

# A suspected case of SARS-CoV-2 persistence with reactivation

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## Article

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# Abstract

Reinfections with SARS-CoV-2 have been verified by the presence of phylogenetically distant viruses in each episode. Here, we report a suspected case of SARS-CoV-2 persistence with reactivation in a 35-years old patient presenting positive RT-PCR on April 7th and August 7th, each episode characterized by mild and severe symptoms, respectively. Sequencing of viral genomes identified only two SNPs indicating the presence of genetically linked viruses for the first time. Subsequently to hospital discharge, the patient presented a positive diagnosis for SARS-CoV-2 in stool, urine and semen samples.

## Main Text

The novel human coronavirus SARS-CoV-2 emerged in Wuhan, province of Hubei in China in December 2019, and since it has infected more than 37 million people worldwide and 1.079.029 deaths (as of October 13th, 2020)<sup>1</sup>. In the absence of vaccines or any specific antiviral treatment, people depend on a protective immune response to fight against SARS-CoV-2 infection and the effects of COVID-19<sup>2,3</sup>. In addition to the new outbreaks occurring in places with previously controlled infections, cases of reinfections have also been reported in patients from Hong Kong<sup>4</sup>, Belgium and Ecuador<sup>5</sup> as well as in health workers from Nevada and India and are expected to accumulate during the following months<sup>4,5,6,7,8</sup>. A hallmark of these cases is phylogenetically distant viruses in samples from both events, which indicate that a new infection and no persistence of the original virus occurred. Here, we report a suspected case of SARS-CoV-2 persistence with reactivation in a healthcare worker at the Teodoro Maldonado Carbo Hospital in Guayaquil, Ecuador.

The patient corresponds to a 35-years old medical doctor diagnosed with SARS-CoV-2 confirmed by RT-PCR on April 7th (Fig. 1 and Table 1). This first episode was characterized by fever (37.7 °C – 37.6 °C), sneezing, nasal obstruction and dry cough. Lung CT scan was normal. No evidence of anti-SARS-CoV-2 IgG/IgM was detected on April 15th and April 22nd by lateral flow test (Fig. 1 and Table 1). Moreover, RT-PCR from a nasopharyngeal swab sample performed on April 17th was negative. Given the mild symptoms and the negative RT-PCR, the patient resumed his activities at the hospital ten days following diagnosis.

On August 4th (i.e., 119 days after the first episode and being asymptomatic during that period of time), the patient presented general malaise, weakness and fever (38.5 °C), which reached 39 °C on August 5th. A Lung CT scan showed diffuse ground glass lung patterns in basal and middle basal fields suggestive of COVID-19. The patient received treatment with azithromycin 500 mg QD, acetylsalicylic acid 100 mg QD, nitazoxanide 500 mg QD, paracetamol 1 gr PRN and Enoxaparin 60 mg SC. On August 6th and 7th, the patient presented arthralgia, myalgia and fever. Confirmatory RT-PCR was performed on August 7th (Fig. 1 and Table 1). On August 8th, the patient presented dry cough and dyspnea on great exertion and on August 9th, the fever raised to 39.9 °C, not responding to intramuscular or intravenous antipyretics. The patient was admitted to the hospital where an oxygen cannula was placed. A Lung CT scan carried out on August 10th showed pulmonary consolidations and atelectasis bands. Treatment with

corticosteroid and broad-spectrum antibiotics (Imipenem 500 mg) were started, and the patient was changed to an oxygen face mask. On August 11th, the patient was transferred to Hospital Luis Vernaza in Guayaquil with severe respiratory failure (Fig. 2A). High flow oxygen cannula was required to maintain proper oxygenation. Antibiotic treatment with meropenem and vancomycin was started. Furthermore, a single-dose of Tocilizumab was prescribed due to high levels of circulating IL-6, ferritin and C- Reactive protein, inflammation biomarkers that continued high until September (Table 1). At 48 hours after admission, blood cultures revealed the presence of *S. aureus*. The patient was transfused with convalescent plasma. Worsening of the respiratory condition led to orotracheal intubation for assisted ventilation on August 13th, at this point clinical evidence of status epilepticus was present with tonic clinic convulsive manifestations. A cerebral magnetic resonance imaging (MRI) was performed and results show evidence of limbic encephalitis (Fig. 2B), which is related to encephalitis neuroimaging reported recently in COVID-19 patients<sup>9,10</sup>. The patient evolved favorably being discharged from the hospital on August 22nd. RT-PCR and IgG/IgM were positive on September 4th. Finally, the patient presented a negative RT-PCR on October 5th (Fig. 1).

Since these mild and severe COVID-19 episodes occurred in the same patient with a 4-months delay where suggestive of reinfection, we performed SARS-CoV-2 whole-genome sequencing using the Oxford Nanopore ARTIC Network protocol for the two different infection episodes (samples 032 and 4477, respectively were stored at -80 °C). Strikingly, we observed that the viral sequence obtained from the second episode was highly similar to the sample obtained on April 7th, only presenting two SNPs (Fig. 3A). As such, phylogenetic analysis of the viral sequences revealed that both viruses are genetically linked being classified together into the B.1.1 lineage, clade GR suggesting the persistence of the original variant (Fig. 3B). A SARS-CoV-2 whole-genome sequence obtained from a health worker colleague also diagnosed on April 7th was near identical in sequence to the suspected case of persistence, while other SARS-CoV-2 sequence obtained from a second colleague partner diagnosed the same day was assigned to clade G, lineage B.1 (Fig. 3B). We also looked at the presence of viral RNA in nasopharyngeal swabs, saliva, urine, stool, blood and semen samples of the suspected case detecting viral genetic material in urine, stool and semen, suggesting viral persistence in this patient (Table 1).

Immunity against SARS-CoV-2 in the population is a major concern for the management of the COVID-19 pandemic. A recent study showed that the presence of antibodies against seasonal human coronaviruses is rather short-lasting<sup>11</sup>. Also, several studies performed in Europe have shown that the presence of anti-SARS-CoV-2 antibodies ranged between 0 and 42.2% in the general population<sup>12</sup>. On the other hand, studies have shown that SARS-CoV-2 can elicit specific CD4 + and CD8 + T-cells responses in individuals exposed to the virus<sup>13,14,15</sup>. Interestingly, these studies showed an important presence of T-cell responses in unexposed and seronegative individuals attributed to cross-reactivity with seasonal coronaviruses. One of these reports also showed the presence of long-lasting CD4 + and CD8 + T-cells from individuals exposed to SARS-CoV in 2003, suggesting that T-cell immunity against SARS-CoV-2 should be long-lasting<sup>13</sup>. Under this scenario, cases of reinfections with genetically distant viruses have been reported at least in Hong Kong, Belgium, India, USA and Ecuador. While some of these new

infections were milder than the first episode, some of them resulted in a severe manifestation of the disease requiring mechanical ventilation. The case presented here represents a warning for the possibility of viral persistence and reactivation in individuals exposed to SARS-CoV-2, which should be considered as a potential risk when assessing policies related to viral resolution and transmission pathways. The results show the possible persistence in the testicles and urinary tract and faeces, suggesting the possibility of sexual transmission and transmit via the orofecal route or in contrast, SARS-CoV-2 could be shed and eliminated by the gastrointestinal tract. However, studies are lacking to verify whether it can remain viable and infectious in faecal, urine and semen samples. The possibility that SARS-CoV-2 could persist in testis and be sexually transmitted is a major concern. In this study, samples were obtained a week after the patient was discharged from the hospital while he was severely affected by his second episode of COVID-19. To date, presence of SARS-CoV-2 in seminal fluid has been investigated in men with positive nasopharyngeal swabs and who still symptoms even 2–3 days of before semen collected <sup>16,17</sup>. In other studies, semen samples diagnosed as negative for SARS-CoV-2 were obtained before the development of symptoms or after developing symptomatology <sup>18,19,20</sup>. Furthermore, patients with severe COVID-19 symptomatology were excluded from the analysis and only included patients with mild to moderate symptoms <sup>21</sup>. It has been proven that some viruses are not able to replicate in the male reproductive system, but testis correspond to an immunoprivileged site with a favorable environment for the virus to escape from the host's immune system, rendering testis a potential site for the establishment of a viral reservoir <sup>22</sup>. Indeed, testis could be a reliable potential viral reservoir site for SARS-CoV-2 due to the high expression levels of ACE2 in spermatogonia, Leydig and Sertoli cells <sup>23,24</sup>. Therefore, further studies are essential to determine the presence of SARS-CoV-2 in the semen including patients with severe COVID-19, re-infection and viral persistence.

## Materials And Methods

Sample from the suspected patient was collected and codified from April 7th (first episode), August 7th (second episode, mild and severe symptoms), September 4th, September 11th and October 05th 2020, in a certified laboratory of the Espiritu Santo University. Ethical approval of this work was given by CEISH-USFQ (Comité de ética de investigación en seres humanos-USFQ): IE-JP067-2020-CEISH-USFQ. Nasopharyngeal swabs preserved in 400 ul of 1X DNA/RNA Shield™ solution (Zymo Research, USA), saliva, urine, blood and semen samples were used for RNA extraction by Quick RNA viral kit (Zymo Research, CA, USA) according to the manufacturer's protocol in a biosafety type II chamber with HEPA filters. The RNA extracted was screened for the presence of SARS-CoV-2 by reverse transcriptase quantitative PCR (RT-qPCR) using Allplex™ SARS-CoV-2 Assay a multiplex real-time PCR assay to detect 4 target genes (E, N RdRp and S gene) for asses the SARS-CoV-2 infection (Seegene Inc, CA, USA).

RNA from the respiratory sample from the suspected patient and colleagues working in the same field, which were diagnosed simultaneously on April 7th were prepared for MinION sequencing protocol. Primer Scheme approach developed by the ARTIC Network for nCoV-2019<sup>25</sup> using the V3 primer sets to generate an amplicon tiling path across the viral genome. cDNA MinION library preparation was performed using

the Rapid 158 Barcoding kit (SQK-RBK004) following manufacturer instructions and then loaded into a MinION flow cell (FLO-MIN 106). Base calling of FAST5 files was performed using Guppy (version 3.4.5)<sup>26</sup>, and the RAMPART software (v1.0.5) from the ARTIC Network (<https://github.com/artic-network/rampart>) was used to monitor sequencing in real-time. Sequence quality scoring, demultiplexing and adapter removal was performed with the NanoPlot and Porechop algorithms respectively<sup>27</sup>. The ARTIC Network bioinformatics pipeline was used for variant calling, and the reads were 165 mapped against the reference strain Wuhan-Hu-1 (GenBank accession number MN908947), to generate consensus genomes. The genomes were uploaded to the GISAID platform and NextStrain.org to determine the epidemiological linkage of circulating SARS-CoV-2 variants (Sequence ID: Sample 032: EPI\_ISL\_527819; Sample 4477: EPI\_ISL\_527818; Sample 128: EPI\_ISL\_491933; Sample 129: EPI\_ISL\_491934).

In order to determine the clade and lineage of the sequenced genomes, they were aligned with another 100 SARS-CoV-2 genomes obtained from GISAID<sup>28</sup>. Genomes were aligned via MAFFT v7.4<sup>29</sup> and phylogenetic reconstruction was performed by maximum likelihood via IQTREE v1.6<sup>30</sup> and the results were visualized using Dendroscope<sup>31</sup>.

## Declarations

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## Table

**Table 1. Summary of Laboratory Examination by RT-PCR and serological tests, Chemiluminescence and ELISA, Inflammatory biomarkers from April to October 2020**

| RT-PCR             |                           |                                      |       |       |        |       |                       | SARS-CoV-2 RT-PCR kit |                                      |              |
|--------------------|---------------------------|--------------------------------------|-------|-------|--------|-------|-----------------------|-----------------------|--------------------------------------|--------------|
| Date of collection | Sample type               | Ct values                            |       |       |        |       | Result interpretation | Developer             | Name of the kit                      | Target Genes |
|                    |                           | E                                    | RdRp  | N     | ORF1ab | IC    |                       |                       |                                      |              |
| April              | 7th Nasopharyngea 1 Swab  | -                                    | -     | 8.8   | 8.7    | 17.2  | 2019-nCoV Detected *  | CetTest Botec SL      | VIASURE SARS-CoV-2 Real Time PCR Kit | ORF1ab & N   |
|                    | 17th Nasopharyngea 1 Swab | -                                    | -     | -     | -      | 20.2  | Negative              |                       |                                      |              |
| August             | 7th Nasopharyngea 1 Swab  | Result obtained from medical history |       |       |        |       | 2019-nCoV Detected *  | Seegene, Inc.         | Allplex 2019-nCoV assay              | E, RdRp & N  |
|                    | 14th Nasopharyngea 1 Swab | 16.13                                | 22.94 | 19.95 | -      | -     | 2019-nCoV Detected    |                       |                                      |              |
| September          | Stool                     | 29.35                                | 33.43 | 29.83 | -      | 32.4  | 2019-nCoV Detected    |                       |                                      |              |
|                    | 8th Urine                 | -                                    | 38.72 | 38    | -      | 26.74 | 2019-nCoV Detected    |                       |                                      |              |
|                    | Semen                     | 14.79                                | 22.74 | -     | -      | -     | 2019-nCoV Detected    |                       |                                      |              |
| October            | 11th Nasopharyngea 1 Swab | -                                    | -     | -     | -      | 24.34 | Negative              |                       |                                      |              |
|                    | 25th Nasopharyngea 1 Swab | -                                    | -     | 38.09 | -      | 25.29 | 2019-nCoV Detected    |                       |                                      |              |
|                    | 5th Stool                 | -                                    | -     | -     | -      | 32.8  | Negative              |                       |                                      |              |
|                    | 5th Urine                 | -                                    | -     | -     | -      | 39.48 | Negative              |                       |                                      |              |
| October            | Semen                     | -                                    | -     | -     | -      | 25.61 | Negative              |                       |                                      |              |
|                    | 8th Nasopharyngea 1 Swab  | -                                    | -     | -     | -      | 25.86 | Negative              |                       |                                      |              |
|                    | 16th Nasopharyngea 1 Swab | -                                    | -     | -     | -      | 26.05 | Negative              |                       |                                      |              |

\* Samples selected for Nanopore library preparation and sequencing

| SEROLOGICAL TESTS |                   |      |       |                 |
|-------------------|-------------------|------|-------|-----------------|
| Sample date       | Test Type         | IgM  | IgG   | Reference value |
| September         | 4th Chemiluminesc | 5.27 | 28.41 | Reactive >1     |
|                   | ELISA             |      | 1.986 | Reactive >1     |
| October           | 8th ELISA         |      | 2.092 | Reactive >1     |
|                   | 16th ELISA        |      | 2.491 | Reactive >1     |

| INFLAMMATORY BIOMARKERS |          |                    |                        |                  |
|-------------------------|----------|--------------------|------------------------|------------------|
| Sample date             | Ferritin | C Reactive Protein | Interleukin - 6 (IL-6) | Reference values |
| August                  | 13th     | 2257               | 42.18                  | 702              |
| September               | 4th      | 377.86             | 3.33                   | 2.75             |
| October                 | 16th     | 276.7              | 3.04                   | <1.5             |
|                         |          | 22 - 275           | 0 - 5                  | 0 - 6.5          |

## Figures

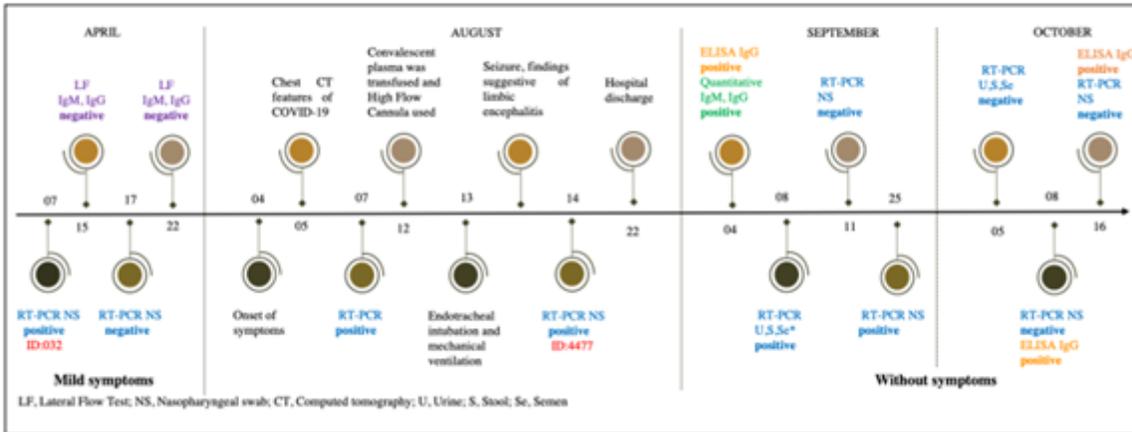


Figure 1

Timeline of the infectious process by SARS-CoV-2; clinical, serological and RT-PCR diagnostic tests performed during April and October 2020. Samples with ID:032 and 4477 were selected for Nanopore sequencing.

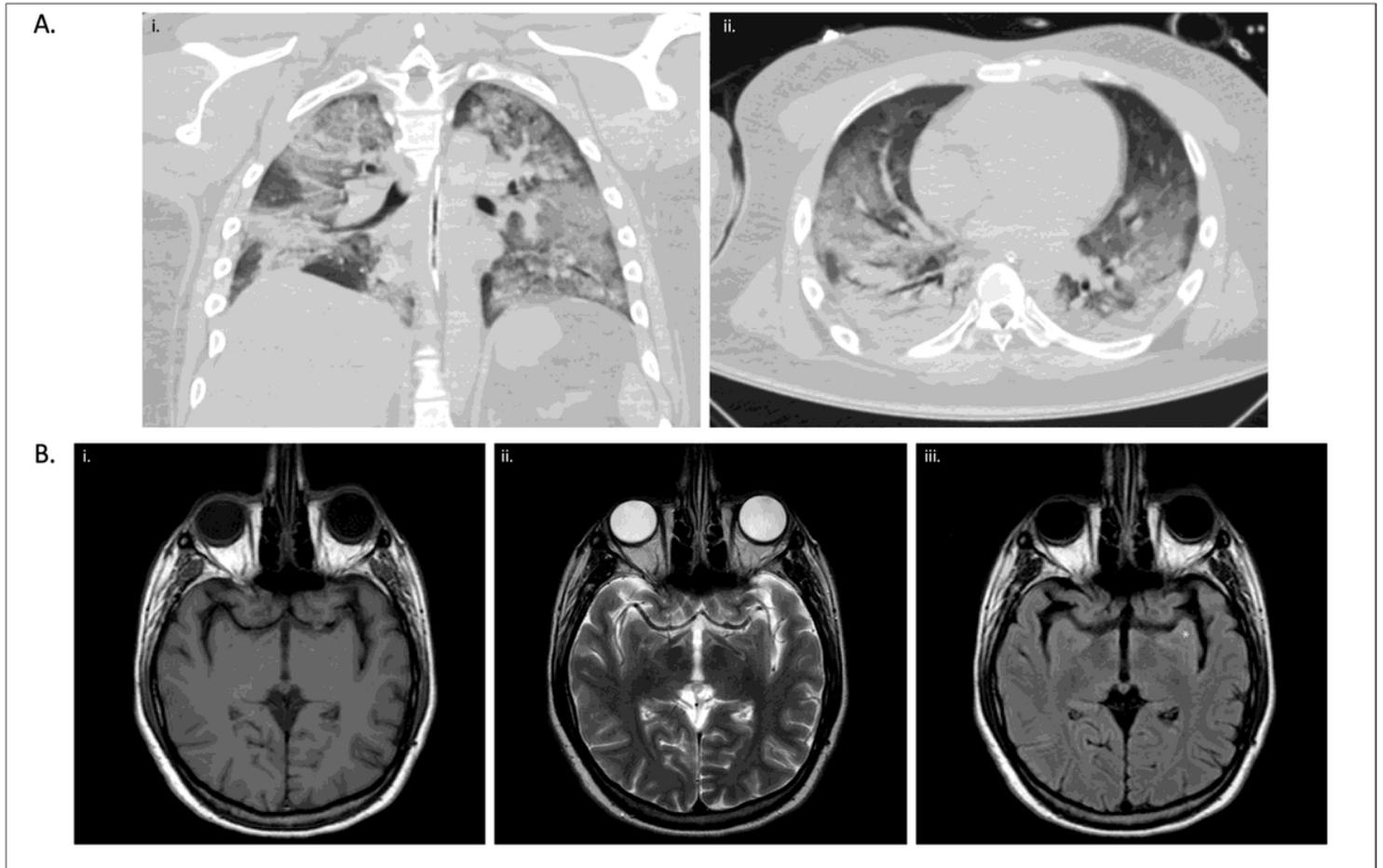
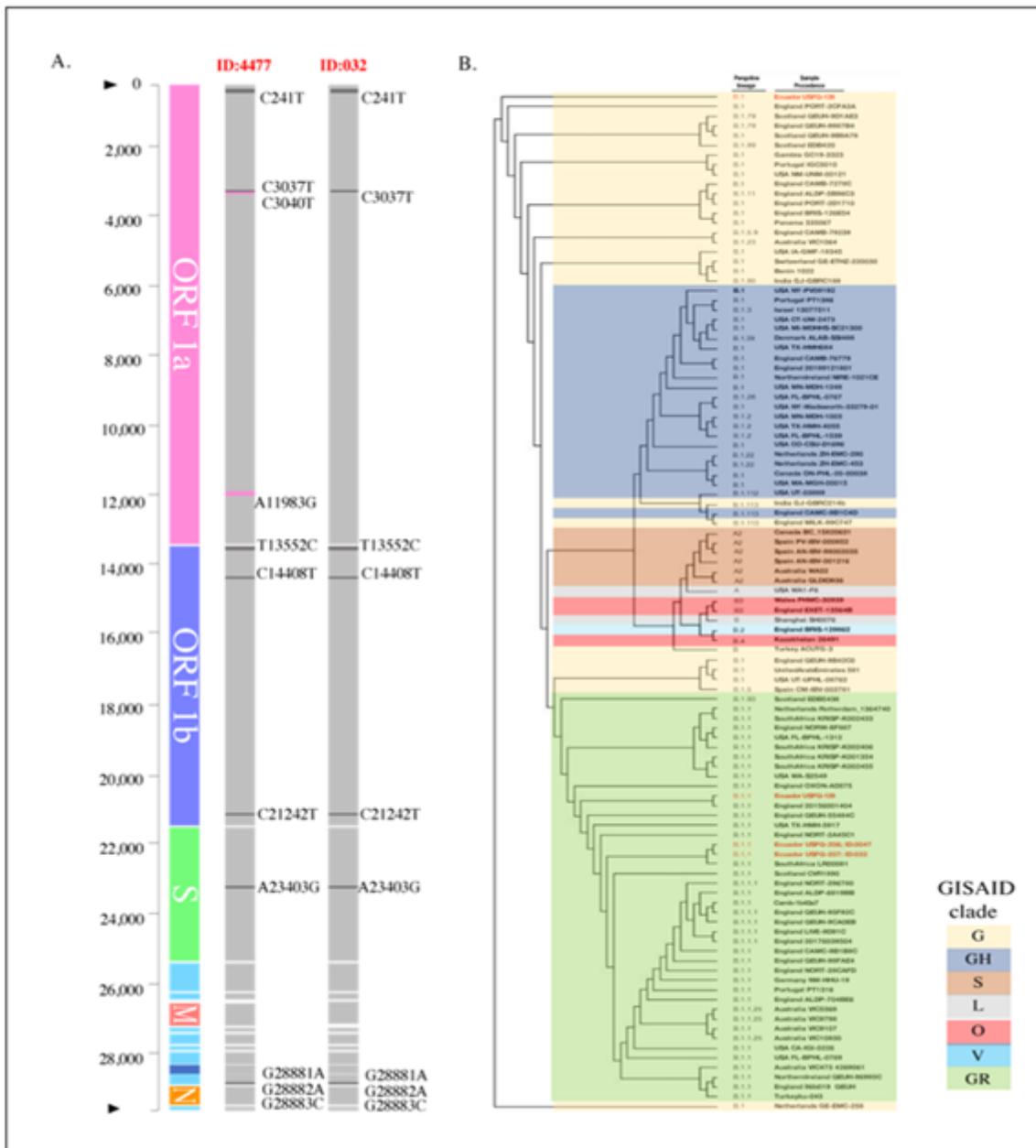


Figure 2

A) Chest computed tomography (CT) revealed ground-glass opacities and consolidations at both lung bases in both lungs Compatible with pulmonary infectious process i: coronal lung, ii: axial lung. B) Axial MR imaging scans the brain of a 35-year-old man intubated with COVID-19 i:T1, ii: T2, iii: T2-FLAIR . (\*) Reported as limbic encephalitis evidence.



**Figure 3**

A) Single Nucleotide Polymorphism of the sequenced SARS-CoV-2 genomes diagnosed by RT-PCR in April (ID: 032) and August (ID: 4477). B) Maximum-likelihood phylogeny of one hundred subsampled SARS-CoV-2 genomes from GISAID website. The columns refer to Pangolin lineage and sample precedence respectively, the colors correspond to which GISAID clade are the genomes related to.