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Relatively rapid evolution rates of SARS-CoV-2 spike gene at the primary stage of massive vaccination

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ABSTRACT

A series of stringent non-pharmacological and pharmacological interventions were implemented to contain the pandemic but the pandemic continues. Moreover, vaccination breakthrough infection and reinfection in convalescent coronavirus disease 2019 (COVID-19) cases have been reported. Further, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants emerged with mutations in spike (S) gene, the target of most current vaccines. Importantly, the mutations exhibit a trend of immune escape from the vaccination. Herein the scientific question that if the vaccination drives genetic or antigenic drifts of SARS-CoV-2 remains elusive. We performed correlation analyses to uncover the impacts of wide vaccination on epidemiological characteristics of COVID-19. In addition, we investigated the evolutionary dynamics and genetic diversity of SARS-CoV-2 under immune pressure by utilizing the Bayesian phylodynamic inferences and the lineage entropy calculation respectively. We found that vaccination coverage was negatively related to the infections, severe cases, and deaths of COVID-19 respectively. With the increasing vaccination coverage, the lineage diversity of SARS-CoV-2 dampened, but the rapid mutation rates of the S gene were identified, and the vaccination could be one of the explanations for driving mutations in S gene. Moreover, new epidemics resurged in several countries with high vaccination coverage, questioning their current pandemic control strategies. Hence, integrated vaccination and non-pharmacological interventions are critical to control the pandemic. Furthermore, novel vaccine preparation should enhance its capabilities to curb both disease severity and infection possibility.

1. Introduction

As of 27 May 2022, the overall number of global coronavirus disease 2019 (COVID-19) confirmed cases has surpassed 525.46 million, including over 6.28 million deaths [1]. To suppress and mitigate the COVID-19 pandemic, a series of stringent non-pharmacological interventions were implemented [2–5]. Moreover, with the rapid development of COVID-19 vaccines [6,7], the mass rollout of vaccination was deployed in many countries. A total of 7.98 billion doses of vaccines have been administered globally, and 54.4 % of the world population has received at least one-dose of the vaccine by 30 November 2021 (https://ourworldindata.org/covid-vaccinations). The COVID-19 vaccination has effectively reduced the number of new infections and hospitalizations [8,9]. However, the causative agent of COVID-19, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has been undergoing genetic evolution and mutations, and multiple virus variants continuously emerged, which challenged the pandemic control and the effectiveness of COVID-19 vaccines designed based on the early isolated SARS-CoV-2 strain (https://nextstrain.org/ncov/gisaid/global) [7]. Five SARS-CoV-2 variants are of great global concerns, including Alpha, Beta, Gamma, Delta, and Omicron variants, due to their high transmissivity, worldwide persistence, and immune and antigenic escape potentials (https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/) [10]. Delta and Omicron variants have replaced other SARS-CoV-2 variants and become the dominant variants of concern currently circulating around the globe thus far.

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HIGHLIGHTS

Scientific question

This study uncovered the evolutionary dynamics of SARS-CoV-2 and epidemiological characteristics of COVID-19 at the primary stage of massive vaccination.

Evidence before this study

Stringent non-pharmacological interventions and massive vaccination were implemented but the pandemic continues. Vaccination breakthrough infection and reinfection in convalescent COVID-19 cases were reported. Further, SARS-CoV-2 variants emerged and exhibited a trend of immune escape. The question that if the vaccination drives genetic or antigenic drifts of SARS-CoV-2 remains elusive.

New findings

Vaccination coverage was negatively related to the infections, severe cases, and deaths of COVID-19, respectively, at the primary stage of massive vaccination. Additionally, with the increasing vaccination coverage, the lineage diversity of SARS-CoV-2 dampened, but the rapid mutation rates (i.e. genetic drift) of the S gene were identified. The vaccination could be one of the explanations for lowering the genetic diversity but driving genetic drift in the S gene of SARS-CoV-2. The resurged new epidemics in several countries (e.g., USA and UK) with high vaccination coverage, questioning their pandemic control strategies and highlighting the cruciality of integrated vaccination and non-pharmacological interventions.

Significance of the study

This study informs massive vaccination could be one of the explanations for driving SARS-CoV-2 evolution and lowering its genetic diversity. This study calls for integrated vaccination and non-pharmacological interventions and a highly effective vaccine design to curb disease severity and infection possibility.

In addition, COVID-19 reinfections have been reported, which raised concerns about the dubitability of antibody protection induced by a prior infection [11]. It has been reported that the antibodies against SARS-CoV-2 in mild syndrome cases vanished after three months post the syndrome onset [12]. Moreover, vaccine breakthrough infections have also been identified [13,14], which are of concern about the effectiveness of vaccines. The mechanism of reinfections and vaccine breakthrough infections remains uncertain; however, these infections potentially increase virus evolutionary rates [13] and may accelerate the emergence of novel virus variants, eventually resulting in a new wave of COVID-19 resurgence.

In this study, to understand the impact of extraordinarily rapid deployment and mass rollout of vaccines at the height of the pandemic, we informed the epidemiological characteristics of COVID-19 infections, and evolutionary dynamics and genetic diversity of SARS-CoV-2 viruses under vaccine pressures in the United States of America (USA) and the United Kingdom (UK), since both are the most-affected countries with a high number of confirmed cases and the relatively high vaccine coverage.

2. Materials and methods

2.1. COVID-19 cases, vaccine coverage, and SARS-CoV-2 genomes

The data about COVID-19 cases, deaths, and vaccine doses administered in different countries around the world were collected from the data repository operated by the Johns Hopkins University (https://github.com/CSSEGISandData/COVID-19; https://github.com/govex/COVID-19). We downloaded all nucleotide genomes and the metadata of SARS-CoV-2 genomes isolated in the USA and the UK from the GISAID database (https://www.gisaid.org; data was retrieved on 14–16 July 2021). We extracted the lineage information defined by the Pangolin nomenclature system (https://pangolin.cog-uk.io) from the metadata from the GISAID database for further analyses.

2.2. SARS-CoV-2 lineage entropy

We calculated the monthly entropy of the lineage probability distribution for the SARS-CoV-2 genomes to describe the genetic diversity of the virus. The sequence lineage was defined according to the previous research [13], as:

$$H(t,c) = -\sum_{l} \frac{N(t,c,l)}{N(t,c)} ln \frac{N(t,c,l)}{N(t,c)}$$

where, t, c, and l represent the specific month, country, and lineage respectively. The $N(t,c,l)$ represents the number of sequences in lineage l in month t in a country c; $N(t,c)$ is the number of sequences in all lineages in month t in a country c. The higher lineage entropy means more lineages occurred in a month and the higher genetic diversity.

2.3. Mutation frequency and entropy on B cell and T cell epitopes of S protein

We downloaded all known neutralizing B cell epitopes and linear MHC I and MHC II T cell epitopes on S protein of the SARS-CoV-2 from the IEDB database (https://www.iedb.org). With respect to the reference Wuhan-Hu-1 isolate (Accession No: NC_045512), we counted the amino acid mutation frequency in epitopes of S gene and calculated mutation entropy for variants of concern and variants of interest defined by the WHO in July 2021 (https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/).

2.4. SARS-CoV-2 evolutionary rate inference

We collated SARS-CoV-2 genome sequences collected from humans, with high coverage (with < 300 Ns) and complete date information, isolated in the USA and the UK from the GISAID database. We aligned SARS-CoV-2 genome sequences using the default parameters by the MAFFT v7.453 [15]. We trimmed the aligned sequences given the reference genome of Wuhan-Hu-1 isolate. We extracted the S gene and coding sequence (CDS) regions except S gene from the alignment as two separate datasets.

Considering the dataset representativity and computational complexity, we generated three subsamples of SARS-CoV-2 genomes for one month in each country by randomly keeping at most 10 virus sequences per day. Later, we performed Bayesian evolutionary reconstruction to infer the evolution rates of SARS-CoV-2 in each month using BEAST v1.10.4 [16]. The general time reversible (GTR) nucleotide substitution model with gamma rate distribution was used to describe rate variation among sites, and a strict molecular model was used to inform the sequence evolution history. We employed a constant tree prior to model the population size dynamic of the virus. Three independent inferences were performed for each sampled dataset using Markov chain Monte Carlo sampling with 50–100 million chain lengths. We also checked the convergence of posterior parameters from the inference in Tracer v1.7.1 [17] and removed adequate burn-in to obtain stable estimates with an effective sample size (ESS) greater than 100. The details of SARS-CoV-2 nucleotide sequences used to infer the evolutionary rates in this study are described in the Supplemental Excel spreadsheet.

3. Results

3.1. Relationship between vaccine coverage and epidemiological characteristics of COVID-19

Based on the Pearson correlation coefficients, we identified a significantly negative association between vaccine coverage proportion

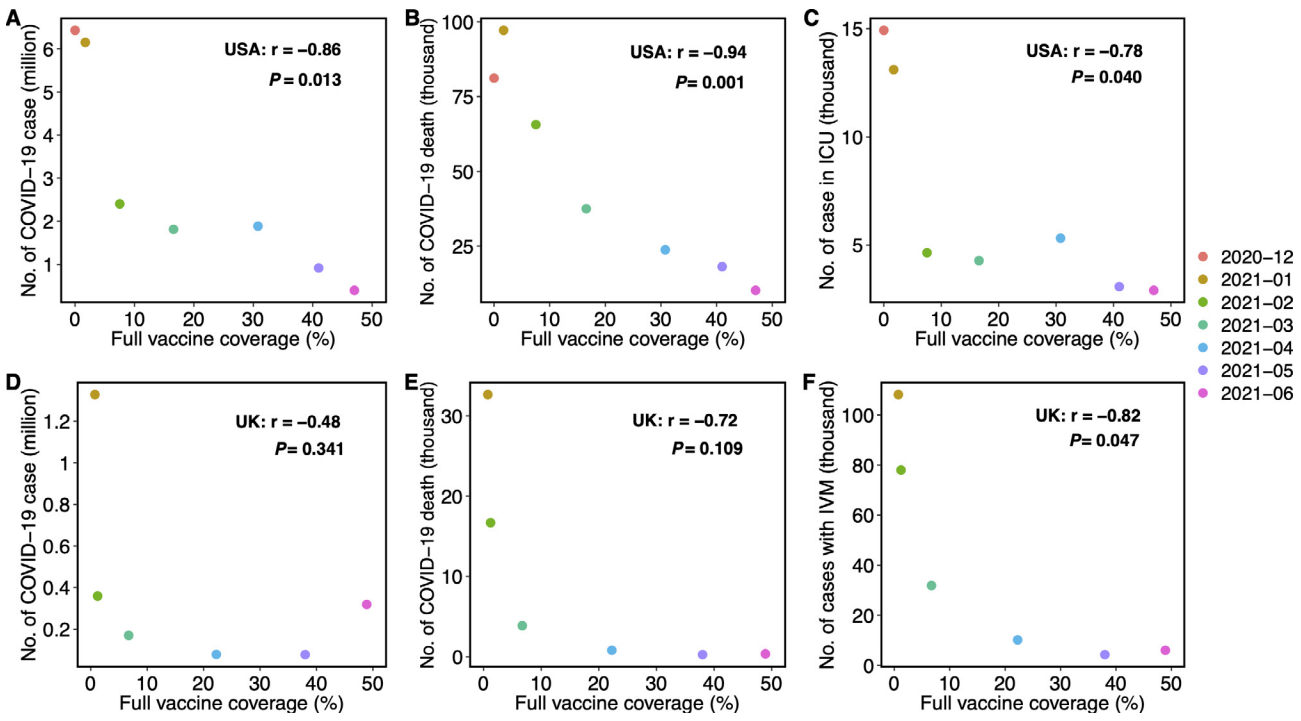

Fig. 1. Negative relationships between fully vaccinated coverage and monthly coronavirus disease 2019 (COVID-19) new cases, deaths, and severity cases, respectively. Scatter plots show the correlation between the proportion of fully vaccinated individuals and the monthly new COVID-19 cases (A)(D), between vaccine coverage proportion and the monthly new deaths (B)(E), and between vaccine coverage proportion and the monthly new cases with severity (C)(F) (IVM represents the invasive mechanical ventilation) in the USA and the UK, respectively.

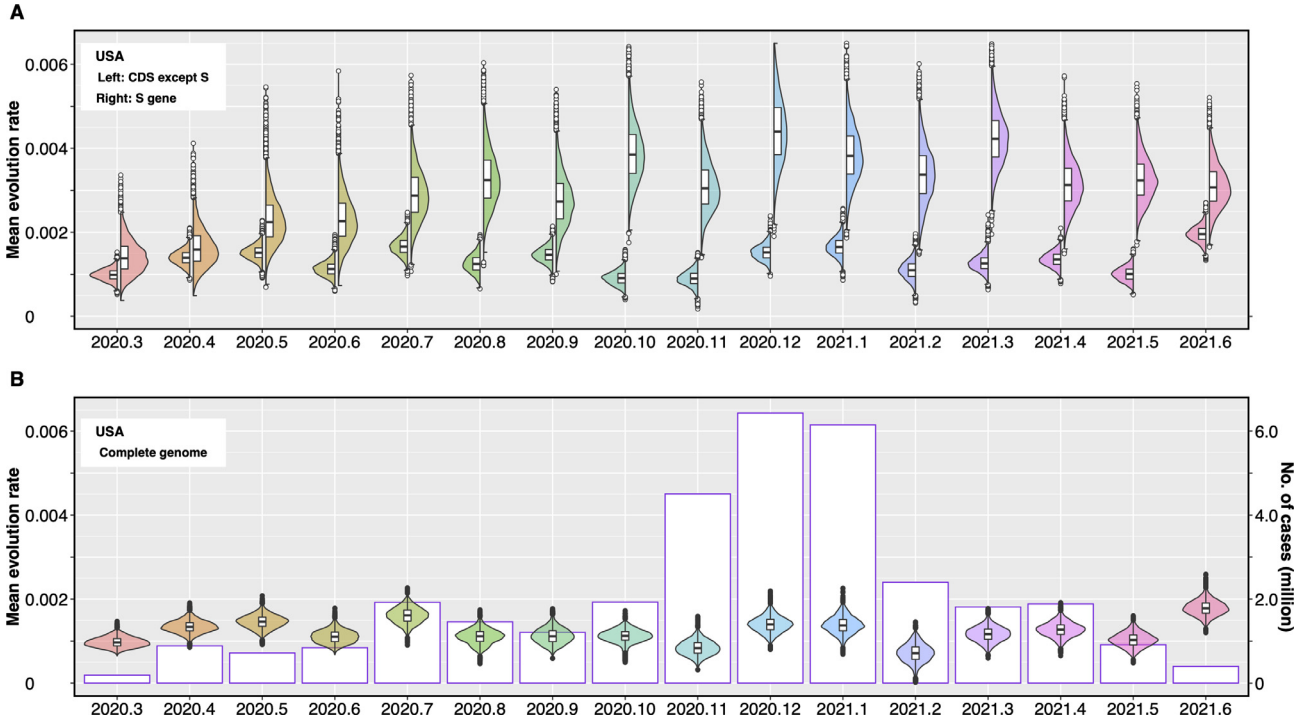

Fig. 2. Dynamics of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) evolutionary rates in the USA. A) The probability density of evolution rates of coding sequence (CDS) regions except the S gene (CDS except S; the left half of the violin plot) and the S gene (the right half) of SARS-CoV-2. The unit of rate is the number of nucleotide substitutions per site per year. B) Evolutionary rates of complete genomes of SARS-CoV-2 through months. The bar plots represent the number of newly confirmed coronavirus disease 2019 (COVID-19) cases in each month.

and the monthly new cases, deaths, and severe cases, respectively, from December 2020 to June 2021 in the USA (Fig. 1). Also, an analogously negative relationship was observed in the UK between January and June 2021, with the exception of statistically insignificant relationships between vaccine coverage and the monthly new cases and deaths respectively. The negative correlations were found either using the two-dose vaccine coverage or at least one-dose vaccine coverage (Figure S1).

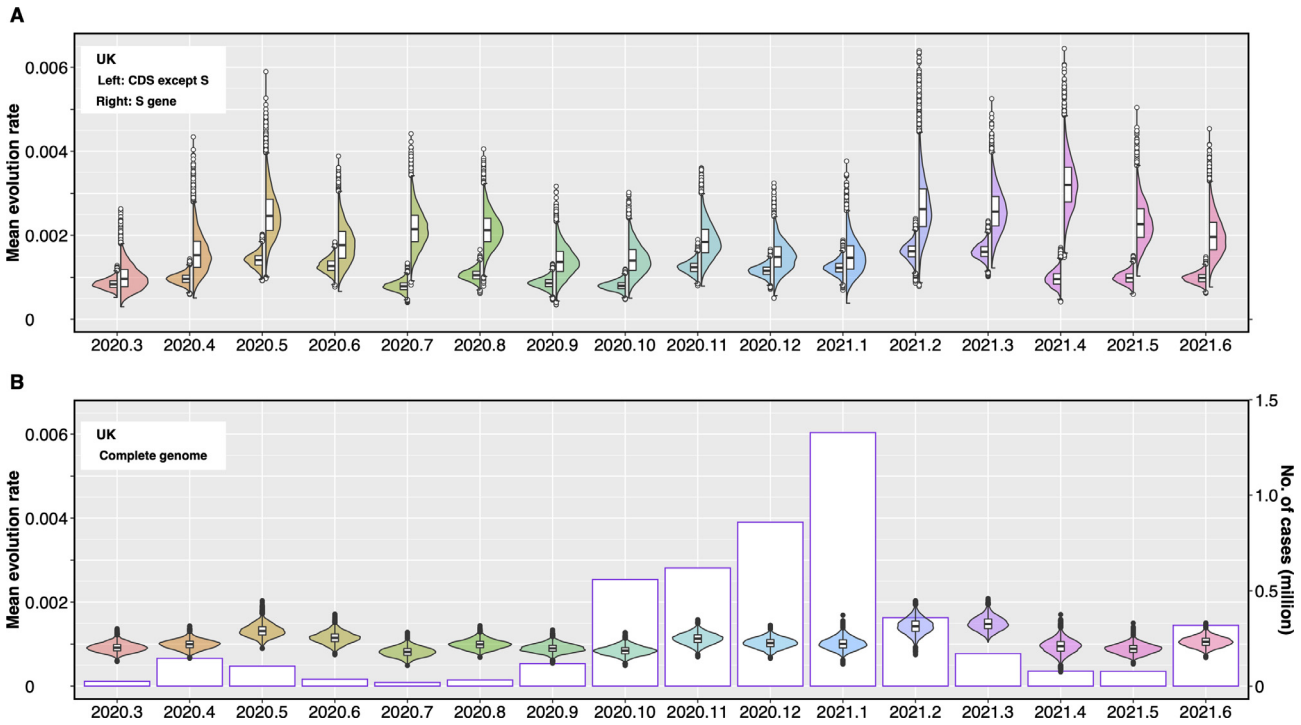

Fig. 3. Dynamics of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) evolutionary rates in the UK. A) The probability density of evolution rates of coding sequence (CDS) regions except the S gene (CDS except S; the left half of the violin plot) and the S gene (the right half) of SARS-CoV-2. The unit of rate is the number of nucleotide substitutions per site per year. B) Evolutionary rates of complete genomes of SARS-CoV-2 through months. The bar plots represent the number of newly confirmed coronavirus disease 2019 (COVID-19) cases in each month.

3.2. Dynamics of SARS-CoV-2 evolution and COVID-19 prevalence

To uncover the evolutionary dynamics of SARS-CoV-2 under the potential immune pressures induced by mass vaccination, we inferred the virus evolution rates through months in the USA and the UK given virus genomes under a Bayesian Markov Chain Monte Carlo (MCMC) framework (Fig. 2 and Fig. 3). Respective evolutionary rates of complete genomes and coding sequence regions except the S gene of SARS-CoV-2 showed no divergent trend between the period before and shortly after the vaccination deployments (Figs. 2A and 3A). However, the virus evolution rates of the S gene in months after the vaccination deployed were slightly higher than the rates in the periods prior to vaccination, especially in the UK (Fig. 3B). Rapid mutation rates of S gene were also recorded in October – December 2020 in the USA (Fig. 2B). In addition, the evolutionary rates of the S gene are relatively higher than those of the complete genomes and coding sequence regions except for S gene.

Regarding the monthly COVID-19 new cases in our study period from March 2020 to June 2021, the disease incidences in the USA were considerably higher than that of the UK (Figs. 2B and 3B). The peaks of COVID-19 cases occurred in November 2020 – January 2021 in the USA and October 2020 – January 2021 in the UK. In addition, a sharp resurgence of COVID-19 infections was reported in June 2021 in the UK but in July 2021 in the USA (Figure S2).

3.3. Genetic mutations and diversity of SARS-CoV-2

To understand mutations related to viral antigenicity, we calculated the mutation frequency and entropy on the B cell and T cell epitopes. Various genetic mutations were recorded on the neutralizing B cell epitopes, and linear The major histocompatibility complex (MHC) I and MHC II T cell epitopes of S protein of SARS-CoV-2 (Fig. 4A-C). These substitutions in epitopes varied in different virus variants. In addition, we used lineage entropy of SARS-CoV-2 to reveal its genetic diversity. We found lineage entropy of SARS-CoV-2 continuously decreased after the vaccination deployment in both USA (starting in December 2020) and UK (starting in January 2021) before June 2021 (Fig. 4D-E). The lineage entropy of SARS-CoV-2 circulating in June 2021 increased slightly but is still relatively low compared to that of May 2021 in the USA and the UK. Further, we uncovered a statistically significant negative relationship between vaccine coverage and lineage entropy in both USA and the UK.

4. Discussion

Utilizing the Pearson correlation coefficients, we identified a negative association between vaccine coverage proportion and epidemiological characteristics (including the monthly new cases, deaths, and severe cases) of COVID-19 from December 2020 to June 2021 in both USA and the UK. Our results indicated the macro-scale vaccine campaigns could decrease COVID-19 infections, deaths, and severe cases. However, the negative and insignificant relationship between vaccine coverage and monthly new infections in the UK probably resulted from a sharp resurgence of COVID-19 infections was reported in June 2021. The COVID-19 infections also rise again in the USA in July 2021 (Figure S2). The rising incidence of COVID-19 in countries despite sustained high vaccine coverage indicated that only using mass vaccination with current vaccines to contain the pandemic is not enough. Hence, non-pharmacological interventions and social distance should be performed simultaneously during rollout of the vaccination programme.

Examining the evolutionary dynamics of SARS-CoV-2 viruses under Bayesian Markov Chain Monte Carlo framework, we found a negligible divergent trend in evolutionary rates of complete genomes and coding sequence regions except the S gene of SARS-CoV-2 between the period before and shortly after deploying vaccination. However, virus evolution rates of the S gene became slightly higher after the vaccination deployment, especially in the UK. Rapid mutation rates of S gene were also recorded in October – December 2020, the peak of COVID-19 cases, in the USA. The surging infections could result in more chances for virus replication errors and mutations although coronaviruses have proofreading capability during replication. Additionally, the evolutionary rates of the S gene are relatively higher compared to that of the complete genomes and coding sequence regions except for S gene. Our results implied that the widely extended use of COVID-19 vaccines

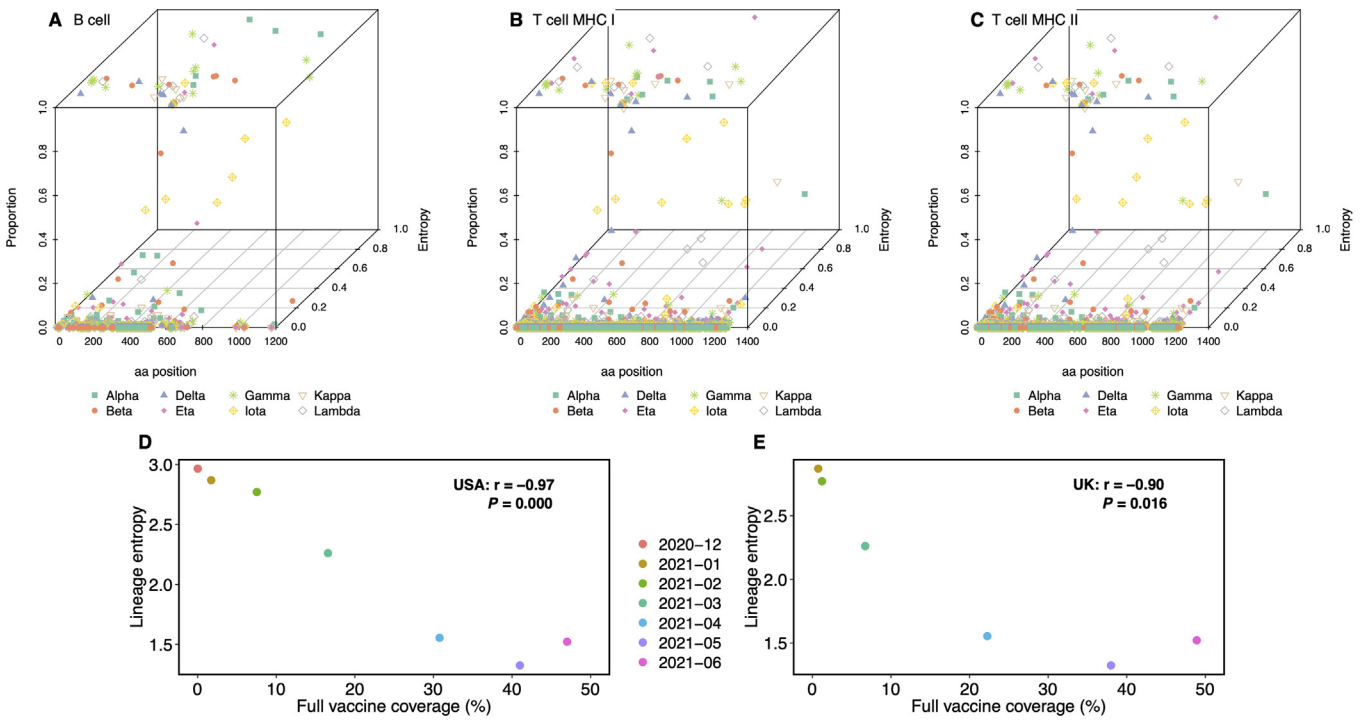

Fig. 4. Genetic mutations and lineage diversity of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) viruses. The frequency and entropy of amino acid substitutions on the A) B cell, B) T cell MHC-I, and C) T cell MHC-II epitopes for different variants of SARS-CoV-2 in the USA, respectively. Scatter plots show the negative correlation between vaccine coverage proportion and lineage entropy of SASR-CoV-2 genomes isolated in the D) USA and E) UK.

and immune pressures in vaccinated individuals could be one of the explanations for driving the genetic mutation and evolution of S gene of SARS-CoV-2. Moreover, S protein is the major protein used as a target in COVID-19 vaccines [18,19], and the interaction between the receptor binding region (RBD) of the S gene and host receptor cells could accelerate the genetic mutation of the S gene of SARS-CoV-2 virus under vaccine pressures.

The rapidly mutated S genes of SARS-CoV-2 could be accelerated by the genetic mutations on the neutralizing B cell epitopes, and linear MHC I and MHC II T cell epitopes of S protein in different virus variants. The mutations on the B cell and T cell epitopes could alter the antigenicity of the virus, affecting interactions between host immunity and virus, changing immune recognition, and then facilitating the virus escape from the immunity protection elicited by both natural infection and vaccination [20]. The neutralization assays with postvaccination sera and convalescent plasma from infected patients suggested several mutations can decrease the neutralizing activity of some variants, such as B.1.351 (Beta variant according to WHO label), relative to the wide-type virus [10,21]. Although rapid evolution of the S gene could be driven by the vaccination, the COVID-19 vaccines may dampen the genetic diversity of SARS-CoV-2 given the decreasing lineage entropies by months (Figs. 2-4 and Figure S3-S4) [13]. Under the immune pressure induced by mass vaccination, the advantaged lineages with higher fitness and adaption may escape the immune recognition and continue to persist along with current vaccines. In addition, the lineage entropy of SARS-CoV-2 circulating increased slightly in June 2021 in the USA and the UK. The slight growth in lineage entropy may result from the evolutionary dynamics of the advantage SARS-CoV-2 variant in June 2021 (Figure S3-S4), which should be further studied.

Some limitations in the present study should be noted. Typically, the COVID-19 vaccines were firstly administered the people ages 18 years and older. Limited by the total population vaccine coverage ratio data that we accessed, we could not differentiate the impact of vaccines on epidemiological characteristics and virus evolution in different age groups. In addition, we used the SARS-CoV-2 genomics from GISAID database to perform our analyses, but the SARS-CoV-2 genomics are far less than the COVID-19 confirmed cases reported by WHO and our results are restricted by available data size. Further, comparing evolutionary dynamics of SARS-CoV-2 under conditions with and without vaccine immune pressure in animal models may help evaluate the impact of the vaccine on virus evolution in an ideal situation without other confounding factors. Moreover, the effect of mass vaccination on the evolutionary trajectory of SARS-CoV-2 in a more extended period, wide geographical range, and refined age groups warrant further study.

In conclusion, the mass vaccination decreased COVID-19 infections and deaths, but most recent epidemics resurged in countries with a relatively high proportion of fully vaccinated individuals, challenging the effectiveness of merely using vaccines but no strict non-pharmacological measures to eliminate human infections. In response to the not complete protection of vaccines against breakthrough infections and re-infections, the timely administration of a third booster dose can increase vaccine effectiveness against the variants and prevent severe clinical outcomes [22,23]. In the long term, a novel vaccine that can curb both disease severity and infection should be developed and deployed to effectively and even thoroughly contain the worldwide spread of the virus and end the pandemic. Further, T cell involvement can contribute to B cell maturation and induce strong and durable antibody responses [24]. T cell response and mucosal immunity may help virus elimination and prevent vaccinated people from a vaccine breakthrough infection. Given this, all correlates of immune protections, particularly T cell responses and mucosal immunity, post vaccination should be evaluated and optimized during vaccine design, in addition to the serum-neutralizing antibodies that are typically considered as a key indicator to assess the vaccine efficacy [25]. In addition, social distancing and wearing masks are still important prevention strategies, even for vaccinated individuals, since the continuing emergence of breakthrough infections and re-infections after recovery and the lagged immune response in the immediately vaccinated persons [24,26]. Hence, only a systematic combination of effective vaccines and non-pharmacological interventions can increase the chance of thoroughly overcoming the pandemic.

Furthermore, the rapid mutation rates of the S gene were identified even after COVID-19 vaccine rollout, although the vaccine-induced immunity should be high enough to slow virus evolution and adaption by reducing the prevalence, incidence, and transmission of COVID-19 [26]. Mutations occurred on the B cell and T cell epitopes of the S gene, which are partially related to some variants exhibiting resistance to antibody-mediated immunity elicited by natural infection or vacci-

nation [20]. Hence, in addition to development for high effective vaccines, real-time surveillance for the emerging variants should be strengthened, especially the mutations related to altering phenotypic characteristics in terms of antigenicity, infectivity, transmissibility, and pathogenicity. Surveillance on functional mutations especially in the immediate period after the mass rollout of vaccination can help us to understand virus evolution characteristics, identify variants with novel phenotypes, and renew vaccine candidates. In addition, expanding vaccination and optimal vaccination strategies should be implemented according to the epidemiological and evolutionary considerations of SARS-CoV-2 [27]. Further, the SARS-CoV-2 could be a long-term persistent pathogen in humans as the influenza virus after the end of the pandemic. If seasonal fluctuations emerged in temporal dynamics of future COVID-19 infections analogous to that of seasonal influenza, vaccines for "seasonal COVID-19" should be evaluated and updated annually to prevent infections from the novel antigenic variants, and the potential next pandemic triggered by a novel variant.

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

Author contributions

Jing Yang: Data Curation, Formal Analysis, Visualization, Writing – Original Draft, Writing – Review & Editing, Funding Acquisition. **Min Han:** Data Curation, Writing – Review & Editing. **Liang Wang:** Writing – Review & Editing. **Likui Wang:** Writing – Review & Editing. **Tianrui Xu:** Data Curation, Writing – Review & Editing. **Linhuan Wu:** Writing – Review & Editing. **Juncai Ma:** Writing – Review & Editing. **Gary Wong:** Writing – Review & Editing. **Wenjun Liu:** Writing – Review & Editing. **George F. Gao:** Writing – Review & Editing. **Yuhai Bi:** Conceptualization, Funding Acquisition, Writing – Review & Editing.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bsheal.2022.07.001.

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