Virus Genes (2021) 57:556–560
https://doi.org/10.1007/s11262-021-01866-5

SHORT REPORT

A SARS-CoV-2 mutant from B.1.258 lineage with ΔH69/ΔV70 deletion in the Spike protein circulating in Central Europe in the fall 2020

Broňa Brejová[1] · Kristína Boršová[2,3] · Viktória Hodorová[4] · Viktória Čabanová[2] · Lenka Reizigová[5,6] · Evan D. Paul[7] · Pavol Čekan[7] · Boris Klempa[2] · Jozef Nosek[4] · Tomáš Vinař[1]

Received: 17 May 2021 / Accepted: 13 August 2021 / Published online: 27 August 2021

Abstract

SARS-CoV-2 mutants carrying the ΔH69/ΔV70 deletion in the amino-terminal domain of the Spike protein emerged independently in at least six lineages of the virus (namely, B.1.1.7, B.1.1.298, B.1.160, B.1.177, B.1.258, B.1.375). We analyzed SARS-CoV-2 samples collected from various regions of Slovakia between November and December 2020 that were presumed to contain B.1.1.7 variant due to drop-out of the Spike gene target in an RT-qPCR test caused by this deletion. Sequencing of these samples revealed that although in some cases the samples were indeed confirmed as B.1.1.7, a substantial fraction of samples contained another ΔH69/ΔV70 carrying mutant belonging to the lineage B.1.258, which has been circulating in Central Europe since August 2020, long before the import of B.1.1.7. Phylogenetic analysis shows that the early sublineage of B.1.258 acquired the N439K substitution in the receptor-binding domain (RBD) of the Spike protein and, later on, also the deletion ΔH69/ΔV70 in the Spike N-terminal domain (NTD). This variant was particularly common in several European countries including the Czech Republic and Slovakia but has been quickly replaced by B.1.1.7 early in 2021.

Keywords SARS-CoV-2 · B.1.1.7 · B.1.258 · Variant · Spike · Deletion

The SARS-CoV-2 mutants carrying the Spike ΔH69/ΔV70 deletion are easily misidentified in routine RT-qPCR as quickly spreading variant B.1.1.7. Here, we demonstrate that many Slovak samples collected in December 2020, originally presumed to contain B.1.1.7, belong to a sublineage of B.1.258, which acquired the Spike N439K substitution followed by the ΔH69/ΔV70 deletion. Here, we denote this sublineage as B.1.258 ΔH69/ΔV70 to distinguish it from sublineages without the ΔH69/ΔV70 deletion (most samples in sublineages B.1.258.1–B.1.258.3 and B.1.258.14–B.1.258.16).

The variant has been highly prevalent in the Czech Republic (~59% sequenced samples between September and December 2020), Slovakia (~25% sequenced samples over the same period), and several other countries. The ΔH69/ΔV70 deletion is associated with increased infectivity and evasion of the immune response [1] and evidence suggests that this mutation has arisen in B.1.258 independently

Edited by Detlev H. Kruger.

Broňa Brejová and Kristína Boršová have contributed equally to this work.

✉ Boris Klempa
boris.klempa@savba.sk

[1] Faculty of Mathematics, Physics and Informatics, Comenius University in Bratislava, Mlynská dolina, 842 48 Bratislava, Slovak Republic

[2] Institute of Virology, Biomedical Research Center of the Slovak Academy of Sciences, Dúbravská cesta 9, 845 05 Bratislava, Slovak Republic

[3] Department of Microbiology and Virology, Faculty of Natural Sciences, Comenius University in Bratislava, Ilkovičova 6, 842 15 Bratislava, Slovak Republic

[4] Department of Biochemistry, Faculty of Natural Sciences, Comenius University in Bratislava, Ilkovičova 6, 842 15 Bratislava, Slovak Republic

[5] Regional Authority of Public Health, Trenčín, Slovak Republic

[6] Department of Laboratory Medicine, Faculty of Healthcare and Social Work, Trnava University, Trnava, Slovak Republic

[7] MultiplexDX, s.r.o., Comenius University in Bratislava Science Park, Ilkovičova 8, 841 04 Bratislava, Slovak Republic

of the B.1.1.7 variant. The deletion is likely to cause a drop-out of the Spike gene target in some RT-qPCR assays [2–5] and thus its carriers can be easily misidentified as B.1.1.7 (as happened in Slovakia [6]). The B.1.258 ΔH69/ΔV70 variant also contains the N439K mutation in the receptor-binding domain (RBD) of the Spike protein, enhancing its affinity to the ACE2 receptor and facilitating escape from monoclonal antibodies and convalescent sera [7]. The lineage B.1.258 is generally characterized by five mutations (one in ORF1a, two in ORF1b, and two in Spike genes) but can be further divided into 21 sublineages differing by various combinations of additional 40 mutations. The ΔH69/ΔV70 deletion is listed as one of the characteristic mutations for 14 of these sublineages (B.1.258.4–7, 9, 11, 12, 17–23) [8]. Most of the B.1.258 ΔH69/ΔV70 samples also include substitutions in the virus replication proteins, namely, NSP9 M101I, NSP12 V720I, and NSP13 A598S.

The ΔH69/ΔV70 deletion has arisen independently at least six times (Fig. 1A), frequently co-occurring with mutations in the Spike receptor-binding domain (RBD) such as N439K, Y453F, and N501Y [9–13]. Besides the B.1.258 ΔH69/ΔV70 and B.1.1.7 variants, the deletion has been observed in B.1.1.298 (Danish mink farm outbreak [10, 14]) and B.1.375 (USA [15, 16]). Recurrent emergence even within well-established clades (such as EU1 (B.1.177) and EU2 (B.1.160)) suggests that ΔH69/ΔV70 can increase overall fitness in concert with mutations that would be otherwise neutral or lower the infectivity [7, 9].

The earliest B.1.258 samples with ΔH69/ΔV70 deletion were observed in Switzerland and in the UK at the beginning of August 2020 (Fig. 1B). The Spike N439K mutation has emerged before the ΔH69/ΔV70 deletion (see sample from Romania as early as May 13, 2020); the outgroup that does not contain N439K has been observed in England on March 22, 2020. During the fall of 2020, B.1.258 variant gained significant prevalence in multiple countries, including Croatia, the Czech Republic, Sweden, Slovakia, Slovenia, Poland, Denmark, and Austria (Fig. 1C). Analysis of disease incidence and hospitalization data did not reveal any clear trends that would be common to all or most of the examined countries when correlated with B.1.258 prevalence. Nevertheless, in several countries (e.g., Austria, the Czech Republic, and Slovakia), the increasing incidence and hospitalization trends started already during the fall of 2020 while the B.1.1.7 variant dominance started later, early in 2021. One can therefore speculate that worsening of the epidemiologic situation in those countries could be at least partially attributed to the spread of the B.1.258 variant.

A newly developed RT-qPCR assay differentiating B.1.1.7 from other ΔH69/ΔV70 variants [17] indicated that out of 122 clinical samples from a mass testing campaign in the city of Trenčín (Slovakia) in December 2020, only 5 (4.1%) belonged to the B.1.1.7 lineage, while an additional 50 (41.0%) samples carried the ΔH69/ΔV70 deletion but were not B.1.1.7 (selected samples were later confirmed as B.1.258 ΔH69/ΔV70 by sequencing). A routine RT-qPCR assay showed significantly lower Ct values in the swab samples for both B.1.1.7 and B.1.258 ΔH69/ΔV70 samples, reflecting higher viral loads in patients carrying these variants (Fig. 1D).

Altogether, we have described a B.1.258 ΔH69/ΔV70 variant of the SARS-CoV-2 virus that contains the S:N439K mutation shown to enhance the binding affinity of the Spike protein to human ACE2 receptor and facilitating escape from immune response, the S:ΔH69/ΔV70 mutation, which is known to increase viral infectivity, as well as several other non-synonymous mutations in NSP9, NSP12, and NSP13 likely affecting viral replication. RT-qPCR analysis on random samples collected during a mass testing campaign indicates that B.1.258 ΔH69/ΔV70 samples carry higher viral loads compared to other strains, similarly as in the case of the B.1.1.7 variant. Interestingly, the B.1.258.17 sublineage has accumulated a higher number of mutations compared to other B.1.258 samples, including additional substitutions in the Spike protein (L189F, V772I), helicase NSP13 (P53L), and a substitution Q185H in ORF3a involved in apoptosis [18].

B.1.258 ΔH69/ΔV70 variant shares many characteristics with quickly spreading B.1.1.7 and is likely responsible for worsening of the epidemiological situation in several countries, including Slovakia and the Czech Republic, in the fall of 2020. While in Slovenia the B.1.258 variant was still one of the major strains as of March 2021, in Slovakia the B.1.1.7 variant has extremely quickly replaced the B.1.258 by the beginning of February 2021 (estimate of ~ 74% of B.1.1.7 and ~ 6% of B.1.258 nationwide on February 3, 2021 by differential RT-qPCR tests [19] compared to 4.1% of B.1.1.7 and 41% of B.1.258 in the city of Trenčín on December 19–20, 2020). The B.1.258 ΔH69/ΔV70 variant, as most of any other variants, was quickly replaced by B.1.1.7 variant early in 2021. Most recently, isolated cases associated with B.1.258 ΔH69/ΔV70 variant were reported from Slovenia and Croatia in June 2021 [20].

Our characterization of the B.1.258 ΔH69/ΔV70 variant that has been circulating in several European countries and appears to result in higher viral loads highlights the importance of vigilant genomic surveillance in properly identifying and tracking SARS-CoV-2 variants that display the potential to derail worldwide efforts to mitigate the pandemic.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11262-021-01866-5.

Acknowledgements We gratefully acknowledge the authors from the originating laboratories responsible for obtaining the specimens, as well as the submitting laboratories where the genome data were

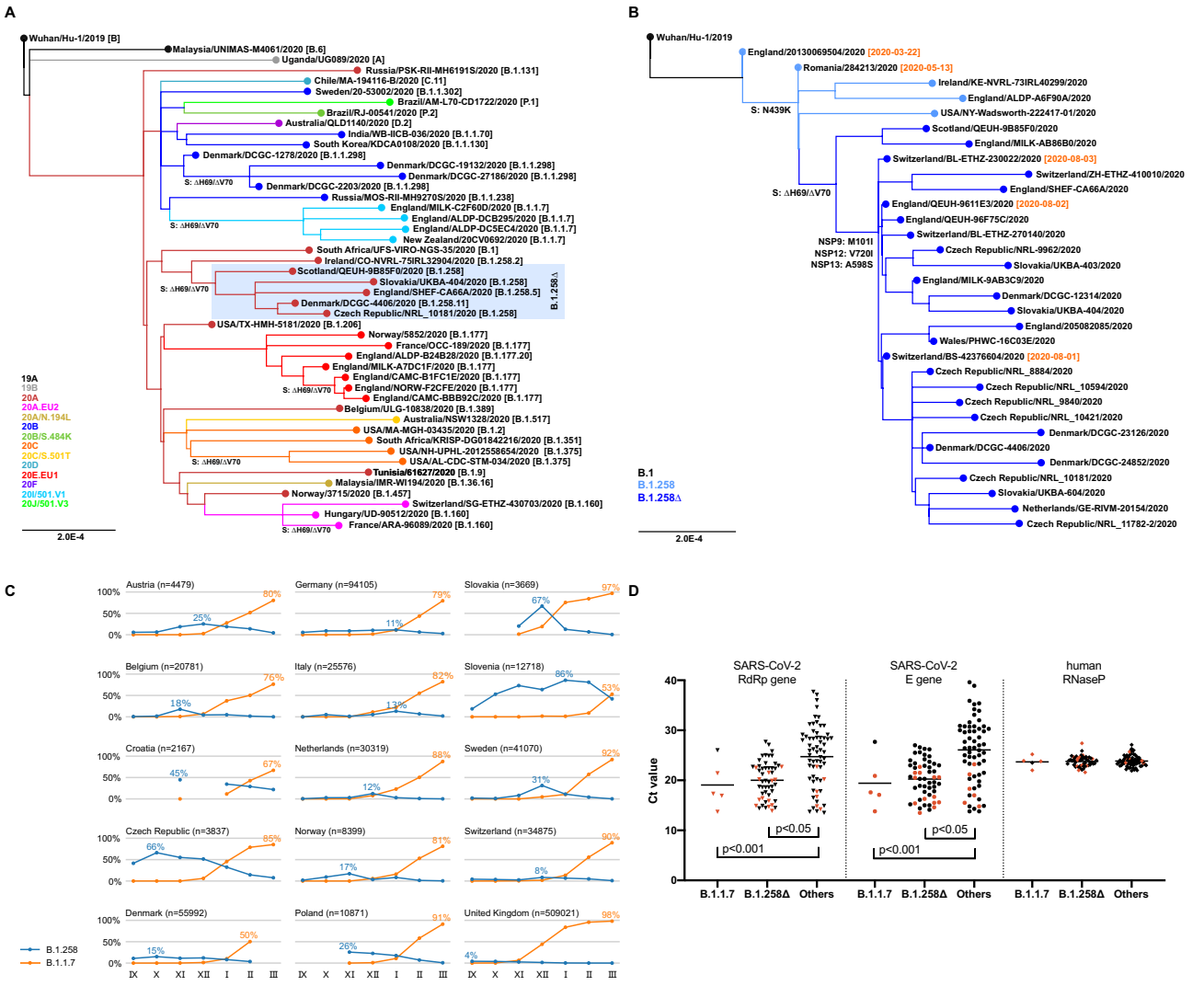

Fig. 1 **A** Points of recurrent emergence of the ΔH69/ΔV70 mutation. Nextclade lineages [20] in color. Pangolin lineages [12] in brackets. B.1.258Δ denotes B.1.258 with ΔH69/ΔV70 deletion. **B** Origins of B.1.258 ΔH69/ΔV70 variant. Mutations S:N439K, S:ΔH69/ΔV70, NSP9:M101I, NSP12:V720I, and NSP13:A598S are marked. Collection dates near to the important branching points are shown. The samples shown in the phylogenetic trees were selected from GISAID database [20] to cover significant lineages of interest (B.1.258 ΔH69/ΔV70 variant in different countries and its outgroups and lineages containing ΔH69/ΔV70 mutation and their outgroups). Phylogenetic trees were built using Augur v. 6 [21]. The prevalence was assessed based on all samples in GISAID (downloaded on April 29, 2021) for a particular country based on PANGO lineage classification [12] provided in GISAID metadata. **C** Prevalence of B.1.258 and B.1.1.7 variants in selected countries out of GISAID samples collected between September 2020 and March 2021. The highest monthly prevalence of both B.1.258 and B.1.1.7 is shown for each country. B.1.258 counts include all sublineages, regardless of the presence of ΔH69/ΔV70 mutation. Only months where at least 20 samples were sequenced in the country are shown. Note that the samples may not be representative, as the sampling strategy differs from country to country, and it also changes over time. **D** Ct values in the swab specimens from the city of Trenčín (Slovakia) mass testing grouped according to the identified lineages. Ct values from routine RT-qPCR assay targeting RdRp, E, and human RNase P genes (used as a control to exclude possible impact of the sample quality) are shown. Classification of samples marked in red was confirmed by sequencing. B.1.258Δ denotes B.1.258 with ΔH69/ΔV70 deletion. RT-qPCR assays were performed on RNA extracted by the Biomek i5 Automated Workstation using the RNAdvance Viral kit (Beckman Coulter, Indianapolis, Indiana, USA) from swab samples previously collected for the primary diagnostics. Besides rTEST COVID-19 RT-qPCR Allplex kit (MultiplexDX, Bratislava, Slovakia) targeting the RNA-dependent RNA polymerase (RdRp) and Envelope (E) genes, the newly developed rTEST COVID-19 qPCR B.1.1.7 kit (MultiplexDX, Bratislava, Slovakia) was used to differentiate B.1.1.7 and B.1.258 ΔH69/ΔV70 variants [17]. The real-time PCR was performed on a QuantStudio™ 5 Real-Time PCR System (Applied Biosystems, Foster City, California, USA). The SARS-CoV-2 sequences were determined on a MinION sequencer (Oxford Nanopore Technologies) using a protocol based on PCR-tiling of 2-kb long amplicons [22]. The horizontal lines represent mean values. The differences in Ct values were statistically evaluated by unpaired *t* test using GraphPad Prism version 8.4.0

generated and shared via GISAID (https://www.gisaid.org/), on which this research is based (GISAID Acknowledgements in Supplementary Information).

Author contributions BB, TV, JN, and BK conceived the study. BB, KB, BK, and TV analyzed the data. VH, KB, and VČ performed the experimental work. LR and BK procured samples for analysis. PČ, EDP, KB, VČ, and BK conceived and implemented the RT-qPCR study. All authors edited and approved the manuscript.

Funding Our research was supported by Grants from the Slovak Research and Development Agency (APVV-18-0239 to JN, PP-COVID-20-0017 to BK, and PP-COVID-20-0116 to PC and BK), the Scientific Grant Agency (VEGA 1/0463/20 to BB, VEGA 1/0458/18 to TV), and the European Union's Horizon 2020 Research and Innovation program (EVA-GLOBAL Project #871029 to BK and PANGAIA Project #872539). The research was also supported in part by OPII project ITMS2014: 313011ATL7.

Data availability All sequences sequenced by the authors have been submitted to GISAID.

Declarations

Conflict of interest MultiplexDX, s.r.o. has developed and manufactured the B.1.1.7 differential RT-qPCR assay kit. Biomedical Research Center of the Slovak Academy of Sciences has entered into collaboration with MultiplexDX, s.r.o. for development and validation of RT-qPCR tests. All other authors declare no competing interests.

Ethical approval All clinical specimens used within this study were previously collected for the purpose of primary diagnosis of SARS-CoV-2 by the Regional Authority of Public Health, Trenčín, Slovakia and were transferred to the Biomedical Research Center of the Slovak Academy of Sciences, Bratislava, Slovakia while made unidentifiable for the researchers performing this study. The study has been approved by the Ethics committee of Biomedical Research Center of the Slovak Academy of Sciences, Bratislava, Slovakia (Ethics Committee Statement No. EK/BmV-02/2020).

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