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A Novel Recombinant Norovirus GII.4 Sydney 2012 Strain Detected from a Food Poisoning Outbreak in the 2017–2018 Season, Japan

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Norovirus (NoV) is a major pathogen that causes infectious gastroenteritis and food poisoning, and belongs to the *Caliciviridae* family. NoV is a positive-sense, single-stranded RNA, with a genome size of 7.5–7.7 kb that 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 the minor structural protein (VP2), respectively (1). NoV is classified into seven different genogroups (GI–GVII); the genogroups GI and GII mainly infect humans (1), and 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 emerge every few years. The latest is the Sydney 2012 variant, which 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 describe 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 such as nausea, vomiting, diarrhea, and fever. Administrative investigations (Tokyo and Toyama) detected NoV GII in 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 in the stool of seven residents of Toyama Prefecture who dined in the canteen. Nested RT-PCR (3) was performed on these 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 *RdRp*

gene and the 5' terminal 269 nt of the *VP1* gene was determined from the amplicons of six strains. A comparison of the nucleotide sequences of these six strains (GenBank accession nos. LC390332–LC390337) revealed 100% identity in five strains, with a single nucleotide differing in the *RdRp* gene in the remaining strain. All 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 on one of the GII.P12–GII.4 Sydney 2012 positive samples (Toyama18048), using specifically designed primers and previously described L1F (4) (Table 1). Nucleotide sequences of full length *RdRp* and *VP1* genes were determined (GenBank accession no. LC390332). Phylogenetic analyses of these sequences were performed by neighbor-joining method, using MEGA6 software (5). The *RdRp* gene (1,533 nt) belonged to the same cluster as the GII.P12–GII.3 strain detected between 2006 and 2016 (Fig. 1A), with a nucleotide sequence that was 98.4% identical to that of the most closely related strain, Hu/Guangzhou/GZ2013-L20/CHN/2013 (GenBank accession no. KY348697). The *VP1* gene (1,623 nt) belonged to the same cluster as the GII.Pe–GII.4 Sydney 2012 strain detected between 2011 and 2017 (Fig. 1B), with a nucleotide sequence that was 98.3% identical to that of the most closely related strain, Hu/GII.4/OsakaSB2-1/2014/JPA (LC133344).

SimPlot analysis (6) was performed to identify the recombination breakpoint between this strain and the aforementioned strains. The breakpoint was identified as nucleotide position 1457 on the *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 near the ORF1–2 junction.

Nucleotide sequences homologous to the partial sequence of Toyama18048 (714 nt), including those of the *RdRp* and *VP1* genes, were obtained from a BLAST search (June 18, 2018). The top 100 homologous sequences were analyzed using the Norovirus Genotyping Tool. A total of 33 sequences from the GII.P12–GII.4 genotype were identified. Of these, four belonged to the GII.P12–GII.4 Sydney 2012 strain. These four sequences were independently detected in Japan during the same

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Table 1. Primers used for PCR amplification and sequencing against the *RdRp* and *VP1* genes in this study

Viral gene	Primers	Sequence (5'–3')	Sense	Position ¹⁾	Function
<i>RdRp</i>	RdRp-1_F	CCGTGGTCACATTACTCATC	+	3270–3289	Forward
	RdRp-1_R	TTGTCACTCTTGACCAGCTC	–	4092–4073	Reverse
<i>RdRp</i>	RdRp-2_F	TTGAGGAGGGAAAGAACATG	+	4023–4042	Forward
	RdRp-2_R	CCAGAGCCATAACCTCATTG	–	5172–5153	Reverse
	RdRp-2_Seq	GGTCGGTTTCAACCCGTA	–	4681–4664	Sequencing only
<i>VP1</i>	Vp1-1_F	CAAGAGCCAATGTTTCAGATG	+	5003–5023	Forward
	VP1-1_R	AAATCTGGAGTTCTTAGAGG	–	6061–6042	Reverse
<i>VP1</i>	L1F ²⁾	TTCCCYATYCCTTTGGAAAAG	+	5808–5828	Forward
	VP1-2_R	CAAAGAAAGCTCCAGCCA	–	6722–6705	Reverse
	VP1-2_Seq	AGTTGGTGTCTCAAGATG	+	6239–6258	Sequencing only

¹⁾: Positions are indicated relative to the Lordsdale strain (GenBank accession no. X86557).

²⁾: Primer in ref. no. 4.

period and have been deposited in GenBank (accession nos. LC375954–LC375957) by the Osaka Institute of Public Health. The nucleotide sequences, including the 3' terminal of the *RdRp* gene and the 5' terminal of the *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 between 2004 and 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 previous strains of GII.P12-GII.3 (six strains available from GenBank) and GII.Pe-GII.4 Sydney 2012 (134 strains available from GenBank). Alignment of the deduced amino acid sequences did not reveal any 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 during 2015–2016 (10). Therefore, the novel recombinant strain of GII.P12-GII.4 Sydney 2012 identified in this study has the potential to 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.

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Fig. 1. Phylogeny of norovirus GII by neighbor-joining 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 $\geq 80\%$ (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.