Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Letter to the Editor

Germline variants of *IGHV3-53 / V3-66* are determinants of antibody responses to the BNT162b2 mRNA COVID-19 vaccine

Dear Editor,

While the effectiveness of COVID-19 vaccines has been established, there is low acquisition of neutralising antibodies (nAbs) after vaccination and waning of the antibody titre over time, along with an increased risk of breakthrough infection.[1,2] Despite the accumulation of information on the confounding factors of antibody titres after vaccination,[3,4] little is known about genetic factors.

It has been recognized that heavy chains of the nAbs against SARS-CoV-2 are frequently encoded by two paralogous immunoglobulin heavy variable (*IGHV*) genes, *IGHV3-53* and *IGHV3-66*.[5,6] We postulated that germline variants affecting the function or expression of these genes may influence antibody acquisition in recipients of COVID-19 vaccines designed to induce nAbs. To validate this hypothesis, we designed and conducted a two-part genetic analysis (Fig. 1). Samples and information from 2,015 healthcare workers at Chiba University Hospital who were going to receive the BNT162b2 mRNA COVID-19 vaccine (Pfizer and BioNTech) from 3 March to 9 April 2021 and had enrolled in our previous study [4] were obtained in this study. The Chiba University Hospital Ethics Committee approved the collection of samples and clinical information from the vaccine recipients (No. HS202101-03), and their genetic analyses (No. HS202105-01) in this study. All participants provided written informed consent for providing their clinical information and blood samples and were also given the opportunity to opt out of this study. First, single-nucleotide variants (SNVs) to be evaluated were determined. We narrowed down candidate SNVs surrounding *IGHV3-53* using expression quantitative loci and linkage disequilibrium information and selected

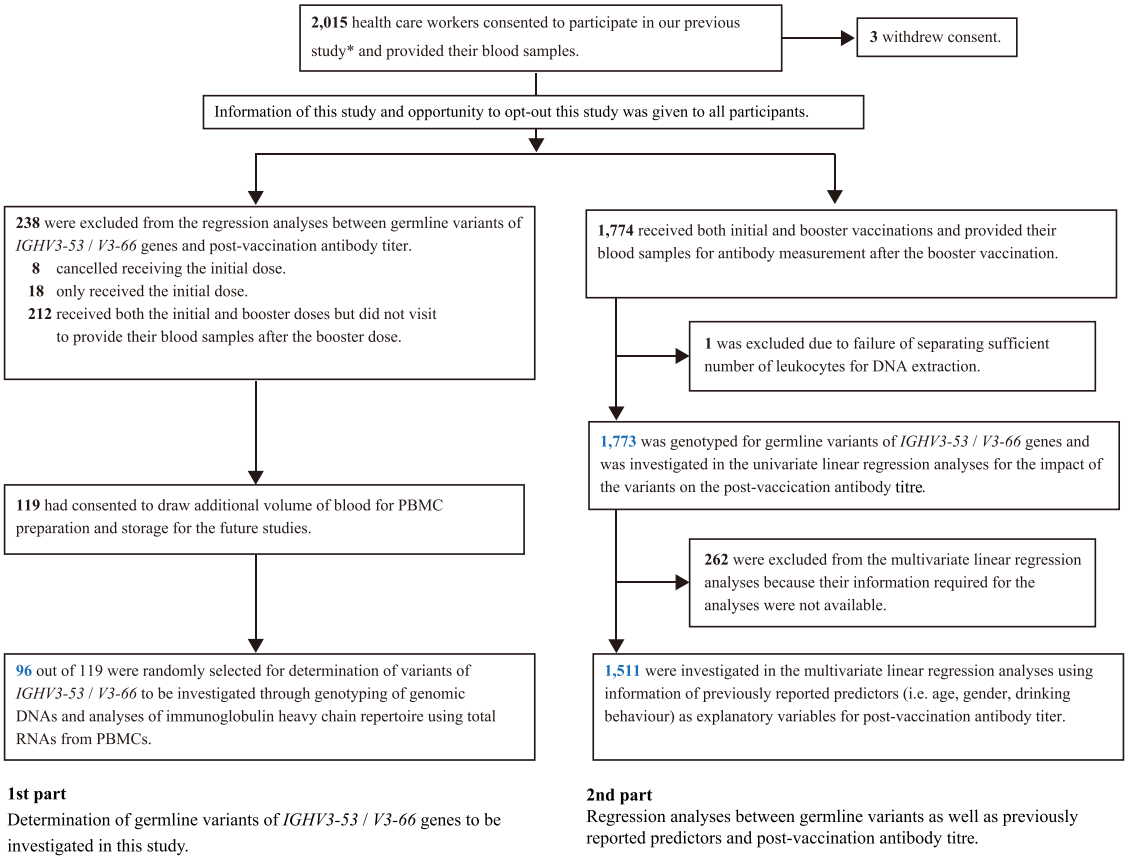

Fig. 1. Selection of the subjects in the two parts of the study. *Kageyama T. *et al.* [4].

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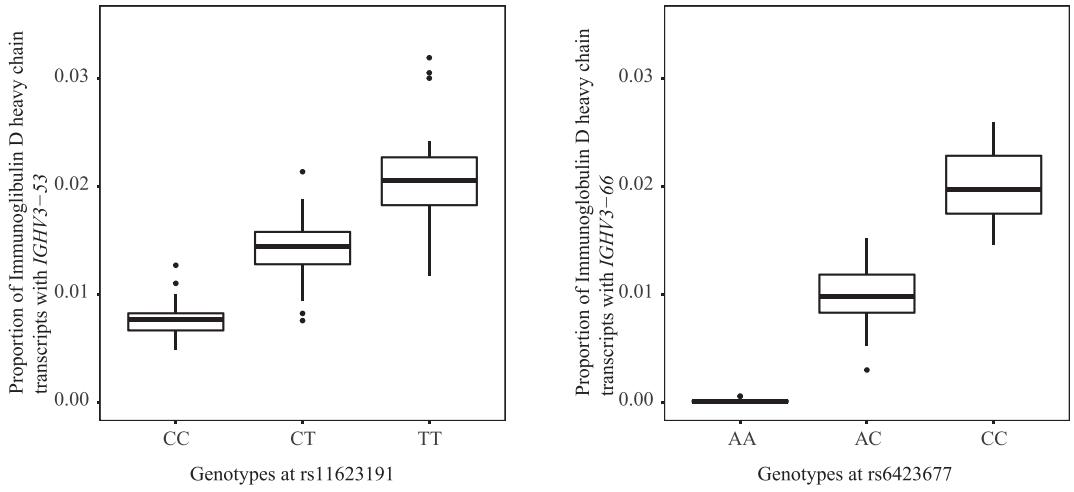
A



B

Variant	Genes	Alleles	Regression Coefficient [B]	95% confidence interval	*P* value
rs11623191	*IGHV3-53*	C/T	0.0021	-0.071– 0.075	0.96
rs6423677	*IGHV3-66*	A/C	0.080	0.0085– 0.15	0.028
2 SNVs[a]	*IGHV3-53/V3-66*	–	0.095	0.018– 0.17	0.015

C

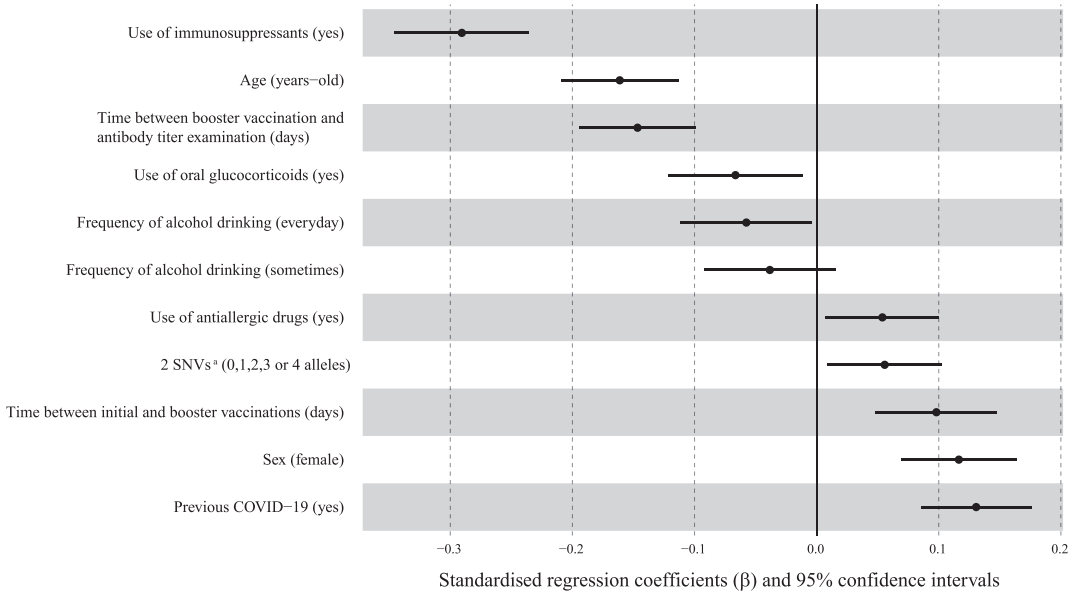


Fig. 2. A. Genotypes at single-nucleotide variants (SNVs) within immunoglobulin heavy variable (*IGHV*) genes and the usage of the genes in IgD heavy chain transcripts. Relationships between genotypes and gene usage in 96 subjects are shown for rs11623191 and *IGHV3-53* (left) and rs6423677 and *IGHV3-66* (right). Regression coefficients B, 95% confidence intervals, and *P* values in linear regression analyses were 0.010, (0.0093 – 0.011), and $<2.0 \times 10^{-16}$ for rs116231191 and 0.0065, (0.0056 – 0.0073), and $<2.0 \times 10^{-16}$ for rs6423677. B. Results of the univariate linear regression analysis for SNV alleles and the $\log_2$-transformed antibody titre against the severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) spike (S) protein after booster vaccination with BNT162b2. For rs6423677 and rs11623191, the number of alleles associated with higher gene usage (C and T alleles, respectively) was used as the independent variable. The sum of the numbers of rs11623191-T and rs6423677-C alleles (0, 1, 2, 3, or 4) was used as the independent variable. C. Results of a multivariate linear regression analysis for various predictors of post-vaccination antibody titres against SARS-CoV-2 S protein. The sum of the number of rs6423677-C and rs11623191-T alleles as well as previously identified predictors of post-vaccination antibody titres (Kageyama T. *et al.*[4]) was used as explanatory variables. Dots and bars represent the standardised regression coefficient β and 95% confidence intervals for the variables, respectively.

rs11623191 as the most promising candidate. The procedure is detailed in Supplementary Figure 1. For *IGHV3-66*, we selected the SNV rs6423677 within the gene that has been reported to impact gene usage in *IGH* chains.[7] To avoid bias induced by clonal expansion in memory B cells, we assessed the influence of SNVs on the usage of *IGHV* genes in IgD heavy chains that are predominantly expressed in naïve B cells. We genotyped 96 participants for SNVs, performed next-generation sequencing of IgD heavy chains expressed by their peripheral blood mononuclear cells, and then conducted univariate linear regression analyses (see Supplementary Methods and Supplementary Table 1). As shown in Fig. 2A, rs6423677-C and rs11623191-T significantly increased the usage of *IGHV3-66* and *IGHV3-53*, respectively. In the second part, we evaluated the effects of these two SNVs on $\log_2$-transformed antibody titres against the anti-SARS-CoV-2 spike protein, which was correlated with the nAb titre [1] among 1,773 study participants after they received initial and booster vaccinations. As shown in Fig. 2B, rs6423677 had an impact on the antibody titre, and, as expected, the number of C alleles was a predictor of a higher value (regression coefficient [B] = 0.080, 95% confidence interval [CI], 0.0085–0.15). This relationship was not observed for rs11623191 (B = 0.0021, 95% CI, -0.071–0.075). Although not significant, the positive influence of the rs11623191-T allele on the antibody titre was consistently observed in subjects stratified by their genotypes at rs6423677 (Supplementary Table 2). Therefore, we evaluated the combined effects of these two SNVs. When the numbers of the rs6423677-C and rs11623191-T alleles were summed and used as independent variables, they appeared to predict a larger titre increase than rs6423677 alone (B = 0.095, 95% CI, 0.018–0.17; Fig. 2B).

A multivariate analysis was carried out for 1,511 (981 females and 530 males) out of 1,773 participants for whom information on predictive factors identified in our previous study,[4] including age, sex, and several factors such as medication and drinking behaviour, was also available. Again, a significant effect of the two SNV alleles on the antibody titre was observed (standardised coefficient [β] = 0.056, 95% CI, 0.0088–0.10; Fig. 2C). Interestingly, the influence of the two SNVs was not uniform among the participant subpopulations stratified by sex and/or age (Supplementary Figure 2 and Supplementary Table 3). The effect of the genotype of both *IGHV3-53* and *V3-66* on the usage of the genes in *IGH* transcripts was observed regardless of sex (Supplementary Figure 3). Therefore, we speculated that the difference in the genetic effect on the final antibody titre may have occurred after naïve B cells developed and matured.

Recently, it was reported that the plasma from BNT162b2 vaccine recipients has less neutralising ability against the Omicron variant compared to the ancestral strain.[8] Given the continuing pandemic and unpredictable breakthrough infections, refinement of preventive strategies for COVID-19, including the development of new vaccines against mutant strains and optimisation of vaccine programmes based on accurate risk prediction, is a public health issue. *IGHV3-53* has been found in potent candidates of broadly nAb (bnAb) for SARS-CoV-2 which are not affected by mutations in the receptor binding domain and cover circulating and emerging variants of SARS-CoV-2.[9] Thus, when the next-generation COVID-19 vaccine, which is designed using information on the epitopes of such bnAbs, becomes available, the significance of genotypes of *IGHV3-53* and its paralogue, *IGHV3-66*, might even increase.

Limitations regarding generalisability exist, because this was a single-site study of a single ethnic group. However, the low admixture of Japanese people and low cumulative incidence of COVID-19 (estimated to be as low as 0.36% - less than 0.45 million PCR confirmed cases in 125.8 million population) at the time of participant recruitment in Japan, might have contributed to the statistical power of detection. Considering the subsequent nationwide spread and progress of the pandemic vaccination program, future opportunities to study adult individuals who will be exposed to SARS-CoV-2 antigens for the first time in their lives will be limited.

This study is the first to reveal the influence of the germline variants of two *IGHV* genes on the antibody response against SARS-CoV-2 after BNT126b2 vaccination. As BNT126b2 is one of the first modified mRNA vaccines used in the real world, further investigations must be carried out to determine the genetic factors associated with the high efficacy of the vaccine or susceptibility to its side effects.

Author contributions

H.I., K. Yokote, H.N., and Y.O. conceived the study. Y.O. and Y.M. designed the study and wrote the main draft of the manuscript. Y.M., T.K., S.T., T.T., K.M., H.I., and H.H. collected samples and clinical information. Y.M. performed the next-generation sequencing and genotyping. Y.M., K. Yamazaki, and Y.O. performed statistical analyses. All authors contributed to and reviewed the final manuscript.

Data availability

The genotypes at rs6423677 and rs11623191, as well as post-vaccination antibody titres of the study participants, are available from the corresponding author upon request. There are restrictions on the availability of clinical information from the study participants for ethical reasons.

Declaration of Competing Interest

None.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2022.10.015.

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Yoichi Mashimo, Keiko Yamazaki
Department of Public Health, Chiba University Graduate School of Medicine, 1-8-1 Inohana, Chuo, Chiba, Chiba 260-8670, Japan

Takahiro Kageyama, Shigeru Tanaka
Department of Allergy and Clinical Immunology, Chiba University Graduate School of Medicine, 1-8-1 Inohana, Chuo, Chiba, Chiba 260-8670, Japan

Toshibumi Taniguchi
Department of Infectious Diseases, Chiba University Hospital, 1-8-1 Inohana, Chuo, Chiba, Chiba 260-8670, Japan
Chiba University Hospital COVID-19 Vaccine Center, 1-8-1 Inohana, Chuo, Chiba, Chiba 260-8670, Japan

Kazuyuki Matsushita
Division of Laboratory Medicine, Chiba University Hospital, 1-8-1 Inohana, Chuo, Chiba, Chiba 260-8670, Japan

Hidetoshi Igari
Department of Infectious Diseases, Chiba University Hospital, 1-8-1 Inohana, Chuo, Chiba, Chiba 260-8670, Japan
Chiba University Hospital COVID-19 Vaccine Center, 1-8-1 Inohana, Chuo, Chiba, Chiba 260-8670, Japan

Hideki Hanaoka
Clinical Research Center, Chiba University Hospital, 1-8-1 Inohana, Chuo, Chiba, Chiba 260-8670, Japan

Koutaro Yokote
Department of Endocrinology, Hematology and Gerontology, Chiba University Graduate School of Medicine, 1-8-1 Inohana, Chuo, Chiba, Chiba 260-8670, Japan

Hiroshi Nakajima
Department of Allergy and Clinical Immunology, Chiba University Graduate School of Medicine, 1-8-1 Inohana, Chuo, Chiba, Chiba 260-8670, Japan
Chiba University Hospital COVID-19 Vaccine Center, 1-8-1 Inohana, Chuo, Chiba, Chiba 260-8670, Japan

Yoshihiro Onouchi*
Department of Public Health, Chiba University Graduate School of Medicine, 1-8-1 Inohana, Chuo, Chiba, Chiba 260-8670, Japan

*Corresponding author at: Department of Public Health, Chiba University Graduate School of Medicine, 1-8-1 Inohana, Chuo, Chiba, Chiba 260-8670, Japan.
E-mail address: onouchy@chiba-u.jp (Y. Onouchi)