DOI: 10.1002/jmv.26997

LETTER TO THE EDITOR

JOURNAL OF MEDICAL VIROLOGY WILEY

Symptomatic reinfection of SARS-CoV-2 with spike protein variant N440K associated with immune escape

Dear Editor,

A number of cases of reinfections in individuals with SARS-CoV-2 have emerged across the globe.[1,2] With the emergence of genetic variants associated with immune escape in many regions of the world, it is being widely believed that immune escape variants could contribute to an increase in reinfections as well as potentially adversely affect the efficacy of vaccines.[3,4] A recent report also describes reinfection in a patient with neutralizing antibodies and identified an immune escape variant in the spike protein.[5]

Here, we describe a case of reinfection in an individual from South India characterized by whole genome sequencing of the virus isolated from both episodes. Analysis shows the presence of an immune escape variant N440K in the Spike protein in both episodes of infection. Incidentally, this variant was also found in a case of reinfection previously reported by us by a healthcare worker from North India.[1]

In the first episode of infection, the 47-year-old male civil official from Andhra Pradesh, India, was identified to be positive for SARS-CoV-2 on July 25, 2020, in nasopharyngeal specimens analyzed as a part of routine surveillance and was asymptomatic. The cycle threshold values (C_t) were 22.3 and 19.1 for ORF1ab and N genes, respectively (Labsystems Diagnostic Inc.). The individual tested negative on August 2, 2020, and tested positive again on September 10, 2020, during routine surveillance, but during this episode, he was symptomatic with fever, cough, and malaise. The C_t values for the probes targeting ORF1ab and N genes were 21.9 and 19.2, respectively (Labsystems Diagnostic Inc.) during this episode and he tested negative after 14 days.

The RNA samples were sequenced using COVIDSeq protocol[6] on Illumina MiSeq (Illumina Inc) generating paired-end sequencing (75 × 2 bp) reads, which were analysed as per standard protocols.[7] Variants were called using VarScan.[8] Only variants having a frequency greater than 50% and a minimum depth of 50 reads were considered for further analysis. Lineages were assigned using PANGOLIN.[9]

The analysis revealed a total of 15 and 17 genetic variants in the genomes from the two episodes E1 and E2, respectively, of which 14 variants were common between the two episodes (Figure 1). A close comparison of the genetic variants with a compendium of immune escape variants revealed a 22882T>G (Spike: N440K) variant in the genomes isolated from both episodes of infection. The variant has previously been shown to emerge in vitro under selective pressure against the human monoclonal antibody C135 and show resistance to it.[3] The variant had a high prevalence of over 33% in the state of Andhra Pradesh[10] and has been reported previously in another case of SARS-CoV-2 reinfection from North India.[1]

Phylogenetic analysis for the two isolates was done using all global genomes having the N440K variant, which includes 92 genomes from India sequenced in-house (BioProject ID: PRJNA655577). The dataset of global genomes was obtained from GISAID (https://www.gisaid.org/) by searching for the "Spike_N440K" substitution in the database. Analysis shows that genomes isolated from the two episodes fell under two distinct clusters of genomes (Figure 1B). The genomes clustered closely with other genomes from Andhra Pradesh. Genome isolates of both episodes were assigned the PANGO lineage B.1.36. The close resemblance of the genome isolates from the two episodes of infection yet the presence of distinct variants in the two genomes suggests that the patient acquired both infections from the same location in Andhra Pradesh at two different time points.

Recent studies exploring the role of SARS-CoV-2 genetic variations in escaping immune response has shed light on the possible mechanisms of the pathogen to evade antibody response and immune reactions. The N440K variant has been reported to be resistant to class 3 monoclonal antibodies (mAbs) C135 and REGN10987 that are candidates for clinical development.[3,11,12] Both C135 and REGN10987 mAbs have been shown to have interactions focused on the N440 residue of the Spike protein and the close proximity of the N440 residue to the structural epitope of the mAbs potentially confers loss of binding and resistance to the neutralizing effect of the mAbs.[3,11] The variant is also reported to have an enhanced binding affinity to the ACE2 receptor in humans.[3,11] Several studies have recently documented cases of reinfection with the presence of the variant E484K in the Spike protein in the second episode of infection, despite the presence of anti-SARS-CoV-2 antibodies in the patient.[5,13,14] The E484K is a defining variant for the two circulating SARS-CoV-2 variants of concern, B.1.351 lineage (20H/501Y.V2) and P.1 lineage (20J/501Y.V3), first identified in South Africa and Brazil, respectively.[15] The variant has also been individually reported to be associated with escape from several mAbs[3,4,16] and a loss of neutralization activity of antibodies elicited by vaccines[17,18] and its increasing prevalence across different regions raises concern about its potential impact. The high prevalence of N440K in India along with the findings presented here and in other related studies highlights the importance of analyzing the potential impact of the variant and additional host factors on reinfections and immune evasion.

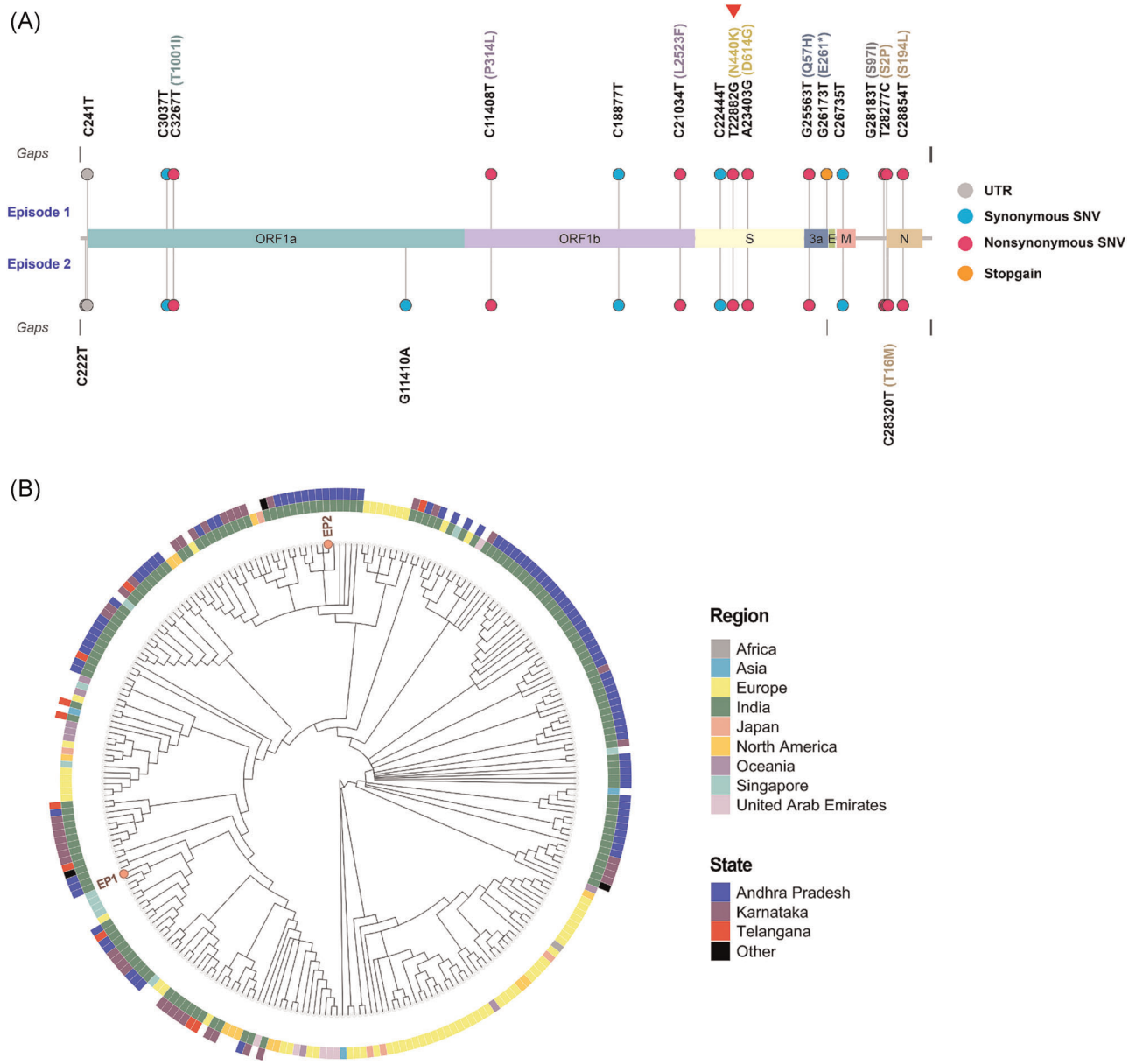

FIGURE 1 (A) Genetic variants in the genome isolates of the two episodes (denoted as Episode 1 and Episode 2) of SARS-CoV-2 infections. The 22882T>G (Spike: N440K) variant is marked with an arrowhead. (B) Phylogenetic context of the virus isolates of the two episodes with other global samples having the N440K variant

ACKNOWLEDGMENTS

The work was supported by the Council of Scientific and Industrial Research (CSIR India) through grant [CODEST and MLP2005]. Bani Jolly, Abhinav Jain, and Mohit Divakar acknowledge a research fellowship from CSIR India.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Pallavali R. Rani, Juturu V. Lakshmi, Avileli Surekha, Pulala Chandrasekhar, Damam Srinivasulu, and P. R. Vanaja performed the sample collection, clinical evaluation, and primary diagnosis of the patient. Mohamed Imran, Vigneshwar Senthivel, Mohit K. Divakar, and Rahul C. Bhoyar performed the library preparation and sequencing of the samples. Bani Jolly and Abhinav Jain performed the analysis of the samples. Vinod Scaria and Sridhar Sivasubbu conceptualized the project, provided the overview and guidance and contributed to writing the manuscript with Bani Jolly, Mohamed Imran, Rahul C. Bhoyar, and Abhinav Jain.

DATA AVAILABILITY STATEMENT

All sequences generated in this study have been submitted to GISAID (accession IDs EPI_ISL_1103556 and EPI_ISL_1103557).

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