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Research paper

Distinguishing the genotype 1 genes and proteins of human Wa-like rotaviruses vs. porcine rotaviruses

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ABSTRACT

Group A rotaviruses (RVAs) are 11-segmented, double-stranded RNA viruses and important causes of gastroenteritis in the young of many animal species. Previous studies have suggested that human Wa-like RVAs share a close evolutionary relationship with porcine RVAs. Specifically, the VP1-VP3 and NSP2-5/6 genes of these viruses are usually classified as genotype 1 with >81% nucleotide sequence identity. Yet, it remains unknown whether the genotype 1 genes and proteins of human Wa-like strains are distinguishable from those of porcine strains. To investigate this, we performed comprehensive bioinformatic analyses using all known genotype 1 gene sequences. The RVAs analyzed represent wildtype strains isolated from humans or pigs at various geographical locations during the years of 2004–2013, including 11 newly-sequenced porcine RVAs from Brazil. We also analyzed archival strains that were isolated during the years of 1977–1992 as well as atypical strains involved in inter-species transmission between humans and pigs. We found that, in general, the genotype 1 genes of typical modern human Wa-like RVAs clustered together in phylogenetic trees and were separate from those of typical modern porcine RVAs. The only exception was for the NSP5/6 gene, which showed no host-specific phylogenetic clustering. Using amino acid sequence alignments, we identified 34 positions that differentiated the VP1-VP3, NSP2, and NSP3 genotype 1 proteins of typical modern human Wa-like RVAs versus typical modern porcine RVAs and documented how these positions vary in the archival/unusual isolates. No host-specific amino acid positions were identified for NSP4, NSP5, or NSP6. Altogether, the results of this study support the notion that human Wa-like RVAs and porcine RVAs are evolutionarily related, but indicate that some of their genotype 1 genes and proteins have diverged over time possibly as a reflection of sequestered replication and protein co-adaptation in their respective hosts.

1. Introduction

Group A rotaviruses (RVAs) are gastrointestinal pathogens of many animal species. In humans, RVAs are a leading cause of childhood diarrheal death, particularly in developing regions of the world (Tate et al., 2012). RVAs are also a major cause of acute viral diarrhea in suckling and weaned piglets, imparting significant financial losses to the pork industry (Chang et al., 2012). The RVA genome consists of 11 segments of double-stranded RNA, which are encapsidated within a non-enveloped, triple-layered virion particle (Estes and Kapikian, 2007). The outermost layer of the RVA virion is made up of VP4 and VP7, while the middle layer is comprised of VP6. The innermost core shell is formed of VP2, and it surrounds the viral genome and RNA processing enzymes (VP1 and VP3). Five or six viral non-structural proteins (NSP1-NSP5/6) are made within infected cells and play various roles during viral replication.

RVAs are classified according to a system that designates a specific genotype for each of the 11 viral genome segments (i.e., genes) based on their nucleotide sequences and established percent identity cut-off values (Matthijnssens et al., 2008). In this system, the genotype constellation of the VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5/6 genes is described as Gx-Px-Ix-Rx-Cx-Mx-Ax-Nx-Tx-Ex-Hx, where x is a number. The vast majority of human RVAs sequenced to date (n > 300) exhibit the genotype constellation of G1/2/3/4/9/12-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1 (Matthijnssens and Van Ranst, 2012). Human RVAs with this genotype constellation are referred to as "Wa-like" because they are genetically similar to the archival reference strain Wa (Wyatt et al., 1980). While fewer porcine RVAs have been sequenced to date (n = 33), the available data suggests that these strains typically exhibit the genotype constellation G3/5/9/11-P[6]/[7]/[13]/[19]/[23]-I5-R1-C1-M1-A8-N1-T1/7-E1-H1 (Kim et al., 2012; Martel-Paradis et al., 2013; Matthijnssens et al., 2008; Monini et

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al., 2014; Nagai et al., 2015; Okitsu et al., 2013; Silva et al., 2015; Theuns et al., 2015). Thus, it seems that while human Wa-like RVAs and porcine RVAs differ in their VP7, VP4, VP6, and NSP1 gene genotypes, they tend to have similar genotype 1 VP1-VP3 and NSP2-NSP5/6 genes. This observation suggested that human Wa-like RVAs and porcine RVAs share an evolutionary relationship and perhaps a common ancestor (Matthijnssens et al., 2008).

In the current study, we sought to more fully characterize the genotype 1 genes and proteins of human Wa-like RVAs and porcine RVAs and to determine whether specific genetic signatures differentiate those from each host. We constructed neighbor-joining phylogenetic trees to reveal the relationships between the genotype 1 genes of typical and atypical human Wa-like and porcine RVAs. We also employed amino acid sequence alignments to identify positions that varied in the genotype 1 viral proteins in a host-specific manner. Furthermore, we documented how several archival/unusual isolates differ at these host-specific variable positions. Altogether, these results enhance our understanding of RVA genetic diversity and elucidate putative evolutionary signatures of genotype 1 viral proteins from human versus porcine strains.

2. Materials and methods

2.1. Nucleotide sequencing of 11 Brazilian porcine RVAs

Near-complete genome nucleotide sequences were determined for 11 porcine RVAs (ROTA02, ROTA03, ROTA04, ROTA05, ROTA13, ROTA16, ROTA18, ROTA25, ROTA27, ROTA30, and ROTA31) using the same approach described in Silva et al., 2015 (Silva et al., 2015). These porcine strains are considered to represent typical modern isolates as they were found in fecal specimens collected from diarrheic nursing and suckling piglets (<60 days of age) on various Brazilian farms during the years of 2012–2013. The 83 new full or partial gene sequences were genotyped using RotaC2.0 (Maes et al., 2009) and were deposited into GenBank (Table S1). Only the sequences for the genotype 1 VP1-VP3 and NSP2-NSP5/6 genes were analyzed in the current study.

2.2. Sub-genotypic neighbor-joining phylogenetic analyses

During the initial stages of the analyses, we downloaded the nucleotide sequences of genotype 1 VP1-VP3 and NSP2-NSP5/6 genes of all known human Wa-like RVAs and porcine RVAs. Alignments were created for each gene using Geneious Pro v5.6.5 (Biomatters) and the ClustalW algorithm and were trimmed so that the sequences were the same length in each alignment. The following nucleotide regions were analyzed for each gene: VP1 (nts 162-1572), VP2 (nts 28-1431), VP3 (nts 52-1034), NSP2 (nts 76-907), NSP3 (nts 59-977), NSP4 (nts 48-577), and NSP5/6 (nts 43-535). Neighbor-joining phylogenetic trees were created for each gene using Geneious Pro v5.6.5 (Biomatters). The trees were out-group rooted to the genotype 2 genes of strain DS-1 and built using three different distance models (Jukes-Cantor, Tamura-Nei, and HKY) and 100 bootstrap replicates. The overall tree topologies and major groupings were identical irrespective of the distance model chosen. Based upon the clustering of sequences in the initial trees, we then selected 102 representative RVAs to include in the final trees, which were built using the Jukes-Cantor distance model (Table S2 and Figs. S1–S7). For selection of the final strains, we also considered the year, G/P-genotype, and geographical location of the strain. To prepare Fig. 1, major groupings were collapsed using FigTree v1.4 and colorized using Adobe Illustrator CS5 (Adobe Systems).

2.3. Identification of host-specific amino acid changes in genotype 1 proteins

The deduced amino acid sequences of genotype 1 proteins from the 102 representative strains (Table S2) were aligned using Geneious Pro v5.6.5 and the BLOSUM-62 matrix of ClustalW. Amino acids that varied according to host species or viral isolate were identified by visual inspection of the alignments and confirmed by NCBI BLAST analysis. For VP2, which varies in length, the documented position numbers are based on those of strain RVA/Human-wt/PRY/1638SR/2008/G1P[8]. The three dimensional locations of the host-specific variable positions were determined using UCSF Chimera v1.8 and the predicted or known atomic structures of viral proteins: strain UK and SA11 VP1 and VP2 (PDB# 4F5X), strain RRV VP3 (PDB# 2IHP), strain SA11 NSP2 (PDB# 1L9V), and SA11 NSP3 (PDB# 1KNZ and PDB# 1LJ2) (Deo et al., 2002; Estrozi et al., 2013; Pettersen et al., 2004; Groft and Burley, 2002; Jayaram et al., 2002; Ogden et al., 2014).

3. Results

3.1. Genetic relationships between the genotype 1 genes of human Wa-like RVAs and porcine RVAs

To investigate the genetic relationships between human Wa-like RVA and porcine RV genotype 1 genes, we constructed individual phylogenetic trees for VP1-VP3 and NSP2-NSP5/6. Initial trees were constructed using all human Wa-like RVA and porcine RVA genotype 1 nucleotide sequences available in GenBank (data not shown). In this study, we also deduced the full or partial gene sequences for 11 porcine RVAs isolated from diarrheic piglets in Brazil during the years 2012–2013. The final trees included the genotype 1 genes of these 11 new Brazilian porcine RVA sequences as well as those of 91 additional human or porcine RVAs that altogether reflected the genetic diversity seen in the initial trees (Fig. 1). The genotype constellations of the 102 representative strains are shown in Fig. 2.

The human Wa-like RVAs chosen as representatives of typical modern strains (Fig. 2A) included those that were isolated during the years of 2004–2011 from diarrheic children at various geographical locations: Thailand (strains CU938-BK, CU747-KK, and CU460-KK), Africa (strains MRC-DPRU1424, MRC-DPRU1723, and MRC-DPRU1262), Belgium (strains BE00030, BE00043, BE00055, B4633, and B3458), the United States (strains 2008747100, 2008747288, 2007719720, VU08-09-20, VU06-07-21, VU06-07-32, VU05-06-2, and VU05-06-47), Australia (strains CK00100, CK00088, CK00034, and CK00005), China (strains R588 and Y128), Italy (strains JES11 and AV21), Germany (strains GER126-08 and GER172-08), India (strains 61060 and 6361), and Paraguay (strains 954SR and 1638SR) (Arora and Chitambar, 2011; Ianiro et al., 2013; Matthijnssens et al., 2008; McDonald et al., 2012; Nyaga et al., 2013; Pietsch and Liebert, 2009; Rahman et al., 2007; Shintani et al., 2012; Theamboonlers et al., 2014; Zeller et al., 2015).

We also included in our phylogenetic analyses the genotype 1 gene sequences of archival/unusual human strains (Fig. 2B) that were either isolated prior to 1992 (strains RV3, 116E, YO, IAL28, DC1476, DC1600, and DC4608) or that were likely the result of pig-to-human interspecies transmission events (strains BE2001, Arg4605, Mc323, BP271, BP1227, BP1547, 1809SR, Dhaka6, Matlab36, Ryukyu-1120, and EC2184) (Banyai et al., 2009; Degiuseppe et al., 2013; Ghosh et al., 2012; Heiman et al., 2008; Komoto et al., 2013; Martinez et al., 2014; Matthijnssens et al., 2008; Matthijnssens et al., 2010; McDonald et al., 2011; McDonald et al., 2009; Papp et al., 2013; Rippinger et al., 2010; Zeller et al., 2012; Zhang et al., 2014).

For the porcine RVAs, we included in the final trees typical modern strains for which complete or near-complete genome sequence information is available (Fig. 2C). These strains were considered to be wildtype porcine RVAs because they had G/P-genotypes normally associated with porcine RVAs and they were found in the feces of diarrheic or non-diarrheic piglets during the years of 2006–2014. The porcine RVAs were also from various geographical locations: Brazil (strains ROTA01-10, ROTA24-25, ROTA27, and ROTA30-31), Belgium (strains 12R022, 12R002, 12R006, 12R005, 12R041, and 12R046), Italy (strains 3BS, 2CR, and 7RE), Thailand (strains CMP45, CMP29, CMP40, and