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## Short Report: Norovirus Genogroup II Gastroenteritis in Hospitalized Children in South India

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**Abstract.** The distribution of norovirus (NoV) genogroup II in children < 5 years of age admitted to a south Indian hospital with diarrhea was investigated. Viral RNA extracted from 282 stool samples were screened for NoV GII and positive amplicons sequenced. Twenty-eight (9.9%) had NoV GII infection with a median age of 6 months, with more severe episodes of diarrhea among infected (median Vesikari score 13, interquartile range [IQR] 10–15) than children without infection (median score 10, IQR 8–13,  $P = 0.002$ ). The study documents NoV GII infections as an important cause of gastroenteritis and the genetic diversity of circulating strains.

### INTRODUCTION

Noroviruses (NoVs) are the most important cause of non-bacterial gastroenteritis in all ages in both developed and developing countries.<sup>1</sup> It has recently been estimated that each year NoVs cause 64,000 episodes of diarrhea requiring hospitalization and 900,000 clinic visits among children in industrialized countries and up to 1.1 million hospitalizations and 200,000 deaths of children < 5 years of age in developing countries.<sup>2,3</sup> NoVs cause outbreaks of acute gastroenteritis in a variety of settings, such as schools, restaurants, hospitals, cruise ships, nursing homes, and military settings.<sup>4</sup> Norovirus infections accounted for 18.2% of outbreaks and 65% of closures of hospital wards during 2008 and 2009 in the United States.<sup>5</sup>

A recent review of reports from both developed and developing countries indicates that the prevalence rates of NoV gastroenteritis in hospitalized children range from 6% to 48%, with an overall prevalence of 14%.<sup>6</sup> There have been recent reports of NoV outbreaks in neonatal nurseries associated with clinical presentations such as necrotizing enterocolitis, diarrhea, vomiting, abdominal distension, and fever.<sup>7–9</sup> The primary objective of this study was to identify the prevalence of NoV GII among children < 5 years of age admitted to the hospital with diarrhea.

### MATERIALS AND METHODS

**Study population.** The study was carried out at the Christian Medical College (CMC), a 2,695-bed tertiary care hospital in Vellore, southern India with 60 neonatal and 84 pediatric beds. There are ~400 pediatric admissions every month. This includes about 40 cases presenting with diarrhea requiring hospitalization for rehydration. Stool samples were collected from children < 5 years of age who were admitted to the pediatric ward with acute gastroenteritis between the years 2005 and 2006 and screened for NoV GII. All stool samples collected were transported within 2 hours to the laboratory and stored at  $-70^{\circ}\text{C}$  until testing. Diarrheal stool samples were screened for parasite ova and cysts by microscopy and for rotavirus by enzyme-linked immunosorbent assay (Rota IDEIA; Dako, Ely, UK).

Written informed consent was obtained from parents of all children before enrollment, and the study was approved

by the Institutional Review Board of the Christian Medical College, Vellore.

**Viral RNA extraction and complementary DNA synthesis.** Viral RNA was extracted from 200  $\mu\text{L}$  of 20% fecal suspension in minimal essential medium (MEM) using the guanidium isothiocyanate-silica method.<sup>10</sup> A known positive control was included in all extractions, to detect polymerase chain reaction (PCR) inhibition. The RNA eluted was converted to complementary DNA (cDNA) by reverse transcription at  $37^{\circ}\text{C}$  for an hour, with random hexamers (Pd[N]6, Pharmacia Biotech, Little Chalfont, Buckinghamshire, UK). The cDNA were stored at  $-20^{\circ}\text{C}$  until further use.

**Detection and characterization of NoV GII strains.** The cDNA was used as a template for the amplification of the ORF 1–2 regions using primers GIIFB1, GIIFB2, GIIFB3/GIISKR to amplify a 468 bp fragment of the ORF 1–2 region.<sup>11</sup> The PCR amplicons were resolved by gel electrophoresis using 1.5% Agarose gel (Medox, Chennai, India) containing ethidium bromide (0.5 mg/mL) in 1X TBE (Tris–Base Boric acid–EDTA, Sigma-Aldrich, St. Louis, MO) buffer. Sequencing of the positive amplicons was carried out by using the ABI PRISM Big Dye Terminator cycle sequencing ready reaction kit (Applied Biosystems, Carlsbad, CA). Sequences were resolved using an automated DNA sequencer ABI PRISM 310 Genetic Analyzer. Nucleotide sequence similarity searches were performed using the National Center for Biotechnology Information basic local alignment search tool (BLAST) server (<http://www.ncbi.nlm.nih.gov/blast/Blast.cgi>). Multiple sequence alignment was then carried out with ClustalW alignment algorithm of the BioEdit Sequence Editor v7.0.9.0 (<http://www.mbio.ncsu.edu/bioedit/bioedit.html>) by including reference sequences obtained from GenBank. Neighbor-joining method (10,000 pseudo replicates) with MEGA software (version 4, <http://www.megasoftware.net/>) was used to assign the samples to a genogroup if the polymerase and/or capsid region shared > 70% homology at the nucleotide (nt) level to those of strains within a recognized genogroup. Genotypes were assigned based on > 90% homology at the nt level with other strains within a given genotype.<sup>12</sup>

**Statistical analysis.** The data were analyzed using STATA 10.0 for Windows (STATA Corp., College Station, TX). Descriptive analysis was performed for all explanatory variables. Statistical significance of the observed differences in outcome between explanatory variables was assessed using the  $\chi^2$  test or Fisher's exact test for categorical variables and two-tailed  $t$  test or Mann-Whitney  $U$  test for continuous variables, depending on the distribution of the data.

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## RESULTS

A total of 282 children < 5 years of age admitted to the hospital between the years 2005 and 2006 with gastrointestinal symptoms were screened for norovirus GII. Clinical information was collected for all children. The median interquartile range (IQR) age of children with diarrhea was 9 (4–16) months. The median interquartile range (IQR) Vesikari score of diarrheal severity was 10 (8–13) and the children required admission for a mean (SD) duration of 3 ( $\pm$ 2) days.

Of the 282 children, 28 (9.9%) had NoV GII infection. Sixteen (57.1%) of these 28 children were males. Of the 254 children without NoV infection 182 (71.7%) were males, although this difference was not statistically significant ( $P = 0.456$ ). The median (IQR) age of NoV diarrhea was 8.5 (7–12) months, whereas for those who did not have NoV diarrhea it was 9 (4–17) months, which was comparable ( $P = 0.676$ ).

When the severity of diarrheal episodes were compared between those with and without NoV infection, the episodes of diarrhea associated with NoV infection were found to be more severe (median Vesikari [IQR] = 13 [10–15]) than those not associated with NoV infection (median Vesikari [IQR] = 10 [8–13],  $P = 0.002$ ). When the associated clinical symptoms were individually compared between children with and without NoV diarrhea, a significantly higher proportion of children with NoV diarrhea had associated vomiting than other children ( $P = 0.044$ ) (Table 1). Other symptoms such as fever and degree of dehydration were comparable between the two groups (Table 1). Of the 282 children, 30 (10.6%) were positive for rotavirus, but none of the NoV positive children had a co-infection with rotavirus. No parasitic pathogens were identified. No significant difference was observed in the clinical features between the rotavirus and norovirus infected children.

The sequencing of the amplified products from the positive samples showed a predominance of NoV GII.4 accounting for 42.9% (12 of 28) followed by GII.3, GII.2, and GII.1 (25%, 17.9%, and 14.3%, respectively) (Figures 1 and 2). A comparison of age, sex, and other clinical symptoms including need for rehydration of children with NoV GII.4 versus other NoV genotypes showed no significant difference. Nucleotide sequences of positive samples from the study have been deposited in the GenBank and the accession numbers are KC594868–KC594888.

## DISCUSSION

This study documents the proportion of NoV GII infections and genetic diversity of circulating NoV strains among Indian children < 5 years of age admitted with diarrhea. The rate of

TABLE 1

Comparison of clinical symptoms and treatment needs of children with and without norovirus gastroenteritis

Clinical symptom	NoV positive (%) N = 28	NoV negative (%) N = 254	P value
Presence of vomiting	22 (78.6%)	150 (59.1%)	0.044
Fever (> 37°C)	14 (50%)	129 (50.8%)	0.937
Dehydration	24 (85.7%)	174 (68.5%)	0.059
Intravenous fluid replacement	27 (96.4%)	237 (93.3%)	1.000

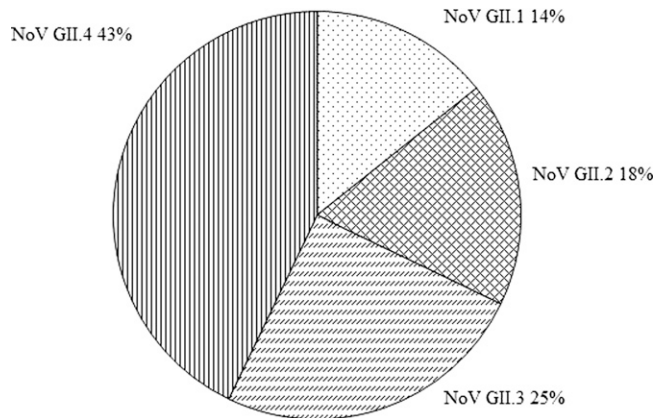


FIGURE 1. Distribution of norovirus genotypes among 28 children hospitalized with noroviral gastroenteritis in Vellore.

9.9% is similar to studies reported from other high- and low-income countries.<sup>13–17</sup>

Previous reports have shown that NoV GII strains are more likely to induce vomiting and severe diarrhea among children; similar observations were made in the current study.<sup>18,19</sup> A recent review that examined NoV outbreaks across the world showed an increase in NoV diarrhea.<sup>20</sup> This could be caused by the rapidity by which NoV has evolved that leads to distinct strains emerging every 2–4 years or wider availability of diagnostic tools.

Hospital-based studies from India between 2003 and 2009 reported a 6% prevalence of NoV associated diarrhea among children  $\leq$  5 years of age,<sup>21–24</sup> which is lower than the current study. This variation could be a result of the detection method used. A recent comparison of different primer sets for the detection of NoV GII showed that a higher detection can be obtained by either using multiple primer sets or using a sensitive nested reverse transcription-polymerase chain reaction assay.<sup>25</sup> Nucleotide sequence analysis of the positive samples showed a predominance of NoV GII.4 (43%) followed by GII.3, GII.2, and GII.1.

A previously published study from the same area showed the prevalence of NoV and sapovirus (SaV), in the community and hospital settings,<sup>23</sup> with predominance of GII, but at a lower level than currently reported. A reason for this increase could be the circulation or evolution of newer strains caused by sequence mutation and/or recombination, a possibility that needs further exploration.

The higher severity of NoV-associated diarrhea and the predominance of the NoV GII strains in hospital and community settings have heightened the need for routine stool testing to identify the etiology of gastroenteritis, particularly to prevent inappropriate antibiotic usage and inform the need for future vaccines. In India, NoVs are not covered in the routine stool testing in most laboratories and hence the viral distribution patterns across the country are not known. The current study shows the NoV GII are an important cause of diarrhea and that GII.4 are the most common.

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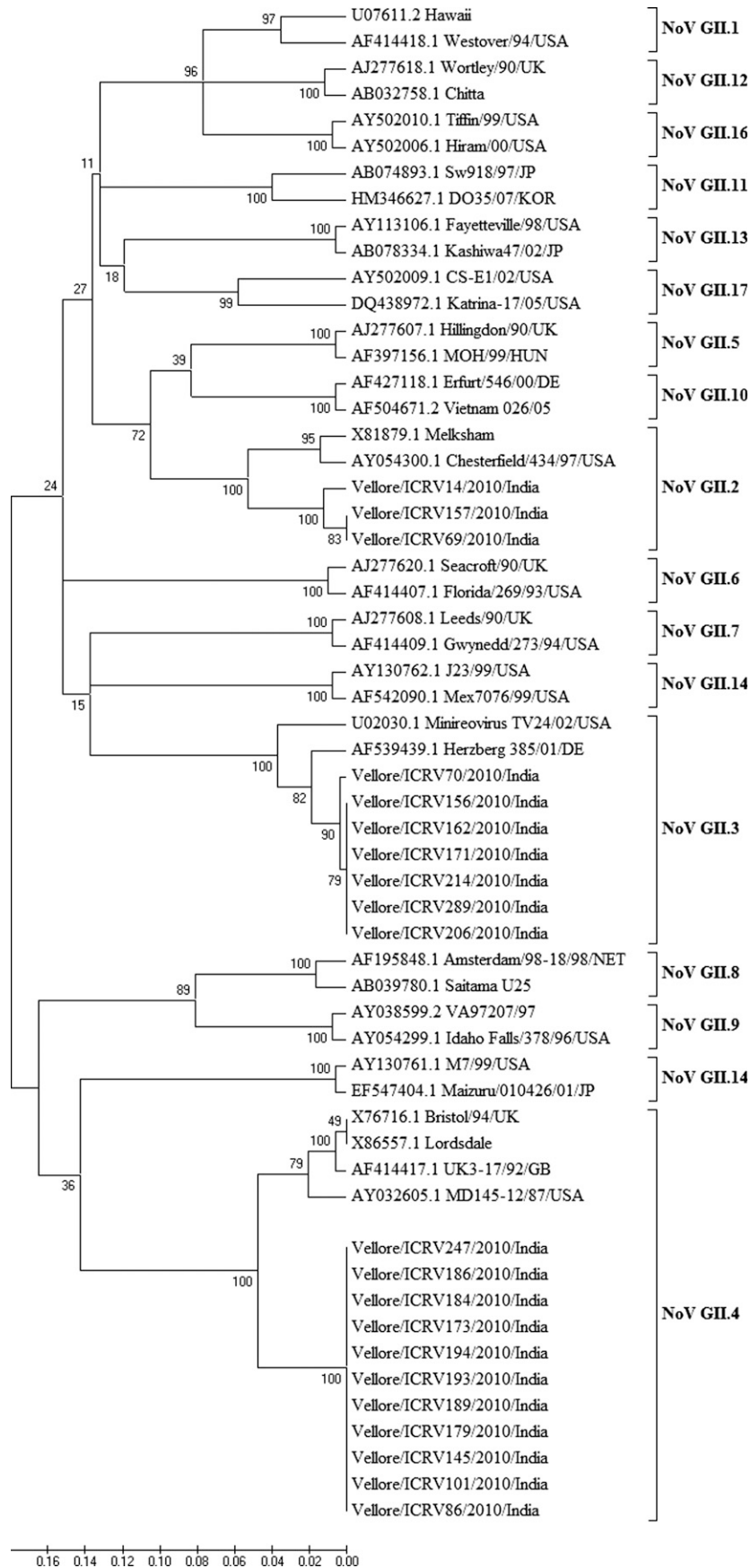


FIGURE 2. Dendrogram constructed using neighbor-joining of nucleotide sequences corresponding to a fragment of 468 bp of the ORF 1-2 region of the NoV capsid encoding gene. Bootstrap *P* values for 10,000 pseudoreplicates are shown.

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