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Letter to the Editor

Genomic evidence of SARS-CoV-2 reinfection case with the emerging B.1.2 variant in Brazil

Dear Editor,

We read with interest the recently published manuscript of Santos et al., about evidence of reinfection and enhanced severity in Brazilian healthcare worker[1] and here we report the first confirmed case of SARS-CoV-2 reinfection of a 29-year-old male, medical doctor, from Minas Gerais state, Southeast Brazil.

The duration of acquired immunity conferred by infection with SARS-CoV-2 is still poorly understood and recently released data suggest that having COVID-19 may not protect against getting infected again with some of the new variants, evoking the nightmare of a never-ending pandemic.

Since the report of the first confirmed case of COVID-19 on 26 February 2020 in São Paulo (SP) state, Brazil, the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has affected more than 12 million people and to date, has caused approximately 300 thousand deaths in Brazil. [2] Infection with SARS-CoV-2 leads to detectable, short-lasting, IgG responses[3,4] likely to provide protection to reinfection. Nonetheless, susceptibility of previously infected individuals to reinfection, due to the circulation of different SARS-CoV-2 variants and lineages,[5–7] is now starting to be considered a growing concern.

Here, we present the first confirmed case of SARS-CoV-2 reinfection in Minas Gerais state, presenting two distinct COVID-19 illnesses from genetically distinct SARS-CoV-2 variants, including the emerging B.1.2 lineage in Brazil. Identifying cases of SARS-CoV-2 reinfection is essential to better understand the course of the COVID-19 pandemic, to monitor the evolution of population herd-immunity, and to guide strategies for vaccine development.

A 29-year-old male, medical doctor, resident in Sabará, Minas Gerais state, southeast Brazil, with no comorbidities, presented two clinical episodes of SARS-CoV-2 infection separated by a 225-day interval (**Fig. 1 panel A**).

In the first episode on May 21st, 2020 the patient presented fever, myalgia, cough, sore throat, and diarrhea for approximatively 10 days (**Fig. 1 panel A**). Two months after testing positive by RT-PCR in the first episode, an IgG test against S1 protein by chemiluminescence, was performed and showed a positive result (index value: 5.07 on 07/08/2020), followed by a negative IgG assay on Mid-December 2020.

The patient's symptoms returned on January 4th, 2021, after returning from holiday from Rio de Janeiro, when a second nasopharyngeal swab (on January 06th, 2021) (**Fig. 1 panel A**) was obtained and presented a positive result for SARS-CoV-2 infection by re-al-time RT-PCR testing.

Viral RNA was extracted from nasopharyngeal swabs and tested for SARS-CoV-2 using the protocol established by the Center for Disease Control and Prevention that targets the Nucleocapsid gene (CDC, Atlanta).[8] On both occasions, results of RT-PCR tests targeting 2 genes (N1 and N2) were positive for SARS-CoV-2. Antibody testing (IgG) after the first and the second episode was performed by chemiluminescence (Alinity^{TM}, Abbott).

Cycle threshold values (Cts) of N1, and N2 targets were 15.7, and 18.9 in the first episode and 17.6, and 19.6 in the second episode. In early February 2021, a second positive IgG assay was also detected (index value: 7,58) (**Fig. 1 panel A**).

Genome sequencing was then conducted by PGM Ion Torrent (Life Technologies, USA) and a total of 1.486.791 mapped reads for sample A and 1.228.341 reads for sample B were obtained, resulting in a sequencing mean depth >1000 for both samples and a coverage > 99%.

The distinct viral origin of the two infections was evaluated by combining our new isolates (EPI_ISL_1182550 and EPI_ISL_1182549) with $n = 3852$ representative full-length viral genomes available on GISAID (https://www.gisaid.org/) up to March 23rd, 2021, with which phylogenetic inference was performed. Low-quality genomes (> 10% of ambiguous positions) were excluded. Sequences were aligned using MAFFT[9] and submitted to IQ-TREE for maximum likelihood (ML) phylogenetic analysis.[10] The statistical robust-ness of individual nodes was determined using the SH- aLTR test.

Sequence data and phylogenetic analysis, indicated that the two COVID-19 episodes, were indeed caused by different SARS-CoV-2 lineages, confirming reinfection. In the first episode, the lineage B.1.1.28 was detected and genomic sequence analysis identified $n = 7$ mutations ORF1ab: P4715L and M6078I; Spike: D614G and V1176F; Nucleocapsid: R203K and G204R; ORF14: G50N. In the second infection, the B.1.2 lineage was detected for the first time in Brazil (**Fig. 1 panel B**) which showed $n = 15$ mutation ORF1ab (**Fig. 1 panel C**): T265I, M2606I, L3352F, P4075S, A4489V, P4715L, N6054D, T6938I, R7014C and T265I; Spike: D614G; ORF3a: Q57H and G172V; ORF8: S24L; Nucleocapsid: P67S.

In conclusion, our case report describes the first individual in Minas Gerais state to have symptomatic reinfection with SARS-CoV-2 with no increases in symptom severity from the first to the second episode. Our study reports the first detection of the B.1.2 lineage in Brazil, which is mainly circulating in North America, reinforcing how the high connectivity of countries can mediate the introduction of new viral strains. Considering the recent concern of the rapid rise (starting from late January 2020) of the B.1.2 infections carrying a substitution affecting amino acid position 677 of the Spike protein,[7] our findings reinforce the need for active monitoring of travelers, to follow the real-time spread of new SARS-CoV-2 variants with possible implications for public health policies and immunization strategies.

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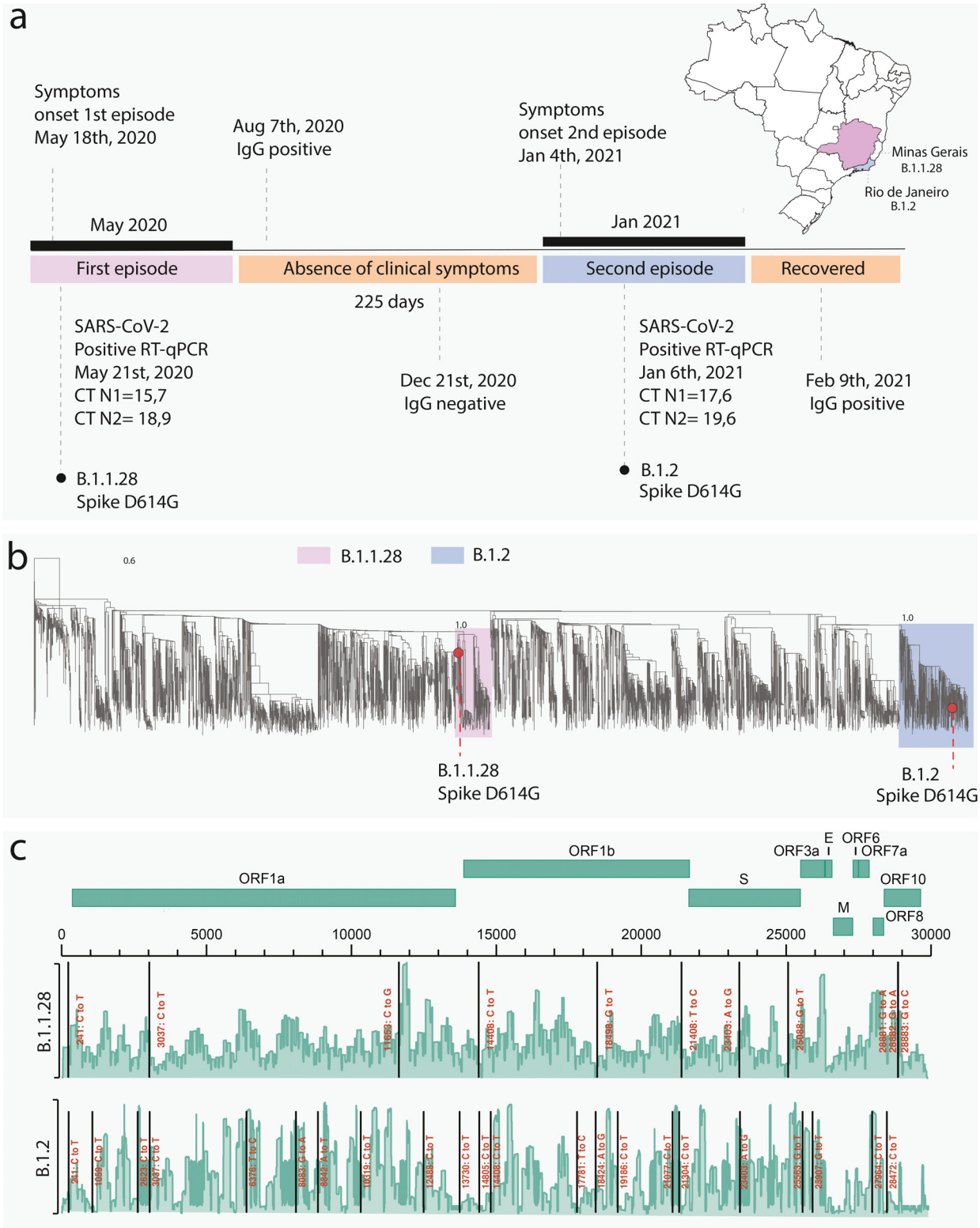

Fig. 1. Genomic characterization of a COVID-19 reinfection case in Minas Gerais state, Southeast Brazil. (A) Timeline of symptom onset, molecular diagnosis, and sequencing of specimens; (B) ML tree including the newly SARS-CoV-2 genomes (EPI_ISL_1182550 and EPI_ISL_1182549) recovered from a 29-year-old male resident in Sabará, Minas Gerais state, Southeast Brazil, with $n = 3852$ representative full-length viral genomes available on GISAID (https://www.gisaid.org/) up to March 23rd, 2021. New genomes are highlighted with red circles. Branch support (SH-aLTR >0.8) is shown at key nodes; (C) Variant mapping of specimens recovered from the first and the second episode (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

Ethical approval

This research was approved by the Ethics Review Committee of the Federal University of Minas Gerais (CEP/CAAE: 32912820.6.1001.5149 approval number). The availability of these samples for research purposes during outbreaks of national concern is allowed to the terms of the 510/2016 Resolution of the National Ethical Committee for Research – Brazilian Ministry of Health (CONEP - Comissão Nacional de Ética em Pesquisa, Ministério da Saúde), that authorize, without the necessity of an informed consent, the use of clinical samples collected in the Brazilian Central Public Health Laboratories to accelerate knowledge building and contribute to surveillance and outbreak response.

Data availability

Newly generated SARS-CoV-2 sequences have been deposited in GISAID under accession numbers EPI_ISL_1182550 and EPI_ISL_1182549.

Declaration of Competing Interest

The authors declare no conflict of interest.

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