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Running head: novel recombinant norovirus
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小渕正次
Norovirus (NoV) is a major pathogen causing infectious gastroenteritis and food poisoning, and belongs to the *Caliciviridae* family. Its approximately 7.5–7.7-kb, plus-sense, single-stranded RNA genome contains three open reading frames (ORFs). ORF1 encodes non-structural proteins, including RNA-dependent RNA polymerase (RdRp). ORF2 and ORF3 encode the major capsid protein (VP1) and minor structural protein (VP2), respectively (1). NoV is classified into seven different genogroups (GI–GVII); GI and GII are the main genogroups infecting humans (1). GI and GII are classified into nine (GI.1–9) and 22 (GII.1–22) different genotypes, respectively, according to their VP1 amino acid sequences. The GII.4 genotype is the predominant strain circulating worldwide (1). New variants of GII.4 occur every few years. The latest is the Sydney 2012 variant that emerged in 2012. NoV reportedly undergoes genetic recombination in the ORF1–2 junction region (2). The main *RdRp* genotypes of the GII.4 Sydney 2012 variant are GII.Pe, GII.P4, and GII.P16. In this study, we report GII.P12-GII.4 Sydney 2012, a genetically recombinant Sydney 2012 variant not reported previously, in patients who developed food poisoning in Tokyo during the 2017–2018 season (September 2017 to August 2018). We also performed *RdRp* and *VP1* gene analysis to investigate this genetically recombinant virus in greater detail.

In December 2017, several groups of people who dined at a university canteen in Tokyo developed symptoms including nausea, vomiting, diarrhea, and fever. Administrative investigations (Tokyo and Toyama) detected NoV GII from the stools of patients and a canteen food handler, and the outbreak was designated as a case of institutional mass food poisoning. NoV GII was detected from seven residents of Toyama Prefecture who dined in the
canteen. Nested RT-PCR (3) was performed on their stool samples to amplify the region containing the NoV GII ORF1–2 junction (805 nt). A sequence (714 nt) including the 3' terminal 465 nt of the \textit{RdRp} gene and the 5' terminal 269 nt of the \textit{VP1} gene was determined from the amplicons of six samples. Comparison of the nucleotide sequences of these six strains (GenBank accession nos. LC390332-LC390337) revealed 100% identity in five, with a difference of a single nucleotide in the \textit{RdRp} gene in the remaining strain. All the strains were classified as GII.P12-GII.4 Sydney 2012 by genotype identification using the Norovirus Genotyping Tool version 2.0 (https://www.rivm.nl/mpf/typingtool/norovirus/).

RT-PCR and sequencing were performed for one of these samples (Toyama18048), using newly designed primers and previously described L1F (4) (Table 1). Nucleotide sequences of full lengths of \textit{RdRp} and \textit{VP1} genes were determined (GenBank accession no. LC390332). Phylogenetic analyses of these sequences were performed by the neighbor-joining (NJ) method, using the MEGA6 software (5). The \textit{RdRp} gene (1,533 nt) belonged to the same cluster as the GII.P12-GII.3 strain detected from 2006 to 2016 (Fig. 1A), with a nucleotide sequence that was 98.4% identical with that of its most closely related strain, Hu/Guangzhou/GZ2013-L20/CHN/2013 (GenBank accession no. KY348697). The \textit{VP1} gene (1,623 nt) belonged to the same cluster as the GII.Pe-GII.4 Sydney 2012 strain detected from 2011 to 2017 (Fig. 1B), with a nucleotide sequence that was 98.3% identical with that of its most closely related strain, Hu/GII.4/OsakaSB2-1/2014/JPA (LC133344).

SimPlot analysis (6) was then performed to identify the recombination breakpoint between this strain and the aforementioned strains. The
breakpoint was identified as nucleotide position 1457 on the \textit{RdRp} gene (nucleotide position 5009 in the Lordsdale strain (X86557)), close to the starting point of ORF2 (nucleotide position 5085 in the Lordsdale strain) (Fig. 2). This suggested that the outbreak strain may have originated by genetic recombination between the GII.P12-GII.3 and GII.Pe-GII.4 Sydney 2012 strains in the ORF1–2 junction region.

Nucleotide sequences homologous to the partial sequence of Toyama18048 (714 nt), including the \textit{RdRp} and \textit{VP1} genes, were obtained from a BLAST search (as of June 18, 2018). The top 100 homologous sequences from that search were analyzed using the Norovirus Genotyping Tool. A total of 33 sequences from the GII.P12-GII.4 genotype were identified. Of these sequences, four belonged to the GII.P12-GII.4 Sydney 2012 strain. They were independently detected in Japan during the same period and have been deposited in GenBank (accession nos. LC375954–LC375957) by the Osaka Institute of Public Health. These nucleotide sequences, including the 3' terminal of the \textit{RdRp} gene and the 5' terminal of the \textit{VP1} gene (1,067–1,104 nt) of these strains, were 100% identical to each other, and 99.6–99.8% identical to that of Toyama18048. The remaining 29 sequences belonged to the GII.P12-GII.4 Asia 2003 (7, 8) or GII.P12-GII.4 Den Haag 2006b (9) strains, which were detected in China, Japan, Thailand, and South Korea in 2004–2009. These results indicate that the GII.P12-GII.4 Sydney 2012 strain in the present outbreak is a novel recombinant norovirus that emerged in the winter of 2017-2018 in Japan.

The full-length amino acid sequences of RdRp and VP1 in this novel strain were compared with those of past strains of GII.P12-GII.3 (6 strains
available from GenBank) and GII.Pe-GII.4 Sydney 2012 (134 strains available from GenBank), respectively. Alignment of the deduced amino acid sequences revealed no amino acid substitutions specific to the novel strain (data not shown). However, a novel recombinant strain of GII.P16-GII.4 Sydney 2012 appeared in the United States in 2015, and replaced GII.Pe-GII.4 Sydney 2012 as the dominant circulating genotype in 2015–2016 (10). Therefore, the novel recombinant strain of GII.P12-GII.4 Sydney 2012 identified in this study might become a dominant strain in coming years. Close surveillance is needed to continuously monitor these norovirus infections.

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**Conflict of interest:** None to declare.
REFERENCES


trends of norovirus outbreaks in the United States from 2013 to 2016
demonstrated emergence of novel GII.4 recombinant viruses. J Clin
**Figure legends**

Fig. 1.
Phylogeny of norovirus GII by the NJ method for the full-length *RdRp* gene (1,533 nt) (A) and *VP1* gene (1,623 nt) (B). The full-length sequences were downloaded from GenBank as reference strains. The numbers on the branching points indicate bootstrap values ≥80% (1,000 replicates). The strain detected in this outbreak is in bold. (A) GII.P12 strains are contained within the broken lines and the *VP1* genotype of each strain is given on the right of the cluster. (B) GII.4 Sydney 2012 strains are contained within the broken lines and the *RdRp* genotype of each strain is given on the right of the cluster. Of the reference strains, those contained within the broken lines are listed in the order of their GenBank accession number and name, and other strains are listed in the order of their genotype, GenBank accession number, and name.

Fig. 2.
SimPlot analysis of the full-length nucleotide sequences of the *RdRp* and *VP1* genes of the reference strains (window size, 200 bp; step size, 20 bp). The X-axis shows the number of nucleotides from the 5' terminus of the *RdRp* gene in the alignment sequence. The Y-axis shows the degree of similarity with the reference strains. The vertical broken line shows the starting point of ORF2. Schematics of the *RdRp* and *VP1* genes, and nucleotide positions in the Lordsdale strain are shown below the graph. Solid and open bars indicate the GII.P12-GII.3 and GII.Pe-GII.4 Sydney 2012 strains, respectively.
Table 1. Primers used for PCR amplification and sequencing against the *RdRp* and *VP1* genes in this study

<table>
<thead>
<tr>
<th>Viral gene</th>
<th>Primers</th>
<th>Sequence (5’→3’)</th>
<th>Sense</th>
<th>Position</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>RdRp</em></td>
<td>RdRp-1_F</td>
<td>CCGTGGTCACATTACTCATC</td>
<td>+</td>
<td>3270 – 3289</td>
<td>Forward</td>
</tr>
<tr>
<td></td>
<td>RdRp-1_R</td>
<td>TTGTCAATCGTTGACACACGCTC</td>
<td>−</td>
<td>4092 – 4073</td>
<td>Reverse</td>
</tr>
<tr>
<td></td>
<td>RdRp-2_F</td>
<td>TTAAGGAGGAAAGAGCCATG</td>
<td>+</td>
<td>4023 – 4042</td>
<td>Forward</td>
</tr>
<tr>
<td></td>
<td>RdRp-2_R</td>
<td>CCAGAGCCATAACCTATTG</td>
<td>−</td>
<td>5172 – 5153</td>
<td>Reverse</td>
</tr>
<tr>
<td></td>
<td>RdRp-2_Seq</td>
<td>GGTCGGTTTCAACCAGTA</td>
<td>−</td>
<td>4681 – 4664</td>
<td>Sequencing only</td>
</tr>
<tr>
<td><em>VP1</em></td>
<td>Vp1-1_F</td>
<td>CAAAGAGCCAATGTTCAGATG</td>
<td>+</td>
<td>5003 – 5023</td>
<td>Forward</td>
</tr>
<tr>
<td></td>
<td>Vp1-1_R</td>
<td>AAATCTGGAGTTCCTAGAGG</td>
<td>−</td>
<td>6061 – 6042</td>
<td>Reverse</td>
</tr>
<tr>
<td></td>
<td>Vp1-2_R</td>
<td>TTCCCYATYCCTTTGGAAAAG</td>
<td>+</td>
<td>5808 – 5828</td>
<td>Forward</td>
</tr>
<tr>
<td></td>
<td>Vp1-2_Seq</td>
<td>AGTTGGTGTCATCCAGAGATG</td>
<td>+</td>
<td>6239 – 6258</td>
<td>Sequencing only</td>
</tr>
</tbody>
</table>

1) Positions are indicated relative to the Lordsdale strain (GenBank accession no. X86557).
2) Primer in ref. no. 4.
Fig. 1
Fig. 1 (continued)
SimPlot - Query: Toyama18048

Fig. 2

Window: 200 bp, Step: 20 bp, GapStrip: On, Kimura (2-parameter), T/t: 2.0