

Detection of SARS-CoV-2 from Patient Fecal Samples by Whole Genome Sequencing

Andreas Papoutsis (✉ papoutsis@progenabiome.com)

ProgenaBiome <https://orcid.org/0000-0002-7035-5101>

Thomas Borody

Center for Digestive Diseases

Siba Dolai

Centre for Digestive Disease

Jordan Daniels

ProgenaBiome

Skylar Steinberg

ProgenaBiome

Brad Barrows

ProgenaBiome

Sabine Hazan

ProgenaBiome, Ventura Clinical Trials

Research

Keywords: COVID-19, SARS-CoV-2

DOI: <https://doi.org/10.21203/rs.3.rs-48863/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

Background SARS-CoV-2 has been detected not only in respiratory secretions, but also in stool collections. Here we sought to identify SARS-CoV-2 by enrichment NGS from fecal samples, and to utilize whole genome analysis to characterize SARS-CoV-2 mutational variations in COVID-19 patients.

Results Study participants underwent testing for SARS-CoV-2 from fecal samples by whole genome enrichment NGS (n = 14), and RT-PCR nasopharyngeal swab analysis (n = 12). The concordance of SARS-CoV-2 detection by enrichment NGS from stools with RT-PCR nasopharyngeal analysis was 100%. Unique variants were identified in four patients, with a total of 33 different mutations among those in which SARS-CoV-2 was detected by whole genome enrichment NGS.

Conclusion These results highlight the potential viability of SARS-CoV-2 in feces, its ongoing mutational accumulation, and its possible role in fecal-oral transmission. This study also elucidates the advantages of SARS-CoV-2 enrichment NGS, which may be a key methodology to document complete viral eradication.

Background

December 2019, the virus, which would cause a worldwide pandemic, was first identified in the city of Wuhan, China [1]. In January 2020, it was implicated in various pneumonia cases, and was rapidly isolated from a bronchoalveolar lavage sample, analyzed via next-generation sequencing (NGS), and identified to be a novel betacoronavirus [2, 3], the same family of viruses responsible for severe acute respiratory syndrome (SARS) and middle east respiratory syndrome (MERS) [4–6]. This new virus, named the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) on February 11, 2020 by the International Committee on the Taxonomy of Viruses (ICTV), has now been implicated in over 13.4 million cases worldwide, and over 580,000 deaths [7, 8]. Over 138,000 of these deaths have been in the United States alone.

Early diagnosis of SARS-CoV-2 has been challenging and may contribute to its high mortality rates. Due to the similarities between SARS-CoV-2 and SARS-CoV, a similar diagnostic approach utilizing real time polymerase chain reaction (RT-PCR) from nasopharyngeal swabs has been adopted as the standard of care [9]. Contributing to the spread of the disease, has been the viral load being below the threshold of detection in some individuals [10]. Additionally, acquired mutations may assist in its evasion of detection from specifically targeted PCR primers [11]. Further, samples collected soon after infection, or after symptoms have resolved, have resulted in high false negative rates. Samples collected in these timeframes have been shown to have false-negative rates as high as 33% [12]. The exact rate of false positives has not been established for SARS-CoV-2, however in a study by Cohen and Kessel, the rate was extrapolated based on known false-positive rates from other viral RT-PCR tests. The mean false-positive rate in the study was found to be 3.2%, ranging from 0–16.7%, and an interquartile range of 0.8 to 4.0% [13].

Symptoms of SARS-CoV-2 infection cover a broad range including fever, malaise, body aches, chills, headaches, cough, and shortness of breath, as well as gastrointestinal symptoms such as diarrhea, nausea, and vomiting [14, 15]. Research from SARS and MERS has shown that coronaviruses can be present in stool of infected patients, and that the fecal-oral route may be a mode of transmission. Wang et al in 2005 demonstrated that SARS-CoV was not only present in stool samples collected from patients, but also in the wastewater of two hospitals [16]. In review of this data, with inclusion of SARS-CoV-2, a meta-analysis by Parasa et al. found that 40% of patients with positive RT-PCR for SARS-CoV-2 from nasopharyngeal swabs or respiratory secretions were also found to be positive from stool samples [17]. This data was supported by two recent studies documenting positive SARS-CoV-2 fecal samples from “recovered” COVID-19 patients with negative nasopharyngeal swabs [18, 19].

In view of the large percentage of SARS-CoV-2 detectible by RT-PCR in stools of infected patients, we sought to identify the presence of the SARS-CoV-2 by NGS of fecal samples from symptomatic study participants positive for SARS-CoV-2 by nasopharyngeal sample RT-PCR, in addition to asymptomatic individuals (with or without prior nasopharyngeal sample RT-

PCR). We also aimed to execute whole genome analysis to characterize SARS-CoV-2 mutational variations to identify potentially significant nucleotide changes.

Results

We evaluated the results from patients that had their stool samples tested by whole genome enrichment NGS, and their nasopharyngeal swabs tested by RT-PCR for the presence of SARS-CoV-2. Of the 14 study participants, ten were symptomatic and tested positive for SARS-CoV-2 by RT-PCR, two asymptomatic individuals tested negative, and two other asymptomatic individuals did not undergo RT-PCR testing (Table 1). Patients 5 and 7, that tested positive by RT-PCR from nasopharyngeal swabs, were treated with Hydroxychloroquine (HCQ), Azithromycin, vitamin C, vitamin D, and zinc for 10 days prior to fecal collection. Similarly, after positive nasopharyngeal swab, patient 13 was treated with vitamin C, vitamin D, and zinc for 10 days before fecal collection. The concordance of SARS-CoV-2 detection by enrichment NGS from stools among positive non-treated patients tested by RT-PCR nasopharyngeal analysis was 100% (7/7). Patient 8, who did not undergo nasopharyngeal analysis, tested positive for SARS-CoV-2 by NGS. The three patients (5, 7, 13) that received treatment prior to providing fecal samples, all tested negative by NGS. Asymptomatic patients 2 and 9, who tested negative by nasopharyngeal swab, were also negative by NGS, as was asymptomatic patient 14.

Table 1
Symptoms and SARS-CoV-2 testing results

Sample ID	Symptoms	Nasopharyngeal Swab (RT-PCR)	Treated	Fecal (NGS)	Patient Location
Patient 1	febrile, diarrhea, anosmia, O2 sat. <90%	+	no	+	PA
Patient 3	febrile, diarrhea, O2 sat. <90%	+	no	+	CA
Patient 4	febrile, diarrhea, anosmia, O2 sat. <90%	+	no	+	AZ
Patient 6	febrile, cough, anosmia	+	no	+	AZ
Patient 8	none	n/a	no	+	CA
Patient 10	febrile, cough, headache	+	no	+	GA
Patient 11	febrile, cough, headache	+	no	+	GA
Patient 12	febrile, cough	+	no	+	GA
Patient 5	febrile, cough	+	yes	-	CA
Patient 7	febrile, cough	+	yes	-	GA
Patient 13	febrile, cough	+	yes	-	GA
Patient 2	none	-	no	-	CA
Patient 9	none	-	no	-	CA
Patient 14	none	n/a	no	-	CA

All fecal samples analyzed by enrichment NGS from positive patients by RT-PCR, achieved 100% genome coverage of SARS-CoV-2 except for patient 3 which had 45%, and patient 10 which had 93% coverage (Table 2). The total number of SARS-CoV-2 mapped reads for patients 1, 3, 4, 6, 8, 10, 11, and 12 were 465645, 5984, 131582, 793603, 496852, 5929, 1270734, and 38256 respectively. The mean read depths of SARS-CoV-2 for patients 1, 3, 4, 6, 8, 10, 11, and 12 were 1129.8x, 31.7x, 318.6x, 1924.6x, 1206.7x, 15.5x, 3075.3x, and 92.7x respectively. The read depths at specific coordinates along the SARS-CoV-2 genome for each patient are captured in Fig. 1.

Table 2
Enrichment NGS metrics

Sample ID	Genome Coverage	Number of Variants	Mapped Reads	Mean Depth
Patient 1	100%	11	465645	1129.8x
Patient 3	45%	11	5984	31.7x
Patient 4	100%	9	131582	318.6x
Patient 6	100%	10	793603	1924.6x
Patient 8	100%	10	496852	1206.7
Patient 10	93%	9	5929	15.6x
Patient 11	100%	10	1270734	3075.3x
Patient 12	100%	10	38256	92.7x

Following alignment and mapping of SARS-CoV-2, patient genomes were compared to the Wuhan-Hu-1 (MN90847.3) SARS-CoV-2 reference genome via One Codex's bioinformatics pipeline to identify mutational variations. This analysis identified nucleotide variants at positions nt241 (C → T) and nt23403 (A → G) across all positive patients, and variants at positions nt3037 (C → T) and nt25563 (G → T) in seven of the eight patients (Table 3). Interestingly, patients 8, 11, and 12 harbored the same set of variants, as did patients 4 and 6 (who were kindreds). Unique variants not identified in any of the other individuals were detected in patients 1, 3, 6, and 10, with patient 3 harboring the most distinct SARS-CoV-2 genome with eight unique variants, followed by patient 1 with seven. Collectively, there were thirty-three different mutations among the patients in which SARS-CoV-2 was detected by whole genome enrichment NGS.

Table 3
SARS-CoV-2 genomic positions, variant changes, and frequencies across the positive patient cohort

Region (ORF)	Position	Variant	Patient 1	Patient 3	Patient 4	Patient 6	Patient 8	Patient 10	Patient 11	Patient 12
5'-UTR	241	C → T	100%	100%	100%	100%	100%	100%	100%	100%
1a	833	T → C	x	x	x	x	100%	x	100%	100%
1a	1059	C → T	x	x	100%	100%	99%	100%	100%	100%
1a	1758	C → T	x	x	100%	100%	x	x	x	x
1a	1973	C → T	x	x	x	87%	x	x	x	x
1a	3037	C → T	100%	x	100%	100%	100%	100%	100%	100%
1a	3078	C → T	x	89%	x	x	x	x	x	x
1a	4866	G → T	75%	x	x	x	x	x	x	x
1a	6720	C → T	93%	x	x	x	x	x	x	x
1a	8102	G → T	x	100%	x	x	x	x	x	x
1a	9401	T → C	x	x	x	x	x	64%	x	x
1a	9403	T → A	x	x	x	x	x	64%	x	x
1a	10870	G → T	x	x	100%	100%	x	x	x	x
1a	11123	G → A	x	x	100%	100%	x	x	x	x
1b	14408	C → T	100%	x	100%	100%	100%	x	100%	100%
1b	14877	C → T	x	100%	x	x	x	x	x	x
1b	16616	C → T	x	x	x	x	100%	x	100%	100%
1b	16848	C → T	100%	x	x	x	x	x	x	x
1b	18652	C → A	x	x	x	x	x	83%	x	x
1b	19989	T → G	x	100%	x	x	x	x	x	x
Spike	21576	T → G	x	83%	x	x	x	x	x	x
Spike	23264	G → A	x	75%	x	x	x	x	x	x
Spike	23403	A → G	100%	100%	100%	100%	100%	100%	100%	100%
Spike	23603	C → T	82%	x	x	x	x	x	x	x
3a	25563	G → T	x	100%	100%	100%	100%	100%	100%	100%
3a	25976	C → A	x	x	x	x	100%	x	100%	100%
8	27964	C → T	x	x	x	x	100%	x	100%	100%
Nucleoprotein	28881	G → A	100%	x	x	x	x	x	x	x
Nucleoprotein	28882	G → A	100%	x	x	x	x	x	x	x
Nucleoprotein	28883	G → C	100%	x	x	x	x	x	x	x
Nucleoprotein	28997	C → T	x	100%	x	x	x	x	x	x

Region (ORF)	Position	Variant	Patient 1	Patient 3	Patient 4	Patient 6	Patient 8	Patient 10	Patient 11	Patient 12
Nucleoprotein	29019	A → T	x	100%	x	x	x	x	x	x
Nucleoprotein	29364	C → G	x	x	x	x	x	85%	x	x

Discussion

Coronaviridae is a family of enveloped, single-stranded, positive-sense RNA viruses [20, 21]. The total length of the genome is 30 Kb, consisting of a 5'-terminal noncoding region, an open reading frame (ORF) 1a/b-coding region, an S region encoding the spike glycoprotein (S protein), an E region encoding the envelope protein (E protein), an M region encoding the membrane protein (M protein), an N region encoding the nucleocapsid protein (N protein), and a -3'-terminal noncoding region [22–25]. Among them, the poly protein encoded in the ORF1a/b region of the nonstructural protein can be cut by 3CLpro and PLpro of the virus to form RNA-dependent RNA polymerase and helicase, which guides the replication, transcription, and translation of the virus genome. The M and E proteins are involved in the formation of the envelope, while the N protein is involved in assembly. The spike protein binds to the receptor of the host cell and confers specificity for viral invasion into susceptible cells.

Once decoded, the SARS-CoV-2 genome was found to share high sequence identity with the bat coronavirus, BatCoV RaTG13 (96.2%) (2). Upon further investigation, it was discovered that SARS-CoV-2 harbored significant sequence homology with the viruses responsible for SARS and MERS, with a notable exception found in the receptor binding domain (RBD) [26]. Shang et al. elucidated the RBD structure of the human ACE2 receptor (angiotensin-converting enzyme 2) [27], demonstrating that the replacement of several residues within the protein caused it to have a much more compact hydrophobic pocket. This change increased the binding affinity of SARS-CoV-2 to ACE2 as compared to SARS-CoV. While this has contributed to its greater virulence, it also represents a potential therapeutic target [28]. This concept was detailed in the mini-review by Yang and Shen, wherein they proposed that SARS-CoV-2 may be susceptible to the inhibitory effect of chloroquine (CQ), a lysosomotropic agent, via its accumulation in the acidic organelles [29]. The therapeutic effect of CQ may be a result of its ability to neutralize the endosome-lysosomal acidic pH and block the protease activity necessary for viral entry [30]. This may be evidenced by the HCQ treated patients in this study that appeared to have cleared the virus having tested negative by enrichment NGS for SARS-CoV-2 while having testing positive by nasopharyngeal analysis prior to treatment.

Two recent studies that support targeting the endocytic pathway and autophagy as therapeutic strategies are by Arshad et al. and Lagier et al., both of which show that HCQ was associated with a significant reduction of in-hospital mortality compared to those not receiving HCQ [31, 32]. Numerous other studies have also reported the efficacy of CQ and/or HCQ in various treatment regimens for COVID-19 [33–39]. Adding to the complexity of COVID-19 treatment and prevention, is that SARS-CoV-2 appears to be mutating at an alarming rate, as reported in the Icelandic study which identified the presence of 291 sequence variants that were not present in the Global Initiative on Sharing All Influenza Data (GISAID) reference database as of March 22 (11).

Although previous studies have identified SARS-CoV-2 in fecal collections by RT-PCR [40, 41], this is the first to our knowledge, to report whole genome sequencing (WGS) of SARS-CoV-2 from stool samples. Herein we were able to identify SARS-CoV-2 in patients that tested positive by nasopharyngeal swab RT-PCR analysis and observed unique genomes in 62.5% of the NGS positive patients. The overall homology among the genomes was high (99.97%), with variations identified in the ORF regions 1a, 1b, S, 3a, 8, and N. Of particular interest, was the adenine to guanine change in the S protein at position nt23403 which converts aspartic acid to glycine. Although the significance of this variation is unclear, it warrants further investigation to understand its effect on spike glycoprotein ACE2 binding and virulence. The conversions of glycine to arginine (nt28883) and proline to arginine (nt29364) in the nucleoprotein also necessitate further examination. While

enrichment NGS is both costly and time consuming, these striking results highlight the potential viability of SARS-CoV-2 in feces, its possible role in transmission, and may accurately document complete eradication of the virus.

Figure 1. Whole genome alignment of SARS-CoV-2 in patients (Pt). The x-axis depicts the genomic coordinates as aligned to the MN908947.3 reference genome, and the y-axis represents the read depth at specific loci.

Conclusion

Next generation sequencing identified the SARS-CoV-2 whole genome sequence in 100% of patients with positive nasopharyngeal RT-PCR and did not detect it in treated patients, or those with negative RT-PCR. These results highlight the importance of metagenomic analysis of the SARS-CoV-2 viral genome. This alternative diagnostic methodology may help with viral identification and tracking its evolutionary progression through the population, as well as documenting its clearance.

Methods

Study participants (n = 14) underwent testing for SARS-CoV-2 from fecal samples by whole genome enrichment NGS. Following fecal collection (Zymo Research Shield Fecal Collection Tubes), RNA was extracted (Qiagen Allprep Power Viral Kit), reverse transcribed (New England Biolabs NEBNext 1st and 2nd Strand Synthesis Modules), library prepped (Illumina Nextera Flex for Enrichment), enriched (Illumina Respiratory Virus Oligo Panel), and sequenced on Illumina's NextSeq 550 System. Sequences were then mapped to the SARS-CoV-2 Wuhan-Hu-1 (MN90847.3) complete genome utilizing One Codex's SARS-CoV-2 bioinformatics analysis pipeline. SARS-CoV-2 positive samples were further analyzed for mutational variants that differed from the reference genome. Of the 14 study participants, 12 also had their nasopharyngeal swabs tested for SARS-CoV-2 by RT-PCR.

Abbreviations

next-generation sequencing, NGS

severe acute respiratory syndrome, SARS

severe acute respiratory syndrome coronavirus 2, SARS-CoV-2

International Committee on the Taxonomy of Viruses, ICTV

real time polymerase chain reaction, RT-PCR

hydroxychloroquine, HCQ

chloroquine, CQ

nucleotide, nt

cytosine, C

adenine, A

guanine, G

thymine, T

open reading frame, ORF

bat coronavirus, BatCoV

receptor binding domain, RBD

angiotensin-converting enzyme 2, ACE2

Middle East respiratory syndrome, MERS

Global Initiative on Sharing All Influenza Data, GISAID

whole genome sequencing, WGS

Declarations

Ethics approval and consent to participate

This study was approved by the institutional review board of Advarra.

Consent to participate in this study from each patient was agreed via signed informed consent

Consent for publication

Agreed to by patients on the signed informed consent, demonstrating that the patient understood the procedures and the purpose of the study.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was funded by ProgenaBiome.

Authors' contributions

Conceptualization, AP and SH; Methodology, AP and SH; Investigation, AP and SH

Writing-original draft, AP; Writing-review and editing, AP, TB, SD, JD, SS, BB, and SH

Supervision, SH; Funding acquisition, SH. All authors read and approved the final manuscript.

Authors

Andreas Papoutsis¹, Thomas Borody², Siba Dolai², Jordan Daniels¹, Skylar Steinberg¹, Brad Barrows¹, Sabine Hazan,^{1,3}

Andreas Papoutsis, PhD

Thomas Borody MD, PhD

Siba Dolai, PhD

Jordan Daniels

Skylar Steinberg

Brad Barrows, DO

Sabine Hazan, MD

Author details

¹ ProgenaBiome, Ventura, CA

² Centre for Digestive Diseases, Sydney, Australia

³ Ventura Clinical Trials, Ventura, CA.

Acknowledgements

We thank Dr. Alan Miller at Alta Pharmaceuticals for contributing to providing patient samples.

References

1. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020; 395:497-506.
2. Zhou P, Yang X-L, Wang X-G, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 2020; 579:270-273.
3. Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. *N Engl J Med* 2020; 382:727-733.
4. Drosten C, Günther S, Preiser W, et al. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. *N Engl J Med* 2003; 348:1967-1976.
5. Ksiazek TG, Erdman D, Goldsmith CS, et al. A novel coronavirus associated with severe acute respiratory syndrome. *N Engl J Med* 2003; 348:1953-1966.
6. Zaki AM, van Boheemen S, Bestebroer TM, Osterhaus ADME, Fouchier RAM. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. *N Engl J Med* 2012; 367:1814-1820.
7. "Coronavirus." *World Health Organization*, World Health Organization, 15 July 2020, [who.int/emergencies/diseases/novel-coronavirus-2019](https://www.who.int/emergencies/diseases/novel-coronavirus-2019).
8. Johns Hopkins University. Coronavirus Resource Center. COVID-19 Dashboard by the Center for Systems Science and Engineering (CSSE) at Johns Hopkins University (JHU). 15 July 2020, www.coronavirus.jhu.edu/map.html.
9. Wang D, Hu B, Hu C. et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus_infected Pneumonia in Wuhan, China. *JAMA* 2020 Feb 07. doi: 10.1001/jama.2020.1585.
10. Tahamtan A, and Ardebili A. "Real-Time RT-PCR in COVID-19 Detection: Issues Affecting the Results." *Expert Review of Molecular Diagnostics*, vol. 20, no. 5, 2020, pp. 453–454., doi:10.1080/14737159.2020.1757437.
11. Gudbjartsson D, et al. "Spread of SARS-CoV-2 in the Icelandic Population." *New England Journal of Medicine*, vol. 382, no. 24, 11 June 2020, pp. 2302–2315., doi:10.1056/nejmoa2006100.
12. Wikramaratna P, et al. "Estimating False-Negative Detection Rate of SARS-CoV-2 by RT-PCR." *MedRxiv*, 14 Apr. 2020, doi:10.1101/2020.04.05.20053355.
13. Cohen A, and Kessel B. "False Positives in Reverse Transcription PCR Testing for SARS-CoV-2." 2020, doi:10.1101/2020.04.26.20080911.

14. Report of the WHO-China joint mission on coronavirus disease 2019 (COVID-19); <https://www.who.int/docs/default-source/coronaviruse/who-china-joint-mission-on-covid-19-final-report.pdf> 2020 2020
15. Kakodkar, Pramath, et al. "A Comprehensive Literature Review on the Clinical Presentation, and Management of the Pandemic Coronavirus Disease 2019 (COVID-19)." *Cureus*, 2020, doi:10.7759/cureus.7560.
16. Wang, Xin-Wei. "Excretion and Detection of SARS Coronavirus and Its Nucleic Acid from Digestive System." *World Journal of Gastroenterology*, vol. 11, no. 28, 2005, p. 4390., doi:10.3748/wjg.v11.i28.4390.
17. Parasa, Sravanthi, et al. "Prevalence of Gastrointestinal Symptoms and Fecal Viral Shedding in Patients With Coronavirus Disease 2019." *JAMA Network Open*, vol. 3, no. 6, 2020, doi:10.1001/jamanetworkopen.2020.11335.
18. Zhang B, Liu S, Dong Y, Zhang L, Zong Q, Zou Y, Zhang S. Positive rectal swabs in young patients recovered from coronavirus disease 2019 (COVID-19). *Journal of Infection*. April 20, 2020;13:37. doi:org/10.1016/j.jinf.2020.04.023.
19. Chen L, Lou J, Bai Y, et al. COVID-19 Disease With Positive Fecal and Negative Pharyngeal and Sputum Viral Tests. *Am J Gastroenterol* 2020 Mar 20. doi:10.14309/ajg.0000000000000610.
20. Weiss SR, Navas-Martin S. Coronavirus pathogenesis and the emerging pathogen severe acute respiratory syndrome coronavirus. *Microbiol Mol Biolo Rev*. 2005; 69: 635-64.
21. Scoeman D, Fielding BC. Coronavirus enveloped protein: current knowledge. *Virology*. 2019; 16: 69.
22. Zumla A, Chan J, Azhar, E. et al. Coronaviruses – drug discovery and therapeutic options. *Nat Rev Drug Discov* 15, 327–347 (2016). doi:org/10.1038/nrd.2015.37.
23. Zhao, Ling, et al. "Antagonism of the Interferon-Induced OAS-RNase L Pathway by Murine Coronavirus ns2 Protein Is Required for Virus Replication and Liver Pathology." *Cell Host & Microbe*, vol. 11, no. 6, 2012, pp. 607–616. doi:10.1016/j.chom.2012.04.011.
24. Belshaw R, et al. "The Evolution of Genome Compression and Genomic Novelty in RNA Viruses." *Genome Research*, vol. 17, no. 10, 2007, pp. 1496–1504., doi:10.1101/gr.6305707.
25. Fehr A, and Perlman S. "Coronaviruses: An Overview of Their Replication and Pathogenesis." *Coronaviruses Methods in Molecular Biology*, 2015, pp. 1–23., doi:10.1007/978-1-4939-2438-7_1.
26. Li, Fang. "Structure, Function, and Evolution of Coronavirus Spike Proteins." *Annual Review of Virology*, vol. 3, no. 1, 2016, pp. 237–261. doi:10.1146/annurev-virology.110615-042301.
27. Shang, Jian, et al. "Structural Basis of Receptor Recognition by SARS-CoV-2." *Nature*, vol. 581, no. 7807, 2020, pp. 221–224., doi:10.1038/s41586-020-2179-y.
28. Yang N, Shen H. Targeting the Endocytic Pathway and Autophagy Process as a Novel Therapeutic Strategy in COVID-19. *Int J. Biological Sciences*. 2020; 16(10) 1724-1731. doi:10.7150/ijbs.45498.
29. Degtyarev M, De Maziere A, Orr C, Lin J, Lee BB, Tien JY, et al. Akt inhibition promotes autophagy and sensitizes PTEN-null tumors to lysosomotropic agents. *J Cell Biol*. 2008; 183: 101-16
30. Wang M, Cao R, Zhang L, Yang X, Liu J, Xu M, et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. *Cell res*. 2020.
31. Arshad S, Kilgore P, Chaudhry Z, et al. Treatment with Hydroxychloroquine, Azithromycin, and Combination in Patients Hospitalized with COVID-19. *International Journal of Infectious Disease*. doi.org/10.1016/j.ijid.2020.06.099.
32. Lagier J-C, Matthieu M, Gautret P, Colson P, Cortaredona S, et al. Outcomes of 3,737 COVID-19 patients treated with hydroxychloroquine/azithromycin and other regimens in Marseille, France: A retrospective analysis. *Travel Medicine and Infectious Disease*. doi:org/10.1016/j.tmaid.2020.101791.
33. Gao J, Tian Z, Yang X. Breakthrough: Chloroquine phosphate has shown apparent efficacy in treatment of COVID-19 associated pneumonia in clinical studies. *Biosci Trends*. 2020;14(1):72–73.
34. Gautret P, Lagier JC, Parola P, et al. Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. *Int J Antimicrob Agents* 2020;105949.

35. Million M, Lagier JC, Gautret P, et al. Early treatment of COVID-19 patients with hydroxychloroquine and azithromycin: A retrospective analysis of 1061 cases in Marseille, 29 France. *Travel Med Infect Dis.* 2020 May 5:101738. doi: 10.1016/j.tmaid.2020.101738.
36. Yu B, Wang DW, Li C. Hydroxychloroquine application is associated with a decreased mortality in critically ill patients with COVID-19. medRxiv 2020. doi:org/10.1101/2020.04.27.20073379
37. Chen Z, Hu J, Zhang Z, Jiang S, Han S, Yan D, Zhuang R, Hu B, Zhang Z. 2020. Efficacy of hydroxychloroquine in patients with COVID-19: results of a randomized clinical trial. medRxiv 03.22.20040758; doi:org/10.1101/2020.03.22.20040758.
38. Huang M, Tang T, Pang P, Li M, Ma R, Lu J, Shu J, You Y, Chen B, Liang J, Hong Z, Chen H, Kong L, Qin D, Pei D, Xia J, Jiang S, Shan H. Treating COVID-19 with Chloroquine. 2020. *J Mol Cell Biol.* doi:org/10.1093/jmcb/mjaa014.
39. Geleris J, Sun Y, Platt J, et al. Observational Study of Hydroxychloroquine in Hospitalized Patients with Covid-19. *N Engl J Med.* 2020 May 7.
40. Chen Y, et al. "The Presence of SARS-CoV-2 RNA in the Feces of COVID-19 Patients." *Journal of Medical Virology*, vol. 92, no. 7, 2020, pp. 833–840., doi:10.1002/jmv.25825.
41. Wu Y, et al. "Prolonged Presence of SARS-CoV-2 Viral RNA in Fecal Samples." *The Lancet Gastroenterology & Hepatology*, vol. 5, no. 5, 2020, pp. 434–435., doi:10.1016/s2468-1253(20)30083-2.

Figures

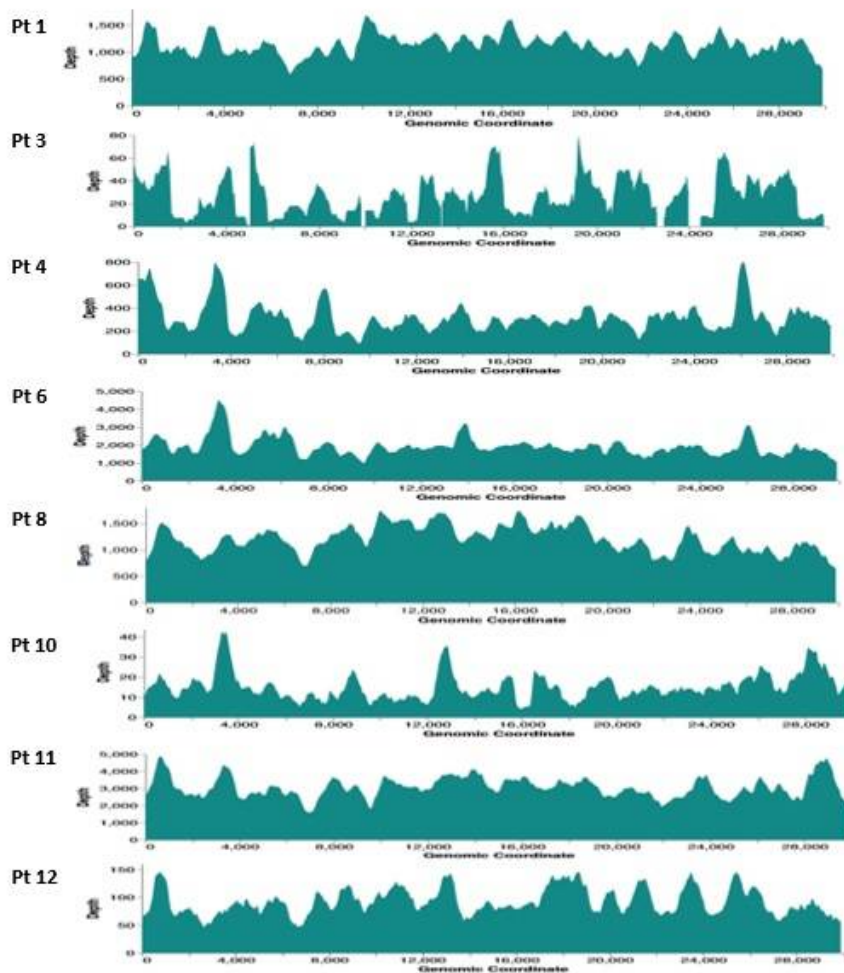


Figure 1

Whole genome alignment of SARS-CoV-2 in patients (Pt). The x-axis depicts the genomic coordinates as aligned to the MN908947.3 reference genome, and the y-axis represents the read depth at specific loci.