Case Report


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None of the authors has a conflict of interest.

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ABSTRACT

During a rotavirus surveillance conducted in Lulong County, Hebei Province, China, a total of 331 stool specimens collected in 2003 from children under 5 years old with diarrhea were screened. We identified a novel group A human rotavirus of G5P[6] genotype. Phylogenetic analysis confirmed that the VP7 protein of this newly identified LL36755 strain was closely related to those of the G5 strains. As such, it has 95.4% homology with its counterparts in the porcine G5 strains C134 and CC117 at the level of amino acid sequences. On the other hand, the VP4 protein of the LL36755 strain was 94.5% homologous to that of the porcine P[6] strains 134/04-10, 134/04-11, 221/04-7 and 221/04-13. Our findings implicate a dynamic interaction between human and porcine rotaviruses.

Keywords: human, novel G5P[6] genotype, porcine, reassortment, rotavirus, China
Group A rotaviruses are a major cause of acute gastroenteritis in infants and young children as well as a wide variety of domestic animals (2,11,36). In humans rotavirus diarrhea results in significant morbidity and mortality, especially in developing countries. Globally, World Health Organization estimated that 611,000 (range 454,000 ~ 705,000) children die each year due to rotavirus diarrhea (33).

Rotavirus, a member of the family *Reoviridae*, has a triple-layered capsid that contains 11 segments of double-stranded genomic RNA. Rotavirus genotypes are defined by genome segment 4 for the P (protease-sensitive protein) type and by genome segment 9 (or 7 or 8, depending on the strain) for the G (glycoprotein) type. Through extensive serological and genomic studies, 15 G and 26 P genotypes have been established for existing human and animal rotaviruses (10,17,18,20,21,22,26,27). The most common rotavirus G genotypes found in humans are G1, G2, G3 and G4. Meanwhile, genotype P[8] is most common in humans, followed by P[4] and P[9]. Recently, rotavirus P[6], which was first detected as an asymptomatic infection in neonates, has been increasingly found.

Human rotavirus strains of unusual G or P types and of rare combinations of G and P types have recently been identified. G6 has been detected in Italy, Australia, India, the United States, Belgium, and Hungary; G8 has been frequently isolated in Africa and sporadically in other countries; G10 has been reported in the United Kingdom, India, Thailand, Paraguay, and Brazil (29,28); G11 has recently been detected in Dhaka, Bangladesh (26); and G12 has been identified in the Philippines (31), Thailand (25), the United States (14), India (8), Japan (30), Korea (6), Argentina (5), and Brazil (32). G5 strains, originally detected only in pigs, have been reported in Brazil, Argentina, Paraguay, Cameroon, and the United Kingdom (1,29,28). Although extensive surveys on the distribution of G genotypes in Asia have been conducted (4),
existence of G5 in humans has not been reported.

We have collected, a total of 331 fecal specimens from children under 5 years old with acute gastroenteritis in Lulong County, Hebei Province, China in 2003. Using an ELISA kit (DAKO), 130 out of 331 (39.3%) stool samples were detected positive for group A rotavirus. Furthermore, viral RNA was extracted from these positive samples by using a QIAamp Viral RNA Mini Kit (QIAGEN) as per the manufacturer's instructions. G3P[8] was found to be the most common genotype in positive samples based on reverse transcription–polymerase chain reaction (RT-PCR) analysis. For specimens in which genotypes could not be determined by RT-PCR, DNA sequencing of VP4 and VP7 genes was performed for genotyping. One rare G/P combination of G5P[6] was identified from specimen LL36755. This strain was isolated from an 18-month-old girl with high fever, vomiting (10 times a day), and watery diarrhea. To the best of our knowledge, this is the first identification of a human G5 rotavirus in Asia and of a G5P[6] genotype combination in the world. RNA PAGE shows that the electropheretic mobilities of genes 10 and 11 of this strain are similar to those of the Wa strain (data not show).

In comparison with representative rotavirus strains of the 15 known G genotypes, the VP7 sequence of strain LL36755 shared high homology of 88.6%-89.9% at the nucleotide level and 92.6%-95.4% at the amino acid level with those of the other G5 strains. To the remaining 14 G genotypes, the amino acid sequence homology ranged from 57.7% (Ch2, G7) to 88% (YM, G11). Since strains sharing an amino acid sequence identity of ≥ 90% belong to the same genotype [10,12,19], LL36755 qualifies as a G5 rotavirus. Phylogenetic analysis using the MEGA3.1 software further confirmed that the VP7 gene of the LL36755 strain was closely related to counterparts in the G5 strains. In particular, it clustered with VP7 of
the porcine G5 strains C134 and CC117 in the phylogenetic tree (Fig. 1). The VP7 protein of LL36755 shared 95.4% identical amino acid residues with the same protein in C134 and CC117. This degree of conservation is slightly higher than compared to human strains MRC3105 and IAL-28 (94.0% and 93.3% identity at the amino acid level, respectively). Moreover, nine hypervariable regions (VR1 to VR9) in the linear amino acid sequence of VP7 were highly conserved within rotavirus strains of the G5 genotype, but were highly polymorphic among strains of different G genotypes. Likewise, hydrophobic and hydrophilic regions in VP7 were also conserved within all G5 strains but not among strains of different G genotypes (data not show).

The deduced amino acid sequence of the VP4 gene of the LL36755 strain encoding 290 residues representing the complete VP8 and the amino terminus of VP5 was compared with counterparts in rotavirus strains of all 26 P genotypes. The LL36755 VP4 had 83.2% to 94.5% amino acid sequence homology with the P[6] rotaviruses, whereas the homology with VP4 proteins from rotaviruses of other P genotypes was less than 74.2%. The VP4 sequence of the LL36755 strain was most closely related to that of the porcine strains 134/04-10, 134/04-11, 221/04-7, and 221/04-13 of P[6] genotype (Fig. 2). Actually it shared 93.0% nucleotide identity and 94.5% amino acid identity with all these four strains. In contrast, the amino acid sequence homology between VP4 proteins of LL36755 and a rotavirus of different P genotype ranged from 42.6% to 74.2%. Phylogenetic analysis demonstrated that the VP4 protein of the strain LL36755 clustered with porcine viruses of the P[6] genotype (Fig. 2).

Globally, G1 to G4 as well as P[8] and P[4] are the most frequently distributed rotavirus G and P genotypes. (9,16)The four most common combinations of VP7 and VP4 are G1P[8], G2P[4], G3P[8], and G4P[8] (13). In addition, unusual G types, P
types, and G-P combinations have also been reported. It is thought that immunity
rotavirus infection is predominantly homotypic initially and is broadening after
subsequent infections (3,15). This eventually leads to protective immunity to all
antigenic types. The reassortant rotavirus vaccine formulations have been targeted at
the most commonly circulating human rotavirus strains (G1 to G4) with the ultimate
goal of providing protection and minimizing severe rotavirus-associated disease.
However, recent epidemiological studies in developing countries have shown
increasing diversity of human rotaviruses. Rotavirus G5, G6, G8, G10 and G12 strains
are more frequently found in humans, but uncommon strains and high regional
diversity among circulating rotaviruses have been increasingly documented over the
past decade. G5 strains have been detected in Brazil, Argentina, Paraguay, Cameroon,
and the United Kingdom. Particularly, in Brazil a surprising prevalence rate of 26%
has been reported for G5 strains (35).

The LL36755 strain we identified has long RNA pattern and G5P[6]
specificity. Epidemiological investigations and genotype analysis based on VP7 and
VP4 proteins are important for developing efficient rotavirus vaccines and elucidating
rotavirus ecology and evolution. The amino acid sequences of the VP7 gene of the
LL36755 strain showed the highest identity to those of porcine G5 strains C134 and
CC117. In addition, the LL36755 VP4 is highly homologous to porcine strains
been detected only in humans and pigs. The prevalence of human P[6] rotaviruses was
low in Lulong County; since only one out of 331 cases of rotavirus infection during
2003 was identified to be a P[6] strain. Lulong is a half-mountainous half-rural county
remote from cities. Direct transmission of the LL36755 strain from other areas
previously recognized to have G5 rotaviruses seems unlikely. Thus, it will be of great
interest to understand the origin of the LL36755 strain of G5P[6] genotype. Similarity of its VP7 and VP4 genes to porcine viruses (Fig. 1 and Fig. 2) suggested that the unusual human strains might be evolved gradually from other porcine and human strains. Cross-species transmission of rotaviruses has been documented (23) and represents one mechanism for genetic diversity of rotaviruses.

LL36755 is the first human G5P[6] rotavirus. The similarity of LL36755 to porcine strains suggests that the flux of genetic material between human and animal rotaviruses under natural conditions might be more common than expected [7,24,30,34]. Extensive surveillance of rotaviruses in humans as well as animals is therefore warranted. Although few G5 isolates have been found to date, further surveillance in different populations and geographical settings will likely reveal additional G5 rotaviruses. Thus, it remains to be determined whether this genotype should be included in future rotavirus vaccines. Rotaviruses are likely spread through different routes in developed and developing countries. Evidence for genetic reassortment between human and animal rotaviruses has been obtained in two particular animal species of cow and pig, which are in close contact with humans. In Lulong County, people and domestic farm animals, especially domestic pigs, live in close proximity. The identification of a human G5P[6] rotavirus supports a dynamic interaction between human and animal rotaviruses. In this regard, further investigates are required to elucidate whether the LL36755 strain represents a natural reassortment between human and porcine viruses and is able to stabilize and spread successfully in humans. Simultaneous surveillance of animal and human rotavirus infections is therefore paramount for studying the evolution of these viruses.

**Nucleotide sequence accession numbers.** The nucleotide sequences of the VP7 and VP4 genes of the human rotavirus LL36755 strain have been deposited in
the GenBank database under accession numbers EF077484 and EF159569.
REFERENCES


Nucleotide sequence of VP4 and VP7 genes of human rotaviruses with subgroup I specificity and long RNA pattern: implication for new serotype specificity. J. Virol. 64:5640–5644.


FIGURE LEGENDS

Fig. 1  Phylogenetic tree of the VP7 protein of the LL36755 strains with other G genotype. Phylogenetic analysis, distance calculations were done by using the Kimura-2 correction for evolutionary rate. The confidence values of the internal rods were calculated by performing 1,000 bootstrap analyses. Evolutionary trees for deduced amino acid (aa) sequence were drawn by using the neighbor-joining method.

Fig. 2  Phylogenetic tree of the VP4 protein of the LL36755 strains with other P genotype. Phylogenetic analysis, distance calculations were done by using the Kimura-2 correction for evolutionary rate. The confidence values of the internal rods were calculated by performing 1,000 bootstrap analyses. Evolutionary trees for deduced amino acid (aa) sequence were drawn by using the neighbor-joining method.