

The F456L mutation of Omicron EG.5.1: Insight of the molecular mechanism

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The E.G.5.1 sub-variant, named "Eris", from the already redoubtable Omicron, has made its way into several nations, creating a new wave of uncertainty. Although its destructive potential has yet to be fully assessed by the World Health Organization (WHO), this strain may have a high potential for transmission following its appearance in 51 countries. The entry point for sarscov2 is via the angiotensin receptor, so in our study we tried to clarify the mutation that raises many questions about its impact. We used computational approaches to try and get as close as possible to an adequate answer. We found that the mutation at position 456 allowed the L456 residue to become more flexible than the F456 residue; we found that there is a 60% gain in flexibility for this variant compared to the ancestral strain. This led to a new π - σ interaction between RBD-ACE2, thus promoting affinity. This linkage advantage may explain the exponential increase in Eris transmission, but the pathogenicity remains to be assessed over the coming months. What makes this mutation unique is that it is the first time it is been seen in this position, which raises uncertainties about vaccine efficacy and viral transmissibility.

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The Omicron strain of SARS-CoV-2, B.1.1.529 was initially recognized by the World Health Organization (WHO) as a variant of concern (VOC) in 2021. By the beginning of 2022, COVID-19 had been mainly caused by the Omicron variability and its five lineages, BA.1, BA.2, BA.3, BA.4, and BA.5.¹⁻² A recombinant of two BA.2 sublineages with a change from the F486V mutation in variants BQ.1.1, BA.4, BA.5 and BQ.1.1. to F486V in EG.5.1. and XBB.1.5., the Omicron XBB.1.5 subvariant is a sublineage of the XBB variety with the F486P mutation in the spike protein. Since the advent of the XBB.1.5 variant there has been an increase in the number of cases linked to this variant following reinforcement of the binding to the angiotensin 2 receptor. On the other hand there has been an escape from the immune system, with a decrease in neutralization capacity to the antibodies of mRNA vaccines with a drop in neutralization capacity from 7 to 17 compared to the BA1.5 variant³.

In this study, we will analyze the difference in affinity between XBB.1.5, EG.5.1 (Figure 1) and the wild-type form of COVID-19 for the ACE2 and more specifically the F486L mutation in the EG.5.1 variant which is positioned in the binding site with ACE2, describing the interactions that govern this binding site and their link with increased transmissibility.

MATERIALS AND METHODS

Structures were extracted from protein data bank the wild type of sarscov-2(PDB: 6MOJ) and XBB.1.5. (PDB: 8SPI). The EG.5.1 mutation has been inserted using the insertion module in discovery studio. All structures have undergone energy minimization to eliminate clashes that may hinder interactions using Chiron, a high-performance tool⁴. The analysis was

done by discovery studio. We used The SPServer as a web server that is simple to use and a protein-protein interaction structure that uses a scoring splitting technique to quantify energy⁵ with a minimum residue-residue distance of <5Å. The quantitative analysis of protein-protein interaction has been performed by the SPServer using pair scoring function.⁶ Interaction residue-residue was achieved by pyDockEneRes server, a free server that uses a linear combination of solvation, electrostatic, van der Waals and hydrogen energies⁷. We used coarse-grained model CABS-flex to get inside the flexibility of residues⁸⁻⁹ with using the root mean square fluctuation (RMSF).

RESULTS AND DISCUSSION

After energy minimization of the structures without and with their mutation. Analysis of the protein-protein interaction of the wild type showed that the major interactions governing the Spike-ACE2 contact are of three types, van der Waals, electrostatic and hydrogen bonds. The key amino acids are found between 20-85 and 330-390 of ACE2 417-505 of RBD. The wild-type strain showed a hydrogen bond number of 11, electrostatic 1 and van der Waals 106. For the XBB.1.5 and EG.5.1 Analysis shows loss of hydrogen bond Q493 of RBD and E35 of ACE2 and 5 non-bonded contacts. The key amino acids are highlighted in Table 1 with their energy values, Qualitative analysis showed that for strain XBB.1.5 the RBD-ACE2 interaction shows no change in binding, whereas the L456 mutation of EG.5.1 shows a break in intra-protein binding with loss of the n-n interaction between F456 of RBD and Y473 of RBD, with acquisition of n-σ interaction between H34 of

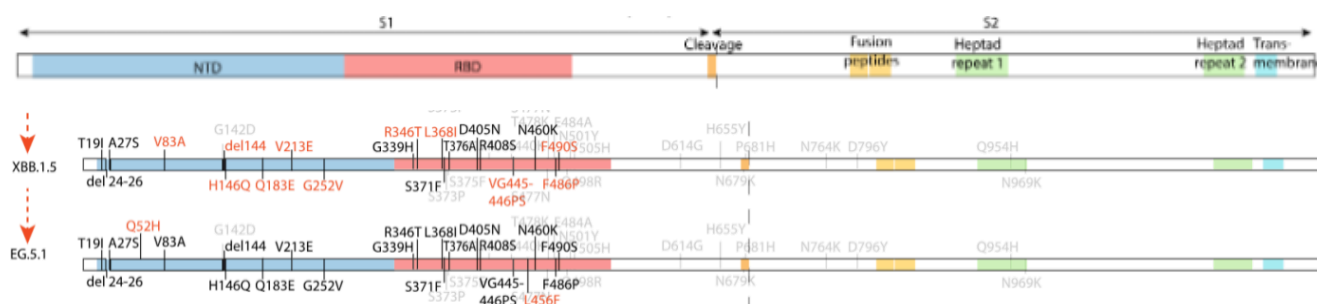


Figure 1 Schematic representation of spike glycoprotein. The figure shows functional S1 and S2 domain(RBD, receptor binding domain; NTD, N-terminal domain) of XBB.1.5 and EG.5.1.14

Table 1 Free energies of residues involved in RBD-ACE2 interaction

Amino Acid	Energy (Kcal/mol)		
	WT	XBB.1.5	EG.5.1
PHE486	-12.09549	-12.09549	-9.33448
PHE456	-6.96575	-6.96575	-5.07683
TYR505	-6.16554	-6.16554	-3.76164
TYR489	-5.03070	-5.03070	-2.65827
ARG403	-3.77155	-3.77155	-2.13665
LEU455	-3.06347	-3.06347	-2.02153
LYS417	-2.71798	-2.71798	-1.87837
ARG408	-1.83313	-1.83313	-1.56525
LYS444	-1.67201	-1.67201	-1.46781
GLN498	-1.64766	-1.64766	-1.41230

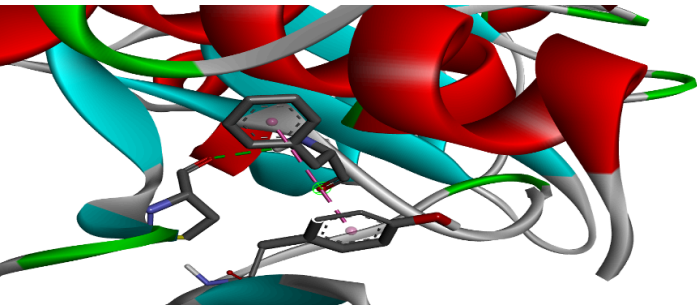


Figure 2 Analysis of the receptor binding domain (RBD) without substitution of the F456. Hydrogen bond linking P491 from RBD and F456 from RBD and n-n stacking between Y473 and F456.

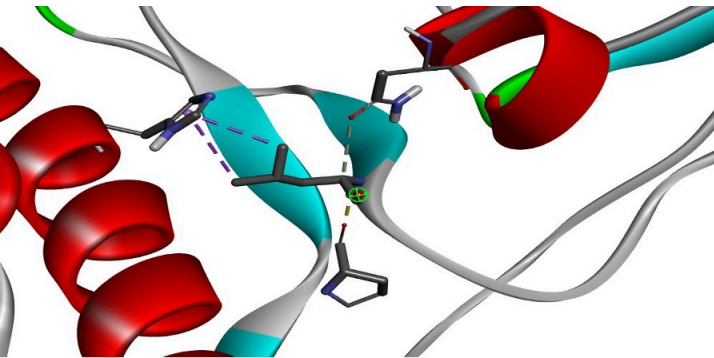


Figure 3 Analysis of the receptor binding domain (RBD) without substitution of the F456 by Leucine. n-si interaction H34 from ACE-2 and L456 from RBD.

ACE2 and L456 and hydrogen bonds between N417 of ACE and L456 of RBD (Figure 2, 3).

The quantitative analysis indicates that the mutation at RBD position 456 alters the interaction energy of EG.5.1 with a loss of affinity from -6.7 Kcal/mol for the wild-type strain and XBB.1.5 to -2 Kcal/mol. For residue-residue the mutation has an impact on energy with -6.96 to -2.65 with a $\Delta G_{mut}/\Delta G_{wt}$ ratio 0.38, however, the bond strength of the most important residue F486 goes from -12.09 to Y501 - 9.33 with a decrease in the number of bonds with an energy ≥ 2 (Table 1) for the EG.5.1. Molecular dynamics analysis was used to assess the flexibility of RBD-ACE2 using RMSF. The information obtained from CABS-flex shows that the molecular dynamics model shows that the mutant forms of the RBD-ACE2 complex of XBB.1.5. and EG.5.1 show a gain in flexibility with respective values of 1.95Å, 1.84Å compared to the wild-type strain 1.80. The position 456 of RBD in EG.5.1 reveals loss of flexibility with RMSF of 0.713Å in comparison of wild type and XBB.1.5Å with 1.15Å and 0.997 (Figure 4). Mutations in amino acids contribute to many changes when the mutation is located in the protein-protein binding site, in which case it directly affects the contact residues with a direct impact on residue-residue affinity, However, mutations outside the contact sites have been shown to exert an effect on structural flexibility, as in the case of D614G, which lies outside the RBD region, with a loss of hydrogen bonding between the two subunits S1 and S2. The loss of bonding induces significant flexibility, favoring the open conformation and resulting in increased thermodynamic stability¹⁰⁻¹¹. Variant XBB.1.5. showed an escape from the immune system, which drew attention. However, studies have shown that vaccination with the wild-type strain loses neutralization capacity against the Omicron sub-variant, hence the interest of a vaccine¹². 7354 Omicron EG.5 sequences from 51 different nations were sent to GISAID as of August 7th, 2023. China accounts for the biggest percentage of EG.5 sequences (30.6%, 2247 sequences). The United

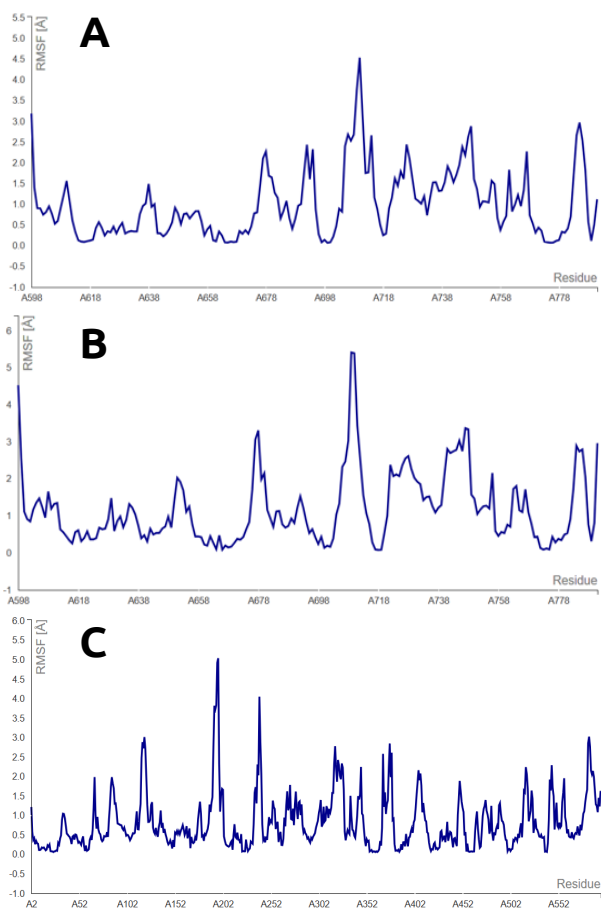


Figure 4 Fluctuation obtained for RBD-ACE2 complex in Å. A. Wild type. B. XBB.1.5. C. EG.5.1

States of America (18.4%, 1356 sequences) and the Republic of Korea (14.1%, 1040 sequences) are the other nations with at least 100 sequences. Australia (2.1%, 158 sequences), Singapore (2.1%, 154 sequences), the United Kingdom (2.0%, 150 sequences), France (1.6%, 119 sequences), Portugal

(1.6%, 115 sequences), Spain (1.5%, 107 sequences), and Japan (11.1%, 814 sequences). The danger to public health posed by EG.5 is rated as low globally based on the evidence available, matching the risk posed by XBB.1.16 and the other VOIs that are currently in circulation. The prevalence and immune escape qualities of EG.5 have all grown, although there have been no observed changes in the severity of the disease to date. In nations like Japan and the Republic of Korea, concomitant rises in the proportion of EG.5 and COVID-19 hospitalizations (lower than prior waves) have been noted; however, no links between these hospitalizations and EG.5 have been found. However, due to its growth advantage and immune escape traits, EG.5 may increase the incidence of cases, become dominant in some nations, or possibly cause a spike in disease rates¹³.

CONCLUSION

The World Health Organization has placed the two variants Omicron XBB.1.5 and EG.5.1 under observation. In terms of transmissibility, the two variants show some stability in position 456 vis-à-vis of the ACE2 compared to the wild state, with a gain in stability of (+61%) for EG.5.1 and (+15%) for XBB.1.5. However, their efficacy is decreasing relative to other variants as they show increasing resistance to the vaccine. However, additional research both in-depth and experimental must be done in order to fully understand the viral neutralization process and to assess how it affects patient survival and vitality prognosis.

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