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A rare G1P[6] super-short human rotavirus strain carrying an H2 genotype on the genetic background of a porcine rotavirus

Loan Phuong Do, Toyoko Nakagomi, Osamu Nakagomi *

Department of Molecular Microbiology and Immunology, Graduate School of Biomedical Sciences, and Global Center of Excellence, Nagasaki University, Nagasaki, Japan

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ABSTRACT

Rotavirus strains with a rearranged 11th genome segment may show super-short RNA electropherotypes. Examples from human strains were limited to seven strains, 69M, 57M, B37, Mc345, AU19, B4106 and BE2001, which have a variety of G and P genotypes. AU19 is a rare G1P[6] human rotavirus strain detected in a Japanese infant with severe acute gastroenteritis. This study was undertaken to better understand the origin of AU19 by determining the genotype constellation of AU19. Upon nearly-full genome sequencing, AU19 had a G1-P[6]-I5-R1-C1-M1-A8-N1-T1-E1-H2 genotype constellation. Possession of I5 and A8 genotypes is indicative of its porcine rotavirus origin, whereas possession of H2 genotype is indicative of its DS-1 like human rotavirus origin. At the phylogenetic lineage level for the genome segments that share the genotype between porcine and human rotaviruses, the VP1-4, VP7, NSP3-4 genes were most closely related to those of porcine rotaviruses, but the origin of the NSP2 gene was inconclusive. As to the NSP5 gene, the lineage containing AU19 and the other three super-short human strains, 69M, 57M and B37, carrying the H2 genotype (H2b) clustered with the lineage to which DS-1- like short strains belonged (H2a) albeit with an insignificant bootstrap support. Taken all these observations together, AU19 was likely to emerge as a consequence of interspecies transmission of a porcine rotavirus to a child coupled with the acquisition of a rare H2b genotype by genetic reassortment probably from a co-circulating human strain. The addition of the AU19 NSP5 sequence to much homogeneous H2b genotypes shared by previous super-short rotavirus strains made the genetic diversity of H2b genotypes as diverse as that of the H2a genotype, lending support to the hypothesis that super-short strains carrying H2b genotype have long been circulating unnoticed in the human population.

1. Introduction

Rotavirus A, a member of the genus *Rotavirus* within the family *Reoviridae*, is a major cause of severe acute gastroenteritis in infants and young children worldwide (Tate et al., 2012), and will hereafter be referred to as rotavirus. The rotavirus virion consists of a triple-layered capsid containing a genome consisting of 11 segments of double-stranded RNA (Greenberg and Estes, 2009). The genome encodes six structural viral proteins (VPs) and six non-structural proteins (NSPs), each genome segment codes for a single viral protein, with the exception of genome segment 11 that encodes two proteins (NSP5 and NSP6) (Greenberg and Estes, 2009). Because of their involvement in virus neutralization and protective immunity, much attention has been paid to the genotypes of the two outer capsid proteins, VP7 and VP4; the genotype of the gene encoding VP7 is termed the G type (for VP7 is a glycoprotein) and that of the gene encoding VP4 is termed the P type (for VP4 is a protease-sensitive protein) (Greenberg and Estes, 2009). As more rotavirus strains were sequenced for the entire genome segments, there was an increasing need for expanding a similar classification system for every genome segment; thus, the Rotavirus Classification Working Group (RCWG) was organized, and it adopted the classification system in which the genome of individual rotavirus strains is given the complete descriptor of

Abbreviations: VP, viral protein; NSP, non-structural protein; G, the suffix for the VP7 genotype deriving from glycoprotein; P, the suffix for the VP4 genotype deriving from Protease sensitive protein; I, the suffix for the VP6 genotype deriving from Intermediate capsid shell; R, the suffix for the VP1 genotype deriving from RNA-dependent RNA polymerase; C, the suffix for the VP2 genotype deriving from Core shell protein; M, the suffix for the VP3 genotype deriving from Methyltransferase; A, the suffix for the NSP1 genotype deriving from Interferon Antagonist; N, the suffix for the NSP2 genotype deriving from NTPase; T, the suffix for the NSP3 genotype deriving from Translation enhancer; E, the suffix for the NSP4 genotype deriving from Enterotoxin; H, the suffix for the NSP5 genotype deriving from pHosphoprotein; NTP, nucleoside triphosphate; dNTP, deoxynucleoside triphosphate; HRVs, human rotaviruses; BRVs, bovine rotaviruses; PRVs, porcine rotaviruses.

* Corresponding author. Address: Department of Molecular Microbiology and Immunology, Graduate School of Biomedical Sciences, Nagasaki University, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan. Tel.: +81 95 819 7063; fax: +81 95 819 7064.
E-mail address: onakagom@nagasaki-u.ac.jp (O. Nakagomi).

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Gx-P[x]-Ix-Rx-Cx-Mx-Ax-Nx-Tx-Ex-Hx ("x" representing the genotype number) for denoting the VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5 genes, respectively (Matthijnssens et al., 2008a,b; Matthijnssens et al., 2011). Thus, the genotype constellations of prototype human strains Wa and DS-1 are described as G1-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1, and G2-P[4]-I2-R2-C2-M2-A2-N2-T2-E2-H2, respectively (Matthijnssens et al., 2008b).

The two prototype strains Wa and DS-1 represent two distinct RNA migration patterns, termed electropherotypes, observed upon polyacrylamide gel electrophoresis: the long electropherotype with faster-migrating 10th and 11th genome segments and the short electropherotype with slower-migrating 10th and 11th genome segments (Kutsuzawa et al., 1982). What actually happened in short electropherotype, however, is the rearrangement of the 11th genome segment (coding for NSP5) by which this genome segment acquired an extra few hundred nucleotide insertion at their 3′ non-coding region; hence migrating slower than the original 10th genome segment (coding for NSP4) which in turn becomes the 11th genome segment as a consequence (Dyall-Smith and Holmes, 1981). Rotavirus strains, albeit infrequently, may show super-short electropherotypes in which the rearranged 11th genome segment becomes even longer than that of short electropherotype strains. To date there are only seven super-short human rotavirus strains reported in the literature, but they carry a variety of G and P genotype combinations: 69M (G8P[10]) detected in Indonesia in 1980 (Heiman et al., 2008; Matsuno et al., 1985; Qian and Green, 1991), 57M (G4P[10]) also in Indonesia in 1980 (Ghosh et al., 2013; Hasegawa et al., 1984), B37 (G8P[10]) in Indonesia in 1978–1979 (Albert, 1985; Hum et al., 1989; Nuttall et al., 1989), Mc345 (G9P[19]) in Thailand in 1989 (Ghosh et al., 2012b; Okada et al., 2000; Urasawa et al., 1992), AU19 (G1P[6]) in Japan in 1997 (Ahmed et al., 2007; Nakagomi et al., 1999), B4106 (G3P[14]) in Belgium in 2000 (Matthijnssens et al., 2006), and BE2001 (G9P[6]) in Belgium in 2009 (Zeller et al., 2012).

The VP4 protein of AU19 was shown to have a P2C serotype by cross-neutralization assays, and the P[6] genotype encoding AU19 VP4 gene was located in a unique position that was distinct from either porcine Gottfried P[6] genotype or human P[6] genotype when it was initially reported (Nakagomi et al., 1999). However, it was later found that the P[6] VP4 genes of two Japanese porcine rotavirus strains were very similar to that of AU19 and that three of them clustered together with a 100% bootstrap probability,

Table 1
The primers used to amplify the genome segments of AU19.

Genome segment	Primer name	Primer sequence	References
VP1	GEN_VP1Fb	5′-GGC TAT TAA AGC TRT ACA ATG GGG AAG-3′	Matthijnssens et al. (2008a)
	GEN_VP1Rb	5′-GGT CAC ATC TAA GCG YTC TAA TCT TG-3′	Matthijnssens et al. (2008a)
VP2	GEN_VP2Fc	5′-GGC TAT TAA AGG YTC AAT GGC GTA CAG-3′	Matthijnssens et al. (2008a)
	GEN_VP2Rbc	5′-GTC ATA TCT CCA CAR TGG GGT TGG-3′	Matthijnssens et al. (2008a)
VP3	M1V3_22F	5′-AGT GCG TTT TAC CTC TGA TGG-3′	This study
	M1V3_2591R	5′-GGT CAC ATC GTG ACT AGT GTG TTA-3′	This study
VP4	BegG4	5′-TGT ATC ATA CGG CTA TAA AAT G-3′	Isegawa et al. (1992)
	EndG4	5′-TAA GGT TAT GTA TGG TCA CAT C-3′	Isegawa et al. (1992)
VP6	GEN_VP6F	5′-GGC TTT WAA ACG AAG TCT TC-3′	Matthijnssens et al. (2008a)
	GEN_VP6R	5′-GGT CAC ATC CTC TCA CT-3′	Matthijnssens et al. (2008a)
VP7	Beg9	5′-GGC TTT AAA AGA GAG AAT TTC CGT CTG G-3′	Gouvea et al. (1990)
	End9	5′-GGT CAC ATC ATA CAA TTC TAA TCT AAG-3′	Gouvea et al. (1990)
NSP1	GEN_NSP1F	5′-GGC TTT TTT TTA TGA AAA GTC TTG-3′	Matthijnssens et al. (2008a)
	GEN_NSP1R	5′-GGT CAC ATT TTA TGC TGC C-3′	Matthijnssens et al. (2008a)
NSP2	GEN_NSP2F	5′-GGC TTT TAA AGC GTC TCA G-3′	Matthijnssens et al. (2008a)
	GEN_NSP2R	5′-GGT CAC ATA AGC GCT TTC-3′	Matthijnssens et al. (2008a)
NSP3	GEN_NSP3F	5′-GGC TTT TAA TGC TTT TCA GTG-3′	Matthijnssens et al. (2008a)
	GEN_NSP3R	5′-ACA TAA CGC CCC TAT AGC-3′	Matthijnssens et al. (2008a)
NSP4	GEN_NSP4F	5′-GGC TTT TAA AAG TTC TGT TCC-3′	Matthijnssens et al. (2008a)
	GEN_NSP4R	5′-GGW YAC RYT AAG ACC RTT CC-3′	Matthijnssens et al. (2008a)
NSP5	Beg11	5′-GGC TTT TAA AGC GCT ACA GTG ATG-3′	Giambiagi et al. (1994)
	End11	5′-GGT CAC AAA ACG GGA GTG GG-3′	This study

All the primers are located at the 5′ and 3′ ends of the respective gene segments, except for primer GEN_VP2_Rbc, which starts at nucleotide 2, primer M1V3_22F, which starts at nucleotide 22. Degenerate bases: R = A/G, W = A/T, Y = C/T.

Table 2
The position and length of the sequence determined for each the 11 genome segments of AU19.

Segment	Positions and length of sequence	Accession number	References
VP7	50–1030 (981)	AB018697	Ahmed et al. (2007)
VP4	23–2312 (2290)	AB770153	This study
VP6	151–1307 (1157)	AB770154	This study
VP1	28–3245 (3218)	AB770155	This study
VP2	65–2657 (2593)	AB770156	This study
VP3	87–2541 (2455)	AB770157	This study
NSP1	198–1363 (1166)	AB770158	This study
NSP2	48–1008 (961)	AB770159	This study
NSP3	58–948 (891)	AB770160	This study
NSP4	51–738 (688)	AB770161	This study
NSP5	25–928 (904)	AB770162	This study