THE OMICRON XBB.1 VARIANT AND ITS DESCENDANTS: GENOMIC MUTATIONS, RAPID DISSEMINATION AND NOTABLE CHARACTERISTICS

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ABSTRACT

The SARS-CoV-2 virus, which is a major threat to human health, has undergone many mutations during the replication process due to errors in the replication steps and modifications in the structure of viral proteins. The XBB variant was identified for the first time in Singapore in the fall of 2022. It was then detected in other countries, including the United States, Canada, and the United Kingdom. We study the impact of sequence changes on Spike protein structure on the subvariants of XBB with particular attention to the velocity of variant diffusion and virus activity w.r.t. its diffusion. We examine the structural and functional distinctions of the variants in 3 different conformations: (i)

Spike glycoprotein in complex with ACE2 (1-up state), (ii) Spike glycoprotein (closed-1 state) and (iii) S protein (open-1 state). We also estimate the transmissibility of the affinity binding between Spike proteins and ACE2. The market binding affinity observed in specific variants raises questions about the efficacy of current vaccines in controlling the spread of these variants. This work may be useful in devising strategies to manage the ongoing COVID-19 pandemic. To stay ahead of the virus evolution, further research and surveillance should be carried out to adjust public health measures accordingly.

***K*eywords** XBB Variant · Omicron · Covid · genomic analysis

1 Introduction

The SARS-CoV-2 genome is composed of 29.9 kilobases [1] and has 14 open reading frames (ORFs). It contains multiple sections that encode four structural proteins: Spike (S), Envelope (E), Membrane (M), and Nucleocapsid (N). Moreover it is characterised by 16 nonstructural proteins (nsp1-nsp16 complexes) and accessory proteins [2, 3, 4].

SARS-CoV-2, like other viruses, undergoes several mutations during the replication process [5, 6], due to errors in replication steps and modifications to the structure of the viral proteins [7, 8]. Mutations determining a competitive advantage of the associated virus are preserved [4, 9, 10, 11]. For this reason, the World Health Organization (WHO) closely monitors SARS-CoV-2 mutations, as reported in Figure 2. Among its activities, WHO is in charge of selecting variants which may require attention from government public health services to rapidly define guidelines and actions for the containment of virus evolution [12, 13]. Variants with similar genetic changes and/or shared attributes and are indicated as: Variant Being Monitored (VBM), Variant of Concern (VOC), or Variant of Interest (VOI).

The Omicron variant represents an important milestone in the evolution of Sars-Cov-2 variants, mainly due to the high mutations rate. It has been proved that Omicron's high number of mutations makes it more contagious than earlier versions [14]. Moreover, it seems to be better equipped to avoid the immune system's response to prior infection or immunization. Nevertheless, the Omicron variant is usually milder than its predecessors, hence the risk of severe illness or death is much lower. The development of the Omicron variant has been intricate and ever-changing.

We focus here on the more recent evolution of the virus, also indicated as XBB family [15, 16]. The original Omicron variant (BA.1) was the leading variant for a few months, later to be replaced by a number of subvariants, such as BA.2, BA.4, and BA.5. These subvariants are thought to be more contagious than BA.1, but they did not appear to pose such a threat to the human defence mechanism.

The current wave of COVID-19 cases is being driven in many countries by the BA.5 subvariant. BA.5 is believed to be even more transmissible than BA.4, and it is also more likely to evade the immune response from prior infection or immunization. Fortunately, the risk of severe illness and death from BA.5 is still relatively low [17].

The Omicron XBB variant [18] is a subvariant of the Omicron variant BA.2.75, which was first identified in South Africa (December 2022) and has then been detected in other countries, including the United States, Canada, and the United Kingdom. The XBB variant is believed to be more transmissible than the original Omicron variant as well as more likely to evade the immune response from previous infection or vaccination [19]. It has 32 mutations, including 10 mutations in the spike protein. Figure 3 reports a summary of the mutations limited to Spike. It may be more likely to overcross the immune response with respect to previous infections or vaccinations [20].

At the time of writing (September 2023), XBB has continued to evolve yielding to the appearance of XBB1.5, XBB1.16, XBB1.91 and EG5.1 subvariants. Figure 1 describes the number of infected people and the related variants. The distribution at national level is reported in Appendix 8. We consider such data as the global scenario of the XBB subvariants at the present time.

We address three questions:

- Is the pattern of the evolution of XBB different from the overall pattern of the evolution of SARS-CoV-2?
- Do XBB and its descendents present any peculiar characteristics which may determine a new outbreak?
- Are the current vaccines able to stop the diffusion of XBB or do we need the introduction of further measures (e.g. wearing masks, gloves, hand sanitisers)?

The obtained results can be summarized as follows:

- In the XBB mutations spike protein variations are similar in terms of speed w.r.t. to the ones present in previous variants;
- The EG.2 variant is radically different from the others and seems to pread much faster than previous variants, probably due to the fact that the number of real cases is greater than the recorded ones [22, 23];
- Mutations of EG.2 make this variant more similar to the original Omicron (in particular Q52H and F456L) which determine a lower net charge and a greater binding affinity w.r.t. the other XBB descendants.

2 The landscape of the Omicron variants

We consider the Omicron variants as the evolution for the XBB variant studied here, which is a subvariant of BA.2. BA.2 can be considered as a BA.1 subvariant containing some unique RBD (Receptor Bind Domain) spike mutations.

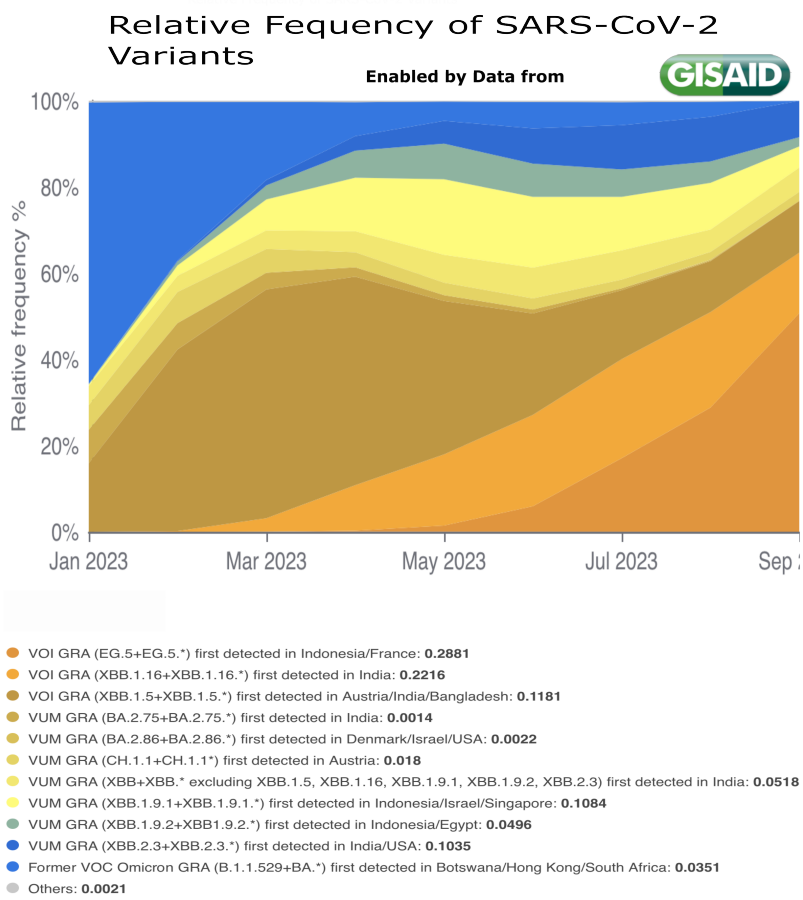

Figure 1: Figure shows the relative frequency of the SARS-CoV-2 variants from January 2023 until September 2023. It highlights the relatively rapid evolution of the virus and the rise of EG.5.1 variant since May 2023. Data Extracted from `https://gisaid.org/hcov-19-variants-dashboard/` [21]

The T376A, D405N, R408S substitutions, located in the strategic antigenic site, are associated with its capacity to evade immunisation and its high transmissibility.

XBB is a recombinant of two Omicron sublineages: (i) BJ.1 (also indicated as BA.2.10) called *Argus* and (ii) BM.1.1.1 (also indicated as BA.2.75) called *Mimas*.

XBB was identified for the first time in Singapore in the fall of 2022 and quickly began to spread across the globe. XBB was considered to be the most immune-evasive COVID variant the time, surpassing the immune-evasiveness of BA.5, which was dominant world wide until the end of August 2021. The XBB variant presents a strong capacity of overcrossing the immune system.

The XBB variant, also named as *Gryphon*, started to dominate the SARS-CoV2 scenario and the majority of circulating variants are XBB descendants (also known as the *Gryphon Family*). As reported in Figure 2, XBB descendant (see node 22f (Omicron, XBB in the descendant tree) can be summarized as follows:

- XBB.1.5 (Kraken) emerges due to a genetic recombination between two BA.2 sublineages (see ancestors of XBB nodes in the tree), combined with the S486P mutations at a significant point in its evolutionary history.
- XBB.1.9.1 (Hyperion) is XBB.1.5 sibling.
- XBB.1.16 (Arcturus) was initially identified in India with a single mutation (K478R) in the RBD of XBB.1.5. Earlier studies demonstrated that K417N, Q498R and N501Y mutations in the RBD region raise the variant's ability to bind to the human ACE2 receptor. Mutations in residue 484 in the loop area have been associated with the virus's ability to evade the immune system.

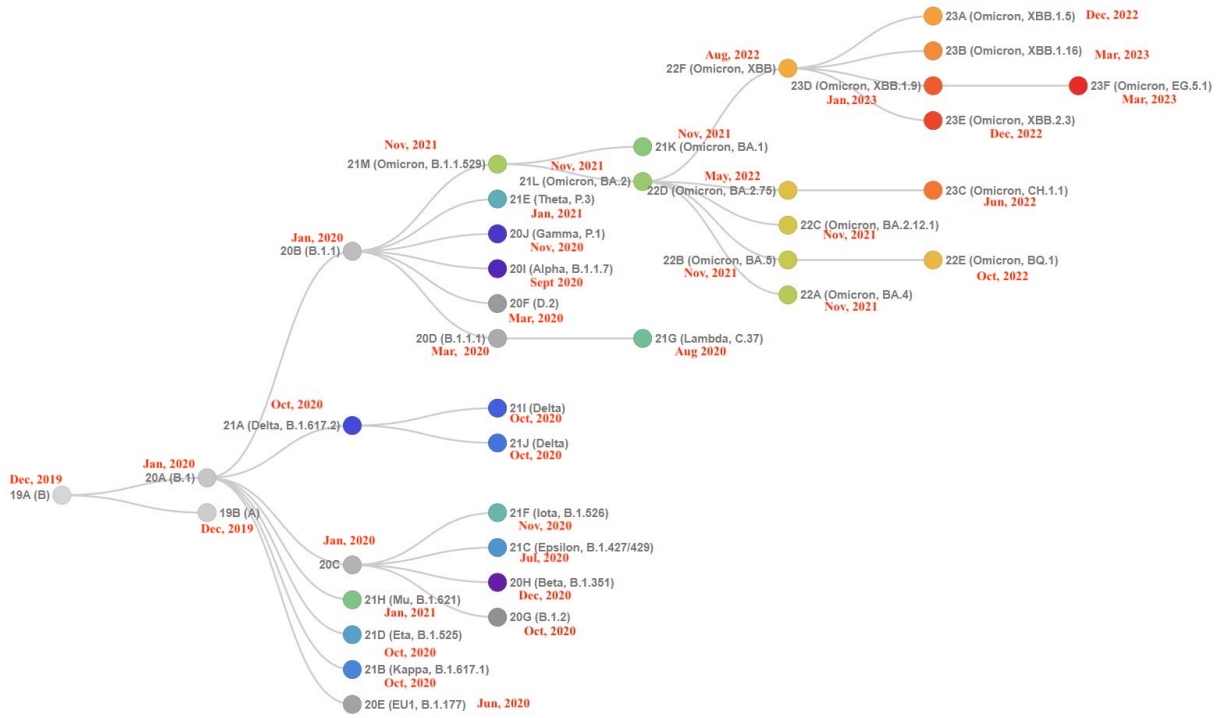

Figure 2: Phylogenetic relationships of SARS-CoV-2 clades. Covariants follow the Nextstrain Clade schema, where variants can descend from other variants. Starting from this figure, it is possible to show how the Omicron variant (**21M clade, B.1.1.529**) gave rise to a greater number of VOI/VOC. The tree has been generated from `https://covariants.org`. The red labels represent the date of first detection of each variant. As highlighted, XBB.1 showed a greater number of subvariants in a relatively short time.

- XBB.2.3 (Acrux) first appeared in late December 2022 in India, even if it didn't begin to spread until March 2023. It presents a highly evasive mutation S:T478K.
- EG.5.1 (Eris) is a direct descendent of XBB.1.9.2, which has the same spike amino acid profile as XBB.1.5. EG.5 was first reported on February 2023, and designated as a variant under monitoring (VUM) on 19th July 2023 [24].

Figure 3 depicts the mutations of the XBB Omicron subvariants considering only mutations of the S protein. Figure 1 shows the relative frequencies in the detected cases from January to September 2023. The detailed landscape of mutation across the whole viral genome is reported in Appendix 1.

We focus on XBB EG.5.1. descendant and compare it with previously identified variants and with active ones. We also pay attention to the evolutionary mutations, speed of variant diffusion and virus activity w.r.t. the spread of infection.

3 Methods

In order to study the XBB family we analyzed the variants to verify binding affinity among ACE2 and the studied variants [1]. We focus on variations (indicated as Delta) to profile the rapidity of the variant's evolution related to their diffusion and also to underscore the aggressiveness of the virus expression associated with the variant.

Measures for variations of binding affinity indicate the capacity to relate to ACE2 and thus justify VOC and VOM indexes. We examined Omicron structure subvariants of SARS-CoV-2 XBB spike glycoprotein, in 3 conformations: (i) spike glycoprotein in complex with ACE2 (1-up state), (ii) spike glycoprotein (closed-1 state) and (iii) spike glycoprotein (open-1 state). Each variant with their mutations is shown in Table 1. Sequence data has been downloaded from the PDB [25].

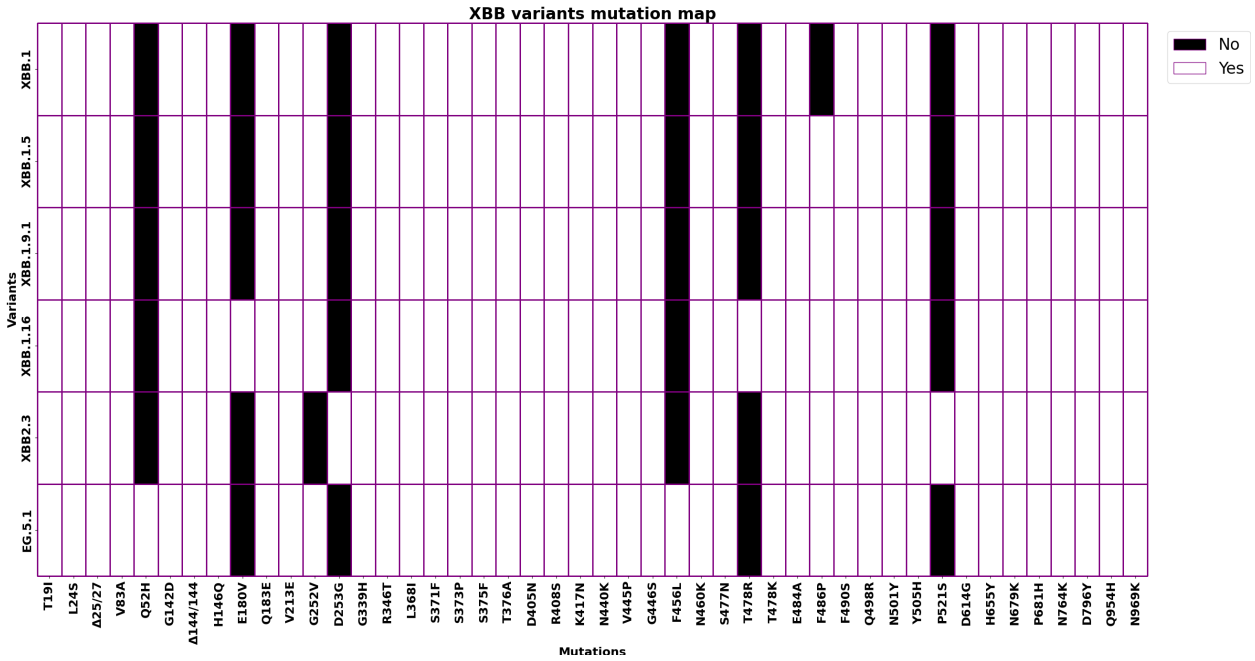

Figure 3: The matrix reports the mutations of XBB variants on the S protein. All the XBB descendants share almost all the XBB mutations. EG2 presents two unique mutations Q52H and F562 L.

Table 1: Mutations in the SARS-Cov-2 XBB Spike variants. Mutations of the discendent of XBB.1.0 are reported considering as reference XBB.1.0

Spike variant	**Mutations**
XBB.1.0	T19I + L24S + DEL25/27 + V83A + G142D + DEL144/144 + H146Q + Q183E + V213E + G252V + G339H + R346T + L368I + S371F + S373P + S375F + T376A + D405N + R408S + K417N + N440K + V445P + G446S + N460K + S477N + T478K + E484A + F490S + Q498R + N501Y + Y505H + D614G + H655Y + N679K + P681H + N764K + D796Y + Q954H N969K
XBB.1.9.1	XBB.1.0 + F486P
XBB.2.3	XBB.1.0 + V252G + D253G + F486P + P521S
XBB.1.5	XBB.1.0 + F486P
XBB.1.16	XBB.1.0 + E180V + T478R + F486P
EG.5.1	XBB.1.0 + Q52H + F456L + F486P

Structural data of Spike protein of the XBB.1 variants were also downloaded from the PDB database.We used the 8IOS structure to model the S protein in closed form and the 8IOU for the human ACE2 – SARS-CoV-2 S complex. The open configuration of the S protein was obtained by removing human ACE2 from the complex.

All the structures (open, closed and complex) for the other variants were obtained by using the *mutagenesis* PyMoL [26] starting from the PDB structures of XBB.1.

We computed TM-scores [27] between pairs of Spike proteins of two different variants by using the US-align (Universal Structural alignment) software [28]. We performed sequence alignment using the CLUSTALW software setting parameters at default values [29].

Binding Affinity of the spike proteins of the variants and human ACE2 was calculated by using PRODIGY, a web server for predicting the binding affinity of protein–protein complexes [30], available at `https://wenmr.science.uu.nl/`

`prodigy/`. We set the temperature of the simulation at **35.8** degrees Celsius. For each complex PRODIGY calculated the δG, i.e. the Gibbs free energy, and the K_d , dissociation constant.

For each variant, we computed the acid dissociation constant pK_a for each amino acid of the analyzed proteins using the PROPKA3 web server [31]. Given a node, the pK_a value equals to $-log_{10}K_a$, where K_a is the acid dissociation constant which measures the amino acid acidity or alkalinity. Following the method proposed in [32], pKa values were used to predict the overall domain charge.

4 Results

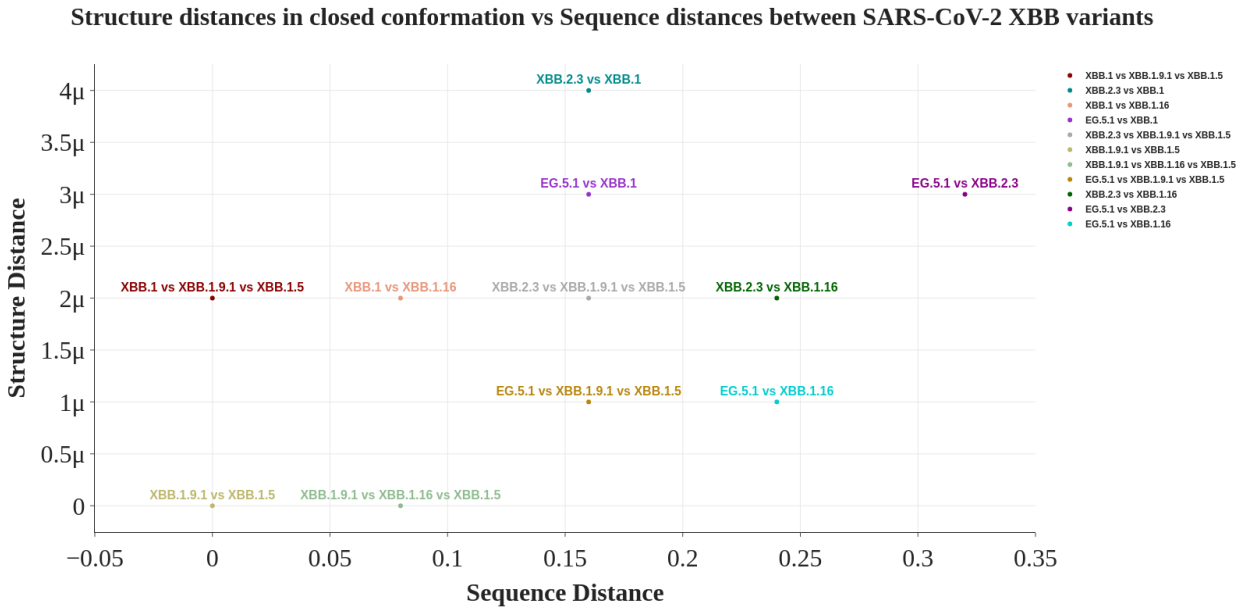

Figure 4: The x-axis of the Figure represents the distance between the sequences, while the y-axis gives the distance between the structures. Each point represents the correlation of a pair sequence/structure. We report the XBBs variants in the closed conformation.

We investigated the relationship between sequence distances and the corresponding structure distances, as depicted in Figure 4. We analysed the relation between sequence and structure to characterise the evolution of the XBB subvariants and compare them with the initial variants as discussed in [14].The x-axis of the graph displays the pair-wise sequence distances calculated on the primary structure, while the y-axis reflects the pair-wise mutual distances of the protein-structures. Each point on the graph represents the correlation between a pair of sequence and structure. The Figure clearly shows that there is no correlation between sequence and structure distance, thus confirming that the evolution of the XBB descendants is similar to the whole SARS-CoV-2 phylogeny. Figure 4 shows no evidence of a structural relationship between sequence and structure. Note that the sequence distance does not influence the results on the structural distance.

This result implies that structural (and thus functional) distinctions are highly dependent on the local structural context when examined at a finer level of detail, making it impossible to extrapolate the same information from the sequences.

We estimate the transmissibility of the affinity binding between Spike protein of the variants and the human ACE2 receptor by measuring biochemical properties of the proteins.

First, we report the net charge of domain NTT in each variant. We report a negative charge in all the variants, indicating affinity to bind to the human ACE2. We report similar values for all the XBB.1 subvariants except for EG.1 due the specific mutations of these variants. Surprisingly, EG.1 is more similar to Omicron than to XBB when the net charge of NTD domain is considered.

For each variant, Figure 6 reports the Gibbs free energy. The subvariants of XBB have a lower $\Delta\mathcal{G}$ and this implies a greater binding affinity. Not surprisingly EG5.1 has the maximum binding affinity. We also reported the difference between the free energy of each variant and the XBB in Figure 7. We measured the $\Delta\Delta\mathcal{G}$ as the difference among the

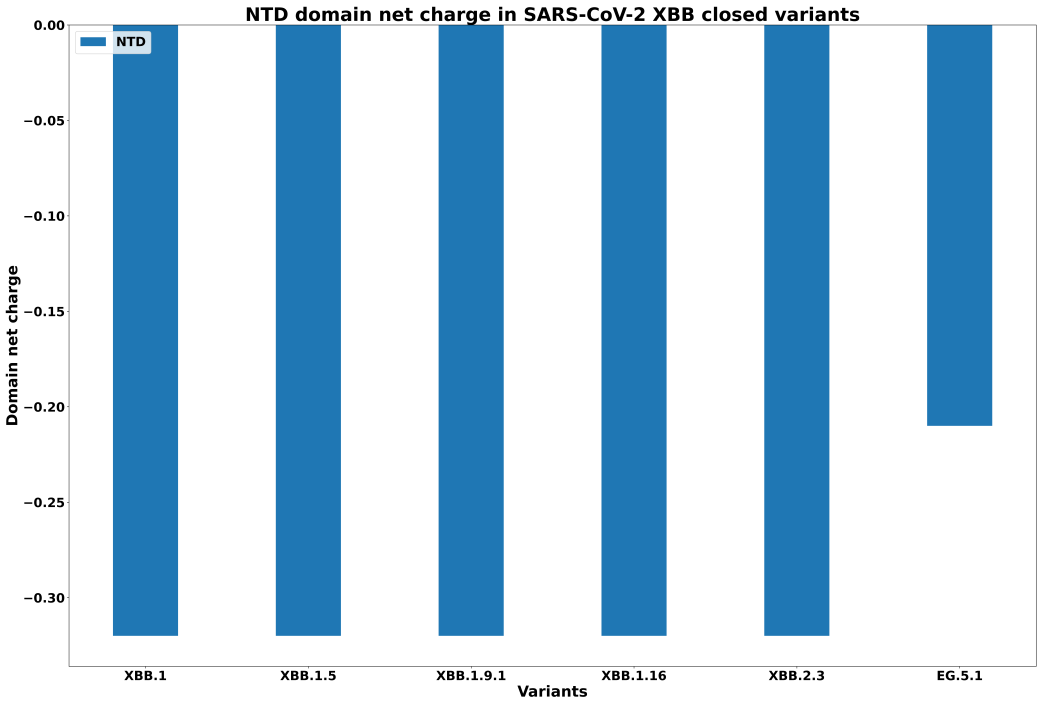

Figure 5: Net charges in the NTD domain for all XBB variants.

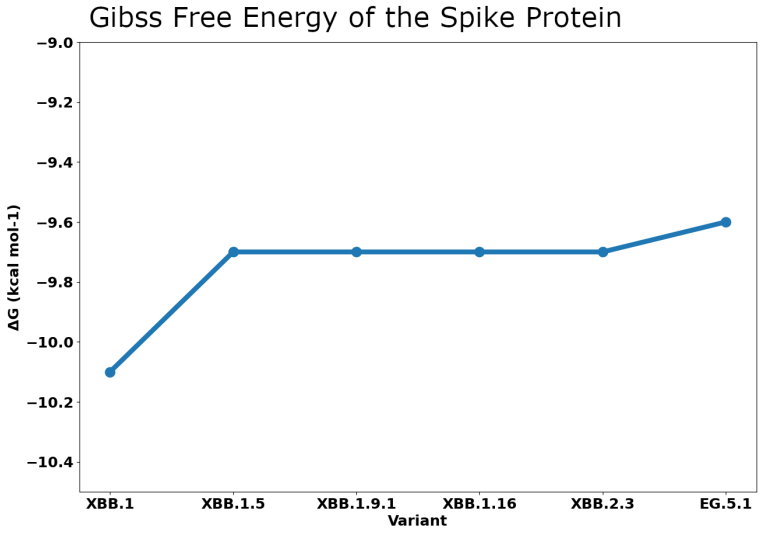

Figure 6: Gibbs free energy ΔG to quantify the binding affinity of each XBB variant with the ACE2 receptor.

calculated values of $\Delta\mathcal{G}$. We compared the binding affinity changes considering both Omicron (in Figure 8) and Wild Type (in Figure 9 as a reference). In particular Figure 9 shows the variation of Gibbs free energy of the Spike Protein with respect to the previous Delta variant.

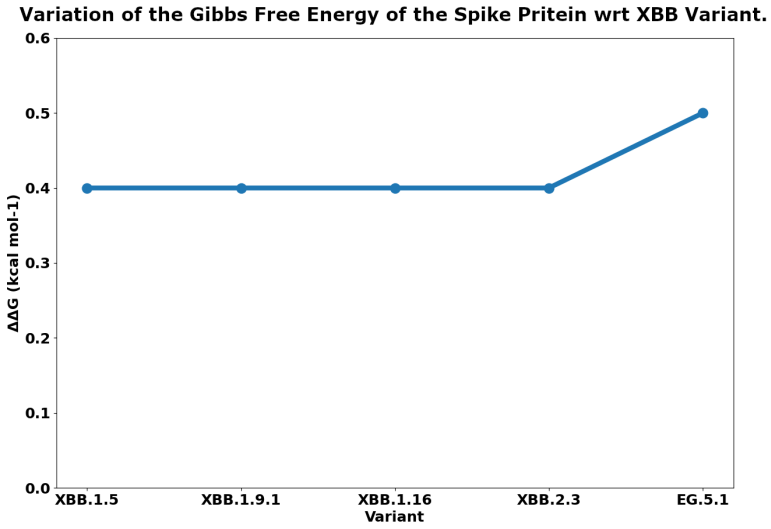

Figure 7: Gibbs free energy difference $\Delta\Delta$G between studied XBB variants and their ancestor XBB.

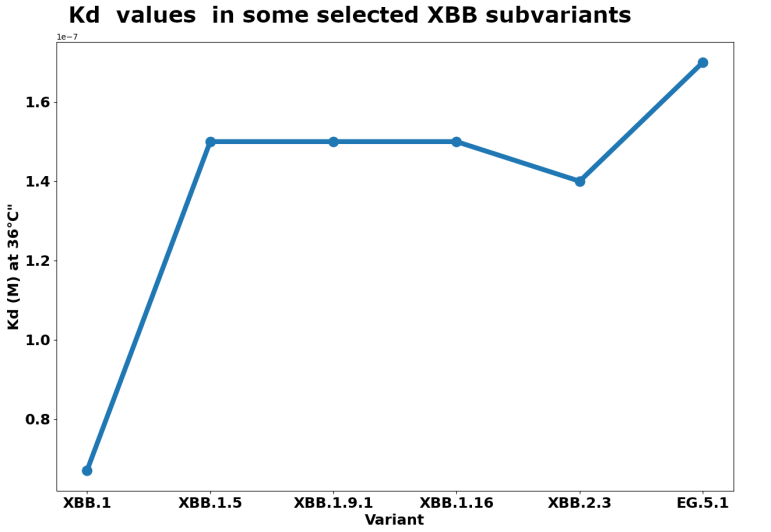

Figure 8: Dissociation constant K_D of the selected XBB variants. A higher value of XBB determines a stronger binding affinity. As evidenced, EG.5.1 has the greatest binding affinity to the human ACE2 receptor.

5 Discussion

Compared to previous ones, the evolution of XBB variant is interesting in terms of speed and number of cases identified in a short time [33]. XBB and XBB.1 have shown the highest levels of immune escape of all the Omicron sublineages currently identified and have shown significant reductions in the capacity of infecting from vaccinated individuals. In particular, several substitutions of XBB.1 (first descendent of XBB) have been shown to confirm significant resistance to BA.2 infections. The immune resistance, conferred by each individual substitution, is relatively minor when compared to the resistance of XBB.1, suggesting that multiple substitutions in the XBB.1 S protein cooperatively contribute to resistance to immunity induced by breakthrough BA.2 infection [34]. This may explain the relatively high frequency of people reinfected by the XBB variant and its descendants jointly with the escape of vaccine immunity [35].

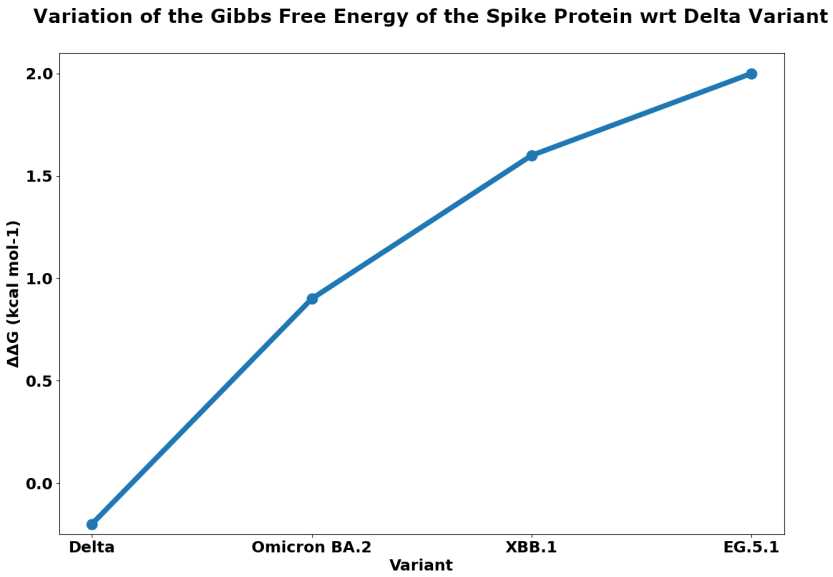

Figure 9: Gibbs free energy difference $\Delta\Delta G$ between Delta, BA.2 Omicron, XBB.1, EG.5.1 variants and the SARS-CoV-2 wild type form.

Moreover, XBB.1.5 has shown an RBD Spike mutation (F486P) that increases infectivity due to increased binding affinity to the angiotensin-converting enzyme 2 (ACE2) receptor [36].

As reported in Figure 7, the EG.5.1 variant has shown increased prevalence, growth advantage, and immune escape properties. This is mainly due to the flip mutations F456L and L455F. Such interesting substitutions (nicknamed FLip-FLop) are of interest for two adjacent amino acids (455-456) of the RBD Spike protein.

6 Conclusion

Focusing on recent modifications, we found a correlation between genetic sequences, protein structures, and binding affinities of the SARS-CoV-2 variants for the XBB lineage. The findings suggest that the evolution of the XBB variants is similar to the overall SARS-CoV-2 phylogeny, implying that the structural and functional distinctions depend on the context. The strong binding affinity observed in certain XBB variants, particularly EG5.1, raises questions about the ability of current vaccines to control the spread of these variants. These results which have implications for understanding the transmissibility and infectivity of these variants, may be of use in devising strategies to manage the ongoing COVID-19 pandemic. To stay ahead of the evolution of the virus, further research and surveillance are essential and should be used to adjust public health measures accordingly.

7 Data and Code Availability.

All the data and the code used for this paper are available at `https://github.com/UgoLomoio/XBBSARSCoV2`. Third part softwares are available on their own websites.

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8 Appendix 1 : Relative Frequency of Omicron Sub variants

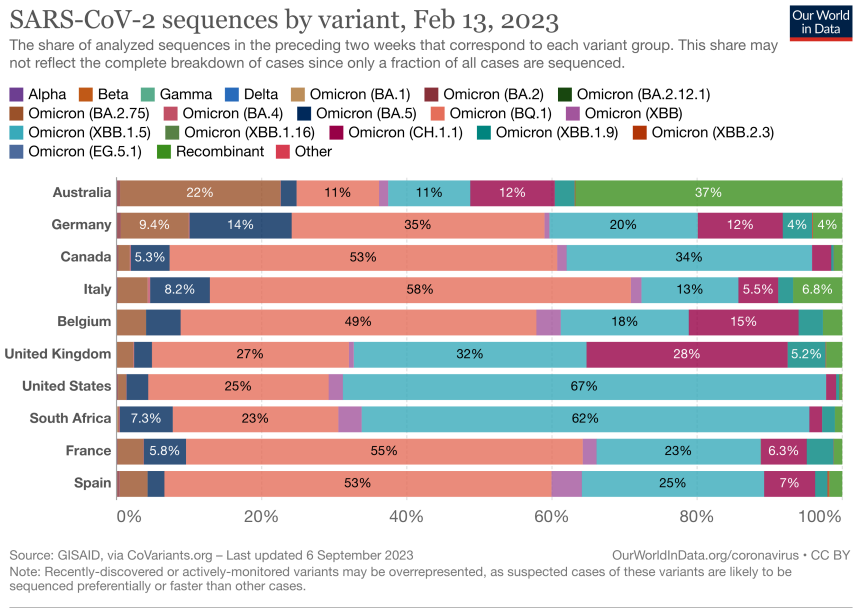

Figure 10: Relative Frequency of Omicron Variants on February. Image generated from `https://ourworldindata.org/` under open access under the Creative Commons BY license.

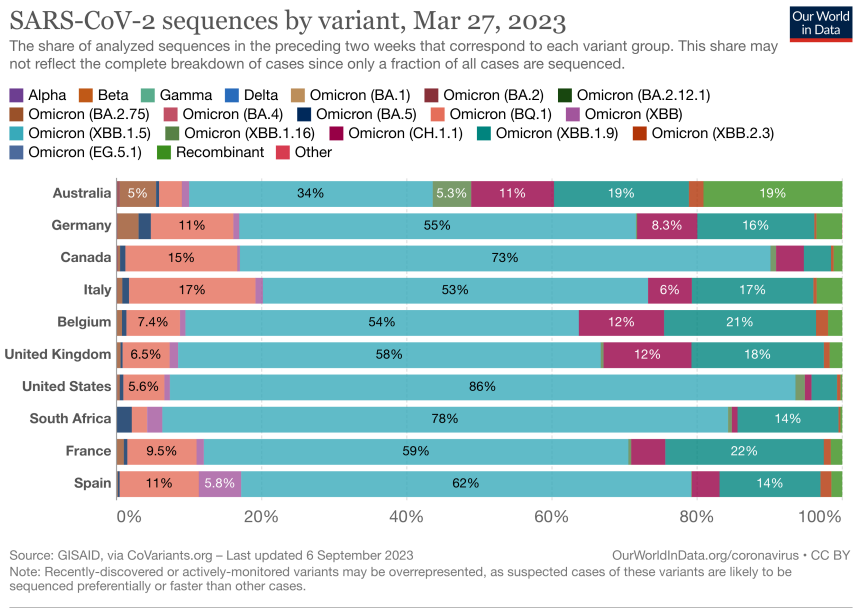

Figure 11: Relative Frequency of Omicron Variants April 2023. Image generated from `https://ourworldindata.org/` under open access under the Creative Commons BY license.

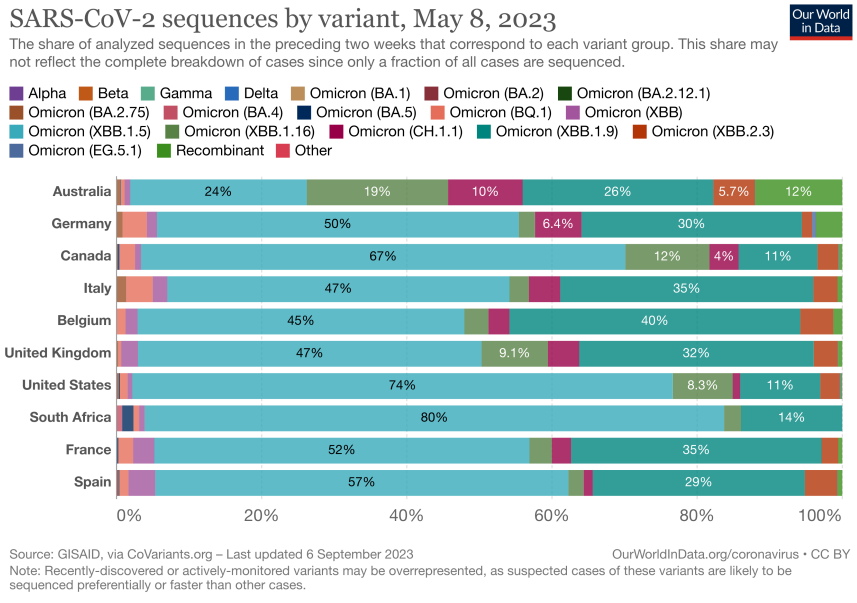

Figure 12: Relative Frequency of Omicron Variants May. Image generated from `https://ourworldindata.org/` under open access under the Creative Commons BY license.

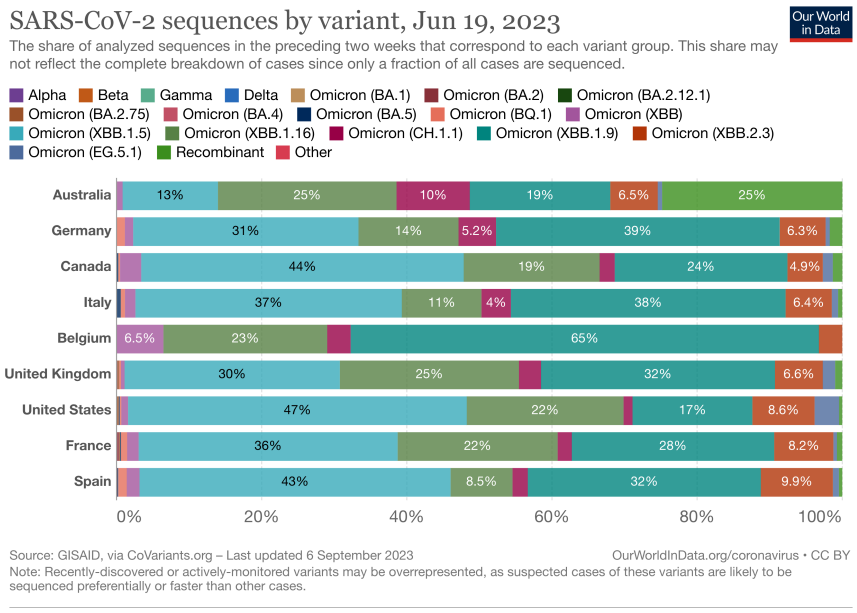

Figure 13: Relative Frequency of Omicron Variants June. Image generated from `https://ourworldindata.org/` under open access under the Creative Commons BY license.

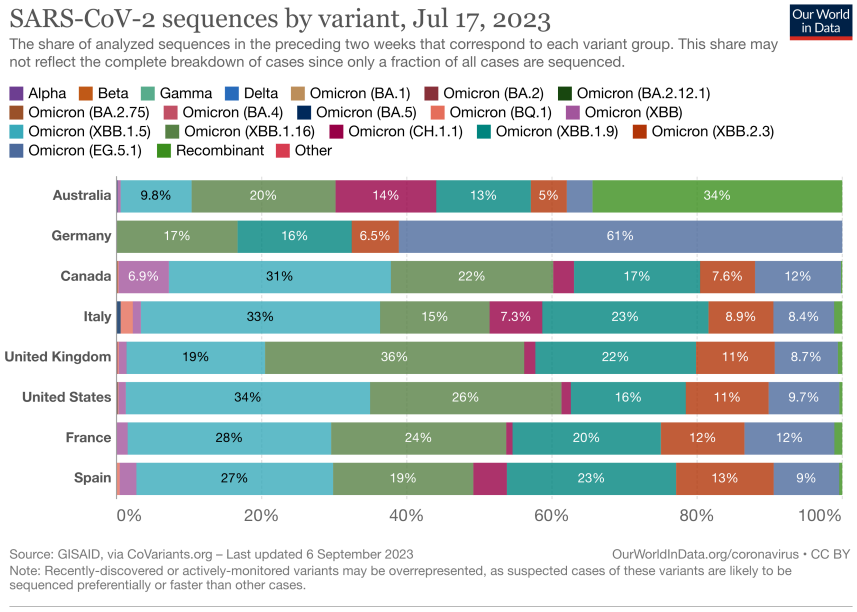

Figure 14: Relative Frequency of Omicron Variants July. Image generated from `https://ourworldindata.org/` under open access under the Creative Commons BY license.

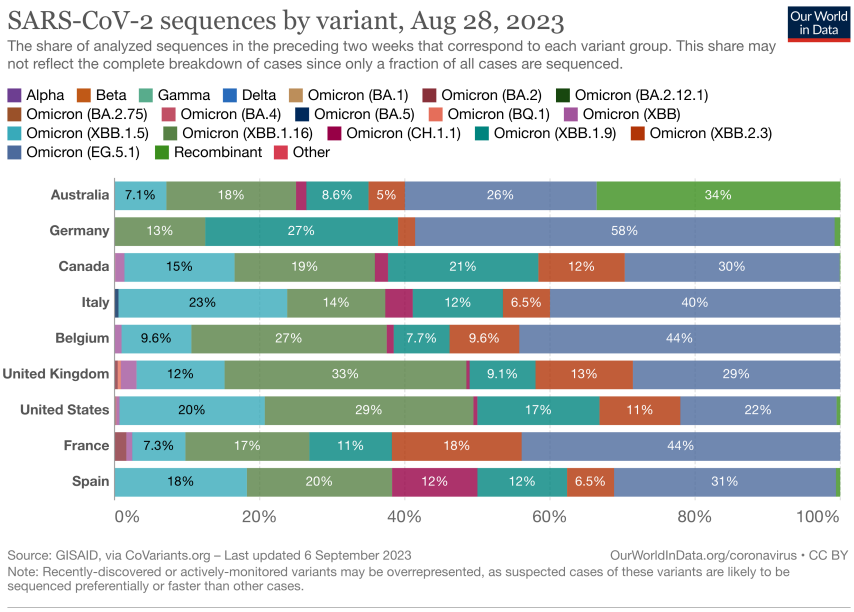

Figure 15: Relative Frequency of Omicron Variants August 2023. Image generated from `https://ourworldindata.org/` under open access under the Creative Commons BY license.

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