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SHORT COMMUNICATION

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Unrecognized introduction of SARS-CoV-2 variants of concern to Central Africa: Import and local transmission of B.1.1.7 in Gabon in the very early stage of the variant spread to the African continent

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Abstract

The rapid spread of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variant of concern with higher infectivity has already resulted in the enormous increase in infection cases worldwide. We report an unrecognized introduction of the variant B.1.1.7 in Gabon in December 2020, which was the initial phase of the variant introduction to Africa. The B.1.1.7 variant was also detected in a hospitalized patient in January 2021, indicating a rapid spread of the variant in Gabon since its first detection. Phylogenetic analysis revealed that the detected B.1.1.7 variants originated from the distinct regions, strongly suggesting that the B.1.1.7 variant had been repeatedly introduced to Gabon since December 2020. These results provide insights on the

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unrecognized risks of infections with variants of concern, and show the necessity to conduct continuous genomic monitoring for immediate alert and control of novel SARS-CoV-2 variant infections.

KEYWORDS
Africa, Gabon, phylogeny, SARS-CoV-2, variant

1 | INTRODUCTION

The emergence of the variants of concern (VOC) of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), such as B.1.1.7 (202012/01 or 20I/501Y.V1) in the United Kingdom and B.1.351 (20H/501Y.V2) in South Africa, has caused a remarkable increase in coronavirus disease 2019 (COVID-19) cases worldwide.[1] Recent reports have suggested that the B.1.1.7 variant is more transmissible than the non-VOC strains,[2] and the B.1.351 variant confers resistance to the neutralizing antibodies acquired by the infection of earlier SARS-CoV-2 isolates.[3] Furthermore, B.1.1.7 infections have been indicated to lead to 40%–80% higher mortality rates than non-VOC infections.[4]

As of April 22, 2021, a cumulative total of 4 467 666 confirmed COVID-19 cases with 118 937 deaths (mortality rate 2.7%) have been reported in Africa.[5] The Africa Centres for Disease Control and Prevention warned that the rising COVID-19 mortality rates across the continent have exceeded the current global average of 2.1%.[6] The continuous introduction of VOCs has been a concern to cause increased cases and mortality in Africa. To date, genomic sequences of the B.1.1.7 and B.1.351 variants have been deposited from 19 to 23 African countries on GISAID (https://www.gisaid.org/), respectively. However, genomic information is significantly limited in Central Africa (only 4.5% of all African sequence data are on GISAID), reflecting a non-negligible bias of the genomic dataset among regions on the continent. To provide an insight into how and when VOC strains were introduced to Central Africa, we performed sequencing analysis for VOC in Gabon. Herein, we report detection of the B.1.1.7 variants in a sample collected in December 2020, which was the very early stage of the variant introduction to the African continent. Moreover, the B.1.1.7 variant was transmitted locally soon after the first detection.

2 | MATERIALS AND METHODS

Viral RNAs were extracted from 140 μl of each sample with a QIAamp Viral RNA Mini Kit (Qiagen) according to the manufacturer's instructions. Extracted viral RNAs were reverse-transcribed by SuperScript IV Reverse Transcriptase (Thermo Fisher Scientific). To efficiently sequence full-length coding regions by a next-generation sequencer, we performed a multiplex PCR method.[7] Multiplex PCR reaction was performed using Q5 High-Fidelity DNA Polymerase (New England Biolabs) as described previously.[8] Detailed methods are available in Supporting Information.

Libraries were prepared using an NEBNext Ultra II FS DNA Library Prep Kit for Illumina (New England Biolabs) in combination with NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 1) (New England Biolabs) according to the manufacturer's instructions. Sequencing was performed using a 300-cycle High Output Kit (Illumina) on a MiniSeq sequencer (Illumina). Mapping of the paired-end reads was performed on CLC Genomics Workbench 11.0.1 software (Qiagen) using whole-genome sequences of the Wuhan-Hu-1 reference strain (GISAID accession number EPI_ISL_402125) as a template.

For phylogenetic analysis of whole-genome sequences, a Maximum-Likelihood tree was inferred using IQ-TREE software (http://www.iqtree.org/) under the condition of a best-fit substitution model GTR+F+I. For better visualization, the phylogenetic tree was modified using FigTree v1.4.2 software (http://tree.bio.ed.ac.uk/software/figtree).

This study was approved by the Institutional Ethical Committee of CERMEL and the Institute of Tropical Medicine at Nagasaki University (approval numbers CEI-007, and 170921177, respectively).

3 | RESULTS

On March 26, 2021, we received SARS-CoV-2-positive swab samples of 3 individuals (AP360, AP264, and JE08) as part of the VOC surveillance activity in Gabon. AP360 and AP264 were samples of returning travelers from outside of Gabon and were collected at the international Léon-Mba airport on December 28, 2020, and February 21, 2021, respectively. These samples were tested at Laboratoire Professeur Daniel Gahouma. JE08, collected on January 12, 2021, was the sample of a patient who was hospitalized in Centre Hospitalier Universitaire Mère-Enfant Fondation Jeanne Ebori in Libreville.

We conducted whole-genome sequencing of SARS-CoV-2 at Centre de recherches médicales de Lambaréné (CERMEL) in Gabon. Obtained reads were mapped to the Wuhan Hu-1 reference sequence (GISAID accession number EPI_ISL_402125). Consequently, 18 941 179 mapped reads were obtained for AP360, 23 713 499 for AP264, and 20 378 740 for JE08. Almost the full-length of the genome was covered at more than 50 000× depth in each sample. These genomic sequences were deposited on GISAID under the accession numbers EPI_ISL_1760554–1760556.

To identify the lineage of SARS-CoV-2 detected in Gabon, we inferred phylogenetic relationships with 143 high-coverage full-length genomes that were selected from the GISAID database to cover the main GISAID clades, including VOC strains. The result

showed that the AP360 and JE08 strains clearly belonged to the B.1.1.7 lineage, whereas the AP264 strain was located in the B.1.351 lineage (Figure 1A–C). Interestingly, the AP360 strain formed a cluster with other African B.1.1.7 strains, indicating that intra-African transmission events greatly contributed to the spread of B.1.1.7 throughout the African continent (Figure 1B). The suspected locally transmitted JE08 strain originated from the European lineage. A table of amino acid substitutions clearly showed the genetic characteristics of the B.1.1.7 African and European lineages: shared substitutions in the S and N genes, whereas different substitution patterns in the ORF1ab region (Table 1). Thus, the B.1.1.7 strains AP360 and JE08 might have been independently introduced into Gabon within a short period. Concerning the B.1.351 lineage, the AP264 strain formed a cluster with European strains, indicating an import event from Europe to Gabon (Figure 1C). As B.1.351 strains of other Central African countries were located independently in the lineage, it is unlikely that B.1.351 strains were transmitted among neighboring countries in Central African (e.g., via land transportation). Amino acid substitutions were highly conserved within the cluster to which the AP264 strain belonged (Table 2).

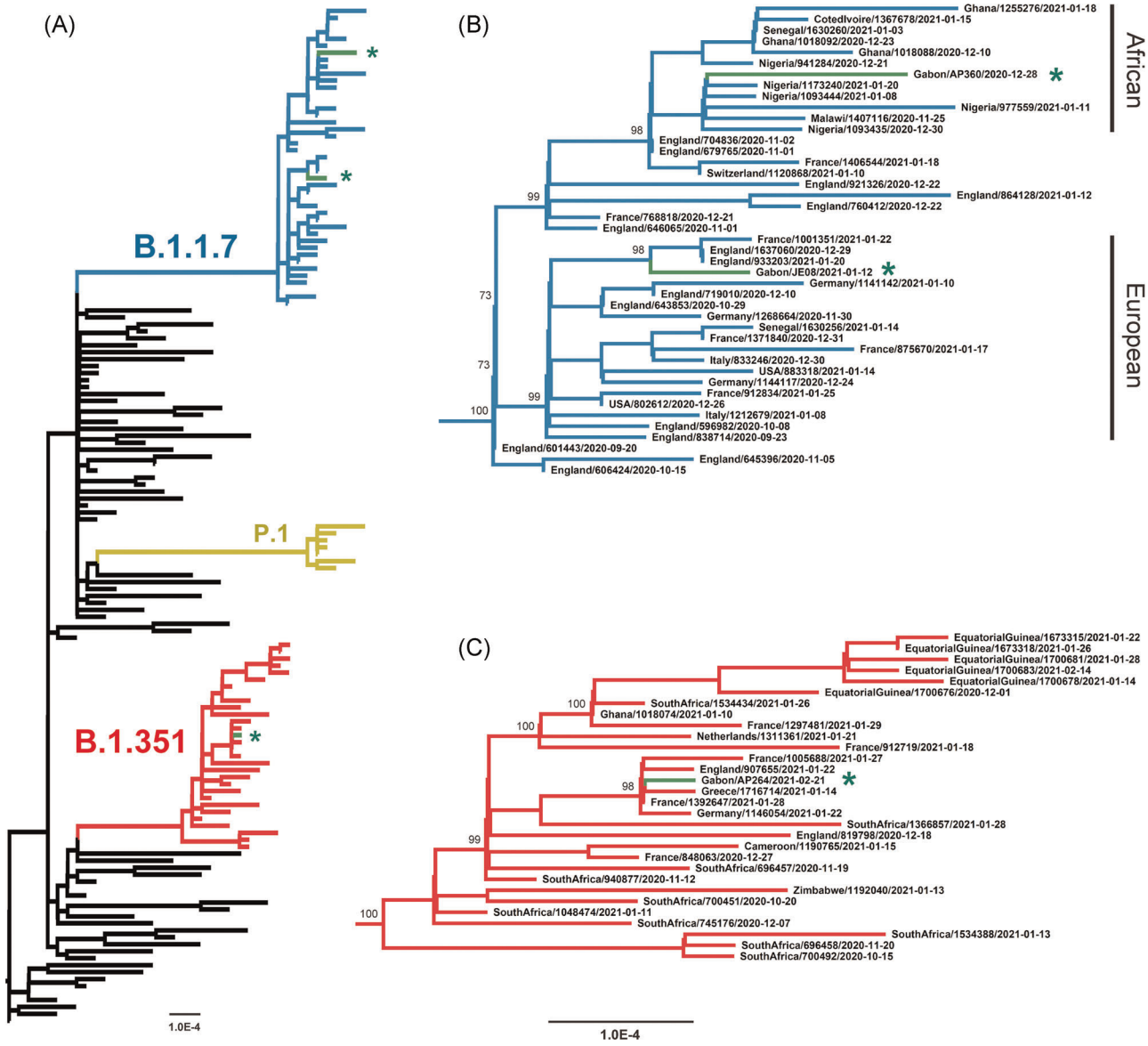

FIGURE 1 Phylogenetic analysis of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants of concern detected in Gabon between December 2020 and February 2021. (A) A maximum-likelihood tree was inferred with a global subsampling of 143 sequences. The enlarged phylogenies of (B) the B.1.1.7 and (C) B.1.351 lineages were shown to display the details of the clade. Colors depict lineages of the variant of concern: blue, B.1.1.7; red, B.1.351; gold, P.1; green, strains detected in Gabon. Numbers and dates in the strain name depict the GISAID accession number and sample collection date, respectively. Bootstrap values of ≥70% are shown at the main nodes. Asterisks show the positions of the AP360, AP264, and JE08 strains. Scale bars indicate nucleotide substitutions per site

TABLE 1 Amino acid substitutions of the B.1.1.7 variants detected in Gabon

Gene/protein		ORF1a NSP3						NSP6	NSP9	RdRp		ORF1b NSP13	ORF1b NSP14	S											ORF8	N		
Amino acid position		1001	1157	1708	2230	2259	2278	3675	4211	4619	4715	5784	6272	6	69	144	501	570	614	681	716	982	1118	1202	27	3	203	235
Wuhan-Hu-1	Reference	T	D	A	I	M	I	SGF	P	P	P	K	E	V	HV	Y	N	A	D	P	T	S	D	E	Q	D	RG	S
Gabon/AP360	African	I	N	D	T	I	*	Del	S	L	L	*	*	*	Del	Del	Y	D	G	H	I	A	H	Q	Stop	L	KR	F
Nigeria/CV743	African	I	*	D	T	I	*	Del	*	L	L	*	*	*	Del	Del	Y	D	G	H	I	A	H	*	Stop	L	KR	F
Gabon/JE08	European	I	*	D	T	*	*	Del	*	*	L	R	G	A	Del	Del	Y	D	G	H	I	A	H	*	Stop	L	KR	F
England/CAMC-10FCE37	European	I	*	D	T	*	V	Del	*	*	L	R	G	*	Del	Del	Y	D	G	H	I	A	H	*	Stop	L	KR	F

Note: *Identical residues to the reference.

TABLE 2 Amino acid substitutions of the B.1.351 variant detected in Gabon

Gene/protein	ORF1a NSP2	NSP3		Mpro	NSP6	RdRp	S								ORF3a		E	ORF7b	N	
Amino acid position	265	1612	1655	3353	3675	4715	80	215	242	417	484	501	614	701	57	171	71	39	205	362
Wuhan-Hu-1	T	S	K	K	SGF	P	D	D	LAL	K	E	N	D	A	Q	S	P	E	T	T
Gabon/AP264	I	L	N	R	Del	L	A	G	Del	N	K	Y	G	V	H	L	L	Stop	I	I
France/PDL-CHUN-0001	I	L	N	R	Del	L	A	G	Del	N	K	Y	G	V	H	L	L	Stop	I	I

4 | DISCUSSION

In the present study, we provide evidence that the SARS-CoV-2 B.1.1.7 variant was introduced to Gabon by December 28, 2020, at the latest, which is the earliest date for the detection of B.1.1.7 in Central Africa. There were only 8 sequences of B.1.1.7 strains detected before December 28, 2020, throughout Africa (Senegal, Malawi, Ghana, and Nigeria), indicating that the B.1.1.7 import into Gabon occurred in the very early stage of the variant introduction to the African continent. In addition, detection of the B.1.1.7 variant from the hospital sample on January 12, 2021, showed a rapid spread of the variant in a community since its introduction into Gabon. However, these B.1.1.7 strains (AP360 and JE08) originated from different regions, strongly suggesting that the B.1.1.7 variant had been repeatedly introduced to Gabon since December 2020. Especially, the first B.1.1.7 introduction to Gabon occurred with the strain belonging to the African lineage, indicating that an intra-African transmission had a critical role in the rapid spread of the variant throughout Africa in the initial phase of the B.1.1.7 introduction. Detection of the B.1.351 variant in Gabon in February 2021 follows its detection in other Central African countries, including Cameroon, Equatorial Guinea, and the Democratic Republic of the Congo. Phylogenetic relationships suggested that the B.1.351 variant was introduced to Gabon via Europe rather than from other African countries.

As of April 18, 2021, the number of deaths (69 deaths) in 2021 has already exceeded the number in 2020 (64 deaths) in Gabon,[9] possibly reflecting the rapid spread of VOC strains. In Gabon, preventive vaccination against SARS-CoV-2 was initiated in March 2021.[10] As the impact of VOCs on clinical significance and vaccination efficacy remains unclear, it is necessary to establish a borderless genomic surveillance system to combine epidemiological information with the genetic characteristics of SARS-CoV-2 in Central Africa.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Haruka Abe and Yuri Ushijima contributed equally to this article; Haruka Abe, Yuri Ushijima, and Jiro Yasuda designed the study; Haruka Abe, Yuri Ushijima, Rodrigue Bikangui, Georgelin Nguema Ondo, Gédéon Prince Manouana, Ayong More, Emilio Skarwan, and Yoric Yali-Assy-Oyamli performed sequencing analysis; Samira Zoa-Assoumou, Bénédicte Ndeboko, Rotimi Myrabelle Avome Houechenou, and Joel Fleury Djoba Siawaya collected samples and performed diagnostic tests; Haruka Abe, Yuri Ushijima, and Jiro Yasuda wrote the manuscript and contributed to interpretation of the data; Bertrand Lell and Ayola Akim Adegnika contributed to management of samples and a critical discussion of the manuscript; Ayola Akim Adegnika and Jiro Yasuda contributed equally to this work as corresponding authors. All authors read and approved the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in GISAID (https://www.gisaid.org/), accession numbers EPI_ISL_1760554–1760556. The supplementary material that supports the detailed methods of this study is available.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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