Poster Number 1064
Phone Number: 216-218-4845
E-mail: elhaddk@ccf.org

Intra-Host Mutation Rate and Diversity of SARS-CoV-2

El Haddad K[1], Adhikari T[2], Tu ZJ[3], Cheng Y[3], Leng X[2], Zhang X[2], Rhoads D[3], Ko J[3], Worley S[4], Rubin B[3], Li J[2], Esper F[1]

[1]Center for Pediatric Infectious Disease, [3]Robert J. Tomsich Pathology and Laboratory Medicine Institute, [4]Department of Quantitative Health Sciences, Cleveland Clinic, Cleveland, OH
[2]Department of Computer and Data Sciences, Case Western Reserve University, Cleveland, OH

Background & Aims

Our understanding of SARS-CoV-2 evolution is limited. Most estimates arise from analysis of global databases populated with unrelated sequences and have ranged from 21.6- 29 substitutions/genome/year over the course of the pandemic. SARS-CoV-2 polymerase contains a proofreading function encoded by NSP-14 limiting change. Additionally, virus evolution may be influenced by patient comorbidity. Intra-host mutational rate during infection remains poorly studied.

Aims: Determine the intra-host mutational rate during the course of infection.

a. Compare mutation rates between isolates with and without mutation in NSP-14

b. Compare mutation rates among patients with different comorbidities

Material and Methods

Inclusion Criteria

Adults with >1 positive SARS-CoV-2 NP sample collected 5 - 60 days apart from 3/17/2020 to 5/27/2020

Exclusion Criteria

Patient paired samples identified with discordant lineage/ clades suggesting re-infection, pairs with CT ≥ 30 and pairs with interval days < 5 or > 60.

Criteria for Sequencing and Mutation Calling

- Viral Genome analysis by MiSEQ
- Map to the reference Wuhan-Hu-1 (NC_045512.2)
- Mutation Calling: Depth ≥ 10, Allele Frequency(AF) ≥ 0.1, AF ≥ 0.5, AF≥ 0.75 and manual review
- Mutation changes at corresponding AF identified in initial and subsequent pairs and quantified.

Calculation of Mutation Rates: F81 and JC69 modeling

Both models assume equal mutation rates across different nucleotides allowing for a smaller number of model parameters.

For both models, mutation rates were estimated by the use of maximum likelihood algorithms.

- JC69 assumes equal base frequencies
- F81 allows for variable base frequencies with equal substitutions providing a more realistic calculation of the mutation rate.

Calculate and Compare mutation rates between isolates with (Δ NSP-14) and without (wtNSP-14) mutation in NSP-14

Calculate and compare mutation rates among patients by age and different comorbidities.

- **Pulmonary, Cardiac, Endocrine, Immune**

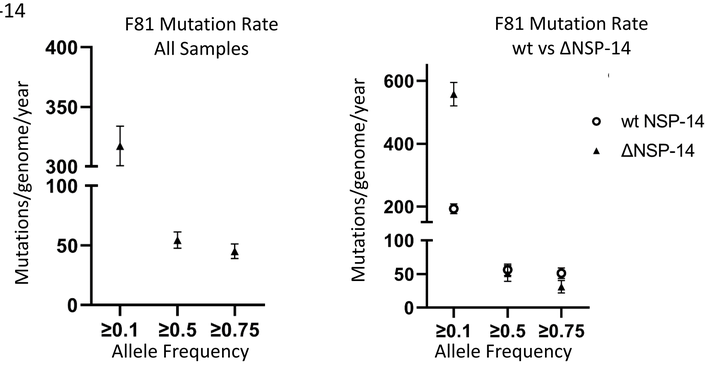
Results

Table 1. Patient Demographics of Paired SARS-CoV-2 Isolates

	Total	wt NSP-14	Δ NSP-14	P-value
Total pairs	40	27 (67.5%)	13 (32.5%)	
Median interval (d) [IQR]	13[8.3-19.8]	13 [8- 21]	13 [8.5-19.5]	0.93[a]
Demographics				
Median Age (yr) [IQR]	54 [30.3-65.8]	65 [30-69]	51 [31-61.5]	0.51[a]
Males	20 (50.0%)	13 (48.1%)	7 (53.8%)	0.74[b]
Race				
White	26 (65.0%)	15 (55.6%)	11 (84.6%)	0.029[c]
African American	10 (25.0%)	8 (29.6%)	2 (15.4%)	
Others	4 (10.0%)	4 (14.8%)	0 (0%)	
Comorbidity				
Any	28 (70%)	18 (66.7%)	10 (77.9%)	0.72[c]
Endocrine	23 (57.5%)	14 (51.9%)	9 (69.2%)	0.30[b]
Cardiac	17 (42.5%)	11 (40.7%)	6 (46.2%)	0.75[b]
Pulmonary	8 (20.0%)	5 (18.5%)	3 (23.1%)	0.99[c]
Immune/Oncologic	6 (15.0%)	5 (18.5%)	1 (7.7%)	0.64[c]

p-values: a=Wilcoxon rank sum test, b=Pearson's chi-square test, c=Fisher's Exact test.

Figure 1. F81 Mutation Modeling by Allele Frequency with and without Alteration in NSP-14

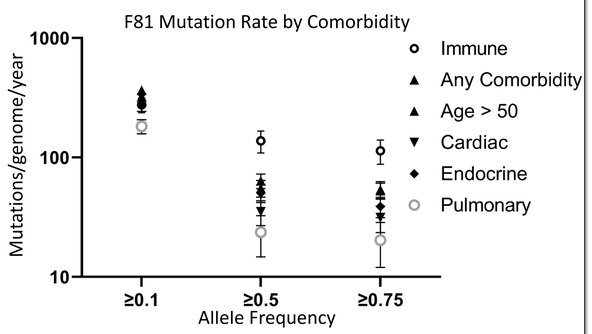


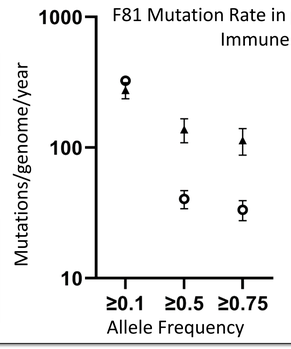
	Total (n=40)	wt NSP-14 (n= 27)	ΔNSP-14 (n=13)	
	Mutation rate (Subs/genome/year) [95% CI]	**Mutation rate (Subs/genome/year) [95% CI]**	**Mutation rate (Subs/genome/year) [95% CI]**	**P- value**
AF ≥0.1	317.2 [312.0-322.3]	193.1 [187.1-199.1]	557.7 [537.2-578.2]	<0.001
AF ≥0.5	54.6 [52.5-56.7]	56.3 [53.1-59.4]	50.8 [44.3-57.3]	0.144
AF ≥0.75	45.1 [43.1-47.0]	51.3 [48.3-54.3]	31.0 [25.9-36.1]	<0.001

Results

Figure 2. F81 Mutation Modeling by Comorbidity

	Allele Frequency	Mutation rate (Mutations/genome/yr)	95% CI
Total (n=40)	AF ≥0.1	317.2	[312.0-322.3]
	AF ≥0.5	54.6	[52.5-56.7]
	AF ≥0.75	45.1	[43.1-47.0]
Age > 50 (n=23)	AF ≥0.1	324.0	[315.0-333.0]
	AF ≥0.5	55.3	[51.7-59.0]
	AF ≥0.75	54.0	[50.3-57.5]
Any Comorbidity (n=28)	AF ≥0.1	365.4	[357.5-373.2]
	AF ≥0.5	63.7	[60.5-67.0]
	AF ≥0.75	52.7	[49.7-55.7]
Endocrine (n=23)	AF≥ 0.1	341.1	[332.1-350.2]
	AF≥ 0.5	50.4	[46.9-53.8]
	AF ≥0.75	38.9	[35.8-41.9]
Cardiac (n=17)	AF≥ 0.1	291.3	[279.9-302.6]
	AF ≥0.5	35.2	[31.2-39.1]
	AF ≥0.75	31.3	[27.6-35.0]
Pulmonary (n=8)	AF≥ 0.1	182.4	[165.2-199.6]
	AF≥ 0.5	23.6	[17.4-29.8]
	AF ≥0.75	20.3	[14.5-26.0]
Immune/Oncologic (n=6)	AF≥ 0.1	275.9	[244.6-307.1]
	AF≥ 0.5	137.6	[114.6-160.5]
	AF ≥0.75	113.7	[92.8-134.5]





Conclusion

- Intra-host mutation rate is over 2 times greater at AF ≥0.75 (WHO standard), 2.5 times higher at AF ≥0.5 and nearly 15 times higher at AF ≥0.1 than those reported through population based surveillance.
- Mutation changes in NSP-14 were found to have significantly elevated mutation rate only at low AF but significantly depressed at high AF.
- Enhanced mutation rates occurred in immunocompromised patients at high AF, while mutation rates in patients with underlying cardiac, pulmonary or endocrine comorbidities were not significantly elevated.
- SARS-CoV-2 intra-host dynamics have crucial implications on current and future pandemic planning, development of vaccines, and antiviral therapy.