BRIEF REPORT

Norovirus GII-4 Causes a More Severe Gastroenteritis Than Other Noroviruses in Young Children

Leena Huhti,[1] Evelin D. Szakal,[1] Leena Puustinen,[1] Marjo Salminen,[1] Heini Huhtala,[2] Oona Valve,[1] Vesna Blazevic,[1] and Timo Vesikari[1,3]

[1]Vaccine Research Center, University of Tampere Medical School, [2]School of Public Health, and [3]Department of Pediatrics, Tampere University Hospital, Tampere, Finland

Norovirus (NoV) GII-4 has emerged as the predominant NoV genotype in outbreaks of gastroenteritis worldwide. We determined clinical features of NoV GII-4 associated acute gastroenteritis (AGE) in comparison with AGE associated with other NoV types in infants during seasons 2001 and 2002. During the prospective follow-up period, 128 primary infections of AGE due to NoV were identified in 405 infants; of these, GII-4 was found in 40 cases (31%). NoV GII-4 was associated with longer duration of diarrhea and vomiting than other NoV genotypes, suggesting greater virulence of NoV GII-4.

Norovirus (NoV) is the second most frequen causative agent of acute gastroenteritis (AGE) in young children after rotavirus [1]. NoV AGE in young children is likely to result from primary NoV infection. An early seroprevalence study in Finland found that 49% of infants were infected with NoV by the age of 2 years [2]; some of those NoV infections were clinically manifest, and others were subclinical. In a prospective study of rotavirus vaccine recipients in Finland during 1993–1995, NoV AGE was observed in 20% of the children in a 1-year follow-up study [1]. In this study, genogroup GII accounted for 90% of the cases of NoV AGE [1].

Since 1995, NoV GII-4 genotype has emerged worldwide, displaced other NoV GII genotypes, and caused an increase in overall incidence of NoV infection [3, 4]. The reason for the surge of GII-4 activity is unknown, and 2 questions arise: is GII-4 inherently more virulent than other NoVs, and if so, what is the underlying mechanism? The polymerase region has been identified to be a marker of major NoV-associated gastroenteritis outbreaks (i.e. virulence) [3]; however, changes in the capsid region also affect the antigenicity, host specificity, host cell binding, and virus entry properties and, thereofore, may affect virulence [4].

A confounding factor in studies of virulence of NoV genotypes is pre-existing immunity, which might ameliorate the clinical course of gastroenteritis caused by old NoV genotypes but not by a newly emerged type, such as GII-4. We therefore conducted a comparative study on the clinical severity of AGE caused by NoV GII-4, compared with other genotypes, in young children. We propose that our study cases of NoV AGE in infants likely represent primary NoV infection in previously naive children. Our study material therefore provides a unique opportunity to examine the relationship between the clinical features of our study cases and the genotypes of causative NoVs and, in the case of GII-4, variants of GII-4 without the influence of pre-existing immunity on the clinical severity of episodes.

METHODS

The clinical material was originally collected for an efficacy trial of rotavirus vaccine RIX4414 during 2000–2002 in Finland, as described elsewhere [5]. The vaccine study protocol and consent forms were approved by the appropriate ethics committees and patients or legal guardians volunteering for the study after informed consent was provided. Children were vaccinated at 2 and 4 months of age and followed up for 2 rotavirus winter-epidemic seasons until age 20–24 months. Information on clinical features was collected for each episode of AGE [5]. Earlier studies indicated that rotavirus vaccination did not have any affect on the occurrence or severity of NoV AGE [1, 6]. Therefore, cases in the vaccine and placebo recipients were pooled for this study. Clinical severity of the AGE episodes was assessed using a 20-point severity score described by Ruuska and Vesikari [7].

NoVs were detected using a reverse-transcription polymerase chain reaction (PCR) assay targeted at polymerase gene region (A) [8, 9]. RNA was extracted from the stool specimens with use of Boom's silica method [10] and transcribed to cDNA with use of SuperScript II RNase H- Reverse Transcriptase (Invitrogen) [1]. The NoV sequences were analyzed by the Food-borne Viruses in Europe Network and aligned to the following EMBL/

Received 26 October 2010; accepted 20 December 2010.

Potential conflicts of interest. TV has received consultation and lecture fees from GlaxoSmithKline. All other authors report no conflicts.

Correspondence: Leena Huhti, MSc, University of Tampere Medical School, Vaccine Research Center, Biokatu 10, FIN-33520 Tampere, Finland (leena.huhti@uta.fi).

The Journal of Infectious Diseases 2011;203:1442–4

0022-1899 (print)/1537-6613 (online)/2011/20310-0001$14.00
DOI: 10.1093/infdis/jir039

Genbank NoV strains: Grimsby/95/UK (AJ004864), FL408/1996/USA (AF080558), EmmenE006/2002/NL (AB303929), Lanzhou/35666/2002/China (DQ364459), Hilversum/1999 (AF365989), Leeds/90/UK (AJ277608), Melksham/1989/UK (X81879), Hawaii/1971/US (U07611), Hesse3/1997/DE (AF093797), Valetta/1995/MT (AJ277616), Birmingham/1993/UK (AJ277612), and Southampton/1991/UK (L07418). NoV GII-4 genotype was then specified using another PCR targeted at the gene encoding for the capsid region (C) [11].

Statistical analyses were performed using the Mann-Whitney *U* test to compare the medians of clinical symptoms (duration of diarrhea and vomiting [days], maximum number of diarrheal stools and vomiting episodes in 24 h, and age months) and SPSS, version 15.0 (SPSS), to analyze the medians of severity score points between NoV GII-4 and the other genotypes. All tests were 2-tailed and were considered to be statistically significant at $P < .05$.

RESULTS

Human caliciviruses were detected in 192 of the 485 episodes of AGE in 405 infants. Of these, 148 cases were associated with NoV and 128 were primary infection due to NoV (in 20 cases, a second NoV infection in the same child was detected during follow-up). Only the primary infections were included in the study material. The yearly observation period was defined from July through June (season). Most (75%) of the cases occurred during the second year of follow-up in 2002 (Table 1).

NoV strains belonging to genogroup GII were found in 110 (86%) of the primary infections, genogroup GI NoV strains were found in 18 cases (14%). Nine different NoV genotypes belonging to either genogroup were detected (GI-2, GI-3, GI-4, GI-6, GIIb, GII-1, GII-2, GII-4, and GII-7). GII-4 genotype was detected in 40 cases (31 %) and the second most common genotype GIIb in 33 cases (26%). GII-4 cases occurred during both epidemic seasons 2001 and 2002 (Table 1). There were no recombinants between GII-4 and other genotypes, as determined from the gene sequences of the polymerase and capsid regions.

The median age of the children was 17 months (interquartile range, 10–19 months). Children infected with norovirus GII-4 were slightly older than children infected with other norovirus genotypes (Table 2), but the age distribution between compared groups was not statistically significant. Clinically, GII-4 was associated with more severe AGE episodes than other NoV genotypes (Table 2). Infants with GII-4 had a longer duration of diarrhea ($P = .006$) and a greater number of diarrheal stools during a 24-h period ($P = .003$) than did those infected with other NoV genotypes. The duration of vomiting in children with GII-4 was longer than in children with infection due to other genotypes ($P = .014$). The overall severity score was also higher in the GII-4–infected cases than in the other cases ($P = .002$).

Table 1. Norovirus (NoV) Genotypes Identified during the 2 NoV Epidemic Seasons 2001 and 2002 in Infants With the First Infection of NoV Gastroenteritis

	Season 1	Season 2	Both years combined
Genotypes	No. (%)	No. (%)	No. (%)
GII-4	7 (22%)	33 (34%)	40 (31%)
GIIb	9 (28%)	24 (25%)	33 (26%)
Other genotypes combined:	16 (50%)	39 (41%)	55 (43%)
GII-1	3 (9%)	2 (2%)	5 (4%)
GII-2	1 (3%)	12 (13%)	13 (10%)
GII-7	8 (25%)	11 (12%)	19 (15%)
GI-2	1 (3%)	1 (1%)	2 (1%)
GI-3	1 (3%)	5 (5%)	6 (5%)
GI-4	2 (6%)	2 (2%)	4 (3%)
GI-6	0 (0%)	6 (5%)	6 (5%)
	32 (25%)	96 (75%)	128 (100%)

DISCUSSION

In outbreaks, GII-4 NoVs have been associated not only with higher attack rate [3] but also with more severe clinical presentation than other NoV genotypes [12]. To our knowledge, this is the first study to examine the severity of primary infection with NoV GII-4 in young children relative to other NoV genotypes. The advantage of studies in young children, compared with adults, is that the severity of episodes can be examined without the effect of any pre-existing immunity. Our results suggest that GII-4 caused more severe disease than other NoV genotypes in primary infections of AGE in young children.

Table 2. Clinical Features of Acute Gastroenteritis in Young Children Associated With GII-4 and Other NoV Genotypes.

	Children with NoV genotype		
Variable	GII-4 (no.= 40)	Others (no.= 88)	*P*-value
Age, months	18 (16-20)*	17 (9-19)	0.094
Diarrhoea			
Days	2 (1–3.75)*	1 (0-2)	**0.006**
Maximum number of times/day	4 (1- 6)*	3 (0-4)	**0.003**
Vomiting			
Days	2 (1–2.75)*	1 (1-2)	**0.014**
Maximum number of times/day	3 (2–5.75)*	3 (1-5)	0.326
Severity score			
Points	7 (6-9)*,**	6 (4-7)	**0.002**

NOTE. *Reported as median value followed by the interquartile range in parentheses (Mann-Whitney *U*-test).

** The severity score points was assessed according to the clinical symptoms (duration of diarrhoea and vomiting (days), maximum number of diarrhoeal stools and vomiting episodes in 24 hours, fever, treatment and dehydration) [7].

We assumed that the primary infection of AGE detected in an infant represents a primary NoV infection in the majority of children. We could not confirm this, because we did not have serum samples for testing pre-existing NoV antibodies. A second infection of NoV AGE was found in only 14% of the children during the 2-year follow-up period. Therefore, it is reasonable to assume that most of AGE episodes detected during the follow-up represented primary NoV infection in these infants.

The next most common genotype after GII-4 was GIIb (26%), as has been reported in outbreaks in adults and older children in Sweden and Finland [13, 14]. In this study, GIIb did not cause more severe infection than the remaining NoV genotypes. We found more cases of NoV AGE during 2002 than during 2001. This may simply reflect the greater likelihood of children to experience NoV AGE in the second than in the first year of life. Alternatively, new GII-4 variants appeared to cause an unusual epidemic peak of outbreaks during 2002 in Europe [3], and such activity might also be associated with the greater number of cases in children during 2002.

In conclusion, the finding of greater clinical severity of the first in lifetime NoV AGE episodes associated with GII-4 NoV, compared with other NoV genotypes, suggests that GII-4 has greater inherent virulence than other NoV types. However, the molecular basis of such enhanced virulence remains speculative and requires further study.

Acknowledgments

We thank the technicians at the laboratory of Vaccine Research Center in Tampere for their excellent assistance.

References

1. Pang XL, Joensuu J, Vesikari T. Human calicivirus-associated sporadic gastroenteritis in Finnish children less than two years of age followed prospectively during a rotavirus vaccine trial. Pediatr Infect Dis J **1999**; 18:420–6.
2. Lew JF, Valdesuso J, Vesikari T, et al. Detection of Norwalk virus or Norwalk-like virus infections in Finnish infants and young children. J Infect Dis **1994**; 169:1364–7.
3. Lopman B, Vennema H, Kohli E, et al. Increase in viral gastroenteritis outbreaks in Europe and epidemic spread of new norovirus variant. Lancet **2004**; 363:682–8.
4. Siebenga JJ, Vennema H, Renckens B, et al. Epochal evolution of GGII.4 norovirus capsid proteins from 1995 to 2006. J Virol **2007**; 81:9932–41.
5. Vesikari T, Karvonen A, Puustinen L, et al. Efficacy of RIX4414 live attenuated human rotavirus vaccine in Finnish infants. Pediatr Infect Dis J **2004**; 23:937–43.
6. Zeng SQ, Halkosalo A, Salminen M, Szakal ED, Karvonen A, Vesikari T. Norovirus gastroenteritis in young children receiving human rotavirus vaccine. Scand J Infect Dis **2010**; 42:540–4.
7. Ruuska T, Vesikari T. Rotavirus disease in Finnish children: use of numerical scores for clinical severity of diarrhoeal episodes. Scand J Infect Dis **1990**; 22:259–67.
8. Jiang X, Huang PW, Zhong WM, Farkas T, Cubitt DW, Matson DO. Design and evaluation of a primer pair that detects both Norwalk- and Sapporo-like caliciviruses by RT-PCR. J Virol Methods **1999**; 83:145–54.
9. Farkas T, Zhong WM, Jing Y, et al. Genetic diversity among sapoviruses. Arch Virol **2004**; 149:1309–23.
10. Boom R, Sol CJ, Salimans MM, Jansen CL, Wertheim-van Dillen PM, van der Noordaa J. Rapid and simple method for purification of nucleic acids. J Clin Microbiol **1990**; 28:495–503.
11. Buesa J, Collado B, Lopez-Andujar P, et al. Molecular epidemiology of caliciviruses causing outbreaks and sporadic cases of acute gastroenteritis in Spain. J Clin Microbiol **2002**; 40:2854–9.
12. Friesema IH, Vennema H, Heijne JC, et al. Differences in clinical presentation between norovirus genotypes in nursing homes. J Clin Virol **2009**; 46:341–4.
13. Maunula L, Von Bonsdorff CH. Norovirus genotypes causing gastroenteritis outbreaks in Finland 1998–2002. J Clin Virol **2005**; 34:186–94.
14. Lindell AT, Grillner L, Svensson L, Wirgart BZ. Molecular epidemiology of norovirus infections in Stockholm, Sweden, during the years 2000 to 2003: association of the GGIIb genetic cluster with infection in children. J Clin Microbiol **2005**; 43: 1086–92.