RESEARCH ARTICLE

Culture and identification of a "Deltamicron" SARS-CoV-2 in a three cases cluster in southern France

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Abstract

Multiple SARS-CoV-2 variants have successively, or concomitantly spread worldwide since the summer of 2020. A few co-infections with different variants were reported and genetic recombinations, common among coronaviruses, were reported or suspected based on co-detection of signature mutations of different variants in a given genome. Here we report three infections in southern France with a Delta 21J_AY.4-Omicron 21K/BA.1 "Deltamicron" recombinant. The hybrid genome harbors signature mutations of the two lineages, supported by a mean sequencing depth of 1163–1421 reads and a mean nucleotide diversity of 0.1%–0.6%. It is composed of the near full-length spike gene (from codons 156–179) of an Omicron 21K/BA.1 variant in a Delta 21J/AY.4 lineage backbone. Importantly, we cultured an isolate of this recombinant and sequenced its genome. It was observed by scanning electron microscopy. As it is misidentified with current variant screening quantitative polymerase chain reaction (qPCR), we designed and implemented for routine diagnosis a specific duplex qPCR. Finally, structural analysis of the recombinant spike suggested its hybrid content could optimize viral binding to the host cell membrane. These findings prompt further studies of the virological, epidemiological, and clinical features of this recombinant.

KEYWORDS
delta, deltamicron, epidemic, lineage, Omicron, recombinant, SARS-CoV-2, variant

1 | INTRODUCTION

The current SARS-CoV-2 pandemic has highlighted since the summer of 2020 the successive or concomitant emergence of numerous viral variants, each causing a specific epidemic.[1–3] Some of these variants spread to become pandemic while others remained epidemic in a restricted geographical area. The variants characterized so far have been shaped by nucleotide substitutions, insertions, or deletions. However, another major evolutionary mechanism of RNA viruses is genetic recombination, which is very common among coronaviruses.[4–8] It requires co-infection of the same host cell by two viruses, which may be two distinct mutants or variants.[9] Therefore, the frequency of creation of recombinants between two variants depends on the duration of their co-circulation, the time until viral clearance, and the number of people exposed to both viruses. Co-infections with two variants were reported including recently with SARS-CoV-2 Delta and Omicron variants.[10–13] Furthermore, genetic recombinations were reported or suspected, based on the concurrent detection in consensus genomes of signature mutations of different mutants or variants.[10,12,14–24] A study detected up to 1175 (0.2%) putative recombinant genomes among 537 360 genomes and estimated that up to 5% of SARS-CoV-2 having circulated in the USA and UK could be recombinants.[16]

Two pandemic variants, Delta and Omicron 21K (Nextclade classification[25,26])/BA.1 (Pangolin classification[27]), recently succeeded each other as the predominant viruses but co-circulated for a period of several weeks, creating conditions for co-infections and subsequently recombinations. This period spanned between November 28th, 2021, and February 14th, 2022 in our geographical area, as assessed by our SARS-CoV-2 genotypic surveillance based on variant-specific qPCR and next-generation genomic sequencing.[3,28,29] In January 2022, genomes harboring mutations from both Delta and Omicron 21K/BA.1 variants were reported in Cyprus but it was questioned whether sequences might have resulted from contamination.[23] Still, 15 genomes as of 27/02/2022 being hybrids of these two variants and highly similar to each other were reported since February 2022 (https://github.com/cov-lineages/pango-designation/issues/444). We herein report three infections by a recombinant SARS-CoV-2 Delta 21J_AY.4-Omicron 21K/BA.1 whose genome is highly similar to the 15 previously reported genomes and the isolation of the recombinant virus from one of the patients.

2 | MATERIALS AND METHODS

Nasopharyngeal samples were collected from two patients in our university hospital institute (Méditerranée Infection; https://www.mediterranee-infection.com/) and tested for SARS-CoV-2 infection by real-time reverse transcription-PCR (qPCR) as previously described.[3,28,29] The third patient was sampled in a private medical biology laboratory in southern France (Inovie Labosud). qPCR assays that screen for SARS-CoV-2 variants were performed as recommended by French public health authorities (https://www.data.gouv.fr/fr/datasets/donnees-de-laboratoires-pour-le-depistage-indicateurs-sur-les-mutations/). In our center this included the detection of spike mutation K417N (Thermo Fisher Scientific), combined with testing with the TaqPath COVID-19 kit (Thermo Fisher Scientific) that target viral genes ORF1, N (nucleocapsid) and S (spike), as previously reported.[3,28,29] The private medical laboratory used the ID SARS-CoV-2/VOC Revolution Pentaplex assay (ID Solutions) that detects spike mutations K417N, L452R, and E484K (Pentaplex assay; ID Solution).

SARS-CoV-2 genomes were sequenced in the framework of genomic surveillance implemented since February 2020[3] in our institute. Next-generation sequencing was performed with the Illumina COVID-seq protocol on the NovaSeq. 6000 instrument that uses the ARTIC nCoV-2019 Amplicon Panel v3 of primers (Illumina Inc.) or with the Oxford Nanopore technology on a GridION instrument (Oxford Nanopore Technologies Ltd.) combined with prior multiplex PCR amplification according to the ARTIC procedure (https://artic.network/), as previously described,[3,28] with the ARTIC nCoV-2019 Amplicon Panel v4.1 of primers (IDT). Then, sequence read processing and genome analysis were performed as previously described.[3,28] Briefly, for Illumina NovaSeq reads, base calling was performed with the Dragen Bcl Convert pipeline [v3.9.3; https://emea.support.illumina.com/sequencing/sequencing_software/bcl-convert.html (Illumina Inc.)], mapping was performed with the bwa-mem2 tool (v. 2.2.1; https://github.com/bwa-mem2/bwa-mem2) on the Wuhan-Hu-1 isolate genome (GenBank accession no. NC_045512.2) then cleaned with Samtools (v. 1.13; https://www.htslib.org/), variant calling was carried out with freebayes (v. 1.3.5; https://github.com/freebayes/freebayes) and consensus genomes were built using Bcftools (v. 1.13; https://samtools.github.io/bcftools/bcftools.html). Nanopore reads were processed with the ARTIC-nCoV-bioinformaticsSOP pipeline (v1.1.0; https://github.com/artic-network/fieldbioinformatics). Nucleotide and amino acid changes relative to the Wuhan-Hu-1 isolate genome were obtained using the Nextclade tool (https://clades.nextstrain.org/).[25,26] Nextstrain clades and Pangolin lineages were determined using the Nextclade web application (https://clades.nextstrain.org/)[25,26] and the Pangolin tool (https://cov-lineages.org/pangolin.html),[27] respectively. The alignment of genomes with next-generation sequencing reads was performed with the ClustalW multiple alignment accessory application using the BioEdit software (v.7.0.9.0; https://bioedit.software.informer.com/).[30] Genome sequences described here were deposited in the GenBank sequence database (https://www.ncbi.nlm.nih.gov/genbank/)[31] (OM990851, OM990852, OM991095, OM991295), and on the IHU Méditerranée Infection website (https://www.mediterranee-infection.com/sars-cov-2-recombinant/). The Simplot software (https://sray.med.som.jhmi.edu/SCRoftware/SimPlot/)[32] was used for recombination analysis. Phylogeny was reconstructed by the IQTree (v2.1.3; http://www.iqtree.org/)[33] or MEGA X[34] (v10.2.5; https://www.megasoftware.net/) tools after sequence alignment with MAFFT (https://mafft.cbrc.jp/alignment/server/)[35] and visualized with MEGA X. As phylogenetic analysis can hardly apply to sequences that have different evolutionary histories, we built two separate trees, the first

one for the regions classified as of the Delta 21J_AY.4 variant (positions 1-22,128 and 25,519-29,903 in reference to the genome of the Wuhan-Hu-1 isolate), and the second one for the regions classified as of the Omicron 21K/BA.1 variant (positions 22,129-25,518). The 10 genomes the closest genetically to these fragments of the genome obtained here were selected through a BLAST[36] search against genomes of the Delta 21J_AY.4 and Omicron 21K/BA.1 variants in the sequence database of our institute that contains approximately 50 000 SARS-CoV-2 genomes; then, they were incorporated into the phylogeny together with the genome of the Wuhan-Hu-1 isolate. As a matter of fact, it should not be acceptable to make a phylogenetic tree based on the concatenation of sequences from different origins.

SARS-CoV-2 culture isolation was performed by inoculating 200 µl of respiratory sample on Vero E6 cells as previously described.[37] Cytopathic effect was observed by inverted microscopy. Viral particles were visualized in the culture supernatant by scanning electron microscopy with a SU5000 microscope (Hitachi High-Technologies Corporation), as previously described.[38] SARS-CoV-2 isolates were serially diluted from 10^{-1} to 10^{-7} in a culture medium for clonal isolate obtention. Each dilution was then inoculated into 8 wells of a 96-well microplate as previously described for median tissue culture infectious dose 50 (TCID$_{50}$) determination[39] and incubated at 37°C. Seven days postinoculation, the presence of a cytopathic effect was observed under an inverted microscope. Then, viral genome was recovered from a culture supernatant showing a cytopathic effect with the Nanopore technology combined with prior multiplex PCR amplification with the ARTIC procedure as explicated above [GenBank Accession No. ON202467; also available from the IHU Méditerranée Infection website (https://www.mediterranee-infection.com/sars-cov-2-recombinant)].

Structural predictions of the spike protein were performed as previously described.[28,40,41] Briefly, amino acid changes were introduced in the framework of a complete 14–1200 structure of the original SARS-CoV-2 20B spike, and missing amino acids were incorporated with the Robetta protein structure prediction tool (https://robetta.bakerlab.org/) before energy minimization through the Polak-Ribière algorithm.

An in-house duplex qPCR assay specific of the SARS-CoV-2 recombinant was designed that targets the genomes of the Delta 21J_AY.4 [targeted mutation: A11201G in the Nsp6 gene (corresponding to amino acid substitution T77A)] and the Omicron 21K/BA.1 [targeted mutations: A23040G, G23048A, A23055G in the spike gene (Q493R, Q496S, Q498R)] variants. The sequences of the primers and probes (in 5′-3′ orientation) are as follows: (i) for the Delta 21J-targeting system: forward primer, CTGCTTTTGCAAT GATGTTTGT; reverse primer, TACGCATCACCCAACTAGCA; probe, 6FAM-CTTGCCGCTGTAGCTTATTTTAAT (primers and probe concentrations in the mix were 200 and 150 nM, respectively); (ii) for the Omicron 21K/BA.1-targeting system: forward primer, CCTTGTAATG GTGTTGAAGGTTTT; reverse primer, CTGGTGCATGTAGAAGTT CAAAAG; probe, 6VIC-TTTACGATCATATAGTTTCCGACCC (primers

and probe concentrations in the mix were 250 and 200 nM, respectively).

3 | RESULTS

The three case patients were SARS-CoV-2-diagnosed on nasopharyngeal samples collected between 07/02/2022 and 16/02/2022. Cycle threshold values (Ct) of diagnostic qPCR were between 20 and 21. The patients were two women and one man below 40 years of age. Two resided in Marseille and one about 150 km west of Marseille, southern France, and they did not travel abroad recently. They presented mild respiratory symptoms. Two were vaccinated against SARS-CoV-2 (with two or three doses administered). Variant screening qPCR for the 2 samples collected in our institute showed positivity for the K417N mutation while the TaqPath COVID-19 kit provided positive signals for all three genes targeted (ORF1, S, and N). The third sample showed positivity for the K417N mutation and negativity for the L452R and E484K mutations. Thus, overall, qPCR carried out on the three samples was indicative of an Omicron variant.

The three viral genomes [GenBank Accession no. OM990851, OM990852, OM991095 (https://www.ncbi.nlm.nih.gov/genbank/)[31]; also available from the IHU Méditerranée Infection website (https://www.mediterranee-infection.com/sars-cov-2-recombinant/)], obtained here with the Illumina technology, were hybrids of Delta 21J_AY.4 and Omicron 21K/BA.1 variant genomes (Figure 1A–C; Table 1). At positions harboring mutations compared to the genome of the Wuhan-Hu-1 isolate, mean sequencing depth was between 1163 and 1421 reads, and the mean prevalence of the majority nucleotide was between 99.4% and 99.9% (with minimum values between 80.3% and 98.7%), ruling out the concurrent presence of two variants in the samples either due to co-infection or to contamination. In this SARS-CoV-2 recombinant, most of the spike gene was replaced in a Delta 21J_AY.4 matrix by an Omicron 21K/BA.1 sequence (Figure 1A,B). Indeed, the recombination sites were located between nucleotide positions 22,034 and 22,194 for the first one, and between nucleotide positions 25,469 and 25,584 for the second one. These regions correspond to amino acids 158–211 of the spike protein and to amino acids 26–64 of the ORF3a protein whose gene is contiguous to the spike gene. The Simplot recombination analysis tool provided congruent results (Figure S1 and Table S1).

Genomes from two of the three patients were identical and clustered in the phylogenetic analysis (Figure 1D,E) despite no epidemiological link being documented between these two patients. The third genome exhibited 5 nucleotide differences. Phylogenetic analyses show that the majority parts of the recombinant genomes were most closely related to Delta 21J_AY.4 variant genomes identified in our institute, while the regions spanning a large part of their spike gene were most closely related to Omicron 21K/BA.1 variant genomes identified in our institute.

The respiratory sample from which the first recombinant genome was obtained was inoculated on Vero E6 cells the day following
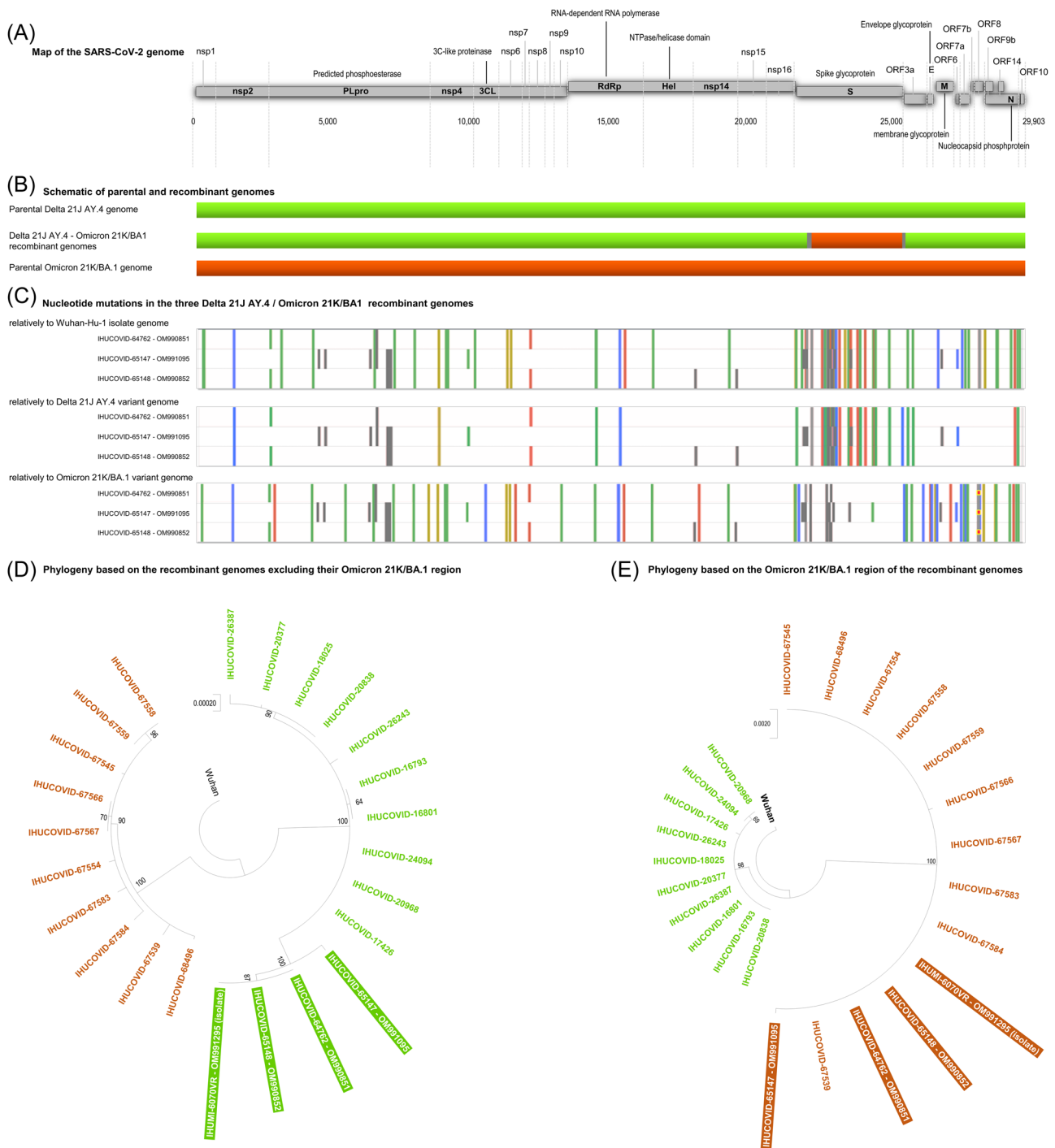
FIGURE 1 Schematic of the SARS-CoV-2 Delta 21J_AY.4-Omicron 21K/BA.1 recombinant genome, and phylogenetic trees based on different regions of the Deltamicron genome reflecting the origin of most of the viral genome from a Delta 21J_AY.4 variant and the origin of a region spanning a large part of the spike gene from an Omicron 21K/BA.1 variant. (A) Map of the SARS-CoV-2 genome. (B) Schematic representation of parental and recombinant genomes. (C) Mutations in the three Delta 21J_AY.4-Omicron 21K/BA1 recombinant genomes. Adapted from screenshots of the nextclade web application output (https://clades.nextstrain.org).[25,26] Color codes for nucleotide mutations are as follows: Green: U; yellow: G; blue: C; red: A; light grey: deletions; dark grey: uncovered regions. Genomes are labeled with the identifiers of the NCBI GenBank (https://www.ncbi.nlm.nih.gov/genbank/)[31] and IHU Méditerranée Infection (https://www.mediterranee-infection.com/tout-sur-le-coronavirus/sequencage-genomique-sars-cov-2/) sequence databases. (D, E) Phylogenetic trees based on different regions of the Deltamicron genomes showing the origin of most of the viral genome from a Delta 21J_AY.4 variant (D) and the origin of a region spanning a large part of the spike gene from an Omicron 21K/BA.1 variant (E). The 10 genomes the most similar genetically to these regions of the recombinant genomes obtained here were selected from the sequence database of our institute through a BLAST[36] search, then were incorporated in the phylogeny together with the genome of the Wuhan-Hu-1 isolate. Sequences from recombinant genomes are indicated by green or orange backgrounds. Sequences are labeled with identifiers from the GenBank (https://www.ncbi.nlm.nih.gov/genbank/)[31] and IHU Méditerranée Infection sequence databases.

TABLE 1 Nucleotide and amino acid changes in the Deltamicron recombinant according to their presence/absence in the Delta 21J_AY.4 lineage and the Omicron 21K/BA.1 variant

SARS-CoV-2 genes or genome regions	Nucleotide changes	Amino acid changes	Present in the Delta 21J_AY.4 lineage	Present in the Omicron 21K/BA.1 variant	Present in the Delta 21J_AY.4-Omicron 21K/BA.1 recombinant
5UTR	G210U	/	X		X
5UTR	C241U	/	X	X	X
ORF1a	A1321C	E352D			X
ORF1a	C3037U	/	X	X	X
ORF1a	G4181U	A1306S	X		X
ORF1a	C6402U	P2046L	X		X
ORF1a	C7124U	P2287S	X		X
ORF1a	C7851U	A2529V	X		X
ORF1a	A8723G	I2820V			X
ORF1a	C8986U	/	X		X
ORF1a	G9053U	V2930L	X		X
ORF1a	C10029U	T3255I	X	X	X
ORF1a	A11201G	T3646A	X		X
ORF1a	A11332G	/	X		X
ORF1b	C14407U	P314F/P314L	X	X	X
ORF1b	C14408U	P314F/P314L	X	X	X
ORF1b	U15264C	/	X		X
ORF1b	G15451A	G662S	X		X
ORF1b	C16466U	P1000L	X		X
ORF1b	C19220U	A1918V	X		X
S	C21618G	T19R	X		X
S	G21641U	A27S			X
S	C21846U	T95I	X	X	X
S	G21987A	G142D	X		X
S	GAGUUCA22028G	EFR156G	X		X
S	AAUU22193A	NL211I		X	X
S	U22204UGAGCCAGAA	Ins214EPE		X	X
S	G22578A	G339D		X	X
S	UC22673CU	S371L		X	X
S	U22679C	S373P		X	X
S	C22686U	S375F		X	X
S	G22813U	K417N		X	X
S	U22882G	N440K		X	X
S	G22898A	G446S		X	X
S	G22992A	S477N		X	X
S	C22995A	T478K	X	X	X

(Continues)

TABLE 1 (Continued)

SARS-CoV-2 genes or genome regions	Nucleotide changes	Amino acid changes	Present in the Delta 21J_AY.4 lineage	Present in the Omicron 21K/BA.1 variant	Present in the Delta 21J_AY.4-Omicron 21K/BA.1 recombinant
S	A23013C	E484A		X	X
S	A23040G	Q493R		X	X
S	G23048A	G496S		X	X
S	A23055G	Q498R		X	X
S	A23063U	N501Y		X	X
S	U23075C	Y505H		X	X
S	C23202A	T547K		X	X
S	A23403G	D614G	X	X	X
S	C23525U	H655Y		X	X
S	U23599G	N679K		X	X
S	C23604A	P681H		X	X
S	C23854A	N764K		X	X
S	G23948U	D796Y		X	X
S	C24130A	N856K		X	X
S	A24424U	Q954H		X	X
S	U24469A	N969K		X	X
S	C24503U	L981F		X	X
S	C25000U	/		X	X
ORF3a	C25667U	S92L			X
ORF3a	G25855U	D155Y			X
M	U26767C	I82T	X		X
ORF7a	U27638C	V82A	X		X
ORF7a	C27752U	T120I	X		X
ORF7b	C27874U	T40I	X		X
ORF8	AGAUUUC28247A	DF119Del	X		X
Intergenic region	UA28270U	/	X		X
N	A28461G	D63G	X		X
N	G28881U	R203M	X		X
N	G28916U	G215C	X		X
N	G29402U	D377Y	X		X
Intergenic region	G29540A	/			X
ORF10	G29645U	/			X
3UTR	G29742U	/	X		X

Note: / = No change; X = present. S gene region is indicated by a grey background.

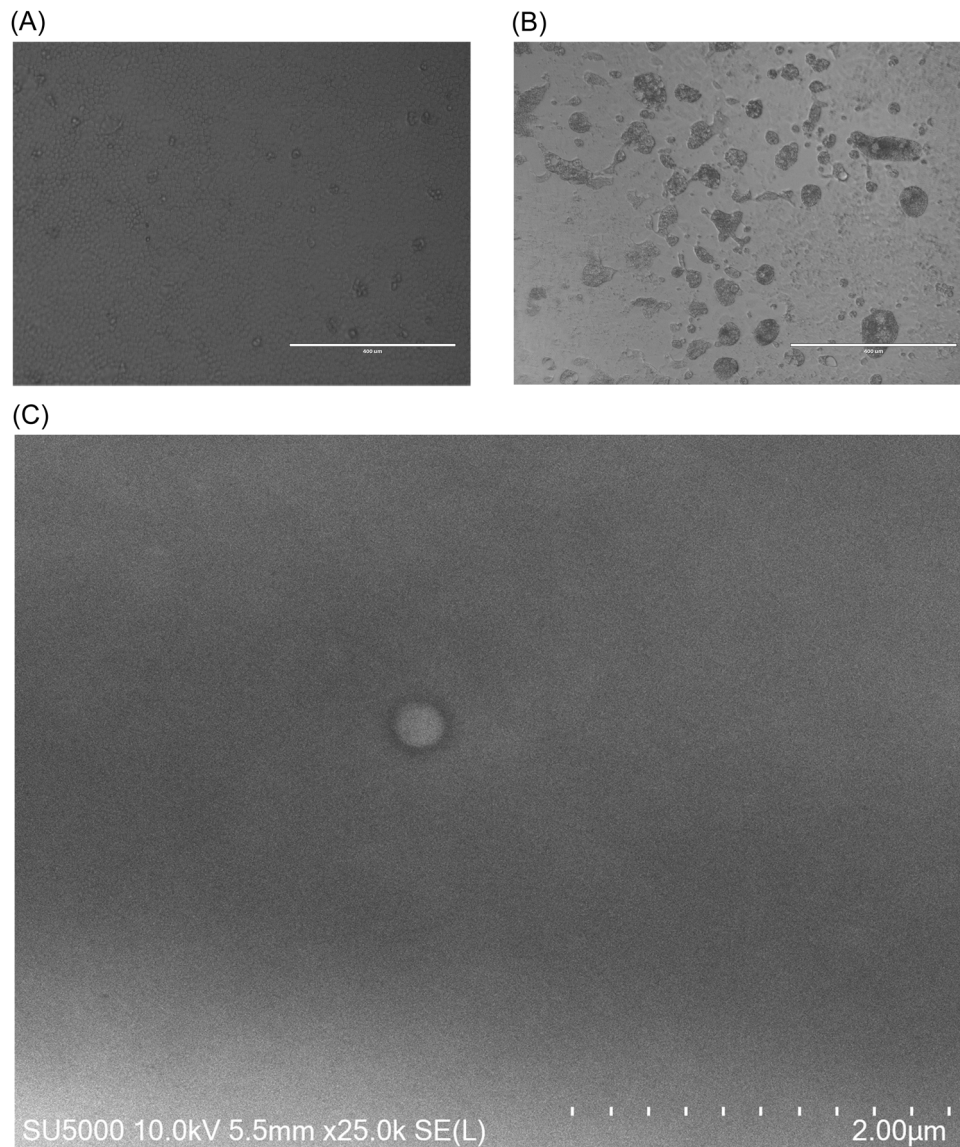Abbreviations: Del, deletion; Ins, insertion; UTR, untranslated region.

(A)

(B)

(C)

SU5000 10.0kV 5.5mm x25.0k SE(L)   2.00μm

FIGURE 2 Microscopy images of the virus cytopathic effect (A, B) and of a viral particle (C) in the culture of the SARS-CoV-2 Delta 21J_AY.4-Omicron 21K/BA.1 recombinant on Vero E6 cells. (A) Absence of cytopathic effect (negative control: absence of virus). (B) Cytopathic effect observed 4 days postinoculation on Vero E6 cells of the respiratory sample of the first patient for whom the SARS-CoV-2 Delta 21J_AY.4-Omicron 21K/BA.1 recombinant was identified. (C) Scanning electron microscopy image was obtained with a SU5000 microscope (Hitachi High-Technologies Corporation)

recombinant identification, and a cytopathic effect was observed after 4 days (Figure 2A,B; collection of strains of IHU Méditerranée Infection, No. IHUMI-6070VR). The same day, the supernatant was collected and next-generation genome sequencing was performed using Nanopore technology on a GridION instrument after PCR amplification with the ARTIC nCoV-2019 Amplicon Panel v4.1 of primers, which allowed obtaining the genome sequence of the viral isolate 8 h later [GenBank Accession no. OM991295 (https://www.ncbi.nlm.nih.gov/genbank/)[31]; also available from the IHU Méditerranée Infection website (https://www.mediterranee-infection.com/sars-cov-2-recombinant/)]. At mutated positions compared to the Wuhan-Hu-1 isolate genome, the mean sequencing depth was 2,771 reads and mean prevalence of the majority nucleotide was 99.1%

(minimum, 95.1%), and the consensus genome was identical to that obtained from the respiratory sample, showing unambiguously that the virus isolated was the Delta 21J_AY.4-Omicron 21K/BA.1 recombinant. In addition, following serial dilution from 10^{-1} to 10^{-7} of SARS-CoV-2 isolates for clonal isolation, a cytopathic effect was observed for 3 of 8 wells at dilution 10^{-5}. Next-generation sequencing was performed from one of these three culture supernatants that showed a cytopathic effect. The genome obtained by next-generation sequencing with the Nanopore technology after PCR amplification with ARTIC primers was confirmed to be that of a Deltamicron recombinant [GenBank Accession no. ON202467; also available from the IHU Méditerranée Infection website (https://www.mediterranee-infection.com/sars-cov-2-recombinant)]. At
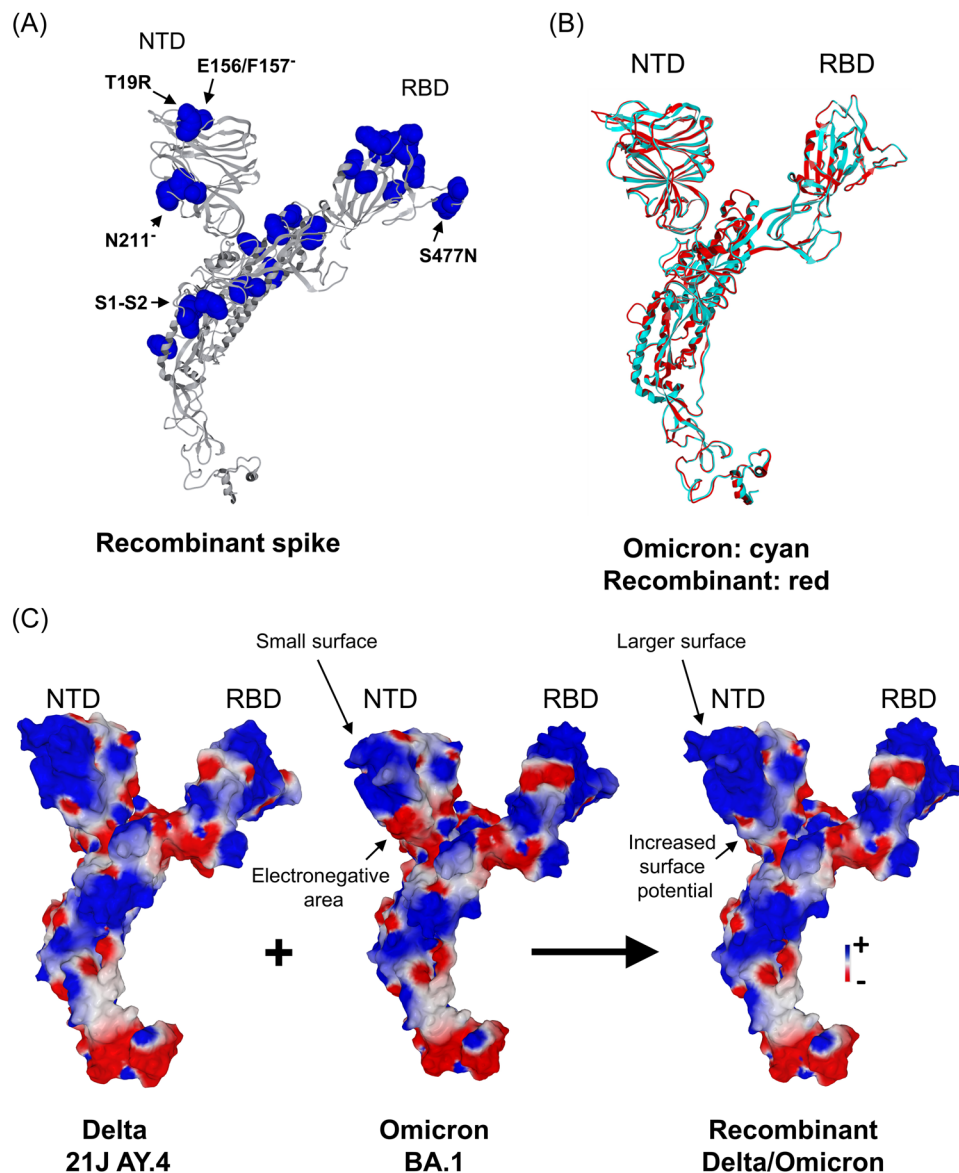
FIGURE 3 Schematic of the predicted structure of the spike protein of the SARS-CoV-2 Delta 21J_AY.4-Omicron 21K/BA.1 recombinant (A) Overall structure of the recombinant spike protein. The secondary structure is in grey, mutated amino acids are in blue. NTD, N-terminal domain; RBD, receptor-binding domain; S1-S2, cleavage site. (B) Superimposition of the secondary structure of the Omicron 21K/BA.1 variant (in cyan) and recombinant (in red) spike proteins. NTD, N-terminal domain; RBD, receptor-binding domain. (C) Comparison of the electrostatic surface potential of the spike proteins in Delta 21J_AY.4 lineage, Omicron 21K/BA.1 variant, and in the recombinant. The color scale (negative in red, positive in blue, neutral in white) is indicated. NTD, N-terminal domain; RBD, receptor-binding domain

positions mutated relatively to the Wuhan-Hu-1 isolate genome, the mean sequencing depth was 72,831 reads, and the mean prevalence of the majority nucleotide was 90.5%. Moreover, we observed that reads overlapping the first recombination site harbored concomitantly signature mutations of the Delta 21J_AY.4 variant toward their 5′ tip and of the Omicron 21K/BA.1 variant toward their 3′ tip, indicating that they were generated from the genome of the Deltamicron recombinant (Figure S1). Overall, Deltamicron genomes were obtained following multiplex PCR amplification either with the ARTIC nCoV-2019 Amplicon Panel v3 of primers (IDT) in case of next-generation sequencing with the Illumina COVID-seq protocol

(Illumina Inc.) or with the ARTIC nCoV-2019 Amplicon Panel v4.1 of primers in case of next-generation sequencing with the Oxford Nanopore technology (Oxford Nanopore Technologies Ltd.). Finally, viral particles were observed in the culture supernatant by scanning electron microscopy with a SU5000 microscope within minutes after supernatant collection (Figure 2C).

The overall structure of the recombinant spike protein was predicted (Figure 3A–C). When superimposed with the spike of the Omicron 21K/BA.1 variant, the main structural changes were located in the N-terminal domain (NTD). In this region, the surface of the recombinant spike protein is enlarged, flattened, and more

electropositive, a property that is characteristic of the Delta 21J_AY.4 NTD (Figure 3C). In the initial interaction of the virus with the plasma membrane of the host cells, the NTD is attracted by lipid rafts, which provide an electronegative landing platform for the spike.[40,41] Thus, an increase in the electrostatic surface of the NTD is expected to accelerate the binding of the virus to lipid rafts, which may confer a selective kinetic advantage against virus competitors.[41] The receptor-binding domain (RBD) of the recombinant is clearly inherited from the Omicron 21K/BA.1 variant (Figure 3C). The consequence is also an increase in the electrostatic surface potential of the RBD, which may facilitate the interaction with the electro-negative interface of the ACE-2 cellular receptor. Overall, this structural analysis suggests that the recombinant virus could have been selected on the basis of kinetic properties conferred by a convergent increase of the electrostatic potential of both the NTD and the RBD, together with an enlargement of the NTD surface, all features that suggest optimization of virus binding to the host cell membrane.

Currently used qPCR screening assays were not able to discriminate this Delta 21J_AY.4-Omicron 21K/BA.1 recombinant and the Omicron 21K/BA.1 variant because the Delta variant signature mutation detected is absent from the recombinant genome. Therefore, we attempted to promptly implement a specific qPCR assay that could be used to detect the recombinant for routine diagnostic use. This was achieved in 1 day by selecting qPCR systems from our toolbox of dozens of in-house qPCR systems that were designed since the emergence of the first variant during summer 2020 to specifically target SARS-CoV-2 variants or mutants.[3,42] Two systems that target either the Delta 21J variant or the Omicron BA.1 variant were combined in a duplex qPCR that can screen for the recombinant. In a preliminary assessment, both tested recombinant-positive samples were positive for the Delta 21J and Omicron BA.1 targets. In addition, 7 Delta non-21J-positive samples were negative for both targets, 4 Delta 21J-positive samples were positive for the Delta 21J target but negative for the Omicron BA.1 target, and 3 Omicron BA.1-positive samples were negative for the Delta 21J target but positive for the Omicron BA.1 target.

4 | DISCUSSION

It is increasingly demonstrated that the genomes of most biological entities, whatever their level of complexity, are mosaics of sequences from various origins.[43–47] The present observations show us in real life the recombination potential of SARS-CoV-2, already largely established for other coronaviruses[4–6,8,48] and reported or suspected for SARS-CoV-2.[10,12,14–17,24] SARS-CoV-2 recombinations were difficult to spot when only genetically very similar viruses were circulating, as was the case in Europe during the first epidemic episode with mutants derived from the Wuhan-Hu-1 virus. The increasing genetic diversity of SARS-CoV-2, the tremendous number of infections at global and national levels, and the unprecedented global effort of genomic sequencing (https://covariants.org/per-country),[3,25,49] raised the probability of detecting recombinants. Such observations will probably make it possible in the short or medium-term to assess the recombination rate of SARS-CoV-2, whether there are recombination hotspots, and to what extent recombinations between different variants can generate new viable, and epidemic variants. They question on the impact of recombina-tions on viral replication and transmissibility, and clinical severity, as well as on the virus's ability to escape neutralizing antibodies elicited by vaccines or a previous infection. In this view, culture isolation of SARS-CoV-2 recombinants as was carried out here with the Deltamicron is of primary importance to investigate their epidemic potential. This will allow studying their phenotypic properties, among which their replicative capacity in various cell lines, their sensitivity to antibodies, or their genetic evolution *in vitro*. Concurrently, a high level of genomic surveillance must be maintained to detect and characterize all recombination events and circulating recombinants, which is a critical scientific and public health issue.

CONFLICTS OF INTEREST

Didier Raoult has a conflict of interest as having been a consultant for Hitachi High-Technologies Corporation, from 2018 to 2020. He is a scientific board member of Eurofins company and a founder of a microbial culture company (Culture Top). All other 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.

DATA AVAILABILITY STATEMENT

The dataset generated and analyzed in the present study is available from the GenBank database (https://www.ncbi.nlm.nih.gov/genbank/)[31] (GenBank Accession No. OM990851, OM990852, OM991095, OM991295, ON202467) and from the IHU Méditerranée Infection website (https://www.mediterranee-infection.com/sars-cov-2-recombinant/) (IHUCOVID-64762, IHUCOVID-65148, IHUCOVID-64762-culture, IHUCOVID-65147, IHUMI-6070VR_Dil_10^-5).

ETHICS STATEMENTS

This study has been approved by the ethics committee of the University Hospital Institute Méditerranée Infection (No. 2022-001). Access to the patients' biological and registry data issued from the hospital information system was approved by the data protection committee of Assistance Publique-Hôpitaux de Marseille (APHM) and was recorded in the European General Data Protection Regulation registry under number RGPD/APHM 2019-73.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.