SARS-CoV-2 genome sequences from Lucknow, Uttar Pradesh, India by Nanopore Sequencing

Abstract:

A new challenge has immerged in the form of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in worldwide. Rapid genome sequencing of SARS-CoV-2 has been a powerful tool to study the pathogenicity of severe acute respiratory syndrome coronavirus 2.During this pandemic situation more genome sequencing of SARS-CoV-2 should be done in order to detect the mutations and genomic modifications across the globe. Here, in this study we have sequenced 23 SARS-CoV-2 positive samples from the state of Uttar Pradesh, India collected during the first pandemic. A range of 2-22 mutations were observed including D614G, L452R, Q613H, Q677H, T1027I in S gene, S194L in N gene, Q57H, L106F, T175I in ORF3a gene as reported previously and also possible novel mutations like P309S in ORF1ab gene, T379I in N gene and L52F, V77I in ORF3a gene were detected.Phylogenetic genome analysis has shown similarity with other SARS-CoV-2 viruses reported in Uttar Pradesh. Mutations in these genes have the potential to affect the severity of the disease. Therefore, identify the mutation is very important to know the pathogenicity of SARS-CoV-2 virus.

Key Words: SARS-CoV-2, mutations, SNPs, Uttar Pradesh

Introduction:

In December 2019, Wuhan, Hubei province, China first reported a numerous pneumonias like cases with unidentified etiology. Later it was identified as a novel coronavirus (COVID-19)[1]**.** According to WHO, as of now 13 August 2021, a total of 205,338,159 confirmed COVID-19cases, including 4,333,094 deaths have been reported worldwide and 32,117,826 Confirmed Cases and 430,254 deaths in India. The state of Uttar Pradesh witnessed 17, 08,876 confirmed cases and 22,782 deaths according to the Government of India.First strain of Wuhan-Hu-1 coronavirus was isolated and the complete genome was sequenced of 29.9 kb[2]. Also other types of coronavirus including SARS-CoV and MERS-CoV has identified previously, who infect humans which are positive-sense RNA genomes with 27.9 kb and 30.1 kb, respectively[3]. To understand the genetic variants of SARS-CoV-2, genome sequencing is an essential tool to track cases and determining microbial provenance[4], [5]**.** First SARS-CoV-2 genome sequence was publicly available on January 10, 2020 (GenBank ID: MN908947.3)[2]**.** Since then multiple sequences have been submitted to the publicly available database such as GeneBank and GISAID globally. Study of this extensive genomic sequencing to identify the mutations which can increase transmissibility and virulence of the virus[6], [7]**.** SARS-CoV-2 is an enveloped positive sense single stranded RNA virus which has multiple genes which code for different proteins such as open reading frames, such as ORF1a, ORF1b ORF3a, ORF6, ORF7a, ORF7b, ORF8, and ORF10, S gene (Surface glycoprotein), N gene (nucleocapsid phosphoproteins), M gene (membrane glycoprotein) and E gene (envelope protein)[8]**.** Viral genome of SARS-CoV-2, mainly placed in the first ORF (ORF1a/b) which translated into two replicase polyproteins (pp1a and pp1ab) and 16 non-structural proteins (NSP),RNA-dependent RNA polymerase which are important for replication and survival in the host[9]. The remaining ORFs such as ORF3a, ORF7

and ORF8 genes code for accessory and structural proteins, however their role is not yet fully understood. ORF3a in SARS-CoV has played a significant role in viral release to the host[10], [11].The exact role of ORF7 and ORF8 is still unknown but several report suggested that they are involving in the viral replication and has immune responses[12], [13]**.** Open-source databases such as the GenBank-NCBI and GISAID has a huge number of SARS-CoV-2 genomic sequencing data to identify the mutation, SNPs (single nucleotide polymorphism) in SARS-CoV-2. SNPs in the SARS-CoV-2 genome leads to the missense variant such as P323L, P4715L in ORF1ab, D614G, N439K in S gene, R203K, R202K and G204R in N gene which are commonly reported. P323L, P4715L mutation in ORF1ab has play an important role in regulating RNNA dependent RNA polymerase[14]**.** D614G, N439K mutation in S gene has associated with Increase infectivity[15]**.** R203K, R202K and G204R mutations in N gene are linked with viral survival and replication in the host[16]. Knowing the variants which could escape the immunity, by genome sequencing is essential to stop the coronavirus across the globe.

In this study, we sequenced SARS-CoV-2 genome from 24 SARS-CoV-2 positive samples received at Dr. Ram Manohar Lohia Institute of Medical Sciences, Lucknow during the first peak during the pandemic in the state of Uttar Pradesh, India. Various mutation were observed in the genomic sequences including previously reported mutations as well as novel mutations.

Materials and Methods:

Collection of Covid-19 positive RNA Samples:

RNA from twenty three Covid-19 positive samples was obtained from Dr. Ram Manohar Lohia Institute of Medical Sciences, Lucknow. The presence of SARS-CoV-2 were detected by COVID-19 RT-qPCR kit ((Labgun, lab, Genomics.co. Ltd, Republic of Korea). Cq values in between 18-35 were taken for sequencing. This study protocol was approved by Institutional Human Ethics Committee SGPGIMS, Lucknow (Ref N. 111 PGI/BE/327/2020)

Whole genome sequencing:

For sequencing, libraries were constructed using a ligation kit (SQK-LSK109) as described in PCR-tiling of COVID-19 virus protocol (PTC_9096_v109_revF_06Feb2020; Oxford Nanopore Technologies). Briefly, 24 SRS-CoV-2 positive RNA samples were isolated from swabs positive for the presence of SARS-CoV-2 in RT-qPCR assay (quantification cycle (Cq) values18-31;(Table 1) were converted into complementary DNA (cDNA). Then the cDNA products were amplified using the primer pools spanning the SARS-CoV-2 whole genome sequence (i.e., 400-bp Artic nCoV-2019 V3 panel (https://github.com/artic-network/artic-ncov2019) purchased from Integrated DNA Technologies according to the manufacturer's instructions. DNA library prepare (SQK-LSK-109, Oxford Nanopore Technologies, United Kingdom), purification using AMPure XP magnetic beads (Beckman Coulter), adaptor ligation and barcoding EXP-NBD104 (barcodes 1-12) or EXP-NBD114 (barcodes 13-24) kits (Oxford Nanopore Technologies, United Kingdom) were done as per the manufacturer's instructions. DNA libraries were pooled and loaded on

R9.4.1 flow cell (FLO-MIN106, Oxford Nanopore Technologies, United Kingdom). The sequencing was performed using a MinION Mk-1b device (Oxford Nanopore Technologies).

Genome Assembly, Alignment, and Phylogenetic Analysis:

Nanopore sequencing data were base called and de-multiplexed using Guppy v.3.4.4. Variant analysis was performed using Artic analysis pipeline v.1.1.3. (https://artic.network/ncov-2019/ncov2019-bioinformatics-sop.html) using recommended settings. Minimum and maximum read lengths in the Artic guppyplex filter were set to 400 and 700 for the 400-bp amplicons. Wuhan Hu-1 (GenBank ID: MN908947.3) used as reference genome.

MAFFT was used to align the whole sequences of the 24 genomes to SARS-CoV-2 reference genome (MN908947.3)[17], [18]. SnpEff v4.3 was used to identify the SNPs and changes in the amino acid produced by the gene in the genome[19].

Phylogenetic analysis was done using Nextstrain and aligned using MAFFT v7.471 to assess to previous reported genomes. Maximum likelihood trees were generated and identified clade as well as lineages to classified the identified variants using Nextclade [20].

Results and Discussion:

Twenty three RT-PCR SARS-CoV-2 positive samples from different time periods of the pandemic in Uttar Pradesh were selected for this study. Genome coverage was obtained in between 1000X to 8500X for all the samples at the end of sequencing. Next consensus genomes were obtained after assembled with the reference genome. All the sequenced genome were submitted to GISAID [Table 2].

The twenty three viral SARS-CoV-2 genome assemblies were aligned to the reference genome (SARS-CoV-2 Wuhan-Hu-1 MN908947.3) using MAFFT and genome similarities to the reference genome were calculated (Table 2). All genomes showed more than 95% similarity.

Individual genomewere aligned to NCBI reference genome to predict the mutations in the genome. Using SnpEff v4.3 tool, various synonymous and missense mutations were detected. A range of 8 to 22 mutations were detected in major genes in twenty three samples (Table 3).

Non-synonymous mutations were detected in ORF1ab, S, N, ORF3a gene (Table 3).Mutations in S gene such as D614G, L452R, Q613H, Q677H, T1027I has identified as previously reported. Non-synonymous mutation D614G has identified in most of the genome sequences, as reported widely in the literature that increase infectivity by adding more functional S protein into the virion causing more severity[21]**.** Otheridentified mutations are also linked with increase viral infectivity. Also several previously reported mutations like S194L in N gene, Q57H, L106F, T175I in ORF3a gene were observed. L52F, V77I mutations in ORF3a were also identified in one and three cases respectively.

A few possible novel mutations were also observed in ORF1ab, N and ORF3a (Table 3). P309S mutation in ORF1ab gene was identified in four samples. T379I mutation in N gene was

observed in one case. These mutations in the genome indicate presence of multiple variations of the virus in Uttar Pradesh.

141 SARS-CoV-2 genome sequences from Lucknow, Uttar Pradesh were downloaded from GISAID to construct the phylogenetic tree with our 23 sequenced SARS-CoV-2 genome sequences. The sequenced 23 SARS-CoV-2 genome sequences were found in clade 20 A and 20B, out of which 16 variants were found related to clade 20 A and remaining 6 variants were found related to clade 20 B. Out of 23 genome sequences, 6 variants were identified as B.1 lineage, 8 variants as of B.1.36 lineage, 6 variants as of B.1.1.216 lineage and one variants was of B.1.456 lineage while the remaining two variants has not shown association with any of lineages.

Conclusion:

We sequenced twenty three SARS-CoV-2 genomes from clinical positive samplescollected from Uttar Pradesh, India during the first wave of Covid-19. Whole genome sequencing studies has identified several possible new mutations as well as previously reported mutations. Most of the samples have D614G non-synonymous mutation. Detection of these mutations in the viral genome by genome sequencing discovers the pandemic in the given geographic region. So, future studies can be done in order to the better understanding if these mutations if they have the potential influence on host susceptibility, pathogenicity and virulence. Phylogenetic analysis of the isolated viral genomes showedhigh similarities with the previously isolated SARS-CoV-2 genomes in the state of Uttar Pradesh. Thus, rapid whole genome sequencing of the clinical samples to identify the genomic variants of SARS-Cov-2 in the targeted geographic regions.

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Figure 1:

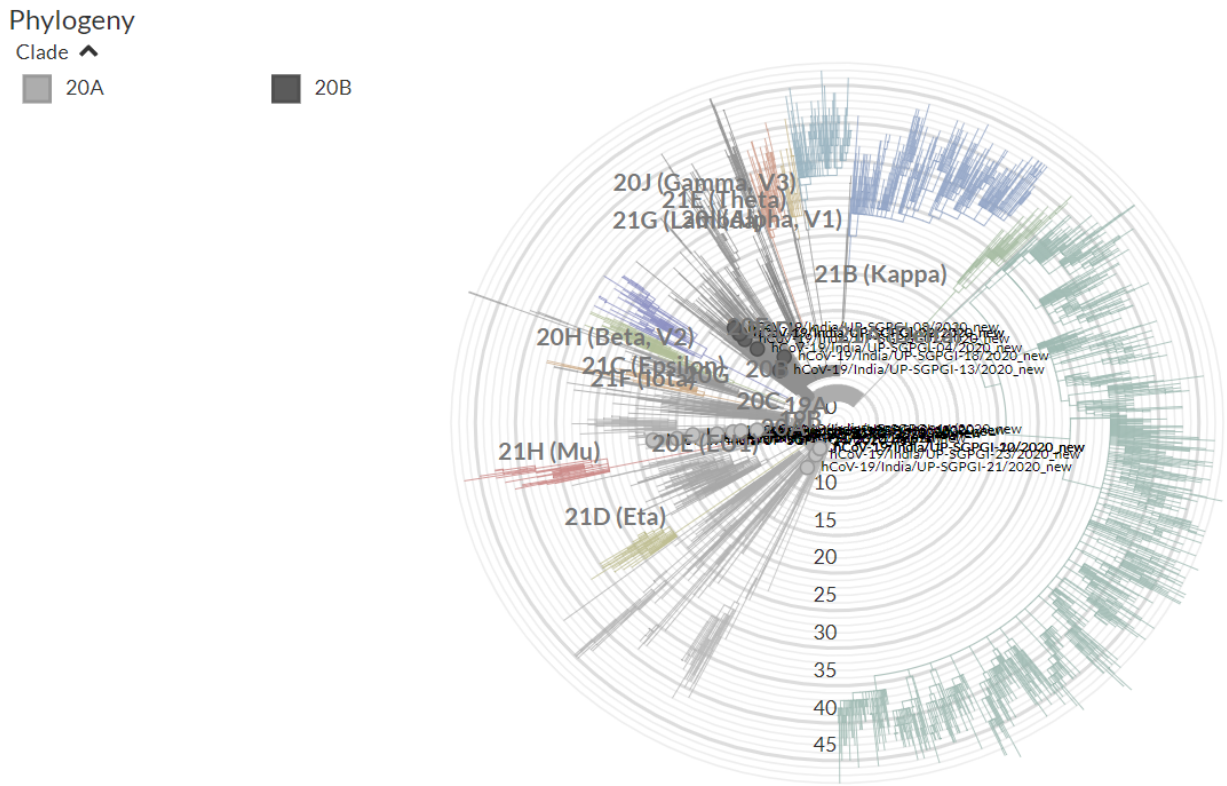

Figure 1: Phylogenetic analysis of 164 SARS-CoV-2 genome sequences: including 23 variants of present study and 141 variants from different regions of Lucknow, Uttar Pradesh, were retrieved from GISAID database. Nextclade was used for phylogentic analysis and nextstain nomenclature of all variants as shown in figure.

Table 1:

Sample ID	Age	Sex	Cq values		
			E gene	N gene	RdRp
hCoV-19/India/UP-SGPGI-01/2020	39	M	23.14	24.37	23.64
hCoV-19/India/UP-SGPGI-02/2020	45	M	22.64	23.68	21.93
hCoV-19/India/UP-SGPGI-03/2020	29	M	25.46	24.48	22.48
hCoV-19/India/UP-SGPGI-04/2020	39	M	26.78	26.14	26.98
hCoV-19/India/UP-SGPGI-06/2020	23	M	28.42	28.03	28.56
hCoV-19/India/UP-SGPGI-07/2020	57	M	18.93	19.43	19.13
hCoV-19/India/UP-SGPGI-08/2020	48	M	29.04	29.46	29.44
hCoV-19/India/UP-SGPGI-09/2020	47	M	27.78	27.16	27.04
hCoV-19/India/UP-SGPGI-10/2020	71	M	27.56	27.86	27.13
hCoV-19/India/UP-SGPGI-11/2020	72	M	28.92	28.48	27.89
hCoV-19/India/UP-SGPGI-12/2020	50	M	29.06	28.06	29.65
hCoV-19/India/UP-SGPGI-13/2020	39	F	29.56	28.18	29.03
hCoV-19/India/UP-SGPGI-14/2020	40	F	31.27	30.56	29.25
hCoV-19/India/UP-SGPGI-15/2020	28	F	24.06	24.56	23.94
hCoV-19/India/UP-SGPGI-16/2020	37	F	26.84	26.12	25.68
hCoV-19/India/UP-SGPGI-17/2020	19	F	24.04	23.68	23.55
hCoV-19/India/UP-SGPGI-18/2020	36	F	17.88	18.12	18.36
hCoV-19/India/UP-SGPGI-19/2020	48	F	26.67	26.44	26.21
hCoV-19/India/UP-SGPGI-20/2020	62	F	21.27	21.36	26.68
hCoV-19/India/UP-SGPGI-21/2020	48	F	18.15	19.34	19.65
hCoV-19/India/UP-SGPGI-22/2020	49	F	28.79	28.41	27.86
hCoV-19/India/UP-SGPGI-23/2020	58	F	30.04	29.67	29.16
hCoV-19/India/UP-SGPGI-24/2020	61	F	30.86	30.64	30.78

Table 1: Cq values of the isolated samples for SARS-CoV-2 detection.

Table 2:

Sample ID	GISAID ID	Total of Reads	Genome Coverage Obtained	Genome Similarity
hCoV-19/India/UP-SGPGI-01/2020	EPI_ISL_4768146	47548	3275X	95.4
hCoV-19/India/UP-SGPGI-02/2020	EPI_ISL_4769080	87770	5329.6X	95.5
hCoV-19/India/UP-SGPGI-03/2020	EPI_ISL_4769421	107414	5906.7X	95.4
hCoV-19/India/UP-SGPGI-04/2020	EPI_ISL_4769787	182878	9977.6X	95.6
hCoV-19/India/UP-SGPGI-06/2020	EPI_ISL_4770946	137188	6826.2X	95.5
hCoV-19/India/UP-SGPGI-07/2020	EPI_ISL_4771322	90296	5151.2X	95.5
hCoV-19/India/UP-SGPGI-08/2020	EPI_ISL_4771568	108639	6172.8X	95.5
hCoV-19/India/UP-SGPGI-09/2020	EPI_ISL_4771854	98729	5114.9X	95.6
hCoV-19/India/UP-SGPGI-10/2020	EPI_ISL_4772199	89936	5347.3X	95.6
hCoV-19/India/UP-SGPGI-11/2020	EPI_ISL_4772715	116880	5986.6X	95.5
hCoV-19/India/UP-SGPGI-12/2020	EPI_ISL_4773080	98995	5832.5X	95.5
hCoV-19/India/UP-SGPGI-13/2020	EPI_ISL_4773776	77121	5003.9X	95.5
hCoV-19/India/UP-SGPGI-14/2020	EPI_ISL_4775580	143542	8404.1X	95.6
hCoV-19/India/UP-SGPGI-15/2020	EPI_ISL_4777224	69930	3897.6X	95.6
hCoV-19/India/UP-SGPGI-16/2020	EPI_ISL_4778343	86119	4998.6X	95.5
hCoV-19/India/UP-SGPGI-17/2020	EPI_ISL_4778982	92708	5211.1X	95.6
hCoV-19/India/UP-SGPGI-18/2020	EPI_ISL_4779221	83562	4598.4X	95.6
hCoV-19/India/UP-SGPGI-19/2020	EPI_ISL_4779775	34542	2631.8X	95.6
hCoV-19/India/UP-SGPGI-20/2020	EPI_ISL_4780686	64034	3427.8X	95.4
hCoV-19/India/UP-SGPGI-21/2020	EPI_ISL_4781620	17817	1257.8X	94.6
hCoV-19/India/UP-SGPGI-22/2020	EPI_ISL_4782798	108963	6378.9X	95.6
hCoV-19/India/UP-SGPGI-23/2020	EPI_ISL_4783944	26242	1560.3X	95.5
hCoV-19/India/UP-SGPGI-24/2020	EPI_ISL_4784200	84284	5123.9X	95.6

Table 2: Genome similarity of twenty four sequenced genomes when compared to NCBI reference genome (MN908947.3) along with total reads generated and genome coverage obtained**.**

Table 3:

				GISAID ID	EPI_ISL_4768146	EPI_ISL_4768146	EPI_ISL_4768146	EPI_ISL_4768146	EPI_ISL_4768146	EPI_ISL_4768146	EPI_ISL_4768146	EPI_ISL_4768146	EPI_ISL_4768146	EPI_ISL_4768146	EPI_ISL_4768146	EPI_ISL_4768146	EPI_ISL_4768146	EPI_ISL_4768146	EPI_ISL_4768146	EPI_ISL_4768146	EPI_ISL_4768146	EPI_ISL_4768146	EPI_ISL_4768146	EPI_ISL_4768146	EPI_ISL_4768146	EPI_ISL_4768146	EPI_ISL_4768146	EPI_ISL_4768146	EPI_ISL_4768146
Position	**Reference**	**Variant**	**Gene**	**Amino acid change**																								
66	C	A						*																				
240	TC	CT										*																
241	C	T				*	*	*		*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
313	C	T	orf1ab	L16L																		*						
874	C	T	orf1ab	D203D									*													*		
1190	C	T	orf1ab	P309S							*								*	*								*
1306	G	T	orf1ab			*							*													*		
1947	T	C	orf1ab							*	*							*	*	*	*							*
1961	G	T	orf1ab									*													*			
2648	G	A	orf1ab			*							*													*		
2973	C	T	orf1ab																		*							
3003	A	G	orf1ab												*													
3037	C	T	orf1ab			*	*	*		*	*		*	*	*	*		*	*	*	*	*		*		*		*
3546	A	T	orf1ab				*																					
3692	G	T	orf1ab																				*					
4198	A	G	orf1ab									*																
4300	G	T	orf1ab																		*							
4510	G	T	orf1ab																									*
4890	C	T	orf1ab			*							*													*		
5700	C	A	orf1ab																			*						
5884	C	T	orf1ab					*				*	*				*									*		
6414	A	C	orf1ab												*													
6145	C	T	orf1ab							*																		

6443	G	A	orf1ab									*														*			
6821	A	G	orf1ab							*																			
6865	G	T	orf1ab							*																			
8114	G	A	orf1ab					*																					
8599	T	C	orf1ab								*								*	*			*						*
8836	C	T	orf1ab																			*							
8858	G	A	orf1ab																		*								
10039	C	T	orf1ab												*														
10323	A	G	orf1ab																			*							
10369	C	T	orf1ab						*																				
11083	G	T	orf1ab								*								*	*									*
11095	CTTTTTACC	C	orf1ab																							*			
11417	G	T	orf1ab										*														*		
11540	G	T	orf1ab								*									*			*						*
12130	A	T	orf1ab				*																						
12312	C	T	orf1ab								*								*	*			*						*
12651	C	T	orf1ab																										*
14408	C	T	orf1ab				*			*	*	*	*	*	*	*	*	*	*	*	*	*		*	*	*	*	*
14809	C	T	orf1ab																	*									
14925	C	T	orf1ab																	*									
15720	C	T	orf1ab												*														
17195	A	G	orf1ab									*																	
17403	C	T	orf1ab				*																						
18877	C	T	orf1ab			*			*	*				*			*	*	*	*				*			*
19029	C	T	orf1ab									*																	
19816	G	T	orf1ab		*								*														*		
20670	G	T	orf1ab								*									*			*						*
20703	C	T	orf1ab																	*									
21034	C	T	orf1ab																										*
21781	C	T	S	T73T											*														
22444	C	T	S	D294D																	*								

22917	T	G	S	L452R											*													
23086	C	T	S	Y508Y								*																
23401	G	T	S	Q613H																								*
23403	A	G	S	D614G				*		*	*	*	*	*	*	*	*	*	*	*	*	*		*		*		*
23593	G	T	S	Q677H							*								*	*			*					*
23608	G	A	S	R682R					*																			
23923	A	G	S	Q787Q											*													
24642	C	T	S	T1027I																	*							
25500	G	A	ORF3a	P36P								*																
25546	C	T	ORF3a	L52F				*																				
25563	G	T	ORF3a	Q57H					*	*	*				*			*	*	*	*							*
25621	G	A	ORF3a	V77I									*				*									*		
25708	C	T	ORF3a	L106F	*						*									*			*					*
25916	C	T	ORF3a	T175I																*								
26167	G	T	ORF3a	V259L																	*							
26735	C	T	M	Y71Y						*	*				*			*	*	*	*							*
28253	C	T	ORF8	F120F									*													*		
28854	C	T	N	S194L	*		*			*	*				*			*	*	*	*							*
28881	GGG	AAC	N			*		*				*	*				*									*		
29409	C	T	N	T379I																*								
29769	C	T										*																

Table 3: | Mutations detected and their respective amino acid changes when compared to NCBI reference genome (SARS-CoV-2, Wuhan Hu-1, MN908947.3) (* marks indicate the presence of mutation)