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Review

The virological impacts of SARS-CoV-2 D614G mutation

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The coronavirus diseases 2019 (COVID-19) caused by the infection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in December 2019 has caused more than 140 million infections worldwide by the end of April 2021. As an enveloped single-stranded positive-sense RNA virus, SARS-CoV-2 underwent constant evolution that produced novel variants carrying mutation conferring fitness advantages. The current prevalent D614G variant, with glycine substituted for aspartic acid at position 614 in the spike glycoprotein, is one of such variants that became the main circulating strain worldwide in a short period of time. Over the past year, intensive studies from all over the world had defined the epidemiological characteristics of this highly contagious variant and revealed the underlying mechanisms. This review aims at presenting an overall picture of the impacts of D614G mutation on virus transmission, elucidating the underlying mechanisms of D614G in virus pathogenicity, and providing insights into the development of effective therapeutics.

Keywords: SARS-CoV-2, COVID-19, D614G, infectivity, virus mutation

Introduction

The pneumonia of coronavirus diseases 2019 (COVID-19) was caused by the infection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a member of β-coronavirus clade, which also contains SARS-CoV emerging in 2002 and Middle East respiratory syndrome coronavirus (MERS-CoV) identified in 2012 (Ksiazek et al., 2003; Christian et al., 2004; Zaki et al., 2012). Most patients with COVID-19 only had mild or moderate symptoms, such as fever and cough. Unfortunately, some patients developed severe chronic respiratory diseases and must be admitted to the intensive care unit (Shi et al., 2020; Forni and Mantovani, 2021). Besides, those patients of severe type generally manifested symptom of lymphopenia likely ascribed to cell-in-cell-mediated elimination of lymphocytes by syncytia resulted from SARS-CoV-2 infection (Zhang et al., 2021b). By the end of April 2021, the pandemic of SARS-CoV-2 had caused >140 million infections and >3 million deaths worldwide, which posed a serious threat to global health.

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SARS-CoV-2 virus encodes four important structural proteins, spike glycoprotein (S protein), M glycoprotein, E glycoprotein, and N phosphoprotein (Chan et al., 2020). The host entry of SARS-CoV-2 is mediated by the S protein that contains the N-terminal S1 fragment and the C-terminal S2 fragment. It was found that S1 is responsible for binding to host cell receptor angiotensin-converting enzyme 2 (ACE2). S2 promotes membrane fusion, which is prominently responsible for the high contagiousness of SARS-CoV-2 (Chan et al., 2020). Compared with other coronaviruses known to infect humans, SARS-CoV-2 likely has a super infection ability and shows a longer incubation period (Qin et al., 2020; Wang et al., 2020). To prevent the spread of SARS-CoV-2, considerable efforts were made to develop safe and effective drugs, antibodies, and vaccines (Dagotto et al., 2020; Dong et al., 2020; Gaebler et al., 2021; Koenig et al., 2021), among which most targeted the S protein (Dagotto et al., 2020; Samrat et al., 2020).

Like the other two β-coronaviruses SARS-CoV and MERS-CoV, SARS-CoV-2 virus is also an enveloped single-stranded positive-sense RNA virus (Chen et al., 2020b; Rabaan et al., 2020). Although the evolution of the RNA virus is considered to be slow due to the self-correcting mechanisms (Robson et al., 2020), the rapid global spread of the SARS-CoV-2 virus provides advantages for natural selection to act on rare but beneficial mutations, which may make the virus infect humans more

effectively and spread widely. Therefore, intensive attentions were paid to emerging mutations accumulated during SARS-CoV-2 spreading, leading to the identification of a number of mutations. Some mutations, such as D614G, N501Y, E484K, L452R, and K417N, turned out to be functionally significant and present in certain highly transmissible variants like B.1.1.7, B.1.351, B.1.1.28.1, and B.1.429 (Bakhshandeh et al., 2021; Starr et al., 2021). D614G, with glycine substituted for aspartic acid at position 614 in the S protein, was one of the most frequent mutations present in majority of the circulating SARS-CoV-2 strains (Jiang et al., 2020; Korber et al., 2020). In this review, we summarized the impact of D614G mutation on the epidemiology of SARS-CoV-2 and the mechanism of its enhanced infectivity and rapid transmission. We also opened up many interesting questions, aiming to provide new insights into future research on SARS-CoV-2 mutations.

Epidemiology of D614G variant

Because of the RNA proofreading ability of the replication mechanism, virus evolution was considered to be slow (Robson et al., 2020). However, the rapid global spread of SARS-CoV-2 has provided a sufficient opportunity for natural selection to make it possible for rare but beneficial mutations to accumulate. It has been found that mutations of SARS-CoV-2 were accumulated throughout the viral genes that encode the nonstructural proteins, including ORF1a, ORF1b, ORF3, and ORF8, and the structural proteins consisting of N and S proteins (Franco-Muñoz et al., 2020; Ozono et al., 2021), among which mutations in S protein are particularly worthy of attention, because the S protein is responsible for viral entry and its mutations can strongly influence host range, tissue tropism, and pathogenesis (Walls et al., 2020; ZHou et al., 2020). The availability of thousands of SARS-CoV-2 genome sequences provides a valuable resource that can help to better understand the evolution of the virus across time and location. It was observed that a viral variant in the S1 domain of S protein with a single amino at position 614 rapidly became the most prevalent variant all around the world (Isabel et al., 2020; Korber et al., 2020). Analyses revealed that D614G mutation was rare before March 2020, but increased rapidly and replaced the original virus as the main variant by April 2020 (Korber et al., 2020; Figure 1). Moreover, compared with other variants, the transition frequency of D614G occurred asynchronously in different regions, which spread more easily and displayed higher frequency in regions of Europe and North America, but not in East Asia (Bhattacharyya et al., 2020; Korber et al., 2020). Up to now, the demographic distribution analysis indicates that there is no significant difference between the D614G variant and other sequenced SARS-CoV-2 variants (Isabel et al., 2020; Korber et al., 2020).

Previous studies on SARS-CoV have shown that the continuous accumulation of S protein mutations affected its affinity for ACE2 and virus transmission and infectivity (Sui et al., 2008; Du et al., 2009). Therefore, it is conceivable that D614G mutation may also affect the transmission and infectivity of SARS-CoV-2. In agreement with this, the COVID-19 patients infected with G614 variant were significantly higher than those infected with D614 variant in viral loads, as demonstrated by the lower cycle threshold (Ct) in quantitative RT-PCR (Korber et al., 2020; Lorenzo-Redondo et al., 2020; Figure 1). However, in contrast with the above conclusion, one study by Isabel et al. (2020) found no significant difference in the average Ct values between D614 and G614 variants. Considering a relatively small selection of samples in the latter study, it is more likely that the patients infected with G614 variant have higher viral loads. Moreover, it was found that SARS-CoV-2 virus with D614G mutation had significantly higher titers than D614 in a variety of cell types no matter for vesicular stomatitis virus or lentiviral pseudotype virus (Korber et al., 2020; Diez-Fuertes et al., 2021). Consistently, higher infection efficiency was observed for G614 mutation in multiple cells that were infected with similar titers of pseudoviruses produced with the S protein or the intact SARS-CoV-2 carrying either D614 or G614 mutation (Hu et al., 2020; Ogawa et al., 2020; Zhang et al., 2020; Daniloski et al., 2021). Thus, D614G mutation likely enhanced the infection efficiency of SARS-CoV-2 virus. In addition, D614G increases infectivity on target cells bearing ACE2 orthologs from multiple species, including human (*Homo sapiens*), Chinese rufous horseshoe bat (*Rhinolophus sinicus*), Malayan pangolin (*Manis javanica*), cat (*Felis catus*), and dog (*Canis lupis*), indicating that the increased infectivity of D614G is not specific for human ACE2 (Yurkovetskiy et al., 2020). Furthermore, it was observed that D614G does not change the process of S protein synthesis, processing, and incorporation into SARS-CoV-2 particles, suggesting that the increased infectivity caused by D614G mutation is primarily manifested during the period when the virus enters the target cells postassembly (Yurkovetskiy et al., 2020). In agreement with this, our research showed that the luciferase activity, an infection reporter, was higher in cells infected with S-G614 pseudovirus than those infected with S-D614 pseudovirus; moreover, the luciferase activities were positively correlated with membrane fusion index (Jiang et al., 2020). These findings suggested that D614G mutation might promote SARS-CoV-2 infection through membrane fusion-mediated host entry. In addition to *in vitro* experiments, researchers also explored the effects of D614G mutation on SARS-CoV-2 viruses in different animal models such as the golden Syrian hamster and ferret (Plante et al., 2021; Zhou et al., 2021). Results showed that the replication, infectivity, and fitness activities of SARS-CoV-2 with D614G mutation were increased. The same conclusion was obtained in a primary human airway tissue model too.

Mechanisms of enhanced infectivity by D614G mutation

Given that D614G mutation can enhance the replication and infectivity of SARS-CoV-2 virus, efforts were carried out to

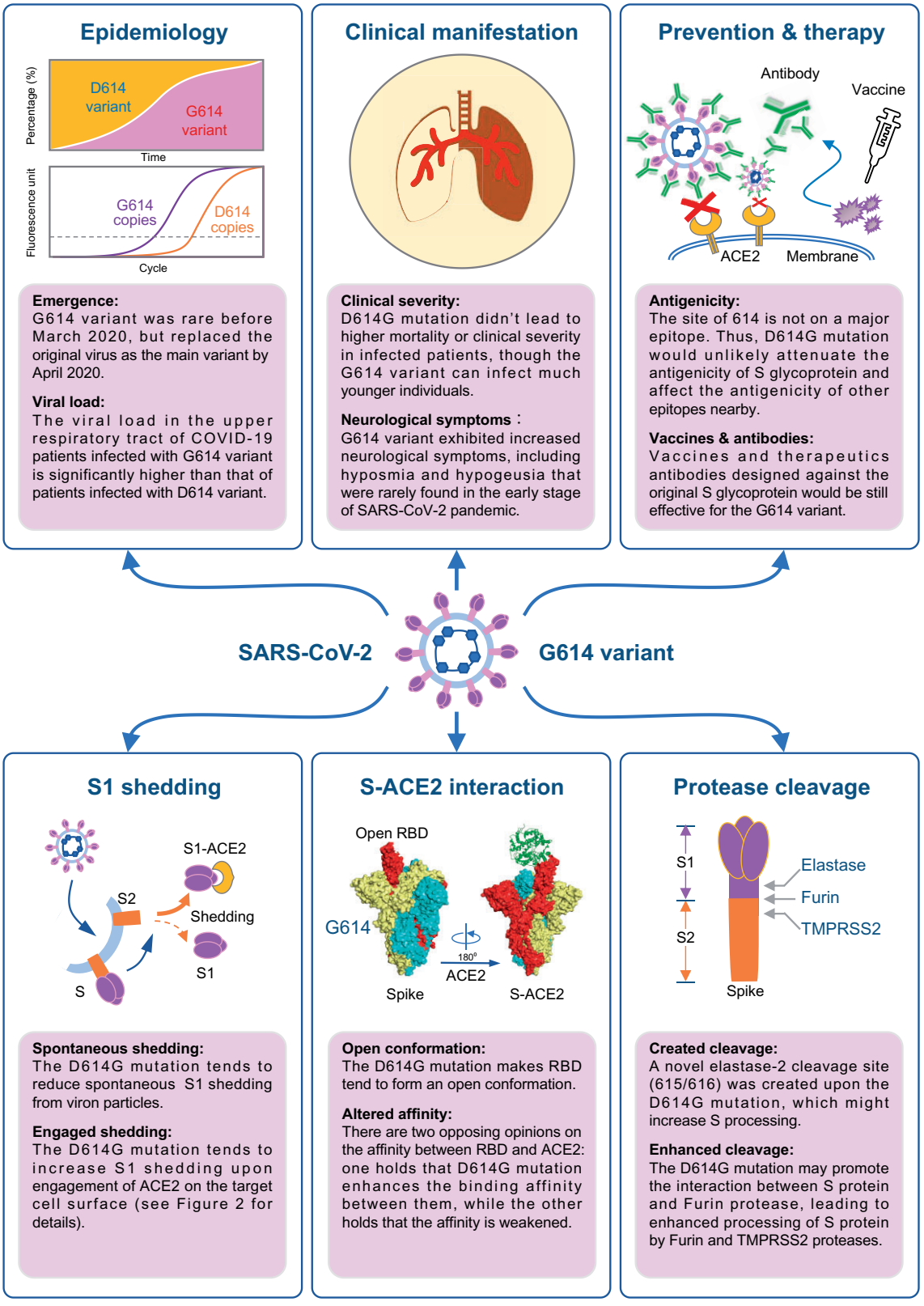

Figure 1 The impacts of D614G mutation on SARS-CoV-2 pathogenesis and the underlying mechanisms.

explore the underlying mechanisms. We summarize the current studies on the mechanisms at three levels: cytological, molecular, and structural.

Effects of D614G mutation on membrane fusion

In the process of SARS-CoV-2 virus infection, the virus first binds to the host cell receptor ACE2, which interacts with S protein to mediate membrane fusion and host entry. It was speculated that D614G mutation in S protein may increase the infectivity of SARS-CoV-2 virus by enhancing membrane fusion. To verify the above view, Ogawa et al. (2020), by coculturing 293T cells expressing S protein (D614 or G614) with cells expressing ACE2, observed an enhanced membrane fusion in S-G614-expressing cells. This was independently validated in our research on syncytia formation in cells stably expressing ACE2 (293T-ACE2 and Hela-ACE2). Results showed that S-G614 exhibited a significantly stronger ability to induce syncytium formation, as evidenced by increased frequency and size (syncytia with more nuclei) of syncytia formation (Figure 2). In line with this, the viruses pseudotyped with S-G614 demonstrated higher infectivity than did S-D614-pseudotyped viruses (Jiang et al., 2020). Other researches also support the above notion that the G614 variant is more effective at membrane fusion than wild-type D614, which is likely to be responsible for the higher infectivity of SARS-CoV-2 and the global transmission (Chen et al., 2020a; Zhang et al., 2021a). Nevertheless, one study notably showed that the difference in fusion activity between D614 and G614 gradually decreased with the increase of the amount of transfected S protein, which indicates that the high expression of S protein may compensate the inferior infectivity of S-D614 protein (Zhang et al., 2021a).

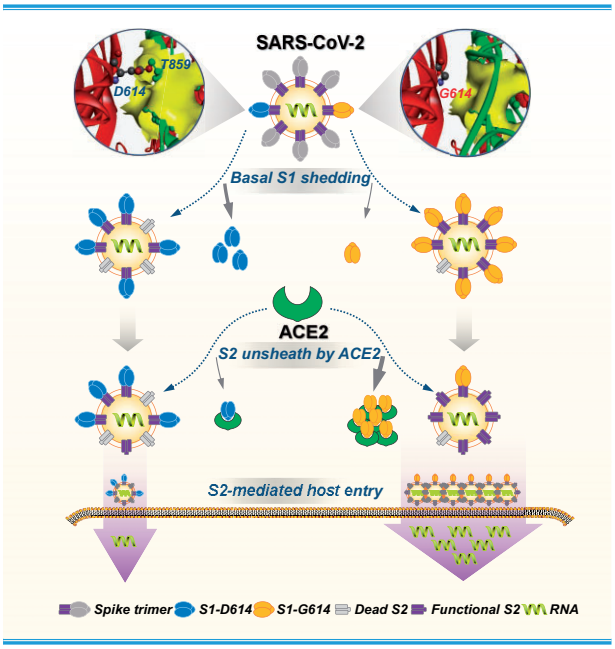

Figure 2 The bimodular effects of D614G mutation on spike protein.

Effects of D614G mutation on S–ACE2 interaction and protease cleavage sites

Similar to SARS-CoV, SARS-CoV-2 binds to ACE2 through the receptor-binding domain (RBD) in the S1 domain of S protein (Walls et al., 2020). The binding affinity between S protein and ACE2 was considered to be associated with virus transmissibility and infectivity (Wan et al., 2020). Compared with SARS-CoV, SARS-CoV-2 was found to be more infectious, and its affinity for ACE2-binding is 10- to 20-fold higher than that of the former (Wrapp et al., 2020). Although D614G is located at the C-terminus of the S1 domain outside of the RBD, this single mutation was believed to enhance S–ACE2 interaction by promoting the open conformation of RBD (Yurkovetskiy et al., 2020), which may conceivably increase S–ACE2 binding. Consistent with this idea, Zhou et al. (2021) reported that S-G614 monomer could bind to ACE2 with higher affinity than did S-D614 monomer (Zhou et al., 2021). However, more studies using S trimer, a functional form of S protein, showed that the interaction between S-G614 and ACE2 was not enhanced or even decreased (Yurkovetskiy et al., 2020; Zhang et al., 2020, 2021a), arguing against that the increased infectivity of D614G mutation may be attributed to altered binding affinity between S and ACE2.

In addition to binding to host cell receptors, the activation of S protein by host cell proteases is another key factor in regulating infectivity and pathogenicity. For efficient host entry of SARS-CoV-2 virus, S protein needs to be cleaved by furin at residues 682–685 into two domains including S1 and S2, which is thought to be important for the conformational transformation required for receptor binding or the exposure of another cleavage site (Walls et al., 2020). The cleavage by TMPRSS2 of S2 domain creating the S2′ fragment, probably subsequent to furin cleavage, is related to the activation of membrane fusion (Bestle et al., 2020). Therefore, the sensitivity of S protein to protease-mediated processing may also be responsible for the enhanced infectivity caused by D614G mutation. It was found that S-G614 was more stable and resistant to proteolytic cleavage in the process of S protein synthesis and virus assembly when the receptor ACE2 was absent, which is consistent with high transmission of the SARS-CoV-2 with D614G mutation (Jiang et al., 2020; Daniloski et al., 2021). However, during SARS-CoV-2 infection when S protein bound to ACE2, D614G mutation increased the shedding of S1, leading to unsheathe of S2 that promotes membrane fusion and host entry of SARS-CoV-2. Thus, D614G mutation may impose a unique bimodular effect on the stability of S protein, i.e. stabilizing S protein on the virions in the absence of ACE2 and increasing S1 shedding upon ACE2 engagement on the cell surface (Figure 2) as proposed previously (Jiang et al., 2020). The increased shedding of S1 upon D614G mutation is believed to result in enhanced cleavage of S protein by proteases (Chen et al., 2020a; Jiang et

al., 2020; Zhang et al., 2020; Gobeil et al., 2021). Consistent with the above results, Mohammad et al. (2020) found that D614G mutation increased the binding affinity of furin and S protein by using PRODIGY server, thermodynamic analysis, and molecular dynamics (MD) simulations. Thus, D614G mutation may improve the S1/S2 cleavage efficiency by increasing the interaction between S protein and furin protease, thereby enhancing viral entry into host cells, and ultimately resulting in higher infectivity (Figure 1). However, it is worth noting that one study showed no difference in S cleavage mode between the two kinds of exocellular SARS-CoV-2 virions in western blotting assay, contrary to the observation reported in the pseudovirus study (Hou et al., 2020). Therefore, the infection modes of pseudovirus and wild SARS-CoV-2 virus might be different, further pointing to essential validation of results from pseudovirus by the authentic SARS-CoV-2 virus.

In addition to the above two cleavage sites, another novel serine protease (elastase-2) cleavage site at residues 615–616 of S-G614 glycoprotein was identified using the method of PROSPER, which is thought to be also important for virus infectivity. Compared with S-D614 pseudovirus, S-G614 pseudovirus more effectively infected 293T-ACE2 cells that had been pretreated with elastase, indicating that D614G mutation can also facilitate elastase-enhanced viral infectivity (Hu et al., 2020; Figure 1). However, McAuley et al. (2020) disagreed with that elastase-mediated S1/S2 cleavage promoted S-G614-mediated infectivity, because MD simulations models suggested that, though furin can access the S1/S2 cleavage site, the elastase cleavage site introduced by D614G mutation cannot be accessed because of its interface positioning and glycan blocking, which can be disturbed by the separation of S1 trimer cap and S2.

Effects of D614G mutation on the structure of S protein

During the process of SARS-CoV-2 virus receptor binding and host entry, S protein undergoes a large-scale structural rearrangement, from a prefusion conformation to a postfusion conformation, which exposes the hydrophobic fusion peptide (FP) promoting cell membrane fusion and virus entry (Cai et al., 2020; Walls et al., 2020; Wrapp et al., 2020). The S1 subunit comprises N-terminal domain (NTD) and RBD, which is responsible for recognizing and binding to ACE2 and contributes to stabilization of the prefusion conformation (Walls et al., 2020; Gobeil et al., 2021). The S2 domain contains FP followed by the second protease cleavage site (S2′), which is responsible for membrane fusion (Hoffmann et al., 2020; Gobeil et al., 2021). In order to interact with ACE2 receptors, the conformation of RBD domains transforms between two states including 'closed' (or down) for the state of receptor inaccessibility and 'open' (or up) for the state of receptor access (Cai et al., 2020; Shang et al., 2020).

The D614 residue of wild-type S protein is located at the surface region, where it interacts with the neighboring subunit. Structural analysis indicated that D614 interacts with the neighboring T859 via a hydrogen bond. However, D614G mutation disrupts the hydrogen bond due to loss of side chains (Gupta et al., 2020; Jiang et al., 2020; Korber et al., 2020; Omotuyi et al., 2020; Yurkovetskiy et al., 2020), possibly enhancing the mainchain flexibility and reducing S1 shedding from viral-membrane-bound S2 (Jiang et al., 2020; Zhang et al., 2020; Figure 1). In addition, the electrostatic bond formed by D614–K854 in the FP proximal region is disrupted by D614G mutation as well, contributing to the fixation of RBD in the prefusion conformation (Gupta et al., 2020). Simultaneously, the D to G mutation may regulate the glycosylation of the nearby N616 site, affecting the dynamics of the spatial proximal FP adjacent to the protomer (Hu et al., 2020; Korber et al., 2020). Moreover, the hydrogen bond between the skeleton amine of the G residue and the carboxyl group of the adjacent amino acid (A647) was shortened, possibly locally stabilizing S protein (Fernández, 2020; Jiang et al., 2020). Hence, D614G mutation may change the structural conformation of S protein, leading to altered stability and related functions, such as membrane fusion.

Though the open conformation of RBD is necessary for ACE2 binding, the binding site of RBD is partially blocked in the closed conformation (Cai et al., 2020). D614 is located on the S1 protomer, close to the RBD, so it is not difficult to infer that the D to G mutation may result in the conformational changes of RBD, thus affect its recognition of ACE2 receptor, and ultimately affect the infection process of SARS-CoV-2. Omotuyi et al. (2020) found that the RBD of G614 mutation was preferentially in an open state, while the wild-type (D614) samples were largely in a closed state (Figure 1). Moreover, compared with the prefusion conformation preferred by D614, the conformation of S2 domain sampling in G614 is mainly postfusion, providing evidence that D614G mutation of S protein is more available for receptor binding and has better fitness than the wild-type (Omotuyi et al., 2020). Cryo-electron microscopy structures of the SARS-CoV-2 trimeric S protein ectodomain have revealed that the RBD of each protomer could adopt the closed or open conformation independently, resulting in asymmetric trimers (Walls et al., 2020). However, D614G mutation can destroy this asymmetry to a certain extent and contribute to symmetrizations from three aspects, such as the interprotomer contacts, the correlations between the RBDs, and the specific interprotomer hydrogen bond. Besides, these symmetrizations of the energetics of D614G mutation lead to the higher population of infection-capable (one-up) conformation, enhancing the exposure of RBD to ACE2 binding and epitope sites (Mansbach et al., 2021). Consistently, while the percentage of D614 RBDs in the open conformation was only 18%, 58% of the protomers had an open conformation in S-G614. Moreover, though G614 RBD overlapped well with D614 RBD in the closed conformation state, G614 showed a significant deviation of all S1 domains away from the S2 subunit compared with D614 of the open conformation. The distribution of RBD conformation was further classified by D614G homotrimers, which showed that 5% of the particles had all

three protomers in the closed conformation, 36% had one open, 39% had two open, and 20% of the particles had all three protomers in the open conformation (Yurkovetskiy et al., 2020). However, in contrast with this, other research found that despite extensive classifications, including searching with low-pass filtered maps, any populations of two or three RBD in the open conformation could not be identified (Gobeil et al., 2021). It is worth noting that although the above studies are inconsistent in some aspects, it is certain that the RBD of G614 variant tends to increase the open conformation, which is preferentially ACE2-binding. Further studies have shown that D614G mutation not only affected the interaction between RBD and ACE2, but also increased furin binding due to the flexibility of S protein, which conceivably promotes SARS-CoV-2 infection by increasing exposure of S protein to cleavage by furin (Mohammad et al., 2020; Figure 1).

Relationship between D614G mutation and severity for COVID-19 patients

A major focus of current research into SARS-CoV-2 genetics is whether any mutations have the potential to significantly alter viral properties, such as transmission, or disease progression. Evolutionary theory predicts that most new viral mutations are deleterious and short-lived, whereas mutations that persist and grow in observed frequency may be selectively or advantageous to viral fitness (Bhattacharyya et al., 2020). Multiple researches have shown that D614G mutation in S protein increased viral fitness, potentially viral loads, and infectivity (Korber et al., 2020; Volz et al., 2021). However, given the rapid expansion and the threat of SARS-CoV-2 virus to the human society, whether D614G mutation causes more severe symptoms for COVID-19 patients caught more attention.

According to the study by Korber et al. (2020) who analyzed 999 individuals with COVID-19 at the Sheffield Teaching Hospitals, the D614G status showed no significant correlation with the disease severity as measured by hospitalization outcomes by regression analysis. Consistent with this conclusion, Mak et al. (2020) tracked and analyzed 113 cases in Hong Kong, from January 21, 2020 to June 12, 2020, and also found that D614G has nothing to do with the severe illness ($P = 1.000$). The latest research also showed that D614G mutation did not lead to higher mortality or clinical severity in infected patients, though the G614 variant can infect much younger individuals (Volz et al., 2021). In summary, current researches so far show that there is no significant correlation between the G614 variant and more severe disease (Figure 1).

Effects of D614G mutation on antigenicity of SARS-CoV-2

The S protein of SARS-CoV-2 is not only related to receptor binding and cell fusion but also can stimulate potent cellular and humoral immune responses, which is an important target for the development of vaccines and therapeutic antibodies (Du et al., 2009; Poland et al., 2020). Up to now, there are multiple vaccines and neutralizing antibodies being explored, and most of them target S protein. Inevitably, there have been mutations of S protein during SARS-CoV-2 virus transmission, and the vaccines and neutralizing antibodies initially developed targeting the original virus in the early stage may not be effective for the later mutant virus.

By evaluating the neutralization activity of serum samples from convalescent patients with COVID-19, no significant difference was identified in protecting against viruses pseudotyped with both S-D614 and S-G614 (Hu et al., 2020; Korber et al., 2020). However, it is unknown what kind of virus initially infected the convalescent patients with COVID-19. Later, to explore the question about whether D614G mutation could reduce the efficacy of the current vaccine based on the original D614 spike sequence, Plante et al. (2021) used a panel of sera collected from hamsters that were previously infected with D614 SARS-CoV-2 to neutralize heterologous G614 virus and homologous D614 virus, respectively. The results showed that all sera exhibited 1.4- to 2.3-fold higher neutralization titers against G614 virus than against D614 virus. After that, they tested the neutralization potency of 11 human RBD mAbs, which further proved that mAbs exhibited 2.1-fold higher potency (mAb18) in protecting against G614 virus or similar neutralization activities (the other 10 mAbs) against both viruses. In order to evaluate the ability of antibodies produced during SARS-CoV-2 infection to react with S-D614 or S-G614, enzyme-linked immunosorbent assay was employed to measure serologic reactivity. As a result, D614G mutation did not alter the performance of IgG, IgM, or IgA seroassay, and all sera from donors who tested positive for D614 antibodies also displayed strong reactivity to G614 spike (Klumpp-Thomas et al., 2020). INO-4800 was a DNA vaccine that had been demonstrated to protect ferrets from SARS-CoV-2 infection. D614G mutation had little influence on the protection efficiency of INO-4800 against SARS-CoV-2 infection (McAuley et al., 2020). So far, all existing studies showed that D614G mutation does not attenuate the antigenicity of S protein, and vaccines and therapeutic antibodies designed against the original S protein would be still effective for the G614 variant (Figure 1). However, it is worth noting that all the experiments that have been conducted were based on the S-protein-pseudotyped viruses. Therefore, these above conclusions warrant further validation with authentic SARS-CoV-2 viruses.

Conclusion and future perspectives

Despite the high mutation rate of RNA virus, many mutations of SARS-CoV-2 were believed to be eliminated by the genetic proofreading mechanism (Sevajol et al., 2014). However, the G614 variant replaced the original D614 variant and became a global pandemic in just two months, indicating that D614G mutation provides a selective advantage for SARS-CoV-2 with more fitness (Hou et al., 2020; Plante et al., 2021; Figure 1). In

multiple assays, D614G mutation was found to replicate more efficiently and increase the infectivity of SARS-CoV-2 virus both in cells and animal models (Jiang et al., 2020; Korber et al., 2020; Plante et al., 2021; Zhou et al., 2021). Mechanistically, the high infectivity of the D614G variant depends partly on its ability to increase host entry, which is largely attributed to altered stability and processing of S protein, leading to enhanced membrane fusion and virus replication (Figure 1). Nevertheless, virus infectivity is not identical to virus transmission, and detailed analysis should be performed on more patients infected with D614 or G614 variants. Although the high infectivity of the G614 variant contributes to its rapid spread, other factors, such as the geographical distribution of host populations, should also be considered. One recent study showed that the high rate spreading of the G614 variant in Europe and North America is related to a single-nucleotide allele deletion that regulates the expression of TMPRSS2 and MX1 host proteins (Bhattacharyya et al., 2020). It should be noted that there are several mutations always coming out together with D614G mutation in SARS-CoV-2 variants, including a C-to-T mutation in the 5′ UTR region at position 241, a silent C-to-T mutation at position 3037, and a C-to-T mutation at position 14408 that results in an amino-acid change in RNA-dependent RNA polymerase (RdRp P323L) (Korber et al., 2020). These mutations, and other accompanying mutations as well, may affect the impacts of D614G mutation and should be taken into account when accessing the infectivity and transmissibility of D614G variants. Last but not least, detection methods and epidemic prevention capabilities are also important factors affecting regional transmission.

Although no significant relationship between D614G mutation and disease severity was identified based primarily on the hospitalization outcomes or the cases of patients with severe symptoms (Korber et al., 2020; Mak et al., 2020), the latest data in western countries indicated that the D614G variant was accompanied by the increased frequency of neurological symptoms, including hyposmia and hypogeusia that were rarely found in the early stage of SARS-CoV-2 pandemic (Alturki et al., 2020; Butowt et al., 2020; Figure 1). Therefore, it is speculated that the enhanced efficiency of virus binding to host cells *in vivo* caused by D614G mutation may be responsible for the high incidence of neurological symptoms (Butowt et al., 2020). Besides, the latest research showed that, even after 6 months of symptom onset, ~76% of the new COVID-19 patients have at least one adverse symptom, 63% of which are mainly fatigue or muscle weakness, and 26% of patients have sleep difficulty (Huang et al., 2021). Overall, these results suggest that, in future research, more attention should be paid to the correlation between certain symptoms, patient prognosis, and D614G mutation or even other mutations such as N501Y (Leung et al., 2021).

At present, most vaccines and antibodies in clinical trials were designed based on S-D614, which is sharply incompatible with the global prevalence of the G614 variant. Given that S-G614 is quite different from S-D614 in structure and biological function, the efficacy of the existing antibodies and vaccines may be carefully tested with the G614 variant before approved for clinical application. Fortunately, most antibodies and sera arose from/against S-D614 were found being able to react with the G614 spike antigens, indicating that the site of 614 is not a major epitope and does not affect the antigenicity of other epitopes nearby, and D614G mutation less likely affects the efficacy of vaccines and antibodies (Hou et al., 2020; Klumpp-Thomas et al., 2020; McAuley et al., 2020; Yurkovetskiy et al., 2020; Guthmiller et al., 2021; Ozono et al., 2021; Plante et al., 2021). Nevertheless, with the ongoing vaccination of larger population, further studies on sera from vaccinees are needed to examine their efficacies on mutated variants including not only D614G but also other newly emerging variants such as the UK VUI-202012/01variant, the South Africa 501.V2 variant, and the Brazil P.1 variant.

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