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GENOME SEQUENCES

Genome Sequences of SARS-CoV-2 P.1 (Variant of Concern) and P.2 (Variant of Interest) Identified in Uruguay

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ABSTRACT Two severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants associated with increased transmission and immune evasion, P.1 and P.2, emerged in Brazil and spread throughout South America. Here, we report genomes corresponding to these variants that were recently detected in Uruguay. These P.1 and P.2 genomes share all substitutions that are characteristic of these variants.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel member of the genus *Betacoronavirus* (family *Coronaviridae*) and the causative agent of the ongoing coronavirus disease 2019 (COVID-19) pandemic (1). The analysis of the SARS-CoV-2 RNA genome has been crucial for understanding its origin and spread and for controlling the virus. The high evolutionary rate of SARS-CoV-2, usual for an RNA virus, has led to a number of mutations that appear to impact virus fitness (2). Genetic variants of SARS-CoV-2 can be classified into variants of concern (VOCs) and variants of interest (VOIs) (3). VOCs have been associated with an increased transmissibility and reduction in neutralization by natural or vaccine-derived antibodies and may cause more severe symptoms. VOCs share the N501Y change in the receptor binding domain (amino acids 319 to 541) of the spike glycoprotein (S) that provides a higher affinity toward the ACE2 host receptor (4, 5). These VOCs or lineages were originally detected in the United Kingdom (B.1.1.7), South Africa (B.1.351), and Brazil (B.1.1.28.1 or P.1). The VOIs have specific genetic markers (i.e., E484K) that might affect infectivity and immune response (6), and they comprise the B.1.536 and B.1.525 variants from New York and B.1.1.28.2 (P.2) from Brazil.

The research described in this study was performed in adherence to the Declaration of Helsinki; no specific authorization was required, because the activities were conducted as part of a routine virological surveillance (anonymously, without identification of patients) by the Uruguayan official Institution for Surveillance of Influenza and Other Respiratory Viruses of the Ministry of Public Health (DLSP-MSP).

The variants P.1 and P.2, which emerged in Brazil, have spread to other parts of South America (7, 8). Here, we describe the genomes of SARS-CoV-2 variants P.1 (SARS-CoV-2/human/URY/374/2021) and P.2 (SARS-CoV-2/human/URY/380/2021) detected in Uruguay.

Nasopharyngeal swab samples were collected in March 2021 in the Uruguayan Rivera department bordering Brazil and came from two symptomatic cases. The samples tested positive for SARS-CoV-2 using a standard quantitative PCR (qPCR) procedure (9); both patients had a threshold cycle (C_T) value of <18. RNA was extracted with a QIAmp viral minikit (Qiagen, USA). Genome amplification was achieved using ARTIC 3 primers (https://artic.network/ncov-2019). First, cDNA strand analysis, Nextera DNA

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TABLE 1 Genetic changes of variants P.1 and P.2 from Uruguay compared to the Wuhan-Hu-1 SARS-CoV-2 reference sequence (GenBank accession number NC_045512)

Variant name	Genome region (position)	Codon change	Amino acid change (position)
P.1	ORF1ab-nsp3 (3827–3829)	TCA→TTA	S→L (370)
P.1	ORF1ab-nsp3 5648–5650	AAA→CAA	K→Q (977)
P.2	ORF1ab-nsp5 (10667–10669)	TTA→GTA	L→V (205)
P.1	ORF1ab-nsp6 (11288–11296)	Del (TCTGGTTTT)	SGF (106–108)
P.2	ORF1ab-nsp7 (12053–12055)	CTT→TTT	L→F (71)
P.1/P.2	ORF1ab-nsp-12 (14407–14409)	CCT→CTT	P→L (323)
P.1	ORF1ab-nsp-13 (17257–17259)	GAG→GAT	E→D (341)
P.1	S (21614–21617)	CTT→TTT	L→F (18)[a]
P.1	S (21620–21622)	ACC→AAC	T→N (20)[a]
P.1	S (21638–21640)	CCT→TCT	P→S (26)[a]
P.1	S (21974–21976)	GAT→TAT	D→Y (138)[a]
P.1	S (22130–22132)	AGG→AGT	R→S (190)[a]
P.1	S (22811–22813)	AAG→ACG	K→T (417)[a]
P.1/P.2	S (23012–23014)	GAA→AAA	E→K (484)[a]
P.1	S (23063–23065)	AAT→TAT	N→Y (501)[a]
P.1/P.2	S (23402–23404)	GAT→GGT	D→G (614)
P.1	S (23525–23527)	CAT→TAT	H→Y (655)[a]
P.1	S (24641–24643)	ACT→ATT	T→I (1027)[a]
P.1/P.2	S (25088–25090)	GTT→TTT	V→F (1176)
P.2	S (25247–25249)	ATG→ATT	M→I (1229)
P.1	ORF3a (26149–26151)	TCC→CCC	S→P (253)
P.2	M (26589–26561)	GTA→TTA	V→L (23)
P.1	ORF8 (28167–28169)	GAA→AAA	E→K (92)
P.1	Intergenic region (28263–28266)	Ins (AACA)	
P.1	N (28515–28517)	CCA→CGA	P→R (80)
P.2	N (28632–28634)	GCT→TCT	A→S (119)
P.1/P.2	N (28844–28846)	AGG→AAA	R→K (203)
P.1/P.2	N (28887–28889)	GGA→CGA	G→R (204)
P.2	N (28997–28999)	ATG→ATT	M→I (234)

[a] P.1-specific substitutions on the spike (S) protein.

Flex library preparation, and 2 × 150-bp sequencing on an Illumina MiniSeq platform were performed following a previous report (10). Adapter/quality trimming and filtering of raw data were performed with BBDuk, and clean reads were mapped using Geneious Prime. Annotation and identification of nucleotide mutations were performed with Geneious software and with CoV-GLUE (http://cov-glue.cvr.gla.ac.uk/). Lineages refer to those assigned using the pangolin tool (https://cov-lineages.org). All tools were run with default parameters unless otherwise specified.

Sample SARS-CoV-2/human/URY/374/2021 (P.1) has a sequence length of 29,835 nucleotides (nt), 1,240,211 total reads, 3,920× mean coverage, and a 38.0% G+C content. Sample SARS-CoV-2/human/URY/380/2021 (P.2) has a sequence length of 29,858 nt, 1,007,202 total reads, 5,877× mean coverage, and a 37.9% G+C content. Their genome sequences lack the outermost nucleotides (<20 nt) of the 5′ and 3′ untranslated regions (UTRs), which are not usually sequenced with the ARTIC protocol.

The VOC P.1 genome is 99.89% identical to the Wuhan-Hu-1 reference genome but has several amino acid substitutions (Table 1). The S protein has 12 replacements, including 10 variant-specific substitutions (7). It also has a codon-aligned deletion (106 to 108) in *nsp6* that is considered a P.1 genetic signature (7). The VOI P.2 genome also has 99.89% identity to the reference genome but has only 4 replacements in the S protein, including the independently acquired E484K marker, and lacks indels (Table 1).

A significant increase in the numbers of cases and deaths has been occurring in Uruguay since March 2021, coinciding with the appearance and increase of P.1 and P.2 variants in the territory. The identification of variants with potentially new biological

properties encourages the efforts of doing genomic surveillance to contribute to controlling the pandemic.

Data availability. These genome sequences were deposited in GenBank under accession numbers MW988204 (P.1, SARS-CoV-2/human/URY/374/2021) and MW988205 (P.2, SARS-CoV-2/human/URY/380/2021). The raw reads and metadata were deposited under the BioProject accession number PRJNA634396 and SRA accession numbers SRX10652818 (SARS-CoV-2/human/URY/374/2021) and SRX10652819 (SARS-CoV-2/human/URY/380/2021).

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We declare no conflict of interest.

All authors revised and approved the manuscript. Y.P., R.P., and H.C. conceived the study. L.C., C.T., S.G., E.F., A.M., and G.T. did the next-generation sequencing (NGS). Y.P. and R.P. analyzed the data. N.G., V.R., M.R.F., N.M., M.N.C., H.C., A.D., N.R., and S.F. carried out the diagnostic and Sanger typification. C.M. is head of the DLSP. J.A. and R.P. got the financial support. R.P. and Y.P. wrote the manuscript.

REFERENCES

1. Zhou P, Yang X-L, Wang X-G, Hu B, Zhang L, Zhang W, Si H-R, Zhu Y, Li B, Huang C-L, Chen H-D, Chen J, Luo Y, Guo H, Jiang R-D, Liu M-Q, Chen Y, Shen X-R, Wang X, Zheng X-S, Zhao K, Chen Q-J, Deng F, Liu L-L, Yan B, Zhan F-X, Wang Y-Y, Xiao G-F, Shi Z-L. 2020. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 579:270–273. https://doi.org/10.1038/s41586-020-2012-7.
2. Volz EM, Hill V, McCrone JT, Price A, Jorgensen D, O'Toole A, Southgate JA, Johnson R, Jackson B, Nascimento FF, Rey SM, Nicholls SM, Colquhoun RM, da Silva Filipe A, Shepherd JG, Pascall DJ, Shah R, Jesudason N, Li K, Jarrett R, Pacchiarini N, Bull M, Geidelberg L, Siveroni I, Goodfellow IG, Loman NJ, Pybus O, Robertson DL, Thomson EC, Rambaut A, Connor TR. 2021. Evaluating the effects of SARS-CoV-2 spike mutation D614G on transmissibility and pathogenicity. Cell 184:64–75.e11. https://doi.org/10.1016/j.cell.2020.11.020.
3. CDC. 2021. SARS-CoV-2 variant classifications and definitions. CDC, Atlanta, GA. https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/variant-surveillance/variant-info.html#Concern.
4. Gaebler C, Wang Z, Lorenzi JCC, Muecksch F, Finkin S, Tokuyama M, Cho A, Jankovic M, Schaefer-Babajew D, Oliveira TY, Cipolla M, Viant C, Barnes CO, Bram Y, Breton G, Hägglöf T, Mendoza P, Hurley A, Turroja M, Gordon K, Millard KG, Ramos V, Schmidt F, Weisblum Y, Jha D, Tankelevich M, Martinez-Delgado G, Yee J, Patel R, Dizon J, Unson-O'Brien C, Shimeliovich I, Robbiani DF, Zhao Z, Gazumyan A, Schwartz RE, Hatziioannou T, Bjorkman PJ, Mehandru S, Bieniasz PD, Caskey M, Nussenzweig MC. 2021. Evolution of antibody immunity to SARS-CoV-2. Nature 591:639–644. https://doi.org/10.1038/s41586-021-03207-w.
5. Guruprasad L. 2021. Human SARS CoV-2 spike protein mutations. Proteins 89:569–576. https://doi.org/10.1002/prot.26042.
6. Nonaka CKV, Franco MM, Gräf T, de Lorenzo Barcia CA, de Ávila Mendonça RN, de Sousa KAF, Neiva LMC, Fosenca V, Mendes AVA, de Aguiar RS, Giovanetti M, de Freitas Souza BS. 2021. Genomic evidence of SARS-CoV-2 reinfection involving E484K spike mutation, Brazil. Emerg Infect Dis 27:1522–1524. https://doi.org/10.3201/eid2705.210191.
7. Naveca F, Nascimento V, Souza V, Corado A, Nascimento F, Silva G, Costa A, Duarte D, Pessoa K, Mejía M, Brandão M, Jesus M, Gonçalves L, da Costa C, Sampaio V, Barros D, Silva M, Mattos T, Pontes G, Abdalla L, Santos J, Arantes I, Dezordi F, Siqueira M, Wallau G, Resende P, Delatorre E, Gräff T, Bello G. 2021. COVID-19 epidemic in the Brazilian state of Amazonas was driven by long-term persistence of endemic SARS-CoV-2 lineages and the recent emergence of the new variant of concern P.1. Res Sq https://doi.org/10.21203/rs.3.rs-275494/v1.
8. Faria NR, Claro IM, Candido D, Franco LAM, Andrade PS, Coletti TM, Silva CAM, Sales FC, Manuli ER, Aguiar RS, Gaburo N, Camilo CDC, Fraiji NA, Crispim MAE, Carvalho MDPSS, Rambaut A, Loman N, Pybus OG, Sabino EC. 2021. Genomic characterisation of an emergent SARS-CoV-2 lineage in Manaus: preliminary findings. https://virological.org/t/genomic-characterisation-of-an-emergent-sars-cov-2-lineage-in-manaus-preliminary-findings/586.
9. Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DKW, Bleicker T, Brünink S, Schneider J, Schmidt ML, Mulders DGJC, Haagmans BL, Van Der Veer B, Van Den Brink S, Wijsman L, Goderski G, Romette JL, Ellis J, Zambon M, Peiris M, Goossens H, Reusken C, Koopmans MPG, Drosten C. 2020. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Eurosurveillance 25:2000045. https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045.
10. Panzera Y, Ramos N, Frabasile S, Calleros L, Marandino A, Tomás G, Techera C, Grecco S, Fuques E, Goñi N, Ramas V, Coppola L, Chiparelli H, Sorhouet C, Mogdasy C, Arbiza J, Delfraro A, Perez R. 2021. A deletion in SARS-CoV-2 ORF7 identified in COVID-19 outbreak in Uruguay. Transbound Emerg Dis https://doi.org/10.1111/tbed.14002.