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A new variant of Norovirus GII.4/2007 and inter-genotype recombinant strains of NVGII causing acute watery diarrhoea among children in Kolkata, India

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ABSTRACT

Background: Noroviruses (NoVs) are one of the major causal agents of acute gastroenteritis among different age groups. Some of the recent studies reveal that NoV genome is highly prone to mutation and recombination which often leads to emergence of new strains.
Objectives: To explore the genetic diversity of human Caliciviruses (HuCVs) among diarrhoeic children in Kolkata.
Study design: The HuCVs were detected by reverse transcription-polymerase chain reaction (RT-PCR) of the partial RNA dependent RNA polymerase gene (RdRp) and capsid gene and confirmed by sequencing. The sequences were analyzed and the recombination point was detected.
Results: Faecal specimens of children ($n = 111$) visiting outpatient department of Dr B. C. Roy Memorial Hospital for Children with acute gastroenteritis were studied: 22 cases were HuCV positive with 21 NoVs. Of these, 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. Three novel NoV GII inter-genotype recombinant strains V1628/IND, V1656/IND and V1737/IND were also detected. The RdRp region of V1628 showed 96% identity with Pont de Roide 673/FRN whereas capsid region resembled GII.7/Osaka F140/JPN strain (98%); the strain V1656 showed 98% identity with RdRp region of GII.4/Monastir 375/TUN but capsid region resembled GII.8/Leverkusen 267/DE (91%); the strain V1737 showed 88% identity with RdRp of GII.5/Minato 6/N1/6/JPN whereas capsid region resembled the GII.12/Gifu 96/JPN (93%). During characterization of Caliciviruses two strains of NoV GII.b and one strain of each NoV GI.1/V1622/06/IND, GI.3/V1707/07/IND, GII.3/V1668/IND, GII.16/V1729/IND, Sapovirus GII.1/V1716/IND were also detected.
Conclusions: The emergence of new variant of GII.4/2007, three novel NoV GII inter-genotype recombinant strains and various other NoVs, indicates the remarkable genetic diversity of the HuCVs as diarrhoeagenic viruses circulating in Kolkata, India.

1. Background

Acute gastroenteritis is one of the most common diseases of humans in developed as well as developing countries.[1] Noroviruses (NoVs) have been identified as one of the major causal agents of sporadic infection or outbreaks of gastroenteritis worldwide among different age groups.[2,3] The two genera namely NoV and Sapovirus (SaV) that belong to the family *Caliciviridae* are referred as human Caliciviruses (HuCVs), consisting of single-stranded, positive-sense, polyadenylated RNA genome of 7.4–7.7 kb.[4] The genome of NoVs comprises of three open reading frames (ORFs) whereas the genome of SaVs consists of two ORFs.[4–7] The NoVs are genetically variable and show divergent phylogeny among different strains within a genogroup and the analysis of their genome has become a primary means of classification.[8] Currently there are seven genogroups of NoVs, out of which five genogroups (GI, GII, GIV, GVI and GVII) are associated with human gastroenteritis.[8–11] The GII.4 strains showing higher prevalence have emerged as the principal strains that cause outbreaks and sporadic infections worldwide.[12] An inter-genogroup recombinant NoV that was involved in causing acute watery diarrhoea, in Kolkata was also reported recently.[13]

2. Objective

The NoVs show high level of genetic diversity, so we attempted to study the genetic changes among NoV strains circulating in Kolkata, India. In this study, in-depth molecular characterization of the new

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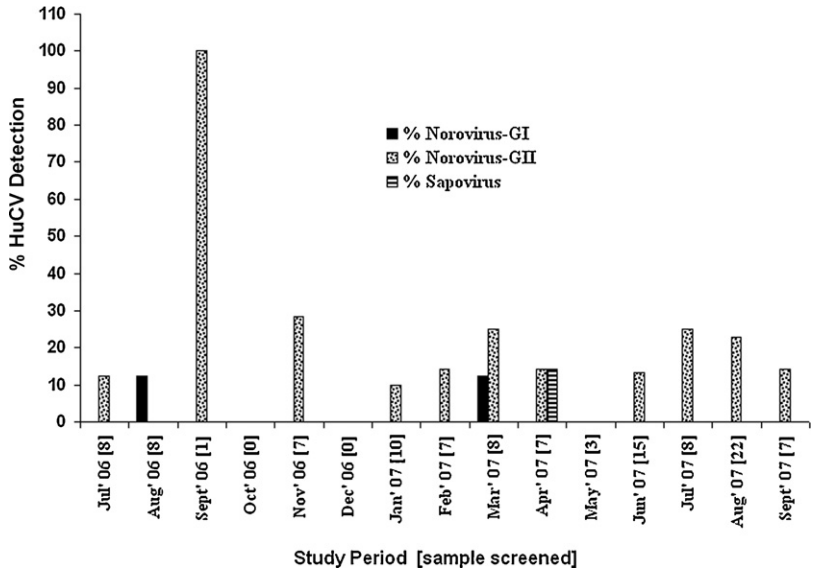

Fig. 1. Graphical analysis of the HuCVs detected in Kolkata, Eastern India between July' 06 and September' 07 among children suffering from acute watery diarrhoea.

variant of GII.4/2007 strain, three novel inter-genotype recombinant strains of NoV GII and other HuCV strains causing acute watery diarrhoea among children in Kolkata is described.

3. Study design

Faecal specimens were collected from children aged 5 months to 3 years suffering from acute watery diarrhoea, visiting the outpatient clinic at Dr. B. C. Roy Memorial Hospital for Children, Kolkata, India, during the period July' 06–September' 07. The viral RNA was extracted from the specimens, by using QIAamp viral RNA kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions.

The reverse transcription (RT) reaction for NoV was preformed by using 100 ng of random hexamers and Avian Myeloblastosis Virus Reverse Transcriptase (AMV-RT) (Promega, Madison, USA). The partial RNA dependent RNA polymerase (RdRp) region (partial ORF1), partial capsid region (partial ORF2) and partial ORF1–ORF2 along with the overlapping junction of NoV genome were amplified by polymerase chain reaction (PCR) by using the primer pair as described previously.[13,14] The RT reaction for SaV was carried out by using 100 ng of random hexamers and AMV-RT (Promega, Madison, USA) and PCR reaction was carried out by using the primer pair Sapp36 and JV33 to amplify the partial fragment of RdRp region.[15,16] The sequencing reaction and sequence analyses were carried out as described previously.[13] The possible recombination and crossing-over point was detected by using the SimPlot version 3.5.1.[17]

The nucleotide sequence of partial RdRp and capsid region with the overlapping junction of ORF1 and ORF2 of the Kolkata strains was submitted to DNA Data Bank of Japan (DDBJ), http://www.nig.ac.jp under the accession number: AB447405, AB447406, AB453773, AB453774 and AB447409–AB447426.

4. Results

A faecal specimen was collected from acute watery diarrhoea patients ($n = 111$) for the detection of HuCVs (Fig. 1). The 814 bp fragment within the RdRp region was amplified by RT-PCR for NoV

Table 1
The clinical symptoms associated with HuCV infections among children aged 5 months to 3 years in Eastern part of India (Kolkata).

Sample no.	Diarrhoea	Dehydration	Vomiting	Anorexia	Onset of diarrhoea	Frequency of diarrhoea	HuCV detected
V1607	+	–	–	–	2 h	6–7 times	NoV G-II
V1622	+	–	–	–	20 h	10 times	NoV G-I
V1628	+	–	+	–	72 h	7–8 times	NoV G-II
V1656	+	–	–	–	28 h	8 times	NoV G-II
V1668	+	–	–	–	72 h	4–5 times	NoV G-II
V1682	+	–	–	–	24 h	9–10 times	NoV G-II
V1699	+	+	–	–	12 h	7–8 times	NoV G-II
V1702	+	–	–	–	14 h	8 times	NoV G-II
V1706	+	–	–	–	72 h	5 times	NoV G-II
V1707	+	–	–	–	72 h	4 times	NoV G-I
V1714	+	–	+	–	24 h	4 times	NoV G-II
V1716	+	–	–	–	24 h	6 times	SaV
V1729	+	–	–	–	51 h	4 times	NoV G-II
V1737	+	+	–	–	27 h	10 times	NoV G-II
V1749	+	–	–	–	24 h	4–5 times	NoV G-II
V1750	+	–	–	–	12 h	2 times	NoV G-II
V1760	+	–	–	–	20 h	4–5 times	NoV G-II
V1766	+	–	–	–	12 h	3 times	NoV G-II
V1772	+	+	–	+	24 h	5–6 times	NoV G-II
V1774	+	–	–	–	72 h	8–10 times	NoV G-II
V1776	+	–	–	–	36 h	4–5 times	NoV G-II
V1783	+	–	–	–	24 h	4–5 times	NoV G-II