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ScienceDirect

Virology 363 (2007) 11 – 14

VIROLOGY

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Rapid Communication

Characterisation of a novel recombination event in the norovirus polymerase gene

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Received 20 January 2007; accepted 8 March 2007
Available online 16 April 2007

Abstract

This communication describes a novel recombination event in the norovirus genome. Similarity plot analysis of a nucleotide fragment (1003 bp) amplified from a norovirus positive clinical specimen (IrlN05771) identified a previously undescribed recombination point in the 3′ region of the polymerase gene (nucleotide position 4889 bp). Nucleotide multiple alignments demonstrated that Irl05N771 shared 78.6% and 94% identity with all other Irish norovirus sequences before and after the recombination point, respectively and confirmed the sequence as a genogroup II/type 4 recombinant. Irl05N771 shared more identity with Asian norovirus sequences. This is the first description of recombination within the norovirus polymerase and highlights the continuous genetic evolution of noroviruses.

Keywords: Norovirus; Recombination; Molecular; Characterisation; Polymerase; Ireland

Introduction

Noroviruses (NoV) belong to the family of caliciviruses, a genetically diverse group of morphologically similar positive stranded RNA viruses, which are the most common cause of gastroenteritis worldwide. The noroviral genome consists of three separate open reading frames (Orf), encoding the non-structural proteins (Orf 1), the capsid structural protein (Orf 2) and a small basic structural protein (Orf 3) (Clarke and Lambden, 2000; Jiang et al., 1993; Lambden et al., 1993; Prasad et al., 1999). A total of 14 different genogroup I (GI) genetic genotypes (GI/genotype1 (GI/1)) to GI/14) and 17 different GII genotypes (GII/1 to GII/17) have been reported to be circulating in Japan between 1997 and 2002 (Kageyama et al., 2004). This huge amount of genetic diversity is a direct result of the virally encoded RNA dependent RNA polymerase and strain recombination (Rohayem et al., 2005). This communication describes for the first time a novel recombination event in the norovirus polymerase gene.

Results

A blinded 2-year prospective study analysed NoV strains circulating in Ireland between 2003 and 2005 (n=976). In the first year of the study (n=478), Bristol-like or GII/4 viruses accounted for the majority of infections (92.2%) and most specimens (95%) received for analysis were derived from the hospital setting, reflecting the epidemiology of NoV-associated outbreaks in Ireland (Waters et al., 2006). During this prospective study, we received a stool specimen (Irl05N771) for laboratory analysis, which was confirmed positive for noroviral RNA by a diagnostic RT-PCR that targeted the polymerase gene (nucleotide positions 4279–4586) (Vennema et al., 2002; Vinje and Koopmans, 1996). The Orf 1/Orf 2 junction region of the NoV genome was amplified in a second PCR and phylogenetic analysis was based on alignments of these sequenced amplicons, which consisted of 203 bp of the 3′ end of the polymerase gene and 250 bp of the 5′ end of the capsid gene (nucleotide position 4880–5332). Two maximum likelihood phylogenetic trees, based on the partial coding regions of both the polymerase and capsid genes were compared. The Irl05N771

Abbreviations: NoV, norovirus; Orf, open reading frame; RT-PCR, reverse transcription-polymerase chain reaction.

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doi:10.1016/j.virol.2007.03.012

NoV sequences exhibited aberrant clustering on the GII/4 branch of the tree, and it was suspected that the NoV strain had undergone a recombinational event. A larger region of the Orf 1/Orf 2 junction was amplified (1003 bp, nucleotide positions 4329–5332) and bootscanning and similarity plot analyses were carried out. The Irl05N771 Genbank sequence accession number is EF219487.

Similarity plot analysis identified a putative recombination site in the Irl05N771 sequence, which was mapped to nucleotide position 4889 (with respect to the Lordsdale virus, accession no. X86557) and is located in the 3′ end of the polymerase gene (Fig. 1). Two neighbour-joining phylogenetic trees, based on sequences found either side of the identified recombination site, were compiled to confirm recombinant strain detection. Irl05N771 clustered as a GII/4 genotype when the phylogenetic tree was built from NoV sequences located after the identified recombination site (Fig. 2).

Basic Local Alignment Sequence Tool (BLAST) analysis identified previously characterised NoV sequences that shared high nucleotide identity with Irl05N771. The Irish recombinant sequence was genetically more similar to Asian NoV sequences than other Irish strains. Irl05N771 shared 97% identity with the Hu/Hokkaido/133/2003/JP NoV strain in the polymerase gene, before the recombination site (nucleotide 4329–4880), although the genotypic origin of this sequence could not be determined. The Irl05N771 sequence clustered with Hu/Hokkaido/133/2003/JP with a bootstrap value of 100% and away from all other characterised reference strains (Table 1). Analysis of sequences located after the newly identified recombination site (nucleotide 4900–5332) showed that the recombinant strain was 98% identical to a Japanese GII/4 strain (Hu/Sakai/04-179/2005/JP) (Table 1, Fig. 2).

Nucleotide multiple alignment of Irish NoV sequences was compiled to investigate the nucleotide identity shared between other Irish GII/4 sequences and Irl05N771. This analysis demonstrated that Irl05N771 shared 78.6% and 94% identity with all other circulating Irish NoV strains before and after nucleotide 4889, respectively, confirming the sequence as a GII/4 recombinant. Vinje et al. have shown that strains belonging to the same genotype present more than 87% and 91% identities to each other for GI and GII strains, respectively (Vinje and Koopmans, 1996).

Discussion

The NoV genus is highly genetically diverse and this is, in part, maintained by homologous recombination. In 2006, Reuter et al. described an ‘epidemic spread of recombinant NoV strains in Hungary’ and estimated that approximately 14.4% of all strains analysed were the newly classified GII/b recombinants (Reuter et al., 2006). NoV recombination usually occurs at the Orf 1/Orf 2 junction of the genome (Ambert-Balay et al., 2005; Rohayem et al., 2005). Recombination within the capsid gene has also been described and shown to occur by homologous recombination at the interface been the P1-1 and P2 sub-domains and within the P2 sub-domain (Ambert-Balay et al., 2005). To date, no inter-genogroup recombinant strain has been identified.

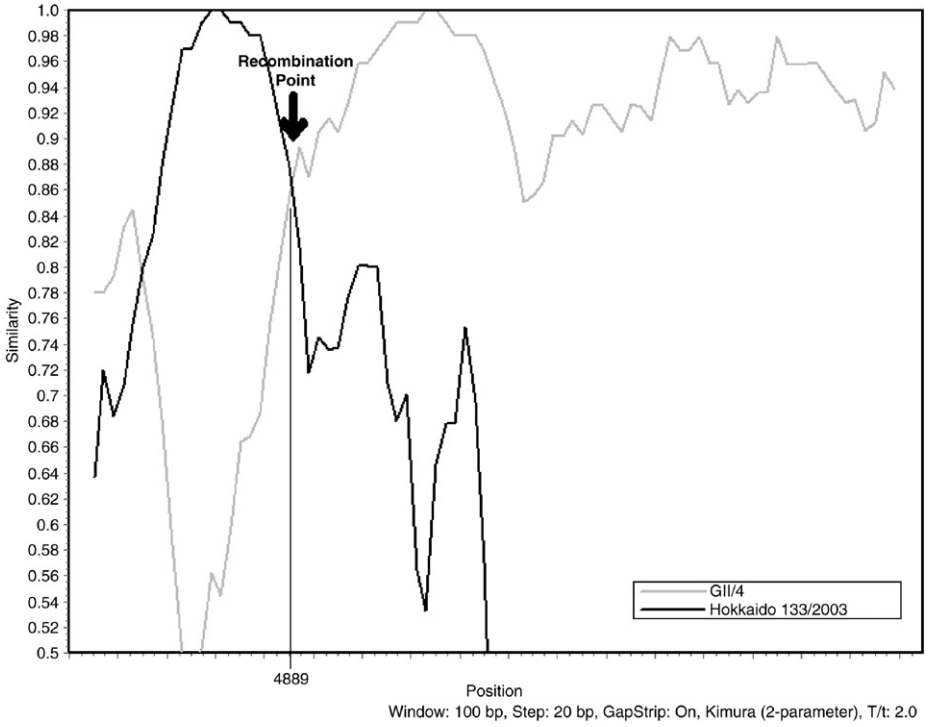

Fig. 1. Sequence similarity plot analysis of the Orf 1/Orf 2 junction (1003 bp) of the strain Irl05N771. The sequence was most similar to the Hu/Hokkaido/133/2003/JP before the recombination site and afterward shares the highest identity with a group of GII/4 viruses. Similarity plot analysis was carried out using the program Simplot (Lole et al., 1999). The recombination site is located at nucleotide position 4889, with respect to the published Lordsdale GII/4 NoV sequence, accession number X86557.

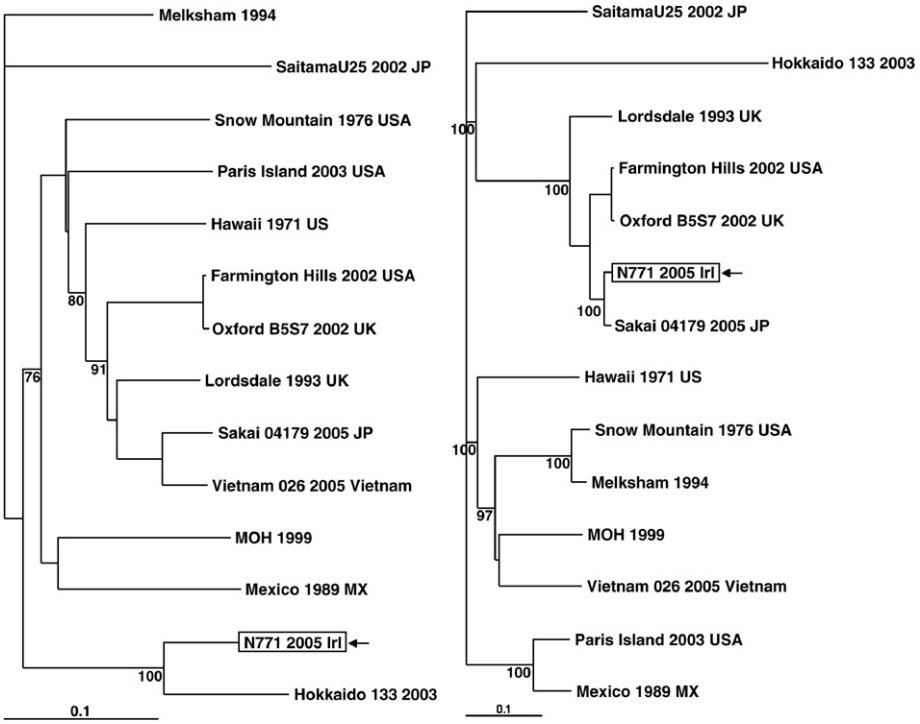

Fig. 2. Neighbour-joining phylogenetic trees based on sequences before and after the identified recombination site (nucleotide positions 4329-5332). (a) The sequence Irl05N771 is most similar to the strain Hu/Hokkaido/133/2003/JP and clusters on a separate branch with a bootstrap value of 100. (b) The sequence Irl05N771 clusters on the GII/4 branch of the tree with a bootstrap value of 100 and specifically with the Japanese GII/4 strain Hu/Sakai/04-179/2005/JPGII/4. Trees were built using the neighbour-joining algorithm in PAUP* and bootstrap resampling was carried out using the neighbour-joining algorithm also. Bootstrap values of less than 70% are not shown.

Genomic recombinational events between RNA viruses are thought to occur at high frequencies. However, the high prevalence of GII/4 strains within the Irish NoV population renders the detection of recombinant strains difficult. The Irish Irl05N771 recombinant strain described in the present study has not been isolated since and likely represents a genetically unstable recombinant virus strain and is probably an unusual case of NoV associated gastroenteritis within the Irish NoV strain population. The genetic similarity of the characterised recombinant strain, with previously published Chinese and Japanese strains, and not Irish and European strains, suggests that the recombinant viral strain may have originated in Asia. Notably the recombination site of the strain Irl05N771 was located in the polymerase gene and is different to the more commonly described Orf 1/Orf 2 junction and capsid regions.

To our knowledge this is the first description of recombination in the NoV polymerase gene and highlights the continuous genetic evolution of noroviruses.

Table 1
Norovirus reference strains

Genotype	Accession number	Taxonomic strain name
GII/1	U07611	Hu/NLV/Hawaii virus/1971/US
GII/2	AY134748	Hu/NV/SMV/1976/US
GII/2	X81879	Hu/NLV/Melksham/1994
GII/3	U22498	Hu/NV/MX/1989/MX
GII/4	AY588014	Hu/NLV/Oxford/B5S7/2002/UK
GII/4	AY502023	Hu/NoV/Farmington Hills/2002/USA
GII/4	X86557	Hu/NV/LD/1993/UK
GII/4	AB220922	Hu/Sakai/04-179/2005/JP
GII/5	AF397156	NLV/MOH/1999
GII/6	AF414416	NLV/Miami/81/1986/US
GII/6	AF414407	NLV/Florida/269/1993/US
GII/7	AF414409	NLV/Gwynedd/273/1994/US
GII/8	AB039780	NLV/SaitamaU25/2002/JP
GII/10	AF504671	NLV/Vietnam026/2005/Vietnam
GII/b	AY773210	Hu/NLV/VannesL169/2000/France
GII/b	AB231359	Hu/Hokkaido/157/2003/JP
GII/b	AY652979	NLV/Paris Island/2003/USA
GII/b	AY588132	NLV/Sydney2212/2004/AUS
Unknown	AB212306	Hu/Hokkaido/133/2003/JP

The genotype, strain and year are listed.

Materials and methods

The specimen, Irl05N771, was collected and analysed during a blinded 2-year norovirus prospective study, as previously described by Waters et al. (2006). The Orf 1/Orf 2 junction of the noroviral genome (450 bp) was amplified according to the following parameters: 200 μM each dNTP (Roche Diagnostics, Lewes, UK), 1.5 mM $MgCl_2$, 500 μM each primer (GIIP2 F: CATGARGACCCATCTGAAAC/G2SKR: CCRCCNGCATR-HCCRTTRTACAT (Kojima et al., 2002)), 2.5 U Taq polymerase (Promega, Southampton, U.K.), 50 mM KCl, 10 mM Tris–HCl and 0.1% Triton X-100. Amplification was as follows: denaturation at 95 °C for 5 min, followed by 40 cycles of 94 °C for 30 s; annealing of primers was at 55 °C for 30 s, elongation at 72 °C for 1 min followed by a final elongation step of 72 °C for 10 min.

A 1003 bp fragment of the Orf 1/Orf 2 junction was amplified in three overlapping fragments according to the cycling parameters outlined above, an annealing temperature of 52 °C and using the following primer sets:

JV12Y/JV13I (amplified nucleotides 4279–4586)
GIINoV4594 F: TATGGTGATGATGAAATNGT and
GIINoV5267 R: GGRGASACTGTAAAYTCSCCA (amplified nucleotides 4594–5267)
P2GIIF (4844): TGGTGGACTCGCGGGCCAAAC and G2SKR (5389) (Kojima et al., 2002) (amplified nucleotides 4844–5389). The nucleotide positions are listed, with respect to the Lordsdale virus GII/4 reference norovirus strain, with accession number X86557.

Maximum likelihood phylogenetic trees were built using PAUP*, version 4.0 beta 10 (Swofford, 2002) and Modeltest (Posada and Crandall, 1998). Similarity and bootscanning were carried out using Simplot (Lole et al., 1999).

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