3. Nakamura S, Yang CS, Sakon N *et al.* Direct metagenomic detection of viral pathogens in nasal and fecal specimens using an unbiased high-throughput sequencing approach. *PLoS ONE*. 2009; 4: e4219.
4. Pruitt KD, Tatusova T, Maglott DR. NCBI reference sequences (RefSeq): acurated non-redundant sequence database of genomes, transcripts and proteins. *Nucleic Acids Res* 2007; 35(Database issue): D61–D65.
5. Heng L, Jue R, Richard D. Mapping short DNA sequencing reads and calling variants using mapping quality scores. *Genome Res* 2008; 18: 1851–1858.
6. Yang J, Yang F, Huang F, Wang J, Jin Q. Subclinical infection with the novel Influenza A (H1N1) virus. *Clin Infect Dis* 2009; 49: 1622–1623.
7. Ren L, Gonzalez R, Wang Z *et al.* Prevalence of human respiratory viruses in adults with acute respiratory tract infections in Beijing, 2005–2007. *Clin Microbiol Infect* 2009; 15: 1146–1153.

First detection of group C rotavirus in children with acute gastroenteritis in South Korea

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Abstract

Group C rotavirus (GpC RV) causes sporadic cases and outbreaks of acute diarrhoea in humans worldwide, but has not been detected among children in South Korea. The present study aimed to detect GpC RV among children hospitalized with gastroenteritis in South Korea and to perform a molecular characterization of GpC RV strains. From November 2003 to January 2006, 434 faecal samples were collected from children <10 years of age who were hospitalized for treatment of acute diarrhoea and screened for group C and A rotaviruses by enzyme immunoassay. GpC RV strains were characterized by sequence and phylogenetic analysis.Of the 434 samples screened, two were positive for GpC RV and one had a mixed GpC and GpA RV infection. One of the strains, Icheon, shared high sequence conservation in VP4, VP6 and VP7 genes with other published GpC RV. This is the first report describing the molecular characteristics of GpC RV among children in South Korea. Additional surveillance is needed to determine the burden of GpC RV gastroenteritis.

Keywords: Diarrhoea, group C rotavirus, molecular epidemiology, phylogenetic analysis

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Rotaviruses are the most important cause of acute gastroenteritis in humans and a variety of animals [1]. Rotaviruses possess a segmented, double-stranded RNA genome and are classified into seven groups (A–G) based on their distinctness with respect to genes and capsid proteins. Group A, B and C rotaviruses cause disease in both humans and animals [1]. Group C rotavirus (GpC RV), first detected in piglets [2] and an infant [3], has been associated with sporadic cases and outbreaks of gastroenteritis among children and adults in many countries [4–9]. GpC RV is generally considered to cause a relatively milder disease, with fewer episodes of vomiting per day and less dehydration compared to group A rotavirus (GpA RV) infection in children [4,7].

All human GpC RV strains detected to date throughout the world have shown high sequence conservation and belong to single G and P genotypes [10,11]. By contrast, human GpC strains are divergent from those in cattle and swine [11–14]. In the present study, for the first time, we detected GpC RV in faecal samples from children with acute diarrhoea in South Korea and determined the genetic relatedness of the Korean isolate to other published strains.

A total of 434 stool samples was collected from patients <10 years of age, who were hospitalized for treatment of gastroenteritis at Sungmo Hospital, Icheon, South Korea, from November 2003 to January 2006. All 434 samples were screened for GpA RV by using a Rotatek™ kit (South Korea Green Cross Corp., Seoul, South Korea) [15] and, for GpC RV, by using an in-house immunoassay developed with hyperimmune sera against porcine GpC RV Cowden strain and human GpC RV VLPs [4,16]. Briefly, 96-well plates were coated with porcine anti-GpC serum (U340; dilution 1:2000) or normal porcine serum (Z1329; dilution 1:2000) in coating buffer (35 mM $NaHCO_3$, 15 mM Na_2CO_3, pH 9.6) and incubated overnight at 4°C. Plates were washed with phosphate buffered saline-Tris (pH 7.6) and incubated with blotto for 1 h at 37°C. Plates were washed and then incubated with diluted stool samples (dilution 1:10) or GpC VLPs (positive

control) for 2 h at 37°C. Plates were washed and then incubated with rabbit hyperimmune serum to GpC VLPs (CD94; dilution 1:4000) for 1 h at 37°C. Plates were washed and incubated with horseradish perxidase-goat anti-rabbit IgG (KPL, Gaithersburg, MD, USA) diluted 1:5000, for 1 h at 37°C. Plates were washed and then reacted with tetramethylbenzidine for 10 min. Reactions were stopped with 1 N HCl and plates were read at 450 nm with an enzyme immunoassay (EIA) reader. A sample was considered positive if the ratio of absorbance with the hyperimmune serum (U340) over the normal serum (Z1329) was ≥2. GpC RV positive samples were further tested by electron microscopy (EM) and RT-PCR and nested PCR with primers specific to human GpC RV VP4, VP6 and VP7 genes [7,17]. PCR products were sequenced using an ABI 3130 XL Sequencer (Applied Biosystems, Foster City, CA, USA) and sequencing and phylogenetic analyses were performed using Lasergene (DNA Star, Inc., Madison, WI, USA) and MEGA, version 4 (http://www.megasoftware.net/).

Of the 434 stool samples examined by EIA specific to GpA RV or GpC RV, 302 (69.6%) were positive for rotaviruses. Two of these were positive for GpC RV and one for GpA RV and GpC RV. By EM analysis, both complete rotavirus and damaged rotavirus-like particles were observed in samples containing GpC RV and mixed GpC and GpA RV (Fig. 1). All three GpC RV patients had symptoms of diarrhoea, vomiting, and fever over 38°C. The two patients infected with only GpC RV were 38 and 71 months of age and had mild dehydration; whereas the 47-month-old patient with mixed GpC RV and GpA RV infection had moderate dehydration.

Of the three GpC EIA-positive samples, only one isolate KUMC04-18, designated Icheon, was amplified by RT-PCR using primers specific to VP4, VP6 and VP7 genes. This sample contained viral particles with intact structural integrity (Fig. 1A). By contrast, the other GpC positive sample that was not amplified by PCR had only damaged rotavirus-like particles (Fig. 1B). Both complete and empty rotavirus particles were seen in the GpA and GpC sample (Fig. 1C). Our findings are in agreement with other studies where human GpC RV often appeared empty or damaged in stool samples [18–20].

To determine the genetic relationship with human GpC RV from other countries, we sequenced VP4, VP6 and VP7 genes of the Icheon strain. Partial VP4 nucleotide (nucleotides 1–330) and deduced amino acid sequences (amino acids 1–104) of the Icheon strain showed the highest identity with the cognate gene of Thai strain CHM004/03 (98.8% nucleotides and 98.1% amino acids) and the Japanese strain AE53 (98.5% nucleotides and 98.1% amino acids) (Fig. 2A). Similarly, the Icheon strain shared the highest identity in VP6 gene with the Thai strain CMH004/03 (99.6% nucleotides and 100% amino acids) and the Chinese strain Wu82 (99.3% nucleotides and 100% amino acids) (Fig. 2B) and in VP7 gene with the strain CMH0004/03 (99.9% nucleotides and 100% amino acids) and the strain AE53 (99.1% nucleotides and 99.4% amino acids) (Fig. 2C). The Icheon strain was slightly more closely related to the human GpC reference strains reported in Asian countries than to those in other regions of the world. By contrast, the Icheon strain showed lower sequence identity (81.6–84.2%) with partial VP6 genes from porcine GpC RV strains recently reported in South Korea [21] (data not shown).

This is the first report of the detection and characterization of GpC RV from children with severe diarrhoea in South Korea. A previous study detected GpC RV in a faecal specimen of a child in South Korea by using PAGE but did not perform a genetic characterization of the strain [22]. The low detection rate of GpC RV reported to date suggests that this virus is uncommon in the country. However, our findings indicating that GpC RV is present and can be a causative agent of severe diarrhoea among children in South Korea should encourage pediatricians and scientists to search diligently for this virus. By using RT-PCR, we observed no PCR product from two samples (KUMC04-57 and KUMC04-164) that were positive for GpC RV by EIA.

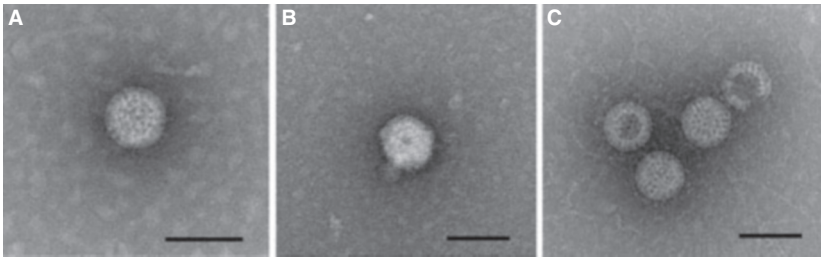

FIG. 1. Electron micrographs of rotavirus particles in fecal samples. Panels A, complete GpC RV particles in sample KUMC04-18 (Icheon); B, GpC RV-like particle in sample KUMC04-57; and C, complete and empty rotavirus particles in sample KUMC04-164 containing GpC and GpA RV. Bar = 100 nm.