Molecular epidemiology of norovirus GII.4 variants in children under 5 years with sporadic acute gastroenteritis in South Korea during 2006–2013

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ABSTRACT

Background: The global emergence of norovirus (NoV) GII.4 variants has raised public concerns in the world including South Korea since 1996.
Objective: We analyzed seasonality and genotypic pattern for sporadic cases by norovirus GII-4 variants.
Study design: To determine the epidemic status of GII.4 variants in South Korea during 2006–2013, 7301 fecal specimens were collected from children who were younger than 5 years and had sporadic acute gastroenteritis (AGE).
Results: During the study period, NoVs were the most prevalent viral agent, detected in 877 (12.0%) of the 7301 fecal specimens from children with sporadic AGE. NoV GII strains predominantly accounted for 97.6% of all sporadic NoV infections. NoV GII.4 was the most prevalent genotype and comprised 67.6% of the NoV GII strains. However, seasonal prevalence of GII.4 strains varied depending on the spread of GII.4 variants. GII.4-2006b variant most predominantly circulated from 2006–2007 to 2009–2010 and persisted during other seasons. GII.4-2009 variant was first detected in January 2010 and predominant in 2011–2012. However, it was rapidly displaced by GII.4-2012 variant, which emerged in May 2012 and substantially circulated in 2012–2013.
Conclusions: The frequent emergence and rapid spread of GII.4 variants significantly affect the magnitude of sporadic NoV infections in children. Hence, to minimize the disease burden of NoV infections, GII.4 strains should be considered as a primary target for vaccine development against NoVs.

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1. Background

Noroviruses (NoVs) are the leading viral agent of acute gastroenteritis (AGE) and are responsible for 12% of sporadic AGE cases in children across the world [1]. The transmission of NoVs occurs year-round, but epidemic peaks are commonly observed during the cold season worldwide [2]. NoVs can infect persons of all ages, and the highest infection rate is observed in young children [3]. The primary transmission route is known to be fecal-to-oral spread [4]. However, NoVs can be rapidly spread through asymptomatic persons shedding virus, infectious vomit, contacts with contaminated environmental surface and aerosolization along with waterborne- and foodborne-transmission [4].

NoVs, belonging to the *Caliciviridae* family, are positive-sense single-stranded RNA viruses [5]. NoVs are classified based on amino acid variation of the major structural protein VP1, divided into five genogroups (GI–GV), and humans can be infected by GI, GII, and GIV, which are further subdivided into genotypes [6,7]. Among them, NoV GII is the predominant genogroup, accounting for 75–100% of sporadic NoV infections [1,8]. Recent molecular epidemiological studies have shown that GII.4 strains had a global

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Table 1
Seasonal prevalence of NoVs detected from hospitalized children with sporadic acute gastroenteritis in Gyeonggi province, South Korea, from 2006–2007 to 2012–2013.

NoV geno group	No. of specimen in sporadic AGE (%)							Total
	2006/2007[a]	2007/2008	2008/2009	2009/2010	2010/2011	2011/2012	2012/2013	
Total	1197	1317	1033	814	947	836	1157	7301
NoV (%)	99 (8.3)	238 (18.1)	167 (16.2)	99 (12.2)	96 (10.1)	66 (7.9)	112 (9.7)	877 (12.0)
GI (%)	2 (2.0)	5 (1.9)	4 (2.4)	–	1 (1.0)	1 (1.5)	4 (3.6)	17 (1.9)
GI + GII[b] (%)	–[c]	1 (0.4)	2 (1.2)	1 (1.0)	–	–	–	4 (0.5)
GII (%)	97 (98.0)	232 (97.5)	161 (96.4)	98 (99.0)	95 (99.0)	65 (98.5)	108 (96.4)	856 (97.6)

[a] Season was defined as 12-month period from the previous September to the following August.
[b] GI + GII indicated a mixed infection that NoV GI and NoV GII strains were detected in a specimen simultaneously.
[c] '–' denoted 'not detected'.

distribution, accounting for approximately 70–80% of sporadic NoV infections [8–10].

Rapid spread of new GII.4 variants has been periodically reported since 1996 [11]. The frequent emergence of novel GII.4 variants has been attributed to rapid evolution and antigenic variation in response to herd immunity [10,12,13]. The protruding (P) 2 domain in ORF2 of NoV genome is the most variable region, and provides potential binding sites for neutralizing antibodies and histo-blood group antigen (HBGA) ligands [8,10,12,14,15].

In South Korea, NoV has been regarded as a major viral agent, with GII.4 strains being the most prevalent genotype in sporadic AGE during the early 2000s [16,17]. Several studies also revealed the emergence of NoV GII.4-2006b and GII.4-2007 variants [18–20]. However, the emergence of GII.4-2009 and recent GII.4-2012 variants have not been documented, and the epidemic changes of GII.4 variants have not been fully estimated on a long-term scale.

1.1. Objectives

This study was performed to analyze the molecular epidemiological characteristics of NoV GII.4 variants in children with sporadic AGE from 2006–2007 to 2012–2013 in the Gyeonggi province, a highly populated area that houses 24% of the South Korean population.

2. Study design

2.1. Collection of fecal specimens

All of the 7301 clinical stool specimens from children younger than 5 years were collected from four collaborating general hospitals in Gyeonggi province, South Korea, from September 2006 to August 2013 (Table 1). Fecal specimens were transported to the division of virology at the Gyeonggi Provincial Research Institute for Public Health and Environment (GIHE) for the identification of viral pathogens as previously reported [16]. The samples were obtained from the KNRRC waterborne virus bank [21].

2.2. RNA extraction and RT-PCR

The fecal specimens were diluted to 10% suspensions with phosphate-buffered saline (PBS) and clarified by centrifugation at $800 \times g$ for 15 min. Viral RNA was extracted from the fecal supernatant using the QIAamp viral RNA mini-kit (QIAGEN, CA, USA) according to the manufacturer's instructions. For the detection and genotyping of NoVs, semi-nested RT-PCR was performed using specific primer sets (NV-GIF1 M/NV-GIR1 M/NV-GIF2 for NoV GI; NV-GIIF1 M/NV-GIIR1 M/NV-GIIF3 M for NoV GII), targeting the capsid gene (Region C) as previously described [22]. To obtain the nucleotide sequence of the P2 domain, we amplified a 674 bp fragment (positions 5730–6403 of NoV AY502023) with the forward primer EVP2F and the reverse primer EVP2R as previously reported [23]. The amplified fragments were purified and then cloned into

the pGEM-T Easy vector (Promega, WI, USA) according to the manufacturer's recommendations. Plasmids that included sequences of the P2 domain were purified and then used for further sequencing.

2.3. Phylogenetic analysis

Genotyping of NoV GII strains was conducted by phylogenetic analysis of the capsid gene, using the neighbor joining algorithm with the Kimura two-parameter model with 1000 bootstrap replicates in the MEGA software v. 5.0 with 17 reference strains reported by Kageyama et al. [6]. GII.4 variants were determined by using the nucleotide sequence of the P2 domain and an automated typing tool (available at http://www.rivm.nl/mpf/norovirus/typingtool) [24]. The nucleotide sequences of the P2 domain used in this study were deposited in the GenBank sequence database (accession nos. KM268114–KM268121).

3. Results

3.1. Seasonal prevalence of sporadic NoV infections

During the study period, NoVs were the most prevalent viral agent, detected in 877 (12.0%) of the 7301 fecal specimens from children with sporadic AGE. On the other hand, group A rotavirus, enteric adenovirus, astrovirus, and sapovirus constituted 10.2% ($n = 747$), 1.5% ($n = 110$), 0.5% ($n = 35$) and 0.02% ($n = 2$), respectively, of the total cases (data not shown). The seasonal prevalence of NoVs varied depending on the season. The highest seasonal prevalence of NoVs was identified as 18.1% in 2007–2008, gradually decreased to 7.9% in 2011–2012 and recently re-increased to 9.7% in 2012–2013 (Table 1). The epidemic peaks of NoVs were apparently observed during the cold season in South Korea, from November to February (Fig. 1). Therefore, to compare the seasonal prevalence of NoVs, an epidemic season was defined as the 12-month period from the previous September to the following August when the epidemic peak of NoVs was centralized.

3.2. Seasonal prevalence of the NoV GII.4 genotype

NoV GII strains significantly contributed to the seasonal epidemics of NoVs throughout the study period. Based on the NoV genogroup (G), NoV GII predominantly accounted for 97.6% of all sporadic NoV infections followed by 1.9% of NoV GI and 0.5% of NoV GI + GII mixed cases (Table 1). Of 860 NoV GII strains, 682 (79.3%) samples were successfully sequenced and used for phylogenetic analysis to determine the NoV GII genotype [6]. NoV GII.4 was the most prevalent genotype and comprised 6.3% (461/7301) of the sporadic NoV prevalence. On the other hand, the total prevalence of other genotypes of NoV GII strain accounted for 3.0% (221/7301). NoV GII.3 was the second most prevalent genotype as 1.6% ($n = 117$), followed by GII.2 ($n = 19$, 0.3%), GII.5 ($n = 13$, 0.2%), GII.6 ($n = 14$, 0.2%), GII.7 ($n = 1$, 0.01%), GII.8 ($n = 16$, 0.2%), GII.11 ($n = 1$, 0.01%), GII.12 ($n = 5$, 0.1%), GII.13 ($n = 3$, 0.04%), GII.14 ($n = 18$,
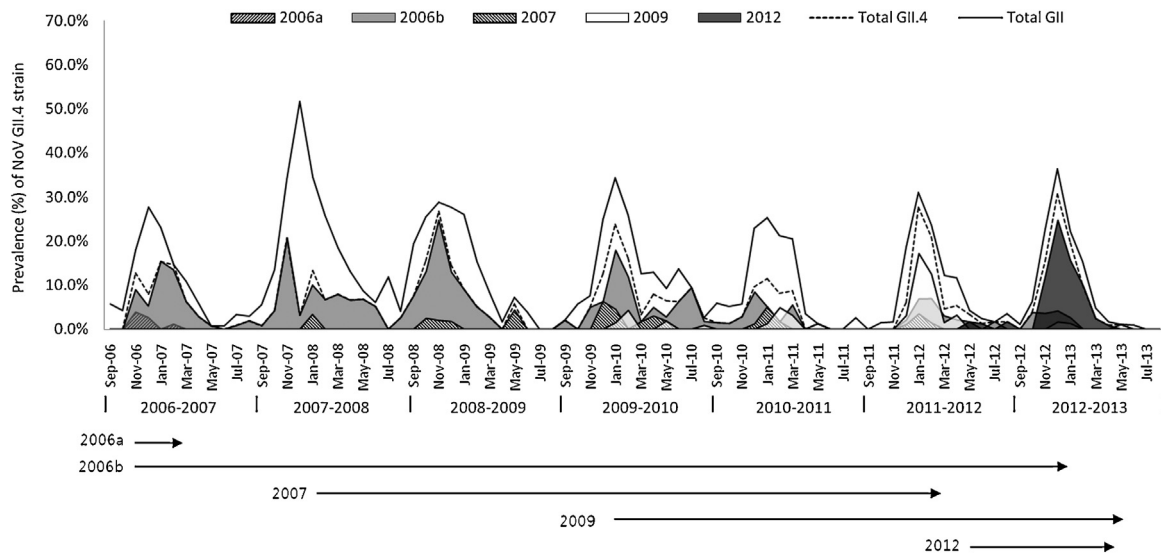
Fig. 1. Epidemic changes of GII.4 variants detected from children with sporadic acute gastroenteritis in Gyeonggi province, South Korea, from 2006–2007 to 2012–2013. The epidemic durations of each GII.4 variant are denoted with arrows below the scheme. The dotted and bold lines indicate the total prevalence of the GII.4 and GII strains, respectively.

0.2%), GII.16 ($n = 3$, 0.04%), GII.17 ($n = 6$, 0.1%) and not-assigned strains (GQ925181-like) ($n = 5$, 0.1%) were observed, respectively. The seasonal prevalence of the GII.4 strains was dynamically changed, ranging from 4.0% to 8.8%. It had been steadily increased to 8.8% in 2009–2010, sharply decreased to 4.0% in 2010–2011 and re-increased to 7.1% in 2012–2013 (Table 2).

3.3. Epidemic changes of the NoV GII.4 variants

To determine the cause of epidemic changes of NoV GII.4 strains, we analyzed the genetic variants of GII.4 strain using the nucleotide sequence of the P2 domain. Five GII.4 variants were identified during the study period (Table 2). GII.4-2006a variant was exclusively identified in the first season as 0.5% in 2006–2007 and its total prevalence, 0.1%, was the lowest among other GII.4 variants. GII.4-2006b was the most prevalent variant and constantly detected during the study period; it extensively circulated from 2006–2007 to 2009–2010, ranging from 3.9% to 7.7%, and then their prevalence steadily reduced to 1.1% in 2012–2013 (Table 2 and Fig. 1). GII.4-2007 variant first emerged in January 2008 and was lastly identified in February 2012 (Fig. 1). However, the seasonal prevalence of GII.4-2007 variant peaked as 1.5% in 2009–2010, relatively

Table 2
Seasonal proportion of NoV GII.4 variants detected from hospitalized children with sporadic acute gastroenteritis in Gyeonggi province, South Korea, from 2006–2007 to 2012–2013.

NoV		Genbank accession no	No. of norovirus strain from sporadic AGE (%)							Total
			2006/2007	2007/2008	2008/2009	2009/2010	2010/2011	2011/2012	2012/2013	
Total		Tested	1197	1317	1033	814	947	836	1157	7301
GII strains[a]		Positive	97 (8.1)	233 (17.7)	163 (15.8)	99 (12.2)	95 (10.0)	65 (7.8)	108 (9.3)	860 (11.8)
		Sequenced[b]	68 (5.7)	156 (11.8)	124 (12.0)	87 (10.7)	84 (8.9)	62 (7.4)	101 (8.7)	682 (9.3)
GII.4	Subtotal (%)		53 (4.4)	83 (6.3)	90 (8.7)	72 (8.8)	38 (4.0)	43 (5.1)	82 (7.1)	461 (6.3)
	2006a[c]	EU078419	6 (0.5)	–	–	–	–	–	–	6 (0.1)
	2006b	EU078417	47 (3.9)	80 (6.1)	80 (7.7)	52 (6.4)	23 (2.4)	16 (1.9)	13 (1.1)	311 (4.3)
	2007	AB445395	–	3 (0.2)	10 (1.0)	12 (1.5)	6 (0.6)	3 (0.4)	–	34 (0.5)
	2009	GU445325	–	–	–	8 (1.0)	9 (1.0)	21 (2.5)	4 (0.3)	42 (0.6)
	2012	JX459908	–	–	–	–	–	3 (0.4)	65 (5.6)	68 (0.9)
GII.others[d]	Subtotal (%)		15 (1.3)	73 (5.5)	34 (3.3)	15 (1.8)	46 (4.9)	19 (2.3)	19 (1.6)	221 (3.0)
	2	U70059	1 (0.1)	2 (0.2)	4 (0.4)	1 (0.1)	2 (0.2)	9 (1.1)	–	19 (0.3)
	3	U02030	3 (0.3)	56 (4.3)	12 (1.2)	1 (0.1)	30 (3.2)	8 (1.0)	7 (0.6)	117 (1.6)
	5	AJ277607	8 (0.7)	3 (0.2)	–	1 (0.1)	1 (0.1)	–	–	13 (0.2)
	6	AJ277620	–	–	4 (0.4)	4 (0.5)	4 (0.4)	1 (0.1)	1 (0.1)	14 (0.2)
	7	AJ277608	–	–	–	–	1 (0.1)	–	–	1 (0.01)
	8	AJ277618	–	–	12 (1.2)	1 (0.1)	2 (0.2)	–	1 (0.1)	16 (0.2)
	11	AY038599	1 (0.1)	–	–	–	–	–	–	1 (0.01)
	12	AY130761	1 (0.1)	3 (0.2)	–	1 (0.1)	–	–	–	5 (0.1)
	13	AF427118	–	3 (0.2)	–	–	–	–	–	3 (0.04)
	14	AY113106	–	–	2 (0.2)	6 (0.7)	4 (0.4)	1 (0.1)	5 (0.4)	18 (0.2)
	16	AB112260	1 (0.1)	1 (0.1)	–	–	–	–	1 (0.1)	3 (0.04)
	17	AY502009	–	–	–	–	2 (0.2)	–	4 (0.3)	6 (0.1)
	NA[e]	GQ925181	–	5 (0.4)	–	–	–	–	–	5 (0.1)

[a] GII strains included the NoV GII strains from both NoV GII and NoV GI + GII mixed infection cases.
[b] Sequenced means the number of NoV GII strains, which was successfully sequenced and genotyped.
[c] GII.4 variants strains.
[d] GII.others includes non-GII.4 genotypes among NoV GII strains [6].
[e] NA denoted 'not assigned'.

lower than those of the other GII.4 variants. GII.4-2009 variant was first detected in January 2010 and its prevalence peaked to 2.5% in 2011–2012, but dramatically decreased to 0.3% in 2012–2013. Recently, GII.4-2012 variant emerged in May 2012 and was predominantly detected as 5.6% in 2012–2013 (Table 2 and Fig. 1).

4. Discussion

Most epidemics of NoV infections have been associated with the emergence of several novel GII.4 variants worldwide during the last decade [8,9,11]. In this study, epidemic changes in NoV GII.4 variants in sporadic NoV infections, especially in the case of children younger than 5 years, were observed in Gyeonggi, South Korea, during 2006–2013. Five GII.4 variants frequently emerged and showed dynamic circulation; GII.4-2006b variant predominantly circulated during the early seasons of the study period and were subsequently displaced by GII.4-2009 variant and the most recent GII.4-2012 variant. On the other hand, GII.4-2006a and GII.4-2007 variants were not predominant during their epidemic seasons.

The emergence of GII.4-2006b and GII.4-2007 variants was reported in different regions of South Korea, in 2007–2008 and in 2008–2009, respectively [18–20]. However, GII.4-2006b and GII.4-2007 variants were first identified in 2006–2007 and in 2007–2008 in this study, which was one season earlier than previous reports [18–20]. Furthermore, GII.4-2009 and GII.4-2012 variants first emerged in South Korea a few months after the first global reports as shown [23,25]. This suggests that, in spites of fecal-to-oral spread, newly emerging GII.4 variants might be rapidly imported into South Korea as like global surveillance data [8,9].

As previously reported in other countries, the seasonal prevalence of the GII.4 strains in South Korea varied depending on the spread of GII.4 variant [9,13]. GII.4-2006a variant was detected with low frequency in 2006–2007, which was consistent with previous reports showing that it was rarely detected in Asian countries [8,9]. GII.4-2006b variant was the most influencing GII.4 variant in this study. They were likely to have successful transmission in 2006–2007, dominantly circulated between 2007–2008 and 2009–2010 and consistently detected during subsequent seasons as reported [8,23,26,27]. However, GII.4-2007 variant never became predominant during the epidemics seasons [27]. Several studies suggested that GII.4-2007 variants might not successfully escape from herd immunity formed by GII.4-2006b variants [10,12,13,28]. GII.4-2009 variant more successfully escaped from pre-existing herd immunity than GII.4-2007 variant as reported [28,29]. Recently, GII.4-2012 variant became predominant in 2012–2013, consistent with what happened globally [25,27]. These suggest that novel GII.4 variants frequently emerged and simultaneously circulated with pre-existing GII.4 variants, but their epidemic levels were substantially different depending on the GII.4 variant.

So far, it is unclear why some GII.4 variants become pandemic whereas others do not [9]. Previous studies suggested that the different epidemic levels of GII.4 variants may be determined by various factors such as antigenic variation, the level of accumulated herd immunity, and host ligand-binding properties [9,12,14,28,30]. Other factors, climate factors like temperature, hygiene level of population, spread of other genotypes and virulence of each GII.4 variant, may also affect the epidemic levels of GII.4 variants [27]. To explore the different epidemic levels of GII.4 variants, we previously reported the antigenic variations of the full VP1 region of GII.4 variants used in this study [31]. We assume that differing antigenicity to the pre-existing GII.4 variants may be the one of determinant factor for the epidemic level of newly emerging GII.4 variants as observed in GII.4-2009 and GII.4-2012 variants [29,32]. The duration of herd immunity caused by pre-existing GII.4 variants may

be another decisional factor to explain the various epidemic levels of GII.4 variants. In fact, a recent study proposed that the duration of herd immunity to NoVs was estimated to 4–9 years, which is longer than that previously reported [3]. However, long-persisted spread of GII.4-2006b variant in this study could not be completely explained by the extended-duration of herd immunity. Different HBGA-binding affinity of GII.4 variants may also affect the spread of each GII.4 variants [30]. However, epidemiological information for host receptor, i.e. ABH or Lewis blood types, was not obtained from clinical samples in this study. Therefore, further epidemiological studies how pre-existing herd immunity or ligand-binding property of GII.4 variants influence the spread of GII.4 variants are needed to elucidate the different epidemic patterns of GII.4 variants.

In conclusion, frequent emergence and spread of GII.4 variants significantly affected the prevalence of sporadic NoV infections in children in South Korea from 2006 to 2013. Recently, Desai et al. [33] reported that NoV GII.4 infections were associated with increased hospitalization and mortality. Hence, to reduce the disease burden of NoV infections, targeted vaccines for GII.4 strains should be developed urgently.

Competing interests

None declared.

Ethical approval

Not required.

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