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A 21L/BA.2-21K/BA.1 "MixOmicron" SARS-CoV-2 hybrid undetected by qPCR that screen for variant in routine diagnosis

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ABSTRACT

Among the multiple SARS-CoV-2 variants identified since summer 2020, several have co-circulated, creating opportunities for coinfections and potentially genetic recombinations that are common in coronaviruses. Viral recombinants are indeed beginning to be reported more frequently. Here, we describe a new SARS-CoV-2 recombinant genome that is mostly that of a Omicron 21L/BA.2 variant but with a 3′ tip originating from a Omicron 21K/BA.1 variant. Two such genomes were obtained in our institute from adults sampled in February 2022 in university hospitals of Marseille, southern France, by next-generation sequencing carried out with the Illumina or Nanopore technologies. The recombination site was located between nucleotides 26,858-27,382. In the two genomic assemblies, mean sequencing depth at mutation-harboring positions was 271 and 1362 reads and mean prevalence of the majoritary nucleotide was 99.3 ± 2.2% and 98.8 ± 1.6%, respectively. Phylogeny generated trees with slightly different topologies according to whether genomes analyzed were depleted or not of the 3′ tip. This 3′ terminal end brought in the Omicron 21L/BA.2 genome a short transposable element of 41 nucleotides named S2m that is present in most SARS-CoV-2 except a few variants among which the Omicron 21L/BA.2 variant and may be involved in virulence. Importantly, this recombinant is not detected by currently used qPCR that screen for variants in routine diagnosis. The present observation emphasizes the need to survey closely the genetic pathways of SARS-CoV-2 variability by whole genome sequencing, and it could contribute to gain a better understanding of factors that lead to observed differences between epidemic potentials of the different variants.

1. Introduction

Multiple SARS-CoV-2 variants have been identified since summer 2020 (Lemey et al., 2021; Colson et al., 2022a; Hodcroft et al., 2021; Harvey et al., 2021). There were periods during which two distinct variants co-circulated with a crossing of their incidence when an old variant was vanishing and a new one was rising. Substantial rates of co-incidence over periods of several weeks have thus been reported recently worldwide for the Delta [WHO denomination (https://www.who.int/fr/activities/tracking-SARS-CoV-2-variants)]/21J [Nextclade classification (Aksamentov et al., 2021) (https://nextstrain.org/)] and Omicron 21K/BA.1 [Pangolin classification (Rambaut et al., 2020) (https://cov-lineages.org/resources/pangolin.html)] variants, and thereafter, to a lesser extent, for the Omicron BA.1 and BA.2 variants (https://covariants.org/per-country) (Hodcroft, 2021; Hadfield et al., 2018), including in our geographical area (Fig. 1). This created the opportunity for coinfections (Rockett et al., 2022; Hosch et al., 2022; Bolze et al., 2022; Belén Pisano et al., 2022), and consequently for homologous genetic recombinations, which constitute a major and very common mechanism of evolution in viruses (Bentley and Evans, 2018). Such genetic recombinations are extremely frequent for viruses of family *Coronaviridae*, and they have already been identified for endemic human

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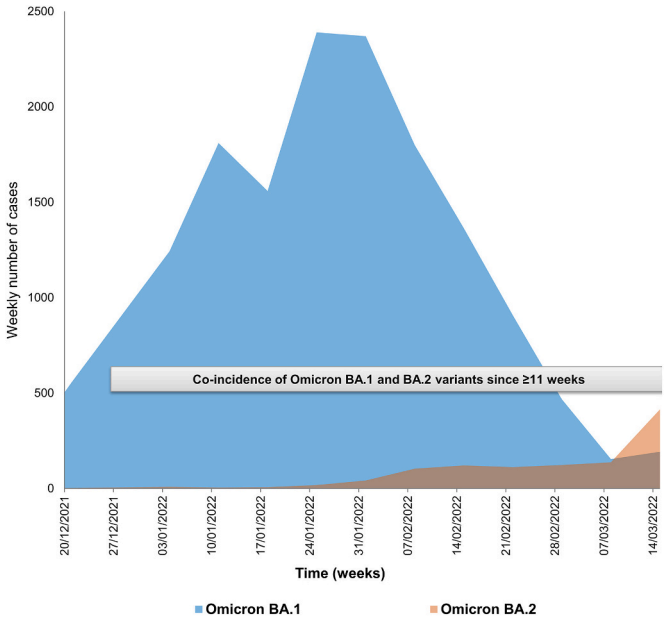

Fig. 1. Incidence and co-incidence of Omicron 21K/BA.1 and Omicron 21L/BA.2 variants among cases diagnosed in our institute.

coronaviruses (Lai, 1996; Zhang et al., 2015; So et al., 2019; Gribble et al., 2021). Regarding SARS-CoV-2, the occurrence of recombinations has been reported or suspected (Yi, 2020; Yeh and Contreras, 2020; Haddad et al., 2021; Ignatieva et al., 2022; Jackson et al., 2021; Taghizadeh et al., 2021; Varabyou et al., 2021; Kreier, 2022; Wertheim et al., 2022; He et al., 2022; Sekizuka et al., 2022; Colson et al., 2022b; Lacek et al., 2022; Lohrasbi-Nejad, 2022; Bolze et al., 2022; Ou et al., 2022; Belén Pisano et al., 2022; Burel et al., 2022). Very recently, we described the identification and culture of two SARS-CoV-2 recombinants, one between the B.1.160 and Alpha/20I variants in a patient chronically-infected with SARS-CoV-2 (Burel et al., 2022), and another between the Delta/21J AY.4 and Omicron 21K/BA.1 variants in patients infected approximately 10 weeks after the start of the period of co-detection of these two variants in our geographical area (Colson et al., 2022b). Here, we describe a new hybrid genome, which consists of a Omicron 21L/BA.2 genome whose 3′ tip originates from a Omicron 21K/BA.1.

2. Materials and methods

Nasopharyngeal samples were tested for the presence of SARS-CoV-2 RNA by real-time reverse transcription-PCR (qPCR) using the BGI real-time fluorescent RT-PCR assay (BGI Genomics, Shanghai Fosun Long March Medical Science Co., Ltd., Shenzhen, China), as previously described (Burel et al., 2022). Subsequently, qPCR assays that screen for SARS-CoV-2 variants were carried out with the detection of mutations among which spike substitution K417N (Thermo Fisher Scientific, Waltham, USA) and the targeting of viral genes S (spike), N (nucleocapsid) and ORF1 with the TaqPath COVID-19 kit (Thermo Fisher Scientific), as previously reported (Colson et al., 2022a; Colson et al., 2022b).

SARS-CoV-2 genomes were obtained and analyzed as described previously (Colson et al., 2022a; Colson et al., 2022b). Briefly, next-generation sequencing was carried out with the Illumina COVID-seq protocol on the NovaSeq 6000 instrument (Illumina Inc., San Diego, CA, USA), or with the Oxford Nanopore technology (ONT) on a GridION instrument (Oxford Nanopore Technologies Ltd., Oxford, UK) following multiplex PCR amplification with the ARTIC nCoV-2019 Amplicon Panel v4.1 of primers (IDT, Coralville, IA, USA) and the ARTIC procedure (https://artic.network/).

Sequence read processing and genome analysis were performed as described previously (Colson et al., 2022a; Colson et al., 2022b). Briefly, for Illumina NovaSeq reads, base calling was carried out using the Dragen Bcl Convert pipeline [v3.9.3; https://emea.support.illumina.com/sequencing/sequencing_software/bcl-convert.html (Illumina Inc.)], mapping was carried out using the bwa-mem2 tool (v2.2.1; https://github.com/bwa-mem2/bwa-mem2) on the Wuhan-Hu-1 isolate genome (GenBank accession no. NC_045512.2) before cleaning with the SAMtools program (v. 1.13; https://www.htslib.org/) (Danecek et al., 2021). Variant calling was performed using FreeBayes (v1.3.5; https://github.com/freebayes/freebayes) (Garrison and Marth, 2012), and consensus genomes were built with the Bcftools program (v1.13; https://samtools.github.io/bcftools/bcftools.html). ONT reads were processed with the ARTIC-nCoV-bioinformaticsSOP pipeline (v1.1.0; https://github.com/artic-network/fieldbioinformatics). Nucleotide and amino acid changes compared to the Wuhan-Hu-1 isolate genome were determined using the Nextclade tool (https://clades.nextstrain.org/) (Hadfield et al., 2018; Aksamentov et al., 2021). Nextstrain clades and Pangolin lineages were identified with the Nextclade web application (https://clades.nextstrain.org/) (Hadfield et al., 2018; Aksamentov et al., 2021) and the Pangolin tool (https://cov-lineages.org/pangolin.html) (Rambaut et al., 2020), respectively.

Phylogenetic analyses were performed with the MEGA X software (v10.2.5; https://www.megasoftware.net/) (Kumar et al., 2018) following sequence alignment with MAFFT (https://mafft.cbrc.jp/alignment/server/) (Katoh et al., 2002), and trees were visualized with MEGA X. We built two separate trees, a first one for the whole recombinant genome and a second one for its part originating from an Omicron 21L/BA.2 variant. The 10 genomes the most similar to these sequences among genomes of the Omicron 21L/BA.2 and 21K/BA.1 variants of the sequence database of our institute were selected by a BLAST search (Altschul et al., 1990) then incorporated in the phylogenies together with the sequence of the Wuhan-Hu-1 isolate.

This study has been approved by the ethics committee of the University Hospital Institute Méditerranée Infection (No. 2022–008). 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. Genome sequences obtained and analyzed here were deposited in the NCBI GenBank nucleotide sequence database (https://www.ncbi.nlm.nih.gov/genbank/) (Sayers et al., 2022) (Accession no. OM993515 and OM993473), on the IHU Méditerranée Infection website (https://www.mediterranee-infection.com/sars-cov-2-recombinant/) (IHUCOVID-063942 and IHUCOVID-068136), and in the GISAID database (https://www.gisaid.org/) (Elbe and Buckland-Merrett, 2017; Alm et al., 2020) (EPI_ISL_10843457, EPI_ISL_10047082).

3. Results

The recombinant genomes were obtained from two adult patients sampled in February 2022 in the university hospitals of Marseille, southern France. Cycle threshold values (Ct) of the diagnosis qPCR performed in our institute were 13 and 19. The TaqPath COVID-19 assay showed positivity for all targeted genes including the S gene, and the spike K417N mutation was detected, which led to suspect an infection with the Omicron 21L/BA.2 variant. However, next-generation sequencing performed in our laboratory allowed obtaining SARS-CoV-2 Omicron 21L/BA.2-21K/BA.1 genomic hybrid forms. These hybrid genomes have a Omicron 21L/BA.2 backbone but their approximately 2500–3000 nucleotide-long 3′ terminal region is that of a Omicron 21K/BA.1 (Fig. 2). The recombination site is located between nucleotides 26,858 and 27,382 (in reference to the genome of the Wuhan-Hu-1 isolate). This 3′ terminal end in the genomes of the Omicron BA.1 variant does not harbor any signature mutations, and therefore a "gain"

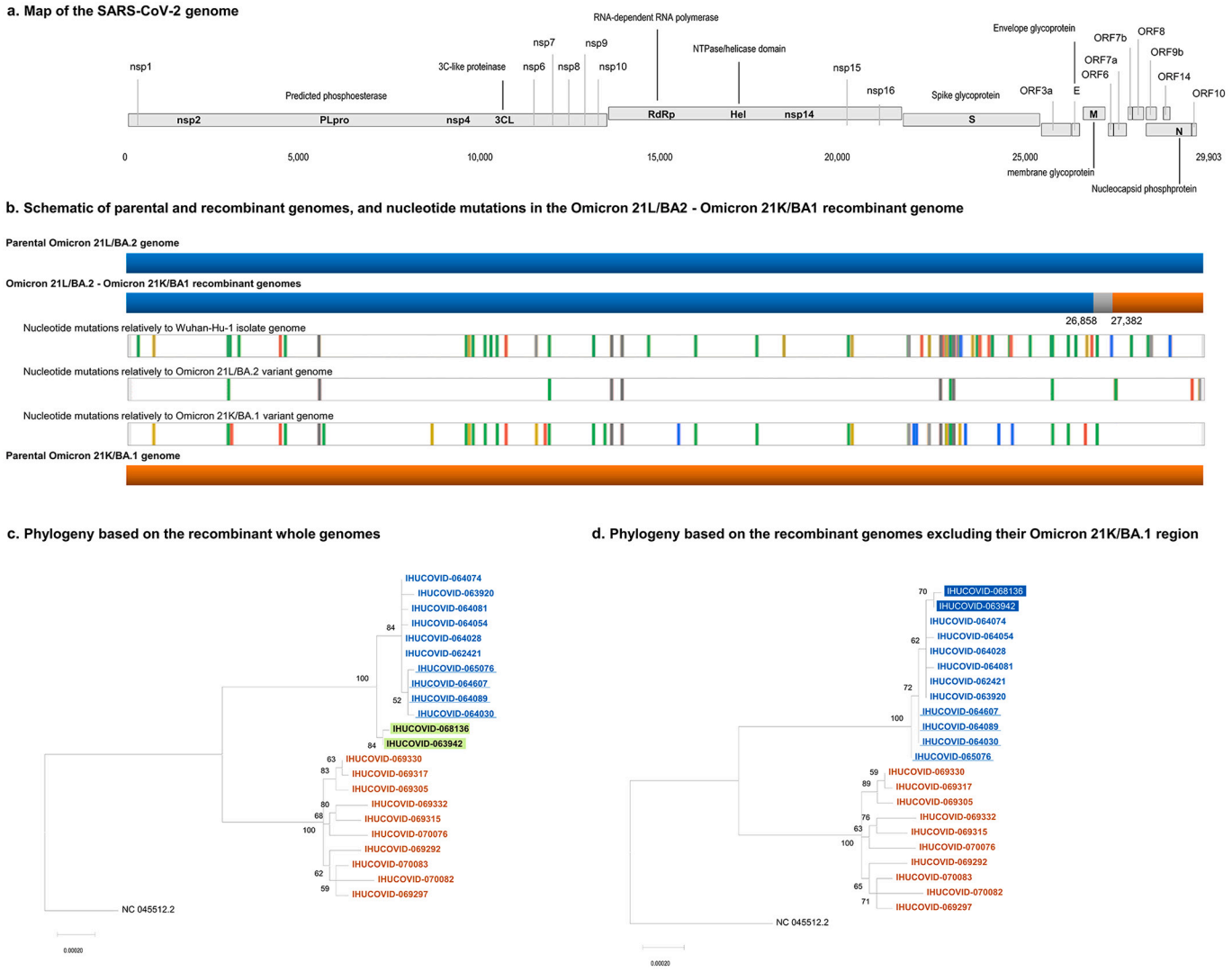

Fig. 2. Schematic of the SARS-CoV-2 Omicron 21L/BA.2-21K/BA.1 recombinant genomes (a, b) and phylogeny reconstruction based on SARS-CoV-2 Omicron 21L/BA.2 and Omicron 21K/BA.1 genomes (c, d).

a. Map of the SARS-CoV-2 genome.

b. Schematic representation of parental and recombinant genomes and mutations in the recombinant genomes. Adapted from screenshots of the nextclade web application output (https://clades.nextstrain.org) (Aksamentov et al., 2021; Hadfield et al., 2018). Color codes for nucleotide mutations are as follows: Green: U; yellow: G; blue: C; red: A; light grey: deletions; dark grey: regions uncovered by sequencing reads.

c, d. Phylogeny reconstructions based on the whole genomes (c) or only on the region of these genomes that corresponds to the region of the recombinant genome that originates from the Omicron 21L/BA.2 variant. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

of mutation could not be observed here. However, we noted the presence and integrity of a sequence corresponding to a short transposable element of 41 nucleotides named S2m (Tengs et al., 2021), which is present in the Omicron 21K/BA.1 variant but is truncated of 26 nucleotides in its central part in the Omicron 21L/BA.2 variant. Current strategies of variant screening by qPCR therefore fail to detect this recombinant as they target mostly mutations in the spike gene, or other genomic regions that do not allow identifying it either. In the two genomic assemblies, the mean (±standard deviation) sequencing depth at positions harboring mutations relatively to the genome of the Wuhan-Hu-1 isolate was 271 ± 164 and 1362 ± 1146 reads and the mean prevalence of the majoritary nucleotide was 99.3 ± 2.2% and 98.8 ± 1.6%, respectively, which rules out the concommitant presence of the sequences of two variants in the samples, either due to co-infection or to contamination.

The two recombinant genomes harbor 65 mutations relatively to the genome of the Wuhan-Hu-1 isolate, including 3 or 4 that are not signature mutations of the parental Omicron 21L/BA.2 or Omicron 21K/BA.1 genomes (Table 1). The spike genes of the two genomes only harbor signature mutations of the Omicron 21L/BA.2 variant, apart from a synonymous mutation in one genome. Besides, these two genomes differ between each other by a single mutation in the ORF1 gene (C2790U; T841I).

The two phylogenetic trees that were built, based either on the whole genomes or only on the region of these genomes that originate from the Omicron 21L/BA.2 variant in the recombinants, did not show exactly the same topology (Fig. 2c, d). The trees incorporated the 10 genomes of Omicron 21L/BA.2 and 21K/BA.1 variants from the sequence database of our institute that were the most similar to the sequences of the recombinants. In both trees, sequences from the recombinants were clustered with sequences of the Omicron 21L/BA.2 variant. Nonetheless, whole recombinant genomes were clustered separately, apart from their best hits, in the Omicron 21L/BA.2 clade that encompassed two sister groups. In contrast, the two partial recombinant genomes were clustered

Table 1
Nucleotide and amino acid changes in the MixOmicron recombinant according to their presence/absence in the Omicron 21L/BA.2 and Omicron 21K/BA.1 variants.

Gene or genome region	Nucleotide changes	Amino acid changes	Omicron BA.2 genomes	IHUCOVID-068136 (or OM993515, or EPI_ISL_10843457)	IHUCOVID-063942 (or OM993473, or EPI_ISL_10047082)	Omicron BA.1 genomes
5UTR	C241T	/	X	X	X	
ORF1a	T670G	S135R	X	X	X	
ORF1a	**C2790T**	**T842I**	**X**		**X**	
ORF1a	A2832G	K856R				X
ORF1a	C3037T	/	X	X	X	X
ORF1a	G4184A	G1307S	X	X	X	
ORF1a	C4321T	/	X	X	X	
ORF1a	T5386G	/				X
ORF1a	AGTT6512A	SL2083I				X
ORF1a	G8393A	A2710T				X
ORF1a	C9344T	L3027F	X	X	X	
ORF1a	A9424G	/	X	X	X	
ORF1a	C9534T	T3090I	X	X	X	
ORF1a	C9866T	L3201F	X	X	X	
ORF1a	C10029T	T3255I	X	X	X	X
ORF1a	C10198T	/				
ORF1a	G10447A	/				X
ORF1a	C10449A	P3395H	X	X	X	X
ORF1a	AGTTTGTCTG11282A	LSG3674-				X
ORF1a	GTCTGGTTTT11287G	SGF3675-	X	X	X	
ORF1a	A11537G	I3758V				X
ORF1a	**C11650T**	**/**		**X**	**X**	
ORF1a	C12880T	/	X	X	X	
ORF1a	T13195C	/				X
ORF1b	C14408T	P314L	X	X	X	X
ORF1b	C15240T	/				X
ORF1b	C15714T	/	X	X	X	
ORF1b	C17410T	R1315C	X	X	X	
ORF1b	A18163G	I1566V	X	X	X	X
ORF1b	C19955T	T2163I	X	X	X	
ORF1b	A20055G	/	X	X	X	
S	C21618T	T19I	X	X	X	
S	TTACCCCCTG21632T	LPPA24S	X	X	X	
S	C21762T	A67V				X
S	ATACATG21764A	HV69-				X
S	C21846T	T95I				X
S	GGTGTTTATT21986G	GVYY142D				X
S	G21987A	G142D	X	X	X	
S	AATT22193A	NL211I				X
S	T22200G	V213G	X	X	X	
S	T22204TGAGCCAGAA	INS214EPE				X
S	G22578A	G339D	X	X	X	X
S	T22673C	/				X
S	C22674T	S371F	X	X	X	X
S	T22679C	S373P				X
S	C22686T	S375F	X	X	X	X

S	A22688G	T376A	X	X	X	
S	G22775A	D405N	X	X	X	
S	A22786C	R408S	X	X	X	
S	**C22792T**	/		**X**	**X**	
S	G22813T	K417N	X	X	X	X
S	T22882G	N440K	X	X	X	X
S	G22898A	G446S				X
S	G22992A	S477N	X	X	X	X
S	C22995A	T478K	X	X	X	X
S	A23013C	E484A	X	X	X	X
S	A23040G	Q493R	X	X	X	X
S	G23048A	G496S				X
S	A23055G	Q498R	X	X	X	X
S	A23063T	N501Y	X	X	X	X
S	T23075C	Y505H	X	X	X	X
S	C23202A	T547K				X
S	A23403G	D614G	X	X	X	X
S	C23525T	H655Y	X	X	X	X
S	T23599G	N679K	X	X	X	X
S	C23604A	P681H	X	X	X	X
S	C23854A	N764K	X	X	X	X
S	G23948T	D796Y	X	X	X	X
S	C24130A	N856K				X
S	A24424T	Q954H	X	X	X	X
S	T24469A	N969K	X	X	X	X
S	C24503T	L981F				X
S	C25000T	/	X	X	X	X
ORF3a	C25584T	/	X	X	X	X
ORF3a	**C25624T**	**H78Y**		**X**	**X**	
ORF3a	C26060T	T223I	X	X	X	
E	C26270T	T9I	X	X	X	X
M	A26530G	D3G				X
M	C26577G	Q19E	X	X	X	X
M	G26709A	A63T	X	X	X	X
M	C26858T	/	X	X	X	
ORF6	A27259C	/	X	X	X	X
ORF6	GAT27382CTC	D61L	X			
ORF7b	C27807T	/	X	X	X	X
/	A28271T	/	X	X	X	X
N	C28311T	P13L	X	X	X	X
N	GGAGAACGCA28361G	ERS31-	X	X	X	X
N	GGG28881AAC	RG203KR	X	X	X	X
N	A29510C	S413R	X			
3UTR	CGAGGCCACGCGGAGT ACGATCGAGTG29733C	/	X			

/, no change; UTR, untranslated region; S gene region is indicated by a grey background; X, present.
Mutations with a bold font are those that are not signature mutations of the Omicron BA.1 or Omicron BA.2 variants.

together but nested inside the Omicron 21L/BA.2 clade.

4. Discussion

In the present work we show a recombination between Omicron BA.2 and BA.1 variant viruses. Cases of detection of recombinant genomes have been increasingly reported in 2022 (Wertheim et al., 2022; Sekizuka et al., 2022; Colson et al., 2022b; Lacek et al., 2022; Bolze et al., 2022; Ou et al., 2022; Belén Pisano et al., 2022; Burel et al., 2022) illustrating that recombination is a significant evolutionary pathway in SARS-CoV-2 (Rochman et al., 2022). The SARS-Cov2 recombination rate has been estimated in previous studies. The proportion of recombinant

genomes was 0.006% (16 out of a set of 279,000 genomes) (Jackson et al., 2021); 0.219% (1175 of 537,360) (VanInsberghe et al., 2021); 0.257% (225 of 87,695) (Varabyou et al., 2021); or 2.7% (43,104 of approximately 1.6 million with 589 recombination events) (Turakhia et al., 2022). In addition, VanInsberghe et al. reported that up to 5% of SARS-CoV-2 that circulated in the USA and UK might have been recombinants (VanInsberghe et al., 2021). Turkahia et al. also reported that the ratio of variable positions contributed by recombination *versus* that contributed by *de novo* mutation was 0.00264, which is about 100-times lower than estimated for MERS-coronavirus (Turakhia et al., 2022). Muller et al. reported that the recombination rate of SARS-like viruses including SARS-CoV-1, SARS-CoV-2 and related bat and pangolin coronaviruses was about 2×10^{-6} per site per year, which is about 0.06 recombination event per lineage per year, being a rate approximately 200 times lower than the evolutionary rate (Müller et al., 2021). In comparison, recombination rates for human seasonal coronaviruses was reported to be a little higher, about 1×10^{-5} per site per year, being approximately 10–20 times lower than the evolutionary rate. Ignatieva et al. estimated that the recombination rate was $>4 \times 10^{-5}$ per site per year (Ignatieva et al., 2022).

As a matter of fact, the SARS-CoV-2 recombination rate depends of several parameters. Recombinations require co-circulation of parental viruses and co-infection of same host cells to occur. Thus recombinants are all the more likely to be generated when different variants co-circulate with high incidence levels. In our geographical area, >15,000 infections with the Omicron 21K/BA.1 variant and > 1000 infections with the Omicron 21L/BA.2 variant were diagnosed over the same period of 11 weeks between late December and mid-March (Fig. 1). The frequency of intra-host recombination is also potentially enhanced when the duration of viral carriage is elongated, which is the case in immunocompromised individuals who can remain infected for several months (Burel et al., 2022). Moreover, recombinants become more easily identifiable during bioinformatic analyses due to the accumulation over time of mutations along SARS-CoV-2 genomes, at intervals of increasingly reduced size. Indeed, the detection of recombinants often uses the identification of combinations of mutations that are hallmarks of a given lineage. Hence, the greater the number of such hallmark mutations within the genomes, the greater the ability to identify combinations of mutations from different lineages indicating recombination events.

In the present work, it is worthy to note that the two phylogeny reconstructions based on whole genomes or on their region that originate from the Omicron 21L/BA.2 variant in the recombinants exhibited slightly different topologies. This emphasizes that phylogenetic analyses do not accurately handle genomes that are hybrids of sequences with different evolutionary histories, which prompts building separate trees for sequences of different origins in the case of recombinants.

The spike gene of the recombinant virus identified here is typical of that of the Omicron 21L/BA.2 variant, which suggests similar phenotypic features regarding immune escape (Yu et al., 2022; Iketani et al., 2022). However, the acquisition by a Omicron 21L/BA.2 genome of the 3′ terminal part of a Omicron 21K/BA.1 genome is of very particular interest as this Omicron 21K/BA.1 fragment contains a short transposable element named S2m.

S2m is a 41-nucleotide long stem loop motif located in the 3′ untranslated region of the SARS-CoV-2 genome. Interestingly, it is present in four different distantly-related families of positive-sense single-stranded RNA viruses including *Astroviridae*, *Caliciviridae*, *Picornaviridae*, and *Coronaviridae* (Robertson et al., 2005; Imperatore et al., 2022). Among coronaviruses, it is present in sarbecoviruses, notably in SARS-CoV-1 and in most of the SARS-CoV-2 genomes available; its sequence in SARS-CoV-1 and SARS-CoV-2 differ by only two nucleotides. Strikingly, s2m also shows high levels of similarity with sequences of multiple insect species among which some belonging to the *Araneae* order (Tengs et al., 2021). Sequence conservation has been deemed to be related to a requirement for the elaboration of the three-dimensional structure (Lulla et al., 2021). The function of the S2m element is currently unclear. It was proposed to be involved in RNA interference pathways through its processing into a mature microRNA (Tengs et al., 2013), and in hijacking of host protein synthesis *via* interactions with ribosomal proteins (Robertson et al., 2005). The S2m of SARS-CoV-2 was recently reported to interact with the human cellular miRNA-1307-3p, which is predicted to regulate the translation of some interleukins (among which IL18), interleukin receptors, and the interferon alpha receptor, being putatively able to manipulate the host immune response (Imperatore et al., 2022). It was also reported that antisense oligonucleotides may interact with this s2m element, inhibit SARS-CoV-2 replication in culture (Lulla et al., 2021), decrease astrovirus titers and reduce the activity of an astrovirus replicon (Lulla et al., 2021; Janowski et al., 2022). However, Janowsky et al. did not observe that deletion or mutation of s2m of SARS-CoV-2 altered viral growth *in vitro* (Janowski et al., 2022). It was also hypothesized that s2m could intervene in the protection of the viral genome from degradation by host ribonucleases (Tengs and Jonassen, 2016). Otherwise, s2m was reported to form a homodimeric structure with the aid of the chaperone activity of the SARS-CoV-2 nucleocapsid protein, which may stabilize a dimerized form of the RNA genome (Imperatore et al., 2022). Also, it was hypothesized that s2m may have a role in genome circularization that was found to be essential for flavirus RNA replication (Tengs and Jonassen, 2016). Finally, S2m was reported to be involved in RNA recombination events (Gallaher, 2020). Although conserved in almost all SARS-CoV-2, s2m is absent or truncated in a few variants including the Eta (B.1.525), Iota (B.1.526) and B.1.640.1 lineages, which all had a low epidemic spread; and it is also truncated in the Omicron 21L/BA.2 variant. Taken together these data suggest that s2m could be considered as the equivalent of a virulence factor. This mobile genetic element could have initiated viral infection and pathogenicity in various animal hosts post-transfer. The consequence of the s2m acquisition by an Omicron 21L/BA.2 genome as reported here is unknown. A possibility would be a gain in transmissibility leading to a larger epidemic, which should be investigated by genotypic surveillance among SARS-CoV-2 diagnoses and phenotypic *in vitro* experiments. Hence, the present observation may contribute to gain a better understanding of factors that enhance SARS-CoV-2 spread and lead to the observed differences between the epidemic potential of the variants (Tao et al., 2021; Campbell et al., 2021).

Overall, the increasing identification of recombinant SARS-CoV-2 genomes worldwide highlights the unpredictable nature of the genetic variability of this virus. The recombinant described here is not detected by current strategies that screen for variants in routine diagnosis by qPCR systems that target a single or at best a few mutations, often very close to each other in the genome, which does not allow detecting hybrid genomes. This emphasizes the interest of the most exhaustive whole-genome based surveillance possible to allow deciphering the genetic pathways of the variability and investigating their phenotypic consequences regarding transmissibility, clinical severity, and escape from neutralizing antibodies.

Author contributions

Conceptualization: Philippe Colson, Pierre-Edouard Fournier, Bernard La Scola, Didier Raoult. Methodology, Investigation, Formal analysis: Philippe Colson, Jeremy Delerce, Elise Marion-Paris, Jean-Christophe Lagier, Anthony Levasseur. Data analyses: Philippe Colson, Jeremy Delerce, Elise Marion-Paris, Jean-Christophe Lagier, Anthony Levasseur, Pierre-Edouard Fournier, Bernard La Scola, Didier Raoult. Writing -original draft of the manuscript: Philippe Colson, Pierre-Edouard Fournier, Bernard La Scola, Didier Raoult. Writing - review & editing: all authors.

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Data availability

Genome sequences generated and analyzed in the present study are available from the NCBI GenBank nucleotide sequence database (https://www.ncbi.nlm.nih.gov/genbank/) (Sayers et al., 2022) (Accession no. OM993515 and OM993473), from the IHU Méditerranée Infection website (https://www.mediterranee-infection.com/sars-cov-2-recombinant/) (IHUCOVID-063942 and IHUCOVID-068136), and from the GISAID database (https://www.gisaid.org/) (Elbe and Buckland-Merrett, 2017; Alm et al., 2020) (EPI_ISL_10843457, EPI_ISL_10047082).

Conflicts of interest

The authors have no conflicts of interest to declare relative to the present study. Didier Raoult was a consultant for the Hitachi High-Technologies Corporation, Tokyo, Japan from 2018 to 2020. He is a scientific board member of the Eurofins company and a founder of a microbial culture company (Culture Top). Funding sources had no role in the design and conduct of the study, the collection, management, analysis, and interpretation of the data, and the preparation, review, or approval of the manuscript.

Ethics

This study has been approved by the ethics committee of the University Hospital Institute Méditerranée Infection (No. 2022–008). 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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