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Coronavirus Pandemic

Whole Genome Sequencing and Phylogenetic Analysis of SARS-CoV-2 strains in Turkey

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Abstract

Introduction: Coronaviruses which are single-stranded RNAs, are members of a large family of viruses that may be important pathogens for humans. SARS-CoV-2 was found to cause the severe respiratory syndrome, and on January 22, 2020 first human-to-human transmission was reported. We aimed to reveal the complete genomes of 19 SARS-CoV-2 isolates from Denizli province and identify Turkish patients' genetic similarities.

Methodology: 15 samples with the highest viral loads resulting from RT-PCR were selected for NGS analysis. Fifteen SARS-CoV-2 complete genome sequences were then subjected to phylogenetic analysis and uploaded to the GISAID database. Phylogenetic trees were constructed by the Neighbor-Joining method using MEGAX software.

Results: Whole-genome sequencing of the viral RNA samples revealed 32 missense, 21 synonymous, and 4 non-coding alleles. In all samples c.1-25C>T (5'UTR), c.14144C>T (ORF1ab), c.2772C>T (ORF1ab) and c.1841A>G(S) mutations were detected. Phylogenetic analysis revealed that most of the present study's genomes are in 20B clade while the two are in 20A. The phylogenetic tree constructed with all complete SARS-CoV-2 genomes of Turkey showed that the viruses were spread nearly homogenous on eastern (around Kars) and western (around Istanbul) sides.

Conclusions: Here, we reported the viral genomes in Denizli comprehensively for the first time. We identified 11 rare missense mutations in the virus compared to the reference genome. Phylogenetic analysis revealed that while most of our isolates were similar to European sequences, some had different sublineages depending on their genomic variants.

Key words: Coronaviruses; SARS-CoV-2; NGS; clade; lineage.

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Introduction

Coronaviruses (CoVs) are members of a large family of viruses that may be important pathogens for humans and vertebrates. Coronavirus is a single-stranded RNA virus that belongs to the family Coronaviridae included in the order Nidovirales [1]. The family members of Coronaviridae can infect a variety of systems, including respiratory, gastrointestinal, hepatic, and central nervous systems of humans and animals such as birds, bat, and mice. Human Coronaviruses known to date are as follows: HCoV-229E, HCoV-NL63, HCoV-OC43, HCoV-HKU1, SARS-CoV MERS-CoV, and SARS-CoV-2. SARS-CoV-2, a new Coronavirus described in mid-December 2019, was reported in China and linked to a pathology name Coronavirus disease 2019 (COVID-19) [2]. The virus that caused severe respiratory syndrome and human-to-human transmission was first reported on January 22, 2020 [3]. With the World Health Organization (WHO) declaration, it has been announced that SARS-CoV-2 infection became pandemic on March 11, 2020. The severity of the SARS-CoV-2 infection is variable. Some people are lethally affected, while others are strikingly asymptomatic for the disease. Patients with COVID-19 usually develop some signs and symptoms such as mild respiratory illness and persistent fever, nausea, diarrhea, skin rash, and loss of taste and smell an average of 5–6 days after infection [4,5]. At the beginning of the pandemic of COVID-19, difficulty in breathing was a typical syndrome for a patient who needs artificial ventilation in intensive care [6]. With the spread of this pandemic all over the world, 59.204.902 confirmed cases of COVID-19 and 1,397,139 deaths were reported by WHO website (https://covid19.who.int) in more than 200 countries and territories and as of November 22, 2020. The first case of COVID-19 in Turkey was confirmed on March

11, 2020. As of March 15, 2021, there have been 2,894,893 cases and 29,552 deaths declared by The Ministry of Health, Turkey. Understanding the transmission patterns and evolution of the SARS-CoV-2 virus is crucial for generating efficient drugs and vaccines for disease prevention [7,8]. Therefore, the analyses of genomic sequences of coronaviruses are essential tools for drug/vaccine design. There are 220,620 SARS-CoV-2 complete genome sequences available on Global Initiative's database on Sharing All Influenza Data (GISAID) (https://www.gisaid.org/epiflu-applications/hcov-19-reference-sequence).

In the GISAID platform, there are 194 complete SARS-CoV-2 sequences from Turkey, and only two sequences among these were found from Denizli province. In this study, we performed next-generation sequencing (NGS) analysis to reveal the complete genomes of 15 SARS-CoV-2 isolates on Turkish patients from Denizli province. To identify their genetic similarity, phylogenetic analyses were performed by considering the complete SARS-CoV-2 genomes of Turkey and worldwide isolates selected from GISAID. Besides, we focused on the variation analysis to show the mutations in SARS-CoV-2 genomes.

Methodology

Ethical considerations

This study was approved by both the Republic of Turkey Ministry of Health COVID-19 Scientific Research Evaluation Commission (Approval date: 18/05/2020; number: 2020-05-14T11_57_29) and the Local Ethics Committee of Pamukkale University Faculty of Medicine (Approval date: 06/05/2020 number: 60116787-020/28659).

Sample selection and viral RNA isolation

Nasopharyngeal swab (NPS) and oropharyngeal swab (OPS) specimens of COVID-19 suspected patients who were sent for routine diagnosis to the Virology Unit, Pamukkale University Hospital, Denizli, Turkey, were selected for viral cultivation. The study was conducted in Denizli, a region in the western part of Turkey. Detailed patient information about the patients is shared in supplementary S1. 15 SARS-CoV-2 positive samples with high viral load (assessed by Real-time PCR) were selected for NGS analysis. Viral RNAs of confirmed COVID-19 cases showed Cq <20 by real-time PCR were extracted by using QIAamp Viral RNA Mini kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions on 2020-07.

NGS Library Preparation

Sequencing libraries were prepared using the CleanPlex® SARS-CoV-2 Research and Surveillance Panel (Paragon Genomics, San Francisco, CA, USA), following the manufacturer's instructions. Briefly, the quantity of viral RNA samples was assessed with the Qubit RNA HS Assay kit. 50ng of extracted viral RNA was used as an input for each sample. As a first step, RNAs were used as a template for reverse transcription. Then genome of each sample was amplified using two primer pool protocol (343 primer pairs separated in two pools) with ten cycle multiplex PCR to cover the entire genome of SARS-CoV-2 with a median size of 149 bp. The reaction was terminated by the addition of 2µl of stop buffer. PCR products were then purified by adding magnetic beads. Indexing primers were introduced by second PCR using twenty-five cycles, followed by final bead purification was performed. PCR products were quantified and qualified by both dsDNA HS Assay Kits with Qubit 4.0 Fluorometer (Thermo Fisher Scientific Inc.) and an Agilent Bioanalyzer 2100 with High Sensitivity DNA Chips (Agilent Technologies Inc.) according to manufacturers' instructions. Finally, 15 libraries were pooled in equimolar concentrations and sequenced on the NextSeq500 instrument with mid-output 2 × 150 bp flowcells (Illumina).

Data Quality and Variant Analysis

Raw sequencing data were obtained in FASTQ format and processed for variant calling and consensus sequences generation. First, the FASTQ sequences' quality was evaluated by the FastQC program, and then adapter sequences were trimmed from reads using Cutadapt software. Processed reads were aligned to the reference genome (NC_045512.2) using BWA mem v.0.7.12. Variant calling and generation of consensus sequences were performed by using Samtools v.1.2.0. Moreover, samples were also analyzed in the SOPHIA platform for SARS-CoV-2 for variant calling and reporting the viral presence.

Phylogenetic Analysis

Fifteen SARS-CoV-2 complete genome sequences were subjected to phylogenetic analysis to reveal the molecular relationship and evolutions. For this purpose, all 160 complete and high coverage sequences isolated in Turkey (last access November 15, 2020) were selected and retrieved from GISAID. To obtain the phylogenetic tree on a global scale, 180 sequences based on major GISAID clades (19A, 19B, 20A, 20B, and 20C) were also selected from the same database.

Table 1. Mutations identified in 15 SARS-COV-2 genome sequences from Denizli/Turkey. See the supplementary S1 for full variant list of each isolate.

Mutations	Protein	Gene	Type of mutation	Detected Patients	Percentage
c.1841A > G	p.(Asp614Gly)	S	missense	All samples	100
c.14144C > T	p.(Met3752Ile)	ORF1ab	missense	All samples	100
c.608G > A	p.(Arg203Lys)	N	missense	1,3,4,5,6,7,8,9,10,11,12,13,15	86,66666667
c.610G > C	p.(Gly204Arg)	N	missense	1,3,4,5,6,7,8,9,10,11,12,13,15	86,66666667
c.418C > T	p.(Leu140Phe)	ORF3a	missense	1,4,6,9,15	33,33333333
c.73G > T	p.(Gly25Cys)	N	missense	1,4,6,9,15	33,33333333
c.171G > T	p.(Gln57His)	ORF3a	missense	2,14	13,33333333
c.17426C > T	p.(Ser5809Leu)	ORF1ab	missense	2,14	13,33333333
c.7616A > G	p.(Asn2539Ser)	ORF1ab	missense	7,13	13,33333333
c.21040C > A	p.(Arg7014Ser)	ORF1ab	missense	10,12	13,33333333
c.21041G > A	p.(Arg7014His)	ORF1ab	missense	10,12	13,33333333
c.13C > T	p.(Leu5Phe)	S	missense	12	6,666666667
c.26T > C	p.(Ile9Thr)	ORF8	missense	2	6,666666667
c.146T > C	p.(Val49Ala)	ORF8	missense	5	6,666666667
c.578G > C	p.(Ser193Thr)	ORF8	missense	14	6,666666667
c.255G > T	p.(Leu85Phe)	ORF3a	missense	4	6,666666667
c.79G > T	p.(Asp27Tyr)	ORF3a	missense	13	6,666666667
c.6368C > T	p.(Ala2123Val)	ORF1ab	missense	8	6,666666667
c.11256G >T	p.(Met3752Ile)	ORF1ab	missense	2	6,666666667
c.10691C > T	p.(Ser3564Leu)	ORF1ab	missense	2	6,666666667
c.16574A > G	p.(Glu5525Gly)	ORF1ab	missense	3	6,666666667
c.11152G > T	p.(Val3718Phe)	ORF1ab	missense	4	6,666666667
c.5074C > T	p.(Pro1692Ser)	ORF1ab	missense	5	6,666666667
c.12544C > T	p.(Leu4182Phe)	ORF1ab	missense	5	6,666666667
c.15646G > T	p.(Asp5216Tyr)	ORF1ab	missense	8	6,666666667
c.9214G > T	p.(Gly3072Cys)	ORF1ab	missense	8	6,666666667
c.5357C > T	p.(Pro1786Leu)	ORF1ab	missense	12	6,666666667
c.686A > G	p.(Gln229Arg)	N	missense	2	6,666666667
c.617C > T	p.(Ser206Phe)	N	missense	8	6,666666667
c.643G > A	p.(Gly215Ser)	N	missense	8	6,666666667
c.1477G > T	p.(Gly493Cys)	N	missense	14	6,666666667
c.436C > T	p.(Arg146Cys)	M	missense	11	6,666666667
c.2772C > T		ORF1ab	synonymous	All samples	100
c.609G > A		N	synonymous	1,3,4,5,6,7,8,9,10,11,12,13,15	86,66666667
c.48C > T		ORF1ab	synonymous	1,3,4,6,7,8,9,10,11,12,13,15	80
c.1062C > T		S	synonymous	1,4,6,8,9,15	40
c.12195A > G		ORF1ab	synonymous	1,4,6,9,15	33,33333333
c.15777C > T		ORF1ab	synonymous	1,4,6,9,15	33,33333333
c.17865C > T		ORF1ab	synonymous	7,13	13,33333333
c.18613C > T		ORF1ab	synonymous	2,14	13,33333333
c.70T > C		S	synonymous	5	6,666666667
c.1854A > T		S	synonymous	14	6,666666667
c.360C > T		ORF8	synonymous	11	6,666666667
c.19155G > T		ORF1ab	synonymous	2	6,666666667
c.14643T >C		ORF1ab	synonymous	2	6,666666667
c.1848C > T		ORF1ab	synonymous	2	6,666666667
c.81C > T		ORF1ab	synonymous	2	6,666666667
c.7500C > T		ORF1ab	synonymous	2	6,666666667
c.4275C > T		ORF1ab	synonymous	5	6,666666667
c.3990G > C		ORF1ab	synonymous	10	6,666666667
c.16746C > T		ORF1ab	synonymous	11	6,666666667
c.9708G > A		ORF1ab	synonymous	13	6,666666667
c.336C > T		M	synonymous	2	6,666666667
c.1-25C > T		5'UTR		All samples	100
c.1-49C > T		5'UTR		7,13	13,33333333
c.1-21C > T		5'UTR		10,12	13,33333333
c.117+156G > T		3'UTR		2	6,666666667

Sequences were aligned using MAFFT [9] with the following settings: Clustal format; FFT-NS-2 strategy. The evolutionary history was inferred using the Neighbor-Joining method [10]. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) [11]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method [12] and are in the units of the number of base substitutions per site. All ambiguous positions were removed for each sequence pair (pairwise deletion option). The evolutionary analyses were conducted in MEGA X [13].

Results

We sequenced 15 samples from Denizli province by using the CleanPlex® SARS-CoV-2 Panel. These samples were collected among 15 patients who were detected positive as a result of RT-PCR. Age intervals of the patients were between 5 and 80. Thus, patients' mean age was approximately 53 years, which is consistent with older adults being more susceptible to COVID-19. The number of raw data reads of 15 samples on the library was obtained as 13,069,752 read pairs, and SARS-CoV-2 genomes (> 98% coverage) were recovered from all samples. We aligned our genomic sequence with the SARS-CoV-2 NCBI Reference Sequence Wuhan (NCBI GenBank, NC_045512) to investigate variants throughout the genome. Depending on genomic alignment, we obtained 57 total and 11 unique variants (Table 1). All detected variants in samples are given in Supplementary Table 2.

Totally 32 missense, 21 synonymous, and 4 non-coding alleles were obtained. The most common detected variants were c.1-25C>T(5'UTR), c.14144C>T (ORF1ab), c.2772C>T (ORF1ab) and c.1841A>G (S) in all our genomes (15/15); c.608G>A (N), c.609G>A (N), c.610G>C (N) in 13 samples (86,67%), and c.48C>T (ORF1ab) in 12 samples (80%) (Table 1). Additionally, 35 variants were detected only once. Most of the variants were detected in ORF1ab (30/57), and all of the isolates contained a signature c.1841A>G (D614G) mutation in the spike glycoprotein (S). D614G substitution in the spike glycoprotein of SARS-CoV-2 strains is now the most prevalent, and patients carrying the D614G variant are found to have higher upper respiratory tract viral loads. According to the GISAID database, a new missense mutation was also identified in the spike protein for the first time in Turkey. c.610G>C (G204R) mutation, one of the common mutations that were also detected in our study, was obtained 79988 times (34.84% of all samples with N sequence). The other one was c.608G>A (R203K) which has already obtained 80702 times (35.15% of all samples with N sequence) in 108 countries. c.171G>T ORF3a mutations was 13,3% in our study. However, in the previous studies from Turkey, it was 40%. Additionally, we determined some missense mutations found for the first time in the samples from Turkey (Table 2).

L140F is one of the rare mutations that was obtained in 5 out of 15 samples. Three patients with L140F mutations were found to come from the same family and they have same variants (Patients 1, 6, and 9). Moreover, we found that these three patients in the same lineage, B.1.1.162. We think that this viral genotype similarity is due to in-family spread. In this study, c.146T>C (V49A) mutation found in one patient was only reported once in the USA (0.00% of all samples with NS8 sequence).

Table 2. Detected rare missense mutations.

Mutation	Gene	Type of mutation	Worldwide frequency	Number of cases in the world	Cases in this study
L140F	ORF3a	missense	0.03%	59	Patient 1,4,7,11 and 20
I9T	ORF8	missense	0.02%	40	Patient 2
Q229R	N	missense	0.01%	19	Patient 2 and 19
S301L	ORF1ab	missense	0.01%	21	Patient 2
G25C	N	missense	0.06%	136	Patient 1,4,7,9,11,16 and 20
V49A	ORF8	missense	0.00%	1	Patient 5
N1721S	ORF1ab	missense	0.00%	6	Patient 8 and 15
G215S	N	missense	0.01%	32	Patient 10
D824Y	ORF1ab	missense	0.01%	27	Patient 10
S193T	N	missense	0.03%	69	Patient 17
G313C	ORF1ab	missense	0.00%	4	Patient 17

Table 3. GISAID accession numbers and GISAID clades and lineages of our isolates.

Patients	Accession ID	GISAID Clade	Lineage
Patient 1	EPI_ISL_707938	GR	B.1.1
Patient 2	EPI_ISL_707939	GH	B.1.9
Patient 3	EPI_ISL_708184	GR	B.1.1.267
Patient 4	EPI_ISL_708185	GR	B.1.1.226
Patient 5	EPI_ISL_707936	GR	B.1.1.243
Patient 7	EPI_ISL_707937	GR	B.1.1
Patient 8	EPI_ISL_708186	GR	B.1.1
Patient 10	EPI_ISL_708188	GR	B.1.1
Patient 11	EPI_ISL_708189	GR	B.1.1
Patient 12	EPI_ISL_708190	GR	B.1.1.44
Patient 13	EPI_ISL_708191	GR	B.1.1.267
Patient 14	EPI_ISL_708195	GR	B.1.1.44
Patient 15	EPI_ISL_708192	GR	B.1.1
Patient 17	EPI_ISL_708193	GH	B.1.255
Patient 19	EPI_ISL_708194	GR	B.1.1

Phylogenetic Analysis

In the present study, domestic and international evolutionary linkage of the isolated sequences were demonstrated in Figures 1 and 2, respectively. Most of the genomes isolated in the present study are in the 20B clade, while the EPI_ISL_707939 and EPI_ISL_708193 are in the 20A clade with respect to the year-letter nomenclature clade of Nexstrain. On the other hand, to determine our sequences' clade and lineages, we uploaded our 15 sequences to the GISAID database. Table 3 demonstrates the clades and sublineages of each virus sequence. Definition of clade and lineage classifications were demonstrated by Tang *et al*. 2020 and Han et al. 2019 [14,15].

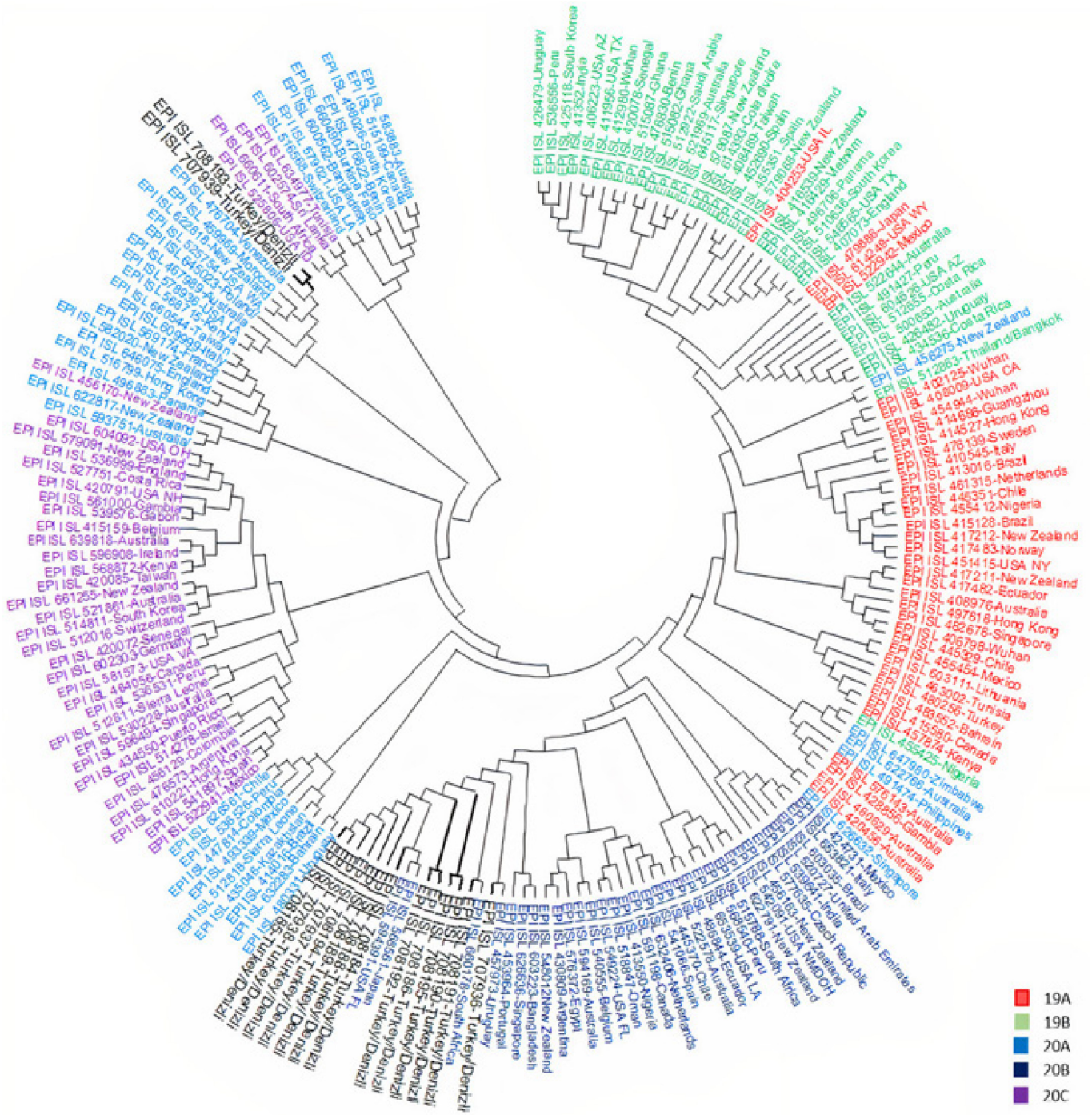
The clades were prominently dominated in the European region, having C3037T - C144408T - A23403 and G28881A - C14408T - A23403G mutations for 20A and 20B, respectively [16]. Like the result, all presented virus genomes were in lineage B.1 of GISAID pangolin clades, the major European lineage (Figure 1). Notably, EPI_ISL_707939 is in B1.9, the Turkish lineage, while EPI_ISL_708193 is in B.1.227, the Irish lineage. Unlike other isolated viruses, EPI_ISL_707939 and EPI_ISL_708193 had both genomes located at different roots in the phylogenetic tree. Considering the phylogenetic tree constructed with all complete SARSCoV2 genomes of Turkey, the viruses were seen to spread nearly homogenous in both eastern (around Kars) and western (around Istanbul) sides of the country (Figure 2). However, the difference of the two EPI_ISL_707939 and EPI_ISL_708193 genomes was also shown in the tree as a diverse root from other strains, which are mainly positioned in the eastern region of the country.

Discussion

SARS-CoV-2 is a novel coronavirus that infected more than 76 million people leading to approximately 1.69 million deaths globally as of December 2020 (https://www.gisaid.org/epiflu-applications/hcov-19-reference-sequence). As of December 2020, more than 17,000 patients died in Turkey due to COVID-19. Herein, we reported 15 virus genomes isolated in Denizli, and to our knowledge, this case series is the first comprehensive study of a COVID-19 sample population from Denizli, Turkey. The emergency departments are less frequented by younger patients and biased toward patients 18 years and older. Patients with higher viral loads detected by RT-PCR (≤20 ct) were also correlated with higher coverage of SARS-CoV-2 genome by sequencing. Besides, we performed variant calling and phylogenetic analysis with these 15 samples. We compared our sequences to the reference genome to identify genomic variants of SARS-CoV- 2 sequences (NC_045512.2). Depending on the variant analysis, all samples contained a D614G mutation in the spike glycoprotein, a widespread mutation in the samples from Turkey [17,18]. In the literature, Zhang *et al*. showed that the D614G mutation was associated with increased viral loads throughout the World [19]. We observed that c.14144C>T (P323L) and c.1841A>G (D614G) mutations co-occur in all samples, correlatively. Besides, we observed that c.608G>A and c.610G>C missense mutations are the second common mutations (86.67%) in our sequences. A study performed by Karamese *et al*. reported that 549 total and 53 unique variants were obtained in 47 isolates. Similar to our study's findings, the previous study showed that D614G was the most frequent mutation which is occurred in spike glycoprotein [17].

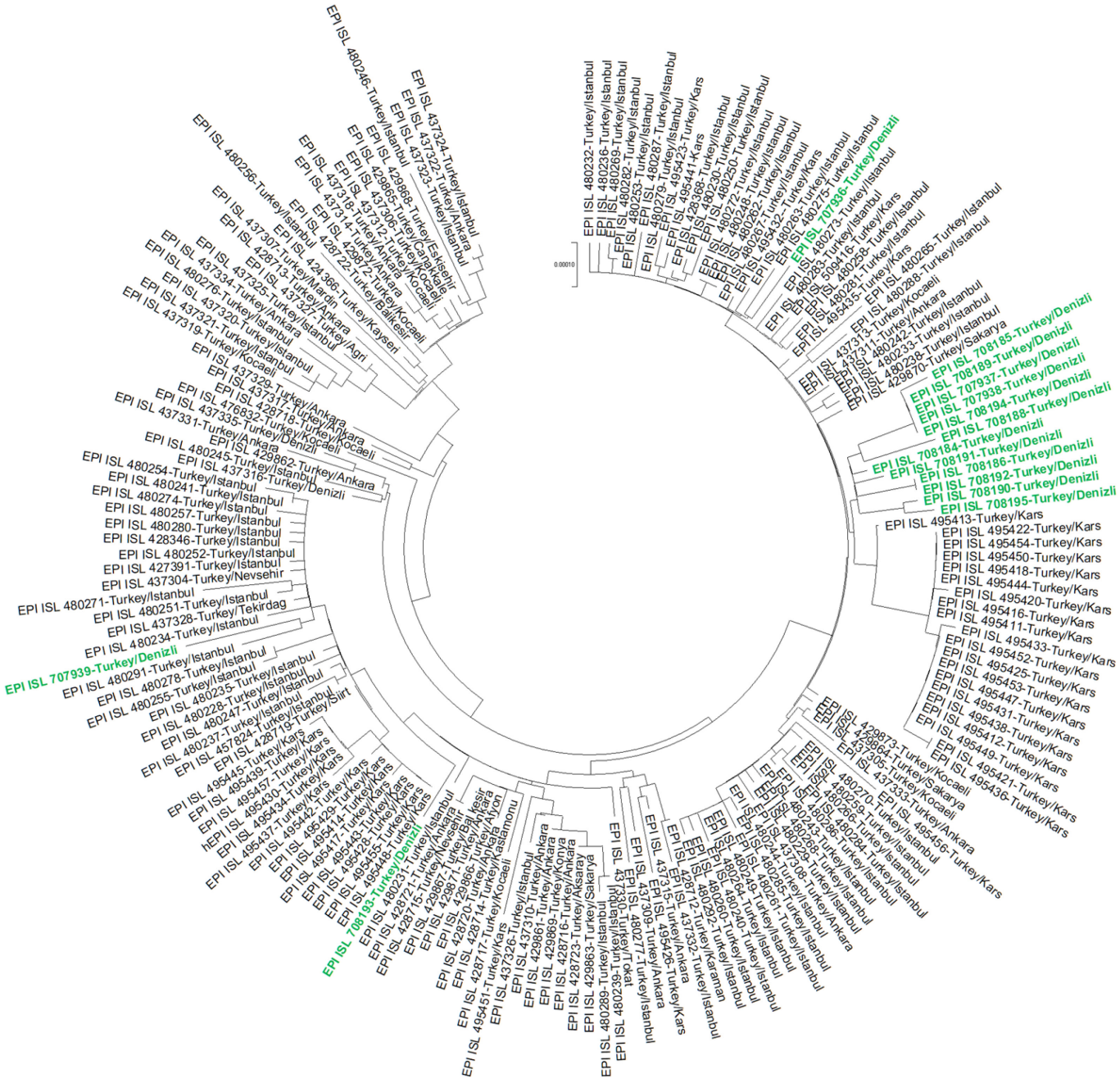
To obtain the global frequency of our genetic variations, we uploaded our sequences to the GISAID database. According to the database results, we obtained 11 sporadic missense mutations. The frequency of these rare mutations was between 0.00 to 0.06 percent. The mutations were not observed before in samples isolated in Turkey. Thus, the rare mutations in these samples may be significant for developing efficient antiviral vaccines or therapeutics. This virus's phylogenetic characterization is vital for contributing to the knowledge of viral variation to specify the most suitable regions to be used as vaccine targets or antivirals. Therefore, to determine the clades and lineages of our results, we uploaded our sequences to the GISAID database. For this purpose, all 160 complete and high coverage sequences isolated in

Figure 1. Phylogenetic tree showing the genomic epidemiology of a Denizli/Turkey focused subsampling of 15 SARS-CoV-2 genomes.



Turkey (Last access November 15, 2020) were selected to generate the local phylogenetic tree. Moreover, 180 sequences based on major GISAID clades (19A, 19B, 20A, 20B, and 20C) were also selected for generating a global phylogenetic tree. According to the local phylogenetic tree, the viruses were spread nearly homogenous in eastern (around Kars) and western (around Istanbul) parts of the country. However, the EPI_ISL_707939 and EPI_ISL_708193 genomes were in diverse root from other strains due to their rare mutations. Depending on the GISAID database, all sequenced virus samples were in major European lineage B.1.(13). Lineage B.1 is the most predominant known global lineage and has been subdivided into >70 sublineages. We identified seven descendant sublineages derived from lineage B.1. 7 out of 15 samples were found in lineage B.1.1, which is defined as European lineage with 28881GA, 28882GA, and 28883GC SNPs. In conclusion, all of our virus sequences are found in lineage B, similar to all Turkish sequences [17,18]. GISAID classifies phylogenetic clusters into currently seven clades (S, L, V G, GH, GR,

Figure 2. Local Phylogenetic tree of samples from Denizli/Turkey.



GV) using specific combinations of genetic variations. Depending on the unique variant profile, 13 out of 15 samples were belonged to the GR clade, whereas the other two samples (EPI_ISL_707939 and EPI_ISL_708193) were in the GH clade. However, most viral isolates in Turkey showed L-type characteristics and formed a monophyletic clade [18]. In conclusion, we analyzed fifteen samples from SARS-CoV-2 positive patients from Denizli, Turkey. Here, we reported the viral genomes circulating in Denizli, Turkey comprehensively for the first time. As a result of our variants' analysis, we identified 11 rare missense mutations in the virus genome compared to the reference genome. According to the phylogenetic analysis, most of our isolates were found to have similar to European sequences. However, some of the sequences had different sublineages depending on their genomic variants. More samples should be included from different Turkey areas to investigate the phylogenetic characteristics of SARS-CoV-2 infections in detail. The SARS-CoV-2 pandemic affected worldwide allows analyzing the progress and improvements of the virus and investigating the mechanisms of selecting appropriate mutations. After more genome analyses have been carried out, further studies should be done to compare strains better; however, we think that these data could help understand the virus's dynamics and help further treatment/vaccine development studies.

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Conflict of interests: No conflict of interests is declared.

Annex – Supplementary Items

Supplementary Table 1. Descriptive information about the patients.

	Age	Gender	Hospitalization
Patient 1	72	Male	Hospitalized
Patient 2	22	Male	Released
Patient 3	58	Male	Released
Patient 4	80	Male	Hospitalized
Patient 5	75	Male	Hospitalized
Patient 6	75	Male	Hospitalized
Patient 7	28	Male	Released
Patient 8	40	Male	Released
Patient 9	27	Female	Released
Patient 10	46	Male	Hospitalized
Patient 11	13	Male	Released
Patient 12	54	Female	Hospitalized
Patient 13	32	Male	Released
Patient 14	77	Male	Hospitalized
Patient 15	77	Male	Hospitalized

Supplementary Table 2. Detected variants in samples.

gene	codingConsequence	refGenome	chromosome	genome_position	depth	var_percent	exon_rank	c.DNA	protein
PATIENT 1									
5'UTR	5'UTR	NC_045512.2	1	241	908	99,6	1	c.1-25C>T	
ORF1ab	synonymous	NC_045512.2	1	313	817	99,9	1	c.48C>T	p.(Leu16=)
ORF1ab	synonymous	NC_045512.2	1	3037	255	99,2	1	c.2772C>T	p.(Phe924=)
ORF1ab	synonymous	NC_045512.2	1	12460	722	99,7	1	c.12195A>G	p.(Thr4065=)
ORF1ab	synonymous	NC_045512.2	1	16041	3668	99,9	1	c.15777C>T	p.(Tyr5259=)
S	synonymous	NC_045512.2	1	22624	43	100	1	c.1062C>T	p.(Asn354=)
N	synonymous	NC_045512.2	1	28882	1638	99,6	1	c.609G>A	p.(Arg203=)
ORF1ab	missense	NC_045512.2	1	14408	660	99,1	1	c.14144C>T	p.(Pro4715Leu)
S	missense	NC_045512.2	1	23403	1305	100	1	c.1841A>G	p.(Asp614Gly)
ORF3a	missense	NC_045512.2	1	25810	90	100	1	c.418C>T	p.(Leu140Phe)
N	missense	NC_045512.2	1	28346	2301	99,8	1	c.73G>T	p.(Gly25Cys)
N	missense	NC_045512.2	1	28881	1629	99,4	1	c.608G>A	p.(Arg203Lys)
N	missense	NC_045512.2	1	28883	1629	99,6	1	c.610G>C	p.(Gly204Arg)
PATIENT 2									
3'UTR	3'UTR	NC_045512.2	1	29830	56	100	1	c.117+156G>T	
5'UTR	5'UTR	NC_045512.2	1	241	1184	99,8	1	c.1-25C>T	
M	synonymous	NC_045512.2	1	26858	2450	100	1	c.336C>T	p.(Phe112=)
N	missense	NC_045512.2	1	28959	3210	99,9	1	c.686A>G	p.(Gln229Arg)
ORF1ab	missense	NC_045512.2	1	14408	660	99,1	1	c.14144C>T	p.(Pro4715Leu)
ORF1ab	synonymous	NC_045512.2	1	19419	37	100	1	c.19155G>T	p.(Val6385=)
ORF1ab	synonymous	NC_045512.2	1	14907	47	100	1	c.14643T>C	p.(Asn4881=)
ORF1ab	synonymous	NC_045512.2	1	2113	791	99,5	1	c.1848C>T	p.(Ile616=)
ORF1ab	missense	NC_045512.2	1	11521	699	97,7	1	c.11256G>T	p.(Met3752Ile)
ORF1ab	synonymous	NC_045512.2	1	18877	1949	100	1	c.18613C>T	p.(Leu6205=)
ORF1ab	missense	NC_045512.2	1	10956	553	100	1	c.10691C>T	p.(Ser3564Leu)
ORF1ab	synonymous	NC_045512.2	1	3037	255	99,2	1	c.2772C>T	p.(Phe924=)
ORF1ab	synonymous	NC_045512.2	1	346	589	100	1	c.81C>T	p.(Leu27=)
ORF1ab	synonymous	NC_045512.2	1	7765	472	99,6	1	c.7500C>T	p.(Ser2500=)
ORF1ab	missense	NC_045512.2	1	17690	2629	99,6	1	c.17426C>T	p.(Ser5809Leu)
ORF3a	missense	NC_045512.2	1	25563	668	100	1	c.171G>T	p.(Gln57His)
ORF8	missense	NC_045512.2	1	27919	7195	99,9	1	c.26T>C	p.(Ile9Thr)
S	missense	NC_045512.2	1	23403	1198	99,7	1	c.1841A>G	p.(Asp614Gly)
PATIENT 3									
5'UTR	5'UTR	NC_045512.2	1	241	908	99,6	1	c.1-25C>T	
N	missense	NC_045512.2	1	28881	1639	99,7	1	c.608G>A	p.(Arg203Lys)
N	missense	NC_045512.2	1	28883	1640	99,9	1	c.610G>C	p.(Gly204Arg)
N	synonymous	NC_045512.2	1	28882	1638	99,6	1	c.609G>A	p.(Arg203=)
ORF1ab	synonymous	NC_045512.2	1	3037	709	99,7	1	c.2772C>T	p.(Phe924=)
ORF1ab	missense	NC_045512.2	1	14408	1356	99,3	1	c.14144C>T	p.(Pro4715Leu)
ORF1ab	missense	NC_045512.2	1	16838	1311	99,8	1	c.16574A>G	p.(Glu5525Gly)
ORF1ab	synonymous	NC_045512.2	1	313	817	99,9	1	c.48C>T	p.(Leu16=)
S	missense	NC_045512.2	1	23403	1305	100	1	c.1841A>G	p.(Asp614Gly)
PATIENT 4									
5'UTR	5'UTR	NC_045512.2	1	241	421	100	1	c.1-25C>T	
N	synonymous	NC_045512.2	1	28882	1629	99,6	1	c.609G>A	p.(Arg203=)
N	missense	NC_045512.2	1	28346	2301	99,8	1	c.73G>T	p.(Gly25Cys)
N	missense	NC_045512.2	1	28881	1629	99,4	1	c.608G>A	p.(Arg203Lys)
N	missense	NC_045512.2	1	28883	1629	99,6	1	c.610G>C	p.(Gly204Arg)
ORF1ab	synonymous	NC_045512.2	1	3037	1209	100	1	c.2772C>T	p.(Phe924=)
ORF1ab	synonymous	NC_045512.2	1	16041	3668	99,9	1	c.15777C>T	p.(Tyr5259=)
ORF1ab	missense	NC_045512.2	1	14408	3345	99,7	1	c.14144C>T	p.(Pro4715Leu)
ORF1ab	synonymous	NC_045512.2	1	313	1618	100	1	c.48C>T	p.(Leu16=)
ORF1ab	synonymous	NC_045512.2	1	12460	722	99,7	1	c.12195A>G	p.(Thr4065=)
ORF1ab	missense	NC_045512.2	1	11417	3960	95,5	1	c.11152G>T	p.(Val3718Phe)
ORF3a	missense	NC_045512.2	1	25810	90	100	1	c.418C>T	p.(Leu140Phe)
ORF3a	missense	NC_045512.2	1	25647	1803	95	1	c.255G>T	p.(Leu85Phe)
S	missense	NC_045512.2	1	23403	902	100	1	c.1841A>G	p.(Asp614Gly)
S	synonymous	NC_045512.2	1	22624	43	100	1	c.1062C>T	p.(Asn354=)
PATIENT 5									
5'UTR	5'UTR	NC_045512.2	1	241	559	99,6	1	c.1-25C>T	
N	synonymous	NC_045512.2	1	28882	1020	100	1	c.609G>A	p.(Arg203=)
N	missense	NC_045512.2	1	28883	1020	99,8	1	c.610G>C	p.(Gly204Arg)
N	missense	NC_045512.2	1	28881	1019	99,4	1	c.608G>A	p.(Arg203Lys)
ORF1ab	synonymous	NC_045512.2	1	4540	703	98	1	c.4275C>T	p.(Tyr1425=)
ORF1ab	missense	NC_045512.2	1	5339	178	100	1	c.5074C>T	p.(Pro1692Ser)
ORF1ab	missense	NC_045512.2	1	12809	1058	99,5	1	c.12544C>T	p.(Leu4182Phe)
ORF1ab	synonymous	NC_045512.2	1	3037	703	100	1	c.2772C>T	p.(Phe924=)
ORF1ab	missense	NC_045512.2	1	14408	1036	99,5	1	c.14144C>T	p.(Pro4715Leu)
ORF8	missense	NC_045512.2	1	28039	1624	99,7	1	c.146T>C	p.(Val49Ala)
S	synonymous	NC_045512.2	1	21632	452	100	1	c.70T>C	p.(Leu24=)
S	missense	NC_045512.2	1	23403	1197	99,8	1	c.1841A>G	p.(Asp614Gly)
PATIENT 6									
5'UTR	5'UTR	NC_045512.2	1	241	577	99,7	1	c.1-25C>T	
N	synonymous	NC_045512.2	1	28882	753	99,7	1	c.609G>A	p.(Arg203=)

gene	codingConsequence	refGenome	chromosome	genome_position	depth	var_percent	exon_rank	c.DNA	protein
N	missense	NC_045512.2	1	28881	753	99,2	1	c.608G>A	p.(Arg203Lys)
N	missense	NC_045512.2	1	28883	753	99,7	1	c.610G>C	p.(Gly204Arg)
N	missense	NC_045512.2	1	28346	1309	99,8	1	c.73G>T	p.(Gly25Cys)
ORF1ab	missense	NC_045512.2	1	14408	1053	98,9	1	c.14144C>T	p.(Pro4715Leu)
ORF1ab	synonymous	NC_045512.2	1	3037	638	100	1	c.2772C>T	p.(Phe924=)
ORF1ab	synonymous	NC_045512.2	1	313	529	100	1	c.48C>T	p.(Leu16=)
ORF1ab	synonymous	NC_045512.2	1	12460	651	100	1	c.12195A>G	p.(Thr4065=)
ORF1ab	synonymous	NC_045512.2	1	16041	2397	99,9	1	c.15777C>T	p.(Tyr5259=)
ORF3a	missense	NC_045512.2	1	25810	218	99,1	1	c.418C>T	p.(Leu140Phe)
S	synonymous	NC_045512.2	1	22624	35	100	1	c.1062C>T	p.(Asn354=)
S	missense	NC_045512.2	1	23403	1269	99,8	1	c.1841A>G	p.(Asp614Gly)
PATIENT 7									
5'UTR	5'UTR	NC_045512.2	1	217	52	100	1	c.1-49C>T	
5'UTR	5'UTR	NC_045512.2	1	241	52	100	1	c.1-25C>T	
N	missense	NC_045512.2	1	28883	4826	99,9	1	c.610G>C	p.(Gly204Arg)
N	synonymous	NC_045512.2	1	28882	4826	99,6	1	c.609G>A	p.(Arg203=)
N	missense	NC_045512.2	1	28881	4826	99,8	1	c.608G>A	p.(Arg203Lys)
ORF1ab	synonymous	NC_045512.2	1	18129	28	100	1	c.17865C>T	p.(Asp5955=)
ORF1ab	missense	NC_045512.2	1	7881	28	100	1	c.7616A>G	p.(Asn2539Ser)
ORF1ab	synonymous	NC_045512.2	1	3037	863	100	1	c.2772C>T	p.(Phe924=)
ORF1ab	synonymous	NC_045512.2	1	313	1491	99,7	1	c.48C>T	p.(Leu16=)
ORF1ab	missense	NC_045512.2	1	14408	1923	99,9	1	c.14144C>T	p.(Pro4715Leu)
S	missense	NC_045512.2	1	23403	40	100	1	c.1841A>G	p.(Asp614Gly)
PATIENT 8									
5'UTR	5'UTR	NC_045512.2	1	241	435	100	1	c.1-25C>T	
N	missense	NC_045512.2	1	28890	879	99,3	1	c.617C>T	p.(Ser206Phe)
N	synonymous	NC_045512.2	1	28882	838	99,5	1	c.609G>A	p.(Arg203=)
N	missense	NC_045512.2	1	28883	838	100	1	c.610G>C	p.(Gly204Arg)
N	missense	NC_045512.2	1	28916	1019	99,8	1	c.643G>A	p.(Gly215Ser)
N	missense	NC_045512.2	1	28881	838	100	1	c.608G>A	p.(Arg203Lys)
ORF1ab	missense	NC_045512.2	1	15910	610	100	1	c.15646G>T	p.(Asp5216Tyr)
ORF1ab	missense	NC_045512.2	1	6633	108	100	1	c.6368C>T	p.(Ala2123Val)
ORF1ab	synonymous	NC_045512.2	1	3037	627	99,7	1	c.2772C>T	p.(Phe924=)
ORF1ab	missense	NC_045512.2	1	9479	677	99,7	1	c.9214G>T	p.(Gly3072Cys)
ORF1ab	missense	NC_045512.2	1	14408	955	100	1	c.14144C>T	p.(Pro4715Leu)
ORF1ab	synonymous	NC_045512.2	1	313	567	100	1	c.48C>T	p.(Leu16=)
S	missense	NC_045512.2	1	23403	1397	99,9	1	c.1841A>G	p.(Asp614Gly)
S	synonymous	NC_045512.2	1	22624	37	100	1	c.1062C>T	p.(Asn354=)
PATIENT 9									
5'UTR	5'UTR	NC_045512.2	1	241	592	100	1	c.1-25C>T	
N	synonymous	NC_045512.2	1	28882	847	99,9	1	c.609G>A	p.(Arg203=)
N	missense	NC_045512.2	1	28881	847	99,6	1	c.608G>A	p.(Arg203Lys)
N	missense	NC_045512.2	1	28883	847	99,8	1	c.610G>C	p.(Gly204Arg)
N	missense	NC_045512.2	1	28346	727	99,7	1	c.73G>T	p.(Gly25Cys)
ORF1ab	missense	NC_045512.2	1	14408	891	99,5	1	c.14144C>T	p.(Pro4715Leu)
ORF1ab	synonymous	NC_045512.2	1	3037	959	100	1	c.2772C>T	p.(Phe924=)
ORF1ab	synonymous	NC_045512.2	1	313	802	100	1	c.48C>T	p.(Leu16=)
ORF1ab	synonymous	NC_045512.2	1	12460	749	100	1	c.12195A>G	p.(Thr4065=)
ORF1ab	synonymous	NC_045512.2	1	16041	2355	99,5	1	c.15777C>T	p.(Tyr5259=)
ORF3a	missense	NC_045512.2	1	25810	259	99,2	1	c.418C>T	p.(Leu140Phe)
S	synonymous	NC_045512.2	1	22624	119	100	1	c.1062C>T	p.(Asn354=)
S	missense	NC_045512.2	1	23403	1322	99,8	1	c.1841A>G	p.(Asp614Gly)
PATIENT 10									
5'UTR	5'UTR	NC_045512.2	1	241	1739	99,7	1	c.1-25C>T	
5'UTR	5'UTR	NC_045512.2	1	245	1739	99,9	1	c.1-21C>T	
N	synonymous	NC_045512.2	1	28882	2858	99,6	1	c.609G>A	p.(Arg203=)
N	missense	NC_045512.2	1	28883	2858	99,6	1	c.610G>C	p.(Gly204Arg)
N	missense	NC_045512.2	1	28881	2858	99,7	1	c.608G>A	p.(Arg203Lys)
ORF1ab	missense	NC_045512.2	1	21304	1685	100	1	c.21040C>A	p.(Arg7014Ser)
ORF1ab	missense	NC_045512.2	1	14408	1537	99,7	1	c.14144C>T	p.(Pro4715Leu)
ORF1ab	synonymous	NC_045512.2	1	4255	4677	100	1	c.3990G>C	p.(Pro1330=)
ORF1ab	synonymous	NC_045512.2	1	3037	1132	99	1	c.2772C>T	p.(Phe924=)
ORF1ab	synonymous	NC_045512.2	1	313	994	99,7	1	c.48C>T	p.(Leu16=)
ORF1ab	missense	NC_045512.2	1	21305	1687	99,1	1	c.21041G>A	p.(Arg7014His)
S	missense	NC_045512.2	1	23403	2895	99,8	1	c.1841A>G	p.(Asp614Gly)
PATIENT 11									
5'UTR	5'UTR	NC_045512.2	1	241	415	100	1	c.1-25C>T	
M	missense	NC_045512.2	1	26958	369	74,5	1	c.436C>T	p.(Arg146Cys)
N	synonymous	NC_045512.2	1	28882	763	99,7	1	c.609G>A	p.(Arg203=)
N	missense	NC_045512.2	1	28883	763	99	1	c.610G>C	p.(Gly204Arg)
N	missense	NC_045512.2	1	28881	763	99,9	1	c.608G>A	p.(Arg203Lys)
ORF1ab	missense	NC_045512.2	1	14408	52	100	1	c.14144C>T	p.(Pro4715Leu)
ORF1ab	synonymous	NC_045512.2	1	313	618	98,1	1	c.48C>T	p.(Leu16=)
ORF1ab	synonymous	NC_045512.2	1	3037	749	99,5	1	c.2772C>T	p.(Phe924=)
ORF1ab	synonymous	NC_045512.2	1	17010	628	77,2	1	c.16746C>T	p.(Ile5582=)
ORF8	synonymous	NC_045512.2	1	28253	594	81,6	1	c.360C>T	p.(Phe120=)

gene	codingConsequence	refGenome	chromosome	genome_position	depth	var_percent	exon_rank	c.DNA	protein
S	missense	NC_045512.2	1	23403	706	99,6	1	c.1841A>G	p.(Asp614Gly)
PATIENT 12									
5'UTR	5'UTR	NC_045512.2	1	241	617	99,8	1	c.1-25C>T	
5'UTR	5'UTR	NC_045512.2	1	245	617	93,5	1	c.1-21C>T	
N	synonymous	NC_045512.2	1	28882	646	99,8	1	c.609G>A	p.(Arg203=)
N	missense	NC_045512.2	1	28881	646	99,8	1	c.608G>A	p.(Arg203Lys)
N	missense	NC_045512.2	1	28883	645	99,4	1	c.610G>C	p.(Gly204Arg)
ORF1ab	missense	NC_045512.2	1	21304	650	84,8	1	c.21040C>A	p.(Arg7014Ser)
ORF1ab	missense	NC_045512.2	1	5622	60	96,7	1	c.5357C>T	p.(Pro1786Leu)
ORF1ab	missense	NC_045512.2	1	14408	1003	100	1	c.14144C>T	p.(Pro4715Leu)
ORF1ab	synonymous	NC_045512.2	1	3037	352	100	1	c.2772C>T	p.(Phe924=)
ORF1ab	synonymous	NC_045512.2	1	313	347	100	1	c.48C>T	p.(Leu16=)
ORF1ab	missense	NC_045512.2	1	21305	651	85,9	1	c.21041G>A	p.(Arg7014His)
S	missense	NC_045512.2	1	21575	3934	87,5	1	c.13C>T	p.(Leu5Phe)
S	missense	NC_045512.2	1	23403	2098	99,8	1	c.1841A>G	p.(Asp614Gly)
PATIENT 13									
5'UTR	5'UTR	NC_045512.2	1	217	593	100	1	c.1-49C>T	
5'UTR	5'UTR	NC_045512.2	1	241	592	99,7	1	c.1-25C>T	
N	synonymous	NC_045512.2	1	28882	1123	99,7	1	c.609G>A	p.(Arg203=)
N	missense	NC_045512.2	1	28883	1123	99,9	1	c.610G>C	p.(Gly204Arg)
N	missense	NC_045512.2	1	28881	1123	99,8	1	c.608G>A	p.(Arg203Lys)
ORF1ab	missense	NC_045512.2	1	7881	838	100	1	c.7616A>G	p.(Asn2539Ser)
ORF1ab	synonymous	NC_045512.2	1	9973	1208	99,5	1	c.9708G>A	p.(Lys3236=)
ORF1ab	missense	NC_045512.2	1	14408	1235	99,4	1	c.14144C>T	p.(Pro4715Leu)
ORF1ab	synonymous	NC_045512.2	1	3037	623	100	1	c.2772C>T	p.(Phe924=)
ORF1ab	synonymous	NC_045512.2	1	313	430	100	1	c.48C>T	p.(Leu16=)
ORF1ab	synonymous	NC_045512.2	1	18129	1156	99,8	1	c.17865C>T	p.(Asp5955=)
S	missense	NC_045512.2	1	23403	1151	100	1	c.1841A>G	p.(Asp614Gly)
PATIENT 14									
5'UTR	5'UTR	NC_045512.2	1	241	1984	99,8	1	c.1-25C>T	
N	missense	NC_045512.2	1	28851	1744	79,7	1	c.578G>C	p.(Ser193Thr)
ORF1ab	synonymous	NC_045512.2	1	3037	56	96,4	1	c.2772C>T	p.(Phe924=)
ORF1ab	synonymous	NC_045512.2	1	18877	280	100	1	c.18613C>T	p.(Leu6205=)
ORF1ab	missense	NC_045512.2	1	14408	164	99,4	1	c.14144C>T	p.(Pro4715Leu)
ORF1ab	missense	NC_045512.2	1	1742	413	89,6	1	c.1477G>T	p.(Gly493Cys)
ORF1ab	missense	NC_045512.2	1	17690	1972	89,7	1	c.17426C>T	p.(Ser5809Leu)
ORF3a	missense	NC_045512.2	1	25471	235	83	1	c.79G>T	p.(Asp27Tyr)
ORF3a	missense	NC_045512.2	1	25563	382	95,3	1	c.171G>T	p.(Gln57His)
S	synonymous	NC_045512.2	1	23416	918	100	1	c.1854A>T	p.(Thr618=)
S	missense	NC_045512.2	1	23403	921	99,3	1	c.1841A>G	p.(Asp614Gly)
PATIENT 15									
5'UTR	5'UTR	NC_045512.2	1	241	979	99,4	1	c.1-25C>T	
N	synonymous	NC_045512.2	1	28882	907	99,9	1	c.609G>A	p.(Arg203=)
N	missense	NC_045512.2	1	28881	907	99,7	1	c.608G>A	p.(Arg203Lys)
N	missense	NC_045512.2	1	28883	907	100	1	c.610G>C	p.(Gly204Arg)
N	missense	NC_045512.2	1	28346	1035	99,8	1	c.73G>T	p.(Gly25Cys)
ORF1ab	missense	NC_045512.2	1	14408	1335	100	1	c.14144C>T	p.(Pro4715Leu)
ORF1ab	synonymous	NC_045512.2	1	3037	655	100	1	c.2772C>T	p.(Phe924=)
ORF1ab	synonymous	NC_045512.2	1	313	497	100	1	c.48C>T	p.(Leu16=)
ORF1ab	synonymous	NC_045512.2	1	12460	706	100	1	c.12195A>G	p.(Thr4065=)
ORF1ab	synonymous	NC_045512.2	1	16041	5053	99,9	1	c.15777C>T	p.(Tyr5259=)
ORF3a	missense	NC_045512.2	1	25810	371	100	1	c.418C>T	p.(Leu140Phe)
S	synonymous	NC_045512.2	1	22624	35	100	1	c.1062C>T	p.(Asn354=)
S	missense	NC_045512.2	1	23403	1243	100	1	c.1841A>G	p.(Asp614Gly)