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SHORT COMMUNICATION

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Serosurvey in SARS-CoV-2 inactivated vaccine-elicited neutralizing antibodies against authentic SARS-CoV-2 and its viral variants

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Abstract

Various variants of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have been emerging and circulating in different parts of the world. Millions of vaccine doses have been administered globally, which reduces the morbidity and mortality of coronavirus disease-2019 efficiently. Here, we assess the immune responses of individuals after two shots of BBIBP-CorV or CoronaVac inactivated vaccine. We measured neutralizing antibody responses after the second vaccination by using authentic SARS-CoV-2 and its viral variants. All the serum samples efficiently neutralized SARS-CoV-2 wild-type lineage, in contrast, a part of serum samples failed to neutralize Alpha, Beta, Gamma, Delta, or Eta lineages, and only several serum samples were able to neutralize Omicron lineage virus strains (BA.1 and BA.2) with low neutralization titer. As compared with the neutralization of SARS-CoV-2 wild-type lineage, the neutralization of all other SARS-CoV-2 variant lineages was significantly lower. Considering that all the SARS-CoV-2 mutation viruses challenged the antibody neutralization induced by BBIBP-CorV and CoronaVac, it is necessary to carry out a third booster vaccination to increase the humoral immune response against the SARS-CoV-2 mutation viruses.

KEYWORDS

immune response, inactivated vaccine, neutralizing antibodies, SARS-CoV-2, viral variants

1 | INTRODUCTION

The epidemic of severe acute respiratory syndrome, coronavirus 2 (SARS-CoV-2) has rapidly spread worldwide. As of July 5, 2022, there have been more than 547 million confirmed cases of coronavirus disease-2019 (COVID-19), including more than 6.3 million deaths, reported to the World Health Organization (WHO).[1] The development of vaccines is urgently needed for the prevention and control of COVID-19, different candidates have been developed since 2020.[2] As of April 30, 2022, more than 12 532 million vaccine doses have been administered globally, which reduces the morbidity and mortality of COVID-19 efficiently. Recently, several new SARS-CoV-2 variants emerged worldwide, including Alpha, Beta, Gamma, Delta, and Omicron, raising concerns that they may evade neutralizing antibodies and pose a threat to the efficacy of current COVID-19 vaccines.[3] BBIBP-CorV and CoronaVac are two COVID-19

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inactivated vaccines developed in China, which have been approved by the WHO and are widely used in several countries.[4] It is an important scientific issue to determine whether the inactivated vaccines are still effective against the respective SARS-CoV-2 variants. An earlier study revealed that the CoronaVac vaccine displayed comparable neutralization reductions against authentic beta variants,[5] but the study did not look at other virus variants besides the beta viruses and the sample size was small. Another article showed that all sera samples were below the lower limit of quantitation against Omicron BA.1 after the two doses of inactivated COVID-19 vaccine vaccination, the study only tested Omicron BA.1, not BA.2, in a sample of 24.[6] To address this question, we collected and tested a panel of human sera from 91 volunteers obtained between 9 and 21 days after the administration of the second dose of BBIBP-CorV or CoronaVac, which was carried out 4 weeks after the first vaccination (Supporting Information: Table 1). All the volunteers in this study were confirmed with no history of natural SARS-CoV-2 infection by negative results from the detection of antibodies against the SARS-CoV-2 Nucleocapsid protein. Authentic neutralization was performed by cytopathic effect-based assay according to a previous study,[7] using the SARS-CoV-2 viruses isolated from our laboratory, which has confirmed their identity by next-generation whole-genome sequencing.

2 | METHODS

2.1 | Data reporting

Statistical analysis was performed using the paired *t* test and the *p* values were shown.

2.2 | Study participants

Eligible participants in this study included adults at least 18 years of age with no known heart, lung, kidney disease, or bleeding disorders, and no history of HIV-1 or malaria infection. All participants were asymptomatic at the time of the study visit and had received a complete two-dose regimen of BBIBP-CorV or CoronaVac inactivated vaccine. Based on previous studies, we divided the individuals into the younger group (aged 20–49) and the older group (aged 50–59).[8] Informed consent was obtained from all participants. Detailed participant characteristics are shown in Supporting Information: Table 1.

2.3 | Serum neutralization assay

Vero cells (10^4) were seeded 24 h before the infection in a 96-well plate (Costar). On the day of infection, the cells were washed twice with a cell culture medium. The sera from mice were incubated at 56°C for 30 min and the sera were diluted to 10-fold in cell culture medium (Dulbecco's modified Eagle medium) as initial concentration and then diluted twofold. Aliquots (40 µl) of diluted sera (from 20- to 5120-fold) were added to 50 µl of cell culture medium containing 100 tissue culture infective dose of wild type or each variant SARS-CoV-2 virus strain (isolated from Guangdong Provincial Center for Disease Control and Prevention) on a 96-well plate and incubated at 37°C for 2 h in CO_2 5% v/v. The virus serum mix was then added to cells in 96-well plates and plates were incubated at 37°C with microscopic examination for cytopathic effect (CPE) after 5-day incubation. The highest dilution of the serum that showed inhibition activity of SARS-CoV-2 was recorded as the NT titer. NT assays were performed in triplicate with negative control sera.

3 | RESULTS

All the serum samples efficiently neutralized wild-type SARS-CoV-2 wild-type lineage with neutralization titer ranging from 4 to 1024, in contrast, a part of serum samples failed to neutralize Alpha, Beta, Gamma, Delta, or Eta lineages, and only several serum samples were able to neutralize Omicron lineage virus strains (BA.1 and BA.2) with low neutralization titer (Figure 1). As compared with neutralization of SARS-CoV-2 wild-type lineage, neutralization of all other SARS-CoV-2 variant lineages, including Alpha, Beta, Gamma, Delta, Eta, Omicron-BA.1 and Omicron-BA.2 lineages were significantly lower (Figure 1). The geometric mean neutralization titers of all the serum samples against SARS-CoV-2 wild type, Alpha, Beta, Gamma, Delta, Eta, Omicron-BA.1, and Omicron-BA.2 lineages were 34.36, 5.94, 2.21, 4.48, 11.62, 3.09, 1.11, and 1.08, respectively (Figure 1). There were no significant differences in neutralization titer against wild type, Alpha, Beta, Gamma, Delta, Eta, Omicron-BA.1, and Omicron-BA.2 lineages between the sera from men and women (Figure 2). A tendency for a better neutralization activity was observed in sera from younger people (aged 20–49) when compared to the older ones (aged 50–59), however, there was not a statistically significant difference between the two groups in neutralization titer (Figure 3), these results consistent with a previous report.[8] Interestingly, there is no obvious tendency of a better neutralization activity against all the viral lineages with sera collected between 15 and 21 than with those collected between 9 and 14 days after the administration of the second dose of the inactivated vaccine (Figure 4). Amino acid sequences of the spike proteins of wild type and variants of SARS-CoV-2 virus strains in the neutralization assays of this study were further confirmed finally, the virus strains were not mutated again (Supporting Information: Table 1).

4 | DISCUSSION

The Omicron lineage virus is the most widespread SARS-CoV-2 virus strain in the world,[9] most of the vaccines with two shots of inactivated vaccine displayed undetectable neutralization titer in the serum samples against the BA.1 and BA.2 Omicron virus strains. Sera from two-doses of inactivated vaccines revealed significant

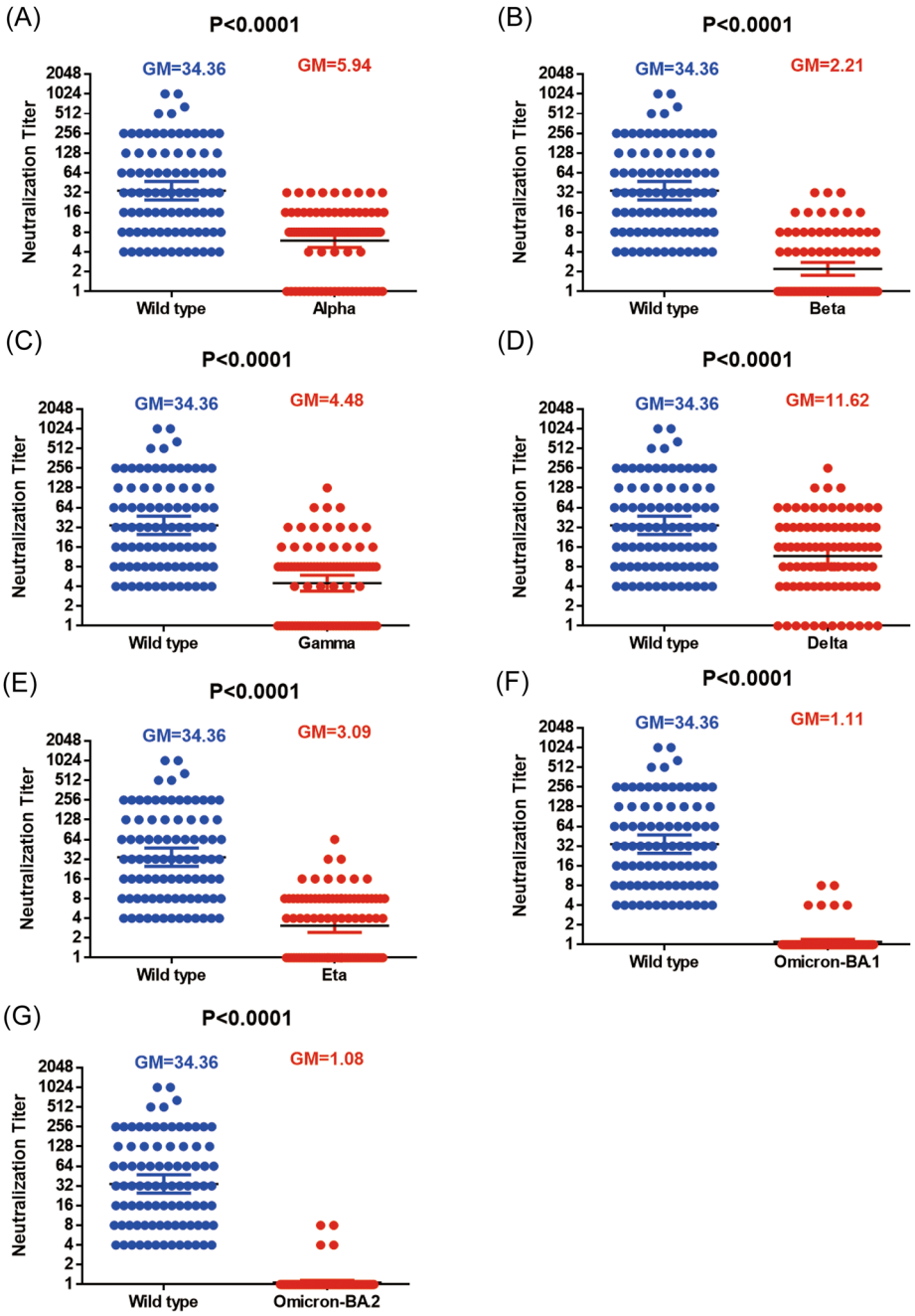

FIGURE 1 Neutralization antibody titers of inactivated vaccines immune sera against wild-type authentic severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and its viral variants. SARS-CoV-2 wild-type lineage and SARS-CoV-2 Alpha lineage (A). SARS-CoV-2 wild-type lineage and SARS-CoV-2 Beta lineage (B). SARS-CoV-2 wild-type lineage and SARS-CoV-2 Gamma lineage (C). SARS-CoV-2 wild-type lineage and SARS-CoV-2 Delta lineage (D). SARS-CoV-2 wild-type lineage and SARS-CoV-2 Eta lineage (E). SARS-CoV-2 wild-type lineage and SARS-CoV-2 Omicron BA.1 lineage (F). SARS-CoV-2 wild-type lineage and SARS-CoV-2 Omicron BA.2 lineage (G). Shown are the results of the neutralization test with the use of 91 samples obtained from 91 volunteers between 9 and 21 days after the immunization of the second dose of SARS-CoV-2 inactivated vaccines (the interval between the two immunizations was 4 weeks). Neutralization of authentic viruses was performed by cytopathic effect (CPE)-based assay using a viral titer of 100 tissue culture infective dose. The highest serum dilution that protected more than 50% of cells from CPE was taken as the neutralization titer. The neutralization titer of over 4 was identified as positive, and the negative values were unified as 1. The bars indicate geometric mean titers (GM) and the I bars indicate 95% confidence intervals. Statistical analysis was performed using the paired *t* test and the *p* values were shown.

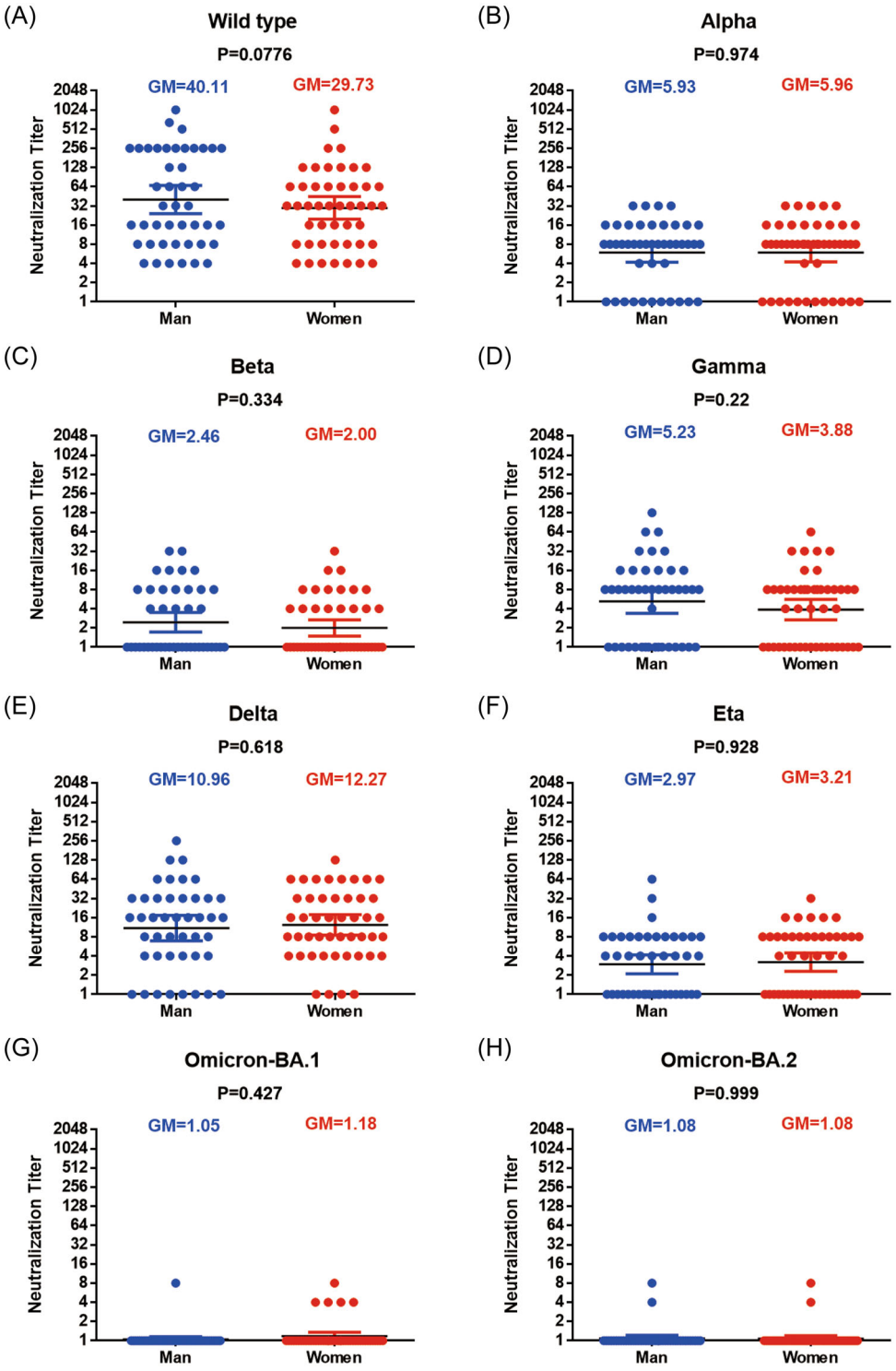

FIGURE 2 Analyses of neutralization titers of sera from volunteers of different genders against authentic severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) wild-type lineage and its variants. Ninety-one human sera obtained by CoronaVac or BBIBP-CorV vaccinated volunteers were tested against wild-type SARS-CoV-2 (A), Alpha (B), Beta (C), Gamma (D), Delta (E), Eta (F), Omicron-BA.1 (G), and Omicron-BA.2 (H) lineages. Shown are the results of the neutralization test divided into two groups ($n = 44$ sera from male volunteers and $n = 47$ sera from female volunteers). Horizontal lines indicate geometric mean titers (GM). The I bars indicate 95% confidence intervals. Statistical analysis was performed using the two-tailed unpaired *t* test and *p* values indicate the statistical significance of neutralization titers between the two indicated groups for each SARS-CoV-2 lineage.

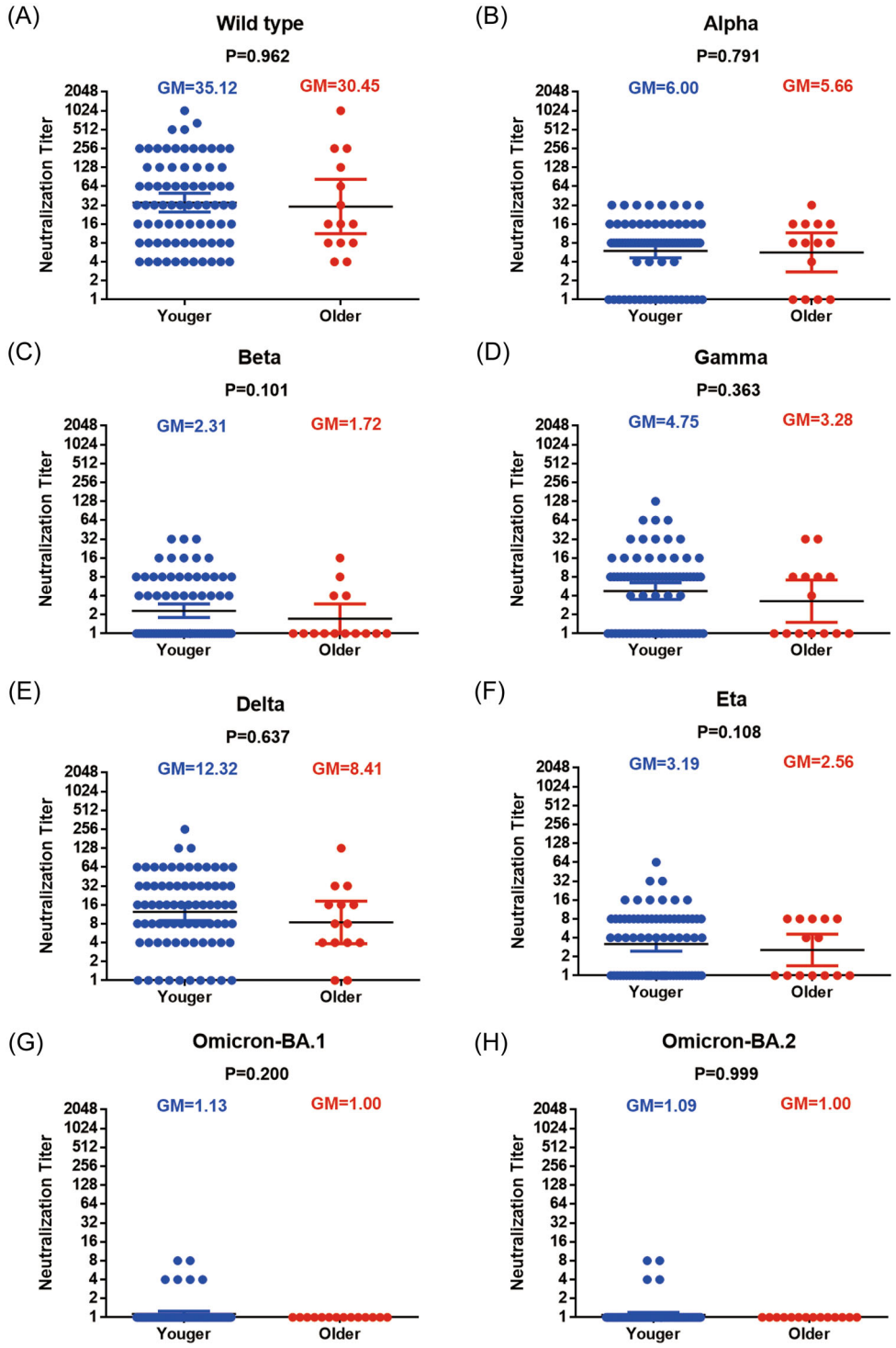

FIGURE 3 Analyses of neutralization titers of sera from volunteers of different ages against authentic severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) wild-type lineage and its variants. Ninety-one human sera obtained by CoronaVac or BBIBP-CorV vaccinated volunteers were tested against wild-type SARS-CoV-2 (A), Alpha (B), Beta (C), Gamma (D), Delta (E), Eta (F), Omicron-BA.1 (G), and Omicron-BA.2 (H) lineages. Shown are the results of the neutralization test divided into two groups (n = 77 sera from younger adults, aged 20–49 and n = 14 sera from older adults, aged 50–59). Horizontal lines indicate geometric mean titers (GM). The I bars indicate 95% confidence intervals. Statistical analysis was performed using the two-tailed unpaired t test and p values indicate the statistical significance of neutralization titers between the two indicated groups for each SARS-CoV-2 lineage.

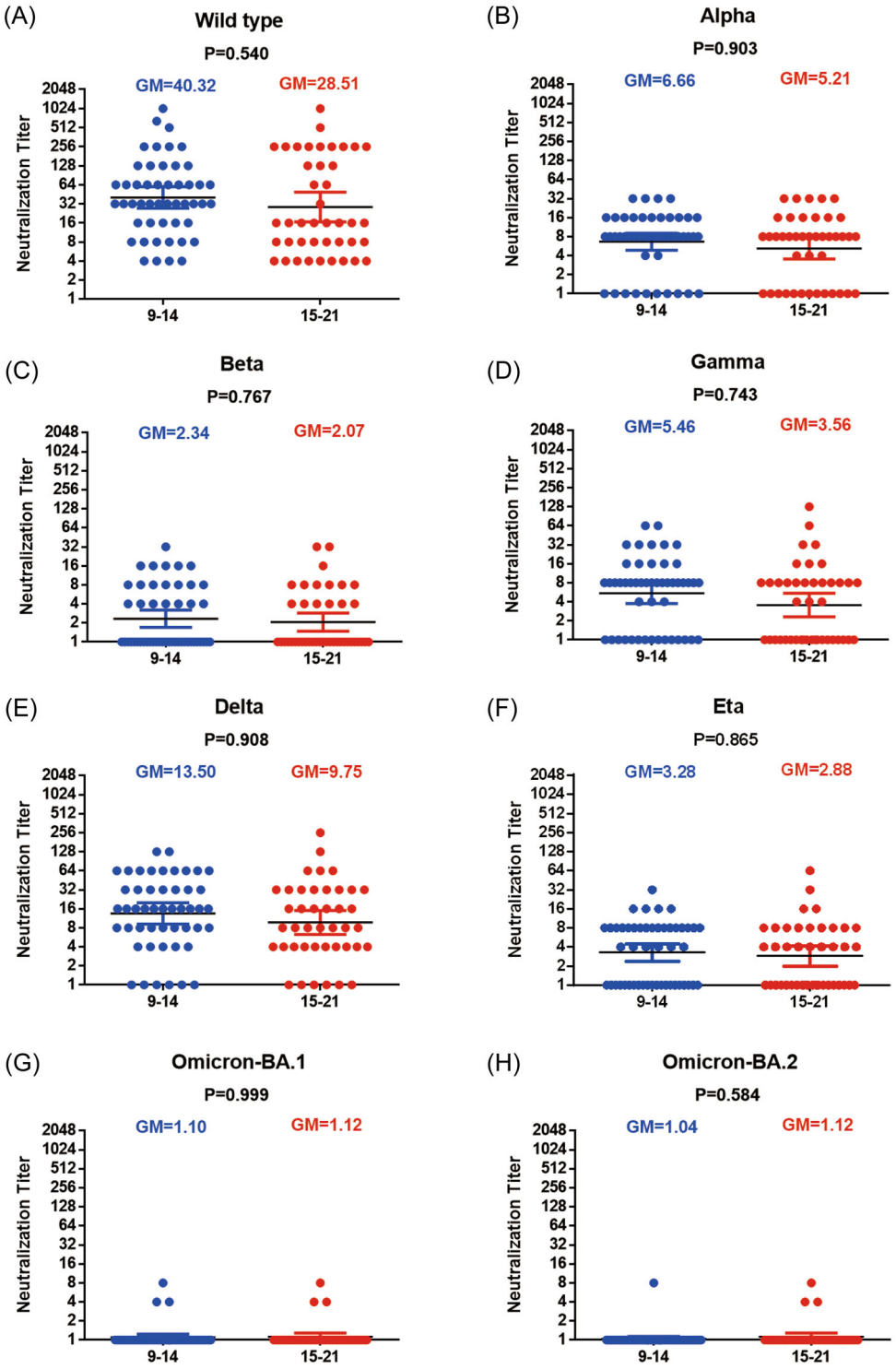

FIGURE 4 Analyses of neutralization titers of sera collected from different days postvaccination against authentic severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) wild-type lineage and its variants. Ninety-one human sera obtained by CoronaVac or BBIBP-CorV vaccinated volunteers were tested against wild-type SARS-CoV-2 (A), Alpha (B), Beta (C), Gamma (D), Delta (E), Eta (F), Omicron-BA.1 (G), and Omicron-BA.2 (H) lineages. Shown are the results of antibody neutralization titers divided into two groups according to the collection period after the administration of the second dose of the inactivated vaccine ($n = 49$ collected between 9 and 14 days postvaccination; $n = 42$ sera collected between 15 and 21 days postvaccination). Horizontal lines indicate geometric mean titers (GM). The I bars indicate 95% confidence intervals. Statistical analysis was performed using the two-tailed unpaired *t* tests and *p* values indicate the statistical significance of neutralization titers between the two indicated groups for each SARS-CoV-2 lineage.

neutralization reductions against authentic Alpha, Beta, Gamma, Delta, Eta, Omicron-BA.1, and Omicron-BA.2 lineage viruses, as compared with neutralization of SARS-CoV-2 wild-type lineage. These results indicated that mutations that occurred in the Omicron lineage virus had dramatically disruptive effects on antibodies induced by inactivated COVID-19 vaccines, while mutations that occurred in the Alpha, Beta, Gamma, Delta, and Eta viruses had less impact on the inactivated vaccine evading. Considering that all the SARS-CoV-2 mutation viruses challenged the antibody neutralization induced by BBIBP-CorV and CoronaVac, it is necessary to carry out a third booster vaccination to increase the humoral immune response against the SARS-CoV-2 mutation viruses. The results in this study show that there is no obvious tendency for a better neutralization activity against all the SARS-CoV-2 strains with sera collected between 15 and 21 than with those collected between 9 and 14 days after the administration of the second dose of the inactivated vaccine. This result may be due to SARS-CoV-2 specific antibody responses reaching a peak within 2 weeks after the second dose of vaccination, which is consistent with reported data.[10] There are some limitations in this study. First, it lacks a correlation study between neutralizing antibody titers and the protective efficacy of the inactivated vaccine against COVID-19 disease, vaccine-mediated protection must be verified by data on clinical effectiveness. Second, the volunteers in this study were vaccinated with BBIBP-CorV or CoronaVac, as the information collection of volunteers was not comprehensive, this study could not analyze the immune effect of the two kinds of vaccines separately, whether vaccination with different vaccines would affect the titer and broad spectrum of neutralizing antibodies in sera need to be investigated in further study. Based on reported data, the humoral immune response titer of these two inactivated COVID-19 vaccines may be similar.[11] Third, the Omicron strain BA.4/5 has become one of the main epidemic strains of COVID-19 worldwide, since this strain has not been isolated in our laboratory, we cannot evaluate the neutralization activity of sera in this study directly against BA.4/5. However, according to the reported data, BA.4/5 may completely escape the immune sera induced by the current vaccine.[12] We will analyze the neutralization potency of the sera from two doses and three doses of inactivated vaccines against BA.4/5 in further study.

AUTHOR CONTRIBUTIONS

Jianfeng He, Chenguang Shen, and Baisheng Li had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. *Study concept and design*: Baisheng Li, Lirong Zou, Huan Zhang, Chenguang Shen, and Xiaoling Deng. *Acquisition of data*: Lirong Zou, Huan Zhang, Zhonghua Zheng, Yushan Jiang, Yushi Huang, Shujian Lin, Jianxiang Yu. *Authentic virus assay*: Lirong Zou, Huan Zhang, and Zhonghua Zheng. *Analysis and interpretation of data*: Jianfeng He, Chenguang Shen, and Baisheng Li. *Drafting of the manuscript*: Baisheng Li and Chenguang Shen. *Study supervision*: Jianfeng He, Chenguang Shen, and Baisheng Li. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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