Putative Canine Origin of Rotavirus Strain Detected in a Child with Diarrhea, Taiwan

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Abstract

Rotavirus G3P[3] strains have been reported from a variety of species including humans, cats, dogs, monkeys, goats, and cows. Here, we report the characterization of the first human G3P[3] rotavirus from East Asia identified in a 2-year-old child who was treated in a hospital's emergency ward in Taiwan in February 2005. Sequence and phylogenetic analysis demonstrated a close genetic relationship between the VP4, VP6, VP7, and NSP4 genes of Taiwanese G3P[3] strain 04-94s51 and an Italian canine strain isolated a decade ago, suggesting a canine origin for the Taiwanese strain. In contrast, the Taiwanese strain was only moderately related to well-characterized canine-origin human G3P[3] strains Ro1845 and HCR3, suggesting a distinct origin for the rotavirus strain from Taiwan.

Key Words: Gastroenteritis—*Rotavirus A*—Zoonosis.

Introduction

GROUP A ROTAVIRUSES ARE a major cause of acute gastroenteritis in humans and animals. Rotavirus is characterized by strong host species restriction; however, it has been demonstrated that direct interspecies transmission or reassortment between heterologous strains are key mechanisms in generating rotavirus strain diversity in new hosts (Matthijnssens et al. 2009). An 11 gene-based classification scheme has recently been proposed to help understand the origin of rotavirus strains (Matthijnssens et al. 2008). Numerous studies have shown that sequence and phylogenetic analysis of the genes encoding the two outer capsid proteins VP7 and VP4, the inner capsid protein VP6, and the enterotoxigenic polypeptide NSP4 is a suitable approach to obtain epidemiologic information and trace the origin of various rotavirus strains (Iturriza-Gómara et al. 2003, Araújo et al. 2007, Martella et al. 2008, Bányai et al. 2009a, Benati et al.

2010). To date, at least 23 VP7 (G) genotypes, 32 VP4 (P) genotypes, 13 VP6 (I) genotypes, and 10 NSP4 (E) genotypes have been described. In humans, five strains are predominant worldwide: G1, G3, G4, and G9 VP7 types in association with P[8] VP4 type, I1 VP6 and E1 NSP4 type and G2 rotavirus often associated with P[4] type, I2 VP6 specificity, and E2 NSP4 type (Matthijnssens et al. 2008). Nonetheless, human rotaviruses are surprisingly diverse, and many of the human genotype specificities are shared with animal rotaviruses. For example, G8P[1] strains that are common in cattle, or G4P[6] and G5P[6] that frequently are detected in swine, have been sporadically identified in children suffering from diarrhea (Martella et al. 2006, Araújo et al. 2007, Bányai et al. 2009a, 2009b). Other studies indicate that dogs may also serve as reservoirs for generation of rotavirus diversity in man. The few rotavirus strains of canine origin identified and characterized in detail to date share common VP7, VP4, VP6, and NSP4 genotype

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configurations, G3-P[3]-I3-E3 (De Grazia et al. 2007, Matthijssens et al. 2008, Tsugawa and Hoshino 2008).

The Case

Rotavirus infection was diagnosed by commercial enzyme immunoassay from a bloody-mucoid stool sample of a 2-year-old boy treated for acute diarrhea overnight on 19–20 February 2005 at the emergency ward of the municipal hospital, in Zhanghua, Taiwan. Initial characterization of the VP7 and VP4 genes by nucleotide sequencing (Wu et al. 2009) demonstrated that strain 04-94s51 belonged to G3P[3] genotype, which is most closely related to canine rotaviruses. To further

characterize this strain, we determined the sequence of the VP6 and NSP4 genes using primers and methods described by Wu et al. (2011). Phylogenetic analyses were done by the maximum likelihood algorithm as implemented in MEGA5 (Tamura et al. 2011) using an appropriate substitution model obtained for each gene by the AIC prediction method. Bootstrap analysis was performed using 500 pseudoreplicates.

In brief, the full-length open-reading frame (ORF) of the VP7 gene for strain 04-94s51 was determined and an 825 nt-long fragment was used to compare this strain to others by phylogenetic analysis. Strain 04-94s51 shared the highest nt similarity with canine RV52/96 and GC/KS05 G3 strains (98.5% and 97.6% nt sequence identity) and with human

FIG. 1. Maximum-likelihood trees of rotavirus VP7, VP4, VP6, and NSP4 genes. Arrows indicate the G3P[3] strain, 04-94s51, identified in Taiwan. Bootstrap values > 60 are indicated.

PA260/97 strain (98.4% nt sequence identity) but demonstrated moderate-to-low nt sequence identity with other G3 strains (91.2% or less). In the VP7 phylogeny, the 4 closely related strains comprised a single monophyletic group that was distantly related to other canine and canine-derived strains (e.g., feline Cat97 and human Ro1845 and HCR3A strains) (Fig. 1). In the VP4 gene sequence analysis, the variable region (822 nt in length) VP8* was compared to other related P[3] strains (Fig. 1). The Taiwanese 04-94s51 strain showed the highest nt similarity to the canine RV198/95 strain (97.2%) and was closely related to other canine and canine-derived zoonotic human strains (range, 93.3–95.4%), but lower degrees of similarity were observed to ruminant and simian P[3] rotavirus strains (range, 78.7–79.7%). The full ORF was determined for the VP6 gene of strain 04-94s51 and a 1225 nt-long fragment was used to determine relatedness to other strains (Fig. 1). The highest nt similarity was seen between 04-94s51 and canine strains RV52/96 (94.7%) and RV198/95 (94%), but the strain was only distantly related to other genotype I3 strains, such as the feline rotavirus-like human strain, AU-1 (85.7%), or the canine rotavirus-like human strains Ro1845 (85.5%) and HCR3A (85.1%). Phylogenetic analysis demonstrated that Taiwanese 04-94s51 strain comprises a single common cluster with the human strain T152, and the canine strains RV52/96 and RV198/95, representing a unique lineage within the I3 genotype of the VP6 gene that is divergent from other known feline, canine, and zoonotic human strains. The NSP4 gene of 04-94s51 shared the highest nt similarity along a 683 nt-long fragment to the canine RV52/96 strain (99.1% nt identity) and the feline FRV348 strain (98.7% nt identity) and was more closely related to some other genotype E3 feline strains (up to 91.5%) than to most of the known canine rotaviruses (86.8% or less). Phylogenetic analysis confirmed the distance-based estimation of relatedness among strains, demonstrating that the NSP4 gene of 04-94s51 forms a single common cluster with RV52/96 and FRV348 (Fig. 1).

Discussion

Interspecies transmission of animal rotaviruses to humans is a major source of strain diversity. To date, at least 12 G types and 15 P types have been described in human rotavirus infections; of these, 11 G and 13 P genotype specificities are shared between animal and human rotaviruses (Matthijnsens et al. 2009). Nonetheless, the marked sequence diversity between human and animal rotaviruses indicates that zoonotic transmission of rotaviruses might be in general an uncommon event. This hypothesis is supported by a rarity of reported human infections with rotaviruses closely related to those found in domesticated cats and dogs, despite the frequent close physical contacts between humans and these companion animals.

Our study demonstrated unexpectedly high sequence conservation between a Taiwanese human G3P[3] strain and an Italian canine strain, RV52/96, isolated 10 years ago. This finding provides strong evidence that the ancestor of 04-94s51 could have been a domesticated carnivore, most likely a canine, rotavirus. However, the Taiwanese strain characterized here showed only moderate genetic relatedness to several other known canine-derived human rotaviruses (e.g., HCR3A and Ro1845), suggesting a different origin and independent transmission of these various strains. Detection and charac-

terization of more transmissible suspected animal-derived rotavirus strains in humans demonstrated that such strains are usually reassortants, which often carry a single outer capsid gene or a few genes from an animal rotavirus in their genome (Matthijnsens et al. 2009). Although molecular analysis of 4 key genomic segments of 04-94s51 helped to identify the probable host species origin for the parental strain, we were not able to predict whether reassortment between this putative canine ancestral strain and potential heterologous strains could have occurred.

In summary, we described a canine-related human rotavirus strain that was identified in a child treated for acute gastroenteritis at a hospital's emergency ward in Taiwan. While genetically related human rotavirus strains have already been detected in the United States, Italy, and Israel (De Grazia et al. 2007, Tsugawa and Hoshino 2008), this is the first report of the occurrence of a canine-derived zoonotic human G3P[3] strain in East Asia. The incidence of rotavirus infections in cats and dogs in this area needs to be determined to better assess the risk of infection with these heterologous rotavirus strains in humans.

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Disclaimer

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Disclosure Statement

No competing financial interests exist.

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