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Emergence of New Recombinant Noroviruses GII.P16-GII.2 and GII.P16-GII.4 in Aichi, Japan, during the 2016/17 Season

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Norovirus (NoV) is recognized as one of the leading causes of acute gastroenteritis outbreaks. NoV belongs to the *Caliciviridae* family and the positive sense ~7.5 kb RNA genome contains 3 open reading frames

(ORFs); ORF1 encodes a nonstructural polyprotein with RNA-dependent RNA polymerase (RdRp), ORF2 encodes a capsid protein VP1, and ORF3 encodes a minor capsid protein VP2. NoVs are a genetically diverse group of viruses divided into 7 genogroups (GI to GVII) based on ORF2 sequence homology (1). Among them, GI and GII predominantly comprise strains that cause significant human disease. Natural recombination is considered a common event between norovirus strains. A recombination hot spot is present near the ORF1/ORF2 junction and a variety of recombinant strains have been detected globally (2).

During the 2016/17 season (from September 2016 to August 2017), new recombinant noroviruses, GII.P16-

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Fig. 1. Phylogenetic analysis of the capsid N/S region (287nt) of norovirus GII.2 from GII.P16-GII.2 associated 17 outbreaks and the strains isolated in the past seasons (2000 to 2015) (A) and the partial RdRp region (665nt) of GII.P16 strains (B). Aichi strains in the 2016/17 season are indicated by bar on the right of the clad. The strains in the past season are indicated similarly. The reference strains of GII.2 (A) and GII.P16 (B) are indicated by underline. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) is shown next to the branches. The evolutionary distances were computed using the Kimura 2-parameter method.

GII.2 (GII.2 capsid with a GII.P16 RdRp) and GII.P16-GII.4 (GII.4 Sydney 2012 capsid with a GII.P16 RdRp), emerged in Aichi Prefecture, Japan. In this report, we described the characterization of these recombinant strains.

From September 2016 to August 2017, 341 stool samples corresponding to 36 food borne outbreaks were investigated. Viral RNA was extracted from a 10% stool suspension and NoVs were detected by real-time RT-PCR as described previously (3,4). Four samples from one outbreak were positive for NoV GI. A total of 155 samples, corresponding to 24 out of 36 outbreaks (67%), were positive for NoV GII. GII-positive specimens were genotyped by sequencing a fragment of the *RdRp* gene and a fragment of the capsid gene. Semi-nested RT-PCR was performed using primers G2F1 (5), G2F2 (5), and G2Rv: 5'-gcataMccRttRtacattct-3', modified from G2R1 (5) by Kobayashi, for a capsid fragment; G2Rv, COG2R (4) and YuriF-ver2: 5'-atgaatgaggatggMcccat-3', modified from Yuri22F (6) by Kobayashi, for an RdRp fragment. The PCR products were purified and sequenced as previously described (3). Genotype was determined using Norovirus Genotyping Tool version 2.0 (<http://www.rivm.nl/mpf/typingtool/norovirus/>). Phylogenetic analysis of the partial capsid region (287 nt) of GII.P16-GII.2 strains and the partial RdRp region (665 nt) of GII.P16 strains was performed using MEGA7 software (<http://www.megasoftware.net/>).

For some samples, an approximately 2.4 kb region

spanning the partial RdRp and the entire capsid region were sequenced to confirm the recombination and to obtain the putative amino acid sequences of the RdRp and the capsid. Two overlapping fragments were amplified using primer pairs of YuriF-ver2/G2Rv, and COG2F (4)/GV132 (7). Complete capsid sequences and partial RdRp sequences were obtained from 4 GII.P16-GII.2 strains (F75_20160913, F120_20161107, F149_20161122, and F158_20161128), and 2 GII.P16-GII.4 Sydney 2012 strains (F104_20161106 and F25_20170531).

GII.P16-GII.2 was identified as the cause of 17 (71%) of the 24 GII-associated outbreaks. GII.Pe-GII.4 Sydney 2012 was detected from 4 outbreaks (16%), GII.P16-GII.4 Sydney 2012 from 2 (8%), and GII.P7-GII.6 from one. All GII strains obtained were recombinant viruses. Furthermore, GII.P16-GII.2 and GII.P16-GII.4 Sydney 2012 strains were identified for the first time in Aichi Prefecture. However, because we had not investigated the RdRp region of NoV GII isolates before 2014, such recombination might have been missed. According to recent reports (8), GII.P16-GII.2 strains have been circulating since the 2009/10 season in Japan. GII.P16-GII.4 strains have been reported to emerge in the 2015/16 season (9). Therefore, the strains reported in the past seasons were also included in the phylogenetic analysis.

Phylogenetic analysis (Fig. 1A) showed that all the GII.P16-GII.2 strains detected in the 2016/17 season clustered into a group separated from the strains of the past seasons. We compared the amino acid sequence of

Fig. 2. Amino acid substitutions in the capsid P2 domain of GII.P16-GII.2 strains (A), and in the partial RdRp (N-terminal 238aa) of GII.P16 strains (B). Asterisk indicates Aichi strain. The top line of each alignment is the sequence of a reference strain: (A) GII.P2-GII.2 genotype, (B) GII.P16-GII.16 genotype. Black arrow in Fig. 2A indicates the mutated amino acid in the 2016/17 season. White arrow indicates the reverse changed amino acid.

the capsid protein of GII.P16-GII.2 strains obtained in the 2016/17 season and that of the strains isolated in the past season. Fig. 2A shows an alignment of the deduced amino acid sequence of the GII.2 capsid P2 domain (aa279 to 405) known to be responsible for antigenic specificity. Three changes in the amino acids from the strains of the past season were commonly observed: V335I, K341R, and T344S. Amino acid residues 354 and 400 were the reverse changes.

Fig. 1B shows the phylogenetic tree of the RdRp region of GII.P16 strains. The GII.P16 RdRp strains carried either the GII.2 or GII.4 capsid. Despite the differences in their capsid sequences, GII.P16 RdRps from the strains isolated in the 2016/17 season were classified into the same cluster, which was different from the one for the GII.P16-containing strains isolated in the previous season. Putative amino acid sequences of the partial RdRp region (carboxy-terminal 238 aa) are shown in Fig. 2B. Sequence alignment indicated several amino acid substitutions in the RdRp and these changes in amino acids were consistent in both the GII.2 and GII.4 capsid strains. Four of the RdRp substitutions—S293T, V332I, K357Q, and T360A—are located on the surface of the polymerase and spatially close to positions known to influence RdRp function and viral transmission (10). Analyses of the nucleotide tree and the amino acid sequence alignment of RdRp indicated that GII.P16 RdRp of the

GII.2 and GII.4 strains are derived from a common ancestor.

These results demonstrated that both the capsid and RdRp of GII.P16-GII.2 strains changed in the amino acid sequence in the 2016/17 season.

In Aichi, GII.P16-GII.4 Sydney strains were less prevalent during the 2016/17 season, accounting for 8% of GII-associated outbreaks, whereas GII.P16-GII.2 strains predominated, accounting for 71% of the outbreaks. There was no difference of the capsid of GII.P16-GII.4 Sydney strains and GII.Pe-GII.4 Sydney strains (data not shown). Modification in both, the RdRp and capsid may play some role in this predominance because the GII.P16-GII.2 strains carrying both modified RdRp and capsid were predominant in the 2016/17 season.

All nucleotide sequences determined in this paper were deposited in GenBank under the accession numbers LC325213-31, LC325821-38, and LC331997.

Conflict of interest None to declare.

REFERENCES

- Vinje J. Advances in laboratory methods for detection and typing of norovirus. *J Clin Microbiol*. 2015;53:373-81.
- Bull RA, Tanaka MM, White PA. Norovirus recombination. *J Gen Virol*. 2007;88:3347-59.
- Nakamura N, Kobayashi S, Minagawa H, et al. Molecular

- epidemiology of enteric viruses in patients with acute gastroenteritis in Aichi Prefecture, Japan, 2008/09–2013/14. *J Med Virol.* 2016; 88:1180-6.
4. Kageyama T, Kojima S, Shinohara M, et al. Broadly reactive and highly sensitive assay for Norwalk-like viruses based on real-time quantitative reverse transcription-PCR. *J Clin Microbiol.* 2003;41: 1548-57.
  5. Kobayashi S, Sakae K, Suzuki Y, et al. Expression of recombinant capsid proteins of Chitita virus, a genogroup II Norwalk virus, and development of an ELISA to detect the viral antigen. *Microbiol Immunol.* 2000;44:687-93.
  6. Saito H, Saito S, Kamada K, et al. Application of RT-PCR designed from the sequence of the local SRSV strain to the screening in viral gastroenteritis outbreaks. *Microbiol Immunol.* 1998;42:439-46.
  7. Tu ET, Bull RA, Greening GE, et al. Epidemics of gastroenteritis during 2006 were associated with the spread of norovirus GII.4 variants 2006a and 2006b. *Clin Infect Dis.* 2008;46:413-20.
  8. Mizukoshi F, Nagasawa K, Doan YH, et al. Molecular evolution of the RNA-dependent RNA polymerase and capsid genes of human norovirus genotype GII.2 in Japan during 2004–2015. *Front Microbiol.* 2017;8:705.
  9. Matsushima Y, Shimizu T, Ishikawa M, et al. Complete genome sequence of a recombinant GIIP16-GII.4 norovirus detected in Kawasaki City, Japan, in 2016. *Genome Announc.* 2016;4(5). pii:e01099-16.
  10. Tohma K, Lepore CJ, Ford-Siltz LA, et al. Phylogenetic analyses suggest that factors other than the capsid protein play a role in the epidemic potential of GII.2 norovirus. *mSphere.* 2017;2(3). pii:e00187-17.