Chronic norovirus infection in a patient with a past history of Burkitt lymphoma

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Abstract The noroviruses are a leading cause of gastroenteritis worldwide. Although the illness is normally mild and self-limiting, there is a growing literature documenting the chronic excretion of norovirus in the immunocompromised. The aim of the current study was to examine the molecular features of chronic norovirus excretion in an immunocompromised patient with a past history of Burkitt lymphoma. During the 241 day course of the study from December 2013 to August 2014, seven faecal specimens were collected from the patient, tested for norovirus by RT-PCR and further analysed in the open reading frame (ORF) 1 and ORF 2 regions. All seven specimens were positive for norovirus by RT-PCR. Molecular sequencing in the polymerase (ORF 1) and capsid (ORF 2) regions indicated that the norovirus could be classified as GII.4 (2006b)/GII.4 (unknown). No significant mutation was found in the ORF 1 or ORF 2 regions analysed over the period of the study. The current report appears to be the first to document chronic norovirus excretion in a patient with a past history of Burkitt lymphoma. It is also the first to indicate long term norovirus excretion in a given individual need not involve major genetic change in key regions of the genome.

Keywords Burkitt lymphoma · Norovirus · Chronic excretion · Immunocompromised

The noroviruses, which are single-stranded positive sense RNA viruses classified in the genus *Norovirus* within the family *Caliciviridae* [6] are now considered a leading cause of non-bacterial gastroenteritis [6]. Noroviruses are currently classified into six genogroups [6], and three of these, genogroups I, II and IV (GI, GII and GIV), occur in human infections [6]. The norovirus genome comprises three open reading frames (ORFs) [6]. ORF 1 encodes the non-structural polyprotein, ORF 2 encodes the major capsid protein and ORF 3 encodes the minor capsid protein [6]. Norovirus genotype classification using nucleic acid sequencing can be based on the ORF 1 region or the ORF 2 region [9]. The GII.4 genotype appears to be the most common in humans [6] and has been further subdivided into “variants” or “strains” [9].

Norovirus infection is usually characterized by symptoms including diarrhea, vomiting and abdominal cramps [6]. The illness is normally mild and self-limiting lasting about 24 to 48 h [6]. However, there is now some literature documenting the chronic excretion of norovirus in the immunocompromised (CENI syndrome) [7] although the topic is still not well understood. The current report examines molecular aspects of norovirus excretion in a patient with a past history of Burkitt lymphoma, an aggressive B cell non-Hodgkin lymphoma in longstanding remission following distant intensive chemotherapy, and appears to be the first to document CENI syndrome in such a patient.

The subject of this study (the ‘patient’) had a history of immunosuppression and ongoing diarrhea. The male ‘patient’, was 25 years old at the commencement of the study (in December 2013). Six years prior he had presented with Burkitt lymphoma and had subsequently received six cycles of chemotherapy. In 2011 he was noted to have hypogammaglobulinaemia and was started on intravenous

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immunoglobulin treatment, which continued for several years. For the period of the current study he was noted to have recurring diarrhea.

Seven sequential faecal specimens were received for norovirus testing at the Victorian Infectious Diseases Reference Laboratory. The dates of collection of the seven specimens were 21 Dec 2013, 31 Jan 2014, 20 Mar 2014, 30 Mar 2014, 31 Jul 2014, 1 Aug 2014 and 18 Aug 2014, i.e. the study spanned a period of 241 days. The specimens are referred to as specimen 1, specimen 2 etc. numbered in the order of their collection.

Faecal specimens were prepared as a 20 % (vol/vol) suspension in Hanks' complete balanced salt solution (Sigma-Aldrich Company, Irvine, UK) and the suspension clarified with a 10 min centrifugation spin at 7000 g. This clarified extract was then used for RNA extraction followed by reverse-transcription PCR (RT-PCR). RNA extraction was carried out using the Corbett automated extraction procedure (now Qiagen Sciences, Germantown, MD, USA). Two RT-PCR protocols, each utilizing two rounds, were used in the study. For the first round of each of the two protocols a commercial kit, the Qiagen (Qiagen GmbH, Hilden, Germany) OneStep RT-PCR kit, that

combined the RT step and the first round of the PCR, was utilized. The second round PCR was performed using the Qiagen *Taq* DNA polymerase kit.

Initially all specimens received for norovirus identification were tested by an ORF 1 RT-PCR for GI and GII norovirus. This was carried out with a two round RT-PCR protocol using primers NV 4562, NV 4611, NV 4692, NV 5296, NV 5298 and NV 5366, as given previously [1]. Nucleotide sequencing analysis of the PCR product derived from this protocol utilised a 440 bp fragment corresponding to nucleotides 185-624 (relative to DenHaag virus, EF126965).

All specimens were also tested by an ORF 2 RT-PCR protocol directed to region C of GII noroviruses. This two round RT-PCR protocol was carried out using primers G2F3 and G2SKR as given previously [2]. Nucleotide sequencing analysis of the PCR product derived from the GII protocol utilised a 195 bp fragment corresponding to nucleotides 834-1028 (relative to DenHaag virus, EF126965). Nucleotide sequencing and phylogenetic analysis were carried out essentially as described previously [3]. Genotype analysis also made use of the norovirus automated genotyping tool (<http://www.rivm.nl/mpf/norovirus/typingtool>) [10].

Fig. 1 a ORF 1 nucleotide sequence alignment of the seven specimens from the CENI syndrome patient. The 440 bp sequence corresponds to nucleotides 185-624 of the GII reference strain DenHaag virus (EF126965). It can be seen that apart from occasional transient base change, no major change in the genome took place.

b ORF 2 nucleotide alignment of four specimens (specimens 3-6) from the CENI syndrome patient. The 195 bp sequence corresponds to nucleotides 834-1028 of the GII reference strain DenHaag virus (EF126965)

Fig. 2 DNA (dist) Kimura phylogenetic tree showing the relationship of the four GII.4 ORF 2 sequences from specimens 3–6 of this study compared with GII.4 variant reference strains as listed in Kroneman et al. [9]. The tree was generated using the software Phylip v3.68. All sequences are 195 bp long. The accession number of the reference

strain used for each GII.4 variant is given in brackets. Patient specimens 3–6 were all identical; the sequence of one of them (specimen 3) has been lodged in GenBank and has accession number KT364262. The *scale marker* represents substitutions per site

All seven specimens collected from the patient were positive for norovirus by the ORF 1 PCR and, in all cases, ORF 1 nucleotide sequencing analysis indicated the norovirus genotype was the genogroup 2 norovirus GII.4 (2006b). All seven sequences were closely related to the sequence for the ORF 1 region (bases 185–624) of the GII.4 (2006b) reference strain DenHaag86/2006/NL (EF126965) (93 % identity).

A visual representation of the seven 440 bp ORF 1 nucleotide sequences analysed is given in Fig. 1a. It can be seen that the sequences for specimens 1–4 were identical. For specimen 5 three bases had changed- at positions 9, 42 and 145 in the sequence of 440 bases. For specimen 6 the changes noted in specimen 5 remained, as well as a change at position at position 129. None of these base changes altered the amino acid sequences. For specimen 7 the sequence reverted to the original sequence found for specimens 1–4.

ORF 2 PCR and nucleotide sequencing were successfully carried out on four of the seven specimens- specimens 3–6; a visual representation of the four 195 bp ORF 2 nucleotide sequences analysed is given in Fig. 1b. All four

ORF 2 195 bp sequences were identical (Fig. 1b). The norovirus genotyping tool classified these ORF 2 sequences as the genogroup 2 GII.4 (unknown) although the four ORF 2 sequences were closely related to the sequence for the ORF 2 region (bases 834–1028) of the GII.4 (2006b) reference strain DenHaag86/2006/NL (EF126965) (94.9 % identity). The construction of a phylogenetic tree which included all major GII.4 variants, as given by Kroneman et al. [9], provided visual confirmation that the GII.4 (unknown) found in the current study was most closely related to the variant GII.4 Den_Haag_2006b (Fig. 2).

There is now growing evidence that CENI is a significant clinical syndrome and it has been documented in a number of European countries [5, 12], as well as the USA [11]. The current report appears to be the first in the mainstream literature emanating from the Southern Hemisphere.

A number of norovirus genotypes have been linked to the CENI syndrome. These include GII.3 (ORF 2) [4], GII.4 (ORF 2) [8, 13, 15], the ORF 1/ORF 2 recombinant GII. 7/GII.6 [12], GII.7 (ORF 2) [8] and GII.17 (ORF 2) [13]. In the current study norovirus genotype GII.4 was

identified in the CENI syndrome case. The norovirus sequences found in the current study accorded closely to GenBank reports of the GII.4 (2006b) ORF 1 and ORF 2 sequences and the findings indicate the chronic infection was not linked to an unusual norovirus strain.

Previous nucleotide sequencing studies in this laboratory have shown that changes in both the polymerase (ORF 1) region and region C of the capsid (ORF 2) can be linked to the development of a new virulent strain of norovirus [2]. Furthermore Setoh et al. [14] have recently provided supporting evidence for the importance of non-structural sequences in determining virulence in that they showed that the non-structural genes made a major contribution to the virulence of West Nile virus. Thus nucleotide sequencing in the current project was carried out in the ORF 1 non-structural polymerase region as well as in the ORF 2 region C of the capsid. No evidence of major genetic change was found in either the ORF 1 or ORF 2 regions.

In contrast to the findings of the current study, a number of sequencing studies carried out in the ORF 2 region on specimens from patients with the CENI syndrome have found evidence of evolutionary change [4, 8, 13, 15]. The norovirus genotypes identified where evolutionary change was noted were GII.3 [4], GII.4 [8, 13, 15], GII.7 [8] and GII.17 [13]. Thus, taken together, the limited sequencing data available suggests that the CENI syndrome may in some cases be accompanied by significant genetic change, whereas in others it is not. A possible explanation of this observation is given by Hoffmann et al. [8] who note that without an immune response mutations would merely result from genetic drift but, if the immune response cannot eliminate the virus but still exerts some selective pressure, evolutionary change can be expected. Further sequencing studies on the effect of the CENI syndrome on norovirus evolution are clearly required.

The current study helps establish the global universality of the CENI syndrome by demonstrating its occurrence in the Southern Hemisphere and appears to be the first to document the CENI syndrome in association with a past history of Burkitt lymphoma. The study also appears to be one of the first to sequence nonstructural as well as structural regions of the norovirus genome in a CENI syndrome case and indicates chronic excretion need not involve significant genetic change.

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