D614G and other mutations are critical for fitness of novel spike variants

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Leonid Yurkovetskiy[1], Thomas Nyalile[1], Ann Dauphin[1] Jeremy Luban[1]
[1]University of Massachusetts Medical School, Worcester, MA, USA

BACKGROUND

- During the beginning of the SARS-CoV-2 pandemic the D614G mutation in Spike protein appeared and became the first dominant variant across the globe. This mutation led to increased infectivity and stability of the spike protein and its importance is highlighted by being the one common mutation between every spike variant of concern that has come after the original. We asked how dependent spikes of subsequent lineages were on the D614G mutation and whether lineages in the absence of D614G had other mutations that increased fitness compared to the ancestral Wu-1 strain

METHODS

- We explore the contribution of D614G and other identified stabilizing mutations on spike mediated infectivity by incorporating them into different spike constructs. Psuedotyped lentiviral and SARS-COV-2 virus like particle (VLP) reporters are utilized along with biochemical analysis probing expression, processing, and incorporation of spike constructs into VLPs

D614G is the only common spike mutation present in every successive variants. Reverting G614 to D in context of Alpha, Beta, Gamma, and Delta Spikes results in critical loss of infectivity. In addition to D614G, Q613H and H655Y increase infectivity on Wu-1 background. Increased furin mediated S1/S2 processing increases dependency on presence of these mutations while deletion of the Furin cleavage site rescued high infectivity of D614 bearing spikes.

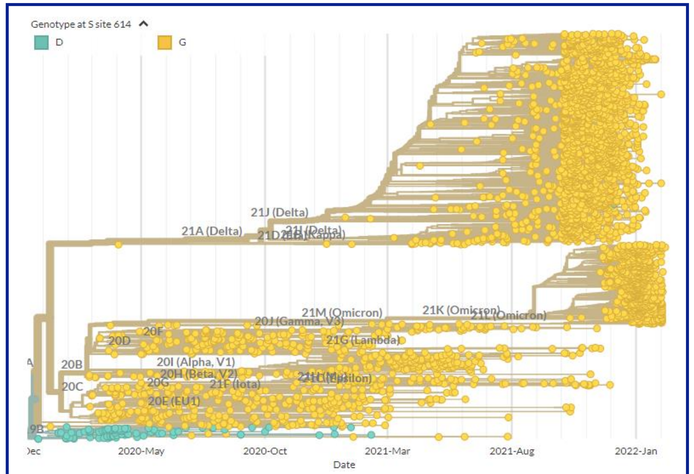


Figure 6. Phylogeny of sequenced SARS-CoV-2 viruses in relation to D614G genotype

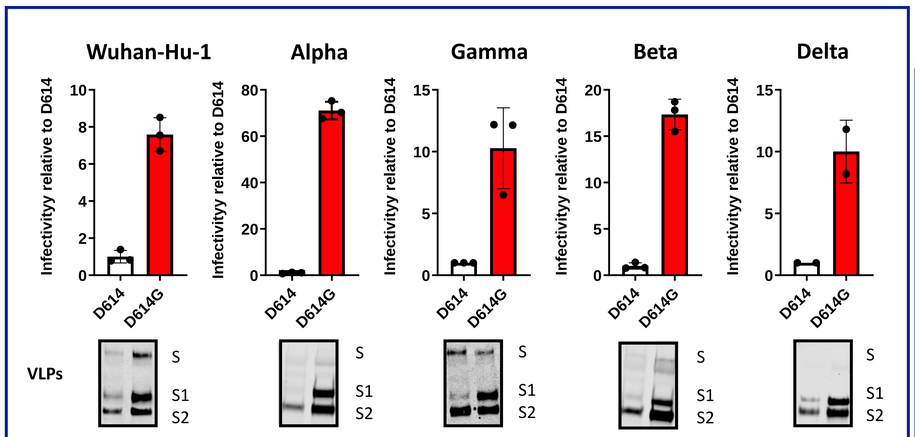


Figure 1. Variant infectivity depends on presence of D614G. Infectivity of SARS-2 glycoprotein psuedotyped HIV-1 bearing D614G or D614.

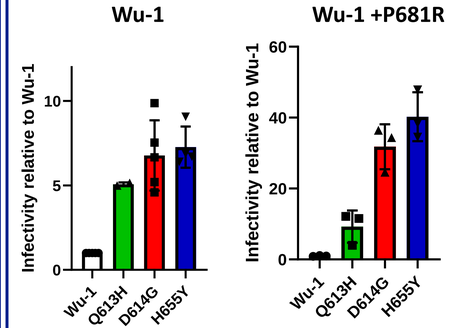


Figure 3. Addition of D614G Q613H or H655Y alone are sufficient to increase infectivity on Wu-1. Dependence on key mutations increases in presence of P681R mutation that increases S1/S2 processing.

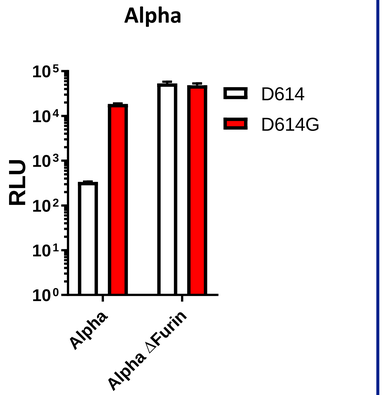
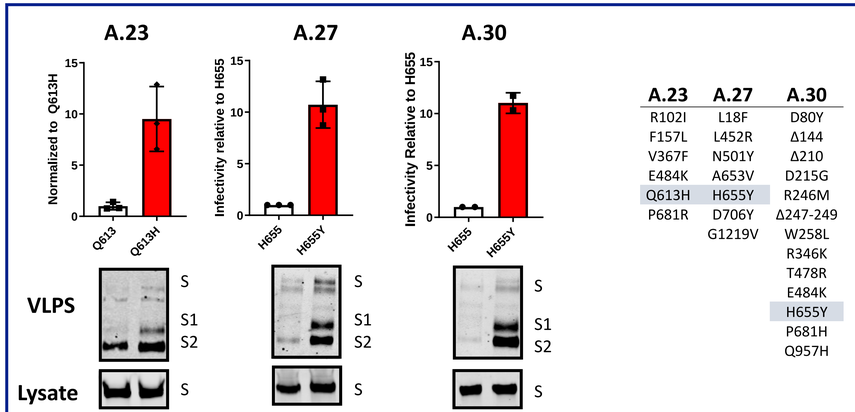


Figure 4. Dependence on D614G for infectivity of Alpha construct is ablated with deletion of furin cleavage site.



A.23	A.27	A.30
R102I	L18F	D80Y
F157L	L452R	Δ144
V367F	N501Y	Δ210
E484K	A653V	D215G
Q613H	H655Y	R246M
P681R	D706Y	Δ247-249
	G1219V	W258L
		R346K
		T478R
		E484K
		H655Y
		P681H
		Q957H

Figure 2. Infectivity of SARS-2 A lineage glycoproteins psuedotyped HIV-1 bearing indicated amino acid. Normalized to amino acid found in original Wu-1

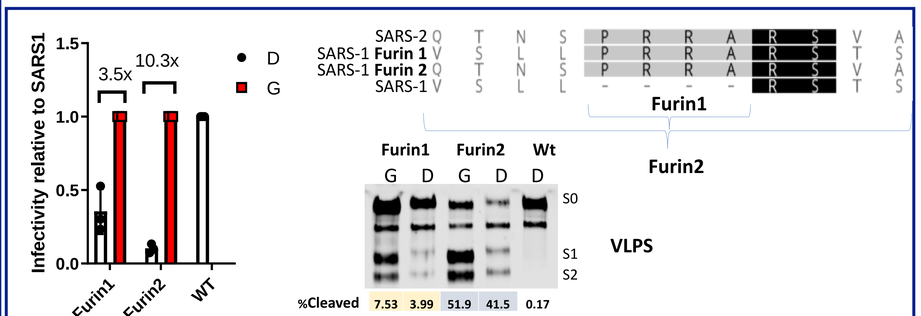


Figure 5. Addition of Furin cleavage site motif from SARS2 onto SARS1 spike results in dependency on D614G mutation for efficient infectivity

CONCLUSIONS

We identify that the D614G mutation is critical for stability, infectivity, and virion loading in a number of prominent variants of concern and dominant lineages. Studying spike variants that were in circulation in absence of D614G mutation led to identification of Q613H and H655Y at the S1 S2 interface performing similar stabilizing spike function as D614G, increasing infectivity compared to the ancestral Wu-1 Spike. The dependence on the presence of D614G or other stabilizing mutations only increased in the presence accumulating S1 mutations and specifically mutations that increase processing of spike by host furin protease, such as P681R. These keystone mutations may have an epistatic role balancing accumulated mutations in new Spike variants that may be detrimental on spike fitness otherwise. It is interesting that currently the most mutated Spikes sequenced all have not only D614G as a common mutation, but in combination with H655Y and Q613H suggesting an additive role in maintaining spike fitness while permitting selection of immune escape mutations.

ADDITIONAL KEY INFORMATION

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