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Norovirus GII/4 Variants Observed in Outbreaks of Gastroenteritis in Miyagi Prefecture between November and December of 2006

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According to the Miyagi Prefectural Epidemiological Surveillance of Infection Agents, the number of weekly reported patients with gastroenteritis caused by Norovirus (NoV) has sharply increased in Miyagi Prefecture since the end of November 2006. The number reached 33.74 cases per clinic, which was about 1.6 times larger than that of last year,

at the end of December 2006.

Between November and December 2006, 16 outbreaks of gastroenteritis were reported to public health and welfare offices in the prefecture and stool specimens were collected from 113 patients. Viral RNA was extracted using a QIAamp Viral RNA Mini Kit (Qiagen, Valencia, Calif., USA) from

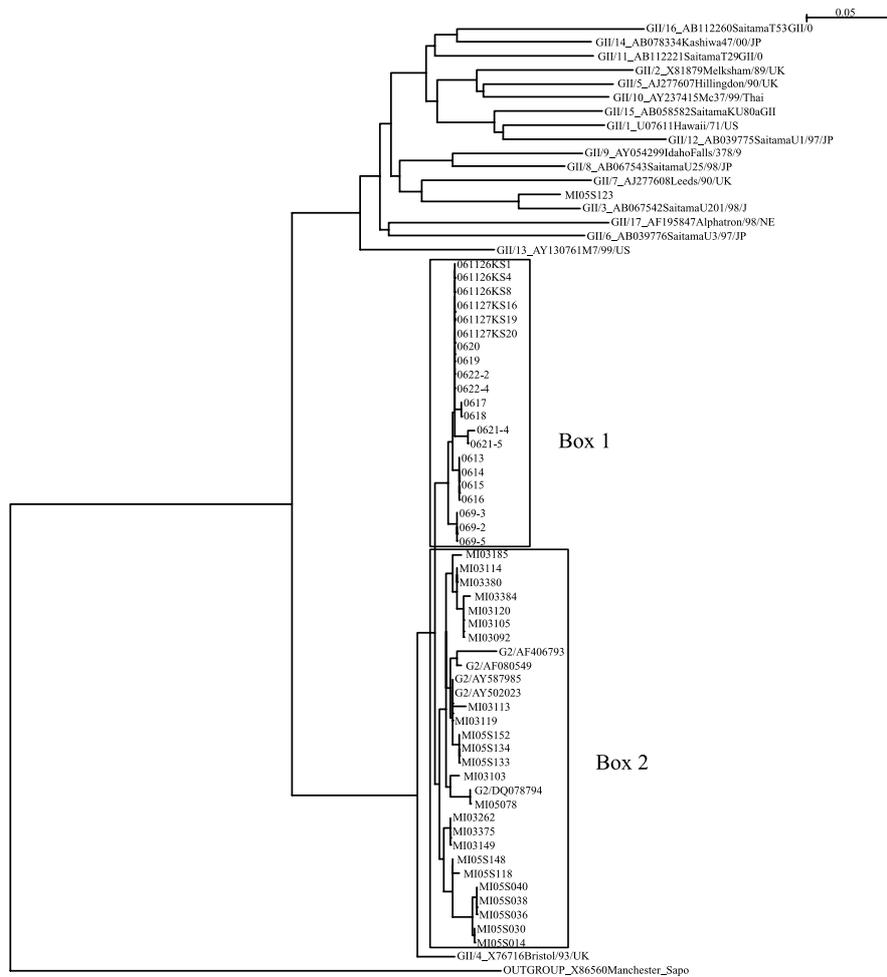


Fig. 1. Phylogenetic tree based on partial nucleotide sequences of the capsid region of NoV genogroup II. The distance was calculated by Kimura's two parameter method, and the tree was plotted by neighbor-joining method. MI03 and MI05 represent the strain of NoV detected in 2003 and 2004.

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10% stool suspensions. Prior to quantitative polymerase chain reaction (PCR) using ABI H7900 (Applied Biosystems, Foster City, Calif., USA), DNase treatment and reverse transcription were performed as previously described (1). Ninety-one of 113 stool specimens were positive for NoV and it was found that all of 16 outbreaks were caused by NoV.

Subsequently, in order to perform phylogenetic analysis of the NoV detected at the outbreaks, 21 stool specimens were randomly selected from 91 positive specimens. Reverse transcriptase (RT)-PCR targeting a partial region of the ORF2 coding viral capsid protein was carried out using a One-step RT-PCR Kit (Invitrogen, Carlsbad, Calif., USA) employing primers of COG2F (1) and G2SKR (2). RT-PCR products were analyzed by 2% agarose gel electrophoresis and visualized by ethidium bromide (EtBr) staining. As a result, gene of NoV GII was detected in all 21 specimens, and gene of NoV GI was done in 2 specimens. Sequences of amplicons were determined using an ABI PRISM 310 (Applied Biosystems) and aligned with Clustal X (free soft). Based on these results, phylogenetic analysis was performed using the neighbor-joining method.

A phylogenetic tree based on partial nucleotide sequences of the capsid region of NoV GII is illustrated in Fig. 1. Box 1 in this figure includes the strains detected in the above specimens collected between November and December 2006. Those strains were GII/4 variants and closely related to each other ($\geq 98.8\%$ nucleotide and $\geq 97.6\%$ amino acid identities). Box 2 in Fig. 1 includes the NoV GII/4 strains detected in patients between 2003 and 2005 in Miyagi Prefecture, and strains detected in patients in the United States (US 95/96;

accession no. AF080549) (3), the United Kingdom (Farmington Hill; accession no. AY502023) (4), Australia (Hunter'04; accession no. DQ078794) (5) and so on. Since the strains shown in Box 1 are obviously distinct from those in Box 2, the outbreaks that occurred in Miyagi Prefecture between November and December 2006 were caused mainly by newly emerging NoV strains belonging to GII/4, which were globally observed (5). This result indicates the necessity of careful surveillance for gastroenteritis caused by NoV GII/4 variants.

This information was widely provided to residents of the prefecture via a website.

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