



Effects of SARS-CoV-2 mutations on protein structures and intraviral protein–protein interactions

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Abstract

Since 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causing coronavirus disease 2019 (COVID-19) has infected 10 millions of people across the globe, and massive mutations in virus genome have occurred during the rapid spread of this novel coronavirus. Variance in protein sequence might lead to a change in protein structure and interaction, then further affect the viral physiological characteristics, which could bring tremendous influence on the pandemic. In this study, we investigated 20 nonsynonymous mutations in the SARS-CoV-2 genome in which incidence rates were all $\geq 1\%$ as of September 1st, 2020, and then modeled and analyzed the mutant protein structures. The results showed that four types of mutations caused dramatic changes in protein structures (RMSD ≥ 5.0 Å), which were Q57H and G251V in open-reading frames 3a (ORF3a), S194L, and R203K/G204R in nucleocapsid (N). Next, we found that these mutations also affected the binding affinity of intraviral protein interactions. In addition, the hot spots within these docking mutant complexes were altered, among which the mutation Q57H was involved in both Orf3a–S and Orf3a–Orf8 protein interactions. Besides, these mutations were widely distributed all over the world, and their occurrences fluctuated as time went on. Notably, the incidences of R203K/G204R in N and Q57H in Orf3a were both over 50% in some countries. Overall, our findings suggest that SARS-CoV-2 mutations could change viral protein structure, binding affinity, and hot spots of the interface, thereby might have impacts on SARS-CoV-2 transmission, diagnosis, and treatment of COVID-19.

KEYWORDS

mutation, protein structure, protein–protein interaction, SARS-CoV-2

1 | INTRODUCTION

The ongoing outbreak of coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has been characterized as a pandemic by the World Health Organization (WHO). SARS-CoV-2 is a positive-sense single-stranded RNA virus and belongs to betacoronavirus,¹ whose genome has ~30,000 nucleotides,

containing 12 open-reading frames (ORFs), encoding 4 structural and 22 nonstructural proteins. The structural proteins include spike protein (S), envelope protein (E), membrane protein (M), and nucleocapsid protein (N), while the nonstructural proteins (nsp) contain nsp1–16 encoded by ORF1ab and six accessory proteins, which are Orf3a, 6, 7a, 7b, 8, and 9b.² It is known a virus that undergo evolution and natural selection, and most of them evolve rapidly. As reported, the

evolution rate of a typical RNA virus is about 10^{-4} substitutes per year per site,³ and mutations could occur during each replication cycle. The high mutation rates of RNA viruses, coupled with short generation times and large population sizes, allow viruses to evolve rapidly and adapt to the host environment. The rapidity of viral mutation also causes problems in the development of successful vaccines and antiviral drugs. With the continued spread of SARS-CoV-2 around the world, thousands of mutations have been identified, some of which have relatively high incidences, but their potential impacts on virus characteristics still remain unknown.

Among all the mutations, nonsynonymous mutations cause amino acid substitutions, then could alter virus protein structures, which might affect viral reproduction and give rise to false-negative diagnoses and drug resistance. It is reported that, after depletion of nine amino acids in SARS-CoV-2 Orf6, its protein structure was dramatically changed and its transmembrane localization was also shifted, which would lead to interferon (IFN) resistance during antiviral therapy.⁴ Additionally, along with the occurrence of S139A and F140A mutations, the structure of SARS-CoV 3CLpro altered remarkably, followed by the decrease of its enzyme activity.⁵ Furthermore, modified protein structure begotten by mutations can also affect protein–protein binding, and intraviral protein–protein interactions are often indispensable in assembly and release of coronavirus. In SARS-CoV, the interactions between structural proteins are essential for its maturation,⁶ while the binding between nonstructural proteins guarantees the completion of virus replication.⁷ Besides, mutations I529T and D510G in MERS-CoV S reduced the binding affinity of the receptor-binding domain (RBD)-CD26, respectively, which impaired the virulence of the virus.⁸ However, it is still unclear whether some SARS-CoV-2 mutations can lead to the changes in protein structures, protein–protein interactions, protein function, and even virus infection, which could strike on COVID-19 epidemic control.

In this study, we selected the nonsynonymous mutations of SARS-CoV-2 with $\geq 1\%$ incidence, predicted and compared their protein structures, and found that four types of mutations had significant impacts on protein structures, which resulted in remarkable changes in binding affinities and hot spots between virus proteins. Besides, statistical analyses on mutation rates exhibited dynamic changes in the process of time, and also demonstrated that these four SARS-CoV-2 variants were widely distributed and had relatively high incidences in certain countries. These findings on SARS-CoV-2 mutations would be very helpful for better understandings of this virus and dealing with the complicated situations in COVID-19 prevention, diagnosis, and treatment.

2 | METHODS

2.1 | Online resources

All information about SARS-CoV-2 genomes were obtained from the China National Center for Bioinformation 2019 Novel Coronavirus Resource (CNCB 2019nCoV; <https://bigd.big.ac.cn/ncov>).

2.2 | Protein structure prediction

Protein structure of control SARS-CoV-2 S was from C-I-TASSER (<https://zhanglab.ccmb.med.umich.edu/C-I-TASSER/2019-nCoV/>),⁹ while other SARS-CoV-2 protein structure models were predicted by I-TASSER.¹⁰ For each protein, five models were generated and the model with the highest C-score was selected as the best one and used for the following analysis.

2.3 | Protein structure alignment

The mutant protein structures were aligned to corresponding control ones by using the TM-align web-server (<https://zhanglab.ccmb.med.umich.edu/TM-align/>).¹¹ Random structural similarity was determined by TM-score between 0.0 and 0.3 and root-mean-square deviation (RMSD) $\geq 5.0 \text{ \AA}$.^{11,12}

2.4 | Molecular docking and hot spots prediction

Protein–protein docking was performed with the HADDOCK web-server (<http://haddock.chim.uu.nl/>).¹³ The structure was chosen according to the HADDOCK score, and a complex binding affinity was calculated by PRODIGY.^{14,15} Hot spots within protein–protein interfaces were predicted using Knowledge-based FADE and Contacts Server (KFC, https://mitchell-web.ornl.gov/KFC_Server/index.php).¹⁶ Data visualization was accomplished by PyMOL.

3 | RESULTS

3.1 | SARS-CoV-2 mutations led to altered protein structures

As the COVID-19 pandemic is spreading around the world, more than 10,000 SARS-CoV-2 mutations have been evolved. To investigate these SARS-CoV-2 nucleotide polymorphisms, first, one of the earliest reported SARS-CoV-2 genome sequences (GenBank accession number: MN908947.3) was chosen as a control, then 20 nonsynonymous mutations with frequency $\geq 1\%$ were selected from CNCB 2019nCoV database (see Section 2), as of September 1st, 2020, which were located in 10 different SARS-CoV-2 protein-coding regions (Table S1). Specifically, there was one amino acid substitution in nsp5, nsp6, or nsp7. Meanwhile, two single substitutions were observed in nsp2, nsp12, nsp13, S, Orf3a, or Orf8, among which mutation D614G in S had the highest incidence (43.27%), and five single ones were found in N. In particular, there were also some mutation combinations, such as P504L/Y541C in nsp13 and R203K/G204R in N (Table S1). Briefly, there were 17 types of mutants composed of 20 mutations with $\geq 1\%$ incidence, which included one nsp5, nsp6, nsp7, or nsp13 mutant, two nsp2, nsp12, S, Orf3, or Orf8 mutants and three N mutants.

To examine whether these mutations had effects on protein structures, three-dimensional (3D) structures of each mutant and its reference protein were predicted with I-TASSER (Table S1),¹⁰ and the structural similarity between mutant and control protein were measured by RMSD.¹¹ After structural alignment, we found that four mutants exhibited a significant difference in protein structural morphology from their control ones (RMSD ≥ 5.0 Å), which were Q57H Orf3a, G251V Orf3a, S194L N, and R203K/G204R N (Figure 1 and Table S1).

3.2 | SARS-CoV-2 mutations resulted in the changes in protein-protein interactions

The SARS-CoV-2 proteins have been shown to display characteristic SARS-CoV features.^{17,18} So here, we referenced SARS-CoV protein

combined patterns and explored the effect of these SARS-CoV-2 mutations related to mutant structure alterations on intraviral protein interaction, which was known as a rate-limited procedure for virus reproduction.^{19–22} Among 16 mutant docking pairs, five mutant complexes had significantly higher binding affinity than control ones (Figure 2). Differently, another four mutant pairs showed weakened binding affinity in comparison with the control, and there was no obvious change in the remaining seven groups in which absolute values of $\Delta\Delta G$ ($=G_{\text{control}} - \Delta G_{\text{mutant}}$) were <1 kcal/mol (Figure 2). Strikingly, the binding affinity of Q57H Orf3a–S complex showed the greatest increase ($\Delta\Delta G = 4.2$ kcal/mol), while the most dramatic decrease was observed in the binding affinity of G251V Orf3a–M complex ($\Delta\Delta G = -2.3$ kcal/mol; Figure 2A). Moreover, in the process of Orf3a interacting with M or S, Q57H mutant pairs showed enhanced binding affinity, while G251V mutant pairs had attenuated ones as just mentioned (Figure 2A), suggesting that diverse amino

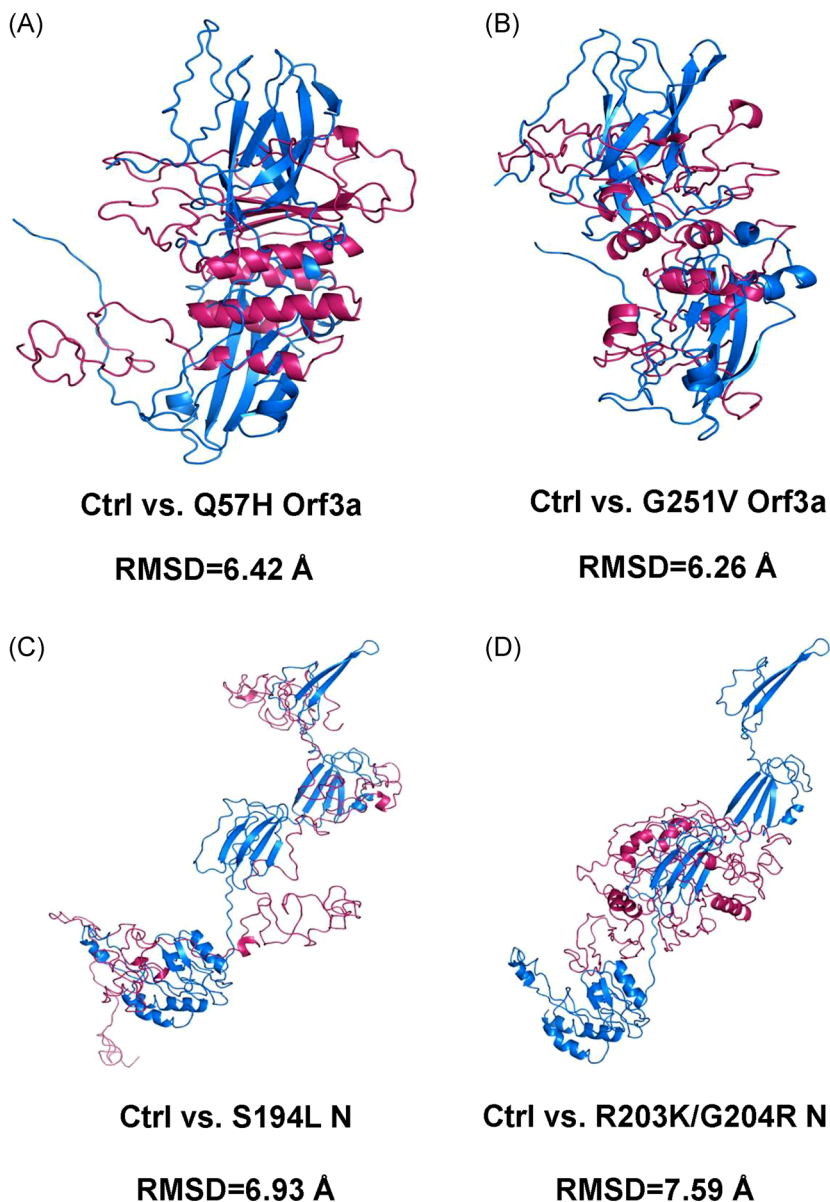


FIGURE 1 Comparisons of protein structures between SARS-CoV-2 control and mutant proteins. The aligned structures of control ones and Q57H Orf3a (A), G251V Orf3a (B), S194L N (C), and R203K/G204R N (D) are shown in blue (control, Ctrl) and warm pink (mutant) with the value of RMSD below. N, nucleocapsid protein; RMSD, root-mean-square deviation; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2

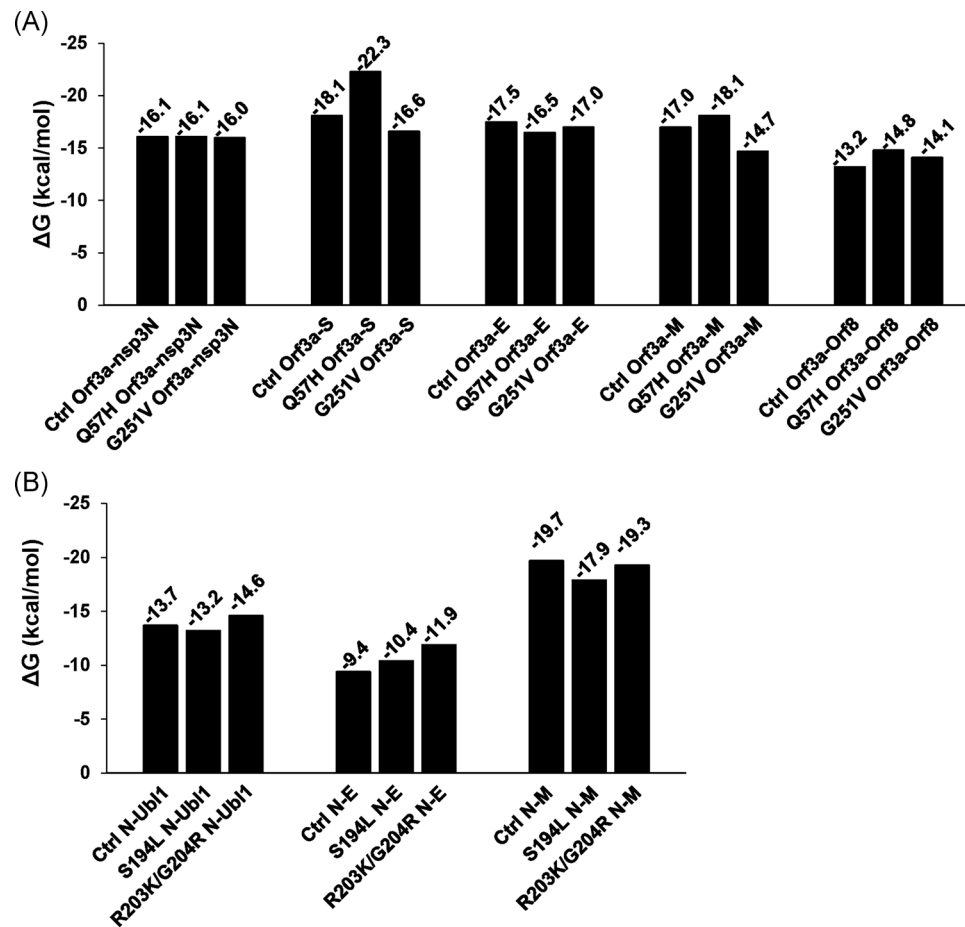


FIGURE 2 Protein-binding affinity in SARS-CoV-2 mutant complexes. Bars show Gibbs-free energy (ΔG) for representing the binding affinity in Orf3a (A) and N (B) relevant complexes. Nsp3N: 1-595aa of nsp3, Ubl1: 1-112aa of nsp3. E, envelope protein; M, membrane protein; N, nucleocapsid protein; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; S, spike protein

acid replacements in the same protein have given different consequences on binding affinity.

sites and lead to therapy failure by shifting the protein-binding interface.

3.3 | SARS-CoV-2 mutations caused varied hot spots within protein complexes

Hot spots are functional sites within protein-interacting interfaces, which are conservative and often taken as attractive drug targets via preventing protein-protein interactions. To further study the influence of SARS-CoV-2 mutations on protein-binding hot spots, we predicted the hot spots in nine mutant complexes with altered bind affinity using the KFC2 server (Figure 3, S1, S2, S3, and S4). Surprisingly, the results showed that the binding hot spots on mutant protein-protein interfaces were notably different from control ones, and few identical ones were shared by both the mutant and control complexes. In particular, Q57 residue in control Orf3a was not involved in the protein-binding interfaces, but amino acid substitution Q57H became a hot spot in both Orf3a-S and Orf3a-Orf8 complexes (Figure 3). These results indicated that SARS-CoV-2 mutations might destroy drug-targeting

3.4 | SARS-CoV-2 mutations were globally distributed with dynamic incidences over time

The changes in SARS-CoV-2 proteins caused by mutations can affect virus transmission, pathogenesis, and immunogenicity. And with the spread of the COVID-19 pandemic, the incidence and lethality of SARS-CoV-2 infection varied from country to country. Hence, to link SARS-CoV-2 mutations and COVID-19 prevalence, we analyzed the occurrence of these four types of mutations (Q57H and G251V in Orf3a, S194L and R203K/G204R in N) based on CNCB 2019nCoV. To avoid deviation caused by insufficient sample size, we screened out the countries or regions where the number of SARS-CoV-2 genome sequences submitted was no more than 100, as of September 1st, 2020. The statistical results showed that SARS-CoV-2 mutations with high incidences were distributed nearly all over the world with dynamic occurrences over time (Figure 4). The frequency of all these mutations was relatively high in some countries

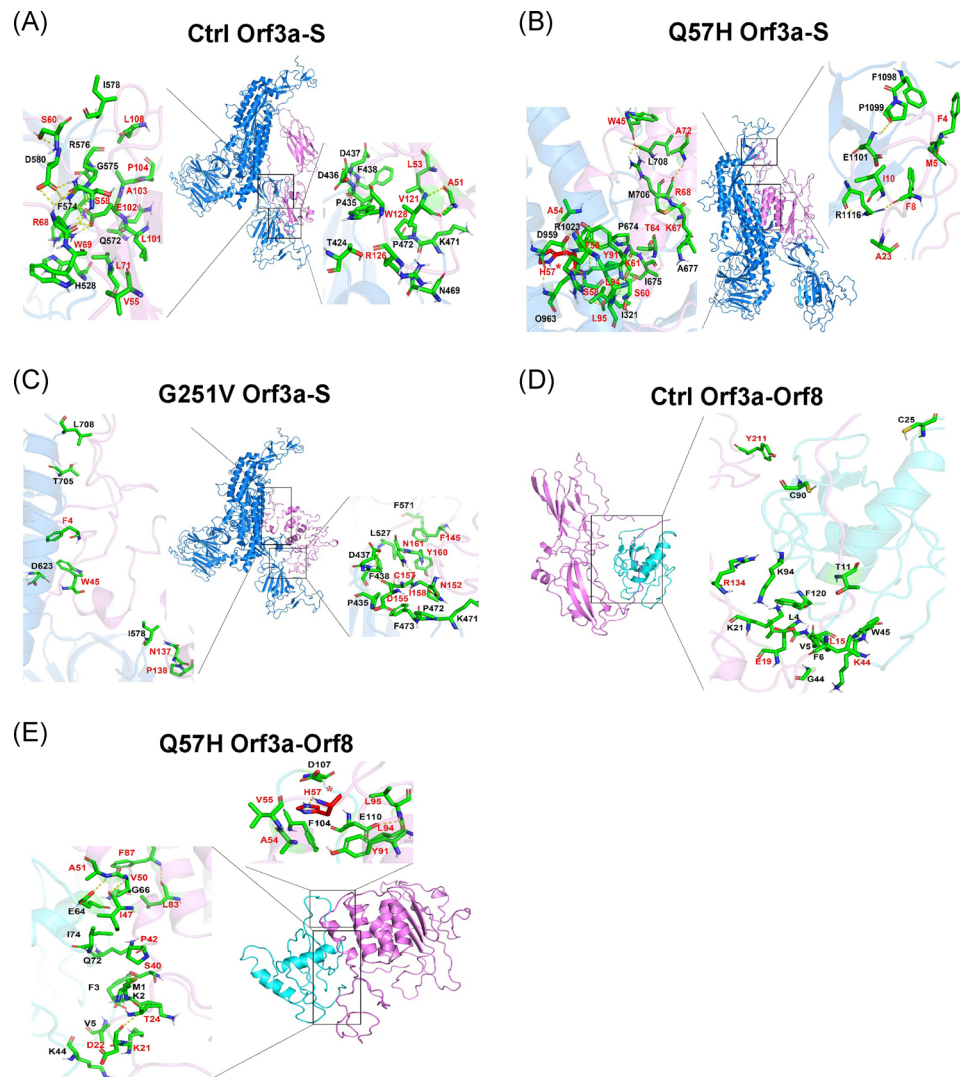


FIGURE 3 Hot spots within interactions between SARS-CoV-2 Orf3a and S or Orf8. The hot spots in Ctrl Orf3a-S (A), Q57H Orf3a-S (B), G251V Orf3a-S (C), Ctrl Orf3a-Orf8 (D), or Q57H Orf3a-Orf8 (E) complexes are shown as sticks. Control and mutant Orf3a are shown in violet, and S or Orf8 is shown in marine or cyan. The residues of Orf3a and docking proteins are colored in red and black, respectively. Asterisk (*) represents a mutated residue. N, nucleocapsid protein; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; S, spike protein

(Figure 4). Among them, we noticed that the incidence of R203K/G204R in N was as high as 86.97% in Bangladesh; meanwhile, it was also over 50% in the other four countries, with a trend of escalation (Figure 4D). Analogously, the frequency of Q57H in Orf3a in Finland, Egypt, South Korea, and Denmark had shown high incidences, which were 69.4%, 67.26%, 59.72%, and 58.03%, respectively (Figure 4A).

4 | DISCUSSION

The mutation is one of the major mechanisms of how viruses undergo continuous change as a result of genetic selection. Although most of the point mutations are neutral and do not change the protein that the gene encodes, yet there are always some occasionally favorable ones that can help viruses confer evolutionary advantages such as an

abrupt epidemic outbreak. In addition, the single amino acid substitution would alter protein structure as well, and it has been reported that G104E mutation in SARS-CoV nsp9 could prevent viral replication via changing protein structure and further destroying the helix-helix interface.²³ In the same vein, masses of single nucleotide polymorphisms (SNPs) have emerged in SARS-CoV-2 genomes. According to the data in CNCB 2019nCoV, as of September 1st, 2020, we screened 20 SARS-CoV-2 nonsynonymous mutations with $\geq 1\%$ incidence, which were categorized into 17 types of mutation combinations. After structural alignment, four protein mutants (Q57H Orf3a, G251V Orf3a, S194L N, and R203K/G204R N) were found displaying different structures from control ones, which corresponding mutations were located in the coding region of N and Orf3a known as essential proteins for coronavirus assembly²⁴ and cytotoxicity.²⁵ Remarkably, the incidences of Q57H in Orf3a and

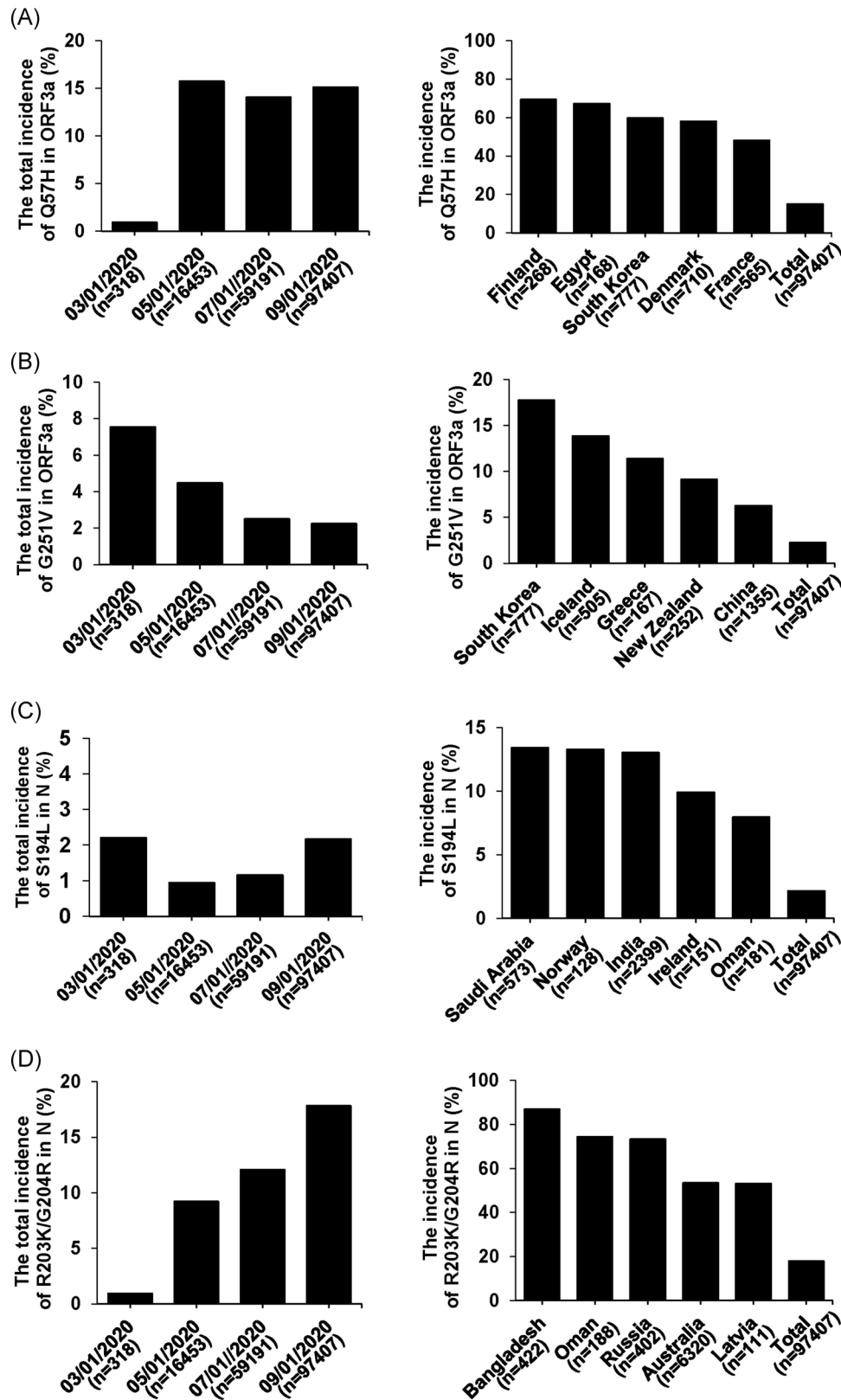


FIGURE 4 Temporal and spatial analyses of SARS-CoV-2 mutation incidences. Bars show the incidences of Q57H (A) and G251V (B) in Orf3a, S194L (C) and R203K/G204R (D) in N at different time periods or in the top five countries and globe (total), as of September 1st, 2020. N represents the all available nucleotide sequences of the country or globe submitted. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2

R203K/G204R in N were quite high (15.12% and 17.84%), which could be a sign that these mutations are correlated with enhanced virulence, evolvability, and traits considered beneficial for the virus. However, it is interesting that some mutations, such as P323L in nsp12 and D614G in S, with even much higher occurrence rates (43.21% and 43.27%) were found with no significant impact on protein structure, which indicates they maybe could affect virus characteristics via altering RNA second structure,²⁶ protein stability,²⁷ or partial structure,²⁸ instead of integral protein structure. For instance, a study reported that D614G in S could lead to increased virus infectivity by eliminating side-chain hydrogen bond, which was only a tiny change in overall protein structure,²⁹ and our data also showed a slight structural difference between control and D614G mutant (RMSD = 2.33 Å). Therefore, more research about SARS-CoV-2 mutations are worth doing to determine whether these structure alterations of Orf3a or N could influence protein function and even virus infectivity.

Besides the protein structure alteration, the mutations in the SARS-CoV-2 genome could also affect protein-protein interactions. With molecular docking and ΔG value calculation, we found that protein-binding affinity changed between each of all four above mutants and their intraviral docking proteins, and these protein interactions in SARS-CoV have been confirmed to play indispensable mediating roles in virus replication and infected ability.^{6,25,30} Moreover, the combination of SARS-CoV Orf3a and S can prevent the release of premature viral RNA,³¹ and Orf3a-M complex is localized in Golgi,³² where virus particles are assembled by budding.³³ Our results showed stronger binding affinities in Q57H Orf3a-M and Q57H Orf3a-S complexes, but weaker affinities in G251V Orf3a-M and G251V Orf3a-S complexes. Meanwhile, considering the great disparity between the incidences of Q57H in Orf3a (15.12%) and G251V in Orf3a (2.25%), it could be speculated that the virus assembly and transmission would be disproportionately affected by different Orf3a mutations. Furthermore, some research indicated that the interaction between N and E had a function in the SARS-CoV virus release,^{21,34,35} and the N-M complex was necessary for the assembly of coronavirus.²² Therefore, the enhanced interaction between each SARS-CoV-2 N mutant (S194L or R203K/G204R) and E might promote virus release, while decreased binding affinity of S194L N-M might attenuate virus assembly. Besides, SARS-CoV N can bind to heterogeneous nuclear ribonucleoprotein A1 (hnRNP A1) of host cells and their interaction plays a regulatory role in the synthesis of SARS-CoV RNA,³⁶ so it would be interesting to detect whether these N mutants would interfere with the combination of proteins in host cells. What is more, the hot spots that play critical roles in protein-protein interactions can be used as drugs target,³⁷ and our results demonstrated that all these selected SARS-CoV-2 mutations (Q57H and G251V in Orf3a, S194L and R203K/G204R in N) had great influences on hot spots within protein combinations, which the changes could have strong impacts on clinical treatment. Nevertheless, scientific experiments are indispensable to clarify the impacts of these mutations on virus characteristics, such as investigating mutated

virus protein interaction with its partners and analyzing the infectivity of the mutated virus to host cells, which it is essential to confirm the interference of SARS-CoV-2 mutations during the development of vaccines or drugs.³⁸ Previous experimental research showed that Y195A mutation in SARS-CoV M was found to disrupt its interaction with S, resulting in a declined ability of virus assembly.³⁹ Accordingly, mutations of the virus could alter intraviral protein-protein interactions and the characteristics of the virus, which illustrate that our results are valuable for providing important proofs for future studies.

In the last several months, populations in more than 180 countries/regions have been affected by SARS-CoV-2, and the emergence of mutations probably already makes important contributions to virus adaption to new environments and selective pressures, which, in turn, can impact transmissibility, pathogenesis, and immunogenicity of SARS-CoV-2.⁴⁰ It has been reported that variant SARS-CoV-2 genomes occurred in different areas.⁴¹⁻⁴⁵ Our results also showed that some mutations were widely distributed, and their occurrence rates showed different dynamic fluctuations. Take R203K/G204R in N as an example; this mutation combination with increased incidence was found mainly in Europe till March 2020,^{46,47} while its high frequency also happened in Asian and African countries as of September 2020. Although there is no reliable evidence for any necessary link between SARS-CoV-2 mutations and epidemic outbreak in specific countries/regions, it is still particularly important to certify the relevance of geographically aggregated mutations to SARS-CoV-2 transmission and pathogenicity for effective containment of COVID-19 outbreak.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Siqi Wu, Chang Tian, and Lijun Liu conceived the study. Siqi Wu, Chang Tian, and Panpan Liu collected and summarized the data of SARS-CoV-2 mutations information. Yang Zhang, Wei Zheng, Chang Tian, Siqi Wu, and Panpan Liu contributed to protein structure prediction. Xiaoqiang Huang, Chang Tian, Siqi Wu, and Panpan Liu contributed to molecular docking. Chang Tian, Siqi Wu and Panpan Liu predicted hot spots within the protein-protein interface. Dongjie Guo contributed to protein structures alignment and data visualizations. Siqi Wu, Chang Tian, and Lijun Liu wrote the paper. Lijun Liu directed the study.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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