Analysis of the Interferon

by Zen Hafy

Submission date: 29-Apr-2023 09:17AM (UTC+0700)
Submission ID: 2078852693
File name: Analysis_of_the_Interferon_Lambda_3_and_4_Gene.25.pdf (386.91K)
Word count: 3399
Character count: 18041

Original Article

Analysis of the Interferon Lambda 3 and 4 Gene Single Nucleotide Polymorphisms and Vaccine Response against COVID-19

Zen Hafy, Ziske Maritska[1], Susilawati[2], Ella Amalia[3], Veny Larasati, Soilia Fertilita

Departments of Histology, [1]Biology Medicine, [2]Parasitology and [3]Microbiology, Faculty of Medicine, Sriwijaya University, Indonesia

Abstract

Introduction: Assessing how various COVID-19 vaccines work against SARS-CoV-2 in different individuals is pivotal to planning better management and coping with the pandemic. Single nucleotide polymorphisms (SNPs) are one of the contributing factors to the immune response. This study investigated interferon lambda 3 and 4 (IFNL3/4) gene polymorphism and their association with an individual's immune response after receiving COVID-19 vaccines. **Materials and Methods:** An exploratory laboratory study to identify SNPs rs1297860 and rs368234815 in IFNL3/4 genes in Indonesian who have received two shots of CoronaVac and further evaluate its association with the COVID-19 vaccine response. The immune response was reflected from the serum titres of SARS-CoV-2 IgG (anti-spike IgG level), quantified using the SARS-CoV-2 IgG II Quant assay, where the IFNL3/IFNL4 SNPs identified using polymerase chain reaction restriction fragment length polymorphism. **Results:** From March to August 2021, this study recruited 47 eligible and healthy persons. None of the subjects in this study have the assumed associated genotypes (TT in IFNL3 or DG/DG in IFNL4). There was also no significant difference in the Mean Fold Rise of anti-spike IgG level between individuals with IFNL3 rs12979860 C/T polymorphism (CT genotype) and those with the homozygous common (wild-type) genotype (TT genotype) ($U = 358$; $P > 0.05$). **Conclusion:** The frequency of IFNL3/4 polymorphisms in this study population was low. Furthermore, the IFNL3/4 polymorphisms do not affect immune response (anti-spike IgG level) in individuals receiving two shots of the COVID-19 vaccine in this study.

Keywords: COVID-19, immune response, single nucleotide polymorphism, vaccine

Introduction

Infection with SARS-CoV-2 that first emerged in China in late 2019 (later referred to as COVID-19) has become a major global threat. It spread vastly and was declared a pandemic by the World Health Organisation (WHO) on 11 March 2020. As of June 2022, more than 58 million confirmed cases were reported in the WHO South-East region.[1] Its wide clinical spectrum which is not always in the form of severe complications leading to death, but also in the form of mild symptoms or even asymptomatic, becomes one of the causes of difficulty stemming the spread of this disease.[2]

Accelerating the launch of various types of COVID-19 vaccines and mandatory vaccinations implemented in many countries has been reported to be able to reduce the number of severe cases and reduce the number of deaths.[3] Vaccination is expected to increase the levels of neutralising antibodies (IgG) among those who have been vaccinated, forming immunity as a result.[4] The effectiveness of the vaccine can be seen from the immune response developed in the form of an increase in antibody titres after the administration of the vaccine. However, the increase in titre of antibodies may vary from person to person. Many factors that affect immune responses against vaccines include the

Access this article online

Quick Response Code:

Website: www.aihbonline.com

DOI: 10.4103/aihb.aihb_113_22

Address for correspondence: Dr. Zen Hafy, Department of Histology, Faculty of Medicine, Universitas Sriwijaya, Jl. Dr. Mohammad Ali, Palembang, South Sumatera 30128, Indonesia.
E-mail: zenhafy@gmail.com

Submitted: 16-Jun-2022 **Revised:** 31-Jul-2022
Accepted: 01-Aug-2022 **Published:** 23-Sep-2022

How to cite this article: Hafy Z, Maritska Z, Susilawati, Amalia E, Larasati V, Fertilita S. Analysis of the interferon lambda 3 and 4 gene single nucleotide polymorphisms and vaccine response against COVID-19. Adv Hum Biol 2023;13:135-8.

quality of the vaccination programme (type of vaccine used, age at first vaccination, the interval between doses, the potential for vaccine use, formulation and stability, vaccine administration and schedule of vaccination) and host-related factors such as age, sex, smoking habit, diet, presence of co-morbidities such as diabetes and hypertension and also one's genetic makeup.[5,6]

Several studies have shown that some single nucleotide polymorphisms (SNPs) contained in genes involved in the physiological process of the immune response such as the Type III interferon, also known as interferon lambdas (IFNLs), not only affect the treatment response of infectious diseases such as influenza and Hepatitis C virus but also affect the response to increased antibody levels after vaccination against the virus.[7] Homozygous variants of rs1297860 (C/T) in IFNL3 and rs368234815 (TT/DG) in IFNL4 have been reported to be strongly associated with viral clearance in children with acute viral infection in Africa,[8] and related to COVID-19, individuals with homozygous rs368234815 DG/DG showed higher viral load which is thought to be related to the defective upregulation of inflammatory pathways.[2]

There has been no study evaluating the effect of SNPs on the IFNL genes on the development of humoral response after two doses of COVID-19 vaccination. The present study aimed to examine the association between SNPs rs1297860 and rs368234815 in IFNL3/4 genes to SARS-CoV-2 anti-spike receptor-binding domain IgG level in individuals who have received the COVID-19 vaccine in Indonesia.

Materials and Methods

Study population

This research was performed at the Faculty of Medicine, Sriwijaya University (FK Unsri) Palembang, Indonesia from March 2021 to August 2021. A total of 46 healthy adult subjects (age range 20–59 years) who received two doses of inactivated COVID-19 vaccine CoronaVac (the most widely administered COVID-19 in Indonesia) from several institutions in Palembang were included in the study. Recruited subjects did not have a history of autoimmune disease and a history of previous COVID-19 infection confirmed by the polymerase chain reaction (PCR) or antigen results, and did not show a reactive antibody test result at the time of screening. All subjects provided written informed consent before enrolment. This study was approved by the Faculty of Medicine's Unsri Research Ethics Board with protocol No. 062-2021 on 23 March 2021.

Sample collection

Approximately 5 ml of venous blood was collected on day 13 after the first dose vaccination to measure SARS-CoV-2 anti-Spike IgG antibody titre at 13 days after the first vaccination. Another 5 ml of blood was collected on day 30 after the second dose vaccination to measure anti-Spike IgG antibody titre and to obtain whole blood DNA for SNPs detection.

Total antibody test for SARS-Cov-2

The serum titres of SARS-CoV-2 IgG (anti-spike IgG level) of all subjects were quantified using the SARS-CoV-2 IgG II Quant assay, a Chemiluminescent Microparticle Immunoassay for determining of antibodies to SARS-CoV-2 in serum and plasma (Abbott Diagnostics, Chicago, IL, USA). We set the cutoff of IgG reactivity (seropositivity) at 50 AU/ml according to the manufacturer's instruction. Results below 50 AU/ml were considered non-reactive. The reactive results were classified as high if their anti-Spike IgG titre were >2150 AU/ml, referring to the plaque reduction neutralisation test (PRNT) where the anti-spike IgG level of 2150 AU/ml is considered to be approximately 80% effective in preventing infection.[9] Seroconversion rate is defined as at least a four-fold increase in anti-spike IgG titres after the 2nd dose over baseline (the anti-spike IgG titre after the 1st dose).

DNA extraction and interferon lambda 3/interferon lambda 4 single nucleotide polymorphisms identification

Genomic DNA was extracted from 200 µl of the whole blood using the QIAamp® DNA Mini Kit (QIAGEN, Germany) according to the manufacturer's recommendation and was stored at − 20°C. The IFNL3 SNP rs12979860C/T and the IFNL4 SNP rs368234815 TT/DG were identified using the PCR amplification followed by restriction fragment length polymorphism assay as previously described.[10,11] For the IFNL3 rs12979860, the extracted DNA was amplified using the following PCR primer pairs; forward: 5'-GCTTAT CGCATACGGCTAGG-3', and reverse: 5'-AGGCTCAGGGTCAATCACAG-3. Then, the 142 bp amplification products were incubated overnight with the Bsh12361 (BstUI) endonuclease enzyme to detect the rs12979860C/T SNP. For IFNL4 rs368234815, the PCR primers were: 5'-GACGCAGGACCC CTTGGGACAGGA-3' (forward) and 5'-TCTGGGCCGCAGTGGCCGCGAGG-3' (reverse). Then, the 227 bp PCR products were introduced into the MspA1 endonuclease enzyme to detect polymorphism at rs368234815.

Statistical method

Statistical analyses were performed using SPSS version 26.0 (IBM SPSS Statistics for Windows, Version 26.0. Chicago, IL: IBM Corp). A Shapiro–Wilk normality test was performed for the continuous variables. Mann–Whitney *U*-test was applied to compare whether there is a difference in anti-spike IgG response between independent genotype groups. IgG response is measured by looking at the total increase in IgG levels after the second dose of vaccination from the IgG value after the first dose of vaccination. Two-tailed $P > 0.05$ was considered statistically significant.

Results

In the present study, blood samples from 46 participants who received the first and second doses of COVID-19 vaccination were collected. Of them, 20 (43.5%) participants were male and 26 (56.5%) were female. The average anti-spike IgG serum

level after the first dose and the second dose vaccination was 221.6 ± 507 AU/ml and 1480 ± 2463 AU/ml, respectively. Of the 46 subjects, 22 subjects (47.8%) showed a reactive anti-spike serum IgG level (>50 AU/ml) after the first dose, and two of them already had titres above 2000 AU/mL. After the second dose of vaccination, all the subjects showed a reactive serum anti-spike IgG level, and five of them a protective anti-spike IgG titres (>2150 AU/ml). The seroconversion rate in this study population after receiving the 2nd dose of vaccination was 84.8% [Table 1].

Association of interferon lambda 3/interferon lambda 4 single nucleotide polymorphisms with anti-spike IgG response

The most prevalent genotype for IFNL3 rs12979860 identified in our population was CC (89.1%), followed by the genotype CT (10.9%). None of the participants has the genotype TT. The frequency of allele C in this study population was 94.6%. Based on the Mann–Whitney test, there was no significant difference in the Mean Fold Rise (MFI) of anti-spike IgG response between subjects with IFNL3 rs12979860 C/T polymorphism (CT genotype) and subjects with the homozygous common (wild-type) genotype (TT genotype) (U = 358; P > 0.05). Of the 46 enrolled subjects, two subjects reported with rs368234815 TT/DG genotype (4.4%) and 44 subjects with TT/TT genotype (95.6%). The two subjects with TT/DG genotype are also harbouring the rs12979860 CT genotype [Table 2].

Discussion

Vaccination against SARS-CoV2 is believed to be one of the main keys to suppressing the spread and reducing morbidity and mortality of COVID-19. Vaccination is expected to trigger the emergence of antibodies against SARS-CoV2, especially anti-spike can inhibit the interaction between the virus S protein and the ACE receptor on the human cell surface.[12] Someone who has received at least two doses of the COVID-19 vaccination is expected to have levels of anti-spike IgG that can provide a protective effect. In this study, seroconversion (a four-fold increase of IgG titre) was detected in all the subjects after the second dose of vaccination. A similar result was reported by other COVID-19 antibody response studies in Brasil and Chile.[13,14] Referring to the PRNT, the anti-spike IgG level of 2150 AU/ml is considered to be approximately 80% effective in preventing infection.[9] This study found that only a small number of subjects (<13%) could achieve IgG levels of more than 2150 AU/ml after the second dose of CoronaVac.

Genetic background can affect someone's immune response to viral infections or vaccinations. The polymorphisms rs 1297860 C/T in IFNL3 and rs 358234815 in IFNL4 have been associated with a reduction of viral clearance in children with acute respiratory infections in Africa.[8] A study conducted in Italy suggested that COVID-19 patients with homozygous polymorphic status of IFNL3 TT and IFNL4 DG/DG showed a higher viral load or a lower ability in SARS-CoV2 clearance[2,15] This is the first study to examine the effect of genetic polymorphisms within the *IFNL3/IFNL4* genomic region on antibody response to COVID-19 vaccination. The results indicated that the MFI of anti-spike IgG titre after second dose vaccination in the subjects with the rs1297860 C/T polymorphism (CT genotype) is lower than the MFI in the subjects with the common genotype (TT), but the difference is not statistically significant. We only identified two subjects with the rs368234815 (TT/DG) polymorphism in this study, and interestingly, both subjects are also with the rs1297860 C/T polymorphism. It was reported that there is a high linkage disequilibrium between the two genotypes, which is linked to a lower ability in viral clearance.[2,16] Unfortunately, this study could not analyse if this haplotype might affect the MFI of the subjects due to the small sample size.

At least three things that need to be considered from the results of this study; First, the COVID-19 vaccine assessed in this study was CoronaVac that based on the previous reports and results of this study showed low effectiveness in triggering an increase in anti-Covid-19 antibodies after administration of the second dose. Second, none of the subjects had homozygous polymorphic genotypes, either IFNL3 TT or IFNL4 DG/DG which were the problematic genotypes as reported by the previous studies.[2][8] Third, frequency of the polymorphisms, especially the rs368234815 (TT/DG) in IFNL4 in this study population were quite low which may affect the analysis result.

Nonetheless, this is the first study to identify the frequency of the rs1297860 C/T polymorphism in IFNL3 in the Indonesian population. A genome-wide association study involving more than 1600 subjects with hepatitis C from different populations in the world demonstrated that the frequency of rs1297860

Table 1: Baseline characteristics and seroresponse

	n (%)
Sex (*n*=46)	
Male	20 (43.5)
Female	26 (56.5)
Age (years)	32±12
BMI (kg/m^2)	20.8±4
Comorbidities	
Hypertension	7 (15.2)
Diabetes	1 (2.2)
Autoimmune diseases	0
Anti-spike IgG	
After the 1st dose	
Average IgG titer (mean±SD)	221.6±507
Seropositive	22 (47.8)
High IgG titre	1 (2.1)
After the 2nd dose (AU/ml)	
Average IgG titer (mean±SD)	1480±2463
Seropositive	46 (100)
High IgG titre	5 (10.9)
Seroconversion rate	39/46 (84.8)

BMI: Body mass index, SD: Standard deviation

Table 2: Genotypes and antibody responses

	rs12979860 IFNL3		rs368234815 IFNL4	
	CC	CT	TT/TT	TT/DG
Frequency, *n* (%)	41 (89.1)	5 (10.9)	44 (95.6)	2 (4.4)
Anti-spike IgG				
After the 1st dose (AU/ml)				
Average IgG titer (mean±SD)	236.3±535	101.04±77	226.4±517	115.5±123
After the 2nd dose (AU/ml)				
Average IgG titre (mean±SD)	1578.2±2590	675.4±497	1526.3±2509	459.6±98
MFI (mean±SD)	26.9±30	10.1±7	25.8±29	10.4±12
U, P	358, >0.05			

MFI: Mean fold increase, SD: Standard deviation

C allele in the IFNL3 gene is lower in Africa, Europe and South-west Asia populations compared to the frequency in South Asia and South East Asia populations.[15] It was reported that rs1297860 C allele frequency in the Cambodians and the Laotians was 97.9% and 93.6%, respectively which is comparable to the frequency of allele C obtained in this study (94.6%).

Conclusion

The study suggested that the rs1297860 C/T polymorphism in IFNL3 does not affect antibody response (anti-spike IgG titre) following the second dose COVID-19 vaccination. The frequency of the rs1297860 C/T IFNL3 and the rs368234815 (TT/DG) IFNL4 polymorphisms in the Indonesian population is low and the heterozygotes polymorphic genotypes are dominant.

Acknowledgement

The authors would like to thank Competitive grant from Universitas Sriwijaya, Palembang, Indonesia.

Financial support and sponsorship

This study was financially supported by Competitive Grant Universitas Sriwijaya.

Conflicts of interest

There are no conflicts of interest.

References

1. World Health Organization. Coronavirus Disease Situation Dashboard. Available from: https://covid19.who.int. [Last accessed on 2020 Jun 07].
2. Amodio E, Pipitone RM, Grimaudo S, Immordino P, Maida CM, Prestileo T, *et al.* SARS-CoV-2 viral load, IFNλ polymorphisms and the course of COVID-19: An observational study. J Clin Med 2020;9:3315.
3. World Health Organization. Statement for Healthcare Professionals: How COVID-19 Vaccines are Regulated for Safety and Effectiveness; 2021. Available from: https://www.who.int/news/item/17-05-2022-statement-for-healthcare-professionals-how-covid-19-vaccines-are-regulated-for-safety-and-effectiveness. [Last accessed on 2022 Jun 07].
4. Duc Ha N, Duc M, Minh Thong P. The current update of vaccines for SARS-COV-2. Electron J Gen Med 2020;17:2-5.
5. Patarčić I, Gelemanović A, Kirin M, Kolčić I, Theodoratou E, Baillie KJ, *et al.* The role of host genetic factors in respiratory tract infectious diseases: Systematic review, meta-analyses and field synopsis. Sci Rep 2015;5:16119.
6. Kaur SP, Gupta V. COVID-19 vaccine: A comprehensive status report. Virus Res 2020;288:198114.
7. Linnik JE, Egli A. Impact of host genetic polymorphisms on vaccine induced antibody response. Hum Vaccin Immunother 2016;12:907-15.
8. Rugwizangoga B, Andersson ME, Kabayiza JC, Nilsson MS, Ármannsdóttir B, Aurelius J, *et al.* IFNL4 genotypes predict clearance of RNA viruses in rwandan children with upper respiratory tract infections. Front Cell Infect Microbiol 2019;9:340.
9. Abbott Diagnostics. Architect SARS-COV-2 IgG II Quant Instructions for Use, H18566R01. USA: Abbott Diagnostics, IL; USA 2020.
10. Daneshvar M, Nikbin M, Talebi S, Javadi F, Aghasadeghi MR, Mahmazi S, *et al.* Role of IL28-B polymorphism (rs12979860) on sustained virological response to pegylated interferon/ribavirin in Iranian patients with chronic hepatitis C. Iran Red Crescent Med J 2016;18:e28566.
11. Pouryasin M, Sharafi H, Behnava B, Alavian SM, Keshvari M, Pouryasin A. A simple PCR-RFLP method for genotyping of IFNL4 rs368234815 polymorphism in patients with chronic hepatitis C. Lab Med 2017;48:51-6.
12. Robek MD, Boyd BS, Chisari FV. Lambda interferon inhibits hepatitis B and C virus replication. J Virol 2005;79:3851-4.
13. Dai L, Gao GF. Viral targets for vaccines against COVID-19. Nat Rev Immunol 2021;21:73-82.
14. Fonseca MH, de Souza TF, de Carvalho Araújo FM, de Andrade LO. Dynamics of antibody response to CoronaVac vaccine. J Med Virol 2022;94:2139-48.
15. Sauré D, O'Ryan M, Torres JP, Zuniga M, Santelices E, Basso LJ. Dynamic IgG seropositivity after rollout of CoronaVac and BNT162b2 COVID-19 vaccines in Chile: A sentinel surveillance study. Lancet Infect Dis 2022;22:56-63.
16. Thomas DL, Thio CL, Martin MP, Qi Y, Ge D, O'Huigin C, *et al.* Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. Nature 2009;461:798-801.

Analysis of the Interferon

ORIGINALITY REPORT

23%	18%	22%	11%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Phey Liana, Zen Hafy, Soilia Fertilita, Ella Amalia, Veny Larasati, Tungki Pratama Umar. "The Polymorphisms of TNF-α Related-gene and Antibody Production Following Third Dose of COVID-19 Vaccination: A Pilot Study.", Vacunas, 2023 Publication	4%
2	virologyj.biomedcentral.com Internet Source	3%
3	iapindia.org Internet Source	3%
4	Submitted to Erie Community College Student Paper	2%
5	Hiroaki Ikezaki, Hideyuki Nomura, Nobuyuki Shimono. "Dynamics of anti-Spike IgG antibody level after the second BNT162b2 COVID-19 vaccination in health care workers", Journal of Infection and Chemotherapy, 2022 Publication	2%
6	qspace.qu.edu.qa Internet Source	2%

7	www.medrxiv.org Internet Source	2%
8	hypothes.is Internet Source	1%
9	www.researchsquare.com Internet Source	1%
10	bmcinfectdis.biomedcentral.com Internet Source	1%
11	Harika Oyku Dinc, Nese Saltoglu, Gunay Can, Ilker Inanc Balkan et al. "Inactive SARS-CoV-2 vaccine generates high antibody responses in healthcare workers with and without prior infection", Vaccine, 2021 Publication	1%
12	Submitted to Universitas Indonesia Student Paper	1%
13	link.springer.com Internet Source	1%
14	trepo.tuni.fi Internet Source	1%
15	www.dovepress.com Internet Source	1%
16	Çetin KILINÇ, Melike YAŞAR DUMAN, Büşra ÇALIŞIR, Nilay ÇÖPLÜ et al. "Evaluation of anti-spike IgG response after inactivated	1%

COVID-19 vaccine in healthcare workers", Kastamonu Medical Journal, 2022

Publication

Exclude quotes	On	Exclude matches	< 1%
Exclude bibliography	On		