Chapter 15
Amplicon-Based Nanopore Sequencing of Patients Infected by the SARS-CoV-2 Omicron (B.1.1.529) Variant in India

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Abstract We report the sequencing of SARS-CoV-2 Omicron variants from 75 patients, using nanopore long-read sequencing chemistry. These data show a range of mutations in spike glycoprotein that are both unique and common to other populations.

Keywords SARS-CoV-2 · COVID-19 · Omicron · Mutation · Sequencing

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1 Introduction

Coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a betacoronaviridae family member, and has been a primary and urgent concern worldwide [1–3]. As of March 4, 2022, over 107 countries had reported infections due to Omicron variants, since the reporting of first case on November 29, 2021 [4]. India saw the first few Omicron cases originating in the state of Karnataka on December 1, 2021 [5], with Delhi reporting a case later from a Tanzania returnee [6]. In this study, we sought to sequence all COVID-19 samples including Omicron variants that were reported in our tertiary care to gain further insights into the mutations occurring in this SARS-CoV-2 variant.

2 Methods

Nasopharyngeal swab samples were collected from 75 patients with a travel history of Africa/Middle East. Here, we randomly analysed samples from 10 representative patients who presented with mild symptoms (fever, cold, cough, sore throat and mild weakness) within 3 days of onset of infection and prior to hospitalization. The samples were used as an input for the ARTIC network "Midnight" protocol (Fig. 15.1) for PCR tiling of SARS-CoV-2, including sequencing with Oxford Nanopore Technologies (ONT) long-read whole-genome sequencing (Rapid Barcoding Kit 96/SQL-RBK-110-96) [7, 8].

3 Results and Discussion

ONT sequencing yielded an average of 25 million reads from all 10 samples, spanning 96.28% of the SARS-CoV-2 genome (20× coverage depth) (Table 15.1). To check the transmissibility associated with the number of mutations in the spike glycoprotein associated with receptor-binding domain (RBD), we compared the 44 common mutations from our samples with the recently emerging mutations of Omicron. Our preliminary analysis indicated that the Omicron variant subcladed with the dominant Delta variant and might have evolved rapidly from multiple mutations (Tables 15.2a, 15.2b, 15.3 and 15.4). A neighbourhood joining tree was constructed using Clustal Omega with the sequences sorted vertically, thereby drawing a circular and unrooted tree (Fig. 15.2a) [9]. We observed that the Indian Omicron variants were clustered together with a root emerging from OL815455, the variant that was first detected from Botswana. The iTOL containing the 75 sequenced samples and Wuhan reference yielded distinct clades in both unrooted and rooted circular tree (data not shown) and the four samples that were claded separately suggested that these were among the first suspected Omicron cases in India (Fig. 15.2a)

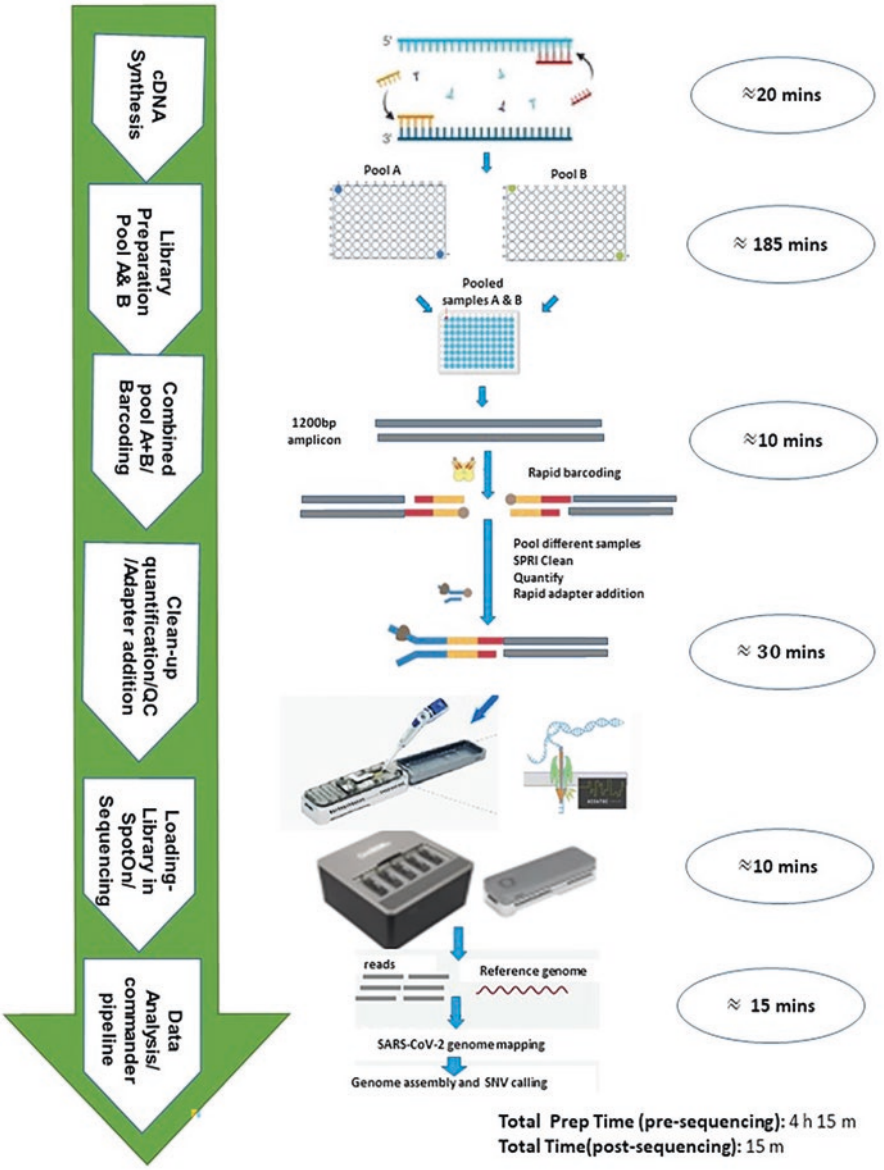

Fig. 15.1 Midnight workflow for preparation of SARS-CoV-2 whole-genome sequencing. This method was similar to the ARTIC amplicon sequencing protocol for MinION for SARS-CoV-2 v3 (LoCost) by Josh Quick and the method used in Freed et al. [8]

[10–12]. We obtained p.Thr614Ile, p.Thr1822Ile, p.Thr6098Ile and p.Asp155Tyr from LNHD9, p.Ala701Val and p.Val1887Ile from LNHD8 and p.Gly667Ser from LNHD1. However, our preliminary observations indicated that none of these are known to confer detrimental properties to the spike (e.g. changes in transmissibility,

Table 15.1 List of the 10 samples with coverage, CT values, clinical symptoms and age/sex

S. No.	GenBank	Size (bp)	GISAID	Age/Sex	Lab ID	CT VALUE	Clinical Symptoms	Coverage 20×
1	ON063250.1	29,746	EPI_ISL_7877026	35Y/M	LNHD4	E gene-25, rdrp gene-26	Fever, cough or mild weakness	99.94
2	ON063249.1	29,768	EPI_ISL_7877093	39Y/F	LNHD5	E gene-19, rdrp gene-22	Fever, cough, cold or mild weakness	98.39
3	ON063248.1	29,742	EPI_ISL_7877115	6Y/M	LNHD6	E gene-27, rdrp gene-27,	Not available	91.31
4	ON063247.1	29,779	EPI_ISL_7877191	40Y/M	LNHD7	E gene-20, rdrp gene-23	Fever, cough, cold, mild weakness	97.16
5	ON063246.1	29,751	EPI_ISL_7877201	18Y/M	LNHD8	E gene-24, rdrp gene-26	Fever, cough, mild weakness	97.27
6	ON063245.1	29,739	EPI_ISL_7877202	57Y/M	LNHD9	E gene-18, rdrp gene-20	Fever, cough, cold, sore throat, mild weakness	97.22
7	ON063244.1	29,780	EPI_ISL_7877203	19Y/F	LNHD10	E gene-30, rdrp gene-30	Not available	97.23
8	ON063243.1	29,737	EPI_ISL_7877297	23Y/M	LNHD11	E gene-21, rdrp gene-23	Fever, cough, cold, mild weakness	97.25
9	ON063242.1	29,739	EPI_ISL_7889640	31Y/M	LNHD12	E gene-20, rdrp gene-22	Fever, cough, cold, mild weakness	94.3
10	ON063241.1	29,743	EPI_ISL_7889641	42Y/M	LNHD13	E gene-17, rdrp gene-19	Fever, cough, cold, sore throat, mild weakness	93.2

S. No. sample number, *bp* base pairs, *CT* cycle threshold

severity or immune evasion). Mutations in the spike proteins (Fig. 15.2b(i–iii)) of SARS-CoV-2 variants of concern have also been compared to the parental SARS-CoV-2 isolate B.1 suggesting that the amino acid substitutions are already found in altered positions but with distinct substitutions (Supplementary Tables 15.1 and 15.2).

Table 15.2a Genome Coverage and mutations in the cohort

Sample	Query	Length (nucleotides)	Length (amino acids)	Total mutations (number)	Unique mutations (number)	Reference
1	hCoV-19/ India/un-LNHD4/2021|EPI_ISL_7877026	29,751	9710	41	0	hCoV19/Wuhan/ WIV04/2019
2	hCoV-19/ India/un-LNHD5/2021|EPI_ISL_7877093	29,779	9710	45	0	hCoV19/Wuhan/ WIV04/2019
3	hCoV-19/ India/un-LNHD6/2021|EPI_ISL_7877115	29,742	9710	43	0	hCoV19/Wuhan/ WIV04/2019
4	hCoV-19/ India/un-LNHD7/2021|EPI_ISL_7877191	29,768	9710	45	0	hCoV19/Wuhan/ WIV04/2019
5	hCoV-19/ India/un-LNHD8/2021|EPI_ISL_7877201	29,746	9710	45	0	hCoV19/Wuhan/ WIV04/2019
6	hCoV-19/ India/un-LNHD9/2021|EPI_ISL_7877202	29,771	9710	49	0	hCoV19/Wuhan/ WIV04/2019
7	hCoV-19/India/ un-LNHD10/2021|EPI_ISL_7877203	29,738	9710	43	0	hCoV19/Wuhan/ WIV04/2019
8	hCoV-19/India/ un-LNHD11/2021|EPI_ISL_7877297	29,752	9710	47	1	hCoV19/Wuhan/ WIV04/2019
9	hCoV-19/India/ un-LNHD12/2021|EPI_ISL_7889640	29,745	9710	44	0	hCoV19/Wuhan/ WIV04/2019
10	hCoV-19/India/ un-LNHD13/2021|EPI_ISL_7889641	29,764	9710	41	0	hCoV19/Wuhan/ WIV04/2019

Table 15.2b Multiple mutations identified in the study cohort

1	NSP3_K38R,NSP3_A1892T,NSP4_T492I,NSP5_P132H,NSP6_I189V,NSP12_P323L,NSP14_I42V,Spike_N679K,Spike_Q493R,Spike_G339D,Spike_G446S,Spike_P681H,Spike_D614G,Spike_N969K,Spike_N764K,Spike_T478K,Spike_H655Y,Spike_G496S,Spike_N856K,Spike_N440K,Spike_A67V,Spike_S371L,Spike_Q498R,Spike_K417N,Spike_T547K,Spike_L981F,Spike_S375F,Spike_Q954H,Spike_S477N,Spike_N501Y,Spike_T95I,Spike_Y505H,Spike_D796Y,Spike_S373P,Spike_E484A,E_T9I,M_A63T,M_D3G,M_Q19E,N_G204R,N_R203K
2	NSP3_S1265N,NSP3_L1266I,NSP3_K38R,NSP3_A1892T,NSP4_T492I,NSP5_P132H,NSP6_I189V,NSP12_P323L,NSP14_I42V,Spike_N679K,Spike_Q493R,Spike_G339D,Spike_G446S,Spike_P681H,Spike_D614G,Spike_N969K,Spike_R346K,Spike_N764K,Spike_T478K,Spike_H655Y,Spike_G496S,Spike_N856K,Spike_N440K,Spike_A67V,Spike_S371L,Spike_Q498R,Spike_K417N,Spike_T547K,Spike_L981F,Spike_S375F,Spike_Q954H,Spike_S477N,Spike_N501Y,Spike_T95I,Spike_Y505H,Spike_D796Y,Spike_S373P,Spike_E484A,E_T9I,M_A63T,M_D3G,M_Q19E,N_G204R,N_P13L,N_R203K
3	NSP3_K38R,NSP3_A1892T,NSP4_T492I,NSP5_P132H,NSP6_G107S,NSP6_I189V,NSP12_P323L,NSP14_I42V,Spike_N679K,Spike_Q493R,Spike_G339D,Spike_G446S,Spike_P681H,Spike_D614G,Spike_N969K,Spike_R346K,Spike_N764K,Spike_T478K,Spike_H655Y,Spike_G496S,Spike_N856K,Spike_N440K,Spike_S371L,Spike_Q498R,Spike_K417N,Spike_T547K,Spike_L981F,Spike_S375F,Spike_Q954H,Spike_S477N,Spike_N501Y,Spike_T95I,Spike_Y505H,Spike_D796Y,Spike_S373P,Spike_E484A,E_T9I,M_A63T,M_D3G,M_Q19E,N_G204R,N_D63G,N_R203K
4	NSP3_S1265N,NSP3_L1266I,NSP3_K38R,NSP3_A1892T,NSP4_T492I,NSP5_P132H,NSP6_G107S,NSP6_I189V,NSP12_P323L,NSP14_I42V,Spike_N679K,Spike_Q493R,Spike_G339D,Spike_G446S,Spike_P681H,Spike_D614G,Spike_N969K,Spike_R346K,Spike_N764K,Spike_T478K,Spike_H655Y,Spike_G496S,Spike_N856K,Spike_N440K,Spike_A67V,Spike_S371L,Spike_Q498R,Spike_K417N,Spike_T547K,Spike_L981F,Spike_S375F,Spike_Q954H,Spike_S477N,Spike_N501Y,Spike_T95I,Spike_Y505H,Spike_D796Y,Spike_S373P,Spike_E484A,E_T9I,M_A63T,M_D3G,M_Q19E,N_G204R,N_R203K
5	NSP3_S1265N,NSP3_K38R,NSP3_A1892T,NSP3_V1069I,NSP4_T492I,NSP5_P132H,NSP6_I189V,NSP12_P323L,NSP14_I42V,Spike_N679K,Spike_Q493R,Spike_G339D,Spike_G446S,Spike_P681H,Spike_D614G,Spike_N969K,Spike_N764K,Spike_T478K,Spike_H655Y,Spike_G496S,Spike_N856K,Spike_N440K,Spike_A67V,Spike_S371L,Spike_Q498R,Spike_K417N,Spike_T547K,Spike_L981F,Spike_V70I,Spike_S375F,Spike_Q954H,Spike_S477N,Spike_N501Y,Spike_T95I,Spike_A701V,Spike_Y505H,Spike_D796Y,Spike_S373P,Spike_E484A,E_T9I,M_A63T,M_D3G,M_Q19E,N_G204R,N_R203K

(continued)

Table 15.2b (continued)

6	NSP2_T434I,NSP3_S1265N,NSP3_L1266I,NSP3_K38R,NSP3_T1004I,NSP3_A1892T,NSP4_T492I,NSP5_P132H,NSP6_I189V,NSP12_P323L,NSP14_T173I,NSP14_I42V,Spike_N679K,Spike_Q493R,Spike_G339D,Spike_G446S,Spike_P681H,Spike_D614G,Spike_N969K,Spike_N764K,Spike_T478K,Spike_H655Y,Spike_G496S,Spike_N856K,Spike_N440K,Spike_A67V,Spike_S371L,Spike_Q498R,Spike_K417N,Spike_T547K,Spike_L981F,Spike_S375F,Spike_Q954H,Spike_S477N,Spike_N501Y,Spike_T95I,Spike_Y505H,Spike_D796Y,Spike_Y145H,Spike_S373P,Spike_E484A,NS3_D155Y,E_T9I,M_A63T,M_D3G,M_Q19E,N_G204R,N_P13L,N_R203K
7	NSP3_K38R,NSP3_A1892T,NSP4_T492I,NSP5_P132H,NSP6_I189V,NSP12_P323L,NSP14_I42V,Spike_N679K,Spike_Q493R,Spike_G339D,Spike_G446S,Spike_P681H,Spike_D614G,Spike_N969K,Spike_N764K,Spike_T478K,Spike_H655Y,Spike_G496S,Spike_N856K,Spike_N440K,Spike_A67V,Spike_S371L,Spike_Q498R,Spike_K417N,Spike_T547K,Spike_L981F,Spike_S375F,Spike_Q954H,Spike_S477N,Spike_N501Y,Spike_T95I,Spike_Y505H,Spike_D796Y,Spike_S373P,Spike_E484A,E_T9I,M_A63T,M_D3G,M_Q19E,N_G204R,N_D63G,N_R203K,N_Q9L
9	NSP2_R46K,NSP3_K38R,NSP3_A1892T,NSP4_T492I,NSP5_P132H,NSP6_I189V,NSP12_P323L,NSP14_I42V,Spike_N679K,Spike_Q493R,Spike_G339D,Spike_N211I,Spike_G446S,Spike_P681H,Spike_D614G,Spike_N969K,Spike_N764K,Spike_T478K,Spike_H655Y,Spike_G496S,Spike_N440K,Spike_A67V,Spike_S371L,Spike_Q498R,Spike_K417N,Spike_T547K,Spike_S375F,Spike_S477N,Spike_N501Y,Spike_T95I,Spike_Y505H,Spike_D796Y,Spike_G142V,Spike_S373P,Spike_L212I,Spike_E484A,NS3_L41F,E_T9I,M_A63T,M_D3G,M_Q19E,N_G204R,N_D63G,N_R203K
10	NSP3_S1265N,NSP3_L1266I,NSP3_K38R,NSP3_A1892T,NSP4_T492I,NSP5_P132H,NSP6_I189V,NSP12_P323L,NSP14_I42V,Spike_N679K,Spike_Q493R,Spike_G339D,Spike_G446S,Spike_P681H,Spike_D614G,Spike_N969K,Spike_N764K,Spike_T478K,Spike_H655Y,Spike_G496S,Spike_N856K,Spike_N440K,Spike_A67V,Spike_S371L,Spike_Q498R,Spike_K417N,Spike_T547K,Spike_L981F,Spike_S375F,Spike_Q954H,Spike_S477N,Spike_N501Y,Spike_T95I,Spike_Y505H,Spike_D796Y,Spike_S373P,Spike_E484A,NS3_L106F,N_G204R,N_D343G,N_R203K

Table 15.3 Amino acid substitutions in the spike region observed in the study cohort

Spike mutation	Occurrences
K417N	9
T478K	9
S477N	9
E484A	9
G339D	9
N440K	9
G496S	9
Q493R	9
T547K	9
G446S	9
S375F	9
D614G	9
S373P	9
N764K	9
N679K	9
S371L	9
Y505H	9
Q498R	9
P681H	9
T95I	9
H655Y	9
N501Y	9
D796Y	9
N969K	9
A67V	8
Q954H	8
N856K	8
L981F	8
R346K	4
S373P	1
A67V	1
N764K	1
N679K	1
Q954H	1
K417N	1
S371L	1
T478K	1
S477N	1
Y505H	1
N856K	1

(continued)

Table 15.3 (continued)

Spike mutation	Occurrences
E484A	1
L981F	1
G339D	1
Q498R	1
P681H	1
N440K	1
H655Y	1
G496S	1
T95I	1
Q493R	1
T547K	1
G446S	1
S375F	1
D796Y	1
D614G	1
N501Y	1
N969K	1
N211I	1
Y145H	1
A701V	1
G142V	1
V70I	1
L212I	1

The limitation of our study is that although the adopted ARTIC sequencing protocol allowed the confirmation of SARS-CoV-2 infections, we did not carry out analyses to determine the probable structural impact of mutations on binding of antibodies produced by existing vaccines or previous SARS-CoV-2 infections, as described by Kannan et al. [13].

In conclusion, our study has demonstrated the utility of nanopore sequencing for SARS-CoV-2 genomes from clinical specimens. We firmly hope that prompt diagnosis and rapid whole-genome analysis would allow a decisive response to the SARS-CoV-2 outbreak that will bring disease control and prevention efforts.

Table 15.4 Common mutations ($n = 44$) seen across the Indian cohort

p.Ala67Val
p.Thr95Ile
p.Gly339Asp
p.Ser371Pro
p.Ser371Phe
p.Ser373Pro
p.Ser375Phe
p.Lys417Asn
p.Asn440Lys
p.Gly446Ser
p.Ser477Asn
p.Thr478Lys
p.Glu484Ala
p.Gln493Arg
p.Gly496Ser
p.Gln498Arg
p.Asn501Tyr
p.Tyr505His
p.Thr547Lys
p.Asp614Gly
p.His655Tyr
p.Asn679Lys
p.Pro681His
p.Asn764Lys
p.Asp796Tyr
p.Asn856Lys
p.Gln954His
p.Asn969Lys
p.Leu981Phe
p.Thr9Ile
p.Asp3Gly
p.Gln19Glu
p.Ala63Thr
p.Arg203Lys
p.Gly204Arg
p.Gly645Ser
p.Lys856Arg
p.Ala2710Thr
p.Thr3255Ile
p.Pro3395His
p.Ile3758Val
p.Ala4409Thr
p.Pro4715Leu
p.Ile5967Val

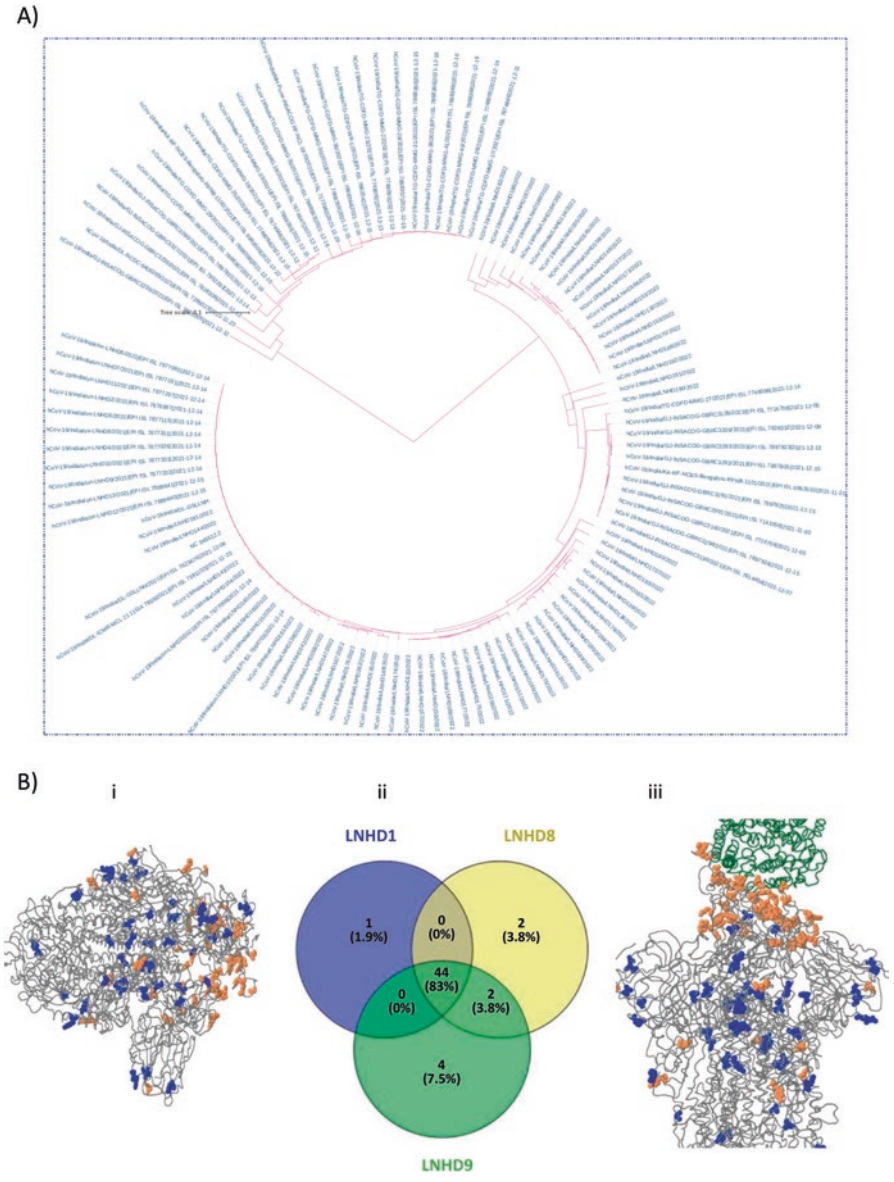

Fig. 15.2 (**a**) Circular phylogenetic tree of all 75 samples from India claded with the Wuhan reference genome. The unrooted tree shows a clear dissection of Wuhan from other lineages. All LNHD accessions are labelled. In the Indian sub-population, spike mutations ($n = 35$) were seen with the nearest residue if in loop/termini region (A67V, V70I(69), T95I, G142V, Y145H(143), N211I, L212I, G339D, R346K, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H T547K, D614G, H655Y, N679K(674), P681H(674), A701V, N764K, D796Y, N856K, Q954H, N969K and L981F). (**b**) (i) Spike glycoprotein (PDB: 6acc, EM 3.6 Angstrom) with RBD in down conformation. (ii) Multi-Venn diagram of three samples LNHD1, LNHD8 and LNHD9 showing unique and common mutations to all the LNHD series. (iii) Spike glycoprotein (PDB: 6acj, EM 4.2 Angstrom) in complex with host cell receptor ACE2 (green ribbon). (Also see links to Supplementary Tables 15.1 and 15.2)

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Ethics Statement Informed consent was judiciously taken before the sample was sequenced. The Institutional Ethics Committee of Maulana Azad Medical College, Delhi, India, had given approval for the study (F.1/IEC/MAMC/85/03/2021).

Data Availability All Omicron variant samples have been uploaded to NCBI-GenBank with accession IDs ON063241–ON063253 (https://www.ncbi.nlm.nih.gov/nuccore/?term=ON063241:ON063253[accn]) and ON060006–ON060067 (https://www.ncbi.nlm.nih.gov/nuccore/?term=ON060006:ON060067[accn]). The same have also been submitted to GISAID.org with hCoV-19/India/un-LNHD**XX**/2021 series

EPI_ISL_7864703
EPI_ISL_7876997
EPI_ISL_7877026
EPI_ISL_7877006
EPI_ISL_7877093
EPI_ISL_7877115
EPI_ISL_7877191
EPI_ISL_7877201
EPI_ISL_7877202
EPI_ISL_7877203
EPI_ISL_7877297
EPI_ISL_7889640

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