Genetic Characterization of Genogroup I Norovirus in Outbreaks of Gastroenteritis

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Abstract

In this study we demonstrate that differences within the P2 domain of GI strains can be used to segregate outbreaks which are unrelated, whereas complete conservation within this region allows tracking of strains that are part of a single outbreak and likely to have a common source.
Noroviruses (NoVs) are members of the *Caliciviridae* family (7) and the leading cause of outbreaks of acute gastroenteritis world-wide (14). NoV outbreaks are frequently associated with semi-closed or closed institutions such as hospitals and homes for the elderly (11, 22), but outbreaks also occur in other settings including eating establishments, cruise ships, concert halls (2, 10, 20) and schools (16).

Transmission of NoVs is usually person-to-person (15), although food and water (1, 3, 5, 9, 17-18, 21) and environmental or airborne contamination have all been implicated in transmission (6, 19).

Human NoVs are genetically diverse and three distinct genogroups (GI, GII and GIV), and many genotypes/genetic clusters exist (8, 13, 26). Diversity among NoVs is generated through the accumulation of point mutations associated with the error prone nature of RNA replication and genetic recombination involving the exchange of sequences between related RNA viruses.

The NoV capsid is divided into the S domain which constitutes the 5’ end (amino acids [aa] 1-225) and the P (protruding) domain aa 226-530 (23). The P domain can be further sub-divided into two sub-domains P1 and P2. The P2 domain is the hyper-variable region of the capsid and corresponds to the most exposed area likely to be involved in immune recognition and attachment. Due to the high diversity within this region it is not possible to design a single cross-reactive primer pair capable of amplifying all genotypes within a genogroup, therefore genotype-specific primers are required in order to amplify this region, as previously seen for NoV genogroup II (25).
Faecal samples were collected from genogroup I outbreaks of gastroenteritis as part of the ongoing National Surveillance Programme of the molecular epidemiology of Norovirus genotypes.

Outbreaks were defined as including two or more cases of gastroenteritis linked in place and time. A new outbreak was arbitrarily defined as occurring at least 7 days after the last case in a previous outbreak or as occurring in a different patient care unit such as a ward or hospital.


Faecal specimens were prepared as previously described and nucleic acid extraction, Norovirus detection through amplification of a small region spanning the Orf1/2 junction and genotyping through sequence analysis of the S domain were all performed as previously described (4).

Oligonucleotide primers for the amplification of a region encompassing the P2 domain of NoV GI genotypes 1 to 7 were designed from alignments of complete Orf2 nucleotide sequence data. See Table 1 for primer sequence and positions and
amplification conditions. Separate monoplex reactions were carried out for each of the
genotypes and amplicons were separated by agarose gel electrophoresis and
sequenced directly after purification using the same P domain genotype-specific
primers.

Sequence analysis of the region encoding the P2 domain (nt 6165-6572) on the GI-1
strain Norwalk/1968/US (M87661) was performed using Bionumerics ver. 3.5
(Applied Maths, Kortrijk, Belgium). Sequence alignments were performed using the
Clustal algorithm. Of the P domain sequences, only the region corresponding to the
P2 domain was used in comparisons.

NoV genogroup I, genotypes 1 to 7 were all successfully amplified using the
genotype-specific primers designed to provide amplicons encompassing the P2
domain (Table 1). Phylogenetic analysis of the P2 domain sequences differentiated
between each genotype (Figure 1).

Genotyping and subsequent analysis of the P2 domain showed that outbreak 414003,
which was linked to the ingestion of faecal contaminated oysters in 2004 (3)
contained a mixture of GI-1 and GI-2 genotypes. Conservation within the P2 domain
of the GI-1 or GI-2 indicated that the customers were infected by a common source
(Figure 1).

Among four GI-4 outbreaks detected between 2005 and 2007, strains within outbreaks
showed 100% identity within the P2 domain but were different among the 4 outbreaks
(Figure 1). Two of these outbreaks occurred in the same geographical region one in
November and the second December 2006. Diversity of the P2 domain between strains in these two outbreaks clearly distinguished them as separate events whereas the strains within each of the outbreaks clearly linked them as having a common source.

Similarly three unrelated outbreaks caused by GI-3, GI-5 or GI-6 demonstrated that strains within an outbreak were identical (Figure 1).

The analysis of sequence from the S domain region of GI NoVs is valid for genotyping and benefits from the use of a single set of consensus primers (4, 12). Previously we reported that sequence identity within the P2 domain among GII strains was a useful tool for outbreak tracking and monitoring transmission events between outbreaks which using then common epidemiological definition were identified as separate events (24-25). Similarly, data from this study shows the validity of using P2 domain sequences to link GI strains within an outbreak and segregate outbreaks which are unrelated.
Nucleotide Sequence Accession Numbers


Acknowledgements

We would like to thank Dr Richard Cooke, Aintree Hospital, Dr Barry Vipond, Bristol HPA, Dr John Cheesbrough, Preston HPA and Hilary Cotterill, Manchester HPA for sending us faecal samples and information from outbreaks.
Table 1. Norovirus genogroup I genotype-specific primers for amplifying a region encompassing the P2 domain. PCR cycling conditions were 94°C for 2 min followed by 40 cycles at 94°C for 30 s, 45°C for 1 min and 72°C for 1 min, and finally 72°C for 5 min.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Primer Names</th>
<th>Primers</th>
<th>Annealing Temperature</th>
<th>Amplicon size</th>
<th>P2 domain size</th>
</tr>
</thead>
<tbody>
<tr>
<td>GI-1</td>
<td>P2 GI-1 F</td>
<td>5'TCN-AAY-TCA-CGT-GCT-CCT-CTT 3'</td>
<td>47°C</td>
<td>682</td>
<td>407</td>
</tr>
<tr>
<td></td>
<td>P2 GI-1 R</td>
<td>5'TCC-GNC-CNG-TAT-CAG-GGT-CAA 3'</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>GI-2</td>
<td>P2 GI-2 F</td>
<td>5'TCC-AAT-TCT-AGG-CTT-CTT-CT-CT-CT 3'</td>
<td>47°C</td>
<td>670</td>
<td>437</td>
</tr>
<tr>
<td></td>
<td>P2 GI-2 R</td>
<td>5'GGG-CTT-GTC-CAC-TGA-CAA-AGT-G 3'</td>
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<tr>
<td>GI-3</td>
<td>P2 GI-3 F</td>
<td>5'TCW-AAY-TCA-AGR-6TC-CTT-CTT-CT 3'</td>
<td>50°C</td>
<td>684</td>
<td>437</td>
</tr>
<tr>
<td></td>
<td>P2 GI-3 R</td>
<td>5'GCT-TCM-CCT-CTA-GTG-GGG-GCC-T 3'</td>
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<td>GI-4</td>
<td>P2 GI-4 F</td>
<td>5'TCT-AAT-TCY-AGG-AGC-CCA-AAT 3'</td>
<td>45°C</td>
<td>665</td>
<td>434</td>
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<td>P2 GI-4 R</td>
<td>5'GCC-TGC-TCA-CTA-ATA-AAG-TGT-G 3'</td>
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<td>GI-5</td>
<td>P2 GI-5 F</td>
<td>5'TCC-AAT-TCC-GTG-GTG-GGC-TTG-TTC-CTT-CTT 3'</td>
<td>47°C</td>
<td>678</td>
<td>437</td>
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<tr>
<td></td>
<td>P2 GI-5 R</td>
<td>5'CCT-NGA-KGG-GGC-TTG-TTC-ACT 3'</td>
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<td>GI-6</td>
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<td>5'TCA-AAT-TCT-CTG-CCT-CTG-CT-CTG-CT 3'</td>
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<td>646</td>
<td>425</td>
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<tr>
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<td>P2 GI-6 R</td>
<td>5'GTT-CAT-TRC-AGA-AGT-GGG-TAA-T 3'</td>
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<td>GI-7</td>
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F = Forward, R = Reverse. The forward primers correspond to nucleotide positions 6081-6101 and the reverse primers to nucleotide positions 56742-56761 on the GI-1 strain Norwalk/1968/US (M87661). The P2 domain region of GI strains corresponds to nucleotide positions 6165-6572 on the GI-1 strain Norwalk/1968/US (M87661). Y = C or T; R = A or G; N = C or G or T or A.
Figure 1.
Figure Legends

Figure 1. Dendrogram of P2 domain sequences derived from strains of 10 outbreaks, constructed using the Neighbor Joining algorithm. Strain denomination indicates the outbreak number, year of isolation and country. Genogroup and genotype is indicated in the second column.
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