Unique mutational changes in SARS-CoV-2 genome: A case study for the largest state of India

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Abstract

COVID-19 is a global pandemic causing more than 8 million deaths till mid-August, 2020. In India, more than 3 million confirmed cases have been reported although with relatively low mortality of 1.8%. In this study, we sequenced 47 genomes of SARS-CoV-2 from 13 districts of Uttar Pradesh (UP), the largest state of India using third-generation sequencing technique. The phylogenetic clustering revealed that no UP sample was aligned with the previously defined USA clade, where the mortality was high. We identified 56 distinct SNP variations in the genomes of UP resulting in a unique mutation rate of 1.19% per sequence, which is greater than the value (0.88% per sequence) obtained for the rest of India. The relatively less death rate in UP indicates that such mutation in the virus is deleterious. Further investigation is required with larger sample size to determine the cause of virulence based on SNP variation.

Keywords

Third Generation Sequencing, COVID-19, India, Uttar Pradesh, SNP, Unique

1. Introduction

COVID-19, a global pandemic [1] has been the focus of many researchers worldwide to identify the potential region(s) of its pathogenicity since its emergence in December 2019 [2]. The causative agent, SARS-CoV-2 belongs to the Coronaviridae family and comprises of single-stranded positive-sense RNA with a genome size of approximately 30 kb [3]. SARS-CoV-2 genome consists of 29 open reading frames (ORFs), among which four are structural proteins (envelope protein, membrane protein, nucleocapsid protein, and spike protein), some accessory proteins translated by ORF3a, ORF6, ORF7a, ORF7b, ORF8, and ORF10, and 16 nonstructural proteins (nsps) [4].

During the spread of COVID-19, the virus evolved continuously resulting in variation in death rates in different countries [5]. As of 20th August 2020, approximately 23 million confirmed cases were reported worldwide with ~800 thousand registered deaths. At present, India ranks 3rd in the total number of COVID-19 cases. However, fatality is 1.8 %, which is considered as one of the lowest death rates in the world [6] [7]. Many reports correlate the fatality to the mutational changes in the virus genome in different geographic regions [8][9,10][11]. D614G substitution at the spike protein has been related to the cause of high mortality in humans [12]. The authors of a study [13] on the western Indian populations have correlated the mutational changes to specific age groups and modes of infection. To assess mutation rates and understand the evolution of the SARS-CoV-2 genome in different continents, many genomes were sequenced by employing different techniques and submitted to GISAID [14]. More than 84 thousand full-length SARS-CoV-2 genome sequences including >2400 viral genomes from India were available on the GISAID platform as on 12th August, 2020. Using this data, the Council of Scientific Industrial Research-Institute of Genomics and Integrative Biology (CSIR-IGIB) recently delineated an India-specific clade [15].

Currently, out of 29 states and 7 Union Territories of India, UP ranks 5th in confirmed cases of COVID-19. As on 12th August, 2020 only 11 sequences were available on the GISAID database for UP that represented only a small population of four districts. Considering the extremely less number of genome for UP despite of high number of infected persons and to understand the genome characteristic in terms of mutation, visa viz the national/global mutation variations we report 47 genome sequences of SARS-CoV-2 virus representing 13 districts of Uttar Pradesh (UP) using third-generation sequencing technique (Oxford Nanopore PromethION). The phylogenetic clustering was undertaken to classify UP genomes into different clades. We investigated the unique mutational features and rates during the

transmission of the virus. We correlated the death rates in different states including UP with their synonymous to nonsynonymous unique mutation ratio.

2. Methods

2.1 RNA Preparation and Sequencing of the viral genome

Total viral nucleic acid was extracted from the clinical sample of SARS-CoV-2 using PureLink DNA/RNA mini kit (Invitrogen, USA) as per manufacturer's instructions and Indian Council of Medical Research (ICMR) guidelines [16]. The Human RnaseP gene was tested in all samples to check the quality of samples and to validate the process of nucleic acid extraction. Real-time RT-PCR assays were performed using the Superscript III One-Step Real-Time PCR kit (ThermoFisher Scientific, USA) as per the protocol[17]. All samples were tested by two stage protocol consisting of screening and confirmation. E-gene and RnaseP were used for screening while RdRp and ORF1b were used as confirmatory assay for SARS-CoV-2. All the samples positive for E-gene, RdRp, and ORF1ab with Ct $\leq$ 25 were further taken for whole-genome sequencing.

Virus genomes were generated by using ARTIC COVID-19 multiplex PCR primers followed by nanopore sequencing on two ONT PromethION flow cell[18]. For PromethION sequencing, libraries were prepared using the ligation sequencing kit (SQK-LSK109) and Native barcoding (EXP-NBD 104 and EXP-NBD 114) of 24 samples in a single flow cell. The selected samples were converted to the first-strand cDNA using reverse transcriptase as suggested in Nanopore protocol “PCR tiling of COVID-19 virus” (Version: PTC_9096_v109_revD_06Feb2020). The multiplex PCR was performed with two pooled primer mixture as recommended and the cDNA was amplified. After 35 rounds of amplification, the PCR products were collected, purified with AMPure XP beads (Beckman coulter), and quantified using Qubit4 Fluorometer (Invitrogen). The double-stranded cDNA

was further subjected to end-repair, A-tailing, and Native barcode ligation individually. The barcoded samples were pooled together and ligated with sequencing adapters followed by loading of 50ng of pooled barcoded material and sequencing on two PromethION flow cells having 24 samples each.

2.2 Genome Assembly

Quality passed nanopore reads were subjected to remove the chimeric reads between the 300 to 500 base pair length according to the amplicon size. Using Artic network pipeline [19], we individually assembled the high coverage (approx. 9000X) filtered long reads of each sample. During the assembly "--normalize" option was used to normalizes the data up to 200 bp coverage. This normalization is useful for the minimization of the computational requirements of our high coverage data. The SARS-CoV2 reference genome (MN908947.3) [20] was used for generating the consensus sequence from the assembled reads. Low complexity or primer binding regions were masked during the consensus sequence generation and variant calling, to avoid the ambiguity sequences.

2.3 Data Preparation

For comparative and explanatory analyses of our assembled genome from UP, we downloaded the whole genome data of all Indian sequences from the GISAID database that was available until the 12th of August. We randomly selected 100 sequences each from Asia, Europe, South America, North America, Central America and Eurasia as an outlier for different analyses of Indian sequences. A total of 2923 processed sequences (2323 from India and 600 from different continents) were further screened based on unrecognized nucleotides (Ns) and the full genome size. We kept only those sequences whose unrecognized base pair (number of Ns) is less than 1% of the total length followed by the masking of the low

complexity region. Along with it, we also considered the 47 sequenced genome of Uttar Pradesh of India for further analysis.

2.4 Phylodynamics clustering of Indian sample

We sequenced and assembled a total of 47 genome sequences of UP, India followed by the phylodynamic clustering, on the basis of nextstrain defined clade [21]. For a comprehensive study of the clade, we took 856 samples of the SARS-CoV-2 genome of India that was available till 10th June. Whole-genome alignment has been carried out by the mafft aligner [22] by taking the Wuhan Hu-1(MN908947.3) sample as a reference genome. IQTREE software [23] was used for the phylogenetic tree with the GTR substitution model. The refinement of the tree was done by the “--timetree" parameters to adjust the branch lengths and filter out the interquartile ranges from regression. The refined time-resolved tree was further screened to derive the ancestral traits, infer nucleotide mutation as well as amino acid variation from the root. The resulting phylodynamics tree was then used to define the clade based on a new classification that signifies the geographic spreading of SARS-CoV-2 in India. The final tree was further visualized through the nextstrain shell by using the auspice interface [24].

2.5 Divergence estimates of the newly assembled genome

Divergence estimation of the newly assembled genome and the most recent common ancestor (tMRCA) were examined using the BEAST v1.10.4 program [25]. Through the MRCA analysis, the origin of the ancestral virus was identified. With the strict molecular clock and the exponential growth model, we processed the Markov chain Monte Carlo algorithm on the masked alignment file for 100 million steps, where the first 30 million steps were used for burn-in to get the effective sample size. Bayesian coalescent analysis of the UP samples was conducted using the GTR substitution model with the kappa scale of 1 to 1.25. Molecular

clock and root divergence time were reconfirmed with the treetime [26] using the same substitution model.

2.6 Variant identification and functional evaluation

We scrutinized the unique mutations in our assembled genome of UP state that was called during the assembly. To identify the SNP variants among all the processed (downloaded and assembled) sequences and to ensure the uniqueness of variants in our assembled data, we aligned each downloaded sequences separately to the reference genome (MN908947.3) by use of mafft aligner [22]. The variation was detected with the snp-sites program and [27] nextstrain. The resulting Variant Call Format (VCF) file was annotated further to find out the position of the mutation that affects the SARS-CoV-2 virus genome though snpEff program [28]. Finally, manual screening of mutation was carried out to define the unique mutation in our processed sequences (2370) along with the other continents of the world. Functional validation of the emerging new mutations in UP sequences was undertaken through the Sorting Intolerant from Tolerant (SIFT) [29]. SIFT evaluated the functional consequences of the variants, where the SIFT score of 0.0 to 0.5 indicates deleterious effect and >0.05 was interpreted as a toleratable mutation. The Identified variants of UP sequences were visualized through the R package [30].

3. Results and Discussion

3.1 Demographic Representation

In this study, a total of 47 genomes of SARS-CoV-2 were sequenced using the long reads PromethION sequencer. All the samples were from UP state of India representing 13 different districts (**Fig 1a**). The samples were collected from symptomatic and asymptomatic patients in which the male-female ratio of patients was 3:1. The age of the patients varied

between 2 and 86 years with a mean age of 35 years. The maximum number of samples was collected in May'2020while samples numbers 2 and 13 were collected in March and April'2020 respectively (**Supplementary File 1**). The average coverage of all the samples was 9000X which was correlated with the ct value of the samples (**Fig. 1b**).

3.2 Assembly of the whole genome

The resulting fastq and fast5 files of the nanopore PromethION sequencer were quality checked by the artic network pipeline [19]. The amplicon reads of 300 to 700 base pair length were removed from the raw fastq file to avoid the generation of chimeric reads. Filtered fastq file and raw fast5 format files of each sample were taken as input for whole-genome assembly. The MN908947.3 genome was used as a reference to generate the consensus sequence for each sample individually. Additionally, medaka parameters were also used to identify the variants or SNPs based on assembled reads during the generation of the consensus sequence. To avoid the ambiguity at the time of SNP calling, the primer binding region in the genome was masked. The resulting Single-nucleotide Polymorphism (SNPs) were considered as true SNPs in our sequenced samples data because they have high-quality read depth (~300) at each base pair. These High-quality SNP reads were used to define the unique mutation features in the SARS-CoV-2 genome sequences of UP state in comparison to the sequences from other states of India as well as from different continents. The assembled whole-genome sequence was submitted to the GISAID with accession ID (EPI_ISL_516940-EPI_ISL_516986) and the metadata information is available in **Supplementary File 1**.

3.3 Phylodynamic analyses of the assembled genome

Phylodynamic clustering was used for clade assignment in our assembled genome based on nextstrain where we could trace the origin of the clade concerning the specific mutation on

the genomic region. A total of 47 assembled genomes of SARS-CoV-2 from 13 different districts of UP in India and 856 genomes from 21 different states of India as available on 10th June was used to perform the phylodynamic clustering according to the new clade classification by taking the Wuhan SARS-CoV-2 genome reference genome (EPI_ISL_406798) as an outlier. The information of the sequences including their accession number, submitting sample name, collection date is mentioned in **Supplementary File 2**.

The augur pipeline [31] was used for the alignment of all the sequences, their phylogenetic clustering, and deciphering the time resolving tree, using SARS-CoV-2 (MN908947.3) genome as the root. The resulting tree was subjected to defining the potential emerging clades based on nucleotide mutation(s). Indian samples were clustered into five clusters viz., 19A, 19B, 20A, 20 B, 20C (**Fig. 2a**) as designated by the nextstrain nCoV global page[32]. According to them, two clades viz., 19A and 19B were primitive and dominated by Asia country, and were delineated by C to T mutation at 8782 positions in ORF1ab region, and T to C mutation at 28144 positions, respectively. A total of 43% of processed sequences of India (380 out of the 856) were aligned with these two clades.

The clades 20A and 20B representing the European clade while 20C is well known for North American clade [32]. A total of 500 out of 856 Indian sequences was aligned with these clades which deviated from the root (19A) in early 2020. Most of the Indian sequences (366) were clustered into 20A clade, followed by 19A clade (333) (**Fig. 2b**). The least number of Indian sequences belongs to 20C clade. The United State of America (USA) is the primary source for 20C clade and only 4 Indian samples (2 from Delhi and Gujarat each) were aligned. The sequences from Delhi (EPI_ISL_435063, EPI_ISL_435064) did not show any nucleotide or amino-acid mutation, while sequences form Gujarat (EPI_ISL_435051, EPI_ISL_435052) had two missense variations (A55C, C241T) in ORF1ab region.

SARS-CoV-2 genome sequences of UP state, obtained in the current study, 17 sequences belong to 19A, 23 to 20A and 7 to 20C clade, and none of the sequences were aligned with 19B and 20C clade **(Fig. 2c)**. A similar pattern of clade distribution has been seen in 11 downloaded sequences from UP. According to the nextstrain global page, these two clades (19B and 20C) show somewhat mix geographical distribution. This could be investigated further with the increasing number of SARS-CoV-2 genome sequences.

3.4 Estimation of the date of origin

The mutation rate of 47 sequenced genomes in UP state was estimated using BEAST software and by using the coalescent model as the prior of the trees [25]. The estimated rate of mutation was predicted as 3.279e-04 substitutions per site per year for the given SARS-CoV-2 genomes sequences. Highest Posterior Density (95 %) intervals are in the range of 2.24e-04 to 4.459e-04. (**Supplementary File 3**). The substitution rate was further confirmed through the treetime software [26] where root to tip regression rate was calculated as 3.078e-04 with the 0.05 correlation value (**Fig. 3**). This correlation value indicates the informative behavior of inputs for temporal information to rationalize the molecular clock approach. The root date of the SARS-CoV-2 genome from UP states was evaluated as 29 December 2019 which substantiated the reported timing of this virus in Wuhan city of China [33].

3.5 Assessment of unique Mutational features in different states of India

We identified the unique mutational features in the SARS-CoV-2 genome of other states of India. The unique mutation rate is around 0.88% of the total sequenced genome with 0.5 synonymous to nonsynonymous mutationratio. For the isolation of unique SNP features, we selected only those states whose SARS-CoV-2 genome samples were available in more than 10 samples. Thus, total 12 states; Gujarat, Maharashtra, Telangana, Delhi, Odisha, Karnataka, West Bengal, Uttarakhand, Madhya Pradesh, Tamil Nadu, Haryana, and Uttar Pradesh were

selected **(Supplementary File 4) (Table 1)** whose average active case is around 2 lacs according to the WHO India tally as on 10th of August, 2020[2]. We strike off the common mutation positions from different continents (Asia, Africa, Eurasia, North America, Oceania, and South America) to check the mutated version of the SARS-CoV-2 genome in the Indian strain. A higher number of unique mutations were seen in Maharashtra followed by the Gujarat, Odisha, Telangana, West Bengal, and Karnataka **(Table 1)**. These unique SNPs information was annotated on the SARS-CoV-2 reference genome (MN908947.3) and classified it into synonymous, missense, stop gain, stop loss, upstream gene variant and downstream gene variant **(Fig. 4)**.

Delhi state has a very low number of unique mutation rate (0.23%) with a comparatively high death rate (2.66%), suggest that Delhi was infected with a fatal or raw version of the virus genome. The phylogenetic clustering by nextstrain also supplemented the information where the genome from Delhi was clustered on 20C clade [21]. On the basis of death rate and the number of genome sequences, Maharashtra and Gujarat were top listed with the 1.11% and 0.89% unique mutation rate, respectively. These two states have an equal number of missense variants **(Fig. 4)** with the stop gain at the envelope protein in Maharashtra and ORF7a in Gujarat **(Supplementary File 5)**. Odisha state has a relatively higher number of unique mutations while synonymous to nonsynonymous mutation ratio is lower (0.39) than the average (0.5). The mortality rate of this state is low as seen in other states like Telangana and Harayana. Through this observation, we can estimate the death rate by calculating synonymous to nonsynonymous unique mutation ratio. This hypothesis could be further validated with the increasing number of SARS-CoV-2 genome sequences of all states.

3.6 Dynamic characterization of Unique SNP variation in UP state

We identified 56 distinctive SNPs in 47 assembled sequences while 11 unique SNPs in downloaded sequences of UP. Out of 56, 36 SNPs were annotated as missense variants which causes the amino acid changes in the SARS-CoV-2 genome. Two nonsense mutations (T18126A, C18431T) show the premature stop gain in the ORF1ab region (**Table 2, Fig 5**). The functional evaluation of nonsynonymous mutations was predicated through Sorting Intolerant From Tolerant (SIFT) server which revealed the deleterious effects of 37% of total unique mutations. A maximum number of deleterious mutations have been seen in the non-structural protein of ORF1ab region (Table 3). Threonine to Isoleucine intolerable mutation at 6056th position in ORF1ab region affects the NSP11 protein in the NBRI-N21 sample. This deleterious mutation might change the polymerase activity of the genomes, as it is associated with the programmed cell death evasion, cell cycle and DNA replication [34,35].

One deleterious mutation was found in ORF3a, ORF7a, and ORF8 regions of the SARS-CoV-2 genome (**Table 3**). The non-structural proteins present in these ORFs act as an accessory factor [36], and assisted for virus-host interactions [37]. ORF3a regulates the interferon signaling pathway, cytokines production [38]. Deleterious mutation in the accessory protein has been found in the latest evolving SARS-CoV-2 genome from Indian samples [39] which is also correlated in our analysis. A structural protein, N encodes nucleocapsid protein shows the deleterious effects by the mutations at D371V, K370N, D371Y, P279Q, and T362I positions. This protein has a highly conserved domain [40] that plays a crucial function in the virus genome by regulating RNA transcription and modulating the infected cell biological processes [41]. Another nonsynonymous, deleterious mutation (V1570L) was observed in NSP3 protein in the NBRI-N42 sample. This protein has papain-like viral protease activity to generate the other replicase subunits from nsp1 to nsp16 [42].

4. Conclusion:

In this study, 47 SARS-CoV-2 genome sequences of the Uttar Pradesh state have sequenced with a large scale of high coverage third-generation sequencing data. We identified 56 peculiar SNPs in our sequenced genome in which a large number of SNPs in the ORF1ab region are deleterious. These SNP variations might affect the replication of the virus genome during the infection which ultimately would be the reason for the less death rate. The identified mutations in SARS-CoV-2 genome of 47 individuals could be used as the potential target for personalized medications or effective vaccine doses to combat the effects of the COVID-19. Additionally, the relation with the synonymous to nonsynonymous unique mutation ratio with the mortality could be studied further to understand the putative region/SNPs that cause fatal to the human being.

Author contributions:

Priti Prasad: Conceptualization; Data curation; Supervision; Formal analysis; Investigation; Methodology; Resources; Software; Visualization; Writing - original draft; Writing - review & editing, Shantanu Prakash: Conceptualization; Methodology; Writing - review & editing, Mehar H. Asif: Conceptualization; Supervision; Writing - original draft; Writing - review & editing, Kishan Sahu: Resources; Writing - review & editing, Suruchi Shukla, Babita Singh, Hricha Mishra, Danish Nasar Khan, Om Prakash, MLB Bhatt: Resources, SK Barik: Conceptualization; Resources; Writing - review & editing, Samir V. Sawant: Conceptualization; Resources; Supervision; Formal analysis; Writing - review & editing, Amita Jain: Conceptualization; Resources; Writing - review & editing, Sumit Kr. Bag: Conceptualization; Resources; Supervision; Writing - review & editing

Conflicts of Interest

Authors declare no conflicts of interest

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Figure legends:

Fig. 1

a) The detailed map of the SARS-CoV-2 genome sequence of different districts of the Uttar-Pradesh state of India. The scale denoted the number of genome sequencing of the virus genome. Grey color means no genome was sequenced while the darker blue color represents the highest number of genome sequencing. b) The Co-linear plot of the Ct value of 47 positive COVID-19 patients to the coverage of the whole genome sequence long-read data. Each sample was represented through the blue dots where higher Ct value shows high coverage data.

Fig. 2

a) The phylogenetic clustering of 889 samples of India into 5 different clades according to the nextstrain based mutation. The 19A and 19B clades are the primitive clades that were sequenced in early 2020; whereas 20A, 20B and 20C clades are emerging clades. The reference genome of the Wuhan sample was implemented in the clustering trees which aligned in 19A clade before the starting of the Jan-2020. b) The distribution of genome sequenced in different clades was visualized through the piechart. In the 20A clade, 366 genomes were clustered followed by the 19A clade. The lowest number of clustering has been seen in the 20C clade which is well known for the USA defining clade. c) Distribution

of UP sequences on different clade. Blue color bar shows the 47 assembled sequences and red color bar represents the 11 downloaded sequences of UP.

Fig. 3

The root-to-tip regression rate of sequenced SARS-CoV-2 genome of Uttar Pradesh state. X-axis describes the timing of the sample collection of COVID-19 infected persons. Y-axis describes the regression rate in comparison to the sample collection date. The origin of infection in Uttar Pradesh samples is estimated by 2019.5.

Fig. 4

Unique mutation features in 12 different states of India whose SARS-CoV-2 genome sequence is present in large amounts in GISAID. The pie chart shows the different types of variants that were annotated on the SARS-CoV-2 genome of different states of India, with their unique mutation variation per high-quality genome sequenced. In the pie chart of Uttar Pradesh, the publically available genome and the assembled genome through this study were mentioned separately with plus(+) sign.

Fig. 5

The nonsynonymous mutation of SARS-CoV-2 genome of the Uttar Pradesh 47 samples. The scale bar shows the length of the genome on which the dashed line with a brown color number represents the mutational points that putatively altered the functionality of the genome. The circled nucleotides are the mutated nucleotide bases, in which black color bases are the missense variation while red color shows the stop gain at the genomic regions.

Table 1

The statistics of the different selected states of India along with their unique mutation rate and the mortality rate for the respective states according to the mid-August, 2020 COVID cases. The sequences data is downloaded from GISAID, available till the 12th of August.

Table 2

Detail of Nonsynonymous SNP mutation of all 47 sequenced UP samples with their genomic and amino acid position on the genome. The different colored Gene color shows different genomic regions while stop codon affecting mutation wee highlighted in green in color.

Table 3

The Putative nonsynonymous mutation with their SIFT score. The SIFT score value less than 0.05 is depicted as a deleterious mutation that is highlighted in yellow whereas unhighlighted SIFT score value for the particular SNPs is predicted as a tolerable mutation.

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Fig. 1a

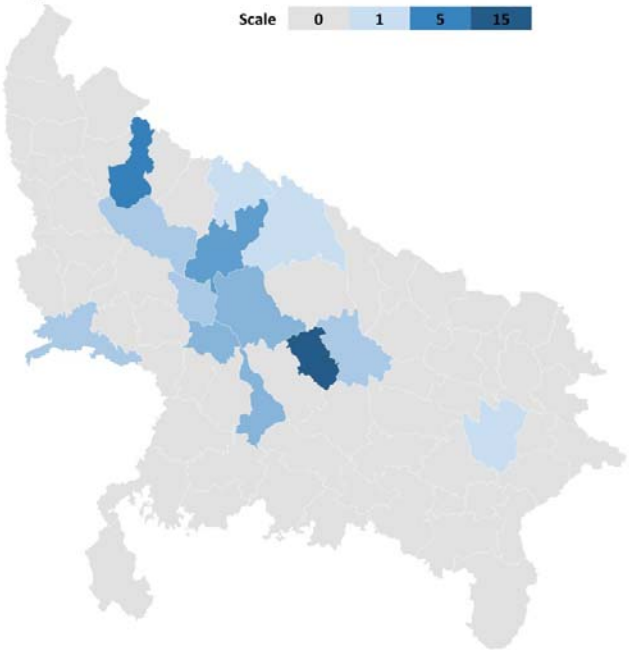


Fig. 1b

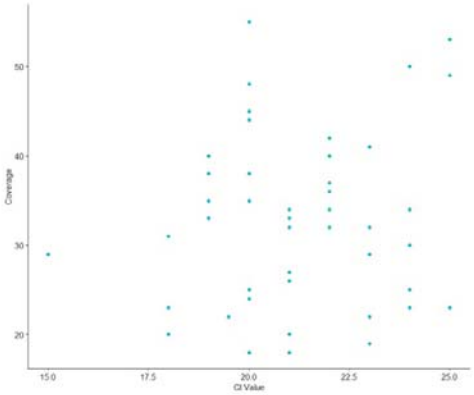
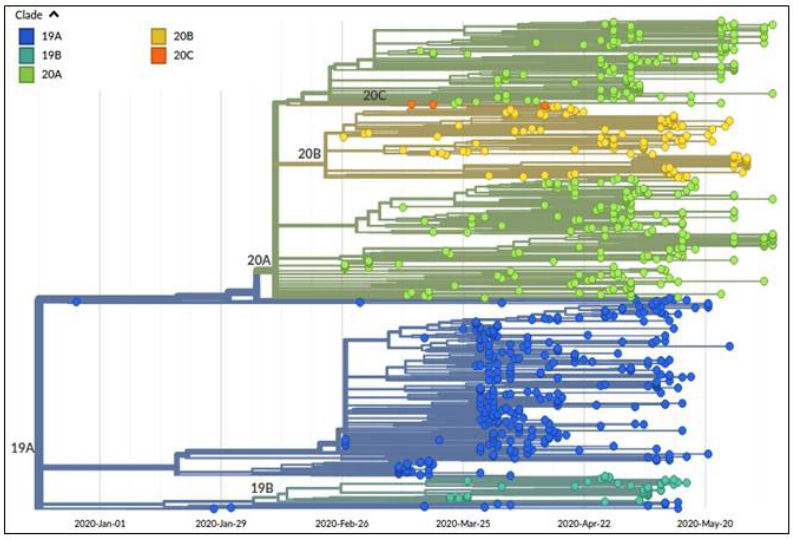


Fig. 2a



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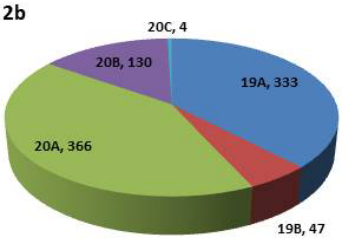

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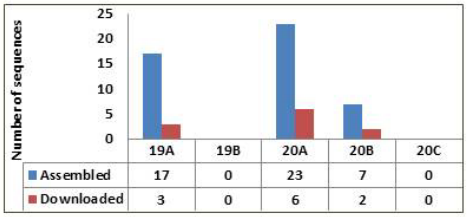
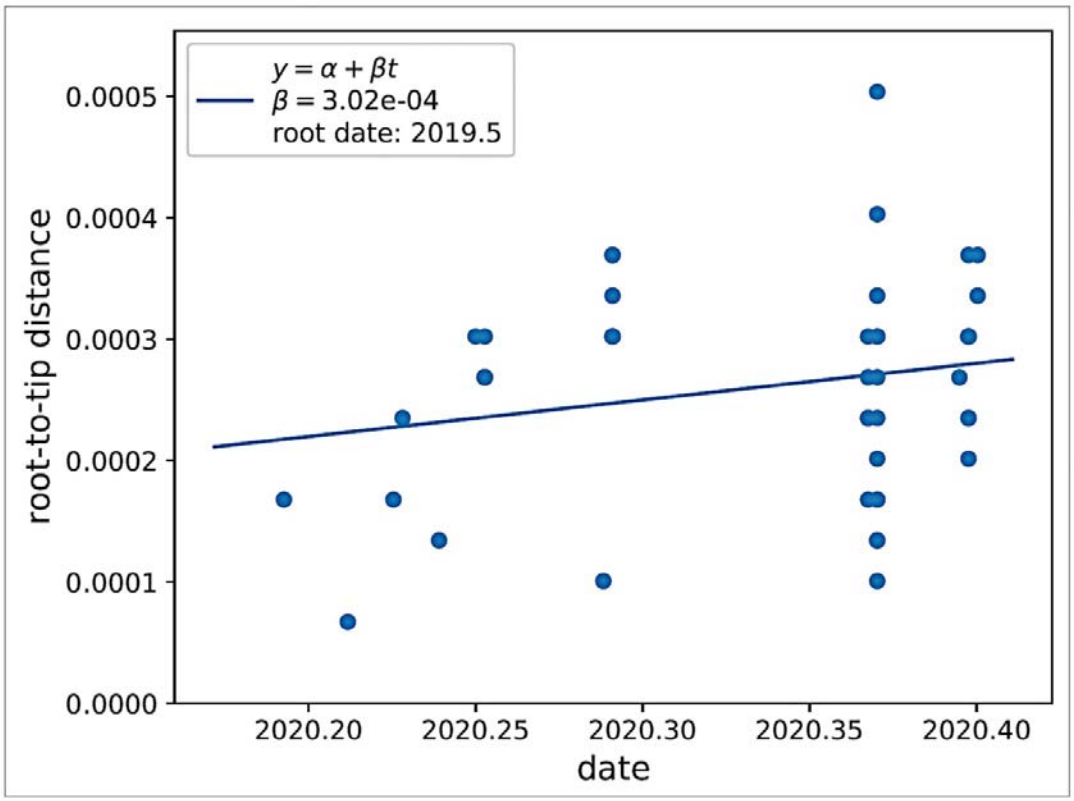


490

Fig. 3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0.0004
0.0005
2020.20
2020.25
2020.30
2020.35
2020.40
date

Fig. 4

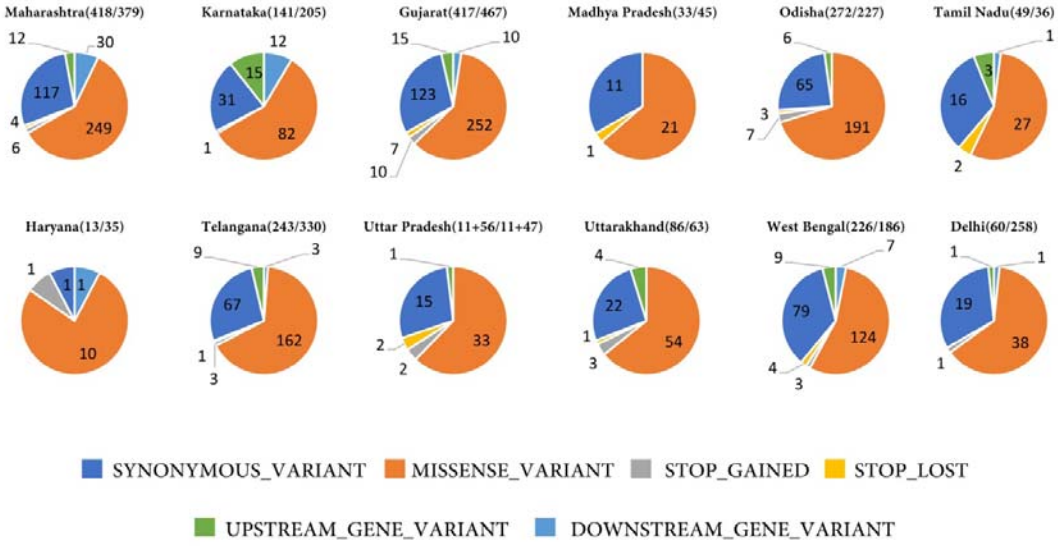
Maharashtra(418/379)
Karnataka(141/205)
Gujarat(417/467)
Madhya Pradesh(33/45)
Odisha(272/227)
Tamil Nadu(49/36)
Haryana(13/35)
Telangana(243/330)
Uttar Pradesh(11+56/11+47)
Uttarakhand(86/63)
West Bengal(226/186)
Delhi(60/258)
SYNONYMOUS_VARIANT
MISSENSE_VARIANT
STOP_GAINED
STOP_LOST
UPSTREAM_GENE_VARIANT
DOWNSTREAM_GENE_VARIANT

Fig. 5

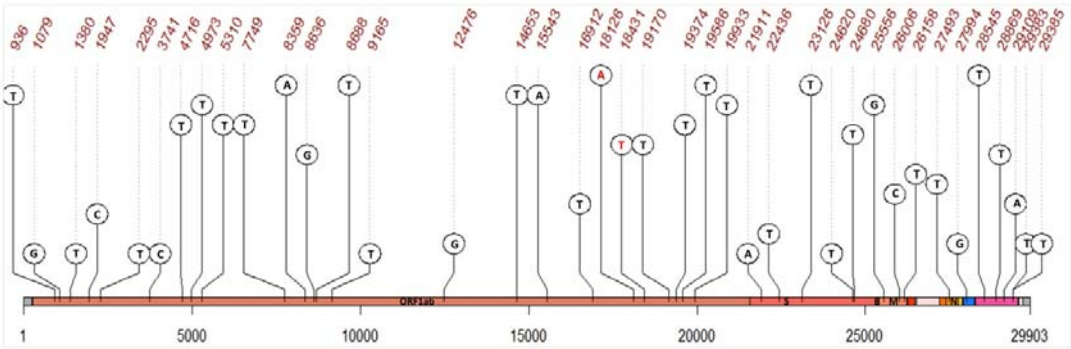

Table 1

State	Genome Sequenced	Unique mutation	Mutation rate (Unique mutation / sequence)	Death rate
Maharashtra	379	422	1.113456464	3.261071861
Gujarat	467	417	0.892933619	3.336060568
Odisha	227	273	1.202643172	0.5883102
Telangana	330	243	0.736363636	0.717308726
West Bengal	186	227	1.220430108	2.011953626
Karnataka	205	141	0.687804878	1.68566019
Uttarakhand	63	87	1.380952381	1.322401481
Delhi	258	60	0.23255814	2.663099352
Tamil Nadu	36	49	1.361111111	1.717780091
Madhya Pradesh	45	33	0.733333333	2.431820632
Haryana	35	13	0.371428571	1.10874122
Uttar Pradesh	11 + 47	11 + 56	1.191489362	1.558198114

Table – 2

Sample_ID	POS	REF	ALT	Gene	Mutation	Type of mutation
NBRI-N24	936	C	T	ORF1ab	Thr224Ile	missense_variant
NBRI-N25	1079	A	G	ORF1ab	Asn272Asp	missense_variant

NBRI-N38	1380	C	T	ORF1ab	Ala372Val	missense_variant
NBRI-N48	1947	T	C	ORF1ab	Val561Ala	missense_variant
NBRI-N51	2295	C	T	ORF1ab	Thr677Ile	missense_variant
NBRI-N22	3741	T	C	ORF1ab	Ile1159Thr	missense_variant
NBRI-N55	4716	C	T	ORF1ab	Thr1484Ile	missense_variant
NBRI-N42	4973	G	T	ORF1ab	Val1570Leu	missense_variant
NBRI-N47	5310	C	T	ORF1ab	Thr1682Ile	missense_variant
NBRI-15	7749	C	T	ORF1ab	Thr2495Ile	missense_variant
NBRI-N39	8359	T	A	ORF1ab	His2698Gln	missense_variant
NBRI-N24	8636	A	G	ORF1ab	Thr2791Ala	missense_variant
NBRI-N26	8688	G	T	ORF1ab	Gly2808Val	missense_variant
NBRI-N42	9165	C	T	ORF1ab	Thr2967Ile	missense_variant
NBRI-N44	12476	A	G	ORF1ab	Met4071Val	missense_variant
NBRI-N30	14653	G	T	ORF1ab	Met4796Ile	missense_variant
NBRI-N48	15543	G	A	ORF1ab	Arg5093Gln	missense_variant
NBRI-N21	16912	G	T	ORF1ab	Leu5549Phe	missense_variant
NBRI-N49	19170	C	T	ORF1ab	Ser6302Leu	missense_variant
NBRI-N50	19374	C	T	ORF1ab	Ser6370Phe	missense_variant
NBRI-N21	19586	C	T	ORF1ab	Leu6441Phe	missense_variant
NBRI-N58	21911	C	A	S	Leu117Ile	missense_variant
NBRI-N32	22436	G	T	S	Ala292Ser	missense_variant
NBRI-N49	23126	G	T	S	Ala522Ser	missense_variant
NBRI-N48	24620	G	T	S	Ala1020Ser	missense_variant
NBRI-N35	24680	G	T	S	Val1040Phe	missense_variant
NBRI-N48	25556	T	G	ORF3a	Val55Gly	missense_variant
NBRI-N39	26006	G	C	ORF3a	Ser205Thr	missense_variant
NBRI-N44	26158	G	T	ORF3a	Val256Phe	missense_variant
NBRI-N24	27493	C	T	ORF7a	Pro34Ser	missense_variant
NBRI-N34	27994	A	G	ORF8	Asp34Gly	missense_variant
NBRI-N20	28545	C	T	N	Thr91Ile	missense_variant
NBRI-N50	28869	C	T	N	Pro199Leu	missense_variant
NBRI-N57	29109	C	A	N	Pro279Gln	missense_variant
NBRI-N29	29383	G	T	N	Lys370Asn	missense_variant
NBRI-N34	29385	A	T	N	Asp371Val	missense_variant
NBRI-N20	18126	T	A	ORF1ab	Leu5954*	stop_gained
NBRI-N50	18431	C	T	ORF1ab	Gln6056*	stop_gained
NBRI-N28	19933	A	T	ORF1ab	Ter6556Cysext*?	stop_lost

Table -3

Sample_ID	Gene	Mutation	SIFT Score
NBRI-N34	ORF1ab	P5496S	0.81
NBRI-N57		T6056I	0.03

NBRI-N15		T2495I	0.55
NBRI-N16		T2016K	0.59
NBRI-N21		V4691F	0.01
		V5550L	0.02
		V5894I	0.59
		T6441I	0.85
NBRI-N22		S5483F	0.02
NBRI-N23		T5300I	0.13
NBRI-N25		N272D	0.06
NBRI-N27		P3395S	1
NBRI-N28		T6557S	0
NBRI-N30		V4797F	0
NBRI-N36		R24S	0.47
		G7063S	0.7
NBRI-N38		A372V	0.05
NBRI-N40		S558F	0.46
NBRI-N42		V1570L	0.02
		T2967I	0.25
NBRI-N47		T1682I	0.2
NBRI-N51		T677I	0.36
NBRI-N55		T1484I	0.05
		V4980L	0
NBRI-N39		H2698Q	0.18
NBRI-N24		T224I	0.03
NBRI-N26		G2808V	0.14
NBRI-N17	N	D343H	0.19
NBRI-N34		D371V	0.01
NBRI-N29		K370N	0.02
		D371Y	0.01
NBRI-N50		P199L	0.11
NBRI-N57		P279Q	0
NBRI-N41		T362I	0.03
NBRI-N48	S	A1020S	0.39
NBRI-N32		A292S	0.64
NBRI-N35		V1040F	0.12
NBRI-N49		L5F, A522S	0.10,0.73
NBRI-N58		L117I	0.76
NBRI-N39	ORF3a	S205T	0.06
NBRI-N48		V55G	0
NBRI-N24	ORF7a	P34S	0
NBRI-N29	ORF8	D34G	0
NBRI-N26		V62L	0.54