Journal of Travel Medicine

International Society of Travel Medicine
Established 1991
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Journal of Travel Medicine, 2021, 1–3
https://doi.org/10.1093/jtm/taab153
Advance Access Publication Date: 27 September 2021
Research Letter

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Multiple Introductions of SARS-CoV-2 C.37 Lambda lineage in the Southern Brazilian region

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Submitted 27 July 2021; Revised 28 August 2021; Accepted 15 September 2021

Key words: SARS-CoV-2, Lambda, South America, Brazil, C.37

The SARS-CoV-2 variant assigned to PangoLineage C.37 was first reported in late December 2020 in Peru and by April 2021 was responsible for 97% of new infections in that country.[1] It is currently expanding in Chile and Argentina, and there is evidence of onward transmission in Colombia, Ecuador, Mexico, the USA, Germany and Israel.[2] C.37 presents 30 molecular signatures, seven nonsynonymous substitutions (G75V, T76I, R246N, L452Q, F490S, D614G, T859N) and a deleted region (Δ247–253) in the Spike protein.[1] Another deleted region is found in the ORF1a gene (Δ3675–3677) also present in variants of concern Alpha, Beta and Gamma.[1] Due to genomic characteristics and epidemiological relevance, on 15 June 2021, WHO designated C.37 as Variant of Interest (VOI) Lambda.[2,3]

Until 26 July 2021, six cases of C.37 were notified in Brazil, two in the southern state of Rio Grande do Sul (RS) and four in the southeastern state of São Paulo (SP). The first case in RS (Patient 01), notified in early June, was a 23-year-old (yo) male truck driver returning from a trip through Argentina on 5 June.[4] An epidemiological investigation was not able to identify potential infection sources, and even though two contacts also tested positive in antigen tests, samples were unavailable for genomic characterization. The second RS case (Patient 02), notified in early June, was a 1-year female resident of Santo Ângelo, near the Argentine border. Only 2 days before the first symptoms, the patient had resumed pre-school classroom classes. The patient's father, mother and 4-yo sister presented symptoms compatible with COVID-19, but only the father had positive antigen results, the other two had them undetectable.

The SARS-CoV-2 whole-genome (>99% coverage) of Patient 2 was recovered using Illumina COVIDSeq Test. FastQ reads obtained were imported into the CLC Genomics Workbench version 20.0.4 (Qiagen A/S, Denmark), trimmed and mapped against the reference sequence EPI_ISL_402124. Pango Lineages tool v.3.1.7 was used for lineage assignment and CoVSurver (https://mendel.bii.a-star.edu.sg/METHODS/corona/beta/) and Nextclade Web v.1.5.2 (https://clades.nextstrain.org/) tools were used to map the nucleotide and amino acid substitutions.

To understand the spatiotemporal dynamic of RS-identified introductions, we constructed a time-scaled Bayesian phylogenetic tree using the Bayesian Markov chain Monte Carlo (MCMC) approach implemented in BEAST 1.10 with BEAGLE library v3 to improve run-time efficiency.[5] To this end, a sub

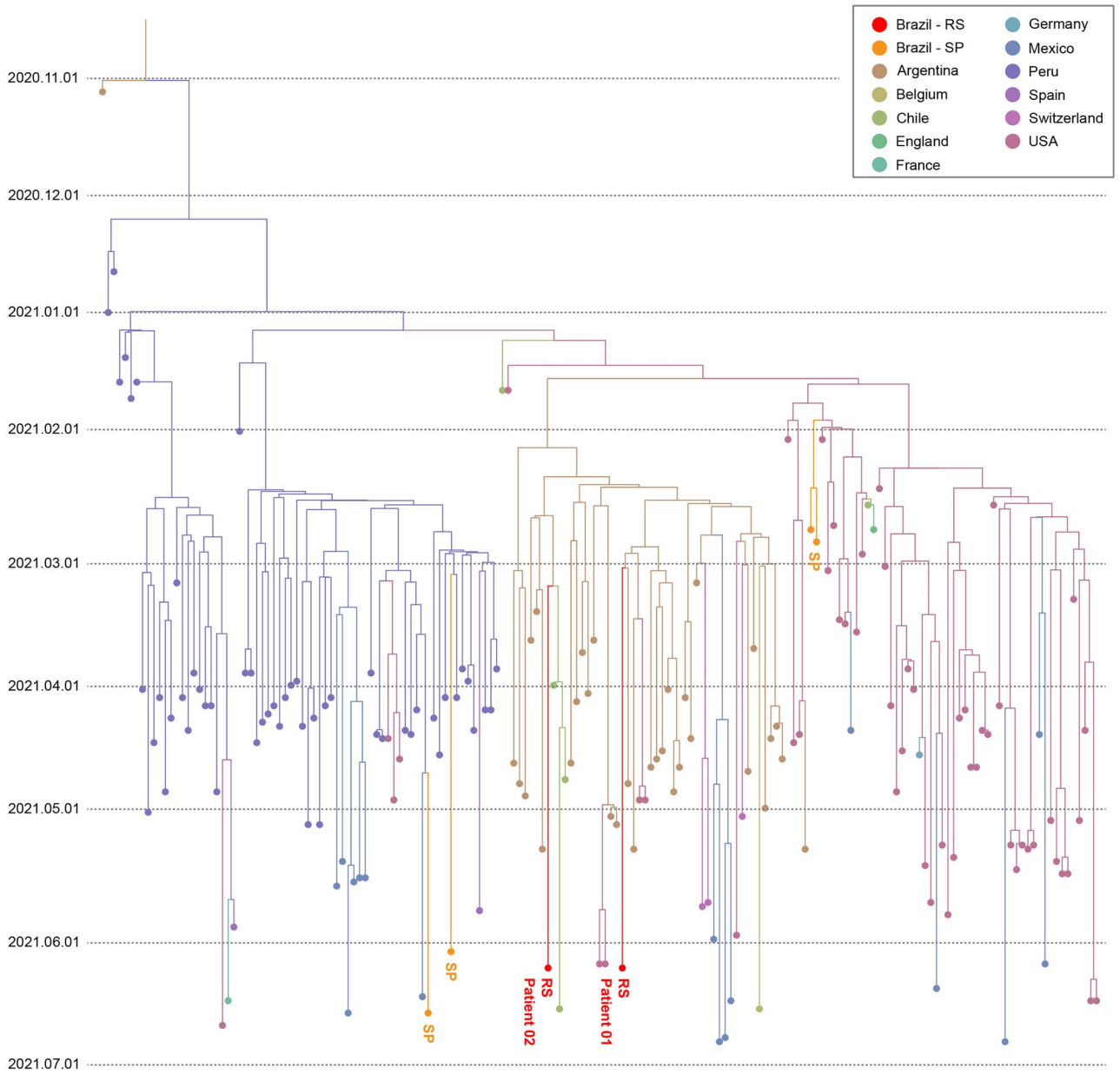

Figure 1. Spatiotemporal characterization of multiple SARS-CoV-2 C.37 lineage introductions in Brazil. Time-scaled Bayesian MCC tree of SARS-CoV-2 C.37 genome sequences ($n = 175$) from Brazil ($n = 6$) and 11 other countries. Branches are coloured according to the most probable location state of their descendent nodes as indicated in the legend on the upper right side.

dataset ($n = 175$) of all C.37 genomes available in the EpiCoV database of the GISAID initiative (https://www.gisaid.org/) until 17 July was constructed by a local blast search followed by trimming of structured clusters in an ML topology inferred with IQ-TREE v2.1.3.[6,7] The Bayesian MCMC analysis was run for 200×10^6 generations and performed using a strict molecular clock model, a constant prior distribution on the substitution rate ($8–10 \times 10^{-4}$ substitutions per site per year), and the non-parametric Bayesian skyline model as a coalescent tree prior.[8] Viral migrations were reconstructed using a reversible discrete phylogeographic model with a continuous-time Markov chain rate reference prior.[9] Sequences were classified according to their country of origin.

Five Lambda introductions were detected in Brazil, in RS ($n = 2$) and SP ($n = 3$) (Supplementary Table 1), with only one introduction in SP aggregating more than one sequence. In the time-scaled Bayesian MCC tree (Figure 1), Patient 01's genome (EPI_ISL_2617911), sampled on 10 June, had its Most Recent Common Ancestor (MRCA) located in Argentina [Posterior State Probability (PSP) = 1.0], emerging on 14 March (95% Highest Probability Density (HPD): 03/28–03/03). This observation was in accordance with Patient 01's known travelling pattern in the days before the onset of its symptoms. Patient 02's genome (EPI_ISL_3751412), sampled on 10 June, had its MRCA located in Argentina/Chile (PSP = 0.48/0.34) on 6 March (95% HPD: 02/18–03/21). The family of Patient 02 lives in a city near the

Argentine border, an epidemiological link that, in the absence of autochthone transmission, could answer for its lack of travelling history. The relatively low PSP value, however, is most probably due to a genomic subsampling. Other introductions in SP were associated with Peru ($n = 2$) or the USA ($n = 1$) (PSP = 1.0). The SP dyad was the one introduction associated with the USA and had its MRCA located in Brazil (PSP = 1.0). The absence of additional identification of C.37 in the state speaks in favour of a limited introduction or limited circulation. The synchronicity of this introduction with the early stages of Gamma dissemination in Brazil, a variant that would lead the largest yet surge of COVID-19 cases in the country, may also have limited its reach in the Brazilian population.

The six Brazilian genomes, as analysed by the Nexclade algorithm (https://clades.nextstrain.org/), exhibited almost all the lineage's 30 synapomorphies.[1] The N gene T366I substitution was not present in three SP sequences (EPI_ISL_2674368, EPI_ISL_1966094 and EPI_ISL_1445272). The genome of Patient 2 bore two additional amino acid substitutions in the Spike protein, T95I and S151I. Both signatures were not found in any of the sequences the genome is clustered with in the MCC tree topology (Figure 1). The Spike protein deletion (Δ247–253) was absent in the SP dyad, one of them (EPI_ISL_1445272) also not exhibiting the deletion in ORF1 (Δ3675–3677).

Given the relevance to which the VOI Lambda rose in South America, and evidence of increased infectivity and immune escape, genomic surveillance of its spread is essential. Brazil is particularly vulnerable, considering its frontiers with 10 out of all 12 South American countries, with land borders extending for $>15 \times 10^3$km. The association of both RS introductions detected here with neighbouring Argentine illustrates the effect of geographical position in the dissemination of infectious diseases such as SARS-CoV-2. Therefore, an increased genomic surveillance across Brazil's neighbouring countries as well as in borderline Brazilian states could lead to the detection of additional introductions and elucidate the structure of clusters that lead to the known occurrences. However, despite questions raised by epidemiological surveys, evidence of autochthone C.37 transmission is yet to be detected. Nonetheless, we demonstrated here that, since February, multiple Lambda introductions have occurred in Brazil.

Supplementary data

Supplementary data are available at *JTM* online.

Acknowledgements

The authors wish to thank all the health care workers and scientists who have worked hard to deal with this pandemic threat, the GISAID team and all the EpiCoV database's submitters (Acknowledgment Table 1). We also appreciate the support of the Fiocruz COVID-19 Genomic Surveillance Network (http://www.genomahcov.fiocruz.br/) members, the Respiratory Viruses Genomic Surveillance. General Coordination of the Laboratory Network (CGLab), Brazilian Ministry of Health (MoH), Brazilian States Central Laboratories (LACENs) for the partnership in viral surveillance in Brazil.

Conflict of interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Author contributions

Conceptualization: IA and PCR; Formal Analysis: I.A. and P.C.R.; Sequencing: A.C.D.P., L.A., R.S.L., A.C.F.M., A.S.B.R., T.M.M. and E.C.P.; Epidemiological Survey: R.S.S., T.S.G., L.G.M., A.L.B. and A.F.M.; Supervision: P.C.R. and M.M.S.; Writing—Original Draft: I.A., P.C.R., R.S.S., T.S.G. and L.G.M. All authors took part in the review of the manuscript.

Funding

Departamento de Ciência e Tecnologia/Secretaria de Ciência, Tecnologia, Inovação e Insumos Estratégicos em Saúde/Ministério da Saúde [402 457/2020-9, 403 276/2020-9]; Inova Fiocruz/Fundação Oswaldo Cruz [VPPCB-007-FIO-18-2-30, VPPCB-005-FIO-20-2-87]; Instituto Nacional de Ciência e Tecnologia-FCx [465 259/2014-6].

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