Asian Flush Gene Variant Enhances Cellular Immunogenicity of COVID-19 Vaccine: Prospective Study in the Japanese General Population

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Article

Asian Flush Gene Variant Enhances Cellular Immunogenicity of COVID-19 Vaccine: Prospective Study in the Japanese General Population

Sudarma Bogahawaththa [1,2], Megumi Hara [3], Takuma Furukawa [3,4], Chiharu Iwasaka [3,5], Takeshi Sawada [6], Goki Yamada [7], Mikiko Tokiya [2], Kyoko Kitagawa [8], Yasunobu Miyake [9], Yoshio Hirota [10] and Akiko Matsumoto [2,*]

[1] Laboratory of Biochemistry, Department of Applied Biochemistry and Food Science, Faculty of Agriculture, Saga University, Japan; sz1944@cc.saga-u.ac.jp
[2] Department of Social and Environmental Medicine, Saga University, Japan; sx4932@cc.saga-u.ac.jp
[3] Department of Preventive Medicine, Faculty of Medicine, Saga University, Japan; harameg@cc.saga-u.ac.jp (M.H.); sr0753@cc.saga-u.ac.jp (T.F.); sy7509@cc.saga-u.ac.jp (C.I.)
[4] Clinical Research Center, Saga University Hospital, Japan
[5] National Institute of Biomedical Innovation, Health and Nutrition, Japan
[6] Department of Histology and Neuroanatomy, Faculty of Medicine, Saga University, Japan; 22624004@edu.cc.saga-u.ac.jp
[7] United Graduate School of Agriculture, Kagoshima University, Japan; 23975002@edu.cc.saga-u.ac.jp
[8] Division of Ultrastructural Cell Biology, Department of Anatomy, Faculty of Medicine, University of Miyazaki, Japan; kyoko_kitagawa@med.miyazaki-u.ac.jp
[9] Department of Immunology, Faculty of Medicine, Saga University, Japan; ymiyake@cc.saga-u.ac.jp
[10] Clinical Epidemiology Research Center, SOUSEIKAI Medical Group (Medical Co., LTA), Japan; hiro8yoshi@lta-med.com
* Correspondence: matsumoa@cc.saga-u.ac.jp; Tel.: +81-952-34-2289

Abstract: Because we observed a reduced humoral immune response to the COVID-19 vaccine and subsequently discovered a lower susceptibility to COVID-19 in individuals carrying the ALDH2 rs671 variant, we hypothesized that rs671 is beneficial for cellular immunity against COVID-19. Using the IFN-γ enzyme-linked immunospot (ELISPOT) assay, we evaluated cellular immunity before and after COVID-19 vaccination in two subcohorts of a previously reported cohort. Subcohort 1 (26 participants) had six repeated observations at baseline and after one to three doses, whereas subcohort 2 (19 participants) had two observations, before and after the third dose. ELISPOT counts at six months after the second dose increased from baseline in carriers of the rs671 variant, but not in non-carriers. A positive effect of rs671 on ELISPOT counts was estimated using a mixed model, including the random effect of subcohort, repeated measures, and fixed effects of vaccine type, age, sex, height, lifestyle, steroid use, and allergic disease. There was no association between ELISPOT counts and specific IgG levels, suggesting a limitation in estimating protective potential by humoral response. There is lower humoral and higher cellular immunity against SARS-CoV-2 in rs671 variant carriers compared to non-carriers, providing a potential basis for optimizing preventive measures and drug discovery.

Keywords: ALDH2; rs67; COVID-19; enzyme-linked immunospot; cellular immunity; T cell

1. Introduction

The COVID-19 pandemic, caused by the SARS-CoV-2 virus, was a global health crisis. The virus spreads primarily through respiratory droplets and causes symptoms ranging from mild respiratory problems to severe pneumonia and acute respiratory distress syndrome [1]. Vaccination plays a central role in the adaptive immunity and management of the COVID-19 pandemic [2] by stimulating the immune system to mount a protective response, including both cellular and humoral immunity [3].

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The rs671 variant, a known East Asian-specific genetic diversity, is a missense mutation in aldehyde dehydrogenase 2 (*ALDH2*). An amino acid substitution from glutamic acid (GAA) to lysine (AAA) at position 504 or 487 of the immature or mature ALDH2 enzyme (Glu504Lys or Glu487Lys) results in a collapsed three-dimensional structure [4]. ALDH2 plays a crucial role in alcohol metabolism by oxidizing acetaldehyde, a toxic product of ethanol oxidation, into the less harmful substance, acetate [5–7]. Individuals carrying the rs671 variant, found almost exclusively in East Asians, have substantially reduced ALDH2 enzymatic activity, leading to accumulation of acetaldehyde in the body when they consume alcohol [8]. This accumulation causes vasodilation, resulting in skin flushing, increased heart rate, nausea, and other unpleasant symptoms [9], a phenomenon known as the Asian flush [10,11]. The variant allele of rs671 is associated with various phenotypes [12–17], including alcohol-irrelevant disease risks, such as vasospastic angina [18] and cognitive impairment [19].

Based on our previous findings on the association between rs671 and immune function (i.e., rs671 influences basal T cell subpopulation [20] and the efficacy of immune checkpoint inhibitors [15]), we launched a prospective cohort study to investigate the immunogenicity of the COVID-19 mRNA vaccine and found an inverse association between the rs671 variant and IgG production [21]. Subsequently, a web-based retrospective cohort study revealed lower susceptibility to COVID-19 among rs671 variant carriers [22]. These seemingly contradictory findings suggest the involvement of cellular immunity which plays a greater role than humoral immunity in protecting against COVID-19 [23] as shown by the successful recovery of patients with X-linked agammaglobulinemia and genetic deficiency of mature B lymphocytes from COVID-19 [24].

To test our hypothesis, we designed an additional study using frozen blood cells from the abovementioned study on vaccine immunogenicity to evaluate SARS-CoV-2-specific T lymphocyte counts before and after COVID-19 vaccination.

2. Materials and Methods

2.1. Study Design and Participants

The current study used a database of frozen blood cells collected from participants of our prospective and observational study [25]. To maximize statistical power with limited financial resources, rs671 minor allele carriers were prioritized over major allele carriers. The participants were employees and students of Saga University, Japan, aged 20 years or older, who voluntarily received the COVID-19 vaccine administered at Saga University. Pregnant women and those who had been infected or suspected of being infected with COVID-19 were excluded from the study.

As shown in Figure 1, university employees (N = 16) and some of the students (N = 10) completed two doses of mRNA-1273 (Moderna Inc., Cambridge, MA, USA/Takeda Pharmaceutical Co., Ltd., Tokyo, Japan) (100 μg) starting August 9–11, 2021, and a booster dose of BNT 162b2 (Pfizer Inc., New York, NY, USA/BioNTech SE, Mainz, Germany) (30 μg) (subcohort-1), and the rest of university students (N = 19) received three doses of BNT 162b2 (Pfizer) starting May 19, 2021 (subcohort-2). Vaccination history was recorded using a vaccination certificate issued by the local government of Saga Prefecture, Japan. mRNA-1273 and BNT 162b2 were completed at an interval of three or four weeks, and booster vaccination with BNT 162b2 was given six or eight months after the second dose for subcohorts 1 and 2, respectively. Blood samples were collected at baseline, three weeks after the first dose, one, three, and six months after the second dose (two, four, and seven months after the first dose), and one month after the third dose (8 months after the first dose) for subcohort 1. For subcohort 2; eight months after the second dose and one month after the second dose. Blood sample collection for subcohort 2 began before the booster dose because of a change in the budget plan.

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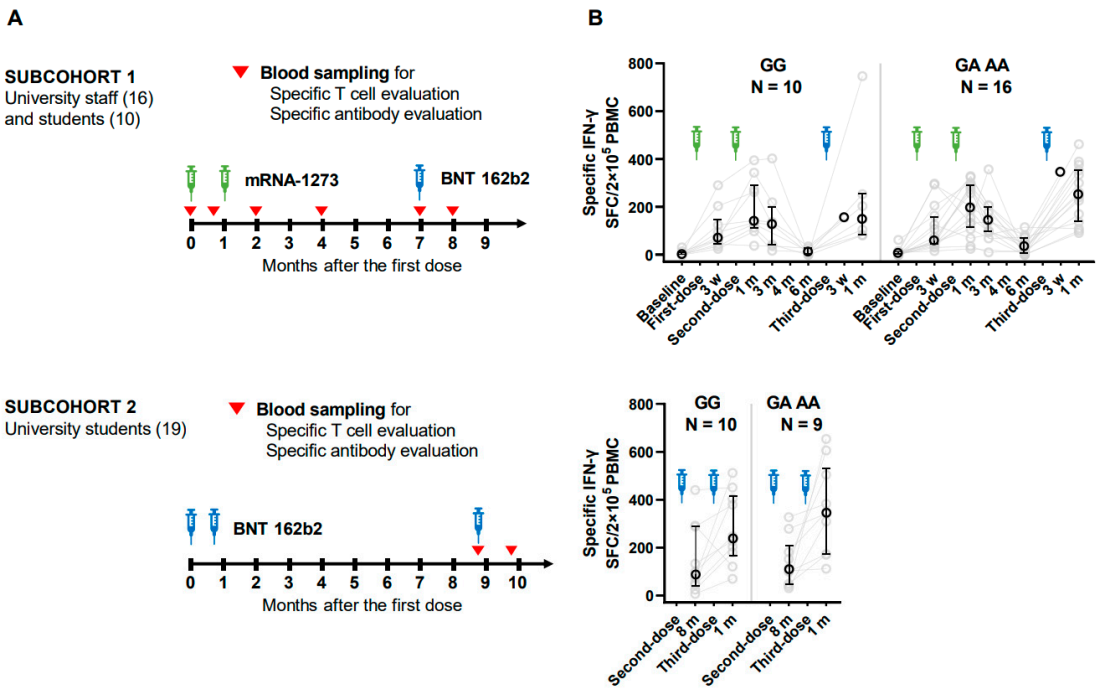

Figure 1. SARS-CoV-2 S1 protein specific IFN-γ+ cell counts after COVID-19 vaccination. (a) study design. Subcohort 1 included 26 participants who completed two doses of mRNA-1273 (100 μg), and a booster dose of BNT162b2 (30 μg). Subcohort 2 included 19 male students who received three doses of BNT162b2. The green and blue syringe icons indicate mRNA-1273 and BNT 162b2, respectively. Blood samples were collected as indicated in red triangles. **(b)** SARS-CoV-2 S1 protein specific IFN-γ+ cell counts. IFN-γ ELISPOT counts after immunological activation with 315-peptide mixtures of SARS-CoV-2 spike glycoprotein of the original strain were plotted. Grey circles indicate the individual ELISPOT counts. Black circles indicate the median ELISPOT count for each time point with error bars representing the interquartile range.

2.2. Questionnaire

A self-administered questionnaire was used to obtain participants' general information, as previously reported [21]. Briefly, sex, date of birth, disease (chronic lung disease, heart disease, stroke, kidney disease, liver disease, blood disease, diabetes, immunodeficiency, malignancy, collagen disease, and allergic disease), treatment history, cigarette smoking, drinking, and exercise habits were recorded. Ethanol intake in the previous six months was estimated, adjusted per 60 kg of body weight, and then categorized into <1, <20, and ≥20 g/day. Exercise habit was chosen from no habit, <1 day/week, 1 to 3 days/week, and ≥3 days/week. The question "Do you feel psychological stress?" was asked to evaluate perceived stress on a five-point scale: no (0), mostly no (1), unsure (2), quite often (3), and yes (4).

2.3. Genotyping

The *ALDH2* rs671 genotype was determined using the DNA extracted from blood clots using a TaqMan® SNP genotyping assay system (Thermo Fisher Scientific, Waltham, MA, USA) as described previously [25].

2.4. Separation of Peripheral Blood Mononuclear Cells (PBMCs)

Peripheral blood was drawn into EDTA-2K vacuum blood sampling tubes. To collect PMBCs, blood samples were subjected to gradient separation with Lymphoprep™ (Serumwerk Bernburg, Burnburg, Germany) within the day of sampling, according to manufacturer's instruction. The cell suspension extracted at the border of the top and second layers was collected, washed with > 5 times

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the volume of saline solution, and dissolved in KM Banker II (Kohjin Bio Co., Ltd., Saitama, Japan), a culture medium suitable for the long-term cryopreservation of lymphocytes. A small portion of the PBMC suspension was subjected to cell counting after 0.4 w/v% trypan blue. Finally, the PBMC samples were gently cooled and stored at −80 °C until the enzyme-linked immunospot (ELISPOT) assay.

2.5. ELISPOT Assay

A human IFN-γ ELISPOT kit (Mabtech AB, Nacka Strand, Sweden) was used to evaluate cellular immunity against SARS-CoV-2 using lyophilized 315-peptide mixtures of SARS-CoV-2 spike glycoprotein, PepMixTM SARS-CoV-2 (JPT Peptide Technologies, Berlin, Germany), the original strain (PM-WCPV-S-2) as antigens. The peptide mixture was dissolved in DMSO at a concentration of 0.5 ug/eptide, and a DMSO addition group was provided as a negative control. Similarly, an NF-kB activator, phorbol myristate acetate, and ionomycin addition group were provided as positive controls. The assay was performed according to the manufacturer's instructions using an ImmunoSpot S6 VERSA spot counter (Cellular Technology Limited, Shaker Heights, Ohio, USA).

2.6. Statistical Analysis

SARS-CoV-2 infection during follow-up was detected by an increase in anti-SARS-CoV-2 S1 protein IgN levels or specific IgG, and T-cell counts without vaccination, and the corresponding observation points were removed after suspected infection. Spearman's rank correlation ϱ was calculated to examine non-linear association between rank or continuous variable, such as rs671 variant number, ethanol intake, ELISPOT counts, and IgG levels. Mixed models were used to compute the association between the rs671 variant allele numbers and the log-transformed specific T cell levels to account for repeated measurements and the random effect of the subpopulation (proc mixed by SAS9.4 TS Level 1M5 for Windows, SAS Institute, Cary, NC, USA). ELISPOT counts were converted into log values to approximate a normal distribution. Statistical significance was set at $p < 0.05$.

3. Results

3.1. Baseline Characters of the Participants

Table 1 shows the baseline characteristics of each subcohort, including 30 males and 15 females with confirmed rs671 genotypes: wild-type homozygous (GG type, N = 20), heterozygous (GA type, N = 17), and variant homozygous (AA type, N = 8). Daily ethanol intake (g/day) was low in the variant allele carriers (GA and AA) ($\varrho = -0.42$, $p < 0.01$). The distribution of exercise habits, perceived stress, and allergic diseases did not differ among the three groups ($p > 0.4$, Fisher's exact test). Steroid use was reported only by two participants of the GG-type.

Table 1. Baseline characteristics of participants by ALDH2 rs671 genotypes.

	Subcohort 1 University employees and students			Subcohort 2 University students		
N	26			19		
Type of vaccine	mRNA-1273 × 2 BNT162b2 × 1			BNT162b2 × 3		
ALDH2 rs671	GG	GA	AA	GG	GA	AA
Males, N	3	7	1	10	4	5
Females, N	7	6	2	0	0	0
Age, years						
Median	39	47	21	23	23	22
(IQR)	(22–56)	(21–55)	(21–42)	(22–23)	(23–24)	(22–23)
Body height, cm						

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Median	162	166	157	171	173	172
(IQR)	(155–166)	(163–170)	(151–163)	(167–175)	(167–178)	(170–173)
Cigarette smoke, yes	0	1	0	0	0	0
Ethanol intake*						
<1 g/d	6 (60%)	9 (69%)	3 (100%)	2 (20%)	2 (50%)	4 (80%)
≥1, <20 g/d	4 (40%)	2 (15%)	0 (0%)	8 (80%)	2 (50%)	1 (20%)
≥20 g/d	0 (0%)	2 (15%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Exercise habit						
No habit	3 (30%)	4 (31%)	1 (33%)	1 (10%)	1 (25%)	2 (40%)
< 1 d/w	2 (20%)	2 (15%)	0 (0%)	4 (40%)	1 (25%)	1 (20%)
1 to 3 d/w	2 (20%)	4 (31%)	1 (33%)	2 (20%)	2 (50%)	2 (40%)
≥ 3 d/w	3 (30%)	3 (23%)	1 (33%)	3 (30%)	0 (0%)	0 (0%)
Perceived stress						
0 (no)	1 (10%)	5 (38%)	1 (33%)	5 (50%)	1 (25%)	1 (20%)
1	3 (30%)	1 (8%)	0 (0%)	1 (10%)	1 (25%)	0 (0%)
2	2 (20%)	2 (15%)	0 (0%)	1 (10%)	2 (50%)	3 (60%)
3	4 (40%)	4 (31%)	0 (0%)	3 (30%)	0 (0%)	1 (20%)
4 (yes)	0 (0%)	1 (8%)	2 (67%)	0 (0%)	0 (0%)	0 (0%)
Steroid use, yes	0 (0%)	0 (0%)	0 (0%)	2 (20%)	0 (0%)	0 (0%)
Allergic disease, yes	2 (20%)	6 (46%)	1 (33%)	5 (50%)	1 (25%)	1 (20%)

GG, GA, and AA represent the genotypes of rs671, i.e., *ALDH2*1/*1*, *ALDH2*1/*2*, and *ALDH2*2/*2*, respectively. IQR, interquartile range. * Ethanol intake was adjusted for body weight (g/day/60 kg body weight). Due to financial limitations, we selected 19 male subjects to include all homozygous variant allele (AA) carriers among the 42 paticipants in subcoohrt 2.

3.2. Changes in Cellular Immune Response after Vaccination

As depicted in Figure 1, we conducted an IFN-γ ELISPOT assay to quantify the number of T cells producing IFN-γ in response to SARS-CoV-2 specific antigens. The variant carriers tended to have higher counts than wild-type carriers at most time points. For example, at baseline in subcohort 1, wild and variant types had median counts of 0.5 and 5.5 spot- forming cells per 2×10^5 PBMC, respectively. One month after the second dose, the medians reached 139.5 and 196, respectively. Six months after the second dose, the number decreased to 36 in the wild-type group, showing an indistinguishable level from baseline ($p = 0.10$, Wilcoxon rank-sum test), whereas 116 in the variant-type group were higher than baseline ($p < 0.01$).

As shown in Table 2, the effects of rs671 were estimated using multivariate mixed models with repeated measures. Model 1, including categorical time-points, vaccine type, age, sex, and the number of variant alleles, estimated a positive effect of the variant ($\beta = 0.27$ per allele, $p = 0.01$). Model 2, additionally including lifestyle, perceived stress, steroid use, and allergic disease history, gave a similar estimation ($\beta = 0.3$, $p = 0.007$).

Table 2. Estimated fixed effects of baseline characteristics on log-transformed IFN-γ ELISPOT count.

	AIC = 486		AIC = 498	
	183 datapoints		183 datapoints	
Fixed effect	β	p-value	β	p-value
BNT162b2 (reference)				
mRNA-1273	-0.58	0.067	-0.64	0.065
Age (per 10 year)	0.08	0.108	0.09	0.137
Female sex	0.10	0.536	0.24	0.358
Height (per 10 cm)			0.04	0.802
Tobacco smoking, yes			-0.52	0.294

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Ethanol intake (per category)			0.10	0.500
Exercise (per category)			0.07	0.337
Perceived stress (per category)			0.02	0.767
Steroid use, yes			0.17	0.759
Allergic diseases			-0.22	0.185
Number of rs671 variant allele	0.27	0.010	0.30	0.007

β, Partial correlation coefficient. All models include the fixed effects of post-vaccination timing as a categorical variable and variables listed in the table.

As reported previously, anti-SARS-CoV-2 spike 1 IgG (anti-S1 IgG) and the rs671 variant were inversely associated in the current cohort [25]. Therefore, humoral and cellular immunity were correlated with rs671 in the opposite direction. As shown in Figure 2, no rank correlation was observed between specific IgG and T cell responses obtained from the same blood samples. In a mixed model to estimate log-transformed specific T cell count by categorical time-points and log-transformed anti-S1 IgG considering repeated measures and subcohorts, anti-S1 IgG was not a predictor of specific T cell count (partial correlation coefficient = −0.05, p = 0.7, 183 observations).

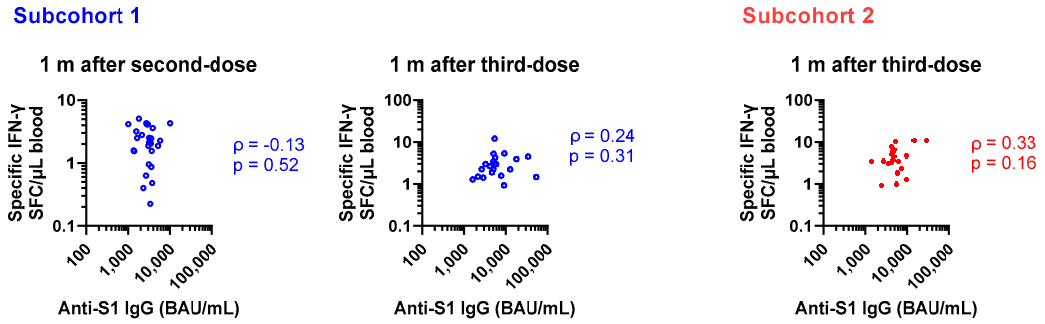

Figure 2. Association between humoral and cellular immunity. Anti-SARS-CoV-2 S1 IgG and IFN-γ+ PBMC count per 1 μL whole blood and anti-S1 IgG levels are plotted for 1 month after the second and third dose of the COVID-19 vaccine (blue and red dots represent subcohort 1 and 2, respectively). SFC: spot-forming cells. PBMC, peripheral blood mononuclear cells. Spearman's rank correlation coefficients (ρ) and p-values are presented.

4. Discussion

Confirming our hypothesis, we found for the first time a positive association between the rs671 variant and cellular immunity characterized by SARS-CoV-2 spike protein specific IFN-γ+ T cell count after COVID-19 vaccination in a prospective observation of the general population in Japan. Cellular immunogenicity remained detectable six months after the second dose only in the rs671 variant group. The humoral immune reaction measured using anti-S1 IgG was not associated with the cellular immunity observed in the current study.

Although ALDH2 is known as an alcohol-metabolizing enzyme, its essential role is the metabolism of endogenous aldehydes, such as formaldehyde and 4-hydroxy-2-nonenal (4-HNE), which are produced in the body as byproducts of normal cellular processes, such as spontaneous generation during one-carbon metabolism [26], demethylation of sarcosine [27,28] for formaldehyde, and peroxidation of arachidonic acid [29] for 4-HNE. The accumulation of these aldehydes due to low ALDH2 activity is a potential pathogen via adduct formation with DNA [30,31] and proteins [32], resulting in carcinogenesis and functional alterations of the molecules [33,34].

Endogenous formaldehyde and 4-HNE inhibit mechanistic target of rapamycin (mTOR) signaling by modulating mTOR complex components [35]. The mTOR protein kinase serves as a central regulator of cell growth, proliferation, metabolism, and survival. It exists in two distinct multiprotein complexes: mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). mTORC1

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primarily regulates the processes related to cell growth, protein synthesis, and metabolism in response to nutrients, growth factors, and energy status. Inhibition of mTORC1 signaling by formaldehyde and 4-HNE may have implications for the maintenance of memory T cells, which are a small population of long-lived immune cells derived from effector T cells that provide rapid and specific responses upon re-exposure to previously encountered pathogens or antigens [36]. In our observation, the variant type showed higher T cell counts than the wild type, however, multiple testing of rank correlation between the variant number and specific T cell count for each time-point gave only significance at six months after second dose ($\varrho = 0.5$, $p = 0.02$). This finding is consistent with the hypothesis that accumulation of endogenous aldehydes inhibits mTOR signaling, providing acquired immunity via survival of memory T cells.

Another possible mechanism is the inhibition of T cell glycolysis by endogenous aldehydes, as observed in acetaldehyde-exposed T cells [37]. By interfering with glycolytic activity, endogenous formaldehyde and 4-HNE may shift cellular metabolism towards alternative pathways such as fatty acid oxidation, the pentose phosphate pathway, and oxidative phosphorylation for energy production. Memory T cells favor these alternative pathways, which are crucial for providing energy, biosynthetic precursors, and maintaining the redox balance, ensuring the metabolic fitness, longevity, and functionality of memory T cells.

Although the COVID-19 vaccines in our study were designed for the original strain, PBMCs in the current study reacted with original, delta, and omicron variants (r squared = 0.71 and 0.82 for original vs delta and original vs omicron, respectively, Figure S1). In contrast, humoral immunity is expected to escape, particularly for omicron variants [38,39]. Given this evidence, our hypothetical benefit of the rs671 variant on the survival of memory T cells may hold throughout the pandemic; the rs671 variant may promote COVID-19 protection via cellular immunity induced by the vaccine or exposure history to similar virus. The lower occurrence of COVID-19 and related hospitalizations in our previous report [22] is thus explainable by the current findings; however, the reported strong effect in the early phase (hazard ratio 0.2) may not be fully explained, suggesting the presence of additional defense mechanisms, such as antimicrobial effects of metabolic intermediate aldehydes [40]. For instance, we propose that the accumulation of formaldehyde in individuals with the rs671 variant may protect against COVID-19 through its bacteriostatic effect at the physiological level (100 μM) [41]. This infection defense phenotype of the rs671 variant is a possible reason for its spread in East Asia following major lifestyle changes associated with the infectious hazards of rice cultivation [42–45].

To address the limitation of the validity of the current study owing to the small sample size, we attempted to maximize the detection power using statistical modeling to obtain all observation points. Targeting single cytokine production, IFN-γ, is another limitation, because multi-cytokine evaluation gives us background information, IL-4 for example, an indicator of antibody production systems [46]. In addition, the use of PBMC without subpopulation sorting limits the specificity of findings because several types of immune cells can produce IFN-γ, including CD4+ T helper cells, CD8+ cytotoxic T cells, and natural killer T cells.

5. Conclusions

This study revealed for the first time a positive association between the rs671 variant and enhanced cellular immunity following COVID-19 vaccination. Reduced ALDH2 activity may favor the development and maintenance of COVID-19 specific memory T cells. The current findings support the previously reported lower susceptibility to COVID-19 infection in rs671 variant carriers. These findings may help in designing effective vaccination strategies; however, the underlying mechanisms require further investigation.

6. Patents

This section is not mandatory but may be added if there are patents resulting from the work reported in this manuscript.

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Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Figure S1: Cross-reactivity to SARS-CoV-2 delta and omicron variants

Author Contributions: Conceptualization, A. M.; Study design, A.M. and M.H.; Investigation, M.H., A.M., C.I., T.S., T.F., G.Y., and M.T.; Data Curation, M.H. and A.M.; Statistical Analysis, A.M. and S.B.; Writing-Original Draft Preparation, S.B.; Writing-Review and Editing, All authors; Visualization, A.M. and S.B.; Supervision, A.M., M.H, K.K., Y.M., and Y.H. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: This study was approved by the Research Review Committee and Human Genome Ethics Review Committee of the Faculty of Medicine, Saga University, Japan (approval numbers: R2-24, R2-44, R3-4, R3-9, and R3-39), and conducted in accordance with the Declaration of Helsinki.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The datasets generated and/or analyzed in the current study are not publicly available to protect the privacy of the participants; however, they are available from the corresponding author upon reasonable request.

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Conflicts of Interest: The authors declare no conflicts of interest.

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