SARS-CoV-2 variants, spike mutations and immune escape

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Abstract

While most SARS-CoV-2 mutations are expected to be either deleterious and swiftly purged or relatively neutral, a small proportion will affect functional properties and may alter infectivity, disease severity or interactions with host immunity. Emergence of SARS-CoV-2, in late 2019, was followed by a period of relative evolutionary stasis lasting about eleven months. Since late 2020, however, SARS-CoV-2 evolution has been characterised by the emergence of sets of mutations, in the context of 'variants of concern', that impact virus characteristics including transmissibility and antigenicity, probably in response to the changing immune profile of the human population. While there is emerging evidence for reduced neutralisation of some SARS-CoV-2 variants by post-vaccination serum, greater understanding of correlates of protection is required to evaluate how this may impact vaccine effectiveness. Nonetheless, manufacturers are preparing platforms for a possible update to vaccine strain, and it is critical that surveillance of genetic and antigenic changes in the global virus population is carried out alongside experiments to elucidate the phenotypic impacts of mutations. Here, we review the literature on mutations to the SARS-CoV-2 Spike protein, the primary antigen, focussing on their antigenic impacts and contextualising them in the protein structure and observed mutation frequencies in global sequence datasets.

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As of April 2021, SARS-CoV-2, the causative agent of COVID-19, accounts for over 136 million infections and approaching three million deaths worldwide (https://covid19.who.int/). Virus genomic sequences are being generated and shared at an unprecedented rate, with over one million sequences available via the Global Initiative on Sharing All Influenza Data (GISAID), permitting near real-time surveillance of the unfolding pandemic [1]. The use of pathogen genomes on this scale to track the spread of the virus internationally, study local outbreaks and inform public health policy signifies a new age in virus genomic investigations [2]. Further to epidemiological understanding, sequencing enables identification of emerging SARS-CoV-2 variants and sets of mutations potentially linked to changes in viral properties.

As highly deleterious mutations are rapidly purged, the majority of mutations observed in genomes sampled from circulating SARS-CoV-2 viruses are expected to be either neutral or mildly deleterious. This is because although high-effect mutations that contribute to virus adaption and fitness do occur, they will tend to be in the minority compared to tolerated low/no-effect "neutral" amino acid changes [3]. A small minority of mutations are expected to impact virus phenotype in a way that confers a fitness advantage, in at least some contexts. Such mutations may alter various aspects of virus biology such as pathogenicity, infectivity, transmissibility and/or antigenicity. While care has to be taken not to confound mutations being merely correlated with growing lineages [4], fitness-enhancing mutations were first detected to have arisen within a few months of the evolution of SARS-CoV-2 within the human population. For example, the Spike amino acid change D614G was noted to be increasing in frequency in April 2020 and to have emerged several times across the global viral phylogeny and exhibiting a high dN/dS ratio suggesting positive selection at the codon [5,6]. Subsequent studies indicated D614G confers a moderate advantage for infectivity [7,8] and transmissibility [9]. Several other Spike mutations of note have now arisen and are discussed in this review, with a particular focus on mutations affecting antigenicity.

The extent to which mutations affecting the antigenic phenotype of SARS-CoV-2 will enable variants to circumvent immunity conferred by natural infection or vaccination remains to be determined. However, there is growing evidence that mutations that change the antigenic phenotype of SARS-CoV-2, are circulating and affect immune recognition to a degree that requires immediate attention. The Spike protein mediates attachment of the virus to host cell-surface receptors and fusion between virus and cell membranes (**Box 1**) [10]. It is also the principal target of neutralising antibodies generated following infection by SARS-CoV-2 [11,12], and is the SARS-CoV-2 component of both mRNA and adenovirus-based vaccines licensed for use and others awaiting regulatory approval [13]. Consequently, mutations that affect the antigenicity of the Spike protein are of particular importance. Here, we review the literature on the antigenic consequences of mutations focusing on the Spike protein and antibody-mediated immunity and discuss these in the context of observed mutation frequencies in global sequence datasets.

Box 1 | Spike protein structure, function and mutation frequency. As with other coronaviruses, the entry of SARS-CoV-2 into host cells is mediated by the transmembrane Spike glycoprotein which forms

homotrimers on the surface of the virion. The SARS-CoV-2 Spike is post-translationally cleaved by mammalian furin into two subunits: S1 and S2 (**Figure 1a**). The S1 subunit largely consists of the N-terminal domain (NTD) and the receptor-binding domain (RBD) and is responsible for binding to the host cell surface receptor, angiotensin-converting enzyme 2 (hACE2), while the S2 subunit includes the trimeric core of the protein and is responsible for membrane fusion (**Figure 1b**). The presence of a polybasic furin cleavage site at the S1-S2 boundary, unique within the *Sarbecovirus* subgenus, is important for infectivity and virulence [14], with furin cleavage facilitating the conformational change required for receptor-binding [15]. Spike transiently undergoes conformational movements between a closed and an open conformation in which a hinge-like movement raises the RBD [15]. The residues comprising the receptor-binding motif (RBM) are revealed on the upright RBD enabling binding to ACE2, which induces a progressively more open structure until a fully open, three-ACE2-bound structure is formed, initiating S2 unsheathing and membrane fusion [16]. The SARS-CoV-2 Spike protein is highly glycosylated, with 66 potential N-glycosylation sites per trimer (violet arrows in **Figure 1a**) [17,18].

The SARS-CoV-2 virus has a positive-sense single-stranded RNA genome consisting a single linear RNA segment of approximately 29,891 nucleotides in length [19]. The Spike protein is synthesised as a 1,273 amino acid polypeptide and the frequency of amino acid variants, including both substitutions and deletions, at each of these positions is shown in **Figure 1a**. These variants, relative to Wuhan-Hu-1, are identified using CoV-GLUE [20] which filters out GISAID sequences [21] identified as being of lower quality or from non-human hosts (sequences retrieved from GISAID on 3 February 2021). Among 426,623 genomes after filtering, 5,106 different amino acid replacements or substitutions across 1,267 Spike positions were identified, of which 320 at 259 positions were observed in at least 100 sequences. In addition to substitutions, several deletions have been observed, particularly within the NTD. The most frequently detected NTD deletion is the two-residue deletion at positions 69 and 70 (Δ69-70), present in 45,898 sequences.

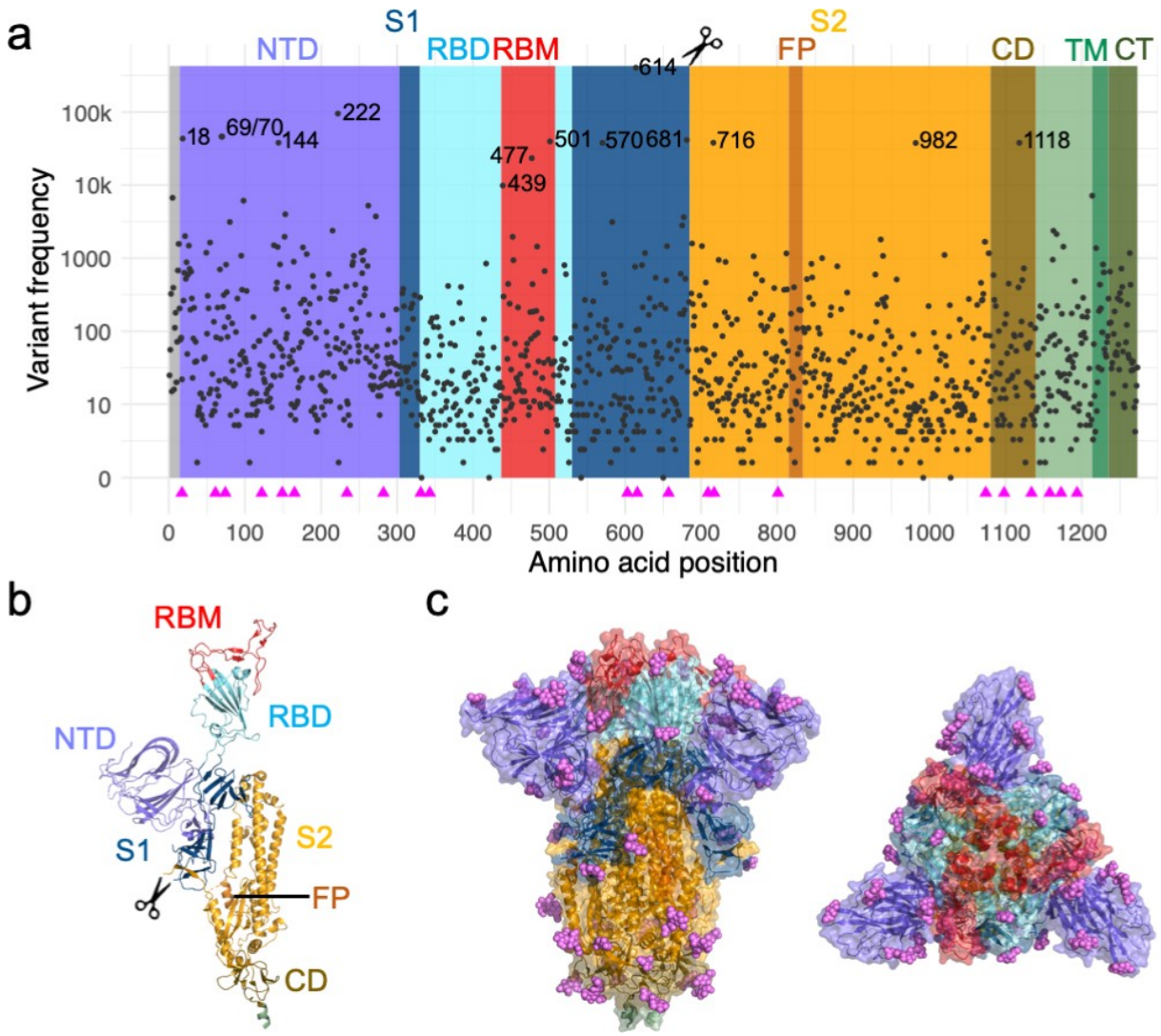

Figure 1. Spike protein sequence variability and structure. (**a**) Schematic representation of SARS-CoV-2 Spike protein's domain organisation showing protein sequence variability. Variant frequency at each amino acid position is the sum of amino acid replacements and deletions and is shown on a log scale (x-axis). Sequence variability based on CoV-GLUE, http://cov-glue.cvr.gla.ac.uk/, processed GISAID Initiative data (downloaded 3 Feb 2021). The labelled structural domains are: NTD N-terminal domain, RBD receptor-binding domain, FP fusion peptide, CD connecting domain, TM transmembrane domain, CT cytoplasmic tail. Scissors mark the S1/S2 boundary at position 685 and potential N-linked glycosylation sites are labelled with magenta triangles. (**b**) Depiction of the Spike monomer displaying upright RBD. (**c**) Spike protein structure in closed conformation overlaid with surface representations shown with a trimer axis vertical view (centre) and an orthogonal top-down view along this axis (right). Domains are coloured consistent with panel **a** and glycans are shown as magenta spheres. Figure prepared using cryo-EM structures with PDB IDs 6ZGG and 6ZGE [15].

Spike mutations receiving early attention. The rate of evolution of SARS-CoV-2 from December 2019 to October 2020 was consistent with the virus accommodating approximately two mutations per month in the global phylogeny [22,23]. While our understanding of the functional consequences of Spike mutations is rapidly expanding, much of this knowledge involves the reactive investigation of amino acid changes identified as rapidly increasing in frequency or being associated with unusual epidemiological characteristics. Following the

emergence of D614G, a substitution within the receptor-binding motif (RBM), N439K, was noted as increasing in frequency in Scotland in March 2020. While this first lineage with N439K (designated B.1.141 using the Pango nomenclature system introduced by Rambaut *et al.* (2020), https://cov-lineages.org) is now extinct, another lineage (B.1.258) also defined by the presence N439K is circulating in many European countries and internationally [24]. N439K is noteworthy as it enhances binding affinity to the ACE2 receptor and reduces the neutralising activity of some monoclonal antibodies (mAbs) and from polyclonal antibody responses present in sera from a sizable fraction of people recovered from infection [25]. Another RBM amino acid change, Y453F – associated with increased ACE2 binding affinity [26]– received considerable attention following its identification in sequences associated with infections in humans and mink; most notably with one lineage identified in Denmark and initially named 'cluster 5' (now, B.1.1.298) [27]. As of 5 November 2020, 214 human COVID-19 cases infected with SARS-CoV-2 related to mink were all carrying the mutation Y453F [28]. The B.1.1.298 lineage also has Δ69-70, an NTD deletion that has emerged several times across the global phylogeny, including in the second N439K lineage, B.1.258. Δ69-70 is predicted to alter the conformation of an exposed NTD loop and has been reported to be associated with increased infectivity [29].

Genomic analyses indicate a change in host environment and signatures of increased selective forces acting upon immunologically important SARS-CoV-2 genes sampled from around November 2020 [30]. This coincided with the emergence of variants with higher numbers of mutations, relative to previous circulating variants. These lineages due to their association with increased transmissibility were named 'variants of concern'. They are defined by multiple convergent mutations that are hypothesised to have arisen either in the context of chronic infectious or previously infected individuals [31-36]. In addition to understanding the transmissibility and pathogenicity of these emerging variants, it is critically important to characterise their antigenicity and the level of cross-protection provided from infection by earlier viruses that are genetically and antigenically similar to the virus that first emerged in December 2019 and which is used in all of the current vaccine preparations. Information on how Spike mutations affect antigenic profiles can be derived from structural studies, mutations identified in viruses exposed to mAbs or polyclonal plasma, targeted investigations of variants using site-directed mutagenesis and deep mutational scanning (DMS) experiments that systematically investigate mutational possibilities.

Structural analysis to inform identification of immunogenic regions of Spike. Several studies have probed the antigenicity of SARS-CoV-2 Spike protein by solving the structure of Spike in complex with the antigen-binding fragment of particular antibodies [12,37-39]. Serological analyses of almost 650 SARS-CoV-2-infected individuals indicated that around 90% of the plasma or serum neutralising antibody activity targets the Spike RBD [11]. A relative lack of a glycan-shielding may contribute to the immunodominance of the RBD [40]. Barnes *et al*. (2020) reported structural, biophysical and bioinformatics analyses of fifteen SARS-CoV-2 RBD-binding neutralising antibodies [38]. Antibody footprints were generated by structural analyses

of the Spike residues considering potential hydrogen bonds and van der Waals interactions with an mAb atom that were less than 4.0 Å. Structural analysis allowed the categorisation of RBD-binding neutralising antibodies into classes (**Figure 2a**-**b**): 1) neutralising antibodies that block ACE2 and bind Spike in the open conformation, 2) ACE2-blocking antibodies that bind RBD in both open and closed conformation, 3) antibodies that do not block ACE2 and bind the RBD both in open and closed configuration, or 4) neutralising antibodies that bind outside the ACE2 site and only in open configuration [38]. Within the RBD, RBM epitopes overlapping the ACE2 site are immunodominant whereas other RBD sites generate lower and variable responses in different individuals [11].

While the RBD is immunodominant, there is evidence for a significant role of other Spike regions in antigenicity, most notably the NTD [12,37,41]. Early structural characterisation of NTD-specific antibodies 4A8 [39] and 4-8 [12] revealed similar epitope locations towards the upper side of the most prominently-protruding area of the NTD. Cryo-electron microscopy was used to determine the antibody footprint of neutralising antibody 4A8 and showed key interactions involving Spike residues Y145, H146, K147, K150, W152, R246 and W258 [39]. Epitope-binning of 41 NTD-specific mAbs led to the identification of six antigenic sites, one of which is recognised by all known NTD-specific neutralising antibodies and has been termed the NTD supersite consisting of residues 14-20, 140-158, and 245-264 [37] (**Figure 2a-b**). The mechanism of neutralisation by which NTD-specific antibodies act remains to be fully determined though may involve the inhibition of conformational changes or proposed interactions with auxiliary receptors such as DC-SIGN/L-SIGN [39,42]. Relatively little is known of antigenicity in S2, with immunogenicity thought to be impeded by extensive glycan shielding [43], and while both linear and cross-reactive conformational S2 epitopes have been described [44,45], the biological significance of these is not yet known.

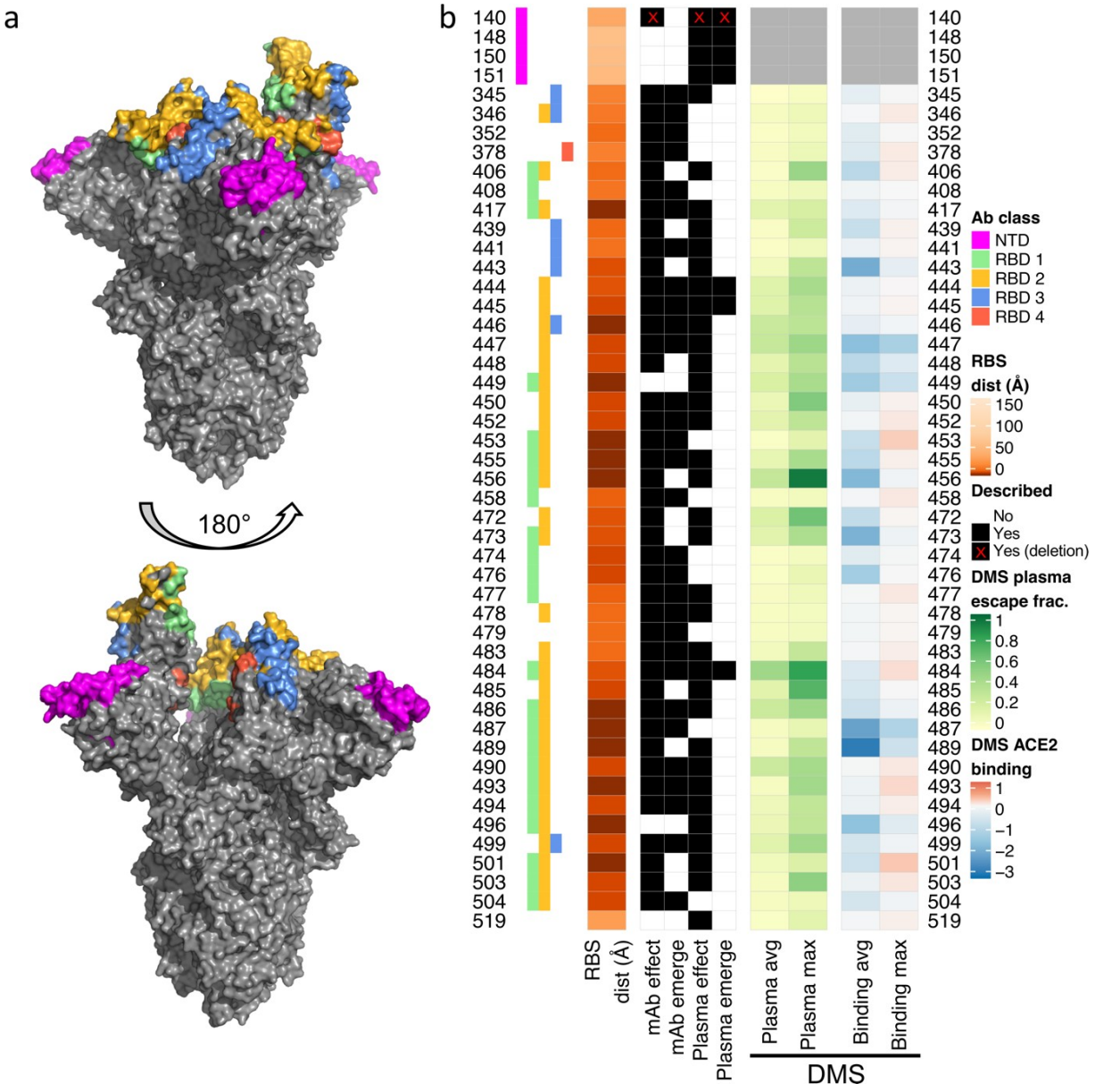

Figure 2. Visualisation of neutralising antibody classes defined by structural analyses and properties of spike protein residues where substitutions are described as affecting polyclonal plasma (**a**) Residues are coloured according to the class of antibody that binds to an epitope. RBD antibody classes 1-4 [38] are depicted by colours: green (class 1: ACE2 blocking, bind open RBD only), yellow (class 2: ACE2 blocking, bind open and closed RBD), blue (class 3: non-ACE2 blocking, bind open and closed RBD) or yellow (class 4: non-ACE2 blocking, bind open RBD only). When residues belong to epitopes of multiple classes, priority colouring is given to Abs that are ACE2 blocking and bind the closed Spike. The NTD supersite [37] is coloured in magenta. (**b**) Aligned heatmaps showing properties of amino acid residues where substitutions affecting recognition or emerging upon exposure to plasma antibodies. From left to right: antibody classes are coloured consistently with panel **a**; distance to the ACE2-contacting residues that form the receptor-binding site (RBS) (Å) is shown in shades of orange; each residue is classified as having evidence for mutations either affecting neutralisation by mAbs [46-49] or polyclonal plasma from previously infected individuals (convalescent plasma) [47-51] or vaccinated individuals [52]. For both mAbs and plasma, a subset of these residues have mutations described as emerging upon exposure (co-incubation) to mAbs [46,48,49] or plasma [49,50] in laboratory experiments. When an observation includes a deletion, this is indicated by a red cross; in shades of green, the results of deep mutational scanning results (DMS) experiments where RBD mutants exposed to multiple samples of human convalescent [51] are shown. The escape

fraction averaged across all amino acid substitutions at a residue (plasma avg) and the maximally resistant substitution (plasma max) are indicated; finally, DMS data on ACE2 binding affinity [26] is shown with red and blue representing higher and lower ACE2 affinity, respectively. The mean change in binding affinity averaged across all mutations at each site (binding avg) and alternatively the maximally binding mutant (binding max) are represented. Scores represent Δlog10 K_D (log10) relative to the wild-type reference amino acid.

Spike RBD mutations affecting antibody recognition. Several studies have contributed to the current understanding of how Spike mutations in the SARS-CoV-2 Spike protein affect neutralisation. These studies include traditional escape mutation work that identify mutations which emerge in virus populations exposed to either mAbs [51] or polyclonal convalescent plasma [49,50], targeted characterisations of particular mutations [25,53], wider investigations of either large numbers of circulating variants [47] or of all possible amino acid replacements in the RBD [51]. For Spike residues where mutations have been shown to influence polyclonal recognition, the observation of an effect on either mAb or plasma is indicated in **Figure 2b** ('mAb effect' and 'plasma emerge'). For a smaller number of residues, escape mutations emerging in virus exposed to mAbs or polyclonal plasma have been described ('mAb emerge' and 'plasma emerge' in **Figure 2b**).

In DMS studies, Greaney *et al*. assessed all possible single amino acid variants using a yeast-display system and detect variants that escape either nine neutralising SARS-CoV-2 mAbs [54] or convalescent plasma from eleven individuals taken at two time points post-infection (shades of green in **Figure 2b**) [51]. The resulting maps provide rich data on the antigenic consequence of RBD mutations with the plasma escape mutations being of particular interest given they impact polyclonal antibodies of the kind the SARS-CoV-2 experiences in infections with significant levels of immunity acquired through prior exposure or vaccination. While significant inter- and intra-person heterogeneity in the impact of mutations on polyclonal serum has been described, the mutations that most strongly reduce antibody binding occur at a relatively small number of RBD residues indicating substantial immunodominance within the RBD [51].

Of all RBD residues to which substitutions affected recognition by convalescent sera, DMS identified E484 as being of principal importance with amino acid changes to K, Q, or P reducing neutralisation titres by more than an order of magnitude [51]. E484K has also been identified as an escape mutation emerging under exposure to mAbs C121 and C144 [49] and convalescent plasma [50], and was the only mutation described by Baum *et al*. (2020) as reducing the neutralising ability of a combination of mAbs (REGN10989/10934) to an unmeasurable level [46]. In an escape mutation study using 19 mAbs, substitutions at E484 emerged more frequently than at any other residue (in response to four mAbs) and each of the four 484 mutants identified (E484A/D/G/K) subsequently conferred resistance to each of four convalescent sera tested [48]. No other mAb-selected escape mutants escaped each of the four sera, though mutations K444E, G446V, L452R and F490S escaped three of the four tested [48].

Mutations at 477 (S477G, S477N and S477R) rank prominently among mAb escape mutations identified by Liu *et al*. (2021) in terms of resistance and S477G escapes two of the four sera

tested [48]. However, substitutions at 477 were not identified as being of importance in DMS with convalescent plasma [51]. N439K is described as increasing ACE2 affinity [26] and is predicted to result in an additional salt bridge at the RBM:ACE2 interface and is described as tending to preferentially reduce neutralisation by already lower neutralising plasma [25]. However, Greaney *et al*. (2021) did not find the mutation N439K to significantly alter plasma recognition in contrast with previous observations that found N439K reduced neutralisation by a range of both mAbs and convalescent plasma [25]. One hypothesis for this inconsistency is that the mechanism of immune escape conferred by N439K is via increased ACE2 affinity rather than by directly affecting antibody epitope recognition and that perhaps the experimental design of Greaney *et al*. (2021) is less sensitive to immune evasion mutations of this kind.

Box 2| Mechanisms of antigenic change. In common with other virus surface glycoproteins responsible for attachment to host cell surface receptors, such as influenza haemagglutinin or the envelope glycoprotein GP120 of HIV, the SARS-CoV-2 Spike glycoprotein is an important target for neutralising antibodies. There are various distinct mechanisms by which mutations can alter the antigenic properties of a glycoprotein.

Amino acid substitutions that alter the epitope. A change in the biophysical properties of an epitope residue directly diminishes antibody binding. For example, the neutralising antibody 4A8 forms salt bridges with Spike residues K147 and K150 and therefore substitutions to these residues are likely to inhibit binding. The E484K substitution that has received attention for its effect on mAbs and convalescent plasma neutralising activity, has been described belonging to the footprint of several antibodies and a change in charge caused by a replacement of a glutamic acid with a lysine has the potential to diminish antibody binding.

Increasing receptor-binding avidity. Substitutions that increase receptor-binding avidity can shift the binding equilibrium between glycoprotein and neutralising antibodies in favour of the interaction between glycoprotein and the cellular receptor [55]. The Spike substitution N501Y, which increases ACE2-binding affinity [26], has been described as emerging in individuals treated with convalescent plasma, potentially as a means of immune-escape.

Change in glycosylation. A substitution can introduce an additional N-linked glycosylation motif (Asparagine-X-Serine/Threonine, where X is not proline). Glycans are bulky sugar molecules that may shield epitopes from antibody binding. The acquisition of epitope-masking glycans during the evolution of human influenza viruses is well described [56].

Deletion or insertion. The deletion or insertion of residues has the potential to alter epitope conformation diminishing antibody binding. Several deletions in the Spike NTD that affect recognition by neutralising antibodies have been described [50,53]. In laboratory experiments, a multi-residue insertion in the Spike NTD has been described as emerging and contributing to escape from polyclonal convalescent plasma [50].

Allosteric structural effects. Similar to deletions or insertions, an amino acid substitution outside of an epitope footprint may affect antibody binding by changing the protein conformation in such a way that an epitope is altered or differently displayed. In the Spike NTD, changes to disulfide bonds are thought to reduce binding by multiple mAbs through this mechanism [37].

Antigenic consequence of Spike NTD mutations. In the NTD, the majority of evidence for a role in immune evasion focuses on a region centred on a conformational epitope consisting of residues 140-156 (N3 loop) and 246-260 (N5 loop) which includes the epitope of the

antibody 4A8 (magenta in **Figure 2**) [39]. In studies that monitor emergence of escape mutations in virus populations exposed to convalescent plasma, mutations are roughly evenly distributed between RBD and NTD (**Figure 2b**). Weisblum *et al*. described the emergence of escape mutations in viruses exposed to two convalescent plasmas, one of which selected for NTD mutations only (N148S, K150R/E/T/Q and S151P). This was despite this plasma being the source of the highly potent RBD-targeting mAb C144 [49]. NTD escape mutations were not observed for the other plasmas investigated and furthermore, the 148-151 mutants only exhibited marginal reductions in sensitivity to these plasmas tested indicating individual immune responses may be differentially affected by mutations to RBD and NTD epitopes [49].

Deletions in NTD have been observed repeatedly in the evolution of SARS-CoV-2 and have been described as changing NTD antigenicity [37,50,53]. McCarthy *et al*. identified four recurrently deleted regions (RDRs) within NTD and tested five frequently observed deletions within these: Δ69-70 (RDR 1), Δ141-144 and Δ146 (RDR2), Δ210 (RDR3), and Δ243-244 (RDR4) [53]. Of the four RDRs, RDR 1, 2 and 4 correspond to NTD loops N2, N3, N5, while RDR 3 falls between N4 and N5 in another accessible loop (starred in **Figure 3a**). Both RDR2 deletions, Δ141-144 and Δ146, and Δ243-244 (RDR4) abolished binding of 4A8 [53]. Further evidence of the importance role of RDR2 deletions in immune escape is provided by Andreano *et al*. (2020) who describe the emergence of Δ140 in virus co-incubated with potently neutralising convalescent plasma, causing a four-fold reduction in neutralisation titre [50]. This Δ140 mutant subsequently acquired E484K resulting in a further four-fold drop in neutralisation titre, thus a two residue change across NTD and RBD can dramatically inhibit the polyclonal response. The Δ140 + E484K mutant next acquired an 11-residue insertion in the NTD N5 loop between Y248 and L249 completely abolishing neutralisation. This insertion, which also introduced a new glycosylation motif in the vicinity of RDR4, is predicted to alter the structure of the antigenic N3 and N5 NTD loops [50]. This further demonstrates the structural plasticity of NTD and indicates that insertions and the acquisition of additional glycosylation motifs are further mechanisms in addition to deletion that the NTD may use to evade immunity. Another example of mutations that impact the epitope/paratope interface indirectly are mutations in the signal peptide region and at the cysteine residue 15 and 136 which engage in a disulfide bond that staples the NTD N-terminus against the galectin-like β-sandwich [37]. Mutations at those sites (e.g., C136Y and S12P, which alter the cleavage occurring between residues C15 and V16) have been shown to affect the neutralising activity of several mAbs, likely disrupting the disulfide bond and therefore dislodging the supersite targeted by several antibodies [37].

Across Spike, some mutations that escape neutralising mAbs also have little impact on serum antibody binding [49,51,57], possibly because those mAbs are rare in polyclonal sera, targeting subdominant epitopes [11,51,57]. Escape mutations emerging in virus exposed to convalescent plasma have been identified in both the NTD (ΔF140, N148S, K150R/E/T/Q and S151P) and the RBD (K444R/N/Q, V445E, E484K) (**Figure 2b**) [49,50]. Notably, mutations emerging under pressure from convalescent plasma may be different to those selected by the most frequent mAb isolated from the same plasma [49]. Potentially, observed differences arise because

mutations selected by convalescent plasma facilitate escape from multiple mAbs. Fewer data on the antigenic effects of S2 mutations exist, though D769H has been described as conferring decreased susceptibility to neutralising antibodies [31]. Residue 769 is positioned in a surface-exposed S2 loop and D769H is described as emerging, in linkage with Δ69-70, in an immunocompromised patient treated with convalescent plasma [31].

Conformational epitope analysis. To evaluate potential antigenicity across the Spike protein, we analysed Spike using BEpro, a program for the prediction of conformational epitopes based on tertiary structure [58]. This approach calculates a structure-based epitope score which approximates antibody accessibility for each amino acid position. For each residue, the calculated score accounts for the local protein structure: half sphere exposure measures and propensity scores each depend on all atoms within 8-16 Å of the target residue, with weighting towards closer atoms. Due to this aggregation, calculated scores are relatively insensitive to the effects of single amino acid substitutions. Scores were calculated for the Spike protein in both closed and open conformation (**Figure 3**). It has been estimated that around 34% of Spike are closed and 27% are open (with the remainder in an intermediate form) following furin cleavage [15]. Scores rescaled between zero and one are plotted for the closed conformation in **Figure 3a** and represented on the structure in **Figure 3b**. A limitation of this approach is it does not account for glycan shielding of residues and likely overestimates scores at the base of the ectodomain for residues closest to the C-terminus.

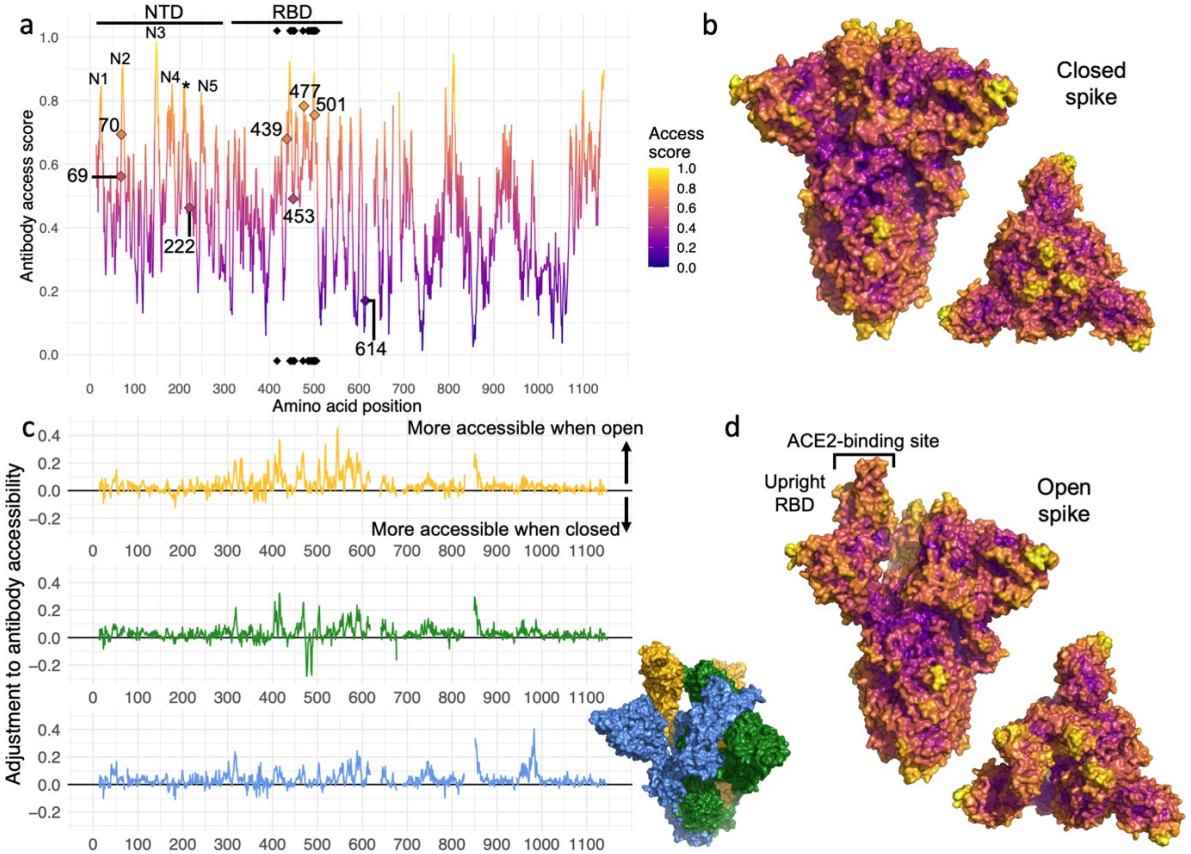

Figure 3. Structure-based analysis of conformational epitopes on SARS-CoV-2 Spike protein. (**a**) Structure-based epitope or antibody accessibility score for each Spike ectodomain residue in the closed form calculated using BEpro [58]. Black diamonds at the top and bottom of the plot indicate the positions of ACE2 contacting residues. Accessible NTD loops N1-N5 are labelled and a loop falling between these is starred. (**b**) Epitope scores are used to colour two surface representations of Spike in closed conformation according to the colour scheme of panel **a**, a trimer axis vertical view (left) and an orthogonal top-down view along this axis (right). (**c**) The extent to which each Spike residue becomes more or less accessible when the Spike is in its open form. For each Spike monomer, the difference relative to score calculated for the closed form (shown in panel **a**): upright RBD in yellow (top), closed RBD clockwise adjacent in green (middle) and closed RBD anti-clockwise adjacent in blue (bottom). (**d**) Two surface representations of Spike in open conformation with a single monomer with an upright RBD are shown coloured according to antibody accessibility, a trimer axis vertical view (left) and an orthogonal top-down view along this axis (right).

Comparison with reporting of antibody footprints and the antigenic impact of mutations, indicates residues with mutations described as affecting recognition by mAbs or convalescent plasma (**Figure 2b**) tend to occur at residues with higher structure-based antibody accessibility scores, compared with other residues belonging to epitope footprints and residues not implicated in antigenicity (**Figure S1b**)**.** Notably, scores for residues with mutations described as affecting plasma recognition are also a little higher on average compared with those described as affecting plasma compared with mAbs only. Epitope scores are particularly high for residues with mutations described as emerging during coincubation with convalescent plasma (**Figure S1b**) [49,50]. Experimental data on emergence under selective pressure from polyclonal antibodies is relatively rare, though these trends indicate approaches of this kind can contribute to the ranking of residues at which substitutions are likely to impact the polyclonal antibody response.

Within the RBD, the two areas with high structure-based antibody accessibility scores for the closed Spike structure (peaks with consecutive residues with scores >0.8 in **Figure 3a**) are centred on residues 444-447 and residues 498-500. These areas are visualised as yellow patches near the centre of the top-down view of the Spike structure in **Figure 3b**. **Figure 3c** shows that, in general, residues become more accessible and likely to form epitopes when Spike is in the open conformation and this is especially true for the RBD, particularly for the upright RBD (**Figure 3c**, yellow). In the open form, residues close to ACE2 binding sites (405, 415, 416, 417, and 468) become much more exposed on both the upright RBD and the clockwise adjacent closed RBD (**Figure 3c**, green). The effect of mutations at these positions is likely to be greater for antibodies belonging to RBD class 1. Residues centred on 444-447 and 498-500 maintain high scores on the upright RBD and are joined by residues in areas 413-417 and 458-465. The only RBD residues that become notably less accessible in the open Spike structure are residues 476, 477, 478, 586, and 487 of the closed RBD clockwise adjacent to the upright RBD which become blocked by the upright RBD (**Figure 3c**, green). Several RBD-specific antibodies are only able to bind the open Spike (classes 1 and 4 [38]) and interestingly, it has been observed that D614G makes Spike more vulnerable to neutralising antibodies by increasing the tendency for the open conformation to occur [59].

Within the NTD the highest scoring Spike residues in the closed form, belong to a loop centred on residues 147-150, which each have scores greater than 0.9 (**Figure 3a**, yellow patch to the extreme right of the structure viewed from the side in **Figure 3b**). This loop, known as the N3 loop, is described as forming key interactions with the neutralising antibody 4A8 [39]. Chi *et al*. (2020) described the structure of five previously unmodelled, protruding NTD loops denoting them N1-5. In addition to N3, high-scoring residues (> 0.7) are found at positions 22-26 (N1), 70 (N2), 173-187 (N4), 207-213 (starred in **Figure 3a**) and 247-253 (N5). Structural analysis indicates NTD-binding antibodies are likely able to bind epitopes when Spike is in either closed or open conformation (**Figure 3c**). Outside of NTD and RBD, the highest scoring residues are 676 and 689 (which lie either side of the loop containing the S1/S2 furin cleavage site, which is disordered in both open and closed conformations [15]), 793-794, 808-812, 1099-1100, and 1139-1146 (**Figure 3a**). When Spike is in the open conformation, increased accessibility results in substantially higher potential epitope scores for S2 residues centred on 850-854 (which follow an unmodelled region) on all monomers and residues 978-984 on the closed, anti-clockwise adjacent monomer (**Figure 3c**, blue).

Structural context of Spike mutations in the globally circulating SARS-CoV-2 variants. In order to assess the impact of Spike mutations and their immunological role in the global SARS-CoV-2 population, we combined structural analyses with the observed frequency of mutations in circulating variants (**Figure 4**). Globally, the highest number of amino acid variants, mapped against reference strain Wuhan-Hu-1 (MN908947), are recorded at residues 614, 222 and 18 (**Figure 1a**) (among 426,623 high quality sequences retrieved from GISAID on 3 February 2021 and processed using CoV-GLUE). Residues at positions 614 and 222 present relatively low antibody access scores and are positioned around 50 Å from the receptor-binding site residues when Spike is in the open conformation (**Figure 4a-b**). As mentioned above, there is evidence that indicates D614G confers a moderate advantage for infectivity [7,8] and increased transmissibility [9]. The Spike substitution with the second highest frequency is A222V which is present in the 20A.EU1 SARS-CoV-2 cluster (also designated as lineage B.1.177). This lineage has spread widely in Europe and is reported to have originated in Spain [60]. There is no evidence for a notable impact of A222V on virus phenotype and so its increase in frequency is generally presumed to have been fortuitous rather than any selective advantage. The substitution L18F has occurred approximately 21 times across the global phylogeny [61] and is associated with escape from multiple NTD-binding mAbs [37].

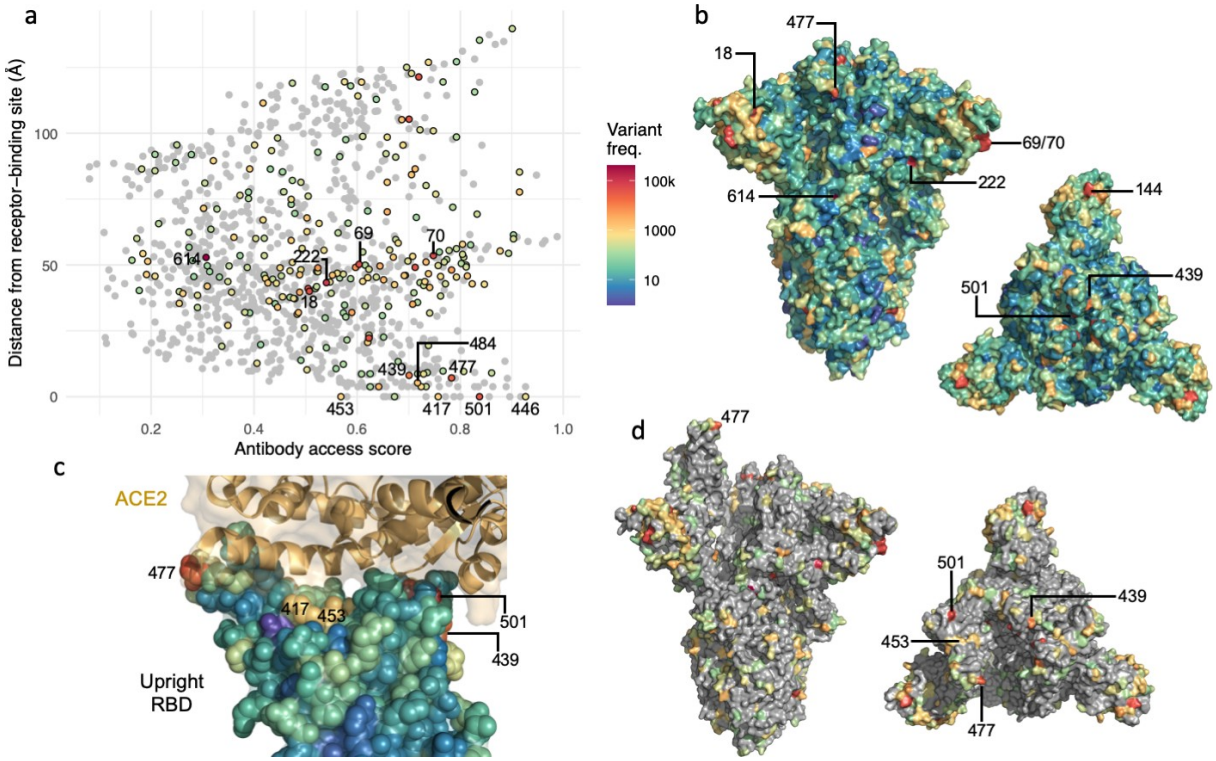

Figure 4. Structural context of Spike amino acid substitutions in globally circulating SARS-CoV-2 data. Spike residues are coloured by the frequency of amino acid variants according to the legend. Variants (retrieved from CoV-GLUE) are based on 426,623 high quality sequences downloaded from GISAID on February 3, 2021. (**a**) Points representing each Spike residue are positioned according to antibody accessibility score and distance to the nearest residue in the receptor-binding site. Residues where at least 100 sequences possessing a substitution are coloured according to the scale, with the remainder shaded grey. (**b**) Spike in closed form with all residues coloured according to scale, a trimer axis vertical view (left) and an orthogonal top-down view along this axis (right). (**c)** Closeup of the RBD bound to ACE2, PDB ID: 6M0J [62], with RBD residues shown as spheres coloured by amino acid variant frequency and ACE2 shown as an overlay and surface representation in gold. Amino acid variants are present at high frequency in positions at the RBD-ACE2 interface. (**d**) Spike in open form with residues where at least 100 sequences possessing a substitution highlighted, a trimer axis vertical view (left) and an orthogonal top-down view along this axis (right).

Among the 5,106 independent substitutions observed in Spike (Box 1), 161 are described as affecting recognition by mAbs or polyclonal sera, of which 22 are present in more than 100 sequences. On average, variant frequency is higher at amino acid positions where mutations are described as affecting antibody recognition compared with positions with no described substitutions of antigenic significance (**Figure S1a**) and high levels of amino acid variants are observed at some residues at which mutations are described as affecting recognition by convalescent plasma including 439 and 484. This indicates that, generally, the amino acid positions at which escape mutations have been detected *in vitro,* tolerate mutations, at least to some degree, *in vivo*.

Within the RBD, the positions at which amino acid variants are present at highest frequency are located in close proximity to the RBD:ACE2 interface (**Figure 4**). Of the three RBD substitutions present in several thousand sequences, N439K and N501Y are described above

and N501Y is discussed in more detail below in the context of variants of concern. The third of these, S477N, is estimated to have emerged at least seven times across the global phylogeny and has persisted at a frequency of between 4-7% of sequences globally since mid-June 2020 [61]. Liu *et al.* (2021) described multiple mAbs selecting for the emergence of S477N and found this mutant to be resistant to neutralisation by the entire panel of RBD-targeting mAbs that they tested. In contrast, when tested with convalescent serum, the neutralisation of the S477N mutant was similar to wild-type [48]. In common with N439K and N501Y, S477N results in increased affinity for the ACE2 receptor, though to a lesser extent [26,63]. As described in Box 2, substitutions may facilitate immune escape by increasing receptor-binding affinity independently of any effect they may have upon antibody recognition of epitopes, therefore it is possible that such a mechanism contributes to the impact of S477N on antibody neutralisation. Variant frequency is also moderately high at RBD:ACE2 interface sites 417, 453 and 446. Of these residues, 446 occurs in a position in the structure predicted to be highly antigenic and substitutions at this site are described as affecting both mAbs and antibodies present in polyclonal antiserum [47,48,51,64]. Substitutions at sites 417 and 453 are described below in the context of variants of concern.

Variants of interest/concern. In addition to single mutations of note, several heavily mutated lineages have emerged. Arguably the first variant of interest defined by the presence of several Spike mutations and referred to as B.1.1.298 (Cluster 5), was detected in Denmark spreading among farmed mink and a small number of people [27]. This lineage is characterised by four Spike amino acid differences, ΔH69-V70, Y453F, I692V and M1229I (**Figure 5**). Of these, the Y453F substitution occurs at a residue within the ACE2 footprint and has been shown using DMS to increase ACE2 affinity [26]. In addition, Y453F has been described as reducing neutralisation by mAbs [46]. In late 2020 and early 2021 the emergence and sustained transmission of lineages possessing mutations that affect the characteristics of the virus received much attention, most notably the lineages B.1.1.7, B.1.351 and P.1 (also known by 501Y.V1, 501Y.V2, and 501Y.V3). The locations of Spike mutations in B.1.1.298 and in these three lineages are annotated on the structure in **Figure 5a** and information on the structural context and consequences of mutations for antibody recognition and ACE2 binding are shown in **Figure 5b**.

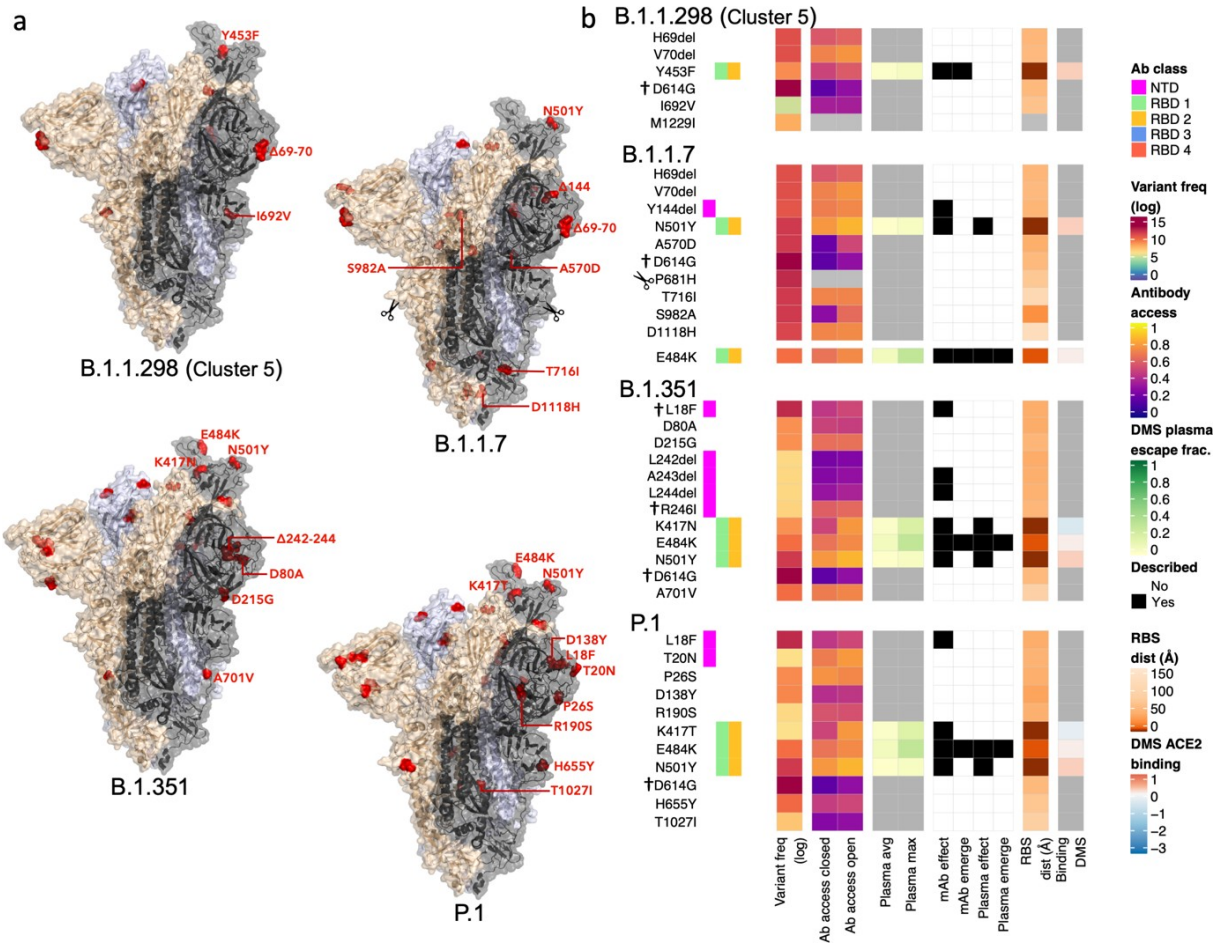

Figure 5. Amino acid variants that characterise variants of concern. (a) Spike heterotrimer in open conformation with overlaid with surface representation, PDB: 6ZGG [15]. Locations of amino acid substitutions and deletions that define variants of concern are highlighted as red spheres. For B.1.1.7, scissors mark the approximate position of substitution P681H within the furin cleavage site which is absent from the structural model. (**b**) Aligned heatmaps showing properties of amino acid residues or of the specific mutation, as appropriate. From left to right: epitope residues are coloured to indicate NTD or RBD class [37]; structure-based antibody access scores for Spike in closed and open conformation; for RBD residues, the results of deep mutational scanning results (DMS) study show the escape fraction for each mutant averaged across plasma (avg) and for the most sensitive plasma (max) [51]; each mutation is classified as having evidence for mutations either affecting neutralisation by mAbs or convalescent plasma [51] or vaccinated individuals [52], and emerging in selection experiments using mAbs [46,48,49] or post-infection serum [46,48,49]; distance to the ACE2-contacting residues that form the receptor-binding site RBS (Å) - (for residue 681, this is estimated using the nearest residues present in published structures); finally, DMS data on ACE2 binding affinity [26] is shown by aggregating scores and averaging across each mutant at a residue and alternatively the maximally binding mutant. Scores represent Δlog10 K_D (log10) relative to the wild-type reference amino acid. Mutations present in a variant but also widespread in the virus population in which a variant emerged are marked with †.

Of the lineages summarised in **Figure 5**, several amino acid replacements are convergent having arising independently in different lineages: N501Y which is present in lineages B.1.1.7, B.1.351 and P.1; E484K which is present in lineages B.1.351 and P.1 and has been detected as emerging within B.1.1.7 [65], and ΔH69-V70 in B.1.1.298 and B.1.1.7. Additionally, B.1.351 and P.1 possess alternative amino acid changes K417N and K417T, respectively. Further lineages possessing these mutations have also been identified. For example, an independent

emergence of N501Y in a lineage labelled B.1.1.70 and largely circulating in Wales. Residue 501 is at the ACE2 interface (**Figure 3c**) and N501Y has been shown experimentally to result in one of the highest increases in ACE2 affinity conferred by a single RBD mutation [26]. E484 has been identified as an immunodominant Spike residue, with various substitutions, including E484K, facilitating escape from several mAbs [46,48,49] as well as convalescent plasma [48-51]. E484K is estimated to have emerged repeatedly across the global phylogeny [61] and has been described in two other lineages originating from B.1.1.28 in addition to lineage P.1 reported to be spreading in the Rio de Janeiro State (lineage P.2) [66] and in the Philippines (lineage P.3) [67]. While K417 is described in the epitopes of class 1 and 2 antibodies [38], substitutions to K417 tend to affect class 1 antibody binding and therefore somewhat less important for the polyclonal response to RBD which is dominated by class 2 antibodies which are more susceptible to substitutions such as E484K [52,57,68]. In addition to their antigenic effect, both K417N and K417T are expected to moderately decrease ACE2 affinity [26] (**Figure 5b**). The ΔH69-V70 deletion has been identified in variants associated with immune escape in immunocompromised patients treated with convalescent plasma [31]. Experiments show ΔH69-V70 does not reduce neutralisation by a panel of convalescent sera, however it may compensate for infectivity deficits associated with affinity-boosting RBM mutations that may emerge due to immune-mediated selection [29].

The first genomes belonging to the lineage B.1.1.7 were collected in the south of England in September 2020. Lineage B.1.1.7 is defined by the presence of 23 nucleotide mutations across the genome that map to a single branch of the phylogenetic tree [2]. Of these 23 mutations, 14 encode amino acid changes and three are deletions, including six substitutions in Spike (N501Y, A570D, P681H, T716I, S982A and D1118H) and two NTD deletions (ΔH69-V70 and ΔY144) [2]. The lineage has been associated with a rapidly increasing proportion of SARS-CoV-2 reported cases and phylogenetic analyses indicates this lineage was associated with a growth rate estimated to be 40 to 70% higher than other lineages [69,70]. There is also evidence that this lineage may be associated with a higher viral load [71]. In addition to N501Y which has some evidence for reducing antibody neutralisation, possibly as a result of its ACE2-affinity boosting impact, there is evidence for an antigenic effect of ΔY144 (**Figure 5b**). This deletion is expected to alter the conformation of the N3 NTD loop (residues 140-156) and has been demonstrated to abolish neutralisation by a range of neutralising antibodies [37]. The B.1.1.7 Spike mutations have been shown to diminish neutralisation by a higher proportion of NTD-specific (9 of 10, 90%), rather than RBD-specific (5 of 31, 16%), neutralising antibodies [72]. Given the immunodominance of RBD, this could explain the modest reductions in neutralising activity of convalescent sera against authentic B.1.1.7 or pseudoviruses carrying the B.1.1.7 Spike mutations [73,74]. The co-occurrence of ΔY144 and E484K is concerning with respect to the polyclonal antibody response as the N3 loop that ΔY144 changes is predicted to be among the most immunogenic regions of Spike (**Figure 3a**) and substitutions to 484 diminish neutralisation by a range of RBD-targeting antibodies.

SARS-CoV-2 variants, spike mutations and immune escape

Reports of lineages with N501Y circulating in the UK were followed by reporting of another lineage possessing N501Y circulating in South Africa (lineage B.1.351), which has been rapidly expanding in frequency since December 2020 [75]. In addition to N501Y, lineage B.1.351 is defined by the presence of five further Spike substitutions (D80A, D215G, K417N, E484K and A701V) and a deletion in the NTD, Δ242-244. High numbers of B.1.351 viruses also have the Spike substitutions L18F, R246I and D614G. A similar NTD deletion, Δ243-244, abolishes binding by the antibody 4A8 [53], while L18F and R246I also occur within the NTD supersite and likely affect antibody binding too [37]. The co-occurrence of K417N, E484K and these NTD substitutions therefore suggests B.1.351 may overcome the polyclonal response by reducing neutralisation by RBD-specific antibodies of classes 1 and 2 and NTD-specific antibodies (**Figure 5b**). Data reported by Wibmer *et al.* showed that nearly half of the examined convalescent plasma (21 of 44, 48%) had no detectable neutralisation activity against B.1.351 virus [68]. Other experiments with pseudotyped viruses showed that the B.1.351 variant was also resistant to the neutralising activity of some mAbs (12 of 17, 70%) [76]. Similarly, Cele *et al.* showed that the neutralising effect of convalescent plasma collected from six individuals was strongly attenuated against the live (authentic) virus B.1.351 (with IC_{50} six to two hundred-fold higher relative to first wave virus) [77].

The P.1 lineage, a sub-lineage of B.1.1.28 was first detected in Brazil [78] and in travellers from Brazil to Japan [79]. Lineage P.1 is characterised by the presence of several amino acid variants in Spike: L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, H655Y and T1027I [78]. In addition to substitutions at 417, 484 and 501 discussed above, the P.1 lineage has a cluster of substitutions in close proximity to the described antigenic regions of the NTD including L18F, which is known to reduce neutralisation by some antibodies [37]. The substitutions T20N and P26S also occur in or near the NTD supersite[37] at residues with high antibody accessibility scores (**Figure 5b**) and T20N introduces a potential glycosylation site which could result in glycan shielding (**Box 2**) of part of the supersite. The P.1 lineage has also been associated with a reinfection case in Manaus [34], indicating it is contributing to antigenic circumvention of what might have been an otherwise effective immune response. More details of frequency and geographic distribution can be found here at https://cov-lineages.org/global_report_P.1.html.

Increasingly, lineages possessing independent emergences of mutations in common with those lineages summarised in **Figure 5** are being detected, suggestive of convergent evolution. For example, viruses of lineage B.1.525 which has been observed in several countries, albeit at low frequency to date, have NTD deletions ΔH69-V70 and ΔY144 in common with B.1.1.7, and E484K in common with the B.1.351 and P.1 lineages, alongside Spike substitutions Q52R, Q677H and F888L [80]. Polymorphisms at position 677 and the independent emergence of Q677H in several lineages in the US provides strong evidence of adaptation, potentially via an effect of this mutation on the proximal polybasic furin cleavage site, though further experiments are required to determine its impact [81]. Other novel variants have been identified spreading in California and New York (B.1.427/B.1.429 and B.1.526 respectively). The B.1.427/B.1.429 variants carry an antigenically significant substitution

L452R [82], which has been shown to reduce neutralisation by several mAbs [47,48,52,54] and convalescent plasma [47]. L452R has independently appeared in several other lineages across the globe between December 2020 and February 2021, indicating this is probably the result of viral adaptation due to increasing population immunity [82]. L452R is also present in the A.27 lineage associated with a cluster of cases identified on the island of Mayotte [83]. The lineage B.1.526 has been described to carry either S477N or E484K among other lineage defining mutations [84,85], both of which are described as antigenically significant above. A new variant carrying E484K designated currently A.VOI.V2 has been recently identified in Angola from cases involving travel from Tanzania [86]. This variant carries several amino acid substitutions in the Spike and three deletions in the NTD, some of which are within the antigenic supersite [86]. Another variant within A lineage whose prevalence is rising in Uganda (A.23.1) shares with B.1.1.7 a substitution at position 681 within the furin cleavage site (P681R rather than P681H found in B.1.1.7) and additionally has the substitutions R102I, F157L, V367F and Q613H. The latter is speculated to be of importance as it occurs at the position neighbouring D614G [87]. Residue 157 has been identified as an epitope residue with F157A reducing neutralisation by the mAb 2489 [41].

New variants will continue to emerge and while it is important to understand the phenotypes of emerging variants, it is also important to dissect the impacts of mutations, both individually and in combination with other mutations. As new variants continue to emerge, such insights will allow inference of phenotypic consequences, while the experimental characterisation of antigenic mutations generated to date will continue to provide extremely useful information on mutations that may not yet have been seen in circulating viruses.

Vaccine efficacy against circulating SARS-CoV-2 variants. To date, several vaccines have been administered very successfully in several countries but this still represents a fraction of the global population (**Table S1**). In order to assess the impacts of mutations on vaccine efficacy, authentic and pseudoviruses possessing particular Spike mutations, either individually or in combination, and larger sets of mutations representing variants of concern and other circulating Spikes have been assessed using neutralisation assays with post-vaccination sera (**Table S1**). Typically, studies report a fold change in variant virus, or pseudovirus, neutralisation relative to wild-type (the serum concentration at which 50% neutralisation, IC_{50}, is achieved with the variant divided by the average IC_{50} for the wild-type).

In a cohort of 20 volunteers, epitope mapping for the mRNA vaccines mRNA-1273 (Moderna) and BNT162b2 (Pfizer-BioNTech) showed high levels of IgM and IgG anti-SARS-CoV-2 RBD binding titres, eliciting similar mAbs to those isolated from naturally infected donors [52]. The plasma neutralising activity and the numbers of RBD-specific memory B cells were found to be equivalent to plasma from patients who recovered from COVID-19 natural infection [52]. Investigation with pseudoviruses possessing RBD mutations in variants of concern demonstrated the neutralising activity of plasma from vaccinated individuals showed small but significant decreases of one- to three-fold against E484K, N501Y or the K417N + E484K + N501Y triple mutation [52]. Other data indicate the effect of N501Y alone on neutralisation to

be relatively modest, with other studies using sera from 20 participants in a trial of the BNT162b2 showing equivalent neutralising titres to the N501 and Y501 pseudoviruses [88]. Other investigations with recombinant viruses carrying either N501Y, ΔH69-V70 + N501Y + D614G or E484K+ N501Y + D614G demonstrated that, compared with the Wuhan-1 reference virus, only E484K + N501Y + D614G resulted in small and modest reduction in neutralisation by post-vaccination sera elicited by two BNT162b2 doses and only modest differences compared with the Wuhan-1 reference virus [89].

As stated above, convalescent plasma from individuals infected with pre-B.1.1.7 viruses shows only modest reduction in neutralisation of authentic B.1.1.7 or pseudovirus possessing B.1.1.7 Spike mutations [72,85], and results with post-vaccination sera are broadly consistent with this. Pseudoviruses carrying the set of B.1.1.7 Spike mutations evaluated with post-vaccination serum BNT162b2 (2 dose) [72,85,90] or mRNA-1273 (2 dose) [72] exhibited only modest reduction in neutralisation titres (less than three-fold). However, B.1.1.7 with the addition of E484K, which has been observed in sequencing of circulating isolates, has been shown to result in larger, more significant drops (6.7-fold) in neutralisation with BNT162b2 post-vaccination sera [91]. In a live-virus neutralisation assay, neutralising titres of ChAdOx1 nCoV-19 post-vaccination sera were nine times lower against the B.1.1.7 relative to a canonical non-B.1.1.7 lineage (Wuhan-1 with the S247R Spike mutation) [92]. Similarly, neutralising activity of sera elicited by inactivated vaccine Covaxin against B.1.1.7 was largely preserved [94]. Pseudovirus and live-virus neutralisation assays showed that the neutralising activity of sera from individuals after the two doses of the ChaAdOx1 against the B.1.351 variant was reduced or abrogated [95]. Post-vaccination sera from individuals receiving two doses of mRNA-1273 (28 days apart) showed reduced neutralisation against the mutations present in B.1.351 (6.4-fold reduction) [96]. In contrast, neutralising activity of sera elicited by inactivated vaccine BBIBP-CorV against the authentic virus B.1.351 showed only a slight reduction (less than two-fold) [97].

Comparison of the differing extents to which variants affect neutralisation by post-vaccination serum is complicated by the different methodologies used by various studies. However, Garcia-Beltran *et al.* tested eight SARS-CoV-2 variants of concern (including each of those in **Figure 5**) including three B.1.351 variants distinguished by their combination of NTD mutations using the same virus pseudotype system and post-vaccination sera from individuals who received two doses of either the BNT-162b2 (n = 30) or mRNA-1273 (n = 35) vaccines [96]. Compared with wild-type, pseudoviruses with D614G, or the mutations defining lineages B.1.1.7, B.1.1.298 and B.1.429 each showed non-statistically significant fold decreases in neutralisation [96]. Lineages P.1 and P.2 each show significant fold decreases with both BNT162b2 (6.7 and 5.8-fold respectively) and mRNA-1273 (4.5 and 2.9-fold respectively) post-vaccination sera [96]. The three B.1.351 variants investigated, representing the majority of deposited B.1.351 sequences, showed much larger decreases in neutralisation ranging from 34 to 42-fold (BNT162b2) and 19.2 to 27.7-fold (mRNA-1273). Taken together, these data indicate E484K is the primary determinant of the decreases in neutralisation titres that

distinguish P.1, P.2, and the three B.1.351 variants from the other pseudoviruses tested. In addition to E484K, the further mutations shared by each of the three B.1.351 variants, but not possessed by P.1. and P.2, were D80A, Δ242-244, K417N (though K417T is present in P.1) and A701V.

To complement the experimental data provided by neutralisation assays, there is emerging evidence from clinical trials on the impact of variants on vaccine efficacy. Early indications are that these are broadly consistent with the laboratory results, with the B.1.351 variant showing greater signs of vaccine escape. The ChAdOx1 vaccine showed clinical efficacy against the B.1.1.7 variant but failed to provide protection against mild-to-moderate disease due to the B.1.351 variant, with vaccine efficacy against the variant estimated at 10.4% (95%, -76.8 to 54.8) [91,92,97]. Preliminary data from clinical trials reported that the NVX-CoV2373 (Novavax) protein-based vaccine gives 95.6% efficacy against the wild-type virus and that this is moderately lower for B.1.1.7 (85.6%) and further reduced for B.1.351 (60%) [98]. Similarly, the single dose covid-19 vaccine JNJ-78436735 (Johnson & Johnson/Janssen) has been shown to provide 72% protection against moderate to severe covid-19 infection in the US, but the proportion significantly decreased to 57% in South Africa (at a time when B.1.351 is ubiquitous) [99]. These data indicate that NVX-CoV2373 and JNJ-78436735 are clinically efficacious against the B.1.1.7 and variants circulating in the US but are consistent in that B.1.351 is associated with a larger reduction in vaccine efficacy.

In addition to vaccine efficacy evaluation against SARS-CoV-2 variants and mutations, the effect of mutations on some mAbs used as therapeutics have been described (**Table S2**). Single mAb treatment can exert a selective pressure that potentially increases the possibility of mutational escape of the targeted antigen. The risk is likely to be reduced with the use of cocktails of two or more mAb targeting non-overlapping epitopes. Regeneron (REGN-COV2) (included in the UK's RECOVERY trial) and AZD7742 are 2 examples of mAbs cocktails that have been developed [100]. Importantly, some mutations in the RBM have already been identified in variants which are circulating in the UK (e.g., N439K, T478I and V483I) and have likely antigenic significance.

Conclusions

There is now clear evidence of the changing antigenicity of the SARS-CoV-2 Spike protein and of the amino acid changes that affect antibody neutralisation. Spike amino acid replacements and deletions that impact neutralising antibodies are present at significant frequencies in the global virus population and there is emerging evidence of variants exhibiting resistance to vaccinated sera. Greater understanding of the correlates of immune protection is required in order to provide context for the results of studies reporting changes in neutralisation. A comprehensive understanding of the consequences of mutations on antigenicity will encompass both T-cell-mediated immunity and non-Spike epitopes recognised by antibodies. To monitor vaccine efficacy, it will be important to collect information on vaccine status associated with genome sequence data for individuals infected with SARS-CoV-2 in order to better understand the implications of antigenic variation for vaccine effectiveness. More

generally, a broader understanding of the phenotypic impacts of mutations across the SARS-CoV-2 genome and their consequences for variant fitness will help to understand drivers of transmission and evolutionary success.

Recent studies have shown the potential pressure exerted by convalescent plasma/mAbs treatments on SARS-CoV-2 evolution in immunocompromised patients [31-33]. Such circumstances, involving long-term virus shedders, may have contributed to the sporadic emergence of viruses with several mutations B.1.1.7 and B.1.351. Given that therapeutics (vaccines and antibody-based) mainly target the SARS-CoV-2 Spike protein, the selection pressures that favour emergence of new variants carrying Spike escape mutations generated in chronic infections [31-33] might be similar to those selecting for mutations that allow reinfections within the wider population [34-36]. Therefore, sequencing of prolonged infections will provide useful information on mutations that could contribute to elevated transmissibility or escape from vaccine-mediated immunity.

As antigenically different variants are continuing to emerge, it is going to become necessary to routinely collect serum samples from vaccinated individuals and from individuals that have been infected with circulating variants of known sequence. Cross-reactivity between circulating lineages can be assessed by measuring the ability of these sera to neutralise panels of circulating viruses. The systematic surveillance of antigenic SARS-CoV-2 variants will be enhanced by the establishment of a network similar to the WHO-coordinated Global Influenza Surveillance and Response System (GISRS), a collaborative global effort responsible for tracking the antigenic evolution of human influenza viruses and making recommendations on vaccine composition. Modelling approaches to predict the evolutionary trajectories of emerging variants based on an understanding of the phenotypic effects of mutations may have the potential to assist this process, as is the case for influenza [100].

Prediction of the mutational pathways by which a virus such as SARS-CoV-2 will evolve is extremely challenging. Nonetheless, there is a rapidly expanding knowledge base regarding the effect of SARS-CoV-2 Spike mutations on antigenicity and other aspects of virus biology. The integration of these data and emerging SARS-CoV-2 sequences has the potential to facilitate the automated detection of potentially concerning variants at low frequency, i.e., before they are spreading widely. Tracking the emergence of these viruses flagged as potential antigenically significant variants will help to guide the implementation of targeted control measures and further laboratory characterisation. An important part of this process will be the preparation of updated vaccines tailored to emerging antigenic variants and that are maximally cross-reactive against other circulating variants. All of these processes will benefit from close international collaboration and the rapid and open sharing of SARS-CoV-2 data.

Definitions

Mutation is used to describe a change of a nucleotide in the virus RNA genome, a subset of which results in a change in amino acid (referred to as a substitution or replacement), or a mutation can refer to a deletion or insertion event in the virus genome. By convention an

amino acid substitution is written N501Y to denote the wild-type (N, asparagine) and replacement amino acid (Y, tyrosine) at site 501 in the amino acid sequence.

Glycoprotein. A protein which contains oligosaccharide chains (glycans) covalently attached to amino acid side chains. Virus surface glycoproteins embedded in the membrane often play a role in interactions with host cells including receptor-binding and are also commonly targeted by host antibodies.

B-cell epitope. The part of the antigen, in this case the Spike protein, that immunoglobins or antibodies bind. Antibodies often recognise conformational epitopes that consist of amino acid residues brought together by the 3-D folding of a protein.

Monoclonal antibody (mAb). An antibody made by cloning a unique white blood cell which usually has monovalent binding affinity for a specific epitope. Virus particles can be saturated with mAbs and the structure solved to determine the antibody footprint or mAbs can also be used to select for mutations that escape recognition.

Convalescent plasma. Convalescent plasma or post-infection serum are often used interchangeably to refer to blood serum from a previously infected individual that contains, usually, a cocktail of different antibodies referred to as polyclonal antibodies. Similarly, post-vaccination serum includes polyclonal antibodies generated by vaccination.

Viral variant refers to a genetically distinct virus with different mutations to other viruses. Variants can also refer to the founding virus of a cluster/lineage and used to refer collectively to the resulting variants that form the lineage. Variants with changed biological characteristics or antigenicity have been termed variants of interest or concern by public health bodies.

Lineages are assigned based on the SARS-CoV-2 global phylogenetic tree. Here we use the nomenclature system introduced by Rambaut et al. (2020) [24], https://cov-lineages.org.

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Competing interests

The authors declare no competing interests

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