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LETTER TO THE EDITOR

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From Alpha to Omicron SARS-CoV-2 variants: What their evolutionary signatures can tell us?

Dear Editor,

Since the first detection of the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in 2019, the continuous interest in gaining insights into its epidemiology and evolution is widespread. Despite showing a proofreading system involving (but not limited to) the non-structural protein 14 exoribonuclease activity,[1] which characterizes an evolutionary pattern with a lower global mutation rate when compared with other RNA viruses, the SARS-CoV-2 has been also undergoing biologically relevant Spike's protein amino acid substitutions in different Variants of Interest (VOI) and Variants of Concern (VOC) as a result of convergent and directional evolution, which means that multiple and specific sites share identical mutations.[2]

Regardless of retrospective circulation in Europe (doi:10.1038/d41586-021-03610-3), the SARS-CoV-2 Omicron VOC was first detected in South Africa through the excellent work conducted by Dr. de Oliveira's research team, together with the Department of Health and scientists from the Network for Genomic Surveillance in South Africa (http://www.sun.ac.za/english/Lists/news/DispForm.aspx?ID=8785). With 35 non-synonymous reported mutations found in the Spike's protein amino acid, of which 15 on the receptor-binding domain (RBD) and 10 of those on the receptor-biding Motif (RBM), the concern about the potential increased spread and virulence of the Omicron variant is justified.

Noteworthily, with the exception of S371L substitution, in a span of 12 months (October 2020–2021), all other mutations in Spike's RBD and RBM had been already described and predicted to promote different phenotypic characteristics (Table 1). Further importance is given to the latter region (RBM) as it mediates the recognition to the human angiotensin-converting enzyme 2 receptors (ACE2) being, therefore, an important neutralizing antibody target[12,13] (Figure 1). It is also worth mentioning that the low frequency of some substitutions in many virus strains is different from the emergence of the combination of these substitutions in a particular strain.

We also found the additional Omicron Spike amino acid substitutions L141F, R346K, and V367F, also previously described.[3] The substitution L141F had not been cataloged by the World Health Organization (WHO) as present in this VOC. Furthermore, the substitution V367F is also not labeled by the WHO (i.e., "n/a") and, according to the European Centre for Disease Prevention and Control (ECDC) (https://www.ecdc.europa.eu/en/covid-19/variants-concern), it was first detected in the United Kingdom in December 2020. Moreover, the substitution R346K, classified by WHO as Mu variant, was first detected in Colombia in January 2021.

Additionally, we draw attention to the following additional mutations found in the Omicron variant such as the substitution G142D (Spike's N-terminal domain, under convergent evolution and present in Kappa and Delta variants), and P681H (Spike's C-terminal domain 2, furin cleavage site, present in Alpha, Gamma, Lambda, Mu and Theta variants),[2] both corresponding to important regions targeted by neutralizing antibodies.[14,15] The substitution Q498R appears to be epistatic to N501Y.[10] This in a general context somehow let us know: how little we are sequencing, how fast the virus evolves, and how far we are behind in bringing it to the end. Nevertheless, such SARS-CoV-2 evolutionary signatures show us a way forward.

Taking into account that SARS-CoV-2 variants carry homoplasy traits from independent evolution,[16] where the viral effective population size has earned identical site-specific mutations in the Spike protein (i); assuming that unidentified SARS-CoV-2 variants and sub-lineages carrying new as well as several previously described mutations are very likely to be circulating worldwide (ii) and considering a high vaccine coverage with two/booster-dose schedules (iii): should we expect a high number of severe infections from different SARS-CoV-2 variants?

Someway, it brings evidence that the currently available immunizers are effective against distinct SARS-CoV-2 variants and a series of further sub-lineages. Even considering a diminished vaccine efficacy, they can still be protective, as supported by a series of well-conducted studies through different vaccine strategies.[17–19]

To better understand the evolution of the SARS-CoV-2 Omicron variant, on December 11 we carried out some additional analysis by collecting whole-genome sequences ($n = 146$) from Hong Kong, Botswana, South Africa, Canada, Australia, Italy, Belgium, Israel, Austria, England, and Germany, which were downloaded from the Global Initiative on Sharing Avian Influenza Data-EpiCoV (GISAID-EpiCoV). Sequences from SARS-CoV-2 Alpha ($n = 1719$), Beta ($n = 5870$), Gamma ($n = 5$), and Delta ($n = 10\,133$) variants isolated from Africa were also included in the analysis. Data sets were filtered out (0% of degenerated bases) via the biopython-based software Sequence Cleaner (https://biopython.org/wiki/Sequence_Cleaner), aligned with the SARS-CoV-2 reference coding-sequence (NC_045512.2) through MAFFT v.7 (https://mafft.cbrc.jp/alignment/software/closelyrelatedviralgenomes.html) and edited by the UGENE v.38.1 platform.[20]

Subsequently, the sequences were submitted to the Datamonkey web-server for the Genetic Algorithm for Recombination Detection (GARD), Single Breakpoint (SPB), and Pairwise Homoplasy Index (PHI,

TABLE 1 Amino acid substitutions present in SARS-CoV-2 Spike (receptor-binding domain) Omicron variant

Substitutions	Span of time from the first date of publication (in months)	Publication month, year	References
S373P	0	Oct, 2020	Long et al.[3]
G496S[a]	5	Mar, 2021	Teng et al.[4]
G339D	6	Apr, 2021	Smaoui and Yahyaoui[5]
S375F	6	Apr, 2021	Chen et al.[6]
G446S,[a] **T478K**,[a] Q493-K,[a] and Y505H[a]	8	Jun, 2021	Verma and Subbarao[7]
K417N	8	Jun, 2021	Barton et al.[8]
N440K,[a] **S477N**,[a] and **N501Y**[a]	9	July, 2021	Gan et al.[9]
Q498R[a]	10	Aug, 2021	Zahradník et al.[10]
E484A[a]	12	Oct, 2021	Laurini et al.[11]

[a]Receptor-binding motif; In bold: shared substitutions in SARS-CoV-2 VOI and/or VOC.
Additional information: ECDC: https://www.ecdc.europa.eu/en/covid-19/variants-concern

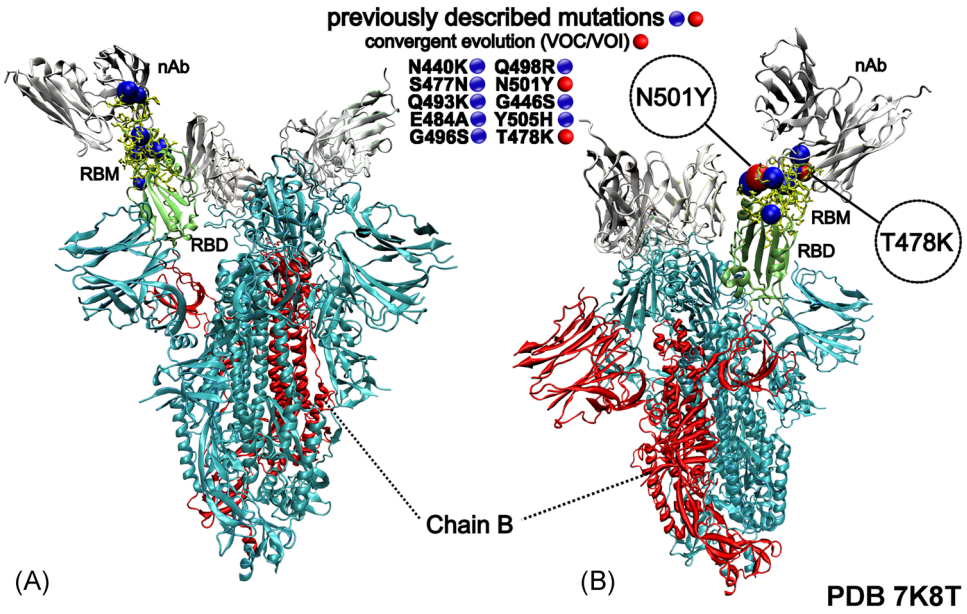

FIGURE 1 Three-dimensional structural representation of the SARS-CoV-2 Spike protein exhibiting the mutations present in Omicron variant receptor-binding motif (RBM). Blue and red spheres represent the RBM's substitutions. nAb, vaccine-induced neutralizing-antibody (silver); RBM, receptor-binding motif (yellow) and RBD, receptor-binding domain (green). The image was created with the Visual Molecular Dynamics (VMD) v.1.9.3 (http://www.ks.uiuc.edu/Research/vmd/)

SplitsTree v.4.17.0, using default settings, available at: http://www.splitstree.org/) algorithms. In silico evidence of recombination was found when only the sequences from the SARS-CoV-2 Beta, Delta, and Omicron VOC were aligned together, indicating that the variants cocirculation can enhance recombination events (Supporting Information). Remarkably, Beta, Delta, and Omicron are the most predominant variants circulating in Africa (https://www.bmj.com/content/375/bmj.n3013).

Finally, giving special emphasis to areas with high COVID-19 immunization coverage plus booster-dose schedules presenting a low ratio of serious diseases, hospitalization, and death from different SARS-CoV-2 variants (https://cdn.who.int/media/docs/default-source/immunization/covid-19/strategy-to-achieve-global-covid-19-vaccination-by-mid-2022.pdf); and through viral molecular evolution approaches, our hypothesis is that the currently

available immunizers from different technologies leveraged for vaccine production, taking into consideration the different levels of vaccines effectiveness, are highly probable to be effective against SARS-CoV-2 Omicron variant, which further increases the urgency of vaccination programs.

In view of the SARS-CoV-2 evolutionary signatures, it is also believed that such a proposition can be also addressed to other unidentified genetic lineages from the Greek alphabet which will prospectively label future SARS-CoV-2 variants. By providing useful insights into a linear vaccine perspective, we believe that these insights add important knowledge to the state of the art of the COVID-19 disease pandemic in general and to vaccine efficacy considerations in particular.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

DATA AVAILABILITY STATEMENT

Sequences downloaded are available as Supporting Information file.

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