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Molecular Epidemiology of Norovirus Infections in Children with Acute Gastroenteritis in South Korea, November 2005 through November 2006

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Running title: Norovirus infections in South Korea
Norovirus infections were detected in 114 of 762 children with acute gastroenteritis in South Korea from November 2005 to November 2006. Seasonality peaks in December, March, and October was also assessed in this study. We identified eight noroviral genotypes (GI/6, GII/2, GII/3, GII/4, GII/5, GII/6, GII/8) and a C1-120 strain showing low identity (79.3%) with GII 13 and GII 17, respectively.

Key words: epidemiology; gastroenteritis; norovirus
Norovirus (NoV) is one of the most important viruses that cause nonbacterial acute gastroenteritis in humans. The mortality of acute gastroenteritis was estimated to be 2.1 dmillion in the year 2000, and mortality due to gastroenteritis in children was higher in developing countries than in the developed countries (2). Recently, NoVs have been recognized as novel emerging pathogens.

NoV is a member of the family *Caliciviridae* and harbors a positive-sense, single-stranded RNA (7.6 kb). The NoVs are classified into 5 genogroups (GI-V) and Human NoV is divided into three genogroups, genogroup I (GI), genogroup II (GII), and genogroup(IV), which are further classified into 8, 17, and 1 genotypes, respectively (15). Previous studies have demonstrated that the GII-4 genotypes are the dominant circulating genotype worldwide (3, 8, 14).

In South Korea, a previous study has been conducted in an effort to characterize the molecular epidemiology of gastroenteritis outbreaks induced by human NoV infection (9); however, no study has yet addressed the molecular epidemiology of human NoV from various districts of South Korea.

Stool specimens were collected from children under 5 years of age suffering from diarrheal disease from 8 domestic hospitals (Our Lady of Mercy Hospital, Kangnam St. Mary’s Hospital, St. Vincent Hospital, Severance Hospital, Wonju Christian Hospital, Jeonju Jesus Hospital, Changwon Fatima Hospital, and Chungnam National Univ. Hospital) in South Korea from November 2005 to November 2006.

The viral genomic RNA was extracted from 140 µL of 10% fecal suspensions via the application of the QIAamp® Viral RNA Mini kit (QIAGEN, Hilden, Germany) in accordance with the manufacturer’s instructions. Reverse transcription PCR (RT-PCR) was conducted using a One Step RT-PCR kit (QIAGEN, Hilden, Germany) for the NoV GI and GII
genotypes. We amplified a 330-bp fragment (5342-5671 of Norwalk virus M87661) of the
capsid gene (GI) with the consensus forward primer NV-GIF1 (5’-CTG CCC GAA TTY GTA
AAT GAT CAT-3’) and the reverse primer NV-GIR1 (5’-CCA ACC CAR CCA TTR TAC
ATY TG-3’) (9). We also amplified a 341-bp (5061-5401 of Lordsdale virus X86557)
fragment of the capsid gene (GII) harboring the consensus forward primer NV-GIIF1 (5’-
GGG AGG GCG ATC GCA ATC T-3’) and the reverse primer NV-GIIR1 (5’-CCR CCI GCA
TRI CCR CCI GCA TTR TAC AT-3’) (9). The reaction was conducted with an initial reverse
transcription step at 50°C for 30 min, followed by PCR activation at 95°C for 15 min, then 35
cycles of amplification (45 s at 94°C, 50 s at 58°C, and 45 s at 72°C), and a final extension
step of 10 min at 72°C in a PCR System Px2 thermal cycler (Thermo Hybaid, Middlesex,
UK). The PCR products were run on 1.5% agarose gel, stained with ethidium bromide and
visualized under UV light. The products were extracted using a QIAquick PCR purification
kit (QIAGEN, Hilden, Germany) and were sequenced by Genotech (Daejeon, South Korea).
The phylogenetic analyses were conducted using the DNASTAR version 5.07 software
package. The DNA sequences were aligned via the Clustal W method. The dendrograms were
constructed via the neighbor-joining method.

Among the 762 stool specimens, 114 (15.0%) fecal samples were identified as NoVs by
RT-PCR and nucleotide sequence analysis. Twelve (10.5%) of the 114 specimens were
determined to belong to genogroup I strains and 102 (89.5%) of the 114 specimens belonged
to the genogroup II strains.

Whereas 12 GI NoVs among the total 114 NoVs were classified further into only one
genotype, GI/6 accounting for 10.5% (12 of 114), 102 GII NoVs were classified further into
GII/2, GII/3, GII/4, GII/5, GII/6, GII/8 as well as a C1-120 genotype accounting for 0.9% (1
of 114), 7.9% (9 of 114), 71.9% (82 of 114), 5.3% (6 of 114), 1.8% (2 of 114), 0.9% (1 of
114), and 0.9% (1 of 114), respectively. The NoVs identified in this study were constructed
via the phylogenetic analyses of nucleotide sequences on the basis of the GI (314 bp) and GII (305 bp) capsid regions (Fig. 1A and 1B)

The difference in NoV infection rate between the males and females was not significant (50.9% and 49.1%, respectively) (data not shown). Distribution of norovirus genogroups in children with acute gastroenteritis by age was as followed; 49 (0-1 age), 36 (1-2 age), 21 (2-3 age) 7 (3-4 age), and 1 (4-5 age). Although NoV infections were detected in all age groups, NoV infections were found most frequently in the <1 year of age group (43.0%; 49 of 114). It was also determined that most of the NoV infections occurred in children < 2 years of age (74.6%, 85 of 114).

NoVs were continuously detected throughout the year, but the principal peaks of detection in South Korea were in December, March, and October (Fig. 2). GI NoV infections exhibited a peak in December, corresponding to the winter season, whereas GII NoV infections evidenced two peaks, in March and October, corresponding to the spring and fall, respectively. The peaks for GI NoV infections preceded those for GII NoV infections by at least 3 months.

NoVs, which cause acute gastroenteritis, have emerged as an increasingly urgent public health problem in societies and hospitals. Due to the absence of a cell culture system and experimental animal model for NoVs, the development of sensitive diagnostic methods for the detection of these viruses is required. With the introduction of RT-PCR and ELISA as a diagnostic method, NoV has become to be understood as an important etiologic agent of acute gastroenteritis worldwide (4). Although another previous report was conducted regarding NoV infections in South Korea, in which outbreaks of NoV in Jeju Island, South Korea were described (9), it remains an open question as to whether many various genotypes of NoVs were actually detected in these outbreaks.

Our results demonstrated that the detected NoV belonged to two distinct genogroups, namely genogroup I and II (GI and GII), and these represented 10.5% and 89.5%,
respectively. These findings were concordant with previous epidemiological studies conducted worldwide; NoV GI was consistently present at low incidence in fecal specimens when compared to NoV GII (11, 12). Among the NoV GII isolates, we detected one strain, C1-120 which showed a low identity (79.3%) with the closest references, Nov GII 13 and GII 17, respectively, on the basis of 305 bp region (5085-5389 of Lordsdale virus X86557) of Nov capsid gene (Figure 1B).

The overall frequency (15.0%) of NoV detected in our study is consistent with previous reports regarding the molecular epidemiology of NoV infection worldwide, in which the prevalence falls within a range of 6% to 19% (1, 5, 6). The highest incidence of NoV infection was in the 1-year old group, and the incidence rate decreased with increasing age over 2 years.

In many countries, NoV infections prevail during the winter months (10, 13), though several studies evidenced a peak of seasonal distribution (7, 13). In our study, the principal peaks of NoV infection were in December (in GI) and in March and October (in GII). Thus, the NoV GI and GII infections evidenced very different seasonality characteristics. Obviously, there are three peaks of NoVs infections that cause acute gastroenteritis in South Korea.

However, the seasonality pattern observed in this study must be analyzed with caution, as our study involved only a 1-year detection period; longer periods will be required in order to determine with more accuracy the possible pattern of seasonality.

Our study will be the first large-scale epidemiological study in South Korea showing diverse NoVs genogroups and a potential novel strain from huge samples of 8 hospitals located in a variety of provinces. Nevertheless, continuous epidemiological studies and monitoring of NoV infections in South Korea will be necessary to effectively and efficiently address and solve public health problems in South Korea communities.

**Nucleotide sequence accession number.** The nucleotide sequence data reported in this
article have been deposited at GenBank (accession numbers EU249129-EU249146 and EU442642).

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References


FIGURE LEGENDS

**FIG. 1.** Phylogenetic analysis of identified norovirus based on GI (314 bp region) and GII (305 bp region) of the norovirus capsid gene. A. phylogenetic tree based on GI (314 bp region) of the norovirus capsid gene. B. phylogenetic tree based on GII (305 bp region) of
the norovirus capsid gene. Comparative strains are GI-1/NV-USA1968 (Norwalk, M87661),
GI-2/SOV-GBR1993 (Southampton, L07418), GI-3/DSV-USA1993 (Desert Shield, U04469),
GI-4/Chiba-JPN2000 (AB042808), GI-5/Musgrov-GBR1989 (Musgrove, AJ277614), GI-
6/Hesse-DEU1998(AF093797), GI-7/Wnchest-GBR1994 (Winchester, AJ277609), GI-
8/Boxer-USA2001 (AF538679), GII-1/Hawai-USA1971 (U07611), GII-2/Melksham-
GBR1994 (X81879), GII-3/Toronto-CAN1993 (U02030), GII-4/Bristol-GBR1993 (X76716),
GII-5/Hillingd-GBR1990 (Hillingdon, AJ277607), GII-6/Seacrof-GBR1990 (Seacroft,
AJ277620), GII-7/Leeds-GBR1990 (AJ277608), GII-8/Amstdam-NLD1990 (Amsterdam,
AF195848), GII-9/VABeach-USA1997 (AY038599), GII-10/Erfurt-DEU2000 (AF427118),
GII-11/SW918-JPN1997 (AB074893), GII-12/Wortley-GBR1990 (AJ277618), GII-
13/Faytvil-USA1998 (Fayetteville, AY113106), GII-14/M7-USA1999 (AY130761), GII-
15/J23-USA1999 (AY130762), GII-16/Tiffin-USA1999 (AY502010), GII-17/CSE1-
USA2002 (AY502009), and C1-120 (EU442642).

**FIG. 2.** Seasonality of GI and GII norovirus infections in South Korea, November 2005-
November 2006.