





Nearly Complete Genome Sequence of a Human Norovirus GII.P17-GII.17 Strain Isolated from Brazil in 2015

Cristina Santiso-Bellón, a Azahara Fuentes-Trillo, b Juliana da Silva Ribeiro de Andrade, c Carolina Monzó, b Susana Vila-Vicent, a Roberto Gozalbo Rovira, a Javier Buesa, a Felipe J. Chaves, b, d Marize Pereira Miagostovich, c D Jesús Rodríguez-Díaza

ABSTRACT Human noroviruses are the most common cause of nonbacterial acute gastroenteritis worldwide. We report here the nearly complete genome sequence (7,551 nucleotides) of a human norovirus GII.P17-GII.17 strain detected in July 2015 in the stool sample from an adult with acute gastroenteritis in Brazil.

uman noroviruses are the most common cause of nonbacterial acute gastroenteritis (AGE) and the leading cause of sporadic cases and outbreaks in children and adults worldwide (1, 2). The Norovirus genus belongs to the Caliciviridae family and is classified into six distinct genogroups (GI to GVI), which are further subdivided into different genotypes (3). Strains belonging to the same genotype share a sequence similarity of higher than 85% (4). Only genogroups I (GI), II (GII), and IV (GIV) have been identified in humans. GII is the most diverse genogroup, being subdivided into more than 20 genotypes and currently with a wide distribution, playing a major role in the etiology of AGE. For decades, the most prevalent genotype worldwide has been GII.4. In the winter of 2014 to 2015, the GII.17 genotype replaced the circulating GII.4 genotype in Asia, the GII.4 Sydney_2012 variant (5, 6). Although the origin of GII.17 strains is still largely unknown, the GII.17 Kawasaki_2014 variant caused large AGE outbreaks in China, Japan, and South Korea (6-9) and sporadic infections in Hong Kong, Taiwan (10), the United States (11), Italy (12), Romania (13), and Australia. In Latin America, this emergent strain was detected in AGE outbreaks and sporadic cases in Brazil (14, 15) and in sporadic cases in Argentina (16).

We report here the complete genome sequence of a human norovirus GII.P17-GII.17 strain detected in feces from an adult with AGE in July 2015 in Brazil.

Viral RNA was extracted from 140 μ l of a 20% fecal suspension in phosphatebuffered saline (PBS), using the QIAamp viral RNA minikit (Qiagen, Hilden, Germany) with the QIAcube automatized system (Qiagen). Total RNA was sent to Macrogen, Inc., for library construction using a TruSeq mRNA library prep kit (Illumina) and sequencing on a HiSeq platform (Illumina). Data were analyzed using different open-source bioinformatics software programs. A total of 68,795,022 paired-end 100-bp-long reads were obtained. Raw read quality control was assessed with FastQC version 0.11.5 (17). Reads were quality trimmed using seqtk trimfq (version 1.2-r101-dirty) (18). One contig covering the Norovirus genome sequence (7,551 nucleotides [nt]) was assembled using SPAdes -meta (version 3.11.1) (19), with autoadjusted k-mer lengths of 21 nt, 33 nt, and 55 nt, containing an average depth of coverage of 455.09 \times (35,098 reads). Only 0.051% of the reads (35,098 of 68,795,022 reads) belonged to the Norovirus genome, and the final coverage of the genome was higher than 99% (7,551/7,569 nt).

The genome sequence (7,551 nt) contains three open reading frames (ORFs). ORF1

Citation Santiso-Bellón C, Fuentes-Trillo A, da Silva Ribeiro de Andrade J, Monzó C, Vila-Vicent S, Gozalbo Rovira R, Buesa J, Chaves FJ, Miagostovich MP, Rodríguez-Díaz J. 2019. Nearly complete genome sequence of a human norovirus GII.P17-GII.17 strain isolated from Brazil in 2015. Microbiol Resour Announc 8:e01376-18. https://doi.org/10.1128/MRA .01376-18.

Editor Jelle Matthijnssens, KU Leuven

Copyright © 2019 Santiso-Bellón et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license

Address correspondence to Jesús Rodríguez-Díaz, jesus.rodriguez@uv.es.

C.S.-B., A.F.-T., and J.D.S.R.D.A. contributed equally to this work.

Received 19 October 2018 Accepted 3 January 2019 Published 31 January 2019

^aDepartment of Microbiology, School of Medicine, University of Valencia, Valencia, Spain

Department of Genomics and Genetic Diagnosis Unit, Hospital Clínico Research Foundation (INCLIVA), Valencia, Spain

^cLaboratory of Comparative and Environmental Virology, Oswaldo Cruz Institute, Rio de Janeiro, RJ, Brazil

dCIBER of Diabetes and Associated Metabolic Diseases (CIBERDEM), Madrid, Spain



is incomplete and extends from nucleotides 1 to 5095 (1,697 amino acids [aa]), ORF2 extends from nucleotides 5076 to 6698 (1623 nt and 540 aa), and ORF3 starts at nucleotide 6698 and finishes at nucleotide 7477 (780 nt and 259 aa). The genotype was assigned using the Norovirus Genotyping tool (20). Afterwards, BLASTn was run, and it was found that this sequence has 99.52% (7,515/7,551) and 99.50% (7,513/7,551) nucleotide similarity with two GII.17 strains (GenBank accession numbers KT380915 and KP998539, respectively). The contig obtained lacked the first 18 nucleotides compared to those sequences (GTGAATGAAGATGGCGTC). SSPACE version 3.0 (21) and AlignGraph (22) were used to try to extend the assembled contig. Reads were mapped using the BWA-MEM version 0.7.15-r1140 software (23) against the identified most closely related reference genome sequences. The first 18 nucleotides at the 5' end were still not found.

Our study describes a nearly complete genome sequence of a *Norovirus* GII.P17-GII.17 genotype strain providing additional information relevant to be used as a reference for phylogenetic and evolutionary studies.

Data availability. The assigned accession number for the present norovirus genome in GenBank is MH997861. The ORFs were given the GenBank accession numbers AYF59857 (ORF1), AYF59855 (ORF2), and AYF59856 (ORF3). The sequence data are available in the Sequence Read Archive (SRA) under BioProject number PRJNA497363.

ACKNOWLEDGMENTS

This work was supported by Spanish Government (Ministerio de Economia y Competitividad) grants AGL2014-52996-C2-2-R and RYC-2013-12442 to J.R.-D. and grant PI16/01471 to J.B. R.G.R. is the recipient of postdoctoral grant APOST/2017/037 from the Valencian Government. S.V.-V. and A.F.-T. are recipients of predoctoral fellowships ACIF/2016/437 and ACIF/2018/303 from the Valencian Government. J.D.S.R.D.A. received financial support from the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior–Brasil (CAPES), finance code 001.

REFERENCES

- Bartnicki E, Cunha JB, Kolawole AO, Wobus CE. 2017. Recent advances in understanding noroviruses. F1000Res 6:79. https://doi.org/10.12688/ f1000research.10081.1.
- Patel MM, Widdowson MA, Glass RI, Akazawa K, Vinjé J, Parashar UD. 2008. Systematic literature review of role of noroviruses in sporadic gastroenteritis. Emerg Infect Dis 14:1224–1231. https://doi.org/10.3201/ eid1408.071114.
- Kroneman A, Vega E, Vennema H, Vinjé J, White PA, Hansman G, Green K, Martella V, Katayama K, Koopmans M. 2013. Proposal for a unified norovirus nomenclature and genotyping. Arch Virol 158:2059–2068. https://doi.org/10.1007/s00705-013-1708-5.
- Zheng D-P, Ando T, Fankhauser RL, Beard RS, Glass RI, Monroe SS. 2006. Norovirus classification and proposed strain nomenclature. Virology 346:312–323. https://doi.org/10.1016/j.virol.2005.11.015.
- Chan MCW, Lee N, Hung TN, Kwok K, Cheung K, Tin EKY, Lai RWM, Nelson EAS, Leung TF, Chan PKS. 2015. Rapid emergence and predominance of a broadly recognizing and fast-evolving norovirus Gll.17 variant in late 2014. Nat Commun 6:10061. https://doi.org/10.1038/ ncomms10061.
- Matsushima Y, Ishikawa M, Shimizu T, Komane A, Kasuo S, Shinohara M, Nagasawa K, Kimura H, Ryo A, Okabe N, Haga K, Doan YH, Katayama K, Shimizu H. 2015. Genetic analyses of Gll.17 norovirus strains in diarrheal disease outbreaks from December 2014 to March 2015 in Japan reveal a novel polymerase sequence and amino acid substitutions in the capsid region. Euro Surveill 20:pii=21173. https://doi.org/10.2807/1560-7917 .ES2015.20.26.21173.
- 7. Lu J, Sun L, Fang L, Yang F, Mo Y, Lao J, Zheng H, Tan X, Lin H, Rutherford S, Guo L, Ke C, Hui L. 2015. Gastroenteritis outbreaks caused by norovirus GII.17, Guangdong Province, China, 2014–2015. Emerg Infect Dis 21: 1240–1242. https://doi.org/10.3201/eid2107.150226.
- 8. Jung S, Hwang B-M, Jung H, Chung G, Yoo C-K, Lee D-Y. 2017. Emergence of norovirus Gll.17-associated outbreak and sporadic cases in Korea from 2014 to 2015. Osong Public Health Res Perspect 8:86–90. https://doi.org/10.24171/j.phrp.2017.8.1.12.

- Lu J, Fang L, Zheng H, Lao J, Yang F, Sun L, Xiao J, Lin J, Song T, Ni T, Raghwani J, Ke C, Faria NR, Bowden TA, Pybus OG, Li H. 2016. The evolution and transmission of epidemic GII.17 noroviruses. J Infect Dis 214:556–564. https://doi.org/10.1093/infdis/jiw208.
- Lee CC, Feng Y, Chen SY, Tsai CN, Lai MW, Chiu CH. 2015. Emerging norovirus Gll.17 in Taiwan. Clin Infect Dis 61:1762–1764. https://doi.org/ 10.1093/cid/civ647.
- Parra GI, Green KY. 2015. Genome of emerging norovirus GII.17, United States, 2014. Emerg Infect Dis 21:1477–1479. https://doi.org/10.3201/ eid2108.150652.
- Medici MC, Tummolo F, Calderaro A, Chironna M, Giammanco GM, De Grazia S, Arcangeletti MC, De Conto F. 2015. Identification of the novel Kawasaki 2014 Gll.17 human norovirus strain in Italy, 2015. Euro Surveill 20:pii=30010. https://doi.org/10.2807/1560-7917.ES.2015.20.35.30010.
- 13. Dinu S, Nagy M, Negru DG, Popovici ED, Zota L, Oprişan G. 2016. Molecular identification of emergent GII.P17-GII.17 norovirus genotype, Romania, 2015. Euro Surveill 21:pii=30141. https://doi.org/10.2807/1560-7917.ES.2016.21.7.30141.
- Andrade JSR, Fumian TM, Paulo J, Leite G, De Assis MR, Bello G, Mir D, Miagostovich MP. 2017. Detection and molecular characterization of emergent Gll.P17/Gll.17 Norovirus in Brazil, 2015. Infect Genet Evol 51:28–32. https://doi.org/10.1016/j.meegid.2017.03.011.
- da Silva LD, Bandeira RD, Junior EC, Lima IC, da Penha Júnior ET, Teixeira DM, Siqueira JA, Resque HR, de Abreu Campos EM, Justino MC, Linhares AC, Gabbay YB. 2017. Detection and genetic characterization of the emergent Gll.17_2014 norovirus genotype among children with gastroenteritis from northern Brazil. Infect Genet Evol 48:1–3. https://doi.org/ 10.1016/j.meegid.2016.11.027.
- Degiuseppe JI, Gomes KA, Hadad MF, Parra GI, Stupka JA. 2017. Detection of novel GII.17 norovirus in Argentina, 2015. Infect Genet Evol 47:121–124. https://doi.org/10.1016/j.meegid.2016.11.026.
- Schmieder R, Edwards R. 2011. Quality control and preprocessing of metagenomic datasets. Bioinformatics 27:863–864. https://doi.org/10 .1093/bioinformatics/btr026.

Volume 8 Issue 5 e01376-18



- 18. Li H. 2015. Seqtk. https://github.com/lh3/seqtk.
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477. https://doi.org/10.1089/cmb.2012.0021.
- Kroneman A, Vennema H, Deforche K, Avoort H, Peñaranda S, Oberste MS, Vinjé J, Koopmans M. 2011. An automated genotyping tool for enteroviruses and noroviruses. J Clin Virol 51:121–125. https://doi.org/ 10.1016/j.jcv.2011.03.006.
- Boetzer M, Henkel CV, Jansen HJ, Butler D, Pirovano W. 2011. Scaffolding pre-assembled contigs using SSPACE. Bioinformatics 27:578–579. https://doi.org/10.1093/bioinformatics/btq683.
- 22. Bao E, Jiang T, Girke T. 2014. AlignGraph: algorithm for secondary de novo genome assembly guided by closely related references. Bioinformatics 30:i319–i328. https://doi.org/10.1093/bioinformatics/btu291.
- Li H. 2013. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. arXiv arXiv:1303.3997. https://arxiv.org/abs/ 1303.3997.

Volume 8 Issue 5 e01376-18 mra.asm.org **3**