Report of SARS-CoV-2 BA.1 Lineage in Morocco

Safae El Mazouri,a Houda Bendani,a Nasma Boumajdi,a Mhammed Chaoui Roqai,b Tarik Aanniz,a Myriam Seffar,a,c Hakima Kabbaj,a,c Ghizlane El Amin,c Amal Zouaki,c Saaïd Amzazi,d Mouna Ouadghiri,a Lahcen Belyamani,e,f Azeddine Ibrahimi

aMedical Biotechnology Laboratory (MedBiotech), Bioinova Research Center, Rabat Medical and Pharmacy School, Mohammed V University, Rabat, Morocco
bEcole des Hautes Etudes de Biotechnologie et de Santé (EHEB), Casablanca, Morocco
cLaboratoire Central de Virologie, Centre Hospitalo-Universitaire Ibn Sina, Hôpital des Spécialités, Rabat, Morocco
dLaboratory of Human Pathologies Biology, Faculty of Sciences, Mohammed V University, Rabat, Morocco
eEmergency Department, Military Hospital Mohammed V, Rabat, Morocco
fRabat Medical and Pharmacy School, Mohammed V University, Rabat, Morocco

ABSTRACT Here, we report the near-complete genome sequence and genetic variations of a clinical sample of SARS-CoV-2 for the newly emerged Omicron variant (BA.1). The sample was collected from a nasopharyngeal swab of a Moroccan patient, and the sequencing was done using Ion S5 technology.

On 26 November 2021, the World Health Organization (WHO) announced the emergence of a new variant of concern (VOC) named Omicron (B.1.1.529), belonging to the Betacoronavirus genus of the Coronaviridae family. Currently, Omicron harbors 32 mutations in the spike protein (S) (1, 2). Moreover, the Omicron variant shows more vaccine evasion and a higher rate of reinfection compared to previous VOCs (3). Monitoring the genomic diversity of SARS-CoV-2 remains pivotal in detecting mutations, their distribution, and their potential impact (4). In this study, near-complete genome sequencing of a SARS-CoV-2 strain was carried out using Ion S5 sequencing technology (5).

The sampling was carried out on 27 December 2021. RNA was extracted from a nasopharyngeal swab sample from a patient in the Rabat-Salé-Kenitra Region, Morocco, at the Hospital Ibn Sina of Rabat, using the MegaPure virus DNA/RNA purification kit in the nucleic acid purification system-32 (Bigfish Bio-tech, Hangzhou, China). The patient was identified as positive for COVID-19 by reverse transcriptase quantitative PCR using a SARS-CoV-2 kit (MAScIR, Morocco) and exhibited cycle threshold (CT) values of 18 and 19 for the RdRp and S genes, respectively. Total cDNA was prepared using a SuperScript VILO cDNA synthesis kit (Invitrogen, Thermo Fisher Scientific, USA) and used for SARS-CoV-2 library preparation with an Ion AmpliSeq kit for Chef DL8 (Thermo Fisher Scientific). The library was adjusted to 30 picomoles (pM) and loaded onto the Ion Chef instrument (Thermo Fisher Scientific) for emulsion PCR, enrichment, and loading onto the Ion S5 530 chip. Whole-genome sequencing (WGS) was performed using the Ion AmpliSeq SARS-CoV-2 research panel (Invitrogen, Thermo Fisher Scientific) for complete viral genome sequencing according to the instructions for use on an Ion GeneStudio S5 Prime series system.

Raw data consisting of 981,092 reads with an average read length of 200 bp were analyzed using Torrent Suite v5.12.0 software. The NGS QC Toolkit v2.3.3 was used to remove low-quality and short reads. The consensus sequence was generated using IRMAreport v1.3.0.2 and mapped to the reference sequence (GenBank accession number MN908947.3) using Minimap (6). The BAM file was sorted using SAMtools sort (7); then, it was used to call the genetic variations in variant call format with SAMtools mpileup and bcftools using the multiallelic-caller option. We then annotated the file, and its impact was predicted using SnpEff (8). All tools were run with default parameters.

Our analysis allowed us to obtain a near-complete SARS-CoV-2 genome with a length of 29,805 bp and an overall DNA G+C content of 37.96%. A total of 974,515 reads were correctly mapped, covering 99.33% of the total genome with a mean depth of 1,995 x.
| Gene   | Nucleotide position | Nucleotide change | Residue change | Effect                                      |
|--------|---------------------|-------------------|----------------|---------------------------------------------|
| ORF1ab | 241                 | -25C>T            | No change assigned | upstream_gene_variant                        |
|        | 2015                | 1750A>G           | Met584Val       | missense_variant                            |
|        | 2229                | 1964G>A           | Cys655Tyr       | missense_variant                            |
|        | 2832                | 2567A>G           | Lys856Arg       | missense_variant                            |
|        | 3037                | 2772C>T           | Phe924Phe       | synonymous_variant                          |
|        | 5386                | 5121T>G           | Ala1707Ala      | synonymous_variant                          |
|        | 6512                | 6248_6250delGTT   | Ser2083_Leu2084delInslle | disruptive_inframe_deletion |
|        | 8393                | 8128G>A           | Ala2710Thr      | missense_variant                            |
|        | 10029               | 9764C>T           | Thr3255Ile      | missense_variant                            |
|        | 10449               | 10184C>A          | Pro3395His      | missense_variant                            |
|        | 11282               | 11022_11030delGTCTGGTT | Leu3674_Gly3676del | disruptive_inframe_deletion |
|        | 11537               | 11272A>G         | Ile3758Val      | missense_variant                            |
|        | 13195               | 12930T>C         | Val4310Val      | synonymous_variant                          |
|        | 14408               | 14144C>T         | Pro4715Leu      | missense_variant                            |
|        | 15240               | 14976C>T         | Asn4992Asn      | synonymous_variant                          |
|        | 18163               | 17899A>G         | Ile5967Val      | missense_variant                            |
|        | 21762               | 200C>T           | Ala67Val        | missense_variant                            |
|        | 21764               | 204_209delACATGT | His69_Val70del  | disruptive_inframe_deletion                |
|        | 21946               | 284C>T           | Thr95Ile        | missense_variant                            |
|        | 21986               | 425_433delGTGTTATT | Gly142_Tyr145delInsAsp | disruptive_inframe_deletion |
|        | 22278               | 1016G>A          | Gly339Asp       | missense_variant                            |
|        | 22763               | 1111T>C          | Ser371Pro       | missense_variant                            |
|        | 22674               | 1112C>T          | Ser371Phe       | missense_variant                            |
|        | 22679               | 1117T>C          | Ser373Pro       | missense_variant                            |
|        | 22686               | 1124C>T          | Ser375Phe       | missense_variant                            |
|        | 22813               | 1251G>T          | Lys417Asn       | missense_variant                            |
|        | 22882               | 1320T>G          | Asn440Lys       | missense_variant                            |
|        | 22898               | 1336G>A          | Gly446Ser       | missense_variant                            |
|        | 22992               | 1430G>A          | Ser477Asn       | missense_variant                            |
|        | 22995               | 1433C>A          | Thr478Lys       | missense_variant                            |
|        | 23013               | 1451A>C          | Glu484Ala       | missense_variant                            |
|        | 23040               | 1478A>G          | Gln493Arg       | missense_variant                            |
|        | 23048               | 1486G>A          | Gly496Ser       | missense_variant                            |
|        | 23055               | 1493A>G          | Gln498Arg       | missense_variant                            |
|        | 23063               | 1501A>T          | Asn501Tyr       | missense_variant                            |
|        | 23075               | 1513T>C          | Tyr505His       | missense_variant                            |
|        | 23202               | 1640C>A          | Thr547Lys       | missense_variant                            |
|        | 23403               | 1841A>G          | Asp614Gly       | missense_variant                            |
|        | 23525               | 1963C>T          | His655Tyr       | missense_variant                            |
|        | 23599               | 2037T>G          | Asn679Lys       | missense_variant                            |
|        | 23604               | 2042C>A          | Pro681His       | missense_variant                            |
|        | 23854               | 2292C>A          | Asn764Lys       | missense_variant                            |
|        | 23948               | 2386G>T          | Asp796Tyr       | missense_variant                            |
|        | 24130               | 2568C>A          | Asn856Lys       | missense_variant                            |
|        | 24424               | 2862A>T          | Gln954His       | missense_variant                            |
|        | 24469               | 2907T>A          | Asn969Lys       | missense_variant                            |
|        | 24503               | 2941C>T          | Leu981Phe       | missense_variant                            |
|        | 25000               | 3438C>T          | Asp1146Asp      | synonymous_variant                          |
| ORF3a  | 25584               | 192C>T           | Thr64Thr        | synonymous_variant                          |
|        | 26270               | 26C>T            | Thr9Ile         | missense_variant                            |
| E      | 26530               | 8A>G             | Asp3Gly         | missense_variant                            |
|        | 26577               | 55C>G            | Gln19Glu        | missense_variant                            |
|        | 26709               | 187G>A           | Ala63Thr        | missense_variant                            |
| M      | 27259               | 58A>C            | Arg20Arg        | synonymous_variant                          |
| ORF6   | 27807               | 27807C>T         | No change assigned | intergenic_region |
| ORF8   | 28311               | 38C>T            | No change assigned | intergenic_region |
| N      | 28361               | 90_98delAGAACGCAG | Gln31_Ser33del | disruptive_inframe_deletion                |
|        | 28881               | 608G>A           | Arg203Lys       | missense_variant                            |
|        | 28882               | 609G>A           | Arg203Arg       | synonymous_variant                          |
|        | 28883               | 610G>C           | Gln204Arg       | missense_variant                            |
Phylogenetic analysis using Phylogenetic Assignment of Named Global Outbreak Lineages (pangolin) (9) revealed that the strain belongs to lineage BA.1. The genetic variation process revealed a total of 61 variations compared to the reference sequence (GenBank accession number MN908947.3) (Table 1).

**Data availability.** This sequence was deposited at GenBank under the accession number OM432158.1. The raw reads were deposited at the NCBI Sequence Read Archive (SRA) under the accession number SRR17818130.

**ACKNOWLEDGMENTS**

This work was carried out under national funding from the Moroccan Ministry of Higher Education and Scientific Research (COVID-19 program) to A.I. This work was also supported by a grant from the Moroccan Institute of Cancer Research and the PPR-1 program to A.I.

All research activities described here were conducted in adherence to the Declaration of Helsinki, as revised in 2013.

**REFERENCES**

1. Gao SJ, Guo H, Luo G. 2022. Omicron variant (B. 1.1. 529) of SARS-CoV-2, a global urgent public health alert! J Med Virol 94:1255–1256. https://doi.org/10.1002/jmv.27491.

2. Pascarella S, Ciccozzi M, Bianchi M, Benvenuto D, Cauda R, Cassone A. 2022. The electrostatic potential of the Omicron variant spike is higher than in Delta and Delta-plus variants: a hint to higher transmissibility? J Med Virol 94:1277–1280. https://doi.org/10.1002/jmv.27528.

3. Pulliam JR, van Schalkwyk C, Govender N, von Gottberg A, Cohen C, Groome MJ, Dushoff J, Miliana K, Moultrie H. 2021. Increased risk of SARS-CoV-2 reinfection associated with emergence of the Omicron variant in South Africa. medRxiv https://doi.org/10.1101/2021.11.11.21266068.

4. Akkiz H. 2021. Implications of the novel mutations in the SARS-CoV-2 genome for transmission, disease severity, and the vaccine development. Front Med (Lausanne) 8:636532. https://doi.org/10.3389/fmed.2021.636532.

5. Lopez-Rincon A, Perez-Romero CA, Tonda A, Mendoza-Maldonado L, Claessen E, Garssen J, Kraneveld AD. 2021. Design of specific primer sets for the detection of B.1.1.7, B.1.351 and P.1 SARS-CoV-2 variants using deep learning. bioRxiv https://doi.org/10.1101/2021.01.20.427043.

6. Li H. 2018. Minimap2: pairwise alignment for nucleotide sequences. Bioinformatics 34:3094–3100. https://doi.org/10.1093/bioinformatics/bty191.

7. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R, 1000 Genome Project Data Processing Subgroup. 2009. The Sequence Alignment/Map format and SAMtools. Bioinformatics 25:2078–2079. https://doi.org/10.1093/bioinformatics/btp352.

8. Cingolani P, Platts A, Wang LL, Coon M, Nguyen T, Wang L, Land SJ, Lu X, Ruden DM. 2012. A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff. SNPs in the genome of Drosophila melanogaster strain w1118; iso-2; iso-3. Fly (Austin) 6:80–92. https://doi.org/10.4161/fly.19695.

9. O'Toole Á, Scher E, Underwood A, Jackson B, Hill V, McCrone JT, Colquhoun R, Ruis C, Abu-Dahab K, Taylor B, Yeats C, Du Plessis L, Maloney D, Medd N, Attwood SW, Anensen DM, Holmes EC, Pybus OG, Rambaut A. 2021. Assignment of epidemiological lineages in an emerging pandemic using the pangolin tool. Virus Evol 7:veab064. https://doi.org/10.1093/ve/veab064.