Brief Report

Limited spread of a rare spike E484K-harboring SARS-CoV-2 in Marseille, France

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ABSTRACT

We detected SARS-CoV-2 of Pangolin lineage R.1 in three patients that harbor spike substitution E484K but had limited spread. Eleven other sequences in France and 8,831 worldwide were available from GISAID, 92% originating from Japan. The 3 genomes from our institution were phylogenetically closest to a genome from Guinea-Conakry, where one of the patients had travelled. These viruses did not exhibit particularity in culture. Spike structural predictions found a 1.3-times higher transmissibility index than for the globally spread B.1.1.7 variant, but affinity loss for gangliosides that might have slowed dissemination. The spread of new SARS-CoV-2 mutants/variants remains little understood.

The diversity of SARS-CoV-2 has expanded considerably since the first detection of this virus in December 2019 in China. Several new SARS-CoV-2 variants and mutants have been classified as of concern regarding their transmissibility, pathogenicity and potential escape to immune responses elicited by infection or vaccine immunization [1,2]. They include viruses of Pangolin lineages B.1.351 (WHO Beta variant) and B.1.1.28/P.1 (Gamma variant) that have spread worldwide, and of the less prevalent lineages B.1.525 (Eta variant) or B.1.1.345, which all harbor substitution E484K in their spike protein [3-5]. This substitution, located in the receptor-binding domain (RBD) of the spike, has been reported to decrease sensitivity to monoclonal neutralizing antibodies and convalescent plasma in several studies [5-8]. We describe here three infections with rare E484K-harboring SARS-CoV-2 that harbor 3 other amino acid substitutions in the spike, including W152L, which is located in the N-terminal domain (NTD) and possibly reduce sensitivity to neutralizing antibodies [9], and D614G and G679V, located in the S2 subunit. Ten additional amino acid substitutions were found in these three genomes, including 9 and 1 in the nucleocapsid and membrane protein (**Figure 1A**).

We classified SARS-CoV-2 from these three patients as Marseille-484K.V1 according to the nomenclature we have implemented locally to ease our monitoring and analyses of the epidemics. Also, they were classified into GISAID [10] clade GR, Nextstrain clade 20B [11], and Pangolin lineage R.1 [12]. The three Marseille-484K.V1 genomes were obtained in our institute from respiratory samples collected between February 11 and April 19, 2021. They accounted for 0.04% of the 8,417 SARS-CoV-2 sequences we deposited in GISAID as of June 17, 2021. As of this date, 8,831 sequences of the Pangolin lineage R.1 were available from the GISAID database (https://www.gisaid.org/) [10]. They were obtained from patients sampled between February 1, 2020 and May 28, 2021, but only four sequences were from samples collected before October 24, 2020 (**Figure 1B**). The 8,831 sequences originated from 34 countries on 5 continents, but 99% originated from 13 countries and 92% originated from Japan (5,890 sequences, 67%) or the USA (2,250 sequences, 26%). In Japan, all but four sequences were retrieved from samples collected between the November 30, 2020 and May 17, 2021 [13,14]. This SARS-CoV-2 lineage was also involved in 46 cases in skilled nursing facilities in March 2021 in Kentucky, USA [15]. In this outbreak, efficacy of immunization with the BioNTech mRNA vaccine against SARS-CoV-2 infection was estimated to be 66% and 76% among the residents and vaccinated health-care personnel, respectively, and four reinfections (two laboratory-confirmed infections separated by >90 days) were reported, including one in a resident who eventually died. In France, only 11 other cases were detected, and together with our three genomes, they accounted for 0.03% of 48,137

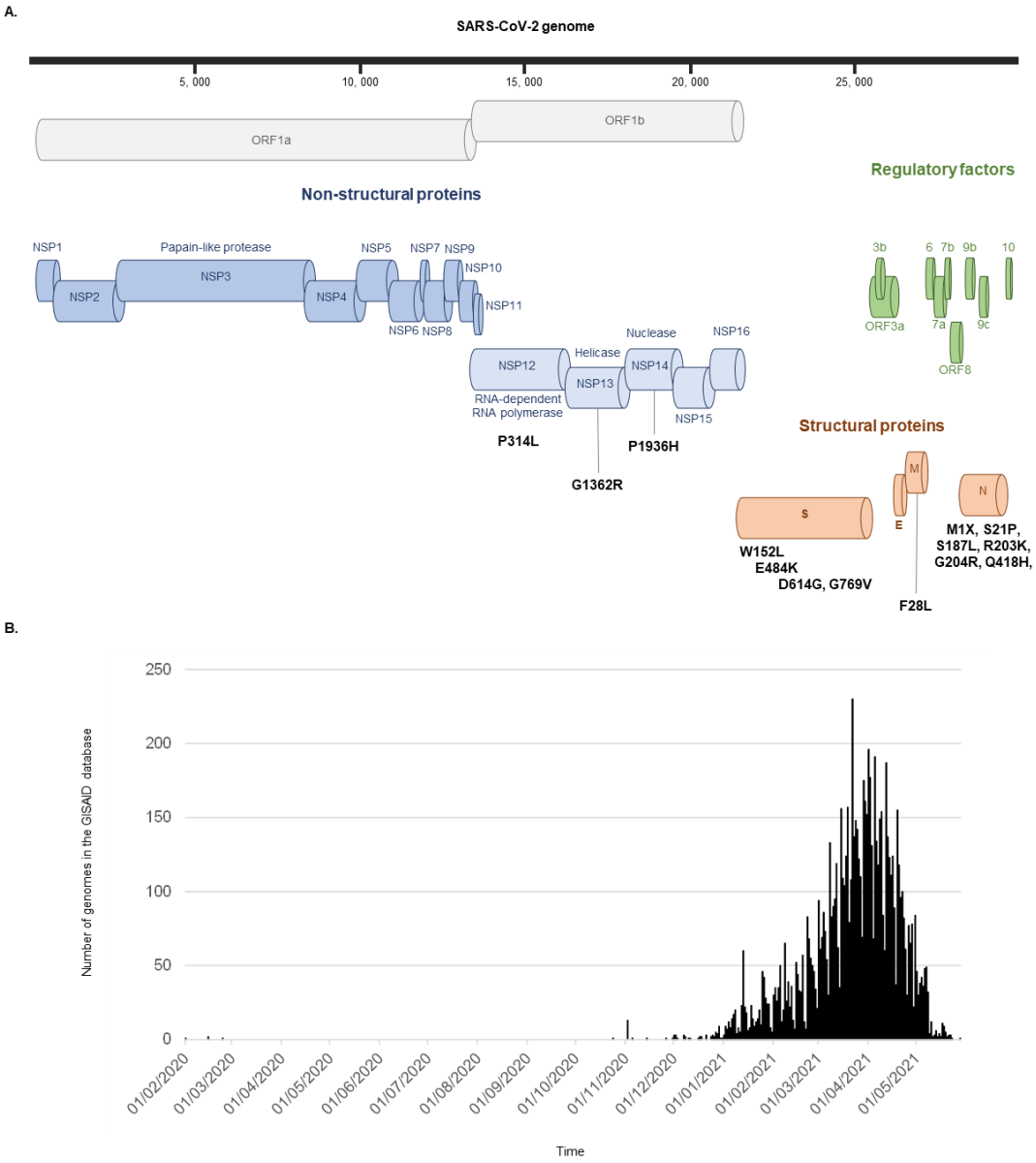

Figure 1. Map of the SARS-CoV-2 genome (A) and number of sequences classified in Pangolin lineage R.1 in the GISAID database (B). The numbers of sequences classified in Pangolin lineage R.1 have been collected from the GISAID database (https://www.gisaid.org/) [10] as of June 17, 2021.

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Phylogenetic analysis of the 3 Marseille-484K.V1 sequences obtained in our institute showed that they were clustered (bootstrap value, 80%) with a genome originating from Guinea-Conakry that was their best BLAST hit in the GISAID database [10] (**Figure 2**). The three patients were women between 24 and 55 years old. One of them reported that she had returned from a trip in Guinea-Conakry. None of the three patients were hospitalized. Culture isolation was performed as previously described [16] by inoculating the respiratory samples from two of the three patients on Vero E6 cells. Cycle threshold values for the qPCR was 15 and 20 for the two inoculated samples. A cytopathic effect was observed after 5 days during the first passage, and no particular phenotypic features were noted, including compared with viruses isolated during the period March-April 2020 [16].

Structural analysis of the Marseille-484K.V1 spike protein showed that amino acid substitutions G679V and D614G are at the same height in the spike protein, in a region considered to play a role in the conformational change required for demasking the RBD in the open state of the trimeric spike (**Figure 3A)**. Interestingly, the surface potential of the RBD was markedly increased by substitution E484K (**Figure 3B**), suggesting a kinetic advantage for this virus for accessing the electronegative surface of the ACE-2 receptor. The electrostatic surface potential of the NTD was also increased by mutation W152L, but by an indirect mechanism of compaction of the domain that decreases the electronegative areas in favor of an enlarged electropositive surface. Although these changes

Fig. 2

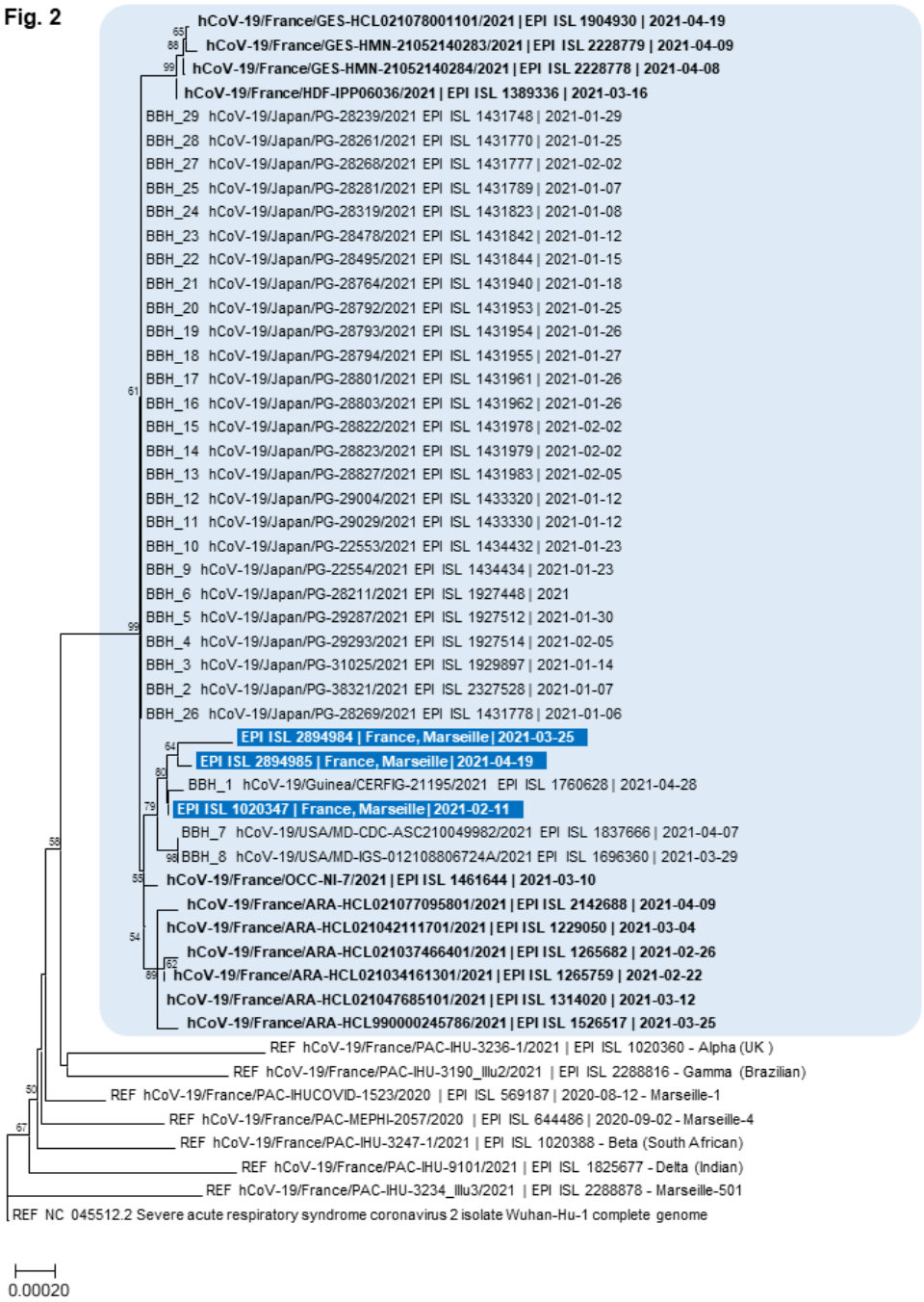

Figure 2. Phylogenetic analysis based on full-length genomes of the three Marseille-484K.V1 viruses. The SARS-CoV-2 phylogenetic tree is based on the full-length SARS-CoV-2 genomes. The 30 sequences with the highest BLAST scores recovered from the GISAID database (https://www.gisaid.org/) [10] were incorporated in the phylogeny reconstruction, being indicated by BBH (for best BLASTn hit) at the beginning of the sequence name. Additional sequences indicated by REF (for reference) at the beginning of the sequence name included the genome of the Wuhan-Hu-1 isolate and genomes obtained in our institute and classified as predominant SARS-CoV-2 variants. Nucleotide alignments were performed using MUSCLE software (http://www.ebi.ac.uk/Tools/msa/muscle/). Evolutionary history was inferred using MEGAX software (http://www.megasoftware.net/) using the neighbor-joining method and the Kimura 2-parameter method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree; the scale bars indicate the number of nucleotide substitutions per site. Bootstrap values > 50% are indicated on the tree.

Fig. 3

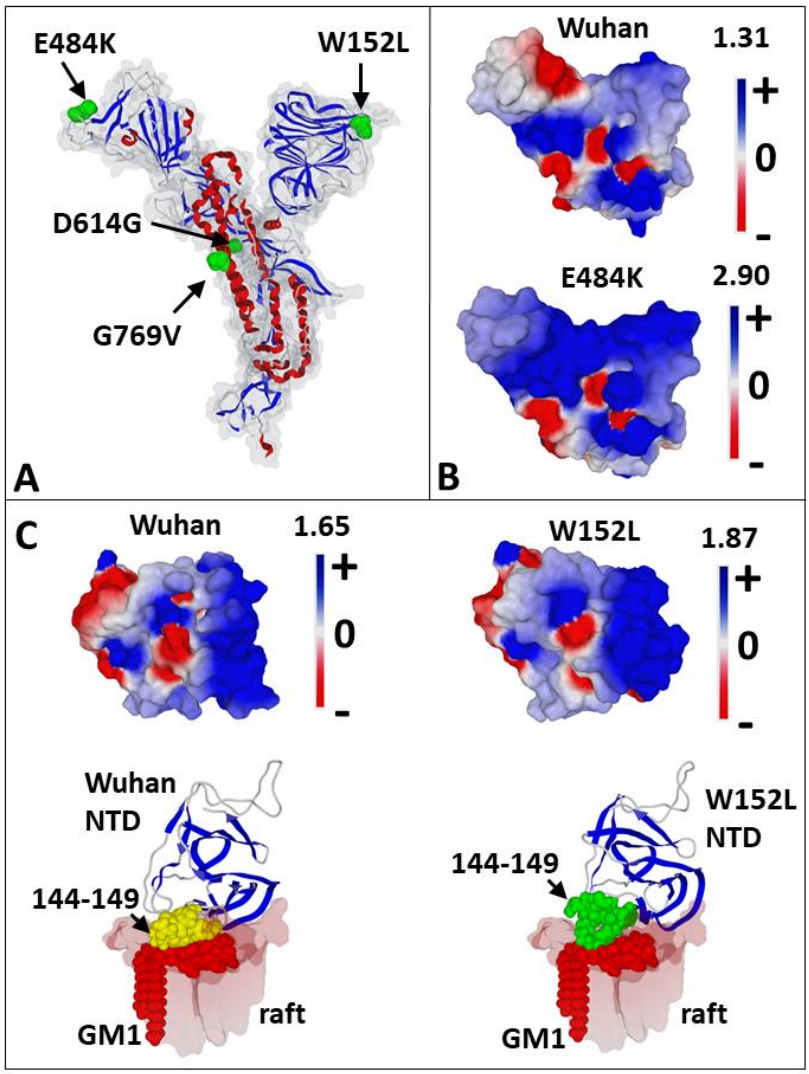

Figure 3. Structural analysis of the Marseille-484K.V1 virus. A. Localization of mutations by molecular modeling of the variant spike. **B.** Effect of the E484K mutation on the electrostatic surface potential of the RBD. The values indicate the estimation of the surface potential of the RBD region facing the host cell membrane, as determined by Molegro Molecular Viewer software. Blue regions are electropositive, red electronegative, and white neutral. **C.** Effect of the W152L mutation on the electrostatic surface potential of the NTD (upper panels). Molecular models of B.1 and mutant NTD binding to lipid rafts (GM1 gangliosides) are shown in the lower panels. Region 144-149 of the NTD is indicated in yellow (B.1 NTD) and green (Marseille_184).

in the surface potential of the RBD and the NTD may potentiate the attraction of viral envelope to lipid rafts containing ACE-2 [17], this kinetic advantage appeared to be tempered by a concomitant loss of affinity of the RBD for ACE-2 (ΔGmut/ΔGwt = 0.93) and of the NTD for lipid raft gangliosides (ΔGmut/ΔGwt = 0.90). Together with surface potential measurements of RBD and NTD surfaces, these affinity estimations obtained by molecular modeling approaches were used to calculate the transmissibility index (T-index) of the Marseille-484K.V1 according to our published protocol [18]. The T-index was 4.53, which is twice the T-index of the Wuhan-Hu-1 virus. In comparison, the T-index of the globally spread B.1.1.7 variant (a.k.a. Alpha variant) is 3.59, suggesting that Marseille-484K.V1 viruses could have been expected to disseminate more widely than observed. However, the loss of affinity of the W152L mutant for gangliosides is not anecdotical. Indeed, the large flat surface of the ganglioside-binding domain of the NTD [19] has a better geometric complementarity in the Wuhan B.1 strain than in the Marseille-484K.V1 viruses, as illustrated in **Figure 3C**. This may have contributed to slow the dissemination of these Marseille-484K.V1 viruses. However, immune neutralization and other host factors may also

explain why they did not outcompete other SARS-Cov-2 that circulated concomitantly.

This Marseille-484K.V1, or R.1 lineage, is another example of convergent evolution for amino acid susbtitution E484K, as also observed for substitutions L452R [20], N501Y [21], Q677H [22] or L18F [23] that occurred independently in various SARS-CoV-2 lineages and geographical areas. The low level of circulation of this variant in France and worldwide, with the notable exceptions of Japan and the USA, contrasts with the dramatic spread of other E484K-harboring viruses such as lineages B.1.351 and B.1.1.28/P.1. Viral culture did not reveal particularities, while structural analyses provided some hints to explain this seemingly low transmissibility. One should also consider the other SARS-CoV-2 that co-circulated and with which Marseille-484K.V1 viruses have competed. Taken together, previous findings show that, despite a tremendous number of epidemiological, genomic, proteomic, structural and culture studies carried out on SARS-CoV-2, the emergence, spread and outcome of new mutants and variants are little understood and therefore unpredictable.

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Declarations

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Conflicts of interest/competing interests

The authors have no conflicts of interest to declare. Funding sources had no role in the design and conduct of the study, collection, management, analysis, and interpretation of the data, and preparation, review, or approval of the manuscript.

Availability of data and material

SARS-CoV-2 genomes generated in our institute have been submitted to the GISAID sequence database (https://www.gisaid.org/) [10].

Code availability (software application or custom code)

Not applicable.

Author contributions

Conceived and designed the experiments: PC, JF, NY, DR, BLS. Contributed materials, data, analysis tools: PC, JF, NY, JD, AL, PEF, JCL, BLS. Analyzed the data: All authors. Wrote the paper: PC, JF. All authors approved the last version of the paper.

Ethics approval

Data have been generated as part of the routine work at Assistance Publique-Hôpitaux de Marseille (Marseille University Hospitals). This study was approved by the ethics committee of Hospital University Institute Méditerranée Infection (No. 2020-016-03).

Consent to participate and for publication

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 in the AP-HM register.

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