Singh et al. Virol J (2021) 18:166
https://doi.org/10.1186/s12985-021-01633-w

# of SARS-CoV-2 and emerging variants

Virology Journal

Evolutionary trajectory

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Abstract

The emergence of a novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and more recently, the independent evolution of multiple SARS-CoV-2 variants has generated renewed interest in virus evolu tion and cross-species transmission. While all known human coronaviruses (HCoVs) are speculated to have originated in animals, very little is known about their evolutionary history and factors that enable some CoVs to co-exist with humans as low pathogenic and endemic infections (HCoV-229E, HCoV-NL63, HCoV-OC43, HCoV-HKU1), while others, such as SARS-CoV, MERS-CoV and SARS-CoV-2 have evolved to cause severe disease. In this review, we highlight the origins of all known HCoVs and map positively selected for mutations within HCoV proteins to discuss the evolution ary trajectory of SARS-CoV-2. Furthermore, we discuss emerging mutations within SARS-CoV-2 and variants of con cern (VOC), along with highlighting the demonstrated or speculated impact of these mutations on virus transmission, pathogenicity, and neutralization by natural or vaccine-mediated immunity.

Keywords: SARS-CoV-2, Coronavirus, Evolution, Mutations, Selection, Variants

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Background

Coronaviruses (CoVs) can infect humans and animals to cause mild to severe disease, including death [1]. CoVs are divided into four genera: ***alpha-*** and ***beta-CoVs*** pre dominantly originate in bats and infect other mammals, while ***gamma-*** and ***delta-CoVs*** originate in and largely infect avian species [2]. CoV infection in animals is gen erally associated with gastric symptoms [3], such as acute diarrhea in young pigs that are infected with porcine epidemic diarrhea virus (PEDV) and swine acute diar rhea syndrome coronavirus (SADS-CoV) [4, 5]. While CoVs mainly circulate in animals, such as pigs, camels, cats, and bats [6], there have been at least seven docu mented instances where these viruses have spilled over into humans [7]. Tese events have led to the emergence of human coronaviruses (HCoVs) that are low and high pathogenic. Te origin of the most recently emerged human coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is speculated to be associ

ated with Rhinolophus bats, but the zoonotic transmis sion pathway remains unknown.

HCoV-229E, HCoV-OC43, HCoV-NL63 and HCoV HKU1 represent endemic and low pathogenic HCoVs, and are responsible for one-third of common cold symp toms [8]. High pathogenic HCoVs such as severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), and SARS-CoV-2 cause or have caused severe disease in humans with case-fatality rates of 10.9%, 34.3%, and 2.1%, respectively [9–11]. SARS-CoV, MERS-CoV and SARS-CoV-2 are ***beta-CoVs*** [12, 13]. MERS-CoV belongs to the ***Merbecovirus*** subgenus, while SARS-CoV and SARS-CoV-2 belong to the SARS-related coronavirus (SARSr-CoV) species within the ***Sarbecovirus*** subge nus [14]. It remains unclear why most HCoVs evolved to largely cause minor illness while MERS-CoV continues to cause severe disease [15–17]. In this

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review, we have

highlighted the origins of HCoVs and mapped positively selected for mutations within HCoV proteins to discuss the evolutionary trajectory of SARS-CoV-2. We have also discussed emerging mutations within SARS-CoV-2 and variants of concern (VOC), along with highlighting the demonstrated or speculated impact of these mutations on virus transmission, pathogenicity, and neutralization by natural or vaccine-mediated immunity.

Origin of human coronaviruses

All known HCoVs are speculated to have an evolutionary origin in bats or rodents [1, 3, 18] (Fig. 1), with five of seven HCoVs originating in bats [3, 19–21] (Table 1). Bats are speculated to be primordial hosts for all CoV lineages due to ubiquitous detection of diverse CoVs and constant CoV population growth, which contrasts epidemic-like growths observed in other animals [22]. Although bats and alpacas can serve as MERS-CoV reservoirs [23, 24], dromedary camels are the major reservoir host and primary contributor to human infections [25–28] (Fig. 1). The full extent of wildlife or intermediate animal reservoirs of SARS-CoV-2 is currently unknown.

SARS-CoV-2 is believed to have originated in a seafood market in Wuhan, Hubei Province, China [29], although limited contact-tracing at the beginning of the pandemic does not allow for definitive characterization of the exact events that led to the first human-to-human transmission, including the index patient or initial animal contact. Nonetheless, it is speculated that the natural reservoirs of SARS-CoV-2 are *Rhinolophus* bats (Table 1) since diverse SARSr-CoVs have been detected in multiple *Rhinolophus* species [22, 30, 31], including RaTG13 in *R. affnis* [32]. RaTG13 is 96.2% identical to SARS-CoV-2 at the whole genome level [32]. Moreover, SARS-CoV-2 contains a polybasic furin-like cleavage site between S1 and S2 spike (S) protein subunits, similar to *Rhinolophus* CoV

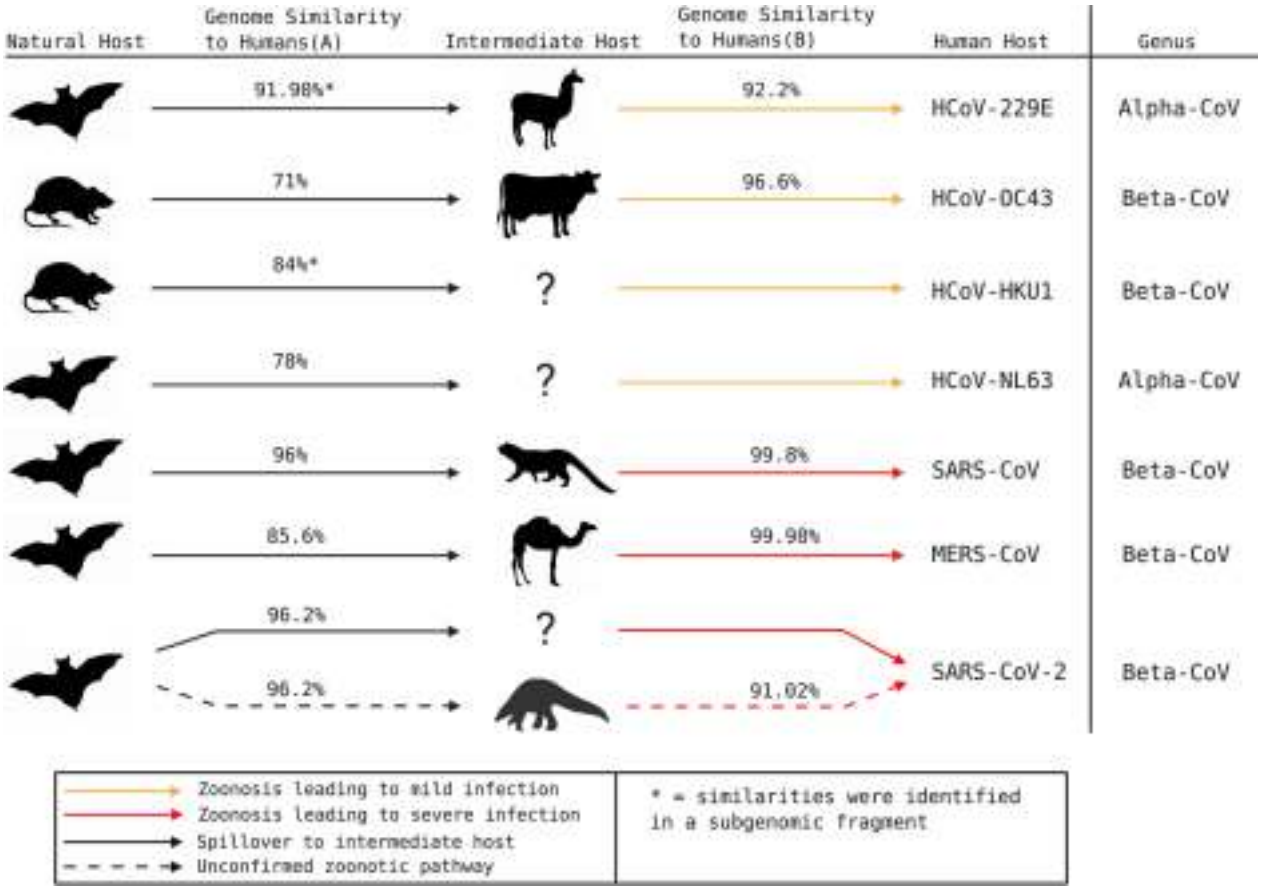

Fig. 1 Speculated animal origins of known human coronaviruses. HCoV species are organized chronologically (top to bottom) by their speculated dates of spill over into humans. Intermediate hosts (top to bottom) shown are alpacas, cattle, civet cats, dromedary camels, pangolins, and unknown (denoted as a question mark). Genome similarity to humans (A) indicates percentage similarity of CoV genomes detected in reservoir species with corresponding human CoV. Genome similarity to humans (B) indicates percentage similarity of CoV genomes detected in intermediate species with corresponding human CoV. Non-human CoVs that are highly pathogenic in animals, such as PEDV and SADS-CoV, are not shown here. Genomic percentage similarities were extracted from existing primary studies [20, 21, 32, 56, 60, 277–283]

Table 1 Speculated timelines for evolutionary origins of known human coronaviruses from bats

Species Discovery in humans Speculated timeline of divergence for human strain	Speculated bat reservoir	References
SARS-CoV-2 2019 Human strain likely diverged from most closely related bat virus in 1969	*Rhinolophus* spp.	[32, 294, 295]
SARS-CoV 2003 Human strain likely diverged from bat strain in 1986	*Rhinolophus* spp.	[22, 53, 280, 296–298]
MERS-CoV 2012 Human strain likely diverged from bat strain before 1990	*Taphozous perforates, Pipistrellus* spp., *Neoromicia* spp.	[282, 299–305]
HCoV-OC43 1967 Human strain likely diverged from bovine strain in 1890	N/A	[276, 306]
HCoV-HKU1 2004 No supported dates of divergence have been established	N/A	[279]
HCoV-229E 1965 Human strain likely diverged from alpaca strain before 1960 and from bat strain between 1686 and 1800 CE	*Hipposideros cafer ruber*	[21, 56, 307]

RmYN02 [33, 34], which shares 93.3% whole genome nucleotide identity with SARS-CoV-2 [34]. However, the receptor binding domain (RBD) of SARS-CoV-2 is only 85% and 61.3% identical to those of RaTG13 and RmYN02, respectively [34–36]. RaTG13 and RmYN02 were discovered in bats of China's Yunnan province, over 1500 km away from Wuhan [34, 35]; however, this does not preclude the possibility of virus spill over as bats can fy long distances. Virus transmission and transport by susceptible intermediate reservoirs or humans is also possible.

Phylogenetic analyses have identifed a possible recom bination-mediated origin for SARS-CoV-2 [37–39]. Neutralizing antibodies to SARS-CoV and SARS-CoV-2 have been detected in Malayan pangolins (**Manis javan ica**), suggesting that SARSr-CoVs have been circulating in pangolins since 2003 [40]. Recombination of CoVs within Malayan pangolins has been suggested given the 97.4% amino acid similarity within the RBDs of pangolin SARSr-CoVs and SARS-CoV-2 [35, 41], including con servation of all critical residues required for successful human ACE2 (hACE2)-mediated cellular entry [35, 39, 41, 42] and the detection of pangolin SARSr-CoVs that bind to hACE2 [43]. Additionally, bats and pangolins may share underground caves [44], facilitating ecological contact in high density areas. However, the lack of robust evidence of direct SARS-CoV-2 emergence from a pan golin CoV precursor [45], along with the reported high pathogenicity of SARSr-CoVs in infected pangolins [41, 42, 45] makes it unlikely that pangolins are intermediate reservoirs of SARSr-CoVs.

Te nucleotide percentage similarity of CoVs detected in reservoir species is generally lower than CoVs detected in intermediate species. Adaptive evolution of CoVs in intermediate species facilitates successful spill over into humans (Fig. 1). Since SARS-CoV-2 is more closely related to bat SARSr-CoVs than to pangolin SARSr-CoVs (Fig. 1), it seems unlikely that pangolins are intermediate hosts, unless we haven't yet detected the full range of SARSr-CoVs in pangolins. It is uncer tain whether an unknown intermediate host provided an opportunistic amplifying role or a stable reservoir for the zoonotic transmission of SARS-CoV-2. While direct human infection with bat SARSr-CoVs has not been reported [46], it is possible that the major ity of adaptive evolution of SARSr-CoVs occurs in bats, prior to spill over into humans [47]. Some notable adaptations include carrying the lowest level of CpG dinucleotides among known **beta-CoV** genomes [48], similar to a mechanism of escaping innate immunity observed in camel MERS-related CoVs strains [49, 50]. Te relatively few SARSr-CoVs detected in the Hubei Province [35] are phylogenetically distant from SARS CoV-2 [51]. Indeed, if SARS-CoV-2 did transmit from animals to humans, further sampling in Hubei Prov ince may identify more closely related SARSr-CoVs in archived animal specimens. Investigating the pos sibility of an infected person travelling to Wuhan and unwittingly spreading the virus will be more difcult in the absence of archived samples and records of travel history.

Despite the abundance of SARSr-CoVs and **beta CoVs** in bat species [52, 53], it is likely that additional reservoirs and intermediate hosts remain undetected [54]. Pigs, alpacas, and dromedary camels also main tain a variety of CoVs with the potential to transmit to humans [3, 12, 20, 55–57]. Independent insertions within RBDs of SARS-CoV, MERS-CoV, and SARS CoV-2 suggest convergent evolution, which will likely lead to emergence of more pathogenic HCoVs [58]. Further sampling of bats, pangolins, and other spe cies that share an ecological niche with bats may help piece together the puzzle surrounding the spill over of SARS-CoV-2 into humans [59] and also help discover other CoVs with potential to infect humans. Aside from consistent spill over of MERS-CoV from camels [60], HCoVs have emerged through limited spill over events, followed by human-to-human transmission [3, 61]. While challenging to predict, future spill over events are likely, due to the long history of CoV host shift ing [62–65]. Anthropogenic factors such as urbanization and deforestation increase habitat overlap of humans and animals, providing increased zoonotic transmission opportunities [57, 66]. Areas of high contact between humans, wildlife, and domesticated animals, such as live animal wet markets provide opportunity for viral recom bination and adaptation to a broader range of animal species prior to transmission to humans [57]. Identify ing existing CoV diversity in such areas will enhance our understanding of ecological opportunities for zoonosis and will help us better predict and prevent the emergence of future HCoVs.

Evolution of SARS-CoV-2 and its variants

Co-evolution of CoVs with their hosts is driven by genetic diversity that is selected through evolutionary pressures. CoV genetic diversity is made possible by a large genome (26.4–31.7 kb) [67], high mutation

rate due to a low fdel ity viral polymerase (~ 10^{-4} substitutions per site per year) [68, 69], and high recombination frequency (up to 25% for the entire genome in vivo) [70, 71]. Mutations that confer greater ftness are selected for, leading to anti genic drift. Ratios of the rates of non-synonymous/syn onymous mutations (dN/dS) greater than one, less than one and equal to one indicate positive selection, negative (purifying) selection and neutral evolution, respectively [72]. SARS-CoV-2 genomes are currently under purify ing selection [73, 74]. Despite observing little viral diver sity at the beginning of the COVID-19 pandemic [75, 76], positive selection with presumed advantages such as increased transmission rates has now been documented [77–79] (Fig. 2, Table 2). However, functional characteri zation of these mutations remains under-investigated.

Antigenic drift is most frequently observed in viral surface proteins that are highly exposed to selection pressures of the immune system, such as neutralizing antibodies [80]. Indeed, CoV spike genes, particularly the S1 and RBD coding regions, have the highest detected non-synonymous mutation rates [81, 82], a trend observed across the majority of HCoVs (Fig. 2). For low pathogenic and endemic HCoVs, multiple positively selected for residues and polymorphic sites are found in the N-terminal domain (NTD) of S [83–88]. A notable exception is HCoV-HKU1, for which there is a shortage of sequencing data outside of the hemagglutinin ester ase (HE) gene. Emerging data suggest that positively selected for and homoplastic sites have been observed within the SARS-CoV-2 NTD as well [78, 89–91]. Given the observations with other HCoVs (Fig. 2) and the detec tion of neutralizing epitopes within the SARS-CoV-2 NTD [91, 92], we speculate that with continued circula tion, vaccination and convalescent sera therapy, further positively selected for mutations in the NTD are likely to occur. Further retrospective research on the evolution of endemic HCoVs may help predict the likely evolutionary trajectory of SARS-CoV-2.

CoV genomic mutations give rise to virus variants, and closely related variants are grouped into clades. SARS CoV-2 variants have been clustered into nine clades: L, V, S, G, GH, GR, GV, GRY and O [93, 94] (Table 3), named after their most representative mutations [95]. Clade L dominated the beginning of the pandemic [38], prior to the appearances of clade S and the less defned clade O in early January, 2020 [73, 93, 96]. Clades V and G appeared in mid-January, followed by clades GH and GR at the end of February, clade GV at the end of June, and clade GRY in September, 2020 [94, 97, 98]. Clades L and V are likely extinct, while clades G, GH, GR, and GRY comprise the majority of global SARS-CoV-2 sequences currently [97, 98]. Clade S has also been declining since the emer gence of clade G [93]. Following rapid dissemination of clade G and its derivatives, such as B.1.1.7, B.1.351, P.1, and B.1.617.2 variants (Table 5), we may see the rise of other variants, selected by mounting population-level immunity and other yet unidentifed factors [89, 99–101], highlighting the need for international genome surveil lance eforts and global data sharing via the established GISAID resource [102].

Clade G is characterized in part by the single nucleo tide polymorphism (SNP) A23403G within subdomain 2 of the S1 gene, resulting in amino acid mutation D614G [103, 104] (Fig. 2, Table 2). D614G is now detected glob ally in B.1.1.7, B,1,351, P.1, B.1.617.2 and other variants [97, 104, 105] and increases the infectivity of SARS CoV-2 by increasing respiratory viral load [106, 107], possibly due to increased S openness [108, 109] or cleav ability [110], causing this mutation to become dominant upon emergence [93, 111, 112]. Tere is also an epide miological correlation between D614G and anosmia (loss of smell) [109], potentially due to greater viral loads in the olfactory epithelium. Preliminary evidence suggests that D614G increases viral susceptibility to neutralization [113], with uncertain impacts on disease severity [104, 114, 115].

D614G is usually accompanied by three other muta tions which represent clade G [104, 116, 117] (Table 3). Of these mutations, P323L in the RNA-dependent RNA polymerase (RdRp), encoded by *NSP12* (Fig. 2, Table 2), is particularly interesting as CoV RdRp tends to be highly

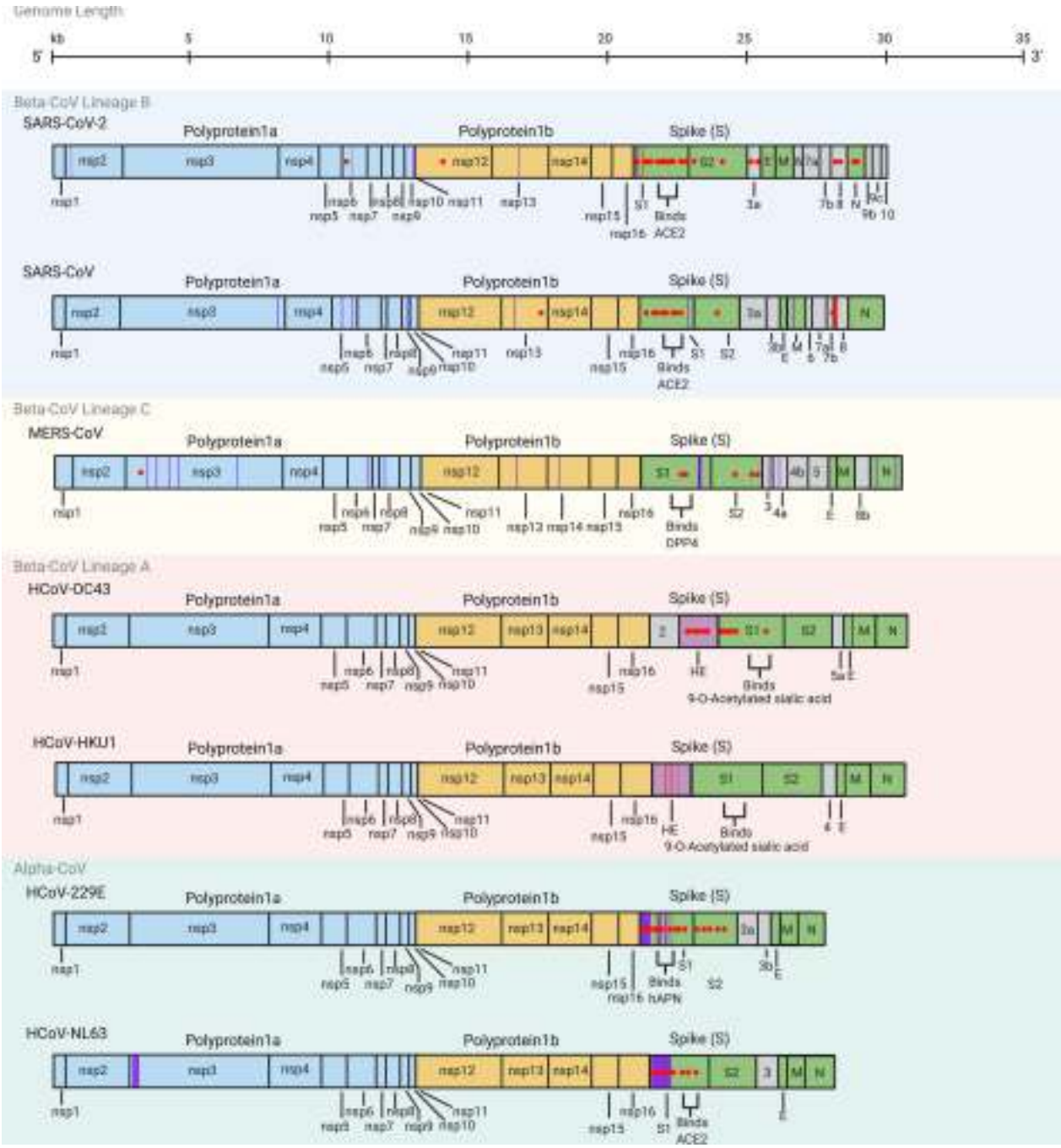

Fig. 2 Mutations identifed in human coronaviruses. Red dots within the genomes correspond to specifc amino acid residues that have been strongly positively selected for such that a specifc mutation has become dominant in the region where it emerged [74, 78, 83–91, 94–96, 99–101, 104, 111, 116, 117, 121, 123–125, 129, 131, 132, 135, 138–140, 146, 151–154, 158, 162, 278, 284–293]. Genomic regions highlighted by red bars correspond to deletions that have been selected for, while purple bars correspond to regions with signifcant polymorphisms within a CoV species. ***Beta-CoV*** Lineage B (***Sarbecovirus***) is represented within the blue shaded area, ***Beta-CoV*** Lineage C (***Merbecovirus***) is represented within the yellow shaded area, ***Beta-CoV*** Lineage A (***Embecovirus***) is represented within the red shaded area, and ***Alpha-CoVs*** are represented within the green shaded area. Genome length in kilobases (kb) is noted on top. See Table 2 for more details

Table 2 Selection sites across various human coronaviruses

Protein SARS-CoV-2 SARS-CoV MERS-CoV HCoV-OC43 HCoV-HKU1 HCoV-229E HCoV-NL63							
Nsp1							

Nsp2	aa85						
Nsp3		nt8441	**R911C**, aa981, aa1099, aa1255, aa1375, aa2119				~nt4000
Nsp4							
Nsp5		nt10384, nt10793					
Nsp6	**L37F,** nt11083		nt11631				
Nsp7							
Nsp8			nt12257				
Nsp9		nt12814					
Nsp10							
Nsp11	nt13402						
Nsp12	**P323L**						
Nsp13	nt16887	nt16177, **E466D**	aa5551				
Nsp14			aa6030				
Nsp15							
Nsp16							
ORF2							
HE				**T114N, T115R, R177P, E178Q, F181S, F247L, H250Y**	**169-176del, 181-182del, 188-194del, 215del, 221-223del**		
S1	nt21575, **S13I, H69del, V70del, Y144del, W152L, A222V, D253G, K417N/T, N439K, N440K, L452R, S477N, T478K, E484K/Q, F490S, N501Y, D614G, Q677P/H, P681H/R**	**D77G, L239S, T244I, R311G, F360S, L472P, D480G, T487S,** nt22797	**D510G, I529T,** nt23722	**N33D, K90L, T93K, D120H, K184N, L195S, Y521H**		**Y26H, Y35H, L88S, D111N, L113S, L121I, T223N, D228del, S229V, D248A, V288A/M/E,** aa308-325, **K314V/P, G321R, D324V,** aa352-359, **V353del, Y354del, Y404L,** aa404-408, **D430K, V444N, K488N**	aa1-200, **50I, 120S, 295A, 310V, 370V, 435K, E471D, I507L, E572A**

S2	**A701V, F888L**	**D778Y**	**Q1020R/H, G1224S, L1267S**			**R642M, N714K, V765A, T871I, I937L**	
ORF3			aa85, aa86				
ORF3a	**Q57H, G251V**						
ORF3b							
ORF4							
ORF4a			aa102				
ORF4b							
ORF5							
ORF5a							
E							
M		nt26428					
ORF6							
ORF7a							
ORF7b		**R17C**					
ORF8	**Q27stop, L84S**	nt27969-27897del					
ORF8b							
N	**R203K, G204R**		aa178, aa300				
ORF9b							
ORF9c							
ORF10							
References	(74,78,94,99,100,121,123,129,135,150,158,196, 205,216–218,227,229,231 ,311)	(74,138,153,286,312–316)	(74,131,152,220 ,287–291,317)	(84,278)	(292)	(83,151,293,318)	(85–88)

Table 2 (continued)

This table illustrates positively selected for residues across multiple human coronaviruses. Shaded boxes represent proteins not encoded by the specifc CoV species. Text in bold highlight mutations and deletions that were positively selected for and showed population-level expansion, while non-bolded text represents highly polymorphic sites. Sites are indicated as nucleotide (nt) position or amino acid (aa) position. Empty cells in the table represent lack of evidence for positive selection or lack of publications on positive selection within these regions

Table 3 Characteristic mutations detected in circulating SARS CoV-2 clades

Clade	Characteristic mutations	References
L	Reference Genome NC_045512.2	[94, 319]
V	Nsp6: L37F ORF3a: G251V	[104, 116, 117] [121, 146]
S	Nsp4: S76S ORF8: L84S	
G	5' UTR: C241T * Nsp3: F106F Nsp12: P323L S: D614G	[123]
GH	5' UTR: C241T * Nsp3: F106F Nsp12: P323L S: D614G ORF3a: Q57H	[97, 150]
GR	5' UTR: C241T * Nsp3: F106F Nsp12: P323L S: D614G N: R203K N: G204R	[89, 146]
GV	5' UTR: C241T * Nsp3: F106F Nsp12: P323L S: A222V S: D614G	
GRY	5' UTR: C241T * Nsp3: F106F Nsp12: P323L S: H69del S: V70del S: Y144del S: N501Y S: D614G N: R203K N: G204R	[93, 94]
O	Variants without mutations characteris tic of other clades	[95, 123, 129, 284] [96, 285]

Characteristic mutations for SARS-CoV-2 clades at the amino acid or nucleotide (*) levels

conserved by purifying selection given its critical role in viral genome replication [118, 119] (Table 4). P323 falls outside of the RdRp catalytic site and within a relatively uncharacterized interface domain that may interact with proteins that regulate viral polymerase function [120]. Te correlation of this mutation with increased point mutations [121] elsewhere in the genome raises an intriguing hypothesis that P323L diminishes RdRp proofreading ability, leading to increased mutation rates. Moreover, P323L downregulates the associa tion of Nsp12 with the Nsp8 primase subunit (Table 4), reducing polymerase activity and viral replication [122]. Decreased replication could decrease symptomology, leading to reduced COVID-19 detection and greater population-level spread. It is important to characterize the cumulative efect of all mutations, as any reduction in transmission due to P323L could be compensated for by the co-existing D614G mutation. Multiple factors may contribute to the success of clade G and its derivatives via rapid spread with low detection in human populations [104].

Positively selected for residues within SARS-CoV-2 Nsp6 [74, 123–126] are intriguing since Nsp6 is rela tively conserved in other coronaviruses [126] (Fig. 2, Table 2). SARS-CoV-2 Nsp6 inhibits IFN-1 responses [127] and may reduce delivery of viral factors to host lys osomes similar to its SARS-CoV ortholog [128] (Table 4). Te Nsp6 L37F mutation may impair Nsp6 function [129], decreasing viral replication and causing increased asymptomatic infections [130]. A similar homoplasy occurs in MERS-CoV Nsp6 [74, 131] (Fig. 2), although the outcome of this mutation is unknown. Te associated clade V mutation (Table 3) in ORF3a (G251V) reduces viral replication through decreased SARS-CoV-2 intravi ral ORF3a-S and ORF3a-membrane protein (M) binding afnity [132]. Nsp6 (L37F) and ORF3a (G251V) muta tions were likely selected to decrease pathogenicity and disease severity. A separate positively selected ORF3a mutation (Q57H) [111] characteristic of clade GH vari ants (Table 3) is speculated to increase ORF3a-S and ORF3a-M binding afnity, promoting virus replication [132]. Te ORF3a viroporin is essential for SARS-CoV-2 pathogenesis [133] and limits apoptosis in infected cells con tributing to less severe disease outcomes.

Another mutation of interest (L84S) lies within ORF8 [123, 124, 135], a protein implicated in evasion of host immune responses [136, 137] (Table 4). ORF8 was under strong directional selection at the beginning of both SARS-CoV-2 [124] and SARS-CoV outbreaks [138], supporting the theory that it facilitates zoonotic trans mission and adaptation in alternate hosts [139, 140]. However, the over-representation of ORF8 deletions in SARS-CoV with no apparent efect on viral survival [138] suggests that ORF8 may be dispensable in humans [139], and L84S mutations may not be signifcant. While relative to its SARS-CoV ortholog [134], potentially

Table 4 Putative functions of SARS-CoV-2 proteins

Gene	Protein	Putative function	References
Nsp1	Leader protein/host translation inhibitor	Inhibits translation of host mRNAs and promotes expression of viral genes	[320]
Nsp2	Non-structural protein 2	Modulates host cell survival signalling pathways	[321]
Nsp3	Papain-like protease	Proteolytic cleavage of polyprotein to generate Nsps 1–3, and inhibi tion of host IFN responses	factors to host lysosomes and inhibits IFN-1 responses
Nsp4	Non-structural protein 4	Interacts with Nsp3 and host proteins to induce cytoplasmic autophagosomes for viral replication	
Nsp5	Chymotrypsin-like protease	Proteolytic cleavage of polyprotein to generate Nsps 4–16 and media tion of Nsp maturation	[322, 323] [324, 325] [326, 327] [127, 128] [120]
Nsp6	Non-structural protein 6	Interferes with delivery of viral	[120]
Nsp7	Primase complex	Forms a complex with Nsp8 which interacts with RdRp (Nsp12) to transcribe viral genome	
Nsp8	Primase complex	Forms a complex with Nsp7 which interacts with RdRp (Nsp12) to transcribe viral genome	
Nsp9	ssRNA-binding protein	Binds to viral ssRNA and promotes replication	[328]
Nsp10	Non-structural protein 10	Interacts with 3′–5′ exoribonuclease (Nsp14) and 2′ O-ribose methyl transferase (Nsp16) and promotes methylation of viral mRNA caps	[328]
Nsp11	Non-structural protein 11	Released from cleavage of pp1a and forms N-terminal sequence of Nsp12 in pp1ab frameshift product. No known function	[328]
Nsp12	RNA-dependent RNA polymerase (RdRp)	Replicates and transcribes viral genome	[326]
Nsp13	Helicase	Unwinds dsRNA and dsDNA in viral replication	[326, 329]
Nsp14	3′–5′ exoribonuclease/N7-guanine methyltransferase	Proofreading during RNA replication (exoribonuclease) and viral mRNA capping (methyltransferase). Interacts with Nsp10	[330]
Nsp15	Nidoviral uridylate-specifc endoribonuclease	RNA processing and inhibition of host IFN responses	[331]
Nsp16	2′ O-ribose methyltransferase	Activated by Nsp10 for methylation of viral mRNA caps	[332]
S	Spike glycoprotein	Cleaved into S1 and S2 subunits. S1 binds host receptor (ACE2) while S2 mediates viral and host membrane fusion	release and may induce necrotic cell death [333]
ORF3a	Orf3a viroporin	Activates NF-kB and NLRP3 infammasome to contribute to cytokine storm. Promotes viral	[334–336]
ORF3b	Accessory protein ORF3b	IFN-1 antagonist	[337]
E	Envelope protein	A viroporin involved in viral assembly, budding, and pathogenesis. Forms CoV envelope	assembly and budding [338, 339] [340]
M	Membrane protein	Forms viral membrane and induces N and S localization to the ER Golgi-Intermediate compartment for virion	
ORF6	Accessory protein ORF6	IFN-1 antagonist	[144]
ORF7a	Accessory protein ORF7a	SARS-CoV ortholog inhibits bone marrow stromal antigen 2 mediated tethering of virions to host plasma membrane	[341]
ORF7b	Accessory protein ORF7b	SARS-CoV ortholog attenuates viral replication	[342]
ORF8	Accessory protein ORF8	Inhibits IFN-1 activity and downregulates MHC-1 expression to evade host immunity	virion assembly [136, 137, 144] [144, 145]
N	Nucleocapsid	Involved in immune evasion through IFN-1 antagonism, nucleocapsid formation, viral RNA replication, and	
ORF9b	Accessory protein ORF9b	Suppresses IFN-1 responses through inhibition of TOM70	[343]
ORF9c	Accessory protein ORF9c	Interferes with IFN signalling, antigen presentation, and complement signalling. Induces IL-6 signalling	[344]
ORF10	Accessory protein ORF10	Interacts with a Cullin 2 RING E3 ligase complex to potentially modu late ubiquitination	[345]

Findings are based on studies with SARS-CoV-2 proteins or SARS-CoV orthologs

L84S may be important in SARS-CoV-2 virulence and pathogenesis given ORF8's role in attenuation of host immunity (Table 4), the continued decline of L84S representation among global SARS-CoV-2 sequences [93] suggests otherwise.

Mutations RG203KR within SARS-CoV-2 nucleoprotein (N) have become dominant and characteristic of clade GR [123]. RG203KR alters N protein morphology, resulting in increased intraviral protein binding afn ity [132]. N-M interactions are necessary for CoV viral assembly [141, 142], while N-envelope (E) interactions potentially increase production of virus-like particles [143]. Terefore, increased intraviral N protein binding afnities could contribute to increased viral replication. RG203KR may also confer immune evasion properties to SARS-CoV-2 considering the rapid expansion of clade GR and the role of N protein in antagonizing human anti viral immune responses [144, 145] (Table 4). Te global prevalence of variant B.1.1.7 has generated clade GRY from clade GR [146].

Clade GV is associated with the European variant 20A. EU1 containing spike NTD mutation A222V [105, 147]. A222 is located within a speculated B lymphocyte epitope [148] that may impact neutralization by human antibod ies, consistent with observed SARS-CoV-2 re-infection with a clade GV variant [149]. Te rise in prevalence of variant 20A.EU1 and clade GV is most likely associated with the relaxing of travel-associated restrictions across Europe near the end of the summer of 2020 consider ing the rapid decline in prevalence of global clade GV sequences in 2021 [97, 150].

Ongoing SARS-CoV-2 evolution and the rise of variants of concern

An aforementioned trend across HCoVs is positively selected residues within RBD [84, 85, 88, 138, 139, 151– 154] (Fig. 2, Table 2), which facilitates interactions with host cellular proteins, providing a crucial target for the host immune response [155]. Accordingly, SARS-CoV-2 RBD is rapidly evolving, leading to novel variants [156, 157] (Fig. 2, Table 2). SARS-CoV-2 variants associated with greater transmissibility, altered virulence, or the ability to escape natural infection- and vaccine-mediated immunity or current diagnostic tests are called Variants of Concern (VOC; Table 5).

Early data suggest that RBD mutation N501Y emerged recurrently in multiple regions due to increased trans missibility, and is associated with multiple VOCs [89, 99, 100, 158] (Table 5). SARS-CoV-2 N501 serves as one of six critical S residues required for binding to ACE2 [159] and N501Y increases viral infectivity through greater S-hACE2 binding afnity, likely due to stronger inter actions with ACE2 residues Y41 and K353 [160]. Other critical residues within the SARS-CoV-2 RBD (L455, F486, Q493, S494, Y505) [73] should be closely moni tored as mutations may increase SARS-CoV-2 transmis sion in humans and facilitate zooanthroponotic transfer to other species.

Early studies of the highly transmissible B.1.1.7 variant [77, 161] originating in the United Kingdom described 17 co-occurring non-synonymous mutations or dele tions [89], which are more than expected since the muta tion rate of SARS-CoV-2 is estimated to be around 2.4×10^{-3} per site per year [135]. In addition to N501Y, spike 69-70del, Y144del, and P681H mutations are specu lated to be of functional signifcance [78, 162] (Table 5). Spike NTD 69-70del variants have shown signifcant

Table 5 SARS-CoV-2 variants of concern (as of July 22, 2021)

Variant	Mutations of interest	Clade	Date of emergence	First detection in human population	Country of likely origin	References
B.1.1.7 (VOC2020 12/01, 501.V1, Alpha)	S: 69-70del S: Y144del S: N501Y S: D614G S: P681H	GRY	September, 2020	December, 2020	United Kingdom	[89, 99, 190]
B.1.617.2 (Delta)	S: L452R S: T478K S: D614G S: P681R	GH	October, 2020	December, 2020	South Africa	[100, 101, 190]
B.1.351 (501.V2, Beta)	S: K417N S: E484K S: N501Y S: D614G S: A701V					
P.1 (501.V3, Gamma)	S: K417T S: E484K S: N501Y S: D614G					

2020 December, 2020 India [196, 201, 202, 346]

GR July, 2020 January, 2021 Brazil [101, 190, 194] G October,

Variant names are based on Rambaut et al.'s classifcation [347]. Other commonly used names are mentioned in brackets. Mutations mentioned here are non synonymous mutations that are speculated to confer some functional signifcance. These variants contain other mutations that may also contribute to viral advantages [89, 99–101]. Updated information about SARS-CoV-2 VOCs can be accessed through the GISAID resource (https://www.gisaid.org). Dates of emergence are based on retrospective analyses. S, spike. del, deletion

transmission expansion, with speculated increased resist ance to antibody-mediated neutralization [92] likely associated with sequestration of a protruding spike loop [78]. Y144del confers antibody resistance due to loss of a negative surface charge [163, 164]. Spike P681 is located in a known CoV mutational hotspot [83, 101] directly adjacent to the SARS-CoV-2 S1/S2 furin cleav age site (aa 681–684) [89, 165, 166] which promotes virus entry into host cells [167]; mutation in this region may increase cleavability and membrane fusion to enhance infectivity. P681 is also within an antigenic epitope recog nized by B and T lymphocytes, implicating host immune response alterations [168]. P681H may therefore repre sent adaptive evolution to evade host immunity, although confrmatory studies are required. Another speculated B.1.1.7 mutation at ORF8 (Q27stop) causes early protein termination [89]. Truncated ORF8 has been associated with milder symptoms [169], although increased mor tality is also associated with the B.1.1.7 variant [79, 170]. Emerging mutations in B.1.1.7 must be monitored and investigated, such as the sub-lineage VOC202102/02 that contains the RBD mutation E484K, which is associated with antibody resistance [171–173].

Another variant containing N501Y is B.1.351, which was frst detected in South Africa in December, 2020 [100], but likely originated in October, 2020 [101]. Tis variant contains eight non-synonymous mutations in S, including three within the RBD (K417N, E484K, N501Y) and three in the NTD that may contribute to increased transmissibility [100, 101]. Both N501Y and E484K are located within the receptor binding motif (RBM) of the RBD. E484 interacts with residue K31 on hACE2 [174], one of two critical hACE2 RBD-interacting residues [159, 175], suggesting that E484K may afect the binding afnity of SARS-CoV-2 with hACE2. However, prelimi nary studies demonstrate contradictory binding afnity observations [176, 177]; further studies are required. In addition, E484K confers some resistance to antibody mediated neutralization of SARS-CoV-2 in vitro [91, 154, 178–181], consistent with the observation that E484 is an important recognition site for neutralizing antibod ies [181, 182], and raising concerns about E484K being an immune escape mutation appearing in multiple inde pendent SARS-CoV-2 lineages [172, 183–186]. Similarly, spike K417 is within a neutralizing antibody epitope [100]. Preliminary evidence suggests K417N reduces rec ognition by human antibodies [187]. K417N may impact RBD-hACE2 binding afnity and stabilize E484K, though these efects remain uncertain [91, 177, 187, 188]. Mutations within the RBD (K417T, E484K, N501Y) have also been observed in the P.1 variant (Table 5) that likely originated in Brazil and has since spread to other countries [101, 189–191]. In contrast, the P.2 variant only contains E484K, likely acquired through convergent evo lution with P.1 [186, 192]. Little is known about the P.1 variant, but based on emerging data [193], we speculate that the RBD mutations likely afect antibody-mediated neutralization and contribute to increased transmission as observed with B.1.351. Mutations shared between the B.1.1.7, B.1.351, and P.1 variants are speculated to have arisen independently, indicating convergent evolution [194] (Table 5). Tese variants also share Nsp6 3675-3677del, with unknown functional signifcance [194, 195].

VOC B.1.617.2 was frst identifed in India in late 2020 and contains positively selected for mutations within the spike protein, namely, L452R, T478K, and P681R, along with the D614G mutation [196] (Table 5). Mutation of the uncharged and hydrophobic leucine (L) residue into the positively charged and hydrophilic arginine (R) resi due at spike position 452 allows for an increased electro static interaction with negatively charged ACE2 residues E35, E37, and D38, likely leading to the observed increase in S-hACE2 complex stability, viral infectivity, and virus replication [196, 197]. Furthermore, abolition of the hydrophobic surface patch through the L452R mutation led to reduced antibody-mediated neutralization and cellular immune recognition [196–198]. Spike muta tion T478K has also been shown to increase electrostatic interactions in the S-hACE2 complex and may increase binding afnity similar to the S477N mutation [199]. Te mutation T478K is within a neutralizing epitope close to the immune evasion

mutation E484K/Q that is present in multiple SARS-CoV-2 variants, including the ances tral B.1.617 lineage and current sub-lineages B.1.617.1 and B.1.617.2 [181, 200, 201]. T478K in combination with L452R may contribute to increased resistance to neutrali zation by monoclonal antibodies, convalescent sera, and vaccinated sera [201, 202]. B.1.617.2 has increased repli cation efciency in human airway systems relative to the B.1.1.7 lineage due to enhanced spike cleavability, which is likely augmented by the P681R mutation [201, 203]. P681R is known to increase cell-to-cell fusion in the res piratory tract, potentially increasing transmissibility and pathogenicity in infected individuals [201, 203]. B.1.617.2 may thus represent a VOC with similar resistance to antibody neutralization as B.1.351 and transmissibil ity beyond B.1.1.7 [200]. Recently discovered B.1.617.2 sequences containing the K417N mutation (AY.1/AY.2 lineages) must be monitored for altered antibody resist ance and increased transmissibility [204].

Circulating variants containing an N439K mutation (e.g. B.1.141 and B.1.258) also show some degree of neu tralization evasion [91, 198, 205], raising speculations about SARS-CoV-2 variants escaping vaccine-mediated immunity. Emerging data suggest that antibodies elicited

by mRNA vaccines (BNT162b2 and mRNA-1273) have 20% and 16.7% reduced neutralization capacity, respec tively, against the B.1.1.7 variant [206, 207] and 67% and 84% reduced neutralization capacity, respectively, against the B.1.351 variant [208, 209]. Neutralization capacity of sera from BNT162b2 and mRNA-1273 vaccinated indi viduals have 87% and 52% reduced neutralization capac ity, respectively, against the B.1.617.2 variant [200, 201, 210]. Te emergence of B.1.1.7 sub-lineages containing the E484K RBD mutation (e.g. VOC202102/02) pose additional challenges for vaccine-mediated immunity [171, 173, 183]. While complete vaccine failure is unlikely [206, 207, 211–215], immune escape variants may create a need to update current SARS-CoV-2 vaccines. Moni toring the emergence of novel SARS-CoV-2 variants is especially important as vaccine-mediated immunity provides stronger selective pressure for SARS-CoV-2 evolution.

Other variants of interest

Multiple emerging SARS-CoV-2 lineages are not con sidered VOCs but are still of interest and may become VOCs in the future. One variant, B.1.525, was frst detected in December, 2020, in the United Kingdom and Nigeria and has since spread internationally. B.1.525 contains spike mutations 69-70del, E484K, Q677H, and F888L. Q677P/H has emerged in disparate variants and may afect spike cleavability similar to P681H [158, 216–218]. F888L lies between the fusion peptide and heptad repeat region of the S2 subunit [219] and may impact host cellular entry, similar to the impact of heptad repeat mutations in MERS-CoV [139, 220].

Variant B.1.526 from New York contains spike muta tions D253G, D614G, and A701V, along with either E484K or S477N, creating two major B.1.526 sub-line ages. NTD mutation D253G reduces antibody-mediated neutralization [163]. A701V, shared by variant B.1.351 [100], is in the S2 subunit adjacent to the furin cleav age site [219] and may impact SARS-CoV-2 cleavability and infectivity. S477N, also found in variant 20A.EU2, increases binding to hACE2 [221, 222] and reduces anti body-mediated neutralization [178, 223], likely due to its position within a neutralizing epitope [224]. D614G and E484K are shared with multiple other variants (Table 5) and likely play a role in B.1.526 expansion.

P681H found in variant B.1.1.207 from Nigeria [162] may enhance infectivity and modulate host immunity as speculated for B.1.1.7. Similar efects are expected for P681R in variant A.23.1 that emerged in Uganda [183, 225]. Te UK A.23.1 sub-lineage VUI-202102/01 also contains immune escape mutation E484K [171, 183]. Preliminary data show increased ACE2 binding afnity and reduced antibody-mediated neutralization for the P.3 variant from Brazil, which contains the spike muta tions E484K, N501Y, and P681H [164]. Data also suggest increased ACE2 binding afnity and reduced neutraliza tion profle for the B.1.620 variant from Central Africa, which contains spike mutations E484K, S477N, D614G, and P681H [226]. Other notable variants include N440K variants from India [227] that have increased transmis sibility, and the R.1 variant from Japan which contains potential immune escape mutations W152L and E484K [228].

B.1.427/B.1.429 are two emerging lineages that origi nated in California in May 2020 [229], however, circulat ing B.1.427/B.1.429 variants are now being replaced by more transmissible variants, such as B.1.1.7 and B.1.617.2 [97, 230]. B.1.427/B.1.429 contains multiple positively selected for mutations within the S protein, such as S13I, W152C, and L452R, all of which contribute to some degree of resistance to antibody-mediated neutraliza tion [229]. L452R has convergently evolved in the B.1.617 lineage and contributed to enhanced SARS-CoV-2 infec tivity [196–198] (Table 5). Spike mutation L452Q was detected in the recently emerged C.37 lineage from Peru and is expected to have similar impacts on virus infectiv ity as the L452R mutation [231]. C.37 also shares Nsp6 3675-3677del with B.1.1.7, B.1.351. and P.1 variants [231], and contains the

spike RBD mutation F490S that has been associated with reduced antibody-mediated neu tralization [91, 178]. Tese variants need to be monitored for transmission expansion and convergent evolution.

Multiple factors will determine the evolutionary trajectory of SARS-CoV-2 and the COVID-19 pandemic

Te future of SARS-CoV-2 and COVID-19 remains uncertain. Many virological, immunological, and social factors will infuence the epidemiological trajec tory of this virus. One particularly intriguing question that remains unanswered is whether SARS-CoV-2 will become endemic in the human population, like HCoVs NL63, OC43, HKU1, and 229E [232–234].

Currently, endemic HCoVs cause seasonal outbreaks [235], with increased circulation observed in the win ter in temperate regions [232]. Cold temperatures are favourable for enveloped viruses [236], as lower tem peratures enhance lipid ordering of the viral envelope, allowing the virus to remain protected outside the host for longer periods of time [237, 238]. Low temperatures also enhance aerosol transmission of respiratory viruses by allowing virions to remain suspended in the air for a longer duration [239]. Furthermore, cold and dry envi ronments can have immunosuppressive efects on a potential host, further increasing the chances of infection [240–242]. Evidence suggests decreased transmission of SARS-CoV-2 in warmer climates [243–246], likely due to degeneration of viral structural stability with increasing temperatures [247]. Decreased transmission of SARS CoV-2 was not observed during the summer of 2020 [11, 248] likely because of the sheer number of cases and an immunologically naïve population. For seasonal ity to have an observable impact on SARS-CoV-2 trans mission, the basic reproduction number (R_0) must drop from its current estimate of around 2.5 to less than 1 [249]. In theory, SARS-CoV-2 R_0 should drop substan tially when population herd immunity is reached through natural infection and vaccination, allowing for meteoro logical factors to infuence viral transmission, leading to seasonal fuctuations. Other intervention mechanisms such as efective social distancing, quarantine, and con tact-tracing will contribute towards reducing the R_0 for SARS-CoV-2 [250, 251].

Multiple studies have demonstrated short-lasting immunity to endemic HCoVs, with waning of protective immunity and re-infections common within 80 days [85] to one year [252–255]. Tere is no observable associa tion between endemic HCoV re-infection and infection severity [254]. Waning of humoral immunity within a year [256–260] and re-infection of immunocompetent patients [149] have been demonstrated for SARS-CoV-2, suggesting the possibility of annual outbreaks [233, 261]. A weaker initial immune response and sharper decline of antibody levels have been reported in individuals with asymptomatic SARS-CoV-2 infections [257, 258]. Tus, multiple exposures to SARS-CoV-2 may be required to develop sufcient immunity to prevent future re-infec tions, which may also be infuenced by adaptive evolution of SARS-CoV-2 in the human population (Table 5). Te duration of protection through vaccination and natural exposures is being closely monitored, along with anti genic evolution of SARS-CoV-2 that may lead to immune escape. Indeed, the evolutionary trajectories of endemic HCoVs suggest that SARS-CoV-2 will evolve to co-exist with the human population. However, with roll-out of the frst ever HCoV vaccines, predicting the evolutionary tra jectory of SARS-CoV-2 remains challenging. An important factor that may infuence ongoing SARS CoV-2 transmission is the potential for cross-protection by humoral and cellular immune responses induced by related endemic HCoVs. Tere is evidence of cross-pro tection within the same genera of HCoVs [233, 262, 263], but not between genera [264]. Tus, immunity against **beta-CoVs** HCoV-OC43 and HCoV-HKU1 may provide some protection against COVID-19 [265–268], while immunity against **alpha-CoVs** HCoV-229E and HCoV NL63 will likely provide little to no protection. Anti body-dependent enhancement has not been observed for SARS-CoV-2 [269, 270], ruling out the possibility of increased disease severity by cross-reactive antibodies generated against endemic HCoVs. Te high frequency of CoV recombination during co-infections raises the addi tional concern that SARS-CoV-2 recombination with sea sonal HCoVs could generate novel CoVs [131, 271, 272]. Te role of HCoV co-infection has not been reported or extensively studied and will be especially important for immunocompromised and elderly individuals.

Conclusions

SARS-CoV-2 continues to evolve and adapt to the human population as highlighted by the emergence of novel vari ants. Mutations within the spike protein of SARS-CoV-2 variants confer increased transmissibility and some degree of resistance to antibody-mediated neutraliza tion. However, recurrent attenuating mutations, such as P323L, L37F, G251V, and Q27stop have also been iden tifed and are speculated to reduce disease severity. Te appearance

of attenuating mutations suggests that SARS CoV-2 is evolving to become less pathogenic in humans. Te current SARS-CoV-2 pandemic is driven by asymp tomatic, pre-symptomatic, or otherwise unrecognized cases [273–275]. Reduced pathogenicity of SARS-CoV-2 combined with mounting population-level immunity will likely cause a reduction of severe cases of COVID-19, leading to an apparent abatement of the pandemic, fol lowed by endemic circulation of low pathogenic SARS CoV-2 variants. A similar evolutionary trajectory may have led to the establishment of current low-pathogenic endemic HCoVs [276].

Monitoring future emerging variants of SARS CoV-2 is critical to determine control measures for the COVID-19 pandemic. Mutations speculated to reduce immune recognition, such as within the spike protein (S13I, 69-70del, W152L, A222V, K417N, N439K, S477N, T478K, E484K/Q, F490S, P681H/R) and nucleoprotein (RG203KR) should be studied for reduced sensitivity to natural or vaccine-induced immunity. Other factors, such as zoonotic and zooanthroponotic transmission of SARS-CoV-2, cross-protection through immunity against endemic HCoVs, and the possible creation of novel ani mal reservoirs through zooanthroponosis should con tinue to be investigated as they may have signifcant implications on the evolutionary trajectory of SARS CoV-2 and the COVID-19 pandemic.

Abbreviations

CoV: Coronavirus; ***dN***: Rate of nonsynonymous mutations; ***dS***: Rate of synony mous mutations; E: Envelope protein; hACE2: Human ACE2; HCoV: Human coronavirus; HE: Hemagglutinin esterase; M: Membrane protein; MERS-CoV: Middle East respiratory syndrome coronavirus; N: Nucleocapsid protein; S: Spike glycoprotein; NTD: N-terminal domain; PEDV: Porcine epidemic diarrhea virus; RdRp: RNA-dependent RNA polymerase; RBD: Receptor binding domain; RBM: Receptor binding motif; R_0: Basic reproduction number; SADS-CoV:

Swine acute diarrhea syndrome coronavirus; SARS-CoV: Severe acute respira tory syndrome coronavirus; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; SARSr-CoV: Severe acute respiratory syndrome related coronavi rus; SNP: Single nucleotide polymorphism; VOC: Variant of concern.

Acknowledgements
None.

Authors' contributions
JS wrote the frst draft. PP, AGM, AB, and KM edited the draft. All authors read and approved the fnal manuscript.

Funding
SARS-CoV-2 studies in the Mossman lab are supported by a Canadian Institutes of Health Research COVID-19 rapid response grant to principal investigator K.M. and co-investigator A.B. A.B. was also supported by a Natural Sciences and Engineering Research Council of Canada fellowship. VIDO receives operational funding for its CL3 facility (InterVac) from the Canada Foundation for Innovation through the Major Science Initiatives. VIDO also receives operational funding from the Government of Saskatchewan through Innovation Saskatchewan and the Ministry of Agriculture.

Availability of data and materials
Not applicable.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent to publication
This article is published with the permission of the Director of VIDO, journal series no. 937.

Competing interests
Authors declare no competing interests.

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Received: 31 March 2021 Accepted: 3 August 2021
Published online: 13 August 2021

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