3b	H6
RVA/Cat-tc/AUS/**Cat2**/1984/G3P[9]	G3a	P[9]b	I3a	R3b	C2	M3b	A3a	N1	T6	E3b	H3
RVA/Cat-wt/ITA/**BA222**/2005/G3P[9]	G3a	P[9]c	I2	R2	C2	M2	A3a	N1	T3a	E2	H3
RVA/Human-wt/ITA/**PAH136**/1996/G3P[9]	G3a	P[9]d	I2	R2	C2	M2	A3a	N1	T6	E2	H3
RVA/Human-wt/ITA/**PAI58**/1996/G3P[9]	G3a	P[9]b	I2	R2	C2	M2	A3a	N2	T6	E2	H3
RVA/Horse-wt/ARG/**E3198**/2008/G3P[3]	G3b	P[3]	I3a	R3a	C3b	M3a	A9	N3d	T3c	E3d	H6
RVA/Simian-tc/USA/**RRV**/1975/G3P[3]	G3b	P[3]	I2	R2	C3b	M3a	A9	N2	T3c	E3d	H6
RVA/Rabbit-wt/CHN/**N5**/1992/G3P[14]	G3b	P[14]	I17	R3a	C3b	M3a	A9	N1	T1	E3d	H2
RVA/Rhesus-tc/USA/**TUCH**/2002/G3P[24]	G3	P[24]	I9	R3a	C3c	M3a	A9	N1	T3d	E3e	H6
RVA/Cow-tc/GBR/**UK**/1973/G6P7[5]	G6	P[5]	I2	R2	C2	M2	A3c	N2	T7	E2	H3
RVA/Human-wt/JPN/**KF17**/2010/G6P[9]	G6	P[9]c	I2	R2	C2	M2	A3a	N2	T3a	E3a	H3
RVA/Guanaco-wt/ARG/**Chubut**/1999/G8P[14]	G8	P[14]	I2	R5	C2	M2	A3a	N2	T6	E12	H3

Various colors have been used to distinguish the major phylogenetic clusters (indicated by a lower case alphabet after the genotype number in this table) observed within the G3, P[9], I3, R3, C3, M3, A3, N3, T3 and E3 genotypes in Fig. 1A–J, respectively.
The H6 genotype is highlighted in pink.
Strains L621, E2451 and AU-1 are underlined.
The common name of a rotavirus strain is highlighted in bold type.