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Distinct evolutionary origins of G12P[8] and G12P[9] group A rotavirus strains circulating in Brazil

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ABSTRACT

G12 group A rotavirus (RVA) are currently recognized as a globally emerging genotype and have been described in combination with several P-types. In Brazil, G12 RVA strains have been described in the Southern (2003) and Northern (2008–2010) regions, in combination with the P[9] and P[6] genotype, respectively. To date, few complete genomes of G12 RVA strains have been described (none from Brazilian strains), considering G12P[9] genotype just one strain, RVA/Human-tc/THA/T152/1998/G12P[9], has their 11 gene segments characterized. This study aims to determine the genomic constellation of G12P[9] and G12P[8] RVA strains detected in Brazil between 2006 and 2011. Therefore, the eleven gene segments of five Brazilian G12 RVA strains were amplified and sequenced, and the genotype of each gene segment was assigned using phylogenetic analysis. Complete genome analyses of G12 RVA strain circulating between 2006 and 2011 in Brazil revealed a conserved Wa-like genomic constellation for three G12P[8] RVA strains; whereas the two G12P[9] strains possessed distinct reassorted AU-1-like genomic constellations, closely related to the reference strain RVA/Human-tc/THA/T152/1998/G12P[9] in most genes. The results obtained in the current study suggest that G12P[9] (AU-1-like) and G12P[8] (Wa-like) strains detected in different regions of Brazil do not share a common origin. Moreover, while Brazilian G12P[8] RVA strains showed a complete Wa-like human constellation, both G12P[9] strains possessed an NSP1 gene of bovine origin (NSP1), and RVA/Human-wt/BRA/PE18974/2010/G12P[9] also possessed a VP3 gene of canine/feline origin.

1. Introduction

Group A rotavirus (RVA) genotype G12 was first detected in children with diarrhea in the Philippines in 1987 (Taniguchi et al., 1990). Recently, this genotype has been described in several studies conducted worldwide, and their global spread was demonstrated in the last decade (Rahman et al., 2007; Matthijnssens et al., 2010a). In Brazil, G12 RVA strains have been described in the Southern (2003) and Northern (2008–2010) regions, in combination with the P[9] and P[6] genotypes, respectively (Pietruchinski et al., 2006; Soares et al., 2011). However, only information about the VP7 encoding gene of these Brazilian G12 strains is available in the GenBank database. Although data on the genetic diversity of the VP7 and/or VP4 genes are important from the point of view of host immunity against RVA disease, information on these genes is not enough to obtain conclusive data on the evolutionary dynamics of RVA strains. Therefore, it is important to perform whole genome analysis of RVA strains to better understand the genetic diversity of these viruses (Ghosh and Kobayashi, 2011).

This work reports the complete genotype constellation of two G12P[9] RVA strains detected in 2008 (RVA/Human-wt/BRA/PE15776/2008/G12P[9]) and 2010 (RVA/Human-wt/BRA/PE18974/2010/G12P[9]) in Pernambuco (PE) state (Northeastern Brazil), and three G12P[8] strains, one detected in Rio de Janeiro (RJ) (Southeastern Brazil) in 2006 (RVA/Human-wt/BRA/RJ12419/2006/G12P[8]) and two in Bahia (BA) (Northeastern Brazil) in 2011 (RVA/Human-wt/BRA/BA20142/2011/G12P[8] and RVA/Human-wt/BRA/BA20144/2011/G12P[8]).

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2. Material and methods

All strains were recovered from children hospitalized with acute gastroenteritis, and strains RVA/Human-wt/BRA/BA20142/2011/G12P[8] and RVA/Human-wt/BRA/PE18974/2010/G12P[9] were collected from children vaccinated with one and two doses of RV1 vaccine, respectively. Nucleic acids extraction and amplicon synthesis were performed using a QIAamp Viral RNA Mini Kit and OneStep RT-PCR Kit (QIAGEN®) following manufacturer's instructions and amplifications conditions as described by Matthijnssens et al. (2010b). PCR amplicons were purified using the QIAquick PCR purification kit (QIAGEN®) and sequenced with the ABI Prism BigDye terminator cycle sequencing reaction kit (Applied Biosystems®) on an ABI Prism 3130 automated sequencer (Applied Biosystems®) (Matthijnssens et al., 2010b).

Phylogenetic analyses were performed using the neighbor-joining method (Kimura-two parameter model, 2000 bootstrap replicates) in MEGA 5.0 (Tamura et al., 2011). Other methods as GTR: General Time Reversible plus Gamma (VP3–4, NSP1) and TVM: Transversion Model plus Gamma (VP7) were also used (data not shown). Sequences obtained in the current study were deposited in the GenBank database under the following accession numbers: KF907251–KF907305.

This research was approved by the Fiocruz Ethical Research Committee (No. 311/06).

3. Results

G12P[9] strains clustered together with other G12P[9] strains detected worldwide inside lineage II for the VP7 gene (Fig. 1a), and showed up to 99% identity with strains detected in South America and Eastern/Southeastern Asia. G12 strains in combination with P[8] grouped in a separate cluster together with the majority of the G12P[8] and G12P[6] strains described worldwide (99% identity) inside lineage III (Fig. 1a).

Phylogenetic analysis based on VP4 gene revealed that G12P[9] Brazilian strains clustered inside lineage II and were closely related to RVA/Human-tc/THA/T152/1998/G12P[9] strain (99% identity) (Fig. 1b). Brazilian G12P[8] strains clustered inside lineage P[8]-III. Strain detected in 2006 was closely related to contemporary G1P[8] and G12P[8] strains detected worldwide (2002–2008), whereas the two strains detected in 2011 were closely related to strains detected in recent years (2009–2010) in the USA, Australia, and Nicaragua (99% identity) (Fig. 1b).

G12P[9] and G12P[8] Brazilian strains revealed a distinct genomic constellation: AU-1-like and Wa-like, respectively (Table 1). Phylogenetic analyses of the NSP2–5, VP1, VP2, and VP6 encoding genes of the G12P[9] Brazilian strains showed a very close genetic relationship with RVA/Human-tc/THA/T152/1998/G12P[9] (data not shown). For the VP3 encoding gene, RVA/Human-wt/BRA/PE18974/2010/G12P[9] strain was closely related to canine/feline P[9] strains, or human strains that are believed to be of canine/feline origin (Fig. 2a). The NSP1 gene of Brazilian G12P[9] strains belonged to A3 genotype clustering with several bovine strains, or strains that are believed to be of bovine origin (Fig. 2b).

Brazilian strain RVA/Human-wt/BRA/RJ12419/2006/G12P[8] clustered together with G1P[8] strains detected in USA (2005), while RVA/Human-wt/BRA/BA20142/2011/G12P[8] and RVA/Human-wt/BRA/BA20144/2011/G12P[8] were nearly identical and clustered with G12P[8] strains detected in Thailand (2009) for most of the RVA genes (data not shown).

4. Discussion

G12 RVA strains have been found in combination with each of the three major human genotype constellations: Wa-like, DS-1-like, and AU-1-like (Rahman et al., 2007; Heiman et al., 2008; Ghosh et al., 2010; Jere et al., 2011; Stupka et al., 2012). However, the majority of G12 strains possess the Wa-like genotype constellation (Freeman et al., 2009; Matthijnssens and Van Ranst, 2012), probably because they were found in combination with the P[8] genotype.

In the current study G12P[8] Brazilian strains analyzed revealed a human Wa-like genomic constellation, whereas the G12P[9] revealed a reassorted AU-1-like genomic constellation. While all G12P[9] strains previously described worldwide possessed a rare

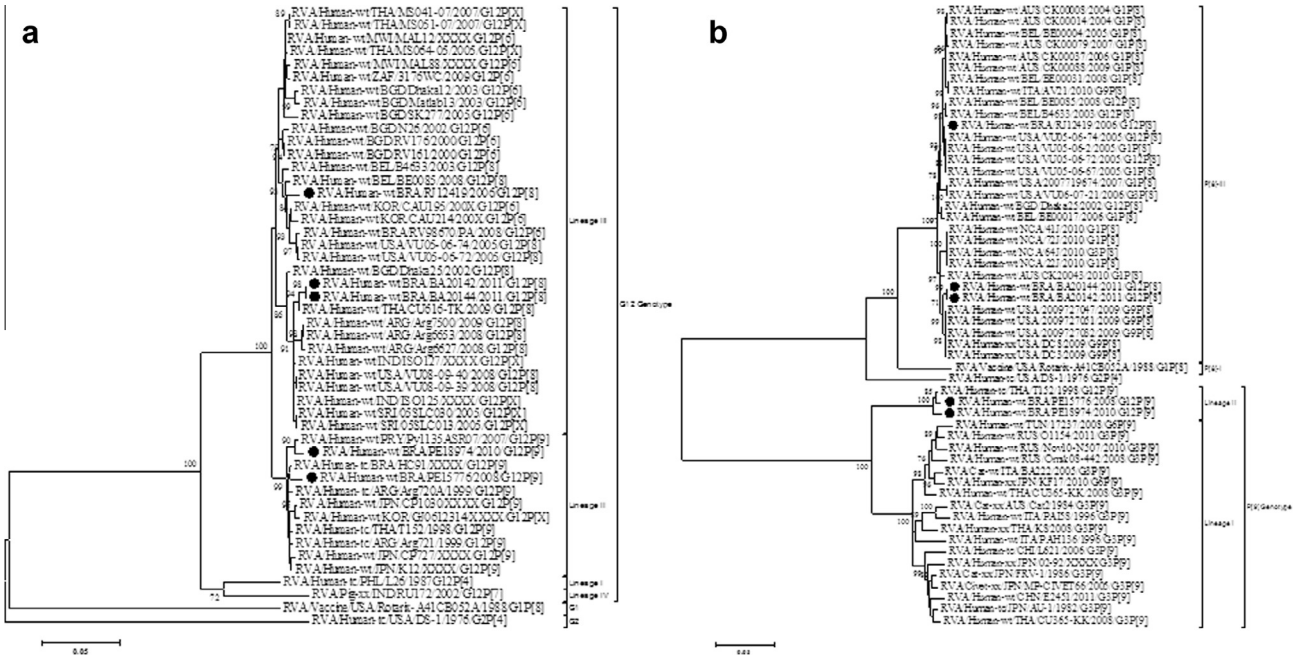

Fig. 1. Phylogenetic analysis based on the VP7 (a) and VP4 (b) gene nucleotide sequences of Brazilian G12 strains, and sequences from the GenBank database. Numbers at the nodes indicate bootstrap values; only values above 70% are shown. The scale bar at the bottom represents 0.05 substitutions per nucleotide position (nt.subst./site). Brazilian G12 strains are marked with a filled circle.

Table 1
Genotype constellation comparison of Brazilian G12 strains, RV1 vaccine strain and group A rotavirus (RVA) prototypes strains. Brazilian strains are shown in bold. Green, red and orange indicate Wa-like, DS-1-like and AU-1-like gene segments, respectively, and blue is used to indicate a gene segment of bovine origin. Gray is used to indicate canine/feline gene segment VP3 found in RVA/Human-wt/BRA/PE18974/2010/G12P[9] strain.

Genogroup	Strain	Origin	Genotypes										
			VP7	VP4	VP6	VP1	VP2	VP3	NSP1	NSP2	NSP3	NSP4	NSP5
Wa-like	RVA/USA/Rotarix-A41CB052A/1988/G1P1A[8]	Human	G1	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1
	RVA/Human-tc/USA/Wa/1974/G1P1A[8]	Human	G1	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1
	RVA/Human-wt/BRA/RJ12419/2006/G12P[8]	Human	G12	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1
	RVA/Human-wt/BRA/BA20142/2011/G12P[8]	Human	G12	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1
	RVA/Human-wt/BRA/BA20144/2011/G12P[8]	Human	G12	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1
	RVA/Human-wt/ZAF/3176WC/2009/G12P[6]	Human	G12	P[6]	I1	R1	C1	M1	A1	N1	T1	E1	H1
	RVA/Human-wt/BGD/Matlab13/2003/G12P[6]	Human	G12	P[6]	I1	R1	C1	M1	A1	N1	T2	E1	H1
	RVA/Human-wt/ARG/6653/2008/G12P[8]	Human	G12	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1
	RVA/Human-wt/ZAF/3133WC/2009/G12P[4]	Human	G12	P[4]	I1	R1	C1	M1	A1	N1	T1	E1	H1
	RVA/Porcine-wt/IND/RU172/2002/G12P[7]	Porcine	G12	P[7]	I5	R1	C1	M1	A1	N1	T1	E1	H1
DS-1-like	RVA/Human-tc/USA/DS-1/1976/G2P1B[4]	Human	G2	P[4]	I2	R2	C2	M2	A2	N2	T2	E2	H2
	RVA/Human-wt/BGD/N26/2002/G12P[6]	Human	G12	P[6]	I2	R2	C2	M2	A2	N1	T2	E6	H2
	RVA/Human-wt/BGD/RV161/2000/G12P[6]	Human	G12	P[6]	I2	R2	C2	M2	A2	N2	T2	E1	H2
	RVA/Human-wt/BGD/RV176/2000/G12P[6]	Human	G12	P[6]	I2	R2	C2	M2	A2	N2	T2	E6	H2
	RVA/Human-tc/PHL/L26/1987/G12P[4]	Human	G12	P[4]	I2	R2	C2	M1/M2	A2	N1	T2	E2	H1
AU-1-like	RVA/Human-tc/JPN/AU-1/1982/G3P[9]	Human	G3	P[9]	I3	R3	C3	M3	A3	N3	T3	E3	H3
	RVA/Human-wt/BRA/PE15776/2008/G12P[9]	Human	G12	P[9]	I3	R3	C3	M3	A3	N3	T3	E3	H6
	RVA/Human-wt/BRA/PE18974/2010/G12P[9]	Human	G12	P[9]	I3	R3	C3	M3	A3	N3	T3	E3	H6
	RVA/Human-tc/THA/T152/1998/G12P[9]	Human	G12	P[9]	I3	R3	C3	M3	A12	N3	T3	E3	H6
	RVA/Human-wt/ARG/Arg720/1999/G12P[9]	Human	G12	P[9]					A12				
	RVA/Human-wt/PRY/Py1135ASR07/2007/G12P[9]	Human	G12	P[9]								E3	H6

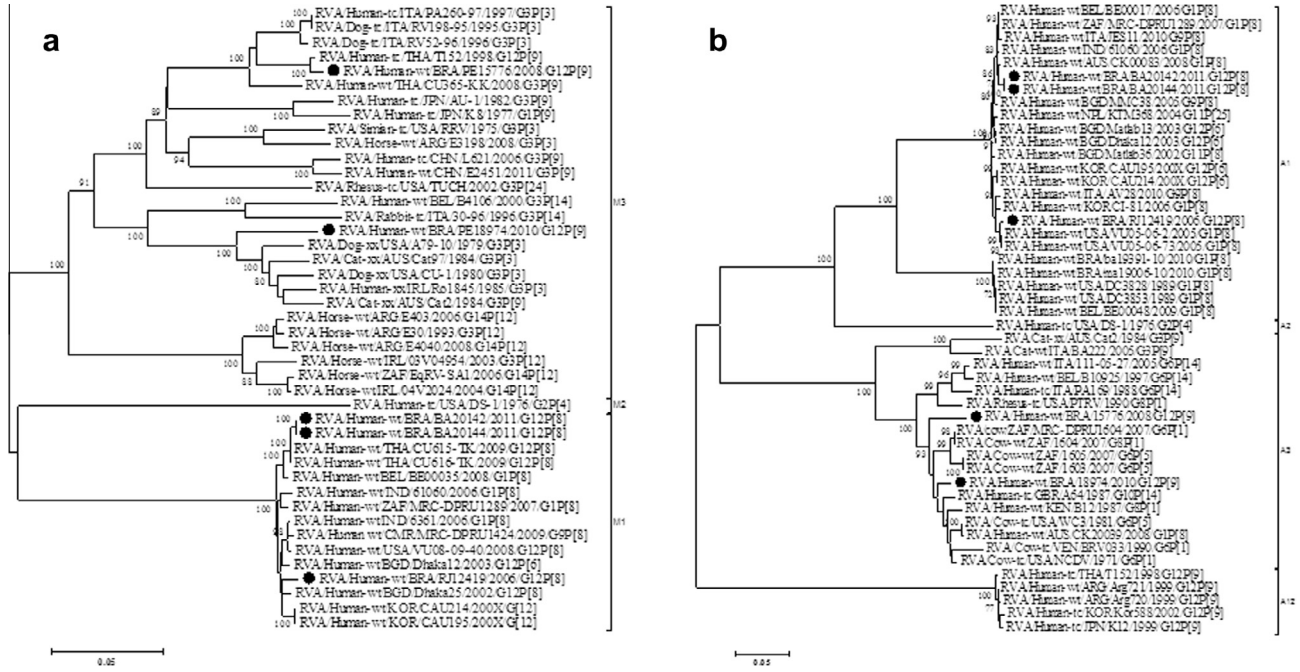

Fig. 2. Phylogenetic analysis based on the VP3 (a) and NSP1 (b) gene nucleotide sequences of Brazilian G12 strains, and sequences from the GenBank database. Numbers at the nodes indicate bootstrap values; only values above 70% are shown. The scale bar at the bottom represents 0.05 substitutions per nucleotide position (nt.subst./site). Brazilian G12 strains are marked with a filled circle.

A12 genotype (Castello et al., 2009), the NSP1 gene of Brazilian G12P[9] strains belonged to the A3 genotype. It has been suggested that G12P[9] strains previously detected in Argentina, Brazil, and Paraguay originated from Eastern Asia (Castello et al., 2009). In this study all genes, except for NSP1 and VP3 (RVA/Human-wt/BRA/PE18974/2010/G12P[9]), were shown to possess a close genetic relationship with strains detected in Eastern and Southeastern Asia. However, it is important to notice that just few G12P[9] strains have been detected and characterized to date.

At the time Brazil introduced the RV1 vaccine in the National Immunization Program (March 2006), G12 specific vaccine effectiveness could not be demonstrated because of the absence of detection of G12 previous to vaccine introduction. Since RV1 possess a human Wa-like genomic constellation, and G12P[9] strains revealed an AU-1-like genomic constellation, a difference in vaccine efficacy would be expected.

5. Conclusion

The results obtained in the current study suggest that G12P[9] and G12P[8] strains detected in different regions of Brazil do not share a common origin. Moreover, while Brazilian G12P[8] RVA strains showed a complete Wa-like human constellation, both

G12P[9] strains possessed a NSP1 gene of bovine origin, and RVA/Human-wt/BRA/PE18974/2010/G12P[9] possessed a VP3 gene of canine/feline origin. It is important to mention that the AU-l-like genotype constellation, sporadically found in humans, is believed to have a close evolutionary relationship with canine/feline RVAs (Matthijnssens et al., 2008).

A more in depth analysis of G12 RVA strains detected worldwide, involving full genomic and antigenic characterization, will help to identify potential mutations and/or reassorted genes that might be correlated with the capacity of RVA strains in evade the immunity caused by RVA vaccines (Gentsch et al., 2009). Availability of such data will be crucial to analyze the efficacy of RVA vaccines.

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