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Genetic Characterization of Rarely Reported GI.Pc_GI.5 Norovirus Strain Detected from a Suspected Foodborne Outbreak in Japan

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Human norovirus is a major causative agent of acute, viral gastroenteritis worldwide. Human norovirus has a positive-sense, single-stranded RNA genome of approximately 7,500 bp (1). The genus *Norovirus* is classified in the family *Caliciviridae* and is divided into 7 genogroups (GI to GVII) based on the *capsid* region (2). Among them, GI and GII are the major genogroups that infect humans and are further classified into 9 genotypes and 22 genotypes, respectively. As recombination frequently occurs in the ORF1/ORF2 overlap region, genotyping of both RNA-dependent RNA polymerase (*RdRp*) in ORF1 and *capsid VP1* in ORF2 is necessary to establish the recombinant identity of the virus (3).

Here, we describe a suspected foodborne outbreak associated with the recombinant norovirus GI.Pc_GI.5 in Fukuoka Prefecture, Japan, in January 2017, where 28 (68.3%) of the 41 individuals who consumed a common meal at a barbecue restaurant were affected. After a food survey, contamination of salad and/or fruits was suspected as the cause of this illness. The symptoms within 12–48 h after consumption included nausea (85.7%), fever (78.6%), diarrhea (67.9%), vomiting (60.7%), stomach ache (50.0%), head ache (46.4%), malaise (42.9%), and chills (25.0%). The age of the patients ranged from 4 to 74 years. None of the 14 staff members of the restaurant reported symptoms.

GI norovirus was detected in the feces of 9 of the 28 patients with diarrheal symptoms and 2 of the 14 asymptomatic staff. Viral RNA extraction was performed as previously described (4). Viral cDNA was synthesized using SuperScript III Reverse Transcriptase (Thermo Fisher Scientific, Waltham, MA, USA). Partial *RdRp* and the *capsid* regions were amplified by RT-PCR using the primers P1/P3 (5) and G1SKF/G1SKR (6), respectively. The norovirus strain detected in this outbreak was classified as GI.Pc (*RdRp*) and GI.5 (*VP1*) using Norovirus Typing Tool version 2.0 (<https://www.rivm.nl/mpf/typingtool/norovirus/>) (7).

In order to understand the genetic characteristics of the GI.Pc_GI.5 norovirus, the entire *RdRp* and *VP1* encoding regions of the GI.Pc_GI.5 norovirus strain detected in the outbreak were amplified using the PrimeScript II High Fidelity One-Step RT-PCR Kit (Takara Bio, Inc., Shiga, Japan) using primers designed in this study (Table 1). These primers were designed based on the complete genomic sequence available for the GI.Pc_GI.5 norovirus SzUG1 strain (Genbank accession number: AB039774) (4). SzUG1 strain was first detected from a patient with a sporadic case of acute gastroenteritis associated with oysters in the late 1990s in Japan (4).

The nucleotide sequences of noroviruses isolated from all 11 samples were identical. The 3,088 bp nucleotide sequence of the representative GI.Pc_GI.5 strain identified in this outbreak was annotated as FAnT99 and deposited in the GenBank/EMBL/DDBJ database under the accession number LC331067.

The lengths of the *RdRp* and *VP1* regions of GI.Pc_GI.5 FAnT99 were 1,395 and 1,629 bp, respectively. By comparing the *RdRp* region of FAnT99 with that of the closest relative SzUG1, the identities of the nucleotide (nt) and amino acid (aa) sequences were found to be 91% (1,269 bp/1,395 bp) and 99% (462 aa/465 aa), respectively. For the *VP1* region, these similarities were 91% (1,479 bp/1,629 bp) and 98% (532 aa/543 aa), respectively.

The phylogenetic analysis of the nucleotide sequence of the *VP1* region revealed that GI.5 strains were divided into a foreign strain cluster and a Japanese strain cluster, and FAnT99 and SzUG1 strain were classified into the Japanese strain cluster (Fig. 1). The nt and aa sequences of the *VP1* region of FAnT99 strain were 91% (nt) and 96%–98% (aa) similar to the Japanese strains and 76%–77% (nt) and 86%–87% (aa) similar to the foreign strains. In this study, the differences between the detection regions and the genetic evolutionary characterization of the 2 clusters could not be clarified. In addition, the phylogenetic analysis of the *RdRp* region could not be conducted owing to the limitation in the number of sequences deposited in GenBank.

In conclusion, we determined and analyzed the sequences of the entire *RdRp* and *VP1* encoding regions of the GI.Pc_GI.5 norovirus responsible for a suspected foodborne outbreak in Fukuoka prefecture in 2017.

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Table 1. Primers used to analyze the entire region of RdRp and capsid in this study

Name	Sequence (5'-3')	nt position ¹⁾	Product size (bp)
GI.5-set1F	CCACAAAGTCTGGCAACACA	3777–3796	424
GI.5-set1R	TGGCCAGAACTGGTGGTTTAA	4180–4200	
GI.5-set2F	GACCAACTGAAGCCATTCGC	4031–4050	895
GI.5-set2R	TAAGCACCTGGGTAAAGCGG	4906–4925	
GI.5-set3F	ACACCAGAATTGGCTTCGGT	4640–4659	691
GI.5-set3R	TGCGATCTCCTGTCCACAAC	5311–5330	
GI.5-set4F	ACACCAGAATTGGCTTCGGT	4640–4659	1,405
GI.5-set4R	AGGGCGTGTCTTTTGTCCA	6025–6044	
GI.5-set5F	GTTGTGGACAGGAGATCGCA	5311–5330	1,395
GI.5-set5R	ACAGGCAAGGGACCAGATTG	6686–6705	
GI.5-set6F	GCTCGTGCCGGAGGTTAATA	5411–5430	634
GI.5-set6R	AGGGCGTGTCTTTTGTCCA	6025–6044	
GI.5-set7F	TGCAATTGGGACCTCACCTC	5599–5618	587
GI.5-set7R	CAGCAGGTGACCGTCAGTAG	6166–6185	
GI.5-set8F	GCAATTGGGACCTCACCTCA	5600–5619	741
GI.5-set8R	TCACACTTCCAAGGTCGGG	6321–6340	
GI.5-set9F	TCCTGGCAGGTAACGCATT	5686–5705	1,022
GI.5-set9R	CAACAGGCAAGGGACCAGAT	6688–6707	
GI.5-set10F	GTGGAACAAAAGACACGCC	6024–6043	1,296
GI.5-set10R	TTGCTGATGAATTCGGGGCT	7300–7319	
GI.5-set11F	ATCTGGTCCCTTGCTGTTG	6688–6707	633
GI.5-set11R	GTTGCTGATGAATTCGGGGC	7301–7320	
GI.5-set12F	CCAATCGGAACCTGGGTGAA	6793–6812	618
GI.5-set12R	GTCATGCCAACAGACCCTGA	7391–7410	

¹⁾: Positions are indicated relative to SzUG1 strain (Genbank accession number: AB039774).

Fig. 1. Phylogenetic analysis of the entire *VP1* region of GI.5. Phylogenetic tree was constructed using GI.5 strains as well as other GI strains as outgroup references. The tree was constructed from 1,629 bp (nucleotide position 5,352–6,980 based on AB039774) and constructed by the maximum likelihood method using MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 software (<http://www.megasoftware.net/>). Bootstrap analyses were performed with 1,000 resamplings of the data sets. Bootstrap values are shown at the nodes of the tree. FAnT99 strain detected in this study is marked with a black circle and GI.5 strains detected in Japan are marked with black squares. Unknown year of detection is indicated as xxxx.

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Conflict of interest None to declare.

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