Epidemiological Characteristics of Norovirus Associated with Sporadic Gastroenteritis among Children from the 2006/2007 to 2011/2012 Season in Nara Prefecture, Japan

Masaki Yoneda Akiko Okayama Yoshiteru Kitahori

Virology and Epidemiology Division, Nara Prefecture Institute of Health, Sakurai City, Japan

Key Words

Norovirus · Sporadic gastroenteritis · Children

Abstract

The present study aimed to describe the epidemiological characteristics of norovirus (NoV) associated with sporadic gastroenteritis in regional populations of Nara Prefecture, Japan, from the 2006/2007 to 2011/2012 epidemic season. Fecal specimens of sporadic gastroenteritis collected between September 2006 and August 2012 in Nara Prefecture were examined for the presence of NoV by reverse transcription-polymerase chain reaction. The NoV genotype was determined by nucleotide sequence analysis. In total, 274 NoVs associated with sporadic gastroenteritis were identified. We detected 10 different NoV genotypes: GI/3, GI/4, GI/8, GII/2, GII/3, GII/4, GII/6, GII/7, GII/12, and GII/13. A high NoV detection rate of 35.9% was identified in 1-year-old children. Among the 274 NoV isolates, 142 were obtained from males and 131 were obtained from females (the source of one was unknown). The most prevalent genotype was GII/4, accounting for 117 of the 192 different NoVs identified by sequencing. More epidemiological data will be required to determine the epidemiological characteristics of NoVs in other areas of Japan.

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Noroviruses (NoVs) (formerly called Norwalk viruses) are a major cause of acute gastroenteritis in children and adults worldwide. Most children have at least one NoV infection within the first 5 years of life [1]. NoVs are nonenveloped viruses with a single-stranded, positive-sense, polyadenylated RNA genome and belong to the family Caliciviridae along with four other genera (*Vesivirus*, *Lagovirus*, *Sapovirus*, and *Nevovirus*). Studies investigating neutralizing antibodies against NoV have not been possible because of the absence of regular tissue culture systems, however recent advances in NoV sequencing have enabled their genomic characterization. The genome of NoV encodes three open reading frames (ORFs): ORF1, ORF2, and ORF3 [2, 3]. ORF1 encodes nonstructural proteins, ORF2 encodes the major capsid protein, and ORF3 encodes a minor structural protein [2, 3]. The majority of human NoVs are classified into two genogroups, GI and GII, which are further subdivided into more than 30 genotypes on the basis of their capsid and/or polymerase genes [4]. Despite this heterogeneity, 1 genotype, GII/4, currently causes the majority of infections worldwide [5]. Four large pandemics (1995–1996, 2002–2003, 2004–2005, and 2006–2007) have been identified, each corresponding to the emergence of one or two new GII/4 variants [6]. As seen in many countries, the

GII/4 variant 2006b became epidemic during the 2006/2007 epidemic season in Japan [7]. Therefore, understanding the molecular epidemiology of GII/4 is very important.

Since the 2006/2007 season, molecular surveillance of NoV infections has been conducted in Nara Prefecture, Japan [8, 9]. In this study, we describe the epidemiological characteristics of NoV associated with sporadic gastroenteritis in regional populations in Nara Prefecture from the 2006/2007 to 2011/2012 epidemic season. Between September 2006 and August 2012, 1,159 stool samples were collected from patients with acute nonbacterial sporadic gastroenteritis from 13 hospitals in Nara Prefecture. All the patients were <15 years old. This study included only fecal specimens from sporadic cases, excluding outbreaks, as assessed by the pediatrician's interview of the patient. For the 1,159 stool specimens, NoVs ($n = 274$), rotaviruses ($n = 256$), adenoviruses ($n = 37$), and other viruses ($n = 51$) were detected. Of the 274 NoV strains, 75, 47, 40, 45, 33, and 33 strains were obtained during the 2006/2007, 2007/2008, 2008/2009, 2009/2010, 2010/2011, and 2011/2012 epidemic seasons, respectively. In this study, the annual observation period for NoV gastroenteritis began in September and ended in August of the following year.

The fecal specimens were diluted in Hank's balanced salt solution to obtain 10% (weight/volume) suspensions and clarified by centrifugation at 1,500 g for 15 min. The aqueous phase was transferred to a new tube for further purification. Chloroform was added, and the suspension was mixed for 15 s before centrifugation at 1,500 g for 15 min. The upper aqueous phase was used for viral RNA extraction. Viral RNA was extracted from the aqueous phase using the QIAmp Viral RNA Mini Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. The extracted viral RNA was dissolved in nuclease-free water and stored at -80° until use for reverse transcription-polymerase chain reaction (RT-PCR). RT-PCR was performed using the PrimeScript One-Step RT-PCR Kit Version 2 (Takara, Shiga, Japan) according to the manufacturer's instructions. Reactions were performed under the following conditions: initial hold at 50° for 30 min and 94° for 2 min, followed by 40 cycles at 94° for 30 s, 60° for 30 s, and 72° for 30 s. The partial N-terminal capsid region was amplified using the primer pair COG1F [10] and G1SKR [11] for GI viruses or COG2F [10] and G2SKR [11] for GII viruses. When the amplification was insufficient for sequencing, semi-nested PCR was performed using the primer pair G1SKF [11] and G1SKR for GI

viruses or G2SKF [11] and G2SKR for GII viruses. To identify NoV genotypes, direct sequencing was performed. PCR amplicons from NoV-positive specimens were separated by 1.5% agarose gel electrophoresis and purified using the NucleoSpin Extract II Kit (Takara). The purified amplicons were used as templates for direct sequencing. Sequencing of the purified amplicons was performed using the BigDye Terminator Cycle Sequencing Kit and Genetic Analyzer 310 System (Applied Biosystems, Foster City, Calif., USA). Sequencing reactions were performed by an initial denaturation step at 96° for 1 min, followed by 25 cycles of 96° for 10 s, 50° for 5 s, and 60° for 4 min. Nucleotide sequences were aligned using ClustalX, and the distances were calculated by Kimura's two-parameter method. Phylogenetic trees with bootstrap analysis from 1,000 replications were generated by the neighbor-joining method as described previously [4]. Genotype numbers in this study were identified according to Kageyama et al. [4]. GII/4 variants were obtained by phylogenetic clustering with 11 reference strains (see fig. 1). The NoV candidate sequences were deposited in the DDBJ database (<http://www.ddbj.nig.ac.jp>) with the following accession numbers: AB751625 to AB751661.

Previously, we reported the results of an epidemiological study of NoVs in Nara Prefecture between April 2006 and March 2008 [8, 9]. The present study extended these findings by investigating the difference in the epidemic patterns until August 2012. In total, 192 of the 274 confirmed NoV-positive specimens were genotyped. Of these 192 strains, 6 (3.1%) belonged to the GI genogroup and 186 (96.9%) belonged to the GII genogroup. In phylogenetic analyses based on the partial N-terminal capsid region with the reference strains, 10 different NoV genotypes were observed. The GI and GII genogroups were further divided into 3 and 7 genotypes, respectively. In each season, 2–6 genotypes were observed. Only GII/4 was observed in every season. Among the 186 GII strains, 117 strains were classified as GII/4 (62.9%), with GII/2 (14.0%), GII/3 (11.8%), GII/13 (6.5%), and GII/6 (3.2%) accounting for fewer strains. NoV GII/7 and GII/12 were detected in less than 2% of the strains (table 1). The high occurrence of the GII/4 genotype was reported in a recent surveillance of NoV epidemics in other areas of Japan [12] as well as in other countries [13]. The NoV detection rate was 35.9% in 1-year-old children between the 2006/2007 and 2011/2012 season. Of the 274 obtained strains, 142 were isolated from males and 131 were isolated from females (the source of 1 strain was unknown), resulting in a male-to-female ratio of approximately 1.08:1. In this

Fig. 1. Phylogenetic analysis based on a 282-bp sequence of the capsid region of 37 GII/4 sequences detected from the 2009/2010 to 2011/2012 season. The numbers on the branches are the bootstrap values for the clusters. The GenBank accession numbers for reference strains used in this analysis are as follows: Hawaii/71/US, U07611; Loadsdaale, X86557; FarmingtonHills/2002/USA, AY502023;

Chiba/04-1050/2005/JP, AB220921; Hunter284E/040/AU, DQ078814; Terneuzen70/2006/NL, EF126964; Yerseke38/2006/NL, EF126963; Nijmegen115/2006/NL, EF126966; DenHaag89/2006/NL, EF126965; OC07138/07/JP, AB434770; Apeldoorn317/2007/NL, AB445395, and NewOrleans1805/2009/USA, GU445325.

study, 117 GII/4 strains were analyzed. As a result, four GII/4 variants were observed in Nara Prefecture from the 2006/2007 to 2011/2012 season. In addition to the pandemic variant GII/4 2006b, GII/4 sequences clustered with the newly reported variant GII/4 2010 after the 2009/2010 season (fig. 1). Only GII/4 2006b was observed in every season.

NoV has been the etiological agent of many sporadic cases and outbreaks of gastroenteritis in Japan [4, 12, 14,

15]. Despite the limited number of positive samples used to determine seasonality, our results illustrated the great diversity of NoV strains in a limited area of Japan because GI/3, GI/4, GI/8, GII/2, GII/3, GII/4, GII/6, GII/7, GII/12, and GII/13 strains were detected. During the 2008/2009 season, GII/6 emerged as the second most common genotype in different regions of Japan [12, 16], and during the 2009/2010 season, a significant increase in the number of GII/2 strains was observed in

Table 1. NoV genogroups and genotypes isolated in Nara Prefecture from the 2006/2007 to 2011/2012 season

	Season						Total
	2006/2007	2007/2008	2008/2009	2009/2010	2010/2011	2011/2012	
NoVs, n	75	47	40	45	33	34	274
Sequence, %	38 (50.7)	34 (72.3)	21 (52.5)	34 (75.6)	33 (100)	32 (97.0)	192 (70.1)
GI total	0	2	0	3	1	0	6
GI/3	–	1	–	–	–	–	1
GI/4	–	–	–	2	–	–	2
GI/8	–	1	–	1	1	–	3
GII total	38	32	21	31	32	32	186
GII/2	–	–	1	12	–	13	26
GII/3	2	2	–	1	17	–	22
GII/4	36	29	14	15	8	15	117
GII/6	–	–	5	–	–	1	6
GII/7	–	–	1	–	–	–	1
GII/12	–	–	–	–	2	–	2
GII/13	–	1	–	3	5	3	12

Osaka City [17]. Our results indicated that a similar pattern was observed in sporadic cases in Nara Prefecture in both the seasons. Regional studies are necessary to elucidate the distribution of NoVs in Japan. In a recent study in Finland, 49% of infants were infected with NoV by the age of 2 years [18]. Although we could not confirm cases of primary infection in this study, 119 of the 192 sequenced strains (62.0%) were detected in children ≤ 2 years of age. Thus, the multitude of young virus-positive patients suggested the possibility of primary infection in this study. A recent report suggested that GII/4 caused more severe disease than other NoV genotypes in cases of acute gastroenteritis in young children associated with primary infection [19]. In this study, 88 of the 119 sequenced strains (73.9%) were GII/4 strains in children ≤ 2 years of age; therefore, it is necessary to provide appropriate care for severe symptoms. The merit of research in children is that episodes can be examined without the effect of preexisting immunity. Thus, further epidemiological investigations are required to clarify the differences between NoV infections in children. GII/4 is the cause of sporadic cases of pediatric gastroenteritis worldwide [5, 20]. In this study, GII/4 was the most prevalent genotype detected and was identified in 117 of the 192 sequenced strains. The high prevalence of GII/4 (62.9%) is consistent with recent epidemiological studies in other areas of Japan and in other countries [5, 12]. Although our study focused

only on the capsid protein, our results revealed the diversity of GII/4 variants in Nara Prefecture. In Belgium, GII/4 2010 variants were identified in December 2009 [21]. More epidemiological data will be required to determine the implication of this variant in other areas of Japan.

Finally, this study analyzed the epidemiological characteristics of NoV in association with sporadic cases among children in Nara Prefecture and revealed the genetic diversity and age distribution of infection in a local area of Japan. Further studies are required to monitor whether GII/4 2010 variants will change in future. In Japan, a few local institutions are conducting detailed genetic analyses of NoVs. Accumulation of such data in local institutions is important for knowing the distribution of the new GII/4 variant not only in Japan but also in other parts of the world.

Acknowledgement

The collection of specimens was strongly supported by participants in the Infection Disease Surveillance in Nara Prefecture. We would like to express our deep thanks to them.

Disclosure Statement

The authors have no conflicts of interest to disclose.

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