GENOME SEQUENCES





Whole-Genome Sequences of SARS-CoV-2 Isolates from Ethiopian Patients

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ABSTRACT Three complete severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) genomes from Ethiopian patients were compared with deposited global genomes. Two genomes belonged to genetic group 20A/B.1/GH, and the other belonged to genetic group 20A/B.1.480/GH. Enhancing genomic capacity is important to investigate the transmission and to monitor the evolution and mutational patterns of SARS-CoV-2 in this country.

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that emerged in Wuhan, China, is an RNA virus that belongs to the genus *Betacoronavirus*, in the family *Coronaviridae* (1). Like most RNA viruses, SARS-CoV-2 is expected to display a relatively high rate of genetic mutations, which may influence viral transmission and pathogenesis, enable escape from host defenses, and negatively affect the efficacy of vaccines and molecular diagnostic tools (2). Thus, enhancing genomic capacity is important to investigate the transmission and to monitor the evolution and mutational patterns of SARS-CoV-2 in this country.

Here, we report three SARS-CoV-2 genome sequences using Illumina NextSeq sequencing technology. The protocol was ethically approved by the ALERT/AHRI Research Ethics Committee. Nasopharyngeal swab samples were collected from subjects with suspected SARS-CoV-2 following routine surveillance and diagnostic procedures. The first two samples (GenBank accession numbers MZ172407 and MZ172408) were collected from a hospital setting, and the last one (GenBank accession number MZ172409) was collected from a health center. Nucleic acid was extracted using a Da An Gene extraction kit (catalog number DA0591) following the manufacturer's protocol. The extracted RNA was reverse transcribed and SARS-CoV-2 was detected using the BGI real-time fluorescent reverse transcription (RT)-PCR kit (catalog number MFG030010). Positive RNA samples were selected for sequencing based on their threshold cycle (C_{τ}) values (C_{τ} values of <24). The RNA was concentrated using SPRI magnetic beads, and reverse-transcribed RNA was sequenced using the shotgun metagenomic workflow outlined by Illumina (3). In short, 200 to 450 ng of input RNA was subjected to ribodepletion, fragmentation, first- and second-strand cDNA synthesis, adenylation, adapter ligation, and amplification, according to the TruSeq stranded total RNA protocol. The prepared libraries were loaded on the NextSeq 500 system for a paired-end 2 imes 76-bp sequencing run. The base call (BCL) files from the NextSeg 500 system were demultiplexed and converted to FASTQ files using Illumina bcl2fastq2 software v2.20. Quality-checked paired-end FASTQ files (4) were trimmed using Trimmomatic v0.36 (5). Taxonomic classification was performed using Kraken2 (6), and the host reads were removed using Bowtie2 (7) and SAMtools (8) with the human reference genome (GRCh38) (ftp://ftp.ccb.jhu.edu/pub/ data/bowtie indexes) to yield unmapped reads. The reads with the host reads removed were aligned to the complete genome of SARS-CoV-2 Wuhan-Hu-1 (GenBank accession number NC_045512.2) using BWA (9), and SAMtools was used for intermediate file conversion and summary. Ivar consensus sequences were used as genome sequences. Variants were called using Snippy (https://github.com/tseemann/snippy) and Nextclade. Local Nextstrain/

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FIG 1 Phylogenetic analysis of representative SARS-CoV-2 genome sequences, including the three current isolates. Available genomes were retrieved from GISAID (https://www.gisaid.org) in January 2021. Sequences with low quality (i.e., ambiguous bases) were discarded. The figure was created using Nextstrain.

Nextclade v0.13.0 was also implemented for clade assignment and variant annotation. The phylogenetic tree was generated with Nextstrain/Augur using its default subsampling scheme and focusing on country Ethiopia, region Africa, where 1,960 samples were subsampled between December 2019 and February 2021; the tree was visualized using the Nextstrain/Auspice tool. Lineage assignments were made using the Phylogenetic Assignment of Named Global Outbreak Lineages (Pangolin) v1.07 tool (https://github.com/hCoV-2019/pangolin) and clades from GISAID (https://www.gisaid.org). All tools were run with default parameters unless otherwise specified. There is 99.68 to 99.92% sequence identity using BLAST between the full genome sequences of the isolates and the reference strain at the nucleotide level and 99.94% identity at the amino acid level. All three isolates have 99.97 to 100% coverage, with 100% coverage of the coding region. The genome sizes were 29,860, 29,856, and 29,871 bp, with GC contents of 53%, 51%, and 49%, for isolates MZ172407, MZ172408, and MZ172409, respectively. Similarly, the average coverage depths were 2,56.7× (range, 1× to 3,183×), 23.8× (range, 1× to 1,110×), and 1,288.3× (range, 4× to 8,002×) for the isolates MZ172407, MZ172408, and MZ172407, MZ172408, and MZ172409, respectively.

Phylogenomic analysis showed that two of the detected SARS-CoV-2 isolates (isolates MZ172408 and MZ172409) belonged to lineage B.1 of the Pangolin lineage, sharing the most common recent ancestor with viruses detected in Germany (Fig. 1). One of the isolates (isolate MZ172407) was found to belong to lineage B.1.480. According to Nextstrain (10), the phylogenetic tree revealed that all of the isolates belonged to Nextstrain clade 20A and GISAID clade GH.

Mutations among the three SARS-CoV-2 strains were identified throughout the whole genome, with reference to the SARS-CoV-2 Wuhan strain (GenBank accession number NC_045512.2), and marked nucleotide differences in some positions were found, as shown in Table 1. In general, several synonymous and nonsynonymous mutations with

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21796 G T G T 21800 G T G G T 21800 G T G G T 23063 A T T A A 23403 A C A A C	S	Spike protein		Missense
21800 G T G G T 23063 A T T A A 23403 A G G G 24070 A C A A	TS	Spike protein		Missense
23063 A T T T A A 23063 23403 A G G G 24070 A C A A C	TS	Spike protein	D80Y	
23403 A G G G G G Z 24070 A C A A C	A S	Spike protein	N501Y	Synonymous
24070 A C A A C	G	Spike protein	D614G	Synonymous
	S	Spike protein	Q836H	Missense
25249 G T T G G G	с S	Spike protein	M1229I	Missense
25563 G T T T T T	T OR	-3a	Q57H	Missense
25844 G T T G G G	G OR	-3a	T1511	Synonymous
25904 C T C T C	COR	⁻ 3a ORF3a protein	S171L	Missense
26416 G C C G G G	U U	E protein	V58L	Synonymous
27484 T C C C T T	T OR	⁻⁷ a ORF7a protein		Synonymous
27546 T C T C T	TOR	-6 ORF 6 protein		
27667 G A G G A	A OR	:7a	E92K	Upstream
28854 C T T C C C	Z U	N protein	S194L	Synonymous
28869 C T C T T	T	N protein	P199L	Missense
29550 C T C T T	Γ	N protein	NA	
29702 G A A G G	G 3′-l	JTR		NA

TABLE 1 Alterations of the SARS-CoV-2 genome

pyrimidine exchanges (C to T or T to C) (55%) were observed in all three genomes (Table 1). Currently, we are sequencing more genomes to further investigate the transmission and to monitor the evolution and mutational patterns of SARS-CoV-2 in this country.

Data availability. The coding-complete sequences were deposited in GenBank with accession numbers MZ172407, MZ172408, and MZ172409 and SRA accession numbers SAMN20692030, SAMN20692031, and SAMN20692032 and in GISAID (https://www.gisaid.org) with accession numbers EPI_ISL_2970353, EPI_ISL_2970354, and EPI_ISL_2970355 for Ethiopia/AHRI-01/2020, Ethiopia/AHRI-02/2020, and Ethiopia/AHRI-03/2020, respectively.

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