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Communication

Variant allele of *ALDH2*, rs671 associates with attenuated post-vaccination response in anti-SARS-CoV-2 spike protein IgG: a prospective study in the Japanese general population

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Abstract: Uncovering the predictors of vaccine immunogenicity is essential for infection control. We have reported that the most prevalent polymorphism of the aldehyde dehydrogenase 2 (ALDH2) gene, rs671, may be associated with an attenuated immune system. To test the inverse relation between rs671 and antibody production after COVID-19 vaccination, the levels of anti-SARS-CoV-2 Spike protein S1 subunit (S1) IgG were repeatedly measured for four months before and after vaccination with BNT162b2 or mRNA-1273, in 88 Japanese workers and students (including 45 females, aged 21–56 years, with an rs671 variant allele frequency of 0.3). The mixed model including fixed effects of the vaccine type, weeks post vaccination (categorical variable), sex, age, body height, smoking status, ethanol intake, exercise habit, perceived stress, steroid use, allergic diseases, and dyslipidemia, indicated an inverse association between log-transformed anti-S1 IgG levels and the number of rs671 variant alleles (partial regression coefficient = -0.15, p = 0.002). Our study indicated for the first time that the variant allele of ALDH2, rs671, is associated with the attenuated immunogenicity of COVID-19 mRNA vaccines. Our finding may provide a basis for personalized disease prevention based on a genetic polymorphism that is prevalent among East Asians.

Keywords: ALDH2; rs671; COVID-19; vaccine; immunogenicity

1. Introduction

Aldehyde dehydrogenase 2 (ALDH2), a member of the ALDH superfamily [1], is expressed in most human tissues, including immune cells, and is crucial for the metabolism of endogenous aldehydes, such as formaldehyde and 4-hydroxynonenal [2]. The rs671 polymorphism, which results from missense mutations in the coding region of the *ALDH2* gene, is the most common ALDH2 deficiency in humans, exclusively observed in the East Asian population, with an incidence of 40–50% in some East Asian populations such as Japanese, Taiwanese, and Han Chinese [3, 4, 5] (https://www.ncbi.nlm.nih.gov/snp/rs671),

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thereby accounting for 5–10% of the world population. Although rs671 polymorphism has never been reported to be associated with vaccine efficacy, it is reported to be involved in various traits, including lifestyle habits, disease risks, and drug sensitivities [2, 6, 7, 8, 9]. Aiming for a proposal of personalized medicine based on rs671, we have performed several investigations on its unique and novel phenotypes [10, 11], including inhibited T cell immunity [12]. Considering intercellular communication in the immune system, B cells and anti-body production may also be affected.

The immune system defends the body against infections from various pathogens including viruses. In the context of the COVID-19 pandemic, it is well known that the extent of the infection as well as the vaccine efficacy varies across individuals. This suggests the need for research on the various factors that affect vaccine immunogenicity, both genetic as well as those pertaining to lifestyle. Numerous studies have indicated that the vaccine efficacy depends on several factors, such as the type of vaccine, number of doses, and the demographical and clinical characteristics of recipients [13, 14, 15, 16, 17]. Although antibody responses against SARS-CoV-2 are characterized by responses against a range of viral proteins, including spike proteins, nucleoproteins, and membrane proteins, the T cell response is a critical component of immune protection against SARS-CoV-2 [18, 19, 20]; T cell responses to these proteins are reportedly correlated with the antibody levels [21, 22, 23]. These findings suggest a relation between rs671 and antibody production.

Therefore, the present study aimed to investigate the immune response in a Japanese population, before and after the administration of COVID-19 vaccination, with the hypothesis that there is an inverse relation between *ALDH2* rs671 and antibody production.

2. Materials and Methods

This study was approved by the Ethics Committee for Clinical Research of the School of Medicine Saga University, Saga, Japan (No R2-44 and R3-9). All participants provided written informed consent before undergoing any study procedure.

2.1. Study Design and Participants

The study group comprised 88 participants from hospitals and a university in Saga prefecture, who were invited to be vaccinated with two mRNA vaccines: 62 participants (20 healthcare workers and 42 students) with two doses of BNT162b2 (Pfizer/BioNTech) (30 μg) and 26 participants (26 university employees and students) with mRNA-1273 (Moderna/Takeda) (100 μg). The first dose was scheduled for April and May 2021, and the second dose was administered 21 and 28 days after the first dose for BNT162b2 and Moderna-mRNA-1273, respectively. None of the participants had a history of COVID-19 infection.

2.2. Serological Tests

Blood samples were collected before the first vaccination and every other week after the second vaccination for healthcare workers; likewise, samples were collected before the first dose, three weeks after the first vaccination, and four weeks after the second vaccination for the university employees and students. Serum was extracted from the samples on the same day and stored at −80 °C until analysis. A high-sensitivity chemiluminescent enzyme immunoassay (CLEIA) platform (Sysmex Co., Kobe, Japan) was used to measure the three anti-SARS-CoV-2 antibodies, the S1 sub-unit of the anti-spike protein (S1) IgG, anti-S1-IgM, and anti-nucleocapsid protein (N)-IgG, as described previously [24].

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2.3. *Self-Administered Questionnaire*

A self-administered questionnaire was employed to ask about sex, age, height, weight, smoking status, alcohol intake, exercise habit, perceived stress, and medical histories. Smoking status (yes) was defined as current smoking of cigarette. None of the participants had changed their smoking habits in the past year. Ethanol intake was calculated based on the amount of alcohol consumed in the last six months, adjusted per 60 kg of body weight, and then categorized into < 1 g/day, ≥ 1 g/day, < 20 g/day, and ≥ 20 g/day. Exercise habit was assessed by asking, "Do you usually exercise?," with possible answers including, 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 5-point scale, no (0), mostly no (1), unsure (2), quite often (3), and yes (4). Steroid use was considered as "yes" if the participants were currently receiving steroids; none of the participants who answered "no" had received steroids in the past 3 years. The allergic disease condition was assessed with the question, "Do you have allergic diseases?" Dyslipidemia was considered as "yes" if currently the participants had dyslipidemia; no one who answered "no" had a history of the disease in the past 3 years.

2.3.1. Covariates

Alongside sex, age, vaccine type, and weeks post vaccination, we included body height, smoking status, ethanol intake, exercise, perceived stress, steroid use, allergic disease, and dyslipidemia as covariate attributes suspected to be associated with vaccine efficacy, immune response, or rs671.

2.3.2. Sensitivity Analysis

Out of the 88 subjects, one participant with dyslipidemia was excluded for the sensitivity analysis (87 subjects, 493 data points). We also performed additional analysis using bodyweight or log-transformed body mass index instead of height. The number of observations resulted in 499 data points for the 87 subjects, because the body weight was unknown for one of the participants.

2.4. Genotyping

The ALDH2 genotype (rs671) was determined using the DNA extracted from blood clots as follows. Approximately 0.1 mL of blood clots was incubated in 0.4 mL of proteinase K solution (proteinase K at 1–10 U/mL in 0.01 M Tris-HCl, pH 8 with 0.01 M EDTA and 0.5% Sodium dodecyl sulfate) at 56 °C overnight, then 0.5 mL of TE-saturated phenol was added. After vigorous mixing for 20 s, the samples were incubated on ice for 10 min, followed by centrifugation at 12,000 rpm for 5 min at room temperature (20–25 °C). The aqueous layer was separated, and 0.5 mL of ethanol (95–100%) was added to it, mixed well, and then incubated at room temperature for 10 min. After centrifugation at 12,000 rpm for 10 min, the precipitated DNA was collected by discarding the supernatant. The DNA pellets were washed with 0.25 mL of 70% ethanol, dried, and dissolved in 20–200 μL DNase-free water. The DNA samples were then genotyped using a TaqMan® SNP genotyping assay system following the manufacturer's instructions (ThermoFisher Scientific, Waltham, MA, USA).

2.5. Statistical Analyses

Mixed models were used to compute the association between the rs671 genotype and the log-transformed antibody 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). $p < 0.05$ was considered to indicate statistically significance. To verify the assumption of the additive effect of the rs671 variant allele, the least squares geometric means and standard errors were computed for each genotype, using mixed

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models that included the interactive terms of weeks post vaccination (categorical) and *rs671 genotype (categorical), and graphically presented.

3. Results

3.1. Baseline Characteristics

Table 1 shows baseline characteristics for the 88 participants, including 45 (51%) females, aged 21–56 years, with the confirmation of the rs671 genotype, namely the wild-type homozygous, *ALDH2*1/*1* (GG-type, N = 44), heterozygous, *ALDH2*1/*2* (GA-type, N = 33), and variant homozygous, *ALDH2*2/*2* (AA-type, N = 11). The variant allele frequency was 0.313, and the genotype frequency did not differ from that expected from Hardy–Weinberg equilibrium ($p = 0.5$ by $\chi 2$ test). Daily ethanol intake (g/day) was low in the variant allele carriers (GA- and AA-types); medians and interquartile ranges were 0.4 (0.1-1.2), 0.06 (0-0.41), and 0 (0-0) for GG-, GA-, and AA-types, respectively ($p < 0.0001$ by Spearman rank correlation). The distribution of exercise habits, perceived stress, and allergic disease was not different among the three groups ($p > 0.4$ by Fisher's exact test). Steroid use was reported only by two participants of the GG type, and dyslipidemia by one participant of the AA type.

Table 1. Baseline characteristics of participants for *ALDH2* rs671 polymorphism.

Participants	**Healthcare workers**			**University students**			**University employees and students**		
n	**20**			**42**			**26**		
First dose	**April 2021**			**May 2021**			**May 2021**		
Type of vaccine	**BNT162b2**			**BNT162b2**			**mRNA-1273**		
Second dose	**Three weeks after the first dose**			**Three weeks after the first dose**			**Four weeks after the first dose**		
	GG	GA	AA	GG	GA	AA	GG	GA	AA
Males, n	3	4	3	11	6	5	3	7	1
Females, n	7	3	0	13	7	0	7	6	2
Age, years									
Median (IQR)	42 (36–49)	36 (25–43)	35 (25–40)	22 (22–23)	22 (22–23)	22 (22–23)	39 (22–56)	47 (21–55)	21 (21–42)
Body height, cm									
Median (IQR)	164 (162–169)	168 (153.8–178)	170 (170–176)	165.5 (158–170.5)	162 (156–174)	172 (170–173)	162 (155–166)	166 (163–170)	157 (151–163)
Smoking status, yes	1	1	1	0	0	0	0	1	0
Ethanol intake*									
<1 g/d	1	4	3	12	8	4	6	9	3
≥1, <20 g/d	7	3	0	12	5	1	4	2	0
≥20 g/d	2	0	0	0	0	0	0	2	0
Exercise habit									
No habit	8	2	1	10	4	2	3	4	1
< 1 d/w	0	0	1	5	1	1	2	2	0
1 to 3 d/w	2	2	1	5	6	2	2	4	1

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≥ 3 d/w	0	3	0	4	2	0	3	3	1
Perceived stress									
0 (no)	2	3	1	12	5	1	1	5	1
1	0	0	1	2	1	0	3	1	0
2	3	1	1	4	4	3	2	2	0
3	4	2	0	6	3	1	4	4	0
4 (yes)	1	1	0	0	0	0	0	1	2
Steroid use, yes	0	0	0	2	0	0	0	0	0
Allergic disease, yes	2	4	0	11	3	1	2	6	1
Dyslipidemia	0	0	1	0	0	0	0	0	0

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).

3.2 Antibody Production Post Vaccination

Anti-N IgG levels were below 0.7 SU/mL during the entire observation period for all participants. The medians and respective interquartile ranges of the anti-S1 IgG levels are shown in Table 2. Antibody titers measured in the third week for all participants were distributed in the range of 19–409 BAU/mL in the BNT162b2 group and 46–1369 BAU/mL in the mRNA-1273 group. In the healthcare worker group, antibody titers peaked in the fifth week (two weeks after the second dose) and ranged from 592 to 7895 BAU/mL.

Table 2. Anti-S1 IgG levels (BAU/mL) according to *ALDH2* rs671 genotype.

	Healthcare workers BNT162b2			University students BNT162b2			University employees and students mRNA-1273		
Genotype	GG	GA	AA	GG	GA	AA	GG	GA	AA
Week 0, n	10	7	3	24	13	5	10	13	3
Median (IQR)	0.37 (0.33–0.5)	0.49 (0.45–0.58)	0.41 (0.4–0.53)	0.39 (0.33–0.52)	0.46 (0.35–0.57)	0.35 (0.28–0.39)	0.49 (0.3–0.64)	0.46 (0.41–0.52)	0.34 (0.3–0.62)
Week 1, n	10	7	3						
Median (IQR)	0.62 (0.37–1.76)	0.54 (0.51–1.15)	0.5 (0.37–7.2)						
Week 2, n	10	6	3						
Median (IQR)	64 (44–89)	46 (18–76)	50 (5.71–95)						
Week 3, n	10	6	3	24	13	5	10	13	3
Median (IQR)	187 (101–295)	92 (70–113)	122 (56–153)	132 (75–210)	134 (91–200)	122 (105–135)	372 (290–393)	214 (143–320)	560 (410–572)
Week 4, n	9	6	3						
Median (IQR)	1498 (691–2529)	629 (576–1271)	898 (241–909)						

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Week 5, n	10	6	3						
Median (IQR)	2482 (1901–2667)	1694 (956–2523)	725 (592–1594)						
Week 6, n	10	6	3						
Median (IQR)	1958 (1507–2113)	1372 (918–1975)	564 (558–1350)						
Week 7, n	10	6	3	24	13	5			
Median (IQR)	1592 (1129–1779)	1109 (619–1658)	510 (433–818)	1597 (1198–2269)	1880 (1461–2292)	1339 (1174–1680)			
Week 8, n							10	13	3
Median (IQR)							3200 (2756–3681)	2959 (1661–3593)	2854 (1362–3565)
Week 11, n	10	6	3	24	12	5			
Median (IQR)	761 (493–854)	526 (419–819)	337 (228–523)	940 (560–1080)	859 (534–1133)	632 (572–752)			
Week 15, n	10	6	3	24	12	5			
Median (IQR)	361 (271–486)	341 (300–402)	162 (113–331)	815 (464–1087)	680 (457–1085)	426 (418–677)			
Week 16, n							10	13	3
Median (IQR)							1579 (1220–1740)	1227 (742–1906)	1268 (983–1746)

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.

3.3. Effect of rs671 on Anti-S1 IgG Post Vaccination.

The correlation coefficients for fixed effects that could explain log-transformed anti-S1 IgG, repeatedly measured for 88 subjects, are shown in Table 3. In Model 1, which included the covariates, vaccine type, weeks post vaccination (categorical variable), sex, and age, the partial regression coefficient (β) was estimated to be -0.11 (p = 0.01), which represents a 20% reduction in the IgG levels of the AA-type. The effect of the rs671 allele was estimated to be stronger when height, lifestyle habits other than ethanol intake, and current medical history were included in the models (β = -0.13, p = 0.002 in model 2). Further adjustment for the alcohol intake suggested an even stronger association, with a β value of -0.15 (p = 0.002 in model 3), indicating a 26% reduction in the IgG titers. Although the vaccine type had a strong effect on antibody levels, no interactive effect between rs671 and the type of vaccine was indicated (p for interaction = 0.5 in a model additionally includes the interactive term to model 3).

Sensitivity analysis excluding one participant who had dyslipidemia, resulted in a similar estimation (N = 493, AIC = 976, β = -0.15, p = 0.002 in model 3). A slightly modified model 3, including weight instead of height, produced similar estimates (N = 499, AIC = 989, β = -0.16, p = 0.001), similar to the model using log-transformed body mass index instead of height (N = 499, AIC = 982, β = -0.16, p = 0.001).

Anti-S1 IgM was not associated with rs671 (Table S1).

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Table 3. Estimated correlation coefficients for fixed effects on log-transformed anti-S1 IgG, BAU/mL.

	Model 1 **AIC = 1007** **503 observations** **88 subjects**		**Model 2** **AIC = 993** **503 observations** **88 subjects**		**Model 3** **AIC = 997** **503 observations** **88 subjects**	
Fixed effects	**β**	***p-value***	**β**	***p-value***	**β**	***p-value***
BNT162b2 (reference)						
mRNA-1273	0.53	*0.0004*	0.50	*<.0001*	0.48	*<.0001*
Week 0 (reference)						
Week 1	1.10	*<.0001*	1.09	*<.0001*	-7.72	*<.0001*
Week 2	4.93	*<.0001*	4.92	*<.0001*	-6.63	*<.0001*
Week 3	5.90	*<.0001*	5.90	*<.0001*	-2.80	*<.0001*
Week 4	8.02	*<.0001*	8.02	*<.0001*	-1.83	*<.0001*
Week 5	8.54	*<.0001*	8.54	*<.0001*	0.30	*0.1691*
Week 6	8.34	*<.0001*	8.33	*<.0001*	0.82	*0.0001*
Week 7	8.18	*<.0001*	8.18	*<.0001*	0.61	*0.0041*
Week 8	8.46	*<.0001*	8.46	*<.0001*	0.46	*0.0087*
Week 11	7.49	*<.0001*	7.49	*<.0001*	0.74	*<.0001*
Week 15	7.24	*<.0001*	7.24	*<.0001*	-0.23	*0.1942*
Week 16	7.72	*<.0001*	7.72	*<.0001*	-0.49	*0.0058*
Age (per year)	-0.01	*0.0087*	-0.01	*0.0007*	-0.01	*0.0030*
Female sex	0.21	*0.0013*	0.07	*0.4522*	0.05	*0.6345*
Body height (per cm)			-0.01	*0.2000*	-0.01	*0.1725*
Smoking status			0.20	*0.1138*	0.20	*0.1039*
Ethanol intake (per category)					-0.05	*0.3908*
Exercise habit (per category)			-0.03	*0.2716*	-0.03	*0.2802*
Perceived stress (per category)			0.07	*0.0043*	0.07	*0.0041*
Steroid use			-0.07	*0.7518*	-0.09	*0.6877*
Allergic disease			-0.04	*0.5208*	-0.04	*0.4938*
Dyslipidemia			-1.02	*<.0001*	-1.02	*<.0001*
ALDH2 (per variant allele)	-0.11	*0.0116*	-0.13	*0.0021*	-0.15	*0.0016*

β, Partial correlation coefficient

The least squares geometric means computed using a mixed model depicted the adjusted correlation between the post-vaccination period and the rs671 genotype (Figure 1 and Figure S1). Anti-S1 IgG levels differed most significantly from week 4 to week 6 (Figure 1). The adjusted geometric means of IgG levels at week 5 were estimated to be 2873, 1800, and 1127 BAU/ml for the participants carrying the GG-, GA-, and AA-type alleles, respectively. No such association was found for the IgM levels (Figure S1).

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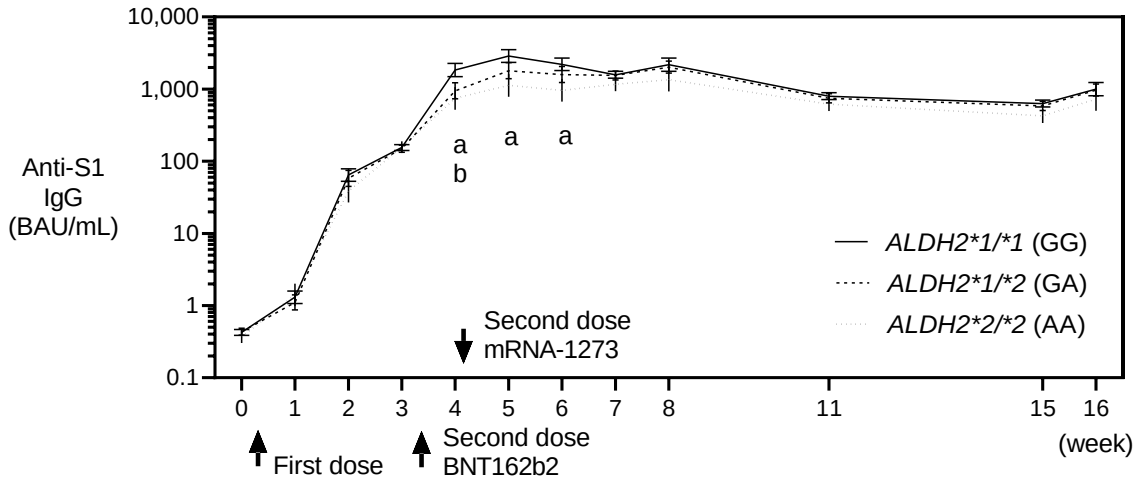

Figure 1. Estimated anti-S1 IgG antibody levels by *ALDH2* rs671 genotype.

Least-squares geometric means and standard errors were computed by a mixed model, which includes all the covariates presented in Table 1, vaccine type, the number of weeks (categorical variables), rs671 genotype, and the interactive terms, rs671*the number of weeks, as fixed effects, and random effects by repeated measures and by the three cohorts. Arrows indicate the time of vaccination. a, $p < 0.05$ for both the comparisons between *ALDH2*1/*1* (GG) and *ALDH2**2/*2 (AA) as well as b, between *ALDH2*1/*1* (GG) and *ALDH2**2/*2 (GA). comparison

4. Discussion

Our study is the first to show the effect of the *ALDH2* polymorphism, rs671, which is carried by nearly half of all East Asians, on vaccine immunogenicity, while no association was found between rs671 and anti-S1 IgM levels. The effect of genetic polymorphisms on immunity after COVID-19 has been well-reported in limited types of genes encoding proteins directly related to the immune response to SARS-CoV-2, such as *ACE* and *HLA* [25, 26]. However, to the best of our knowledge, the genetic polymorphisms, such as rs671, that may strongly affect large populations have never been reported.

In our study, we included multiple covariates to improve the validity and rigor of our statistical assumption based on previous research. Previous studies indicated glucocorticoids, allergy, and ethanol consumption to be associated with vaccine efficacy [13], whereof glucocorticoid is a marker of perceived stress [27]. Exercise is known to affect the immune system [28]. Statin, an effective lipid-modifying drug is suspected to be associated with clinical outcomes of COVID-19 [29, 30]. Additionally, rs671 is reportedly associated with cigarette smoking behavior [31, 32], with a strong effect on drinking behavior, as confirmed in our cohort.

The results of this study should be interpreted with careful consideration of a few limitations. Most importantly, the number of subjects used in the study was low. In particular, the estimated IgG levels at weeks 4, 5, and 6 post vaccination, which were most affected by rs671, were based on the measurements taken from health workers (20 subjects). Furthermore, the number of participants that carried homozygous variants was small (3 health workers and 11 participants overall); we tried to address this issue by

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computing a linear regression between the number of variant alleles and the log-transformed IgG values. Another important limitation is the generalizability; personalizing medication strategies is more important in immunogenically disadvantaged populations such as for patients with autoimmune diseases or those on medication with immunosuppressive drugs [23]; however, this study only included healthy Japanese students and workers.

There are several possible mechanisms for the inhibition of the increase in post-vaccination IgG by rs671. First, since our previous study suggested the suppression of T-cell mediated immune response in patients with thoracic malignancies carrying the rs671 variant allele [11], it can be speculated that the current findings are a consequence of the functional disturbance in CD4+ T cells. The absence of such an effect on anti-S1 IgM is consistent with this speculation because IgG is produced by class-switched memory B cells which are triggered by cytokines released by CD4+ T cells, while IgM production is independent of this signaling. Moreover, we reported that the rs671 variant allele and CD4+ T cell count are inversely related in Japanese workers (N = 328 with 48% of males, *p trend = 0.07*, by a generalized linear model including covariates of sex, age, year of survey, alcohol consumption, rs671, and the interactive term of alcohol consumption*rs671) (conference proceeding) [12]. Therefore, the association between the anti-S1 IgG and rs671 could be because of the low CD4+ T cell count, thus requiring further validation. Second, Brunsdon *et al*. (2022) reported that ALDH2 deficiency delays melanocyte differentiation owing to a deficiency of endogenous formic acid (a metabolite of endogenous formaldehyde), which is required for nucleic acid synthesis [33]. Furthermore, it is well-known that progenitor hematocytes express ALDH [34], and there has been evidence that indicates dependency of those cells on ALDH2 among other ALDH isozymes [35]. These findings indicate that ALDH2 deficiency may inhibit the differentiation of naïve T cells to effector T cells, which promotes the class-switching of B cells, consequently accelerating the increase in IgG production.

5. Conclusions

Our study indicated for the first time that the variant allele of *ALDH2*, rs671, which is prevalent in East Asians, is associated with the attenuated immunogenicity of the COVID-19 mRNA vaccine. However, further experiments are necessary to elucidate the mechanistic details of the effects of this allele on CD4+ T cell functioning. This finding may provide evidence for personalized medicine based on a common genetic polymorphism, in addition to promoting a basic understanding of immunology. While this study focuses on healthy individuals, further research on the immune responses in larger, more varied populations, including subjects with prior health conditions, could lead to a better understanding of vaccine immunogenicity.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Figure S1: Estimated anti-S1 IgM antibody levels for ALDH2 rs671 genotype; Table S1: The estimated correlation coefficients for fixed effects on log-transformed anti-S1 IgM, BAU/mL.

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