Infection, Genetics and Evolution 43 (2016) 338–342

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Research paper

Molecular characterization of the first G24P[14] rotavirus strain detected in humans☆

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ARTICLE INFO

Article history:
Received 22 March 2016
Received in revised form 23 May 2016
Accepted 24 May 2016
Available online 26 May 2016

Keywords:
Rotavirus
G24P[14]
Bovine
Reassortment

ABSTRACT

Here we report the genome of a novel rotavirus A (RVA) strain detected in a stool sample collected during routine surveillance by the Centers for Disease Control and Prevention's New Vaccine Surveillance Network. The strain, RVA/human-wt/USA/2012741499/2012/G24P[14], has a genomic constellation of G24-P[14]-I2-R2-C2-M2-A3-N2-T9-E2-H3. The VP2, VP3, VP7 and NSP3 genes cluster phylogenetically with bovine strains. The other genes occupy mixed clades containing animal and human strains. Strain RVA/human-wt/USA/2012741499/2012/G24P[14] most likely is the product of interspecies transmission and reassortment events. This is the second report of the G24 genotype and the first report of the G24P[14] genotype combination in humans.

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1. Introduction

Rotavirus A (RVA) is a leading cause of diarrheal death and morbidity among children <5 years of age worldwide, particularly in populations not having robust rotavirus vaccination programs (Estes and Greenberg, 2013). RVA are members of the family *Reoviridae* and possess a genome composed of 11 segments of double-stranded RNA (dsRNA) encoding six viral structural proteins (VP1–VP4, VP6 and VP7) and 5 or 6 non-structural proteins (NSP1–NSP5/6) (Fields et al., 2013). The classification nomenclature for the structural and non-structural proteins of RVA is Gx-P[x]-Ix-Rx-Cx-Mx-Ax-Nx-Tx-Ex-Hx, with x indicating the numbers of the corresponding genotypes (Matthijnssens et al., 2011). Currently, there are 32 distinct G genotypes and 46 distinct P genotypes recognized among RVAs in humans and animals (http://rega.kuleuven.be/cev/viralmetagenomics/virus-classification). The majority of human RVA strains possess either the Wa-like genogroup 1 constellation (Gx-P[x]-I1-R1-C1-M1-A1-N1-T1-E1-H1; porcine origin) or the DS-1-like genogroup 2 constellation (Gx-P[x]-I2-R2-C2-M2-A2-N2-T2-E2-H2; bovine origin) (Matthijnssens et al., 2008a). Occasionally human RVA strains have the AU-1-like genogroup 3 constellation (Gx-P[x]-I3-R3-C3-M3-A3-N3-T3-E3-H3; feline origin) (Matthijnssens et al., 2008b).

RVA strain surveillance and characterization studies from around the world have documented the diversity of RVA strains infecting humans. Whole-genomic analyses are essential for understanding the genetic diversity of RVA strains (Ghosh and Kobayashi, 2011). Uncommon G and P genotypes along with novel genotype combinations are emerging that cause disease in humans; whole-genome analyses of these strains provides evidence of interspecies transmission and reassortment between human and animal RVA strains (Chitambar et al., 2011; Gautam et al., 2015; Martella et al., 2010; Matthijnssens et al., 2009; Matthijnssens and Van Ranst, 2012; Mijatovic-Rustempasic et al., 2015; Tacharoenmuang et al., 2015; Tam et al., 2014). During the 2011–2012 RVA season of the CDC New Vaccine Surveillance Network, one case of pediatric acute gastroenteritis associated with a bovine-like RVA strain was identified in a stool sample from Houston, Texas. Here we report the full genome characterization of the first G24P[14] RVA strain detected in humans.

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http://dx.doi.org/10.1016/j.meegid.2016.05.033
1567-1348/Published by Elsevier B.V.

Table 1
Comparison of complete ORF sequences between RVA/human-wt/USA/2012741499/2012/G24P[14] and closely related strains.

Gene	Genotype	Closest strain(s)	Predominant host of closely related strain(s)	% identity to closest related strain(s)
VP7	G24	Dai-10	Bovine	96.3
VP4	P[14]	A64	Human	96.4
VP6	I2	Dai-10, AzuK-1	Bovine	96.4, 95.9
VP1	R2	RF-TOPORF11	Bovine	95.3
VP2	C2	Sun9	Bovine	97.1
VP3	M2	Tottori-SG	Bovine	98.4
NSP1	A3	NCDV-Sapporo	Bovine	96.5
NSP2	N2	RRV	Simian	97.8
NSP3	T9	Dai-10, AzuK-1	Bovine	95.1, 94.9
NSP4	E4	CHLY	Bovine	97.0
NSP5	H3	ARG Chubut	Guanaco	96.3

2. Materials and methods

2.1. Patient history

The patient was a previously healthy two-year-old Hispanic male who presented with a three-day history of diarrhea, a two-day history of non-bilious vomiting, intermittent abdominal pain, subjective fever and one day of decreased urine output. The patient was admitted to a hospital in Houston, Texas and was given fluid resuscitation and antipyretics for fever control. A stool specimen was obtained three days after the onset of illness. The patient had received no doses of RotaTeq® or Rotarix® vaccine. The patient's four-year-old sister was ill with gastrointestinal symptoms one day prior to the patient's symptom onset. Furthermore, the case had household contact with an 82-year old grandfather who had returned from Mexico two months earlier. Of note, at the time of enrollment in this study, the patient's parent reported no history of exposure to animals, including farm animals, or attendance at petting zoos. Institutional review board approvals were obtained from the CDC and from the Houston study site.

2.2. Sequencing and genotype assignment

Full-genome next-generation sequencing was performed using an Illumina MiSeq following a previously published protocol (Mijatovic-Rustempasic et al., 2014). The genotype assignment for each gene was done using the RotaC online classification tool (http://rotac.regatools.be/) and BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

2.3. Phylogenetic and genetic analyses

For phylogenetic analyses, nucleotide sequences of related strains for each gene were retrieved from GenBank and aligned using the MUSCLE program within MEGA version 5 software (http://www.megasoftware.net/). The optimal evolutionary models that best fit the aligned sequence datasets (NSP1-TIM3 + I; NSP2-TrN + G; NSP3-TrN + I + G; NSP4-TPM1uf + I + G; NSP5-HKY + I + G; VP1-GTR + I + G; VP2-TIM2 + I; VP3-TIM1 + I + G; VP4-TIM3 + I + G; VP6-TIM2 + I; VP7-GTR + G) were determined using the JModelTest 2 program (Posada, 2008). Using the optimal models identified by the corrected Akaike Information Criterion (AICc), maximum likelihood trees were constructed using PhyML 3.0 with aLRT statistics computed for branch support (Guindon et al., 2010). Nucleotide distance matrices were prepared using the p-distance algorithm of MEGA version 5 software.

3. Results and discussion

Here we present the full sequence of strain RVA/human-wt/USA/2012741499/2012/G24P[14] which represents the second report of the G24 genotype and the first G24P[14] RVA strain detected in humans. This strain exhibits close relationships to bovine RVA strains in the majority of genes.

3.1. Sequencing results

A combination of *de novo* assembly and subsequent mapping to reference strains was used to obtain the full-length genome of strain RVA/human-wt/USA/2012741499/2012/G24P[14]. The sizes of full-length segments 1 to 11 were 3302, 2687, 2591, 2361, 1578, 1356, 1078, 1059, 1067, 751, 667 bp, and the open reading frames (ORFs) lengths for these segments were 3267, 2643, 2508, 2331, 1476, 1194, 942, 954, 987, 528, 597 bp, respectively. The complete ORFs for all 11 genes of G24P[14] were deposited in GenBank under accession numbers KT281120–KT281130 for VP1, VP2, VP3, VP4, NSP1, VP6, NSP3, NSP2, VP7, NSP4, and NSP5, respectively.

3.2. Genetic distances

Nucleotide distance matrices show that strain RVA/human-wt/USA/2012741499/2012/G24P[14] is closely related to bovine strains for the VP7, VP6, VP1, VP2, VP3, NSP1, NSP3, and NSP4 genes (range: 95.3–98.4%) (Table 1). The VP4 gene is closely related to human strain A64 (96.4%), while the NSP2 gene is closely related to simian strain RRV (97.8%) and the NSP5 gene is closely related to guanaco strain ARG Chubut (96.3%) (Table 1).

3.3. Results of phylogenetic analyses

Phylogenetic analyses of the eleven gene segments revealed that genes VP7, VP6, VP1, VP2, VP3, NSP1, NSP3, and NSP4 were related to bovine strains, in particular AzuK-1(G21P[29]-I2-R2-C2-M2-A13-N2-T9-E2-H3) and Dai-10 (G24P[33]-I2-R2-C2-M2-A13-N2-T9-E2-H3) (Fig. 1A-D). The VP6 gene occupies the basal position in a cluster containing bovine, simian, and human strains (Fig. 1E). The VP1 gene occupies a mixed clade with bovine, simian and human strains (Fig. 1F). The NSP1 gene clusters with bovine and human strains (Fig. 1H). The NSP2 gene occupies a mixed clade with simian, bovine, human, and canine strains (Fig. 1I). Also in mixed clades, the NSP4 and NSP5 genes cluster with bovine, simian and equine strains and guanaco (a camelid native to South America), human, bovine, and simian strains, respectively (Fig. 1J-K).

The G-genotype of strain RVA/human-wt/USA/2012741499/2012/G24P[14] is G24 and shares 96.3% sequence identity with Dai-10 (Table 1). The genes of Dai-10 possess typical bovine genotypes except for VP7 and VP4, which were assigned new G24 and P[33] genotypes by the RCWG (Abe et al., 2011). Abe and co-workers surveyed asymptomatic calves for RVA infection in Japan from 2006 to 2007 (Abe et al., 2009). It was during the survey that a non-typeable strain (AzuK-1) was detected and sequenced. The AzuK-1 strain demonstrated low nucleotide sequence identity with reference G and P genotypes and was assigned new G21 and P[29] genotypes by the Rotavirus Classification Working Group (RCWG) (Abe et al., 2009). In a subsequent study, the complete ORF sequences of all 11 genes of the AzuK-1 strain and another new strain, Dai-10, were determined (Abe et al., 2011). While AzuK-1 appears to be endemic in cattle in Japan, the Dai-10 strain was detected in only two asymptomatic cows in the survey, a mother and calf, suggesting that this strain is rare in cattle (Abe et al., 2011).

The P-genotype of strain RVA/human-wt/USA/2012741499/2012/G24P[14] is P[14], lineage I (Tam et al., 2014), and like the P[14] strains sequenced by Matthijnssens and co-workers (Matthijnssens et al., 2009), phylogenetic analysis shows the close genomic relatedness of

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