MAJOR ARTICLE

Impact of Genotype-Specific Herd Immunity on the Circulatory Dynamism of Norovirus: A 10-Year Longitudinal Study of Viral Acute Gastroenteritis

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(See the editorial commentary by Vesikari and Blazevic on pages 853–5, and the major article by Bernstein et al on pages 870–8.)

Human norovirus is a major cause of viral acute gastroenteritis worldwide. However, the transition of endemic norovirus genotypes remains poorly understood. The characteristics of natural immunity against norovirus are unclear because few studies have been performed in the natural infection setting. This prospective 10-year surveillance study of acute gastroenteritis in the province of Osaka, Japan, revealed that norovirus spread shows temporal, geographic, and age group–specific features in the humans. Genogroup II genotype 4 (GII.4) was detected in most sporadic pediatric cases, as well as in foodborne and nursing home outbreaks, respectively. The dominant genotypes in outbreaks at childcare facilities and schools shifted every season and involved GI, GII.2, GII.3, GII.4, and GII.6. Evidence at both the facility and individual levels indicated that genotype-specific herd immunity lasted long enough to influence the endemic norovirus genotype in the next season. Thus, norovirus circulates through human populations in a uniquely dynamic fashion.

Keywords. genotype; herd immunity; longitudinal surveillance; norovirus; reinfection.

Norovirus (NoV) is the most common causative agent of viral acute gastroenteritis (AG) worldwide. People of all ages are at risk of NoV infection, and it spreads easily by person-to-person contact via the fecal–oral route. Thus, much research has investigated the controlling of NoV outbreaks in school settings, hospitals, and nursing homes [1–4]. The genus *Norovirus* has >30 genotypes [5, 6]. Epidemiological studies show that the pandemics in recent years were caused by NoV genogroup II genotype 4 (GII.4) [3, 7, 8]. These pandemics are associated with antigenic drift in NoV, and molecular epidemiological studies suggest that this drift is promoted by the mutation-prone nature of NoV [9–11].

Human immunity against NoV is relatively short-lived, as volunteer studies show that protective immunity against NoV lasts 4–16 weeks [12–15]. However, infected volunteers do not develop symptoms when challenged with the same strain 6 months after infection [16], indicating that anti-NoV immunity may last longer than half a year. Humoral immunity against NoV is difficult to study because no tissue culture system to grow NoV is currently available. Nevertheless, high anti-NoV antibody titers correlate with protection from subsequent infection with the same genotype [17]. Moreover, longitudinal studies in children reveal that the titer of antibodies against NoV GI.1 (Norwalk virus) increases with age [17, 18]. Also, norovirus has been detected repeatedly in some children <2 years old who remained asymptomatic [19]. Despite this, NoV repeatedly causes pandemics,

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probably because of antigenic drift [20]. Thus, the extent to which herd immunity influences the epidemics of other NoV genotypes remains unclear.

To improve our understanding of NoV infection, a long-term (10-year) molecular epidemiological study involving systematic surveillance of all age groups and routes of infection was performed in the province of Osaka, Japan.

MATERIALS AND METHODS

Specimens

Stool specimens were collected in the Osaka prefecture, Japan, under 3 surveillance systems: a sporadic pediatric infection surveillance system established in 1981, an AG outbreak surveillance system established in April 2005 to monitor human-to-human disease outbreaks, and a foodborne-disease outbreak surveillance system for viral gastroenteritis established in 1997. The last 2 systems were based in regional healthcare centers. The pediatric infection surveillance network was performed with 13 sentinel hospitals and about 200 hospitals. In this study, AG of both sporadic and outbreak NoV cases was analyzed between April 2002 and March 2012.

A human-to-human disease outbreak was defined as an outbreak in various facilities where >10 residents a day or more than half of all residents developed severe symptoms. All nurseries, educational institutions, and nursing homes in Osaka prefecture were obligated to report such outbreaks to their regional healthcare center, and the healthcare center officials are required to collect stool specimens from the affected individuals for investigation. Between April 2005 and March 2012, 52 902 patients with AG who were involved in 1852 outbreaks due to human-to-human contact were reported to healthcare centers. Of these, 489 outbreaks involving at least 15 819 patients in childcare and educational facilities and 312 outbreaks involving 10 240 patients in nursing homes were analyzed in this study. For the foodborne NoV outbreaks, we analyzed 366 outbreaks (2907 specimens) between April 2002 and March 2012 in individuals aged 15–64 years.

Correlation Analysis of Age and the Duration of Symptoms

A total of 1468 children aged 0–14 years were involved in 63 human-to-human NoV outbreaks that occurred in childcare and educational facilities between 2005 and 2010. Symptoms were defined as diarrhea, nausea, vomiting, and abdominal pain. The NoV genotype of each outbreak was determined by representative specimens. The correlation between age and the duration of symptoms was examined by Pearson correlation coefficient test.

Detection of Viruses

Laboratory diagnosis was performed at Osaka Prefectural Institute of Public Health. Each stool specimen was diluted with physiological saline to generate a 10% stool suspension and clarified by centrifugation at 15 000 rpm for 8 minutes at 4°C. The nucleic acids were extracted from 200 μL of stool suspension using the Magtration System Viral RNA Extraction Kit (Precision System Science) with Magtration 6GC or 12GC equipment (Precision System Science). NoV RNA was detected by reverse transcription polymerase chain reaction (RT-PCR) with the G1SKF/G1SKR and G2SKF/G2SKR primers, which target the capsid regions of the virus [21]. The RT and PCR reactions were performed using MuLV Reverse Transcriptase (Life Technologies) and EX Taq (Takara), respectively. To detect rotavirus A and adenovirus 40/41, the clarified 10% stool suspensions were subjected to ELISA (Fujirebio Inc.). Sapovirus and group C rotavirus were detected by RT-PCR as described previously [22, 23]. Mamastrovirus 1 was detected by RT-PCR using the AC4/AC6 primers [24] and the in-house primer AC4′ (5′-tcc agr cct cga ctg aag ag-3′).

Sequence and Phylogenetic Analysis of NoV

From each NoV outbreak, 2 randomly selected specimens known to be NoV-positive were amplified by PCR as described above for sequence analysis. The nucleic acid sequence of the PCR product was determined using the Dye-Deoxy Terminator Cycle Sequencing kit version 1 (Life Technologies). The sequence spanning the N/S domain of the capsid gene was used for phylogenetic analysis [6, 25]. The NoV genotyping tool version 10 was also used to identify the NoV genotype called NoroNet (http://www.rivm.nl/mpf/norovirus/typingtool).

Real-time RT-PCR

Real-time quantitative PCR (qPCR) was performed by adopting the TaqMan probe RT-PCR system using the ReverTra Ace qPCR RT kit (Toyobo) and Real-time PCR Master Mix (Toyobo) as described previously [26]. For quantitation, a plasmid containing the cloned target region was used.

Ethical Approval

The study protocol was approved by the ethics committee of the Osaka Prefectural Institute of Public Health (No. 0710-03).

RESULTS

Overview of the Epidemiological Data and Classification of the Specimens

We categorized specimens into 4 groups depending on patient age and the mode of infection (Table 1, Figure 1, and Supplementary Data 1). The peaks in norovirus outbreak occurred during the winter (Supplementary Data 2). Our analyses likely reflect the general trend of norovirus endemics because the number of examined specimens paralleled the total number of reported cases (Supplementary Data 2).

Group A included 2644 specimens of sporadic pediatric diarrhea cases (<15 years of age), 48.9% (1293/2644 specimens) of which contained viral pathogens. NoV was the most common

Table 1. Summary of the Surveillance Data After Categorization Into 4 Age and Transmission Mode Groups

Group	Age, y	Infection Route	No. of Analyzed NoV Outbreaks in This Study (%)	No. of Analyzed Specimens	No. of Specimens Positive for Norovirus (%)	Norovirus Genotypes	
						GI	GII
A	0–14	Sporadic	. . .	2644	683 (25.8)	1, 3, 4, 6	1, 2, 3, 4, 5, 6, 12, 14
B	0–14	Human-to-human	489 (63.0)	2437	1973 (81.0)	3, 4, 6, 7	2, 3, 4, 5, 6, 7, 12, 13, 14, 16, 21
C	15–64	Foodborne	366 (58.0)	2907	1901 (65.4)	1, 2, 3, 4, 5, 6, 7, GI/12[a]	2, 3, 4, 5, 6, 7, 9, 10, 12, 13, 14, 16, 21
D	>65	Human-to-human	312 (29.3)	1783	1186 (66.5)	3, 6	1, 2, 3, 4, 6, 14

Abbreviation: NoV, norovirus.

[a] GI/12 that was typed by Kageyama et al [5] is not assigned by NoroNet.

viral pathogen, accounting for 52.7% of the group A specimens (682/1293), followed by rotavirus (26.2%).

Group B included 2437 specimens from the pediatric cases in the 489 human-to-human contact AG outbreaks in the child-care facilities and schools. All group B subjects were <15 years old. Of these, 65.2% (319/489) occurred in nursery schools or kindergartens and 34.8% (170/489) occurred in elementary or junior high schools. Contained NoV was 81.0% (1973/2437). Analysis of the seasons shows that the number of NoV outbreaks was particularly high in 2009–2010 and 2010–2011.

Group C included 2907 specimens from the subjects involved in the 366 foodborne outbreaks aged 15–64. NoV was detected in 65.4% (1901/2907) of the specimens collected from the affected individuals and food handlers.

Group D included the 1783 specimens from 312 human-to-human contact disease outbreaks that occurred mainly in nursing homes (foodborne outbreaks were excluded). All subjects were >65 years old. NoV was detected in 66.5% (1186/1783) of the specimens collected from affected individuals.

Annual NoV Genotype Trends

The most prevalent genotype in group A (children, sporadic) was GII.4. The genotype was predominant in 8 of the 10 seasons (Figure 1), as 46.7%–90.1% of the specimens tested positive for GII.4 among those 8 seasons. The second most prevalent genotype was GII.3. This genotype dominated the 2005–2006 and 2010–2011 seasons, with GII.3-positive specimen frequencies of 50.0% and 47.7%, respectively. GII.2 was the second most common NoV genotype (21.7%) in the 2009–2010 season. Other genotypes, including GI, GII.6, and GII.14, were in the minority in each of the studied seasons. GII.14 outbreaks were first detected in the 2005–2006 season.

Group B (children, human-to-human) was characterized by alterations in the dominant genotype almost every season (Figure 1). GII.2 was the most prevalent genotype in the 2004–2005, 2005–2006, 2009–2010, and 2011–2012 seasons, with 36.4%–100% of the specimens tested positive for GII.2 during these seasons. This is notable because endemic GII.2 has rarely been reported since the GII.2 genotype representative strains were first discovered by Melksham in 1989 [27]. GII.3 was predominant in the 2010–2011 season (40.7% of the outbreaks) and was responsible for 10%–20% of the NoV-positive outbreaks in the 2003–2004, 2005–2006, and 2007–2008 seasons. GII.2 and GII.3 spread in a regional fashion in some seasons. For example in 2005–2006, 60.0% of the specimens from the northern area of Osaka province contained GII.2. In the same season, only 22.6% of the specimens tested positive for GII.2 in the southern areas of Osaka province whereas GII.3 dominated. The GII.3 detection rates showed the opposite pattern in the 2010–2011 season. A total of 23.1% and 73.5% of the specimens from the northern and southern areas, respectively, harbored GII.3, and GII.4 dominated in the northern area (data not shown). GI and GII.6 were the most prevalent genotypes in the 2007–2008 and 2008–2009 seasons, respectively. GII.4 was notable in the 2002–2003 and 2006–2007 seasons, which is when new GII.4 strains spread worldwide [3, 8]. GII.14 was first detected in group B in 2006–2007, a year after this genotype was first detected in group A. In both groups A and B, GII.2 was relatively dominant in the 2009–2010 season, and GII.3 was relatively dominant in the 2003–2004, 2005–2006, and 2010–2011 seasons.

In group C (adults, foodborne), GII.4 was the dominant genotype (Figure 1), as 7.5%–70.3% of the specimens were positive for this genotype over the 10 seasons. Group C was unique in that multiple minor genotypes were detected, as we identified 9 GI and 12 GII genotypes during the study period (Table 1). Consistent with these findings, various NoV GI and GII genotypes were detected in the sewerage water of Japan between 2006 and 2008 [28]. Of these, GI, GII.2, and GII.3 were detected constantly at rates of 4%–29%. In group C, GII.14 outbreaks were detected in the 2007–2008 season.

In group D (elderly, human-to-human), GII.4 was the most prevalent genotype throughout the study period and was responsible for 72.2%–100% of the NoV-positive specimens in each season (Figure 1) [29]. Non-GII.4 genotypes were detected in a small number of outbreaks, although no apparent trend was observed. Outbreaks of GII.14 were detected in the 2010–2011 season.

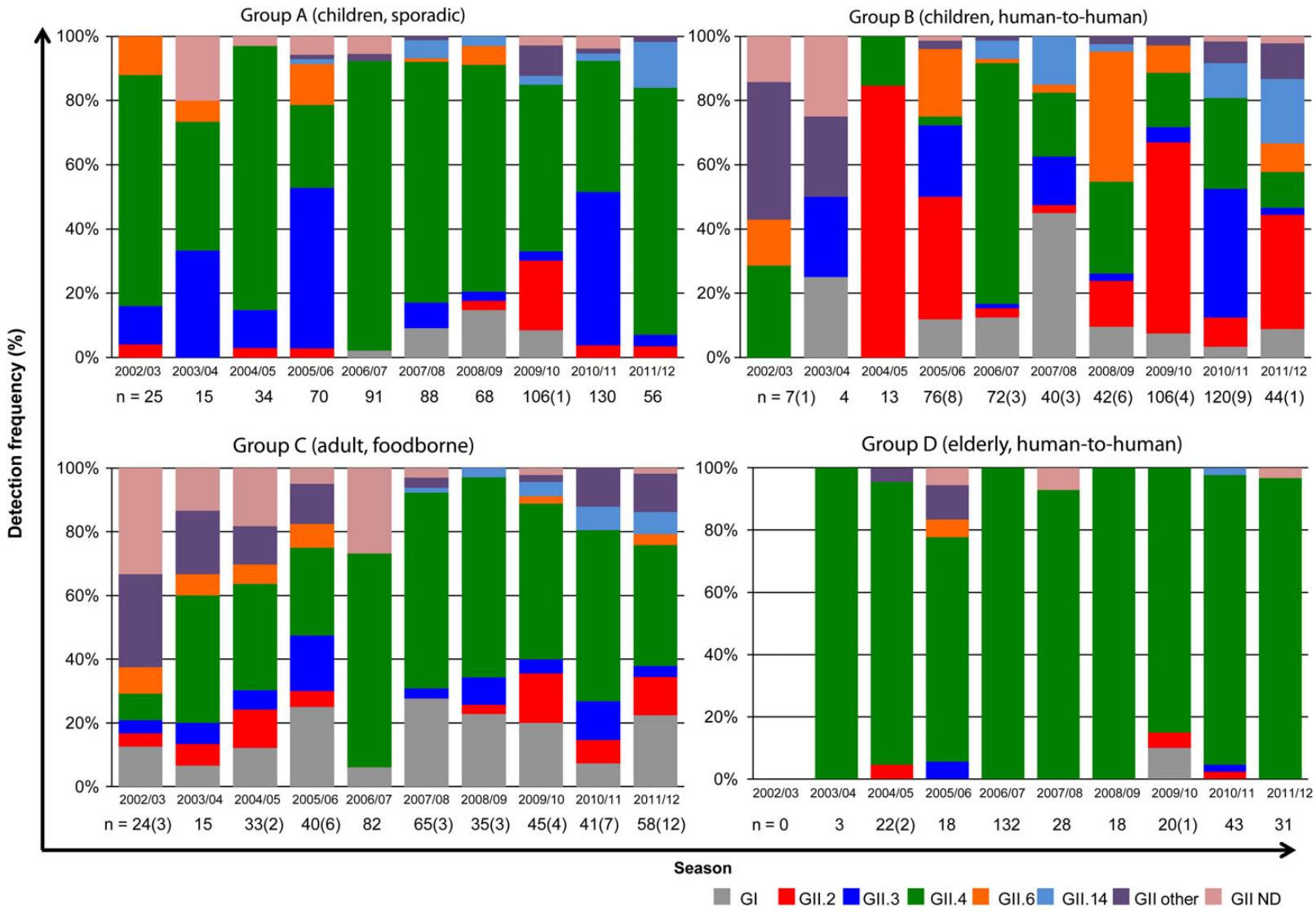

Figure 1. Norovirus epidemiology from the 2002–2003 season to the 2011–2012 season in the province of Osaka. The norovirus-positive cases were categorized into 4 groups depending on the age and mode of infection. Group A included sporadic pediatric diarrhea cases (<15 years of age). Group B included the cases from the outbreaks in childcare facilities or schools. Group C included the adult cases (aged 15–64 years) involving foodborne outbreaks. Group D included the cases involved in nursing home outbreaks (excluding foodborne outbreaks) in subjects >65 years old. The x-axis indicates the season. The y-axis indicates the percentage of norovirus genotypes that were detected in each season. The number of cases or outbreaks in each season is shown below each bar. The number of outbreaks in which there were >2 genotypes is shown in parentheses. Each norovirus genotype is color-coded (see the legend). The minor norovirus genotypes, which are not shown in this figure, are listed in Table 1. Data for group D in the 2002–2003 season are lacking. Abbreviation: ND, not defined.

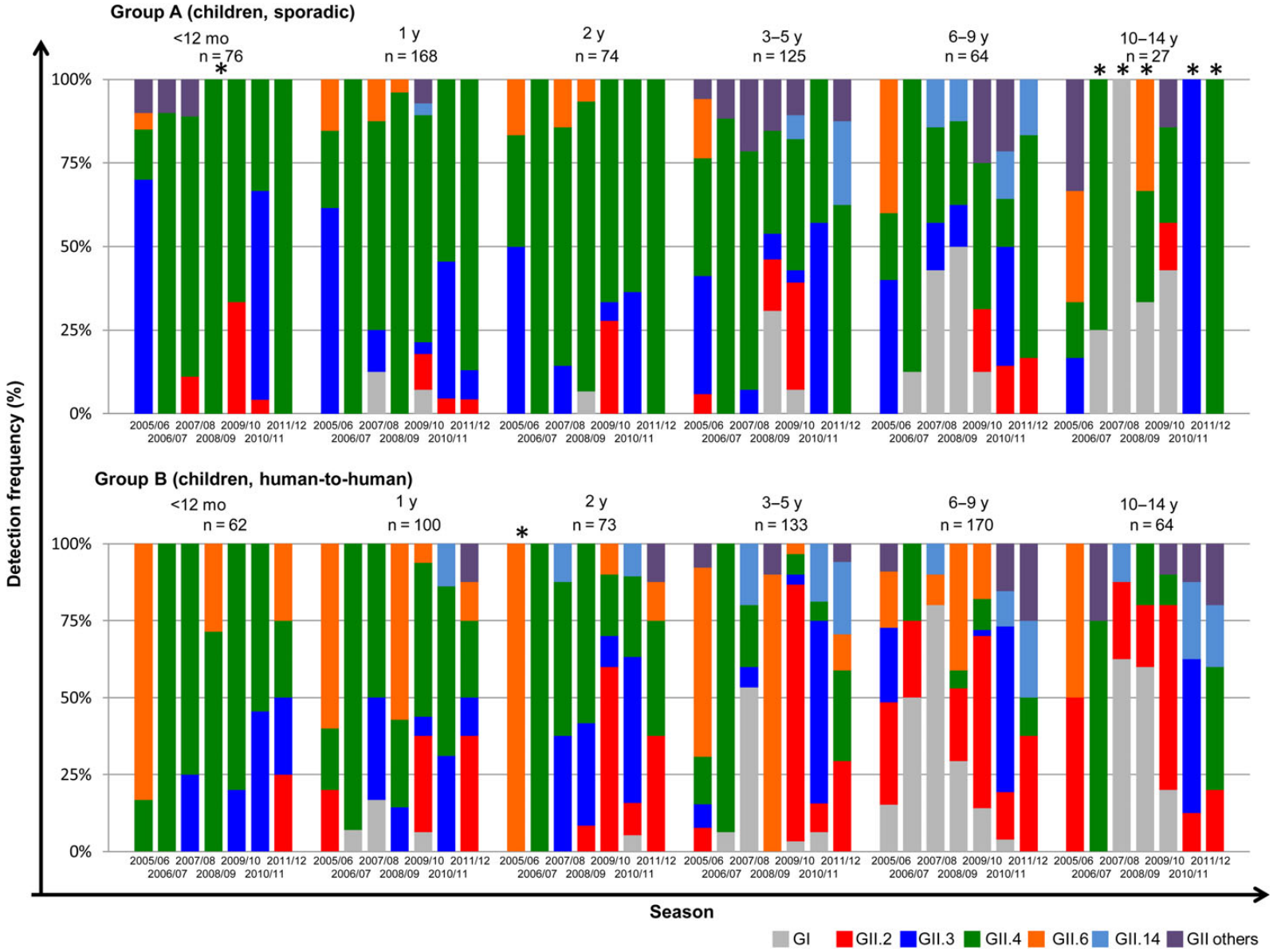

Figure 2. Analysis of the norovirus genotypes in distinct pediatric age groups from the 2008–2009 season to the 2011–2012 season. The subjects in groups A (sporadic) and B (human-to-human) were divided into 6 age groups: <12 months (0 year) and 1, 2, 3–5, 6–9, and 10–14 years. The total numbers of specimens analyzed were 534 and 602 for groups A and B, respectively. The x-axis indicates the seasons. The y-axis indicates the percentage of norovirus genotypes that were detected each year. Each norovirus genotype was color-coded as shown in Figure 1. Asterisks indicate the number of patients was <5.

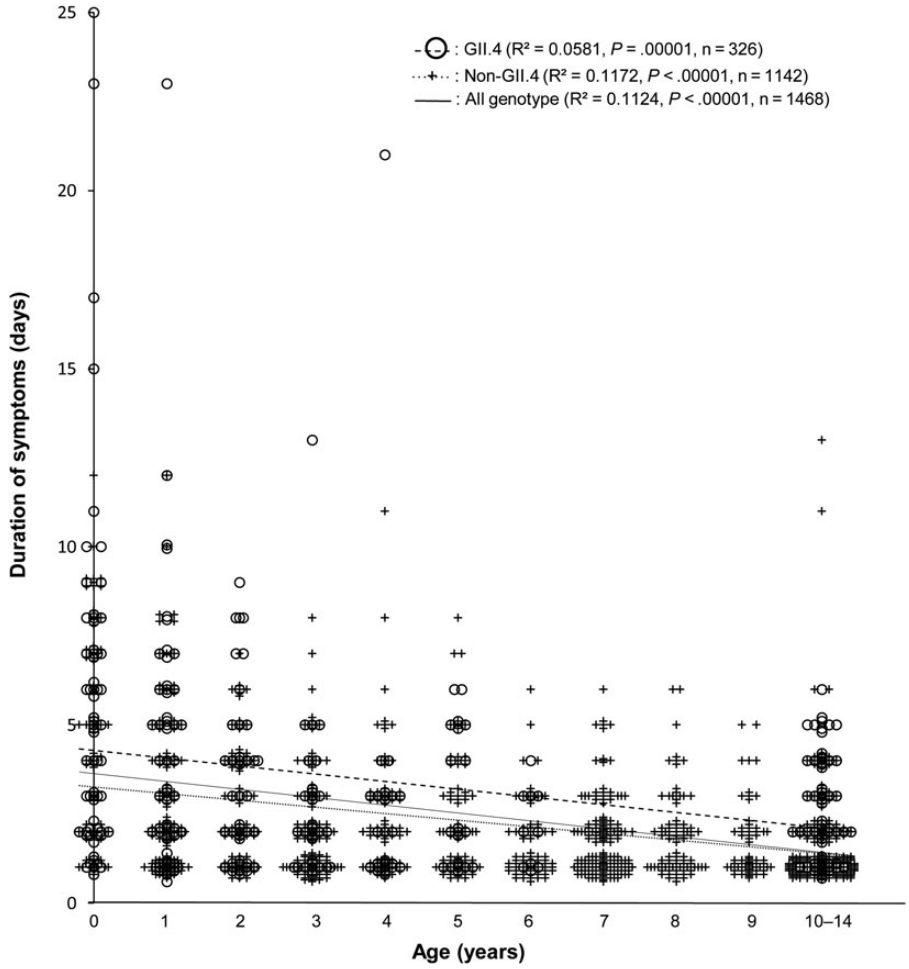

Figure 3. Correlation between age and the duration of symptoms associated with norovirus infection. The 1468 subjects from group B (children, human-to-human) involved in 63 outbreaks from 2005 to 2010 were analyzed. The number of cases with GII.2, GII.3, GII.4, and other genotypes were 612, 157, 326, and 373, respectively. The correlation was statistically significant as assessed by Pearson correlation coefficient (solid line, $P < .0001$).

NoV Infection Trends in Children

To assess whether there was a correlation between age and genotype among children, the subjects of groups A and B were divided into 6 age groups: <12 months and 1, 2, 3–5, 6–9, and 10–14 years of age (Figure 2). The norovirus GII.4 was detected more often in children aged <2 years than in other age groups (group A: $P = .00008$, group B: $P < .0001$, Fisher exact test).

We analyzed the correlation between age and the duration of symptoms in 1468 cases from group B involved in 63 outbreaks. We observed that older children had shorter symptom duration ($P < .00001$, Pearson correlation coefficient test; Figure 3). Duration of symptoms was slightly longer in subjects with GII.4 than in those with non-GII.4 NoV, including GII.2, GII.3, and other genotypes.

Analysis of Repeated Outbreaks in the Same Facilities

In total, 38 group B facilities (nurseries and educational facilities) had repeated outbreaks (93 outbreaks in total) over 4 seasons (Supplementary Data 3). Table 2 shows the details of 5 representative outbreaks in nurseries. Ten facilities reported 2 NoV outbreaks in 1 season. In group B, the initial and subsequent infections in the repeat outbreaks were almost always caused by different NoV genotypes (94.6%). The observed frequency of consecutive infection by the same NoV genotype (3/55 incidence) was significantly lower than the probability estimated from the detection frequency of NoV genotypes in group B throughout this study ($P = .0002$, Z test). These results support the hypothesis that genotype-specific herd immunity may influence NoV outbreaks at the facility level. Only 3 of the consecutive outbreaks were caused by the same genotypes (Table 2 and Supplementary Data 3). In 1 of these 3 cases (GpB-F14), GII.4 was detected twice, with the second episode occurring 4 months later in a different fiscal year. The initial outbreak mainly affected the children in the nursery (age 1–4 years), whereas the second outbreak affected children aged 0–2 years. This suggests that distinct age groups were affected in the 2 outbreaks in the GpB-F14 facility.

Table 2. Representative Repeated Norovirus Outbreaks in the Nurseries

Facility No.	Reported Date	Norovirus Genotype	Interval From the Last Outbreak	Affected Individuals					
				Total No. of Patients	Residents			Staff	
					No. of Specimens	% Positive for Norovirus	Age, y	No. of Specimens	% Positive for Norovirus
GpB-F4	Jan-09	GII.4, GII.6		23	6	83.3	1–2	1	100
	Dec-09	GII.2	11 m	29	5	100	2–5	1	100
	Dec-10	GII.4, GII.14	12 m	17	3	66.7	1–5	NT	NT
	Dec-11	GII.6	12 m	31	3	100	0–2	NT	NT
GpB-F14[a]	Jan-10	GII.2		21	3	100	2–6	NT	NT
	Feb-10	**GII.4**	**1 m**	**14**	**2**	**100**	**1–4**	NT	NT
	Jun-10	**GII.4**	**4 m**	**12**	**5**	**80.0**	**0–2**	NT	NT
	Jul-11	GII.2	13 m	20	5	80.0	1–5	2	100
GpB-F17	Mar-10	GII.2		24	2	100	3–5	NT	NT
	May-10	GII.4, GII.12	2 m	32	2	100	1–2	1	100
	Feb-11	GII.3	11 m	14	3	100	0–5	NT	NT
	Dec-11	GII.14	10 m	20	2	100	4	1	100
GpB-F35[a]	Feb-09	GII.6		26	3	100	1–2	1	100
	Jun-09	GII.3	4 m	15	3	100	0–3	1	100
	Jan-10	**GII.2**	**7 m**	**25**	**4**	**100**	**1–6**	NT	NT
	Feb-11	**GII.2**	**13 m**	**50**	**8**	**62.5**	**1–5**	NT	NT
GpB-F37[a]	Jan-09	GII.6		20	3	66.7	2–6	NT	NT
	Apr-09	**GII.4**	**3 m**	**29**	**5**	**100**	**0–2**	**3**	**100**
	Jan-10	**GII.4**	**9 m**	**27**	**2**	**100**	**4–6**	**NT**	**NT**
	May-10	GII.2	4 m	18	6	50.0	2–5	NT	NT
	Dec-10	GII.4	7 m	22	3	33.3	1	1	100

Abbreviation: NT, not tested.

[a] Boldface text indicates outbreaks that were caused by the same genotype.

Four cases of repeated outbreaks in the same group D facilities were also reported (Supplementary Data 3). In all cases, both the first and subsequent outbreaks were caused by GII.4, and the repeat outbreaks occurred at 2-year intervals. These data also suggest that effective herd immunity against NoV lasts for at least 1 year in the elderly.

NoV Reinfection in Young Children in Group A

We followed 806 outpatients from the 2008–2009 to 2011–2012 seasons in a pediatric clinic and found that 187 infants in group A had attended the clinic repeatedly with diarrhea. NoV was detected in 32.4% of the subjects (260/806 patients) and in 24.4% of the specimens (280/1150 stool specimens). NoV was detected on 2 or 3 visits in 16 patients (16/187 [8.6%]), and 20 incidences of repeat detection were identified (Table 3). The interval between the NoV infections ranged from 22 days to 23 months. In most of the cases, the patient was <3 years of age (81.2% [13/16]). Significantly, the NoV genotypes detected in the first infections were different from the genotypes detected in the subsequent infections in 16 of the 20 incidences.

In the remaining 4 subjects, the same genotypes were detected in both the first and subsequent infections (Table 3). This could be due to either persistent excretion of NoV or viral reinfection. When these 4 cases were grouped according to the detection interval, 2 cases (GpA-P6 and GpA-P10) had a short interval (36 and 46 days, respectively) and 2 cases (GpA-P4 and GpA-P11) had a long interval (11 and 10 months, respectively). In GpA-P6 and GpA-P10, the infections involved GII.2 and GII.4, respectively. Sequencing analysis revealed that the genome sequence of the NoVs in the initial infections were identical to the NoVs in the following infections. Thus, these cases may have been caused by long-term excretion of NoV. Notably, the NoV genome copy number of the second GpA-P10 infection exceeded 1.5×10^6 copies/g stool. This indicates that young children, like adults [30], exhibit extensive and continuing NoV excretion. The 2 long-term excretion cases were negative for rotavirus, adenovirus 40/41, sapovirus, and mamastrovirus 1. Therefore, the cause of the clinical symptoms exhibited by these patients at the second clinic visits was unclear.

The infections in the patients GpA-P4 and GpA-P11 were of the GII.4 genotype. The viruses detected in the initial infections clustered genetically with the 2006b class, while those in the following infections clustered with the New_Orleans_2009 class. Thus, these patients were likely reinfected with NoV rather

Table 3. Children With Repeated Episodes of Norovirus-Positive Acute Gastroenteritis

Patient No.	Age	Sex[a]	Norovirus Genotype[b]	Sampling Date	Interval From the Last Episode	Symptoms[c]	Duration of Symptoms, d
GpA-P1	7 m	M	GII.4 2006b	25/11/2008		Diarrhea (37.1)	14
	2 y		GII.2	30/04/2010	17 m	Vomiting, diarrhea (38.8)	1
GpA-P2	9 m	M	GII.4 2006b	07/03/2011		Vomiting, diarrhea	
	1 y		GII.14	24/06/2011	3 m	Vomiting, diarrhea (38.0)	
GpA-P3	1 y	F	GII.3	13/12/2010		Vomiting, diarrhea	
	1 y 6 m		GII.14	19/06/2011	6 m	Vomiting, diarrhea (37.1)	5
GpA-P4[d]	**1 y**	**M**	**GII.4 2006b**	**31/01/2009**		**Diarrhea, influenza B (37.9)**	**6**
	1 y 10 m		**GII.4 2009**	**04/12/2009**	**11 m**	**Vomiting, diarrhea (38.2)**	**5**
	3 y		GII.14	01/05/2011	16 m	Vomiting, diarrhea	4
GaA-P5	1 y 1 m	F	GII.14	18/03/2010		Vomiting, soft stool (37.5)	
	1 y 2 m		GII.2	10/04/2010	22 d	Vomiting, soft stool[e]	6
	1 y 10 m		GII.3	18/12/2010	8 m	Vomiting	4
GpA-P6[f]	1 y 2 m	M	GII.4 2006b	09/01/2009		Vomiting, diarrhea	5
	2 y 2 m		*GII.2*	*23/01/2010*	*12 m*	*Vomiting, diarrhea*	*6*
	2 y 3 m		*GII.2*	*27/02/2010*	*36 d*	*Anorexia, vomiting, diarrhea*	*7*
GpA-P7	1 y 2 m	F	GII.4 2006b	25/02/2008		Vomiting, diarrhea	5
	2 y 3 m		GI.3	01/03/2009	11 m	Vomiting, diarrhea	3
	3 y		GII.2	14/12/2009	9 m	Vomiting, diarrhea	2
GpA-P8	1 y 3 m	M	GII.14	28/01/2010		Vomiting, diarrhea	
	1 y 5 m		GI.7	12/03/2010	43 d	Diarrhea	7
GpA-P9	1 y 5 m	M	GII.4	25/12/2010		Vomiting, diarrhea	4
	1 y 8 m		GII.3	25/03/2011	3 m	Vomiting, diarrhea (37.0)	3
GpA-P10[f]	*1 y 6 m*	*F*	*GII.4 2010*	*03/03/2009*		*Vomiting, diarrhea (37.7)*	*7*
	1 y 7 m		*GII.4 2010*	*18/04/2009*	*46 d*	*Vomiting, diarrhea (38.6)*	*2*
GpA-P11[d]	**1 y 6 m**	**M**	**GII.4 2006b**	**14/01/2009**		**Vomiting, diarrhea**	**7**
	2 y 4 m		**GII.4 2009**	**19/11/2009**	**10 m**	**Vomiting, diarrhea (38.2)**	**3**
GpA-P12	1 y 7 m	M	GII.2	21/02/2011		Vomiting, diarrhea (37.0)	3
	2 y 5 m		GII.4	25/12/2011	10 m	Diarrhea	4
GpA-P13	2 y	F	GII.12	19/05/2009		Vomiting, diarrhea (37.6)	
	2 y		GII.4 2010	25/01/2010	8 m	Vomiting, diarrhea	
GpA-P14	3 y 4 m	F	GII.12	14/05/2009		Vomiting, diarrhea (37.9)	7
	4 y 10 m		GII.3	8/11/2010	18 m	Vomiting, diarrhea (37.1)	3
GpA-P15	3 y 9 m	F	GII.14	17/03/2010		Vomiting	2
	4 y 5 m		GII.3	20/12/2010	9 m	Vomiting, diarrhea (37.3)	2
GpA-P16	11 y	M	GII.12	06/02/2010		Vomiting, diarrhea	4
	13 y		GII.4 2006b	31/01/2012	23 m	Diarrhea, vomiting (38.1)	3

[a] M, male. F, female.

[b] Variant type was indicated for GII.4 typing.

[c] Fever is indicated by °C and the extent of fever is indicated in brackets.

[d] Boldface text indicates patients who were reinfected with the same genotype.

[e] Poliovirus type 1 was codetected.

[f] Italic text indicates long-term shedder.

than being cases of persistent excretion. Moreover, consecutive infections by the same NoV genogroup are only possible if the reinfecting virus is genetically distant from the initial virus.

Finally, 15 patients with 18 incidences had confirmed NoV reinfections. The observed frequency of consecutive infection by the same NoV genotype (2/18 incidence) was significantly fewer than the probability estimated from the detection frequency of NoV genotypes in group A throughout this study ($P = .0085$, Z test).

DISCUSSION

This is the first large-scale prospective field study on NoV epidemics in Japan, and it confirmed that anti-NoV immunity is

genotype specific and that herd immunity lasts long enough to influence the NoV epidemics in young children in school settings. The characteristics of the NoV epidemics were also quite distinct among different age groups during the same time period, and the virus spreads in different ways. In particular, the NoV genotypes that affected young children in nurseries and schools changed every year. Moreover, GII.2 and GII.3 still triggered outbreaks in children, despite the fact that recent studies suggest that they are not endemic [31–33]. GII.14 initially invaded the study geographic region via children and slowly spread to the elderly. By contrast, GII.4 predominated in children aged <3 years. This is probably because this age group is in closer contact with adults than with older children, and the GII.4 is dominant in adult populations. According to the monthly trends of NoV outbreaks and genotypes from 2008–2009 to 2011–2012, the GII.6 genotype predominated in group B in the winter of 2008–2009 but not in other groups (Supplementary Data 2). The GII.3 genotype was dominant in both groups A and B in the 2010–2011 season. The GII.4 strains 2006b, 2009_New_Orleans, and 2012_Sydney class were first detected in groups D, A, and B at the end of the 2005–2006, 2008–2009, and 2011–2012 seasons, respectively, and were detected frequently in other groups in the following seasons (data not shown). These observations clearly suggest that NoV spread in the distinct human populations via different routes in Osaka.

The present study also provided insights into the presence of herd immunity in infants and young children. At the population level, the dominance of NoV genotypes in group B changed every year. At the facility level, the majority of repeated outbreaks were caused by distinct NoV genotypes. This suggests that genotype-specific herd immunity in infants and young children lasts for at least a few years, thereby influencing the endemic NoV genotype in the next season. Children who have not encountered genotypes other than GII.4 enter nurseries and primary schools every year. This may allow nonendemic genotypes to persist in this population and thereby cause repeat endemics in young children over the next few years. Exceptionally, GII.4 was dominant in group B only in the 2006–2007 season when GII.4 2006b spread globally [3, 34]. A recent report suggesting that antibodies against NoV persist for 4–8 years [35] supports the notion that anti-NoV herd immunity exits. Dynamic antigenic mutations are probably an important trigger of NoV endemics.

GII.4 was always dominant in the adults and the elderly, which may be in part because immunological escape mutants of GII.4 emerge every season, as indicated by previous studies [36, 37]. However, if this was the only reason, GII.4 would have been the dominant genotype in groups A and B as well. Additional factors should be considered to explain our surveillance data. One possibility is that because the population of adults is much larger than that of infants and children, a proportion of adults are exposed to an endemic GII.4 strain during a given season.

Reinfection of NoV among young infants has been unclear in the natural setting. The present study identified 18 incidences of NoV reinfection in a clinic. Most of the patients involved were <3 years old at the time of their first and subsequent infections, and most of the reinfections were caused by genotypes that were distinct from the initial virus. These data are in agreement with our population analysis. The repeated NoV infection with various genotypes in children aged <2 years has been shown in Peru [19], suggesting a unique NoV circulation in young children. Similar to findings reported by Murata et al [38], we showed an inverse relationship between age and the duration of symptoms upon NoV infection in children, suggesting that the infection of one NoV genotype elicits some level of immunity against other genotypes. NoV infection increases the immunoglobulin A titer against the genotype of the previously infected NoV [39], and this cross-genotype booster effect may explain the immunity against NoV. However, repeated asymptomatic infection of various NoV genotypes may also boost the genotype-specific immunity.

We also showed that children can be reinfected by a different NoV genotype in as short a period as 3 weeks and a child shed NoV for 6–7 weeks, consistent with a previous report [38]. These observations suggest that coinfection of NoVs with distinct genotypes is likely to happen in young children, which promotes the genesis of recombinant viruses [40, 41].

Phylogenetic analysis suggested that each genotype formed a genetic cluster every few years and that the rate of mutation per year was similar for GII.3 and GII.4, which is in agreement with a previous report [42]. The genetic distance of each genotype from the respective reference strain ranged from 93% to 98% (data not shown), and the nucleotide to amino acid homology ratio for GII.4 isolates relative to the reference strain was substantially higher than for isolates of other genotypes. Further studies are warranted to analyze our molecular epidemiological data [43].

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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