

RESEARCH PAPER

Omicron variants bind to human angiotensin-converting enzyme 2 (ACE2) much stronger due to higher number of charged-charged interactions

Sibel Kalyoncu¹ 

¹Izmir Biomedicine and Genome Center, 35340 Izmir, Turkiye

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Corresponding Author

Tel.: +90 232 2994168
E-mail: sibel.kalyoncu@ibg.edu.tr

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Abstract

Since the start of COVID-19 pandemic, several mutant variants of SARS-CoV-2 have emerged with different virulence and transmissibility patterns. Some of these variants have been labeled as variants of concern (VOC). There are mainly five strain clades with VOC status: Alpha, Beta, Gamma, Delta, and Omicron. Omicron sub-variants have been currently in circulation around the world, and they show faster transmissibility and lower virulence compared to others. Receptor binding domain (RBD) of SARS-CoV-2 spike protein is the region where it binds to human angiotensin-converting enzyme 2 (hACE2) on the host cell. Mutations on RBD might have direct or indirect effects on differential disease patterns of these variants. In this study, we analyzed sequence and structures of SARS-CoV-2 variants' RBD domains and documented their predicted affinities and contact interactions with hACE2. We found that Omicron sub-variants have much higher hACE2 affinities compared to other VOC strains. To understand reasons behind this, we checked biophysical characteristics of RBD-hACE2 contacts. Surprisingly, number of charged-charged interactions of Omicron sub-variants were on average 4-fold higher. These higher charged residue mutations on epitope region of Omicron sub-variants leading to stronger affinity for hACE2 might shed light onto why Omicron has less severe disease symptoms.

Introduction

On December 2019, the first report of coronavirus disease 2019 (COVID-19) was announced ([Li et al., 2020](#)), and more than 6.8 million people worldwide were killed by COVID-19 as of 11 April 2023 ([Dong et al., 2020](#)). Although the first vaccinations against the original Wuhan strain started in late 2020, new mutant variants of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are emerging and spreading where early-developed vaccines might not protect as expected ([Eyre et al., 2022](#); [Kalyoncu et al., 2023](#); [Markov et al., 2023](#)). There are numerous variants of SARS-CoV-2 of which are labeled as variants of concern (VOC) by World Health Organization (WHO) due to either its increase in transmissibility or virulence ([Aleem](#)

[et al., 2022](#)). Up to now, five main VOCs have been reported with their related strain clades: (i) Alpha (B.1.1.7), (ii) Beta (B.1.351), (iii) Gamma (P.1), (iv) Delta (B.1.617.2), and (v) Omicron. While the former four of them were previously circulating VOCs, Omicron sub-variants are the currently circulating VOCs ([Rambaut et al., 2020](#)). The symptoms of Omicron variants are not as severe as other previous VOCs but its transmissibility is higher ([Chatterjee et al., 2023](#)).

SARS-CoV-2 is a single-stranded RNA-enveloped virus ([Lu et al., 2020](#)). It uses its spike protein for receptor recognition and cell membrane entry. The receptor binding domain (RBD) of its spike protein binds to angiotensin-converting enzyme 2 (ACE2), a cellular receptor, for its viral entry into the host cell ([Jackson et al., 2022](#); [Letko et al., 2020](#)). Because the RBD domain of

spike protein is indispensable for the host cell entry, mutations on RBD for emerging new variants should be analyzed in detail to see whether those mutations affect its receptor binding kinetics. It is not known whether ACE2 binding kinetics of variants' RBD domain affect SARS-CoV-2's transmissibility and/or virulence. We hypothesize that stronger binding of RBD-ACE2 decreases the virulence and increases the transmissibility. We tested this hypothesis by investigating the RBD structures of all VOCs and binding kinetics between their RBDs and human ACE2. We found that Omicron strains mostly have a stronger affinity for ACE2 (dissociation constant- K_D in picomolar range) while previous VOCs have much less affinity (K_D in nanomolar range). We also showed that this stronger binding was caused by a higher number of (four fold) charged-charged amino acid interactions and higher positive charges in the RBD-ACE2 interface.

Materials and Methods

Sequence analysis

Amino acid sequences of RBD domains were extracted from NCBI GenBank database with "Severe acute respiratory syndrome coronavirus 2 isolate" term (with organism tax id of txid2697049). First RNA sequences were downloaded from NCBI Genbank (Genbank IDs: OQ415315.1 for Alpha, OM286905.1 for Beta, OK091006.1 for Delta, OM367886.1 for Gamma, OM366054.1 for Kappa, OX315743.1 for BA.1, OX315675.1 for BA.2, OP603965.1 for Omicron BA.4&5, OM739178.1 for Omicron BA.2.12.1, OQ300138.1 for Omicron BQ.1, OQ300139.1 for Omicron XBB). Regions coding for RBD domain of Spike protein were analyzed and translated to corresponding amino acid sequences. GISAID Database was used to confirm the extracted amino acid sequences (Elbe & Buckland-Merrett, 2017). There were some discrepancies between NCBI extracted data and GISAID database, GISAID Database was used to correct these discrepancies because it consolidates many isolates (>200) for each reported variant. SnapGene software (www.snapgene.com) was used to align amino acid sequences. EMBL-EBI Simple Phylogeny tool was used to create phylogram plot (Madeira et al., 2022). The graph for the selected country (Turkiye in here) was represented as the proportion of the total number of each strain sequences over time (<https://covariants.org/per-country>) (Elbe & Buckland-Merrett, 2017). Variants were represented in the graph if they have at least 70 sequences over a period of at least four weeks.

Structure analysis and homology modelling

Three dimensional RBD domain structures of many variants used in this study were already in RCSB PDB database (PDB IDs: 7EKF for Alpha, 7EKG for Beta, 7EKC for Delta, 7SO9 for Gamma, 7VX9 for Kappa, 7UB0 for BA.2, 7NXQ for Omicron BA.4&5, 7XNS for Omicron BA.2.12.1), only Omicron BA.1, BQ.1 and XBB variants

were not in the database. Therefore, a homology modeling was used for these three variants. Their structural coordinates were modeled using SWISS-MODEL (Waterhouse et al., 2018). The aim of homology modeling is to get the lowest energy conformation for the given amino acid sequence based on a template structure. There are numerous experimental structures of other SARS-CoV-2 variants which can be used as templates in RCSB PDB database. The template with the highest sequence identity was chosen as the homology modeling template for the selected variant (7WBP for Omicron BA.1, 7XNQ for Omicron BQ.1 and 7YQW for Omicron XBB).

RBD-human ACE2 (hACE2) interaction of the Wuhan strain (PDB ID: 6LZG) was used to generate variants' RBD-hACE2 complex structure. PyMOL was used to align each variant's RBD domain structures onto WT RBD (The PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC). Root Mean Square Deviation (RMSD) values were used to assess quantitative measures of the similarity between two superimposed/aligned structures. Then, structures of aligned RBD-hACE2 complexes were exported as the final structures for binding interaction analysis.

Electrostatic potential calculations

Adaptive Poisson-Boltzmann Solver (APBS) under PyMOL was used to calculate and display the electrostatic potentials of each molecular surface (Miller et al., 2012). Results were represented as a color-coded electrostatic surface in units of $K_b T / e_c$ where K_b is Boltzmann's constant, T is temperature and e_c is electric charge.

Affinity and interaction prediction

The affinities and interfacial contact numbers of the interacting proteins were calculated using PRODIGY web server (Vangone & Bonvin, 2015; Xue et al., 2016). The PDB file of each variant's RBD-hACE2 complex was imported into PRODIGY, the model was selected as protein-protein, interactor 1 was set to the chain of RBD, interactor 2 was set to the chain of the hACE2, and the temperature was set to 25 °C. The affinities and dissociation constants of each RBD-hACE2 complex were reported. Also, the number of interfacial contacts based on their types (Charged-Charged, Charged-Polar, Charged-Apolar, Polar-Polar, Polar-Apolar, Apolar-Apolar) were reported. Binding affinity were reported in two terms: predicted free energy (ΔG) and dissociation constant (K_D) according to the equation below:

$$\Delta G = RT \ln K_D$$

where R is the ideal gas constant (kcal/K mol), T is the temperature (K) and ΔG is the predicted free energy.

Results and Discussion

Since the start of the COVID-19 pandemic, several mutant variants have emerged around the world, and each showed different transmissibility and disease

symptoms. Currently, Omicron sub-variants have been circulating in the population. While Omicron variants show lower disease severity, their transmissibility increases (Rana et al., 2022). The molecular mechanism underlying these evolutionary patterns of Omicron variants is still not known. Here, we try to investigate structural mechanisms behind the virus-host cell interaction of Omicron sub-variants by comparing them to early variants of the SARS-CoV-2. After the SARS-CoV-2 virus enters the body, it directly binds to the human ACE2 receptor on the host cell. The interacting domain of SARS-CoV-2 virus is RBD located on the Spike protein. First, we extracted the coding amino acid sequence information of RBD domains for all variants tested (Figure 1). Amino acids 317-540 of Spike protein were selected as RBD domain according to three dimensional structural patterns. On the sequence alignment, epitope regions where it directly contacts with hACE2 were

highlighted along with mutated sites. According to our results, four epitope positions (417, 446, 498, 501) out of 21 showed mutational patterns among different variants (Figure 1). Among these positions, Omicrons sub-variants (BA.1, BA.2, BA.4&5, BA.2.12.1, BQ.1, XBB) showed enriched mutational patterns compared to early variants. For example, positions 440, 498, and 505 clearly evolved to a more basic pattern for all Omicron sub-variants.

The emergence of SARS-CoV-2 variants in Turkey has been parallel to the global pattern (Figure 2A). Evolutionary relationship between variants seemed to align well with their emergence sequence in the population (Figure 2B). And there was a clear distinction between early variants and Omicron sub-variants. From a structural point of view, three dimensional structures of each variants' RBD domain were needed. For most of them, their atomic coordinates were readily available in

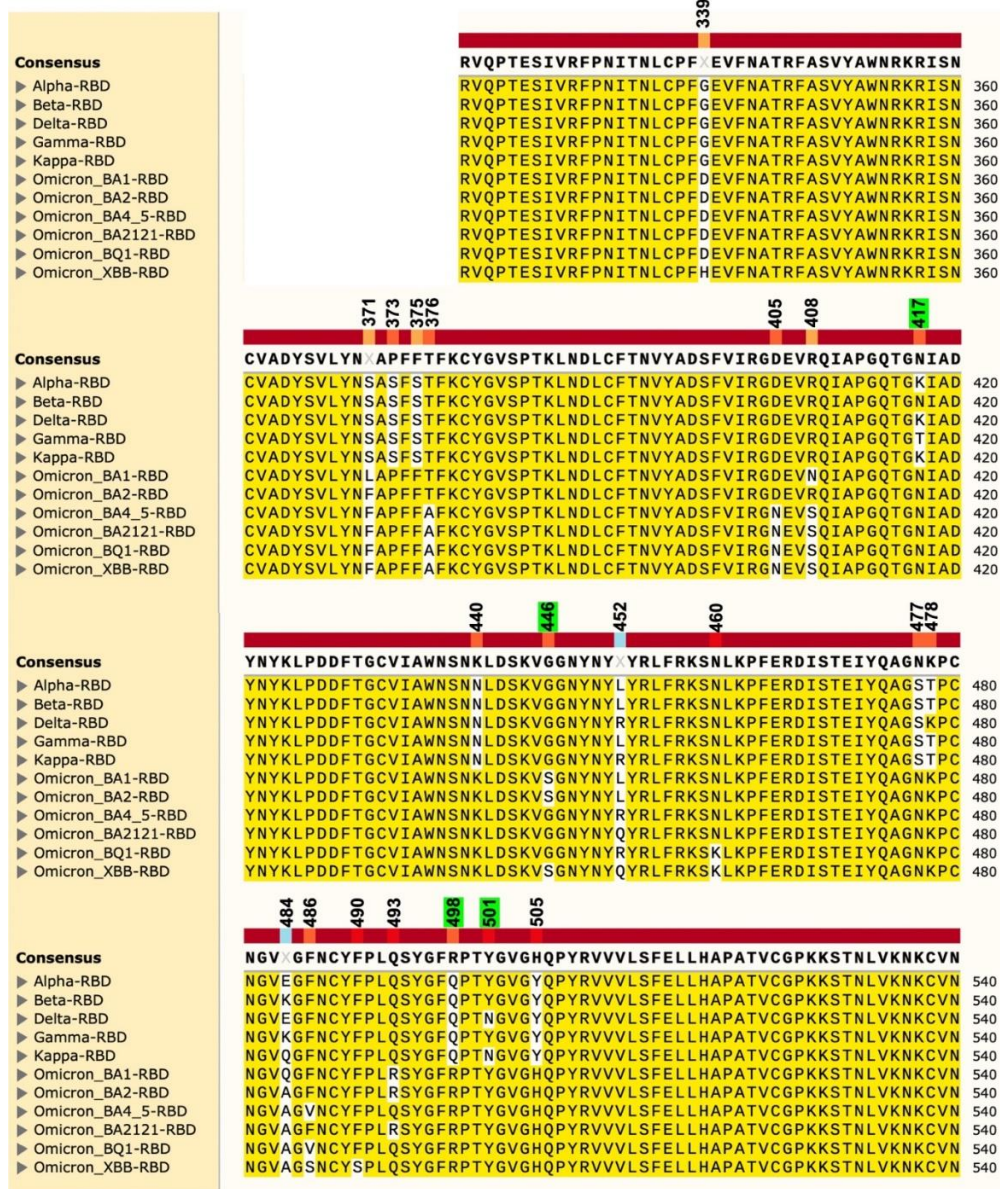


Figure 1. Amino acid sequence alignment of receptor binding domains of SARS-CoV-2 strains. Mutation sites are marked with the residue number, and epitope residues on mutation sites are highlighted with green.

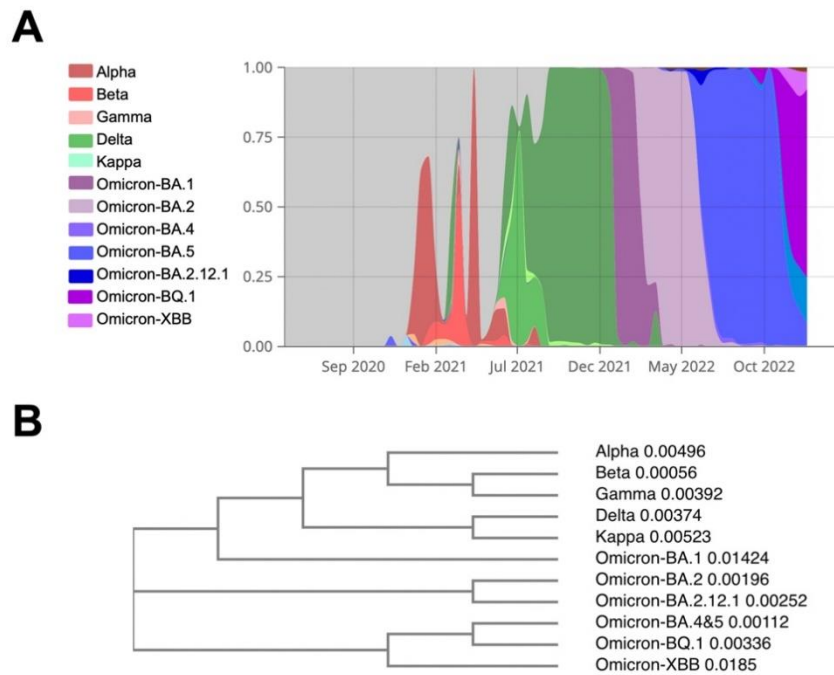


Figure 2. Lineage and structure of SARS-CoV-2 strains. **(A)** Proportion of total number of sequences over time, that fall into strain groups in Türkiye. Data was retrieved from <https://covariants.org/> (Elbe & Buckland-Merrett, 2017). **(B)** Phylogenetic tree showing an evolutionary relationship of SARS-CoV-2 strains.

the protein databank, but those of three variants (Omicrons BA.1, BQ.Q, XBB) were absent. Therefore, homology modeling was performed for each of these three variants with templates of >98% sequence identity (Table 1). In comparison to the original Wuhan strain, RMSD values of early variants were clearly lower than those of Omicron sub-variants with the highest structural distance of Omicron BQ.1 (RMSD of 1.904 Å). Most strikingly, the number of amino acid mutations in Omicron sub-variants was 4x-19x times more than early variants.

Epitope surfaces of RBDs where they bind to hACE2 were first analyzed electrostatically by Poisson-Boltzmann Surface Area (PBSA) method. It is commonly used to calculate free energies of various molecules with a solvation contribution and electrostatic analysis (Wang et al., 2017). There was a clear difference in epitope surfaces of VOC strains and Omicron sub-strains (Figure 3). VOC strains, especially early variants (Wuhan, Alpha, Beta, Gamma), have slightly negative to neutral surfaces, while all Omicron sub-variants have

Table 1. Structure information for RBD domains of all strains. Structure similarity to Wuhan RBD was represented as Root Mean Square Deviation (RMSD) and the number of amino acid mutations compared to Wuhan RBD was reported. PDB IDs used were given for each RBD structure. The strains with no published structure (Omicron-BA.1/BQ.1/XBB) were homology-modelled by Swiss-Model and their model template PDB IDs along with % sequence identity to those templates were given

	RMSD (Å ²) from Wuhan strain	Structure or homology model template (PDB ID)	% Sequence identity, if homology modelled	Number of mutations compared to Wuhan strain
Alpha	0.209	7EKF	-	1
Beta	0.143	7EKG	-	3
Gamma	0.161	7EKC	-	3
Delta	0.456	7SO9	-	2
Kappa	0.840	7VX9	-	2
Omicron-BA.1	0.234	7WBP	98.6	13
Omicron-BA.2	0.612	7UB0	-	16
Omicron-BA.4&5	1.899	7XNQ	-	17
Omicron-BA.2.12.1	1.671	7XNS	-	17
Omicron-BQ.1	1.904	7XNQ	99.5	18
Omicron-XBB	1.129	7YQW	98.6	19

dominantly positive surfaces at varying patch locations on the epitopes. Among early variants, only Kappa strain has a comparable positive epitope surface. This positive charge dominance in epitope surface probably changes the biophysical characteristics of RBD-hACE2 binding interaction.

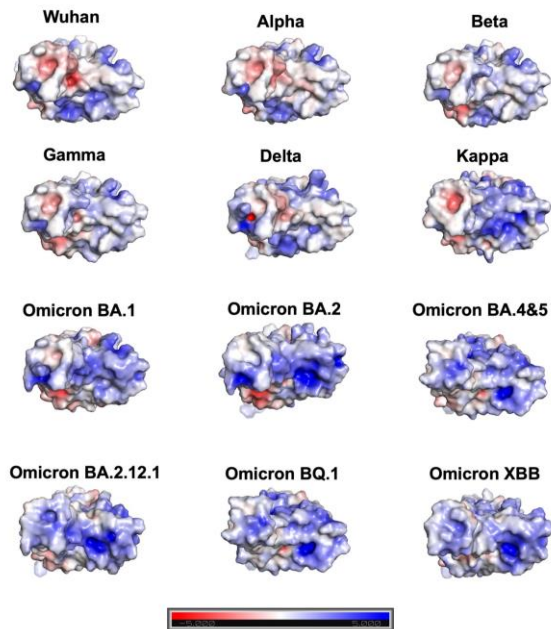


Figure 3. Poisson-Boltzmann Surface Area (PBSA) results of each variant RBD to analyze their electrostatic behaviors. Epitope surfaces where they directly interact with hACE2 were shown for each. More positively charged interaction surfaces of Omicron variants are obvious with more blue patches.

Next, we focused on interaction contacts and affinities of RBD-hACE2. Epitope region of Wuhan strain where hACE2 directly binds was highlighted in the complex structure of Wuhan RBD-hACE2 (Figure 4A). Among those, K417, G446, Q498, and N501 were mutated epitope residues in most of the variants. When RBD domains of all variants were aligned on Wuhan RBD domain with a stationary hACE2, the main regions on the epitope interface seemed to be conserved (Figure 4B). Therefore, we can assume that the same epitope residues dominate the RBD-hACE2 interaction with a possible contribution from nearby conformational residues.

The affinity of protein-protein interactions is an important indicator for association/dissociation kinetics and functional changes. Stronger binding patterns might lead to lower dissociation rates resulting in functional improvements or impairments depending on the mechanism of action (Kastritis & Bonvin, 2013). When we estimated affinities of RBD-hACE2 interaction by Prodigy, there was a clear distinction between Omicron sub-variants and early variants (Table 2). Omicron-subvariants had stronger binding affinities to hACE2 with the highest affinity of Omicron-BA.2.12.1 (K_D of 0.01 nM). While early variants were in nanomolar range for the K_D , Omicron variants showed dissociation constants in picomolar ranges. When averages were taken, Omicron-variants showed more than two times better affinities.

There could be several reasons behind the stronger binding affinities of Omicron variants, but the most obvious cause should be related to changes in epitope-paratope interactions. When we checked epitope-

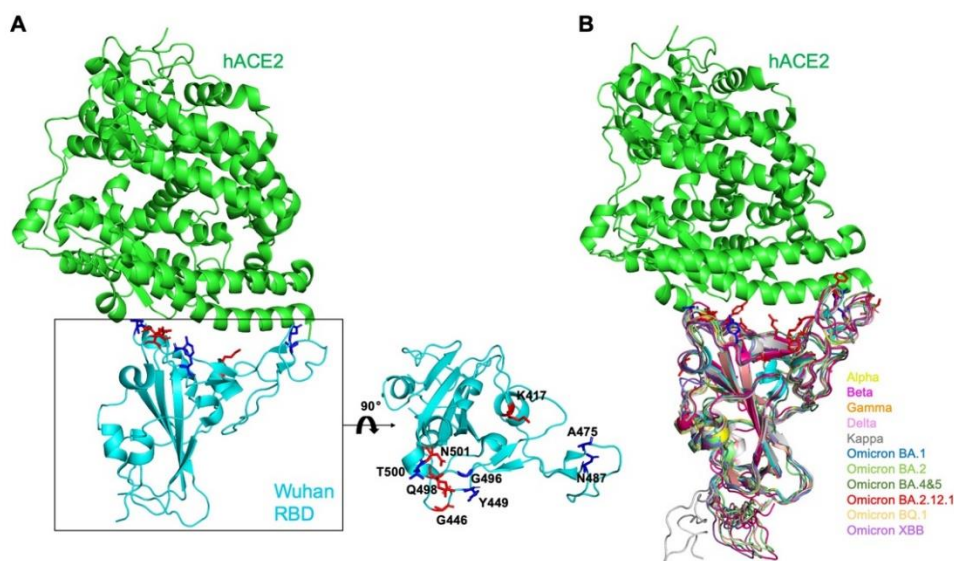


Figure 4. Complex structures of ACE2-RBD. (A) Cartoon representation of human ACE2-RBD of Wuhan strain interaction. Epitope residues of Wuhan RBD were labeled as red and blue sticks which represent mutated and unmutated residues, respectively. (B) Cartoon representation of human ACE2 with overlaid RBD structures of all other strains.

paratope interactions by physicochemical content types, there was a clear difference in the number of charged-charged contacts (Table 2). While early variants had a frequency of 1.8 charged-charged contacts, Omicron variants had 7.0 of those (more than 3.5 times higher frequency). Other interfacial contact types did not have that much of a significant change. Therefore, we concluded that stronger binding patterns of Omicron sub-variants are mostly due to its increased charged-charged interactions between RBD-hACE2 interface, especially through positively charged surfaces of Omicron RBDs. Accumulation of charged mutations around the epitope region of RBD could be an indicator of this affinity increase.

There are several molecular dynamics (Jawad et al., 2021; Kim et al., 2022) and experimental studies on RBD-hACE2 binding kinetics (Barton et al., 2021; Kim et al., 2022). However, they mostly focus on either the Wuhan strain or early strains, none of those studies discuss recently circulating omicron strains such as BQ.1 and XBB. Our general finding of Omicron strains' higher binding affinity was experimentally confirmed by one of these studies (Kim et al., 2022) but they only discuss the first strain of Omicron.

The first Omicron variant (BA.1) first appeared at the end of 2021 in South Africa and its several sub-variants have been emerging until today (Das et al., 2022). Although there are several mutations (>60) in their genome, all Omicron variants show higher transmissibility along with less disease severity. This is good news for the population because the COVID-19 pandemic might have started to converge into an endemic status (Are et al., 2023). We hypothesized that bio-physical/chemical properties of RBD-hACE2 interaction of Omicron variants might have effects on

their disease and transmissibility patterns. We found out that Omicron variants have more positive epitope surfaces, and they also have an overall higher binding affinity to hACE2. The affinity was measured as dissociation constant (K_D), higher the affinity means lower dissociation constant. The affinity is directly related to the dissociation constant (k_{off}) and inversely related to the association constant (k_{on}) (Kastritis & Bonvin, 2013). The higher the affinity is the lower the dissociation and the higher the association of interacting proteins ($K_D = k_{off}/k_{on}$) (Wang et al., 2019). When the association rates are higher, it can attach to the host cells at a higher rate, in this case, hACE2 expressing epithelial cells in the airways (J. Liu et al., 2021). These factors might lead to faster transmissibility due to faster rates of association. On the other hand, lower dissociation rates can cause prolonged actions on the cell probably leading to less disease severity (H. Liu et al., 2021). More experimental research is needed to confirm this theory by investigating the relationship between these RBD-hACE2 affinities and virus variants' transmissibility/virulence patterns.

Conclusion

The COVID-19 pandemic has affected all nations in the world since the start of 2020, but its convergence to an endemic state started after Omicron strains emerged. Omicron strains showed lower disease severity along with rapid transmissibility. There are more than 60 mutations in Omicron variants compared to the original Wuhan strain, but mutations on RBD domain are notable due to their direct contact with the host cell via hACE2. Here, we focused on RBD domains of variants of concern and performed a structural and

Table 2. Interaction information for hACE2 – RBD of each SARS-CoV-2 strain. Prodigy was used to predict affinities/contact numbers for hACE2 based on their three dimensional structures (Vangone & Bonvin, 2015; Xue et al., 2016)

	Affinity to hACE2			Number of interfacial contacts per type				
	ΔG (kcal/mol)	K_D (nM)	Charged- Charged	Charged- Polar	Charged- Apolar	Polar- Polar	Polar- Apolar	Apolar- Apolar
Wuhan	-12.4	0.83	3	10	19	5	23	9
Alpha	-12.2	1.00	3	7	22	5	21	12
Beta	-11.8	2.10	0	9	23	5	20	12
Gamma	-12.1	1.40	0	8	23	5	21	13
Delta	-12.7	0.47	2	9	21	5	24	10
Kappa	-12.2	1.20	3	9	20	7	24	12
Omicron-BA.1	-12.5	0.67	7	7	23	5	20	11
Omicron-BA.2	-13.3	0.17	9	5	27	4	21	15
Omicron-BA.4&5	-12.3	0.88	5	8	26	6	21	17
Omicron-BA.2.12.1	-15.2	0.01	11	7	32	4	27	17
Omicron-BQ.1	-12.3	0.91	5	8	26	6	21	17
Omicron-XBB	-12.6	0.61	5	10	24	6	22	11
Average for initial strains	-12.2	1.17	1.8	8.7	21.3	5.3	22.2	11.3
Average for Omicron strains	-13.0	0.54	7.0	7.5	26.3	5.2	22.0	14.7

functional analysis of their RBD-hACE2 interaction. We found out that Omicron sub-variants bind to hACE2 at least two times stronger. Also, we showed that this affinity increase is mainly due to a higher number of charged-charged contacts especially with positively charged epitope surfaces between RBD and hACE2. We speculate that stronger affinity of Omicron variants might lead to higher transmissibility and lower disease severity patterns due to their faster rates of association and/or slower rates of dissociation.

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