Letter to the Editor

Francesco Broccolo*, Marta Giovanetti, Alessio Colombo, Nicola Perrelli, Adelaide Bussini, Silvia Cavalli, Massimo Ciccozzi and Massimo Zollo

Molecular detection of SARS-CoV-2 eta VOI in Northern Italy: a case report

<https://doi.org/10.1515/cclm-2021-1230>

Received November 24, 2021; accepted November 24, 2021;

published online December 2, 2021

Keywords: B.1.525; COVID-19; eta variant; genomic epidemiology; SARS-CoV-2.

To the Editor,

During the course of the pandemic, variants of SARS-CoV-2 that harbor constellation of mutations into the receptor binding domain (RBD) of the Spike (S) protein have brought concern all over the world [1–4]. These have been called ‘variants of concern’ (VOC) and ‘variant of interest’ (VOI) as it has been suggested that their genome mutations might impact transmission, immune control, and virulence [5].

The eta variant, also known as VUI-21FEB-03 and B.1.525, was first identified in Nigeria and the United Kingdom in December 2020 [5] and as of June 2021, it had been already detected in 69 countries [5]. It has been classified as a VOI due to the presence of some genetic signatures, also shared by other VOCs, including: i) the E484K mutation present in the beta, gamma, and zeta variants; ii) the Q677H, associated with an enhanced transmissibility; iii) (Δ 144) associated with immune

escape; (iv) Δ 106-108 already detected in the alpha, beta, and gamma variants; v) the N439K also found in Y453F, B.1.141, B.1.258 variants; and (vi) two deletions Δ H69/ Δ V70 also detected in B.1.1.7.3 variant. It does not carry the N501Y mutation, but differently from all other variants it carries both the E484K mutation and a new F888L mutation (a substitution of phenylalanine [F] with leucine [L] in the S2 domain of the spike protein) [5]. Currently, this virus strain is not as concerning as other variants, but recent reports suggested that it might be linked with a significant community transmission or multiple COVID-19 clusters, with increasing number of cases over time, enhancing the emerging risk to the global public health [5].

Here, we report genomic evidence of a SARS-CoV-2 eta VOI in Northern Italy (Novara province). A 50-year-old female, resident in Novara province, Lombardy region, reported symptoms compatible with viral infection on March 10th, 2021, including prostration, loss of appetite, taste and smell, and headache. The patient was admitted to the Santa Monica Emergency Department of the Hospital of Arona in Novara and received paracetamol 1.000 mg for five days. However, since disease was classified as mild, she was treated at home, not requiring hospitalization.

Viral RNA was extracted from nasopharyngeal swab and tested for SARS-CoV-2 by multiplex real-time PCR Allplex™ SARS-CoV-2 assay (Seegene Inc, Seoul, Korea) and then submitted to the COVID-19 Variant Catcher assay (Clonit), a qualitative test that allows the identification of the S gene mutations HV 69-70del, E484K, and N501Y for discrimination of SARS-CoV-2 (Wuhan strain) from SARS-CoV-2 strains B. The RT-PCR-positive sample was then submitted to viral genomic amplification and posterior sequencing using the Myseq Illumina system using the CleanPlex SARS-CoV-2 Panel (Sophia genetics), according to the manufacturer’s instructions. Consensus sequence was generated using MEGAHT v1.2.9 [6].

To accurately establish evolutionary relationships among the newly generated sequence and other isolates of SARS-CoV-2, we then subjected a combined data set (including 5,531 SARS-CoV-2 full-length viral genomes available on GISAID [<https://www.gisaid.org/>] up to Oct

***Corresponding author: Francesco Broccolo**, Department of Medicine and Surgery, School of Medicine, University of Milano-Bicocca, Monza, Italy; and Laboratory Cerba Healthcare, Milan, Italy, E-mail: francesco.broccolo@unimib.it. <https://orcid.org/0000-0003-1297-2598>

Marta Giovanetti, Laboratório de Flavivírus, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, Rio de Janeiro, Brazil

Alessio Colombo, Nicola Perrelli, Adelaide Bussini and Silvia Cavalli, Laboratory Cerba Healthcare, Milan, Italy

Massimo Ciccozzi, Medical Statistic and Molecular Epidemiology Unit, University of Biomedical Campus, Rome, Italy

Massimo Zollo, CEINGE, Biotecnologie Avanzate, Naples, Italy; and Dipartimento di Medicina Molecolare e Biotecnologie Mediche (DMMBM), ‘Federico II’ University of Naples, Naples, Italy. <https://orcid.org/0000-0002-0970-7243>

Figure 1: Genomic characterization of a B.1.525 SARS-CoV-2 strain isolated in an Italian patient in Novara Lombardy province in 2021.

(A) Maximum likelihood (ML) tree including the newly complete genome isolate obtained in this study plus $n=5,531$ SARS-CoV-2 Italian strains collected up to June 1st, 2021. New isolate is highlighted in orange (circles). (B) Zoom of the new isolate clade. Branch support (SH-aLTR >0.8) is shown at key nodes. (C) Variant map of the B.1.525 lineage-defining-mutations. Lineage-defining mutation is highlighted in red.

31st, 2021) to phylogenetic inference. Only genomes $>29,000$ bp and $<1\%$ of ambiguities were retrieved, low-quality genomes ($>10\%$ of ambiguous positions), were excluded. Sequences were aligned using MAFFT (FF-NS-2 algorithm) employing default parameters [7]. The alignment was manually curated to optimize number and location of gaps using Aliview [8]. Lineage assessment was conducted using the Phylogenetic Assignment of Named Global Outbreak LINeages tool available at <https://github.com/hCoV-2019/pangolin> [9]. Phylogenetic analysis was performed using the maximum likelihood (ML) method implemented in IQ-TREE-2, employing the best-fit model of nucleotide substitution according to the Bayesian Information Criterion (BIC), as indicated by the Model Finder application implemented in IQ-TREE 2 [10]. The statistical robustness of individual nodes was determined using the SH-aLTR branch support.

Cycle threshold values (Cts) of N, E, and RdRp/S targets were 28.97, 27, and 29 respectively. COVID-19 Variant Catcher assay revealed that the sample was positive for the

E484K mutation with a Ct value of 32.29. Viral genomic RNA amplification and sequencing produced a total of 1,500,514 mapped reads, with corresponding coverage of 99.8% and a mean sequencing depth of 9,701.4X. We then constructed phylogenetic trees to explore the relationship of the sequenced B.1.525 strain from Novara province, to those of other isolates.

Our phylogeny combined with the lineage assignment (<https://github.com/hCoV-2019/pangolin>) showed that the new viral strain obtained in this study belonged to the B.1.525 lineage (Figure 1A), and clustered together with a strong support (SH-aLTR $>80\%$) with other Italian isolates sampled in March 2021 (Figure 1B). Further, we analyzed the mutational profile of the newly generated strain to determine its lineage-defining-mutations. As expected, the identified lineage harbored all the B.1.525 lineage-specific mutations (Figure 1C) with the exception of the del:21765:6. The mutational patterns was also investigated using NextClade (Figure 1C), and no newly acquired mutations were identified.

The current COVID-19 pandemic is a global health emergency that makes monitoring the molecular evolution of SARS-CoV-2 extremely urgent in order to prepare prevention strategies, limit its spread and counter the effects that any viral variants may have on the effectiveness of the vaccine and antiviral therapies. In Italy, there is little information on the genetic variability of SARS-CoV-2. Molecular characterization of viral strains present in the various geographic areas would be extremely useful for the management of the current health emergency. This would allow a better understanding of the spread of the epidemic, to trace the evolution and methods/routes of spread as well as the identification of drug-resistant strains. Furthermore, the emergence of new viral variants could hinder the effectiveness of a vaccine.

Taking into consideration the recent concern due to the rapid rise of this novel variant and considering the constellation of its lineage-specific mutations including the ones into the S protein, our findings reinforce the need to speed up the immunization strategies in order to prevent the emergence of potential newly SARS-CoV-2 variants with possible implications for public health.

Acknowledgments: Authors would like to thank Dr. Angelo Boccia dell' informatic Core Ceinge per data handling, and Dr. Alice Urbani for sequence analysis. Authors would also like to thank all the authors who have kindly deposited and shared genome data on GISAID. A table with genome sequence acknowledgments can be found in Table S1.

Research funding: MZ was supported by the project “CEINGE TASK-FORCE COVID-19,” code D64I200003800 by Regione Campania for the fight against COVID-19 (DGR n. 140 del 17 marzo 2020). MG was supported by Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) and in part through, the CRP-ICGEB RESEARCH GRANT 2020 Project CRP/BRA20-03.

Author contributions: Conception and design: FB and MC; data collection: FB, AC, NP, AB, MZ; investigations: AC, NP, AB, SC, MG, MZ; data analysis: FB, MC, MZ, MG; writing – original: MG, MC, and FB; draft preparation: MG, MC, and FB; revision: FB, MZ, MG, MC.

Competing interests: The authors declare that they have no known competing financial interests or personal

relationships that could have appeared to influence the work reported in this paper.

Informed consent: Informed consent was obtained from all individuals included in this study.

Ethical approval: Not applicable.

References

1. World Health Organization, WHO; 2021. Available from: <https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/>.
2. Rambaut A, Loman N, Pybus O, Barclay W, Barret J, Carabelli A, et al. Preliminary genomic characterisation of an emergent SARS-CoV-2 lineage in the UK defined by a novel set of spike mutations. Available from: <https://virological.org/t/preliminary-genomic-characterisation-of-an-emergent-sars-cov-2-lineage-in-the-uk-defined-by-a-novel-set-of-spike-mutations/563> [Accessed 21 Dec 2020].
3. Tegally H, Wilkinson E, Giovanetti M, Iranzadeh A, Fonseca V, Giandhari J, et al. Detection of a SARS-CoV-2 variant of concern in South Africa. *Nature* 2021;592:438–43.
4. Faria NR, Mellan TA, Whittaker C, Claro IM, Candido D, Mishra S, et al. Genomics and epidemiology of the P.1 SARS-CoV-2 lineage in Manaus, Brazil. *Science* 2021;14:24–6.
5. Public Health England. SARS-CoV-2 variants of concern and variants under investigation in England. 2021. Available from: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/972247/Variants_of_Concern_VOC_Technical_Briefing_7_England.pdf.
6. Li D, Luo R, Liu C-M, Leung C-M, Ting H-F, Sadakane K, et al. MEGAHIT v1.0: a fast and scalable metagenome assembler driven by advanced methodologies and community practices. *Methods* 2016;102:3–11.
7. Nakamura T, Yamada KD, Tomii K, Katoh K. Parallelization of MAFFT for large-scale multiple sequence alignments. *Bioinformatics* 2018;34:2490–2.
8. Larsson A. AliView: a fast and lightweight alignment viewer and editor for large datasets. *Bioinformatics* 2014;30:3276–8.
9. O'Toole Á, McCrone J. Phylogenetic assignment of named global outbreak lineages; 2020. Available from: <https://github.com/hCoV-2019/pangolin>.
10. Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A, et al. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Mol Biol Evol* 2020;37:1530–4.

Supplementary Material: The online version of this article offers supplementary material (<https://doi.org/10.1515/cclm-2021-1230>).