

Increased Binding Affinity of Furin to D614G Mutant S-glycoprotein May Augment Infectivity of the Predominating SARS-CoV-2 Variant

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Abstract

COVID-19 pandemic has inflicted serious challenges to both global health and economy. The disease is caused by a +ssRNA zoonotic coronavirus named SARS-CoV-2, known to have four structural proteins named spike (S), envelope (E), membrane (M), and nucleocapsid (N). Given the critical role in host immunity and virus attachment to ACE2 receptors on target cells, S-glycoprotein mutations are of significant concern. Dynamic tracking and phylogenetic analysis show that S-glycoprotein variants of SARS-CoV-2 (G clade) are emerging globally, particularly the D614G S-glycoprotein variant has been fast spreading through human transmissions in all over Europe since March 2020. Herein, we review a recently published study which characterizes the D614G mutation in SARS-CoV-2 S-glycoprotein and deciphers its impact on furin binding and viral infectivity using bioinformatics tools and molecular dynamic simulations. The findings of this study are reviewed in light of evidence from other studies. In the end, consistent need for epidemiological tracking of other S-glycoprotein variants as well as future perspectives have been addressed.

Keywords: COVID-19, SARS-CoV-2, D614G, S-glycoprotein, Furin, ACE2, Infectivity

Background

The coronavirus disease (COVID)-19 pandemic has profoundly devastated human health and wellbeing all over the world, along with colossal setback to global economy in terms of soaring new infections, hospitalizations, ICU admissions, work losses, closures of businesses and institutions, bankruptcies, and precautionary measures involving social distancing, hygiene, and travel restrictions across the globe. COVID-19 was declared by the World Health Organization (WHO) as a public health emergency of international concern in January 2020, and then as a pandemic in March 2020. There are over 154.64 million confirmed coronavirus infections and more than 3.23 million deaths reported to the WHO globally until date (as of 11:21 a.m. CEST, 6 May, 2021) [1]. The disease is caused by a zoonotic positive-sense single-stranded

ssRNA virus called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is known to have four structural proteins: spike (S), envelope (E), membrane (M), and nucleocapsid (N), with close genetic similarity to bat coronaviruses. The global science initiative source called “Global Initiative on Sharing Avian Influenza Data” (GISAID) has reported seven SARS-CoV-2 clades as G, GH, GR, L, O, S, and V [2].

The trimeric S-glycoprotein of SARS-CoV-2 is central to host cell binding and entry, whereby S1 subunit mediates the attachment of virions to host cell receptor known as angiotensin-converting enzyme 2 (ACE2) and S2 subunit containing fusion peptide promotes the fusion between viral and host cell membranes to allow viral entry in a process which is orchestrated by proteolytic cleavage at the S1/S2 site [3]. Following cleavage by furin protease at

the polybasic RRAR furin recognition site, the structural rearrangement in SARS-CoV-2 S-glycoprotein allows the receptor-binding domain (RBD) in S1 subunit to interact with ACE2 transmembrane protein on target cells [4].

Despite the presence of genetic proof reading mechanisms in coronaviruses, continued passaging and host to host transmission and natural selection processes favor mutations to occur. As opposed to antigenic drift reported in SARS-CoV-1 [5,6], there is still no such evidence available for SARS-CoV-2. Notably, SARS-CoV-2 G clade S-glycoprotein variants are emerging globally, especially, the D614G S-glycoprotein variant has been marked by consistent predominance and human transmission over time in Europe since March 2020. The 23403 A>G genetic mutation encodes replacement of aspartic acid (D) with glycine (G) at position 614 in SARS-CoV-2 S-glycoprotein [7]. Of note, a structural analysis study proposed that the D to G mutation could induce a structural change in SARS-CoV-2 S-glycoprotein, favoring more proteolytic cleavage and emergence of more virulent and infectious G614 variant [8].

Further characterization studies of the D614G mutation remain highly desirable as the changes in the S-glycoprotein are pivotal to viral attachment and entry in host cells. Importantly, a recent study titled: "Higher binding affinity of furin for SARS-CoV-2 spike (S) protein D614G mutant could be associated with higher SARS-CoV-2 infectivity" by Mohammad et al. [9] characterizes the D614G mutation in SARS-CoV-2 S-glycoprotein and assesses impact on viral infectivity by using an array of bioinformatics tools and molecular dynamic (MD) simulations. A brief review of this study predicting the biological significance of D614G S-glycoprotein mutation in SARS-CoV-2 is presented below.

Methodological Approach Used

To analyze and visualize the D614G mutant S-protein dynamics by sampling conformations and predict the effects of D614G mutation on S-protein stability and flexibility, DynaMut, a web server based tool was used to integrate the graph-based signatures together with normal mode dynamics for generating a consensus prediction of the impact of the mutation on S-glycoprotein stability. The SCOOP algorithm was used for accurately predicting the temperature-dependent stability of D614G S-glycoprotein. Of note, this tool helps predict all the thermodynamic values associated with the folding transition, as for instance, the melting temperature (T_m), standard folding enthalpy measured at T_m (ΔH_m), and standard folding heat capacity (ΔC_p). Since the stability curve prediction of target protein is fast, SCOOP can reliably be used on a structurome scale as well. HDock web server-based docking algorithm was used for template-based modeling

on the protein-protein and protein-nucleic acid molecular docking analysis, thus sampling all possible binding modes of one structure in reference to the other. Intrinsic scoring was used to rank the sampled binding modes during/after sampling. The authors have also modeled the furin-to-S-glycoprotein binding for measuring the binding affinities and predict virulence of the G614 variant.

Recapitulating the Main Findings

The authors describe that the D614G S-glycoprotein mutation represents the change from a negatively charged aspartate to glycine in the S1 domain of S-glycoprotein which can impact electrostatic interactions to make the loop region more flexible and accessible for furin cleavage. Regarding thermodynamic analysis of D614G mutation, S-glycoprotein stability was assessed and it was found that mutant G614 loop, compared with wild type (WT) D614 loop, was a slightly more dynamic structure, marked by relatively less thermal stability and higher vibrational entropy. In agreement with this notion, G614 was found to be less stable than D614 at 25°C, as inferred from standard folding enthalpy [10]. Next, the authors analyzed the interatomic interactions in the G614 mutant S-glycoprotein and showed that the short 2.7-Å H-bond that existed between S1 (Chain A) and S2 (Chain B) side chains in the WT D614 was lost in the mutant G614 S-glycoprotein. The authors argue that the loss of the H-bond in the G614 S-glycoprotein variant is expected to confer more flexibility to S2 subunit, and promote S1 accessibility for furin cleavage, thus giving rise to more cytopathic and infectious D614G mutant virions.

To consolidate findings of thermodynamic analysis, the authors modeled the binding between furin and RRAR cleavage site in the D614 and G614 S-glycoproteins. For assessing binding affinities (Kd), model 1 was selected from HDock analysis and binding affinities were determined at different temperatures using PRODIGY server. Furin came up with a stronger binding affinity for G614 than D614 at 38°C, as was expected from the enhanced accessibility of the RRAR site for furin cleavage in the mutant G614 S-glycoprotein due to loss of the H-bond. These data support a higher infectivity predicted for the D614G mutant.

To further characterize the structural stabilities and dynamic features of D614-apo and G614-apo S-glycoproteins as well as their complexes with furin, dynamic protein data were acquired at atomic spatial resolution, running 100 ns simulations using GROMACS molecular dynamics simulator. The effect of the D614G mutation on furin binding was further determined from the total binding free energies using MM/GBSA approach. The binding free energy data again supported higher binding affinity of furin for G614 mutant S-glycoprotein

(-61.9 kcal/mol) as compared to D614 WT S-glycoprotein (-56.78 kcal/mol). It was, therefore, inferred that furin could bind more favorably to RRAR cleavage site in the G614 mutant S-glycoprotein as compared to D614 WT S-glycoprotein. The authors opined that a more efficient furin-mediated cleavage of the G614 mutant S-glycoprotein and its interaction with ACE2 transmembrane protein on target cells could result in more infectious G614 virions.

Supporting Evidence from the Existing Literature

The corroborating evidences, aligned with the predictive underpinning of this study by Mohammad et al., implicate both clinical and *in vitro* data published independently by other groups. In this regard, phylogenetic tracking of SARS-CoV-2 variant frequencies by Korber et al. [11] identified a recurrent pattern and predominance for the G614 mutant at multiple geographic levels, even involving the local epidemics that started from the D614 WT strain which was originally isolated from the first human cases in Wuhan, China. It represents a fitness advantage for G614 over D614. Although, disease severity differed non-significantly between G614 and D614 strains, the G614 variant isolated from infected individuals was found to have lower RT-PCR cycle thresholds, indicating higher upper respiratory tract viral loads (VL) of G614 compared to D614 in patients. The elevated VL may explain why the G614 variant is more readily transmissible than D614 strain. The authors also established the laboratory evidence of the intrinsic fitness of D614G mutation by using two different infection models in various cell types, viz. pseudotyped single-cycle vesicular stomatitis virus (VSV) and lentiviral particles carrying G614 or D614 SARS-CoV-2 S-glycoprotein. The G614 pseudoviruses had significantly higher titers than D614 pseudoviruses. Similarly, Zhang et al. [12] showed that pseudoviruses carrying the G614 mutant S-glycoprotein entered ACE2-expressing cells more efficiently than those with D614 WT S-glycoprotein. Similar results were obtained by using virus-like particles (VLPs) produced with SARS-CoV-2 E, M, N, and S proteins. The authors concluded that the D614G mutation could enhance infectivity by incorporating more functional S-protein into the virions. Next, another study by Plante et al. [13] compared the replication kinetics between G614 and D614 virions in the human lung epithelial cell line Calu-3 and found that the D614G mutation enhanced the infectivity of SARS-CoV-2 that were produced from this cell line. The authors directly compared the fitness of G614 and D614 viruses using competition experiments in hamsters co-infected intranasally with equal amounts (10^4 PFU) of each virus and found that all respiratory tissues had a G614/D614 ratio >1 , indicating that G614 virus had a consistent replication advantage over D614 virus. Moreover, using a primary human airway tissue culture model, the G614

virions outcompeted the D614 virions, based on their increased viral infectivity and replication. The D614G substitution also enhanced the temperature stability of the mutant virus. Altogether, these studies addressing the functional aspects and significance of the D614G mutation in S-glycoprotein of SARS-CoV-2 are concordant with the findings of the study herein reviewed.

Concluding Remarks and Perspectives

In conclusion, the D614G S-glycoprotein mutation in G clade of SARS-CoV-2 has been shown to induce flexibility in S-glycoprotein, leading to enhanced accessibility for furin binding and S1/S2 cleavage at the RRAR site to enable efficient membrane fusion and increased viral infiltration into target cells, consistent with the attributes of a more virulent strain. It is recommended that the future studies continue dynamic tracking of the SARS-CoV-2 variants at all geographic levels. Given the immunogenic significance of S-glycoprotein, enhanced vigilance and spike mutation data monitoring are warranted. Also, more comparative studies of G614 and D614 strains will be required to investigate whether there are varying sensitivities to neutralization by polyclonal (convalescent sera, vaccinal antisera) and monoclonal antibodies. It would be of interest to explore whether the D614G variants could elicit antibody-dependent enhancement responses in humans. Besides, other reported mutations in the S-protein of SARS-CoV-2, such as P681H, D1118H, S982A, N501Y, A570D, Δ H69/ Δ V70, and Δ Y144 also require incessant genomic characterization and close monitoring. Of note, P681H S-protein variant (B.1.1.7 lineage) has been implicated with the fast emerging SARS-CoV-2 infections in the United Kingdom, South Africa, and Nigeria [14,15]. Since this particular mutation is also adjacent to the furin cleavage site, it may have pathobiological impact in amplifying the viral infectivity and transmission as observed for the G614 variant. The infectivity and immunogenicity investigations of P681H S-glycoprotein variant would be highly desirable.

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