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TITLE PAGE

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Spreading of a new SARS-CoV-2 N501Y spike variant in a new lineage

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ABSTRACT

Objectives: Our surveillance of the SARS-CoV-2 genomic epidemiology led us to detect several variants since summer 2020. We report the recent spread of a new SARS-CoV-2 spike 501Y variant.

Methods: SARS-CoV-2 sequences obtained from human nasopharyngeal samples by Illumina next-generation sequencing were analyzed using Nextclade and an in-house Python script and compared using BLASTn to the GISAID database. Phylogeny was performed using the IQ-TREE software.

Results: We identified that SARS-CoV-2 genomes from four patients diagnosed in our institute harbored a new set of amino acid substitutions including L18F;L452R;N501Y;A653V;H655Y;D796Y;G1219V±Q677H. These spike N501Y genomes are the first of Nextstrain clade 19B. We obtained partial spike gene sequences of this genotype for an additional 43 patients. All patients infected with this genotype were diagnosed since mid-January 2021. We detected 42 other genomes of this genotype in GISAID, which were obtained from samples collected in December 2020 in four cases and in 2021 in 38 cases. The 89 sequences obtained in our institute or other laboratories originated from the Comoros archipelago, Western European countries (mostly metropolitan France), Turkey and Nigeria.

Conclusion: These findings warrant further studies to investigate the spread, epidemiological and clinical features, and sensitivity to immune responses of this variant.

TEXT

Introduction

SARS-CoV-2 has now spread worldwide for >1 year (<https://www.ecdc.europa.eu/en/geographical-distribution-2019-ncov-cases>). The emergence of major viral variants expanded dramatically since summer 2020 although this only recently came into the spotlight. Thus, we have implemented since SARS-CoV-2 occurrence in France in late February 2020 a surveillance based on their genomes obtained by next-generation sequencing [1]. During summer 2020 we detected the emergence of 10 viral variants, concomitantly with the re-increase of SARS-CoV-2 diagnoses [2]. These variants have been since then responsible for juxtaposed or successive epidemics and accounted in various proportions over time for the total burden of cases. Since January 1st, 2021, we also performed a systematic screening of variants that predominated or arose in our geographical area with variant-specific in-house qPCR assays (<https://www.mediterranee-infection.com/procedure-pour-la-detection-par-qpcr-des-variants-sars-cov-2-marseille-4/>; <https://www.mediterranee-infection.com/procedure-pour-la-detection-par-qpcr-des-variants-sars-cov-2-n501y/>). These variants include the Marseille-4 variant (Nexstrain clade 20A.EU1) [3], which has been the most prevalent one between August 2020 and January 2021, and the rapidly-spreading variants 20I/501Y.V1, 20H/501Y.V2, and 20J/501Y.V3, which harbor in their spike the amino acid (aa) substitution N501Y [4]. We describe herein the first SARS-CoV-2 spike 501Y variant belonging to Nextstrain clade 19B.

Materials and methods

SARS-CoV-2 genomes were obtained directly from nasopharyngeal swab fluid by next-generation sequencing (NGS) with the Illumina Nextera XT paired-end strategy on a

MiSeq instrument (Illumina Inc., San Diego, CA, USA), as previously described [5]. Genome consensus sequences were generated through mapping on the SARS-CoV-2 genome GenBank accession no. NC_045512.2 (Wuhan-Hu-1 isolate) with the CLC Genomics workbench v.7. In addition, sequences of the spike gene 5'-region (nucleotides 1-1,854 in reference to NC_045512.2) were obtained by NGS after PCR amplification with an in-house protocol (Supplementary Material Data). Sequences were analyzed using Nextclade (<https://clades.nextstrain.org/>) [6] and an in-house Python script. Other SARS-CoV-2 sequences of this spike 501Y clade 19B genotype were searched using BLASTn [7] among all 674,762 sequences available in the GISAID database (<https://www.gisaid.org/>) [8] as on March 3rd, with genomes obtained in our institute as queries. Phylogeny reconstruction was performed using the IQ-TREE software with the GTR Model and 1,000 ultrafast bootstrap repetitions after alignment of genomes using MAFFT v.7; the tree was visualized with the iTOL (Interactive Tree Of Life) software [11,12,21]. SARS-CoV-2 culture was performed by inoculating nasopharyngeal samples on Vero E6 cells, as previously described [9].

Ethics

Data have been generated as part of the routine work at Assistance Publique-Hôpitaux de Marseille (Marseille university hospitals). This study has been approved by the ethics committee of our institution (N°2020-016-03). According to European General Data Protection Regulation No 2016/679, patients were informed of the potential use of their medical data and that they could refuse the use of their data. The analysis of collected data followed the MR-004 reference methodology registered under No. 2020-151 and No. 2020-152 in the AP-HM register.

Results

Our SARS-CoV-2 genomic surveillance allowed us to detect in four patients SARS-CoV-2-diagnosed in our institute a variant harboring a new combination of eight mutations in the spike protein. These mutations include aa substitution N501Y, associated with aa substitutions L18F, L452R, A653V, H655Y, D796Y, and G1219V, and a synonymous mutation at nucleotide position 22,468 [in reference to NC_045512.2]; Figures 1a, 1b]; an eighth spike aa substitution, Q677H, was identified in one of these genomes. Other nucleotide or aa substitutions or deletions are present in 14 other proteins; these changes notably include deletions in ORF3a and ORF8 genes (Figure 1a). Overall, 24-31 nucleotide substitutions, 14-19 aa substitutions and three deletions are present in these four SARS-CoV-2 genomes (Figure 1a; GISAID accession numbers: EPI_ISL_1097023; EPI_ISL_1097024; EPI_ISL_1201045; EPI_ISL_1201046). Viral isolates were obtained by culturing the four patients' nasopharyngeal sample, which will allow further analyses of the phenotypic features of this variant. In addition, sequences that span codons 1-618 of the spike gene and allow the identification of this variant by covering 4 of its hallmark mutations (C21614T (substitution L18F), G22468T, T22917G (L452R), A23063T (N501Y)) and by being devoid of the Nextstrain clade 20 A23403G mutation, were obtained for 43 other patients (deposited at: <https://doi.org/10.35081/43r6-sz33>).

We first detected in our institute this variant we named Marseille-501 in a patient sampled on January 18th, 2021, and its number increased from week 5 (Supplementary Material Figure S1). During the January 18th-February 23rd period (37 days), SARS-CoV-2 genotype was obtained by sequencing for 1,016 patients. We identified in the GISAID database 42 other SARS-CoV-2 genomes, all from humans, with the same spike aa substitution set (Figure 2). Eight originated from Mayotte, a French overseas department in Comoros archipelago. The others originated from France (n=22), Denmark (n=2), Netherlands (n=2), Belgium (n=2), England (n=1), Turkey (n=4), and Nigeria (n=1). The

most ancient samples were collected mid-December in Denmark and late December in the Netherlands. All 38 other SARS-CoV-2 genomes were obtained from samples collected in 2021 (Supplementary Material Figure S1).

Phylogenetic analysis conducted with the four genomes from our institute and the 42 other GISAID genomes showed that all 46 sequences were clustered and delineated a strongly supported (bootstrap value: 100%) 19B subclade that stands apart from other clade 19B genomes (Figure 2). Moreover, 21 genomes, one from our institute, the 8 ones from Mayotte, and 12 other genomes from France or Turkey were clustered and all harbored the spike Q677H substitution.

The 47 patients we diagnosed as infected with this variant had a mean age of 39 ± 19 years (range=16-92); 26 (55%) were male. Epidemiological and/or clinical characteristics were available for 12 patients (Supplementary Table S1): three patients had travelled to or originated from the Comoros, another one originated from Guinea-Conakry but had not travelled abroad recently (Supplementary Table S1). Clinical symptoms were available for 9 patients: 4 were asymptomatic and 34 (85%) had mild symptoms including fever, rhinorrhea, cough, headache, asthenia, or myalgia. A 94-year-old male patient developed SARS-CoV-2-associated hypoxemic pneumonia 4 days after the second administration of the Pfizer/BioNTech vaccine and died 7 days later. Another patient with severe obesity was admitted in intensive care unit.

Discussion

We report herein the spread of the first SARS-CoV-2 501Y variant belonging to clade 19B. Its spike protein harbors several aa substitutions recently reported to emerge in various lineages and/or to be associated with immune escape (L18F, L452R, N501Y, H655Y, Q677H) (Supplementary Material Data). For instance, N501Y is part of several variants that

belong to distinct lineages and have been detected worldwide in association with various combinations of mutations [4], while L18F has been reported in 20E strains in England, in a 20I/N501Y.V1 substrain with an increased replicative fitness, in 20H/501Y.V2 strains with a faster propagation in presence of convalescent plasma, and in a majority of 20J/501Y.V3 variants [10].

It is currently unresolved how such strains harboring new blocks of mutations in the spike emerge. Overlooked genome evolution following transmission to minks and back to humans [3], or promotion and selection of mutations during administration of remdesivir or convalescent plasma [11] have been suspected. Also, recombinations known to be common amongst coronaviruses may occur between SARS-CoV-2 strains [12]. Anyway, this new 501Y variant is another example that same aa substitutions can occur in distinct lineages and follow convergent evolution [4]. Epidemiologically, this new variant infected patients in diverse countries, but with a predominance in the Comoros archipelago (11/47 documented cases) and in metropolitan France. Based on limited data available, it is unknown if this new variant is more or less transmissible compared to other strains and associated with any particular clinical feature.

Overall, these data highlight the need for genomic epidemiology surveillance of SARS-CoV-2 strains. The incidence of this new variant and its epidemiological and clinical features deserve being closely monitored. Moreover, considering its particular association of numerous spike aa substitutions, its sensitivity to neutralizing antibodies and to plasma from convalescent or vaccinated persons should be investigated, which will be done for the isolates cultured in our institute.

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Author contributions

Conceived and designed the experiments: PC, DR. Contributed materials/analysis tools: PC, AL, JD, LP, PD, CD, PEF, BLS, JCL. Analyzed the data: PC, AL, JD, JCL, BLS, DR. Wrote the paper: PC, DR.

Transparency declaration

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FIGURE LEGENDS

Figure 1. Map of the nucleotide and aa substitutions and deletions of the new variant along the SARS-CoV-2 genome (a) and three-dimensional structure of the spike protein showing aa substitutions and deletions in various spike N501Y variants (b)

a. Genes that harbor mutations are indicated by a red frame.

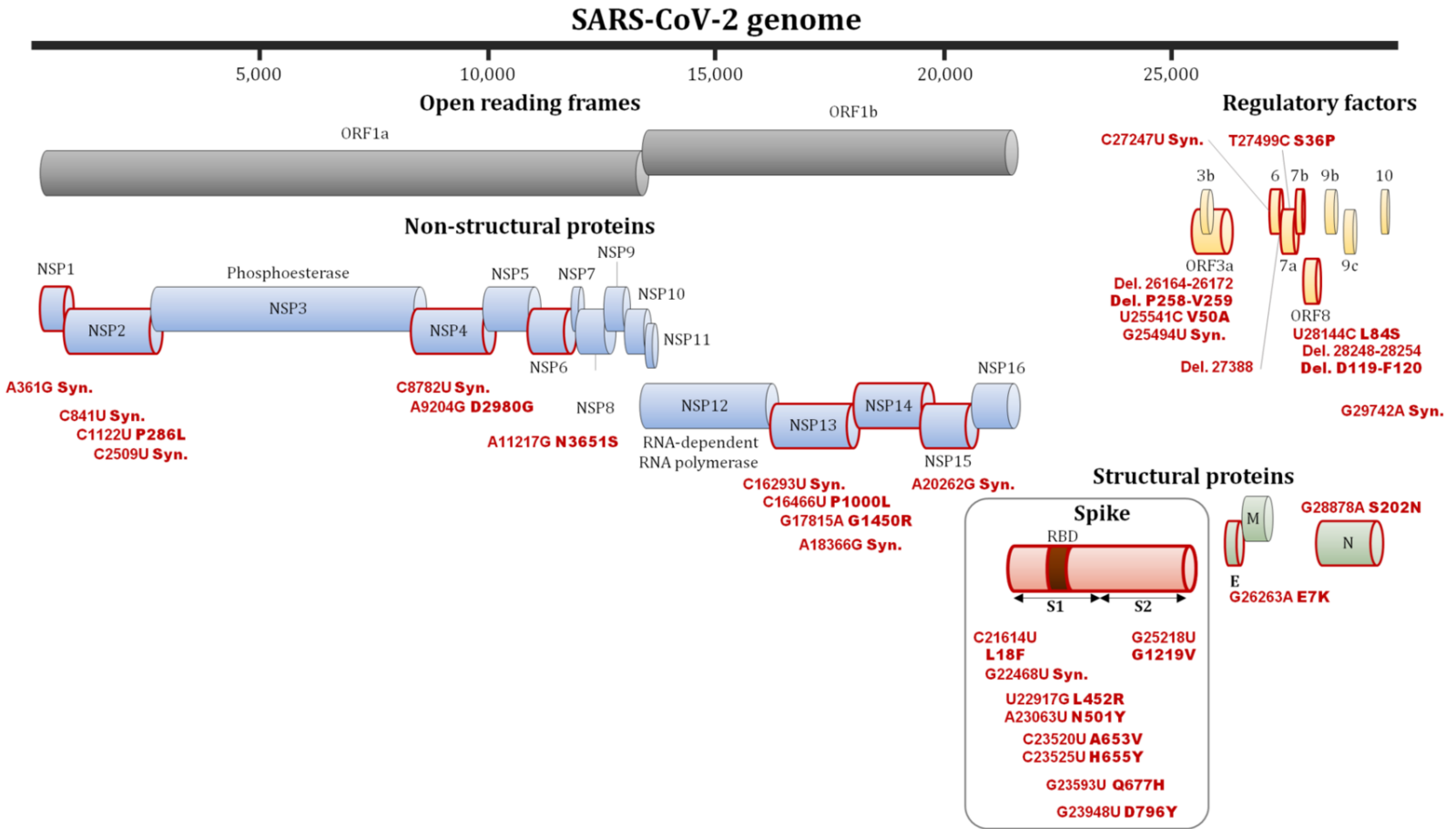
b. See supplementary Material Methods for structure prediction.

Figure 2. Phylogenetic analysis of the SARS-CoV-2 full-length genomes of the new variant recovered from the IHU Méditerranée Infection sequence database (doi) and from the GISAID sequence database.

The SARS-CoV-2 phylogenetic tree is based on the full-length SARS-CoV-2 genomes. The SARS-CoV-2 sequences obtained by next-generation sequencing from four patients are indicated by a white bold font and a dark blue background. The sequences with the highest BLAST scores recovered from the NCBI GenBank nucleotide sequence databases (<http://www.ncbi.nlm.nih.gov/nucleotide/>) including the 42 that are clustered with these four query sequences, were incorporated in the phylogeny reconstruction, being indicated by a dark blue bold font. Additional sequences included the genome of the Wuhan-Hu-1 isolate and genomes obtained in our institute and classified as predominant variants. The 67 genomes of SARS-CoV-2 were aligned by using MAFFT version 7 with default parameters (Nguyen et al.2015). Phylogenetic tree was performed by using iQ-TREE under the GTR model and visualized with iTOL [13-15].

GISAID accession numbers: IHUCOVID-3234: EPI_ISL_1097023; IHUCOVID-3151: EPI_ISL_1097024; IHUCOVID-3377: EPI_ISL_1201045; IHUCOVID-3446: EPI_ISL_1201046.

Fig. 1.
a.



b.

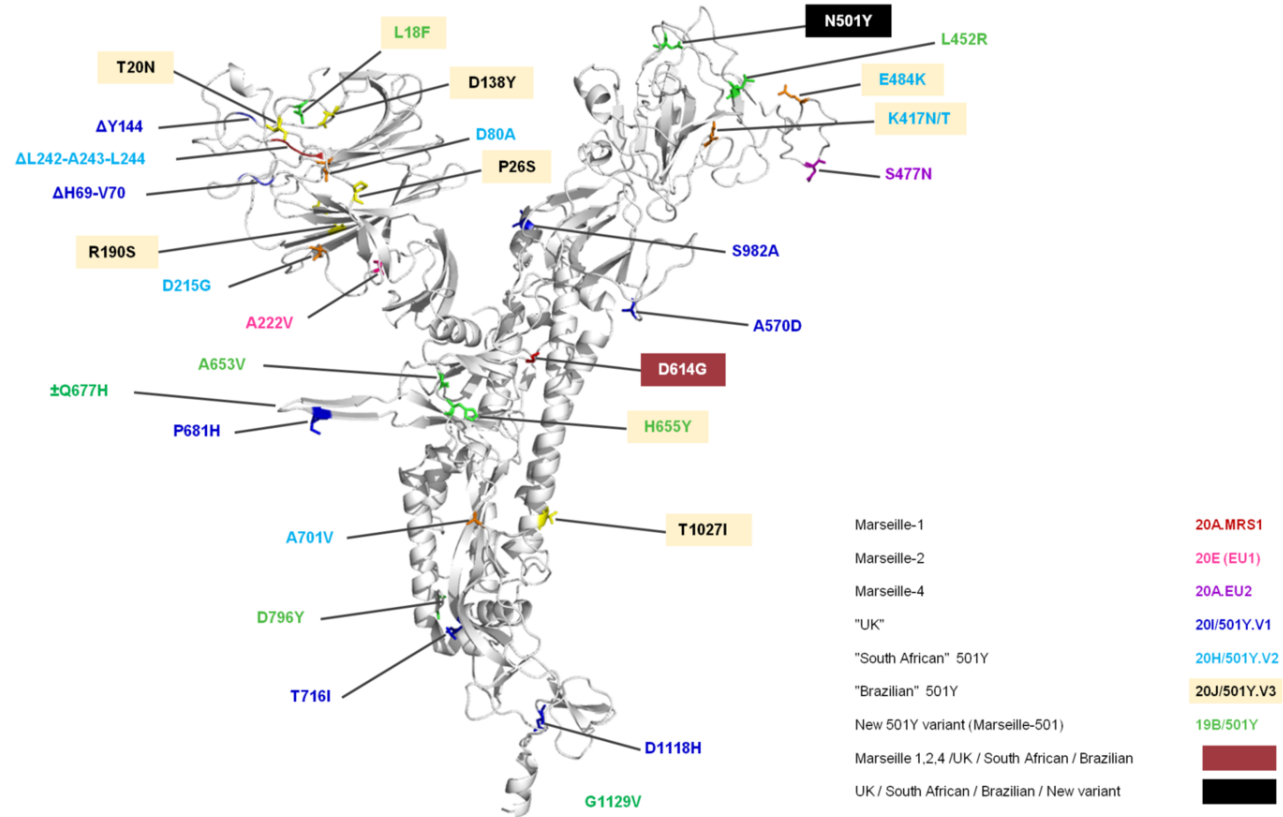


Fig. 2

Tree scale: 0.001

