Infection, Genetics and Evolution 44 (2016) 303–312

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

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

Molecular characterization of two rare human G8P[14] rotavirus strains, detected in Italy in 2012

Roberto Delogu [a], Giovanni Ianiro [b], Anna Morea [c], Maria Chironna [c], Lucia Fiore [a], Franco M. Ruggeri [b,*]

[a] National Center for Immunobiologicals Research and Evaluation, Istituto Superiore di Sanità, Rome, Italy
[b] Department of Veterinary Public Health and Food Safety, Istituto Superiore di Sanità, Rome, Italy
[c] Department of Biomedical Sciences and Human Oncology, University of Bari "Aldo Moro", Bari, Italy

ARTICLE INFO

Article history:
Received 8 May 2016
Received in revised form 30 June 2016
Accepted 18 July 2016
Available online 19 July 2016

Keywords:
Rotavirus
Gastroenteritis
G8P[14]
Zoonotic transmission
Italy

ABSTRACT

Since 2007, the Italian Rotavirus Surveillance Program (RotaNet-Italy) has monitored the diversity and distribution of genotypes identified in children hospitalized with rotavirus acute gastroenteritis.
We report the genomic characterization of two rare human G8P[14] rotavirus strains, identified in two children hospitalized with acute gastroenteritis in the southern Italian region of Apulia during rotavirus strain surveillance in 2012.
Both strains showed a G8-P[14]-I2-R2-C2-M2-A11-N2-T6-E2-H3 genomic constellation (DS-1-like genomic background). Phylogenetic analysis of each genome segment revealed a mixed configuration of genes of animal and zoonotic human origin, indicating that genetic reassortment events generated these unusual human strains. Eight out of 11 genes (VP1, VP2, VP3, VP6, VP7, NSP3, NSP4 and NSP5) of the Italian G8P[14] strains exhibited close identity with a Spanish sheep strain, whereas the remaining genes (VP4, NSP1 and NSP2) were more closely related to human strains. The amino acid sequences of the antigenic regions of outer capsid proteins VP4 and VP7 were compared with vaccine and field strains, showing high conservation between the amino acid sequences of Apulia G8P[14] strains and human and animal strains bearing G8 and/or P[14] proteins, and revealing many substitutions with respect to the RotaTeq™ and Rotarix™ vaccine strains. Conversely, the amino acid analysis of the four antigenic sites of VP6 revealed a high degree of conservation between the two Apulia strains and the human and animal strains analyzed.
These results reinforce the potential role of interspecies transmission and reassortment in generating novel rotavirus strains that might not be fully contrasted by current vaccines.

1. Introduction

Group A Rotaviruses (RVA) are the major cause of acute gastroenteritis (AGE) in young children and many animal species worldwide, and are estimated to cause 453.000 deaths every year among children aged 0–5 years, mostly in developing countries (Parashar et al., 2009; Tate et al., 2012).

In 2009 the World Health Organization recommended the introduction of rotavirus vaccines in all National Vaccine Plans globally to reduce the high mortality and morbidity caused by RVA. Currently, two oral live attenuated vaccines, Rotarix™ (GlaxoSmithKline) and RotaTeq™ (Merck), have been licensed and included into the National Vaccine Plan in many countries (Linhares et al., 2008; Madhi et al., 2010; Ruiz-Palacios et al., 2006; Vesikari et al., 2006). Recently, a locally manufactured low cost vaccine (Rotavac) has been licensed and applied in India, showing high efficacy against rotavirus disease (Bhandari et al., 2014).

Rotavirus, genus *Rotavirus* in the *Reoviridae* family, is a non-enveloped virus with a genome composed of 11 segments of double stranded RNA (dsRNA), which encode six structural viral proteins (VP1-4, VP6, VP7) and six non-structural proteins (NSP1-5/6) (Estes and Kapikian, 2007). The outer layer of the virion is made of VP7, through which 60 VP4 spikes protrude.

Based on genetic characteristics of the inner capsid protein (VP6), rotavirus strains can be classified into 9 groups (A–I), causing gastroenteritis in humans (A, B, and C) and animals (A–I) (Estes and Kapikian, 2007; Matthijnssens and Van Ranst, 2012).

The traditional binomial RVA classification system is based on the two outer capsid proteins VP7 and VP4, and it is the most widely used scheme in molecular epidemiology and surveillance programs (Estes and Kapikian, 2007; Iturriza-Gomara et al., 2011).

Although at least 27 G-types and 37 P-types have been identified (Matthijnssens et al., 2011a; Trojnar et al., 2013), the five most common genotypes (G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8]) represent over

* Corresponding author at: Dept. Veterinary Public Health & Food Safety, Istituto Superiore di Sanità, Viale Regina Elena, 299, 00161 Rome, Italy.
E-mail address: francomariaruggeri@gmail.com (F.M. Ruggeri).

http://dx.doi.org/10.1016/j.meegid.2016.07.018
1567-1348/

90% of the strains analyzed worldwide (Santos and Hoshino, 2005). Emerging G12 rotavirus strains have also increased globally in the last years, becoming an important genotype (Delogu et al., 2015; Freeman et al., 2009; Matthijnssens et al., 2010; Rahman et al., 2007).

Given the segmented structure of rotavirus genome, reassortment events between human and animal strains can occur for each dsRNA segment, representing one of the major processes of genetic evolution of rotaviruses. Therefore, a new classification system has been proposed to better understand the evolution of reassortants and viral inter-species transmission (Matthijnssens et al., 2011a). Following this new classification system, the notations of Gx-P[x]-Ix-Rx-Cx-Mx-Ax-Nx-Tx-Ex-Hx are used for the VP7, VP4, VP6, VP1, VP2, VP3, NSP1, NSP2, NSP3, NSP4, and NSP5/6 encoding genes, respectively.

In addition to the five most common genotypes, unconventional and new emerging rotavirus strains have also been described in many countries, most of which share the background genotype configuration with common strains. Examples are G6, G8, and G12 with P[4], P[6], P[8], P[9] and P[14] strains with either Wa-like or DS1-like genetic background (Delogu et al., 2015; Delogu et al., 2013; Esona et al., 2009; Ianiro et al., 2014; Ianiro et al., 2013; Matthijnssens et al., 2008b; Matthijnssens et al., 2006; Matthijnssens et al., 2009) or unusual G and P combinations derived from common genotypes (Collins et al., 2015; Lennon et al., 2008; O'Halloran et al., 2000; Ruggeri et al., 2011).

Since 2007, the Italian Rotavirus Surveillance Program (RotaNet-Italy), which includes the Istituto Superiore di Sanità and several public hospitals and universities in different Italian regions, has monitored the diversity and distribution of genotypes identified in children presenting to hospitals with severe acute gastroenteritis (Ruggeri et al., 2011). The Italian network is part of the European surveillance network EuroRotaNet (Iturriza-Gomara et al., 2011), the members of which share common analytical protocols and gather genotyping results in a common European database.

During the Italian Rotavirus Surveillance Program in 2011–12, two rare G8P[14] rotavirus strains (BA01/2012 and BA02/2012) were identified in two children hospitalized with acute gastroenteritis and mild dehydration in the Southern Italian region of Apulia. Human G8 rotaviruses in combination with P[1], P[2], P[4], P[6], P[8] types have been reported to infect humans in many countries (Delogu et al., 2013; Esona et al., 2010; Gentsch et al., 2005; Pietsch et al., 2009; Santos and Hoshino, 2005). P[14] strains are most commonly found in combination with G3 or the typical bovine-like G-genotypes as G6, G8 (Chitambar et al., 2011; De Grazia et al., 2011; Medici et al., 2008; Okada and Matsumoto, 2002; Parreno et al., 2004).

To better understand the origin and evolution of strains BA01/2012 and BA02/2012, a full genomic characterization was performed and compared with rotavirus sequences present in GenBank. The amino acid sequence comparison of VP8*, VP7 and VP6 antigenic regions between G8P[14] Apulia and other global G8 or P[14] strains and vaccine strains was also performed.

2. Materials and methods

2.1. Patients and rotavirus testing

The stool samples investigated were collected within 12 h of admission from two female children less than 2 years old, presenting with acute gastroenteritis to the Infectious Diseases Unit of the Giovanni XXIII regional pediatric hospital in Bari (located in the southern Italian region of Apulia), which is involved in the RotaNet-Italia surveillance program. Patients (BA01/2012 and BA02/2012) were admitted to the hospital on two successive days in June 2012 due to feeding difficulties and fever.

Rotavirus infection was diagnosed at the Hygiene Unit of Policlinico Hospital in Bari using commercial enzyme-linked immunoassay kits for antigen detection (Monostep Adeno + Rota - Biolife Italiana Srl, Italy), confirmed by real time PCR (FTD viral gastroenteritis, Arrow Diagnostics, Genoa, Italy), and full molecular characterization was performed at the Istituto Superiore di Sanità, Rome. The children had not been vaccinated against rotavirus and had no history of significant underlying medical conditions. The children's families lived in two distinct rural areas near the city (Bari), both with possibility of contact with swine, bovines and ovines. No other family members reported symptoms of gastroenteritis.

Written informed consent and clinical information were obtained from a parent before any study procedure was undertaken.

2.2. Nucleic acid extraction

Total viral RNA was extracted from 140 μl of suspension (10%) by using QIAamp Viral RNA Mini Kit (Qiagen, Milan, Italy) according to the manufacturer's instructions. RNA was eluted in 50 μl of RNase-free water, and stored at −80 °C.

2.3. Reverse transcription – polymerase chain reaction (RT-PCR)

After viral RNA extraction, samples were initially genotyped for VP7 (G-type) and VP4 (P-type) genes by reverse transcription and multiplex-PCR, using type-specific primers, in accordance with the standardized EuroRotaNet methods (Gentsch et al., 1992; Iturriza-Gomara et al., 2004) (http://www.eurorota.net/docs.php).

In addition to VP7 and VP4 genes, the other 9 genomic segments were reverse-transcribed and amplified by specific consensus primers as published previously (Esona et al., 2009; Gentsch et al., 1992; Ianiro et al., 2015; Iturriza-Gomara et al., 2011; Matthijnssens et al., 2006; Matthijnssens et al., 2008a). Briefly, extracted RNAs were denatured at 97 °C for 5 min, and RT-PCR was carried out using a One-Step RT-PCR kit (Promega, Madison, WI) according to the manufacturer's instructions.

RT-PCR was carried out with an initial reverse transcription step at 45 °C for 45 min, followed by a PCR activation step at 95 °C for 15 min, and 30 cycles of PCR in a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, Inc., Foster City, CA) using the following conditions: 45 s at 94 °C, 45 s at 50 °C, 3 min at 72 °C (6 min for larger segments), followed by a final extension step for 7 min at 72 °C.

For genotype assignment, the size of the PCR products, separated on 2% agarose gel stained with ethidium bromide, was determined using a Molecular Imager Gel Doc XR with the Quantity-One software (BioRad, Segrate, Italy).

2.4. Sequence and phylogenetic analysis

Nucleotide sequencing of gene segments was performed at Macrogen Inc. (Seoul, South Korea). The sequencing reaction was based on BigDye chain termination chemistry, using the same primers used for RT-PCR as sequencing primers (Esona et al., 2009; Ianiro et al., 2015; Matthijnssens et al., 2006; Matthijnssens et al., 2008a). Sequencing data were edited and aligned with the corresponding reference strains using ChromasPro 2.23 software packages (Technelysium, Queensland, Australia) and compared against the NCBI GenBank database (http://www.ncbi.nlm.nih.gov).

Phylogenetic analyses were performed using the MEGA5 software (www.megasoftwares.com) (Tamura et al., 2011). Maximum likelihood phylogenetic trees were built using T92 + G (VP7, VP1, VP3, VP6, NSP2), HKY + G (VP2, VP4, NSP4) and TN93 + I (NSP1, NSP3, NSP5), as a nucleotide substitution model, as suggested by MEGA5 ModelTest, with the statistical support of 1000 bootstrap repetitions. The best-fit nucleotide substitution models selected were based on the lowest Bayesian Information Criterion (BIC) scores.

The genotypes of each of the 11 genome segments were determined using the RotaC online classification tool (v2.0, http://rotac.regatools.be) (Maes et al. 2009).