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BRIEF REPORT

Variants of Concern Are Overrepresented Among Postvaccination Breakthrough Infections of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in Washington State

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Across 20 vaccine breakthrough cases detected at our institution, all 20 (100%) infections were due to variants of concern (VOCs) and had a median Ct of 20.2 (IQR, 17.1–23.3). When compared with 5174 contemporaneous samples sequenced in our laboratory, VOCs were significantly enriched among breakthrough infections ($P < .05$).

Keywords. SARS-CoV-2; vaccine breakthrough; variants of concern; sequencing.

Vaccination against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) elicits an immune response capable of potently neutralizing the virus. Multiple SARS-CoV-2 vaccines have been approved for use in humans through emergency use authorization or are in phase 3 clinical trials. Most of these vaccines use a recombinant spike protein derived from the first sequenced (Wuhan) strain from January 2020. The most widely used vaccines (mRNA-based mRNA-1273 and BNT162b2) have shown up to 95% efficacy at preventing clinical cases and up to 100% efficacy in preventing severe disease [1]. However, there is growing concern that emerging variants may escape vaccine-induced immunity and result in breakthrough infections in fully vaccinated individuals [2].

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Since emerging in humans, SARS-CoV-2 is now defined by a variety of lineages harboring distinctive genetic changes in the spike protein. Variants of concern (VOCs) are those strains that show evidence of increased transmissibility, more severe disease, reduced neutralization by antibodies elicited by past infection or vaccination, reduced efficacy of treatments, or failures in diagnostic detection. As of 20 May 2021, there are currently 4 VOCs identified by the US SARS-CoV-2 Interagency Group (P.1, B.1.351, B.1.427, and B.1.429) that show reduced neutralization by convalescent and postvaccination sera [3].

Although in vitro studies have shown that some VOCs are less effectively neutralized by sera from vaccinated individuals [4], the clinical implications for postvaccination breakthrough infection remain largely unknown. In this study, we examined SARS-CoV-2 genomes isolated from individuals identified as vaccine breakthrough cases and compared them with the background of SARS-CoV-2 sequences from Washington over the same time interval.

METHODS

This work was approved by the University of Washington (UW) Institutional Review Board. The UW Virology Lab (UWVL) routinely performs SARS-CoV-2 whole-genome sequencing of specimens received for clinical testing. In this study, cases were defined as patients who were fully vaccinated against SARS-CoV-2 (>2 weeks post-second dose of Pfizer or Moderna vaccine) who subsequently tested positive by reverse transcription–polymerase chain reaction (RT-PCR). The control group included samples collected in Washington from the same time period as the case samples that were sequenced at UWVL. SARS-CoV-2 was detected by RT-PCR as previously described using the emergency use–authorized UW Centers for Disease Control and Prevention (CDC)–based laboratory-developed test, Hologic Panther Fusion, or Roche Cobas SARS-CoV-2 tests [5]. Sequencing was attempted on all specimens with cycle threshold (Ct) less than 36 using a multiplexed amplicon sequencing panel from Swift Biosciences [6] or Illumina COVIDSeq (Illumina, Inc). Consensus sequences were assembled using a custom bioinformatics pipeline described previously (https://github.com/greninger-lab/covid_swift_pipeline [6]). We excluded SARS-CoV-2 genome sequences that were incomplete (length <29 kbp) or of low quality (>10% ambiguous bases (Ns)). SARS-CoV-2 lineages and clades were assigned using the PANGOLIN (Phylogenetic Assignment of Named Global Outbreak LINeages; https://pangolin.cog-uk.io/) and NextClade (https://clades.nextstrain.org/) tools. Amino acid changes were annotated according to NextClade. Analysis

and calculations were performed in R (R Foundation for Statistical Computing). Significant differences in case distributions according to VOCs were determined using Fisher's exact test.

RESULTS

Beginning in February 2021, SARS-CoV-2 genome sequencing was requested at UW Medicine hospitals and partners as part of investigations into postvaccine breakthrough infections. A total of 20 cases were sequenced in this study, including 13 females, 6 males, and 1 with unknown sex. Ages ranged from 26 to 65 years (median, 43 years; interquartile range [IQR], 28–58 years) (Supplementary Table 1), and Ct values ranged from 16.00 to 35.86 (median, 20.2; IQR, 17.1–23.3). These specimens were collected between 23 February and 27 April 2021. The control group consisted of genomes sequenced by UWVL (n = 5174) during the same period as the vaccine breakthrough cohort (Supplementary Table 1).

All 20 of 20 vaccine breakthrough cases were classified as VOCs: 8 (40%) B.1.1.7, 1 (5%) B.1.351, 2 (10%) B.1.427, 8 (40%) B.1.429, and 1 (5%) P.1 (Figure 1A, Supplementary Table 2). In contrast, during the same time interval, 68% of Washington cases sequenced at UWVL represented VOCs, with 31% B.1.1.7, 1% B.1.351, 3% B.1.427, 27% B.1.429, and 7% P.1 (Figure 1B). Overall, VOCs were proportionally overrepresented in breakthrough cases, with the frequency of all VOCs in breakthrough cases increased by 1.47-fold compared with the control group (95% confidence interval [CI], 1.45–1.50; $P = .001$). Variants B.1.427, B.1.429, and B.1.1.7 were 3.38-fold (95% CI, .90–12.71; $P = .119$), 1.51-fold (95% CI, .88–2.59; $P = .203$), and 1.29-fold (95% CI, .75–2.20; $P = .468$) more common in breakthrough cases compared with controls (Supplementary Figure 1, Supplementary Table 2). The overall distribution of variants was significantly different between breakthrough and control groups ($P = .001$, Fisher's exact test) (Supplementary Table 2). Variants that have been reported to be less effectively neutralized by antibodies from individuals postvaccination (P.1, B.1.351, B.1.427, and B.1.429) were identified in 60% of breakthrough cases and 36.7% of control cases, a 1.63-fold change (95% CI, 1.14–2.34; $P = .037$).

We compared the occurrence of individual mutations in the spike protein in the breakthrough versus control groups. A single mutation, W258L, was 15.22-fold enriched in breakthrough cases (95% CI, 3.91–59.10; $P = .008$) (Figure 1D) but was present in only 2 cases. No other mutations showed significant enrichment in the breakthrough group.

Clinical data, including comorbidities and vaccination type and dates (n = 19), were available for a subset of subjects (Supplementary Table 3). All patients received mRNA-based vaccines (14 BNT162b2, 5 mRNA-1273); 15 out of 18 reported symptoms, and none required hospitalization. Specimens were collected at an average of 67.7 days after vaccination (range, 39–112 days; SD, 18.1 days).

DISCUSSION

In this study, SARS-CoV-2 VOCs were found to be overrepresented in vaccine breakthrough cases when compared with cases circulating in the general population of Washington State over the same time interval. Although no single VOC was significantly enriched in the breakthrough cases, subgroup analysis revealed that variants that have shown reduced antibody neutralization in vitro (B.1.351, B.1.427, B.1.429, and P.1) were overrepresented compared with the B.1.1.7 VOC lineage, which is not associated with reduced neutralization. The 20 vaccine breakthrough cases described here also had a substantially stronger viral load than 22 breakthrough cases among Chicago nursing facility staff and residents recently reported by the CDC [7].

The B.1.427/B.1.429 variants were first identified in Los Angeles County, California, in July of 2020 [8] and quickly became the dominant form of the virus circulating in that state. These variants were first seen in Washington State in December 2020 and comprised 30% of cases during the study period. The B.1.427/B.1.429 variants are characterized by key spike protein changes, with L452R in the receptor binding domain and S13C and W152C in the N-terminal domain (NTD). Recent studies suggest that neutralizing antibody titers from plasma collected from vaccinated or convalescent individuals were reduced 2–7-fold against B.1.427/B.1.429 variants relative to wild-type [8, 9]. Similarly, the P.1 variant identified in Brazil and the B.1.351 variant identified in South Africa also show reduced antibody neutralization efficiency: 4–5- and 5–40-fold, respectively [10, 11]. Both P.1 and B.1.351 were uncommon in the control group, with P.1 comprising 6% of cases and B.1.351 less than 1% of cases. In contrast, the B.1.1.7 variant, first identified in the United Kingdom and known to increase infectivity by approximately 50%, is neutralized as efficiently as wild-type or only minimally decreased in most studies [11–13]. B.1.1.7 was the major variant circulating in our area during the study period. While it is noted that the introduction of E484K into the B.1.1.7 background has been shown to introduce reduced neutralization efficiency [14], this mutation was not present in any of our breakthrough B.1.1.7 cases, so we considered this strain as having similar neutralization efficiency as wild-type. These data therefore suggest that in vitro neutralization assays may predict variants more likely to escape antibodies from vaccinated individuals.

Most mutations thought to lead to antibody neutralization resistance occur in the receptor binding domain of the spike protein [15]. Of note, 2 of 2 B.1.427 cases identified in the breakthrough group contained a spike mutation, W258L, outside of the receptor binding domain, compared with 50 of 208 (24.03%) in the control group. W258L resides in the so-called NTD-antigen supersite.

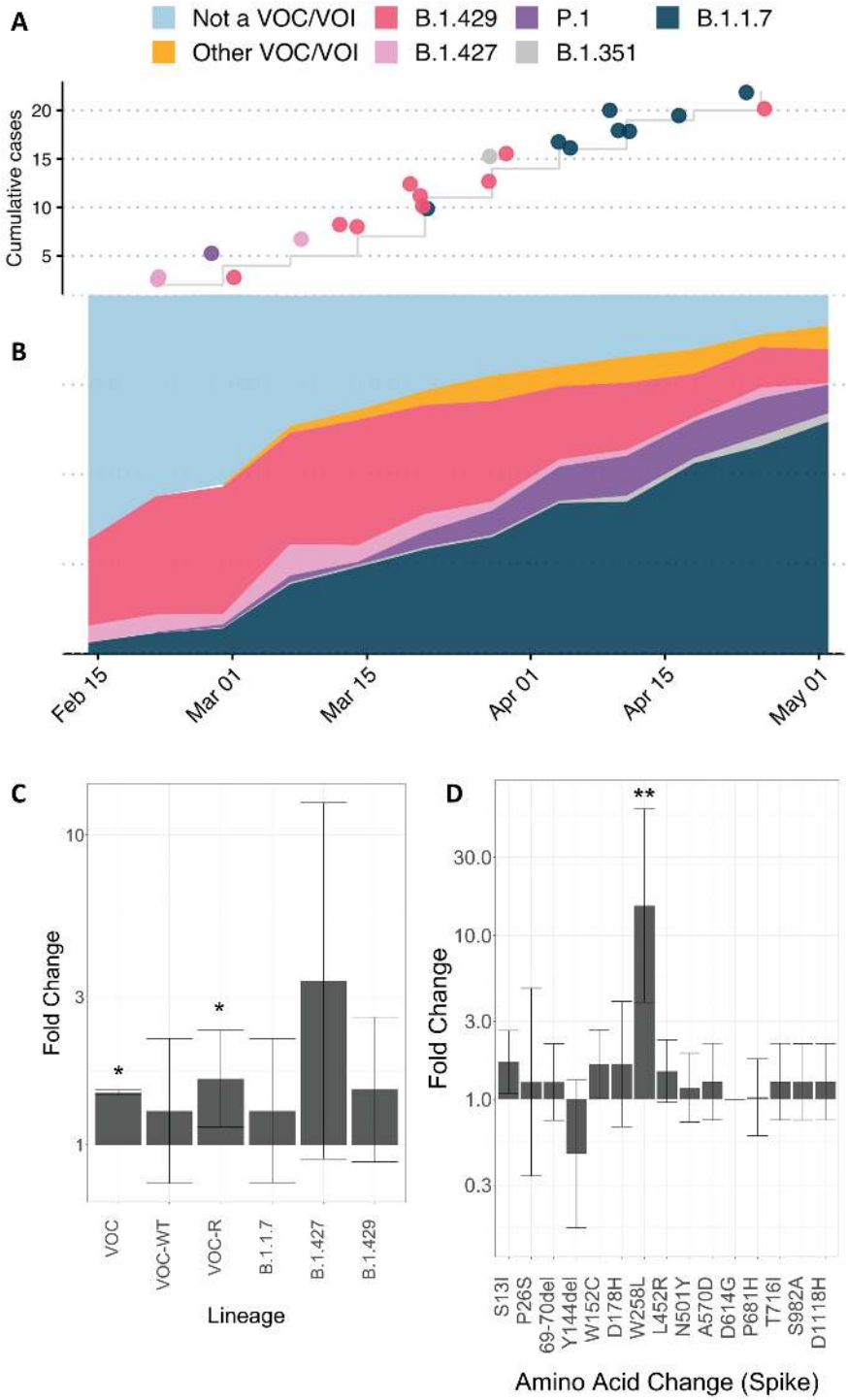

Figure 1. Variants of concern are overrepresented in vaccine breakthrough SARS-CoV-2 genomes. *A*, Cumulative number of breakthrough cases and their Pangolin lineages. *B*, Proportions of lineages for UWVL sequences collected during the same time period. Relative risk of vaccine escape for each VOC (*C*) and spike amino acid change (*D*). Error bars represent 95% confidence intervals. **P* < .05, ***P* < .01 (Fisher's exact test). Abbreviations: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; UWVL, University of Washington Virology Lab; VOC, variant of concern (total); VOC-R, variant of concern with reduced neutralization; VOC-WT, variant of concern without reduced neutralization; VOI, variant of interest.

While antibodies that bind the receptor binding domain may target different epitopes, a single site of vulnerability to antibody neutralization exists in the NTD [16]. The impact of NTD alterations in antibody neutralization efficiency is largely unstudied. Our data suggest that in vitro studies of antibody neutralization residence should include NTD alterations such as W258L.

This study is limited by the small number of subjects in the vaccine breakthrough group, and we cannot exclude the presence of additional breakthrough cases in the control groups due to a lack of clinical data. Moreover, our breakthrough cohort was mostly composed of healthcare workers who were identified by infection control or employee health. In contrast, sequences from UWVL were sampled from across the state and include samples from various testing sites, including hospitals, outpatient clinics, and community testing sites. In addition, testing among healthcare workers was symptom-based or after known/suspected exposure, rather than random sampling. Therefore, asymptomatic breakthrough cases may be underestimated.

All 20 breakthrough cases in this study were assigned a lineage without conflicts. This contrasts with a recent study that demonstrated a novel variant in a postvaccination individual that fell between clades 20B and 20C, raising concern that viral evolution may drive neutralization antibody resistance [2]. Overall, our results are consistent with a recent report from the CDC [17] showing that, while breakthroughs are rare, a majority involve VOCs and can result in symptomatic infection. Continued surveillance of postvaccine breakthrough cases may help target VOCs for inclusion in new or booster SARS-CoV-2 vaccines.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Potential conflicts of interest. A. L. G. reports contract testing from Abbott Laboratories and research support from Gilead and Merck, outside of the described work, and reports a Fast Grants 2244, paid to their institution, during the conduct of the study. A. E. M. reports support from the Precision Medicine Clinical Labs granting program ($10,000, payment made to the institution) from Brotman Baty Institute, outside the submitted work. K. R. J. reports payment to his institution for virologic testing in support of vaccine trials from the HIV Vaccine Trials Network, outside the submitted work. J. L. reports support for infection prevention and control implementation in Kenya from the CDC; fees for serving as a consultant for COVID-19 prevention from Port of Seattle, Vertical World, and the National Football League (NFL); and serves on the IDSA Board of Directors, all outside the submitted work. C. L. reports receiving a National Institutes of Health (NIH) subcontract award (1R01AI151038–01); reports honoraria paid to them by Washington State Medical Oncology Society for lectures; and reports honoraria for Antibacterial Resistance Leadership Group (ARLG) Committee participation paid to them from ARLG, all outside the submitted work. S. A. P. reports receiving grant support for a clinical trial from Global Life Technologies; reports receiving payment for a Continuing Medical Education lecture from Pall Medical; participated in the Advisory Committee on Immunization Practices Zoster Working Group (unpaid); reports serving as an International Council Member for International Immunocompromised Host Society (unpaid); reports serving on the Conference Committee for IDSA (unpaid but provided with travel support); reports serving on the Vaccine and Related Biologic Products Advisory Committee for the Food and Drug Administration (compensated for time); participated in a clinical trial for Chimerix; participated in a clinical trial through another institution for Merck & Co.; and was provided influenza vaccines for an NIH-supported clinical trial for which they are a co-investigator by Sanofi Aventis, outside the submitted work. E. B. reports receiving grants to their institution from the National Institute of Allergy and Infectious Diseases (NIAID), during the conduct of the study; reports receiving grants to their institution from NIAID and the Bill and Melinda Gates Foundation; reports consulting fees paid to them by the University of North Carolina; and participation in Data Safety and Monitoring Boards/Advisory Boards for Merck (paid to them) and NIAID (unpaid), all outside the submitted work. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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