

Novel SARS-CoV-2 Variant Derived from Clade 19B, France

Appendix

Methods

The full-length SARS-CoV-2 genomes from the 4 infected patients were sequenced by next-generation sequencing. Viral RNA was extracted from nasopharyngeal swabs with a NucliSENS easyMAG kit on an EMAG device (bioMérieux, <https://www.biomerieux.com>). Sequencing was performed with the COVIDSeq Test (Illumina, <https://www.illumina.com>), which uses 98-target multiplex amplifications along the full SARS-CoV-2 genome (Bhoyar et al. unpub. data, <https://doi.org/10.1101/2020.08.10.242677>). The libraries were sequenced with NextSeq 500/550 High Output Kit 2.5 on a NextSeq 500 device (Illumina). The sequences were demultiplexed and assembled as full-length genomes with the DRAGEN COVIDSeq Test Pipeline on a DRAGEN server (Illumina). Lineages and clades were interpreted with Pangolin and NextClade (1). Phylogeny was performed after full-length genome alignment with Muscle 3.8.31 (maximum-likelihood model general time-reversible plus invariant sites; 1,000 bootstrap replicates) by using IQ-Tree version 1.3.11.1 and iTOL (2,3).

References

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2. Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A, et al. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Mol Biol Evol.* 2020;37:1530–4. [PubMed <https://doi.org/10.1093/molbev/msaa015>](https://doi.org/10.1093/molbev/msaa015)
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Appendix Table. Amino acid substitutions observed in the spike protein of the 19B.HMN variants*

Lab identification number submitted to GISAID	Amino acid position in spike protein													
	18	49	70	346	452	501	653	655	677	796	798	1113	1191	1219
hCoV-19/France/FR-HMN-21022180538/2021	L18F				L452R	N501Y	A653V	H655Y		D796Y	G798D			G1219V
hCoV-19/France/FR-HMN-21022180543/2021	L18F				L452R	N501Y	A653V	H655Y		D796Y	G798D			G1219V
hCoV-19/France/ARA-0026043/2021	L18F				L452R	N501Y	A653V	H655Y		D796Y				G1219V
hCoV-19/France/ARA-0021076/2021	L18F				L452R	N501Y	A653V	H655Y		D796Y				G1219V
hCoV-19/France/ARA-0028071/2021	L18F				L452R	N501Y	A653V	H655Y		D796Y				G1219V
hCoV-19/France/ARA-0027021/2021	L18F	H49Y			L452R	N501Y	A653V	H655Y		D796Y				G1219V
hCoV-19/France/ARA-0028055/2021	L18F	H49Y			L452R	N501Y	A653V	H655Y		D796Y				G1219V
hCoV-19/France/IDF-0015100/2021	L18F				L452R	N501Y	A653V	H655Y		D796Y				G1219V
hCoV-19/France/IDF-0016022/2021	L18F				L452R	N501Y	A653V	H655Y		D796Y				G1219V
hCoV-19/France/IDF-0015099/2021	L18F				L452R	N501Y	A653V	H655Y		D796Y				G1219V
hCoV-19/France/ARA-0028026/2021	L18F				L452R	N501Y	A653V	H655Y		D796Y				G1219V
hCoV-19/France/FR-HMN-21022180542/2021	L18F				L452R	N501Y	A653V	H655Y		D796Y				G1219V
hCoV-19/France/ARA-0019006/2021	L18F			R346I	L452R	N501Y	A653V	H655Y	Q677H	D796Y			K1191N	G1219V
hCoV-19/France/ARA-0019013/2021	L18F			R346I	L452R	N501Y	A653V	H655Y	Q677H	D796Y			K1191N	G1219V
hCoV-19/France/NAQ-0023004/2021	L18F				L452R	N501Y	A653V	H655Y	Q677H	D796Y			K1191N	G1219V
hCoV-19/France/NAQ-0033090/2021	L18F		V70F		L452R	N501Y	A653V	H655Y	Q677H	D796Y			K1191N	G1219V
hCoV-19/France/NAQ-0023008/2021	L18F				L452R	N501Y	A653V	H655Y	Q677H	D796Y			K1191N	G1219V
hCoV-19/France/NAQ-0023005/2021	L18F				L452R	N501Y	A653V	H655Y	Q677H	D796Y			K1191N	G1219V
hCoV-19/France/FR-HMN-21012220289/2021	L18F				L452R	N501Y	A653V	H655Y	Q677H	D796Y				G1219V
hCoV-19/France/ARA-0025030/2021	L18F				L452R	N501Y	A653V	H655Y	Q677H	D796Y				G1219V
hCoV-19/France/ARA-0033097/2021	L18F				L452R	N501Y	A653V	H655Y	Q677H	D796Y		Q1113K		G1219V
hCoV-19/France/FR-HMN-21012210228/2021	L18F				L452R	N501Y	A653V	H655Y	Q677H	D796Y				G1219V
hCoV-19/France/FR-HMN-21022180546/2021	L18F				L452R	N501Y	A653V	H655Y	Q677H	D796Y				G1219V
hCoV-19/France/FR-HMN-21022230288/2021	L18F				L452R	N501Y	A653V	H655Y	Q677H	D796Y				G1219V
hCoV-19/France/FR-HMN-21012220498/2021	L18F				L452R	N501Y	A653V	H655Y	Q677H	D796Y				G1219V
hCoV-19/France/FR-HMN-21022180551/2021	L18F				L452R	N501Y	A653V	H655Y	Q677H	D796Y				G1219V
hCoV-19/France/FR-HMN-21022150436/2021	L18F				L452R	N501Y	A653V	H655Y	Q677H	D796Y				G1219V
hCoV-19/France/FR-HMN-21012250624/2021	L18F				L452R	N501Y	A653V	H655Y	Q677H	D796Y				G1219V
hCoV-19/France/FR-HMN-21022160243/2021	L18F				L452R	N501Y	A653V	H655Y	Q677H	D796Y				G1219V
hCoV-19/France/FR-HMN-21022230167/2021	L18F				L452R	N501Y	A653V	H655Y	Q677H	D796Y				G1219V
hCoV-19/France/FR-HMN-21022120468/2021	L18F				L452R	N501Y	A653V	H655Y	Q677H	D796Y				G1219V
hCoV-19/France/FR-HMN-21022150491/2021	L18F				L452R	N501Y	A653V	H655Y	Q677H	D796Y				G1219V
hCoV-19/France/FR-HMN-21022190457/2021	L18F				L452R	N501Y	A653V	H655Y	Q677H	D796Y				G1219V

*HMN.19B spike sequences are colored based on geographic region of origin of patients: greater Paris area (IDF or HMN) in red, southeast France (ARA) in blue, and southwest France (NAQ) in green.