Tracking Changes in SARS-CoV-2 Spike: Evidence that D614G Increases Infectivity of the COVID19 Virus

Korber, B., Fischer, W. M., Gnanakaran, S., Yoon, H., Theiler, J., Abfalterer, W., ... & Hastie, K. M. (2020). Tracking changes in SARS-CoV-2 Spike: evidence that D614G increases infectivity of the COVID-19 virus. Cell, 182(4), 812-827.

LMU Seaver

Nathan Beshai, Owen Dailey, Kam Taghizadeh, Ian Wright

BIOL 368: Bioinformatics Lab

November 19th, 2020

LOYOLA MARYMOUNT UNIVERSITY · FOUNDED 1911 ·

Outline

1. Mutations in the SARS-CoV-2 Genetic Sequence Can Alter the Effectiveness of Vaccines and Therapeutics
2. GISAID's SARS-CoV-2 Sequences Were Regionally Segmented and Filtered for Complete Sequence
3. There were Multiple Spike Sites and Mutations of Interest, but the D614G Mutation had the Highest Global Frequency
4. The D614G Mutation Arose Independently in Different Regions of the World
5. G614 frequencies in a variety of geographic locations has significantly increased
6. G614 Mutation Alters Interactions Between S1 and S2 Subunits of Separate Spike Protomers
7. G614 Mutants Grow to a Higher Concentration in Cell Cultures and a Higher Viral Load *in vivo* than their D614 Counterparts
8. Some outliers to the G614 variant frequency trend do exist
9. In trimmed sequences, about half of the sequence produce a variant

Outline

1. **Mutations in the SARS-CoV-2 Genetic Sequence Can Alter the Effectiveness of Vaccines and Therapeutics**
2. GISAID's SARS-CoV-2 Sequences Were Separated Into Regional Alignments for Validation
3. There were Multiple Spike Sites and Mutations of Interest, but the D614G Mutation had the Highest Global Frequency
4. The D614G Mutation Arose Independently in Different Regions of the World
5. G614 frequencies in a variety of geographic locations has significantly increased.
6. G614 Mutation Alters Interactions Between S1 and S2 Subunits of Separate Spike Protomers
7. G614 Mutants Grow to a Higher Concentration in Cell Cultures and a Higher Viral Load *in vivo* than their D614 Counterparts
8. Some outliers to the G614 variant frequency trend do exist.
9. In trimmed sequences, about half of the sequence produce a variant.

Mutations in the SARS-CoV-2 Genetic Sequence Can Alter the Effectiveness of Vaccines and Therapeutics

- SARS-CoV-2 has genetic proofreading mechanisms
- The long duration of the pandemic is giving SARS-CoV-2 time to mutate
    - Antigenic drift is exhibited by Coronavirus OC43, Coronavirus 229E, and SARS-CoV-1
- “Most current SARS-CoV-2 immunogens and testing reagents are based on the Spike protein sequence of the Wuhan reference sequence” (Wang et al., 2020)
- Korber et al. created a bioinformatics database that tracked genetic variance in SARS-CoV-2 spike sequences in the GISAID database
    - If 0.3% of the sequences exhibited a mutation when compared to the Wuhan reference sequence, the lab began monitoring it
- By April of 2020, one mutation was exhibiting a rapid increase in frequency: D614G

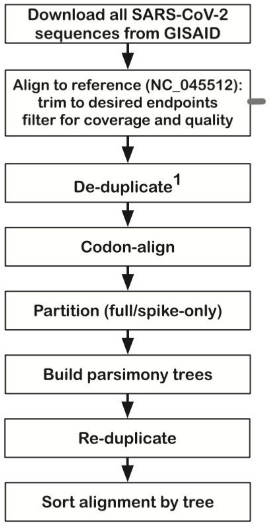
Outline

1. Mutations in the SARS-CoV-2 Genetic Sequence Can Alter the Effectiveness of Vaccines and Therapeutics
2. **GISAID's SARS-CoV-2 Sequences Were Separated Into Regional Alignments for Validation**
3. There were Multiple Spike Sites and Mutations of Interest, but the D614G Mutation had the Highest Global Frequency
4. The D614G Mutation Arose Independently in Different Regions of the World
5. G614 frequencies in a variety of geographic locations has significantly increased.
6. G614 Mutation Alters Interactions Between S1 and S2 Subunits of Separate Spike Protomers
7. G614 Mutants Grow to a Higher Concentration in Cell Cultures and a Higher Viral Load *in vivo* than their D614 Counterparts
8. Some outliers to the G614 variant frequency trend do exist.
9. In trimmed sequences, about half of the sequence produce a variant.

GISAID’s SARS-CoV-2 Sequences Were Separated Into Regional Subalignments for Validation

Sequence Processing Pipeline

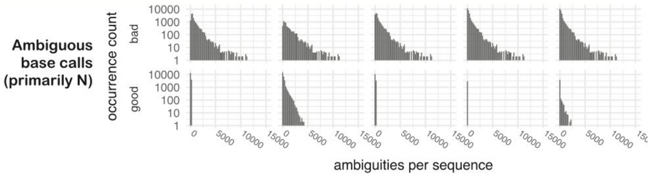
A



B

Sequence filtering results

category	CODING REGIONS[2]	SPIKE	NEAR-COMPLETE	COMPLETE	5' UTR
"good"	17222	28576	14105	3019	4228
"bad"	17172	6382	19607	28984	27886
5' end gaps	73	160	437	2321	26733
3' end gaps	142	35	1546	624	9
5' end gaps + 3' end gaps	148	12	733	9391	125
fragmented matching	15091	4541	15115	15132	6
Excess "N" stretches	323	400	270	9	6
Excess mismatches (>30)	1323	1065	1159	57	10
5' end gaps + mismatches	48	33	114	1229	72
3' end gaps + mismatches	21	11	174	51	21
5' + 3' end gaps + mismatches	0	0	57	0	5
No matching detected	3	122	2	2	920



Notes:
1. Multiply occurring identical sequences are reduced to 2 occurrences to so that parsimony-informative sites do not become unique.
2. "Coding regions" subset includes sequences passing error filtering, bounded from the orf1ab start codon to the ORF10 stop codon (NC_045512 genome positions 266-29674).

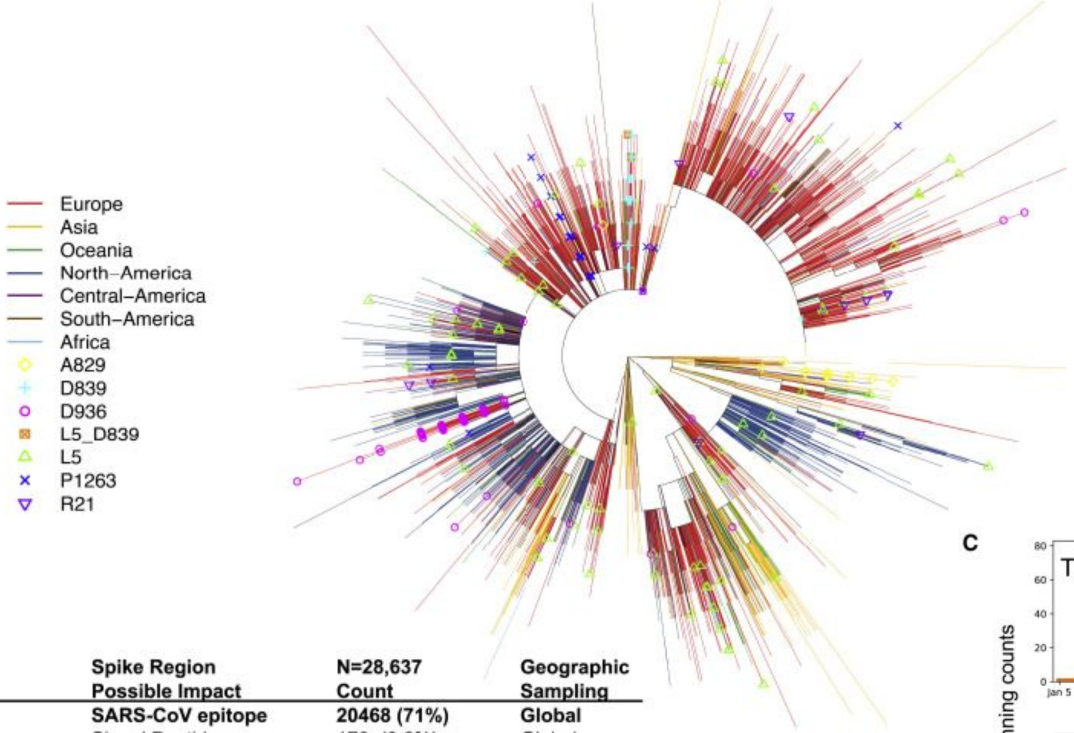
- **For 5 regions of the viral genome, a collection of contiguous sequences was formed**
- **Alignments then created from the ‘good’ sequences**
- **In general, the smaller the region, the more sequences included in alignment analysis**

Outline

1. Mutations in the SARS-CoV-2 Genetic Sequence Can Alter the Effectiveness of Vaccines and Therapeutics
2. GISAID's SARS-CoV-2 Sequences Were Separated Into Regional Alignments for Validation
3. **There were Multiple Spike Sites and Mutations of Interest, but the D614G Mutation had the Highest Global Frequency**
4. The D614G Mutation Arose Independently in Different Regions of the World
5. G614 frequencies in a variety of geographic locations has significantly increased.
6. G614 Mutation Alters Interactions Between S1 and S2 Subunits of Separate Spike Protomers
7. G614 Mutants Grow to a Higher Concentration in Cell Cultures and a Higher Viral Load *in vivo* than their D614 Counterparts
8. Some outliers to the G614 variant frequency trend do exist.
9. In trimmed sequences, about half of the sequence produce a variant.

There were Multiple Spike Sites and Mutations of Interest, but the D614G Mutation had the Highest Global Frequency

A

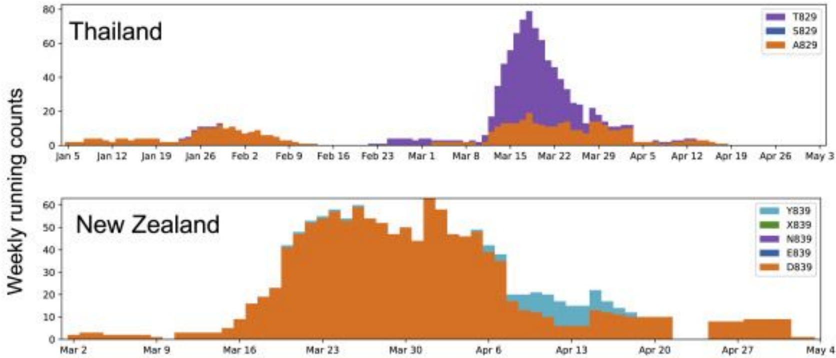


- **17,760 D614G mutations were found in the GISAID database by June 2, 2020.**
- **Many mutations were contained to one geographic region, but D614G was a global mutation**

B

Spike Mutation	Spike Region Possible Impact	N=28,637 Count	Geographic Sampling
D614G	**SARS-CoV epitope**	**20468 (71%)**	**Global**
L5F	Signal Peptide	170 (0.6%)	Global
R21I/K/T	S1 NTD domain	133 (0.5%)	Wales, England, others
A829T/S	Fusion Peptide	91 (0.3%)	Thailand
D839Y/N/E	Fusion Peptide	149 (0.5%)	Portugal, New Zealand, others
D936Y/H	Heptad Repeat 1(HR1)	257 (0.9%)	Sweden, UK, others
P1263L	Cytoplasmic Tail	201 (0.7%)	UK, others

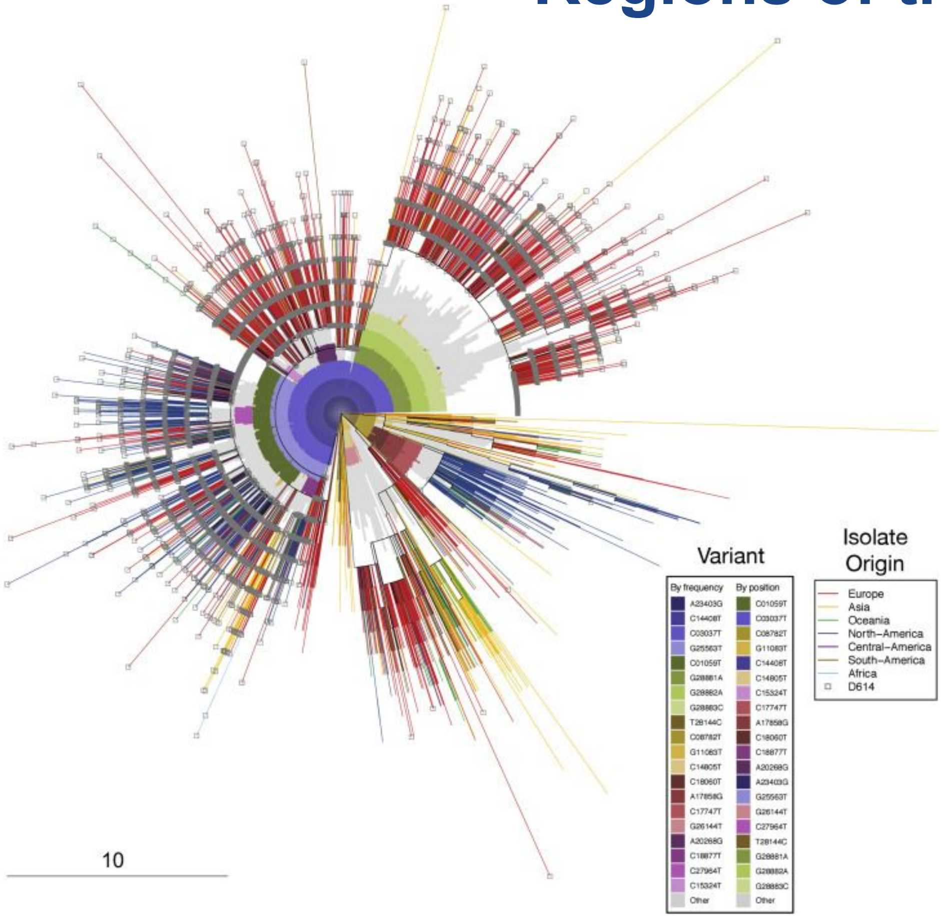
C



Outline

1. Mutations in the SARS-CoV-2 Genetic Sequence Can Alter the Effectiveness of Vaccines and Therapeutics
2. GISAID's SARS-CoV-2 Sequences Were Separated Into Regional Alignments for Validation
3. There were Multiple Spike Sites and Mutations of Interest, but the D614G Mutation had the Highest Global Frequency
4. **The D614G Mutation Arose Independently in Different Regions of the World**
5. G614 frequencies in a variety of geographic locations has significantly increased.
6. G614 Mutation Alters Interactions Between S1 and S2 Subunits of Separate Spike Protomers
7. G614 Mutants Grow to a Higher Concentration in Cell Cultures and a Higher Viral Load *in vivo* than their D614 Counterparts
8. Some outliers to the G614 variant frequency trend do exist.
9. In trimmed sequences, about half of the sequence produce a variant.

The D614G Mutation Arose Independently in Different Regions of the Worlds



- **There are a large number of sequences from Europe.**
- **The Order of the D614 to G614 Transition: Europe -> North America -> Oceania -> Asia**

Outline

1. Mutations in the SARS-CoV-2 Genetic Sequence Can Alter the Effectiveness of Vaccines and Therapeutics
2. There were Multiple Spike Sites and Mutations of Interest, but the D614G Mutation had the Highest Global Frequency
3. The D614G Mutation Arose Independently in Different Regions of the World
4. **G614 frequencies in a variety of geographic locations has significantly increased.**
5. G614 Mutation Alters Interactions Between S1 and S2 Subunits of Separate Spike Protomers
6. G614 Mutants Grow to a Higher Concentration in Cell Cultures and a Higher Viral Load *in vivo* than their D614 Counterparts
7. Some outliers to the G614 variant frequency trend do exist.
8. In trimmed sequences, about half of the sequence produce a variant.

Two One Sided T-Tests Show that the Increasing of G614 is Highly Significant with a Staircase line showing the G614 Daily Frequency Increasing in Cities.

A

GISAID level 3: Subcounty/State

Of 31 regions with a clear direction, 30 are increasing: Binomial p = 2.98e-08

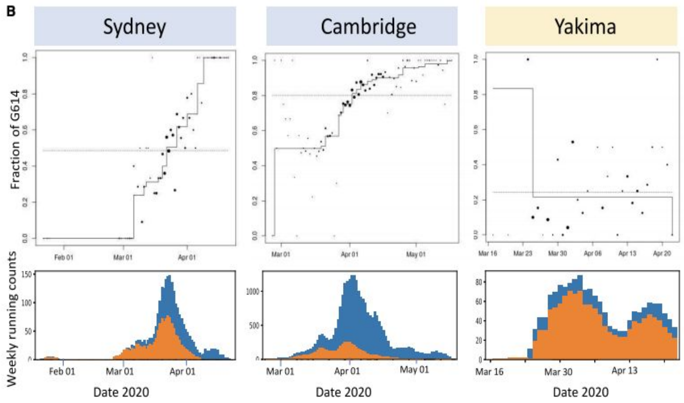
Level 2: Region	# D614	# G614	# of days	Time window days	G614 increasing p-value	G614 decreasing p-value
Australia_New-South-Wales	189	180	51	90	0.00025	0.99
Australia_Victoria	226	306	43	80	0.00025	0.93
Belgium_Ghent	12	37	18	26	0.00025	0.77
China_Beijing	46	25	35	66	0.00025	0.98
Germany_North-Rhine-Westphalia	16	37	16	27	0.00025	0.91
Spain_Comunitat-Valenciana	73	110	32	34	0.00025	0.95
Taiwan_Taoyuan	14	12	16	85	0.00025	0.51
United-Kingdom_England	1904	6338	88	109	0.00025	1.00
United-Kingdom_Scotland	433	1125	73	75	0.00025	0.68
United-Kingdom_Wales	717	1792	45	54	0.00025	0.95
USA_Arizona	11	64	22	71	0.00025	0.99
USA_California	267	199	70	111	0.00025	0.001*
*USA_California excluding Santa Clara	102	175	53	107	0.00025	0.99
USA_Michigan	31	382	38	53	0.00025	0.85
USA_New-York	91	1163	52	55	0.00025	1.00
USA_Utah	24	253	38	45	0.00025	0.98
USA_Virginia	27	220	43	47	0.00025	0.99
USA_Washington	926	683	69	101	0.00025	1.00
USA_Wisconsin	156	197	49	93	0.00025	0.07
India_Maharashtra	30	35	30	48	0.0005	0.97
Thailand_Bangkok	26	12	19	70	0.0005	0.72
Chile_Santiago	19	63	27	34	0.00075	0.99
USA_Illinois	38	56	26	88	0.00075	0.98
United-Kingdom_Northern-Ireland	47	60	22	27	0.00325	0.42
Australia_South-Australia	15	58	23	32	0.0035	0.84
USA_Minnesota	51	96	35	47	0.004	0.76
Taiwan_Taipei	17	26	26	94	0.0053	0.45
Australia_Queensland	12	13	14	58	0.0058	0.96
USA_Florida	11	35	18	69	0.009	0.82
USA_Connecticut	22	127	34	71	0.0095	0.93
Belgium_Liege	19	140	24	44	0.016	0.47
Denmark_Unknown	32	493	34	34	0.026	0.56
Canada_Ontario	26	32	15	57	0.062	0.91
India_Gujarat	10	110	22	36	0.085	0.08
Austria_Vienna	10	108	33	41	0.12	0.53
Spain_Madrid	20	56	16	33	0.15	0.89
Netherlands_Noord-Brabant	44	32	18	23	0.21	0.64
USA_Texas	75	169	29	53	0.26	0.72
Netherlands_Zuid-Holland	15	75	23	29	0.28	0.69
Netherlands_Utrecht	18	52	31	55	0.30	0.54
India_Delhi	24	13	16	32	0.93	0.00075

GISAID level 4: County/City

Of 19 cities with a clear direction, 17 are increasing: Binomial p = 0.0007

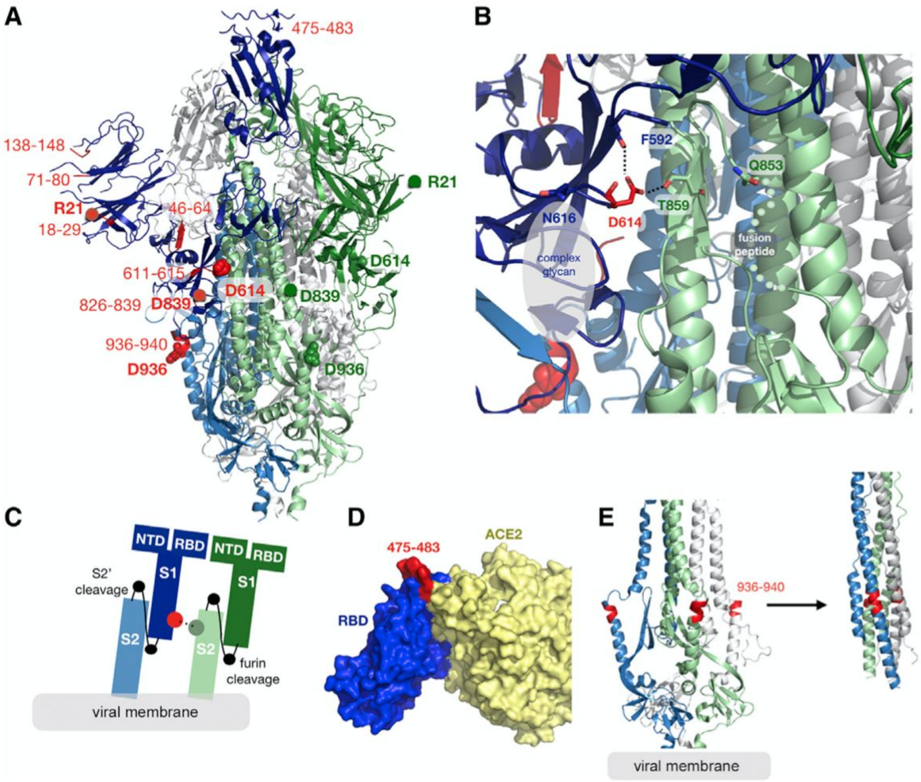
Level 3: County/City	# D614	# G614	# of days	Time window days	G614 increasing p value	G614 decreasing p-value
Australia_New-South-Wales_Sydney	189	179	51	90	0.00025	1.00
Spain_Comunitat-Valenciana_Valencia	72	97	30	34	0.00025	0.64
United-Kingdom_England_Bristol	240	629	35	37	0.00025	0.28
United-Kingdom_England_Cambridge	751	3020	81	81	0.00025	1.00
United-Kingdom_England_Liverpool	97	484	46	45	0.00025	0.71
United-Kingdom_England_Nottingham	204	386	67	76	0.00025	0.99
United-Kingdom_England_Sheffield	120	431	44	51	0.00025	1.00
USA_Washington_King	173	75	58	69	0.00025	0.99
USA_Washington_Pierce	32	35	21	38	0.00025	1.00
USA_Washington_Snohomish	35	32	27	93	0.00025	1.00
USA_Wisconsin_Milwaukee	66	30	32	45	0.00025	0.97
United-Kingdom_England_Norwich	29	269	26	28	0.00075	0.97
USA_California_San-Diego	11	75	33	58	0.002	0.95
United-Kingdom_England_London	36	357	19	24	0.0085	0.91
USA_Wisconsin_Madison	13	43	26	35	0.030	0.39
USA_New-York_Manhattan	38	339	30	45	0.036	0.90
USA_California_San-Francisco	59	83	21	48	0.049	0.34
USA_New-York_Brooklyn	13	292	31	46	0.070	0.87
USA_Washington_Yakima	184	59	31	36	0.073	0.00025
USA_California_Santa-Clara	165	24	50	76	0.49	0.00025

B



- Significant increasing G-614 p-values highlighted in blue.
- The daily fraction of G614 increases in Sydney and Cambridge but maintains relatively low in Yakima.

Spike D614G Mutation Has Structural Implications in the Interactions Between Spike Subunits

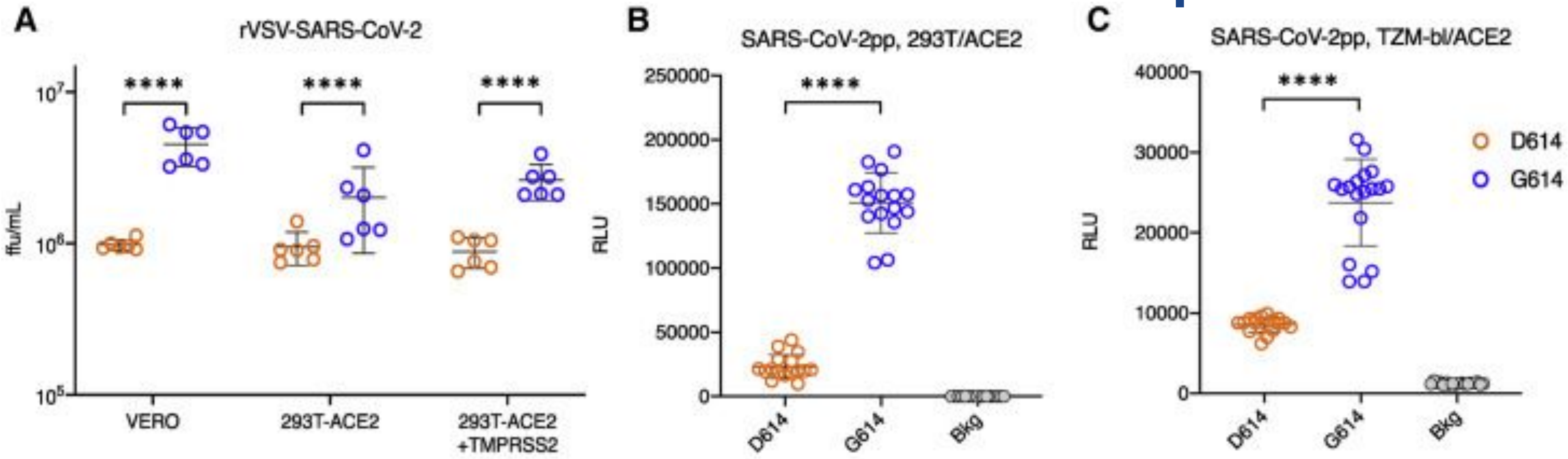


- **D614 forms hydrogen bond with T859 connecting the S1 and S2 subunits of two protomers**
- **The G614 mutation removes this H-bond which leads to increased mainchain flexibility and alters interactions between protomers**

Outline

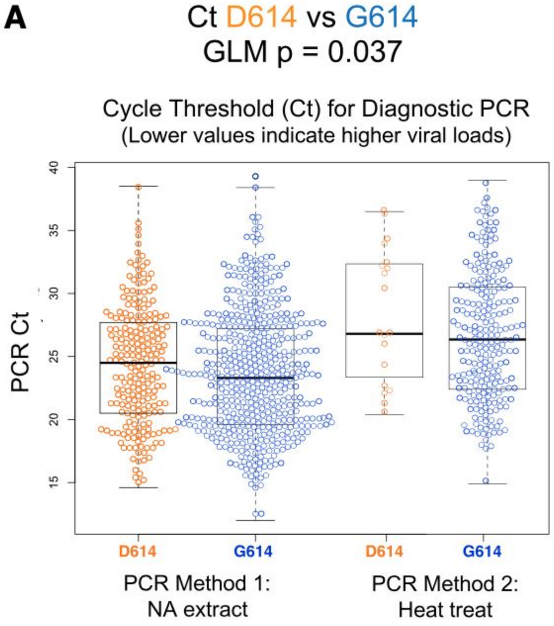
1. Mutations in the SARS-CoV-2 Genetic Sequence Can Alter the Effectiveness of Vaccines and Therapeutics
2. GISAID's SARS-CoV-2 Sequences Were Separated Into Regional Alignments for Validation
3. There were Multiple Spike Sites and Mutations of Interest, but the D614G Mutation had the Highest Global Frequency
4. The D614G Mutation Arose Independently in Different Regions of the World
5. G614 frequencies in a variety of geographic locations has significantly increased.
6. G614 Mutation Alters Interactions Between S1 and S2 Subunits of Separate Spike Protomers
7. **G614 Mutants Grow to a Higher Concentration in Cell Cultures and a Higher Viral Load *in vivo* than their D614 Counterparts**
8. The D614G distribution has increased across the globe throughout time.
9. Some outliers to the G614 variant frequency trend do exist.
10. In trimmed sequences, about half of the sequence produce a variant.

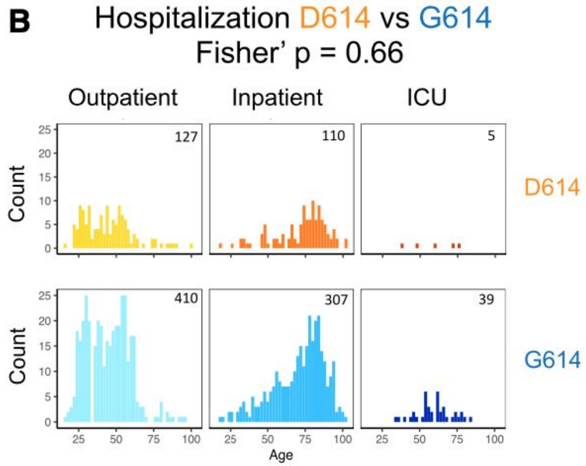
G614 Mutants Grow to a Higher Concentration in Cell Cultures than their D614 Counterparts



- This higher titer for the G614 SARS-CoV-2 strains suggests that G614 mutants are more infectious than D614 mutants.
- There is no difference in the way D614 and G614 interact with TMPRSS2.
- Is there an increased concentration of G614 mutants in lung cells?

Clinical Studies Reveal Higher Viral Loads in G614 Patients But No Change In Disease Severity



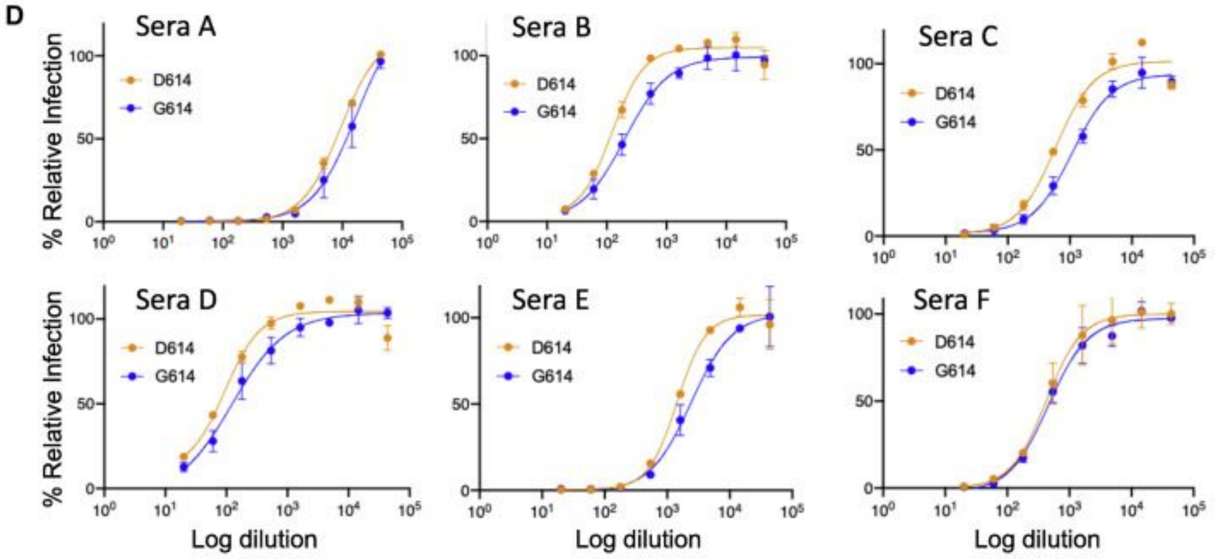


	OP	IP	ICU
D614	127	110	5
G614	410	307	39

Fisher's exact, 2x2: (OP+IP) x ICU = 0.047
Fisher's exact, 2x2, OP x (IP+ICU): p = 0.66

- **Lower Cycle threshold (Ct) in PCR means higher viral load**
- **Hospitalization status was used as disease severity indicator**
- **Neither severity nor age of hospitalized patients changes between D614 and G614**

Despite Being More Infectious, G614 Mutants are Not More Resistant to Polyclonal Antibodies than their D614 Counterparts

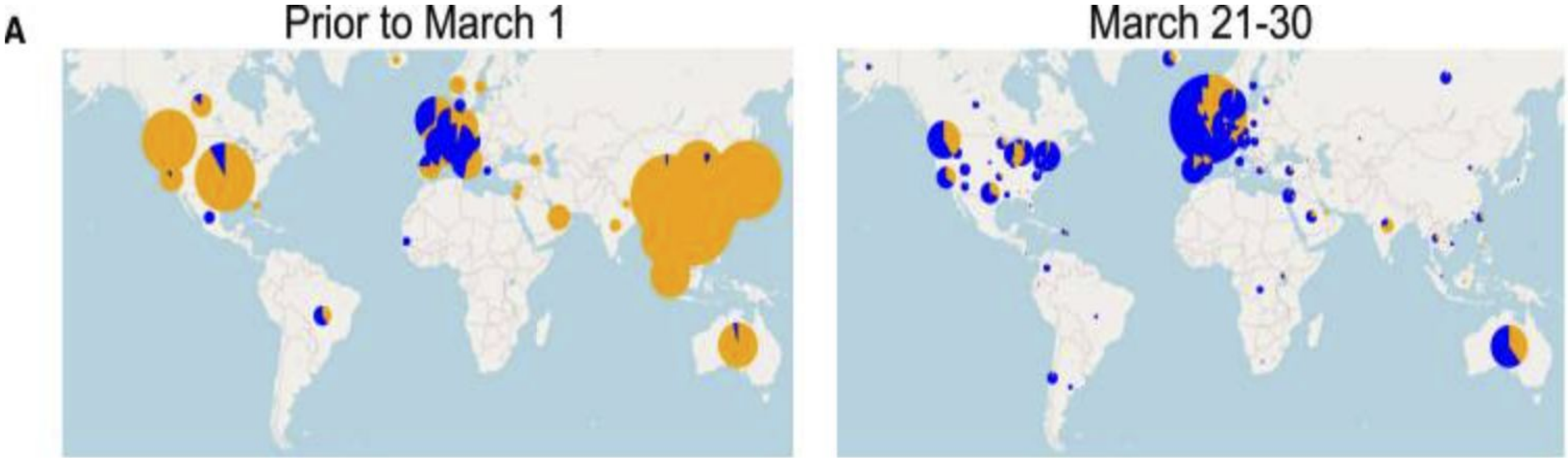


- The convalescent serum was collected from six people in San Diego.
- It is uncertain what form of the virus they were infected with.
- "G614 is associated with potentially higher viral loads in COVID-19 patients but not with disease severity" (Korber et al., 2020)

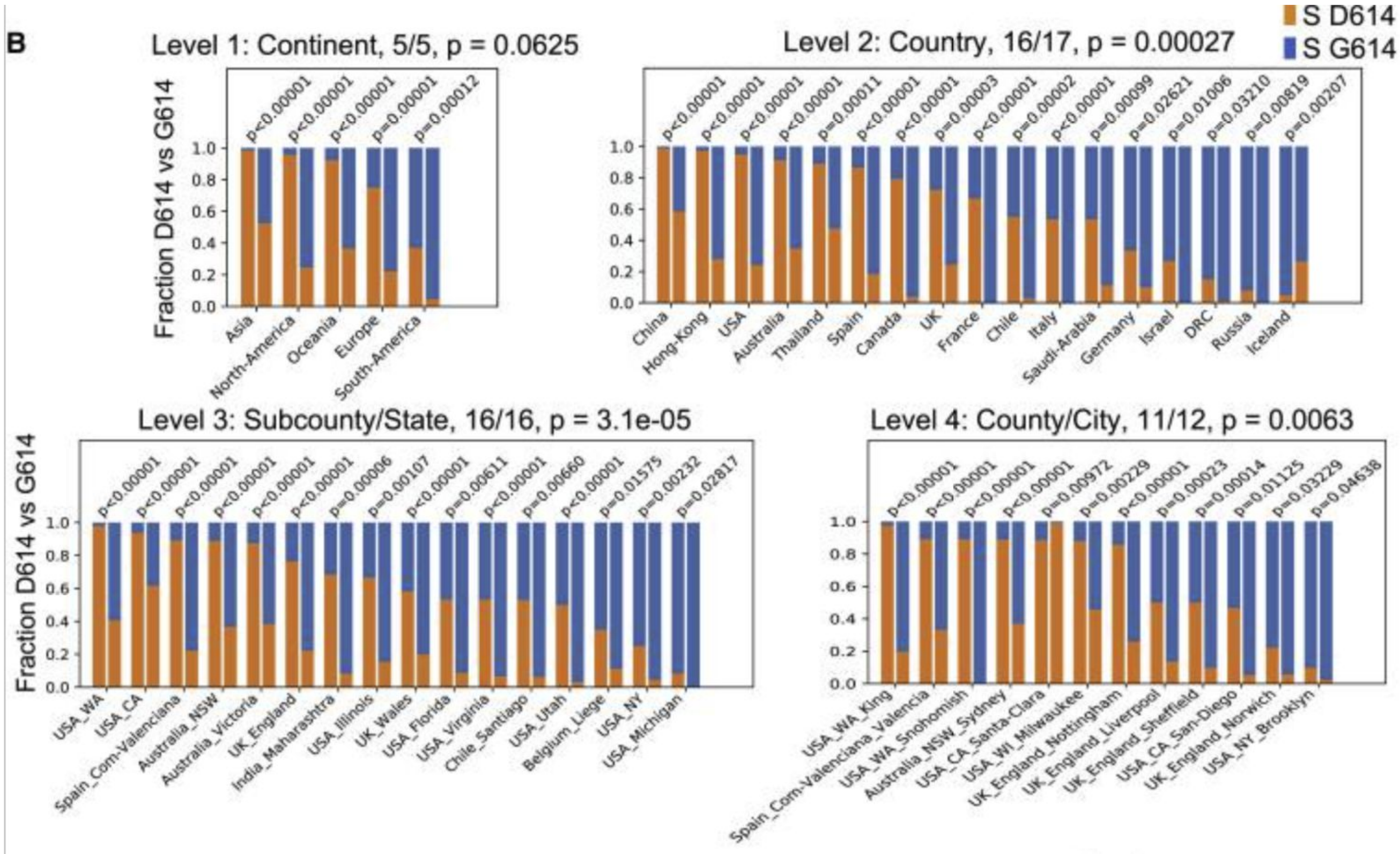
Outline

1. Mutations in the SARS-CoV-2 Genetic Sequence Can Alter the Effectiveness of Vaccines and Therapeutics
2. GISAID's SARS-CoV-2 Sequences Were Separated Into Regional Alignments for Validation
3. There were Multiple Spike Sites and Mutations of Interest, but the D614G Mutation had the Highest Global Frequency
4. The D614G Mutation Arose Independently in Different Regions of the World
5. G614 frequencies in a variety of geographic locations has significantly increased.
6. G614 Mutation Alters Interactions Between S1 and S2 Subunits of Separate Spike Protomers
7. G614 Mutants Grow to a Higher Concentration in Cell Cultures and a Higher Viral Load *in vivo* than their D614 Counterparts
8. **The D614G distribution has increased across the globe throughout time.**
9. Some outliers to the G614 variant frequency trend do exist.
10. In trimmed sequences, about half of the sequence produce a variant.

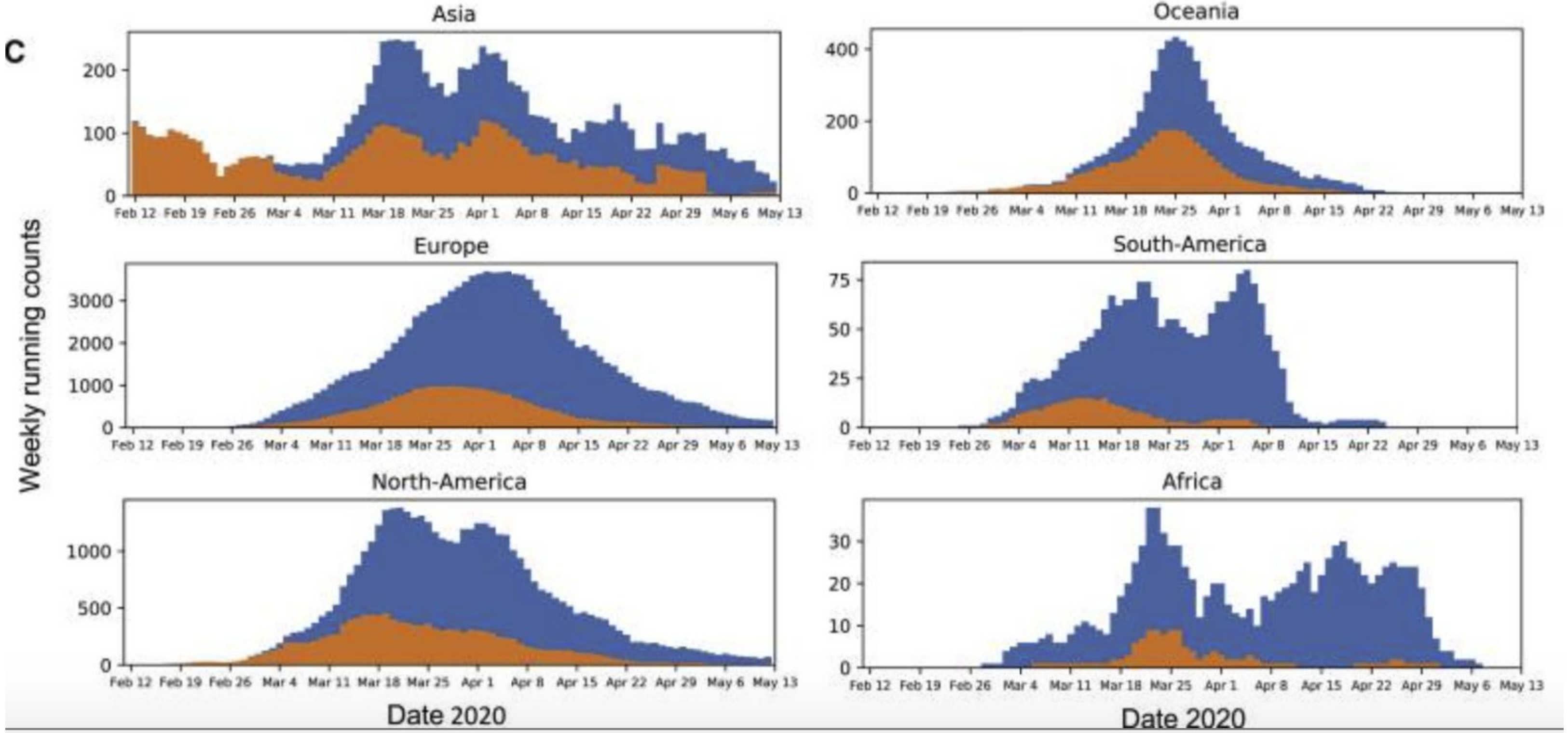
The Global Distribution of the Relative Frequencies of the D614 (orange) and G614 (blue) variants in Two Time Frames



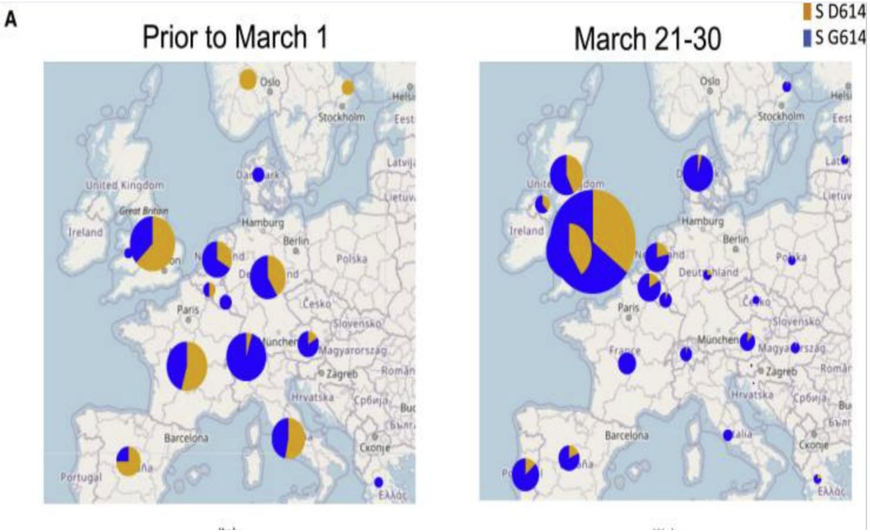
Paired Bar Charts Compare the Fraction of Sequences with D614 and G614 for Two Time Periods



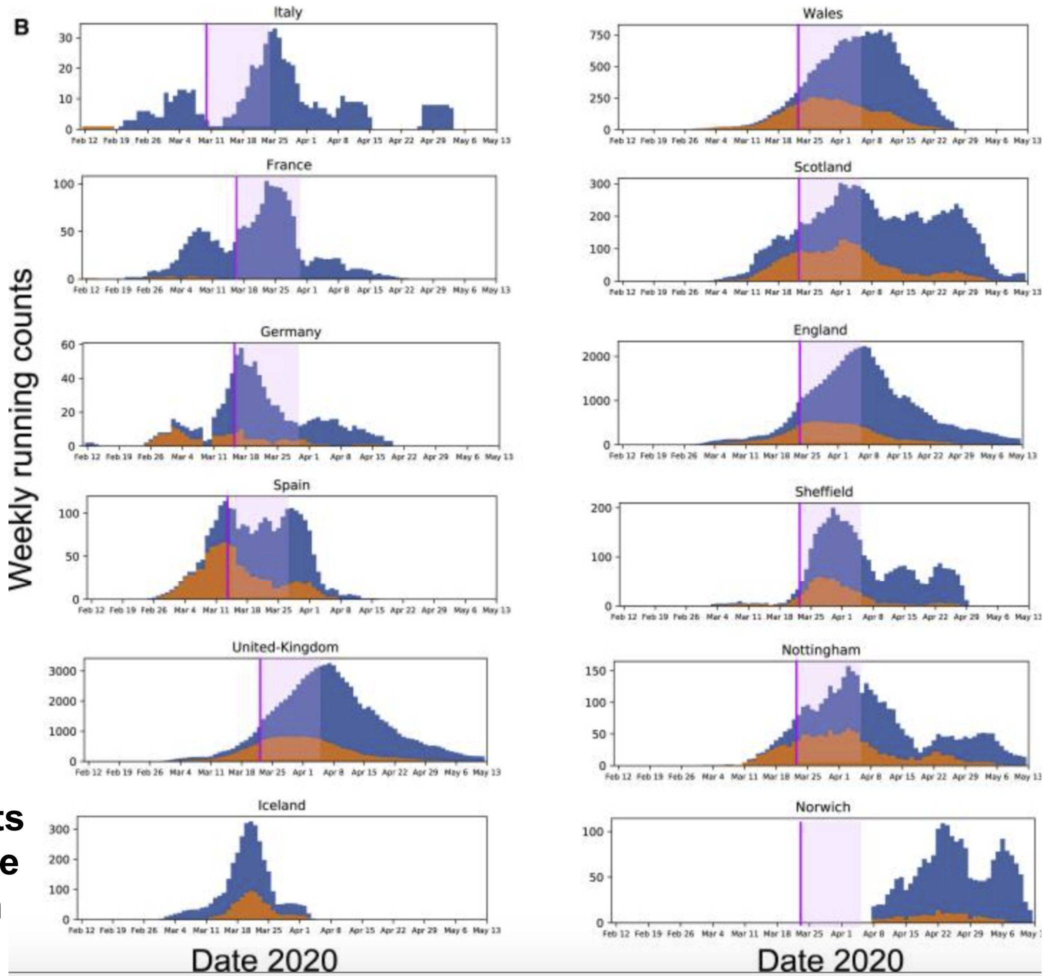
Weekly Average counts of Sampled Sequences exhibiting the D614 (orange) and G614 (blue) variants on different continents



Increasing Prevalence of G614 in Europe

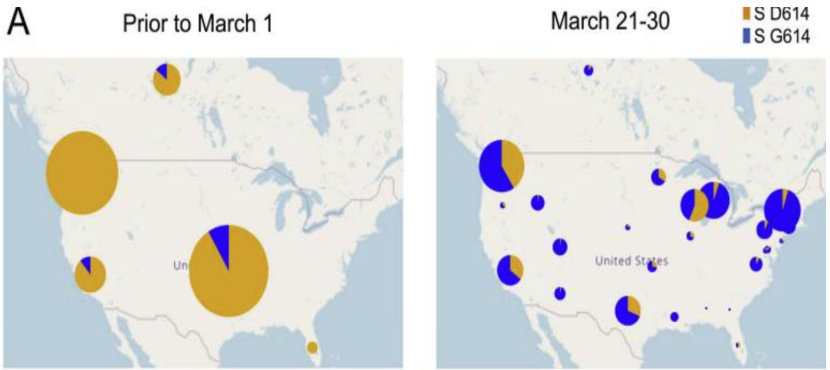


Maps of relative D614 (orange) and G614 (blue) frequencies in Europe in two time frames

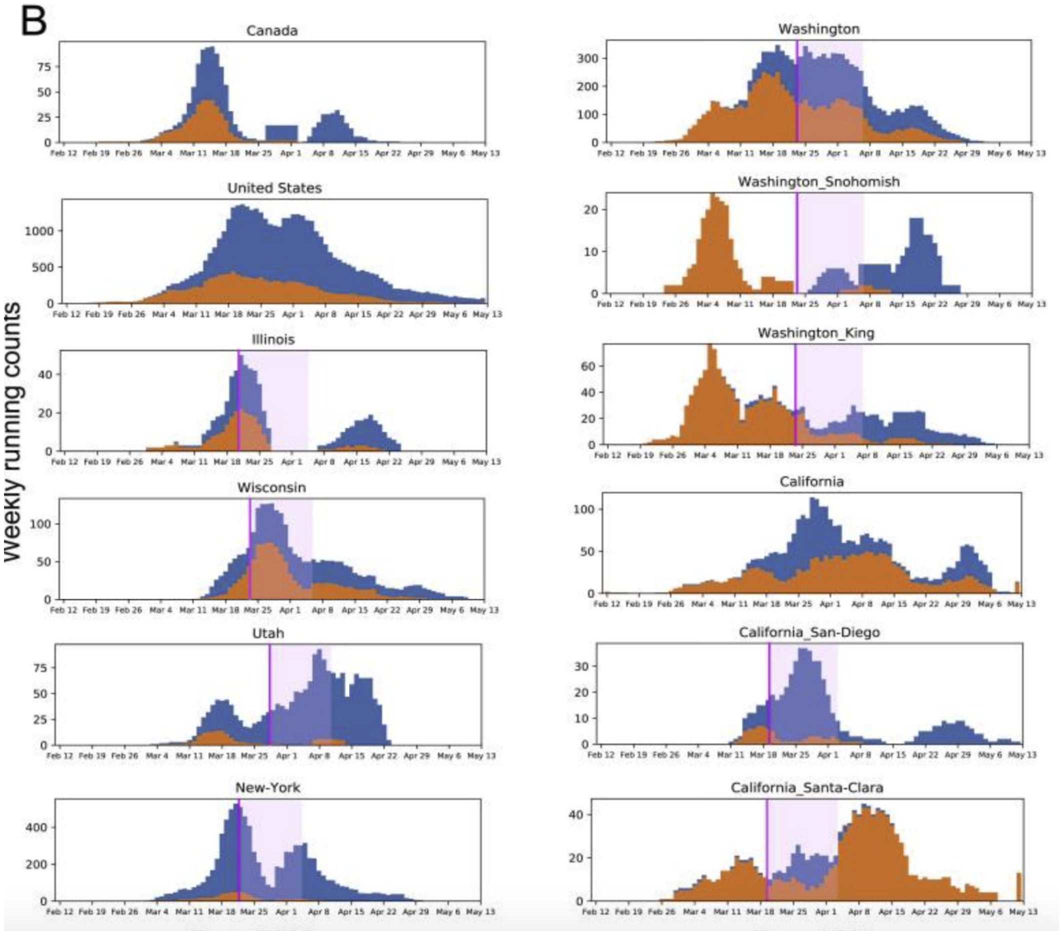


Weekly running counts of G614 illustrating the timing of its spread in Europe

Increasing Prevalence of G614 in North America

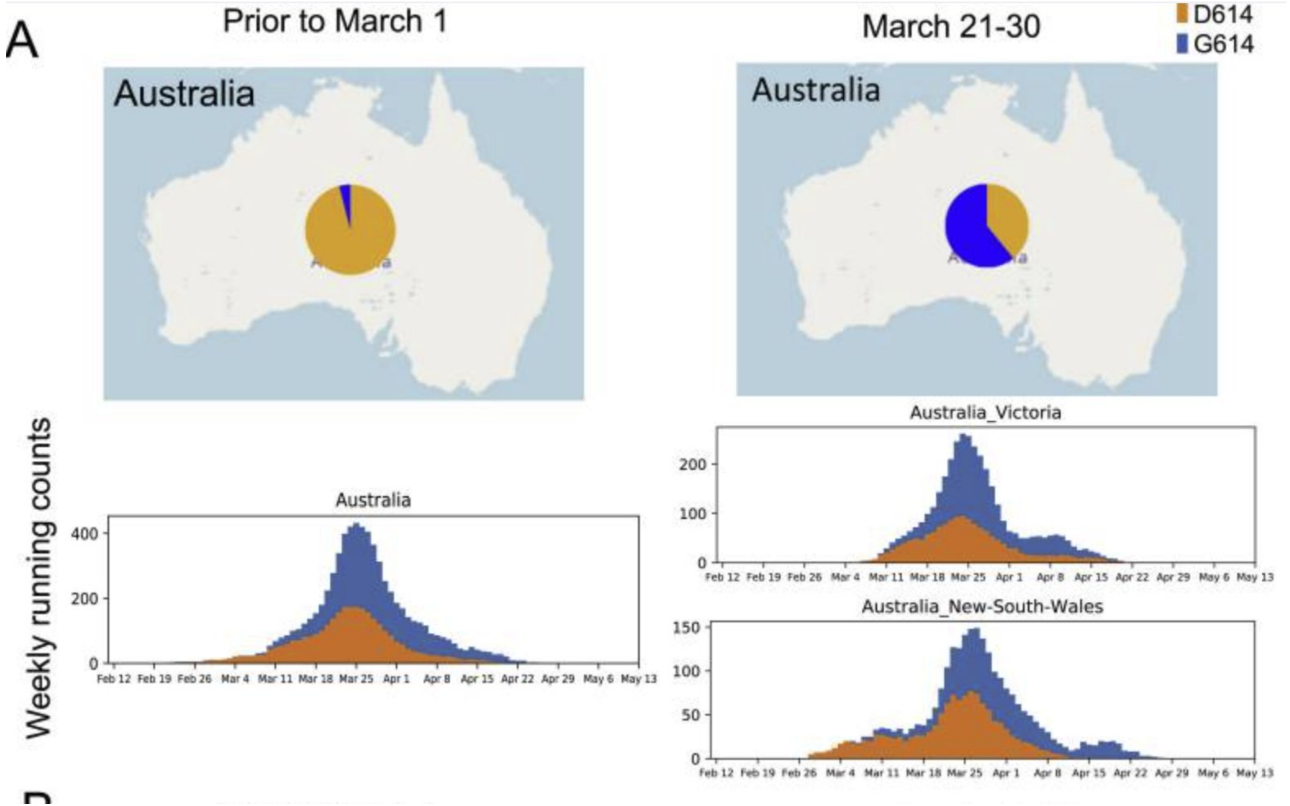


The Increasing Frequency of the D614G Variant over Time in North America

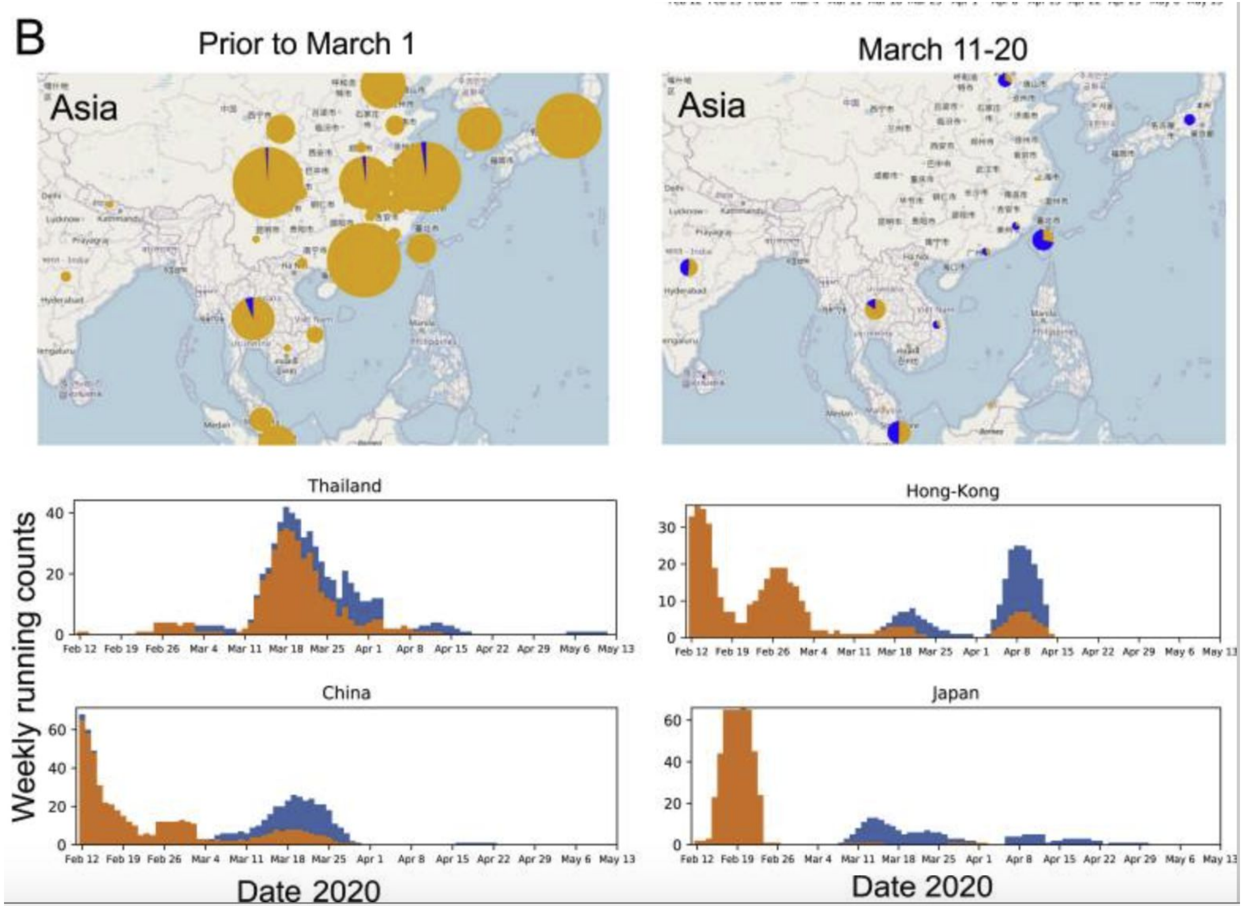


Weekly running counts of G614 illustrating the timing of its spread in North America.

The Increasing Frequency of the D614G Variant over Time in Australia



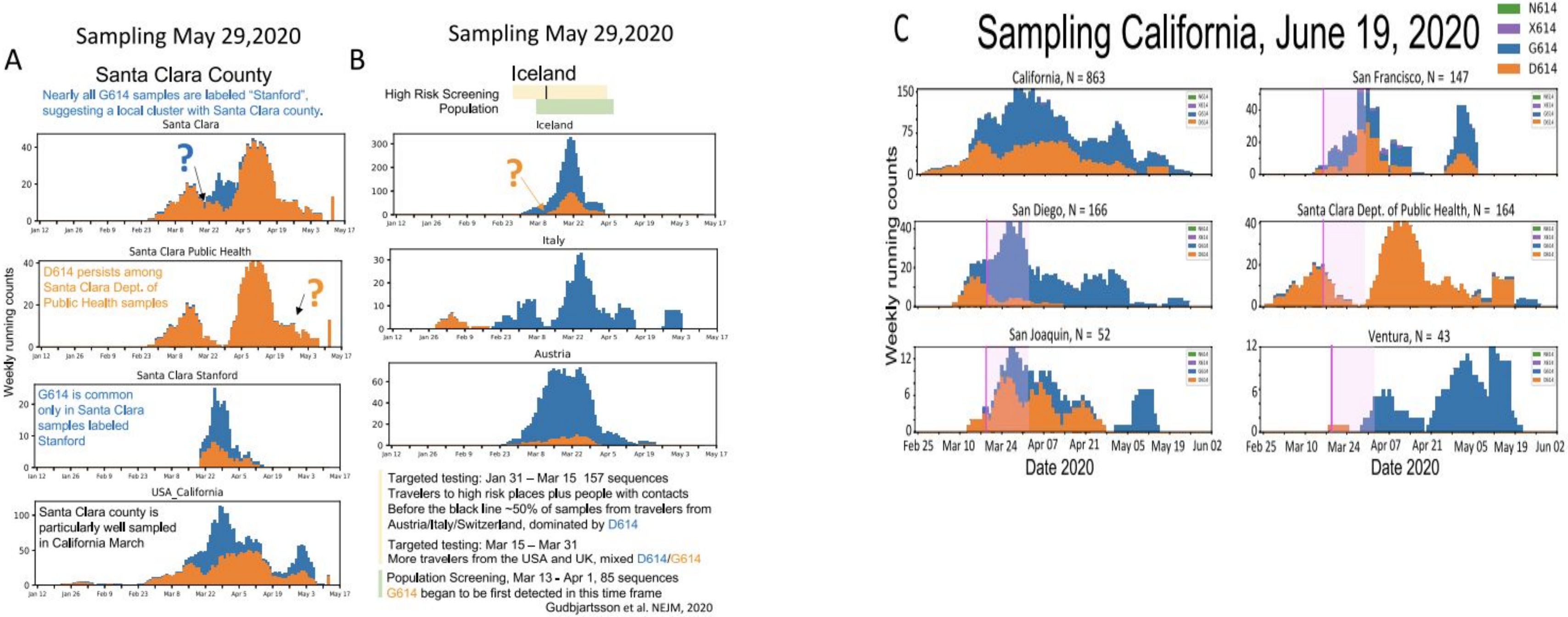
The Increasing Frequency of the D614G Variant over Time in Asia



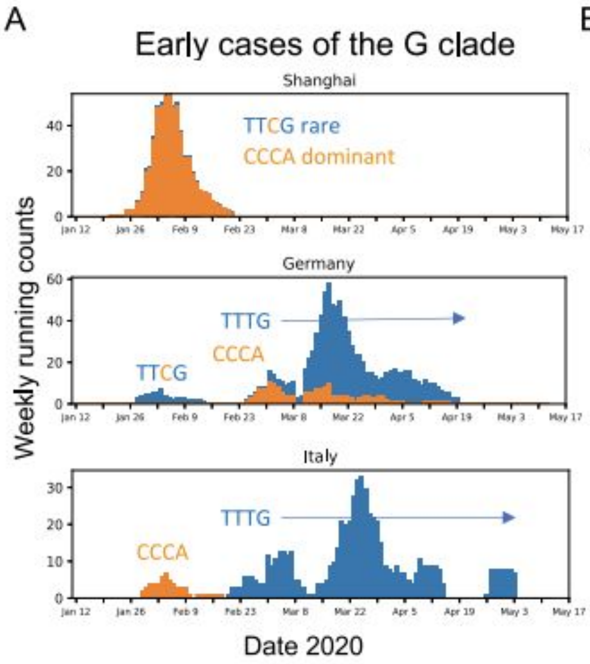
Outline

1. Mutations in the SARS-CoV-2 Genetic Sequence Can Alter the Effectiveness of Vaccines and Therapeutics
2. There were Multiple Spike Sites and Mutations of Interest, but the D614G Mutation had the Highest Global Frequency
3. The D614G Mutation Arose Independently in Different Regions of the World
4. G614 frequencies in a variety of geographic locations has significantly increased.
5. G614 Mutation Alters Interactions Between S1 and S2 Subunits of Separate Spike Protomers
6. G614 Mutants Grow to a Higher Concentration in Cell Cultures and a Higher Viral Load *in vivo* than their D614 Counterparts
7. **Some outliers to the G614 variant frequency trend do exist.**
8. In trimmed sequences, about half of the sequence produce a variant.

Some Geographical Regions do not Follow the Same G614 Trends as the Rest of the World.



The more frequent G-Clade "TTTG" was first sampled in Italy, USA, and Mexico.



B

G-clade mutations (C3037T, C14408T, A23403G) CCA -> TTG
Plus the linked mutation in the UTR: C241T CCCA -> TTTG

	11805	TTG	(72.03%)	9692	TTTG	(71.65%)	
	4582	CCA	(27.96%)	3835	CCCA	(28.35%)	
Variants:							
	53	CTG		51	TCTG	5	CTCG
	39	TCG		32	TTCG	4	CCTA
	16	CCG		13	CTTG	3	TCTA
	9	TTA		11	TCCA	2	CTTA
	8	CTA		9	TCCG	2	CTCA
	5	TCA		7	CCCG	1	TTCA
	1	ACA		6	TTTA	1	CCTG

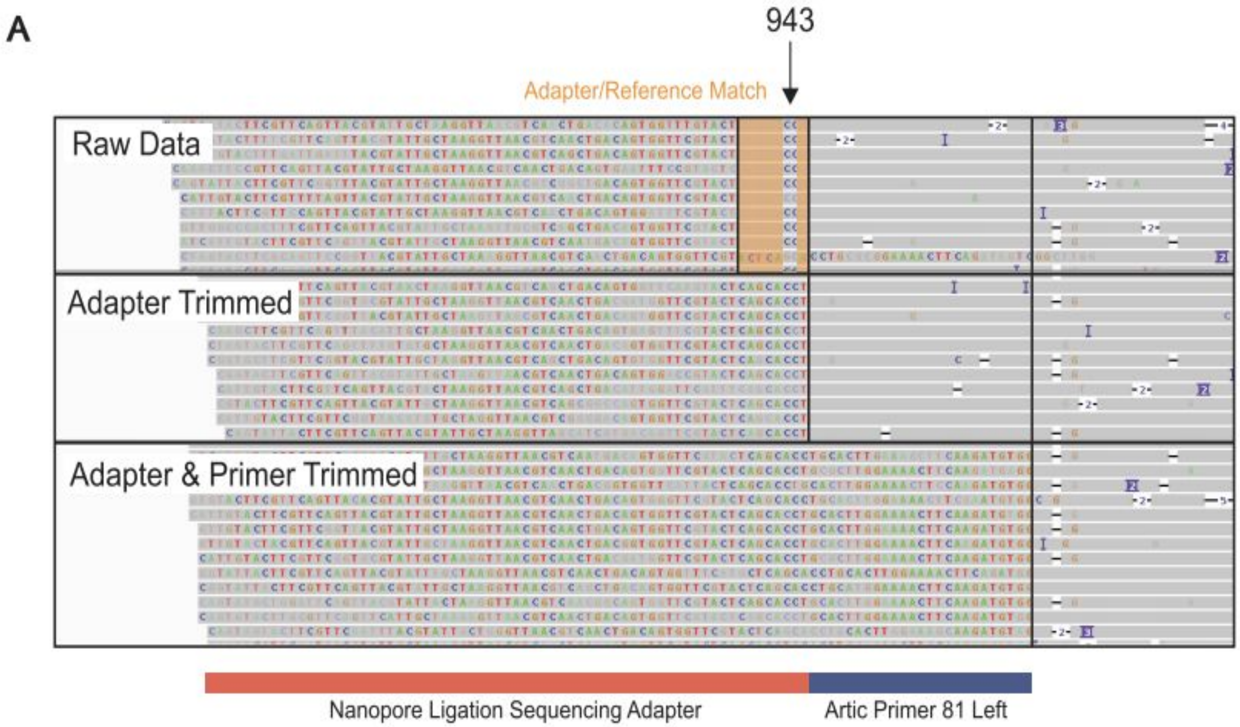
Earliest examples in GISAID:
TTCG: Germany, Jan 2020: cluster of cases late Jan.-Feb.
One example: Germany/BavPat1/EPI_ISL_406862|2020-01-28
TTCG: Sampled several times in China, e.g.:
Sichuan/SC-PHCC1-022/EPI_ISL_451345|2020-01-24
Shanghai/SH0025/EPI_ISL_416334|2020-02-06
Guangzhou/GZMU0019/EPI_ISL_429080|2020-02-05
CCCG: Sampled twice in early Feb., Wuhan and Thailand
Thailand/Samut_prakarn_840/EPI_ISL_447919|2020-02-04
Wuhan/HBCDC-HB-06/EPI_ISL_412982|2020-02-07
TTTG: First identified in Italy; within 10 days sampled in many in countries in Europe, the USA, Mexico
First sample: Italy/CDG1/2020|EPI_ISL_412973|2020-02-20

- **Other forms of the G-Clade exist.**
- **The less frequent TTCG variant can be seen in Germany and a couple of cases in Shanghai.**

Outline

1. Mutations in the SARS-CoV-2 Genetic Sequence Can Alter the Effectiveness of Vaccines and Therapeutics
2. There were Multiple Spike Sites and Mutations of Interest, but the D614G Mutation had the Highest Global Frequency
3. The D614G Mutation Arose Independently in Different Regions of the World
4. G614 frequencies in a variety of geographic locations has significantly increased.
5. G614 Mutation Alters Interactions Between S1 and S2 Subunits of Separate Spike Protomers
6. G614 Mutants Grow to a Higher Concentration in Cell Cultures and a Higher Viral Load *in vivo* than their D614 Counterparts
7. Some outliers to the G614 variant frequency trend do exist.
8. **In trimmed sequences, about half of the sequence produce a variant.**

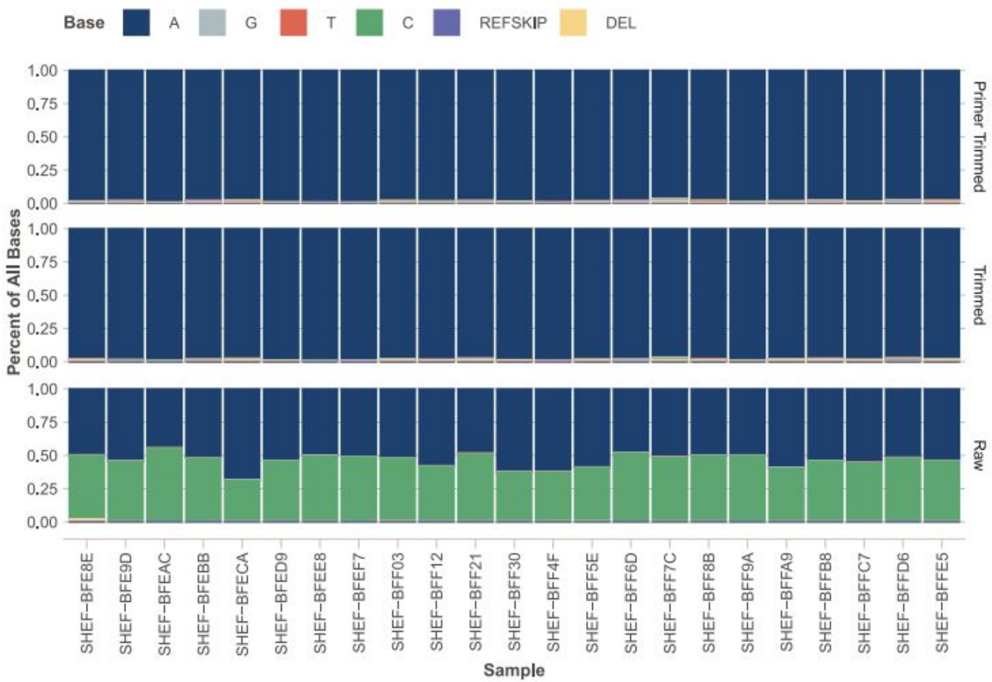
IVG plots show that in Adaptor Trimmed plots half the data shows a C variant at the 24,389 location.



- The variant of C at the 24,389 location can lead to a S943P mutation call.

IVG plots show that in Adaptor Trimmed plots half the data shows a C variant at the 24,389 location

B



- **In 23 samples from the Sheffield data, the half of the sequences had the C variant at the 24,389 location.**
- **This is not seen in the trimmed and primer trimmed data.**

The Overall Frequency of the G614 Mutation has Increased.

- **The study finds that in the GISAID data the overall frequencies of the G614 mutations have increased.**

- **Other studies confirm that the G614 Mutation has increased the viral load and caused cross antibody neutralization (Amadpour et. al, 2020).**

- **Their work sheds light on the evolving nature of SARS-CoV-2 to a mutation with more fitness.**

- **The G614 mutation has significantly increased in frequency over the D614 variant (figure 3).**

Databases have helped share SARS-CoV-2 sequence information and known variants.

- **The researchers have developed and uploaded their data analysis and spike evolution warnings that can be accessed through GISAID and the COVID-19 Viral Genome analysis Pipeline.**

- **This allows other researchers to learn more about the widespread growth of the G614 variant.**

- **Their work has been used by others who have monitored the effects of the G614 variant (Li et. al. 2020).**

Future Experiments and Limitations to their Work

- We recommend the researchers continue to update their data as time progresses and more sequences are made available.

- We also recommend that the researchers compare the fitness mutations of SARS-CoV-1 to SARS-CoV-2 to observe the similarities in progression.
- The researchers stated that one major limitation to their work is a bias in the geographical locations they chose to monitor specific data.

- The researchers also stated that a lot of their work was not up to date and they used up to date information for a couple of the results but not all.

Overview

- Mutations in SARS-CoV-2 alter the effectiveness of vaccines and therapeutics.
- G614 variant frequencies across the globe and have increased.
- G614 mutation alters the viral load and cross-neutralized antibody.
- The researchers have monitored changes in sequence changes through global mapping and descriptives.
- They have uploaded their work to GISAID and to a pipeline which allows other researchers to access their work.

Acknowledgments

- We wanted to thank Dr. Dahlquist for helping us find a resourceful article and aiding us with a couple figures.

- We wanted to thank our T/A Annika Dinulos.

- We also wanted to thank the LMU library for giving us access to articles and databases and the LMU biology department.

References

- Ahmadpour, D., Ahmadpoor, P., & Rostaing, L. (2020). Impact of Circulating SARS-CoV-2 Mutant G614 on the COVID-19 Pandemic. *Iranian journal of kidney diseases*, *14*(5), 331-334.

- Korber, B., Fischer, W. M., Gnanakaran, S., Yoon, H., Theiler, J., Abfalterer, W., ... & Hastie, K. M. (2020). Tracking changes in SARS-CoV-2 Spike: evidence that D614G increases infectivity of the COVID-19 virus. Cell, 182(4), 812-827, retrieved from https://doi.org/10.1016/j.cell.2020.06.043.
- Li, Q., Wu, J., Nie, J., Zhang, L., Hao, H., Liu, S., ... & Qin, H. (2020). The impact of mutations in SARS-CoV-2 spike on viral infectivity and antigenicity. *Cell*, *182*(5), 1284-1294.
- Wang, C., Liu, Z., Chen, Z., Huang, X., Xu, M., He, T., and Zhang, Z. (2020). The establishment of reference sequence for SARS-CoV-2 and variation analysis. J. Med. Virol. *92*, 667–674.