Genetic diversity in three bovine-like human G8P[14] and G10P[14] rotaviruses suggests independent interspecies transmission events

Maria Cristina Medici,1 Fabio Tummolo,1 Melisa Berenice Bonica,1 Elisabeth Heylen,2 Mark Zeller,2 Adriana Calderaro1 and Jelle Matthijnssens2

1Unit of Microbiology and Virology, Department of Clinical and Experimental Medicine, University of Parma, Parma, Italy
2KU Leuven – University of Leuven, Department of Microbiology and Immunology, Laboratory for Clinical and Epidemiological Virology, Rega Institute for Medical Research, B-3000 Leuven, Belgium

The group A rotavirus (RVA) P[14] genotype has been detected sporadically in humans and is thought to be acquired through zoonotic transmission. The present study describes the full-length genome analysis of two G8P[14] and one G10P[14] human RVAs detected in Italy. The strains possessed the typical bovine-like I2-R2-C2-M2-A3/A11-N2-T6-E2-H3 genotype constellation. All the segments of the two G8P[14] RVAs were most closely related to bovine(-like) strains but were relatively distant to each other, suggesting two independent interspecies transmission events. Likewise, the G10P[14] RVA gene segments were most similar to bovine(-like) RVAs but distinct from the G8 strains. The history of these strains probably involved the interspecies transmission of these viruses to humans from an as-yet-unidentified animal host, without evidence of reassortment events involving human RVAs. These results reinforce the potential of animal viruses to cross the host-species barrier, causing disease and increased viral genetic diversity in humans.

Group A rotavirus (RVA), a member of the genus Rotavirus in the family Reoviridae, is the leading aetiologic agent of severe gastroenteritis in the young of humans and many animal species worldwide (Estes & Greenberg, 2013; Martella et al., 2010).

The RVA genome is protected by the viral inner core, and is made up of 11 segments of double-stranded RNA, which encode six structural viral proteins (VP1–VP4, VP6 and VP7) and five or six non-structural proteins (NSP1–NSP5 and sometimes NSP6) (Estes & Greenberg, 2013). Because of the segmented nature of the genome, reassortment between and among human and animal strains is one of the major processes of genetic evolution of rotaviruses.

The traditional binomial RVA classification system was based on the two outer capsid proteins VP7 and VP4, and at least 27 G-types and 37 P-types, respectively, have been identified (Matthijnssens et al., 2011; Trojnar et al., 2013). Extending this classical binomial genotyping system, a new RVA classification system was proposed in which all 11 segments are considered (Matthijnssens et al., 2011). Following this new classification system, the notation of Gx-P[x]-Ix-Rx-Cx-Mx-Ax-Nx-Tx-Ex-Hx is used for the VP7-, VP4-, VP6-, VP1-, VP2-, VP3-, NSP1-, NSP2-, NSP3-, NSP4- and NSP5/6-encoding genes, respectively. Whole-genome sequencing has become widely established in RVA research, because it offers a platform providing a better insight into RVA diversity and evolution.

In human RVAs, G1, G2, G3, G4, G9 and G12 combined with P[4], P[6] and P[8] are frequently detected throughout the world (Bányai et al., 2012; Gentsch et al., 2005; Patel et al., 2011; Santos & Hoshino, 2005). Together with these typical human G- or P-genotypes, a plethora of uncommon genotypes have also been identified in humans, such as G6P[14], G8P[14] and G10P[14] (Bányai et al., 2009, 2010; Matthijnssens et al., 2009a). The detection of uncommon RVA genotypes in humans has been linked to the frequent intersections between the evolution of human and animal RVAs, as a result of numerous interspecies transmission events, sometimes accompanied by reassortment or adaptation to the new host (Degiuseppe et al., 2013; Martella et al., 2010; Weinberg et al., 2012). G6, G8 and G10 are the most common RVA G-genotypes
Table 1. Comparison of the genotype constellation of Italian P[14] RVAs with selected animal RVAs, human bovine-like RVAs and human reference RVAs

The Italian P[14] RVAs in this study are shown in bold type.

<table>
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<td>RVA/Human-tc/USA/Wa/1974/G1[8]</td>
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encountered in cattle (Bányai et al., 2003; Matthijnssens et al., 2008; Papp et al., 2013). The P[14] RVA genotype has been detected sporadically in humans and is thought to be acquired through zoonotic transmission as it has been found in RVA strains from several distinct animal species, such as rabbits, cows, goats, sheep and guanaco. P[14] strains are found most commonly in combination with G3 or the typical bovine-like G-genotypes: G6, G8 and to a lesser extent G10 (Chitambar et al., 2011; Esona et al., 2009; Okada & Matsumoto, 2002; Parreño et al., 2004).

In the area of Parma, northern Italy, continual uninterrupted hospital-based surveillance for RVA infection has been conducted over the last 27 years (Medici et al., 2007, 2014). RVA genotyping data were generated during a 2-year molecular epidemiological survey in 2004–2005 and from 2008 onwards, by sequence characterization of the VP7 and VP4 gene segments. Three unusual RVA strains were found, including one G10P[14] strain, RVA/Human-wt/ITA/PR457/2009/G10P[14] (PR457/2009), and two G8P[14] strains, RVA/Human-wt/ITA/PR1300/2004/G8P[14] (PR1300/2004) and RVA/Human-wt/ITA/PR1973/2009/G8P[14] (PR1973/2009). Preliminary sequence and phylogenetic analyses of the partial VP6, VP4 and VP7 genes of the P[14] RVA strain PR1300/2004 showed that this strain may have been of animal origin (Medici et al., 2008).

In the present study, we analysed the full genome of two G8P[14] and one G10P[14] RVAs detected in the area of Parma during an epidemiological survey in order to obtain conclusive data on the overall genetic makeup and to elucidate the origin of these rare human RVA strains. RVA strain PR1300/2004 (G8P[14]) was detected in the stool of a 5-year-old child in 2004, PR1973/2009 (G8P[14]) in an 8-month-old child in 2009 and PR457/2009 (G10P[14]) in a 1-year-old child in 2009. The children were admitted with acute gastroenteritis to the University Hospital of Parma during the epidemic period (December–April). All 11 gene segments of the Italian RVA strains were submitted to reverse transcription-PCR (RT-PCR), Sanger sequencing and phylogenetic analysis. Briefly, the RVA RNA was subjected to RT-PCR (OneStep RT-PCR kit; Qiagen), using primers for all 11 RNA segments as described previously (Matthijnssens et al., 2008). After visualization in ethidium bromide-stained 6% PAGE gels, PCR products were purified and sequenced. Primer walking was used for full-length sequencing of longer genes (VP1–VP4 and NSP1). Sequences were corrected with
ChromasPro2.23 (Technelysium). Multiple alignments were made for each gene segment with appropriate reference sequences available in GenBank. The sequences included the human reference strains Wa, DS-1 and AU-1, and RVA strains with G/P-genotype combinations equal to those of the analysed Italian strains. The BLAST web-based analysis tool with default parameters was used to find homologous hits in the sequence database (GenBank) and to select closely related strains for phylogenetic analyses.

Multiple sequence alignments and phylogenetic tree constructions were performed with MEGA6 (Tamura et al., 2013), applying the maximum-likelihood method. GenBank accession numbers (VP7, VP4, VP6, VP1–VP3, NSP1-NSP5) for each individual genomic segment of the three Italian P[14] strains are KP198625–KP198635 (PR457/2009), KP198636–KP198646 (PR1300/2004) and KP198647–KP198657 (PR1973/2009), respectively.

**Fig. 1.** Phylogenetic dendrograms based on the nucleotide sequences of all 11 RVA gene segments. •, Italian G8P[14] strains; ▲, completely sequenced G8P[14] strains; ▲, Italian G10P[14] strain; ◊, other completely sequenced G10P[14] strains. Trees were built with the maximum-likelihood method, and bootstrapped with 1000 repetitions. Bootstrap values >70% are indicated. Bar, number of nucleotide substitutions per site.

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In total, 11 phylogenetic trees were built using the nucleotide sequence of the 11 ORFs of PR457/2009, PR1300/2004 and PR1973/2009. The obtained nomenclature and genotype constellations of the three RVA strains are provided in Table 1 and the phylogenetic trees are shown in Fig. 1.

The three Italian P[14] RVAs analysed possessed the typical bovine-like I2-R2-C2-M2-A3/A11-N2-T6-E2-H3 genotype constellation. The G8P[14] RVA strains PR1300/2004 and PR1973/2009 were found to have an identical genotype constellation, G8-P[14]-I2-R2-C2-M2-A3-A11-N2-T6-E2-H3, and, compared with other G8P[14] strains, they were most similar to the human RVA strain RVA/Human-wt/HUN/BP1062/2004/G8P[14] detected in Hungary (Bányai et al., 2010) and the ovine strain RVA/Sheep-tc/ESP/OVR762/2002/G8P[14] detected in Spain (Matthijssens et al., 2009a), sharing the same genotype constellation except for NSP1 (A3 vs A11), and to a guanaco RVA strain (RVA/Guanaco-wt/ARG/Chubut/1999/G8P[14]) (Matthijssens et al., 2009a), sharing the same genotype constellation except for VP1 and NSP4 (Table 1).

The Italian RVA PR457/2009 possessed the G10-P[14]-I2-R2-C2-M2-A11-N2-T6-E2-H3 genotype constellation, which is identical to that found in RVA/Human-wt/AUS/V585/2011/G10P[14] (Cowley et al., 2013). The only other completely sequenced G10P[14] strain (RVA/Human-tc/GBC/A64/1987/G10P10[14]) (Heiman et al., 2008) showed different genotypes for VP3 (M2 vs M1), NSP1 (A11 vs A3) and NSP3 (T6 vs T1) compared with strain PR457/2009.

In the phylogenetic tree of VP7, the two Italian G8P[14] RVAs (PR1300/2004 and PR1973/2009) segregated in the same subcluster of the G8 genotype, with a 2% nucleotide
difference between them. Both strains had a very close relationship with artiodactyl and artiodactyl-like human strains (similarity up to 98 % on the nucleotide level), segregating closely with two porcine/bovine reassortant RVA strains detected in Korea [RVA/Porcine-wt/KOR/07-109-8/2007/G8P[7] and RVA/Porcine-wt/KOR/174-1/2006/G8P[7] (Kim et al., 2010) and two guanaco RVA strains detected in Argentina [RVA/Guanaco-wt/ARG/Chubut/1999/G8P[14] and RVA/Guanaco-wt/ARG/RioNegro/1998/G8P[1] (Matthijnsens et al., 2009a), and slightly more distantly related to a human strain isolated in Kenya (RVA/Human-wt/KEN/B12/1997/G8P[14]), which also has an artiodactyl origin (Ghosh et al., 2011). The analyses of the VP1–VP4, VP6 and NSP2–NSP5 gene segments showed that both G8 strains clustered with bovine and bovine-like G6P[14] and G8P[14] RVAs, but in contrast with the phylogenetic tree of VP7, PR1300/2004 and PR1973/2009 were only distantly related to each other for the other gene segments, with the exception of NSP5, suggesting that both strains are the result of two independent interspecies transmission events.

In the phylogenetic tree of VP7, the Italian G10P[14] RVA strain (PR457/2009) was most similar to equine, porcine and bovine RVA strains (96.4–99 %), while the most closely related human RVA strain was an old unusual bovine-like human strain (A64), showing only 87.3 % similarity at the nucleotide level. For the VP4, VP6 and NSP1–NSP5 gene segments, PR457/2009 was phylogenetically closely related...
with bovine-like strains, showing nucleotide identities ranging from 95.6 to 99.8%. The VP2 segment was most closely related with two goat RVA strains, RVA/Goat-tc/ BGD/G034/1999/G6P[1] and RVA/Goat-xx/CHN/XL/ 2010/G10P[15], and two human bovine reassortant RVA strains, RVA/Cow-wt/GHA/GH018-08/2008/G8P[6] and RVA/ Cow-wt/GHA/GH019-08/2008/G8P[6], recently detected in Ghana (Dennis et al., 2014). For VP1 and VP3, PR457/2009 showed a close clustering with RVA/Human-wt/ITA/PAI11/ 1996/G2P[4] and RVA/Human-tc/SWE/1076/1983/G2P[6], respectively, which were reported to have a bovine-like VP1 and VP3 in a human RVA genetic backbone (Ghosh et al., 2013; Giammanco et al., 2014). In addition, the PR457/2009 VP3 segment clustered closely to another bovine-like strain, RVA/Human-wt/HUN/BP1062/2004/G8P[14], identified during a surveillance study in Hungary (Bányai et al., 2010).

The three Italian P[14] RVA strains analysed shared a typical bovine-like genotype constellation. They clustered with animal RVA strains or human RVA strains believed to be of (partial) artiodactyl origin. Our data strongly suggest that these three P[14] strains are the result of independent zoonotic transmissions from an animal belonging to the order Artiodactyla to a human, as has been described on multiple occasions in the past (Bányai et al., 2009; Cowley et al., 2013; Donato et al., 2014; El Sherif et al., 2011; Ghosh et al., 2007; Matthijnssens et al., 2009a; Mullick et al., 2013).

To the best of our knowledge, this paper describes the first complete genome analyses of unusual human G8P[14] and G10P[14] RVA strains in Italy, further complementing our previous partial genome analyses of the G8P[14] strain (Medici et al., 2008). To date, in Italy P[14] RVAs have been reported sporadically, for example in Sicily during 1987–1988, and in 2003 (De Grazia et al., 2011, 2014) in conjunction with the G6 genotype (bovine-like). G8 and G10 strains were found exclusively during 2008–2009 in different Italian territories, associated with P[4] or P[8], without showing evidence of zoonotic reassortment events (De Grazia et al., 2014; Ianiero et al., 2014; Ruggeri et al., 2011). Furthermore, G6, G8 and G10 RVAs are common in bovines and buffaloes in southern Italy (Pisanelli et al., 2005).

Full-genome sequencing of G6 strains has revealed a close genetic relatedness between human G6P[14] and animal G6P[14] and G8P[14] viruses, and reinforces the idea that animal RVAs impact on the evolution of human RVAs (Bányai et al., 2010; De Grazia et al., 2011; Matthijnssens et al., 2009b). The accumulation of whole genome sequencing data will provide new insights into the mechanisms driving the evolution of RVAs.

It is currently unclear why interspecies transmitted strains are unable to further spread in the human population. Some recent findings indicated that the VP8* region of the P[14] VP4 protein interacts with the type A histoblood-group antigens of humans (Hu et al., 2012; Liu et al., 2012) as well as bovine and porcine mucins (Liu et al., 2012), and this is thought to play a role in cross-species transmission of P[14] RVAs. This study re-emphasizes that humans are susceptible to infection with bovine-like RVA strains, but the further efficient spread of these viruses in the human population is likely prevented by other unknown factors. Continued surveillance of RVA strains will be essential to determine the extent of similar strains in the population in the context of whether or not vaccine-induced heterotypic immunity is sufficient to protect against strains that express the P[14] RVA genotype.

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References


