



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



Case Report

SARS-CoV-2 reinfection with a virus harboring mutation in the Spike and the Nucleocapsid proteins in Panama



Yamilka Díaz^a, Anyuri Ortiz^b, Adriana Weeden^c, Daniel Castillo^a, Claudia González^d, Brechla Moreno^a, Mabel Martínez-Montero^e, Marlene Castillo^a, Grettel Vasquez^a, Lisbeth Sáenz^a, Danilo Franco^a, Yaneth Pitti^a, Oris Chavarria^d, Jessica Gondola^d, Ambar M. Moreno^d, Leyda Ábrego^a, Davis Beltrán^a, Ilka Guerra^a, Jim Chang^a, Zumara Chaverra^a, Isela Guerrero^a, Alejandra Valoy^a, Melissa Gaitán^a, Dimelza Araúz^a, Ernesto Morán^a, Maria Chen-Germán^a, Elimelec Valdespino^a, Rita Rodríguez^a, Rita Corrales^a, Roderick Chen-Camaño^a, Juan Miguel Pascale^c, Alexander A. Martínez^{d,1,**}, Sandra López-Vergès^{a,1,*}, on behalf of the Gorgas COVID19 Team

^a Department of Research in Virology and Biotechnology, Gorgas Memorial Institute of Health Studies, Panama

^b Department of Occupational Health, Gorgas Memorial Institute of Health Studies, Panama

^c Unit of Diagnosis, Clinical Research and Tropical Medicine, Gorgas Memorial Institute of Health Studies, Panama

^d Department of Genomic and Proteomic, Gorgas Memorial Institute of Health Studies, Panama

^e Immunovirology Division, Public Health Reference Central Laboratory, Gorgas Memorial Institute of Health Studies, Panama

ARTICLE INFO

Article history:

Received 13 March 2021

Received in revised form 2 June 2021

Accepted 3 June 2021

Keywords:

SARS-CoV-2

COVID-19

Panama

Reinfection

Mutation

ABSTRACT

We report a case of reinfection by SARS-CoV-2 with the second virus harboring amino acid changes in the Spike protein (141-143del, D215A, ins215AGY, L452R, D614G), orf1a, helicase, orf3a, and Nucleocapsid. The virus associated with the reinfection, from an endemic lineage containing the S:L452R immune escape mutation, was circulating in Panama at the time.

© 2021 The Authors. Published by Elsevier Ltd on behalf of International Society for Infectious Diseases. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction

In March 2020, the World Health Organization declared the COVID-19 pandemic. As it is evolving, more cases of reinfection by SARS-CoV-2 are being reported (Gupta et al., 2020; To et al., 2020), even if it is considered a rare event. New variants with the potential to spread more efficiently have been described (Naveca et al., 2019; Tegally et al., 2021). Some mutations in the Spike (S) protein could

increase viral infectivity (Korber et al., 2020; Tegally et al., 2021; Volz et al., 2021) or decrease sensitivity to neutralizing antibodies (Hoffmann et al., 2020; Li et al., 2020a,b; Naveca et al., 2019; Resende et al., 2021; Tchesnokova et al., 2021) and have been associated with secondary SARS-CoV-2 infections. Here, we report a case of reinfection with SARS-CoV-2 in Panama, confirmed by complete genome sequencing, in which the second virus contained new and previously described mutations implicated in immune escape and higher transmission.

Methodology

SARS-CoV-2 diagnosis

Viral RNA was extracted from the first nasopharyngeal swab sample using Qiagen viral RNA extraction kit and from the second using Chemagic360 kit. SARS-CoV-2 was detected using the RT-qPCR Powercheck kits version 1.0 and version 2.0 for the first and

* Corresponding author at: Department of Research in Virology and Biotechnology, Gorgas Memorial Institute of Health Studies, Justo Arosemena Avenue and 35th Street, 0816-02593, Panama.

** Corresponding author at: Department of Genomic and Proteomic, Gorgas Memorial Institute of Health Studies, Justo Arosemena Avenue and 35th Street, 0816-02593, Panama.

E-mail addresses: almartinez@gorgas.gob.pa (A.A. Martínez),

slopez@gorgas.gob.pa (S. López-Vergès).

¹ These senior authors contributed equally to this article.

second samples, respectively. The cut-off value for both was cycle threshold (Ct) < 35.

Serology

Antibodies evaluation was performed using Virclia IgM and IgG monostest. The cut-off index for positive samples was calculated as >0.6 for immunoglobulin(Ig)M and >1.6 for IgG.

Viral isolation

Samples were used for inoculation on Vero cells (ATCC CCL81). Once cytopathic effect was observed in 75% of cells, virus isolation was confirmed by reverse transcription-quantitative polymerase chain reaction (RT-qPCR).

Genome characterization and phylogenetic analysis

SARS-CoV-2 genome sequencing was performed using ARTIC network protocol. New mutations were confirmed at depth of 250× and allele frequency higher than 15%. Lineage classification was performed using pangolEARN version 2021-01-30. A maximum-likelihood tree was drawn using a GTR substitution model (Supplementary methods).

3. D protein modeling

RNAs were translated into proteins using MEGA-7 software for 3D modeling (Supplementary methods).

Results

Case description

On June 8, 2020, a 36-year-old man with no reported comorbidities exhibited chest pain since the day before,

myalgia, fever, cephalaea, and rhinorrhea (Figure 1A). SARS-CoV-2 RNA (GMI-PA376271) was detected by RT-PCR with a Ct = 19 for RdRp gene. The patient lives with 4 persons; one of them had COVID-19 symptoms and was RT-qPCR positive at the same time. During the 14 days of home quarantine isolation, the patient developed hyposmia and ageusia. On December 5, 2020, 181 days after the first symptoms onset, he developed cephalaea, myalgia and rhinorrhea. SARS-CoV-2 (GMI-PA584303) was detected with a Ct = 27 for gene E and a Ct = 28 for RdRp. On both infections, the patient had total recovery with no sequelae. SARS-CoV-2 (GMI-PA584303) was isolated and induced a cytopathic effect on cells. Serology tests between the 2 infections were not done. Forty-four days after the second infection, serology tests IgM and IgG positive index results were 0.695 and 11.04, respectively.

The patient signed the Gorgas informed consent for clinical cases, approved by the ICGES IRB (N.1079/CNBI/ICGES/17).

SARS-CoV-2 genome characterization

Whole-genome sequencing showed that both viruses belong to 2 endemic lineages (Figure 1B, Supplementary Figure S1): GMI-PA376271 (EPI_ISL_1001457) being A.2.4, previously named A.2.1. (Franco et al., 2020). The second genome GMI-PA584303 accumulated 33 nucleotide mutations compared with reference MN908947.3 and 23 (21 nucleotide substitutions, 1 deletion, 1 insertion) compared to the first sample GMI-PA376271 (Figure 1C, Supplementary Table S1). Of these nucleotide substitutions, 15 induced amino acid changes: 3 in the S protein (D215A, L452R, D614G), 5 in ORF1a (1 in NSP1, 2 in NSP3, 1 in NSP4, 1 in NSP6), 4 in the Nucleocapsid (N), 2 in helicase and 1 in ORF3a. Additionally, we detected a nucleotide deletion (21984-21993del) inducing a loss of 3 amino acids (L141del, G142del, V143del) and a nucleotide insertion (22005-22013 in.) associated with the insertion of 3 amino acids (A215, G216, Y217), balancing the reading frame of the translated S protein.

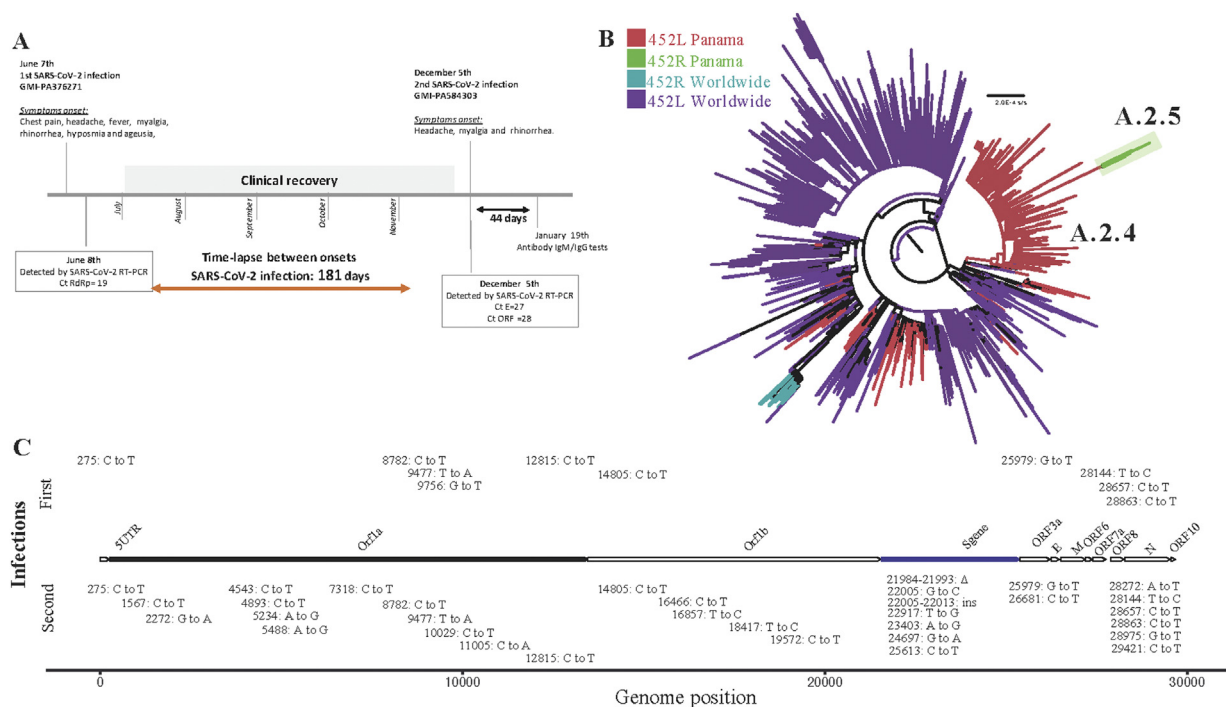


Figure 1. Timeline of SARS-CoV-2 reinfection case and genomic findings. (A) Time-lapse of SARS-CoV-2 reinfection case with the description of symptoms and laboratory findings. (B) Phylogenetic tree of SARS-CoV-2 complete genome from the viruses related to the first and second infections. (C) Mutations identified in the SARS-CoV-2 viruses from the first and second infections compared with the sequence of reference MN908947.3.

Mutations in the Spike and Nucleotide proteins

The amino acid mutations identified in the S protein are located in the subunit S1 (Supplementary Table S1, Supplementary Figure S2A, [Li et al., 2020b](#); [Vilar and Isom, 2021](#)), responsible for receptor binding. The deletion of amino acid LGV (Leucine, Glycine, Valine) at positions 141–143 and the insertion of AGY (Alanine, Glycine, Tyrosine) inducing the substitution D215A are in the N-terminal domain (NTD). In contrast, the L452R mutation is located in the region binding domain (RBD) for ACE2 receptor, D614G being between the RBD and the amino acid bridge between the S1 and S2 subunits.

The mutations S197L, M234I are located in the link region between the RNA-binding domain and the dimerization domain, whereas the P383L is in the C-terminal domain of the N protein (Supplementary Table S1, Supplementary Figure S2B, [Cubuk et al., 2021](#)).

Discussion

We describe the first confirmed SARS-CoV-2 reinfection in Panama in an immunocompetent patient who presented mild symptomatic disease during both infections. Reinfections of seasonal coronaviruses have been described in settings where a high rate of community infection and/or incomplete immunologic protection took place ([Edridge et al., 2020](#)). Previous SARS-CoV-2 reinfection reported cases showed that an induced neutralizing antibody response generated by the first infection is not sufficient for protection ([Tohidinia and Sefid, 2020](#)). Several variables could be implicated in reinfection including the absence of a proper immune response, low amount of neutralizing antibodies or viruses with mechanisms to evade the immune response. A caveat of this clinical case is that there is no serological sample between both infections to determine if the patient developed neutralizing antibodies after the first exposure. However, the mild symptoms after the reinfection could indicate some partial protection, and the genetic characterization of the viruses showed that the second virus harbored some mutations already described as implicated in escape from neutralizing antibodies. The fact that the second virus was isolated could suggest no or low neutralizing antibodies in the sample that could have inhibited the infection of cells. The specific reasons for this reinfection remain an open question.

SARS-CoV-2 genome analysis showed that both infections were induced by viruses from Panamanian endemic lineages, A.2.4 ([Franco et al., 2020](#)) for the first, and A.2.5, containing Spike mutations D614G and L452R, for the second. Most of the 15 induced amino acid changes in the second virus, as with those in ORF1a, have not been previously described. Most mutations detected in S and N, whose coding genes have the highest degree of variability in the SARS-CoV-2 genome ([Vilar and Isom, 2021](#)), have been previously associated with features that could facilitate reinfection. D614G mutation in S, with a higher affinity for ACE2 receptor, results in increased infectivity and higher virus transmission ([Korber et al., 2020](#); [Li et al., 2020b](#)); probably explaining why this mutation, detected in most dominant variants, has overtaken the entire globe ([Vilar and Isom, 2021](#)). Spike mutation L452R, decreasing sensitivity to neutralizing antibodies, has been associated with immune escape and reinfection and is present in the variant of interest (VOI) B.1.526.1, and the variants of concern (VOC) B.1.427 and B.1.429 ([Hoffmann et al., 2020](#); [Li et al., 2020a,b](#); [Naveca et al., 2019](#); [Resende et al., 2021](#); [Tchesnokova et al., 2021](#)). Currently, lineage A.2.5 is the only A lineage with this mutation which is widely spread in B lineages. The observed 3 amino acid deletion (L141del, G142del, V143del) in S was located in an epitope involved in the generation of neutralizing antibodies ([Tohidinia and Sefid, 2020](#)) and Y144del detected in the VOC B.1.1.7. D215A in

the NTD of Spike has not been described; however, D215G has been detected in the VOC B.1.351. As NTD has a role in interaction with the receptor, future studies are needed to analyze the role of these Spike mutations. In the N protein, residues S194 and M234 are some of the most mutated ([Vilar and Isom, 2021](#)), suggesting a purifying selection, and mutation P365S was associated with B cell epitopes ([Tohidinia and Sefid, 2020](#)), suggesting that it could also let to an immune escape phenotype. These changes may reflect the ability of the second virus to escape neutralizing antibodies that could have been produced during the previous infection.

Future studies associated with the genomic surveillance of the virus should be done to determine the prevalence of the mutations described, their role in SARS-CoV-2 transmission and immune escape, their contribution to the reinfection cases in the country and whether they should be considered VOI.

Authors' contribution

Study design: YD, JMP, AAM, SL-V; Data Collection: YD, AO, AW, DC, CG, BM, MM-M, MC, GV, LL, DF, YP, OC, JG, AM, LA, DB, IG, JC, ZC, IG, AV, MG, DA, EM, MC-G, EV, RR, RC, RC-C, AAM, SL-V, Gorgas COVID19 Team; Patient follow-up: AO, AW; Data analysis: YD, AAM, SL-V; Writing: YD, AO, AW, JMP, AAM, SL-V

Ethical approval

The patient signed the Gorgas informed consent for clinical cases, approved by the ICGES IRB (N.1079/CNBI/ICGES/17).

Funding

This work was supported by the COVID-19 Panama's response funds, grant [SINIP:0199.001] of the Ministry of Economy (YD), the Sistema Nacional de Investigación SNI grants 21-2020 (S.L-V.) and 191-2017 (A.A.M.), as well as funds from Sus Buenos Vecinos Foundation.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

S.L-V., A.A.M., J.M.P., L.E.A., A.M.M., are members of Sistema Nacional de Investigación (SNI) from SENACYT, Panama. We gratefully acknowledge the authors, originating and submitting laboratories of the sequences from GISAID's EpiFlu Database on which this research is based (Supplementary Table S2).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.ijid.2021.06.004>.

References

- Cubuk J, Alston JJ, Incicco JJ, Singh S, Stuchell-Brereton MD, Ward MD, et al. The SARS-CoV-2 nucleocapsid protein is dynamic, disordered, and phase separates with RNA. *Nat Commun* 2021;12:1–17. doi:<http://dx.doi.org/10.1038/s41467-021-21953-3>.
- Edridge AWD, Kaczorowska J, Hoste ACR, Bakker M, Klein M, Loens K, et al. Seasonal coronavirus protective immunity is short-lasting. *Nat Med* 2020;26:1691–3. doi:<http://dx.doi.org/10.1038/s41591-020-1083-1>.
- Franco D, Gonzalez C, Abrego LE, Carrera JP, Diaz Y, Caisedo Y, et al. Early transmission dynamics, spread, and genomic characterization of SARS-CoV-2 in Panama. *Emerg Infect Dis* 2020;27;. doi:<http://dx.doi.org/10.1101/2020.07.31.20160929>.
- Gupta V, Bhoyar RC, Jain A, Srivastava S, Upadhyay R, Imran M, et al. Asymptomatic reinfection in 2 healthcare workers from India with genetically distinct severe

- acute respiratory syndrome coronavirus 2. *Clin Infect Dis* 2020;ciaa1451, doi: <http://dx.doi.org/10.1093/cid/ciaa1451>.
- Hoffmann CA, Sarturi JO, Weindorf DC, Darren D, Ramirez HAR, Jackson S, et al. Confirmed reinfection with SARS-CoV-2 variant VOC-202012/01. *Clin Infect Dis* 2020;1–27, doi: <http://dx.doi.org/10.1093/cid/ciab014/6076528>.
- Korber B, Fisher WM, Gnanakaran S, Yoon H, Theiler J, Abfalterer W, et al. Tracking Changes in SARS-CoV-2 Spike: Evidence that D614G Increases Infectivity of the COVID-19 Virus. *Cell* 2020;182(4):812–27, doi: <http://dx.doi.org/10.1016/j.cell.2020.06.043>.
- Li J, Liao C, Wang Q, Tan Y, Luo R, Qiu Y, et al. The ORF6, ORF8 and nucleocapsid proteins of SARS-CoV-2 inhibit type I interferon signaling pathway. *Virus Res* 2020a;286:198074, doi: <http://dx.doi.org/10.1016/j.virusres.2020.198074>.
- Li Q, Wu J, Nie J, Li Zhang, Hao H, Liu S, et al. The impact of mutations in SARS-CoV-2 spike on viral infectivity and antigenicity. *Cell* 2020b;182:1284–1294.e9, doi: <http://dx.doi.org/10.1016/j.cell.2020.07.012>.
- Naveca F, Costa C, Nascimento V, Souza V, Nascimento F, Costa Á, et al. SARS-CoV-2 reinfection by the new variant of concern (VOC) P.1 in Amazonas. [138_TD\$DIFF] virological.org; 2019.
- Resende PC, Bezerra JF, Teixeira de Vasconcelos RH, Arantes I, Appolinario L, Mendonça AC, et al. Spike E484K mutation in the first SARS-CoV-2 reinfection case confirmed in Brazil. *Oxford J* 2021;33:.
- Tchesnokova V, Kulakesara H, Larson L, Bowers V, Rechkina E, Kisiela D, et al. Acquisition of the L452R mutation in the ACE2-binding interface of Spike protein triggers recent massive expansion of SARS-Cov-2 variants. *bioRxiv* 2021;., doi: <http://dx.doi.org/10.1101/2021.02.22.432189>.
- Tegally H, Wilkinson E, Lessells RJ, Giandhari J, Pillay S, Msomi N, et al. Sixteen novel lineages of SARS-CoV-2 in South Africa. *Nat Med* 2021;27(3):440–6, doi: <http://dx.doi.org/10.1038/s41591-021-01255-3>.
- To KK-W, Hung IF-N, Ip JD, Chu AW-H, Chan W-M-M, Tam AR, et al. Coronavirus disease 2019 (COVID-19) re-infection by a phylogenetically distinct severe acute respiratory syndrome coronavirus 2 strain confirmed by whole genome sequencing. *Clin Infect Dis* 2020;ciaa1275, doi: <http://dx.doi.org/10.1093/cid/ciaa1275>.
- Tohidinia M, Sefid F. Identification B and T-Cell epitopes and functional exposed amino acids of S protein as a potential vaccine candidate against SARS-CoV-2/ COVID-19. *Microb Pathogen* 2020;148:104459, doi: <http://dx.doi.org/10.1016/j.micpath.2020.104459>.
- Vilar S, Isom DG. One year of SARS-CoV-2: how much has the virus changed?. *Biology (Basel)* [144_TD\$DIFF]2021;10(2):91, doi: <http://dx.doi.org/10.3390/biology10020091>.
- Volz E, Mishra S, Chand M, Barrett JC, Johnson R, Geindenberg L, et al. Assessing transmissibility of SARS-CoV-2 lineage B.1.1.7 in England. *Nature* 2021;593(7858):266–9, doi: <http://dx.doi.org/10.1038/s41586-021-03470-x>.