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Molecular epidemiology of norovirus infections in children and adults: sequence analysis of region C indicates genetic diversity of NVGII strains in Kolkata, India

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SUMMARY

Faecal specimens of diarrhoea cases ($n = 2495$, collected between November 2007 and October 2009) from Infectious Diseases and Beliaghata General (ID&BG) Hospital, Kolkata, India, were screened by RT–PCR using specific primers targeting region C of the capsid gene of noroviruses (NoVs) to determine the seasonal distribution and clinical characteristics of NoVs associated with diarrhoea. NoV infection was detected in 78 cases, mostly in children aged <2 years. In 22/78 positive cases, the virus was detected as the sole agent; others were as mixed infections with other enteric pathogens. Sequencing of NVGII strains showed clustering with GII.4 NoVs followed by GII.13 and GII.6 NoVs. Clinical characteristics of the diarrhoeic children and adults in Kolkata indicated that NoV infections were detected throughout the year and were associated with a mild degree of dehydration.

Key words: Diarrhoea, Kolkata, molecular epidemiology, Norwalk agent and related viruses, public health emerging infections, region C, RT–PCR.

INTRODUCTION

Norovirus (NoV) is the leading cause of non-bacterial, acute gastroenteritis and has been associated with gastroenteritis in infants, young children and adults in developed as well as developing countries [1–4]. NoVs are non-enveloped, icosahedral viruses and possess a linear, positive-sense, single-stranded RNA (ssRNA) genome of about 7·4–7·7 kb. NoV belongs to the Caliciviridae family. The RNA genome of NoV is organized into three major open reading frames (ORF1, -2, -3) with a polyadenylated 3′-end [5]. Furthermore, ORF1 encodes a large polyprotein that is proteolytically processed into the mature non-structural proteins [6]. ORF2 encodes the major capsid protein, VP1, and ORF3 encodes a minor structural protein, VP2. NoV has five major phylogenetic clades, or genogroups, designated GI to GV [7]. The prevalence of antibody to the GII viruses (Mexico, Hawaii, or Lordsdale) appears to be greater than that of the GI viruses in most studies [4, 8]. Like all enteric viruses,

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NoVs are associated with several modes of transmission. The predominant modes of transmission are person-to-person contact and food- or water-borne transmission [1, 9]. Clinical manifestations associated with NoV infections include nausea, vomiting, diarrhoea, abdominal cramps, headache, fever (subjective), chills, myalgia and sore throat. Vomiting was more frequently associated with diarrhoea in children, whereas in adults the reverse was observed. The duration of illness may range from 2 h to several days, with a mean or median of between 12 h and 60 h [10]. RT–PCR, coupled with sequence analysis of amplicons, has been used extensively to detect and characterize NoVs in various outbreaks. A preliminary report in 2000 showed that NoVs were associated with diarrhoea in the coastal region of southern India [11]. Since then many studies from different parts of the country showed NoV as another important cause of gastroenteritis. This study reports the detection and molecular characterization of NoVs, which play an important role as a viral aetiological agent of acute watery diarrhoea besides rotavirus in eastern India. The objectives of the study were (1) to describe the epidemiological behaviour of NoVs in Kolkata during a 2-year study including seasonal and clinical patterns, (2) to show co-infections with other pathogens such as bacteria, parasites and other viruses, namely rotaviruses, adenovirus, astrovirus or sapovirus, and (3) to understand the phylogenetic relationship of NoV detected in Kolkata with other NoV strains reported from other countries.

MATERIALS AND METHODS

Study design

This hospital-based study was performed by collecting stool specimens from patients admitted to Infectious Diseases & Beliaghata General (ID&BG) Hospital, Kolkata, India, with acute gastroenteritis from November 2007 to October 2009. The ID&BG Hospital provides treatment to about 20 000–25 000 acute diarrhoea patients annually. Patients admitted to the hospital with diarrhoeal complaints were included in the study using a systematic sampling process: on two randomly selected days each week every fifth patient with diarrhoea or dysentery was enrolled. The system remained unbiased for sex and age of the patient at the time of selection. A faecal sample from each of the enrolled patients was collected, processed for the study and total of 2495 faecal samples were analysed during the study period. After informed consent was given epidemiological information from each case was obtained from patients or guardians. Clinical data involving such disease manifestations as fever, vomiting, abdominal pain, or bloody diarrhoea were collected for all patients. Severity criteria, such as duration of diarrhoea, number of stools or vomiting episodes, range of body temperature, and degree of dehydration, were determined for all enrolled patients. This study was conducted with prior approval of the ethics committee of National Institute of Cholera and Enteric Diseases, Kolkata.

Sampling and preliminary analysis

Faecal specimens were collected in sterile glass containers and transported at 4 °C to the Division of Virology, National Institute of Cholera and Enteric Diseases, Kolkata. All faecal specimens were collected within <24 h after admission. In a 2-ml sterile Eppendorf tube 30% faecal suspension was prepared with 1× PBS (pH 7·2). The tubes containing faecal suspension were stored at 4 °C for further processing. All samples were simultaneously screened for *Vibrio cholerae* O1, *V. parahaemolyticus, V. fluvialis, Escherichia coli*, *Shigella* spp., *Aeromonas* spp., *Campylobacter coli and Salmonella* spp. by conventional bacterial culture procedures, and diarrhoeagenic *E. coli* (*DEC*) types [enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), and enterohaemorrhagic *E. coli* (EHEC)] were studied by PCR-based methods. Parasites (*Cryptosporidium*, *Entamoeba histolytica*, *Girardia lamblia*) were screened by using conventional microscopy and staining methods.

RNA extraction

Extraction of viral RNA was performed using QIAamp viral RNA mini kit (Qiagen, Germany) according to the manufacturer's instructions. Sixty microlitres of molecular biology grade viral RNA was eluted for use in RT–PCR experiments. The viral RNA was stored at −20 °C (for immediate use) or at −80 °C for long-term storage.

Reverse transcription

Briefly, 10 μl RNA was placed in a 0·2-ml Eppendorf tube and 1 μl of random primer (150 ng/μl;

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Invitrogen, USA) added. The Eppendorf tube containing the RNA and random primer was incubated in a thermal cycler at 65 °C for 5 min and then kept on ice to snap chill for 10 min, followed by addition of 9 μl RT mix to adjust the final volume to 20 μl. RT mix was comprised of 4 μl of 10× reverse transcriptase buffer (New England BioLabs, USA), 1 μl of 0·1 M DTT (dithiothreitol), 2·5 μl of 10 mM dNTPs (New England BioLabs), 1 μl RNase inhibitor (40 U/μl, Ambion, USA), 0·5 μl Moloney murine leukaemia virus reverse transcriptase (MMLV, 200 U/μl, New England BioLabs). The RT reaction was performed for 60 min at 42 °C to produce cDNA; an aliquot was used directly in the PCR amplification; excess was stored at −20 °C or −80 °C for immediate or later use, respectively.

Region C PCR amplification

Five microlitres of cDNA was added to the PCR mix containing 2·5 μl of 10× PCR buffer (Invitrogen, USA), 0·75 μl of 50 mM $MgCl_2$ (Invitrogen), 0·5 μl of 10 mM dNTPs (New England BioLabs), 1 μl of 10 pmol of each GI-specific primer (G1SKF and G1SKR) or GII primer (G2SKF and G2SKR) [12], 0·25 μl *Taq* DNA polymerase (5 U/μl, Invitrogen) and 14 μl RNase-free water to a final volume of 25 μl. PCRs in separate tubes for NVGI and NVGII were performed under the following conditions: 94 °C for 3 min followed by 40 cycles of 94 °C for 30 s, 46 °C for 30 s, and 72 °C for 1 min, with a final extension step at 72 °C for 7 min. The 330-bp and 344-bp amplicons obtained from NVGI and NVGII viruses, respectively, were visualized by 2% agarose gel electrophoresis followed by ethidium bromide staining.

PCR product purification

Twenty-four NoV GII-positive samples showing expected amplicon size (GII: 344 bp) were subjected to 50 μl PCR reaction and the final PCR product was purified using QIAquick PCR Purification kit (Qiagen). The PCR purified product was then used for sequencing.

Nucleotide sequencing

The purified PCR products were subjected to cycle sequencing using the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction kit, version 3.1 (Applied Biosystems, USA). Sequencing was performed for region C in order to identify the exact genogroup as well as to identify recombinant viruses. For region C, sequencing was carried out by using both forward and reverse primers separately. The sequences were collected from an automated DNA sequencer (ABI 3100, Applied Biosystems). The nucleotide sequences reported in this study were deposited at the DNA Data Bank of Japan (DDBJ).

Phylogenetic analysis

Sequence identity was determined through BLAST (www.ncbi.nlm.nih.gov/blast) and multiple sequence alignment was carried out with the CLUSTAL W program [13]. The phylogenetic analysis of aligned sequences was carried out using MEGA 4.0 [14]. The phylogenetic tree was generated with neighbour-joining algorithm [15]. The reliability of the phylogenetic tree was tested by applying the bootstrap test with 1000 bootstrap replications.

Nucleotide sequence accession number

The partial nucleotide sequences of the gene encoding the capsid region of the NoV strains from Kolkata (present study) were submitted to DDBJ, under accession numbers AB539140–AB539163.

Statistical analysis

The data from the above identification procedures and the epidemiological survey were entered into a pre-designed pro-forma in the SQL server with a built-in entry validation checking facilitated program. Data were randomly matched and checked to establish consistency and validity. SPSS version 14.0 (SPSS Inc., USA) was used for statistical analysis.

RESULTS

During the period of study, NoVs ($n=78$, 3·12%) were detected by RT–PCR in diarrhoeic patients ($n=2495$, enrolled in the surveillance) at ID&BG Hospital. NoVs were sporadically detected throughout the year, and the number of positives detected was insufficient to determine seasonality (Fig. 1). Six (7·7%) out of 78 positive specimens belonged to NVGI and 72 (92·3%) specimens belonged to NVGII strains. NVGI infections were detected in November 2007 (1), February 2008 (3); March 2008 (1) and May 2009 (1), respectively. Of the diarrhoea cases, NoV

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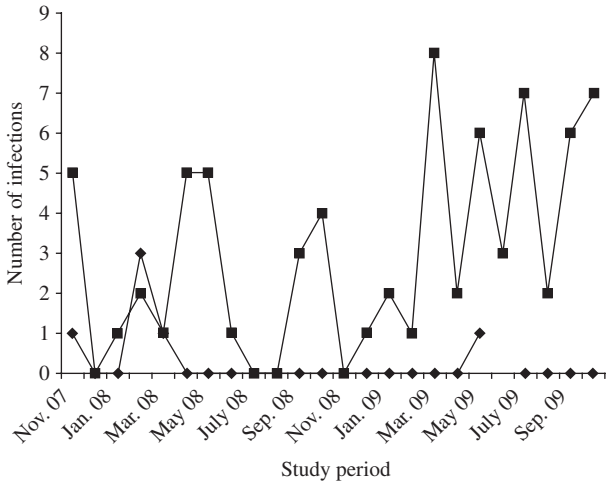

Fig. 1. Detection of NVGI (–◆–) and NVGII (–■–) infections in diarrhoea cases in Kolkata, India from November 2007 to October 2009.

was seen as either a single aetiological agent in 22/78 cases (28·2%) or found in association with other diarrhoeagenic pathogens, e.g. viral, bacteria, or parasitic agents in the other 56/78 cases (71·8%). The faecal specimens collected from all NoV-positive cases were cross-checked for any other co-infection with other enteric pathogens, such as diarrhoeagenic viruses, e.g. rotavirus, astrovirus, adenovirus, sapovirus or bacteria and parasites during the study.

Of the 78 NoV-positive cases, at least one more pathogen was detected in 56 cases with rotavirus being the most common co-infection followed by *Vibrio cholerae* O1, *Cryptosporidium* spp. and *E. coli* (ETEC). The different combinations of mixed infection with different enteric virus, bacteria and parasites is shown in Table 1. Six instances of NoV infection showed co-infection with bacteria and parasites.

Prevalence in different age groups and sex

Distribution of NoV gastroenteritis in children aged <1 and >1–2 years was similar, with the highest predominance of NoV in this age group. In the 14–30 years age group comprising older children and adults, the prevalence of NoV was marginally higher than that of children aged <2 years. Children aged between 2 and 14 years and adults aged between >30 years and >60 years showed lower rates of NoV positivity (Fig. 2). The difference in the NoV infection rate between males and females was significant (64·1% and 35·9%, respectively). Urban people of Kolkata showed more positivity for NoV infection than their rural counterparts (data not shown).

Comparison of clinical symptoms during NoV infections

Clinical symptoms associated with diarrhoea such as number of stools, number of vomiting episodes and degree of dehydration were recorded in all diarrhoea cases, and relevant data for diarrhoea cases with NoV as the only detectable pathogen are shown in Table 2. All the patients had diarrhoea associated with some dehydration, two patients had fever and 16 (72·7%) patients showed no abdominal cramp or pain. Eighteen patients (81·8%) had >5 vomiting episodes in a 24-h period; two patients had no vomiting. Of 22 patients, seven (31·8%) were aged <5 years; interestingly the severity of NoV infection was greater in five (71·4%) of these cases who suffered >10 vomiting episodes and >8 stools during a 24-h period.

Molecular characterization of partial capsid gene

It was observed that phylogenetic analysis of the 282-bp region of the capsid gene of the Kolkata strains reported in this study revealed they were divergent and occupied several branches of the phylogenetic tree (Fig. 3). It was observed that eight Kolkata strains clustered with Bristol/GII.4 (CAA54134) followed by the seven Kolkata strains with Fayetteville/GII.13 (AAM56034) and five Kolkata strains with Seacroft/GII.6 (CAB89101). Strain IDH340 clustered with Hawaii/GII.1 (AAB97768), IDH1521 clustered with Tiffin/GII.16 (AAS86789) and IDH495 and IDH500 clustered with Toronto24/GII.3 (AAA18930).

DISCUSSION

The disease burden of NoVs has been well characterized for developed countries; however, there is little information about this virus in the Indian subcontinent. In this study, we report on the surveillance of NoV from 2495 cases of diarrhoea enrolled in the study following their hospitalization at ID&BG Hospital for treatment. In the present study, 78/2495 diarrhoea cases collected during the study period were found to be positive for NoVs by RT–PCR. In a study from Pune, India, it was reported that the detection of NoV in children aged <7 years was 10·7% [16]. In a study from Djibouti (Horn of Africa) researchers were able to detect eight NoV positives out of 75 cases in adults presenting with acute diarrhoea [17]. In a 2-year study from outpatient clinics in Sapporo, Japan, Nakanishi *et al.* [18] found 13·2% positivity for NoVs

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Table 1. *Co-infection of norovirus with other enteric pathogens, i.e. viruses, bacteria and parasites in Kolkata, India during the study* (*November 2007 to October 2009*)

IDH	Norovirus	Rotavirus*	Astrovirus	Sapovirus	Adenovirus	*Vibrio cholerae* O1	*V. parahaemolyticus*	*V. fluvialis*	*E. coli* (EAEC)	*E. coli* (EPEC)	*E. coli* (ETEC)	*Shigella* spp.	*Aeromonas* spp.	*Campylobacter coli*	*Cryptosporidium* spp.	*E. histolytica*	*Giardia lamblia*
48 (Nov'07)	GII		+														
60	GII		+			+											
268 (F'08)	GI		+												+		
269	GI														+		
274	GI	+													+	+	
280	GII														+		
439 (A'08)	**GII**			+							+						+
443	GII								+								
470 (M'08)	GII																+
495	GII														+		
500	**GII**		+								+				+		
587 (Jun'08)	GII																+
877 (S'08)	GII					+											
883	GII					+											
929	GII					+											
949 (O'08)	GII														+		
1021	GII	+			+												
1285 (Dec'08)	GII									+							
1302 (J'09)	GII					+			+								
1390 (F'09)	**GII**	+			+						+				+		+
1478 (M'09)	GII	+									+						
1481	GII			+							+						
1501	GII					+											
1519	GII					+			+								
1562	GII			+													
1674 (A'09)	**GII**	+			+			+	+						+		
1751 (M'09)	GI	+			+												
1756	GII	+			+			+									
1774	**GII**										+						+
1781	GII								+								
1801	GII					+											
1854 (J'09)	GII				+												+
1857	GII	+															+
1873	**GII**	+			+						+	+				+	
2018 (Jul'09)	GII								+			+					
2051	GII					+											
2081	GII					+											
2310 (Aug'09)	GII	+	+		+												
2418 (S'09)	GII												+				
2428	GII														+		
2454	GII					+											
2485 (O'09)	GII													+			
2486	GII					+											
2503	GII										+						
2505	GII										+						
2534	GII						+			+							
Total	56	20	5	3	8	12	1	2	6	2	9	2	1	1	10	2	7

* Rotavirus co-infection only with norovirus was seen in IDH 41, 186, 278, 434, 969, 2035, 2328, 2373, 2443, 2490.

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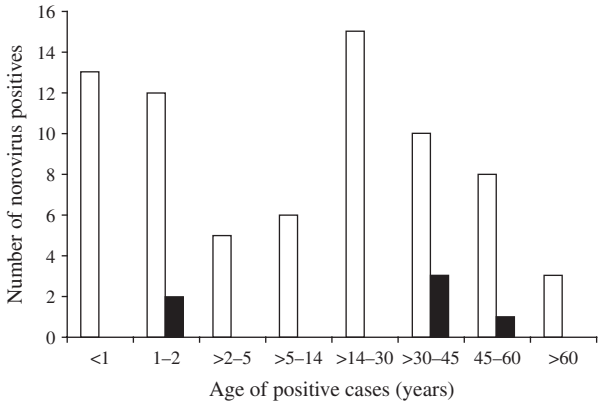

Fig. 2. Age distribution of norovirus positives detected in Kolkata, India from November 2007 to October 2009. ■, NVGI; □, NVGII.

from 877 rectal swabs collected from patients and tested by RT–PCR; frequent vomiting was prominent in NoV gastroenteritis [18]. Another study covering eight different cities of the Russian Federation described the results of 2-year surveillance for group A rotaviruses and other enteric agents, in patients hospitalized with acute gastroenteritis; whereas in the <5 years age group NoV positivity was 12·5%, while NoV was the pathogen most commonly detected in adults at a positivity rate of 11·9% [19]. In 2008 a study from Tunisia showed 17·4% of NoV in acute infantile gastroenteritis cases in Tunisia [20]. To date, in India most of the studies on NoV gastroenteritis were performed on children. This study focuses on the NoV prevalence on both children and adults. Although reports from developed countries indicate that 5–12% of cases of sporadic gastroenteritis are associated with NoVs we observed 0·9% positivity (22/2945) for NoVs as the only detectable pathogen in this study. It is presumed that this may be related to immunity and the resultant mild or asymptomatic infection associated with many exposures to the virus through close contact with infected children, impurity of the water supply and environmental contamination. Of the 78 NoV-positive cases at least one more pathogen was detected in 56 cases with rotavirus being the most common co-infection followed by *V. cholerae* O1, *Cryptosporidium* spp. and *E. coli* (ETEC). In a study from Nicaragua, NoV was detected in 12% (65/542) of children; of these an important proportion (57%) of NoV-infected children were co-infected with diarrhoeagenic *E. coli* [21]. In our study we too found an important proportion of diarrhoeagenic *E. coli* co-infection with the NoV-positive cases. In a Korean study of children with acute diarrhoea it was reported that 33 (58·9%) NoV-positive cases showed mono-infection and 23 (41·1%) had co-infection. Co-infection with rotavirus and NoV was most common, and occurred in 20/155 cases (12·9%) including co-infection with adenovirus [22]. Information on the co-infection of NoV with *V. cholerae* O1 is limited from other parts of the world. In our study a significant number of NoV were co-infected with *V. cholerae* O1. Our study showed that in 22/78 cases, NoV alone accounted for the diarrhoea, with diarrhoea episodes ranging from 4 h to $\geqslant 48$ h with varying ($n=2$–12) number of stools per day. The incidence of NoV infection in children aged <5 years and adults was quite similar. All the patients had diarrhoea associated with some dehydration, two patients had fever and 16 (72·7%) patients showed no abdominal cramps or pain. Eighteen patients (81·8%) had >5 vomiting episodes in a 24-h period and two patients had no vomiting. Similar results were found in a study from Vilnius University Children's Hospital in 2005 [23]. It was observed that in young children, NoV infection manifested as vomiting (94%), diarrhoea (81%), and fever (66%), and presented as gastroenteritis with fever (47%) or without fever (30%) [23]. In our study, we found that six (7·3%) of 78 positive specimens belonged to NVGI strain and 72 specimens belonged to NVGII strains. Similar studies in the population of Cairo, Egypt, found predominance of GII.4 genotypes. The phylogenetic analysis of the capsid gene suggested that GII.4 strains from Cairo were similar to those circulating elsewhere [24]. In our study we found six genotypes, i.e. GII.4, GII.13, GII.6, GII.1, GII.16 and GII.3; with predominance of GII.4 ($n=8$) and GII.13 ($n=7$) genotypes. In a study from Chiang Mai, Thailand, Khamrin *et al.* [25] observed that GII.4 was the most predominant genotype of NVs, followed by GII.15, GII.6, and GII.12. In a study from Nicaragua, nucleotide sequence analysis of NoV-positive samples of the N-terminal and shell region in the capsid gene revealed that at least six genotypes (GI.4, GII.2, GII.4, GII.7, GII.17, and a potentially novel cluster termed 'GII.18-Nica') circulated during the period of that study, with GII.4 virus being predominant [21]. An earlier study from Kolkata, found that 12 NoV cases (54·5%) were GII.4 and six cases showed 99% identity with the new variant Japanese strain Hu/NoV/GII.4/OC07138/JP, that study also detected three novel NVGII inter-genotype recombinant strains in children [26]. In a study from Pune, India, it was shown that the phylogenetic analysis of partial

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Table 2. *The clinical details of patients showing sole norovirus infection in Kolkata, India during November 2007 to October 2009*

Sample no.	Date of interview	Virus	Age (yr. mo.)	Sex	Diarrhoea duration (h)	Type of diarrhoea	Number of stools during last 24 h or since onset	Number of vomiting episodes during last 24 h or since onset	Vomiting duration (h)	Dehydration	Fever	Abdominal pain
IDH 46	13 Nov. 2007	NVGII	50·00	F	26	Watery	9	26	3	Some	No	No
IDH 58	15 Nov. 2007	NVGII	20·00	M	10	Watery	2	9	5	Some	No	No
IDH 96	28 Nov. 2007	NVGI	40·00	M	4	Watery	3	3	2	Some	Yes	No
IDH 321	10 Mar. 2008	NVGI	48·00	M	24	Watery	6	24	4	Some	No	No
IDH 340	13 Mar. 2008	NVGII	21·00	M	5	Loose	5	4	1	Some	No	No
IDH 414	16 Apr. 2008	NVGII	29·00	M	7	Watery	10	6	5	Some	No	No
IDH 422	22 Apr. 2008	NVGII	1·03	M	48	Watery	8	24	7	Some	No	No
IDH 489	13 May 2008	NVGII	0·07	F	12	Loose	10	10	5	Some	No	No
IDH 510	21 May 2008	NVGII	52·00	F	24	Loose	9	24	3	Some	No	No
IDH 1064	22 Oct. 2008	NVGII	2·00	F	18	Loose	8	18	3	Some	No	Yes
IDH 1305	6 Jan. 2009	NVGII	4·00	M	48	Watery	12	0	0	Some	No	No
IDH 1510	16 Mar. 2009	NVGII	70·00	M	8	Loose	8	8	2	Some	No	No
IDH 1511	16 Mar. 2009	NVGII	28·00	F	5	Watery	12	5	3	Some	No	Yes
IDH 1521	18 Mar. 2009	NVGII	3·00	F	6	Watery	6	6	4	Some	No	No
IDH 1671	20 Apr. 2009	NVGII	0·10	F	48	Loose	8	48	4	Some	No	No
IDH 1770	7 May 2009	NVGII	38·00	M	5	Loose	9	5	4	Some	No	No
IDH 1776	11 May 2009	NVGII	3·00	M	24	Loose	8	24	2	Some	No	No
IDH 2021	1 July 2009	NVGII	45·00	M	7	Watery	12	0	0	Some	No	Yes
IDH 2040	6 July 2009	NVGII	20·00	M	12	Watery	12	12	8	Some	No	Yes
IDH 2138	22 July 2009	NVGII	39·00	M	24	Watery	12	24	4	Some	No	Yes
IDH 2381	14 Sept. 2009	NVGII	52·00	M	12	Watery	14	12	4	Some	No	Yes
IDH 2493	13 Oct. 2009	NVGII	30·00	M	24	Watery	8	24	7	Some	Yes	No

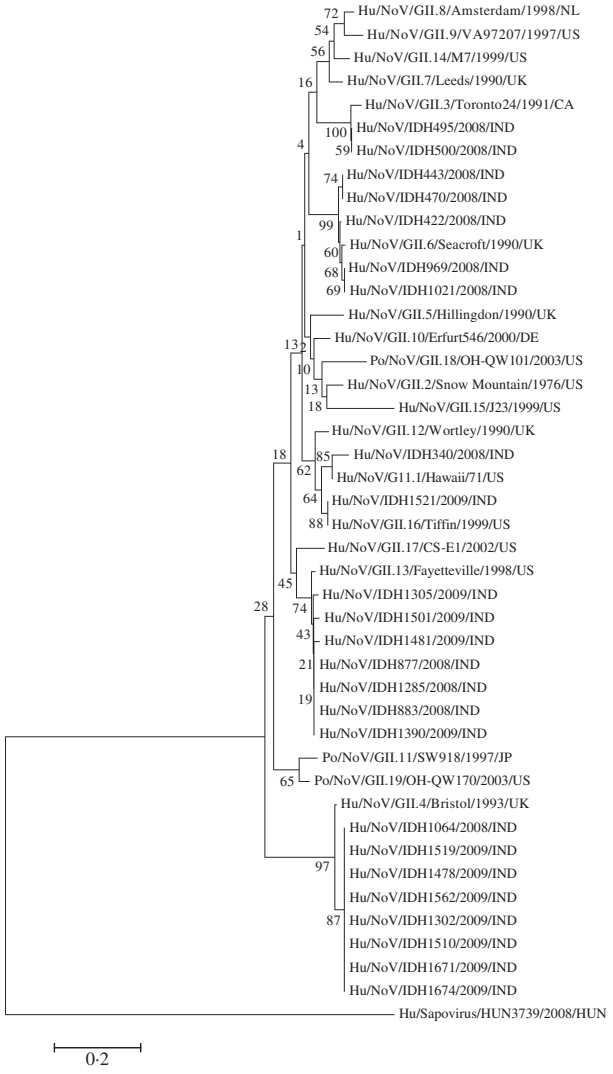

Fig. 3. Phylogenetic analysis based on deduced amino-acid sequences corresponding to 282-bp nucleotide fragment of the capsid gene of Kolkata NoV strains (shown in bold face) and other NoVs. The accession numbers of the capsid fragments for NoV strains shown on the tree is as follows: GII.8/Amsterdam (AAF05820); GII.9/VA97207 (AAK84676); GII.14/M7 (AAN05735); GII.7/Leeds (CAB89089); GII.3/Toronto24 (AAA18930); **IDH495** (AB539144); **IDH500** (AB539145); **IDH443** (AB539142); **IDH470** (AB539143); **IDH422** (AB539141); GII.6/Seacroft (CAB89101); **IDH969** (AB539148); **IDH1021** (AB539149); GII.5/Hillingdon (CAB89088); GII.10/Erfurt546 (AAL18874) GII.18/OH-QW101 (AAX32877); GII.2/Snow Mountain (AAB16915); GII.15/J23 (AAN05736); GII.12/Wortley (CAB89099); **IDH340** (AB539140); GII.1/Hawaii (AAB97768); **IDH1521** (AB539160); GII.16/Tiffin (AAS86789); GII.17/CS-E1 (AAS86786); GII.13/Fayetteville (AAM56034); **IDH1305** (AB539153): **IDH1501** (AB539157); **IDH1481** (AB539156); **IDH877** (AB539146); **IDH1285** (AB539151); **IDH883** (AB539147); **IDH1390** (AB539154); GII.11/SW918 (BAB83516); GII.19OH-QW170 (AAX32883); GII.4 Bristol (CAA54134); **IDH1064** (AB539150); **IDH1519** (AB539159); **IDH1478** (AB539155); **IDH1562** (AB539161); **IDH1302** (AB539152); **IDH1510** (AB539158); **IDH1671** (AB539162); **IDH1674** (AB539163); out group strain Sapovirus Kecskemet/HUN3739 (ACO72592).

RNA polymerase and VP1 (capsid) genes identified five NVGII strains (GII.4, GII.6, GII.7, GII.8, and GII.14 genetic clusters) with the possible occurrence of a '2007 new-variant' of GII.4 [16]. A study from Delhi, India, found 36 (61%) positive for NoV (34 NVGII, 2 NVGI) and identified three genotypes (GII.4, GII.3, GII.b) in children with acute sporadic gastroenteritis [27]. Our study reports first time on the appearance of GII.13, GII.6, GII.1, GII.16 and GII.3 NoVs in children and adults in Kolkata. From the present study, it is clear that NoV is an important viral aetiological agent having a significant role in gastroenteritis, in children and adults other than rotavirus in Kolkata. Therefore, routine surveillance for NoV infections in different settings and molecular epidemiological studies will provide interesting information that will enable us to understand the nature and spread of NoV infection.

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DECLARATION OF INTEREST

None.

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