Several viral strains have been associated with the development of acute gastroenteritis in humans. Norovirus (NoV), rotavirus, and adenovirus 40/41 are the most common causes of sporadic cases of this disease. NoV can be divided into two distinct genogroups, group I (GI) and group II (GII), which can be divided into at least 14 and 17 genotypes, respectively (GI/1-14 and GII/1-17) (1). According to a surveillance report by the Foodborne Viruses in Europe Network, two new GII/4 variants (EU2006a and EU2006b) appeared during the 2005/06 season and were observed globally (2,3). From November to December in 2006, in Japan, outbreaks of acute gastroenteritis caused by NoV GII/4 variant strains were recorded in hospitals, hotels, schools, and nursing homes. In 2006/07, we similarly reported a major increase in the prevalence of GII/4 variant strains were recorded in hospitals, hotels, schools, and nursing homes. In 2006/07, we similarly reported a major increase in the prevalence of GII/4 variant strains (EU2006b > EU2006a) in Nara Prefecture surveillance (4). We phylogenetically analyzed nucleotide sequences of a partial region of the ORF2 to clarify the genetic characteristics of sporadic NoV in Nara Prefecture from April 2007 to March 2008.

Between April 2007 and March 2008, a total of 216 stool specimens were collected from patients with sporadic acute gastroenteritis in Nara Prefecture. NoV was detected in 53 (24.5%) of these specimens, and human group A rotavirus and adenovirus 40/41 were detected in 39 (18.0%) and 13 (6.0%) specimens, respectively. NoV RNAs were extracted using the QuickGene SP kit (FUJIFILM Corp., Tokyo, Japan), and then reverse transcription was performed with ReverTra Ace (TOYOBO, Osaka, Japan). Polymerase chain reaction (PCR) was performed with the COG1/2F and SKG1/2R primer pair. Three of the GI- and 46 of the GII-positive specimens were sequenced using a sequencing kit (Thermo Sequenase Cy5.5 Dye Terminator Cycle sequencing kit; GE Healthcare UK Ltd., Buckinghamshire, UK). We performed a BLAST search for genotyping, and then phylogenetic analysis was performed using the neighbor-joining method with the Kimura 2-parameter model. For phylogenetic analysis of the capsid sequences (282 bp), we included the GenBank sequences with the following accession numbers for reference strains: X76716 (Bristol), AJ004864 (Grimbsby), AY502023 (Farmington Hills), DQ078794 (Hunter284E), EF126964 (Terneuzen70), EF126966 (Nijmegen115), AB294794 (Narashino), EU366113 (Beijing), AB294775 (Matsudo), EU096512 (Mosonmagyarvar2594), AB291542 (Kobe034), AB220925 (Chiba), AB240187 (Hokkaido), and DQ095875 (Nagano).

The distributions of patients with NoV-associated acute gastroenteritis are shown in Figure 1. A peak in NoV activity was observed in early November. Unlike the 2006/07 season, a peak of NoV activity was observed in December 2007, and 18/53 (33.9%) patients had NoV in that month. A similar pattern was observed in the NoV-associated gastroenteritis outbreaks occurring in hospitals, schools, nursing homes, and food contamination cases (data not shown). NoV GII genogroup samples were classified into four types; samples included 1 case of GII/2, 2 of GII/3, 46 of GII/4, and 1 of GII/13. A BLAST search revealed that the strains shared high homology (>98.0% nucleotide) to the GII/Nov750/2004/CAS (EF078288), GII/Yamaguchi18/2004/JP (DQ372862), GII/Narashino/061281/JP, and GII/Osaka659/2006/JP (EF363697) strains, respectively. In detail, the GII/4 NoV gene had high homology to the Nijmegen115/2006/NL, Narashino/061281/2006/JP, and Mosonmagyarvar2594/2006/HUM strains sequence. A phylogenetic tree based on partial nucleotide sequences of the capsid region of NoV GII/4 is illustrated in Figure 2. All NoV GII/4 strains were identified with a GII/4-EU2006b variant. Since November 2006, this EU2006b variant strain has been the dominant strain; it was detected in more than 90% of the cases of GII/4-associated gastroenteritis. Why the GII/4 strains continue to be dominant is unknown. Interestingly, NoV GI was detected in 3 of these 53 specimens, and these were 2 cases of GI/3 strain and 1 of GI/8. This is a change from the lack of
detection of GI strains in 2006/07.

These results show that the infection of GII/4-EU2006b variant strains continued between the 2006/07 and 2007/08 seasons. Our results indicate the necessity for careful monitoring of gastroenteritis caused by the GII/4 variant.

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