

## First detection of SARS-CoV-2 spike protein N501 mutation in Italy in August, 2020

A new variant of SARS-CoV-2, known as VOC-202012/01, is spreading in the UK and is rapidly becoming a global threat.<sup>1,2</sup> VOC-202012/01 is characterised by multiple mutations in the spike protein. Among them, N501Y is of major concern because it involves one of the six key amino acid residues determining a tight interaction of the SARS-CoV-2 receptor-binding domain (RBD) with its cellular receptor angiotensin-converting enzyme 2 (ACE2).<sup>3</sup>

On Nov 10, 2020, a 59-year-old man with a history of SARS-CoV-2 infection persistence presented for molecular testing. Infection was laboratory confirmed; therefore, genetic characterisation of viruses detected in the sample collected in November (MB61-Nov) and in a previous sample collected in August (MB61-Aug) was done by metagenomic sequencing.<sup>4</sup> The two complete genomes were compared with full-length viral genomes available on GISAID (715 Italian isolates plus 1422 reference sequences representative of the global SARS-CoV-2 epidemic). EPI\_ISL\_717978 and EPI\_ISL\_728343 strains, carrying the mutation N501Y in the spike RBD, and recently characterised in Italy and in the UK as belonging to the rapidly emerging B.1.1.7 lineage, were also included in the analysis.<sup>2,5</sup> Sequence alignment and editing were done as previously described.<sup>6</sup>

Bioinformatic analyses showed that the MB61-Aug SARS-CoV-2 isolate had accumulated ten amino acid changes compared with early Italian

isolates, and three more had emerged along its evolution by the end of November (appendix). Phylogeny showed that the viral strains obtained from the patient belonged to the B.1.1 lineage and clustered together with strong support (bootstrap value 1.0). The time-stamped phylogeny was consistent with a persistent infection and accelerated viral evolution (appendix).

Compared with the Wuhan reference strain, both MB61 variants showed four mutations and one deletion in the spike protein, two of which were located within the RBD (appendix).

It is worth noting that the N501T substitution was detected in both MB61-Nov and MB61-Aug SARS-CoV-2 isolates, highlighting that a mutation at the critical amino acid residue 501 was already present in Italy in August, 2020. Our time-scaled maximum likelihood tree suggests that these spike N501T variants emerged in early August (95% highest posterior density early July to end of August) in northern Italy (appendix), and therefore that SARS-CoV-2 strains harbouring a substitution at position 501 might have circulated unnoticed even before the end of September, 2020, when the rapidly emerging B.1.1.7 lineage (carrying the N501Y mutation) was first reported.<sup>2</sup> Interestingly, differently from VOC-202012/01, the MB61 variants showed a second mutation within the RBD at the amino acid position Q493K that, together with N501T, might alter the binding affinity of the spike protein to the ACE2 receptor.

Recent findings about SARS-CoV-2 evolution, especially within the RBD, call for a massive effort in the scientific community to identify new variants that might increase viral spreading,

as well as allow escape from natural or vaccine-induced neutralising immunity.

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*Simona Fiorentini, Serena Messali, Alberto Zani, Francesca Caccuri, Marta Giovanetti, Massimo Ciccozzi, \*Arnaldo Caruso*  
arnaldo.caruso@unibs.it

Section of Microbiology, Department of Molecular and Translational Medicine, University of Brescia, 25123 Brescia, Italy (SF, SM, AZ, FC, AC); Laboratório de Flavivírus, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil (MG); Laboratório de Genética Celular e Molecular, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil (MG); Unit of Medical Statistics and Molecular Epidemiology, University Campus Bio-Medico of Rome, Rome, Italy (MC)

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See Online for appendix

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