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# Asymptomatic reactivation of SARS-CoV-2 in a child with neuroblastoma characterised by whole genome sequencing

*To the editor*

COVID-19 in children with cancer is usually mild and most children recover without any treatment [1,2]. To et al. and others [3–6] have recently reported reinfections with SARS-CoV-2 confirmed by genome sequencing. A few recent cases of potential reactivation with SARS-CoV-2 have been reported in cancer patients on chemotherapy [7–9], however were not confirmed by genetic characterization of the virus. Here we report results of genome sequencing in child with neuroblastoma reported previously by us as re-infected with SARS-CoV-2 after recovering fully and developing IgG antibodies [8]. Genetic characterization of the virus from both episodes confirmed reactivation.

The 3-year-old boy with stage 4 neuroblastoma was being treated as per OPEC/OJEC protocol. After 4 cycles of chemotherapy and surgery he was found to be positive on real time-PCR(RT-PCR) assay for SARS-CoV-2 on on nasopharyngeal & throat swabs (Thermofisher TaqPath; Ct values N-14.59 ORF-16.84 S-17.48). As he was asymptomatic, was isolated at home and the chemotherapy was postponed. Three weeks later, the repeat RT-PCR assay was negative and was commenced on the next course of chemotherapy OJEC. Six weeks later, prior to course of next chemotherapy, the child tested positive again without any symptoms, on routine testing for SARS-CoV-2 RT-PCR assay (Siemens Labgun ExoFast; Ct values, N-22.37 RdRp-22.15). Due to non-availability of the previously used RT-PCR kit, we had to use commercial kit of another company to test for SARS-Cov-2. On the next day, the quantitative IgG antibody assay (by CLIA for anti S1 and anti S2 specific IgG; 306 AU/mL) was also positive. The gap between first and second episodes was 42 days. He was isolated at home for another 2 weeks, and chemotherapy was resumed after testing negative on RT-PCR [8]. A possibility of reactivation was kept in view of low Ct values in both episodes and presence of IgG antibody.

The archived RNA samples were used for the library preparation using the COVID Seq assay kit which utilizes the set of 98 primers specific for the SARS-CoV-2 genome [10]. The synthesized libraries were sequenced on the MiSeq sequencer (Illumina, Inc) on a 75 × 2 paired end sequencing recipe. Genomes were assembled using a reference-based approach following a previously published protocol [11]. Briefly, quality control of the FASTQ files was performed followed by adapter/base trimming at a Phred quality score of Q30 and length cut-off 70 bps. Reads were aligned to the SARS-CoV-2 reference genome (NC_045512.2) The genomes were assembled at a depth of 12615.92X and 7834.78X, with a genome coverage of 99.84 % and 99.70 % for the 2 episodes E1 and E2 respectively (Fig.1 ). Analysis revealed 14 variants in the genome isolate from E1. 13 out of the 14 variants from E1 were also present in the isolate from E2 while 1 variant 12,793 G > A was present in E2 with a read count proportion of 35.8 %. Of the 14 variants, 6 were non-synonymous.

Put together, the genomic analysis suggests that the virus isolated from the two episodes were not distinctly different, suggesting reactivation of the virus during the course of chemotherapy. Other possibility is prolonged shedding of the virus in an immune suppressed child. Children tend to shed virus for prolonged period [12]. False-negative results mainly occurred due to inappropriate timing of sample collection in relation to illness onset and deficiency in sampling technique, especially of nasopharyngeal swabs. Specificity of most of the RT-PCR tests is 100 % because the primer design is specific to the genome sequence of SARS-CoV-2 [13]. To the best of our knowledge, this is one of the first cases of SARS-CoV-2 reactivation corroborated by genetic sequence data.

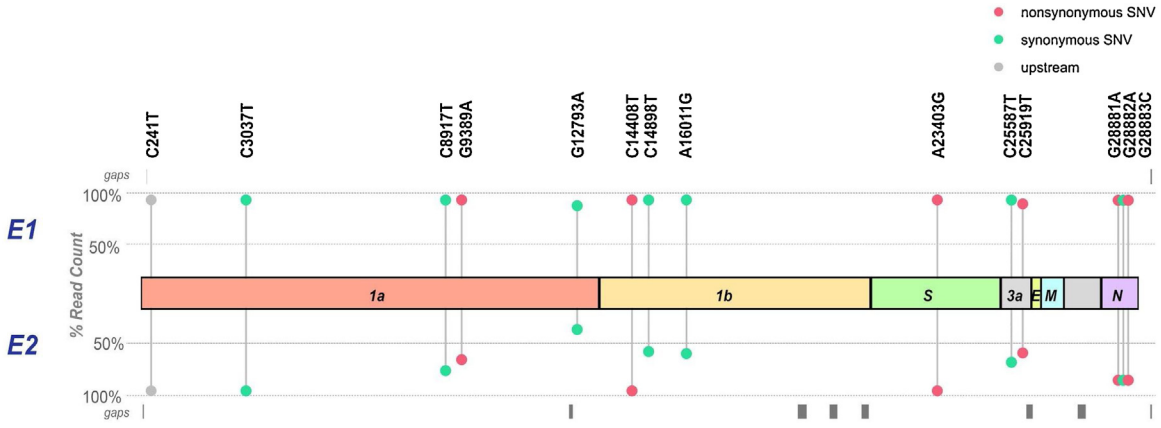


**Fig. 1.** Variants in the genome isolates from the two episodes E1 and E2 with their respective read count proportions.

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## Ethical approval

Not applicable.

## Declaration of Competing Interest

The authors reported no declarations of interest.

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