Impact of Circulating SARS-CoV-2 Mutant G614 on the COVID-19 Pandemic

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Coronavirus family has caused several human illnesses, the latest caused by SARS-CoV-2, has led to COVID-19 pandemic posing serious threat to global health. A SARS-CoV-2 variant encoding a D614G mutation in the viral spike (S) protein has now become the most prevalent form of the virus worldwide, suggesting a fitness advantage for the mutant. The G614 variant is associated with higher upper respiratory tract viral load, higher infectivity, increased total S protein incorporation into the virion, reduced S1 shedding and a conformational change leading to a more ACE2-binding and fusion- competent state. However, it does not seem to be correlated to increased disease severity or escape neutralizing antibodies.

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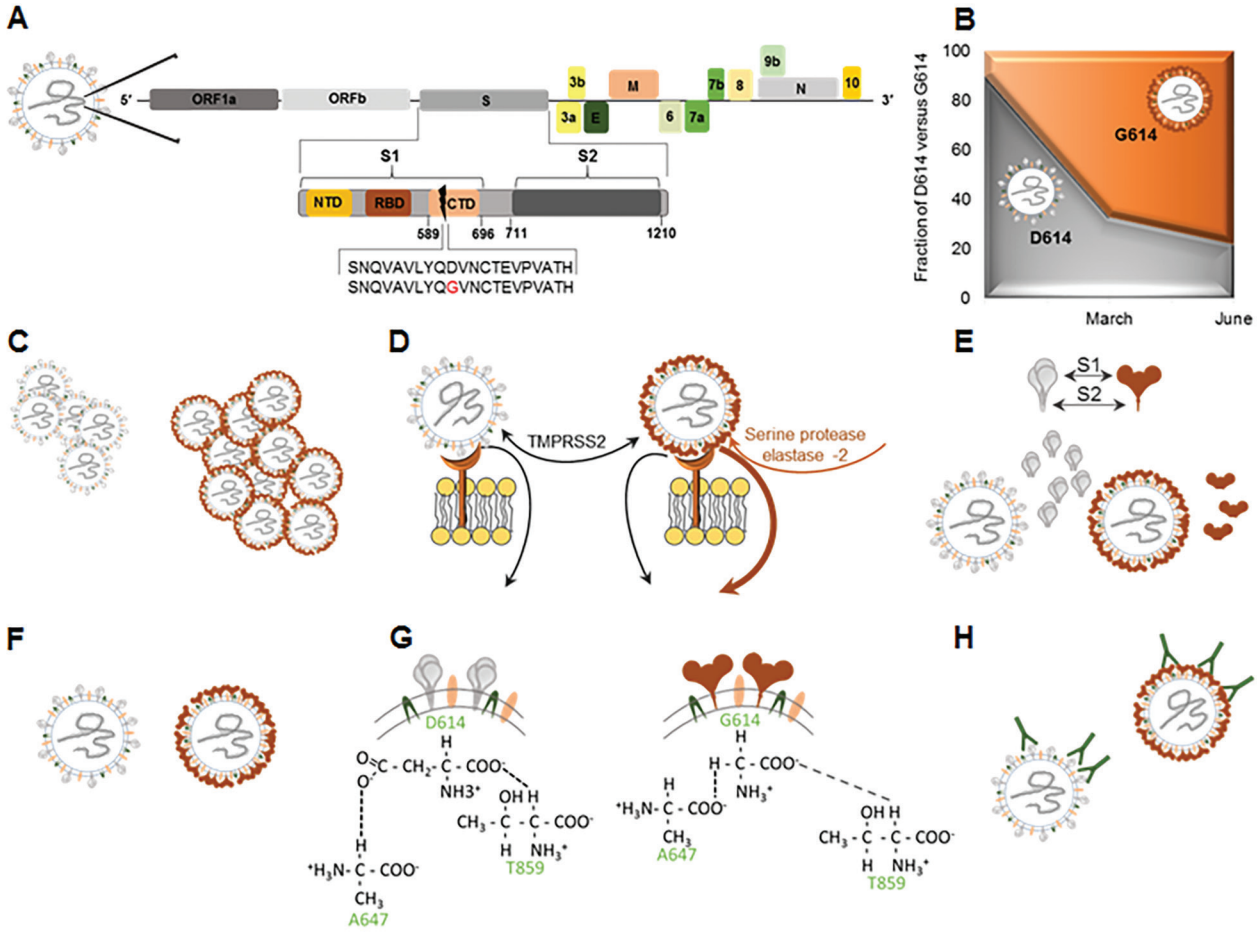
After SARS emerging in 2002, infecting about 8000 people with a 9.5% mortality and MERS emerging in 2012, infecting about 2300 people with 34.4% mortality,[1] the latest severe disease caused by beta coronaviruses first reported in China in December 2019[2] caused severe acute respiratory disease COVID-19. The disease very soon turned to a pandemic and apart from very mild or asymptomatic undocumented cases, as of July 24th 2020, nearly 16 million cases were documented, of which more than 600 000 lost their lives.

The coronavirus SARS-CoV-2 is an enveloped, positive sensed, single stranded RNA genome with about 29.8 to 29.9 kb.[3,4] The SARS-CoV-2 has a typical gene characteristics known for SARS CoVs, with two overlapping ORFs; ORF1a and ORF1b located at 5′ end occupying about two- thirds of genome, encoding proteins which are auto-proteolytically processed into 16 non-structural proteins Nsp1 to 16.[3,5,6] The 3′ end of genome encodes structural proteins spike (S), envelope (E), membrane (M) and nucleocapsid (N) proteins and 9 accessory proteins, encoded by ORF3a, ORF3b, ORF6, ORF7a, ORF7b, ORF8, ORF9b, ORF9c, and ORF10 (Figure 1A).[7] Although the structural proteins are required for a structurally complete viral particle, the ~200 kDa glycoprotein S plays the vital role in attachment of the virus to the host cell surface receptors, membrane fusion and cell entry.[8,9] The S protein binds to the cellular receptor angiotensin-converting enzyme 2 (ACE2)[10] through its S1 subunit, which also further contributes to stabilization of the prefusion state mediated by S2 subunit. Thereafter, employing serine protease TMPRSS2, S will be cleaved at S2′ site located immediately upstream of fusion peptide, which allows viral and cellular membrane fusions.[11,12]

RNA viruses have high mutation rates due to the RNA-dependent RNA polymerase (RdRP) lacking the proofreading activity,[13] which are usually correlated with enhanced transmission, virulence and evolvability, considered beneficial for viruses.[14] In general, mutation rates inferred for SARS-CoVs are considered to be moderate



A) Schematic representation of the genome organization of SARS-CoV-2, spike protein domain arrangement and the site of point mutation, B) The global frequency of S protein D614- and G614-bearing viruses over time, C) Representation of increased viral load in G614 version versus D614, D) Effect of TMPRSS2 compared to serine protease elastase-2 on entry of D614 and G614-bearing viruses, E) Effect of D614G on S1 shedding. F) The S protein incorporation into the virion in D614- versus G614 counterpart, G) The inter-protomer hydrogen bond between aspartic acid 614 and threonine 859 in D614 variant compared to disrupted hydrogen bond between glycine 614 and threonine 859 in G614 variant and strengthened intra-domain interaction with Alanine 647, H) D614 and G614 bearing viruses' antibody cross neutralization

due to the independent proofreading activity.[15,16] However, recent analysis of SARS-CoV-2 sequence reveals several regions of increased variation. Hu *et al.* reported 10 most abundant non synonymous mutations in SARS-CoV-2 S protein, one of which is D614G located in the carboxy (C)-terminal region of the S1 domain (Figure 1A), directly associated with S2 (17-20). D614G represented in 64.6% of their analyzed sequences compared to the next most abundant mutation; A829 to T829, presented in only 0.8% of all analyzed sequences.[17] Upon a dynamic tracking of variant frequencies based on GISAID SARS-CoV-2 sequence database, Korber and co-workers also provided evidence for a clear increase in G614 at multiple geographic levels in which, prior to March, G614 was found in only 10% of 997 global sequences and rare outside of Europe, during March it increased to 67% and up to mid-May it represented 78% of 12,194 sequences, a trend, which is also confirmed by Zhang *et al.* and continued increasing (Figure 1B).[19,20] The fact that D614G is becoming the most predominant form in the global pandemic around the world suggests that G614 may have a fitness advantage.[18] Data indicates a lower RT-PCR cycle threshold in individuals infected by G614 variant suggesting a higher upper respiratory viral load (Figure 1C).[18,21] Additional data in multiple cell types confirms that G614-bearing viruses have significantly higher infectious rate and enhanced infectivity.[18,21,22] Korber *et al.* show that entry of G614-bearing viruses in 293T-ACE2 cells, as

compared to D614-bearing viruses, is not enhanced by TMPRSS2 (Figure 1D), an observation, which needs to be tested in lung cells, with a more credible native protease expression levels.[18] In other hand, serine protease elastase -2 seems to participate in the proteolytic activation of S G614 on a novel serine protease elastase-2 cleavage site on S1-S2 junction region, thereby enhancing the viral entry to 293T-ACE2 cells (Figure 1D),[17] however whether this affect the viral entry and infectivity in vivo is still unclear. Yurkovetskiy and co-workers very recently have shown that D614G is three to nine-fold more infectious than the ancestral form on human lung and colon cell lines and other human cell lines rendered permissive by ectopic expression of human ACE2 and TMPRSS2, or by ACE2 orthologues.[22] Furthermore, 614G bearing viruses infecting ACE2 expressing cells strikingly more efficiently,[17,19] does not seem to be due to increased affinity or better access to RBD but due to two related mechanisms; reduced S1 shedding (Figure 1E) and increased S1 domain and total S protein incorporated into the virion (Figure 1F).[19,20] Interestingly, structural features obtained by cryo-electron microscopy has revealed a similar overall structure compared to D614.[20] However, D614G reveals conformational changes within individual S protomers as well as within trimers leading to disruption of the otherwise stabilizing inter-protomer hydrogen bond between D614 in S1 and T859 in S2. This in turn results in weakening of the inter-protomer contacts, increased distance between the protomers and a dramatic alteration in the ratio of open to closed S protein particles, from 82% closed and 18% open to 42% closed and 58% open respectively, driving the S protein trimer conformation toward an ACE2-binding and fusion-competent state (Figure 1G).[20,22]

However, the impact of this mutation on the severity of the disease, mortality, antigenicity and vaccine and therapeutic development is yet to be elucidated. While early reports indicated a correlation between SARS-CoV-2 viral load and disease severity,[23,24] Korber *et al.* found no significant difference between the two variants D614 and G614 measured by hospitalization outcomes.[18] This finding was supported by another independent study on 88 SARS-CoV-2 genome sequences from Chicago.[21] Although, the unrooted phylogenetic tree provided by Eaaswarkhanth and co-workers, showed that significant numbers of strains bearing G614 were from countries like Belgium, Spain and Italy with higher mortality rate, while Germany with lower death toll had most strains with D614.[25]

Summary of Differences Between New Circulating G614 Versus D614

Higher viral load/higher infectivity
Increased total S protein incorporation into the virion
Reduced S1 shedding
S cleavage by elastase-2
More open S protein conformational change facilitating ACE2 binding and fusion
Similar disease severity
Cross antibody neutralization

While a single mutation in SARS-CoV-1, the Spike D480A/G in the receptor binding domain (RBD) escapes neutralizing antibody 80R,[26] the spike D614G is not located at the RBD, therefore it is believed to not affect the immunogenicity of RBD epitopes, considered to be critical for antibody neutralization.[27] In support of this notion, several groups have provided evidence showing that antibodies produced from natural infection with D614 or G614 bearing viruses could cross neutralize (Figure 1H),[17,18,22,28] suggesting that the locus is not critical for antibody mediated immunity. Therefore, the D614G mutation is believed to unlikely have a major impact on the efficacy of vaccines that are currently in the pipeline, some of which exclusively target the RBD. Although, it remains to determine whether the D614G alters neutralization sensitivity to other classes of anti-Spike monoclonal antibodies.[22]

Taken together, data indicates that G614 non synonymous mutation in the SARS-CoV-2 spike results in higher viral load in upper respiratory tract, is associated with significantly higher infectious rate than the D614 variant, facilitates the S cleavage by serine protease elastase-2 and thereby infects ACE2 expressing cells more efficiently in vitro but is not enhanced by TMPRSS2. The 614G bearing viruses infect ACE2 expressing cells much more efficiently not as a result of the increased affinity for ACE2 or better access to RBD but perhaps due to reduced S1 shedding and increased total S protein incorporated into the virion. Furthermore, the D614G mutation drives the S protein trimer conformation toward an ACE2-binding and fusion-competent state. However, these phenotypes have not been so far associated with the severity of disease. Moreover,

many studies indicate that the mutation has not led to neutralizing antibody escape (Table). Therefore, it is hypothesized that the rapid spread of G614 is due to the more infectious nature of the G614 compared to the wild type.

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