Asymptomatic reinfection in two healthcare workers from India with genetically distinct SARS-CoV-2

Vivek Gupta[1,$], Rahul C. Bhoyar[2,$], Abhinav Jain[2,3,$], Saurabh Srivastava[1], Rashmi Upadhayay[1], Mohamed Imran[2], Bani Jolly[2,3], Mohit Kumar Divakar[2,3], Disha Sharma[2,3], Paras Sehgal[2,3], Gyan Ranjan[2,3], Rakesh Gupta[1], Vinod Scaria[2,3], Sridhar Sivasubbu[2,3]

[1]Government Institute of Medical Sciences, Greater Noida, INDIA

[2]*CSIR Institute of Genomics and Integrative Biology (CSIR-IGIB), Mathura Road, New Delhi, INDIA*

[3]*Academy of Scientific and Innovative Research (AcSIR), Ghaziabad, INDIA*

$ contributed equally to this manuscript

Address for Correspondence

Vinod Scaria, G. N. Ramachandran Knowledge Center for Genome Informatics, CSIR- Institute of Genomics and Integrative Biology, Sukhdev Vihar, New Delhi, India, 110025, Email vinods@igib.in

Dear Editor,

To et al [1] recently reported a case of SARS-CoV-2 reinfection confirmed by genome sequencing. Additional reports of genetically characterized reinfections have emerged [2, 3] raising pertinent questions on the longevity of immune response in SARS-CoV-2 infection . In all previous reports, patients had symptoms in one or both of the episodes. Here we report asymptomatic SARS-COV-2 reinfection in two healthcare workers detected during routine surveillance. The report highlights the possibility of undetected SARS-CoV-2 reinfections and the need for surveillance of SARS-CoV-2 reinfections in healthcare systems.

We describe two individuals, 25 year-old male (I1) and 28 year-old female (I2) healthcare workers posted in the COVID-19 unit of a tertiary hospital in North India, who tested positive for SARS-CoV-2 by reverse transcription-polymerase chain reaction (RT-PCR) on 5th May and 17th May, 2020 respectively. Though both individuals were asymptomatic, they were hospitalised as per institutional policy on 5th May and 18th May, respectively. Subsequently, they tested negative for SARS-CoV-2 by RT-PCR on 13th May and 27th May, respectively. After resuming duties in the hospital, the two individuals tested positive again for SARS-CoV-2 on 21st August and 5th September and further tested negative on the 14th and 6th days respectively. Both individuals were again asymptomatic but had a higher viral load on the second episode of reinfection (C_T values of 36 and 16.6 for I1 and 28.16 and 16.92 for I2 for the first and second episodes, respectively). The timeline of the two episodes of infection in the individuals are summarised in Figure 1A.

Since RNA from the nasopharyngeal/oropharyngeal swabs were archived, after informed consent (IHEC-CSIR-IGIB/IHEC/2020-21/01) the sequencing-ready libraries were prepared using capture-based (TWIST Biosciences) as well as amplicon-based (COVIDSeq, Illumina) approaches. The libraries were sequenced on 75 bp x 2 paired-end recipe on Illumina MiSeq. Genomes were assembled at an average of 13,684X coverage after merging the datasets, partially covering the SARS-CoV-2 reference genome (NC_045512.2) at 89.08% and 99.96 % respectively for the two episodes for I1 and 85.60%

and 92.14% for I2. Analysis of the genomes using a previously published protocol [4] for loci covered in both the genomes revealed 9 and 10 unique variant differences between the virus isolates from the two episodes of infection for I1 and I2 respectively (Figure 1B**)**. Of the unique variants between the pair of samples, seven variants each for the two individuals mapped to predicted immune epitopes [5].

Taken together our analysis suggests that asymptomatic reinfection may be a potentially under-reported entity. Genetically distinct SARS-CoV-2 rules out persistent viral shedding or reactivation. Both individuals had a higher viral load during reinfection highlighting the need for continuous surveillance. It is noteworthy that a genetic variant 22882T>G (S: N440K) found during reinfection in I2 possibly confers resistance to neutralising antibodies [6]. To the best of our knowledge, this is one of the earliest reports of genetically characterized reinfection from India.

ACKNOWLEDGEMENT

Authors acknowledge Kirishnalata, Ravi, Abhinav for assistance in data collection and Anjali Bajaj for assistance in preparing the manuscript. Authors acknowledge Mercy Rophina, Afra Shamnath and Mohit Mangla for the compilation of functionally relevant variants used in this analysis. AJ, BJ, MD and PS acknowledge a research fellowship from CSIR India. DS acknowledges a research fellowship from Intel.

FUNDING

This work was supported by the Council of Scientific and Industrial Research (CSIR India) through grant [CODEST and MLP2005].

CONFLICT OF INTEREST

Vivek Gupta - No Conflict

Rahul C. Bhoyar - No Conflict

Abhinav Jain - No Conflict

Saurabh Srivastava - No Conflict

Mohamed Imran - No Conflict

Bani Jolly - No Conflict

Mohit Kumar Divakar - No Conflict

Disha Sharma - No Conflict

Paras Sehgal - No Conflict

Gyan Ranjan - No Conflict

Rakesh Gupta - No Conflict

Vinod Scaria : VS reports grants from SANOFI GENZYME, technology licensing from Dr LAL PATH LABS and GENIQUE LIFE SCIENCES, outside the submitted work; .

Sridhar Sivasubbu - No Conflict

REFERENCES

1. To KK-W, Hung IF-N, Ip JD, et al. COVID-19 re-infection by a phylogenetically distinct SARS-coronavirus-2 strain confirmed by whole genome sequencing. Clin Infect Dis **2020**.

2. Van Elslande J, Vermeersch P, Vandervoort K, et al. Symptomatic SARS-CoV-2 reinfection by a phylogenetically distinct strain. Clin Infect Dis **2020**.

3. Tillett R, Sevinsky J, Hartley P, et al. Genomic Evidence for a Case of Reinfection with SARS-CoV-2. **2020**; Available at: https://papers.ssrn.com/abstract=3680955. Accessed 14 September 2020.

4. Poojary M, Shantaraman A, Jolly B, Scaria V. Computational Protocol for Assembly and Analysis of SARS-nCoV-2 Genomes. Research Reports, 2020; 4: e1-e14

5. Grifoni A, Sidney J, Zhang Y, Scheuermann RH, Peters B, Sette A. A Sequence Homology and Bioinformatic Approach Can Predict Candidate Targets for Immune Responses to SARS-CoV-2. Cell Host Microbe **2020**; 27:671–680.e2.

6. Weisblum Y, Schmidt F, Zhang F, et al. Escape from neutralizing antibodies by SARS-CoV-2 spike protein variants. bioRxiv **2020**.

Figures Legend

Figure 1. A) The timelines of SARS-CoV-2 infection in the two individuals I1 and I2. **B)** The genetic variants in isolates for the 2 episodes (E1 and E2) for individuals I1 and I2. Non-synonymous variants have been underlined and the gaps in the genome are marked in gray.

Figure 1