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Detection of a novel intergenogroup recombinant Norovirus from Kolkata, India

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ABSTRACT

Mutation and recombination are recognized as important driving forces of evolution among RNA viruses. An intergenogroup recombinant norovirus strain [Hu/Kol/NLV/L8775/*AB290150*/2006/India] was detected in the faecal specimen of a 17 year old male, who had suffered from acute watery diarrhea and severe dehydration. Sequence analysis confirmed that this novel recombinant strain had a polymerase gene fragment that closely resembled a Norovirus (NoV) genogroup-I genotype-3 virus (HuCV/NLV/GI.3/VA98115/*AY038598*/1998/USA) and a capsid gene resembling NoV genogroup-II genotype-4 virus (NoV/Hu/GII.4/Terneuzen70/*EF126964*/2006/NL). The crossing over and recombination was observed at nucleotide (nt) 790 of NoV GI VA98115 strain and nt808 of NoV GII Terneuzen70 strain. In both parent strains conserved nucleotide sequence and hairpin structure (DNA secondary structure) were reported at the junction point of ORF1 and ORF2, exhibiting the mechanism of recombination in these viruses. Thus this novel recombinant NoV is another step in evolution among NoVs, indicating that constant surveillance is important to successfully monitor emergence of these strains.

Introduction

Norovirus (NoV) belonging to family *Caliciviridae* was the first diarrheagenic virus detected by Kapikian et al. (1972). NoVs are nonenveloped, 27 to 40 nm in diameter with single-stranded, positive-sense, polyadenylated RNA genome of 7.4 to 7.7 Kb (Atmar and Estes, 2001). The genome of NoVs comprises of 3 ORFs viz. ORF1 encodes six nonstructural polyproteins [p48, NTPase, p22, VPg, 3CL and RdRp], ORF2 encodes the major capsid protein [VP1] and ORF3 encodes the minor structural protein [VP2] (Hardy, 2005; Jiang et al., 1993; Lambden et al., 1993). NoV is chiefly associated with food borne gastroenteritis, including outbreaks in developed countries (Mead et al., 1999; Okitsu-Negishi et al., 2004). In developing countries, the prevalence rate of NoV associated gastroenteritis is higher and is often associated with severe illness (Atmar et al., 2001; Lopman et al., 2002).

NoVs have been divided into seven genogroups based on genome sequence variability of their RNA dependent RNA polymerase (RdRp) and capsid (Phan et al., 2007), of which 5 genogroups (G) viz. NoV GI, GII, GIV, GVI and GVII have been associated with human gastroenteritis (Ando et al., 2000; Koopmans et al., 2002; Vinje et al., 2004; Phan et al., 2007). To date, molecular characterization of NoVs has revealed 16 genotypes of GI, 23 genotypes of GII, 2 genotypes of GIII and GVI, but only 1 genotype of GIV, GV, and GVII (Phan et al., 2007). Recently Bull et al. (2007) reported intergenotype recombination among 20 different NoV strains. The site of recombination was found mainly at the junction point of ORF1 and ORF2 (Bull et al., 2005; Jiang et al., 1999).

RNA viruses show extremely high mutation rates, owing to lack of proofreading activity in their replicases. The genome of these viruses often undergoes recombination and segmentation (Domingo and Holland, 1997); many genera of positive strand viruses show genome scale ordered RNA structure (GORS), that could play an important role in RNA virus replication and rapid evolution (Simmonds et al., 2004). It has been recognized that these are some of the driving forces of RNA virus evolution that could probably lead to the origin of a new recombinant strains that may be suitable as a multivalent vaccine candidate against these viruses in future (Suzuki et al., 1998). A large number of recombinant strains of NoVs have been reported viz., the NoV strain Arg320 from Norfolk, USA with RdRp region like Lordsdale virus (GII.4) and capsid region of the Mexican NoV (GII.3) strain (Jiang et al., 1999); the NoV strain Mc37 from Thailand showed that its ORF1 sequence has 97.2% nucleotide identity to that of Saitama U1 virus but only 71.3% and 67.9% nucleotide identity in ORF2 and ORF3, respectively (Hansman et al., 2004). The NoV strain MD145-12 has hybrid genome comprising stretches from Lordsdale virus, Gifu96, SaitamaU1, U3, U4, U16, U17, and U25 (Etherington et al., 2006).

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In the course of this study, a recombinant NoV strain [Hu/Kol/NLV/L8775/2006/India] was detected that showed a hitherto unobserved recombination event among NoVs from two different genogroups viz. GI.3/VA98115/1998/USA and GII.4/Terneuzen70/2006/NL. The recombination crossover point was determined by using the RAT program. Furthermore, the DNA secondary structure of both parental NoV strains showed similar secondary structure that affirmed possible mechanism of recombination that gave rise to the L8775 NoV strain.

Results

Detection of recombinant human Norovirus

The faecal specimen (L8775/2006/Kolkata/India) was obtained from a 17 year old male hospitalized for treatment of acute watery diarrhea and severe dehydration at the Infectious Diseases and Beliaghata General Hospital, Kolkata, India. Acute watery diarrhea was associated with other clinical symptoms such as abdominal pain and vomiting. The specimen was negative for *Escherichia coli*, *Vibrio cholerae*, *Shigella,* Rotavirus, Adenovirus, Astrovirus, Sapovirus and Picobirnavirus. The specimen L8775 was positive for NoV RNA by RT-PCR. The monoplex PCR was carried out with different primers (Table 1) that showed the following results: (1) NV1F-NV1R amplified 814 bp of the RdRp gene which was confirmed as a GI nucleotide fragment after sequencing, and (2) JV24–JV21 amplified 300 bp of the capsid gene which was confirmed as a GII nucleotide fragment after sequencing. The NV1F-JV21 amplified 1098 bp of the partial RdRp, ORF1–ORF2 overlap and the partial capsid gene of the L8775 strain which was confirmed after sequencing. The primer pair NV1F-JV21 eliminates the possibility of co-infection with two different NoV genogroups and locates the crossover site in the nucleotide sequence of L8775 NoV. The PCR amplicons of the L8775 strain were sequenced and the partial nucleotide sequence was aligned with other NoV reference strains available in GenBank using the BLAST program. The RdRp region showed 86% similarity to the US NoV strain (Hu/NLV/GI.3/VA98115/98/US), 85% to that of other NoVs viz. NoV/Hu/Vesoul576/03/France, NLV/LittleRock/316/94/US, and NoV Hu/GI/Otofuke/79/JP, and 83% with Desert Shield virus DSV395. The capsid region showed 99% similarity with the Netherland strain (NoV/Hu/GII.4/Terneuzen70/06/NL) and 98.9% similarity to the Japanese strain (NoV/Hu/GII.4/Isumi/060936/06/JP). The comparison of nucleotide sequence similarity between different NoVs and the partial sequence of the L8775 strain is shown in Table 2. Moreover, the nucleotide sequence (1098 bp) of the L8775 strain (the nt1–nt820) showed similarity with NoV GI.3 strains (VA98115, Otofuke and DSV), whereas the nt544 to nt1095 (551nt) stretch closely resembled that of GII.4 NoVs (Oxford, Isumi, and Terneuzen70).

Detection of breakpoint

The RAT program was executed by comparing the percentage nucleotide similarity of seven strains [GI.3/NLV/VA98115/98/US, GI.3/Otofuke/79/JP, GII.4/Terneuzen70/NL, GII.4/Chiba/04-899/04/JP, GII.4/Oxford/B2S16/02/UK, GII.4/FarmingtonHills/02/USA, GII.4/Isumi/060936/06/JP] with the L8775 strain as a query; a recombination point was observed at nt881 in the L8775 NoV strain that was 62 bases downstream from junction point of ORF1–ORF2. But the GII strain shows similarity with the L8775 strain before the recombination point, suggesting that the point is significantly earlier than nt881 which is located at nt819 and 6nt downstream from the junction of ORF1–ORF2. The recombination point was observed at nt790 of NoV VA98115 strain reported from USA and at nt808 of NoV Terneuzen70 strain reported from Netherlands, which are 6nt and 3nt downstream from the overlapping region respectively. Before the breakpoint, the L8775 NoV strain showed maximum similarity with NoV GI, but beyond this point maximum similarity was observed with NoV GII (Fig. 1) which affirms the occurrence of a prominent recombination between two genogroups of NoVs.

Table 1
Primers used for detection of different NoV genogroups during the study

Primer	Sequence (5′–3′)	Ref
NV1F(+)	G A T G C A G A T T A T A C A G C A T G G G A	This study*
NV1R(−)	C T T K G A C G C C A T C W T C A T T R AC	This study*
NV2F(+)	T C A G C T C T A G A A A T C A T G G T T	This study**
NV2R(−)	T T C G A C G C C A T C T T C A T T C A C A	This study**
JV22(+)	G T A A A T G A T G A T G G C G T C T A	de Bruin et al. (2006)
JV23(−)	A T A T T I C C M A C C C A R C C A T T	de Bruin et al. (2006)
JV24(+)	G T G A A T G A A G A T G G C G T C G A	de Bruin et al. (2006)
JV21(−)	C C N R C M Y A A C C A T T R T A C A T	de Bruin et al. (2006)

K-G or T, W-A or T, R-G or A, I-inosine, M-A or C, N-A or T or G or C, Y-T or C.
*The position is indicated relative to Noroviruses *M87661* for NV GI from 4562–4584 to 5354–5375 and ** indicate position related to *AY587984* for NV GII from 4340–4360 to 5080–5101.

Table 2
Comparative nucleotide sequence similarity (%) of the RdRp and capsid region of Kolkata strain L8775 with different Genogroup I and Genogroup II NoVs*

		RdRp								
	IND	A_GI	B_GI	C_GI	D_GI	E_GII	F_GII	G_GII	H_GII	I_GII
IND		**86.2**	**85.4**	**84.5**	**76.9**	62.6	64.8	63.9	64.7	63.9
A_GI	65.7		84.9	97.3	75.6	64.3	64.5	64.7	64.5	62.1
B_GI	65.2	85.8		84.7	78.3	64.2	65.6	65.0	65.9	65.4
C_GI	66.0	98.3	86.8		90.2	64.7	65.1	65.3	65.1	46.9
D_GI	65.2	89.2	83.0	73.9		58.7	60.0	59.9	60.5	59.9
E_GII	**99.0**	64.3	64.5	64.6	64.5		96.3	98.4	95.9	98.5
F_GII	**98.2**	65.0	65.2	65.3	65.2	97.5		97.2	99.4	96.2
G_GII	**97.9**	64.6	64.5	65.0	64.5	98.9	96.7		96.3	98.3
H_GII	**97.5**	64.3	64.5	64.6	64.5	98.6	98.9	98.2		95.8
I_GII	**98.9**	65.0	65.2	65.3	65.2	98.9	98.6	90.6	90.2	
		Capsid								

* IND = Hu/NLV/L8775/06/*AB290150*/IND; A_GI = Hu/NLV/VA98115/*AY038598*/1998/USA; B_GI =Hu/GI/Otofuke/*AB187514*/1979/JP; C_GI = Hu/NLV/Little Rock/316/*AF414405*/1994 /USA; D_GI =Hu/GI/DSV395/DesertShield/*U04469*/2000/USA; E_GII = Hu/GII.4/Terneuzen70/*EF126964*/2006/NL; F_GII = Hu/NoV/Farmington Hills/*AY502023*/2002/USA; G_GII = Hu/Chiba/04-899/*AB220924*/2004/JP; H_GII = Hu/NLV/Oxford/B2S16/*AY587989*/ 2002/UK; I_GII = Hu/NLV/Isumi/060936/*AB294790*/2006/JP.

Phylogenetic analysis of the partial sequence of RdRp and Capsid region

The phylogenetic tree generated with the 814 bp nucleotide sequence of RdRp region showed that the L8775 strain clustered with Otofuke, DSV, Djibouti, Vesoul and Mougon strains of NoVs which belong to GI and genotype-3 (GI.3) (Fig. 2A). However the phylogenetic tree of capsid sequences with 300 bp fragments showed that the L8775 strain clustered with Terneuzen70 and Isumi strains that belong to GII and genotype-4 (GII.4) (Fig. 2B). The phylogenetic analysis of 1098 bp nt sequences [spanning the partial sequence of RdRp region as well as the capsid region, together with its junction point] of L8775 NoV strain and several NoV GI and GII strains, as proposed by Zheng et al. (2006), revealed two distinct clades — A and B and relationship among them near the overlapping region. The clade A was found to comprise of few genotypes of NoV GI (GI.5, GI.7 and GI.8) and NoV GII (GII.5, GII.6, GII.7, GII.8, GII.10, GII.12, GII.13, G.II14 and GII.15), while in clade B a sub cluster was quite distinct with GI.3 NoV strain DSV that appeared to be distantly related to other NoV strains. The Kolkata strain L8775 clustered with GI.3/VA98115 strain to form a distinct sub cluster I; within the sub cluster II GII.1, GII.2, GII.3, GII.4, GII.16, GII.17 and GII.18, were observed and within the sub cluster III GII.9, GII.11, and GII.19 NoVs were observed. The sub cluster IV consisted of GI.1, GI.2, GI.4 and GI.6. Clade B shows the relationship