Association of E484K spike protein mutation with SARS-CoV-2 infection in vaccinated persons--- Maryland, January – May 2021

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Abstract

Among 9,048 people infected with SARS-CoV-2 between January-May, 2021 in Maryland, in regression-adjusted analysis, SARS-CoV-2 viruses carrying the spike protein mutation E484K were disproportionately prevalent among persons infected after full vaccination against COVID-19 as compared to infected persons who were not fully vaccinated (aOR 1.96, 95% CI, 1.36 to 2.83).

Keywords: COVID-19; COVID-19 vaccine effectiveness; SARS-CoV-2; variants of concern; sequencing; genomic surveillance; E484K; L452R; B.1.526, B.1.351, R.1, B.1.526.1, B.1.429, B.1

The Centers for Disease Control and Prevention (CDC U.S. Government SARS-CoV-2 Interagency Group) has highlighted two SARS-CoV-2 spike protein mutations as concerning for possible impact on protection from acquired immunity: substitution of lysine for glutamic acid at the 484 position (E484K); and substitution of arginine for leucine at the 452 position (L452R) [1]. Both mutations occur in the spike protein's Receptor Binding Domain (RBD), a key antigenic target. Both evolved independently in multiple virus lineages, including variants of concern B.1.351 "Beta" and P.1 "Gamma" for E484K and variant of concern B.1.617.2 "Delta" for L452R [2–5]. Both are associated with reduced neutralization by monoclonal antibodies, convalescent plasma, sera from vaccinated persons in laboratory studies, and infections in vaccinated persons in certain lineages, with stronger associations for E484K than L4524R [2–11].

This retrospective study – conducted while the prevalence of lineage B.1.617.2 "Delta" in Maryland was less than 1% – examines whether the E484K and L452R substitutions were associated with infection in vaccinated persons using genetic sequences from SARS-CoV-2 specimens collected in the U.S. State of Maryland.

Methods

This retrospective analysis uses all specimens on GISAID's public online repository of SARS-CoV-2 sequences collected between the start of January 2021 and the second week of May of 2021 that were collected from Maryland residents and could be successfully linked to a case in Maryland Department of Health's COVID-19 surveillance systems (n=9,048; 89% of 10,116 Maryland resident specimens published on GISAID).

Infection in a person who was fully vaccinated was defined as having a SARS-CoV-2 positive (by nucleic acid amplification [NAAT] or antigen test) respiratory specimen collected 14 or more days after receiving the final scheduled dose of a COVID-19 vaccine series (2 doses for BNT162b2 mRNA [Pfizer/BioNTech] and mRNA-1273 [Moderna]; 1 dose for Ad26.COV2.S [Janssen]), regardless of

symptoms. Infection in a person with any vaccination was defined as having a positive specimen collected 14 or more days after receiving a first dose of any vaccine, regardless of symptoms. Hospitalization of a person with full or any vaccination was defined the same, with the added condition that the patient was admitted to the hospital within 28 days following collection of their first positive specimen.

Using logistic regression, we estimated the respective crude and adjusted associations of of E484K and L452R being present in a sequence with the corresponding SARS-CoV-2 specimen being collected from a vaccinated person (as defined above). Logistic regression was used to adjust for confounding by the following characteristics: age group, gender, region of residence, laboratory submitting to GISAID, and week of specimen collection.

The analysis was repeated for each of three vaccine types separately. For vaccine-specific analysis, persons vaccinated with a vaccine other than the type examined in that analysis were excluded (e.g., persons vaccinated with mRNA-1273 or Ad26.COV2.S vaccines were excluded from analysis of the BNT162b2 vaccine).

Regression coefficients were exponentiated, and interpreted as odds ratios. Wald confidence intervals (95%) were estimated for all regression coefficients; odds ratios with confidence intervals not containing 1 are statistically significant at the $p<0.05$ level.

Three sensitivity analyses are described in online supplemental Appendix A.

Results

Of 9,048 SARS-CoV-2 specimens on GISAID collected from Maryland residents during the study period and included in this analysis, 1,187 (13.1%) carried the E484K substitution and 731 (8.1%) carried the L452R substitution. Trends in common lineages containing these mutations by week of collection are shown in Supplemental Figure 1. There were 265 (2.9%) instances of infection in persons who were fully vaccinated and 554 (6.1%) instances of infection in persons with any vaccination (including the aforementioned 265 post-full infections). Trends in infection following vaccination are shown in Supplemental Figure 2. A complete description of the sample is shown in Supplemental Table 1.

Viruses carrying the E484K mutation were significantly more likely to have been collected from fully vaccinated persons (OR 1.48, 95% CI 1.08 to 2.04; aOR 1.96, 95% CI, 1.36 to 2.83) and from persons with any vaccination (OR 1.44, 95% CI, 1.15 to 1.81; aOR 1.68, 95% CI, 1.30 to 2.18) (Table 1).

Viruses carrying the L452R mutation were not significantly more likely to be collected from fully vaccinated persons (OR 1.30, 95% CI, 0.87 to 1.95; aOR 1.07, 95% CI, 0.69 to 1.68); or from persons with any vaccination (OR 1.30, 95% CI, 0.98 to 1.74; aOR 1.12, 95% CI, 0.81 to 1.53) (Table 1).

Viruses carrying the E484K mutation were significantly more likely to have been collected from hospitalized fully vaccinated persons (OR 2.30, 95% CI 1.08 to 4.93; aOR 2.56, 1.09 to 6.01) and hospitalized persons with any vaccination (OR 2.09, 95% CI, 1.33 to 3.29; aOR 2.15, 95% CI 1.28 to 3.60) (Table 1).

Viruses carrying the L452R mutation were not significantly more likely to be collected from hospitalized fully vaccinated persons (OR 0.33, 95% CI, 0.05 to 2.44; aOR 0.27 95% CI, 0.04 to 2.06);

or from persons with any vaccination (OR 1.07, 95% CI, 0.54 to 2.12; aOR 0.85, 95% CI, 0.41 to 1.78) (Table 1).

In adjusted analysis, for both E484K and L452R, associations with infection in fully vaccinated persons were comparable for all vaccine types (Table 1).

Discussion

In Maryland, in the first half of 2021, infections in vaccinated persons who received some or all scheduled doses of a COVID-19 vaccine were uncommon. When they did occur, infections in vaccinated persons were disproportionately likely to be viruses that carried E484K. This is consistent with past research that hypothesized E484K reduces the effectiveness of acquired immunity more than other mutations, possibly in part because that mutation is located in core RBD epitope of the spike protein; by contrast L452R is in a loop epitope [2, 10]. Importantly, while hospitalizations following SARS-CoV-2 infection in vaccinated persons were rare in this study, we find that the association of E484K with infection in vaccinated persons persisted even if we restricted the outcome only to persons who were hospitalized. Additional studies focused on severe illness are needed.

We did not find an association of L452R with infections in vaccinated persons. This is in contrast to *in vitro* studies showing L452R is associated with reduced neutralization from polyclonal antibodies, albeit less strongly than E484K [10], and to epidemiologic evidence of reduced vaccine efficacy for lineage B.1.617.2 “Delta”, which carries L452R [11]. B.1.617.2 was less than 1 percent of all sequenced specimens in Maryland during this study period. Past studies examined lineages, rather than specific mutations. If L452R was one of several mutations in those lineages contributing to reduced neutralization, its individual contribution might be too small to detect in this study.

During the period of this study, the rate of confirmed COVID-19 cases in Maryland fell 10-fold [12]. At the same time, the fully vaccinated increased from fewer than 1,000 to approximately

2.8 million in a population of approximately 6 million (22). Nothing here should be interpreted to suggest that mass vaccination campaigns will not be effective for reducing the incidence and mortality of COVID-19.

This analysis has a number of limitations. First, this study, which included only vaccinated persons, does not directly estimate vaccine effectiveness; that would require a comparison of the rate of infection between vaccinated and unvaccinated people. Second, the population of people infected with SARS-CoV-2 in this study may not be representative of the population of people infected with SARS-CoV-2 in Maryland. Only some laboratories either conduct sequencing; populations tested by other laboratories would not be. Further, infections in vaccinated persons were preferentially selected for sequencing by Maryland's public health lab. Third, this study examined two specific SARS-CoV-2 spike protein mutations. SARS-CoV-2 mutations are not randomly assorted; if there is some other spike protein mutation that commonly co-occurs with E484K or L452R in Maryland, that mutation could be responsible for the associations observed here. Fourth, data on patients' underlying health conditions were not available. Fifth, in analyses of hospitalization, the cause of hospitalization was not available. Sixth, data on symptoms were not available. Seventh, no clinical or immunological data were available. Finally, 1,068 Maryland SARS-CoV-2 sequences on GISAID could not be linked to Maryland's other surveillance systems, and therefore could not be included in the analysis. Unlinked sequences may come from viruses collected from non-residents of Maryland tested in Maryland, or may have metadata errors that caused the link algorithm to fail.

This study underscores the importance of integrating SARS-CoV-2 genomic surveillance with other surveillance data to identify mutations associated with infections in vaccinated persons: to provide public health agencies the ability to quickly enhance non-pharmaceutical interventions in response to transmission of suspected vaccine-evasive SARS-CoV-2 variants, and to provide vaccine developers time to update vaccines if necessary.

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Human Subjects Research Determination

- Case investigation, data collection, and analysis were conducted for public health purposes. This project was reviewed by the Division of Scientific Education and Professional Development within the Center for Surveillance, Epidemiology, and Laboratory Services at the Centers for Disease Control and Prevention (CDC). The project was determined to meet the requirements of public health surveillance covered by the U.S. Department of Health and Human Services Policy for the Protection of Human Research Subjects as defined in 45 C.F.R. part 46.102(l)(2), 21 C.F.R. part 56; 42 U.S.C. Sec. 241(d); 5 U.S.C. Sec. 552a; 44 U.S.C. Sec. 3501 et seq. In., and the decision was made that this project was nonresearch and did not require

ethical review by the CDC Human Research Protection Office. Ethical approval was waived and informed consent was not required.

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Tables

Table 1. Crude and adjusted odds ratios for association of E484K and L452R mutations with post-vaccination SARS-CoV-2 infection, by hospitalization status and vaccine type

Outcome	E484K		L452R	
Any infection in vaccinated person	Crude Odds Ratio (95% Confidence Interval), p-value	Adjusted Odds Ratio (95% Confidence Interval), p-value	Crude Odds Ratio (95% Confidence Interval), p-value	Adjusted Odds Ratio (95% Confidence Interval), p-value
Any vaccination	**1.44 (1.15 to 1.81), 0.002**	**1.68 (1.3 to 2.18), 0.000**	1.3 (0.98 to 1.74), 0.071	1.12 (0.81 to 1.53), 0.496
Full vaccination	**1.48 (1.08 to 2.04), 0.015**	**1.96 (1.36 to 2.83), 0.000**	1.3 (0.87 to 1.95), 0.202	1.07 (0.69 to 1.68), 0.754
Infection resulting in hospitalization				
Any vaccination	**2.09 (1.33 to 3.29), 0.001**	**2.15 (1.28 to 3.60), 0.004**	1.07 (0.54 to 2.12), 0.852	0.85 (0.41 to 1.78), 0.673
Full vaccination	**2.3 (1.08 to 4.93), 0.032**	**2.56 (1.09 to 6.01), 0.03**	0.33 (0.05 to 2.44), 0.28	0.27 (0.04 to 2.06), 0.207
In persons vaccinated with BNT162b2				
Any vaccination	1.23 (0.92 to 1.64), 0.169	**1.47 (1.07 to 2.03), 0.019**	1.34 (0.95 to 1.9), 0.094	1.13 (0.78 to 1.64), 0.51
Full vaccination	1.38 (0.93 to 2.06), 0.109	**1.88 (1.19 to 2.96), 0.006**	1.3 (0.79 to 2.13), 0.296	1.09 (0.63 to 1.86), 0.761

In persons vaccinated with mRNA-1273				
Any vaccination	**1.95 (1.34 to 2.82), 0.000**	**2.24 (1.49 to 3.37), 0.000**	1.32 (0.8 to 2.20), 0.282	1.22 (0.71 to 2.09), 0.479
Full vaccination	1.78 (0.98 to 3.21), 0.057	**2.54 (1.30 to 4.94), 0.006**	1.57 (0.75 to 3.29), 0.235	1.36 (0.61 to 3.03), 0.457
In persons vaccinated with Ad26.COV2.S				
Full vaccination	1.6 (0.54 to 4.76), 0.399	2.87 (0.89 to 9.26), 0.078	0.58 (0.08 to 4.32), 0.594	0.61 (0.08 to 4.63), 0.63

Associations statistically significant at the p<0.05 level are bolded.

Adjusted analyses adjusted for age, sex, region of residence, sequencing laboratory, and week of collection.

Note: For vaccine-specific analysis, persons vaccinated with a vaccine other than the type examined in that analysis were.