Emergence of SARS-CoV-2 variant B.1.575.2 containing the E484K mutation in the spike protein in Pamplona (Spain) May-June 2021

Running title: Emergence of B.1.575.2 SARS-CoV-2 lineage in Pamplona (Spain)

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Abstract

With the emergence of new SARS-CoV-2 variants and the acquisition of novel mutations in exiting lineages, the need to implement methods capable of monitoring viral dynamics arises. We report the emergence and spread of a new SARS-CoV-2 variant within B.1.575 lineage containing the E484K mutation in the spike protein (named B.1.575.2) in a region of Northern Spain between May and June 2021. SARS-CoV-2 positive samples with cycle threshold value less than or equal to 30 were selected to screen of presumptive variants using the TaqPath™ COVID-19 RT-PCR kit and TaqMan™ SARS-CoV-2 Mutation Panel. Confirmation of variants was performed by whole genome sequencing. Of the 200 samples belonging to the B.1.575 lineage, 194 (97%) corresponded to the B.1.575.2 sub-lineage, which was related to the presence of the E484K mutation. Of 197 cases registered in GISAID EpiCoV database as lineage B.1.575.2, 194 (99.5%) were identified in Pamplona (Spain).

This report emphasizes the importance of complementing surveillance of SARS-CoV-2 with sequencing for the rapid control of emerging viral variants.

Introduction

During the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, several variants were catalogued as variants of concern (VOC) and variants of interest (VOI) by the European Centre for Disease Prevention and Control has emerged in different countries. As of June 23, 2021, the four important lineages with evidenced impact on transmissibility, severity and immunity are lineage B.1.1.7 (Alpha), B.1.351 (Beta), B.1.617.2 (Delta), and P.1 (Gamma) (1-4). Lineages B.1.351 and P.1 were of specific concern because they present the spike mutation E484K, which has been associated with the reduced neutralizing activity of antibodies and may be associated
with the reduced efficacy of the vaccine (2,3,5,6). Initially, the B.1.1.7 lineage had mutations N501Y and D614G and the characteristic ΔH69/ΔV70 deletion in the spike protein; however, in early 2021, Public Health England reported the first B.1.1.7 SARS-CoV-2 cases that had acquired the E484K mutation (3,7).

In this regard, concerns about the emergence of new SARS-CoV-2 variants and the acquisition of new mutations in existing lineages, such as the accumulation of mutations in the spike gene in B.1.1.7, have been developing since the onset of the pandemic.

The lineage B.1.575 emerged in the United States and since its emergence, two new sub-lineages have been identified. The B.1.575.1 classified in Phylogenetic Assignment of Named Global Outbreak (PANGO) Lineages such as Spanish sub lineage of B.1.575 with spike mutations P681H, S494P and T716I and the B.1.575.2 lineage also originating in Spain whose main characteristic is the presence of the E484K spike mutation (8).

This study identified the emergence and spread of the E484K spike mutation within the SARS-CoV-2 B.1.575.2 lineage that has increased in the circulating virus population in Pamplona (Spain) between May and June 2021. Additionally, we share our experience with the prospective surveillance of novel SARS-CoV-2 variants by implementing a two-step laboratory strategy: reverse transcription quantitative real-time polymerase chain reaction PCR (RT-qPCR) screening and whole genome sequencing.

Materials and methods

The Microbiology Department of the Complejo Hospitalario de Navarra, located in Pamplona, the capital city of Navarra (Spain), is the reference laboratory of the public health system for SARS-CoV-2 (approximately 650,000 inhabitants). Upper respiratory specimens for SARS-CoV-2 detection are routinely collected at the hospital and primary care centers, and processed by commercial RT-qPCR methods. Since the end of
2020, when variant B.1.1.7 became predominant in the United Kingdom, prospective sample-based surveillance has been conducted in our community to identify novel emerging SARS-CoV-2 variants. A two-step laboratory procedure included all positive SARS-CoV-2 samples from hospital patients and community settings with a cycle threshold (Ct) less or equal to 30. Occasionally, targeted samples are also included according to epidemiological criteria.

Screening of presumptive SARS-CoV-2 variants carrying ΔH69-ΔV70 deletion was performed using the TaqPath™ COVID-19 RT-PCR kit (Thermo Fisher Scientific, USA) following the manufacturer’s instructions. Then, all those samples non-B.1.1.7 variants were subjected to a second RT-qPCR, TaqMan™ SARS-CoV-2 Mutation Panel (Thermo Fisher Scientific, USA). At that moment, we customed TaqMan assay for detecting SARS-CoV-2 spike protein with the N501Y, E484K, K417N, K417T mutations. All samples were sequenced.

Whole-genome sequencing was performed using Illumina COVIDSeq Test (Illumina Inc, USA) on the Illumina NovaSeq 6000 located in the public company NASERTIC, following the manufacturer’s instructions. The viral lineages classifications were performed by the Global Initiative on Sharing Avian Influenza Data (GISAID) ([https://www.gisaid.org/](https://www.gisaid.org/)) [GISAID EpiCoV] database, Nextstrain ([https://nextstrain.org/](https://nextstrain.org/)) [Nextstrain] and Phylogenetic Assignment of Named Global Outbreak (PANGO) Lineages ([https://cov-lineages.org/](https://cov-lineages.org/)) [Pango] (9-11).

**Results**

A total of 4,728 SARS-CoV-2 genomes have been sequenced in Navarra until the 1st of August 2021. Our sequencing analysis identified 200 samples (4.2% out of the total) related to the B.1.575 lineage: four (2%) B.1.575, two (1%) B.1.575.1 and 194 (97%) B.1.575.2. Among the common substitutions present in these lineages, four occurred in
the spike protein (S494P, D614G, P681H, T716I) (12). All samples showed a gene S
positive (not carrying ΔH69-ΔV70 deletion) in TaqPath. In TaqMan, all samples
identified by sequencing as B.1.575.2 showed the E484K mutation.

The first case with B.1.575 lineage to be identified in Pamplona dates back to January
20, 2021; since that date, no other case was identified until March 15, 2021, where three
isolates showing mutations common to the B.1.575 lineage were recorded.

Between weeks 20 to 26 of 2021, we identified 194 cases with lineage B.1.575, which
had acquired another S mutation, E484K, classified in the GISAID EpiCoV and
Pangolin databases as the sub-lineage B.1.575.2. The first case with B.1.575.2 lineage
was identified in a sample isolated on May 19 (week 20, 2021), and the number of cases
grew up to 48 cases in weeks 23 and 24 and declined suddenly at the end of June due
to the Delta (B.1.617.2) variant emergence (Figure 1). This variant is more infectious
and is leading to increased transmissibility when compared with other variants.

Nowadays Delta is the predominant variant in Spain. The beginning of the outbreak was
detected in a car repair shop located in a district of Pamplona. These cases could be
related to another more significant outbreak of variant B.1.575.2, which was found in a
mosque. Since these first cases, the variant has spread throughout Pamplona and its
surroundings without affecting the rest of Navarra. The median age of patients was
33±17 years old, 43.8% women, 56.2% men and approximately 50% Arabic origin.

Eighty-two (42.3%) patients acquired the infection at domiciliary ambit, the most
common cause. Only 14 (7.2%) acquired it at the workplace. One hundred and fifty-six
(80.4%) patients showed symptoms, and only four (2.1%) were admitted to hospitals,
no one suffered a severe form of the disease. Twelve (6.2%) of patients have been fully
vaccinated for COVID-19, six (3.1%) two doses of Comirnaty (BNT162b2 mRNA,
BioNTech-Pfizer, Mainz, Germany/New York, United States (US)) and a dose of
Janssen vaccine (Ad26.COV2-S, Janssen-Cilag International NV, Beerse, Belgium). Twenty-four (12.4%) were partially vaccinated with Comirnaty, Spikevax (mRNA-1273, Moderna, Cambridge, US) or Vaxzevria (ChAdOx1 nCoV-19, Oxford-AstraZeneca, Cambridge, United Kingdom) (Table 1). Among fully vaccinated patients most of them (9/12, 75%) showed symptoms. To know the distribution of SARS-CoV-2 B.1.575 lineage, we searched in the GISAID EpiCoV and PANGO lineages databases. From May to July, the lineage and sub-lineages of B.1.575 have increased exponentially in different countries. The B.1.575 lineage was predominant in the United States of America (90%), while the B.1.575.1 and B.1.575.2 sub-lineages dominated Spain with 86% and 92%, respectively. The B.1.575.2 sub-lineage was predominant in Navarra since 99.5% (194/197) of the cases registered in the GISAID EpiCoV database were identified in this region. By contrast, we did not identify any genomes with B.1.575 and B.1.575.1 lineage carrying the E484K mutation.

Conclusions

In this study, we observed the emergence of a lineage B.1.575.2 to acquire the spike E484K mutation circulating in Pamplona associated an outbreak. Pamplona is a small city located in the north of Spain, near to France and it could serve as a spread model for other cities in the world. The new lineage displayed a low prevalence (4.10%) among SARS-CoV-2 genomes analyzed between March 23, 2020, and June 30, 2021. Still, it is already dispersed in our city and comprises 97% of the B.1.575 sequences detected in that period. The E484K mutation is considered one of the most important substitutions associated with reduced antibody neutralization potency and efficacy of the SARS-CoV-2 vaccine (13-15). The E484K mutation has been identified in SARS-CoV-2 variants considered VOC such as B.1.351, P.1 and B.1.1.7+E484K and in VOI
variants such as B.1.525, B.1.620, and B1.621 among others (1-3), so the presence of
this mutation should be supervised and monitored.
Screening PCR is a useful tool for detecting mutations, mainly because of its rapidity.
Future identifications with this method could include new mutations characteristic of
the lineage could serve as a rapid method of variant identification. However, whole
genome sequencing remains the gold standard technique for pandemic control.
In our knowledge, this is the first study that describes the emergence of the lineage
B.1.575.2. This genetic variation includes a mutation in the spike protein (E484K). This
SARS-CoV-2 genetic variation was discover in Pamplona associated an outbreak,
demonstrating how important is the genetic sequencing, especially when it comes to
new community outbreaks.
This brief report emphasizes the importance of exhaustive surveillance for circulating
variants of the SARS-CoV-2 virus to reduce community transmission, asses the
COVID-19 vaccine effectiveness and prevent the emergence of more transmissible
variants that could further increase the severity of the epidemic in the country.

Conflict of interest
The authors declare no conflict of interest.

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Access to data
All genomes generated in this work were deposited in the GISAID EpiCoV database (http://gisaid.org).

**Author contributions**

CTS, AM, MEP, MFH AN, CE conceived and designed the study. GRO was responsible for the whole genome sequencing interpretation. PSS, PLM, JC provided epidemiology data. CTS wrote the manuscript, and all authors critically revised the manuscript. All authors approved the final version of the manuscript and were accountable for its accuracy.
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Table 1. Characteristics of the patients with B.1.575.2 lineage included in the study.

Figure 1. Timeline of SARS-CoV-2 B.1.575, B1.575.1 and B.1.575.2 linages emergence in Pamplona between January and June 2021.
Table 1. Characteristics of the patients with B.1.575.2 lineage included in the study.

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