

1 **A novel B.1.1.523 SARS-CoV-2 variant that combines many spike mutations**
2 **linked to immune evasion with current variants of concern**

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21 Abstract

22 In the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic several variants
23 have emerged that are linked to increased transmissibility and immune evasion. These variants are
24 recognized as variants of concern (VOC). In this study, we describe a B.1.1.523 variant that shares
25 many spike mutations with current VOC. Receptor-binding domain mutations E484K and S494P were
26 observed but also a deletion (position 156-158) in the N-terminal antigenic supersite that is similar
27 to the delta-variant. These mutations are linked to immune evasion in VOC that could lead to less
28 effective vaccines. This variant has been reported in various different countries and continents
29 despite the dominance of B.1.1.7 (alpha) and B.1.617.2 (delta) variant. Furthermore, the B.1.1.523
30 pangolin lineage as a whole is recognized as a variant under monitoring since 14th of July 2021.

31 Introduction

32 Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infected millions of people during
33 the pandemic ^{1,2}. During this pandemic, various variants of this virus were detected in surveillance
34 and were linked with increased infectivity or immune evasion ^{3,4}. Data of these variants are shared
35 via the GISAID (Global initiative on sharing all influenza data) database that helps to understand
36 spread and evolution of SARS-CoV-2. The Centers for Disease Control and Prevention (CDC) and
37 European Centre for Disease Prevention and Control (ECDC) assign certain variants as “variant of
38 concern” (VOC) because of an increase in transmissibility, more severe disease, or immune evasion
39 ^{5,6}. Current VOC are B.1.1.7 (alpha-variant), B.1.351 (beta-variant), P1 (gamma-variant), and
40 B.1.617.2 (delta-variant). These VOC harbor several spike protein mutations linked to immune
41 evasion. The receptor-binding domain (RBD) and N-terminal domain (NTD) are frequently targeted
42 by neutralizing antibodies mostly directed to the NTD ^{7,8}. In addition, a so called antigenic supersite
43 is described in the NTD with three regions. Potent neutralizing antibodies in convalescent plasma
44 target this antigenic super site. Still, mutations in the RBD are important as for example E484K
45 mutation is also strongly linked with immune evasion ^{3,4}. This short communication describes a new
46 variant with a new combination of various concerning spike mutations shared with VOCs and is
47 already spread across many countries. The pangolin lineage of this variant is B.1.1.523 and is
48 recognized as a variant under monitoring since 14th of July 2021 ⁹.

49 Methods

50 GISAID data download and SARS-CoV-2 sequencing

51 All sequences and metadata of B.1.1.523 variant cases with spike mutations S:E156del, S:F157del,
52 S:R158del, S:E484K, and S:S494P were downloaded from www.gisaid.org on 19 August 2021 (n=551).
53 These mutations were chosen because of links with immune evasion. Of these cases 18 were
54 removed based on bad quality score in Nextclade (clades.nextstrain.org). In routine SARS-CoV-2
55 surveillance one case of this B.1.1.523 variant was identified with nanopore sequencing as described
56 in von Wintersdorff et al. 2021 ¹⁰.

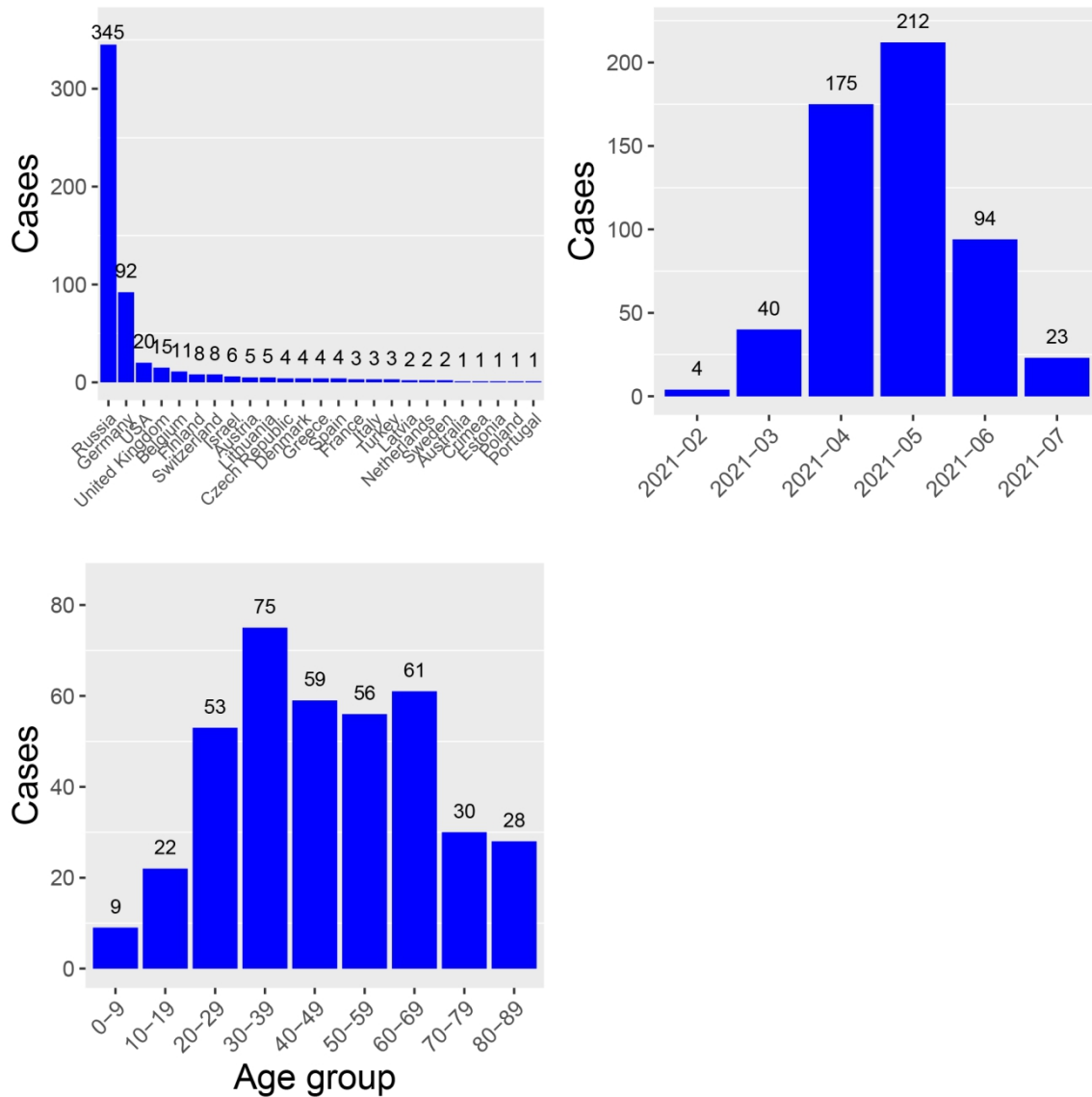
57 Data-analysis

58 Visualization of the filtered data by country, month, and age group were made in R statistical
59 software, version 3.6.2 (R Foundation for Statistical Computing, Vienna, Austria). Figures were made

60 using the R package ggplot2 version 3.3.2. Phylogenetic analysis was performed as described in von
61 Wintersdorff et al. 2021 but with nextstrain/ncov version 7 (<https://github.com/nextstrain/ncov>)¹⁰.
62 The amino acid sequence of Wuhan strain, VOC, and B.1.1.523 variant were aligned in MEGA v10.0.5
63 with Cluster Omega algorithm, with the Wuhan strain as a reference. The 3D structure of the spike
64 protein of the B.1.1.523 variant was predicted using CoVServer ([https://www.gisaid.org/epiflu-
65 applications/covserver-mutations-app/](https://www.gisaid.org/epiflu-applications/covserver-mutations-app/))

66 Results

67 In total, 533 cases of B.1.1.523 with spike mutations S:E156del, S:F157del, S:R158del, S:E484K, and
68 S494P are reported in GISAID till 19 August 2021. Most cases are reported in Russia followed by
69 Germany but cases have also been seen in the USA and Australia (figure 1A). The first few cases were
70 collected in February 2021 and increased in numbers to 203 cases in May 2021 (figure 1B). Number
71 of cases is lower in June and July 2021 as sequenced-based surveillance data is typically lagging. This
72 variant did not show an age related pattern (figure 1C).

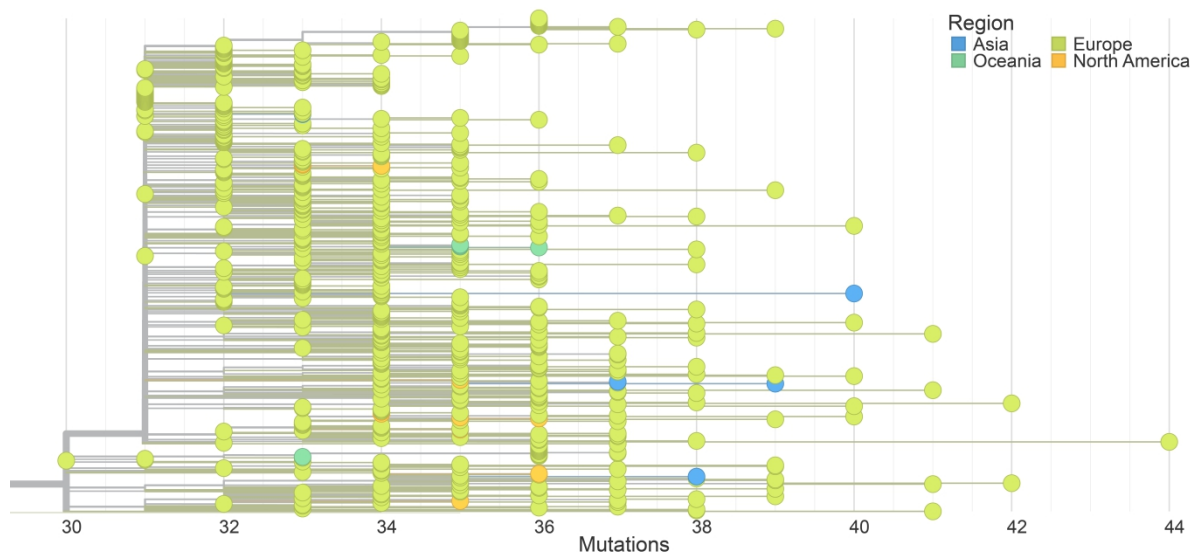


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74 *Figure 1* (A) Number of cases per country. (B) number of cases per month, note that data of June and July are incomplete
 75 as surveillance is typically lagging. (C) Cases per age group.

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77 Sharing of the pangolin lineage and some spike mutations does not necessarily imply a single origin
 78 of this variant. Also, recognition of the first cases in Russia does not necessarily implicate that this
 79 variant originates from this country. To address both issues a phylogenetic tree was constructed and
 80 showed that all cases are similar as they are in the same branch (figure 2). Based on this
 81 phylogenetic analysis the origin of this variant is likely Russian as the first strain is reported from
 82 Moscow (green circle at 30 mutations (EPI_ISL_1823183), figure 2).



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84 *Figure 2* Phylogenetic analysis of cases of B.1.1.523 lineage with S:E156del, S:F157del, S:R158del, S:E484K, and S:S494P.

85 One of the reasons of concern about this variant is a three amino acid deletion in the NTD antigenic
86 supersite and E484K mutation of the spike protein. Therefore a multiple sequence alignment (MSA)
87 was performed with the amino acid sequence of VOC and Wuhan-Hu-1 (figure 3A). Three VOC,
88 B.1.1.7, B.1.351, and B.1.617.2, have deletions in one of the regions of the NTD antigenic supersite.
89 The deletion of B.1.1.523 is similar to the one of B.1.617.2 and has the E484K mutation that is shared
90 in many VOC. In figure 3B the predicted spike structure and interaction with human ACE2 receptor is
91 shown. Spike mutations E484K and S494P are both in the RBD with the ACE2 receptor.

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124 also present B.1.351 (beta) and P.1 (gamma) variants that are strongly linked with reduced efficacy
125 of vaccines^{3,4}. These findings are supported in studies where the effect of spike mutations on
126 efficacy of monoclonal antibodies and convalescent plasma was investigated¹². Similar studies were
127 performed to investigate the antigenic super site of the NTD^{7,8}. In the β -hairpin region of the
128 antigenic super site the B.1.1.523 variant has a deletion (position 156-158) similar to the currently
129 dominant B.1.617.2 (delta) variant (S:E156G and 157-158del)⁵⁻⁸. Other VOC also have deletions in
130 one of the regions of the antigenic super site, B.1.1.7 (alpha-variant) position 144 and B.1.351 (beta-
131 variant) position 241-243. As the mutations observed in this B.1.1.523 variant are strongly linked to
132 immune evasion and disseminated to different continents, despite the dominance of both the
133 B.1.1.7 (alpha) and B.1.617.2 (delta) variants, this could be a variant of interest and should be
134 monitored closely. However, as this is the first study that describes this variant of the B.1.1.523
135 lineage, there is yet no information on transmissibility that contributes to the need of actions
136 required to prevent dissemination.

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