

Rapid Communication

Isolation and characterization of the new SARS-CoV-2 variant in travellers from the United Kingdom to India: VUI-202012/01 of the B.1.1.7 lineage

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Since its emergence in China during December 2019, severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has marked its presence all across the globe. During this pandemic phase, the new genetic mutations acquired by the virus have led to new variants, indicating that the virus is evolving. This is indicated by the emergence of two SARS-CoV-2 variants, B.1.1.7 lineage (*a.k.a.* 20B/501Y.V1 variant of concern [VOC] 202012/01) and B.1.351 lineage (*a.k.a.* 20C/501Y.V2) identified from UK and South Africa, respectively. The B.1.1.7 lineage has eight mutations in Spike receptor-binding domain which mediates the attachment of the virus to the angiotensin-converting enzyme 2 receptor on the surface of human cells, whereas the B.1.351 lineage has the N501Y but not the 69/70 deletion.^{1,2} B.1.1.7 lineage phenotype has also attracted attention, as it proves to be much more transmissible among humans than the other known SARS-CoV-2 lineages.^{1,2} The genetic mutations in these new variants are associated with rapid transmission of the infection.³ However, its effect on the severity of the disease and vaccine efficacy has not yet studied.

The rapid spread of the new variants has alerted the public health system of all the countries and necessitates the active molecular and genomic surveillance of the SARS-CoV-2 with respect to the disease transmission and pathogenicity. As per Ministry of Health and Family Welfare, Government of India, advisory all the international travellers/passengers were screened at the point of entry and at community level that had travelled through UK in the past 4 weeks (from 25th November 2020). Indian SARS-CoV-2 Genomics Consortium was also

established to understand the evolution and spread of new variant.⁴

Five cases with recent travel history from UK to India on 22 December 2020 were tested positive by real-time Reverse Transcriptase - Polymerase Chain Reaction.⁵ They were kept in an isolation facility of respective state health authorities. Four cases (aged: 39, 32, 50 and 35 years) had low-grade fever with mild headache from 2 days before testing of samples; while one case was asymptomatic (aged: 25 years). All the cases were followed for 14 days and no new symptoms developed in any of the cases and all had recovered completely. Here, we report the (VOC) 202012/01 isolation and its molecular characterization from these human cases.

One hundred microliter of oropharyngeal and nasopharyngeal swab specimen of these cases was inoculated onto 24-well cell culture monolayers of Vero CCL-81 and incubated for an hour at 37°C to allow virus adsorption, with rocking every 10 min. The detailed steps followed are described in the earlier published literature.⁶ Cell rounding and detachment along with syncytial cells formation was observed for five samples of four different cases on post-infection day (PID)-4. No cellular changes were observed in the cell control during two passages (Figure 1B). The tissue culture fluids of two passages were tested using real-time RT-PCR targeting SARS-CoV-2 specific E and RDRP genes.⁵ All the isolates were found to be positive for SARS-CoV-2 (Figure 1A).

In order to characterize the SARS-CoV-2 isolates, next-generation sequencing was performed as described earlier.⁷

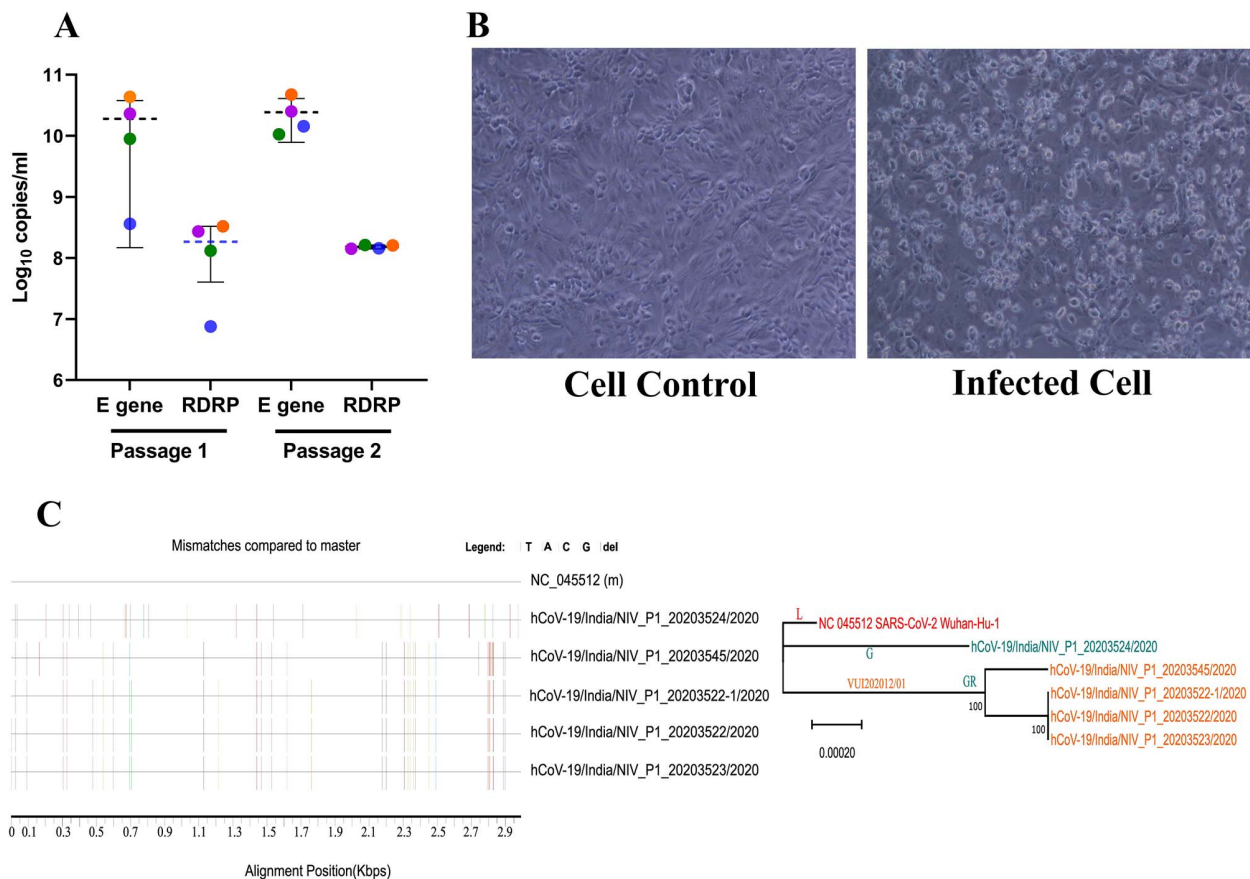


Figure 1. Isolation and characterization of (VOC) 202012/01: (A) The log₁₀ viral RNA copy number per ml of the E and RDRP genes for two different passages. Different colours are used to indicate individual isolate. (B) The cytopathic effect observed in the Vero CCL81 cell culture at passage-1 PID-4 compared to the cell control. (C) The aligned SARS-CoV-2 sequences from the UK variant isolate were compared with the reference isolate of Wuhan-HU-1 (Accession No.: NC_045512.2). The mismatches in the nucleotide position were generated using the highlighter plot (<https://www.hiv.lanl.gov/cgi-bin/HIGHLIGHT/highlighter.cgi>). The alignment positions are in kbps. The changes are marked in different colours.

SARS-CoV-2 isolate Wuhan-HU-1 (Accession No.: NC_045512.2) was used as the reference sequence to retrieve the viral genome. The amino acid and the nucleotide variation between the reference and retrieved SARS-CoV-2 sequences had all the indicated mutations of the (VOC) 202012/01, as published earlier.¹ Four sequences from three cases have all the hallmarks for the (VOC) 202012/01, whereas the fifth sequence varied. According to Global Initiative on Sharing All Influenza Data (GISAID) nomenclature, the four sequences of three cases having (VOC) 202012/01 hallmarks belonged to GR clade and the fifth sequences belonged to G clade. The variation in the retrieved sequences were generated using the highlighter plot (<https://www.hiv.lanl.gov/cgi-bin/HIGHLIGHT/highlighter.cgi>) and are depicted in Figure 1C. The percentage nucleotide difference between the hCoV-19/India/NIV_P1_20203524/2020 and the other GR clade SARS-CoV-2 sequences in this study, was observed to be 0.05%, indicating variation in the isolate sequences.

In conclusion, the isolation of the SARS-CoV-2 Variant Under Investigation (VUI)-202012/01 variant was confirmed using the sequencing method. The isolation of these variants may shed light on vaccine efficacy of the currently

Restricted Emergency-approved coronavirus disease 2019 vaccine in India. Further the efficacy of different SARS-CoV-2 vaccine candidates need timely evaluation against this new variant.

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Conflicts of Interest

Authors do not have any conflict of interest.

Authors' Contributions

P.D.Y. contributed to study design, data collection, data analysis, interpretation and writing and critical review. D.A.N. contributed to data analysis and interpretation, writing and critical review. R.R.S. contributed to data collection, writing and critical review. P.S., J.P., S.P. and S.B. contributed to data collection.

V.P. contributed to data analysis. D.Y.P. contributed to data interpretation, writing and critical review.

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