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Evolution of human G4P[8] group A rotavirus strains circulating in Italy in 2013

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ABSTRACT

Group A rotaviruses (RVA) are the leading cause of acute gastroenteritis in young (<5 years of age) children, causing up to 450.000 deaths worldwide, mostly in developing countries. VP7 (G-type) and VP4 (P-type) genotypes are the basis for the binary RVA classification. Although at least 27 G-types and 37 P-types of rotavirus are presently known, most RVA infections in humans worldwide are associated with the five major G/P combinations G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8].

During RVA gastroenteritis surveillance in Italy, a total of 1112 samples collected from children hospitalized with acute gastroenteritis in 2013 were RVA positive and were genotyped following standardized protocols from the EuroRotaNet. Most strains analyzed belonged to the five major human genotypes. Among these common strains, 22 G4P[8] RVA strains from different Italian regions were subjected to nucleotide sequencing of their VP4, VP6, VP7 and NSP4 genes to investigate their evolution.

The phylogenetic analysis showed that the Italian strains belonged to lineage G4-I for VP7 and to lineage P[8]-III for VP4, in line with the modern G4P[8] RVA strains detected in children worldwide. The phylogenetic trees revealed high degrees of nucleotide identity between the RVA strains involved in this study and G4P[8] strains detected previously in Europe, Asia and Africa, but also demonstrated at least three separate evolution clusters within the same lineage.

Based on the amino acid sequences deduced for their hypervariable regions, both the VP7 and VP8* proteins of the Italian G4P[8] RVA strains presented amino acid substitutions near known neutralizing epitopes.

1. Introduction

Group A rotaviruses (RVA) are the leading cause of severe acute gastroenteritis (AGE) in young children and animals worldwide, and are estimated to cause between 196.000 and 453.000 deaths per year among children, mostly in developing countries (Tate et al., 2012; Walker et al., 2013). Rotaviruses form a genus of the *Reoviridae* family, and their genome is composed of 11 segments of double-stranded RNA, encoding six structural proteins (VP) and five or six non-structural proteins (NSP) (Estes and Cohen, 1989). The accumulation of point mutations generated by the error-prone viral RNA-dependent RNA-polymerase is one mechanism to generate the genetic diversity among human rotaviruses. In addition, the nature of their genome enables RVAs strains to reassort during co-infection of different strains and to generate diverse progeny via exchange of one or more genome segments, which can involve either strains of similar or different genotypes (Desselberger, 1996; Hanada et al., 2004; Ianiro et al., 2013; Parra et al., 2004).

The gene sequences of the two outer capsid proteins VP7 and VP4 are used to differentiate rotaviruses on a binary classification base by G and P genotypes, respectively (Estes and Greenberg, 2013). Currently, 27 G-genotypes and 37 P-genotypes have been described (Matthijnssens et al., 2011; Trojnar et al., 2013), and, despite the high number of possible G/P genotype combinations, approximately 75% of RVA infections worldwide are caused by the five common human genotypes G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8] (Armah et al., 2010; Gentsch et al., 2005; Iturriza-Gomara et al., 2011). The rest of infections are caused by strains that present uncommon or rare genotypes or are untypable in either

Abbreviations: RVA, Group A rotaviruses; AGE, acute gastroenteritis; VP, viral protein; NSP, non-structural protein; dsRNA, double-stranded RNA; RT-PCR, reverse transcription-PCR.

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Table 1
Distribution of rotaviruses by G and P genotype detected by the RotaNet-Italy surveillance system, Italy, 2012–2013.

Strain Genotypes	No of cases	%
G1P[8]	548	49.1
G2P[4]	80	7.2
G3P[8]	100	9.0
G4P[8]	88	8.0
G9P[8]	139	12.4
Uncommon	60	5.6
Mixed infections	83	7.5
Untypable	14	1.3
Total	1112	100.0

G or P genes, or are associated with multiple RVA strains. In African countries RVA circulating in the human population often exhibit genotypes markedly different from those observed in the rest of the world (Armah et al., 2010; Todd et al., 2010).

In 2006, two rotavirus vaccines were licensed for human use. Specifically, the monovalent vaccine Rotarix® is made of an attenuated human G1P[8] viral strain, whereas the bovine-derived pentavalent reassortant vaccine Rotateq® contains G1, G2, G3, G4 and P[8] antigens. Both vaccines have proven highly efficacious in decreasing both severe and milder cases in an increasing number of countries worldwide (Anderson et al., 2011; Giaquinto et al., 2011; Linhares et al., 2008; Patel and Parashar, 2009; Payne et al., 2013; Ruiz-Palacios et al., 2006; Vesikari et al., 2006). The vaccines are based on the most common RVA serotypes circulating worldwide and also induce significant cross-protection against serotypes not included in the vaccines themselves.

As in other countries, a nationwide molecular epidemiological surveillance of RVA from hospitalized gastroenteritis cases is being conducted also in Italy since 2007 (RotaNet-Italy), monitoring the RVA genotypes circulating among children <5 year-old admitted with severe rotavirus diarrhea (Iturriza-Gomara et al., 2011; Ruggeri et al., 2011). During the 2013 RVA surveillance season, a total of 1112 RVA positive samples were analyzed; the predominant genotype was G1P[8] (49.1%), followed by G9P[8], G3P[8], G4P[8] and G2P[4], which ranged from 12.4 to 7.2% of cases (Table 1). Uncommon genotypes were found in 60 samples, and 14 samples could not be genotyped for either VP7 or VP4 genes.

In this study, we performed a molecular analysis of 22 of 88 RVA strains classified as "common" G4P[8] by RotaNet-Italy in 2013, to investigate their origin and evolution. G4 RVA strains have been previously studied in several countries (Bucardo et al., 2007; Feeney et al., 2006; Khetawat et al., 2001; Trinh et al., 2010), but only in limited areas of Italy (Arista et al., 2005; Medici et al., 2014), and were found mostly in combination with either P[6] or P[8].

Nucleotide sequencing and phylogenetic analyses of VP7, VP4 (VP8*), VP6 and NSP4 genes were carried out for the 22 strains selected. More detailed molecular analyses of the hypervariable antigenic amino acid regions of both capsid proteins were performed.

2. Materials and methods

2.1. Sample collection and preparation

Stool specimens were collected in 2013 from children admitted with acute gastroenteritis to public hospitals throughout Italy, in the framework of the molecular surveillance system of RotaNet-Italy (Ruggeri et al., 2011).

RNA was extracted from 140 μl of 10% fecal suspensions in H_2O using the Viral RNeasy Mini Kit (Qiagen, Milano, Italy), according to the manufacturer's instructions. Final elution was performed in 60 μl of RNase-free water, and RNA was stored at −80 °C until use.

2.2. Genotyping and sequencing of RVA strains

RVA genotyping was performed by reverse transcription nested polymerase chain reaction (RT-nPCR), as previously described (Gentsch et al., 1992; Iturriza-Gomara et al., 2004). RT-nPCR reactions were performed following the protocols of EuroRotaNet (http://www.eurorota.net/docs.php). PCR products were electrophoresed on 2% agarose gel and visualized by staining with ethidium bromide.

Nucleotide sequencing of the VP7 (nt 162-697), VP4 (VP8*) (nt 229-787), VP6 (nt 231-880) and NSP4 (nt 147-566) genes amplified was performed at the Macrogen Inc. (Seoul, South Korea), with the same primers used for the RT-PCR, using the BigDye chemistry.

2.3. Phylogenetic analysis of RVA genes

Sequences generated were analyzed and corrected with ChromasPro2.23 (Technelysium, Queensland, Australia). Nucleotide and amino acid sequence similarity searches were performed using the BLAST (Basic Local Alignment Search Tool) server on the GenBank database of the NCBI (National Center for Biotechnology Information, National Institute of Health, Bethesda, MD). Multiple sequence alignments and phylogenetic tree construction were performed with MEGA6 (www.megasoftwares.com), applying the Maximum-Likelihood (ML) method. Among the strains compared in each tree,

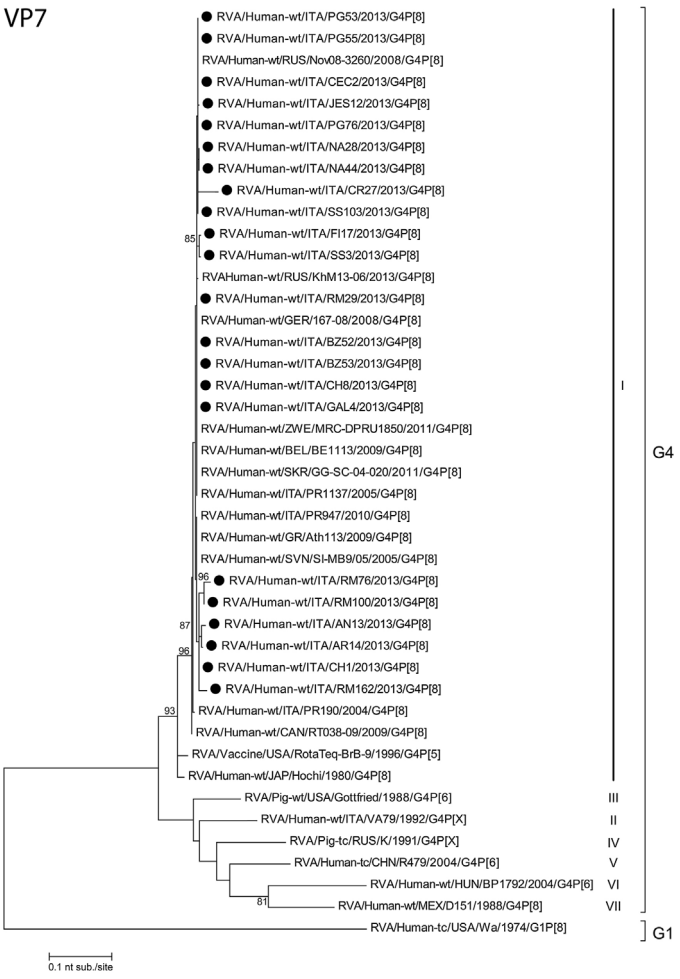

Fig. 1. Phylogenetic tree based on the partial ORF of VP7. Italian G4P[8] RVA strains are marked with a filled circle. Trees were built with the maximum likelihood method (Tamura-3 parameter), and bootstrapped with 1000 repetitions; bootstrap values below 70 are not shown.