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Review Article

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SARS-CoV-2 Omicron Mutation Is Faster than the Chase: Multiple Mutations on Spike/ACE2 Interaction Residues

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ABSTRACT

Recently, a new severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (B.1.1.529) Omicron variant originated from South Africa in the middle of November 2021. SARS-CoV-2 is also called coronavirus disease 2019 (COVID-19) since SARS-CoV-2 is the causative agent of COVID-19. Several studies already suggested that the SARS-CoV-2 Omicron variant would be the fastest transmissible variant compared to the previous 10 SARS-CoV-2 variants of concern, interest, and alert. Few clinical studies reported the high transmissibility of the Omicron variant but there is insufficient time to perform actual experiments to prove it, since the spread is so fast. We analyzed the SARS-CoV-2 Omicron variant, which revealed a very high rate of mutation at amino acid residues that interact with angiostatin-converting enzyme 2. The mutation rate of COVID-19 is faster than what we prepared vaccine program, antibody therapy, lockdown, and quarantine against COVID-19 so far. Thus, it is necessary to find better strategies to overcome the current crisis of COVID-19 pandemic.

Keywords: COVID-19 Omicron; SARS-CoV-2; *Spike* (*S*) gene; Mutation; Receptor binding motif (RBM)

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Conflict of Interest
The authors declare no potential conflicts of interest.

Abbreviations
ACE2, angiotensin-converting enzyme 2; COVID-19, coronavirus disease 2019; NTD, N-terminal domain; RBD, receptor binding domain; RBM, receptor binding motif; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; S, spike; S, subunit; WT, wild type.

Author Contributions
Conceptualization: Kim S[1], Kim S[2], Nguyen TT, Jhun H, Park HY, Kim SH, Kim YG, Song EY, Lee Y, Yum H, Shin KC, Song CS, Yeom SC, Kim B, Choi YK, Netea M; Funding acquisition: Kim S[1], Jhun H; Project administration: Kim S[1]; Supervision: Kim S[1]; Validation: Kim S[2], Nguyen TT; Writing - original draft: Kim S[1]; Writing - review & editing: Kim S[1], Kim S[2], Nguyen TT, Taitt AS.

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INTRODUCTION

Currently, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Omicron (B.1.1.529) variant is the highest transmissible variant compared to the previous 10 SARS-CoV-2 variants. SARS-CoV-2 Delta variant was the dominant transmissible variant before the SARS-CoV-2 Omicron variant had occurred (1-4). The mutation sites of the SARS-CoV-2 Omicron and Delta variant were obtained from the United State of Centers for Disease Control and Prevention (https://www.cdc.gov/coronavirus/2019-ncov/variants/variant-info.html). Recent studies suggested that SARS-CoV-2 variants contributed to severe pathogenesis and death, particularly in unvaccinated people (5,6). SARS-CoV receptor on the surface of the host cell was identified as an angiostatin-converting enzyme 2 (ACE2) about 19 years ago (7). In the absence of biochemical data, ACE2 was considered to be a receptor of SARS-CoV-2 since spike (*S*) gene of SARS-CoV shares 76% identity with that of SARS-CoV-2 (8-15).

The *S* gene is composed of 1,273 amino acid residues that are divided into 16 subdomains by more structural information than functional property except for the receptor binding domain (RBD). The suggested amino acid residue of the RBD is varied by different studies (9,10,13,16-18). Our previous study found that the critical amino acid residues in the receptor binding motif (RBM) of *S* gene were varied in 4 different reports (19). The analysis of ACE2 binding residue from 4 different studies revealed that only 6 amino acid residues (Y449, Y453, F486, N487, Q498, and T501) in RBM are common binding residues among 21 suggested interacting residues within 69 amino acid residues of RBM (19). These 6 amino acid residues elucidate only 28% of the 21 suggested interacting residues, which is an unexpected result because the protein complex structure has been generated by identical spike and ACE2 protein (9,13,14,18).

In the present review, we compared the highest transmissible SARS-CoV-2 Omicron variant to the previously dominant SARS-CoV-2 Delta variant. Omicron has more than twice the number of mutation sites compared to that of Delta including severe mutations on spike/ACE2 interaction residues in RBM. These mutation sites are found in the ACE2 binding residues of SARS-CoV-2 Omicron, which may contribute to the high transmissibility of Omicron.

ANALYSIS OF SARS-CoV-2 OMICRON VARIANT SEQUENCE

New SARS-CoV-2 Omicron (B.1.1.529) variant was first detected from South Africa on the 16th of November 2021. It rose rapidly from 273 patients on the 16th of November to more than 1,200 patients by the 25th of November. More than 80% of SARS-CoV-2 Omicron cases were in the Northern province of Gauteng, where the first cases were seen (20). Currently SARS-CoV-2 Delta variant is the dominant variant however researchers speculate that the SARS-CoV-2 Omicron variant will take over the SARS-CoV-2 Delta variant since the transmissibility of SARS-CoV-2 Omicron variant 1.8–7.0 folds higher than the variant of concern such as SARS-CoV-2 Alpha, Beta, Gamma, and Delta (21).

The whole amino acid sequence of *S* gene from the SARS-CoV-2 Omicron variant was aligned with that of the SARS-CoV-2 wild type (WT) in **Fig. 1A**. The result showed that 39 mutations were highlighted by different colors with bold letters. The mutation residues are also indicated by the absence of an asterisk at the bottom of the alignment. Several

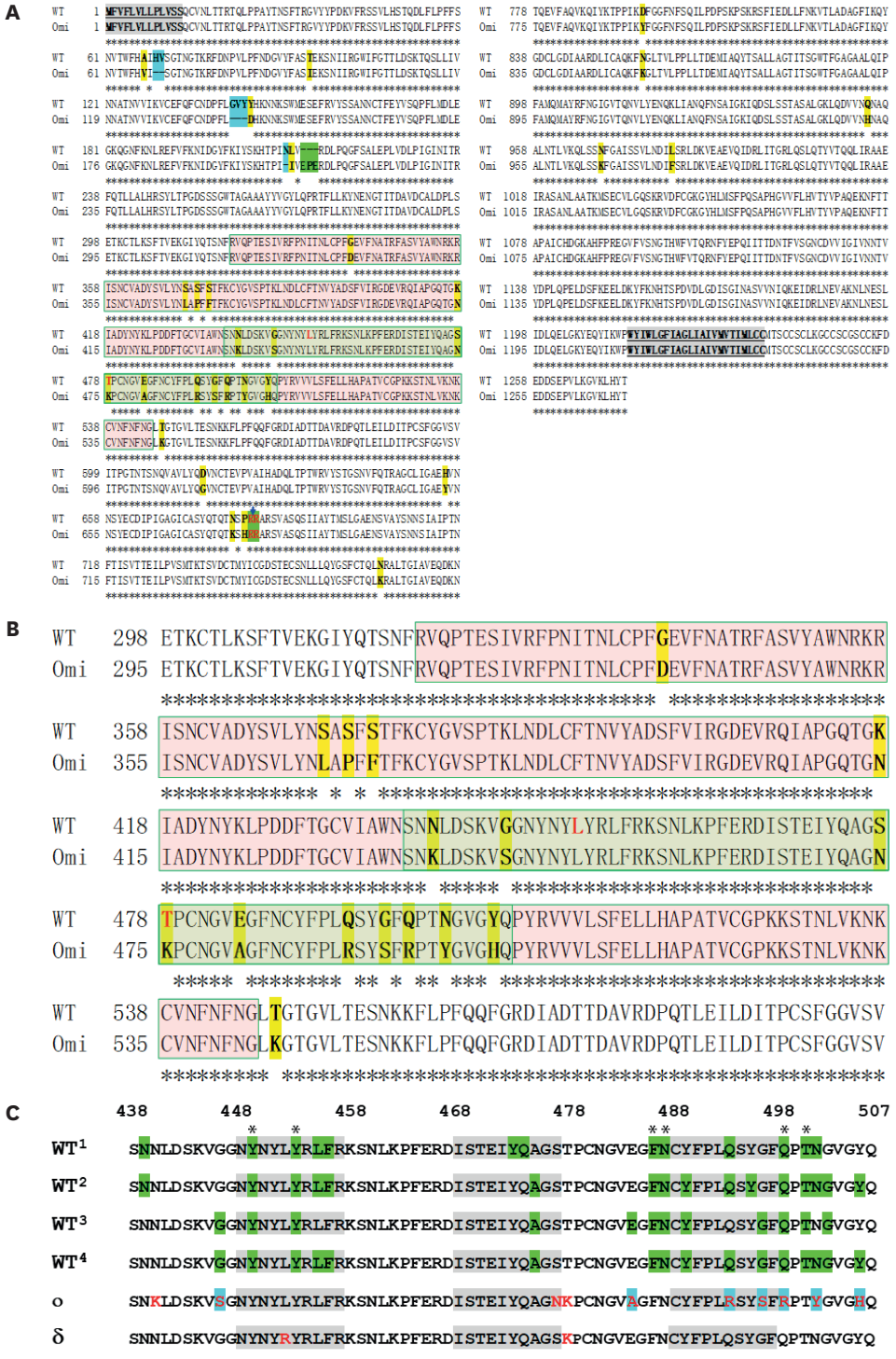

Figure 1. Comparison of SARS-CoV-2 WT to SARS-CoV-2 Omicron *S* protein. (A) The whole *S* protein sequence of SARS-CoV-2 Omicron was compared to that of SARS-CoV-2 WT. The mutated residue was highlighted by different colors such as yellow for amino acid change, blue for deletion, and green for insertion. RBD was highlighted by light pink and RBM was highlighted by light green. The signal sequence and transmembrane are in bold letters and underlined, it is also highlighted in gray. The absence of an asterisk at the bottom of alignment indicates a mutation site. (B) The RBD and RBM region was enlarged for further analysis. There are 15 mutations in RBD and 10 mutations in RBM of SARS-CoV-2 Omicron, whereas only 2 mutations (red bold letter) in RBM of SARS-CoV-2 Delta variant. (C) The alignment of Omicron and Delta *S* protein was compared to the ACE2 receptor interaction sites, which were reported by WT[1] (13), WT[2] (14), WT[3] (18), and WT[4] (9). The 21 receptor binding residues were indicated by the green highlight. The 6 common ACE2 interaction sites were marked by an asterisk on the top to indicate the location (9,13,14,18). The mutation residues in the RBM of Omicron and Delta were indicated by a red letter. The 7 receptor binding residues of Omicron in RBM were highlighted by blue color.

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Table 1. *S* mutation residue of SARS-CoV-2 Omicron and Delta variants

Pango lineage	Origin	Variant name (greek alphabet)	*S* protein mutations	Classification (WHO/CDC)
B.1.617.2	India	Delta, δ	T19R, V70F*, T95I, G142D, E156del, F157del, R158G, A222V*, W258L*, K417N*, L452R, T478K, D614G, P681R, D950N	VOC (3-6)
B.1.1.529	South Africa	Omicron, o	A67V, H69del, V70del, T95I, **G142del, V143del, Y144del, Y145D, N211del, L212I, ins214E, ins215P, ins216E, G339D, S371L, S373P, S375F,** K417N, **N440K, G446S,** S477N, T478K, E484A, **Q493R, G496S, Q498R,** N501Y, **Y505H, T547K,** D614G, H655Y, **N679K,** P681H, **N764K, D796Y, N856K, Q954H, N969K, L981F**	VOC (24)

The mutation sites in *S* gene of SARS-CoV-2 Omicron and Delta VOC were shown. Pango lineage, origin, variant name, mutation residue, and classification of SARS-CoV-2 Omicron and Delta variants were listed. The mutation residues in RBM were highlighted by green and the mutation residues in RBD was highlighted by orange. The RBM is located within the RBD. The 3 new mutations in Delta and 27 new mutations in Omicron were indicated by red and red bold letter, respectively.
VOC, variant of concern; WHO, World Health Organization; CDC, Centers for Disease Control and Prevention. *Detected in some sequences but not all.

announcements said that SARS-CoV-2 Omicron has 34 mutations, but it has 39 mutations in *S* gene (**Table 1**). This discrepancy has happened since serial mutations such as double or triple amino acid residues were indicated as a single mutation, for example, del69-70, del142-144, and ins214EPE.

The del69-70 is 2 residues of H69del and V70del deletion. The del142-144 is 3 residues of G142del, V143del, and Y144del deletion. The ins214EPE is 3 residues of ins214E, ins215P, and ins216E insertion (**Table 1**). These 3 occasions add 5 additional mutations resulting in those 34 mutations becoming 39 mutations. Unique mutation sites of SARS-CoV-2 Delta and SARS-CoV-2 Omicron variant's residues were indicated by red bold letters. SARS-CoV-2 Delta variant has only 3 new mutation residues among 15 mutations whereas SARS-CoV-2 Omicron variant has 27 new mutation residues among 39 mutations in **Table 1**. In addition, the mutation residues of RBD and RBM were highlighted by orange and green color, respectively (**Table 1**). These massive new mutations probably contribute to the high infectivity of the SARS-CoV-2 Omicron variant.

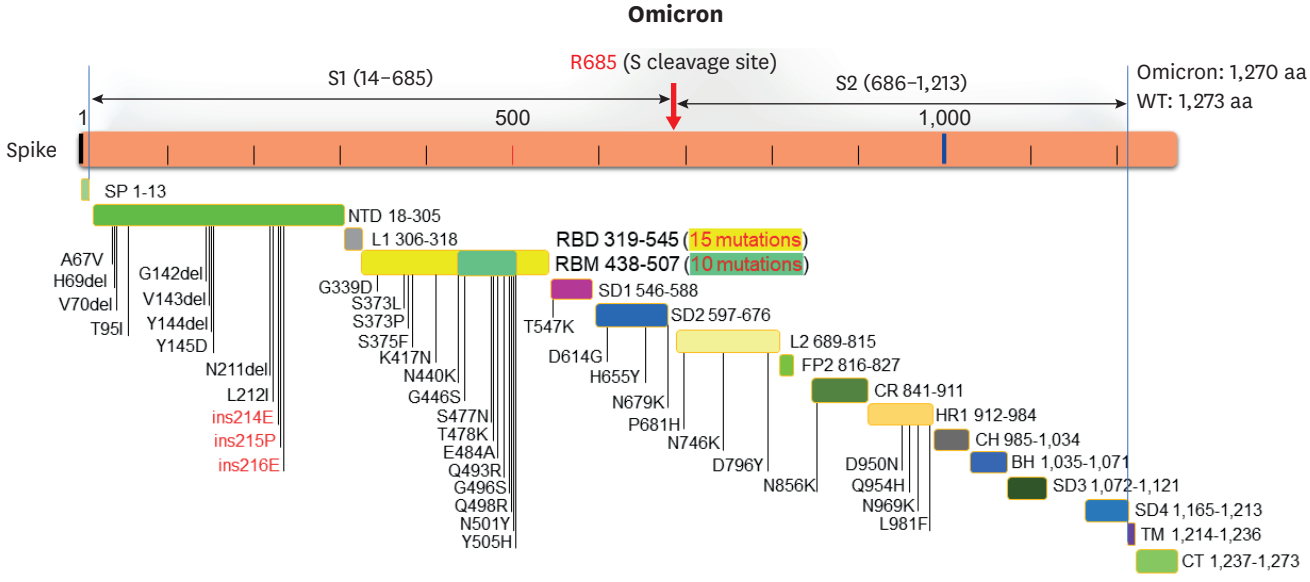
In **Fig. 1A**, the 39 mutation residues were highlighted by yellow for amino acid change, blue for amino acid deletion, and green for amino acid insertion. Interestingly, the deletions and insertions have occurred in the N-terminus of RBD. The insertion of ins214E, ins215P, and ins216E happened for the first time among the 11 SARS-CoV-2 variants. The RBD and RBM were highlighted by a light pink and a light green color, respectively (**Fig. 1A**). The signal peptide and transmembrane domain were indicated by bold underlined letters with gray highlight. The subunit 1 (S1) and subunit 2 (S2) cleavage sites were marked by green highlight with red bold letters that were indicated by a dark blue arrow (**Fig. 1A**, the left down). Interestingly, 2 mutations, N679K and P681H, present just in the N-terminus of the cleavage site. These adjacent mutation sites could influence the cleavage of S1 and S2 resulted in the entry of the SARS-CoV-2 Omicron variant into the host cells (22-25).

The RBD and RBM highlighted regions were enlarged for more detailed analysis (**Fig. 1B**). The 15 mutation sites with yellow highlight are present in the RBD of *S* gene, which is 38% of the total 39 mutations in the SARS-CoV-2 Omicron variant. Surprisingly, the 10 mutations present in critical RBM within 69 amino acid residues resulted in that 14% of the amino acid residue being changed in RBM. However, only 2 mutations were present in the RBM of the SARS-CoV-2 Delta variant that was indicated by a red bold letter. The mutation sites in the RBM of Omicron is 5 folds higher than that of Delta variant (**Fig. 1B**). In addition to this, the 69 amino acid residues of RBM are only 0.05% of the whole *S* gene containing 1,273 amino acid residues. The frequency of mutation in RBM of SARS-CoV-2 Omicron is about 760 folds higher than that of the whole *S* gene. The high frequency of mutation in the critical RBM may

enhance the transmissibility of the SARS-CoV-2 Omicron variant, which has about 8.4 folds higher transmissibility than the known SARS-CoV-2 D614G original strain (21).

The direct binding residue in the RBM of SARS-CoV-2 Omicron, Delta, and previous studies (9,13,14,18) was analyzed by protein sequence alignment (**Fig. 1C**). The specific binding residues were highlighted by green in RBM and the upper 4 lines WT^{1}–WT^{4} from these different reports (9,13,14,18). These 21 residues directly interact with ACE2 on the surface of host cells. Interestingly, each study suggested binding residue was different and this result is unanticipated since the protein complex structure was obtained from an identical spike and ACE2 proteins (9,13,14,18). The analysis of ACE2 binding residue found only 6 binding residues (Y449, Y453, F486, N487, Q498, and T501) were common binding residue among 21 suggested binding residues indicated by an asterisk on the top (**Fig. 1C**). Astonishingly, the 7 mutations (blue highlight; G446S, E484A, Q493R, G496S, Q498R, N501Y, and Y505H) in the RBM of Omicron have corresponded to the ACE2 binding sites whereas the 2 mutations (red letter; L452R and T478K) in the RBM of Delta did not correspond to any of ACE2 binding sites. The Q498R mutation is one of the 6 common binding sites (Y449, Y453, F486, N487, Q498, and T501) from 4 different reports (9,13,14,18). This critical residue Q498R with severe mutations on spike/ACE2 interaction residues in RBM may contribute to the high transmissibility of the SARS-CoV-2 Omicron variant.

The 39 mutations in the SARS-CoV-2 Omicron variant were illustrated by geographical location in *S* gene that was divided into 16 subdomains by different colors (**Fig. 2**). The specific amino acid residue of each domain was shown on the right such as RBD 319–545



Omicron (S. Africa) – 39 amino acid residues (27 new mutation sites)
A67V, H69del, V70del, T95I, G142del, V143del, Y144del, Y145D, N211del, L212I, ins214E, ins215P, ins216E, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F

Figure 2. Geographical drawing of mutation sites in SARS-CoV-2 Omicron *S* gene. The *S* protein contains 16 subdomains that were shown by different colors with specific residues on the right. The S1 (R685) cleavage site was indicated at the top with a red arrow. The 15 mutations in RBD were indicated in red letters with a yellow highlight. The 10 mutations in RBM were indicated in red letters with a green highlight. Each amino acid change was illustrated at the bottom of the domain bar by geographical drawing. The 3 unique insertion sites were indicated by red letters in NTD.
SP, signal peptide; L, loop; SD, subdomain; FP, fusion peptide; CR, connected region; HR, heptad repeat; CH, central helix; BH, β-hairpin; TM, transmembrane domain; CT, cytosolic domain.

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and RBM 483–507. The 15 mutations present in the crucial RBD (yellow bar; 319–545), which was shown by red letters with a yellow highlight. Furthermore, the 10 mutations present in the critical RBM (green bar; 438–507), which was shown by red letters with a green highlight (**Fig. 2**). Interestingly, the SARS-CoV-2 Omicron variant has an insertion site (red letters) in the N-terminal domain (NTD; 18–305) that was found for the first time among the 11 SARS-CoV-2 variants. The 6 deletion sites also present in NTD resulted in the SARS-CoV-2 Omicron variant containing 1,270 amino acid residues which is 3 residues less than SARS-CoV-2 WT (**Fig. 2**, right on the top).

CONCLUSION

Currently, there is a large concern about coronavirus disease 2019 (COVID-19) because the highly transmissible COVID-19 Omicron variant has been reported from different countries. In this review, we analyzed the mutation of the SARS-CoV-2 Omicron variant to understand the current crisis of the COVID-19 pandemic as well as to explain the high transmissibility of Omicron. The analysis of Omicron mutation sites revealed the crucial ACE2 binding residues in the RBM of SARS-CoV-2 Omicron was heavily mutated. Probably the mutation rate of SARS-CoV-2 is much faster than any other infectious respiratory virus. Therefore, the conventional lockdown, quarantine, vaccine program, and antibody therapy are not sufficient to prevent the transmission of COVID-19. We must find alternative approach to overcome the crisis of COVID-19 pandemic.

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