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A cluster of the new SARS-CoV-2 B.1.621 lineage in Italy and sensitivity of the viral isolate to the BNT162b2 vaccine

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Dear Editor,

In this study we show a 7-individual cluster belonging to the SARS-CoV-2 B.1.621 lineage, imported in Italy by traveler from abroad. We isolated, for the first time, the authentic virus from one of these infected individuals and challenged it against 37 sera of BNT162b2 vaccinated volunteers. Here we demonstrate that neutralization of SARS-CoV-2 B.1.621 lineage was robust, even if significantly lower than that observed on SARS-CoV-2 B.1. Our findings underline that vigilance of SARS-CoV-2 genomic evolution is fundamental to limit the spread of new SARS-CoV-2 lineages to different countries.

Since autumn 2020 SARS-CoV-2 variants emerged and spread globally. The Centers for Disease Control and Prevention (CDC) classified them as variant of interest (VOI), variant of concern (VOC) and variant of high consequence.¹ In particular, VOI present specific genetic markers predicted to affect transmission, diagnostic, therapeutics or immune escape and are responsible of unique outbreak clusters or increased proportion of cases.²

A new SARS-CoV-2 VOI, defined as B.1.621 lineage, emerged in January 2021 in Colombia. This lineage carries several Spike mutations, some are common with other VOC (E484K, N501Y, P681H) while others are new (R346K, Y144T, Y145S and 146N insertion).^{3,4} Up to date, B.1.621 lineage is predominantly retrieved in Colombia, USA, Spain, Netherlands and Denmark.⁵

To monitor SARS-CoV-2 variants spread in the Brescia area (Northern Italy), a random genomic surveillance program of SARS-CoV-2 positive samples was implemented through Sanger sequencing. On April 20th 2021 during SARS-CoV-2 genomic surveys, we detected a sequence characterized by B.1.621 lineage typical Spike mutations. A whole genome sequencing (WGS) was performed on this sample confirming the B.1.621 lineage. Hence, all the contacts of this patient, suffering from unspecific symptoms, were traced allowing the identification of other six SARS-CoV-2 positive patients, whose samples underwent WGS and were assigned to the B.1.621 lineage. These data define the

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first Italian cluster of the SARS-CoV-2 B.1.621 lineage. The introduction of SARS-CoV-2 B.1.621 lineage in the Brescia area was ascribed to a traveler coming from Colombia.

To assess the evolutionary relationships among these seven Italian SARS-CoV-2 B.1.621 lineage sequences on a global scale, a maximum likelihood tree was employed. All our sequences form a monophyletic cluster with the SARS-CoV-2 B.1.621 sequence from USA (EPI_ISL_1581369) (Figure 1A). Figure 1B shows key mutations in the SARS-CoV-2 B.1.621 Spike protein.

In this study, we isolated for the first time the virus from the sample of the earliest positive patient and carried-out a neutralization assay using the isolated virus soon after con-firmation of its identity by WGS, and human sera collected between 10 and 20 days after the administration of the second dose of the BNT162b2 vaccine, which occurred 3 weeks after the first immunization. All sera efficiently neutralized the SARS-CoV-2 B.1.621 isolate (Figure 1C), demonstrating that this VOI is not a concern for vaccine efficacy. Indeed, neutralization of SARS-CoV-2 B.1.621 was robust, even if significantly lower than that observed on SARS-CoV-2 B.1.

Our data show that despite several mutations in Spike, SARS-CoV-2 B.1.621 is neutralized by the BNT162b2 vaccine-elicited antibodies. Moreover, they highlight the importance of properly quarantine people after abroad travel to avoid spreading of newly emerging SARS-CoV-2 lineages to different countries.

DATA AVAILABILITY STATEMENT

Data have been deposited in Global Initiative on Sharing All Influenza Data (GISAID) database (accession numbers: EPI_ISL_3098720; EPI_ISL_3098721; EPI_ISL_3098722; EPI_ISL_3098723; EPI_ISL_3098724; EPI_ISL_3098725; EPI_ISL_3098726; EPI_ISL_3098727).

CONFLICT OF INTEREST STATEMENT

No conflict of interest declared.

AUTHOR CONTRIBUTION STATEMENT

Conceptualization, F.C. and A.C.; methodology, S.M., A.B., A.Z. and F.C.; investigation, S.M., A.B., A.Z. and F.C.; data curation, S.M. and G.C.; formal analysis, S.M., A.B. and G.C.; supervision, F.C. and A.C.; writing – original draft, A.B. and F.C.; writing – review & editing, A.C.

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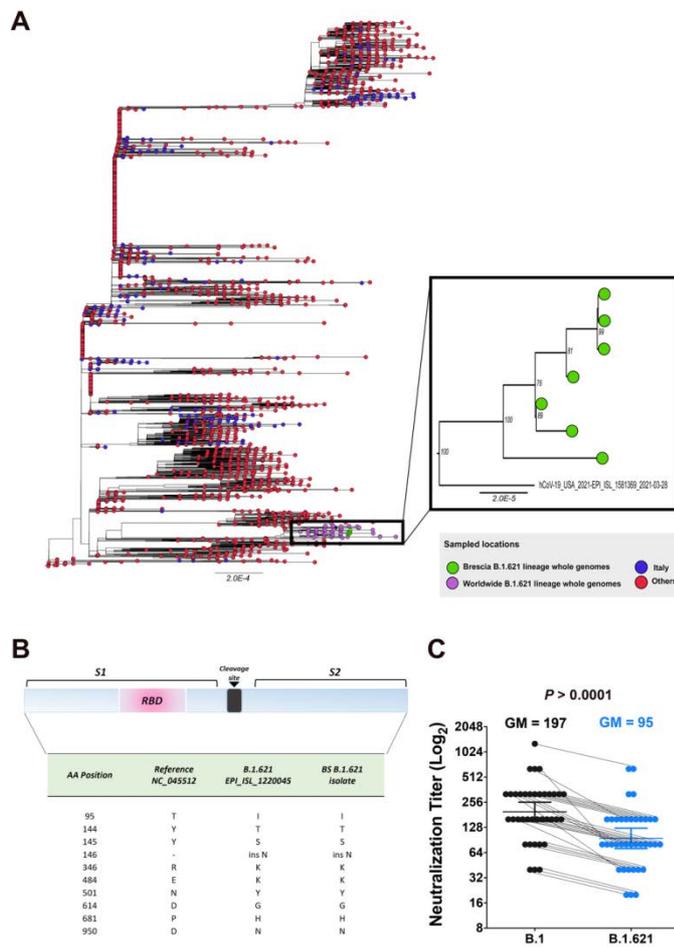


Figure 1. Sequence analyses of SARS-CoV-2 B.1.621 lineage in Italy and its sensitivity to the BNT162b2 vaccine.

(A) Maximum likelihood phylogenetic tree including the 7 SARS-CoV-2 B.1.621 lineage whole genomes from Brescia and 3176 whole genome sequences which are representative of the globally circulating SARS-CoV-2 strains until April 2021 retrieved from GISAID database. Brescia genomes of B.1.621 lineage are highlighted in green while other B.1.621 worldwide whole genomes are marked with violet circles; blue circles and red circles represent genomes not belonging to B.1.621 lineage detected in Italy and abroad, respectively. Support for branching structure is shown by bootstrap values at nodes; Brescia cluster of B.1.621 lineage is zoomed in bold. (B) Amino acid (AA) comparison in the spike region of the Brescia (BS) B.1.621 isolate toward the Wuhan-Hu-1 (Reference, Genbank accession number: NC_045512) and SARS-

CoV-2 B.1.621 (EPI_ISL_1220045) sequences. The one letter AA code has been used; ins indicates the presence of AA insertion; RBD, receptor binding domain. (C) Serum neutralization of authentic SARS-CoV-2 B.1 and B.1.621. Shown are the results of neutralization test using sera obtained from 37 BNT162b2 vaccinated volunteers. Neutralization of the two authentic viruses was performed by cytopathic effect (CPE)-based assay using a viral titer of 10² TCID₅₀. The neutralization titer of each serum sample was calculated as the reciprocal of the highest dilution that protected more than the 50% of cells from CPE. Sera with different neutralization titers against SARS-CoV-2 B.1 and B.1.621 isolates are connected by lines. Horizontal lines and the numbers over the bars indicate geometric mean titers (GM). The I bars indicate 95% confidence intervals. Statistical analysis was performed using the paired t-test and two-tailed P values were calculated.