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Characterization of a G11,P[4] Strain of Human Rotavirus Isolated in South Korea▽

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A novel human rotavirus strain, CUK-1, containing a G11 type combined with a P[4] type was isolated from a 1-year-old female patient with fever and severe diarrhea at Our Lady of Mercy Hospital in Incheon, South Korea. This CUK-1 strain showed the highest degree of nucleic acid similarity (98.7% and 93%) to G11 Dhaka6 and P[4] RV 5, respectively. This novel combined type of CUK-1 rotavirus strain (G11,P[4]) was uncovered from humans and is reported on here for the first time.

Rotavirus infections cause acute gastroenteritis accompanied by watery diarrhea, fever, and vomiting in humans and a variety of animals (13) and are more likely to be associated with dehydration (22) and hospitalization (2). Rotavirus is classified into seven groups (groups A to G), based on the VP6 protein (11). Group A has been the most frequently discovered group worldwide. It is classified into G (glycoprotein) types by protein VP7 and P (protease-sensitive) types by protein VP4, and these types are considered important for vaccine development. So far, strains of at least 16 G genotypes and 27 P genotypes of rotavirus strains (11, 14) have recently been isolated from humans and a variety of mammalian and avian species. The major human G types are G1, G2, G3, G4, and G9, combined with the P types P[8] and P[4] (19). G11 rotavirus (strain YM, G11,P[7]) was first isolated from pigs in Mexico in 1983 (17) and was also reported in Venezuela in 1988 (3). It was subsequently identified in combination with P[6] and P[8] (15) and P[25] (21) from humans in Bangladesh.

A total of 3,275 stool specimens were collected from children under 5 years of age with diarrheal disease from eight domestic hospitals (Our Lady of Mercy Hospital, Kangnam St. Mary's Hospital, St. Vincent Hospital, Severence Hospital, Wonju Christian Hospital, Jeonju Jesus Hospital, Changwon Fatima Hospital, and Chungnam National University Hospital) in South Korea from June 2005 to May 2006. Among the 3,257 stool samples collected, 835 samples were found to be positive for rotavirus antigen. CUK-1 was isolated from 1 of those 835 samples and was from a 1-year-old female patient hospitalized with fever and severe diarrhea at Our Lady of Mercy Hospital in Incheon, South Korea. CUK-1 was identified as G11,P[4] by multiplex reverse transcriptase PCR (RT-PCR), and its identity was confirmed by nucleotide sequencing and alignment analysis.

RT-PCR was carried out with a One Step RT-PCR kit (QIAGEN/Westburg) for rotavirus G and P genotypes. We amplified a 1,062-bp fragment of the VP7 gene with the consensus forward primer Beg9 (5′-GGCTTTAAAAGAGAGAATTTCCGTCTGG-3′) and the reverse primer End9 (5′-GGTCACATCATACAATTCTAATCTAAG-3′) (9). We also amplified an 876-bp fragment of the VP4 gene with the consensus forward primer Con3 (5′-TGGCTTCGCCATTTTATAGACA-3′) and the reverse primer Con2 (5′-ATTTCGGACCATTTATAACC-3′) and then performed the nested PCR (7).

The complete open reading frame (978 bp) and deduced VP7 amino acid sequence of the CUK-1 human rotavirus strain were determined. From comparisons of the VP7 nucleotide and amino acid sequences of CUK-1 with the corresponding VP7 sequences of the prototype strains (G1 to G16), the VP7 sequence of the CUK-1 strain was found to have close relationships to G11 rotavirus strains A253, Dhaka6, and YM (86.9, 98.7, and 91.97% nucleotide sequence identities, respectively, and 95.1, 98.5, and 95.4.5% amino acid identities, respectively). Overall, the CUK-1 strain showed the highest degree of similarity with the Dhaka6 strain. This strain had fewer corresponding identities with the other G types (types G1 to G16), with the exception of G11. We constructed the dendrogram containing VP7 of CUK-1 and the 16 G types described above (Fig. 1A). This phylogenetic analysis indicated that

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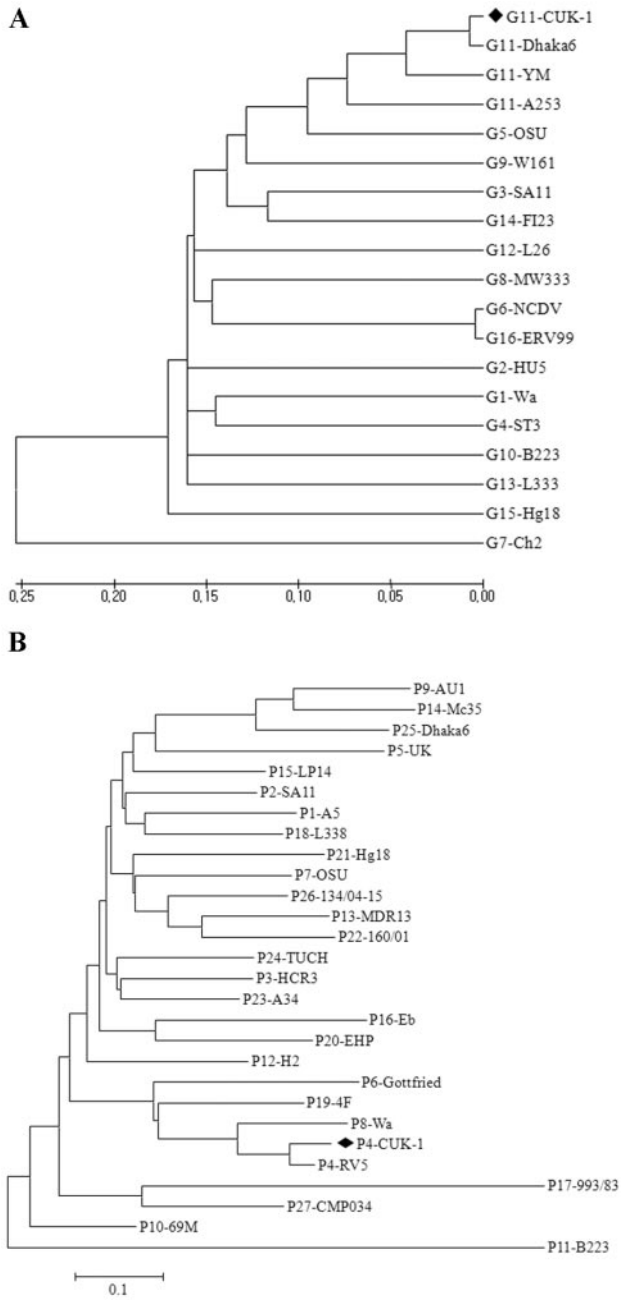

FIG. 1. (A) Phylogenetic tree analysis based on the nucleotide sequences of the VP7 genes (nt 49 to 1026) of strain CUK-1 and strains of other rotavirus G types by use of the neighbor-joining method. The following strains have the indicated GenBank accession numbers: Wa, KO2033; HU5, A01028; SA11, K02028; ST3, X13603; OSU, X04613; NCDV, M12394; Ch2, X56784; MW333, AJ278257; 116E, AB180969; B223, X57852; A253, L24163; Dhaka6, AY773003; YM, M23194; L26, M58290; L338, D13549; F123, M61876; Hg18, AF237666; and Evr99, DQ981478. (B) Phylogenetic tree analysis based on nucleotide sequences (nt 65 to 482) of the VP8* fragments of the VP4 genes of strain CUK-1 and strains of other rotavirus P types by use of the neighbor-joining method. The following strains have the indicated GenBank accession numbers: A5D13395; SA11X14204; HCR3L19712; RV5M32559; UK, M22306; Gottfreid, M33516; OSU, X13190; Wa, L34161; AU1, D10970; 69 M, M60600; B223, D13394; H2, L04638; MDR13, L07886; Mc35, D14032; LP14, L11599; Eb, L18992; 993/83, D16352; L338, D13399; 4F, L10359; EHP, U08424; Hg18, AF237665; 160/01, AF526374; A34, AY174094; TUCH, AY596189; Dhaka6, AY773004; 134/04–15, DQ061053; and CMP043, DQ534016.

CUK-1 was clustered with the Dhaka6 G11 type in a monophyletic branch.

We found that the deduced amino acid sequences of four intragenotype-conserved antigenic regions were aligned within CUK-1 and the other 16 G types. The CUK-1 strain showed a close relationship with G11 strains A253, Dhaka6, and YM in these regions. This CUK-1 strain showed only 1 amino acid change (V218I) in region C compared with the sequence of the Dhaka6 strain. Previous reports on comparative analyses of VP7 gene sequences among rotaviruses of different serotypes had identified a high degree of sequence divergence in several discrete regions, and these regions were highly conserved within the serotypes (10). Also, the A, B, C, and F antigenic regions were involved in virus neutralization (8, 12). The sequences of the CUK-1 strain were nearly similar to those of the Dhaka6, YM, and A253 strains within these regions, with 1, 2, and 3 amino acid changes, respectively. The Asp at position 96 within region A was probably responsible for its G11 specificity, because Asp is also present at this position in the VP7 of the G11 strain but not in the G5 strain (4). The amino acid at this position has been reported to be critical to the conformation of the major antigenic site of VP7 (4, 8, 12), although a single amino acid substitution may not be enough to completely affect the serotype reactivity (4). Therefore, it can be stated that the CUK-1 strain retains its G11 specificity because it has Asp at position 96. Here, the CUK-1 strain showed a high degree of sequence divergence (more than 30%) compared with the antigenic region sequences strains of other serotypes, except G11.

The partial 418-bp gene sequences (nucleotides [nt] 65 to 482) of the VP8* fragments within VP4 of the CUK-1 human rotavirus strain and the deduced amino acid sequences were determined. The CUK-1 strain then presented the highest degrees of identity of 93% and 94.2% with corresponding nucleotide sequences and the deduced amino acid sequences of the VP8* fragments of rotavirus type 5 (RV5) of the P[4] genotype, respectively (Table 1). In addition, phylogenetic tree analysis of the VP8* fragments of the VP4 genes of strain CUK-1 and other established rotavirus P types by the neighbor-joining method also confirmed that strain CUK-1 belonged to type P[4] (Fig. 1B). The type G11 strains A253, CUK-1, Dhaka6, and A253 and the G5 strain may have originated from a common ancestor of VP7 specificity (4), as shown in Fig. 1A. For VP4 specificity, however, the G5 viruses have been detected not only in association with P[7] but also in conjunction with P[6] and P[8] (19), while G11 was combined with P[25] (21) and type G11 strain CUK-1 was combined with P[4]. Therefore, the CUK-1 strain showed a common lineage with G11 and G5 in the molecular evolution of VP7 but a diversity from these strains in the molecular evolution of VP4. The G-type rotaviruses combined with P[4] that have been reported to date are G1,P[4] (1), G2,P[4] (19), G3,P[4] (16), G4,P[4] (1), G8,P[4] (5), G9,P[4] (20), and G12,P[4] (18). On the basis of these results, one might expect a possibility that CUK-1 occurred by a natural reassortment event.

This incidence of a novel recombinant G11,P[4] rotavirus strain in South Korea illustrates the large diversity of rotavirus strains occurring worldwide. Advanced research with this strain will promote the investigation of diverse rotavirus strains in the molecular, genetic, evolutionary, and epidemiological fields. Moreover, studies and surveillance of animal-to-human transmission events

TABLE 1. Nucleotide and amino acid VP4 sequence similarities of strain CUK-1 with those of strains of different P genotypes[a]

P type	Strain	Origin	Similarity (%)	
			Nucleotide	Amino acid
P[1]	A5	Bovine	60.3	55.8
P[2]	SA11	Simian	62.3	57.2
P[3]	HCR3	Human	61.3	54.3
P[4]	RV5	Human	93.0	94.2
P[5]	UK	Bovine	55.8	54.3
P[6]	Gottfreid	Porcine	66.1	65.9
P[7]	OSU	Porcine	59.4	54.3
P[8]	Wa	Human	82.0	81.2
P[9]	AU1	Human	59.1	54.3
P[10]	69M	Human	64.2	58.7
P[11]	B223	Bovine	49.0	39.1
P[12]	H2	Equine	64.4	58.7
P[13]	MDR13	Porcine	62.5	61.6
P[14]	Mc35	Human	56.0	51.4
P[15]	LP14	Ovine	59.4	54.3
P[16]	Eb	Murine	60.3	52.9
P[17]	993/83	Bovine	51.9	35.5
P[18]	L338	Equine	60.1	53.6
P[19]	4F	Porcine	71.4	70.3
P[20]	EHP	Murine	59.9	60.1
P[21]	Hg18	Bovine	59.6	53.6
P[22]	160/01	Lapine	58.9	57.2
P[23]	A34	Porcine	55.0	52.9
P[24]	TUCH	Rhesus macaque	61.8	57.2
P[25]	Dhaka6	Human	58.7	54.3
P[26]	134/04–15	Porcine	62.7	58.7
P[27]	CMP043	Porcine	58.4	54.3

[a] The gene sequences of the VP4 sequences from nt 65 to 482 were compared. The partial VP4 sequences of strain A34 (nt 69 to 481) were compared.

will help to provide an understanding of and prevent rotavirus disease. Some rotavirus strains may have arisen by interspecies transmission or by reassortment between human and animal rotaviruses (6). This enormous diversity among rotavirus strains allows the study of the evolution of rotavirus strains and creates new challenges for rotavirus vaccine development (6). Finally, the discovery of this novel combinant rotavirus strain will play an important role in future vaccine development.

Nucleotide sequence accession number. The complete open reading frame (978 bp) and deduced VP7 amino acid sequence of the CUK-1 human rotavirus strain were enrolled in GenBank under accession number EF121951.

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