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Fixation and reversion of mutations in the receptor-binding domain of the SARS-CoV-2 spike protein: A 2020–2024 analysis

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Dear Editor,

Reversion of amino acid mutations in structural proteins has been a common phenomenon in viral evolution. SARS-CoV-2, with the massive number of whole genome sequences supplied by modern technologies, provides an unprecedented opportunity for ecological studies.

Much attention has been dedicated to the SARS-CoV-2 spike (S) protein, being the target of neutralizing antibodies elicited by infection and/or COVID-19 vaccination. The 223-amino acid region between residues 319 and 541 has been identified as the receptor-binding domain (RBD), with the internal 72 amino acid portion (residues 437–508) identified as the receptor-binding motif (RBM).[1] Deletion of residues 69–70 has been the most widely known example of reversion so far (with diagnostic implications for S-gene target failure in several PCR kits), but other spike residues, both inside and outside the RBD, have been previously reported to reverse their mutations.

At the end of 2023, we charted mutations in each of the RBD residues across the 15,479,677 SARS-CoV-2 genomes sequenced over the 4-year course of the pandemic (December 1, 2019 to August 1, 2023) using the CoV-Spectrum.org web interface[2]). At that time we found[3] that 88 % (196/223) of RBD residues had never mutated from wild-type, 9 % (20/223, 11 inside and 9 outside the RBM) exhibited single mutations that had remained fixated in >50 % of the global population, and 3 % (7/223) exhibited repeated cyclical mutations and reversions (dubbed "yo-yo residues") towards the wild type (each achieving >50 % prevalence for >2 months). At that time "yo-yo residues" were just 7 (K444X, G446S, L452R, Q493R, G496S and N501Y, plus R346X outside the RBM).

We have now replicated the analysis as of October 15, 2024 (Fig. 1). Unmutated residues account now for 86 % (192/223) of the RBD, but this goes down to 72 % (52/72) within the RBM. Fixated mutations (Fig. 1) now account for 11 % (24/223) of the RBD, which goes up to 17 % (12/72) within the RBM: they affect residues 440,445,455,460,477,478,481,483,484,486,498, and 505 within the RBM and 332,339,371,373,375,376,403,405,408, and 417 outside the RBM. Finally, "yo-yo" residues (Fig. 2) now represent 4 % (10/223) of the RBD, which goes up to 11 % (8/72) within the RBM: affected residues include 444, 446,452,456,490,493,496,501 within the RBM and 346, 368 outside the RBM.

Unchanged residues and fixated mutations are likely to identify the most durable candidate vaccine epitopes, while resonating "yo-yo residues" likely represent residues that the virus can change when the following occurs, i.e., during increased selective pressure, while accommodating the reduced fitness. As soon as the immune pressure reduces (e.g., with declining vaccine-elicited immunity[4]), those mutations

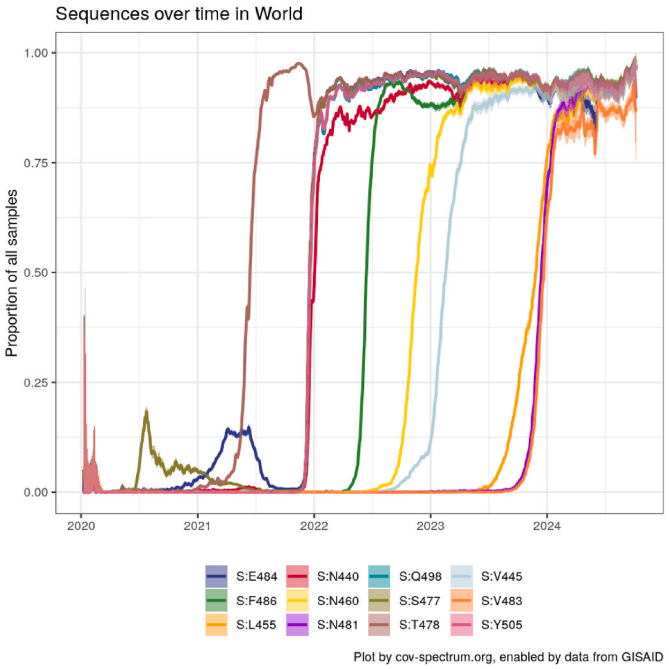

Fig. 1. Summary of mutations fixated within the spike receptor-binding domain (RBD) of SARS-CoV-2 in 2019–2024.

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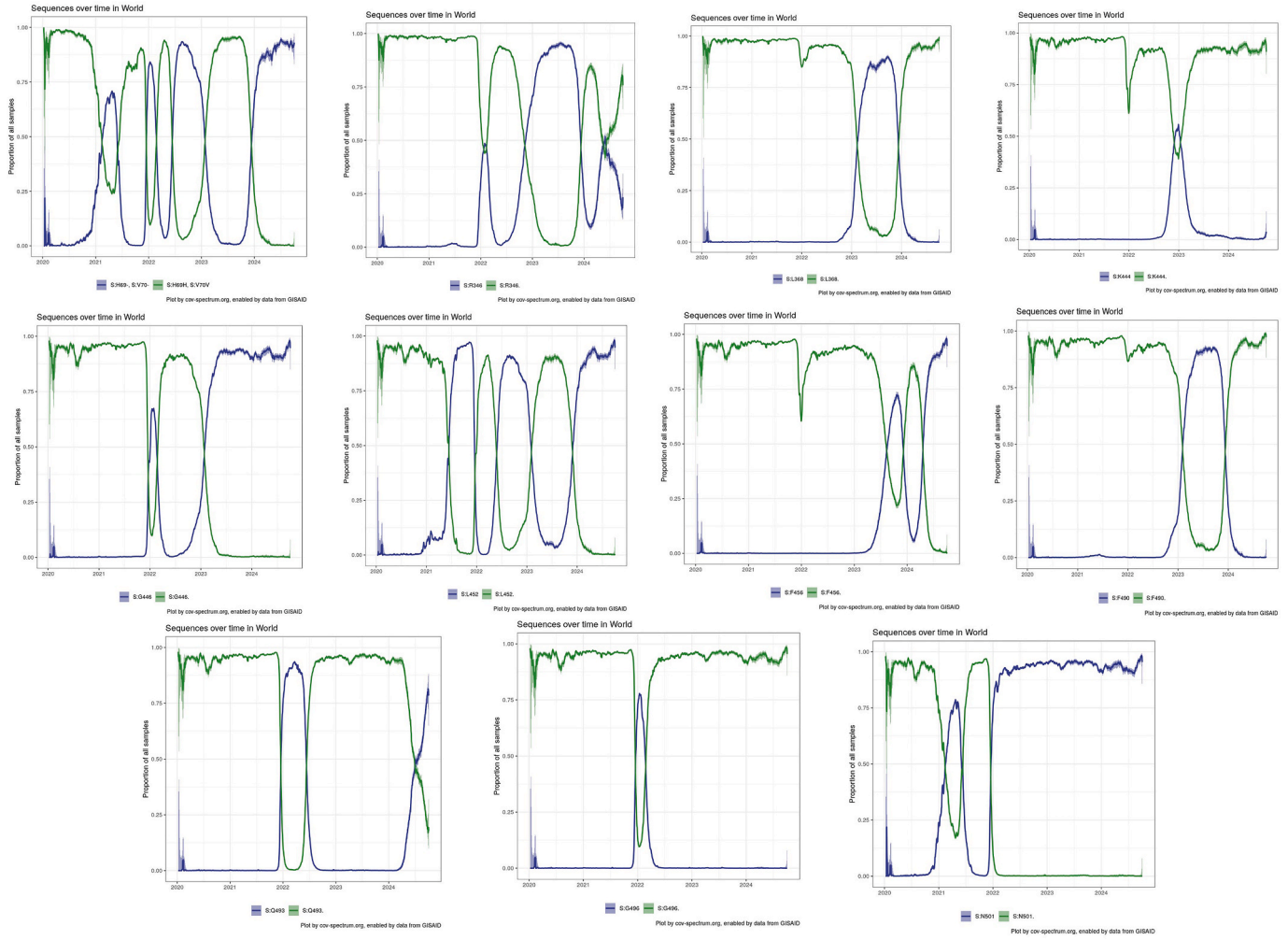

Fig. 2. Spike receptor-binding domain (RBD) residues that have undergone cyclic mutation and reversion, compared to cyclic fluctuations in del69-70. Individual charts were generated using the CoV-Spectrum.org compare variants tool. As per CoV-Spectrum query syntax, "S:XXX" represents an unmutated amino acid residue, while "S:XXX." represents a mutated residue.

can quickly revert, perpetuating multiple cycles of mutations and reversions. Our findings have implications for vaccine and therapeutic anti-spike monoclonal antibody (mAb) manufacturing; predictive models could help avoid the recent failures in mAb development.[5]

CRediT authorship contribution statement

Daniele Focosi: Formal analysis, Data curation, Conceptualization. **Pietro Giorgio Spezia:** Validation, Supervision. **Fabrizio Maggi:** Visualization, Validation, Supervision.

Data availability statement

This study did not generate any new dataset. GISAID analysis was performed using the CoV-Spectrum.org web portal.

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Declaration of competing interest

We declare we have no conflict of interest related to this manuscript.

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