



# Whole-Genome Sequences of Two Human Norovirus GII.4 Variants Isolated in the United States

Yuan Hu,<sup>a</sup> Haifeng Chen<sup>b</sup>

<sup>a</sup>Office of Regulatory Affairs, Northeast Food and Feed Laboratory, Food and Drug Administration, Jamaica, New York, USA

<sup>b</sup>Division of Molecular Biology, Office of Applied Research and Safety Assessment, Center for Food Safety and Applied Nutrition, Food and Drug Administration, Laurel, Maryland, USA

**ABSTRACT** Variants of human noroviruses belonging to the genogroup II genotype 4 (GII.4) lineage have accounted for most norovirus outbreaks in the world since the mid-1990s. We report here the complete genome sequences of two historical human norovirus GII.4 variants isolated from norovirus-positive patient stool specimens in the United States.

Human norovirus is a major cause of acute nonbacterial gastroenteritis in persons of all ages and is considered to be responsible for most foodborne viral outbreaks worldwide (1, 2). Transmission occurs through ingestion of contaminated food (particularly oysters), water, and person-to-person contact by the fecal-oral route, contact with contaminated surfaces, and aerosol transmission (3). The virus genome is composed of a single-stranded, positive-sense, approximately 7.6-kb RNA in 3 open reading frames (ORFs). ORF1 encodes nonstructural proteins, including RNA-dependent RNA polymerase (RdRp), while ORF2 and ORF3 encode major and minor capsid proteins (VP1 and VP2), respectively (4). Noroviruses belong to a genus of genetically diverse viruses within the family *Caliciviridae*. Based on the difference in the complete VP1 sequences, noroviruses are divided into different genogroups, and within each genogroup, divided into specific genotypes (genetic clusters). Human strains are classified into three genogroups (GI, GII, and GIV) and at least 25 genotypes (5, 6). Despite this great genetic diversity, variants of a single genetic lineage, GII.4, have been associated with global epidemics of acute gastroenteritis and have caused 62 to 80% of norovirus outbreaks since the mid-1990s (7, 8).

For better understanding of the genomic information and phylogeny of the etiological agent, we report here the whole-genome sequences of two GII.4 virus variants isolated from clinical fecal specimens collected in 2008 and 2010 in the United States (termed specimens 2008 and 2010, respectively). Whole-genome sequencing was performed for the two norovirus samples using previously described strategies (9). Viral RNA was directly extracted from the fecal samples using a QIAamp viral RNA minikit (Qiagen, USA) as previously described (10, 11). The resulting RNA was then subjected to sequence-independent single-primer isothermal amplification using Ovation RNA-sequencing system v2 (NuGen, USA). Indexed sequencing libraries were then constructed from the amplicons by using an Illumina Nextera XT library prep kit and were sequenced on an Illumina MiSeq 500-cycle paired-end run, according to the manufacturer's instructions. Sequencing reads were trimmed to remove the low-quality reads and adapter sequences using CLC Genomics Workbench 10.1 (Qiagen). Quality-trimmed reads were *de novo* assembled and mapped against norovirus reference sequences using the same software. Full genome coverage was observed in both samples, and the genome sequences obtained for specimens 2010 and 2008 were 7,554 bp (with 3,543-fold read depth) and 7,558 bp (with 245-fold read depth), respec-

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tively. Phylogenetic genotyping analysis of the full-genome sequences was performed using Norovirus Typing Tool 2.0 (12). The analysis revealed that the complete genotypes of the viruses from specimens 2010 and 2008 were GII.P4\_GII.4 (New\_Orleans\_2009) and GII.P4\_GII.4 (Hunter\_2004), respectively, indicating that they were closely related to the New Orleans virus from 2009 (GenBank accession number [JN595867](#)) and the Hunter virus from 2004 ([DQ078814](#)). Whole-genome comparison confirmed that the former shared 98.2% nucleotide identity (NI) with the New Orleans virus, while the latter shared 98.9% NI with the Hunter virus.

The whole-genome information presented here will be useful for epidemiological analysis of the GII.4 noroviruses and future development of molecular diagnostics.

**Data availability.** The genome sequences of the norovirus samples have been deposited in GenBank under the accession numbers [MH413069](#) and [MH413070](#).

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