

Genotypic distribution of rotavirus strains causing severe gastroenteritis in Gyeonggi province, South Korea, from 2003 to 2005

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Abstract Rotavirus was present in 1,367 of 7,060 stool samples (19.4%) collected in Gyeonggi province of South Korea from 2003 through 2005. The predominant genotypes were confirmed as G4/P2A[6] (19.0%) followed by G3/P1A[8] (15.6%), G2/P1B[4] (9.3%), and G1/P1A[8] (6.5%). The predominant types of rotavirus by year were G3/P[8] in 2003, G4/P[6] in 2004, and G1/P[8] in 2005. The prevalent rotavirus genotypes changed constantly from 2003 to 2005.

One of major symptoms of gastroenteritis is diarrhea, which is one of the most common childhood illnesses in both developing and developed countries. Globally, approximately 1.6–2.5 million children below 5 years of age die each year from diarrhea [11]. The etiologic agents of diarrhea are viruses, bacteria, and parasites, with viral infection being the most common cause. Rotavirus, enteric adenovirus, astrovirus, and norovirus are the major viral agents [2]. Among these, rotavirus is the most common cause of diarrhea worldwide [17], being responsible for up

to 600,000 deaths annually of children under 5 years of age [18]. G1P[8], G2P[4], G3P[8], and G4P[8] are the most common types found globally [2, 6]. The most prevalent genotypes have a global distribution, although some genotypes occur endemically [1, 3, 16].

We describe here the genotypic distribution of rotavirus causing gastroenteritis in Gyeonggi province of South Korea. Approximately 11 million people, 25% of the country's population, live in this province, a region located around the capital city.

Fecal samples were collected from 7,060 outpatients with diarrhea from 5 general hospitals in Gyeonggi province from 2003 to 2005. Among the total 7,060 patients assessed in this study, 64.1% were below 5 years old. Rotavirus was isolated from 1,367 (19.4%) samples using Rotavirus Ag capture ELISA (BioinCell, Houston, TX). To survey the prevalence of group A rotavirus genotypes, reverse transcription-polymerase chain reaction (RT-PCR) was conducted on the rotavirus-positive samples using AMV reverse transcriptase (Roche Diagnostics, Mannheim, Germany) and the primer set (for detailed primer information, see Supplementary Material). The primers Beg9 and End9 were selected to produce full-length copies of gene 9 (or gene 8) from any group A rotavirus strain [7]. The primers Con2 and Con3 amplified the highly conserved regions among rotavirus strains from VP4 genetic groups 1–4 [5]. VP7 and VP4 genes were sequenced with the amplified RT-PCR products using ABI 3730XL (Applied Biosystems, Foster City, CA). Sequence data for the reference strains were obtained from GenBank (National Center for Biotechnology Information, NCBI).

The predominant G genotypes detected from 2003 to 2005 in Gyeonggi province were G3 (30.0%) followed by G4 (24.0%), G2 (14.1%), G1 (13.7%), and G9 (8.3%) (Table 1). G1 was the most prevalent strain until 1996;

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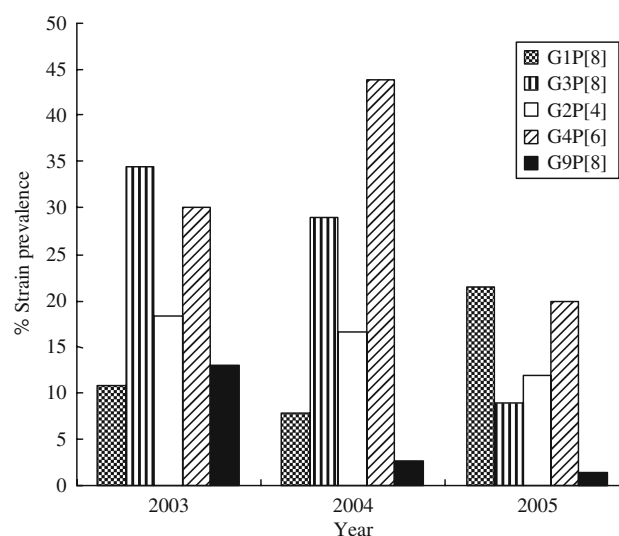
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Table 1 Distribution of P and G genotypes of human group A rotavirus from 2003 to 2005 in Gyeonggi province, South Korea

P genotype	G genotype (number (%))							
	G1	G2	G3	G4	G9	Mixed	Non-typable	Subtotal
P[8]	89 (6.5)	17 (1.2)	213 (15.6)	35 (2.6)	53 (3.9)	3 (0.2)	17 (1.2)	427 (31.2)
P[4]	40 (2.9)	127 (9.3)	75 (5.5)	24 (1.8)	23 (1.7)	6 (0.4)	26 (1.9)	321 (23.5)
P[6]	28 (2.0)	42 (3.1)	62 (4.5)	259 (18.9)	21 (1.5)	5 (0.4)	40 (2.9)	457 (33.4)
P[3]	0 (0)	0 (0)	1 (0.1)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.1)
Mixed	12 (0.9)	2 (0.1)	21 (1.5)	4 (0.3)	5 (0.4)	1 (0.1)	14 (1.0)	59 (4.3)
Non-typable	18 (1.3)	5 (0.4)	38 (2.8)	6 (0.4)	12 (0.9)	1 (0.1)	22 (1.6)	102 (7.5)
Subtotal	187 (13.7)	193 (14.1)	410 (30.0)	328 (24.0)	114 (8.3)	16 (1.2)	119 (8.7)	1,367 (100)

since then, G4, G2, and G3 successively became the most prevalent strain. Elsewhere in South Korea, a similar trend was observed during the last 10 years, with G1 being isolated 89% of the time until 1997 [10], with prevalence shifting to G4 (41%) in the following years from 1998 to 2000 [21, 22]. From 1999 to 2002 in Gyeonggi province, the prevalence pattern was G2 (51.3%) followed by G1 (28.6%), G4 (8.7%), and G3 (0.9%) [15]. However, G9 was not detected in that period although this genotype was continuously found in many countries including China and Japan during the same period [4, 12, 25], and it has become one of the major types in worldwide [20]. In South Korea, G9P[8] strains first emerged in 2002 [8]. Presently, G9P[8] was constantly detected during the study period. We observed that the predominant P genotypes were P2A[6] (33.4%) followed by P1A[8] (31.2%) and P1B[4] (23.5%) in the same period. P[6], P[8], and P[4] were the major groups, consistent with the worldwide trend. A single P type with mixed G types, a single G type with mixed P types, and one strain of mixed G and P types were identified (Table 1). Although their occurrence is low, we have to pay attention to mixed infections because they can represent reassortants. The incidences of P types with non-typable G types, G types with non-typable P types, and non-typable G and P types were 7.1, 5.9, and 1.6%, respectively. The incidence of non-typable isolates is quite high and may suggest the emergence of unusual serotypes.

The predominant types in rotavirus-infected patients by year were G3P[8] in 2003, G4P[6] in 2004, and G1P[8] in 2005 (Fig. 1). Four common G types (G1–4) combined with P[8] or P[4] have been identified from over 88% of the rotavirus strains reported worldwide, and serotypes G9P[8] or G9P[6] have emerged as important strains [20]. G1P[8] strains have been reported as most prevalent in South Korea during 2004–2006 in young children with acute diarrhea [13]. That study identified unusual types such as G8P[8], G12P[6], G2P[9], G1P[9], and G3P[9] that were not identified in our study. Presently, one strain of the unusual G3P[3] type in South Korea was identified. Rotavirus G3P[3] strains are usually recovered from infants

**Fig. 1** Yearly percentage of prevalence of predominant rotavirus strains from 2003 to 2005

with asymptomatic infections, although the G3P[3] strain was recovered from children with gastroenteritis in several countries including in this study [19, 23]. In South Korea, G3P[3] was first identified in a fecal specimen of a Korean native black goat with diarrhea in 1998 [14] and it was also isolated from the stool of a puppy with mild diarrhea [9]. However, to our knowledge, the present finding of a human G3P[3] strain in South Korea is novel. The G3P[3] is closely related to the human rotavirus strain AU-1, and the V4 and V7 gene sequences of the G3P[3] strain show 97.3 and 97.8% identity with the AU-1 strain, respectively. G1P[8] (64.7%) was the most prevalent type from the surveillance of the global distribution of human group A rotavirus during 1989–2004 [20].

To investigate sequence variation and phylogenetic relationship among genotypes distributed in Gyeonggi province from 2003 to 2005, nucleotide sequences of the G and P genotypes of the 297 isolated strains and the sequences in the NCBI GenBank database were analyzed using MegAlign (DNASTAR, Madison, WI). Strains

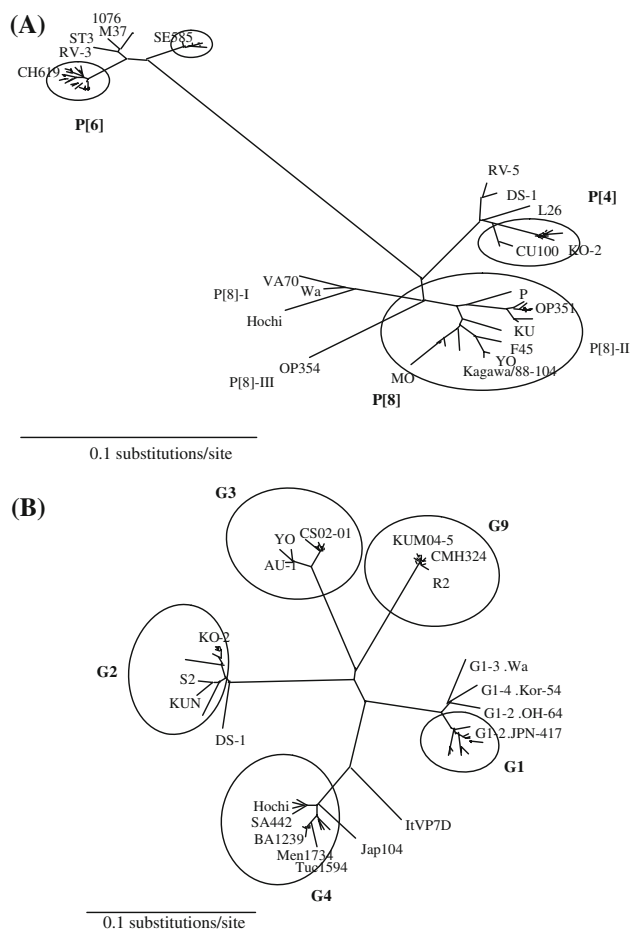


Fig. 2 Phylogenetic trees of the VP4 (A) and VP7 (B) genes of rotavirus. Phylogenetic trees and sequence pair distances of the nucleotides were obtained. The sequenced regions are shown in Table S1. Phylogenetic clusters are circled. The accession numbers of the VP7 and VP4 amino acid sequences are as follows: Wa, P03532; Kor-54, AAB47889; OH-64, AAB47899; JPN-417, BAA03835; KO-2, AAK94067; S2, P04510; KUN, BAA08809; DS-1, BAC82356; CS02-01, AAN86986; YO, BAA23304; MO, BAA23300; AU-1, BAA23292; HOCHI, BAB32864; SA442, AAD42236; BA1239, AAL99414; Men1734, AAL99405; Tuc1594, AAL99417; Jap104, BAA83694; KUM04-5, AAY56328; CMH324, AAU06377; R2, AAO59333 for VP7 and RV-5, P30214; DS-1, CAD62680; L26, P21284; KO-2, AAK94068; CU100, ABB84925; SE585, CAC43311; 1076, P11198; M37, P11197; ST3, AAA66952; RV-3, AAC68883; CH619, AAF14295; OP351, AJ302147; KU, BAE76025; F45, AAB05651; YO, BAA77544; Kagawa/88-104, BAB32844; MO, BAA77543; OP354, AJ302148; HOCHI, BAB32852; Wa, AAA66953; VA70, CAD62682 for VP4

identified as P[8] were genetically more diverse than the P[4] and P[6] types. The viral P[8] isolates were VP4-P[8]-II. In the case of the G type, the isolated strains were grouped into five categories (Fig. 2). All strains were human lineages, and amino acid sequences showed a high homology within each genotype.

Diversity within the G and P types plays a role in immunity, especially in young children, making vaccine development critical. In this study, although the

predominant G and P combination changed by the year, the most common G and P combinations isolated worldwide were also isolated as major combinations, and several uncommon genotype combinations including G3P[3] were detected. This study shows that phylogenetic analysis of sequences of outer capsid genes obtained from the isolated strains reveals genetic diversity in South Korea, indicative of constant genetic reassortment.

Currently, two live oral attenuated vaccines, Rotarix (GlaxoSmithKline) and Rotateq (Merck) are available. Rotarix is a monovalent vaccine derived from the most common human rotavirus strain, G1P[8], and provides cross-protection against most other globally common serotypes, for instance, G3P[8], G4P[8], and G9P[8] strains [24]. Rotateq is a pentavalent bovine (WC3)-human reassortant vaccine. This vaccine showed efficacy against all G1–G4 and G9 serotypes. In view of our results, the two available vaccines are expected to effectively prevent infant diarrhea caused by common rotavirus serotypes in South Korea. However, we also identified unusual serotypes, and the efficacy of the vaccines has not been clearly proved against those serotypes. Moreover, the results showed that the trend of prevalent genotypes has been constantly changing in South Korea. Therefore, strain surveillance should be constantly performed to monitor circulating rotavirus strain diversity and to identify possible new variant strains that cannot be controlled by the vaccines.

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